EFFICACY OF DIFFERENT VACCINATION PROGRAM IN BROILER CHICKEN AGAINST INFECTIOUS BURSAL DISEASE

A THESIS

BY

A.T.M. IFTAKHER MORSHED
Registration No. 1505038
Session: 2015-2016
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MASTER OF SCIENCE (MS)

IN

MEDICINE

DEPARTMENT OF MEDICINE, SURGERY AND OBSTETRICS
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

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[Submitted to the Department of Medicine, Surgery and Obstetrics, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur for partial fulfillment of the requirement of the degree]

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JUNE, 2017

Dedicated to.....

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

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The Author

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ABSTRACT

An experiment was conducted to check the maternally derived antibody in offspring, compare the effect of existing and imposed vaccination program and compare the immune status induced by vaccine under both vaccination programs. Seven different broiler farms in the Pabna District were taken dividing the farms into two groups- Group I for existing vaccination and Group II for imposed vaccination. Blood samples were collected from all the experimental farms before vaccination to check maternal antibody level. Infected as well as dead birds were undergone through necropsy examination properly in spot as well as Nourish Poultry Diseases Diagnostic Laboratory, Unit-2, Bogra, Bangladesh. The present study revealed that chicken aged between 17-25 days was affected with IBDV with 38-57% morbidity and 11.7-33.5 % mortality, Concurrent occurrence of IBD with colibacillosis and other diseases like salmonellosis, coryza, newcastle disease and aspergillosis was noticed in four farms of group I, the birds that survived the diseases lost weight ranges from 1185-1370 g (Group I) than those did not face Gumboro 2115-2228 g (Group II). Thus, there is a significant variation in body weight in Gumboro affected broilers due to existing and imposed vaccination programme under farm condition.

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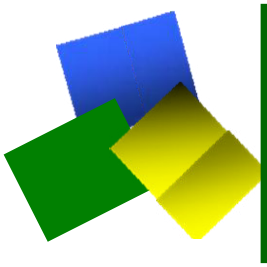
AA	Amino acid
Ab	Antibody
AC-ELISA	Antigen-capture enzyme-linked Immunosorbent assay
AGID	Agar gel immunodiffusion test
Bp	Base pair
BF	Bursa of Fabricius
BALT	Bronchial-associated lymphoid tissues
CALT	Conjunctiva-associated lymphoid tissues
CAM	Chorioallantoic membrane
CEB	Chicken embryo bursa
CEF	Chicken embryo fibroblasts
CEKC	Chicken embryo kidney cell
CMI	Cell mediated immunity
CPE	Cytopathic effect
CSA	Central statistical agency
DFA	Direct Fluorescent-Antibody Assay
ELLISA	Enzyme linked Immunosorbent Assay
FAO	Food and agriculture organization
GALT	Gut-associated lymphoid tissues
GC	Germinal centre
GMEM	Glasgow minimum essential medium
HBSS	Hanks balanced salt solution
IBD	Infectious Bursal disease
IBDV	Infectious Bursal disease virus
Ig	Immunoglobulin
IFA	Immunofluorescence Assay
IFN	Interferon
IIF	Indirect immunofluorescence on section of infected organs
IL	Interleukin
ILTV	Infectious laryngo tracheitis virus
MAB	Maternal antibody
MDAB	Maternally derived antibodies

MHC	Major Histocompatibility complex class
NCD	Newcastle disease
NO	Nitric oxide
NVI	National Veterinary Institute
NCD	Newcastle disease
NOS	Nitric oxide synthesis
OD	Optical density
OIE	International Animal Health Organization
ORF	Open reading frames
PBS	Phosphate buffered saline
PCR	Polymerase Chain Reaction
PI	Post infection
qRT-PCR	Quantitative real time RT-PCR
RE	Restriction enzymes Digestion with different
RNA	Ribonucleic acid IBD
RFLP	Restriction fragment length polymorphism.
RPM	Rotation per minute
RT-PCR	Reverse transcription polymerase chain reaction
SAN	Specifically IBDV antibody free
SNNP	Southern Nation and Nationalities of people
S/P	Sample positive
SPF	Specific pathogens free chickens
SsRNA	Single stranded RNA
TCID50	Tissue culture infective dose 50
USA	United States of America
VIBD	Virulent IBDV
VNT	Virus Neutralization Test
VP	Virus Protein VP
VP2	Virus Protein2
VP1-VP4	Virus Protein1-4
VvIBDV	Very Virulent Infectious bursal disease virus
WRL	World Referral Laboratory



CHAPTER I

INTRODUCTION

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INTRODUCTION

Poultry sector is one of the most important sectors of agriculture in Bangladesh. It plays a vital role in meeting the protein demand, creates employment opportunity and alleviates poverty. Contribution of poultry meat is about 37% of total animal protein supply of Bangladesh (Ahmed, 2008). Poultry industry can contribute to the GDP growth rate by managing food security as well as ensuring employment and reducing poverty at a large scale. Poultry meat and egg production provides more than 30% of all animal protein worldwide and it is increasing day by day (Salauddin *et al*., 2012). Poultry meat especially chicken meat is the most desirable animal protein and is acceptable to most of the people belonging to all castes and religions. Per capita requirement for meat in Bangladesh is 120 g meat/day but per capita consumption is only 16.5 g/day with a deficiency of 50.15 % (Amin, 2005). The availability is quite inadequate for normal growth and development of the body. Whereas, world per capita consumption of poultry meat is 30.14g /day, which is 95.89 g/day for USA (Farrell *et al.*, 2003). Per capita meat consumption in Bangladesh was 5.1, 5.2 and 5.3kg for the years 1998-1999, 1999-2000 and 2000-200lrespectively (BBS, 2002).

Poultry production in tropical countries is based on the traditional scavenging system and chickens are the most important poultry species (FAO, 2000). In fact, 80% of the total poultry population in the world is in traditional village-based production systems, being "low input–low output" systems (Permin *et al.,* 2000). Rural poultry is the dominant form of poultry production in the developing world. In some countries, scavenging and backyard chicken production systems are more important than the modern intensive poultry production (Hailu *et al.,* 2009). Therefore, intensification and upgrading of the potential of poultry will be inevitable to provide surplus products (Hailemariam *et al.,* 2006). However the industry is confronted with a variety of problems, particularly the diseases of viral origin.

Infectious bursal disease (IBD) and Newcastle disease (NCD) have remained as two most important infectious diseases threatening the village chicken and commercial poultry production in most parts of the world (El-Yuguda *et al.,* 2005; El-Yuguda *et al.,* 2009). Infectious bursal disease and also known as Gumboro disease is acute highly

contagious infectious and immunosuppressive (Rauf, 2011) viral disease of young susceptible chicks (Lukert and Saif, 1997 and Hair-Bejo *et al.,* 2004). It affects the poultry industries worldwide (Toro *et al.,* 2009) caused by an RNA virus, infectious bursal disease virus (IBDV) (Mahgoub, 2012; Muller *et al.,* 2012) with a primary target organ of the bursa of Fabricius (Abu-Tabeekh and Al-Mayah, 2009). IBD virus remains infectious for a very long period of time and has resistance to commonly used disinfectants (OIE, 2012). The emergence of antigenic variant as well as very virulent strains in vaccinated flocks considerably stimulated research efforts on both, IBD and IBDV. The disease causes heavy economic losses in poultry industries due to immunosuppression in subclinical cases (Jackwood and Sommer-Wagner, 2010) and in acute cases; it is associated with mortalities, haemorrhages and also bursal damage (Jackwood *et al.,* 2009). The disease is mainly affects young chickens of between 3 and 6 weeks of age characterized by enlarged bursa of fabricus, watery diarrhoea, accumulation of urate in the urinary structure, and severe depression (Saif and Barnes, 2003). In Bangladesh, poultry farmers first tasted a better experience of high morbidity (about 100 %) and mortality (80 %) from IBD causing serious losses (Chowdhury *et al.,* 1996 and Rahman *et al.,* 1996). IBD is a clinical disease solely in chickens but also turkeys, ducks, guinea fowl and ostriches may be infected.

Vaccination of breeding hens with live attenuated or inactivated virus vaccine most effectively controlled the disease. Induced antibodies are transferred to the young chicks via the egg yolk and protect the newly hatched chicks for the critical first few weeks of their life (Wyeth *et al.,* 1976). No vaccine against IBDV has yet developed in Bangladesh using local isolates. To meet the requirement, several live and killed vaccines against IBDV are being imported in Bangladesh from abroad. There are many vaccine manufacturing companies and they have their own specification about utilization of vaccine and the farmers used these vaccines in commercial poultry farms from day old to onward, without knowing knowing the status of maternally derived antibody (MDA) in offspring. Knezevic *et al.,* (1999) mentioned that vaccination of day old chicks with high level of MDA against IBD virus failed to produce primary immune response. However, revaccination provoked delayed primary immune response. So, it is very important to know the persistence of MDA in offspring from vaccinated parent stock (PS). Despite of extensive use of vaccines, outbreaks of gumboro are still recorded in Bangladesh. The possible cause of outbreak in the immunized flocks were failure of

vaccine to protect chicken against virulent type of virus strain (Zorman *et al.,* 1997), MDA interference, poor husbandry, improper vaccination (Zhuo-ZhengQi, 1998), improper storage and failure of maintenance of cold chain, antigenic diversity among the vaccine strains and local field strains or timing of vaccination of chickens depending on the degree of MDA (Voss *et al.,* 1994). As the vaccination is effective means of prevention of the IBD in chicken, it is very important to know when the vaccine should be administered to the chicken and the level of antibody production induced by live vaccine and how long it gives protection to the chicken against the disease.

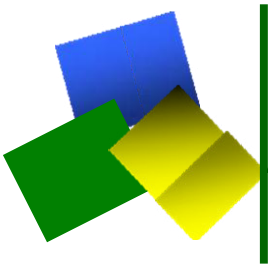
IBDV can be detected by detection of viral antigen or indirectly by detection of antibody in the serum. Direct detection is performed by direct enzyme linked immunosorbent assay (ELISA), polymerase chain reaction, cloning, sequencing, latex agglutination test and tissue culture and also by propagating in chicken embryo. Antibody or directed detection is performed by indirect ELISA (IELISA), serum neutralization test, agar gel precipitation test, fluorescent antibody technique (FAT), passive hemagglutination test, plaque reduction assay, dot blot hybridization assay, coagulation test and western blotting etc.

Despite of extensive use of different types of vaccines the disease is prevailed in our country especially in study area and economically loosing of farmers. Why they are facing the disease? Is there any problem in their managment system or in vaccination program? Considering the above mentioned facts the present study was designed with the following specific objectives.

i. Detection of the maternally derived antibody in offspring against the disease.

ii. Determination of the effect of existing and imposed vaccination program on prevention of disease under farm condition.

iii. Comparative study of the immune status of chicken induced by vaccine used under both vaccination programs.



CHAPTER II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

The available literatures, pertinent to the present study, are reviewed in this part of the thesis with special emphasis on the immunosuppression, effect of IBD and antibody titre of infectious bursal disease (IBD) after a brief overview on the epidemiology, clinical manifestations, pathology and diagnosis of IBD.

2.1 History and distribution of infectious bursal disease virus

Infectious bursal disease (IBD) was first recognized as a distinct clinical entity in 1957 (Cosgrove, 1962). Cosgrove initially described the malady as "avian nephrosis" on account of the tubular degenerative lesions found in the kidneys of infected broiler chickens. The syndrome adopted the name "Gumboro disease" since the first outbreaks occurred in and around the area of Gumboro, Delaware, USA. Predominant signs of illness included trembling, ruffled feathers, watery diarrhoea, anorexia, depression, severe prostration, and death. In addition, hemorrhages in the thigh and leg muscles, increased mucus in the intestine, liver lobe infarction, renal damage, and enlargement of the bursa of Fabricius were lesions commonly observed at necropsy (Cosgrove, 1962). Early studies suggested that the causative agent was a nephropathogenic strain of infectious bronchitis virus due to similar gross changes observed in the kidney by (Winterfield and Hitchner, 1962). Subsequent studies (Pejkovski *et al.,* 1979) however, revealed that IBV immunized birds could still be infected with the "infectious bursal agent" (IBA) and develop changes in their cloacal bursas specific for the disease. Following successful isolation of IBA in embryonated chicken eggs (Hitchner, 1970), proposed that the disease be termed "infectious bursal disease" due to its pathognomonic bursa lesions.

The immunosuppressive effects of infectious bursal disease virus (IBDV) infections were first disclosed by Allen *et al.,* (1972). In 1980, a second serotype was reported (Mc Ferran*et al.,* 1980). These factors, along with the high tendency for IBD infections to recur in successive flocks, emphasized the need for stringent measures of prevention and control. Prior to 1984, spread of both the clinical and subclinical forms of the disease was satisfactorily controlled by vaccination programs. However, in 1984 and 1985, a significant increase in mortality, condemnations, and vaccine failures were reported in

the Delmarva Peninsula broiler growing area (Rosenbergerand Cloud, 1986). These newly emergent viruses were capable of breaking through maternal immunity against classic strains of IBDV (Rosenberger and Cloud, 1986). *In vivo* reciprocal cross-challenge tests showed that unlike classic or standard strains of IBDV, the field isolates caused rapid atrophy and minimal inflammation of the cloacal bursa when inoculated into susceptible SPF leghorns (Rosenberger and Cloud, 1986). Studies suggested that a major antigenic shift in serotype I viruses had occurred in the field (Snyder *et al.,*1992).The IBDV field isolates were characterized as antigenic "variants" of serotype 1 IBDV, while the older serotype 1 viruses discovered prior to these newly emergent viruses were called classic strains of IBDV (Rosenberger and Cloud, 1986). Currently in the United States, clinical cases are rarely reported and these variant strains are the predominant viruses circulating in the field (Eterradossi and Saif, 2008). Outbreaks of very virulent IBDV (vvIBDV) were first reported in Europe in 1987 to 1988 (Eterradossi, *et al.,* 1992). Highly virulent IBDV (vvIBDV) infections are characterized by a per acute onset of severe clinical disease and high mortality (Van den Berg and Meulemans, 1991). Although these new serotype 1 viruses demonstrate increased virulence in their ability to break through the existing level of maternal immunity; they are antigenically similar to the classic strains of IBDV (Van den Berg and Meulemans, 1991). Strains of vvIBDV have rapidly disseminated to every poultry-producing country, except Canada, Mexico, Australia, and New Zealand (Van den Berg, 2000). During the 63rd General Session of the Office International des Epizooties (OIE, 1995), it was estimated that IBD has considerable socio-economic importance at the international level, as the disease is present in more than 95% of the Member Countries (Eterradossi, 1995).

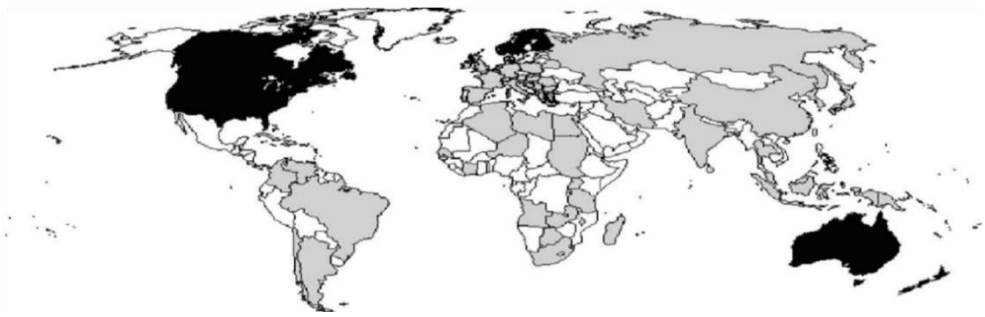


Figure. 1. Worldwide geographical distribution of the acute forms of IBDV**.**

In gray, countries where acute forms have been reported. In black, countries where no acute forms have been reported. In white, countries with no report (Eterradossi, 1995).

2.2 Disease Definition

Infectious bursal disease also called as Gumboro disease (OIE, 2004) is economically important an acute, highly contagious immunosuppressive viral infection of young chickens (Rauf, 2011) caused by infectious bursal disease virus (IBDV) (Mahgoub, 2012; Muller *et al.,* 2012), which belongs to a genus Avibirnavirus (Fauquet *et al.,* 2005), of family Birnaviridae (Delmas *et al.,* 2004) that causes disease and mortality in young chickens mainly 3–6-week-old (Van den Berg *et al.,* 2000; Lukert and Saif, 2003) with a worldwide distribution (Sharma *et al*., 2000). Although turkeys, ducks, guinea fowl and ostriches may be infected, clinical disease occurs solely in chickens. The main clinical signs include watery diarrhea, depression, ruffled feathers, anorexia, trembling, prostration and death after two to three days of clinical signs onset (OIE, 2012). The major post-mortem lesions may include dehydration of the muscles with numerous ecchymotic hemorrhages, swelling and discoloration of the kidneys, with urates in the tubules, inflammation, edema and bursal hemorrhages or atrophy (Chansiripornchai and Sasipreeyajan, 2009). Disease severity depends on the age and breed of the affected birds, the degree of passive immunity and the virulence of the strain of virus (Van den Berg *et al.,* 2000) and secondary infections associated with the immunosuppressive effects of the disease.

2.3 Etiology

Infectious bursal disease virus (IBDV) is an etiology of infectious bursal disease "Gumboro disease", (Mahgoub, 2012; Muller *et al.,* 2012), which belongs to a genus Avibirnavirus (Fauquet *et al.,* 2005), of family Birnaviridae (Delmas*et al.,* 2011). It is a double strand an RNA virus (dsRNA) virus (Eterradossi and Saif, 2008) and a non-enveloped, icosahedral capsid with bi-segmented genome (Wu *et al.,* 2007; Zhu *etal.,* 2008). The larger segment, A, is 3261 nucleotides long and contains two open reading frames (ORF) and encodes four viral proteins designated as VP2, VP3, VP4 and VP5 and also the smaller segmentB encodes only VP1 which has polymerase activity(Van den Berg, 2000; Lukert and Saif, 2003). The two viral proteins, VP2 and VP3 are structural proteins which form the viral capsid. The epitopes responsible for the

induction of neutralizing and protective antibodies are located on the VP2 protein (Abdel and Saif, 2001).

2.3.1 Taxonomy of the Virus

IBDV is classified as a genus Avibirnavirus (Fauquet *et al.,* 2005), family Birnaviridae (Delmas*et al.,* 2011). The family includes 3 genera: *Aquabirnavirus* whose type species is infectious pancreatic necrosis virus (IPNV), which infects fish, mollusks, and crustaceans; *Avibirnavirus* whose type species is infectious bursal disease virus (IBDV), which infects birds; and *Entomobirnavirus* whose type species is *Drosophila* X virus (DXV), which infects insects (Delmas,2004).

IBDV has two serotypes of the virus. IBD virus serotype 1 and IBD virus serotype 2. IBD virus serotype 1 is an important pathogen of chickens (Muller *et al.,* 2003; Van den berg *et al.,* 2004). Serotype 2 viruses are immunologically distinct from serotype 1 viruses since vaccination with serotype 2 viruses did not confer protection against serotype 1 (Van den berg *et al.,* 2004). Antibody has been detected but no clinical disease has been reported in chickens or turkeys as a result of infection with IBD virus serotype 2 (Lukert and Saif, 2003). Serotype 1 IBD viruses can be classified in a number of ways, based on phenotypic traits (such as antigenicity and pathogenicity) and genetic molecular traits (nucleotide sequence of the gene coding for the viral protein VP2) (Lukert and Saif, 2003). Based on their phenotypic traits serotype 1 IBD viruses can be classified in increasing order of virulence as attenuated (vaccine strains), classical (standard), antigenic variant, and very virulent (also known as hypervirulent) strains (Van den Berg *et al.,* 2000;Sapats and Ignjatovic, 2000; Muller *et al.,* 2003). Currently, serotype I IBDV viruses are antigenically grouped as classic (also known as standard) and variant strains based on virus neutralization (Wu*et al.,* 2007; Eterradossi and Saif, 2008). Antigenic variation two serotypes of IBDV are described and distinguished by cross neutralization and cross-protection tests.

IBDV strains can be defined as apathogenic (serotype 2); mild, intermediate or ‘‘hot’’ (serotype 1 vaccines); classical virulent (IBDV), variant, or very virulent (serotype 1). Serotype 2 strains cause neither mortality nor bursal lesions in specified pathogen free birds. Serotype 1 vaccines cause no mortality but possess residual pathogenicity with bursal lesions varying from mild to moderate or even severe. Virulent serotype 1 strains induce both mortality and bursal lesions.

2.3.2 Morphology of the virus, structural protein and functions of IBDV proteins

Infectious bursal disease virus (IBDV) is a double strand RNA virus (dsRNA) and a nonenveloped, icosahedral capsid with bi-segmentedgenome (Wu *et al.,* 2007; Zhu *etal.,* 2008). The capsid shell exhibits icosahedral symmetry composed of 32 cashmeres and a diameter ranging from 55 to 65 nm Its structure is based on a T = 13 lattices composed of trimeric subunits. Cryoelectron microscopy and image processing analysis showed that the outer surface of the viral capsid is made up of 260 trimeric VP2 clusters, while the inner surface is composed of 200 Y-shaped trimeric VP3 structures (Caston *et al.,* 2001). The larger segment A encodes four viral proteinsdesignated as VP2, VP3, VP4 and VP5 and also the smaller segmentB encodes only VP1 which has polymerase activity(Van den Berg, 2000; Lukert and Saif, 2003). The two viral proteins, VP2 and VP3 are structural proteins which form the viral capsid. The epitopes responsible for the induction of neutralizing and protective antibodies are located on the VP2 protein (Abdel *et al.,* 2001).

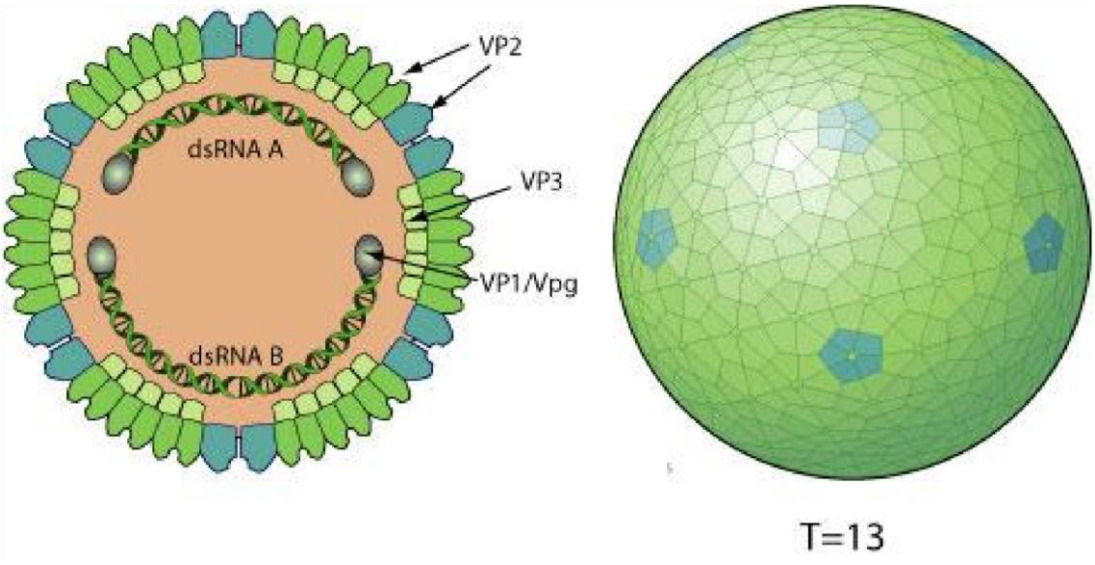


Figure 2. Morphology of the IBDV Virus.

2.4 Epidemiology

2.4.1 Infectious bursal disease and prevalence

Lukert end Hitchner (1988) reported that IBD affected chicken showed dehydration with darkened discoloration of the pectoral muscles. Haemorrtages were frequently found in

the thigh and pectoral muscles. Bursa of fabricius (BF) become swollen, edematous and haemorrhagic.

Rosaes *et al.,* (1988) isolated IBDV from broiler farms in two major broiler producing areas of the State of Georgia. These farms had a history of subclinical IBD associated with respiratory problems and poor performance. These isolates were identified by means of agar gel precipitation test, virus neutralization tests, direct immunofluorescence, histopathology and electron microscopy.

A total of 779 dead chicken were examined from three rural districts of West Bengal, India, over a period of l8 months by Pramanik and Bhattacharya (1984). They found that 171 birds died due to IBD.

Elmuberak and Abutegasim (1990) collected blood samples from the IBD affected flocks aged between 6 to 7 weeks and observed that the serum samples contained precipitin when known IBD antigen was used for test.

Genesan *et al.,* (l990) studied the tissue samples, which were collected from the bursae of 40 broilers showed classic lesions of IBD. Viral antigens were detected in 32 (80%) samples using agar gel immunodiffusion test and in 4 (10%) samples by counter immuno electrophoresis method.

Jhala *et al.,* (1991) collected serum samples of fowls of different age groups and breeds from 4 district of Gujarat. They detected antibodies to IBD by gel precipitation test in 116 (27.61%) of the 420 samples.

Alogninouwa *et al.,* (1992) experimentally introduced the Gradus strain of IBDV to the chicken of three weeks age. The experimental group showed significantly slower growth than the control group.

Serological examination of 350 sera from local chicken and 26 domestic pigeons by agar gel diffusion test was conducted by Maidugu *et al.,* (1992). Among 350 chicken 200 were positive to antibodies against IBDV. None of the pigeons were positive. The prevalence rate was high (73.3 %) in growers than in adult chicken (43.5%).

Rejeswar € *et al.,* (1992) first detected the IBDV in broiler chicks in Kanyaumari district of Tamil Nadu and mortality was 23.2%.In the dead chicken they found enlarged bursa

contain gelatinous materials. Positive reaction in gel precipitation tests were obtained by taking samples from bursa, spleen and kidney.

Rao *et al.,* (1992) found that then immunosuppressive activity of IBDV to Ranikhet disease was maximum at six weeks of age.

Immunodiffusion test for IBD was conducted by Singh *et al.,* (1992). They found that 27% of 1176 serum samples from 32 flocks in Madhya Pradesh were positive. The flock prevalence ranged from 9 to 45 %. 7-11 weeks of aged birds showed most prevalence of disease.

IBD was increases from 19.14% to 26.83% during 1988-1990 to l99l-1992 in Pakistan stated by Anjum *et al.,* (1993). In layers and broilers it was the second largest cause of mortality after Newcastle disease. They found that layers were affected more than broilers. The Fayoumi breed was found to be highly susceptible.

Khurshid Ahmad *et al.,* (1993) stated that 122 (17.2 %) flocks of 700 broiler flocks in Tehsil Gujar Khan were infected with IBDV. They found 4 %, l3.3 %, 43.4 % and 10.7 % incidence during the 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} weeks.

Borzemska *et al.,* (1994) studied the Postmortem findings of infectious bursal disease included large haemorrhages in skeletal muscles and proventriculi as well as an enlargement and necrosis of bursa of fabricious (BF). No immunosuppressive effects of IBD were observed during an outbreak in a flock of 9500, 18 weeks old Messa chicken. They also found high antibody titers 3-9 log 2 (average 6 log 2) in surviving 16 days after the beginning of mortality.

Marked bursal enlargement was taken place in IBDV infected one day old chicks along with significant increased bursal body weight ratio than the non-infected White Leghorn chicken at 3 weeks of age observed by Singh *et al.,* (1994).

Vengadabady *et al.,* (1995) investigated the incidence of IBD in Kerala during the period of 1994-1995. They collected 140 sera samples and 90 bursa from poultry farms suspected to be infected with IBD. They found that 74 (32 %) samples were positive for IBDV in the agar gel diffusion test. The incidence of IBD in Ernakulam, Thissur and Palakked districts was 43 %, 30 % and 28 %, respectively.

Chowdhury *et al.,* (1996) recorded 3 outbreaks of IBD on a broiler farm and 5 outbreak on 1 white Leghorn and 4 Fayoumi pullet raising poultry farms of Mymensingh and Tangail districts of Bangladesh. About 80 to l00 % of the chicken were affected at the age of 26 to 45 days and mortality varied from 20 to 30 in broilers and 40 to 80% in layer chicken. Signs, symptoms, gross and microscopic lesions were typical of acute IBD.

Bhettacharjee *et al.,* (1996) conducted an investigation on the diseases of chicken at the central disease investigation laboratory (CDIL), Dhaka, over a period of one year. A total of 8947 chickens were examined of which diagnosis was confirmed in 83.08 % cases (14.36%) remains undiagnosed due to lack of facilities and 2.56 % materials were found unfit for laboratory examination). Several disease were encountered, among these 14.36 % was Gumboro. They mentioned that Fayoumi breed was most sensitive to IBD (26.32 %) and coccidiosis (18.42 %).

7-20 days of age immunized healthy chicks with IBD was propelled by Liu Jixing *et al.,* (1996) for inactivated vaccine at a dose of 0.5ml. After 28 days, 59 of 60 chicks (98.3 %) were positive for antibodies. They also found that the antibody titer against IBD in the yolk was 1:64, 21 days later of subcutaneous injection al the dose of l ml to the 180-200 days laying hen.

Rahman *et al.,* (1996) observed eight outbreaks of IBD at the central poultry farm of Mirpur, Dhaka, Bangladesh over an 11 months period (April l992 to March 1993). The age of the affected chicks was 2-16 weeks, and found 7.2-16.73% mortality. The course of the disease was 8-12 days. Vaccination of a chick with a live vaccine of an intermediate strain gave at 14.20 days old chicken in drinking water was unsuccessful, but vaccination through ocular route with the same vaccine at 18-28 days old successfully controlled the outbreak.

Qyewola *et al.,* (1996) conducted a serological survey to determine the prevalence of IBD by qualitative agar gel precipitation test in free-range chicken in Ibadan. Among 221 sera screened, 44.34 % were positive for IBD. They mentioned that the chicken had no history of vaccination and the antibodies were due to natural infection.

Zhang *et al.,* (1996) conducted a serological survey from 120 non-immunized rare chicken (20 species) to know the disease affect the rare chicken. They detected that the

rate chickens were affected with the diseases of domestic poultry. IBD, avian adeno virus (other than EDS) and avian influenza were not detected, in any of the samples.

Rai *et al.,* (1997) reported that the most vulnerable age in broiler to IBDV was 4 weeks (3-7 weeks) of age and in layer chicken 6 weeks (5-12 weeks). Morbidity reached 80 % in layers in 1994 and 1996 and 90 % in broilers in 1995.

Serum samples from 135 healthy adult guinea fowls reared semi-intensively in the Dutsin-Ma area of Katsina State, Nigeria, was collected by Nwagu *et al.,* (1997). They assayed the serum for the presence of IBDV by agar gel precipitation test of the 135 sera examined, 16 (11.35 %) were positive for IBDV antibodies. They concluded that although the prevalence was low, the presence of IBDV in guinea fowls represents a risk in areas where different species of poultry live together (especially under the rural small holder system) as guinea fowls could serve as reservoirs for IBDV.

Sami *et al.,* (1997) observed an outbreak of IBD in broilers on 37 poultry farms in Assam between April 1993 to March 1995 and found 4.3 % mortality. They confirmed the disease in 187 of 1123 chicken by postmortem. Incidence was 30.6 % in chicken of 3.4 weeks of age, 24.2 % at 4-5 weeks and 14.2 % at 2.3 weeks of age. Finally they concluded that IBD occurred round the year and was widespread in Assam.

Srithar *et al.,* (1997) made an analysis of postmortem records of 43484 White Leghorn layers over 16 years (1980-1995) and mentioned that the most common disease was Newcastle. During 1987 they first recorded the IBD, thereafter infection level fluctuated, partly, because of vaccination.

Wang *et al.,* (1997) studied the ecology of IBDV by using the anti-IBDV monoclonal antibody sandwich block ELISA. 54 sparrows, 380 ducks and 117 geese, were collected from different regions in china and were serologically examined for IBDV infections. 7.4 % of sparrow 95.5 % duck and 9.4 % of goose samples reacted positively to the test.

A histological, bacteriological and parasitic survey from October 1993 to May 1994 on poultry from 52 infectious bursitis outbreaks in Dakar, Senegal, was conducted by Cardinale *et al.,* (1998). They found that the disease prevalence was 26 % and 7 % in broiler and pullet firms, respectively. In 23% and 8% cases, the disease t as associated with coccidiosis and colibacillosis, respectively. A serological study revealed that disease prevalence reached 69 % and 46 % during the rainy and dry seasons,

respectively. Clinical manifestations were reported in 11 % of infected broiler flocks. Vaccination against infectious bursitis had been correctly performed in only 5 % of the pullet flocks and 11 % of the broiler flocks. Antibody kinetics showed that 52.6 % of chicks from Dakar had a low protection threshold starting at week three. It was suggested that a vaccination between days 9-12 often ensured an early and speedy seroconversion. Finally they concluded ignorance of basic hygiene such as poor cleaning, disinfection and inadequate underfloor space were the cause of high prevalence.

Necropsy of chicken in the department of Pathology, Bangladesh Agricultural University, Mymensingh, was conducted by Islam *et al.,* (1988) over a period of 20 months from January 1997 to October 1998. Single or multiple samples from 139 outbreaks of diseases in 69 small or medium scale commercial poultry farms were received from Mymensingh and neighboring districts. They diagnosed 13 diseases in which IBD was l6.0 % in chicken of up to eight weeks of age.

Ramen *et al.,* (1993) investigated the seasonal pattern of mortality in broilers in Dharmapuri, Tamil Nadu, between March 1993 to February 1995 and found that the commonest cause of death of chicken during 1993-1994 was IBD (45.1%). Between 1994 to 1995 also IBD was the commonest cause of death (41.9 %) followed by Newcastle disease (17.9 %) and mixed infections of IBD and Newcastle disease (8.5 %).

Zhou-ZhengQi *et al.,* (1998) observed an outbreak of IBD in a layer flock 20 days after second vaccination at 28 days old. They mentioned that the possible causes of the outbreak in the immunized flocks were maternal antibody interference, poor husbandry and improper vaccination.

Talha *et al.,* (2001) conducted a pathologic investigation on the mortality of chickens in, different thanas of Mymensingh district during the period from July 1998 to October 1999. A total of 381 chickens of different ages and breeds were subjected to necropsy. The diagnosed diseases included infectious bursal disease 19- l6 %.

Sil *et al.,* (2002) conducted an experiment to find out the disease occurring in cockerels and its relationship with the management of cockerel's farm from day old chicks upto marketing at three different cockerel farms in Mymensingh district. They recorded in the farm I, infectious bursal disease (IBD) appeared at the age of 3l to 35 day, the morbidity being 100 % and mortatity 28.57 %. In the farms 2, the morbidity and mortality of yolk

sac infection, hypovitaminosis-E, coccidiosis and miscellaneous conditions were recorded 3.6 and 2.4 %; 4.4 and 2.0 %; 8.4 and 0.8 %; 0.4 and 0.4 % , respectively. In the farms 3, the morbidity and mortality rates of the diagnosed diseases liked IBD and hypovitaminosis E were 8.6 and l.4 %; l.2 and 0.4 %, respectively. The proportionate mortality of cockerels in these farms were 8l.6 % for IBD, 8.04 %, for hypovitaminosis E, 6.89 % for yolk sac infection, 2.29 % for coccidiosis and 1.14 % for miscellaneous conditions. IBD occurred in age group of >2 to 8 weeks, hypovitaminosis E in 0 to 2 and >2 to 8 weeks group, yolk sac infection in 0 to 2 weeks, coccidiosis in >8 to 20 weeks and the miscellaneous conditions in 0 to 2 weeks group.

2.4.2 Age and mortality

Brown *et al.,* (1994) observed that infection with the newly emerged very virulent strains of IBDV result in l00o/o morbidity and over 70 % mortality.

Rejesware *et al.,* (1995) reported that birds in between 3 and 8 weeks of age are most susceptible. Flock mortality ranges from 5 to 3V/o mortality ranged from I2o/nl5Yo due to IBD alone, 16 to 4l% inter-current infections of IBD, 28-36% in IBD with Marek's disease, 15-30 % in IBD with E coli infection, 26.0 % in IBD with RD and E. coli infection and 2l % in IBD with Fowl cholera affected farm.

Rahman *et al.,* (1996) studied eight outbreaks of infection bursal disease of chickens in central poultry farm at Mirpur, Dhaka over a period of 11 months and observed that the age of the affected chicks varied from 2-16 weeks, mortality ranged from 7.2 to 16.73 % with unchanged morbidity of 100%.

Lukert end Saif (1997) observed that all breeds were affected but White Leghorn chicks exhibited the most severe reaction and highest mortality.

Saha and Majumder (1997) reported that IBD occurred in grower chickens of 11 weeks of age in a layer farm in Tripura India The mortality percentage reached upto 48.84 %.

Farooq *et al.,* (2000) reported that overall incidence of IBD was 3.05 ± 0.04 % and mortality caused by IBD was higher in birds aged 23 to 34 days than in other age groups.

2.5 Occurrence, courses and clinical findings of the disease

Infectious bursal disease virus infection severity of clinical signs and immunosuppression correlate with the status of immunity, age and genetic background of affected chickens and with the virulence of the infecting virus strain (Van den Berg, 2000). IBD occurs in both layer and broiler birds and although it has been found in turkeys (Eterradossi and Saif, 2008). The age of maximum susceptibility is between three and six weeks (Muller, 2003) corresponding to the period of maximum bursa development, during which the acute clinical signs are observed.

Infections occurring prior to the age of three weeks are generally subclinical and immunosuppressive. Some studies have shown that age of infection is directly related to the degree of immunosuppression, (Ivanyi and Morris., 1976) demonstrated that no immunosuppressive response after 3 weeks of infection despite the manifestation of a clinical disease. Variant IBDV strains do not produce overt clinical signs, but cause immunosuppression, which is the most significant economic losses, result from subclinical infections and may cause mortality due to secondary opportunistic infections in immune compromised birds (Van den Berg *et al.,* 2000; Rodriguez, 2002; Eterradossi and Saif, 2008). In contrast, vvIBDV strains cause mortality of 50-60% in laying hens, 25-30% in broilers, and 90-100% in susceptible SPF leghorns (Van den Berg, 2000). In fully susceptible flocks, mortality associated with classic strain infections may range from 1-60%, with high morbidity of up to 100% (Van den Berg, 2000; Muller, 2003).

The disease has an acute and per acute course. The incubation period is very short 2-3 days. However, (OIE, 2004) recommends an incubation period of 7 days for regulatory purposes. Virus excretion can begin as early as 24 hours after infection. Mortality will peak and recede usually in a period of 5-7 days (OIE, 2012). Accompanying symptoms include the disease has been described worldwide (Van den Berg *et al.,* 2000) as acute onset of depression, trembling, white watery diarrhoea, ruffled feathers, severe prostration, vent picking, vent feathers soiled with urates, anorexia, dehydration, and elevated water consumption.

2.6 Gross pathology

Cosgrove (1962) first reported the gross pathology of infectious bursal disease which were dehydration, haemorrhages in leg and thigh muscles, hepatic infarction enlarged kidneys with pronounced tubules, ureters filled with urates and an enlarged bursa.

Cheville (1967) studied the pathologic features of infectious bursal disease and noted that the changes were most severe in the bursa of fabricius, spleen and thymus. There was degeneration and necrosis of bursal follicles replaced by heterophils, pyknotic debris or haemorrhage or severe edema hyperaemia and marked accumulation of heterophils; hyperplasia of RE cells around the adenoid sheath heterophils, hyperplasia of the reticuloendotheial cells and fibrous tissue were observed in the lymphoid organs.

Lukert end Hitchner (1984) observed that infectious bursal disease affected birds showed dehydration with darkened discolouration of the pectoral muscles; swollen, edematous and haemorhagic bursa of fabricius.

Moradian *et al.,* (1998) observed the pathological changes of infectious bursal disease in chickens which included edematous, yellowish or atrophied bursa in severe infection; slightly enlarged spleen; moderately distended intestine with foamy contents.

Sam end Barueh (1998) described the gross lesions of IBD. The bursa was enlarged, grayish white, edematous and covered by slimy material; in some cases haemorhages were marked. The liver and kidneys were enlarged and haemorhagic and sometimes pale. Sometimes haemorhages found in the lungs and at the junction between proventriculus and gizzard. Haemorhages particularly in the thigh and breast muscles were detected. Congestion and haemorhages in the intestine and caecal tonsils were also noticed.

Talha *et al.,* (2001) described the pathological change of infectious bursal disease in bbirds. Grossly, he reported haemorhages in the thigh and pectoral muscles; increascd mucus in the intestine; swollen bursa of fabricius with tncmorrhages, nccrosis and sometimes yellowish caseous mass in the center and also observed haemorhages at the junction of proventriculus and gizzard.

Sil *et al.,* (2002) observed the gross lesions of infectious bursal disease in chickens which were hemorrhages in the thigh and pectoral muscles; increased mucus in the intestine; swollen, edematous, haemorhagic and necrosed bursa of fabricius containing caseous mass; haemorhage at the junction between proventriculus and gizzard; and congested lungs.

2.7 Live vaccine and vaccination schedule

Snedeker *et al.,* (1967) used eighth passaged embryo homogenate to vaccine 7-10 days old chicken through drinking water. The agent produced a mild disease and an immunity to challenge which lasted at least nine weeks.

Winterfield (1969) successfully sued 27^{th} and 41^{st} passaged embryo homogenate through drinking water, spraying or eye drop, without producing any clinical signs. The agent was back passed six times through chicken without evidence of reversion to a virulent state.

Faragher, (1972) stated that live vaccines consists of attenuated strains of live IBDV are used to produce a primary immune response in chicken not vaccinated previously.

Edgar and Cho (1995) first used the bursal suspension containing IBDV to vaccinate chicks three to seven days old on a farm applying through intraocular or drinking water route.

Ismail end Saif (1991) studied the cross protection properties of IBDV and concluded that protection was depended on the strain and dose of both the vaccine and challenged virus and that the virulent strains and standard strains shared a common protective antigen.

Ven den Berg *et al.,* (1991) stated that conventional intermediate IBD vaccinc (D78, SAL) was probably antigenically more related to the highly pathogenic European strain than mild ones (Winterfield, PBG 1998) and the formers were effective in protecting specific pathogen free chicken against the challenge.

Otaki (1993) mentioned that the MDA to IBDV markedly interfered with the efficacy of live vaccines of mild type. In Japan, the disease has been brought under control by applying so called intermediate type live IBD vaccine in breeding flock aged 10-16 weeks.

Chettle *et al.,* (1994) mentioned that virulent virus was able to overcome the maternal immunity before protection with live vaccine.

Coletti *et al.,* (1994) studied the efficacy of live IBDV vaccines in broiler chicken having different level of MDA. The result showed that the live vaccine was able to break

through MDA and stimulate an active immune response. The antibody response was higher chicken having low levels of MDA at vaccination.

Voss *et al.,* (1994) compared four IBD live vaccines of intermediate virulence and one of high virulence with or without the use of inactivated vaccines, in the trials in Germany. They found that there was little difference in residual pathogenicity between the live vaccines. Timing of vaccination of chicks depended on the degree of material immunity.

Whifill *et al.,* (1995) studied that A novel vaccine against IBDV was constructed by mixing bursal disease antibody (BDAO contained in whole antiserum with live IBD before lyophilization A vaccine constructed of l00 EID_{50} mixed with 24 units of BDA was selected as the release dose. The addition of BDA to the IBDV was thought to result in a complex vaccine that allowed for safer immunization in specific pathogen free chicken than the administration of vaccine virus without BDA.

El-Mehdy *et al.,* (1996) screened 80 serum samples from vaccinated chicken for detection of anti-IBDV antibodies after administration of a single dose and after a second dose of live attenuated IBDV vaccine (D7e). They found that AGPT was useful for general screening but the ELISA test was more sensitive, specific and less time consuming. The serum neutralization test appeared equal to the ELISA test in indicating flock immunity, but was more expensive and less time consuming.

Sofei *et al.,* (1996) studied a live vaccine GUMBOVIVAC-CC against IBD. They administered the vaccine by eye drop route or in the drinking water to 14 days old chicken, induced an immune response detectable by indirect ELISA 15 days after vaccination. The antibodies could be detected 40 days after vaccination, by

Zorman *et al.,* (1997) conducted that the efficacy of three vaccination programs against IBD were examined by using two live vaccines on 8 commercial broiler farms in Slovenia- They mentioned that neither vaccine could fully protect broilers against very virulent IBDV strains.

Zulkifli *et al.,* (1997) studied the relationship among short term fasting heat stress and response€ to IBD vaccination. Live IBD vaccine (Nobilis strains D78) was administered intraocularly at 14 and 28 days old chicken. At day 35 they found that the heat treatment suppressed antibody titers. At 42 days of age, IH restricted chicks had a higher antibody response than that fed ad libitum in response to the heat exposure.

Thengavelu *et al.,* (1998) evaluated the pathogenicity and immunosuppressive properties of two field isolates and five commercia.t IBD live virus vaccines marketed in India- They observed that N 35/93 field isolates were more pathogenic and immunosuppressive than the other N45/92. But one of the commercial mild "Lukert" type vaccine was found to be pathogenic.

Wit *et al.,* (1998) stated that working group of the Veterinary Institute and Animal Health Science of the Netherlands recommended that the general use of a live vaccine of intermediate virulence after 14 days of age, reversing stronger vaccines for the flocks with high pressure of infection.

Yehunda *et al.,* (2000) performed two vaccination experiments using 35 commercial hens (including controls). A baculovirus derived recombinant vp2 subunit vaccine elicited anti-IBDV antibodies. They mentioned that the induced antibody levels were similar to those evoked against IBDV by a commercial vaccine. The antibodies were also transferred to the offspring of the hens and were found to be detected in the blood of the progeny for a least 20 days after hatching.

Phan and Pham (2001) administered the oil emulsified vaccine VN-97 against infectious bursal disease (IBD) to 1 day-old chicks. Neutralizing antibody titre were determined 7, 14, 21 and 28 days after vaccination and the efficacy of this vaccine was compared with that of Gumboriffa (Merial, France) vaccine. The chickens showed a high titre of neutralizing antibodies and the efficacy wasg5Yoin 28 days old chickens.

Alam *et al.,* (2002) conducted an experiment to determine the effect of maternally derived antibody on live vaccine. A total of 100 of 100 day old chicks (50 from vaccinated parent stock and 50 from non-vaccinated parent stock) were used in this study. A preset vaccination schedule was followed for chicken and blood samples were collected to find out the actual effect. It was observed that day old chicks contain high level (6294.14 ± 24.95) of maternally derived antibody which gradually decline below positive level within 15-20 days (390.45*, 19.42) and half-life was about 5 days. Vaccination of chicken with high level of maternally derived antibody interferes with the vaccine virus results no immune response but revaccination provokes immune response. Better immune response was found in chickens vaccinated at day 2l and boosted at day 28. But there may be chance of infection because maternal antibody declined below positive level within l5-20 days. Chickens from non-vaccinated parent stock showed

good immune response€ from first time that was from primary vaccination at day 7 and boosting at day 14.

2.8 Enzyme linked immunosorbent assay (ELISA)

Martins *et al.,* (1992) found that antibody response in chicken against a virulent strain of IBDV followed a typical pattern. Indirect ELISA was used to monitor the IgM response.

Cadman *et al.,* (1994) conducted a serological survey with Horseradish peroxidase conjugated goat antibodies IgG for commercial ELISA kit- Antibodies were detected to IBDV (15%) in Ostrich from Zimbabwe.

Cao Yong Chang *et al.,* (1995) evaluated the immunological efficiency of IBD in 3 flocks from 2 chicken farms by ELISA kit. They found that the MDA level of the 3 flocks was similar, and relatively high on day l, declined to day 8 and continued to decline after the first vaccination. The antibody level' reached high on day 30 in 2 simple and accurate in evaluating the immune statues and useful for the establishment of a vaccination program.

Perera *et al.,* (1996) used an ELISA to test 360 serum samples from chicks before inoculation and at different times after vaccination with one dose of a live IBD vaccine, and 120 serum samples from offspring of vaccinated fowls 4, 7 and 10 months after vaccination. They mentioned that the percentage of reactors in offspring of fowl vaccinated l0 months previously was only 58-3% (compared to 89.7%, and 92.1 % offspring of fowls vaccinated 4 and 7 months previously) and concluded that replacement fowls should be revaccinated.

Sofei *et al.,* (1996) determined the serum antibody level against IBD from immunized hens by indirect ELISA and IgY antibodies in the eggs of the vaccinated hen. The cut of value for giving a positive reactions was OD=0.42.

Hueng *et al.,* (1997) evaluated the immunization effect in chicken against IBD by using ELISA. They vaccinated the three groups of day old chicks l, 2 and 3 times, respectively against IBDV starting from 7 days of age; at 7 days interval. Sera were collected l4 days after the last vaccination and tested for IBDV. BF was taken 72 hours after challenge for virus examination- Low titer of virus found when antibody titer was high and no virus was detected when the antibody titer reached 1: 1600.

Cardoso *et al.,* (1998) used a double antibody sandwich ELISA (DAS-EI.ISA) to detect IBDV from bursal samples and to measure the humoral response by same immune reagents. The DAS-ELISA was applied to both 80 bursal suspensions and 724 corresponding serum samples from vaccinated and non-vaccinated commercial flocks. Serum titers obtained in indirect ELISA and serum neutralization test were compared with those in DAS-ELISA. The agreement was 80 % between DAS-ELISA and the conventional techniques with high sensitivity (87 %) and specificity (90 %).

Xylouri *et al.,* (1998) measured the antibody titer against IBD in approximately 12000 layers in Greece using ELISA. The average titer of antibody was low and coefficient of variation was high. This was attributed to random vaccinations without evaluation of immunization criteria.

2.9 Maternally derived antibody (MDA)

Hitchner (1971) gave an experimental infection of both maternally immune and susceptible chicken and observed that some protective affect in chicks less than 2 weeks old. He mentioned that gross lesions in the bursae of all susceptible chicken infected l. 7. 14 and 2l days of age, although no clinical signs or mortality were evident until 28 days of age and older. No lesions were evident in any of the maternally immune chicken until 4 weeks of age. He proposed that there was an early age resistance to infection regardless of MDA status.

Ulbrich *et al.,* (1971) collected 339 blood samples from chicken (6-8 weeks) of 20 poultry farms and tested for antibodies against IBDV by gel precipitation test. They mentioned 127 sera gave positive reaction. The percentage of positive cases was 37.5 %. They inoculated infected bursal homogenate in chicks of four weeks old. The material killed the chicks between 32 hours and 9 days later. Precipitating antigen was demonstrated in bursal tissue between 32 hours and 5 days after infection.

Wyeth *et al.,* (1978) noted that vaccination of parent chicken with a commercial live IBD vaccine under field conditions at varying ages and by different routes resulted in variable susceptibility to the disease and levels of transferred immunity decreased with the age of the parents. The precipitin had disappeared in all flocks by 17 days of age.

Wyeth end Cullen (1979) inoculated an inactivated IBD oil emulsion vaccine at the age of 2l weeks into broiler breed parent chicken which had earlier received commercial live

vaccine produced very high levels of antibody four or five weeks later. They observed from their studies that MDA to IBD persisted in the Progeny of chicken gave inactivated vaccine until at least 29 days of age.

Solano *et al.,* (1986) measured the MDA titer in White Leghorn chicks against IBD by computer assisted single serum dilution, indirect kinetic based enzyme linked immunosorbent assay (KELISA) and virus neutralization (VN) test in order to predict pullets vaccinated within four times or twice with IBDV commercial vaccines at 1, 15 or 28 days of age. Effective initial immunization was confirmed by at increase in antibody of IBDV that occurred when MDA decreased to 8 to 9 on a log2 scale. The compute assisted IBDV KELISA increased the sample processing speed for detecting IBDV antibody, and it was as sensitive as the VN test for predicting the of initial MDV vaccination.

Hahnewald *et al.,* (1989) immunized white Leghorn hens at 18 weeks of age against IBD with Gumborak "Dessau" vaccine and blood samples were taken at 4 weeks intervals between week 30 and week 50. The samples showed a 100 % serum conversion rate in terms of serum neutralization test on chick embryo fibroblast cell cultures and 60-.88% conversion rate in terms of agar gel-precipitation-tests.

Azad *et al.,* (1991) carried out an investigation on determination of MDA against IBD in broiler chicks. Maternal immunity against IBD in broiler chicks of introduced and local breeds was determined by agar gel diffusion technique. The chicks were hatched from eggs derived from dams previously vaccinated at 18 weeks of age by inactivated oil emulsion IBD vaccine. Maternal immunity was found on the first day of life in both the breeds. In the first group (foreign breed) the immunity lasted for 18 days after hatching and in the second group (local breed), the immunity lasted for 14 days after hatching.

Iordanides *et al.,* (1991) reported from their studies that MDA protected chicks upto about 30 days of age preventing typical lesions in the bursa.

Kouwenhoven *et al.,* (1994) said that the MDA of chicks hatched from the eggs of vaccinated hens could not withstand infection with a virulent strain of IBDV, which appeared in Netherlands in 1987, and they developed the disease at 14-28 days of age. Vaccination of broilers at 14-21 days of age solved the problem only partly.

Tsai *et al.,* (1995) vaccinated four broiler breeder flocks using the same vaccination programs against IBD with an intermediate type IBD live vaccine at 2 weeks of age and were boosted with an oil based inactivated vaccine at 18 weeks old. To measure the protection conferred by MDA, chicks hatched from eggs laid by these breeders at 29-61 weeks of age were challenged with a highly virulent IBDV. Chicks surviving the challenge and showed no signs of bursal atrophy were regarded as protected.

Tsei-Hsirng Jung (1995) found in 4 broiler breeder farms that the I day old chick had virus neutralizing MDA (VNMDA) levels 56-68 % of that of hens and ELISA maternal antibody (MDA) levels of 46-81%. The hall life of VNMDA was 3.3-6.3 days and of the ELISAMDA 4.2-9.7 days. No immune response to IBD vaccine was observed in progeny flocks with mean VNMDA titers of over l:5000 whereas there was good immune response in flocks with VNMDA of less than l: l50.

Bidin *et al.,* (1996) administered the IBDV vaccine to the broiler at the age of 10 days. They found that this vaccination ensured lasting of humoral immunity throughout the whole rearing cycle. They mentioned that stress condition had no significant influence on the humoral response against the vaccine viruses.

Ceo Yong Chang *et al.,* (1996) measured the level of MDA (specific to IBDV) by ELISA in day old Shiqiza broiler chicks for the development of an immunizarion program against IBD. They recommended that" for control IBD in shiqiza chicken, inactivated IBDV vaccines in oil emulsion should not be used in parent chicken, and that young broiler should be vaccinated with live IBDV vaccines at the age of day 1 and re-vaccinated through drinking water at 8 and 15 days of age.

Deshpande *et al.,* (1996) tested MDA in progeny from vaccinated parent stock by avidin-biotin ELISA and quantitative agar gel precipitation test. They mentioned that the chicks had positive titers upto 24 days of age most chicken was susceptible to IBDV by 27 days of age. They also mentioned that the ELISA was sensitive, rapid and increased the speed of sample processing when compared with agar gel precipitation test. Malay Mitra *et al.,* (1998) studied on MDA levels against IBD, Newcastle disease and infectious bronchitis in broilers. They mentioned that the antibody level was significantly lower at 12 days of age at one day old.

Saijo *et al.,* (1998) said that half-life of maternally derived antibodies in chicks to IBD was 3.46 days.

Knezevic *et al.,* (1999) mentioned that vaccination of 1 day old chicks with high level of MDA against infectious bursitis virus failed to produce primary immunogenic response. However, revaccinated at 14 days of age provoked delayed primary immune response at 28 days of age.

Wisniewska *et al.,* (1999) examined the MDA to IBDV in 1 to 3 days old chicks in 34 flocks using an ELISA. Mean antibody titer ranged from 194.8 to 712.4. Broilers in flocks 1 and 3(5.9 % of all examined flocks) showed low antibody titers (201.2 and 194.8, respectively) and coefficient of vaccination was >40 % (68-9 and 112.2%, respectively). Emergency vaccinations were carried out in flock I and 3. MDA lasted until day 11 to 19 in 73.5 % of examined flocks where as in one flock (2.9 %) on day 23.

They concluded that the optimum timing of the first vaccination against IBDV was between days 3-9 after hatching. They also suggested that the coefficient of variation <40 % indicates even distribution of antibody titers amongst chicken in flock.

2.10 Effect of infectious bursal disease (IBD)

2.10.1 Effect on body weight

Nerita *et al.,* (1991) conducted that the pathogenicity of avian nephritis virus (ANV) was studied in 7 days old chicks previously infected with infectious bursal disease virus (IBDV) at 1 day of age. Of 20 IBDV + ANV infected chicks, 3 were found dead with no apparent clinical signs within l0 days of inoculation with ANV and they hard marked urate deposits throughout the body. All of the swiveling IBDV +ANV infected chicks had reduced body weight gain.

Tsukamoto *et al.,* (1992) reported that the virulence of strains of infectious bursal disease virus isolated from 5 outbreaks in Japan in 1990-1991, which cause high mortality (4+19.9%), was investigated in groups of SPF chickens. Infected birds developed diarrhoea within 24 hours of infection and they showed depression, trembling, ruffled feathers and were prostate. The 5 isolates caused 30-70% mortality with the Yamaguchi strain causing the highest rate. Those that survived the disease lost weight or showed no weight gain. Atrophy of the bursa Fabricii was seen in all surviving birds.

Singh *et al.,* (1994) mentioned that between February 1990 and May I 993. 218 (27 .7 %) of 881 serum samples collected from 37 poultry flocks in Bihar were positive for participating antibodies to infectious bursal disease virus (IBDV). The disease was found in acute and subclinical forms. The acute form was characterized by high morbidity and mortality (36-65 %). The subclinical form was characterized by marked immunosuppression lowered flock resistance, poor weight gain, increased feed conversion ratio (FCR).

Abdel-Fattah *et al.,* (1999) examined oral administration of thymus extract to 1 day old chicks at 1ml/kg significantly increased concentrations of total. Protein, albumin, globulin, triiodothyronine, thyroxine and body weight gain. Lymphocyte count also increased over 4 weeks after thymus extract administration. It also prevented decrease in relative weights of the bursa, spleen and thyroid land, which commonly occurred during vaccination against infectious bursal disease (IBD). A significant increase in antibody titres was detected in thymus treated chicks using ELISA. Administration of thymus extract and vaccination showed 100 % protection in a challenge experiment with IBD virus, while vaccinated non-thymus treated group showed 80 % protection. It is concluded that thymus extract potentiate immune response in chicks.

Kwon-Jung Taek *et al.,* (1999) studied the objective of this experiment was to investigate the effect of Newcastle disease virus (NDV) and infectious bursal disease virus (IBDV) vaccination on performance of broiler chicks for 5 weeks. Two types of poultry houses and 3 methods of vaccination (NDV/IBDV-NDV/IBDV AND NDV/IBDV) were factorially assigned to 6 treatments. NDV, B1 strain and IBDV, Bursin-2 vaccine were orally administered at 5, 14 and, 7, 18 days, respectively. 4800 chicks were grouped into 4 replications with 200 hydro X hybro chicks per each treatment. Weight gain feed conversion ratio (FCR), mortality and product index were determined at the end of experiment. Bursa index and IBDV antibody titre of chicks were measured weekly.

Herdt *et al.,* (2000) recorded infectious bursal disease virus (IBDV) antibody titres and performance data were recorded in a vertically integrated monitoring scheme between 1993 and 1997 to make a follow up from day old parents down to the broilers at slaughter. High: or uniform antibody titres in the parents were correlated with increased daily weigh gain and decreased mortality and condemnation of broilers. Antibody titres were negatively correlated in broiler parents and their offspring at 1 day of age and at

slaughter. High and uniform antibody titres against IUDV in broiler parents are important for good performance of the broiler offspring.

Trlukder *et al.,* (2000) concurrent coccidiosis in chickens with other bacterial and viral diseases is a major threat to the growing poultry industry in Bangladesh. Seventeen day old Starbrow broiler chicks receiving concurrent experimental infections with *Eimeria tenella* and *Escherichia coli* (Group A), and *Eimeria lenella*, *E. coli* and *Staphylococcus aureus* (Group B) had more intense watery diarrhoea along with bloody faeces on the 3rd day post infection compared with groups receiving *E. tenella* alone (Group E) concurrently infected with *S. oureus* (Group C), infectious bursal disease virus (IBDV) (Group F). Newcastle disease virus (NDV) (Croup G), and with NDV and IBDV (Group H). The faecal oocysts count in Group A was significantly higher ($p<0.05$, $p<0.01$ with some groups) compared with all other infected groups suggesting that the presence of f. coli possibly creates an environment that enhances penetration of the developing stages of Eimeria and their subsequent development. Chicks concurrently infected with IBDV and IBDV with NDV were severely depressed with 41.66 and 33.33 % mortality observed, respectively. However, these two groups produced fewer Oocysts compared with the chicks infected only with *E. tenella* and those receiving concurrent bacterial infection. The chicks receiving concurrent infections with bacteria or viruses had apparently less weight gain compared with the chicks receiving only E. *tenella*, although these differences were not significant (P0.05). However, the uninfected control chicks had a significantly higher ($p<0.05$) weight gain compared with all the infected chicks.

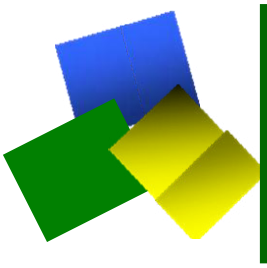
2.10.2 Immunosuppressive effects

IBDV drew the attention of avian virologists mostly because of its severe immunosuppressive effects (Allan *et al.,* 1997). Actively dividing (Lasher and Shane, 1991; Lukert and Saif, 1997; Nagarjan and Kibenge, 1997) or growing (Lukert andSaif. 1997) or differentiating (Hirai.1979) or IgM bearing (Hiral and Calnek, 1979; Rodenberg *et al.,* 1994) B lymphocytes are the target cells of IBD. Alteration immunoglobulin production (Lvanyi 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 imrnune 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.,* 1914: l979). The localization

of viral replication and the immunosuppressive effect of IBDV on the humoral immune response may differ between strain (Rosales *et al.,* 1989 a, b, c; Mazaregos *et al.,* 1990; Tsukanoto *et al.,* 1995b; Thangavelu *et al.,* 1998; Abdel-Alim and Saif, 2001).

IBDV multiplies in the lymphocytes, macrophages, heterophages, and reticular epithelial cells of the bursa (Mandell *et al.,* 1972; Kaufer and Weiss, 1980). IBDE does bot multiply in T lymphocytes or in peripheral B-lymphocytes (Cuniefen, 1980). Depression of the humoral antibody response in IBDV infected chicken-s (Allan *et al.,* 1972; Faragher *et al.,* 1914 and 1979) transformation assay (Sivanandan and Maheswaran, 1981) have already been documented. IBDV affects the Harderian gland influencing the local immune system (Dohms *et al.,* 1981 and Rosenberger, 1994) but IBDV infection leads to the accumulation of T cell 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.



CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1 Study area and period

The research work was carried out in seven different broiler farms in Pabna district during the period of January to February 2017.

3.2 Experimental design

A total of seven broiler farms were selected to conduct this research. All the farmers collected day old chick from same hatchery and in same day. The flock size varied in farm to farm ranges from 600-1300. More or less similar management system was existed in all farms. The farms were visited regularly. Information about management, vaccination, biosecurity, clinical signs exhibited by individual birds during illness and medication etc. were recorded in a prescribed form. Besides, flock size, rearing system, immunization records, types of feed supplied, date of outbreak occurred, number of birds affected, counter measures taken etc. were recorded. The birds suspected to be infected with Gumboro were collected for postmortem examination.

Seven farms were divided into two groups. Group I consisted of five farms and group II consisted of two farms. Farmers of group I practiced their own vaccination program for Gumboro whereas imposed vaccination schedule was implemented in farms of group II (Table 2). From all farms blood sample were collected using 1ml syringes (Hi-tech disposable syringe manufactured in collaboration with SAMSUNG Corporation South Korea) and 2.5 ml syringes (Steripack disposable syringe manufactured by Opso Saline Ltd. Bangladesh) from the heart and wing vein respectively before vaccination and 7 days after vaccination to check antibody level. Infected and dead birds were collected from individual farms and postmortem examination was conducted on spot and in Nourish laboratory, Bogra. Disease and other problems faced during study period especially after outbreaks of Gumboro were studied.

Figure 3: Experimental Birds

3.3 Diagnosis of disease

The diagnosis of disease/conditions was based on the history (provided by the respective owner of the farm), clinical signs, and characteristic postmortem lesions on laboratory examination of the infected or dead birds. A total number of 150 chickens of different age groups suspected to be infected with IBD were collected from four (04) different poultry farms of Group I and 60 chicken from Group II subjected to postmortem examination either in the spot or in the laboratory for the study of pathological lesions For confirmation, dead birds also submitted to Nourish laboratory, Bogra. Cold chain was maintained during transportation of samples.

3.3.1 Postmortem examination

Collected chickens were examined in the laboratory for the evidence of IBD Information about relevant history, clinical signs and symptoms exhibited by the individual chicken during illness were recorded in derail in a prescribed form provided by the respective poultry farm's owner/attendant- Besides flock size, date of outbreak occurred number of

chickens affected, number of chickens died and immunization history etc. were also recorded.

External examination of chickens was performed firstly for detection of any watery diarrhoea soiled vent feathers or any other changes like sign of vent picking etc. then the chickens were dipped into disinfectant solution (Liquid chlorine bleach contain Sodium hypochlorite, manufactured by Reinhart International Foods, Inc. La Crosse) followed by careful opening of visceral organs. During skinning condition of thigh and breast muscles were examined for detection of any haemorrhages in that area. Visceral organs like, liver, spleen, kidney, intestine and bursa were examined.

The evidence of IBD was proved on the basis of history, clinical signs and symptoms, characteristic gross lesions recorded from necropsy. The characteristic gross lesions of IBD were considered as congestion of bursa exudate filled in bursa, swollen bursa and atrophy of bursa (Figure-8), haemorrhage in the thigh and breast muscles (Figure-7) and also in the kidney and proventriculus (Figure-9) etc. The other changes also considered were swollen and atrophy of the spleen with white and dark red sports.

3.3.2 Immunization Program

3.3.2.1 Vaccination program practiced by the farmers

Farmers of group I was free to use their own vaccination program. (Nobilis® Gumboro 228E, Intervet South Africa Ltd.) One of the most important information is that no farmers of group I used booster dose for Gumboro. They vaccinated without consideration of maternal antibody status. Three out of five farmers were used primary dose at day 5, at 7 and at day 8.

3.3.2.2 Imposed vaccination program

The farmers of group II were restricted to use imposed vaccination program (Nobilis® Gumboro 228E, Intervet South Africa Ltd.) for Gumboro. The vaccination Schedule used is presented in Table 1.

Table 1: Imposed vaccination schedule used for Group II

Age (Days)	Vaccine	Route
14 days	IBD Live	Eye (1 drop)
28 days	IBD Live	Eye (1 drop)

3.3.3 ELISA for detection of MDA and antibody induced by vaccination

IELISA test (kit manufactured by IDEXX Laboratories, Inc. USA.) was used for the detection of antibody to IBDV after vaccination in chicken serum. IELISA at a single dilution (l: 500) of serum was applied for the detection of either MDA or IBDV-specific antibody induced by vaccination from the chicken of groups I and II.

3.3.3.1 Collection of blood and preparation of sera for ELISA

Ten birds were reared separately to continue the collection of blood at the age of day 20 and 25 to check maternal antibody up to the age 25 days. Blood samples were collected from farms of group I just before vaccination and 7 days after vaccination. Blood samples were collected (Figure-3) from two farms of group II at day 1, 5, 10, 14 before vaccination and 7 days after primary and booster dose. At day 1 and 5, samples were collected directly from the heart using 1 ml disposable sterile syringes and 2.5 ml syringes were used for collection of blood at day 10, 15, 20 and 25 from the wing vein.

After the collection the syringes with blood were kept slantly at 4 -8°C for overnight, so that blood can clot in one side of the syringe. Then the clotted blood was removed carefully with sterile needle and sera were kept into sterilized eppendorf tube (Figure-4). For each syringe separate needle was used. The sera were subjected to centrifugation at 1000 rpm for l0 minutes for clarification. Finally the clarified sera were stored at -20'c until tested. IELISA was used for the detection of either MDA or antibody induced by vaccination in chicken

Figure 4: Blood Collection at Different ages of birds

Figure 5: Processing of Blood for further investigation

3.3.3.2 Materials supplied in the kit

The reagents and buffers used in the test included infectious bursal disease antibody test kit, manufactured by IDEXX Laboratories, Inc. USA. It contains 5 IBDV antigen coated 96 well micro plates (Figure-10) IBD positive control-hyperimmune chicken sera and negative control specific pathogen free sera non-reactive for anti-IBD in buffer with protein stabilizers, preserved with sodium azide, goat anti-chicken horseradish peroxidase conjugate in tris buffer with protein stabilizers, preserved with gentamicin. It also contain sample diluent buffer with protein v, preserved with sodium azids, TMB substrate with citrate-phosphate buffer containing hydrogen peroxide and stop solution-0.12% hydrofluoric acid.

3.3.3.3 Other materials not supplied in the kit

Multi-channel pipette ranged from 50-250 µl and single channel pipette, ranged from 1-10 µl and 100 - 1000 µl were used, for handling of liquids, manufactured by Labsystem. Tips of 200 µl and 1000 µl, manufactured in Elkay Eireann, Unit 6, Costelloe Industrial Estate, Costelloe. Co, Galway, USA collected from Trades Worth Limited (local agent of Sigma), 78 Motijheel, C/A, First floor, Dhaka Bangladesh and computerized BDSL Immunoskan PLUS microplate reader with an interference filter of 650 nm were used. Labsystem manufactured reader in Finland. Timer was used for preferably cut down type with an audible alarm. Glassware/ plastic ware like, beaker (20-400 ml), flask (50-100 ml) graduated cylinder (10-100- ml) and racks, reservoir for either PBS or deionized distilled water were also used.

Dulbecco's PBS was used in this study. Deionized distilled water was used for washing of microplates.

3.3.3.4 Test Procedure

3.3.3.4.1 Preparation of sample

1 µl of serum was mixed with 500 µl diluent (1:500) provided in the ELISA kit. This mixed sample was used as test sample.

3.3.3.4.2 The basic protocol for the IELISA using a single dilution of serum

In a 96 well plate, precoated with IBDV antigen, the wells with the numbers Al and A2 were selected and used for the negative control serum and well A3 and A4 for positive control serum. The remaining 92 wells were used for 46 samples (one sample in two well). 100 µl of negative control serum and 100 µl of positive control serum (without dilution) was taken in to each selected wells Al, A2 and A3, A4 respectively. Then l00 µl of diluted 46 test samples was taken in to appropriate wells. The plate was incubated at room temperature for 30 minutes and then was washed with deionized distilled water four times and each time 200 µl deionized distilled water in to each well. 100 µl of conjugate was taken in to each well and was incubated at room temperature for 30 minutes. The plate was washed again with deionized distilled water four times and each time 200 µl deionized distilled water in to each well.l00 µl substrate was added in to each well and was kept for 15 minutes.l00 µl of stopping solution was added in to each well.

Reading was taken by ELISA reader, using 650nm filter. The rest of the test samples were tested following the same procedure.

Figure 6: ELISA kit

3.3.3.4.3 Calculation of results

The presence or absence of antibody to IBDV is determined by relating the A (650) value of the unknown to the positive control mean- The positive control has been standardized and represents significant antibody levels to IBD in chicken serum. The relative level of antibody in the unknown can be determined by calculating the sample to positive (S/p) ratio.

The equation of calculation provided in ELISA kit was used for the calculation of antibody titer

a) Negative control mean(NC $\bar{x}$) = $\frac{\text{well Al (650) + well A2 (650)}}{2} = \text{NC}\bar{x}$

b) Positive control mean (PC$\bar{x}$)= $\frac{\text{well A3 (650) + well A4 (650)}}{2} = \text{PC}\bar{x}$

c) S / P Ratio = $\frac{\text{Sample mean} - \text{NC}\,\bar{x}}{\text{PC}\bar{x} - \text{NC}\bar{x}}$

d) Titer Relates S/P *at a*1:500 *dilution to an endpoint titer:* Log_{10} Titer =1.09 (Log_{10} *S/ P*)+3.36

3.3.3.4.4 Interpretation of results

Serum samples with S/P ratios of less than or equal to 0.2 was considered negative. S/P ratios greater than 0.2 (titers greater than 396) should be considered positive and indicates vaccination or other exposure to IBDV.

3.3.3.4.5 Effect of Gumboro on performance (body weight gain)

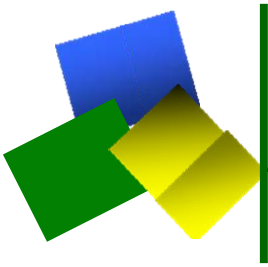
This is done by very simple way. Body weight of individual birds of seven farms (Group I and II) were measured just before marketing. All the birds were in full of their stomach. Then average body weight was calculated.

3.3.3.4.6 Occurrence of concurrent infection

All the firms were closely monitored to check the concurrent infection. Soon after occurrence of any disease the samples (dead and infected live birds) were collected. For the proper diagnosis of disease necropsy was conducted at Nourish Poultry Diseases Diagnostic Laboratory, Unit-2, Bogra. During transportation of samples all protective measures were taken.

3.4 Statistical analysis

The raw data were entered and sorted into MS Excel spread sheet, then analyzed using analytical software Statistical Package for the Social Sciences (SPSS, version 20). To evaluate the differences between two groups students “t” test was used. All significant differences were set at least $P<0.05$.



CHAPTER IV

RESULTS

CHAPTER IV

RESULTS

Profitable poultry farming depend on many factors, Disease problem is one of the most important problems that cause major economic loss by killing the chicken. In this study only, one factor, vaccination program, is studied to check how this factor contributes either positively or negatively to the farmers.

4.1 Result of Laboratory examination of infected and dead birds

Clinical findings: The clinical manifestation in most of the affected flocks were anorexia, depression, ruffled feathers, soiled vent feathers, picking of vent, reduced feed conversion ratio, whitish watery diarrhoea severe prostration and finally death. In few cases there was sudden death. Usually the mortality reached the peak within 4-5 days of outbreak and then gradually declined and the declination continued for a week or so. Emaciation and dehydration were evident in the surviving chicken, which suffered for few days.

Necropsy findings: Congestion, petechial and ecchymotic haemorrhages were often observed in the thigh and breast muscles. ln most cases the bursa of fabricius were swollen, soft, edematous with creamy and contained yellow color cheesy materials, sometimes blackish discoloration due to severe haemorrhages in the bursa.Gelatinous materials were also seen in the lumen of the enlarged bursa in few cases. In some chicken swollen kidney was observed. Liver showed congestion, spleen became congested, swollen and in some cases mottled or pale and atrophied.

Effect of existing vaccination program for Gumboro in broiler

The effects of existing vaccination program for Gumboro in broiler are presented in table 1. It was observed that outbreaks of Gumboro disease were taken place in four farms out of five of group I. The chickens were affected between 17-22 days old with 38-57% morbidity but mortality ranges from and 11.7-33.5%. From table 1 it is also observed that high percentage of mortality was taken places in farms vaccinated at day five than vaccination at day seven. Farm No. 5 faced no Gumboro during rearing cycle although they vaccinated the birds at day eight.

Table 2. Effect of use of existing vaccination program for Gumboro in broiler in terms of disease outbreaks, morbidity and mortality

Farm no.	Flock size	Age of vaccination (days)	Age of outbreak of IBD	Morbidity (%)	Mortality (%)
1	1300	5	17	57	31.4
2	900	5	20	38	33.5
3	900	5	18	48	22.0
4	750	7	21	52	11.7
5	600	8	-	-	-

(-) means no

Effect of imposed vaccination program for Gumboro in broiler

The effects of imposed vaccination program for Gumboro in broiler are presented in table 2. It was observed that no outbreaks of Gumboro occurred in farms of group II during the study period.

Table 3. Effect of use of imposed vaccination program for Gumboro in broiler in terms of disease outbreaks, morbidity and mortality

Farm no	Flock size	Morbidity (%)	Mortality (%)
1	900	-	-
2	800	-	-

Maternally derived antibody in offspring:

The level of antibody in offspring in Group I and II is presented in Table 3. From the result it is observed that birds contain high level of maternal antibody at day l which ranges from 7800-8050 (7928±18.60). The titer level decreases gradually by the day 5. 10. 15.20 and 25 and the level for each day was ranges from 3042-3994 (3673.40±78.63), 1901-2055 (2005.30±16.41), 935-1100 (1017.40±27.84), 348-383 (367.10±10.25) and 175-258 (221.00 ± 28.88) respectively (table 3). The rate of declination was about half in each five days interval. The titer level decreased to negative level by the day 20. According to the instructions mentioned in the manual provided with ELISA kit the serum samples with S/P ratios of less than or equal to 0.2 is considered negative.

Table 4. Persistence of maternally derived antibody in broiler offspring before vaccination in group I and II

Age (days)	Maternal antibody level
	Mean ± SEM
Day 1	7928.20 ± 18.60
Day 5	3673.40 ± 78.63
Day 10	2005.30 ± 16.41
Day 15	1017.40 ± 27.84
Day 20	367.10 ± 10.25
Day 25	221.00 ± 28.88

4.2 Sero-conversion after vaccination practiced by the farmers in group I and II

Sero-conversion after administration of vaccine in two groups of farms is presented in table 4 and table 5. The Table 4 shows that the titer was high at day 5 and then gradually decreases at day 7 and day 8. The titer level before vaccination at the age of day 5 in three farm ranged from were 4247.50 ± 65.98-4700.20 ± 85.41, at age of day 7 was 2703.00 ± 35.53 and at age of day 8 was 2248.45±39.32. However, 7 days after primary vaccination the titer level decrease significantly ($p<0.05$) in all farms instead of increasing trend. In farm number 4 and 5 who vaccinate their chicken at day 7 and day 8 respectively the titer level decreases sharply than other three farms.

Table 5. Seroconversion after vaccination with live vaccine practiced by the farmers of group I

Farm no.	Age of vaccination (days)	Antibody level		Level of significance
		Before vaccination (Mean ± SEM)	7 days after vaccination (Mean ± SEM)	
1	5	4700.20 ± 85.41	1711.40 ± 28.96	P>0.05
2	5	4205.30 ± 45.26	1899.30 ± 21.54	P>0.05
3	5	4247.50 ± 65.98	1727.70 ± 31.40	P>0.05
4	7	2703.00 ± 35.53	602.10 ± 10.59	P>0.05
5	8	2248.45 ± 39.32	750.21 ± 15.32	P>0.05

From the- table 5 it is observed that the titer before primary vaccination was (1268.60 ±38.40) but seven days after vaccination it increases (1447.20 ± 18.57) non significantly

(P>0.05) and this increasing trend continued up to 14 days after vaccination that is up to age of 28. Booster dose was administered at day 28. Seven days after booster dose that is at the age of day 35 the titer increases (2899.70 ± 68.98) significantly (P<0.05).

Table 6. Seroconversion after vaccination with live vaccine imposed to the farmers of group II

Age	Titre level Mean ± SEM	Level of significance
14 days old before primary vaccination	1268.60 ± 38.40	P>0.05
21 days old 7 days after primary vaccination	1447.20 ± 18.57	
21 days old 7 days after primary vaccination	1447.20 ± 18.57	P>0.05
28 days old before booster dose	1560.54 ± 45.21	
28 days old before booster dose	1560.54 ± 45.21	P<0.05
35 days old 7 days after booster dose	2899.70 ± 68.98	

4.3 Effect of Gumboro on bodyweight gain

Effect of Gumboro on bodyweight gain is presented in table 6. In this study Gumboro outbreaks occurred only in four farms of group I. From the table 6 it is observed that the body weight of birds at marketing age, those facing Gumboro, is lower (1185-1370 g) than those not face the disease and their body weight at marketing age ranges from 2115-2128 g.

Table 7. Effect of Gumboro on performance (body weight) of birds

Farm no.	Age of outbreaks of Gumboro (days)	Average body weight at marketing age (g)
Farmers of group I		
1	18	1337
2	20	1290
3	17	1185
4	22	1370
5	-	1652
Farmers of group II		
1	-	2115
2	-	2228

4.4 Concurrent infections occurred during and after the course of Gumboro

In table 8 a list of concurrent diseases, which occurred in four farms of group I during the study period, is presented. It is observed that bacterial, viral and fungal diseases namely salmonellosis, colibacillosis, infectious coryza and aspergillosis affect the birds secondarily during and after the course of infection.

Table 8. Concurrent infections occurred during and after the course of Gumboro

Farm no.	Name of disease (s)
1.	Salmonellosis
2.	Colibacillosis
3.	Newcastle disease
4.	Aspergillosis
5.	Infectious coryza

Figure 7: Gumboro infected bird

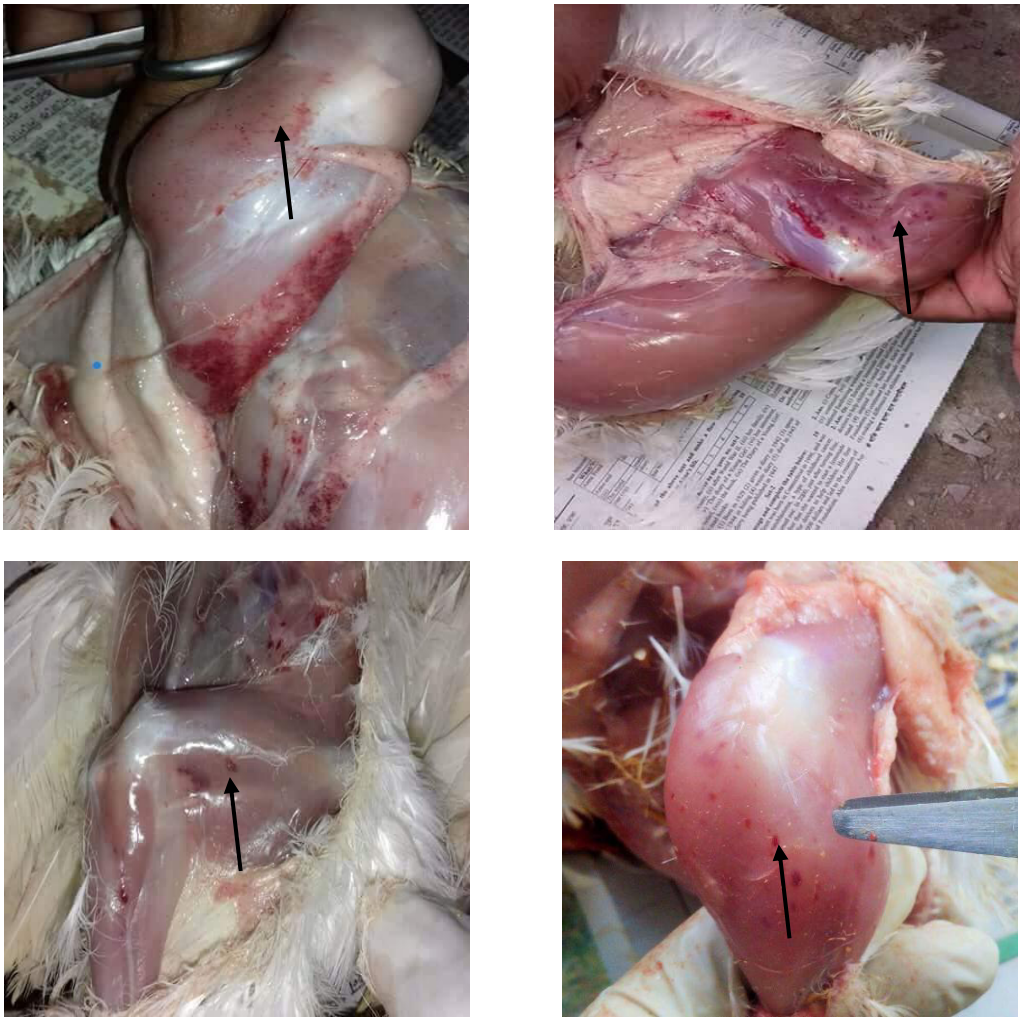


Figure 8: Hemorrhagic leg, breast and thigh muscle of Gumboro infected chicken

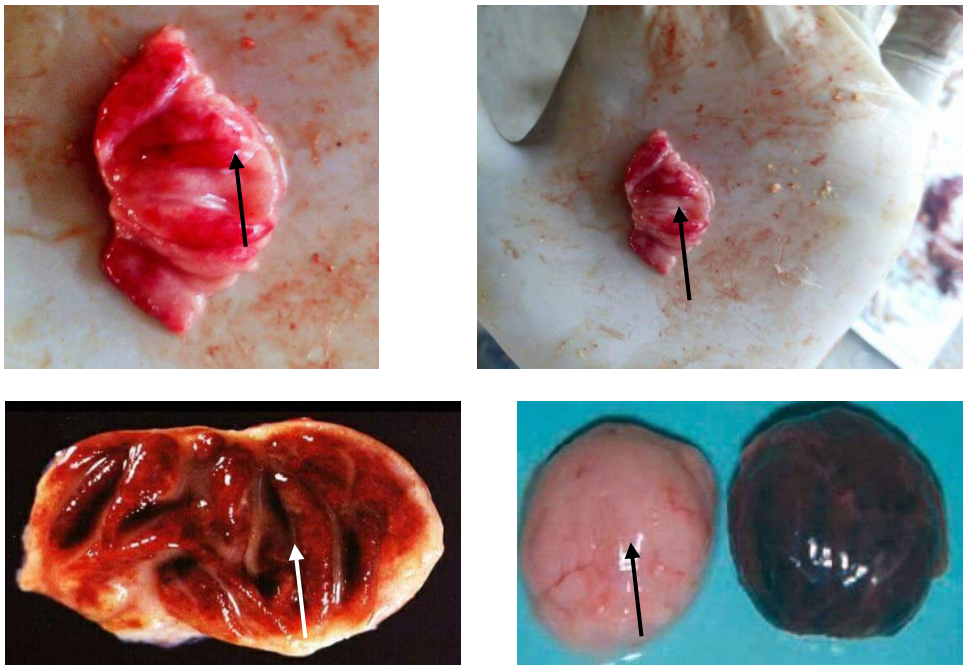
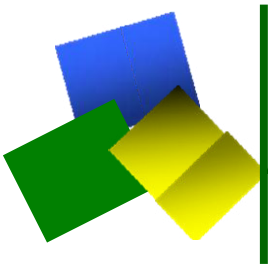


Figure 9: Hemorrhagic and edematous bursa of Gumboro infected chicken

Figure 10: Hemorrhagic proventriculus of Gumboro infected chicken



CHAPTER V

DISCUSSION

CHAPTER V

DISCUSSION

The objective of the present study was to compare the existing and new imposed vaccination program for IBD in terms of its ability to protect the chicken from the disease and its effect on maternal antibody.

To render the poultry industry sustainable emphasis should be given first on the prevention and control of diseases that causes heavy mortality especially Gumboro. Anjum *et al.,* (1993) reported that Gumboro is the second highest cause of mortality of chicken in Pakistan. Outbreaks of various diseases causes about 30 % mortality of chicken in every year (Ali, 1994), is one of the major problems in the development of poultry industry in Bangladesh. Among these diseases Gumboro in chicken is most important. The disease has been occurring in Bangladesh since March 1992 with a very high morbidity and mortality (Islam *et al.,* 1994d. 1994b; Rahman, 1994; Rahman *et al.,* 1996 and Chowdhury *et al.,* 1996).

The findings of high morbidity and mortality of chicken has similarities with the recent observations of (van den Berg *et al.,* 1991; Brown and Grieve 1992; Otaki, 1993; Rahman *et al.,* 1996). Chowdhury *et al.,* (1996) also reported that IBDV causes 20-30 % mortality in broiler that also supports the findings of present study. The morbidity of the IBD following infection with classical strains may be higher than 80 % (Mohanty *et al.,* 1971) or may reach up to 25 % in broilers (Lukert and Hitehner 1984). However, infection with the newly emerged virulent strain of IBDV may result in 100 % morbidity and over 70 % mortality.

During this study a total of four outbreaks of IBD was taken place in all farms except one farm of group I who practiced their own vaccination program for Gumboro. But farmers of group II who practiced new imposed vaccination program, did not face the disease Gumboro. Chicken aged between 17-25 days was affected with IBDV with 38-57% morbidity and 11.7-33.5 % mortality. Similar result is observed by Rai *et al.,* (1997) who reported that broilers are most vulnerable at 4 weeks (3-7 weeks) of age.

The most common clinical signs mentioned by the poultry farms owner/attendant and found in the laboratory- before postmortem examination in the chicken suffering from IBD included depression ruffled feathers, anorexia, white watery diarrhea, picking of vent, soiled vent, feathers, extreme weakness, prostration and death. Major postmortem lesions found during necropsy were swelling or atrophy of bursa of fabricius, with haemorrhages and creamy or yellowish discoloration or enlargement or atrophy of the spleen with white and dark red spots, haemorrhages in the thigh and breast muscles, swelling of kidney and sometimes haemorrhages in the proventriculus. The findings of postmortem changes have similarities with those reported by Cheville (1967); Mohanty *et al.,* (1971); Cho and Edgar (1972); Hirai *et al.,* (1973); Dongaonkar *et al.,* (1979) and Nunoya *et al.,* (1992). Helmboldt and Garncr (1964); Rinaldi *et al.,* (1965) reported mottled appearance of the spleen with dark red and white or gray spots, as observed in the present study. Either atrophy or swelling of the spleen was seen in some cases in the present study. Cho and Edgar (1972) observed atrophy of the spleen, while Craigo *et al.,* (1979) found enlarged spleen, haemorrhages in the kidney and proventriculus are in agreement with the report of Hanson (1967) and Borzemska *et al.,* (1994).

5.1 Maternally derived antibody in offspring:

Maternally derived antibody in offspring blood samples were collected according to the schedule mentioned earlier. After collection all the sera tested using ELISA. The level of antibody in offspring presented. It is observed that birds contain high level of maternal antibody at day 1 which ranges from 7865-8078 (7987.60±27.90). The titre level decreases gradually by the day 5, 10. 15, 20 and 25. From the chart it is also observed that antibody level decreasing about half within five days and decreased to negative level (367.10 ± 10.25) by the day 20. According to the instructions mentioned in the manual provided with ELISA kit the serum samples with S/P ratios of less than or equal to 0.2 is considered negative. The findings of the present study about the maternal antibody have the similarities with the findings of Cao Yong Chang *et al.,* (1995) who evaluated immunological efficiency of IBDV by ELISA and reported MDA level was high at day l. Mitra *et al.,* (1998) found that MDA level were significantly lower at 12 days of age than at one day old. Azad, *et al.,* (1991) carried out an investigation on determination of maternal antibodies against IBD in two groups of exotic and local broiler chicks. They found that maternal antibody lasted 18 days and 14 days after hatching in exotic and local chicken respectively. MDA lasted until 11-19 days and sometimes 23 days after

hatching (Wisniewska and Stosik 1999). Accorcling to Hirchner (l97l); Wyeth and Cullen (1979); Iordanides *et at.,* (1991) and Yehu da *et al.,* (2000) maternal antibody persist up to 28, 29, 30 and 20 days after hatching, respectively. Tsai-Hsing Jung (1995) mentioned that the half-life of ELISA maternal antibody ranges from 4.2-9.2 days. The half-life MDA to IBD in chicks was 1.46 days found by Saijo *et al.,* (1998).

To control IBD and other diseases different types of vaccines is being imported from different manufacturing companies abroad. Usually they have their own instruction about dose, route and age of administration of vaccine to the chicken. Without concern about maternal antibody in offspring farmers are utilizing Gumboro vaccine from day old to onward. Whereas Tsukamoto *et al.,* (1995) reported that the optimum vaccination time could be estimated by titration of MDA against IBDV in day old chicks by an ELISA or agar gel precipitation test. Vaccination with live vaccine of chicken containing high level of maternal antibody has negative effect. Live vaccine neutralizes the maternal antibody and decreases the antibody level quickly instead of stimulation to immune system to produce antibody. It is observed that birds vaccinated with live vaccine at day 5, 7 and 8 have negative result in terms of immune response or antibody production. The birds contained high level of maternal antibody before vaccination but 7 days after vaccination the titer decreases significantly ($p<0.05$) in all farms of group I. Cao Yong Chang *et al.,*(1995) reported that maternally derived antibody declined to day 8 and continued to decline even after the first vaccination supports the findings of present study. Knezevic *et al.,* (1999) also reported that vaccination of chicken using live vaccine with high level of MDA against IBDV failed to produce primary immunogenic response. The declination or no immunogenic response may be due to interaction of MDA and virus of live vaccine.

Farmers of group II vaccinate their chicken using imposed vaccination program. They vaccinate chicken primarily at day 14 and boostered at day 28. The level of MDA at day 14 was 1268.60 ± 38.40. Seven days after vaccination the level of antibody increases significantly 0>0.05) and after booster dose it increases (2899.70 ± 68.98) significantly ($p<0.05$) (table -1). Similar result is observed by Snedker *et al.,* (1967) who found that primary vaccination with live vaccine produce mild immunity. Findings of the present study have the similarities with titre findings of Knezevic *et al.,* (1999) who mentioned that vaccination of chicken using live vaccine with high level of maternal antibody failed to produce primary immune response but revaccination provoked immune response.

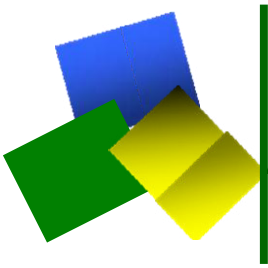
Gumboro has drawn the attention of avian virologist not only because of their high mortality from the disease proper but also due to the profound immunosuppression induced by the virus resulting in subsequent secondary infections, vaccination failure and reduced weight gain (Allan *et al.,* 1972, Earagtrer *et al.,*1972, Hirai *et al.,* 1974 and Tsukamoto *et al.,* 1992). IBD is a major economic problem facing poultry industry worldwide. The economic loss by IBD is manifested by two ways. First by the disease itself due to high mortality and reduced weight gain. The second by its immunosuppressive effect. The fact of immunosuppression includes Marek's disease (Cho, 1970), aflatoxicosis (Somvanshi *et al.,* l992), anemic syndrome, gangrenous dermatitis, inclusion body hepatitis, Salmonellosis and E coli infection, coccidiosis and finally vaccination failure (Calnek *et at.,* 1997). In the present study it is observed that the birds survived the disease lost weight ranges from 1185-1370 g than those did not face Gnmboro 1642-1675 g (Table 6). Similar findings reported by Narita *et al.,* (1991), Tsukamoto *et al.,* (1992). Gumboro in chicken occurred in acute and subclinical forms. High morbidity and mortality (36-65 %) characterized acute form and subclinical form was characterized by marked immunosuppression, lowered flock resistance, poor weight gains and increased feed conversion ratio (Singh *et al.,*1994).

Gumboro is an immunosuppressive disease and when the birds fall in this suppression other disease agent become more active because protective mechanism of the body failed to fight with them. Concurrent occurrence of IBD with colibacillosis and other respiratory disease like coryza was noticed, in four farmers of group I (table 7). Colibacillosis as concurrent infection with Gumboro also reported by several other authors (Wyeth, 1975; Ahmed *et al.,* 1993 and Binta *et al.,* 1995). Lesions resembling coryza and salmonellosis were reported by Wyeth, 1975 and Binta *et al.,* 1995, Ahmed *et al.,* 1993. Pathological changes similar to aspergillosis was seen to occur concomitantly with IBD in one farm that has similarities with the reports of Chowdhury *et al.,* (1996); Okoye (1984) and Faragher (1972).

In the present study a good relation is observed in vaccination, immune status, maternal antibody and disease outbreak. Farmers of group I vaccinate their chicken in early age and they use no booster doses for Gumboro. Early vaccination with live vaccine neutralizes maternal antibody up to certain level instead of stimulation for antibody production (Table 4). The maternal antibody decreases gradually below positive level within l5-20 days. And then outbreaks of the disease occurred within l7-22 days (Table-

l). As they use no booster doses the birds get no stimulation for further antibody production before outbreak of disease. Similar result was observed by Zhou-ZhengQi *et al.,* (1998) who reported thx the possible cause of outbreaks in immunized chicken flocks were maternal antibody interference, poor husbandry, and improper vaccination.

The source and age of birds of farmers of group I and II was same. So they have borne with more or less similar level of maternal antibody. Farmers of group II did not vaccinate their chicken earlier as a result maternal antibody protect them up to certain period before they decline negative level. Primary vaccination at day 14 stimulates immune system and little immune response taken place and after booster dose titer increases sharply which gives protection- Voss *et al.,* (1994) reported that timing of vaccination of chickens dependent on the degree of maternal antibody and in the present study this important message was taken consideration before preparation of vaccination program. It is noted here that immunization by vaccination could not give 100% protection against IBD.



CHAPTER VI

SUMMARY AND CONCLUSION

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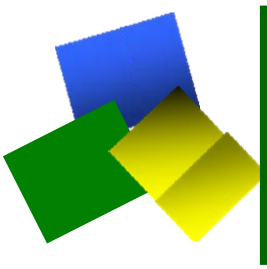
Gumboro is an acute highly contagious immunosuppressive disease of young chicken, characterized by white watery diarrhea, soiled vent, ruffled feather, trembling and death. Double stranded RNA virus causes the disease. The virus has predilection site to replicate in the lymphoid cell specially B cells of BF leading to lymphoid depletion.

To compare an existing and a new imposed vaccination program for gumboro in broiler; a total of seven farms were selected. Five farmers (group I) out of seven practiced their own vaccination program for Gumboro whereas rest two farmers (group II) follow the imposed vaccination program. The effect of these two vaccination strategies was studied in terms of their ability to protect the chicken from the disease. The effect on maternal antibody, immune response induced by vaccination and performances of birds.

During this study four outbreak of Gumboro occurred in four farmers of group I that is birds vaccinated within first week of age experienced the disease Gumboro. One of the important characteristics of the farmers of group I is that they did not conduct booster dose. No outbreaks recorded in farms of group II who vaccinate their birds primarily at day 14 and boostered at day 28. The disease was diagnosed on the basis of history, clinical signs, symptoms and postmortem lesions. The disease mostly occurs in chicken of 3-4 weeks of age with 38-57 % morbidity and 11.7-33.5 % mortality. Those survived the disease showed less weight gain than those did not faced the disease. Concurrent occurrence of IBD with colibacillosis and other diseases like salmonellosis, coryza, Newcastle disease and aspergillosis was noticed in four farms of group I.

It is found that early vaccination with live vaccine decreases the maternal antibody significantly ($p<0.05$) instead of stimulation to immune system for antibody production. But first vaccination at the age of day 14 results mild immune response that is not significant ($p>0.05$). The titre increases significantly ($p<0.05$) after booster dose. It is fact that vaccine cannot ensure 100 % protection. To control the disease stringent hygienic measures are required, day old chicks should be provided with high and uniform levels of maternal antibody by immunizing breeders with oil-based vaccines and day old chicks in a flock should not be mixed with chicks from breeder flocks of

different ages or vaccination program. Use of vaccine must be chosen to agree with vaccination history, appropriate serological monitoring. Although the timing of primary vaccination depends on the level of maternally derived antibody in offspring, from the present study it may be concluded that broiler birds may primarily vaccinate at the age of around day 14 and booster dose must be conducted accordingly- Because from this study it is observed that level of maternally derived antibody decline below positive level within 15-20 days after hatching.



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