STUDIES ON MATERNAL IMMUNITY TO INFECTIOUS BURSAL DISEASE VACCINES

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CERTIFICATE

I certify that **JYOTHSNA NUTENKI** has satisfactorily prosecuted the course of research and the thesis entitled **"STUDIES ON MATERNAL IMMUNITY TO INFECTIOUS BURSAL DISEASE VACCINES"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination.

I also certify that the thesis or part thereof has not been previously submitted by her for a degree of any University.

Date:
Place: Hyderabad

Dr. K. DHANALAKSHMI
(Major Advisor)

DECLARATION

I, **JYOTHSNA NUTENKI** hereby declare that the thesis entitled **"STUDIES ON MATERNAL IMMUNITY TO INFECTIOUS BURSAL DISEASE VACCINES"** submitted to **P.V. NARSIMHA RAO TELANGANA VETERINARY UNIVERSITY** for the degree of **MASTER OF VETERINARY SCIENCE** is the result of original research work done by me. It is further declared that the thesis or any part thereof has not been published earlier in any manner.

Date:

Place: Hyderabad (**JYOTHSNA NUTENKI**)

CERTIFICATE

This is to certify that the thesis entitled **"STUDIES ON MATERNAL IMMUNITY TO INFECTIOUS BURSAL DISEASE VACCINES"** submitted in partial fulfillment of the requirements for the degree of **MASTER OF VETERINARY SCIENCE** of the **P.V. NARSIMHA RAO TELANGANA VETERINARY UNIVERSITY, HYDERABAD** is a record of the bonafide research work carried out by **JYOTHSNA NUTENKI** under my guidance and supervision. The subject of the thesis has been approved by the student's advisory committee.

No part of the thesis has been submitted for any other degree or diploma. The published part has been fully acknowledged. All the assistance and help received during the course of the investigations have been duly acknowledged by the author of the thesis.

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ABSTRACT

The study was conducted to determine the optimum age of Infectious bursal disease (IBD) vaccination in Vanaraja breed chicks for effective protection based on transfer rate of maternal antibodies from parent birds to chicks and decaying rate of IBD antibody titre in the chicks.

Serum samples were collected at weekly intervals from 31^{st} – 40^{th} week of age randomly from each of 20 birds in a flock of 1000 Vanaraja female parent birds which were vaccinated at 18^{th} - 20^{th} week of age. Infectious bursal disease vaccine antibody titres were estimated by using indirect ELISA. The observed persistence of IBD antibody mean titres from 31^{st} - 40^{th} week were 2707, 3121, 3550, 2613, 3085, 3365, 2486, 3054, 2391 and 1553, respectively and that vary significantly ($P<0.05$) among different ages.

Serum samples were collected from day-old chicks of 31^{st}, 35^{th} and 40^{th} week parent birds and IBD maternal antibody transfer rate was calculated. The estimated transfer rate in the chicks was found to be 96%, 38% and 68%, respectively and which significantly ($P<0.05$) varied at different ages of parent birds.

Serum samples were collected periodically from chicks at ages 1, 3, 5, 7, 10, 14, 18, 21, 24, 28, 31 and 35 days hatched from 31^{st} week parent birds. The decaying mean of IBD maternal antibody titre was calculated by using indirect ELISA. The highest antibody titre was observed in day-old chick (2604), least or almost no mean antibody titre was observed on 35^{th} day. Break through titre of maternal antibody titre for intermediate vaccine was 125 and found to be on 12-13^{th} day. Hence, the predicted day suitable for vaccination was calculated as 12-13^{th} day.

Serum samples were collected from day-old chicks of 35th week parent flock and observed antibody titre was 1161. Based on initial titre, estimated day of vaccination in chicks of 35th week-old parents was 9-10th day. Chicks hatched from 35th week parent birds were divided into two groups, one group was vaccinated on 9th day and the other on 14th day (routine practice at DPR). Serum samples were collected from both groups at 7, 14 and 21 days post vaccination (dpv). The antibody response was observed at 14 dpv in chicks vaccinated at 9th day while in 14th day vaccinated chicks, the response was delayed and observed at 21dpv.

Therefore, it may be concluded that correct time of vaccination will produce faster protective titres compared to standard vaccination schedule that is followed currently. Based on this study, the age of IBD vaccination in chicks cannot be decided using antibody titres of parent birds.

The same serum samples were also used for estimation of efficacy of Newcastle Disease (ND) vaccine. The persistence of mean antibody titre from 31st- 40th week parent birds (9.4, 7.9, 8.0, 8.0, 8.8, 7.0, 7.5, 8.4, 7.3, and 8.9) was significantly different ($P<0.05$). The estimated transfer rate of maternal antibodies from parent birds to chicks was 88%, 90% and 80% at 31st, 35th and 40th week of age, respectively. The decaying mean maternal antibody titre (log2) in chicks of 31st week parent birds was 8.3 at day one and became approximately half (3.5) at 14th day. Subsequently, the titre decreased to 1.8 at 21 day and became zero at 31st day.

LIST OF ABBREVIATIONS

Ab	Antibody
AGPT	Agar Gel Precipitation Test
ANOVA	Analysis of variance
CD	Cluster of differentiation
cDNA	Complementary DNA
CMI	Cell Mediated Immunity
CpG	Cytosine and Guanosine are connected by a phosphodiester bond
CV	Coefficient of variation
°C	Degree Celsius
DIVA	Differentiate Infected from Vaccinated Animals
DNA	De oxyribonucleic acid
dpi	Days Post infection
dpv	Days post vaccination
DPR	Directorate of Poultry Research
Ds-RNA	Double stranded RNA
ELISA	Enzyme Linked Immunosorbent Assay
g	Gram (s)
HA	Haemagglutination
HI	Haemagglutination Inhibition
HRPO	Horseradish peroxidase
HVT	Herpes virus of turkey
IBD	Infectious bursal disease
IBD-ICx	IBD Immune complex
IBDV	Infectious bursal disease virus
ICAR	Indian Council of Agricultural Research
IFN-γ	Interferon-gamma
Igs	Immunoglobulins
IL	Interleukin
iNOS	Inducible Nitric Oxide Synthesis
KDa	Kilo Daltons
Log10	Logarthmic value to base 10
MDAbs	Maternal derived antibodies
MD	Marek's Disease
MHC	Major Histocompatability Complex
µl	Microlitre
ml	Millilitre
m-RNA	Messenger-RNA
NaCl	Sodium chloride
ND	New Castle Disease
NDV	New Castle Disease Virus

NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
nm	Nanometer
NS	Normal saline
OIE	Office International des Epizooties
ORF	Open reading frame
pH	Power of hydrogen
%	Per cent
RBC	Red blood cell
RNA	Ribonucleic acid
rpm	Revolutions per minute
RT-PCR	Reverse transcription-polymerase chain reaction
s/c	Subcutaneous
SE	Standard Error
S/P	Sample positive
TMB	3, 3', 5, 5'-Tetramethylbenzidine
UK	United Kingdom
USA	United states of America
VN	Virus neutralisation
VP	Viral porotein
v/v	Volume/volume
vvIBDV	Very virulent Infectious bursal disease virus

CHAPTER I

INTRODUCTION

The production and consumption of eggs and poultry meat has been increasing worldwide over the last three decades as the consumption of eggs has doubled and that of chicken meat has tripled (Jordan and Pattison, 2001). At the same time, several problems interfere with the development of poultry industry of which emergence of new diseases and failure to control the existing diseases are considered as vital. Among them, infectious bursal disease (IBD) also called Gumboro disease is considered as one of the killer diseases in poultry industry**.**

Infectious bursal disease is an acute highly contagious viral disease of young chickens caused by *Avibirnavirus* of *Birnaviridae* family (Dobos *et al*., 1979). It is a non enveloped, icosahedral, bi-segmented, double stranded RNA virus with a diameter of about 55-60 nm (Ismail and Saif, 1990). The virus replicates in differentiating lymphocytes of the bursa causing immune suppression and often fatal condition. Infectious bursal disease virus consists of two serotypes, 1 and 2. Serotype 1 viruses are infectious for chicken and are classified as avirulent, classical, variant and very virulent (vv) strains based on their pathogenicity. Serotype 2 is more prevalent in turkey (Muller *et al*., 2003).

Infectious bursal disease is most commonly recognized in three-six weeks-old chicks. The virus is very stable and resistant to many disinfectants. Once established in a poultry-rearing area, this virus infection may recur in subsequent flock, if proper disinfection and hygienic conditions are not observed. Therefore, vaccination is considered as the best way to control the disease. Currently, hyper immunizing parent birds with live and inactivated IBD vaccines are used to achieve protection against IBD in chicks during

the most susceptible period. The parent birds develop a high level of antibodies which are transmitted to the progeny chicks in the form of maternal antibodies (Sharma *et al*., 1989). The duration of the parental immunity is variable in progeny and outbreaks have been reported at different development stages of progeny (Khan *et al*., 1988).

In practice, different vaccination schedules have been recommended and used. Despite these vaccination schedules, outbreaks are still recorded. Hence, the study was taken up to predict the optimum age of vaccination in vanaraja breeder chicks based on transfer rate of maternal antibodies from parents to chicks and decaying of IBD antibody mean titre in chicks with the following objectives.

1. To determine the persistence of antibody titres in IBD vaccinated parent chicken.
2. To estimate the rate of maternal antibodies transfer from the parent flock to their chicks and to predict the day-old chick titre against IBD.
3. To determine the decay rate of maternal antibodies against IBD in the progeny chicks.
4. To predict the optimum date of vaccination.

CHAPTER II

REVIEW OF LITERATURE

2.1 INFECTIOUS BURSAL DISEASE (IBD)

Infectious Bursal Disease (IBD) is a viral infection affecting the immune system of poultry. The disease is highly contagious affecting young chicken and characterised by the destruction of the lymphoid organs, and in particular the bursa where B lymphocytes mature and differentiate. The target cell of the virus is the B lymphocyte in an immature stage and the infection when not fatal causes an immune suppression in most cases temporary the degree of which is often difficult to determine. Infectious Bursal Disease is defined as a list B disease by the Office International des Epizooties (OIE, 2000).

2.2 HISTORY AND DISTRIBUTION

The first report of a specific disease affecting the bursa of fabricius in chickens was made by Cosgrove in 1962. The cases were observed in the area of Gumboro, in Delaware (United States of America [USA]) hence it was named “Gumboro disease’’. It was also referred to as “avian nephrosis’’ because extreme kidney damage was found in birds that succumbed to the infection.

Winterfield and Hitchner (1962) described a virus isolate (Gray) from field case of nephrosis. Because of similarity between kidney lesions induced by Grayvirus and those seen in avian nephrosis as described by Cosgrove, it was believed that Grayvirus was the causative agent. Later, studies revealed that birds immune to Grayvirus could still be infected with the IBD agent and would develop changes in the cloacal bursa specific for the disease.

In subsequent studies on IBD, Winterfield *et al*. (1962) succeeded in isolating an agent in embryonating eggs. The mortality pattern was irregular and the agent was difficult to maintain on serial passages. The isolate was referred as "infectious bursal agent'' and was identified as the true cause of IBD. Gray virus was identified as an isolate of infectious bronchitis virus with nephropathogenic tendencies.

Allan *et al*. *(*1972) reported that IBD virus (IBDV) infections at an early age were immune suppressive. The recognition of immunosuppressive capability of IBDV infections greatly increased the interest in the control of these infections.

Hitchner (1976) subsequently proposed the term IBD as the name of the disease causing specific pathognomic lesions of the cloacal bursa.

. The existence of a second serotype was reported in 1980. Control of IBD has been complicated by the recognition of "variant'' strains of serotype I IBDV (Saif, 1984). These strains were breaking through maternal immunity against "standard'' strains and they also differed from standard strains in their biological properties (Rosenberger *et al*., 1987). These variants or subtypes were either already present in the nature but unrecognized or were new mutants that have arisen, possibly due to immune pressure.

In the late 1980s, very virulent strains of IBDV were isolated in Netherlands (Chettle *et al*., 1989). These strains quickly spread to Africa, Asia and then to South America (Difabio *et al*., 1999). Australia, New Zealand, Canada and the USA were unaffected with vvIBDVs (Proffitt *et al*., 1999). It is estimated that vvIBDVs are present in 95% of the Office International des Epizooties member countries (Van Den Berg, 2000).

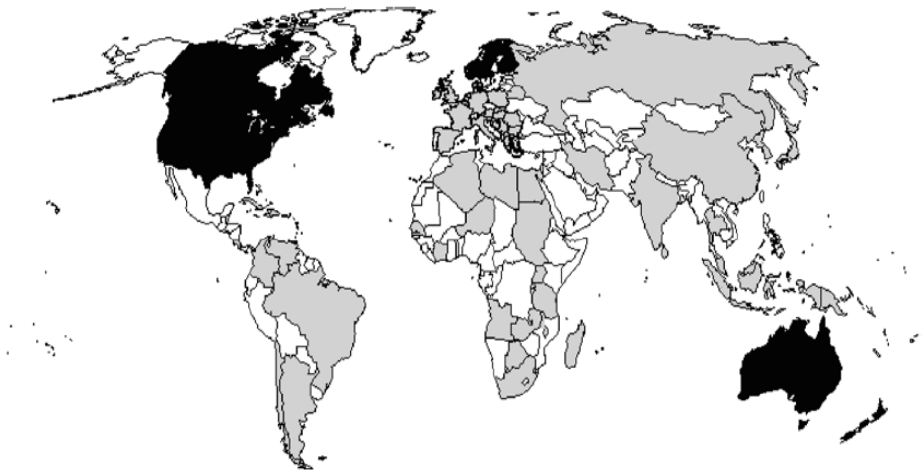

Fig.1: *Worldwide geographical distribution of the acute forms of IBDV (Van den berg, 2000). In gray, countries where acute forms have been reported. In black, countries where no acute forms have been reported. In white, countries with no report.*

2.2.1 DISTRIBUTION IN INDIA

In India, the disease was first reported by Mohanty *et al*. (1971). It was observed in its classical form until early 1990's with mortality upto 30%. Considerably high mortality upto the tune of 70% due to IBD was recorded in late 1990's with the emergence of very virulent (vv) strains of IBDV that resulted in heavy economic losses to the poultry industry in the country (Sah *et al*., 1995).

According to OIE annual animal disease status, 281, 185, 167, 247, 260 and 177 outbreaks of IBD were recorded during the years 2002, 2003, 2004, 2005, 2006, 2007, respectively in India (Sunil *et al*., 2010). In Andhra Pradesh, IBD infection was first reported by Chetty in 1975.

2.3 ECONOMIC IMPORTANCE

Infectious Bursal Disease is an important immunosuppressive viral disease of chickens. It has been described throughout the world and its economic significance is recognized worldwide. The economic loss due to this disease could be due to direct

mortality of chicken during acute lethal course or by the immune suppression. Most of the economic devastation associated with IBD is due to its immunosuppressive effect that leads to poor vaccination response, secondary bacterial, viral, protozoan infection and poor performance and economic return (Van Den Berg *et al* ., 2004)

2.4 ETIOLOGYAND CHARACTERISTICS

2.4.1 Classification and structure

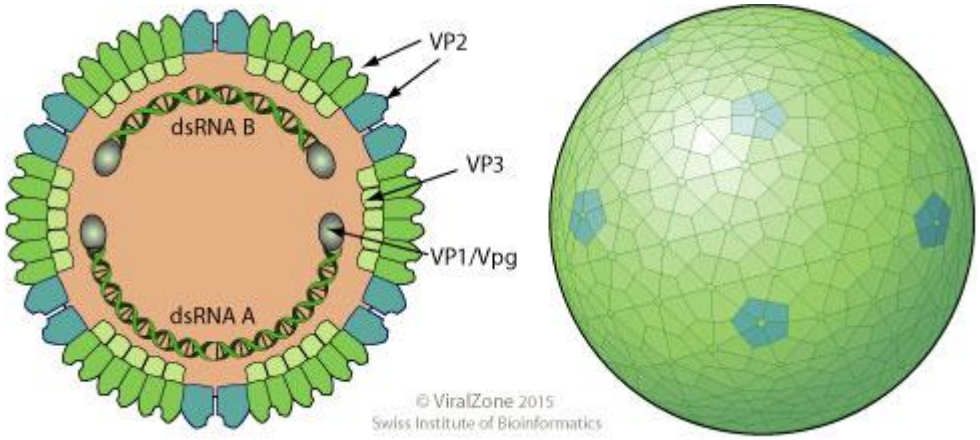

Fig. 2: *Structure of IBD virus*

Infectious bursal disease virus is a member of *Birnaviridae* family and *avibirnavirus* genus. Virions are non-enveloped, hexagonal in outline with icosahedral symmetry composed of 32 capsomeres and diameter of 60-70nm. The genome consists of two segments (A and B) of linear double stranded RNA (Murphy *et al*., 1999).

The smaller genomic segment B encodes viral protein (VP1) of 98,000 Daltons and is the viral polymerase. The larger segment A encodes three proteins namely VP2, VP3 and VP4 of which VP2 and VP3 are structural proteins while VP4 is viral protease. Neutralizing monoclonal antibodies (Mab) have been shown to bind to VP2 whereas VP3 doesn't carry neutralizing epitopes (Fahey *et al*., 1991).

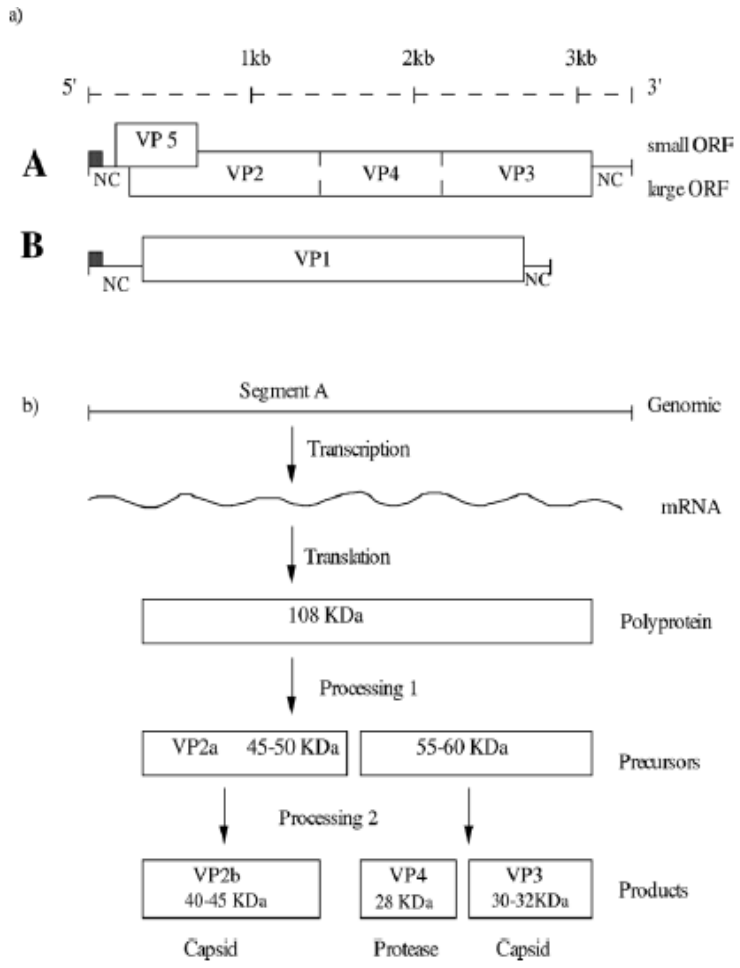

Fig.3: *(a) Genomic organization of IBDV; (b) Post translational modifications in the ORF (Open Reading Frame) of segment A; Source*: (Van Den berg, 2000).

Two recognized serotypes of IBDV are established. Serotype 1 includes pathogenic viruses capable of causing disease in chickens. Serotype 2 is pathogenic to turkey and, so far, its pathogenicity to chicken has not been described. At least six antigenic subtypes of IBDV serotype 1 have been identified by *in vitro* cross neutralization assay (Jackwood and Saif, 1987). Serotype 1 viruses can be further categorized into 4 groups on the basis of their pathogenicity as avirulent, classical, variant, very virulent (Muller *et al*., 2003).

2.5 EPIDEMIOLOGY

2.5.1 Host range

Only chickens develop IBD after infection by serotype 1 viruses. Turkeys may be asymptomatic carriers of serotype 2 (Ismail *et al*., 1988). Pathogenicity of serotype 1 virus for turkeys is ill-defined (Reddy and Silim, 1991).

The Pekin duck is also an asymptomatic carrier of serotype 1 viruses (McFerran *et al*., 1980). Anti-IBDV antibodies have been detected in guinea-fowl (Adewuyi *et al*., 1989), common pheasants (Louzis *et al*., 1979) and ostriches (Cadman *et al*., 1994), which have also been demonstrated to carry serotype 2 viruses (Guittet *et al*., 1981).

Neutralizing antibodies have been detected in various species of wild duck, goose, tern, puffin, crow and penguin, which may mean that wild birds act as reservoirs or vectors (Wilcox *et al*., 1983).

2.5.2 Susceptibility factors

The age of maximum susceptibility was between three to six weeks, corresponding to the period of maximum bursa development, during which the acute clinical signs were observed. Infections occurring prior to the age of three weeks were generally subclinical and immunosuppressive. Clinical cases might be observed up to the age of fifteen to twenty weeks (Okoye and Uzoukwu, 1981). Light strains of laying stock were more susceptible to the disease than the heavy broiler strains (Hassan and Saif, 1996).

2.5.3 Route of excretion and transmission

Infected birds excreted the virus in their droppings at least for 14 days (Baxendale, 2001). It is excreted in the faeces which contaminates water, feed and litter, where it persists and from where it commonly spreads. The most common mode of infection is through the oral route, conjuctival and respiratory routes may also be involved (Sharma *et*

al., 2000). Virus is highly contagious, transmitted by direct contact with excreting subjects, or by indirect contact with any inanimate or animate objects, contaminated vectors between infected and susceptible flocks (OIE, 2008).

The high tenacity of the virus and its resistance to several disinfectants and virucidal procedures might be contributed to the rapid distribution of the virus (Van den berg *et al*., 2000). There was no evidence to suggest that IBDV is spread via transovarial transmission (Eterradossi and Saif, 2008). No specific vectors or reservoirs of IBDV were established, but the virus was isolated from mosquitoes, rats, and lesser meal worms (Eterradossi and Saif, 2008). Viable vvIBD virus was recovered after two days from the faeces of a dog that had been fed tissues from experimentally infected chickens, indicating that dogs might act as mechanical vectors for the virus (Pages-Mante *et al*., 2004).

2.5.4 Resistance to disinfectants

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

2.5.5 Potential risk of spreading infectious bursal disease virus through trade

Vertical transmission of the disease has not been reported. Horizontal transmission due to external contamination of egg shells has not been documented. As a result, the most likely sources of contamination during commercial trade of poultry products are live animals and poultry meat. The IBD-free status of imported live animals can only be established by a negative serological test, repeated after quarantine sufficiently long to allow for the eventuality of seroconversion (at least three weeks). Although imported meat

has not been demonstrated to be responsible for the spread of IBDV, this remains a theoretical possibility.

Contaminated meat may be produced, either by the slaughter of viraemic asymptomatic chickens (Winterfield *et al*., 1972), or by the slaughter of convalescent chickens which, ten to sixteen days after infection, are no longer symptomatic, but continue to carry pathogenic virus in the digestive tract, and thus may constitute a viral source of cross-contamination along the slaughter line.

The resistance of infectivity of IBDV to temperatures below freezing (at least three years at -20°C) (Cho and Edgar, 1969), and to heat (Benton *et al*., 1967), is another factor in the spread of IBD through trade in poultry meat derivatives.

2.6 PATHOGENESIS

The virus affects lymphoid tissue mainly bursa causing destruction of B lymphocytes and the spleen and caecal tonsil-lymphocyte are relatively unaffected. Four to five hours after oral infection virus can be detected in macrophages and lymphoid cells in the caecum, duodenum, jejunum and kupffer cell of the liver. The bursa is infected via the blood stream and by 11^{th} hour many cells in this organ contain antigen. Viraemia follows when the virus infect other organs including spleen, the harderian gland and the thymus lymphocyte and appearance of viral antigen can be found in the bursa up to 14 days post infection (Jordan *et al*., 2002).

In some birds, the kidneys appear swollen and may contain urate deposit and cell debris which was probably as a result of blockage of ureters by severely swollen bursa (Sharma *et al*., 2000). Histological lesions in the cloacal bursa resembled an Arthus reaction (necrosis, hemorrhage, and large number of polymorphonuclear cells). This reaction is a type of localized immunologic injury induced by antigen–antibody complexes

and complement (Ivan and Morris, 1976). The haemorrhagic lesions which were observed with the disease were suggested to be due to an increased clotting time in chicks infected with IBDV (Skeels et *al*., 1980).

2.7 CLINICAL SIGNS

Infectious bursal disease virus has a short incubation period of two-three days and the infection generally last five-seven days. One of the earliest signs of IBDV infection is the tendency for bird to engage in vent pecking. Clinical signs are described as acute onset of depression, trembling, white and watery diarrhoea, anorexia, prostration, ruffled feathers and vent feather solids with urates. In severe cases, bird became dehydrated and in terminal stages subnormal temperature and death (Zelleke *et al*., 2005).

Mortality commenced on the third day of infection, reached a peak by day four, then drop rapidly and the surviving chicken recovered a state of apparent health after five to seven days. Disease severity depends on the age and breed sensitivity of the infected birds, the virulence of the strain and the degree of passive immunity. If the virus persists on the farm and is transmitted to successive flocks, the clinical forms of the disease appear earlier and are gradually replaced by subclinical forms. Moreover, a primary infection may also be inapparent when the viral strain is of low pathogenicity or if maternal antibodies are present (Van Den Berg *et al*., 2004).

2.8 GROSS LESIONS

The gross lesions of IBD includes dehydration, dark discoloration, petechial haemorrhages of the pectoral, thigh muscles and in junction between proventriculus and gizzard, increased mucus in the intestine and renal changes (cosgrove,1962).

The primary target organ for IBDV is the bursa. The third day post infection showed increased weight and size due to oedema and hypaeremia and the normal white color changed to cream. By the fourth day, it was double its normal weight then started to decrease in size. By the fifth day, it returned to normal size and weight, and became gray but continued to decrease, and on the eight day onwards it was approximately one third its normal size (cheville, 1967).

2.9 SUBCLINICAL AND CLINICAL IBD

Infectious bursal disease follow one of the two courses, depending on the age at which chicken are infected. The subclinical form of disease occurs in chicken less than three weeks age. Chicken present no clinical sign of disease, but experience permanent and severe immune suppression, which occurs due to damage of bursa (Jordan *et al*., 2002). The affected birds show poor body weight and feed conversion and high mortality.

The clinical form of IBD usually occurs in chicken from three-six weeks of age. The clinical disease has a sudden onset and mortality rate in the flock increases rapidly. Clinical form of disease includes dehydration, trembling, ruffled feathers, vent pecking and depression (Saif and Barnes, 2003).

2.10 IMMUNE RESPONSE TO IBD

Infectious Bursal Disease infection in chickens activates all branches of the immune system. However, the level of activation varies depending on the virulence of infecting strains, age, immune status and genetic background of affected chickens. The immune response can be altered by maternal antibody, the more virulent vaccine strains can break through higher levels of antibody. Progeny of parent flocks vaccinated with classical strains of IBD virus may have poor maternal immunity against more virulent strains of the virus (Ignjatovic *et al*., 2001).

A high level of maternal antibodies will protect the youngest chickens against challenge by vvIBD virus for up to three weeks after hatching (Van Den Berg, 2000). The half-life of the passive antibodies varies between three days (for broilers) and five days (for laying hens) (Brandt *et al*., 2001). Thus, if the antibody titre of a chick at hatch is known, then the time of maximum flock susceptibility to the wild or vaccinal virus can be determined. This information is very important when establishing the timing of vaccination programmes (Van Den Berg, 2000).

2.10.1 Innate immunity

Influx of macrophages, heterophils and mast cells in the bursa constitutes the early innate immune response to IBDV. The influx of these cells may be mediated by chemokines (IL-8, iNOS) (Khatri *et al*., 2005).

The release of these cytokines was suggested to be tightly regulated by NF-κB, whereby its expression was found to be elevated in the bursa during the early phase of IBDV infection (Guo *et al*., 2012). Nitric oxide released by macrophages may constitute an early host defence against IBDV and promotes the killing of IBDV-infected cells (Khatri *et al*., 2005).

2.10.2 Humoral immunity and cellular immunity

Humoral immunity plays a significant role in protection against IBDV. All classes of immunoglobulins can be produced, but the Ab response may not protect chickens from antigenetically different IBDV strains. Neutralizing Antibodies are directed against the conformation dependent neutralizing epitopes of VP2 (Snyder *et al*., 1992). Significant titres of systemic IBDV specific antibodies have been detected in the convalescent sera of

chickens that are naturally or experimentally infected with IBDV (Eterradossi and Saif, 2008).

Although Ab mediated immunity is crucial against IBDV, maternal derived antibodies (MDAbs) provide passive protection in the first few weeks after hatch (Alnatour *et al*., 2004). Maternal antibody positive chickens developed significantly less bursal lesions than MDAb negative chickens after IBDV challenge supporting the role of passive immunity in protection (Hassan *et al*., 2002). MDAbs may interfere with the development of an active immune response after IBDV vaccination (Rautenschlein *et al*., 2005a). Live and inactivated IBDV vaccines might be induce vigorous Ab responses in the first few weeks post vaccination (Maas *et al*., 2001).

During acute IBD, bursal follicles are B-cell depleted, T-cells accumulate at the site of virus replication (Sharma *et al*., 2000). A notable influx of CD4+ and CD8+ T-cells was detected as early as one day post infection (dpi) and peaked at around seven dpi (Kim *et al*., 2000). Although viral Ag was cleared by week 3 pi, T-cell influx and activation continued to week 12 dpi. No T-cell depletion was detected from the bursa during IBDV infection. However, IBDV particles were detected in intra bursal T-cells (Mahgoub *et al*., 2012). Infiltrating T-cells in the bursa showed markers of activation such as up regulated IL-2, major histocompatibility complex (MHC) class II molecules, and IFN-γ mRNA expression (Rauf *et al*., 2011).

T-cells are not only involved in bursal recovery by killing virus infected cells, but also contribute to bursal lesions. An important role of the cell mediated immunity (CMI) is suggested by several groups (Rautenschlein *et al*., 2002). The significance of virus-specific antibodies (indicated that antibody alone was not adequate in inducing protection against IBDV and that T cell involvement is critical for protection (Rautenschlein *et al*., 2002).

2.11 IMMUNOSUPPRESSION MECHANISM OF IBD

Infectious Bursal Disease virus causes severe immuno suppression in young chickens by its lymphocytolytic effects on surface IgM bearing B-cells. The involvement of other mechanisms such as altered antigen presenting and helper T cell functions had been proposed (Sharma *et al*., 2000).

Chickens infected with IBDV at one day of age were found to be completely deficient in serum immunoglobulin G and produced only a monomeric immunoglobulin M (Van Den Berg *et al*., 2004). Immunoglobulin-G levels varied depending on the age at the time of infection, virus replication during the acute lytic phase resulted in a dramatic reduction in circulating IgM + cells and a prolonged suppression of the primary antibody response. The number of B cells in peripheral blood was reduced after infection with IBDV but T cells were not appreciably affected or T cells are resistant to infection by IBDV (Sharma *et al*., 2000).

2.12 TREATMENT

No therapeutic treatment has been found to have an effect on the course of the viral infection. However, birds might be helped with drugs to treat symptoms so as to control secondary agents and the effects of immunosuppression (Muller *et al.,* 2003).

2.13 PREVENTION AND CONTROL

2.13.1 Management and hygiene procedures

Infectious bursal disease virus is highly contagious and very resistant to inactivation, which accounts for its persistent survival on poultry farms, despite disinfection. Therefore, strict bio-security programmes (e.g. ‘down time’ between broods, all-in/all-out production, cleaning and disinfection of the premises and equipment) and

vaccination are important to reduce the incidence and impact of IBD in the poultry industry (Van den Berg *et al*., 2000; Teshome and Admassu, 2015).

2.13.2 Vaccine and Vaccination

Immunization of chicken is the principal method used for the control of IBD. The most important is the immunization of breeder flocks so as to confer parental immunity to their progeny. Such maternal antibodies protect the chick from early immunosuppressive infections. Maternal antibody will normally protect chicks for one-three weeks, but boosting the immunity in breeder flocks with oil-adjuvant vaccines, passive immunity in chicks may be extended to four-five weeks (Baxendale and Lutticken, 1981).

The major problem with active immunization of young maternally immune chicks is determining the proper time of vaccination. A universal vaccination programme cannot be offered because of the variability in maternal immunity, management and operational conditions that exist. If very high levels of maternal antibody are achieved and the field challenge is reduced, then vaccination of broilers may not be needed. (Darteil *et al*., 1995).

Vaccination timing with attenuated live vaccines varied from as early as seven days to two to three weeks. Monitoring of antibody levels in a breeder flock or its progeny (flock profiling) could aid in determining the proper time to vaccinate. It should be mentioned that although they produced correlated antibody titres, the ELISA and VN (Virus Neutralisation) tests might result in predicting different dates for vaccine susceptibility in progeny chicks (Darteil *et al*., 1995).

2.14 TYPES OF VACCINES

Most of the commercially available IBDV vaccines are live attenuated and inactivated and also IBD Immune complex (IBD-ICx) vaccines.

2.14.1 Live IBDV vaccines

Live vaccines are produced from classical and variant IBDV strains by passaging these viruses in tissue cultures or embryonated chicken eggs (Jackwood and Sommer-Wagner., 2011). Several live-attenuated virus vaccines that differ according to their virulence and antigenic characteristics are available commercially. With regard to virulence or residual virulence for SPF chickens and the level of attenuation, vaccine strains are classified as mild, mild intermediate, intermediate, intermediate plus or hot vaccines. Live-attenuated vaccines are administered via drinking water application or nebulisation between the ages of seven days and two or three weeks (Eterradossi and Saif, 2008).

Live vaccines are favourable for mass application through drinking water and can induce strong humoral and cellular immunity (Muller *et al*., 2003). The proven reversion to virulence (Yamaguchi *et al*., 2000) and their residual immunosuppressive effects (Rautenschlein *et al*., 2007) are major safety concern of their extensive field applications.

Highly virulent (hot), intermediate, and avirulent strains break through maternal VN antibody titres of 1:500,1:250, and less than 100, respectively (Skeeles *et al*., 1979). De Wit (1998) clearly stated that chickens vaccinated using live intermediate IBD vaccine had a capacity to breakthrough maternally derived antibody titre of 125 whereas those vaccinated using intermediate plus IBD had the capacity to break through maternal antibody titre of 500.

2.14.2 Killed IBDV vaccines

Killed-virus vaccines in an oil adjuvant are often used to boost levels of maternal antibodies and confer long-lasting immunity in breeder hens. The duration and uniformity of this immunity might be influenced by the concentration and antigenic specificity of the vaccine strain (Van den Berg *et al*., 2000). These vaccines are not ideal for stimulating a

primary antibody response, they tend to be most effective in chicks that have been "primed" with a live virus vaccine or naturally infected through field exposure to IBDV. Currently, many oil-adjuvant vaccines contain both classic and variant IBDV strains (Eterradossi and Saif. 2008).

Killed-virus vaccines are administered by subcutaneous or intramuscular injection at sixteen to twenty weeks of age (Van den Berg *et al*., 2000). Traditionally, breeder flocks are hyper immunized by priming with live vaccines. Passive immunity protect chicks against early immunosuppressive infections for one to three weeks and this protection might be extended to four or five weeks by boosting the immunity in breeders with oil-adjuvant vaccines (Eterradossi and Saif, 2008).

Serological monitoring of the antibody level in a breeder flock or its progeny was aid in determining the right time to vaccinate (Eterradossi and Saif, 2008; OIE, 2012). According to literature (Van den Berg, 2000), oral, nasal or ocular mild vaccines were effective only in immunizing chicks that had passively acquired neutralizing antibody titres lower than 100.

2.14.3 IBD Immune complex (IBD-ICx) vaccines

Infectious Bursal Disease immune complex (IBD-ICx) vaccines were found to be safe and efficacious for in-ovo and post hatch vaccination of broilers (Giambrone *et al*., 2001). They were prepared by combining an IBDV-hyper immune serum with live intermediate plus IBDV (Johnston *et al*., 1997).

The working mechanism was investigated by comparing the infectivity of the IBDV-Icx and the virus alone at various time points after in-ovo injection (Jeurissen *et al*., 1998). With both vaccines, IBDV was associated with B lymphocytes, macrophages and

follicular dendritic cells in the bursa and spleen, although IBDV complex with specific antibodies caused a delay in virus detection of approximately five days.

In-ovo injection of an intermediate IBD vaccine alone experimentally resulted in fast recovery of bursal lesions, as compared with post hatch vaccination and similar protection against challenge (Rautenschlein and Haase, 2005). Recently, recombinant neutralizing antibodies had been developed and used in an experimental IBDV-Icx vaccine (Sapats *et al*., 2006).

2.14.4 Subunit vaccines

Production of IBD subunit vaccines had been attempted mainly from Baculovirus (Synder *et al*., 1994; Vakharia *et al*., 1994) or yeast (Pitcovski *et al*., 2003) expressed proteins, with a report of expression using a Semliki forest virus vector (Phenix *et al*., 2001). Several studies reported that Baculovirus expressed recombinant VP2 conferred good protection against IBDV challenge (Pitcovski *et al*., 1996).

Baculovirus expressed VP2 protein had been used commercially in broiler breeders and immunity was transferred to their progeny. An oil adjuvant vaccine based on purified recombinant VP2 antigen expressed in the *Pichia pastoris* yeast was developed. This vaccine induced a protection level similar to conventional inactivated IBD vaccine and was used in broilers in the field in Israel (Pitcovski *et al*., 2003).

A fusion protein consisting of VP2 and chicken interleukin-2 considered to enhance immunogenicity had been developed and was tested as a potential vaccine (Liu *et al*., 2005).

2.14.5 DNA vaccines

DNA vaccination is another approach, based on plasmids expressing the polypeptide gene (Chang *et al*., 2001) or the VP2 gene alone (Wang *et al*., 2003). Immune

response to a foreign antigen could be induced by transfer of naked DNA, encoding the target gene, into host cells. This procedure overcamed the problems posed by the presence of specific antibodies to the target antigen in the organism and promoted the induction of specific antibodies and also of cytotoxic T- cells after intracellular expression of the antigen (Hsieh *et al*., 2010).

Significant levels of protection were observed when high amounts of plasmid DNA were repeatedly injected. Attempts had been made to increase the efficacy of IBD DNA vaccines, by co-administrating IL genes (Sun *et al*., 2005) or synthetic oligodeoxynucleotides containing unmethylated CpG dinucleotide motifs (Wang *et al*., 2003) with an adjuvant effect, or by changing the route of administration of the DNA vaccine (Haygreen *et al*., 2006).

The use of DNA vaccination via the in-ovo route was also described (Oshop *et al*., 2003; Park *et al*., 2009). The results showed that the in-ovo delivery without a boost vaccination was not sufficient to induce protective immunity. Bacteria, including *Lactococcus lactis*, *Salmonella typhimurium* and *E. coli*, have been used to deliver IBDV cDNA vaccine orally but with variable success. Here, difficulties in the secretion or translocation of the expressed viral protein across the bacterial cell wall could be the limiting factor (Li *et al*., 2006).

2.14.6 Vectored vaccines

Vector vaccines are genetically engineered vaccines in which a gene from one organism (donor) is inserted into the genome of another organism (vector) to elicit a protective immune response against both organisms. Among others, fowlpoxvirus (Heine and Boyle, 1993), Newcastle disease virus (Huang *et al*., 2004), herpesvirus of turkey (HVT) (Darteil *et al*., 1995), Marek's disease virus (Tsukamoto *et al*., 1999), avian

adenovirus (Francois *et al.,* 2004) and T4 bacteriophage (Cao *et al*., 2005) had been used as vector viruses for expressing VP2, the only antigen inducing protective immunity to IBDV.

Herpesvirus of turkey has been used as a safe and effective vaccine against Marek's disease for decades; as it is poorly sensitive to interference with maternally derived antibodies, HVT has been proposed as a vector for IBD (Darteil *et al*., 1995) and other diseases. Meanwhile, several ''HVT plus IBDV-VP2'' vector vaccines had been developed for application in-ovo or by the subcutaneous route in one-day-old chickens. Some had been licensed in various countries and data on field efficacy trials were reported (Le Gros *et al*., 2009).

As with recombinant subunit vaccines, vector vaccines that express VP2 alone could also allow the development of a DIVA strategy.

2.15 VACCINATION FAILURES AND POTENTIAL CAUSES

In general, vaccine efficacy highly depends on the dose and strains of the vaccine, challenge viruses, the route of administration, the appropriate vaccination time, and the levels of maternal antibodies (OIE, 2012).

The potential causes that affect the outcome of an IBDV vaccine are largely based on the gap on correlation between strains of the vaccine with pathogenicity and antigenicity type of the circulated virus, the appropriate vaccination time, the age and the breed of the bird, and the presence or absence of neutralizing antibodies (MDAbs) and the vaccination history of the progeny of parent flocks, which determine the efficacy of IBD vaccination. In addition to this, vaccination is not a usual practice in small holder poultry and control is further complicated by the regular emergence of new strains that may not be covered by existing vaccine. Most control strategies designed in the country do not take into

consideration the local chickens and this may lead to the failure of most strategies (Tadelle and Ogle, 2001).

The causes of failure of live virus vaccinations are numerous. Interference with MDAbs (AI-Natour *et al.,* 2004) is one of the most frequent causes of failure. The duration and uniformity of this immunity might be influenced by the concentration and antigenic specificity of the vaccine strain (Van den Berg *et al*., 2000). Therefore, it requires continuous monitoring of the antibody level in a breeder flock or its progeny to aid in determining the right time to vaccinate (Eterradossi and Saif, 2008).

Classical live attenuated vaccines might be induce broad, lifelong protection, but they also carry residual pathogenicity and the potential to revert to virulence (Van Den Berg *et al*., 2000). But, in case of inactivated vaccine, failure is rare, but might be occur, either due to the absence of previous contact of some of the birds with a live virus (vaccine virus), or the existence of antigenic variants not present in the vaccine. These vaccines are not ideal for stimulating a primary antibody response and therefore, they tend to be most effective in chicks that have been "primed" with a live virus vaccine or naturally infected through field exposure to IBDV (Eterradossi and Saif, 2008).

ELISA test for detecting maternal immunity in young chicks had been increasingly used as an aid in predicting the date at which the chicks would become sufficiently susceptible to enable efficient vaccination. The concept was first investigated in White Leghorns by Wil Solano *et al.* (1986).

Differing target titres can be chosen depending on the degree to which the vaccine in use is affected by maternal antibody. The target titres which have been used in the UK are shown below Table.1

Table 1. The targeted IBD maternal antibody titres to live intermediate plus and intermediate vaccines

Titre	Vaccine
500	LZ228E (Intervet), Bursa-plus (Solvay)
125-250	Bursine II (Solvay), D78 (Intervet), Bur 706 (Merieux)

Kramer and Cho (1970) were investigated the presence of immunoglobulins Ig-G in the fertile hen's egg during embryogenesis through immunoelectrophoretic studies. The results of this study indicate that the efficacy of deposition of antibodies and other proteins in yolk also increases as the flock matures.

Fletcher *et al.* (1981) were conducted to determine the influence of layer flock age on egg component yields and solids content of the yolk and albumen. Results indicated that as layer flock age increases, egg weight, dry shell weight, deformation and percent yolk increased, percent shell, percent albumen and percent albumen solids decreased, and percent yolk solids exhibited no consistent patterns.

Naqi *et al.* (1983) conducted VN studies on IBDV maternal antibody in unvaccinated and IBDV-vaccinated chicks and showed that the vaccine virus did not accelerate the antibody depletion rate in vaccinated chicks. Chicks carrying high IBDV maternal antibody showed no active immune response to vaccination with commercial IBDV vaccines. They were also refractory to a pathogenic field isolate of IBDV. Chicks with low levels of maternal antibody responded to both vaccine virus and the field virus, although their response to vaccine virus was milder and delayed.

Burkhardt and Muller (1987) were infected chickens in vitro by the pathogenic strain of infectious bursal disease virus (IBDV). Six hours after infection 32.5 per cent of the bursal cells reacted immunocytologically with IBDV antiserum and had high infectivity titres in plaque assays and an electron microscopy, virus particles arranged in a crystalloid patter detected in bursal cells. The results of this study indicate that proliferating lymphoid cells at a certain stage of cellular differentiation are the target cells for IBDV.

Fahey *et al.* (1987) evaluated maternal antibody to infectious bursal disease (IBD) virus using ELISA in the circulation of one-day-old layer strain chickens was found to be on average, 45% of the antibody titre in their respective dam. Maternal antibody was found to disappear from the circulation of these crossbred chickens with a half-life of 6.7 days.

Sajio and higashihara (1998) the optimal time of initial vaccination with a mild-type live IBD vaccine in chicks with maternally derived antibody (MDAb) was studied. The half-life of MDAb was 3.46 days, and critical MDAb titre to protect from infection with field virus was 1:28. The protective immunity was acquired 6 days after vaccination, and an MDAb tirre of 1:28 interfered with active immunization.

Kumar *et al.* (2000) calculated the decaying mean of MDAb titre by using quantitative AGPT and concluded that antibody titre was highest in day-old chicks of broiler breed and least in 21 day-old chicks. Greatest immune response was seen in 21 day-old chicks and poorest in day-old chicks after vaccination with an intermediate vaccine.

Alam *et al.* (2002) determined the effect of maternally derived antibody on efficacy of live vaccine. Better immune response was found in vaccinated parent stock chicks when vaccinated at day 21 and booster dose at 28 days. Chickens from non-vaccinated parent

stock showed good immune response with vaccination at day 7 and boosting at day 14 because of absence of MDAb interference.

Emikpe *et al.* (2002) vaccinated the chicks at 7 and 14 days for IBD and conducted a study on vaccine immune response using qualitative and quantitative AGPT by giving different routes of vaccination and concluded that maternal antibodies in the broiler chicks were low, variable and waned completely by 12 days. The subcutaneous route of vaccination gave better immune response (protection (100%) compared to oral and intramuscular (90%) routes.

Ahmed and Akther (2003) reported that maternal antibodies in unvaccinated chickens persisted up to 21 days as determined by ELISA with complete decay by 28 and 35 days.

Ahmed *et al. (*2003) evaluated the immune response and challenge studies to IBD vaccines and concluded that those chicks having sufficient IBDV maternal antibodies or the birds challenged at least two weeks post vaccination were refractory to the virus. They also reported that immune response against different vaccines varied in accordance with the vaccination schedule and level of maternal antibodies present in chicks.

Abdalla (2005) made a study on maternal antibodies and revealed that the maternally derived antibodies against IBD virus in unvaccinated chicks persisted up to 6^{th} week as determined by ELISA and protective level of these antibodies expired by the 4^{th} week. He also reported that vaccine given via nasal drops at various ages was the best among other routes.

Paul *et al.* (2005) carried out a study to evaluate the vaccination programme using ELISA against Gumboro disease using D78 vaccine in broiler chicken, they concluded that maternal antibody level decreased to about half within five days and decreased to negative

level (364±8.25) by the day 20 and demonstrated that mortality of chickens occurred in farms in which the birds were vaccinated in between five to seven days of age. They finally suggested that broiler birds are to be vaccinated at the age of around day 14 with booster at 28 days-old for better immune response.

Hamal *et al.* (2006) investigate maternal antibodies are transferred from hens of 39 week old to the chicks via the egg using ELISA. They were find that Ig-Y levels in the dams' plasma or eggs were found to be a direct indicator of maternal antibody transfer to the chick circulation.

Moneim and Gawad (2006) evaluated the immune responsiveness to infectious bursal disease virus (IBDV) in four native and crossbred chicken lines was compared. ELISA IBDV antibody titres in hen serum samples, yolk from matched eggs and sera from matched 1-day-old chicks from each chicken line with an identical vaccination program. There was considerable variation between lines in the measured IBDV specific antibodies, in vaccinated parent hens and in the amounts of inherited maternally derived antibodies in both yolk and progeny chicks. Concluded that serum titre of hen and/or progeny chicks from yolk are varied among chicken lines.

Gharaibeh *et al.* (2008) evaluated the rate of antibody transfer from hens to their day-old chicks by using ELISA. The transfer percentage of mean IBD antibodies was found to be 73.6% which was the average of 37^{th} week (83.4%), 40^{th} week (60.3%) and 45^{th} week (77%) from broiler type of parent birds to their chicks.

Ostyina *et al.* (2009) conducted a challenge studies in chicks. Serum samples were obtained from all chicks before vaccination and virus challenge. Antibody titres were measured by Enzyme-linked Immunosorbent assay (ELISA). All vaccinated chicks were

fully protected from the vvIBD virus, as neither morbidity nor mortality was observed in the vaccinated chicks after challenge.

Daniela botus *et al.* (2010) collected serum samples from different age group of chickens for the detection antibodies using ELISA against IBD and they noticed that chickens of 1-13 days-old descending from vaccinated birds had protective level of antibodies (>1500), but the chickens of 18 days-old did not have protective titre.

Bart van Leerdam and Arts (2011) estimated the half-life of maternal antibodies in the serum samples using ELISA (Biocheck) and observed that the highest half-life values were during 1st and 2nd day of age being 4.6 days .Thereafter, the half-life values were around a fairly constant decaying rate of about four days between three to ten days-old.

Leandro *et al.* (2011) two experiments were conducted to examine the effects of broiler breeder dietary grain source and cage density on maternal antibody transfer to progeny in 2 genetic strains. Progeny of breeders fed corn-based diets had smaller spleen white pulp only when hens were housed at 2 hens/cage compared with 1 hen/cage. The results of these experiments suggest that breeder strain and cage-density conditions affected maternal antibody transfer to progeny and embryo development of spleen and bursa.

Besseboua *et al.* (2014) conducted a study to determine the effect_of maternally derived antibody on live vaccine against IBD using ELISA and suggested that the chickens should be vaccinated at day 21 and boosted at day 28 for better immune response.

Fantay *et al.* (2015) conducted a study on rate of maternal antibodies transfer from parent birds to chicks by using ELISA and determined proper age of vaccination of chickens against IBD and concluded that transfer rate was 56.5% and proper time for

administration of live intermediate IBD vaccine was 18 days. They suggested that chicks should not be vaccinated without determining the maternal antibody levels.

CHAPTER III

MATERIALS AND METHODS

3.1: MATERIALS

3.1.1: Glassware

Neutral glassware (Borosil) were used throughout the study.

3.1.2: Plasticware

Plastic ware of 'Tarsons' brand microfuge tubes (1.5 ml), micropipette tips and 96-well plates were used in the study.

3.1.3: Chemicals

HiMedia chemicals and reagents were used during the study. Double glass distilled water was used for the preparation of reagents.

3.1.4: Serum samples

Serum samples were collected from Vanaraja female parent birds and chicks at DPR, Rajendranagar, Hyderabad.

3.2: METHODS

3.2.1: Sterilization of Glassware and others

Glassware were soaked in neutral detergent (Labolene) overnight and washed thoroughly under running tap water. Then, they were washed with de-ionized water followed by single distilled water and finally rinsed with double distilled water. Air dried, packed and were sterilized in hot air oven at 160°C for two hours. Micropipette tips, troughs, microfuge tubes and normal saline (NS) were sterilized by autoclaving at 121°C at 15 lbs for 15 minutes.

3.2.2: Housing of parent birds

A flock of thousand Vanaraja female parent birds were reared in isolated room with cage system at the Directorate of Poultry Research, Rajendranagar, Hyderabad. All birds were fed, watered and kept under the same environmental conditions throughout the experiment.

3.2.3: Vaccination of birds:

The Vanaraja female parent birds at DPR (Directorate of Poultry Research) were vaccinated for IBD and ND as follows.

Table.2 Vaccination schedule of IBD, ND at DPR

Disease	Day/week	vaccine	Route /Dose	Manufacturer
ND	7^{th} day	Lasota strain live	Eye drops	Indovax
IBD	14^{th} day	Intermediate live vaccine (Georgia)	Oral drops	Indovax
IBD booster	24^{th} day	Intermediate live vaccine (Georgia)	Oral drops	Indovax
ND booster	28^{th} day	Lasota strain live	Eye drops	Indovax
ND	9^{th} week	R2B strain live	S/c 0.5mL	Indovax
ND+IBD	18-20^{th}week	Killed vaccine	S/c 0.5mL	MSD
ND+IBD	40^{th} week	Killed vaccine	S/c 0.5mL	MSD

3.2.4: Collection of eggs from parent birds

A total of 120 eggs were collected randomly each time from parent birds at the age of 31st, 35th, and 40th week and kept for hatching. On the previous day of egg collection, serum samples were collected randomly from the same flock of parent birds.

3.2.5: Housing of chicks

A total of 80 chicks were reared in each batch in an isolated condition with cage sytem at Directorate of Poultry Research, Rajendranagar, Hyderabad. All chicks were fed, watered and kept under the same environmental conditions throughout the experiment. Experimental chicks were vaccinated at the age of day-old with Marek's disease vaccine.

3.2.6: Blood collection and serum separation

Blood was collected from the heart or jugular vein of chicks and the wing vein of adult parent birds. Sterile disposable 2 mL syringe (Dispovan) with 21 gauge needle was used for collection. An amount of 1.5 mL of blood from 20 parent birds and 1.0 mL of blood from 10 chicks were collected each time. The serum was separated and clarified by centrifugation at 10,000 revolutions per minute (rpm) for 10 minutes and was stored in microcenrifuge tube at -20°C until use.

3.3: EXPERIMENTAL DESIGN

3.3.1: Experiment 1

The serum samples were collected randomly from the vaccinated female parent chicken at weekly intervals from 31st week to 40th week and persistence of IBD antibody titre was calculated.

3.3.2: Experiment 2

Eggs were collected randomly from vaccinated Vanaraja female parent birds at 31st, 35th and 40th week. Chicks were hatched; serum samples were collected from day-old chicks. The rate of IBD maternal antibodies transfer from parents to chicks was calculated.

3.3.3: Experiment 3

Chicks of 31st week parent birds were maintained up to 35 days. Serum samples were collected periodically (1, 3, 5, 7, 10, 14, 18, 21, 24, 28, 31 and 35th day). The decaying pattern of mean antibody titre and optimum age of vaccination was determined.

3.3.4: Experiment 4

Chicks of 35th week parent flock were divided into two groups: one group was vaccinated as per the estimated date and the other in the normal vaccination schedule as followed at Directorate of Poultry Research. Serum samples were collected at 7 days, 14 days and 21 days post primary vaccination from both groups and antibody titres were compared.

3.3.5: Experiment 5

The same samples were used for screening of antibodies for New Castle Disease vaccines.

3.4: ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

Infectious bursal disease antibody test kit was obtained from IDEXX laboratories (USA) for detection of antibodies in serum samples.

3.4.1: Kit reagents

1. IBD antigen coated plate
2. Positive control (PC): diluted chicken anti – IBD serum, preserved with sodium azide

3. Negative control (NC): diluted chicken serum non reactive to IBD, preserved with sodium azide
4. Conjugate – (Goat anti-chicken: HRPO (Horseradish peroxidase) Conjugate, preserved with gentamicin and Kathon)
5. Sample diluent –Preserved with sodium azide
6. TMB substrate (3, 3', 5, 5'-Tetramethylbenzidine)
7. Stop solution

All the reagents were stored at 2 - 8°c until use.

3.4.2: Materials used

Micropipettes and multi dispensing micropipetts (eppendorf)

Disposable pipette tips

96 -well micro plate reader equipped with 650 nm filter (BIO-TEK Instuments.Inc)

Double distilled water

3.4.3: Preparation of samples

Serum samples were kept at room temperature before testing**.** They were diluted to five hundred fold (1:500) with sample diluents prior to assay in microtitre plates. Controls were used directly without dilution. Samples were thoroughly mixed prior to dispensing into the microtitre plate.

3.4.4: Test method

The antigen coated plates (inactivated viral antigen on microtitre plates) and the ELISA kit reagents were kept to room temperature prior to the test and sample position recorded on a work sheet.

1. One hundred microlitre of diluted serum was added into each well of the antigen coated plate except in positive and negative control wells.

2. This was followed by the addition of 100 µL of undiluted negative and positive controls in each of duplicate wells. The plate was incubated 30 minutes (±2 minutes) at 18-26°C
3. Each well was then washed with approximately 300 µL of distilled or deionized water three-five times. Drying of plates was avoided. The plates were tapped onto absorbent material after the final wash to remove any residual wash fluid.
4. One hundred mirolitre of conjugate was added into each well. The plate was incubated for 30 minutes (±2 minutes) at 18-26°C
5. Repeated step 3.
6. One hundred mirolitre of substrate was dispensed into each well. The plate was then incubated for 15 minutes (±1 minutes) at 18-26°C
7. Finally, 100 µL of stop solution was dispensed into each well to stop the reaction. The absorbance values were measured and recorded at 650 nm wavelength using an ELISA microtitre plate reader.
8. Titre values were calculated using following formulae.

$$\mathrm{NCx} == \frac{\mathrm{NC1\ A(650)} + \mathrm{NC2\ A(650)}}{2}$$

$$\mathrm{PCx} = \frac{\mathrm{PC1\ A(650)} + \mathrm{PC2\ A\ (650)}}{2}$$

PCx-NCx> 0.075 NCx ≤ 0.150

3.4.5 Data analysis

IBD antibody titre was calculated using software xchek (IDEXX)

The ELISA data presented as:

S/P ratio: of the samples where (S) represented the absorbance value of the test serum divided by the absorbance value of the positive control (P) serum.

$$S/P = \frac{\text{Mean of the test sample} - \text{mean of negative control}}{\text{Mean of positive control} - \text{mean of negative control}}$$

The following equation relates the S/P of a sample at a 1:500 dilution to an end point titre

Log 10 Titre = 1.09 (Log 10 S/P) + 3.36 Antilog = Titre

Table.3 IDEXX ELISA standard for IBD antibody titre

S/P value	**Titre range**	**Antibody status**
<0.20	<396	negative
≥0.20	≥396	positive

Coefficient of Variation (CV): is an indicator of individual value dispersal with regards to titre mean.

$$\text{Co efficient of variation} = \frac{\text{Standard deviation}}{\text{Arithmetic mean titre}}$$

CV values were made according to the following threshold

< to 30%	:	Very homogenous
30 to 50%	:	Homogenous
50 to 80%	:	poorly homogenous
> 80 %	:	Heterogeneous
> to 150 %	:	Very heterogeneous

One-way ANOVA was used in order to find out the relationship between different age groups administrations with regard to S/P and CV%.

3.5: HAEMAGGLUTINATION INHIBITION TEST (HI)

Haemagglutination inhibition test was used to assess the antibody response to ND vaccine and to detect the persistence of mean antibody titre in ND vaccinated Vanaraja female breeder chicken, transfer rate and mean of decaying pattern of ND maternal antibodies in their progeny.

3.5.1 Preparation of reagents/chemicals

3.5.1.1: 1% chicken RBC

Sterilized Alsever's solution was taken in a15mL centrifuge tube. Chicken blood was collected in equal amount of Alsever's solution and then centrifuged 3000 rpm/10 minutes, supernatant was removed. Red Blood Cell (RBC) pellet washed with 10 mL of Normal saline (NS) three times. Then finally, one percent of RBC was prepared by adding one millilitre of sedimented RBC to 99 mL of NS.

3.5.1.2: 0.89% Normal saline

0.89g of NaCl was dissolved in 100mL of distilled water. The pH of the reagent was adjusted to 7.2.

3.5.1.3: 8HA units of ND virus

ND virus having a HA (Haemagglutination) titre upto 10^{th} dilution (1:1024) was used for the HI test study. An eight HA unit of virus was prepared by adding one millilitre of virus in 127 mL of NS and was used for screening of serum samples for HI (OIE, 2012).

3.5.2: Materials used

Plastic V – bottomed Microtitre plates

Micropipettes and multi dispensing micropipetts (eppendorf)

Disposable pipette tips

3.5.3: Test procedure:

1. Recorded the sample position on a work sheet and serum samples were adjusted at room temperature before testing.
2. Fifty microlitre of o.89% normal saline was added into all the wells of a plastic V – bottem micro titre plate.
3. Fifty microlitre of serum samples was added into the first well of the each row.
4. Two fold dilutions of the serum were made across the plate.
5. An eight HA units ND virus in 50 µL was added to each well and the plate was left for a minimum of 30 minutes at room temperature.
6. Fifty microlitre of one percent (v/v) chicken RBC was added to each well of the plate and gently tapped the plate and allowed the RBCs to settle for about 30-40 minutes at room temperature until control RBCs were settled to a distinct button.
7. Recorded the HI titre of the serum samples. The HI titre is the highest dilution of serum causing complete inhibition of 8 HA units of antigen. The agglutination was assessed by tilting the plates. Only those wells in which the RBCs stream at the same rate as the control wells (positive serum, virus/antigen and NS controls) were considered to show inhibition.

3.6 STATISTICAL ANALYSIS

The data were analyzed using SPSS 15^{th} version (Statistical package for social sciences). Means were compared using Duncan's multiple range tests and significance was considered at $P<0.05$.

CHAPTER IV

RESULTS

4.1 SERO MONITORING STUDIES ON INFECTIOUS BURSAL DISEASE VACCINES

4.1.1 Persistence of mean antibody titre in IBD vaccinated female parent chicken from 31st week to 40th week

Sera were collected at weekly intervals from 31st to 40th week (n=20). The samples were screened for antibodies using indirect ELISA. The observed persistence of mean IBD antibody titre in 31st to 40th weeks were 2,707±454.7, 3,121 ±361.7, 3,550 ±491.9, 2,613 ±391.3, 3,085±487.3, 3,365±426.3, 2,486±270.7, 3,054±330.6, 2,391±380.1, 1,553^{b}±191.1. The data on the persistence of IBD vaccine antibody titre in female parent chicken are shown in Table.4 and Fig.4 and mean titres varied significantly ($P<0.05$) among the groups.

4.1.2. Rate of IBD maternal antibody transfer from parents to chicks

Serum samples were collected from day-old chicks of 31st, 35th and 40th week parent birds and transfer rate was calculated. The observed transfer percentage of IBD antibody titres (Table 5 and 6, Fig.5) were significantly different ($P<0.05$) among chicks from different age group of parent birds. The estimated transfer rate was found to be 96.1%, 38% and 68.1%, respectively

4.1.3 Decaying mean of IBD maternal antibody titre in unvaccinated chicks

Sera were collected from chicks of 31st week parent birds from day-old to 35 days in a periodical interval (1, 3, 5, 7, 10, 14, 18, 21, 24, 28, 31 and 35th day) and decaying rate of antibody titre was calculated. The decaying mean of IBD maternally derived antibody titres decreased significantly ($P<0.05$) from day-old to 35 days (Table .7 and Fig.6).

A low level of antibody titres was observed from 14th day onwards and complete decay was observed nearing 35th day. As per IDEXX, ELISA standard break through titre for intermediate plus vaccines is 500 and for intermediate live vaccine 125 (as for UK standard it was 125-250). Hence, estimated age of IBD vaccination is 12-13thday with intermediate live vaccine.

4.1.4 Vaccination of chicks based on predicted date of vaccination

The fertile eggs were collected from 35th week-old Vanaraja parent birds and chicks were hatched. Antibody titre were estimated in day-old chicks. The mean MDAb titre in day-old chicken was 1161±297. Based on the maternal antibodies decay observed in the above experiment (4.1.3), the date of vaccination calculated was 9th day with break through titre 205±51. The chicks of 35th parent birds were divided into two groups, one group was vaccinated at 9thday and the other group was given on 14th day (as it was followed previously at DPR) of age with intermediate strain of IBD vaccine. The observed antibody titres after 7, 14, 21 days post vaccination (dpv) were significantly different ($p<0.05$) among the groups of chicks.

In 9th day vaccinated chicks, the observed IBD titres were 87 at 7 dpv, 740 at 14 dpv and 1027 at 21 dpv (Table.8 and Fig.7). In 14th day vaccinated chicks, the observed IBD titres were 46 at 7 dpv, 45 at 14 dpv and 984 at 21 dpv (Table.9 and Fig.8)

Table 4. Persistence of mean antibody titres in IBD vaccinated female parent chicken from 31st to 40th week of age

Age in weeks	n	Mean ± S.E (Titre)	CV (%)
31	20	2,707^{ab} ±454.7	75.1
32	20	3,121^{a} ±361.7	5 1.8
33	20	3,550^{a} ±491.9	61.9
34	20	2,613^{ab} ±391.3	66.9
35	20	3,085^{a} ±487.3	70.6
36	20	3,365^{a}±426.3	56.6
37	20	2,486^{ab} ±270.7	48.6
38	20	3,054^{a} ±330.6	48.3
39	20	2,391^{ab} ±380.1	71
40	20	1,553^{b}±191.1	55

n = no.of random serum samples

Values with different superscripts vary significantly.

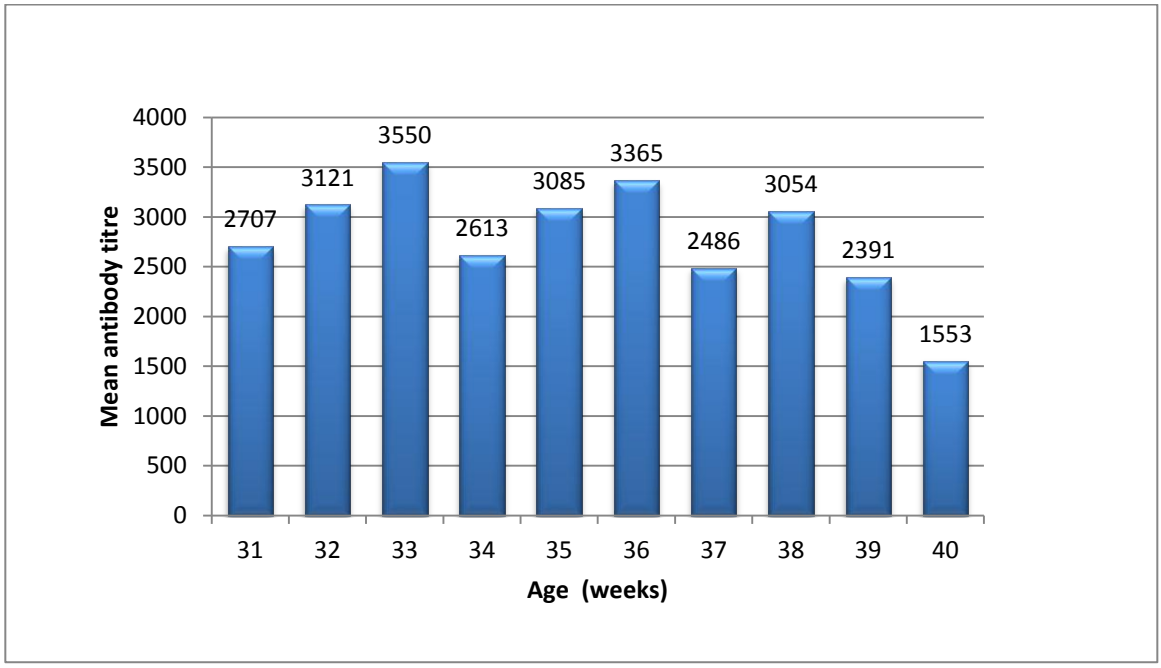

Fig.4: Persistence of mean antibody titre in IBD vaccinated female parent chicken from 31st to 40th weeks of age.

Table 5. Mean of maternal antibodies transfer from Parents to Chicks

Age in weeks	n	Mean±S.E	CV (%)
31 week parent birds	20	2707±454.7	75.1
Chicks of 31st week parent birds	10	2604[a]±629	76.3
35th week parent birds	20	3085±487.3	70.6
Chicks of 35th week parent birds	10	1161[b]±297	80.9
40th week parent birds	20	1553±191.1	55
Chicks of 40th week parent birds	10	1058[b]±213	41.9

Table 6. Percentage of maternal antibodies transfer from parents to chicks

Age in weeks (parent birds)	Female parent birds Ab mean titre	Chicks mean Ab titre	Transfer percentage
31	2706	2604	96.1
35	3084	1161	38
40	1553	1058	68.1

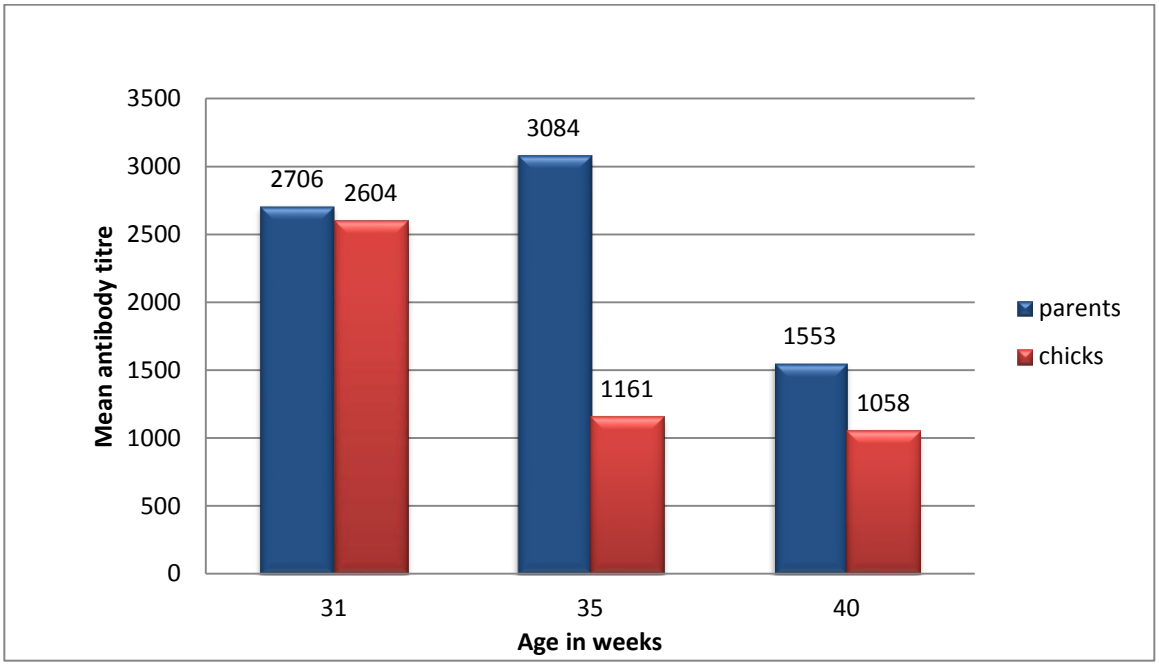

Fig.5: Mean titres of IBD maternal antibodies transfer from parents to chicks

Table7. Decaying mean of maternal antibody titre in unvaccinated chicks from day-old to 35 days of age.

Age in days	n	Mean ±S.E	CV(%)
1	10	2,604 [a] ±629	76
3	10	1,355 [a] ±475	111
5	10	1,203 [a] ±384	101
7	10	648 [ab]±202	98
10	10	406 [c] ±155	121
14	10	91 [c] ±47	163
18	10	89 [c] ±67	239
21	10	15 [c] ±9	193
24	10	11[c] ±9	193
28	10	6 [c] ±4	200
31	10	3 [c] ±2	267
35	10	2 [c] ±1	100

n = no.of random serum samples

Values with different superscripts vary significantly.

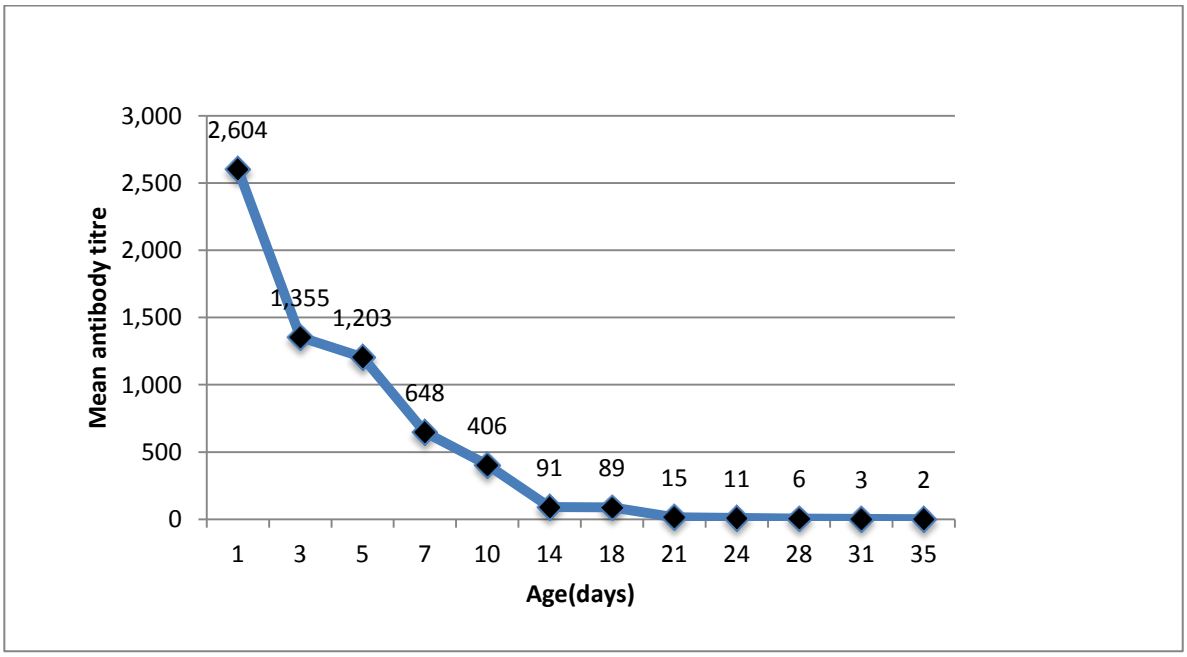

Fig.6: Decaying mean of maternal antibody titre in unvaccinated chicks from day-old to 35 days of age.

Table 8. Seroconversion in chicks vaccinated at 9th day

Age	n	Mean	CV (%)
Day-old	10	1,161[a] ±297	81
9th day	10	205[b]±51	79
9th day	Vaccination		
After 7 (16th day of age)	10	87[b] ±37	134
After14 (23rd day of age)	10	740[a] ±157	67
After 21 (30th day of age)	10	1,027[a] ±168	52

Table 9. Seroconversion in chicks vaccinated at 14th day

Age	n	Mean	CV (%)
Day-old	10	1,161[a] ±297	81
9th	10	205[b]±51	79
14 day	10	181[b] ±61	107
14th day	vaccination		
After 7 (21st day of age)	10	46[b] ±14	98
After 14 (28th day of age)	10	45[b] ±29	207
After 21 (35th day of age)	10	984[a] ±297	95

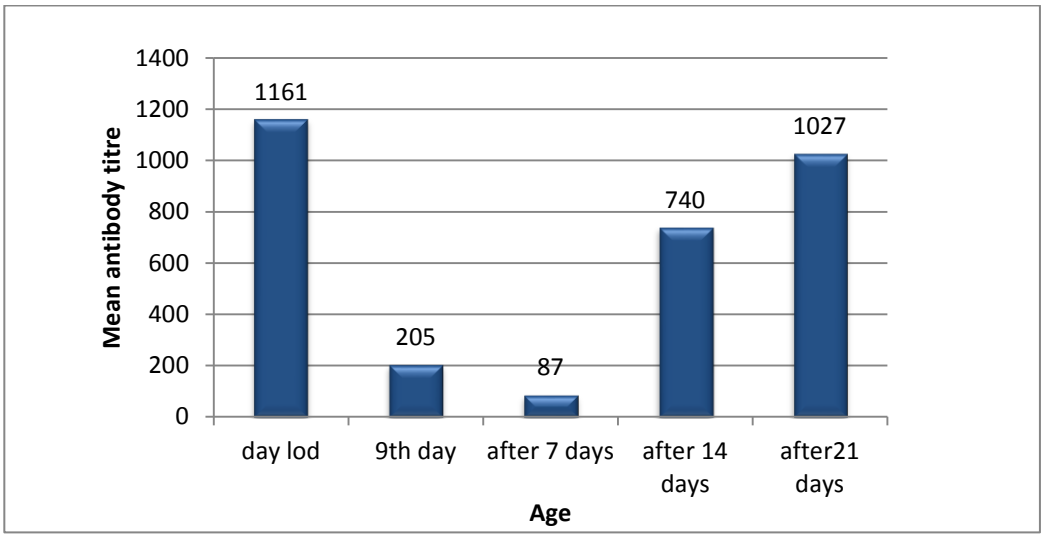

Fig.7: Seroconversion after 7, 14, 21 days post vaccination in 9th day IBD vaccinated chicks

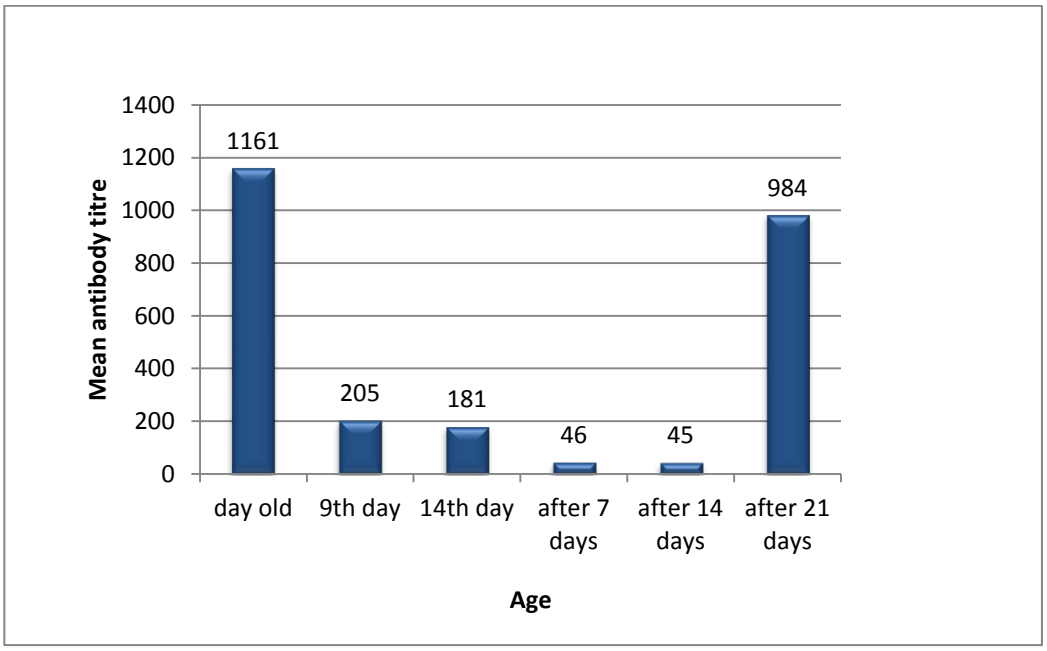

Fig.8: Seroconversion after 7, 14, 21 days post vaccination in 14thday IBD vaccinated chicks

4.2 SERO MONITORING STUDIES ON NEW CASTLE DISEASE (ND) VACCINES

4.2.1 Persistence of mean antibody titre in ND vaccinated female parent chicken from 31st week to 40th week

Serum were collected from ND vaccinated Vanaraja female parent chicken at weekly interval from 31stweek to 40th week and persistence of mean antibody titre was calculated by using Haemagglutination Inhibition (HI) test. The observed persistence of mean ND antibody titre in 31st to 40th weeks parent chicken varied significantly ($P<0.05$) among the groups. The data on the persistence of ND vaccine antibody titre in female parent chicken are shown in Table.10 and Fig.9

4.2.2 Rate of maternal antibody transfer from parents to chicks

Blood samples were collected from day-old chicks of 31st, 35th and 40th week parent birds and Ab transter rate was calculated by using HI test. The estimated transfer rate from parent birds to chicks (shown in Table11 and 12, Fig.10) were 88.2%, 90.2% and 80% at 31st, 35th and 40th week, respectively.

4.2.3 Decaying mean of maternal antibody titre in unvaccinated chicks

Blood was collected from day-old chicks of 31st week parent birds up to 35 days in a periodical interval (1, 5, 7, 10, 14, 18, 21, 24, 28, 31 and 35th day) and decaying rate of antibody titre was calculated. The observed ND maternal derived antibody titre significantly ($P<0.05$) decreased from day-old to 35 days. The ND Ab titre (log2) was 8.3 at day one and became approximately half (3.5) at 14th day. Subsequently, the titre decreased to 1.8 at 21 day and become zero at 31st day (Table.13, Fig: 11).

Table 10. Persistence of mean antibody titre in ND vaccinated female parent chicken from 31st week to 40th week.

Age in weeks	n	Mean	CV (%)
31	8	9.4 ^{a}±0.4	12.8
32	8	7.9^{bcd} ±0.4	12.7
33	8	8.0^{bcd} ±0.5	16.3
34	8	8.0^{bcd} ±0.3	11.3
35	8	8.8 ^{ab}±0.4	11.4
36	8	7.0 ^{d} ±0.3	12.9
37	8	7.5 ^{cd} ±0.5	18.7
38	8	8.4 ^{abc}±0.4	13.1
39	8	7.3 ^{cd} ±0.3	12.3
40	8	8.9 ^{ab}±0.2	6.7

n = no.of random serum samples

Values with different superscripts vary significantly.

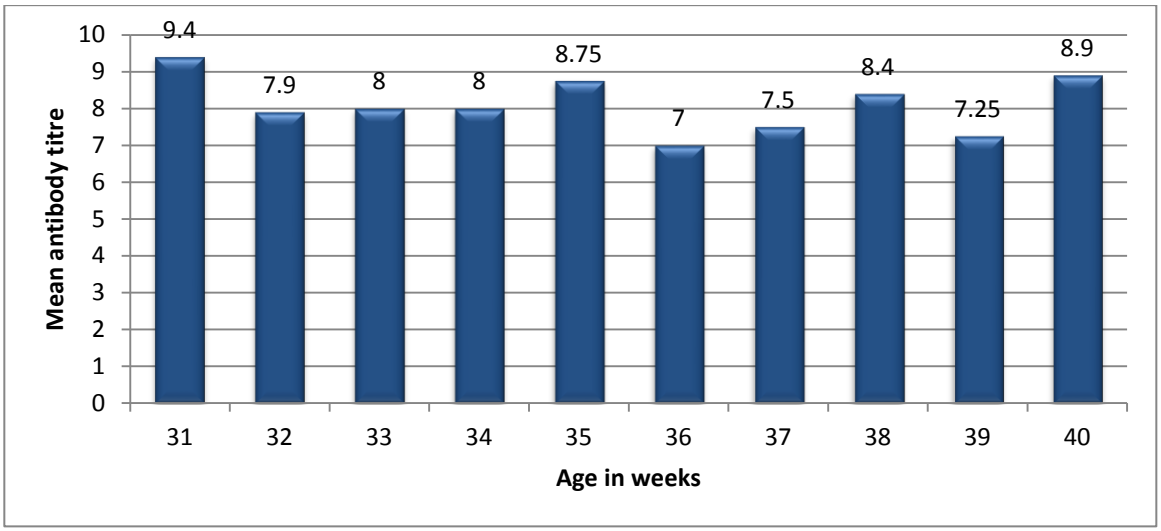

Fig.9: Persistence of mean antibody titres in ND vaccinated female parent chicken from 31st week to 40th week.

Table 11. Mean titres maternal antibodies transfer from parents to chicks

Age in weeks	n	Mean±S.E	CV (%)
31st week parent birds	8	9.4 ±0.4	13
Chicks of 31st week parent bidrs	8	8.3 [a] ±0.5	17
35th week parent birds	8	8.8 ±0.4	11
Chicks of 35th week parent birds	8	7.9 [a] ±0.4	14
40th week parent birds	8	8.9 ±0.2	7
Chick of 40th week parent birds	8	7.1 [a] ±0.5	21

Table 12. Persentage of maternal antibodies transfer from parents to chicks

Age in weeks (parent birds)	Parent birds	chicks	Transfer percentage
31	9.4	8.3	88.2
35	8.75	7.9	90.2
40	8.9	7.1	80

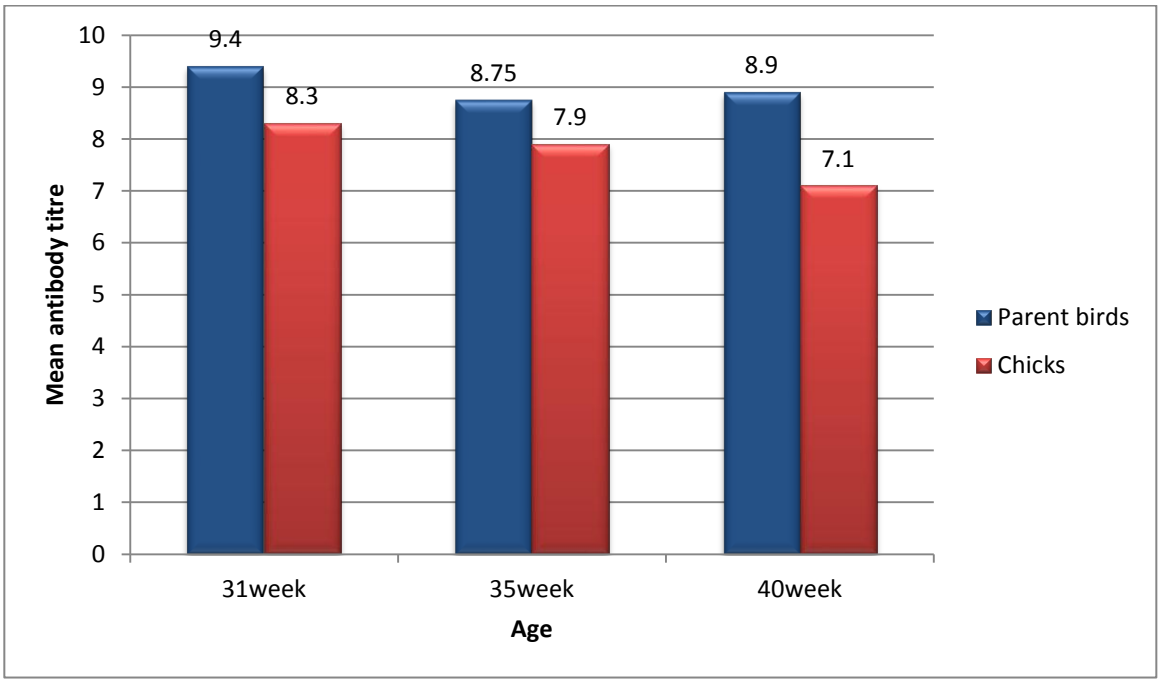

Fig.10: Mean titres of maternal antibodies transfer from parents to chicks

Table 13. Decaying mean of maternal antibodies in unvaccinated chicks from day-old to 35 days

Age in days	n	Mean ±S.E	CV (%)
1	8	8.3 [a]±0.5	17
3	8	8.1 [a]±0.4	14
5	8	6.6 [b] ±0.4	18
7	8	6.4 [b] ±0.6	25
10	8	5.0 [c]±0.3	18
14	8	3.5 [d]±0.5	37
18	8	2.0 [e]±0.3	40
21	8	1.8 [e]±0.4	56
24	8	1.3 [e]±0.2	38
28	8	0.1 [f]±0.1	40
31	8	0.0 [f]±0.0	0
35	8	0.0 [f]±0.0	0

n= no.of random serum samples

Values with different superscripts vary significantly.

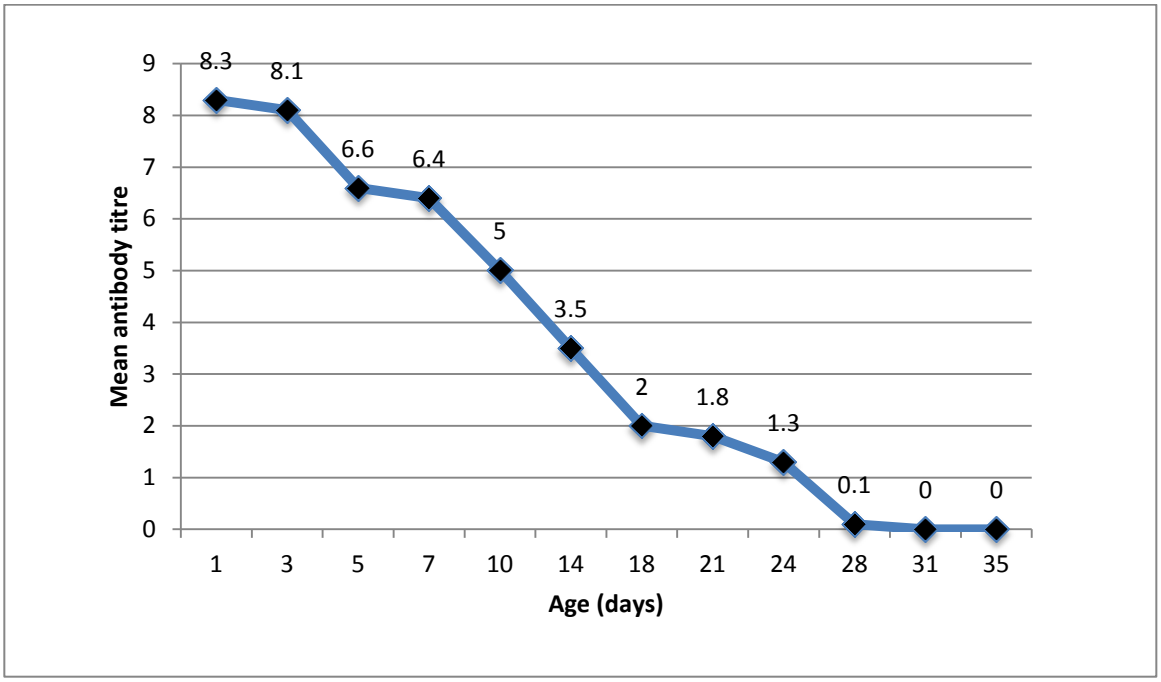

Fig.11: Decaying mean of ND maternal antibody titres in unvaccinated chicks from day-old to 35 days of age.

CHAPTER V

DISCUSSION

During the last two decades, poultry industry in India has reached a stage of self-sufficiency. Rapid growth of the industry has encouraged many farmers to adopt poultry farming as the main source of their income. However, in-spite of the prevailing favourable agro-climatic conditions and availability of high producing strains of birds, the poultry industry is witnessing a series of problems mainly due to recent spurt of various diseases like Infectious bursal disease (IBD).

Infectious bursal disease virus belongs to the genus *Avibirnavirus* with in the family *Birnaviridae*. It is a non-enveloped, icosahedral, bi-segmented, double stranded RNA virus with a diameter of about 55-60 nm (Ismail and Saif, 1990).

Infectious bursal disease is an acute viral disease mainly affecting young chickens and is of great economic importance. The disease causes considerable morbidity and mortality mainly by causing immune suppression. The virus targets B-lymphocytes in bursa causing their depletion, which increases the susceptibility of IBD affected birds to other concurrent infections and poor response to vaccines.

The epidemiology of infection is not extensively studied but it is known that the virus is contagious. Contact with the infected birds and contaminated fomites could result in the spread of infection. Rigorous biosecurity measures have to be implemented in order to stop the spread of virus from one flock to the next. The virus is environmentally stable and resistant to many physical and chemical agents. Integrated nature of commercial poultry operations and vectors like lesser mealworm, mosquitoes and rats pose extra problems for the control of this infection. No therapeutic treatment has been found to have

an effect on the course of the viral infection. There are no reports of the use of the antiviral compounds and interferon inducers for the treatment of IBD (Lukert and Saif, 2003).

Infectious bursal disease virus is highly infectious and very resistant to inactivation therefore, despite strict hygienic measures; an effective IBD prevention and control program must involve an effective breeder vaccination program, an effective bio-security program and effective broiler vaccination programs are recommended to prevent IBD (Teshome and Admassu, 2015).

The major problem with active immunization of maternally immune chickens is to determine the proper time of vaccination that allows adequate replication of the vaccine virus at the same time protects young chicken from disease. The time of vaccination varies with the level of maternal antibodies, route of vaccination and virulence of the vaccine virus. For a successful vaccination programme the factors like environmental stress, management and flock profiling for the presence of maternal antibodies should be taken into account (Lukert and Saif, 2003).

The present study was taken up to predict the optimum age for vaccination in chicks based on IBDV antibody flock profiling in vaccinated Vanaraja female parents and their chicks for prevention of disease. Parent birds and their chicks were maintained separately in an isolated condition and parent birds were vaccinated against all the contagious diseases and chicks were vaccinated against only for marek's disease at the age of day-old. Sera samples were collected from female parent birds were at weekly interval from 31^{st} – 40^{th} week and persistence of mean antibody titre was calculated. Sera samples also collected from day-old chicks of 31^{st}, 35^{th} and 40^{th} week parent chicken and mean antibody transfer rate from parents to chicken was calculated. Chicks of 31^{st} week parent flock were maintained from day-old to 35 days and serum samples were collected

periodically (1, 3, 5, 7, 10, 14, 18, 21, 24, 28, 31 and 35th day) and decaying mean antibody titre was calculated. Indirect ELISA test was used for estimation of IBD antibody titre in sera samples of parent birds and chicks. Based on transfer rate and decaying mean of antibody titre, predicted the optimum day of vaccination in Vanaraja breed chicks.

SERO MONITORING STUDIES OF INFECTIOUS BURSAL DISEASE VACCINES

1. Persistence of mean antibody titre in IBD vaccinated female parent chicken from 31st week to 40th week

The reason for selecting the particular age (31st week - 40th weeks) was the parent birds were having higher egg production and hatchability at that age. The observed persistence of antibody mean titres in parent birds (2707, 3121, 3550, 2613, 3085, 3365, 2486, 3054, 2391 and 1553) were significantly ($P<0.05$) varied at different ages. All age group of parent birds were having protective level of antibodies >1500 (Ostyina *et al*., 2009) but there was no particular pattern of increasing or decreasing observed. The possible reason for variation of IBD antibodies could be due to random selection of breeder birds taken randomly every time from a flock of 1000 birds. The titre also depends on the immune status and genetics of the birds.

2. Rate of maternal derived antibody transfer from parents to chicks

In this study, the observed IBD antibody transfer rate from parent birds to their chicks was (96.1%, 38% and 68.1%) significantly different ($P<0.05$) at 31st, 35th and 40th weeks, respectively.

Bart van Leerdam and Arts (2011) and Fantay *et al.* (2015) reported antibody transfer rate in chicks of 38th week parent birds was 57.7% and 56.57%, respectively.

Infectious bursal disease virus antibodies transfer rate was 45% in layers (Fahey *et a*., 1987) and ranged from 30-53% in native Egyptian chickens (Moneim and Gawad, 2006).

Gharaibeh *et al.* (2008) reported the rate of mean antibody transfer as 73.6% [(Average of 37^{th} week (83.4%), 40^{th} week (60.3%), 45^{th} week (77%)] from broiler type of parents chicken to their chicks and they also observed that maternal antibody transfer rate was higher in IBDV than other poultry diseases like avian encephalomyelitis, avian influenza, chicken anaemia, infectious bronchitis, lnfectious laryngotracheitis, Mycoplasma gallisepticum infection, Mycoplasma synoviae infection, Newcastle disease, and reovirus.

The IBDV targets B lymphocytes (Burkhardt and Muller, 1987); it is believed that this is why IBDV had highest rate of transfer compared with other pathogens mentioned above. This high transfer rate should be taken into account when vaccinating the hatching chicks because chicks with high maternal antibodies against IBDV showed no active immune response to vaccination at early ages (Naqi *et al*., 1983).

The variation in maternal antibody transfer from parents to chicks could be attributed to breeder strain, cage density and diet (Leandro *et al*., 2011). Fletcher *et al.* (1981) reported that probable reason lies on the egg yolk weight, as a proportion of chick weight, increases as the parent's age. Kramer and Cho (1970) reported that the efficacy of deposition of antibodies and other proteins in yolk also increases as the flock matures. Other reasons that affect maternal antibody transfer might be the seasonal variation and rate of egg production.

3. Decaying mean of maternal derived antibody titre in unvaccinated chicks

In the present study, the observed decaying mean of antibody titre in chicks of 31st week parent flock was significantly (P<0.05) decreased from day-old to 35 days. But there was no particular pattern of decreasing in antibody titre observed. Highest titre of antibody (2604) was seen in day old-chicks and was least or almost absent at 35th day chicks. A low level of antibody titres was observed from 14th day onwards followed by a complete decay at nearly 35th day. As per IDEXX, ELISA standard break through titre for intermediate plus vaccines is 500 and for intermediate vaccine 125 (as per UK standards it was 125-250). Hence, the estimated age of vaccination in chicks of 31st week parent birds was 12-13thday with intermediate live vaccine.

The results are in agreement with Ahmed and Akther, (2003). They reported that maternal antibodies in unvaccinated chickens persisted up to 21 days as determined by ELISA with complete decay by 28 and 35 days. In contrary Abdalla (2005) reported that the antibodies were diminished after the first week but persisted up to six weeks of age in progeny chicks.

The results are in agreement with Mitra *et al.* (1998) found that maternal derived antibody (MDA) levels were significantly lower at 12 days of age than in one-day-old chickens.

According to Fantay *et al.* (2015), antibody transfer rate was 56.5%, and proper time for administration of live intermediate IBD vaccine was 18 days instead of 21 days in chicks. Suzuki *et al.* (2009*)* reported that, 15th day is the optimal vaccination time for Gumboro disease which was three days earlier than reported by Fantay *et al.* (2015). This

difference might be due to variation in the type of vaccine used as they used live intermediate plus IBD vaccine instead of intermediate IBD vaccine only.

De Wit (1998) clearly stated that chickens vaccinated using live intermediate IBD vaccine had a capacity to breakthrough maternally derived antibody titre of 125 whereas those vaccinated using intermediate plus IBD had the capacity to break through maternal antibody titre of 500. These substantial differences could be ascribed to the amount of antibodies transferred from hen to chick through the egg (Hamal *et al*., 2006).

Lukert and Saif (1997) noticed that the half-life of MDA to IBDV is between three and five days. Similarly, other studies have reported that the half-life of MDA to IBD in chicks was 3.46 days (Saijo and Higashihara 1998) and decreased every five days (Alam *et al*., 2002).

Fahey *et al*. (1987) reported a half-life of 6.7 days for IBDV-specific MDA. This divergence may be explained by the influence on the half-life of MDA of the vaccine type, its time of application in hens (Alam *et al*., 2002) and probably the immune status of the hen (Kouwenhoven and Van den Bos 1992). Moreover, whilst the antibody titres may not vary greatly amongst hens in a single flock of similar age, the offspring of different vaccinated flocks may show different IBDV MDA titres.

When offspring of different parent flocks are raised together, this may result in different levels of MDA and compartmentalisation of the herd into individuals with low or high susceptibility to virulent IBDV (Tsukamoto *et al*., 1995). Under field conditions, however, the decay pattern of IBDV-specific MDA was proved to be more complex, as it depends largely on initial antibody levels, which may vary between batches and also within a batch, making it difficult to predict the optimal time for vaccination (De Wit, 1998).

Bart van Leerdam and Arts (2011) estimated half-life values of IBD maternal antibodies in commercial broiler breeder by the producers of the BioChek ELISA, to be ranging from 4.6 to 4.0 days, for samples taken at 01D to 10D, respectively. The results in this study confirmed that these approximate half-life values are correct, varying from 4.6 to 4.0 days in the age interval of 01D to 10D. An important point is that half-life time values vary and depend on the age of the bird when the sample is taken. This is important, because correct vaccination date prediction, also depends on the correct use of half-life values, which should correspond with the age at which the serum samples are taken.

The highest half-life observed was during 1st and 2nd day of age, being 4.6 days. Thereafter, the halflife values fluctuated around a fairly constant decline rate of about 4.0 days, between 03D and 10D of age. The higher half-life values during the first two days in the life of a young chick are thought to be the result of rising MAb titres, due to the "Yolk-sac effect". The resorption of yolk, containing maternal antibodies, is highest during the first three days in the life of a young chick, resulting in rising (or stable) titres, rather than decreasing titres.

4. Vaccination of chicks based on predicted date of vaccination

Serum samples were collected from day-old chicks of 35^{th} week parent birds and observed antibody titre was 1161. Based on initial titer, estimated day of vaccination in chicks of 35^{th} week parents was $9\text{-}10^{th}$. Chicks hatched from 35^{th} week parent birds were divided into two groups, one group was vaccinated on 9^{th} day and the other on 14^{th} day (which was normally followed at DPR). The observed antibody mean titres in both groups at 7, 14, 21 days post vaccination varied significantly ($p<0.05$) between groups.

In the present study, earlier seroconversion was observed at 14 days post vaccination (dpv) in 9^{th} day vaccinated chicks. The early seroconversion in 9^{th} day

vaccinated chicks might be due to low maternal antibody interference with the vaccine efficacy and also there may be chance of decrease in the disease incidence by decreasing window period to exposure to causative agent compared to regular vaccinated chicks.

Seroconversion was slow in 14th day vaccinated chick, as compared to 9th day vaccinated chick, almost low or absence of maternal antibody interference with immune response in 14th day vaccinated. However, there may be risk of susceptibility to disease during window period (9-14th day). The delayed seroconversion in the 14th day vaccinated chicks may be due to IBD vaccine virus neutralization of MAbs with cross-infection from 9th day vaccinated chicks because chicks of both groups are maintained in the same shed simultaneously.

CHAPTER VI

SUMMARY

Infectious bursal disease (IBD) is a highly contagious disease of young chicken caused by Infectious bursal disease virus (IBDV), characterized by immune suppression and mortality generally at three to six weeks of age. It is economically important to the poultry industry worldwide due to increased susceptibility to other diseases and negative interference with effective vaccination.

Infectious bursal disease has been endemic in many poultry flocks around the world since 1960s. It was controlled effectively until recently by vaccination of the young birds as well as the parent flocks with live and inactivated oil emulsion vaccines. Progeny chicks from these dams have a high level of maternal antibodies and are protected from bursal damage and immunosuppression induced by field strains of IBDV. Despite many vaccination schedules, outbreaks are still recorded. This study was conducted to predict the optimum day of IBD vaccination in Vanaraja breed chicks based on transfer rate of maternal antibodies from parents to chicks and decaying mean of maternal antibody titre in chicks.

The experiment was conducted at ICAR, DPR, Rajendranagar, Hyderabad from February to May 2016. Parent birds and chicks were maintained separately in an isolated condition.

Serum samples were collected at weekly intervals from 31^{st} – 40^{th} week of age randomly from each of 20 birds in a flock of 1000 Vanaraja female parent birds which were vaccinated at 18^{th} - 20^{th} weeks of age. The antibody titres were estimated by indirect ELISA. The observed persistence of IBD antibody mean titres from 31^{st} - 40^{th} week were

2707, 3121, 3550, 2613, 3085, 3365, 2486, 3054, 2391 and 1553, respectively and were significantly different ($P<0.05$) among different ages.

Serum samples were collected from day-old chicks of 31st, 35th, 40th week parent birds and transfer rate of IBD maternal antibodies was calculated. The estimated transfer rate in the chicks was found to be 96.1%, 38% and 68.1%, respectively and varied significantly ($P<0.05$) at different ages of parent birds.

Serum samples were collected periodically from chicks hatched from 31st week parent birds (1, 3, 5, 7, 10, 14, 18, 21, 24, 28, 31 and 35th day) and decaying mean of IBD MDAb titre was calculated using indirect ELISA. The highest antibody titre was observed in day old-chick (2604), least or almost no mean antibody titre was observed at 35th day. Break through titre of maternal antibody titre (125) for intermediate vaccine was found to be on 12-13th day. Hence, the day of vaccination for efficient protection is predicted to be on 12-13th day.

Serum samples were collected from day-old chicks of 35th week parent birds and observed antibody titre was 1161. Based on the titre, the estimated day of vaccination in chicks of 35th week old parents was 9-10th day. Chicks hatched from 35th week parent birds were divided into two groups, one group was vaccinated on 9th day and the other group was vaccinated on 14th day (which was normally done at DPR). Sera were collected from both groups at 7, 14 and 21days of post vaccination (dpv) and Ab titre was estimated using indirect ELISA. The antibody response was observed at 14 dpv in chicks vaccinated at 9th day while in 14th day vaccinated chicks, the response was delayed and observed at 21dpv.

The same serum samples were also used for estimation of efficacy of ND vaccine. The persistence of mean antibody titre from 31st- 40th week parent birds (9.4, 7.9, 8.0, 8.0,

8.8, 7.0, 7.5, 8.4, 7.3, and 8.9) was significantly different ($P<0.05$). The estimated transfer rate of ND maternal antibodies from parent birds to chicks was 88.2%, 90.2% and 80% at 31st, 35th and 40th week of age respectively. The ND Ab titre (log2) was 8.3 at day one and became approximately half (3.5) at 14th day. Subsequently, the titre decreased to 1.8 at 21 day and became zero at 31st day.

In conclusion, the results of the present study showed that the rate of maternal antibodies transferred from parents to chicks was not the same every time from hatch to hatch and also transfer rate does not depend on their parental antibody titre. The decaying rate of antibody titre in chicks depends on initial titre of chick but does not depend on their parents titre. Appropriate vaccination time in chicks also depends on initial titre of chick. Correct time of vaccination will produce protective vaccine efficacy faster compared to standard vaccination schedule that is followed currently.

The study indicates that there is no straight relationship between maternal antibody titre and passing on of protective antibody titre to chicks.

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