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ABSTRACT

The present study was taken up to evaluate the efficacy of different Infectious Bursal Disease (IBD) vaccines using different vaccination schedules employing different strains of IBD vaccines. The response to vaccination in terms of seroconversion, the extent of bursal damage and immunosuppression caused by these vaccines were studied.

A total of 280 layer chicks were randomly divided into seven groups each consisting of 40 chicks. Six different IBD vaccination schedules were tested using different combinations of IBD vaccines. Group I was maintained as a control without giving any IBD vaccination. All the birds including control received Newcastle Disease (ND) vaccination as per the standard schedule.

The serum samples were collected from all the groups at weekly intervals for 20 weeks (maximum period tested). The IBD antibodies were estimated using IDEXX - ELISA (Flockcheck) kit. A gradual decline of IBD maternal antibodies was noticed in the control group and the samples were found to be negative by sixth week of age. Vaccination of birds with IBD vaccines did not interfere with the decay of maternal immunity. In group II which received IBD vaccines at day old age, the immediate sero conversion was absent and the antibody titres raised only after subsequent vaccinations. The results of ELISA in other groups showed that, lowest titres were recorded during the third week after which the titres increased gradually reaching peak between eight to twelve weeks of age and a slight decline was observed.
by the end of 20th week. But they were all found to be above protective levels till 20 weeks of age. No significant difference was noticed between the titres of different treatment groups. The titres in group III which received an oil emulsion IBD vaccine after initial live virus priming also showed similar response in comparison to other groups.

Significant difference was noticed in the bursa body weight (B-BW) ratios of IBD vaccinated groups in comparison with the control group. During third week of age, the B-BW ratios recorded were more in vaccinated birds while reduced B-BW ratios were recorded during fourth and fifth weeks in comparison with the control. Histopathology of the bursal sections revealed varying degrees of bursal damage in the vaccinated groups while the bursae collected from the control group were normal. Bursal lesion scores were calculated in terms of the lymphoid depletion in the follicles and were graded between 0 to 4. Highest bursal lesion scores were noticed in groups vaccinated with intermediate plus strain of IBD vaccines.

The immunosuppressive effect of different IBD vaccines was evaluated in terms of response to ND vaccination. The results revealed that there was a gradual increase in the NDV-HI titres from third week onwards in all the groups including the control. But a moderate suppression of vaccine response was noticed in groups V, VI and VII which received either one or few doses of intermediate plus vaccines. But all the HI titres were found to be well above the protective level.
Based on the results obtained in the present study it is concluded that even though live IBD vaccines caused significant bursal damage, the immunosuppression caused by these vaccines was only moderate. Hence, they can be used at the field level by selecting a least immunosuppressive strain. Vaccination of day old birds with IBD vaccines and usage of an oil emulsion vaccine in the early days of life did not confer any special advantage. Also no additional advantage was observed by giving a third dose of IBD vaccine after fourth week of age.
CHAPTER - I

INTRODUCTION

Infectious Bursal Disease (IBD) or Gumboro disease is an acute highly contagious viral disease of young chickens of three to six weeks of age, characterised by destruction of lymphoid cells in the bursa of Fabricius causing immunosuppression in addition to losses due to impaired growth and death and excessive condemnations of carcass because of skeletal muscle haemorrhages.

The disease is a major problem in concentrated poultry production areas throughout the world. The affected chickens have reduced antibody response to vaccinations, strong post vaccinal reactions and increased susceptibility to concurrent or secondary infections. Extreme resistance of infectious bursal disease virus to environmental conditions and lateral transmission of virus by direct and indirect contact between infected and susceptible flocks has been responsible for rapid spread of virus resulting in wide spread occurrence of severe IBD.

The IBD virus consists of two known serotypes but only serotype I and its variants were pathogenic to chickens. The classical type I conventional strain was responsible for low, that is upto a maximum of 20 percent mortality in susceptible flocks. However, the immunosuppression caused by this virus adversely affected growth rate, livability and productivity due to subsequent exposure to wide range of viral, bacterial, fungal and protozoal agents. In India, classical IBD was recorded for
the first time in 1971 by Mohanty et al. Until early 1990s, the IBD infection was less alarming with sporadic outbreaks.

Emergence of very virulent form of the IBD (vvIBD) characterised by high morbidity and mortality was first reported in Belgium and Netherlands in 1987, followed by its incidence in France, Germany and UK by 1989-91, middle and southeast Asia by 1992. In India, during November 1992, vvIBD outbreaks were recorded in West Bengal. The disease gained proportion of an epidemic and the wave of vvIBD spread across the length and breadth of India. In the initial phase, the vvIBD virus swept through thickly populated poultry belts of India and took a very heavy toll in layer birds with spiking mortality.

Measures to control the IBD outbreaks were initiated using mild vaccines. Studies conducted have demonstrated that mild vaccines of IBD were totally ineffective against challenge with vvIBD virus. Hence, intermediate IBD vaccines replaced the mild IBD vaccines in the field. Intermediate vaccines provided some protection, but were unable to completely protect the flocks. Therefore intermediate plus (hot) strains of vaccines were introduced which are more invasive and can be effective even in the presence of high maternal antibody titres.

Live intermediate plus vaccines, though confer immunity in chicks, found to cause bursal atrophy and bursal damage with immunosuppression leading to non responsiveness to other live attenuated vaccines against respiratory infections such as ND and Infectious Bronchitis in the flock. These vaccination practices even though
logical, have not been tested adequately either under controlled laboratory conditions or field situation.

In Andhra Pradesh farmers in different regions are following different vaccination schedules against IBD that are suggested by local hatcheries. In these farms inspite of routine vaccination with ND vaccines, there have been reports of breakdown of immunity due to IBD.

Keeping all the above problems in view, the present study was undertaken with the following objectives.

1. To assess the level of antibodies produced by different commercial infectious bursal disease vaccines using different vaccination schedules.

2. To assess the extent of bursal damage caused by different IBD vaccines.

3. To study the effect of IBD vaccines on immunity to ND vaccination.
CHAPTER - II

REVIEW OF LITERATURE

Infectious bursal disease (IBD) is an acute, highly contagious viral disease of young chickens characterized by severe damage to the bursa of Fabricius and immunosuppression (Lukert and Saif, 1991).

2.1 HISTORY

This disease was first reported by Cosgrove (1962) in three to four weeks old broilers from a country town of Gumboro, Southern Delaware, U.S.A. There after, the disease has been reported in almost all parts of the world (Saif, 1998).

In India, the disease was first reported in Uttar Pradesh by Mohanty et al. (1971). Since then, the disease has been reported from Karnataka, Andhra Pradesh and Tamil Nadu (Jayaramaiah and Mallick, 1975), Madhya Pradesh and Maharashtra (Dongaonkar and Rao, 1979), Bihar (Chauhan et al., 1980), Haryana (Verma et al., 1982), West Bengal (Bhattacharryya et al., 1983), New Delhi (Panisup and Verma, 1986), Gujarat (Jhala and Kher, 1991), Andaman and Nicobar Islands (Rai et al., 1997).
2.2 STRAINS OF IBDV/IBDV VARIANTS

Infectious bursal disease is caused by a bi-segmented double stranded RNA virus (Hirai and Shimakura, 1974) belonging to the family Birnaviridae (Dobos et al., 1979).

Mc Ferran et al. (1980) were the first to report antigenic variations among IBDV isolates of European origin. They presented the evidence for the presence of two serotypes designated 1 and 2. They reported only 30 percent relatedness between several strains of serotype 1 and the designated prototype of that serotype. Jack Wood et al. (1982) have observed similar results in the USA and the American serotypes were designated as I and II.

Rosenberger and Cloud (1985) described variant viruses of serotype 1. They observed that the vaccine strains available at that time did not protect the poultry against the variants, which were antigenically different from the standard serotype 1.

Rosales et al. (1989a) reported that isolates U-28 and 3212 differed in antigenicity and pathogenicity from known serotype-1 IBDV isolates. Sharma et al. (1989) have further confirmed the presence of variants in IBDV.
Ohio State University has divided serotypes of IBD virus into six sub-types using cross-virus neutralization testing. The first five sub-types were closely related and vaccination with commercial vaccine provided 80 percent protection, which was referred to as ‘standard sub-type’. Whereas sixth and last sub-type was called as variant of serotype 1 and the protection conferred was in the range of only 20 to 70 percent (Mohiuddin, 1994). Pahar and Rai (1997) reported the presence of three strains with in serotype 1.

2.3 PROPERTIES OF THE VIRUS

Infectious bursal agent is transmitted by contact, contaminated litter and the environment remains infected for at least 122 days after infection (Benton et al., 1967a). Mc Ferran et al. (1980) and Jack Wood et al. (1982) reported that serotype 1 replicated preferentially in B-lymphoid cells of the bursa of Fabricius and was the causative agent of IBD of chicken, whereas serotype 2, which was originally isolated from turkeys does not have selective tropism for B-lymphocytes and was not pathogenic for chicken.

The target cells for the IBD virus were actively dividing B-lymphocytes (Muller, 1986). In bursectomised and non-bursectomised chickens following oral inoculation initial viral replication occured in gut associated lymphoid cells and secondary replication in the bursa of Fabricius was responsible for high titres of virus and mortality. Infection of B-lymphocytes in the bursa was cytolytic leading to immunosuppression. Mortality and clinical signs of IBD have also been associated with immune complexes (Ley et al., 1979; Skeelels et al., 1979a) and a depletion in
circulating levels of hemolytic complement (Skeele et al., 1979b) and clotting abnormalities (Skeele et al., 1980).

Infectious bursal disease virus was very stable persisting in poultry houses even after thorough cleaning and disinfection (Lukert and Hitchner 1984). Virus was resistant to heat, ultraviolet radiation, photodynamic inactivation (Petek et al., 1973) ether, chloroform and the virus gets inactivated at pH 12.0, but unaffected at pH 2.0 (Benton et al., 1967b). Exposure to one percent phenol or one percent cresol for one hour inactivated the virus (Cho and Edgar, 1969) and virus infectivity was markedly reduced by exposure to 0.5 percent formalin for six hours (Benton et al., 1967b) or to one percent formalin for one hour (Cho and Edgar, 1969).

The serotype 1 caused immunosuppressive disease in chicken (Jack Wood et al., 1985). The serotype 2 was infectious but not pathogenic to chickens (Ismail et al., 1988). However, Karunakaran et al. (1993) has reported that both the serotypes were pathogenic to chicks with more or less same morbidity and mortality.

Chicks with maternally derived antibody to either serotype 1 or 2 had good protection against homologous challenge but not against the heterologous virus (Chettle and Wyeth, 1989).

2.4 SERODIAGNOSIS OF IBD
2.4.1 Enzyme-Linked Immuno Sorbant Assay

Marquardt et al. (1980) reported that ELISA test was found to be safe, highly reproducible and sensitive. Results can be read visually or by spectrophotometry. Antibodies could be detected as early as four days post infection.

Howie and Thorsen (1981) reported that ELISA was more sensitive than virus neutralization (VN) test for detecting IBDV antibodies, resulting in positive readings with sera that were negative by the VN test.

The ELISA detected positive sera earlier than virus neutralization (VN) test, which in turn was more sensitive than the Quantitative agar gel precipitin (QAGP) test. The ELISA and QAGP tests were less variable, more reproducible and easier to perform than the VN test (Nicholas et al., 1985)

Solano et al. (1986a) reported that computer assisted kinetic-based Enzyme-linked Immunosorbant Assay (KELISA) was more sensitive, faster and more reliable than the virus neutralisation test for measuring the decay of maternal antibody in chicken. Asi and Iyisan (1991) reported that ELISA was more sensitive and rapid, detecting antibodies 14 days after vaccination compared with the Quantitative agar gel precipitin (QAGP) method, which can detect after 21 days of vaccination.
Amiya Kumar et al. (1991) developed ELISA test system for the first time in India to detect and quantify IBD viral antibodies and reported that ELISA was able to establish a temporal response to infection from one to six weeks post infection and it was possible to correlate the OD values with antibody levels. The results on microplate can be read visually and the test can be run in less than three hours.

The ELISA was very simple and accurate in evaluating the immune status of chicks against IBD and useful for the establishment of a vaccination programme (Cao-Yongchang et al., 1995).

Jack Wood et al. (1996) reported that commercially available ELISA kits detected antibodies to all the antigenic subtypes of IBD virus, while (Ohio-state University) OSU-ELISA detected antibodies to a subgroup of IBDV strains.

An ELISA titre of 400 and above was reported to be protective against challenge with IBDV while the titres of 150 resulted in susceptibility to IBDV (Deshpande and Muniyappa, 1996).

Gowri et al. (1997a) compared four diagnostic tests for the detection of IBD virus. The sensitivity of Dot-ELISA and Avidin-Biotin Dot ELISA was higher than that of Agar Gel Immuno Diffusion (AGID) and Counter Immuno Electrophoresis (CIEP), but there was no difference in the specificity and reliability among the four
tests. Dot-ELISA, Avidin-Biotin Dot ELISA and CIEP can be performed in much more shorter time than AGID.

Dewit et al. (2001) compared the validity of five commercially available ELISAs for detection of antibodies against IBD virus (Serotype 1). The specificity of the ELISAs varied from 63.8 to 100 percent and all ELISAs showed a sensitivity of 100 percent when tested sera collected at 21 days post vaccination. The specificity of the IDEXX standard ELISA kit was 100 percent and sensitivity was 77 and 88 percent at 11 and 14 days post vaccination respectively.

2.4.2 Other Diagnostic Tests

Infectious bursal disease virus antigen and antibody were demonstrated by different researchers, using various tests such as Agar gel precipitation test (Dennet and Bagust, 1980; Mackenzie and Spadbrow, 1981; Wyeth and Chettle, 1988; OIE, 1992; Vijaya 1993); Quantitative agar gel precipitation test (Wood et al., 1983; Wyeth and Chettle, 1990; Pahar and Rai, 1997; Counter immuno electrophoresis technique (Berg, 1982; Durajaiye et al., 1985; Ganesan et al., 1990, Dhinakarraja et al., 1994); Immunoperoxidase method (Cho et al., 1987); Immunodot assay (Lee, 1992); Latex agglutination test (Nakamura et al., 1993; Gowri et al., 1997b); Passive haemagglutination test (Dhinakarraja et al., 1995; Joshi et al., 1998); Double antibody sandwich competitive ELISA (Patnayak et al., 1997); Restriction endonuclease assay (Jackwood and Nielsen, 1997); Nucleic acid hybridization and Reverse Transcription Polymerase chain reaction (Tiwari et al., 1999).
2.5 IMMUNOSUPPRESSIVE EFFECT OF IBDV

Faragher et al. (1974) reported that immunosuppression was very marked when the infectious bursal agent was given at day-old, less so when given at seven days and barely detectable when given at 14 days or more.

Infectious bursal disease virus depressed the humoral antibody response of chickens to various vaccines. The disease primarily effects lymphocytes of bursa of Fabricius, although the spleen, thymus and cecal lymphoid tonsils are also damaged. It does not clinically affect neonatal or adult chicken but is limited to the age range in which the bursa of Fabricins is at its greatest development (Hirai et al., 1974).

Giambrone et al. (1977a) initiated a study to determine whether prior exposure to IBDV influenced the susceptibility of young broiler chicks to Eimeria tenella infection. They found that when day-old chicks infected with IBDV were subsequently challenged with E.tenella, they suffered significantly higher mortality than their hatch mates, which were not exposed to IBDV.

Giambrone et al. (1977b) reported that chicks that have been inoculated with IBDV at one day of age had a severe depression of bursa-dependent humoral immune function by 42nd day, but chicks inoculated with IBDV at 21 days of age produced
nearly normal antibody responses as compared with the responses in uninfected control chicks.

Anderson (1979) reported that IBDV had an immunosuppressive effect when inoculated into Specific-Pathogen-Free (SPF) chicks at one day old or half way through a two week immunization programme against Eimeria tenella, but was not immunosuppressive when inoculated after the immunization programme was complete.

Hopkins et al. (1979) observed severe immunosuppression and bursal damage in chicks vaccinated with IBDV vaccines at one day of age. The infected birds developed poor or delayed serological response to Brucella abortus strain-19 antigen. Further, they also observed depletion of lymphocytes in the bursa of Fabricius and bursal atrophy.

A severe immunosuppression to infectious bronchitis virus in chicks that were infected with IBDV at one or five day old was reported by Pejkovski et al. (1979). Edwards (1982) reported that immunosuppression caused by IBD vaccine virus given to chicks at one day old was shown to persist for four weeks in terms of response to *Brucella abortus* strain-19.

Response to vaccination for infectious bronchitis virus and Newcastle disease virus at one day of age was either blocked or significantly delayed by moderate levels
of maternal antibody and/or was suppressed by an apparent field outbreak of IBD (Snyder et al., 1986).

Antibody titres to both *Brucella abortus* and sheep red blood cell (SRBC) antigen were lower in Gland of Harder and serum of IBDV-infected broilers than uninfected controls (Dohms and Jaeger, 1988).

Onaga *et al.* (1989) reported that infection with pathogenic IBD virus impaired the development of immunity to coccidiosis. Mazariegos *et al.* (1990) compared the immunosuppressive properties of six IBD intermediate vaccine strains. Immunosuppression with strains B and D was more, it was moderate with strains A and C and mild with strains E and F.

Experimental studies revealed that IBD virus makes chicks susceptible to septicaemic infections produced by *E.coli* strains of high and low virulence. Infectious bursal disease virus on *E.coli* may induce additionally marked lymphocytic depletion in the bursa and thymus (Nakamura *et al.*, 1990).

Ramm *et al.* (1991) reported that immunosuppression arise as a direct consequence of infection of B-lymphocytes, but T-lymphocytes appeared to be non-susceptible in IBD affected chicken. Rao and Rao (1992) reported that extent of immunosuppression caused by IBD virus was dependent upon the age of the birds and the interval between the vaccination and entry of IBDV infection.
Lack of IBD booster vaccination favoured the establishment of subclinical IBD, which suppressed immunity and predispose the birds to colisepticaemia (Igbokwe et al., 1996).

### 2.6 MATERNAL IMMUNITY

Solano *et al.* (1986a) reported that day-old maternal antibody titre in the progeny corresponds to the levels of IBDV antibody of vaccinated pullets at the time of lay and the titre decreases rapidly after vaccination of chicks at one day of age.

Maternal antibody decreased to 8 and 9 on a log2 scale between 24 and 28 days, which is an effective period for initial immunization of chicks (Solano *et al.*, 1986b).

Maternal antibodies were found to disappear from the circulation of crossbred chickens with a half-life of six to seven days (Fahey *et al.*, 1987). Chicks from breeders vaccinated twice with live IBD vaccines were seronegative to IBD at 22 days of age (Adene *et al.*, 1989).
Lack of uniformity in the MDA of chicks leads to a critical period where immune and susceptible birds coexist in the same flock (Vanden Berg and Meulemans, 1991). Zajac and Novotna (1991) demonstrated that IBD maternal antibody titres of chicks decreased in the first four weeks after hatching. IBD-MDA concentration decreases to non-protective levels by 16-20th day in broiler chicks (Homer et al., 1992).

Sudhakaran et al. (1993) concluded that high concentration of maternally derived antibodies in chicks at the time of vaccination interfered with the development of protective immunity against IBD.

Tsai-Hsiangjung et al. (1995a) reported that neutralizing maternal antibody titre of 1:256 or higher could confer full protection, while chicks with a titre of 16 or lower were susceptible to IBD. The protection rate of chicks with maternal antibody titres at 128, 64 and 32 were 85, 47 and 14 percent respectively.

No immune response to IBD vaccine was observed in flocks with mean virus neutralizing maternal antibody (VN MA) titres of over 1:500, but there was good immune response in flocks with VN MA of less than 1:500 (Tsai-Hsiang Jung et al., 1995b).
Deshpande and Muniyappa (1996) reported that all the chicks have MDA against IBD up to 24 days of age and most of them were susceptible to IBDV by 27 days of age.

Saijo and Higashihara (1998) reported that half life of MDA in chicks to IBD was 3.46 days, initial antibody titre of 1:28 interfered with the live vaccination and vaccination was efficient when the MDA titre decreased to 1:80.

There was wide variation in the IBD MDA titres between and within the farm and they completely disappeared by 21 days of age after vaccination at seven days. Sero conversion occurred between 42 and 49 days after vaccination in all the birds (Makesh et al., 1999).

Balakrishnan (1999) reported that the IBD MDA titres in chicks decreased gradually and they became undetectable by 42nd day. Reddy and Koteswaran (1999) demonstrated that maternal antibody QAGID titre of 3.9 was found to be protective against challenge with 102 CID50 of field IBDV.

Reddy and Koteswaran (2001) reported that decay of MDA was slower up to 10th day of age and subsequently there was a rapid fall of titres at 14th day of age. The persistency of maternal antibodies depends upon the initial titres of chicks at day old and exposure of chicks to IBD contaminated environment hastens the decay of MDA.
2.7 VACCINATION AGAINST IBD

2.7.1 Live Vaccines

Naqi et al. (1980) evaluated three commercially available IBD vaccines and reported that at five weeks of age mean virus neutralizing antibody titres of the three vaccinated groups did not differ significantly.

Giambrone and Clay (1986a) reported that most efficacious initial vaccination programme for IBD was live vaccine followed by inactivated combined ND-IBD vaccine given sub cutaneously.

Solano et al. (1986b) reported that primary IBDV antibody response was not detected in chicks vaccinated at one or 15 days of age with live intermediate vaccine, but an immediate primary IBD antibody response was detected in chicks vaccinated at 28 days of age.

The degree of bursal damage produced by intermediate IBDV vaccine strain was significantly less severe than the damage caused by the field isolates in maternal antibody free chicken. The active immune response induced by vaccination was cross
protective against the pathological effects produced by the different field isolates (Rosales et al., 1989b).

Early (7 days) IBD vaccination was superior to vaccination at 14 or 28 days in terms of antibody response and protectivity against mortalities and bursal lesions (Adene et al., 1989).

Single inoculation with Gumbovak to pullets at three weeks of age produced antibody titres capable of protecting them against IBD until at least 100 days of age (Hahnewald et al., 1990).

Nakamura et al. (1993) reported that immunization with attenuated live IBD vaccines in commercial chicken flocks, have been successfully employed after decline of maternal antibodies.

Tsukamoto et al. (1995) reported that live attenuated intermediate strain of IBD protected 100 percent of the vaccinated commercial chicks against highly virulent IBDV, while mild attenuated strains A and B protected three fourths and none of the vaccinated commercial chickens from development of severe bursal lesions.

Cao Yong Chang et al. (1996) studied the immunization programme of IBD vaccines in chickens with different levels of maternal antibodies against IBDV and
suggested that young broilers can be vaccinated with live IBD vaccines at the day old age and revaccinated with IBD vaccines in drinking water at eight and 15 days of age.

Bekhit (1997) reported that two doses of IBD vaccination during broiler period was more protective (lower mortalities) than a single vaccination. Liu-Jue et al. (1999) reported that IBDV neutralizing antibody titres of (2968 provide protection against virulent IBDV.

A study on the evaluation of different IBD vaccines in layer chicks showed that the less attenuated 228 E strain induced higher protection (90 percent) than a combined vaccination with BUR 706 and an inactivated vaccine (Abd-El-Aziz, 2000).

Live attenuated IBD virus vaccine prepared from recent Egyptian isolate elicited high neutralizing antibody titres (18 log2) for a stationary phase of about seven months and the vaccine had superior potential immunogenic effect than commercial live mild and intermediate vaccines. Vaccinated chicks were well protected against challenge with highly virulent IBDV after three weeks of post vaccination (Madbouly et al., 2001a).

2.7.2 Inactivated Vaccines
Cullen and Wyeth (1976) reported that chicks inoculated with inactivated oil emulsion IBDV vaccine to groups of three weeks and six weeks of age produced high and uniform antibody response than the response induced by natural or experimental infection with live virus.

Wyeth and Cullen (1978) reported that resistance of the chicks from parents vaccinated with inactivated oil emulsion vaccine was higher than the chicks from the dams, which were exposed to live field virus.

Wyeth and Cullen (1979) reported that mean titres of the chicks vaccinated with inactivated IBD oil emulsion vaccine at 8 and 21 weeks of age were higher than the chicks which were vaccinated with commercial live IBD vaccine only. Eidson et al. (1980) reported that Leghorn breeder chickens, which had received, live IBDV vaccine at 12 weeks of age and inactivated IBD oil-emulsion vaccine at 20 weeks of age, had very high persistant antibody titres for at least nine months.

Naqi et al. (1983) reported that chickens vaccinated with IBDV vaccine early in life and revaccinated with an inactivated oil adjuvant IBDV vaccine at 18 weeks of age produced and maintained high levels of virus neutralizing antibodies through out the ten months of lay.

Wyeth and Chettle (1990) reported that inoculation of IBD oil emulsion vaccine to day old chicks with maternally derived IBD antibodies, protected at least
85 percent of the chicks when they were challenged at four weeks of age with virulent IBDV and at least 90 percent of those challenged at seven weeks of age. There was no benefit in using a combination of oil-emulsion vaccine and a live IBD vaccine.

Best immunity against IBD was produced by inactivated monovalent vaccines than with that of inactivated bivalent vaccine against IBD and ND (Kavazovic et al., 1991).

Wyeth et al. (1992) reported that full dose (0.5 ml) of inactivated IBD oil-emulsion vaccine at seven days of age gives complete protection against challenge with very virulent IBD virus.

Killed vaccines can be used to produce high and uniform levels of antibody in parent chickens so that the progeny will have high and uniform levels of MDA (OIE, 1992).

Nayak et al. (1995) concluded that parenteral injection of oil emulsified (half normal dose) vaccine along with intermediate vaccine intraocularly had conferred protection against very virulent IBD virus inspite of the fact that the bursa had been severely damaged.

Padmanaban (1997) reported that the use of inactivated oil-emulsion IBDV vaccine as primary as well as booster was found to be effective and the residual
pathogenicity was minimum. Makesh et al. (1998) reported that the bursal derived oil emulsified vaccine (OEV) given at full and half the dose provided higher immune response and better protection than chick embryo derived OEV, when vaccinated at seven days of age.

Makesh et al. (1999) reported that vaccination of commercial chicks with inactivated bursal derived oil emulsified vaccine (OEV) at seven days of age resulted in 100 percent sero conversion between 42 and 49 days of post vaccination and the peak titres were above the protective titre.

Inactivated IBD virus vaccine prepared from recent Egyptian virus isolate was proved to be highly immunogenic and elicited high titres of neutralizing antibody (17-20 log2) at weekly intervals until seven months of post vaccination and the vaccine was able to protect vaccinated chickens when challenged at 21 days post vaccination (100 percent protection) (Madbouly et al., 2001b).

2.8 BURSA-BODY WEIGHT RATIO

The bursa body weight ratio is determined by calculating the ratio of bursal weight to the body weight of chicken in grams multiplied by 1000. It was used to assess the virulence of various IBDV strains and also to assess the safeness of the live IBD vaccines (Muskett et al., 1979; Ismail and Saif, 1991; Reddy and Koteeswaran, 2002).
Allan et al. (1972) reported that there was significant difference in the bursa body weight ratio of infectious bursal agent inoculated and uninoculated groups of chicken. Faragher et al. (1974) reported that mean bursa weight of the chicken inoculated with infectious bursal agent was significantly less than that of the uninoculated groups.

The bursa body weight ratios of the chicken vaccinated with 1-65 PV strain of IBD, were not significantly different from those of unvaccinated controls (Zanella et al., 1977).

Muskett et al. (1979) reported that out of the two vaccine strains tested, vaccine A caused severe damage to the bursa and resulted in low bursa body weight ratios where as vaccine B caused no damage to the bursae of chickens examined at nine and 20 days after vaccination.

The bursa body weight ratio was high during acute phase indicating bursal enlargement and it was below 1.5 during the convalescent phase suggesting bursal atrophy (Makesh et al., 1999). Reddy and Koteeswaran (2002) reported that bursa body weight ratios in IBD affected birds during acute phase was very high compared to healthy birds indicating hypertrophy of bursa.
2.9 HISTOPATHOLOGY

Ajinkya et al. (1980) observed characteristic "starry sky" appearance of the follicles with lymphoid depletion and necrotic changes comprised of karyorrhexis and accumulation of amorphous material in the follicles. In regressed follicles reticular cell hyperplasia, plasma cell infiltration were noticed. Proliferation of corticomedullary epithelium in many bursae, cystic degeneration of the follicles in severely affected cases and edema in the interfollicular connective tissue were also reported.

Verma et al. (1981) found both acute and chronic lesions in the IBD affected four to nine week old chicks. These authors found peribursal haemorrhages, inter and intrafollicular heterophilic infiltration, degeneration and necrosis of lymphocytes and metaplasia of plical epithelium in acute cases. In chronic cases proliferation of intrafollicular reticuloendothelial cells, cystic follicles and interfollicular fibroblastic proliferation in the bursal sections.

In five week old broiler chicks inoculated with IBDV, Prajapati and Jalnapurkar (1981) reported inter and intra follicular oedema and heterophilic infiltration or necrosis of the follicles in the bursa by third day, more severe lymphoid necrosis and cystic degeneration by seventh day Post Infection (PI) followed by interfollicular connective tissue proliferation by ninth day PI and adenomatous transformation of the bursal follicles by 11th day PI.
Edwards et al. (1982) examined the bursal sections from chicks for five weeks after IBD vaccination. Seven days after vaccination severe damage was seen to the bursa with destruction of follicular architecture, deflection of lymphocytes, increased connective tissue mucous cysts and thickening and corrugation of the epithelium and few bursae had over 50 percent of the bursal area depleted of lymphocytes. Fourteen days after vaccination bursal sections had a single, large follicle with defined cortex and medulla and normal population of lymphocytes. Twenty one days after vaccination majority of the plicae in the bursa contained at least one apparently normal follicle. Regeneration of the bursal follicles was noticed 28 days and 35 days after vaccination. They concluded that bursae from vaccinated birds were always smaller than those of controls, with corrugated and thickened epithelium.

Okoye and Shoyinka (1983) conducted histological examination of a flock which had experienced sub clinical IBD and reported that the follicles of the bursa were depleted of lymphocytes, had many large cavities and were being repopulated by newly formed healthy lymphocytes.

Mohanty and Rao (1984) found predominant changes in the lymphoid follicles of bursa characterised by destruction of lymphocytes in the medullary region. These authors noticed eosinophilic granular material or haemorrhagic exudate and heterophils in some follicles and in others empty crater like structures. Oedema and heterophilic infiltration in the interfollicular tissue in acute cases and interfollicular fibrosis and mononuclear infiltration in chronic cases were the other lesions noticed.
Ezeokoli et al. (1990) observed marked lymphocyte depletion and cellular infiltration with mononuclear cells in the bursa of Fabricius of birds vaccinated at different ages with live IBD virus vaccine.

Tsukamoto et al. (1995) reported that lymphoid cells were reduced in numbers in 25-50%, 50-75%, >75% and almost 100% follicles in chickens vaccinated with mild vaccines A and B, intermediate vaccine C and highly virulent Ehime/91 strain respectively.

In experimentally infected three week old chicks with IBDV field isolates Singh et al. (2002) noticed enlargement of the bursa, edema and pin-point haemorrhages by three days PI, marked reduction in size of bursa from five days PI. By seven days PI cystic degeneration, heterophilic infiltration, formation of vacuoles, hypertrophy and hyperplasia of lining epithelium were observed. Atrophy of bursal follicles was observed by nine day PI with increase in interfollicular connective tissue. By 11 days PI development of small glandular structures in the place of bursal follicles and 14 days PI lymphoid depletion and plical atrophy were noticed. By 21 days PI, multifocal follicular lymphoid repopulation and recovery of follicular architecture was noticed.

2.10 BURSAL SCORES
To find out the degree of damage to bursa, Muskett et al. (1979) graded the bursa from 0-5 according to the following criteria.

0. No damage

1. Mild necrosis in isolated follicles

2. Moderate generalised lymphocyte depletion or isolated follicles with severe depletion

3. Over 50 percent of follicles with severe lymphocyte depletion.

4. Outline of follicle only remaining with a few lymphocytes and increase in connective tissue, cysts and thickened corrugated epithelium.

5. Loss of all follicular architecture with fibroplasia.

Bursal lesion scores were evaluated on a scale of 1-4 by Rosales et al. (1989a).

1  =  normal, no lesions

2  =  mild, scattered cell depletion in few follicles

3  =  moderate, one-third to one-half of the follicles with atrophy or depletion of cells.
Severe atrophy of all follicles, inflammation and acute necrosis.

Sharma et al. (1989) and Nakamura et al. (1992) scored the sections of bursa on the basis of lymphoid necrosis and/or depletion as follows:

\[
\begin{align*}
0 & = \text{less than 5\% of the lymphoid follicles affected} \\
1 & = \text{5 - 25\% of the lymphoid follicles affected} \\
2 & = \text{25 - 50\% of the lymphoid follicles affected} \\
3 & = \text{50 - 75\% of the lymphoid follicles affected} \\
4 & = \text{greater than 75\% of the lymphoid follicles affected}
\end{align*}
\]

Tsukamoto et al. (1995) scored the histologic lesions of bursa on the basis of lymphoid necrosis and/or depletion as follows:

\[
\begin{align*}
0 & = \text{less than 5\% of the lymphoid follicles affected} \\
1 & = \text{5 - 25\% of the lymphoid follicles affected}
\end{align*}
\]
2 = 25 - 50% of the lymphoid follicles affected

3 = 50 - 75% of the lymphoid follicles affected

4 = greater than 75% of the lymphoid follicles affected

5 = nearly 100% affected follicles with acute inflammatory infiltration.

2.11 EFFECT OF IBD VIRUS ON RD IMMUNITY

Allan et al. (1972) reported that the primary and secondary serological responses (HI titres and Ig G levels) against NDV were significantly reduced in chickens inoculated with infectious bursal agent.

The geometric mean titres of NDV antibody was lower in white leghorn chicks raised from one day old in an environment contaminated with IBDV than controls reared in uncontaminated environment. The reduction was most pronounced at 35-56 days of age for leghorns vaccinated with NDV at one and 28 days (or) at 28 days. This study also confirmed that IBDV infection in chickens increased the susceptibility to ND (Giambrone et al., 1976).
Almassy and Kakuk (1976) reported that a naturally acquired subclinical infection of IBD was the probable causative factor of immunosuppression to ND vaccine in young chicks. However, older hens maintained on the same contaminated premises did not exhibit immunosuppression.

The importance of IBDV vaccine in helping the immune system for an effective immunological response to other vaccines was studied by Zanella et al. (1976). Their studies revealed that prior vaccination against IBD in chicks resulted better immune response to New castle disease virus vaccine. These workers also emphasized the need for vaccinating the chicks against IBD in order to prevent immune breakdowns to other vaccines.

Meulmans and Halen (1977) reported that HI response to ND vaccine was lower and mortality rate after challenge with virulent ND vaccine was statistically higher in a group of chicks infected with IBDV at one day of age and vaccinated against ND on the fifth day than those which were given the IBDV and ND vaccine simultaneously at one day of age or those vaccinated on the first day of life and subsequently infected on the fifth day. They concluded that the immunosuppression due to IBDV infection depended on the age of the birds, the time of ND vaccination and the IBDV strain used.

Zanella et al. (1977) reported that vaccination at either one, seven or 15 days of age with attenuated strain 1-65 PV of IBD virus did not suppress the immune response to ND vaccination.
The serum antibody titres to NDV in chickens infected with Australian strain 002/73 of IBDV was significantly lower than that of birds infected with NDV alone (Westbury, 1978). Cursiefen et al. (1979) demonstrated that initial vaccination with mutant strain Cu-1 M of IBD virus could not suppress the immune response against highly virulent 'Italian' strain of NDV.

Muskett et al. (1979) compared the efficacy of two infectious bursal disease vaccine strains. Chickens vaccinated with vaccine A showed lowered response to ND vaccine, whereas the performance of the ND vaccine was not affected in chicken given vaccine B.

Ajinkya et al. (1980) observed heavy losses in seven broiler farms due to ND. In all the farms chicks aged three to seven weeks, which had been vaccinated against NDV, were affected. Postmortem and serological investigations revealed IBD in all the cases. They concluded that vaccination failure was due to the immunosuppressive effect of IBD.

Panigrahy et al. (1982) found that the geometric mean titres of HI antibody to NDV in chickens infected with IBDV at one day of age, was lower than in those infected at 28 days of age. Infection with IBDV had no influence on secondary immune response to NDV.
Chicks vaccinated with live IBD vaccines had lowered HI antibody response to Newcastle disease vaccine (Bastami et al., 1986; Jhala et al., 1990; Elham et al., 1995 and Das et al., 1996).

An atypical form of ND was recorded by Panisup et al. (1983) in an organised farm, affecting five week old broiler chickens vaccinated against ND. These workers attributed the outbreak of giving ND to immunosuppression resulting from field infection with IBDV.

Okoye (1985) reported that Nigerian strain of IBDV caused severe depletion of bursal lymphocytes in chicks inoculated at two days of age. When B1 vaccine was given seven days after infection, much lower HI antibody titers developed than in controls. When challenged with virulent NDV 14 days after vaccination 70 percent mortality was observed.

Voeten et al. (1985) reported that vaccination with live vaccine against IBD at 8-10 days of age had no influence on ND antibody development after giving ND vaccination at seven days.

Giambrone and Clay (1986b) compared the efficacy of four commercial live IBD vaccines. Birds receiving any of the four IBD vaccines were able to produce high NDV antibody titres and were resistant to NDV clinical challenge infection similar to the birds that were vaccinated only against ND.
Ezeokoli *et al.* (1990) reported that vaccination of chicks against ND at day old age reduced the immunodepressive effect of IBD. Rao and Rao (1992) concluded that immunosuppressive action of IBD virus in chicken against Ranikhet Disease (RD) vaccination was maximum when IBDV was inoculated at six weeks of age and comparatively less at day-old or three weeks of age when subsequent RD vaccination was done at eight weeks of age.

Emenike *et al.* (1992) reported that, two doses of IBD vaccine at zero and 21 days rather than one single dose at either zero day or 21st day was more beneficial, to broiler fowls with specific reference to humoral immune response to ND virus.

Nakamura *et al.* (1992) reported that highly virulent IBDV field isolate 90:11 severely suppressed antibody response to ND vaccination and protective vaccinal immunity against ND. Prabhakaran *et al.* (1995) demonstrated that vaccine-induced immunosuppression was mostly seen in the birds vaccinated against IBD after second week of life.

Sofei *et al.* (1996) reported that Gumbovivac-CC live vaccine against IBD did not induce any immunosuppressive effect on other avian vaccines.

Christopher *et al.* (1997) reported that the observed RD vaccinal titres of samples collected after the outbreak of very virulent IBD were significantly lower than the statistically predicted HI titres for that age.
Makesh et al. (1999) observed that mean NDV HI titre of chicks in IBD affected farms decreased from the day of outbreak and the titre during the convalescent period was significantly lower in IBD unaffected farms. Kumar et al. (2002) reported that immunosuppressive effect was most marked when the interval between the experimental IBD virus infection and RD (F-strain) vaccination was shortest (4 days) and the effect was lowest when the interval was maximum (21 days).
CHAPTER - III

MATERIALS AND METHODS

3.1 MATERIAL

3.1.1 Chicken

Day old white leghorn layer chicks from a single hatch vaccinated against Marek’s disease were procured from Balaji Hatcheries, Chittoor. The birds were maintained in the experimental animal house in the Department of Pathology with standard managemental and feeding conditions throughout the period of experiment.

3.1.2 Vaccines

The different commercial vaccines used in the present study include IBD (Intermediate strain), IBD (Intermediate plus strain), oil adjuvant combined IBD-ND vaccine, ND (Lasota) and ND (R2B) vaccines. These vaccines were procured from Ventri Biologicals, Vaccine Division, Pune and stored at -20°C temperature until they are used.
3.1.3 Embryonated Chicken Eggs

Fertile hen eggs were obtained from Department of Livestock production and Management (poultry), College of Veterinary Science, Tirupati. The eggs were swabbed with 70 percent alcohol and incubated for nine days at 37(C temperature and 85 percent of Relative humidity to facilitate proper embryonic growth.

3.1.4 Chicken Red Blood Cells (RBC)

Pooled chicken blood was collected in equal volume of Alsever's solution and stored at 4(C. The RBC were washed thrice with phosphate buffer Saline (PBS), pH 7.2 and were adjusted to required concentration in PBS as and when required.

3.1.5 IBD - Antibody Test Kit

Commercially available IDEXX-IBD virus Antibody Test Kit (Flockchek) was procured from Polchem Hygiene Laboratories, Pune. The Kit consisted of the following components.

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Reagent</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>IBD antigen coated plates</td>
<td>5</td>
</tr>
<tr>
<td>2.</td>
<td>IBD positive control-Diluted chicken Anti-IBD serum preserved with sodium azide</td>
<td>1.9 ml</td>
</tr>
<tr>
<td>3.</td>
<td>Negative control - Diluted chicken sera non-reactive</td>
<td>1.9 ml</td>
</tr>
<tr>
<td></td>
<td>for Anti-IBD, preserved with sodium azide</td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---------------------------------</td>
<td>---</td>
</tr>
<tr>
<td>4.</td>
<td>(Goat) Anti-chicken : Horse radish peroxidase conjugate, preserved with gentamicin</td>
<td>50 ml</td>
</tr>
<tr>
<td>5.</td>
<td>Sample Diluent buffer preserved with sodium azide</td>
<td>235 ml</td>
</tr>
<tr>
<td>6.</td>
<td>TMB substrate</td>
<td>60 ml</td>
</tr>
<tr>
<td>7.</td>
<td>Stop solution</td>
<td>60 ml</td>
</tr>
</tbody>
</table>

The components were stored at refrigeration temperature (4°C) until they are used.

3.1.6 Glass and Plasticware

During the course of this study, Borosil and corning brand of glassware and "Analar" or guaranteed grade chemicals were used. Triple glass distilled water (pH 7.0-7.2) was employed for the preparation of the reagents. Glassware, syringes and needles, rubber corks were prepared following standard methods and sterilized by usual procedure. Microtitre plates supplied by Laxbro, Pune, India were used for microtitration.

3.2 METHODS

3.2.1 Experimental Design
Two hundred and eighty white leghorn layer chicks were randomly divided into seven groups of forty chicks each and were kept in separate pens. The birds in Group I were vaccinated against New castle disease only and they acted as IBD unvaccinated control. Birds in Group II were vaccinated on first and 15th day by using Intermediate strain and Intermediate plus strain of IBD vaccines respectively. Birds in Group III were vaccinated on 13th day with intermediate strain of IBD and on 28th day with oil adjuvant combined IBD-ND vaccine.

Birds in group IV were vaccinated on 13th day and 21st day with intermediate strain and intermediate plus strain of IBD vaccines respectively.

Birds in group V were vaccinated on 13th, 21st and 29th day with intermediate strain, intermediate plus strain and intermediate strain of IBD vaccine respectively. Birds in group VI were vaccinated on 15th and 30th day with intermediate plus strain of IBD vaccine. Birds in group VII were vaccinated on 15th, 30th and 45th day with intermediate plus strain of IBD vaccine. All the vaccines were administered intra ocularly one drop per bird, except oil adjuvant combined IBD-ND vaccine which was administered 0.5 ml per bird sub cutaneously.

All the birds in seven groups including controls were vaccinated with ND (Lasota) on 7th and 35th day, one drop per bird intra ocularly and with ND (R2B) during 8th and 16th week, at the dose rate of 0.5 ml per bird sub cutaneously.
All the vaccines were reconstituted and administered as per the manufacturer's instructions.

3.2.2 Collection of Serum Samples

Blood samples were collected at weekly intervals from ten birds in each group for 20 weeks and serum was separated. The ten serum samples from each group were pooled into two pools (Five samples in one pool). A drop of thiomersal (1:10000) was added to each sample as a preservative and were stored at -20°C until tested.
TABLE 1: Experimental Design

<table>
<thead>
<tr>
<th>Group</th>
<th>Age of Vaccination</th>
<th>Type of IBD vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Control</td>
<td>No IBD vaccine</td>
</tr>
<tr>
<td>Group II</td>
<td>1 day</td>
<td>Intermediate strain</td>
</tr>
<tr>
<td></td>
<td>15th day</td>
<td>Intermediate plus strain</td>
</tr>
<tr>
<td>Group III</td>
<td>13th day</td>
<td>Intermediate strain</td>
</tr>
<tr>
<td></td>
<td>28th day</td>
<td>Oil adjuvant IBD + ND</td>
</tr>
<tr>
<td>Group IV</td>
<td>13th day</td>
<td>Intermediate strain</td>
</tr>
<tr>
<td></td>
<td>21st day</td>
<td>Intermediate plus strain</td>
</tr>
<tr>
<td>Group V</td>
<td>13th day</td>
<td>Intermediate strain</td>
</tr>
<tr>
<td></td>
<td>21st day</td>
<td>Intermediate plus strain</td>
</tr>
<tr>
<td></td>
<td>29th day</td>
<td>Intermediate strain</td>
</tr>
<tr>
<td>Group VI</td>
<td>15th day</td>
<td>Intermediate plus strain</td>
</tr>
<tr>
<td></td>
<td>30th day</td>
<td>Intermediate plus strain</td>
</tr>
<tr>
<td>Group VII</td>
<td>15th day</td>
<td>Intermediate plus strain</td>
</tr>
<tr>
<td></td>
<td>30th day</td>
<td>Intermediate plus strain</td>
</tr>
<tr>
<td></td>
<td>45th day</td>
<td>Intermediate plus strain</td>
</tr>
</tbody>
</table>

3.2.3 Bursa body Weight Ratio (B-BW)

The bursa body weight ratio was calculated for all the treatment groups including the control group. Six birds in each group were sacrificed at weekly intervals during the first five weeks of age and their body weight and bursa of Fabricius weight were measured using a sensitive balance.

Bursa-body weight ratio was assessed as described by Ismail and Saif (1991).
Bursa weight of chicken

Bursa body weight ratio = \[\frac{\text{Bursa weight}}{\text{Body weight of chicken}}\] \times 100

Body weight of chicken

3.2.4 Histopathology

The six bursae that were collected from each group in weekly intervals were fixed in 10 percent neutral buffered formalin for histopathological studies. They were dehydrated in graded alcohols, cleared with xylene and infiltrated and embedded in paraffin. Embedded tissues were sectioned at four to six micro metre thickness and were stained with hematoxylin and eosin (H & E). Tissue sections were coded before microscopic evaluation to eliminate examiner bias. The different tissue changes noticed in each bursal section were recorded.

3.2.5 Bursal Score

The degree of damage caused by IBD vaccine virus to bursa of Fabricius of birds was assessed by estimating the bursal score values. The bursal lesion score was assessed as per the method described by Sharma et al. (1989). Sections of bursa were scored on the basis of lymphoid necrosis and/or depletion as follows.
0 - Less than 5% of lymphoid follicles affected

1 - 5-25% of lymphoid follicles affected

2 - 25-50% of lymphoid follicles affected

3 - 50-75% of lymphoid follicles affected

4 - Greater than 75% of lymphoid follicles affected

The bursal score values of different treatment groups were compared with the values obtained for the control group.

3.2.6 Cultivation of New Castle Disease Virus

The stock NDV (R₂B) was obtained from Department of Microbiology, College of Veterinary Science, Tirupati.

The stock NDV was diluted 1:100 with sterile PBS, pH 7.2. The virus was treated with 250 i.u. of benzyl penicillin and 250 μg of streptomycin per ml at room temperature for one hour and filtered through 0.45 mm membrane filter. Nine to ten day-old chicken embryos were cleaned with 70 percent alcohol and 0.1 ml of filtered virus suspension was inoculated into allantoic cavity and incubated at 37°C. The embryos died during first 24 hours were considered as due to non-specific causes and discarded. The embryos found dead between 48-72 hours after inoculation were
chilled overnight to avoid mixing of RBCs with allantoic fluid. The allantoic fluid was tested with 10 percent chicken RBC for haemagglutinating activity and those giving positive reaction were harvested, pooled, centrifuged and stored at -20°C until use.

3.2.7 Haemagglutination (HA) Test

Haemagglutination test was performed in U shaped microtiter plates as per Cunningham (1966). Serial two-fold dilutions of the virus (50 μl) were made in PBS (50 μl), starting from 1:2. An equal volume of one percent chicken RBC were added to each well. The plates were shaken gently to facilitate proper mixing of the RBC with the virus and incubated for 30 minutes at room temperature before reading the results. Appropriate controls were included in the test. The reciprocal of the highest virus dilution showing clear haemagglutination (mat) was considered as the HA titer.

3.2.8 Haemagglutination Inhibition (HI) Test

Haemagglutination inhibition test was performed as per the method of Cunningham (1966). Serial two-fold dilutions of the test serum (50 μl) were made in PBS, pH 7.2 starting from 1:2. Equal volume (50 μl) of 4HA units of virus was added to each well and after gentle shaking, kept at room temperature for 30 minutes. Fifty microliters of one percent chicken RBC were added to each well and kept at room temperature for 30 min before reading the results. The reciprocal of the highest serum
dilution at which there is a clear button formation was taken as the HI titer. Appropriate controls were included in the test.

3.2.9 Enzyme-Linked Immunosorbent Assay (ELISA)

3.2.9.1 Test Procedure

An indirect enzyme linked immunosorbent assay was performed to detect the IBD antibody levels using IDEXX-IBD-ELISA kit (Flockchek). The test was performed as per the manufacturer’s instructions.

Serum samples were diluted five hundred fold (1:500) with serum diluent and 100 μl of the sample was added to the antigen coated wells. Samples were tested in duplicate wells and positive and negative controls were included every time in the test. The plates were incubated at 37°C for 30 min. The contents of the wells were removed and the wells were washed three times with distilled water. The plates were dried and 100 μl of (Goat) Anti chicken Horse radish peroxidase conjugate was added to each well. The plates were incubated at 37°C for 30 minutes. The plates were emptied and washed thrice with distilled water. The residual droplets were removed by lightly tapping the plate over a pad of filter paper. One hundred microlitres of TMB substrate was added to each well and incubated in dark at room temperature for 15 min. Later 100 μl of stop solution was added to each well to stop the color reaction. The absorbance values were measured at 650 nm wavelength in an ELISA reader.
3.2.9.2 Interpretation of results

Assay was considered to be valid only when the difference between positive control mean and negative control mean (PCX - NCX) was greater than 0.075. The negative control mean absorbance should be less than or equal to 0.150. Serum samples with S/P ratios of less than or equal to 0.2 (titers greater than 396) were considered as positive. The presence or absence of antibody to IBD was determined by relating the A(650) value of the unknown to the positive control mean.

Calculations

1. Negative control mean (NCX)

2. Positive control mean (PCX)

   Sample mean - NCX

3. S/P Ratio = ________________

   PCX - NCX

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

3.2.10 Statistical Analysis

The data was analysed statistically by student's t-test and analysis of variance as per the methods of Snedecor and Cochran (1967).
CHAPTER - IV

RESULTS

4.1 QUANTITATION OF IBD ANTIBODIES

4.1.1 Decline of maternally derived antibodies (MDA)

The decay of maternally derived infectious bursal disease antibodies was assessed by testing the pooled serum samples collected from the control group birds at weekly intervals. The MDA levels were quantified by ELISA and the results are shown in the Table (2) and Fig(1). An ELISA titre of 2034.31 was obtained on day one, which gradually declined during subsequent weeks. The serum maternal antibody levels were found to be negative by sixth week of age.

4.1.2 Post vaccination immune response

The vaccine response to different commercially available vaccines given in different schedules was assessed by testing the pooled serum samples collected from different groups at weekly intervals for 20 weeks. The quantification of the antibody response was done by checking the ELISA titres. The results of the ELISA are presented in the Table (2) and Fig (2 and 3).
There was a gradual decrease in the IBD antibody titres in all the vaccinated groups from day old age to third week. Lowest ELISA titres were observed during third week which ranged between 1514.47 to 1591.32. Even in the group II which received vaccination on the first day itself, the vaccination response was not seen immediately interms of sero conversion.

Primary immune response to vaccination was noticed at fourth week of age in all the vaccinated groups. The titres began to raise and reached to the peak by 8\textsuperscript{th} week in group IV, by 10\textsuperscript{th} week in group VII and by 12\textsuperscript{th} week in group II, III and V. Again there was a gradual decrease in the IBD antibody titres and by the end of the 20\textsuperscript{th} week they ranged between 1864.18 to 1945.73 in all the vaccinated groups.

The data was subjected to statistical analysis using analysis of variance for different groups. The results revealed that there was no significant difference (P<0.01) in the titres of different treatment groups.

4.2 ASSESSMENT OF BURSAL DAMAGE

4.2.1 Bursa body weight ratio (B-BW)
The bursa body weight ratios of the experimentally vaccinated chicken and also healthy unvaccinated control birds were calculated. The B.BW ratio was calculated from first week to fifth week at weekly intervals. The weight of bursa of chicken at birth was about 0.02 grams.

4.2.1.1 B-BW ratios of Group I

The B-BW ratios of the chicken in control group are presented in Table (3). The average values recorded were 3.47, 4.73, 5.49, 5.94 and 5.74 during 1\textsuperscript{st}, 2\textsuperscript{nd}, 3\textsuperscript{rd}, 4\textsuperscript{th} and 5\textsuperscript{th} week of the experiment respectively. There was a gradual increase in the ratio from first to fourth week and a slight decrease was noticed during the fifth week.

4.2.1.2 B-BW ratios of Group II

The B-BW ratio of the chicks in group II which were vaccinated on day old age against IBD are shown in the Table (4). The mean B-BW ratio of 2.99, 6.57, 7.09, 1.68 and 1.84 were recorded during first to fifth week respectively. During the first week the value was less in comparison with the control and during second and third weeks a higher B-BW ratio was recorded than the control birds and again decreased significantly during fourth and fifth weeks.

4.2.1.3 B-BW ratios of Group III, Group IV and Group V
In the group III, IV and V where the intermediate IBD vaccines were administered during 13 to 15 days of age the maximum increase in the B-BW ratio was observed during third week (6.78 to 6.97) and during fourth and fifth weeks the ratio was decreased (1.53 to 2.82) in comparison with the control. (Tables 5 to 7).

4.2.1.4 B-BW ratios of Group VI and Group VII

The bursa body weight ratios of the groups VI and VII which received intermediate plus (hot) strain of IBD vaccines are detailed in the Tables 8 and 9. The highest average B-BW ratios were recorded in these two groups during third week of age (7.40 and 7.56) in comparison to other groups as well as the control group. In the subsequent fourth and fifth weeks the values were ranging between 1.42 and 1.87.

The statistical analysis of the data using students t test revealed that there was significant difference (P<0.05, P<0.001) in the B.BW ratios of the vaccinated groups in comparison with the control group during third, fourth and fifth weeks as the birds were vaccinated between 13-15 days of age and in the group II which received IBD vaccine on day old age the values differed significantly even from the first week in comparison with the control birds.

4.2.2 Histopathology of the bursa of Fabricius
The bursae collected from different groups were examined microscopically to know the tissue changes that occurred because of vaccination in comparison to control group.

4.2.2.1 Microscopic lesions

Group I

The bursal sections from control group did not reveal any histopathological abnormalities and the normal bursal architecture was noticed during all the weeks (Fig.5).

Group II

The microscopic changes in the bursae during the first week include severe depletion of lymphoid follicles and mild areas of congestion and haemorrhages. In few birds, thinning of cortex and medulla of the bursa was seen. During second week in addition to mild depletion of lymphoid follicles, inter follicular haemorrhages were noticed in some areas. By third week there was development of mild interfollicular and subepithelial fibrosis, with thinning of cortex, fragmentation of nucleus of
lymphocytes and edema of the bursal follicles. Foam cells were noticed in few follicles.

By fourth week, cystic spaces in the lining epithelium were conspicuously noticed in all the birds. Prominency of cortico medullary epithelial region with metaplastic changes in medulla were seen apart from the other changes as seen in the third week. The changes during fifth week were more or less the same as in fourth week with few bursae showing severe metaplastic changes and mild depletion in lymphoid follicles.

**Group III**

The birds in group III which received an intermediate vaccine on 13\textsuperscript{th} day and oil adjuvant IBD vaccine on 28\textsuperscript{th} day showed the following bursal lesions.

During first week no histopathological lesions were seen. Interfollicular haemorrhages and mild depletion of lymphoid follicles were observed during the second and third week of age. The bursal changes during the fourth week include cystic spaces in lining epithelium and in few birds metaplastic changes, thinning of cortex and appearance of foam cells were noticed.
The bursal lesions during the fifth week include interfollicular fibrosis, prominency of corticomедullary junction, thinning of cortex, mild depletion of follicles, intrafollicular cystic spaces, metaplastic changes and foam cell appearance in some areas.

**Group IV**

Birds in group IV received two doses of IBD vaccines, one at 13\(^{th}\) day (Intermediate Strain) and another at 21\(^{st}\) day (Intermediateplus Strain). The bursal changes that were noticed are similar to group III for the second, third and fourth weeks. During fifth week there was more pronounced interfollicular fibrosis with thinning of cortex and medulla.

**Group V**

The birds in group V were given three doses of IBD vaccines. First one on 13\(^{th}\) day (Intermediate Strain), second on 21\(^{st}\) day (Intermediateplus Strain) and third on 29\(^{th}\) day (Intermediate Strain). The bursal lesions seen during second, third, fourth and fifth weeks were similar to that of the changes noticed in group III during the same period.

**Group VI and VII**
Birds in group VI (on 15th and 30th day) and group VII (on 15th and 45th day) were vaccinated with intermediate plus (hot) strain of IBD vaccines.

No histopathological lesions were observed upto third week of age. The bursal changes during fourth week include cystic spaces in lining epithelium and sub epithelial infiltration, thinning of cortex, severe metaplastic changes in medulla, foam cell appearance in few follicles. During fifth week in addition to the above changes prominency of corticomedullary junction and inter follicular fibrosis were noticed.

4.2.3 Bursal score

The bursal scores were given to each individual bursa collected from different treatment and control groups during weekly intervals based on the extent of lymphoid depletion in the follicles. The bursal score values of different groups are depicted in Table 3 to 9.

The control group showed zero values during all five weeks of the study indicating normal healthy bursal follicles. In group II, the average scores recorded were 1, 2.33, 1.83, 3.33 and 1.83 from first to fifth week respectively. In group III bursal score of zero was recorded during first week and in the subsequent weeks the average scores recorded were 1, 0.83, 1 and 1.66.
In group IV and V the average bursal scores of zero and one were recorded during first and second weeks respectively. In the subsequent weeks scores of 0.83, 4 and 2 were recorded in group IV and 1, 4 and 2.5 were recorded in group V during 3rd, 4th and 5th weeks respectively.

In groups VI and VII bursal score was zero upto third week and a score of 4 was recorded during fourth and fifth weeks for all the bursal sections.

4.3 IMMUNOSUPPRESSIVE EFFECT OF IBD VACCINES

In order to evaluate the probable immunosuppressive effect of different IBD vaccines due to the bursal damage caused by these vaccines, the response of birds to Newcastle disease vaccination was compared between the different treatment groups and the control group.

4.3.1 Cultivation of Newcastle disease virus

The standard `R2B' strain of NDV was cultivated in the embryonated hen eggs. Death of the embryo occurred between 48-72 hours after inoculation. The allantoic fluid collected from the chilled embryos showed a HA titre of 1024 after
three serial passages in the embryos, which was used further in the HI test after preparing the 4HA units of the virus.

4.3.2 Haemagglutination inhibition test

All the birds in the treatment groups and the control group received four doses of ND vaccination as per the standard schedule. The pooled serum samples which were collected from different groups during weekly intervals were also tested to quantify the NDV antibody titres by HI test.

The results of the HI test are shown in Table (10) and Fig (11). The maternal antibody titre of the day old birds was found to be 256. As the first dose of ND vaccine was given at 7th day the titres were slightly reduced by 2nd week due to neutralization of the antibodies by vaccine virus and by third week there was a gradual increase in the HI titres ranging between 256 to 512. Only in group II which received IBD vaccine on day old age there was a transitory reduction in the NDV-HI titres during second and third week but after fourth week there was not much difference in the NDV-HI titres among different groups in comparison with the control. A slight reduction in the HI antibody titres were noticed immediately succeeding each vaccination of ND than the preceeding titres because of partial neutralisation of the existing antibodies by the vaccine virus. Thereafter gradual increase of the titres were noticed. There was only a slight reduction in the titres of treatment groups especially in group VI and VII when compared to the control.
4.4 Mortality

The general health status of the birds was maintained by regular deworming, appropriate usage of anticoccidial, antibacterial drugs and incorporation of vitamin supplements to reduce the stress. However, apart from the birds which were sacrificed during the initial five weeks for calculating the B-BW ratio and bursal score values, a total of nine birds died due to different causes. Maximum mortality was noticed in the groups VI and VII which received intermediate plus vaccines. Three birds in group VI, four birds in group VII and one bird in group I which died during the experimental period were confirmed as deaths due to coccidiosis as per the postmortem examination. One bird in Group 7 was died due to E.coli infection. No mortality was encountered in all the other groups during the entire course of the experiment.
Infectious bursal disease virus continues to cause health problems for chicken flocks world wide. The appearance of highly pathogenic form of IBD (vvIBD) has dictated new approaches to vaccination involving use of less attenuated vaccine strains (intermediate and intermediate plus strains) which can stimulate immunity in young chicks even in the presence of significant levels of maternal antibodies. With the failure of mild and intermediate vaccines, producers started using intermediate plus (hot) vaccines to protect their flocks from very virulent field virus. Although, these hot vaccines are more invasive and overcome higher levels of maternal antibodies, they are supposed to be causing immunosuppression by severely damaging the bursa resulting in vaccine failures and out breaks of RD, Fowl cholera etc. In the present study, the efficacy of different IBD vaccines was tested using different vaccination schedules interms of inducing higher levels of antibodies and also the extent of bursal damage and the immunosuppressive effect of these vaccines on RD immunity were studied.

A total of 280 layer chicks were procured from a local hatchery, (Balaji Hatcheries, Chittoor) and they were randomly divided into seven groups consisting of 40 chicks each. Six different vaccination schedules were tried using intermediate and intermediate plus strains of IBD vaccines either alone or in combination (Table 1). A control group was maintained which was vaccinated only against Newcastle disease.
The experiment was conducted for a period of 20 weeks during which the birds in different groups were bled at weekly intervals and the serum was separated for testing.

The levels of IBD maternal antibodies were tested by checking the ELISA titres in the control group. The day old birds showed a titre of 2034.31 and the MDA levels declined gradually in the subsequent weeks. (Fig 1). The serum samples were found to be negative in ELISA test by sixth week of age. Even in the group II which received IBD vaccine on day one and in the other groups which were vaccinated initially between 13 to 15 days of age, the rate of decline was similar to the control group birds. Hence, vaccination of birds with IBD vaccines did not interfere with depletion of maternal antibodies.

A sequential study of the decline rates of IBD MDA in unvaccinated and vaccinated chicks showed that the vaccine virus did not accelerate the antibody depletion rates in vaccinated chicks (Naqi et al., 1983). On the contrary Solano et al. (1986) reported that the titres decreased rapidly after vaccination of chicks at one day of age.

Makesh et al. (1999) reported that MDA levels showed wide variation between and within farms and completely waned by 21 days of age. The results of Tsukamoto et al. (1995) and Balakrishnan (1999) were in accordance with the results obtained in our study. They reported that the MDA levels became undetectable by 5th week and 6th of week of age respectively.
However, in the present study it was observed that vaccination of birds with high IBD maternal antibody status did not interfere with the vaccine response even though immediate sero conversion was not observed in the group II which was vaccinated at day old age. So, intermediate and intermediate plus vaccines were found to be highly invasive and could be given even in the presence of maternal antibodies. In a study conducted by Cursiefen et al. (1979), it was concluded that maternally derived antibodies do not interfere with the establishment of immune protection in young chickens. The same observation was also made by Mazariegos et al. (1990) in their experimental study.

The post vaccinal antibody status against IBD was monitored throughout the period of study by quantifying the antibodies by an IDEXX IBD ELISA standard Kit in different groups. Though different serological tests were used by different authors, ELISA was found to be easier, sensitive and specific for the sero monitoring of IBD antibodies. Dewit et al. (2001) reported that IDEXX standard ELISA showed no false positive results and the sensitivity and specificity of the test was found to be 100%.

There was no immediate sero conversion of the IBD vaccine virus which was given at day old age in the group II. However, the birds in group II responded to the subsequent vaccines of IBD given at 15 days of age. So no special advantage could be ascertained in day old vaccination against IBD in our study. In a study conducted by Snyder et al. (1986), it was reported that response to day old IBD vaccination was either blocked or delayed by declining levels of maternal antibody titres.
In group III which received a combination of live and inactivated oil adjuvant vaccines against IBD, the response to vaccination was on par with the response seen in other groups with high IBD antibody titres. This schedule of vaccination was mainly included to assess the extent of bursal damage caused by this combined vaccines in comparison with the groups which received two or three doses of live vaccines. Nayak et al. (1995), in their study concluded that parenteral injection of oil emulsified vaccine along with intermediate vaccine intra ocularly had conferred protection to a considerably high level even against vvIBD virus. Though vaccination with oil adjuvant vaccines can be practiced in layer chicks, as they require a live virus priming for initial induction of the immunity, and taking into account the cost factor into consideration, oil adjuvant vaccines were mainly found effective in breeder stock to maintain a sustainable immunity, than in young layer chicks.

No significant difference could be seen in the ELISA titres in the other groups which received different schedules of intermediate and intermediate plus vaccines. In all the groups the titres were found to be above protective level. Fahey et al. (1987) had estimated that an ELISA titre of 400 or above can induce protective immunity in chicks against exposure to field IBD virus attacks. In the present study, the ELISA titres were calculated as per the instructions given by manufacturer and found to range between 1514.47 to 2104.92. So all the birds in different vaccine groups were found to be having titres above protective levels as indicated in the Table (2). According to Singh et al. (2003), the antigenic structure of vvIBD is similar to the type I classic strain but the virus possesses an additional antigenic site denoted by a monoclonal antibody designated MAb21. AS the intermediate plus (hot ) vaccines also possess this antigen, they are very effective in the control of vvIBD outbreaks. Hence, usage of hot strains of IBD vaccine is justified in view of the endemicity of the IBD virus in different areas of Andhra Pradesh.
Although, the live intermediate and intermediate plus vaccines are found to be effective in inducing an optimum immune response against IBD, these vaccines were also reported to be causing some bursal damage as a result of multiplication of the virus in the bursal tissue. Hence in the present study an attempt was made to estimate the degree of bursal damage produced in different vaccinated groups in comparison with the unvaccinated control group. Many workers have used the bursa body weight ratios and the bursal score values as the criteria to evaluate the relative bursal damage. (Muskett et al., 1979; Ismail and Saif, 1991). For this purpose, six birds from each group were sacrificed during weekly intervals in the initial five weeks of the experimental study. The experiment was so designed because the most susceptible age group for IBD outbreaks was reported to be 2-5 weeks of age, which corresponds to the maximum activity of the bursa of Fabricius in the life of chicks.

The bursa body weight (B-BW) ratios of the different vaccinated and the control groups are shown in Tables 3 to 9 and Fig 4. The B.BW ratios of group II showed a significant difference when compared to the control group during all the five weeks of the experiment as calculated by the student's t-test. In other groups i.e group III to group VII, the B-BW ratios differed significantly from the control group during 3rd to 5th week of the experiment as the vaccines were given between 13 to 15 days of age.

In general, in all the groups, there was an increase in the B-BW ratios in the week subsequent to the vaccination when compared to control and later on the B-BW ratios decreased during fourth and fifth weeks. Our results are in agreement with the
result of Faragher et al. (1974) and Mazariegos et al. (1990) who also reported that vaccinated groups had low B-BW ratios. Makesh et al. (1999) and Reddy and Koteeswaran (2002) reported that B-BW ratios of IBD affected birds was high during acute phase indicating bursal enlargement and less during convalescent phase suggesting bursal atrophy. Based on these observations, it can be concluded that the vaccine virus also behaves similar to the field virus with relevance to the B-BW ratios although the extent of damage may be less with vaccine virus.

Mazariegos et al. (1990) were of the opinion that the B-BW ratio was not always an indication of the effect of IBD virus. Some chickens showing no effect by the B-BW ratio did not respond normally to ND vaccination and had bursal lesions. Therefore several different tests should be used to determine the bursal damage and immunosuppressive effect of IBD virus. Hence, in the present study, the bursae collected from different groups were further examined by histopathological studies.

The sequential changes in the bursa that were noticed in different groups are detailed in the results (4.2.2). The important observations included edema, congestion, inter follicular hemorrhages, depletion of lymphoid follicles, thinning of cortex and medulla of the bursa followed by presence of cystic spaces in lining epithelium, metaplastic changes, appearance of foam cells, inter follicular and sub epithelial fibrosis (Fig 5 to 10). The extent of damage was comparatively more in groups which received intermediate plus vaccines. In group III also the changes were noticed as the birds were primed with intermediate live vaccine prior to oil adjuvant vaccine. The bursal sections collected from the control group did not show any
histological abnormalities. The normal architecture of the bursal follicles was maintained in all the sections (Fig.5).

Similar histopathological changes were noticed by several workers in the bursae of the IBD affected birds though the severity of the lesions depends on the virulence of the field virus (Ajinkya et al., 1980; Verma et al., 1981; Mohanty and Rao, 1984)

In a study conducted by Edwards et al. (1982), it was reported that the bursal sections from chicks seven days after IBD vaccination revealed severe damage with destruction of follicular architecture, depletion of lymphocytes, increased connective tissue, mucous cysts and thickening and corrugation of the epithelium. After 28 days of vaccination there was 50% of bursal area repopulation. Regeneration continued until 70 days with majority of the follicles being repopulated.

Marked lymphocyte depletion was observed in the bursa of Fabricius of birds vaccinated at different ages with live IBD vaccines (Ezeokoli et al., 1990). Varying levels of lymphoid depletion was observed by Tsukamoto et al. (1995) when different strains of IBD vaccines were used in chicks.

In the present study apart from the histopathological changes, bursal lesion score was also estimated for different groups based on the extent of lymphoid depletion that was observed in the bursal follicles. The values are indicated in Tables...
3 to 9. The bursal lesion score of zero was observed in the control group as there were no histological abnormalities of the bursal follicles. In group II, the scores were 1, 2.33, 1.83, 3.33 and 1.83 during 1st to 5th week respectively, indicating maximum depletion during 4th week. In group III, the bursal scores recorded were minimum (less than 2) when compared to other vaccinated groups in all the weeks. In groups IV and group V, maximum bursal score of four was noticed during fourth week of age. During second and third weeks, the scores were less than 1. During fifth week there was again less scores (2-2.5) probably because of lymphoid regeneration. In groups VI and VII, the bursal lesion score of four was recorded during fourth and fifth weeks indicating maximum damage caused by the intermediate plus vaccines.

Mazariegos et al. (1990) studied the pathogenicity and immunosuppressive effects of seven different commercially available IBD intermediate strain vaccines based on the B-BW ratios and the bursal lesion score. The scores ranged between 1.4 and 4.0 in different vaccines tested. They grouped the vaccines into highly pathogenic, moderately pathogenic and mild varieties. Singh et al. (2002) stated that bursal lesion score could be used as a pathogenicity index for IBD virus.

As most of the IBD vaccines are known to produce immunosuppression as a consequence of severe bursal damage, in the present study, the immuno depressive potential of the IBD vaccines was determined by examining the ability of IBD vaccinated birds to respond to Newcastle disease virus vaccination. All the birds in the IBD vaccinated and control groups were vaccinated against ND as per the Standard Schedule. The response to ND vaccination was quantified by HI test. The results of HI test are presented in the Table (10) and Fig.(11). The NDV-HI maternal
antibody titre of day old birds was found to be 1:256. As the first ND vaccine was given on seventh day of age, there was a gradual increase in the HI titres from third week onwards. In group II which was vaccinated on day one of age, during second week least HI titres of 1 in 32 were observed but after third week, there was a gradual increase in the HI titres.

A moderate decline in the HI antibody titres were observed immediately succeeding the week, when ND vaccination was done probably because of partial neutralization of the antibodies by vaccine virus, which was followed by a gradual increase in the titres. When compared to the control, the response to ND vaccination was not much altered in the groups II, III and IV. In groups V, VI and VII there was a moderate suppression of the vaccine response as evident from the data given in Table (10). However, at no stage of the experiment during the 20 week period, the NDV-HI titres fell below the protective levels. The protective NDV-HI titres were found to be 1 in 32 in pooled serum samples as suggested by Bankowski (1958) and PHilips (1973).

In a similar study conducted by Faragher et al 1974 they stated that the immunosuppressive effect was very marked when the IBD agent was given at day old, less so when given at seven days and barely detectable when given at 14 days or more. Some authors reported that there was a marked reduction in the primary and secondary serological responses against ND vaccination in those chicken inoculated with infections bursal agent (Allan et al., 1972, Westbory, 1978, Jhala et al, 1990; Das et al, 1996).
On the contrary some authors have reported that the immunosuppression observed was not of high order and all IBDU inoculated birds could withstand challenge with virulent RD virus (Rao et al, 992, Giambrone and day, 1986). The results in the present study are in agreement with these findings.

In a study conducted by Nakamora et al, 1992, they observed that certain stairs of IBD cause immunosuppression in terms of response to Newcastle disease virus vaccination. In the present study, the intermediate and intermediate plus IBD vaccines that were administered were procured from ventri biologicals, Pune. And the manufacturer's claim that they have used a strain of IBD which is highly antigenic, though invasive, immunosuppressive effect is least hence it is always preferred in the VVIBD cases and the flock will not show much variation in the vaccinal response of ND vaccine. The results obtained in the present study are also supporting the data given by the manufacturers.

Although only moderate decline was noticed in the RD titres in the groups V, VI and VII, in general higher mortality rate was observed due to coccidiosis and E.cold infection in Groups VI and VII in comparison with the other groups which did not exhibit any mortality, when all the birds were maintained under uniform managerial practices. No significant difference could be found in groups which received more than two IBD vaccines in terms of vaccine response. However, as vaccination of breeds hens with IBD vaccines has become a common practice and chicks with different NDA levels will be present in the same flock, multiple vaccinations will help in protecting a greater proportion of the bids as they become susceptible at different intervals of time.
Based on the observations made in the present study, it is concluded that vaccination of day old chicks does not confer any special advantage compared to vaccination at 13-15 days of age. Maternally derived antibodies in chick goes to negligible levels by sixth week of age. Although, the intermediate plus vaccines cause some bursal damage, their immunosuppressive effect on RD immunity was not so alarming that their usage in the field should be curtailed. As the disease has become endemic in India, with most farms harbouring the IBD virus, use of stronger live IBD vaccines is justified in places where highly virulent form of IBD is present. Usage of appropriate vaccines and stringent bio security measures can prevent the occurrence of major outbreaks of the disease.
Infectious bursal disease is an important viral infection of chicken characterised by high mortality and severe immunosuppression. For the control of the disease different vaccination patterns were being adopted under field conditions. In the present study the efficacy of six different IBD vaccination schedules were studied using intermediate and intermediate plus strains of vaccines either alone or in combination. The serum antibody levels of IBD were quantitated using IDEXX-ELISA kit (Flockcheck). The maternal antibody levels of day old chicks were found to be 2034.31 (log\textsubscript{10}). The decline of maternally derived antibodies (MDA) was checked by ELISA at weekly intervals in the control group. The serum samples were found to be negative for IBD MDA by six weeks of age.

In vaccinated groups there was a gradual decrease in the ELISA titres from day old to third week. The sero conversion of the vaccine virus was noticed during fourth week, reaching to the peak between eight to twelve weeks of age in different groups. Afterwards, there was a gradual decrease in the titres, by the end of 20\textsuperscript{th} week (maximum period tested) titres ranged between 1864 to 1945. There was no significant difference in the titres of different treatment groups. However, the protective antibody titre was reported to be 400 or above in ELISA and also all the groups showed titres above protective level during the entire period of study.
The bursal damage caused by different vaccines in different groups was studied by measuring the bursa body weight (B-BW) ratios during the initial five weeks of the experiment. There was significant difference in B-BW ratios of vaccinated groups in comparison with control group. The bursal sections were subjected to histopathological studies which revealed depletion of lymphoid follicles, presence of cystic spaces in addition to edema and hemorrhages. The birds vaccinated with hot strain of IBD vaccine showed metaplastic changes, presence of foam cells with pronounced interfollicular fibrosis. The bursal lesion scores were calculated based on lymphoid depletion. The bursal scores were maximum in the groups vaccinated with hot strains of IBD vaccines.

The immunosuppressive effect of IBD vaccines was estimated based on the response of birds to Newcastle disease vaccination. The HI titres of different treatment groups were compared with the control. There was only a moderate reduction in the HI titres of IBD vaccinated groups especially in group VI and VII when compared to the control. At no stage of the experiment the NDV-HI titres were found to be below protective levels.

In the present study it is concluded that even though the intermediate plus vaccines cause some amount of bursal damage and a moderate immunosuppression, they can be used at the field level in the areas where IBD is endemic as they can protect the flocks against vvIBD outbreaks. Selection of appropriate strain of vaccine virus is most important as certain strains were reported to be more invasive, highly antigenic but least immunosuppressive which can be used safely at the field level.


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