EFFICACY OF CERTAIN AGENTS IN CONTROLLING IMMUNO SUPPRESSIVE EFFECTS OF INFECTIOUS BURSAL DISEASE VIRUS IN CHICKEN

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IN
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BY

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2002

Dedicated to
My
Parents

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CERTIFICATE-I

This is to certify that the thesis entitled "**EFFICACY OF CERTAIN AGENTS IN CONTROLLING IMMUNO SUPPRESSIVE EFFECTS OF INFECTIOUS BURSAL DISEASE VIRUS IN CHICKEN**"; submitted in partial fulfilment of the requirement for the degree of master of veterinary Science in Microbiology'' of the Faculty of Post Graduate Studies, Rajendra Agricultural University ; Bihar is the record of bonafide research carried out by ***Dr Manoj Kumar*** under my supervision and guidance. No part of the thesis has been submitted for any other Degree or Diploma.

It is further certified that such help or information received during the course of this investigation and preparation of the thesis have been duly acknowledged.

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CERTIFICATE – II

We , the undersigned, members of the Advisory Committee of **Dr.Manoj Kumar** a candidate for the degree of Master of Veterinary Science with major in Microbiology have gone through the manuscript of the thesis and agree that the thesis entitled "**EFFICACY OF CERTAIN AGENTS IN CONTROLLING IMMUNO SUPPRESSIVE EFFECTS OF INFECTIOUS BURSAL DISEASE VIRUS IN CHICKEN**" may be submitted by Dr. Manoj Kumar in partial fulfillment of the requirement for the Degree .

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Place: Patna.

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LIST OF ABBREVIATIONS

AGPT	Agar gel precipitation test
ANOVA	Analysis of variance
BF	Bursa of Fabricius
B:BW	Bursa:Body weight
oC	Degree Centigrade
CS	Course Spray
Co.	Company
DNCB	Dinitro chlorobenzene
Dr.	Doctor
dpv	Days post-IBD vaccination
d.f.	Degree of Freedom
D.W.	Drinking Water
ELISA	Enzyme Linked Immuno Sorbent Assay
Edn.	edition
Fig.	Figure
FCR	Feed Conversion ratio
g	Grams
gr.	Groups
H&E	Haemotoxyline and Eosin
Hb	Haemoglobin
HA	Haemagglutination
HI	Haemagglutination inhibition
Homeo. dr.	Homeopathic drugs
Hrs.	Hours

i.o.	intraocular
iIELs	intestinal intraepithelial leucocytes
IU/Kg	International unit per kilogram
IBDV	Infectious Bursal Disease Virus
IgG	Immunoglobulin G
IgM	Immunoglobulin M
Ltd	Limited
Lb	Pound
MDA	Maternally derived antibody
MAb	Maternal antibody
Micro.	Microbiology
M.S.	Mean sum of squares
Mg	Miligram
Ml	Mililiter
Mr.	Mister
No.	Number
NDV	New Castle Disease Virus
P.	Page
PBS	Phosphate Buffer Saline
PC	Post challenge
PI	Post infection / post inoculation
PCV	Packed Cell Volume
PHA	Phyto haemagglutinin
PPD	Purified protein derivative
Pvt.	Private

QAGPT	Quantitative agar gel precipitation test
RBC	Red Blood Cells
RDV	Ranikhet disease virus
Rpm	Revolution per minute
S.E.	Standard Error
SPF	Specific pathogen free
SRBC	Sheep red blood cells
Thuja oc.	Thuja occidentalis
Uv	unvaccinated
V	Vaccinated
vvIBD	Very virulent infectious bursal disease
WLH	White Leghorn
Wt.	Weight
W/V	Weight by volume
%	Percent

INTRODUCTION

Infectious bursal disease; synonymously called Gumboro disease, is an acute, highly contagious, immunosuppressive viral disease of young chicken. Clinically the disease is characterized by on acute onset of depression, anorexia, white and watery diarrhoea and ruffled feathers. Terminally, the affected birds show sternal or lateral recumbency with a course of tremors. Gross lesion includes feverish and dehydrated carcasses with ecchymotic haemorrhages in breast and thigh muscle. The changes in bursa of Fabricius include turgid and haemorrhagic bursa with increase in size. Petechiae are also noticed at the juncture of proventriculus and gizzard. enteritis and pallor with accumulation of urates in kidney. The etiological agent of the disease is IBD virus which belongs to family *Birnaviridae* and genus *Avibirnaviradae* having double stranded, bisegmented RNA genome. The virus has icosahedral symmetry and non-enveloped with diameter of 50-60 nm.

Initially, the disease was caused by classical virus, producing mild clinical or sub-clinical infection which resulted in impaired growth, poor feed conversion ratio and acquired immunodeficiency with low mortality. Sound bio-security and vaccination through mild attenuated and killed vaccine has been effective control measure.

At the end of nineties the scenario of the disease become more complicated due to production of different clinical pictures such as heavy morbidity as well as mortality rising upto 70-100% Despite solid bio-security

and repeated vaccination the disease perpetuated unabatedly. Later in 1989 the virus was identified as 'very virulent strain' (Box, 1989), being antigenically similar to classical strain but was able to penetrate the maternal antibody barrier establishing the disease in susceptible flock. Erstwhile, use of vaccine, using mild or intermediate strain, was found inadequate. Therefore less attenuated (invasive/moderate hot) vaccine strain capable of evoking immune response, even in presence of maternal antibody, was introduced Albeit, invasive vaccine strain could put the disease under control, they cause immunosuppression and mild bursal lesions (Ezeokoli *et al.*, 1990).

Hence, to ameliorate the deleterious effect of invasive intermediate strain various immunomodulating agents have been studied scientifically. Among them chemical compounds such as levamisole (Mohanty *et al.*, 2000). ascorbic acid (Amakye, *et al.*, 2000), Vitamins (Franchini. 1995; Shadakshrappa *et al.*, 1998) administered concomitantly with IBD vaccine gave encouraging results.

Moreover, contaminated feed also attribute as a possible cause of the disease. In India where there is lack of stringent system for supply of mycotoxins free feed. obviously there is every chance of availability of contaminated feed by mycotoxins such as aflatoxin. ochratoxin and other unknown factor whose feeding even at very low concentration leads to decreased production. reduced growth rate, enhanced mortality, impairment of immune system and vaccine failure (Singh *et al.*, 1996). IBD in concomitance with mycotoxins have been reported

by many workers (Chang and Hamilton, 1982; Burn and Dwivedi, 1986; Mangat *et al.*,1987; Somvanshi and Mohanti, 1991). Therefore introduction of vaccines with minimum immunosuppressive property and contamination free feed is desired for economic poultry farming. Additionally, in a scenario where the vaccine itself is harmful and congenially with unknown or known causes makes in the condition more worse. Hence, utmost precaution should be taken to prevent these deleterious effects.

Therefore, present, investigation has been undertaken with following objectives:

- To study the immunosuppressive effects of invasive intermediate IBD vaccine and local field isolate of IBD virus employing such study like antibody response to RD vaccine by HI test, histopathological changes in the bursa of Fabricius, bursa: body weight ratio, body weight gain and feed conversion ratio (FCR).

- To study the immune responses to invasive intermediate strain IBD vaccine in chickens treated with any of the selected drugs/agents or combination of agents.

- To study the immunopotentiating effect of selected drugs / agents or combination of agents in broiler chicks immunocompromised by the vaccine strain of IBD virus / field virus by determining HI titer to RD

vaccine, bursa: body weight ratio, body weight gain and FCR.

- To study the effectiveness of vaccinal response in IBD vaccinated broiler chicks which have received different treatments with selected drugs / agents or combination of agents by conducting challenge studies.

CHAPTER II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Cosgrove (1962) was first to document infectious bursal disease (IBD) at Gumboro district, a place near South Delaware in United States of America. Hence the eponym Gumboro disease has been given. The clinical picture of disease was ruffled feathers, watery diarrhoea, severe prostration, trembling, oedema and enlargement of bursa of Fabricius and renal damage. The renal damage being the most prominent lesion, the condition was described as "avian nephrosis."

Since its first report, the disease was found to be widely distributed, occurring in essentially all major poultry producing areas of the world (Okoye, 1984). In India, the disease was first reported from Uttar Pradesh by Mohanty *et al.* (1971) on the basis of histopathological studies. Since then several workers have reported the incidence of this disease in different part of country (Ajinkya *et al.*, 1980; Chauhan *et al.*, 1980; Ray and Sarkar, 1984; Sulochana and Lalithakunjamma, 1991; Maherchandani *et al.*, 1992; Singh *et al.*, 1994; Joshi and Shakya, 1996).

Cheville (1967) investigated the cytopathological changes in the bursa, spleen and thymus of chickens following experimental infection with IBDV, intraocularly at 28 days of age. Necrosis of lymphocytes in the medulla of bursal follicles was the initial lesion. This was followed by the destruction of all lymphoid tissues in the bursa, phagocytosis of the necrotic lymphoid cells by large reticular cells resulted in severe reticular hyperplasia.

Initial atrophy was followed by proliferation of the corticomedullary epithelium and the formation of mucous secreting glands.

Del Bono *et al.* (1968) observed lesions in the bursa as necrosis of the lymphoid cells and corticomedullary layer with regression of follicles. The epithelial lining of the plica became hyperplastic and hypertrophic with the development of goblet type cells in an active secretory state. Pseudocystic structures were seen frequently within the follicles.

Hirai *et al.* (1974) demonstrated that antigens from IBDV were specific and formed three precipitation lines due to the differences in the diffusion rates of the viral particles, PA-1, PA-2 and PA-3. The precipitating antigens, PA-1, migrated most slowly and PA-3 migrated most rapidly.

Cullen and Wyeth (1975) described the quantification of IBDV antibodies by AGPT. Antigen was prepared from the bursa of three to five weeks old chicks. The bursal homogenate was treated with Arcton 113. The quantitative agar gel precepitation test (QAGPT) was used to measure the maternal antibody level in chicks from IBDV infected parents (Wyeth and Cullen, 1976). Wood *et al.*, (1979) standrized the QAGPT for determination of IBDV antibodies level in chickens. They reported that the antigen concentration was of no significance within certain limits, but for clarity, high antigen concentration was recommended.

Thornton and Pattison (1975) undertook a comparative study of nine products from seven different sources intended for use as vaccines against infectious bursal disease of chickens. No vaccine caused clinical disease after administration to chicks at seven day of age, however, one of them caused a significant impairment of weight gain, and when given to 1 day old chicks caused some morbidity and deaths. Most vaccines affected the bursa of Fabricius and histological examination of this organ revealed varying degree of tissue damage, which correlated with the reduction in size of this organ. The affects with the different products ranged from no damage to damage almost as rapid and severe as that produced by a fully virulent field strain of the agent. Selected product which differed in their effect on the bursa were tested for their immunosuppressive properties by assessing the response to live Newcastle disease vaccine administered after the IBD vaccine. The effect on the response to New Castle disease vaccine was found to be correlated with the degree of tissue damage . Variation in the ability of the vaccines to protect against IBD challenge was also found, but these did not depend on degree of damaged to the bursa. These studies enable proposals for standard tests for IBD vaccines to be formulated in repect of safety, potency and lack of immunosuppressive effect. Only 2 of 9 vaccinated satisfied these standards.

Siegel and Mortan (1977) have reported a three-fold increase in interferon level following Poly I / Poly C addition to mouse cell cultures containing ascorbate. The interferon inducing capacity of ascorbate has also been reported. Interferon levels were reported to be increased in

mice provided with ascorbic acid in drinking water and exposed to a meurine leukemia virus (Anderson, 1981).

Winterfield and Thacker (1978) compared the usefulness of AGPT and VN test to study the immune responses of different strains of IBD applied as vaccines. They observed that even precipitin negative chickens were often protected, whereas AGPT positive chickens always protected.

Mohanty *et al.* (1981) reported maximum antigen concentration in the bursa collected at 48 hrs. PI. Bursa collected at 12 hours PI has only faint preciptin lines. Two distinct preciptation lines were seen with bursa collected at 24 hours PI. Where as those of 48 hours PI had three lines.

Edwards *et al.*(1982) investigated the duration of immunosuppression and the relationship between bursal damage and depression of humoral response caused by an IBD vaccine strain administered at one day of age. Examination of bursal sections from chicks seven days post 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 in eight out of 10 bursa examined. The remaining two bursa showed over 50 percet of the bursal area depleted of lymphocyts. Subsequently, bursa were collected continuous repopulation of lymphocytes and majority of apparently normal plical cells were observed. Regeneration continued until the end of the 70 days observation period when the

majority of the bursa were repopulated, but evidence of the earlier damage remained. The bursa from the vaccinated birds were always smaller than those of the controls, with epithelium corrugated and thickened.

McFerran *et al.*(1982) conducted field studies with an inactivated vaccine against infectious bursal disease. Vaccination using an inactivated infectious bursal disease vaccine stimulated long lasting neutralizing antibodies. Highest titres were produced in the birds which had previously been infected with a field strain, but satisfactory titres were achieved after priming with an attenuated vaccines. Bursal lesions were delayed by about 2 weeks in the progeny of vaccinated birds. However no economic advantage could be demonstrated from the use of the vaccine. It is concluded that the depressed feed conversion seen in broiler was not due to IBD virus.

Ley *et al.* (1983) detected serologic, histopathological and biochemical changes in 35 days oldspecific pathogen free chickens inoculated with IBDV. A detectable precipitin antibody response occurred between 3 and 5 days post inoculation. Histopathological examination of lymphoid and non lymphoid tissues from IBDV infected SPF chickens affirmed that the predominant lesion was lymphoid necrosis in the bursa of Fabricius. Other lymphoid organs were much less severely affected and possessed greater regenerative potential. Nonspecific and relatively mild changes were found in the liver and kidney.

Lukert and Hitchner (1984) reported that the histopathological lesions in the bursa commenced with

degeneration and necrosis of the lymphocytes in the medullary area of the follicles. The lymphocytes were soon replaced by heterophils, debris, and reticuloepithelial cells. All of the follicles were affected by three to four day PI. As the inflammatory reaction decreased, cystic cavity and fibroplasia of the interfollicular connective tissue developed.

Giambrone and Clay (1986a) compared the immunogenicity, stability, pathogenicity, and immunodepressive potential of four commercial live infectious bursal disease vaccines. Although all vaccines were capable of spreading to in contact controls, only two intermediate vaccines produced slightly atrophied bursae and moderate microscopic bursal lesions. Also, all the vaccines were stable because they failed to revert back to increase in virulence and also did not result in morbility or mortality associated with virulent IBDV after successive passage. None of the four vaccines was found to be immunodepressive, as all IBD vaccinated birds responded to NDV vaccination.

Giambrone and Clay (1986b) compared the efficacy of two intermediate infectious bursal disease vaccines (clone vac D-78 and S-706) for immunizing specific pathogen free white leghorn chickens by coarse spray (CS) against sub-clinical IBD. Both intermediate IBD vaccines were equally capable of immunizing day old SPF chickens by CS and were safe as evidenced by the absence of morbidity, mortality, or severe gross and microscopic bursal pathology at 28 days of age.

Panigrahy *et al.* (1986) conducted an experiment in which five weeks old chickens were inoculated with IBDV and bled five days post inoculation for analysis of haematological changes and serum biochemical changes. From this experiment he concluded that in IBD infected group there were significant decrease in the total erythrocyte count, packed cell volume, haemoglobin concentration, albumin, albumin: globulin ratio, uric acid and glucose. Serum globulin and chotesterol increased significantly. There was no alteration in the levels of sodium, potassium and calcium in IBD affected birds. Other cellular changes that occur in the acute stage of IBD are lymphocytopenia and panleucopenia (cheville 1967). Histological lesions in the bursae of Fabricius of chickens five day post inoculation were characterized by massive cystic degeneration of follicle with depletion of lymphocytes, reticuloendothelial hyperplasia. interfollicular edema and fibrosis and infilteration by heterophils and macrophages. No lesions was present in the bursa of Fabricius of control chicken.

Ezeokoli *et al.* (1990) evaluated the effect of IBD live virus vaccine of the immune response of chickens by the assessment of the antibody response following vaccination as well as persistence to challenge with virulent virus. Birds were vaccinated at various ages and later challenged with a heterologus vaccine or wild type IBD virus. The BF was examined for histopathological changes at regular intervals. Antibody levels to NDV were monitored. Significantly higher mortality rates were observed in birds vaccinated with IBD vaccine than unvaccinated birds following challenge. The bursa of Fabricius from

vaccinated birds showed marked lymphocytic depletion and cellular infiltration with mononuclear cells. NDV vaccine given intraocularly at day old largely prevent the immunodepressive effect of IBD vaccination. Groups that received IBD vaccine on day 14 but no NDV had higher mortality (41.2%) and showed lower antibody response than those vaccinated on day 1 (0%) or controls which did not receive IBD virus challenge (11.8%).

Jhala *et al.* (1990) infected four weeks old broiler chicks with infectious bursal disease virus isolate. The birds were sacrificed 48 hours and 5 days after infection. They did not observe any clinical symptoms in experimentally infected birds during the five days observation period. All the birds showed significant enlargement of bursa of Fabricius at 48 hours PI and atrophy at 5 days PI. Other organs collected were grossly normal at both the occasions. Histopathologically there were necrosis and depletion of lymphocytes in bursal follicles at 48 hours PI. Bursal lesion became severe at five days PI, where bursal follicles were found atrophied with regression on size. There was severe depletion of lymphocyte in the cortex and medulla. The corticomedullary epithelium showed formation of cyst severe proliferation of fibrous connective tissue was observed in the interfollicullar space. They also observed mild depletion of lymphocyte in the lymphoid follicles of caecal tonsil at 48 hours and five days PI. Thymus, spleen and kidney collected at 48 hours and 5 days PI, did not reveal any significant histopathological lesion.

Mazariegos *et al.* (1990) conducted the study to test the pathogenicity and immunosuppressive effects of seven commercially available infectious bursal disease vaccine. The vaccine strains were intermediate in their pathogenicity in susceptible spècific pathogen free chickens. One day old and three week old SPF chickens were vaccinated with these vaccine. Two weeks after IBD vaccination they were vaccinated with Newcastle disease virus. The pathogenic and immunosuppressive effects of the IBD vaccines were evaluated by the antibody response to NDV vaccination, the bursa: body weight index, and histopathological lesions of the bursa. In chicks vaccinated at day old, bursa: body weight index varied from 0.23 to 1.0 whereas in case of chicks vaccinated at 3 weeks of age ratios ranged from 0.24 to 1.0. The HI antibody titre to NDV ranged from 3.7 to 61.7 and 13.0 to 59.2 respectively in chicks vaccinated at day old and those vaccinated at 3 weeks of age. It was found that these strains were highly variable in their virulence and immunosuppressive properties. Three of the strains tested were found to be highly virulent and immunosuppressive, two others were moderate and two could be classified as mild.

Vanden Berg *et al.* (1991) isolated a highly virulent strain of IBDV from the field and propagated in SPF chickens, causing up to 100 percent mortality. Although the virus belonged to the standard serotype 1, serological typing with monoclonal antibodies showed an antigenic drift in this pathogenic strain.

Khafagy *et al.* (1991) reported the isolation, identification and characterisation of 9 field isolates of

very virulent infectious bursal disease virus prevalent on 9 chickens farms in Egyptial Governates. The disease was characterized by sudden appearance with high morbidity usually nearly 100 percent with mortality of upto 70 percent. The isolates were identified as normal Gumboro strain (Faragher) with no variants.

Somvanshi *et al.* (1991) conducted a study on bursa of Fabricius inoculated with infectious bursal disease field virus through light and electron microscope. Studies of Bursa of Fabricius was conducted in chickens of 24, 48 and 72 hours post inoculation. Histological examination revealed marked oedema, hyperaemia, necrosis of lymphoid cells, formation of vacuoles or cystic spaces in the medulla of bursal follicles. At 72 hours, complete depletion of lymphoid cells, atrophy of bursal follicles and proliferation of collagen fibers were seen. Macrophages were observed in and around bursal follicles. Immuno-histochemistry revealed localizational of IBDV antigens in lymphocyte and more frequently in vacuoles of macrophages. Antigen was more in the medulla than in cortical parts of follicles. Higher quality of IBDV antigen was seen at 48 hours interval than at other periods. Wide intercellular spaces, edema and marked degenerative and necrotic changes were observed in bursal lymphocytes. Abnormal shaped or pyknotic nuclei and pronounced darker heterochromatin were the main changes in the nuclei.

Nakamura *et al.* (1992) compared the immunosuppressive effect of highly virulent infectious bursal disease among 2,3 and 4 weeks old chickens. The

virus suppressed antibody responses to ND vaccination in above three groups.

Nunoya *et al.* (1992) reported the occurrence of acute infectious bursal disease virus with high mortality in Japan. They isolated highly virulent infectious bursal disease virus from field outbreaks and studied the pathogenicity of the field isolates in SPF chickens. The experimentally infected chickens developed severe clinical disease with a high mortality rate. The histopathological changes were marked by bursal and thymic necrosis, aplastic anaemia and acute hepatitis.

Tsukamoto *et al.* (1992) reported isolation of virulent infectious bursal disease virus from field outbreaks with high mortality in Japan. In all cases the parent flock were inoculated with IBD vaccine but their progeny were not. The virulence of the isolates from such outbreaks was investigated in SPF chickens. Infected birds developed diarrhoea within 24 hour of infection and showed depression, trembling, ruffled feathers and were prostate. The 5 isolates caused 30-70 percent mortality with Yamaguchi strains. Those that survived the disease, lost weight or showed no weight gain. Atrophy of the bursa of Fabricii was seen in all surving birds.

Wyeth *et al.* (1992) studied the usefulness of inactivated infectious bursal disease oil emulsion vaccine to control very virulent strain of IBD virus in commercial layer chickens having varying levels of maternal antibodies. The QAGPT titres of Mab ranged between 2^0 to 2^5. The chicks were vaccinated at 7,10,14 or 28 days old

with varying doses of vaccines intramuscularly. The birds were challenged by eye drop with 100 CID_{50} of the CS88 strains of IBD in 0.1 ml of inoculum and sacrificed fifty six hours later and their bursae of Fabricius were examined for the presence of viral antigens using the agar gel precipitation test. The partial doses given at 7 or 10 days old gave only partial protection. A full dose given at 10,14 or 28 days old fail to give full protection but a full dose administered at 7 days old protected all the chicks after each challenge with virulent virus.

McIlroy *et al.* (1992) attempted to reduce the economic losses due to sub clinical IBD. Broilers reared in 23 houses known to have been infected with IBD virus were vaccinated with live, attenuated IBD virus vaccine at 20 days of age. Vaccination of either one, two or three consecutive flocks resulted in significant increases in net income and average body weight, and in a decrease in mortality. An improvement in broiler performance was not restricted to vaccinated flocks., but was also apparent for at least 1 year following placing of the first vaccinated flock in subsequent flock reared in the same houses.

McIlroy *et al.* (1993) found improved performance in commercial broiler flocks with sub clinical infectious bursal disease when fed diets containing increased concentration of vitamin E. The economic effects of increased vitamin E supplementation in 79 commerical broiler flock incorporating over 1.5 million birds was assessed. Approximately half of the flocks were fed on either a high (178 IU/Kg) or normal (48IU/Kg) vitamin E containing diet. In addition, in approximately half of the

flocks sub clinical IBD was present. Analysis of the performance data showed that flocks with sub clinical IBD were consistently worse for net income, feed conversion ratio and average weight per bird than flocks sub clinical disease. The trial also indicated that the average net income of flocks with sub clinical IBD and fed a high vitamin E containing diet was of better than that from flocks with sub clinical IBD and fed a normal vitamin E containing diet. The trial also showed that the difference between the average net income achieved by flocks without sub clinical IBD and being fed on either a high or a normal vitamin E containing diet was only 2% and not significantly different. It was suggested that the increased improved performance from high vitamin E containing diet recorded in flocks with sub clinical IBD is due to enhanced immunocompetence and increased resistance to disease. It was also suggested that under field conditions high dietary inputs of vitamin E are most beneficial where there is a challenge to the defence system of the host and that significantly improved performance would occur more predictably under such conditions.

Singh and Dhawedkar (1993) observed that levamisole treatment of IBDV infected chicks was able to restore their immune responses to sheep red blood cells to a level comparable to that or uninfected control. Immunomodulatory effect of levamisole was observed only in birds which had undergone immunosuppression due to prior IBDV infection. This drug did not increase the immune response above the normal level in immunologically competent hosts. Thus the treatment of

birds with levamisole may prevent the disease arising from immunosuppression as a result of sub clinical IBD.

Chatterjee *et al.* (1994a) investigated the immunomodulatory effect of herbal product IMMU-21 (research name) in different laboratory animals. He found that animals treated with IMMU-21(20 mg/kg) significantly increased the microbicidal activity of neutrophils in the experimental animals which may be due to its decreasing effect on circulating level of corticosteroids. Increase in soluble immune complex in the serum of the experimental animals also indicate immunopotentiating action of IMMU-21. At a dose of 20 mg/kg it caused a significant increase in the antibody titres in both the primary and secondary immunity assay while a higher dose (200 mg/kg) of IMMU-21 did not significantly alter the antibody titre and showed slight immunosuppressive effect.

Coletti *et al.* (1994) studied a vaccine strain of infectious bursal disease virus with residual pathogenicity in the fowl. Antibody levels were highest in those chicks which had the lowest maternal antibody levels at the time of vaccination.

El-Zanty (1994) studied the effect of virulent IBDV on 1-week old broiler chicks fed diets containing different concentration of ascorbic acid (AA) (group 1, 100 mg AA/Kg; group 2, 220 mg AA/kg; group 3, 330 mg AA/kg and group 4, 660 mg AA/kg diet respectively). At 4 weeks of age broiler chicks in different groups were infected intraocularly with 105.7 EID_{50} / 0.1 ml virulent IBD virus. The severity of the clinical signs, bursal lesions and other

pathological lesions were reduced in ascorbic acid fed chicks. When bursal homogenate tested by AGPT, titre were high in chicks receiving no AA but low in chicks from group 2 to 4. It was concluded that AA increases the resistance in broiler chicks to virulent IBD infection.

Kouwenhoven and Vanden Bos (1994) controlled the very virulent IBD in the Netherlands with more virulent vaccine. The maternal immunity of chicks hatched from the eggs of vaccinated hens could not withstand infection with a virulent strain of IBD virus which appeared in the Netherland in 1987, and they developed the disease at 14-28 days of age. Vaccination of broiler at 14-21 days of age solved the problems only partly. Trials of three new more virulent live vaccines the Bursa vac (Sterwin Laboratories, U.S.A) LZ228E (Mycoform Nederland B.V the Netherlands) and Bursa plus (Solvay Duphar, B.V. the Netherlands) were conducted on 29 million birds in 96 replacement layer flocks and 714 broiler flocks between October, 1990 and November 1991 with satisfactory results. However, they found the Bursa vac in slightly more virulent than the other two 'hot' vaccines. They also observed that 'hot' vaccines were slightly more pathogenic than the Intermediate vaccines.

Pande and Vijay Kumar (1994) studied the immunomodulatory effect of zeetress in chicken vaccinated against ND (F strain) virus. Zeetress was administered at the rate of 5 g/1000 chicks through the drinking water for first 10 consecutive days and thereafter at the rate of 10 g/1000 birds from 24 to 35 days. On 35 day serum samples were collected for HI test. It was found that antibody

titres, body weight gain and feed efficiency were significantly higher than untreated vaccinated control.

Panda and Rao (1994) observed the effect of a vitamin E Selenium combination in chickens infected with infectious bursal disease virus. Ninety male chicks from a single hatch were divided into 6 groups and infected with infectious bursal disease virus by intraconjunctival inoculation at one day old. Two groups of IBD infected birds and 2 of uninoculated birds were stimulated with a subcutaneous injection of Brucella abortus antigen at the end of the second weeks (Primary stimulation) and third week (Secondary stimulation). A vitamin E selenium supplement (E care Se), at the rate of 25 mg/bird orally in drinking water on alternate days from one day old throughout the experiment, was given to 2 groups of birds (one IBD infected and B. abortus stimulated and one non-IBD infected but B. abortus stimulated). Serum samples were collected weekly from one week after the secondary stimulation for 4 weeks and the humoral response measured by the tube agglutination test. Birds were killed by the end of the seventh week. Birds with a bursa: body weight index lower than 0.85 were considered to have bursal atrophy. Antibody titres were detected only in response to B. abortrus stimulation. The immunosuppressive effect of the IBD virus was indicated by the fact that the IBD infected untreated (without Vitamin E–selenium) birds had the lowest geometric mean titres. The vitamin E–selenium treatment significantly boosted both the GMT in IBD infected birds in comparison with untreated, infected birds. There was no significant difference between the GMT of untreated uninfected birds and treated, infected

birds. The supplementation had no effect on bursa: body ratio in IBD-infected birds and the bursa of these birds showed IBD specific lesions in about 50% of the follicles. The findings strongly suggest the enhancement of immune responses due to vitamin E–selenium supplementation in IBD infection.

Singh *et al.* (1994b) reported the occurrence of infectious bursal disease in chickens between February, 1990 to May, 1993 in Bihar. The disease occurred in both acute and sub clinical forms. The acute IBD was marked by high morbidity and high mortality ranging between 35-65 percent. Three virus isolates were recovered from the affected tissue. Majority of acute IBD outbreaks followed revaccination with RDF vaccine.

Suresh and Vasudevan (1994) administered orally, *Phyllanthus emblica*, an excellent source of vitamin C (ascorbate), has been found to enhance natural killer (NK) cell activity and antibody dependent cellular eytotoxicity (ADCC) in syngeneic BALB/C mice bearing Datton's lymphoma ascites (DLA) tumor. *P. emblica* elicited a 2 fold increase in splenic NK cell activity on day 3 post tumor inoculation, Enhanced activity was highly significant on days 3,5,7 and 9 after tumor inoculation with respect to the untreated tumor bearing control. A significant enhancement in ADCC was documented on days 3,7,9,11 and 13 in drug treated mice as compared to the control. A increase in lifespan (ILS) of 35% was recorded in tumor bearing mice treated with *P. emblica*. This increased survival was completely abrogated when N K cell and killer (k) cell activites was depleted either by

clyclophosphamide or anti-asialo-GM, antibody treatment. These results indicate: (a) an absolute requirement for a functional NK cell or K cell population in order that *P. emblica* can expert its effect on tumor bearing animals, and (b) the antitumor activity of *P. emblica's* mediated primarily through the ability of the drug to augment natural cell medicated cytotoxicity.

Kembi *et al.* (1995) compared the effect of three different routes of administation *viz.* oral, intramuscular and ocular on the immunogenicity of infectious bursal disease vaccine. They recommended the ocular route as the most effective for vaccination.

Rao *et al.* (1995) studied immune responses due to zeetress in infectious bursal disease vaccinated chicks. 120 kalinga brown chicks (layer) were divided into 8 equal groups which included appropriate controls. When Newcastle disease vaccine virus was used as indicator system there was higher level of antibody titres in both IBD vaccinated and unvaccinated birds administered zeetress. There was also a marked increase in rosette forming T lymphocytes suggesting stimulation of cell medicated immunity which was further strengthened by significantly severe delayed type of hypersensitivity reaction in the DNCB (2, 4 – dinitrochlorobenzene) skin sensitivity test. The spleenic macrophage activity was increased as shown by increase in number of formazan positive cells in nitroblue tetrazolium test as a result of administration of zeetress. Zeetress was also responsible for significantly higher body weight in IBD vaccinated birds. Histological examination of bursa revealed that

majority of the follicles which were damaged as indicated by atrophied follicles due to destruction of lymphocyte and intra- and inter follicular oedema as a result of live IBD vaccine were partially protected/spare due to administration of zeetress. It was concluded that there is a significant improvement in immune status of IBD vaccinated chickens receiving zeetress.

Tsukamoto *et al.* (1995) reported the occurrence of highly virulent infectious bursal disease virus in the vaccinated flocks in Japan which produced high mortality. They studied the efficacy of three vaccines available in Japan, two mildly attenuated strain and one intermediate strain in SPF chicks and in commercial chicks with maternal antibody against IBD. Chicks were vaccinated at 20 days old and challenged with highly virulent IBDV 10 days after vaccination. Protection was measured at 7 days after challenge. All the three live vaccines protected SPF chicks, however, only Intermediate strain vaccine protected (100%) commercial chicks against highly virulent IBDV.

Franchini *et al.* (1995) established that vitamin E added to inactivated and emulsified vaccine enhanced the immune response to viral antigens in chickens. Vaccines with vitamin E especially when replacing 20 or 30% of mineral oil, induced a more rapid and higher antibody response than control vaccines. An adjuvant effect of vitamin E as present in viral vaccine lacking bacterial antigens.

Kouwenhoven and Vanden Bos (1996) conducted vaccination trial using conventional intermediate vaccine and more invasive hot vaccines on 95 replacement layer farms and 26 broiler flocks that did not suffer from the disease. They did not find significant difference between the two vaccines on the performance of the vaccinated broilers in respect of mortality, average growth, feed conversion ratio and condemnation percentage.

Kurtoglu *et al.* (1996) studied the effect of vitamin A or E or both on antibody titres and blood T lymphocyte percentage value in chickes vaccinated with Gumboro disease vaccine. Blood immunoglobulin IgG increased considerably after vaccination, Vitamin A excess (80, 000 IU/kg diet) suppressed antibody titres and increased T-lymphocyte and IgG values. Vitamin A and E given together reduced the level of increase in T-lymphocyte values.

Mahesh and Muniyappa (1996) studied the immunogenicity, pathogenicity and immunosuppressive potential of one less attenuated, three intermediate, one mild and combination of mild and inactivated IBD vaccines strain, and also monitored the maternally derived antibody response in both experimental and field conditions. The chicks were vaccinated against IBD according to manufacturers recommendations and also against Newcastle disease on day seventh. The evaluation of immune response and MDA was determined by employing indirect ELISA. The pathogenic and immunosuppressive effect of IBD vaccines were evaluated by bursa: body weight ratio and antibody response to ND vaccination. MDA level decreased

below protective level by 14 days and their half life was 3.6 and varied among breeds under field contidions. The less attenuated strain and one of three intermediate strains induced significantly lower titres followed by other two intermediate strains and significantly lower titres for mild (Lukert) and combination of mild and inactivated strains.

Rao *et al.* (1996) concluded from the study that zeetress had a sparing effect on the damage to lymphoid follicles of bursa in IBD vaccinated group but of no consequence in the challenged/virulent IBD virus infected birds. It was concluded that zeetress had stimulated the rosettle forming T lymphocytes and enhanced the macrophage activity in the spleen. However it had no beneficial effects in virulent IBD virus infection and birds vaccinated with intermediate live vaccine challenged with virulent IBD virus which was indicated by severe bursal atrophy associated with low bursa: body weight index and numerical decrease in number of lymphoid follicles.

Yamaguchi *et al.* (1996) studied the potency of a new vaccine in controlling highly virulent infectious bursal disease virus (HV-IBDV) infection. They adapted some isolates of HV-IBDV through serial passage in embryonated eggs. The embryonated egg adapted HV-IBDV was adapted to grow in chicken embryo fibroblast (CEF). The embryonated egg and cell culture adapted strains showed reduced pathogenicity and did not kill any young chickens after experimental infection. The bursal lesion of the adapted strain chickens. Cross-virus neutralization analysis showed antigenic diversity between the cell culture adapted HV-IBDV strains and classical strain. In

immunization tests, the adapted strain immunized chickens showed good protection against the fatal infection of HV-IBDV. At 3 day after immunization the adapted strains showed effective immunogenicity against challenge infection.

Zorman Rojs *et al.* (1996) observed the immune responses of two live IBDV vaccines (mild and intermediate strain) in broiler chicken with maternal antibody in field condition. Two groups of 7060 broiler chickens obtained from a farm in which a possible infections of IBDV was expected and had low level (ELISA GMT<500) of maternal antibody were vaccinated with each of the vaccine separately at 8 days of age. The level of specific antibody against IBDV was monitored at weekly interval by immunodiffusion and ELISA test. Practically no antibody was found 14 days after vaccinations. No clinical singns of IBD were seen.

Ziauddin *et al.* (1996) evaluated the immunomodulatory activity of an Indian Ayurvedic medicinal preparation, Ashwagandha (*Withania sofnifera*) L. Dunal in mice with myelosuppresion induced by one or more of the three compounds – cyclophosphamide azathioprin, or prednisolone. The assessment of immunomodulatory activity was carried out by haematological and serological tests. A significant modulation of immune reactivity was observed in all the three animal models used. Ashwagandha prevented myelosuppression in mice treated with all three immunosuppressive drugs tested. A significant increase in haemoglobin concentration ($P<0.01$), red blood cells count

(P<0.01), white blood cells count (P<0.05), platelet count (P<0.01), and body weight (P<0.05) was observed in Ashwagandha treated mice as compared with untreated (control) mice. A immunostimulatory activity was also found in treatment accompanied by significant increase in hemolytic antibody response toward human erythrocytes.

Ali *et al.* (1997) observed, that spleenic lymphocyte of chickens proliferated with high when vaccinated with NDV following stimulation by all antigens (NDV, IBDV and FPV). This activity was also seen in IBDV vaccinated and FPV vaccinated chicks following stimulation by their respective antigens. The result suggested strong ability of NDV antigens to stimulate lymphocyte proliferation without interfering with immune system.

Al-Muffarrej and Saraq .(1997) studied the immunostimulating effects of Royal jelly (secretion of nurse bee) in chickens when immunized with sheep red blood cells and re immunized 10 days later of primary immunization. Royal jelly treated and immunized group showed increased antibody production as compared with untreated immunized chickens. It is concluded that royal jelly is an effective immunostimulant and that antibody production is more pronounced following subcutaneous administration compared with oral administration and during secondary immunization compared with primary immunization.

Bekhit (1997) reported highly virulent form of infectious bursal disease from Egypt in outbreak of IBD during 1989-1993. He observed severe outbreaks of IBD

with usually high mortalities (56%). He suggested that the present outbreaks of IBD were attributed to very virulent IBDV belonging to standard serotype I. He also suggested that the failure of the different vaccination programmes to give adequate protection against IBDV field challenge may be attributed to many factors other than antigenic variance such as proper timing of vaccination handling and administration of vaccines, type of vaccine as well as vaccine dose.

Christopher *et al.*(1997) observed the influence of vvIBD on immunity to Ranikhet disease at the field level. They statistically analysed the seroepidemiological data of Ranikhet disease and infectious bursal disease, before (during 1991-92) and after (during 1993-94) the outbreak of very virulent form of IBD (vvIBD) in Tamilnadu. During 1993-94 the half life (1/2) of RD maternally derived antibody was 3.2 days and the IBD-MDA was 4.11 days in clean premises. In the infected premises half life of the RD-MDA was 2.69 days and the $T_{1/2}$ of RD vaccinal titres of samples collected during 1993-94 are significantly lower than the statistically predicted HI titres for that age. Mathematical conclusions indicate that vvIBDV could be the cause of this perceived difference in the RD-HI titre values.

Cos 'Kun *et al.* (1997) observed the effect of four different dietary levels of vitamin E (0,5,35 or 70 I.U./kg of feed) on laying hens for a year. The mean egg production of the four groups was 79.9, 80.6, 77.2 and 79.2 percent respectively and the feed consumption for 1 kg of eggs was 2.23, 2.23, 2.36 and 2.20 kg. There was no

differences in blood vitamin E levels, T-lymphocyte percentage, spleen plasma cell counts and antibody titres to Newcastle disease vaccination. The chicks did not differed in maternal antibody titres or in the histological finding in the spleen, bursa of Fabricii, thymus or ileum.

Haddad *et al.* (1997) observed the efficacy of a novel IBD virus vaccine which was prepared by mixing IBDV strain 2512 with bursal disease antibodies. The result suggested that the one day of age administered IBDV-BDA complex vaccine can induce active immunity and protection against a standard IBDV challenge in the face of variable levels of maternal IBDV immunity.

Panda and Kar (1997) investigated the significance of Ashwagandha (*Withania somnifera*) rood extract in the thyroid function of cockerel and found that its root extract (20 mg/day/bird for 30 days) increased serum thyroxins (T4) concentration significantly. The drug also increased the serum protein significantly. Interestingly liver and muscle protein concentration decreased following the drug administration. No significant change in body weight was observed between the treated and control groups.

Pahar and Rai (1997) observed the immunogenicity of IBD virus strain isolated in India. Out of the three isolates given to 7 days old chicks intramuscularly, only strain 394 gave 100 percent protection, while IBDV strain 494 and IBDV strain 194 gave 88 percent and 76 percent protection respectively after challenge with 10^2 CID_{50} IBD virus. They opined that IBDV S394 may serve as a prophylactic

agent against IBD in poultry without any immunosuppressive effect and mortality in day old chicks.

Vervelde and Davidson(1997) characterized leucocytic changes and determined tropism of infecious bursal disease virus following infection of newborn and 3 week old chickens. In the bursae of both age groups rapid depletion of B lymphocytes and an influx of $CD4^+$ TCR – $a\beta_1^+$ and $CD8^+$ TCR – $a\beta_1^+$ cell was detected within 4 days after inoculation. Leucocytic changes in the spleen, thymus and harderian glands were similar in both groups. From 8 days after inoculation and onward all the lymphoid organs became repopulated with leucocytes and tissue architecture was gradually restored. Virus neutralizing antibodies developed more slowly in newborn birds and 21 days after inoculation the titres was much lower compared to older birds. Lack of clinical signs in newborn chickens was neither due to a failure to respond to the virus nor to a lack of viral replication. It is concluded that age related susceptibility to IBDV in chickens may be due to immunological factors, cytokine release, or blood factor.

Zormon-Rojs and Cajavec (1997) reported the efficacy of different vaccination programmes against infectious bursal disease. The trial was conducted with 2 live vaccines (mild and intermediate streain) on 8 commerical farms in Slovenia. IBD outbreaks were diagnosed in all 8 flocks after vaccination with the mild strain at 8 days of age with mortality of 5.03 percent. After vaccination of 2 flocks with the intermediate strain at 8 days of age, IBD was diagnosed in one flock. IBD was diagnosed in 6 or 8 flocks after administration of intermediate vaccine strains

on 15 and 22 days of age with mortality of 2.5 percent. It was concluded that neither vaccine can fully protected broiler against very virulent IBD virus strains.

Ghosal *et al.* (1998) investigated immunomodulatory effect of a rodent bone marrow cytokine in improving antibody reponse in Newcastle disease vaccinated chicks. One drop of a non-species specific 12.7 KD immunomodulatory cytokine (concentration 0.3 µg/25 µl) was applied via nasal or ocular route post R_2B vaccination. Mean antibody titre of the BIM treated chicks was more as compared to only vaccinated group.

Jeurissen *et al.* (1998) observed the working mechanism of an immune complex vaccine that protects chicken against infectious bursal disease. An immune complex vaccine developed by mixing live intermediate plus infectious bursal disease virus with hyperimmune IBDV chicken serum (IBDV-ICX vaccine). It was compared to the native IBDV (uncomplexed) vaccine for differences in target organs, target cells, spread of virus and replication. Specific pathogen free chicken eggs were inoculated on day 18 of incubation with either one dose of virus alone or the IBDV-ICX vaccine. The replication of IBDV and the frequency of B cells and other leucocyte populations were examined in the bursa of Fabricius, spleen and thymus using immunocytochemistry on day 3,5,7,10,14, and 21 after inoculation with both vaccines. IBDV was detected in association with B cells, Macrophage and follicular dendritic cells (FDC) in the bursa and spleen, although complexing IBDV with specific antibodies caused a delay in virus detection of about five

days. There was low level of depletion of bursal and spleenic B cells in IBDV-ICX vaccinated chickens. Inoculation with the IBDV-ICX vaccine induced more germinal centers in the spleen and larger amounts of IBDV were localized on both spleenic and bursal FDC. It was suggested that the working mechanism of the IBDV-ICX vaccine is related to its specific cellular interaction with FDC in the spleen and bursa.

Kumar *et al.* (1998) studied the influence of immunostimulation with *Mycobacterium phlei* (ISMP) and bone marrow culture spernatent (BMCS) on decreased iIELs cytotoxic activity of day old white leghorn chicks, and found that when the chickens were primed with ISMP a week before IBD infection the cytotoxic activity was approximately restored. However BMCS did not restore cytotoxic activity. It was concluded that immunostimulation may potentiate and restore the functional activity of iIELs in chicken infected with IBD virus.

Khaliel and El-Manakhly (1998) observed the pathologic, immunocytochemical and immunologic studies on a new infectious bursal disease vaccine "Intermediate Plus" in chickens. One-day old chicks vaccinated against Newcastle disease virus and challenged with a local virulent strain of IBD virus were examined for pathological and immunological effects of 2 types of IBD live vaccine (Intermediate and Intermediate plus). Grossly, moderate transient bursal atrophy was seen one week after immunization with the intermediate plus vaccine. Microscopically, the severity of bursal lymphoid cell

necrosis and the intensity of immunoperoxidase staining reaction correlated with the degree of bursal atrophy. Ultrastructurally, the necrotic lymphocytes appeared shrunken with nuclear fragmentation and chromatin condensation or margination. Immunologically, the highest antibody titres were seen in birds immunized with the intermediate plus vaccine, however, cell mediated response was temporarily reduced. Intermediate plus vaccine showed a slight transient immunosuppressive effect against NDV vaccine. Protection against IBDV challenge was highest following immunization with Intermediate plus vaccine particularly when given after the Intermediate vaccine. It was concluded that, despite the state of immunosuppression and the encountered bursal lesions following immunization with the Intermediate plus IBD vaccine, it provided better protection against IBDV challenge. Both immunosuppressive and immunological effects of the vaccine were transient and within safe limit.

Shadaksharappa *et al.*(1998) evaluated the immunomodulatory effect of vitamin E, vitamin C and levamisole hydrochloride on immune response against IBD vaccination in broilers. He observed that the mean antibody titre were comparatively higher but non-significant in both the vitamin E and vitamin C treated and levamisole treated and vaccinated groups than vaccinated control group. The mean antibody titres showed appreciable increase when combindly treated with both vitamin E and levamisole hydrochloride as compared to that of either vitamin E or levamisole hydrochloride alone. This observation indicated the synergistic action of these compounds.

Sadekar *et al.* (1998a) reported the usefulness of *Ocimum sanctum* (tulsi) dry leaves as immunomodulator in poultry, naturally infected with IBD virus. He found that HI titre against ND vaccination in *Ocimum sanctum* treated group was significantly higher in comparison to unvaccinated and untreated control as well as vaccinated untreated control groups. Attainment of significantly high titers at the end of 45 days of *Ocimum sanctum* administration seemed to have overcome the immunosuppressive effect of IBD on lymphoid organs and has stimulated antibody production in these birds.

Sadekar *et. al.* (1998b) evaluated immunopotentiating effects of *Azadirachta indica* (Neem) dry leaves powder in broilers, naturally infected with IBD virus. Commercial broilers were divided into 3 groups at 6 weeks of age. The birds had been vaccinated with NDV (LaSota strain) at one day of age and had survived a natural outbreak of IBD. Group, 1 were control group, 2 were given a booster vaccination (NDV strain R_2B) and group, 3 were given a booster vaccination and fed with powdered neem leaves (125 mg / bird) daily for 2 weeks. Treatment with neem leaves significantly enhanced the antibody titres against NDV antigen and also potentiated inflammatory reactions to dinitrochlorobenzene in skin test. It is concluded that feeding neem leaves to immunosuppressed birds increases their humoral and cell mediated immune responses. It is suggested that neem leaves may be useful for treatment of immunosuppressive diseases, such as IBD in birds.

Szigeti (1998) evaluated a new type of immunostimulant to increase antibody production in response to viral and bacterial vaccines. An experimental product (IM-326) containing feed acidifiers, garlic and microbial cell extracts, was added to the drinking water of poultry at 1ml/litre 2-3 days before vaccination and for 17-20 days there after. It resulted in a 38-226 percent increase in GMT after parentral administration of inactivated vaccines against goose parvovirus, Newcastle disease and avian infectious bursitis and vaccines containing live egg drop syndrome aviadenovirus and killed *Salmonella enteritidis, Pasteurella multocida and Leptospira pamona.*

Thangavelu *et al* (1998) studied the pathogenicity and immunosuppressive properties of two field isolates of IBD virus and five commercial IBD live virus vaccines marketed in India. The pathogenicity of the wild type viruses and vaccines were based on mortality, the bursa: body weight ratio and microscopic lesions in the bursa in 3-week-old chicks that received these viruses were evaluated by measuring the antibody responses to sheep red blood cells, Brucella abortus plain antigen and NDV vaccine in one day old chicks. One field isolate (N35/93) was found to be more pathogenic and immunosuppressive than the other (N45/92) while none of the commercial mild lukert type vaccines were found to be pathogenic. One of the vaccine strains marked as mild lukert type was highly immunosuppressive; one was moderate and one could be classified as mild. Both the intermediate vaccines tested were highly immunosuppressive.

Barbour *et al.* (1998) studied the humoral and cell mediated immunopotentiation in vaccinated chicken layers by thymic hormones and zinc. The birds were vaccinated with trivalent killed vaccine (IBV, IBDV, NDV) and immunopotentiated by various combinations of thymic hormones and zinc group wise. First group received thymopoietin and $ZnCl_2$, second group recived thymolulin and $ZnCl_2$, in the third group each bird received thymulin, thymopoietin and $ZnCl_2$, while each bird of the fourth group received only $ZnCl_2$. Among all combinations, the thymulin – $ZnCl_2$, resulted in birds with the highest humoral immunopotentiation to IBV, IBDV and NDV antigens. The highest cell mediated delayed hypersensitivity reaction was obtained in chickens immunopotentiated by the thymulin – thymopoietin $ZnCl_2$ combination.

Abdel–Fattah *et al.* (1999) studied the effects of crude thymus extracts on the immune response and protection against challenge with virulent IBDV in one-day old chicks. Oral administration of thymus extract (1 ml/kg) markedly and significantly increased the total protein, albumin, globulin, tri-iodothyronine (T3), Thyroxine (T4) and the body weight gain in chickens. In addition, it increased the total lymphocytic count over four weeks after administration. Although vaccination also increased total protein, globulin, T4 and the total lymphocytic count but it significantly decreased the body weight gain of the chicks and administration of thymus extracts, before, during or after vaccination markedly improved the vaccination effectiveness with significant elevation of the globulin level and body weight gain of the chicks. It also

prevented the decrease in the relative weights of bursa, spleen and thyroid glands which commonly prevailed during vaccination. Chickens administered thymus extract and vaccinated with IBD vaccine showed 100% protection against challenge with IBDV. Meanwhile the vaccinated non thymus treated group exhibited 80 percent protection against IBDV challenge. These results indicate a potentiating effect of thymus extract on the immune system in baby chick. These findings are supported by ELISA results that showed a marked increase in antibody titres in thymus treated groups. Additionally microscopical examination of the bursa showed lymphoid hyperplasia in thymus treated group but not in vaccinated group supported these findings.

Kim *et. al.* (1999) studied the long term effect of IBDV in chickens. Specially, the restoration of virus-induced bursal lesions and the duration of humoral immunodeficiency were examined. One-week-old specific pathogen-free chickens were intraocularly inoculated with an intermediate vaccine strain (IBDV-vac) or a virulent strain (IM-IBDV). At different intervals post inoculation chickens were examined for histopathologic lesions. The chickens were injected with a mixture of antigens, and primary antibody responses were examined at 10 dyas postimmunization. Initially, the virus caused extensive necrosis of bursal B lymphocytes. This lesion was accompanied by an infiltration of T lymphocytes with time. the necrotic lesion in the bursa was resolved, the follicles became partly repopulated with B lymphocytes. The repopulation occurred faster in the chickens exposed to IBDV-vac than in the chickens exposed to IM-IBDV. By 7

week PI, 40 percent and 80 percent of bursal follicles in IM-IBDV and IBDV-vac inoculated chickens respectively were repopulated with IgM plus B lymphocytes. Both IBDV-vac and IM – IBDV caused suppression of the primary antibody response to antigens. However, the antibody responses of the chickens exposed to either of the two IBDV strains used were compromised only during the first 6 weeks of virus exposure. Subsequently antibody response returned to near normal level.

Kolte *et al.* (1999) evaluated the immunomodulatory effect of dry powder of *Ocimum sanctum* (Tulsi) and leaf gall of *Ficus racemosa* (Gular) leaves in broilers, stunted and immunosuppressed by IBD virus. Result indicated that HI titre against NDV was lower in all groups before drug treatment. The titre was found significantly raised in drug treated groups. Birds which received a combination of both the birds revealed the highest HI antibody titre as compared to other treatment group. These observations were clearly indicative of the fact the all the tested plant preparations have specific immunostimulatory effect on humoral immune response. Cellular reaction at the DNCB skin contact site revealed that reaction was intense in *O. sanctum* treated and *O. sanctum* plus leaf gall treated group. This observation indicated that the said plant preparations also potentiated the non specific cell mediated immunity in IBD affected birds.

Saravanabava *et al.*(1999) conducted an experiment to asses the effect of Tuftsin (a tetrapeptide) on immune response of birds immunosuppressed to IBD virus. The result indicated that the seroconversion to NDV vaccine as

assessed by HI and ELISA were found to be higher in the birds vaccinated along with Tuftsin as compared to the birds vaccinated without tuftsin both in the immunosuppressed and immunoconmpetant birds. The percentage of leucocyte migration inhibition was also found to be more in the tuftsin administered birds as compared to the birds without tuftsin administered birds as compared to the birds without tuftsin. Percentage of survivability was found to be more in birds vaccinated along with tuftsin as compared to the birds vaccinated without tuftsin. All the unvaccinated birds succumbed to Newcastle disease. Administration of tuftsin alone (without vaccine) did not produce any significant reversing effect in birds. It was concluded that tuftsin produced significant reversing effect of immunosuppression caused by IBDV infection and significant immune enhancement in immunocompetent birds irrespective of the schedule of vaccination and type of vaccine virus used.

Gromov (1999) evaluated the immunogenesis and immunostimulating effect of timalin, a thymic product in chicken that were immunized with inactivated vaccine against infectious bursal disease. 14 days after vaccination antibody titres against IBD increased by 1.8 time in chicken received timalin (1 mg/kg) as compared with vaccinated birds that did not recived timalin .

Kalita and Dutta (1999) studied the immunomodulatory effect of levamisole upon Newcastle disease,Pigeon pox(PP) and Marek's disease (MD) vaccination in broilers. For the study day-old chicks were vaccinated against ND, PP and MD. Levamisole was given

daily for 7 days at 1 mg/50 g body weight orally to treated group while levamisole untreated group served as control. Blood samples were assessed for antibodies to the 3 viruses by Passive Haemagglutination (PHA), and immunoprecipitation (IP) tests, respectively on 14,28,42 and 56 days later. Mab were detected in most of the birds in the first week of life, but were detected in most of the birds in the first week of life, but were not detected again in unvaccinated controls, until 8 weeks of age, in the vaccinated groups however, antibody levels rose steadily. Levamisole had an enhancing effect on the HI titres of the Newcastle disease vaccinated birds. Variable effect on PHA tests in the birds given poxvirus vaccine and also no observable effects on precipitation tests for MD virus vaccination.

Kwon *et al.*, (1999) investigated the effect of Newcastle disease virus (NDV) and infectious bursal disease virus (IBDV) vaccination performance in broiler chicks for 5 weeks. Two types of poultry houses and three methods of vaccination ($NDV^-/IBDV^-$, $NDV^+/IBDV^-$ and $NDV^+/IBDV^+$) were factorially assigned of 6 treatments. NDV, BI strain and IBDV Bursin – 2 vaccine were orally administered at 5,14 and 7,18 day respectively. Weight gain, feed conversion ratio (FCR), mortality and product index were determined at the termination of experiment. Bursa index and IBDV antibody titre of chicks were measured weekly. Weight gain of chicks vaccinated with $NDV^+/IBDV^+$ was significantly increased compared with that of other treatments in both windowed and windowless poultry houses. Chicks vaccinated with $NDV^+/IBDV^+$ also showed significant improvements in the FCR and mortality

compared with those of other treatments at both poultry houses. The bursa indices of both poultry houses were high in one-day-to three-week-old chicks, but were low for the rest of 2 weeks. IBDV antibody was detected in all chicks by agar gel preceipitation test (AGPT) at one-day-old, but was not detected in $NDV^-/IBDV^-$ and $NDV^+/IBDV^-$ treatments at 4 weeks of age. However, it was 100% after $NDV^+/IBDV^-$ treatment. Antibody titre using ELISA showed a similar trend to that of the AGPT. It is concluded that IBDV and NDV combined vaccine. Significantly improved the performance of broiler chicks.

Sivaseelan and Balachandran (1999) studied on the pathology of infectious bursal disease under natural and experimental conditions by collecting the serum samples from clinically affected and recovering birds (21 days after infection) during 15 outbreaks of infectious bursal disease (IBD) in Tamil Nadu in which the disease caused 3 – 15 % mortality. While clinically affected, the birds were seronegative but the IBD virus titre rose from 64 to 230 during the recovery period. Regeneration of lymphocytes in the bursa fabricii was not observed 15 days after infection in birds aged 5-6 weeks when they become infected and these birds showed severe lesions; the histopathological findings are described. A tendency towards higher pathogenecity of the prevailing serotype 1 IBD virus was indicated.

Wu *et al.* (2000) evaluated the effect of ascorbic acid supplementation on the immune response of chickens vaccinated and challenged with infectious bursal disease virus. In the study number of anti-IBDV antibody secreting

cells, production of interleukin (IL-2) by splenocytes, number of CD_4^+, CD_8^+ and IgM^+ cells in the spleen and IgM^+ cells in bursa of Fabricius were compared between the control and treatment (ascorbic acid) groups at 7 days (before vaccination), 21 days (14 days after vaccination and before challenge) and 31 days (10 days after challenge) of age. The number of CD8+ is spleen at 7 days of age and IgM^+ cells in the bursa at 7,21 and 31 days of age were significantly higher in the ascorbic acid supplemented group. Production of IL-2 by splenocytes was higher as indicated by higher stimulation indices in the supplemented group. The number of anti-IBDV IgG antibody secreting cells in the spleen at 21 and 31 days of age were significantly higher in ascorbic acid supplemented group. It is concluded that dietary supplementation of ascorbic acid may ameliorate the immunosuppression caused by IBDV vaccination and improve humoral and cellular immune responses.

Amakye *et al.* (2000) assessed the effect of supplementation of ascorbic acid in protection from IBD vaccination chicks were fed 1000 ppm of ascorbic acid. The effects were determined in terms of vaccination and challenge, serum ascorbic acid concentation, serum corticosterone concentration, ELISA antibody titre to IBDV, body weight, bursa-to-body weight (B:B) ratio and bursal histological score (BHS). Non-vaccinated chicken fed a diet supplemented with ascorbic acid (AA) did not exhibit clinical signs or mortality following challenge whereas AA – unsupplemented counterpart had 100% cumulative morbidity and 30% cumulative mortality. Serum AA levels of AA – supplemented and vaccinated chickens

were significant higher than AA – unsupplemented counterparts. Ascorbic acid – supplemented chickens, especially those also vaccinated had higher body weight gains as compared to the AA – unsupplemented chickens. Ascorbic acid – supplemented chickens challenged with IBDV did not show any clinical signs or mortality. The results suggest that supplementation of AA at 100 ppm in the diet has beneficial effects on antibody response to IBD vaccination and body weight gain.

Sofei and Bucur (2000) assessed the serum antibody immune response in chickens inoculated with immunomodulators – associated live vaccine against IBD For the study, SPF chickens (36 days old) were vaccinated with 3-IBDV strains with or without one of 3-immunomodulators: CUPROSEL, ZINCOSEL and SELERETARD. The serum antibodies – expressed immune response was investigated by indirect ELISA and by agar gel immunodiffusion. The bursa of fabricius (BF) reaction was examined by histopathology. Mean serum antibodies titres to IBD ranged between 132.2 and 152 in the vaccine only groups and from 183.14 to 199 in the groups simultaneously given the vaccine and an immunomodulator. The bursa fabricii showed hyperplasia of lymphoblasts, macrophages and plasmocytes which was more severe in the chickens inoculated with vaccines with immunomodulators.

Mohanty *et al.* (2000) studied the immomodulating effect of levamisole in IBD infected chickens through cell mediated and humoral immune responses. 1 days old chickens were infected with IBD virus in the form of

bursal suspension ($10^{5.542}$ / CID_{50} per gram of infected bursa) at 0.05 ml/bird by inoculation through oculo-rasal route. Levamisole hydrochloride was administered orally is drinking water at 20 mg at weekly intervals for 3 weeks. Immunosuppression caused by IBD virus was demonstrated by low antibody titre against ND virus. Administration of levamisole before IBD infection boosted humoral immune response and significantly increased the bursa: body weight index indicating immunopotentiating activity of levamisole. However, immunostimulation by levamisole was insufficient to protect against infection as IBD specific lesions persisted. There was no significant effect of IBD virus on thymus dependent cellular responses and levamisole did not appear to play significant role in IBD infection, though the cortical lymphocytes in the thymus were stimulated.

Khopde *et al.*(2001) assessed the antioxidant activity of amla. For the study aqueous amla extract was examined for its ability to inhibit γ-radiation-induced lipid peroxidation (LPO) in rat liver microsomes and superoxide dismutase (SOD) damage in rat liver mitochondria. For LPO experiment, amla extract was added as its aqueous solution; and irradiation was carried out at different intervals. The extent of LPO was measured in terms of thiobarbituric acid reactive substances. It was observed that the amla extract acts as a very good antioxidant against γ-radiation induced LPO. Similarly, it was found to inhibit the damage to anti-oxidant enzyme SOD. The antioxidant activity of the amla extract was found to be both dose and concentration dependent. It was also found that microsomes containing similar amount of ascorbic

acid as was present in amla, no inhibition in LPO was observed. It was found that that reactivity of both amla and ascorbic acid towards ABTS a stable free radical, were similar. Based on these results it is concluded that amla is a more potent antioxidant than Vitamin C.

Satturwar *et al.*(2002) studied the immunomodulatory effect of a polyherbal formulation, *Haridradi ghrita*, a ghee based formulation claimed to be an immunopotentiator and hepatoprotetive. The ingredient in the drug contained Cow's ghee, *Embilica officinalis*, *Terminalaia Chebula, Terminalaia bellirica, Azadiracta indica, Sida cordifolia* and *Glycorrhiza glarbra*. The trial was carried out in wistar rats, where the formulation was fed orally at a dose of 100 mg/kg and 200 mg/kg daily. The assessment of the immunomodulatory action was carried out by testing the haemagglutinating antibody titre ((HA titre) for homoral and delayed type hypersensitivity (DTH response) for cellular immune responses to the antigenic challenges with sheep RBCs and by Neutrophil adhesion test. Increase in both, HA titre and DTH response indicated that the *Haridradi ghrita* potentiates humoral as well as cellular immunity. The neutrophil adhesion was increased as compared to control. It was concluded that *Haridradi ghrita* promises strong utility in clinical practice.

Mazhar *et al.*(2002) studied the phytochemical properties of *Ocimum santum* and its various active principle associated with it through aqueous extract. The study revealed presence of saponin, the only active

principle after various test was applied for qualitative determination.

Vetrivel and Verma (2002) Studied the histopathalogical relation to infectious bursal disease virus – immune complex (IBDV – ICX) vaccination in day old broiler chicks. Three groups were designed in which two groups were vaccinated with infectious bursal disease virus – immune complex (IBDV-ICX) vaccine subcutaneously and commercial live intermediate plus IBDV vaccine. The third group remained as control. Representative chicks from each group were challenged with field strain IBDV at 21st day of immunization . On third day post challenged infection, bursae were collected and histopathological study was carried out. Focal follicular atrophy, cortical and medullary lymphocyte depletion and epithelial infolding were noticed in control group. In commercial live IBDV vaccine inoculated group. Significant B-lymphocyte depletion was observed with lesser degree of other patho-morphological changes. In IBDV ICX vaccinated group, the B-cell depletion was found to be of lesser degree as compared to other group and no other pathological changes were noticed.

Khopde *et al.*(2001) assessed the antioxidant activity of amla. For the study aqueous amla extract was examined for its ability to inhibit γ-radiation-induced lipid peroxidation (LPO) in rat liver microsomes and superoxide dismutase (SOD) damage in rat liver mitochondria. For LPO experiment, amla extract was added as its aqueous solution; and irradiation was carried out at different intervals. The extent of LPO was measured in terms of

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CHAPTER III

MATERIALS AND METHODS

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Chicks :

Three to four weeks old, apparently healthy broiler chicks, without vaccination against IBD and also free from antibodies to IBD virus, were procured from local farm at Patna and routinely used for virus propagation and antigen production.

3.1.2 Antigen

Poona strain of IBD virus being maintained in the Department of Veterinary Microbiology. Bihar Veterinary College, Patna in the form of 50% bursal homogenate was used as a reference antigen throughout this study.

3.1.3 Antiserum

The hyperimmune serum against a vaccine strain of IBD virus (Georgia strain, Indovax Pvt. Ltd., Siswala, Haryana, India) raised in this laboratory was used throughout the study as reference antiserum. The serum was inactivated at 56^{0c} for 30 minutes and stored at 0^{0c}.

3.1.4 Vaccines

3.1.4.1 F- Strain RDV vaccine

A commercially available F- strain vaccine manufactured by Indovax Pvt. Ltd., Siswala, Haryana, India was used for vaccination of five – day-old chickens after proper reconstitution. F-Strain RD virus was further used as antigen in HA and HI tests after propagation in embryonating eggs by allantoic route.

3.1.4.2 LaSota Strain RDV Vaccine

A commercially available LaSota strain vaccine manufactured by Indovax Pvt. Ltd., Siswala, Haryana, India was used for vaccination of 25-day-old chickens after proper reconstitution.

3.1.4.3 IBD Vaccine

Cell culture adapted live invasive intermediate IBD virus vaccine (IV 95 strain) available in freeze dried form and manufactured by Indovax Pvt. Ltd., Siswala, Haryana, India was used during the study. The vaccine was reconstituted in diluent supplied with vial and used within few hours after reconstitution.

3.1.5 Chicken red blood cells

0.8% suspension of chicken RBC in phosphate buffer saline (PBS) was used for HA and HI test.

3.1.6 Buffers

3.1.6.1 For agar gel precipitation test (Aziz, 1985)

Solution A:

$Na_2HPO_4.2H_2O$	1.4 g
Double distilled water	100 ml

Solution B :

NaH_2PO_4	1.4 g
Double distilled water	100 ml

Composition of the agar gel :

Solution A	84.1 ml
Solution B	15.9 ml
Sodium chloride	8.0 g
Agarose (Hi-media)	1.0 g
Sodium azide	0.01 g

The mixture was autoclaved at 15lb pressure for 15 minutes.

3.1.6.2 Phosphate Buffer Saline (Aziz, 1985)

NaCl	2.0 g
KCl	0.05 g
$Na_2HPO_4 . 2H_2O$	0.14 g
KH_2PO_4	0.05 g
Double distilled water	250 ml

PH : 7.2 to 7.4

This solution was autoclaved at 15 lb pressure for 15 minutes and stored at refrigerated temperature till use. This buffer was used for

reconstitution and preparation of red blood cells suspension.

3.1.6.3 Alsever's Solution:

Dextrose	5.125 g
Sodium citrate	2.0 g
Sodium chloride	1.05 g
Citric acid	0.137 g
Distilled water	250 ml

This solution was autoclaved in running steam for one hour and kepet at 4^{0c} for 1-2 days.

3.1.7 Drugs / Agents

3.1.7.1 Lemasol – P

A commercially available preparation of Levamisole hydrochloride manufactured by Ranbaxy India Pvt. Ltd., New Delhi was used as immunopotentiator in the present study.

3.1.7.2 Polyzyme

A multienzyme feed supplement formulation of Zeus Biotech Pvt. Ltd., Mysore, India, produced from the fungus *Trichoderma longibrachiotum Rifai* by solid state fermentation containing Endoxylanase, 2000 U/g; ß – gluconase, 600 U/g; Pectinase, 60 U/g; Amylase.

1500 U/g; Cellulase, 15 U/g; Protease, 600 U/g; Phytase, 20 U/g was used during this experiment.

3.1.7.3 DL a – tocopherol acetate

A synthetic vitamin–E preparation from Himedia Laboratories Ltd., 23 Vadhani Industrial Estate, LBS Marg, Mumbai, containing DL a – tocopherol acetate as its constituent, was incorporated as one drug / agent for the study.

3.1.7.4 Bhang (*Cannabis sativa*)

The dry leaves of *C. sativa* was purchased from a local market and air dried. The air dried leaves were powdered mechanically and kept in a air tight container till used.

3.1.7.5 Amla (*Phyllanthus emblica*)

Dried fruits of *P. emblica* was purchased from local market and pulverized using a mechanical grinder, and kept in air tight container till used.

3.1.7.6 Homeopathic Medicine

Carbo animalis 200 (B&T original) was given twice a week at the dose rate of 20 ml /100 birds.

3.1.8 Mitogen used for Cutaneous Basophilic hypersensitivity reactions

3.1.8.1 DNCB (1- chloro – 2,4 – Dinitrobenzene)

One percent DNCB solution was prepared in acetone as 10 mg/ml (w/v).

3.1.8.2 PHA - P (Phytohaemagglutinin)

PHA- P is a plant lectin and is prepared from red kidney bean (*Phaseolus vulgaris*) and was procured from Hi-media Laboratories Ltd., Mumbai It was used as mitogen for Cutaneous basophilic hypersensitivity reaction. A concentration of 1mg/ml of PHA- P in PBS solution was prepared.

3.1.8.3 PPD (Tuberculin)

A commercial preparation of tuberculin (Purified Protein Derivative) available in the diluted form of 10 TU/0.1 ml manufactured by Beacon diagnostics Ltd., 424 New GIDC, Kabilpore, Navsari was procured.

3.2 METHODS

3.2.1 Preparation of Antigen

Poona strain of IBDV in the form of 10% bursal suspension in PBS solution was inoculated

into three to four weeks old broiler chicks at the rate of 0.2 ml of suspension per chick by intra ocular route. The chicks were sacrificed 48 hours post–inoculation and bursae were collected aseptically and homogenized in a sterile mortar using glass wool as an abrasive.

The homogenate was diluted in the ratio of 1:1 (w/v) in PBS (pH 7.4) and treated with 10,000 units of penicillin and 10 mg Streptomycin per ml of suspension. The suspension was frozen and thawed thrice and centrifuged at 4,000 rpm for 15 minutes. The supernatant was collected and tested for the presence of IBDV antigen by agar gel precipitation test (AGPT). Thereafter it was distributed in small aliquots and stored at 0^{0c} as antigen. The normal (uninfected) bursal suspension prepared in the same manner served as negative antigen control.

3.2.2 Production of Hyperimmune Serum

Hyperimmune serum against IBDV was raised in 20 weeks old apparently healthy chickens. Each bird was given Georgia strain of IBDV through occulo-nasal route at weekly intervals. Two weeks after the fourth inoculation the birds were test bled and the serum was tested for the presence of IBDV antibody by AGPT. This serum was stored at 0^{0c} for further use.

3.2.3 Collection of serum samples from chickens

Two to three ml of blood was taken from the wing vein of each bird with the help of 5 ml sterilized disposable syringe using 24 gauze needle. The blood thus drawn was transferred immediately into sterilized test tube which thereafter was kept in slanting position and left for 4 to 5 hours at room temperature. The separated serum was collected in a clean and sterilized vials of 2 ml capacity and were preserved by adding sodium azide (1:10,000) and stored at 0^{0c} until used.

3.2.4 Chicken red blood cell suspension

Two adult chickens were used as donar of blood. One to 1.5ml of blood was collected from each bird in Alsever's solution (1:1). Supernatant fluid was removed after centrifugation at 500 rpm for 10 minutes. The packed cells were washed three times with PBS. Finally, 0.8% RBC suspension was made in PBS and stored at refrigerated temperature (4^{0c}). This RBC suspension was used only for four day from the day of preparation and further fresh RBC suspension was prepared.

3.2.5 Assessment of immune response following IBD vaccination in chicken after administration of different drugs/agents.

3.2.5.1 Humoral immune response

3.2.5.1.1 Agar gel precipitation test

The test was done following the method of Hirai *et al.*(1972) with some modification. The glass microscopic slide (75 x 25 mm) were pre-coated by dipping them in 0.3% agar solution and dried in open air . Approximately 4 ml of molten agar gel was poured on each glass slide with the help of glass pipette and allowed to solidify. After setting , the slides were kept at 4^{0c} for over night to facilitate punching of gels. Hexagonal well patterns consisting of a central well and six peripheral wells of 3.5 mm in diameter, 8 mm apart were punched with the help of a template. The central well was charged with the antigen and one of the peripheral wells with the reference antiserum. The remaining four wells were used for test sera . The slides were incubated in humidified chamber at room temperature and observed daily for three days.

3.2.5.1.2 Quantitative agar gel precipitation test (QAGPT)

The level of precipitating antibody was determined as per the method of Cullen & Wyeth (1975) with some modifications. A two fold serial dilutions of the test serum was made in the same buffer as that was used in the preparation of gel. The central well contained reference antigen and peripheral wells contained two fold dilution

of sera. The volume of reagents put in each well was 0.2 ml . Three replicate of each test were carried out simultaneously. The reciprocal of the highest dilution of serum which gave precipitating line was taken as the titre of the serum. The mean antibody titre of the positive samples were calculated according to Villegas and Purchase (1980).

3.2.5.1.3 Haemagglutination (HA) test

The HA test was performed in perspex plate to prepare 4HA units RD virus as described by Beard (1980). Taking 0.5 ml of virus material two fold serial dilutions were made in PBS. except in control well in which only PBS (0.5 ml) was added. In next step 0.5 ml of 0.8% RBC suspension was added to all the wells. A known positive and negative control was also included. The plate was stirred gently for mixing and uniform distribution of erythrocytes and left at room temperature for 40 minutes. The RDV produced sheet of agglutinated RBC covering the bottoms of the wells. Negative well showed circumscribed compact button at the bottom. The HA pattern was read with the aid of reading mirror and result of HA titre was recorded as reciprocal of the highest dilution showing 100% HA.

3.2.5.1.4 Haemagglutination Inhibition (HI) test

The HI test was performed in U- shape bottomed microtitation plate as per the method suggested by Beard (1980). Four HA units of virus antigen and 0.8% chicken RBC suspension were used in this test. Using 0.25 ml of serum sample two fold serial dilution were made in PBS. To each serum dilution 0.25 ml (4 HA units) of virus antigen was added. After a reaction time of 20 minutes at room temperature , 0.5 ml of 0.8% RBC suspension was added to each well containing serum virus mixture. In each test, a known positive and negative serum samples were also included as controls. The plate was shaken gently to mix the serum virus RBCs , and incubated at room temperature for 40 minutes. The HI antibody titre was taken as the reciprocal of the highest dilution of serum showing 50% inhibition of agglutination of RBCs.

3.2.5.2 Cell mediated immune response

3.2.5.2.1 DNCB skin sensitivity test

The test was done as per the method described by Chauhan and Verma (1983) with minor modifications. Five birds from each group were randomly selected on 14 days post IBD vaccination. Featherless area of about 15 cm^2 was chosen on left and right lateral abdomen for DNCB application. These areas were cleared with acetone and dried. 0.25 ml of DNCB (10 mg/ml) in acetone vehicle was applied on right side. On

left side 0.25 ml of acetone was applied which served as control. The sensitized birds were challenged on 14th day after sensitization by applying 0.25 ml of DNCB (1mg/ml) in acetone on right side and 0.25 ml acetone on left side at the same site of first application. The skin thickness was measured with the help of micrometer at 12, 24 and 48 hours post challenge. The CMI response was calculated by subtracting the thickness of right side from left side. Skin biopsies from these birds were collected in 10% buffered formalin for histopathological examination at the termination of experiment.

3.2.5.2.2 PHA - P skin sensitivity test

The test was done as per the method described by Corrier and Deloach (1990) with minor modifications. Five birds from each group were selected on 14th day of post IBD vaccination. About 10-15 cm^2 areas of abdominal skin were selected and cleaned with acetone. After drying the skin 0.1 ml of PHA-P in 0.1 ml PBS was injected intradermally on the right side of abdomen. The left side received 0.1 ml of sterile PBS and served as control. After 14 days post sensitization , a second injection of PHA-P (0.1 ml) was given on right side and left side was injected with only PBS. The PHA-P stimulation index was calculated as the difference in swelling on PHA-P injected and PBS injected site. Pieces of PHA-P and PBS inoculated skin were collected

separately in 10% buffered formalin for histo-pathological examination at the termination of the study.

3.2.5.2.3 Purified Protein Derivative (PPD) skin sensitivity test

The test was done as per the method described by Singh (1987). Five chicks were selected from each group 14 days post IBD vaccination. About 10-15 cm2 areas of abdomen were selected and cleaned with acetone and dried. 0.3 ml of PPD (tuberculin) emulsified in 0.7 ml of Freundt's complete adjuvant was injected intradermally on right side of abdomen and similar volume of PBS was inoculated on left side. Fourteen days post sensitization 0.1ml of PPD (tuberculin) was injected on right side of abdominal skin and left abdominal skin received 0.1ml PBS. Measurement of skin thickness was made by the aid of micrometer 12, 24 and 48hour post injection. The results were expressed as the difference of swelling on PPD injected site and PBS injected site at 12, 24 and 48 hour post injection

3.2.6 Experimental design

Day-old chicks , numbering 450 were obtained from Prakash hatcheries, Varanasi. On 5th day of age the chicks were given F-strain RDV vaccine intraocularly at the dose rate of

0.05ml per bird. The chicks were wingbanded and divided randomly into nine groups, each consisting of 50 chicks. Identical management condition was provided to all birds . The duration of experiment was of 45 days. All the birds from group I to VII received IBD vaccine (IV95 strain).

Altogether six drugs/agents namely Levamisole hydrochloride, Polyzyme, Vit-E, Bhang, Amla and *Carbo animalis* were studied for their role in combating immunosuppressive effects of IBD vaccine virus. The dose, route and duration of treatment in respect of each of these drugs are shown in table-1.

From each group of chickens pre IBD vaccinated blood and serum samples were collected on nine-day of age and post IBD vaccinated blood and serum samples were collected on 7, 14, 21, 28 and 35 days post IBD vaccination. Serum samples were evaluated for determination of antibody titres to IBD vaccine and RD F-strain vaccine. Five birds from each group were sacrificed at 96 hours of post IBD vaccination and bursa : body weight ratio was determined. Five birds from each group was taken for challenge study of IBD field virus on 35th day of age. Mortality pattern and positivity of bursa of IBD virus antigen by AGPT was determined till one week post challenge. Five birds from the challenged group was sacrificed after 7 day post

challenge to determine bursa : body weight ratio and blood was collected to determine antibody titres to IBD vaccine, RD F–strain and LaSota strain vaccine. Five birds from each group were used for determination of cell mediated immunity by employing DNCB, PHA-P and PPD (tuberculin) skin sensitivity test 14 days post vaccination.

Body weight gain and feed conversion ratio (FCR) of birds from each group will be determined at the termination of experiment (45 days of age).

Body weight and feed conversion ratio (FCR)

The body weight gain of chickens were recorded as the difference between live weight at 45 day and at day second. FCR per bird at 45 day of age was determined as follows:

$$FCR = \frac{\text{Total feed consumption of chicken (g)}}{\text{Total weight gain of chicken (g)}}$$

The bursa – body weight ratio was determined to evaluate the immuno suppressive effect of IBD vaccine. This was calculated as

$$\text{Bursa : Body weight ratio} = \frac{\text{Bursa weight (g)} \times 1000}{\text{Body weight (g)}}$$

3.2.7 Challenge study

Twelve chickens from each group at the age of 25 –day post IBD vaccination were selected for challenge study. Table – 5 depicts the plan of experiment. The chickens were housed separately and maintained under the same nutrition and management conditions. Each chicken was innoculated 0.5ml of 50% bursal suspension of IBDV (field strain).

The chickens were observed for the development of clinical signs as well as mortality for 7 days PI. Gross pathological changes were recorded. The bursa – body weight ratio for each bird was calculated (Ismail and Saif, 1991). Five birds from each group were sacrificed at 7-day PI. Aportion of bursa from each bird was fixed in 10% buffered formalin for histopathological examination. The remaining portion of bursa was processed to make 50% suspension for which AGPT was performed to detect IDBV antigen. The bursal score was determined (Winterfield and Thacker, 1978). The statistical analysis was performed (Snedecor and Cochran, 1967).

3.2.8 Histopathology

The tissue samples for histopathological examination were processed in acetone benzene (Lillie and Fullmer, 1970), and embedded in paraffin wax (melting point 62^0 C). Five micron thick sections were cut and stained by haematoxylin and eosin (Drury and Wallington, 1980).

3.2.9 Scoring of bursal lesions.

The scoring system suggested by Winterfield and Thacker (1978) was followed with slight modifications. Bursal lesion score was done on a scale of 0 (none), 1 (minimal), 2 (mild), 3 (moderate) and 4 (marked) by the following criteria: Lymphoid necrosis, Lymphoid depletion, reticuloepithelial hyperplasia, Vacuolar degeneration, follicular cyst, interfollicular oedema, epithelial changes, interstitial fibrosis and cellular infiltration.

3.2.10 Statistical analysis

The mean and standard errors of the values obtained were determined. Analysis of variance (ANOVA) was performed as per Snedecor and Cochran (1967).

TABLE – 1
Experimental Design

Group	No.of chicks	Treatment; route & dosage	Period of treatment	Age at vaccination (days)			No. of chicks sacrificed at 96 hours post IBD vaccination	Observation planned
				IBD Vaccination (route)	RD vaccination (route)	RDV (LaSota strain) (route)		
I	50	Levamisole (D.W.);7.5 mg / Kg b.wt.	5 – 45 days	10 (i.o.)	5 (i.o.)	25 (D.W.)	5	1. Collection of preIBD vaccinated blood samples on 9th day of age for determination of MDA level of IBD, Ab titre of RDV. 2. Collection of blood samples of 7, 14, 21, 28 and 35th days post IBD vaccination for : i) determination of antibody titre to IBDV by QAGPT ii) determination of Ab titre to RDV (F-strain & LaSota strain) by HI test. 3. Collection of bursae: (i) to determine B:BW ratio (ii)in 10% buffered formalin for histopathological examination 4.Study of CBH response to DNCB,PHA-P and PPD (tuberculin)and collection of skin samples in 10% buffered formally for histo-pathological examination. 5. Body weight gain and FCR recorded on termination of experiment.
II	50	Polyzyme (in feed) ; 30mg/ 100 kg feed	5 – 45 days	10 (i.o.)	5 (i.o.)	25 (D.W.)	5	
III	50	Vitamine–E (orally); 125 mg/bird	5 – 45 days	10 (i.o.)	5 (i.o.)	25 (D.W.)	5	
IV	50	Bhang 40mg / bird (in feed)	5 - 45 days	10 (i.o.)	5 (i.o.)	25 (D.W.)	5	
V	50	Amla 80mg / bird(feed) (in feed)	5 – 45 days	10 (i.o.)	5 (i.o.)	25 (D.W.)	5	
VI	50	*Carboanimalis* (D.W.); 20 ml /100 bird	Weekly starting from 7day of age	10 (i.o.)	5 (i.o.)	25 (D,W.)	5	
VII	50	-	-	10 (i.o.)	5 (i.o.)	25 (D.W.)	5	
VIII	50	-	-	10 (i.o.) *	5 (i.o.)	25 (D.W.)	5	
IX	50	-	-	-	5 (i.o.)	25 (D.W.)	5	

* Received field virus instead of vaccine strain

D.W. – represents drinking water.
i.o. – represents intra-ocular.

Table - 2

Plan of Experiment for CBH reaction to DNCB in IBD vaccinated chickens.

Group	Treatment	No. of birds	DNCB Sensiti-zation days post vaccination	Dose / chicken and route (ml)	Test injection Of DNCB days Post sensitization	Dose/Chick And route (ml)	Measurement of skin Thickness hrs. post challenge		
							1st	2nd	3rd
I	Levamisole	5	14	0.25 ; i.d.	14	0.25; i.d.	12	24	48
II	Polyzyme	5	14	0.25; i.d.	14	0.25; i.d.	12	24	48
III	Vit – E	5	14	0.25; i.d.	14	0.25; i.d.	12	24	48
IV	Bhang	5	14	0.25; i.d.	14	0.25; i.d.	12	24	48
V	Amla	5	14	0.25; i.d.	14	0.25; i.d.	12	24	48
VI	Carbo-animalis	5	14	0.25; i.d.	14	0.25; i.d.	12	24	48
VII	-	5	14	0.25; i.d.	14	0.25; i.d.	12	24	48
VIII	-	5	14	0.25; i.d.	14	0.25; i.d.	12	24	48
IX	-	5	14	0.25; i.d.	14	0.25; i.d.	12	24	48

Table - 3

Plan of Experiment for CBH reaction to PHA-P in IBD vaccinated chickens.

Group	Treatment	No. of birds	PHA-P sensitization; days post vaccination	Dose / chicken and route (ml)	Test injection of PHA-P; days post sensitization	Dose/ chicken and route (ml)	Measurement of skin Thickness hrs. post challenge		
							1st	2nd	3rd
I	Levamisole	5	14	0.10;i.d.	14	0.10;i.d	12	24	48
II	Polyzyme	5	14	0.10;i.d	14	0.10;i.d	12	24	48
III	Vitamin-E	5	14	0.10;i.d	14	0.10;i.d	12	24	48
IV	Bhang	5	14	0.10;i.d	14	0.10;i.d	12	24	48
V	Amla	5	14	0.10;i.d	14	0.10;i.d	12	24	48
VI	*Carbo-animalis*	5	14	0.10;i.d	14	0.10;i.d	12	24	48
VII	-	5	14	0.10;i.d	14	0.10;i.d	12	24	48
VIII	-	5	14	0.10;i.d	14	0.10;i.d	12	24	48
IX	-	5	14	0.10;i.d	14	0.10;i.d	12	24	48

Table - 4

Plan of Experiment for CBH reaction to PPD (tuberculin) in IBD vaccinated chickens.

Group	Treatment	No. of birds	PPD Sensitization days post vaccination	Dose / chicken and route (ml)	Test injection of PPD days post sensitization	Dose/ chicken and route (ml)	Measurement of skin Thickness hrs. post challenge		
							1st	2nd	3rd
I	Levamisole	5	14	1.0;i.d.	14	0.1;i.d.	12	24	48
II	Polyzyme	5	14	1.0;i.d.	14	0.1;i.d.	12	24	48
III	Vitamin-E	5	14	1.0;i.d.	14	0.1;i.d.	12	24	48
IV	Bhang	5	14	1.0;i.d.	14	0.1;i.d.	12	24	48
V	Amla	5	14	1.0;i.d.	14	0.1;i.d.	12	24	48
VI	*Carbo animalis*	5	14	1.0;i.d.	14	0.1;i.d.	12	24	48
VII	-	5	14	1.0;i.d.	14	0.1;i.d.	12	24	48
VIII	-	5	14	1.0;i.d.	14	0.1;i.d.	12	24	48
IX	-	5	14	1.0;i.d.	14	0.1;i.d.	12	24	48

Table – 5.

Plan of experiment to evaluate the efficacy of drugs/agents on countering the immune suppressive effects of IBDV (Vaccine strain / Field strain)

Group	Treatment	No. of chicken	Vaccination age (days)			Dose & route of inoculation	Period of observation (days)	No. of birds sacrificed 7 day post inoculation	Observation planned for :-
			IBD (IV 95 strain)	RDV (F- strain)	RDV (LaSota strain)				
I	Levamisole	12	10	5	25	0.5ml ; i.o.	7	5	i) Clinical symptoms ii) Mortality iii) Gross lesions iv) Histopathological examination V) AGPT
II	Polyzyme	12	10	5	25	0.5ml ;i.o.	7	5	
III	Vit. – E	12	10	5	25	0.5ml ;i.o.	7	5	
IV	Bhang	12	10	5	25	0.5ml ;i.o.	7	5	
V	Amla	12	10	5	25	0.5ml ;i.o.	7	5	
VI	Carbo-animalis	12	10	5	25	0.5ml ;i.o.	7	5	
VII	-	12	10	5	25	0.5ml ;i.o.	7	5	
VIII	-	12	10*	5	25	0.5ml ;i.o.	7	5	
IX	-	12	-	5	25	0.5ml; i.o.	7	5	

* Received field virus instead of vaccine strain.

i.o. – represents intra-ocular

CHAPTER IV

RESULTS

4.1 Effect of selected drugs/agents on immune responses to IBDV (IV95 Strain) in broiler chicken.

The mean QAGPT titres on day before IBD vaccination (IV95strain) and of various treatment groups are expressed in table-6. The Perusal of table revealed the occurence of seroconversion by 35 day post IBD vaccination in group of birds that received IBD vaccine except group II which received polyzyme as well as control group (group IX) which did not received any treatment Further the comparison of QAGPT titres among different groups (I-IX) demonstrated a higher titre in group which received drugs agents (I-VI) than titres observed in group VII which received only IBD vaccine and group VIII which received field virus but no drug treatment overall the intervals post IBD vaccination. Again on comparison of precipitating antibody titres to IBDV among different drug treated groups (I to VI) revealed the highest titre in bhang treated group (gr.IV) followed by amla, *carbo animalis*, Vit-E, levamisole and polyzyme treated groups.

The overall pictures were suggestive of the fact that all the six drugs / agents employed in this study showed immune enhancing effect on responses to IBD virus vaccine in chicken when compared with the precipitating antibody titres of control birds (group VII) and group which received field virus (group VIII) at all intervals post IBD vaccination . Further the precipitating antibody titres in the different

Table – 6.

Effect of drugs / agents on immune responses to a vaccine strain of IBD virus in chicken.

Group	Age at IBD vaccination(days)	Treatment	Mean# ± S.E. of QAGPT titre (log_2) to IBD vaccine					
			Pre-IBD vaccinated	Days post IBD - Vaccination				
				7	14	21	28	35
I	10	Levamisole	-	$3.2^{ab} \pm 0.374$	$4.0^{abc} \pm 0.447$	$4.6^{b} \pm 0.245$	$4.8^{b} \pm 0.374$	$5.2^{cd} \pm 0.374$
II	10	Polyzyme	-	$3.8^{b} \pm 0.200$	$4.4^{c} \pm 0.245$	$4.8^{b} \pm 0.374$	$5.0^{bc} \pm 0.447$	$4.8^{bc} \pm 0.200$
III	10	Vit – E	-	$3.0^{a} \pm 0.316$	$4.2^{bc} \pm 0.374$	$4.8^{b} \pm 0.200$	$5.4^{bc} \pm 0.245$	$5.6^{de} \pm 0.400$
IV	10	Bhang	-	$3.8^{b} \pm 0.200$	$4.0^{abc} \pm 0.316$	$5.2^{b} \pm 0.374$	$6.4^{d} \pm 0.245$	$6.8^{f} \pm 0.316$
V	10	Amla	-	$2.6^{a} \pm 0.245$	$3.6^{ab} \pm 0.400$	$4.8^{b} \pm 0.374$	$5.6^{c} \pm 0.245$	$6.4^{f} \pm 0.245$
VI	10	Carbo-animalis.	-	$2.8^{a} \pm 0.374$	$3.6^{ab} \pm 0.245$	$4.6^{b} \pm 0.400$	$5.8^{d} \pm 0.374$	$6.2^{ef} \pm 0.200$
VII	10	-	-	$3.2^{ab} \pm 0.489$	$3.4^{a} \pm 0.400$	$3.6^{a} \pm 0.245$	$3.8^{a} \pm 0.400$	$3.8^{a} \pm 0.374$
VIII	10*	-	-	$3.8^{b} \pm 0.200$	$3.6^{ab} \pm 0.245$	$3.6^{a} \pm 0.400$	$4.0^{a} \pm 0.316$	$3.0^{a} \pm 0.316$
IX	-	-	-	-	-	-	-	-

- • Received field virus instead of vaccine strains.
- # Means of five observations

Means of atleast one common superscript(a,b,c,d,e,f) do not differ significantly ($P < 0.05$)

treatment groups demonstrated increasing treand till the last day of observation (35 dpv). On the contrary . in untreated control group (Group VII) and polyzyme treated group (group II) the QAGPT titres showed increasing trend only till 28 dpv (table – 6).

The prevaccinated serum samples collected at 9-day of age were negative for precipitating antibody titre to IBD virus. The unvaccinated and untreated control bird (group IX) remainded negative for antibody to IBD virus throughout the period of experiment.

4.2 Effect of selected Agents/Drug on immune responses to RDV (F & LaSota strain) in IBD vaccinated broiler chickens

The immune response to RD virus in IBD vaccinated chickens are presented in Table–7. the perusal of the table showed that the IBD vaccine strain (IV95) virus as well as field virus strain had immunosuppressive effect as evident from lower HI titres in IBD vaccinated but untreated group (Gr. VII) and field virus inoculated (group VIII) over all periods post IBD vaccination when compared with HI titres for the corresponding intervals in birds which neither received IBD vaccine nor any drug treatment (Group IX). The immunosuppressive effect of IBD vaccine (IV95) were countered to varying extent in the group of birds which had received different drugs/agents (group I to VI) as evident from higher HI

Table 7.

Immune responses of RD vaccine (F-Strain / LaSota strain) in IBD vaccinated broiler chicken after administration of different drugs / agents.

Group	Treatment	Vaccination age (days)			Mean# ± S.E. of HI antibody titre (log_2) to RD virus vaccine					
		IBD (IV95 strain)	RDV (F-strain)	RDV (LaSota Strain)	Pre-IBD vaccination	Days post IBD Vaccination				
						7	14	21	28	35
I	Levamisole	10	5	25	$3.4^{a} \pm 0.400$	$4.2^{bc} \pm 0.200$	$5.0^{bc} \pm 0.447$	$5.8^{b} \pm 0.374$	$6.8^{cde} \pm 0.200$	$7.0^{cde} \pm 0.447$
II	Poly-zyme	10	5	25	$4.4^{c} \pm 0.244$	$5.6^{c} \pm 0.244$	$6.2^{c} \pm 0.374$	$7.2^{c} \pm 0.200$	$6.0^{b} \pm 0.447$	$5.8^{b} \pm 0.374$
III	Vit – E	10	5	25	$3.4^{a} \pm 0.245$	$4.6^{bcd} \pm 0.245$	$5.2^{bcd} \pm 0.374$	$6.4^{bcd} \pm 0.400$	$6.6^{bcde} \pm 0.245$	$6.8^{cd} \pm 0.489$
IV	Bhang	10	5	25	$3.6^{ab} \pm 0.400$	$5.0^{dc} \pm 0.316$	$6.4^{f} \pm 0.245$	$6.6^{cde} \pm 0.400$	$7.0^{de} \pm 0.316$	$7.6^{e} \pm 0.245$
V	Amla	10	5	25	$4.2^{bc} \pm 0.200$	$4.8^{cd} \pm 0.374$	$5.6^{cde} \pm 0.245$	$6.4^{bcd} \pm 0.245$	$6.8^{cde} \pm 0.489$	$7.0^{cde} \pm 0.447$
VI	Carbo-animalis	10	5	25	$3.4^{a} \pm 0.245$	$4.8^{cd} \pm 0.374$	$5.4^{bcd} \pm 0.245$	$6.0^{bc} \pm 0.316$	$6.4^{bcd} \pm 0.245$	$6.8^{cd} \pm 0.374$
VII	-	10	5	25	$3.6^{ab} \pm 0.400$	$4.0^{b} \pm 0.316$	$4.8^{b} \pm 0.374$	$5.8^{b} \pm 0.200$	$6.2^{bc} \pm 0.200$	$6.4^{bc} \pm 0.245$
VIII	-	10*	5	25	$3.2^{a} \pm 0.200$	$3.4^{a} \pm 0.244$	$3.4^{a} \pm 0.400$	$3.6^{a} \pm 0.245$	$3.6^{a} \pm 0.400$	$3.8^{a} \pm 0.200$
IX	-	-	5	25	$3.4^{a} \pm 0.245$	$4.6^{bcd} \pm 0.274$	$5.8^{cde} \pm 0.374$	$6.8^{de} \pm 0.489$	$7.2^{e} \pm 0.374$	$7.4^{de} \pm 0.400$

* Received field virus instead of vaccine strain.

\# Means of five observation

Means with atleast one common superscript (a,b,c,d,e) do not differ significantly ($P<0.05$)

titres in these groups at all intervals The comparison of HI titres of different treatment groups (group I to VI) and the values in IBD vaccinated but untreated group (group VII) revealed relatively higher titres in all the treatment groups overall periods post IBD vaccination. The field virus inoculated group (VIII) showed a marked depression in HI titres in comparison to all groups. Further the administration of LaSota strain of RD virus vaccine at 7 day post IBD vaccination showed booster response on HI titres to RD vaccine in all the groups (group I to IX) overall the periods as evident from titres recorded 7 days post LaSota vaccination and afterwards. However, the booster responses due to LaSota vaccine were not marked on last day of observation.

4.3 Effects of different drugs/agents on histopathology in IBD vaccinated broiler chicken

The bursal lesion score in chickens sacrificed at 96 hrs. post IBD vaccination and at termination of experiment are shown in table 8 and table 11, respectively. The histopathological changes produced by the vaccine strain of IBD virus in the bursa of Fabricius were marked by lymphoid necrosis, lymphoid depletion, epithelial invagination, cellular infiltration as well as interfollicular oedema. Additionally, changes such as hyperplasia and vacuolation in epithelial lining and interstitial fibrosis were also noticed. By and large the changes were suggestive of mild to moderate effect of vaccine

Table - 8

Mean[#] ± S.E. of bursal lesion score in different treatment groups of broiler chickens sacrificed at 96 hours post IBD vaccination

Group & Treatment	Inter-Follicular oedema	Follicular Changes					Epithelial Changes				Interstitial fibrosis	Cellular Infiltration	Total lesion score
		Lymphoid necrosis	Lymphoid depletion	Reticulo epithelial hyperplasia	Vacuolar degeneration	Follicular cyst	Hyperplasia	Epithelial invagination	Cyst formation	Vacuolation			
I Levamisole	0.4^{a} ± 0.245	1.6^{a} ± 0.400	1.8^{bcd} ± 0.200	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.6^{a} ± 0.245	1.4^{ab} ± 0.245	0.6^{b} ± 0.245	0.0 ± 0	0.6^{abc} ± 0	0.4^{a} ± 0.245	0.62^{a} ± 1.016
II Polyzyme	0.8^{abc} ± 0.200	2.0^{bc} ± 0.447	2.0^{cd} ± 0.316	0.0 ± 0	0.0 ± 0.0	0.0 ± 0.0	0.8^{ab} ± 0.200	1.8^{bc} ± 0.374	0.8^{b} ± 0.374	0.6^{a} ± 0.245	1.0^{cd} ± 0.316	0.8^{ab} ± 0.374	0.58^{a} ± 0.78
III Vitamin E	0.6^{ab} ± 0.245	1.6^{ab} ± 0.245	1.6^{abc} ± 0.245	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.6^{a} ± 0.245	1.6^{b} ± 0.245	0.6^{b} ± 0.245	0.4^{a} ± 0.245	0.8^{bcd} ± 0.374	0.4^{a} ± 0.245	0.68^{a} ± 0.88
IV Bhang	0.6^{a} ± 0.245	1.8^{ab} ± 0.200	1.4^{ab} ± 0.245	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.4^{a} ± 0.245	1.0^{a} ± 0	0.0 ± 0	0.0 ± 0	0.2^{a} ± 0.447	0.6^{ab} ± 0.0	0.50^{a} ± 1.089
V Amla	0.4^{a} ± 0.245	1.4^{a} ± 0.245	1.2^{a} ± 0.200	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.4^{a} ± 0.245	1.0 0.316	0.20^{a} ± 0.0	0.0 ± 0	0.4^{ab} ± 0.245	0.4^{a} ± 0.295	0.45^{a} ± 800
VI Carbo animalis	0.6^{ab} ± 0.245	2.0^{bc} ± 0.316	1.8^{bcd} ± 0.374	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.6^{a} ± 0.245	1.6^{b} ± 0.245	0.60^{b} ± 0.245	0.0 ± 0	0.6^{abc} ± 0	0.8^{ab} ± 0.374	0.56^{a} ± 0.977
VII No Medic-Ation	1.0^{b} ± 0.316	2.2^{c} ± 0.200	2.2^{d} ± 0.374	0.4^{a} ± 0.245	0.0 ± 0	0.0 ± 0	0.8^{ab} ± 0.200	2.0^{c} ± 0.316	1.00^{b} ± 0.316	0.4^{a} ± 0.245	0.6^{abc} ± 0.400	1.0^{b} ± 0.316	0.82^{a} ± 1.110
VIII* No Medic-ation	1.2^{c} ± 0.200	3.0^{d} ± 0316	3.2^{e} ± 0.200	0.0 ± 0	0.6^{a} ± 0.245	0.0 ± 0	1.2^{b} ± 0.200	2.6^{d} ± 0.245	1.8^{c} ± 0.200	1.6^{b} ± 0.245	1.2^{d} ± 0.200	1.6^{c} ± 0.245	1.5^{b} ± 1.358
IX No. Med./ No. Vac.	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0

* Received file virus instead of vaccine strain.

\# Mean of five observation

Mean bearing atleast one common superscript (a,b,c,d,e) did not differ significantly.

Table - 11

Mean[#] ± S.E. of bursal lesion score in different treatment groups of broiler chickens sacrificed at termination of experiment (45 days of age)

Group & Treatment	Inter-Follicular oedema	Follicular Changes					Epithelial Changes				Interstitial fibrosis	Cellular Infiltration	Total lesion score
		Lymphoid necrosis	Lymphoid depletion	Reticulo epithelial hyperplasia	Vacuolar degeneration	Follicular cyst	Hyperplasia	Epithelial invagination	Cyst formation	Vacuolation			
I Levamisole	0.2^{a} ± 0.200	1.0^{ab} ± 0.316	1.0^{abc} ± 0.0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.00 ± 0	0.400^{a} ± 0.245	0.00	0.0 ± 0	0.00 ± 0.0	0.0	0.22^{a} ± 0.5
II Polyzyme	0.4^{a} ± 0.245	1.2^{ab} ± 0.200	1.0^{abc} ± 0.316	0.0 ±0	0.0 ± 0	0.0 ± 0	0.60^{ab} ± 0.240	0.600^{ab} ± 0.245	0.00	0.0 ± 0	0.400^{a} ± 0.245	0.2^{a} ± 0.20	0.307^{a} ± 118
III Vitamin E	0.0 ± 0	1.0^{ab} ± 0.316	0.8^{ab} ± 0.200	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.00 ± 0	0.400^{a} ± 0.245	0.00	0.0 ± 0	0.2^{a} ± 0.200	0.00 ± 0	0.200^{a} ± 0.083
IV Bhang	0.0 ± 0	1.0^{ab} ± 0.000	0.6^{a} ± 0.245	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.00 ± 0	0.200^{a} ± 0.200	0.00	0.0 ± 0	0.00 ± 0.0	0.0 ± 0	0.15^{a} ± 0.77
V Amla	0.0 ± 0	0.8^{a} ± 0.200	0.6^{a} ± 0.245	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.00 ± 0	0.200^{a} ± 0.200	0.00	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.133^{a} ± 0.079
VI *Carbo animalis*	0.0 ± 0	1.0^{ab} ± 0.316	1.0^{abc} ± 0.0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.20^{a} ± 0.200	0.400^{a} ± 0.200	0.00	0.0 ± 0	0.20^{a} ± 0.245	0.0	0.23^{a}
VII No Medication	0.60^{a} ± 0.245	1.4^{bc} ± 0.245	1.2^{bc} ± 0.200	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.4^{a} ± 0.245	1.00^{ab} ± 0.200	0.400^{a} ± 0.245	0.0 ± 0	0.40^{a} ± 0.245	0.6^{a} ± 0.245	0.48^{a}
VIII * No Medication	1.00^{b} ± 0.200	1.8^{c} ± 0.200	1.4^{c} ± 0.245	0.0 ± 0	0.0 ± 0	0.0 ± 0	1.00^{b} ± 0.200	1.80^{c} ± 0.245	1.00^{b} ± 0.200	0.600^{a} ± 0.245	0.60^{b} ± 0.400	1.6^{b} ± 0.245	0.90^{b}
IX No. Med./ No. Vac.	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0

* Received file virus instead of vaccine strain.

\# Means of five observation

Means with atleast one common superscript (a,b,c) did not differ significantly ($P<0.05$)

virus on bursa of Fabricius in experimental chickens. The overall lesion score suggested that present vaccine strain still retained residual pathogenic effect.

On perusal of these table it appeared that the total lesion score was below one in IBD vaccinated chickens. The virus produced considerable effect on lymphoid cells of the bursal follicles as evident from changes such as lymphoid depletion and lymphoid necrosis. The mean lesion score was highest in birds which received only IBD vaccine but no medication (gr. VII). The different drugs/agents given in treatments groups (gr. I to VI) helped in reduction of bursal lesion score as depicted (table 8 & 11). Among the treatment groups the lowest lesion score was detected in amla treated group followed by bhang, *carbo animalis*, Polyzyme, levamisole, and Vitamin E group during 96 hours post IBD vaccination. However at termination of experiment (45 day of age) lesion score was lowest in amla treated group followed by bhang, Vitamin E, levamisole *carbo animalis*, polyzyme treated group. No changes were noticed in the control group (gr. IX) which neither received IBD vaccine nor any medication.

4.4 Effect of selected drugs/agents or Bursa: Body weight ratio in IBDV vaccinated broiler chickens.

The bursa weight, body weight and bursa: body weight ratio which ranged from 1.895 to 2.290 in

Table 9.

Effect of different drugs on Bursa weight , Body weight and Bursa : Body weight ratio of IBD vaccinated chickens 96 hours post IBD vaccination.

Group	Treatment	Vaccination age (in days)			Bursa weight (g)	Body weight (g)	Bursa : Body wt. ratio
		IBDV (IV 95 strain)	RDV (F-strain)	RDV (LaSota strain)			
I	Levomisole	10	5	25	$0.527^{b} \pm 0.009$	$250^{bcd} \pm 9.140$	$2.106^{bcd} \pm 0.040$
II	Polyzyme	10	5	25	$0.528^{b} \pm 0.011$	$264^{cd} \pm 10.587$	$2.006^{abc} \pm 0.030$
III	Vit. – E	10	5	25	$0.567^{c} \pm 0.008$	$255^{bcd} \pm 8.318$	$2.223^{dc} \pm 0.046$
IV	Bhang	10	5	25	$0.530_{b} \pm 0.008$	$232^{ab} \pm 9.014$	$2.284^{dc} \pm 0.055$
V	Amla	10	5	25	$0.604^{d} \pm 0.010$	$270^{d} \pm 10.390$	$2.237^{dc} \pm 0.052$
VI	*Carbo animalis*	10	5	25	$0.557^{c} \pm 0.006$	$262^{cd} \pm 6.340$	$2.125^{cd} \pm 0.031$
VII	-	10	5	25	$0.478^{a} \pm 0.006$	$243^{abc} \pm 7.778$	$1.967^{ab} \pm 0.050$
VIII	-	10*	5	25	$0.514^{b} \pm 0.007$	$223^{a} \pm 7.899$	$2.304^{c} \pm 0.046$
IX	-		5	25	$0.489^{a} \pm 0.009$	$259^{cd} \pm 9.544$	$1.891^{a} \pm 0.035$

* Received field virus instead of vaccine strain

* Means having one common superscript (a,b,c,d,e) do not differ significantly ($P < 0.05$)

Table - 10.

Effect of different drugs / agents on bursa weight , body weight and bursa : body weight ratio of IBD vaccinated chicken on 35 days post IBD vaccination

Group	Treatment	Age at vaccination (days)			Bursa weight (g)	Body weight (g)	Bursa : body Weight ratio
		IBDV (IV95 strain)	RDV (F- strain)	RDV (LaSota strain)			
I	Levamisole	10	5	25	$1.654^{cf} \pm 0.019$	$1446^{b} \pm 25.56$	$1.144^{c} \pm 0.017$
II	Polyzyme	10	5	25	$1.514^{d} \pm 0.050$	$1464^{bc} \pm 64.91$	$1.034^{bc} \pm 0.024$
III	Vitamin–E	10	5	25	$1.666^{f} \pm 0.013$	$1447^{b} \pm 45.73$	$1.151^{c} \pm 0.028$
IV	Bhang	10	5	25	$1.843^{g} \pm 0.015$	$1565^{c} \pm 33.54$	$1.177^{d} \pm 0.018$
V	Amla	10	5	25	$1.807^{g} \pm 0.005$	$1526^{bc} \pm 21.76$	$1.184^{d} \pm 0.014$
VI	*Carbo animalis*	10	5	25	$1.590^{e} \pm 0.011$	$1457^{bc} \pm 34.51$	$1.091^{bc} \pm 0.018$
VII	-	10	5	25	$1.130^{c} \pm 0.039$	$1246^{a} \pm 37.20$	$0.907^{ab} \pm 0.022$
VIII	-	10*	5	25	$1.042^{b} \pm 0.031$	$1175^{a} \pm 48.50$	$0.886^{a} \pm 0.017$
IX	-	-	5	25	$0.946^{a} \pm 0.014$	$1246^{a} \pm 30.76$	$0.760^{a} \pm 0.016$

* Received field virus instead of vaccine virus.

Means with atleast one common superscript (a,b,c,d,e,f,g) did not differed significantly ($P<0.05$)

different groups of birds (group I to IX) shown in table 9. Again the B:BW ratio recorded at termination of experiment is shown in Table 10. The bursa : body weight ratio at 45 days (termination of experiment ranged from 0.761 to 1.185. The perusal of table revealed that B:BW ratio at 96 hours post IBD vaccination in all the group, differ significantly from each other. The B:BW ratio was higher for drug treated groups when compared with the value in case of group VII (untreated but vaccinated) and group IX (untreated and unvaccinated). However, the group VIII (received field virus instead of vaccine strain) had the highest B:BW ratio among all groups. Similar trend were observed in case of birds sacrificed at the termination of experiment (Table 10) except the values were markedly lower than the values recorded at 96 hours post IBD vaccination (as evident from table 9 and 10).

4.5 Effect of selected drugs/agents on body weight gain and feed conversion ratio in IBD vaccinated broiler chicken

The body weight gains and feed conversion ratios in different treatment groups are shown in table 12. The body weight gain was lowest in case of group VIII (received field virus instead of vaccine)group VIII (received field virus instead of vaccine) followed by control group (Gr. VII) and group IX. Whereas, the values were invariably higher in case of different treatment groups (group I to VI). When compared with

Table 12.

Effect of different agents on certain growth parameters in chickens.

Group	Treatment	Age of vaccination (days)			Mean# ± S.E.			FCR	Percent Mortality
		IBDV (IV95 Strain)	RDV (F – Strain)	RDV (Lasota strain)	Initial body weight (g) on 2nd day of age	Final body weight (g) on 45 day of age	Body weight gain (g) on 45 day of age		
I	Levamisole	10	5	25	$46.23^{NS} \pm 0.269$	$1501.49^{c} \pm 31.29$	$1455.26^{c} \pm 18.572$	2.28	2.5
II	Polyzyme	10	5	25	$47.10^{NS} \pm 0.500$	$1453.72^{c} \pm 47.40$	$1406.66^{c} \pm 16.05$	2.33	5.0
III	Vit- E	10	5	25	$44.44^{NS} \pm 1.230$	$1506.04^{cd} \pm 58.11$	$1461.57^{cd} \pm 28.217$	2.38	2.5
IV	Bhang	10	5	25	$49.21^{NS} \pm 0.428$	$1616.33^{c} \pm 64.00$	$1567.11^{c} \pm 16.950$	2.45	2.5
V	Amla	10	5	25	$48.21^{NS} \pm 0.322$	$1561.46^{dc} \pm 39.21$	$1513.25^{dc} \pm 20.450$	2.39	0.0
VI	Carbo animalis	10	5	25	$48.20^{NS} \pm 0.122$	$1488.74^{c} \pm 24.24$	$1440.52^{c} \pm 21.479$	2.30	2.5
VII	-	10	5	25	$43.92^{NS} \pm 0.311$	$1283.64^{eb} \pm 31.12$	$1239.72^{eb} \pm 22.900$	2.40	5.0
VIII	-	10*	5	25	$45.36^{NS} \pm 0.831$	$1237.37^{e} \pm 39.89$	$1192.00^{e} \pm 27.267$	2.52	7.5
IX	-	-	5	25	$47.22^{NS} \pm 0.512$	$1330.10^{b} \pm 38.42$	$1282.88^{b} \pm 26.21$	2.31	12.5

* Received field virus instead of vaccine strain.

\# Means of five observation.

Means with atleast one common superscript (a,b,c,d,e) did not differed significantly (P<0.05)

NS – Non-Significant

group VII, VIII and IX. The body weight gain was highest for bhang treated froup followed by amla, Vit. E, levamisole, *Carbo animalis* and polyzyme treated group. Analysis of variance showed the body weight gain in bhang treated group was significantly higher than all other groups.

The feed conversion ratio was highest in group IV followed by group VI and was lowest in group IX. The general trend suggested that whereas the ratios were poor in bhang treated group and the group which received field virus, it was comparatively better in birds receiving different treatments other than bhang treated group. Within the treatment groups lowest FCR was visible in levamisole treated group.

4.6 Cutaneous basophilic hypersensitivity (CBH) reaction

4.6.1CBH reaction of broiler chicken to DNCB

The skin sensitivity response to Dinitrochlorobenzene (DNCB), being evaluated as cutaneous basophilic hypersensitivity (CBH), at 12,24 and 48 hours post challenge was recorded (Table -13) The CBH response developed gradually and attained its peak at 24 hours post challenge and declined thereafter. The perusal of table revealed a significant increase in skin thickness 24 hours post challenge among the treatment groups. Further, on comparison the group which received drugs/agents along with IBD

Table - 13

Effect of different drugs/agents on CBH reaction to DNCB in chicken.

Group	Mean* ± SE of increase CBH after DNCB challenge (mm)			Mean ± SE of skin (hours post challenge) after DNCB challenge (mm)					
	12 Hours	24 Hours	48 Hours	12 Hours		24 Hours		48 Hours	
I Levamisole	0.49^{NS} ± 0.036	0.61^{c} ± 0.067	0.54^{NS} ± 0.039	1.675 ± 0.121	1.185 ± 0.042	2.018 ± 0.091	1.408 ± 0.028	1.852 ± 0.056	1.312 ± 0.036
II Polyzyme	0.38^{NS} ± 0.023	0.53^{bc} ± 0.089	0.41^{NS} ± 0.023	1.370 ± 0.093	0.99 ± 0.029	1.841 ± 0.204	1.311 ± 0.074	1.241 ± 0.120	1.200 ± 0.990
III Vitamin E	0.42^{NS} ± 0.030	0.58^{bc} ± 0.032	0.50^{NS} ± 0.023	1.608 ± 0.121	1.188 ± 0.058	1.992 ± 0.216	1.402 ± 0.192	1.789 ± 0.512	1.289 ± 0.037
IV Bhang	0.45^{NS} ± 0.027	0.59^{bc} ± 0.050	0.49^{NS} ± 0.031	1.659 ± 0.042	1.209 ± 0.066	1.979 ± 0.008	1.389 ± 0.042	1.766 ± 0.611	1.276 ± 0.811
V Amla	0.53^{NS} ± 0.012	0.68^{c} ± 0.076	0.60^{NS} ± 0.032	1.732 ± 0.087	1.202 ± 0.022	2.091 0.072	1.411 0.074	1.988 0.312	1.388 ± 0.120
VI Carbo animalis	0.48^{NS} ± 0.029	0.60^{bc} ± 0.084	0.46^{NS} ± 0.035	1.616 ± 0.171	1.136 ± 0.114	1.934 ± 0.190	1.334 ± 0.220	1.751 ± 0.009	1.291 ± 0.128
VII No Medic-ation	0.30^{NS} ± 0.042	0.42^{ab} ± 0.063	0.31^{NS} ± 0.039	1.249 ± 0.096	0.949 ± 0.052	1.589 ± 0.136	1.169 ± 0.068	1.408 ± 0.019	1.098 ± 0.082
VIII No Medic-ation	0.21^{NS} ± 0.016	0.30^{a} ± 0.034	0.20^{NS} ± 0.018	1.143 ± 0.017	0.933 ± 0.096	1.312 ± 0.052	1.012 ± 0.186	1.186 ± 0.322	0.986 ± 0.127
IX No. Med./ No. Vac.	0.36^{NS} ± 0.033	0.62^{c} ± 0.034	0.49^{NS} ± 0.037	1.715 ± 0.128	1.355 ± 0.116	2.12 ± 0.121	1.504 ± 0.310	1.923 ± 0.009	1.433 ± 0.132

CBH – Cutaneous Basophilic Hypersensitivity

* Mean of five observation

Means with common superscript (a,b,c) did not differed significantly ($P<0.01$)

vaccine revealed an improvement over the response shown by the group which received only IBD vaccine alone (gr. VII). When compared the group which did not received either treatment or IBD vaccine (Gr. IX), with group which received only IBD vaccine (Gr. VII), it was found that there was some degree of T-Cell activity suppression in IBD vaccinated and untreated group as evidenced by low CBH response. The treatment effect using different drugs/agents on the CBH response showed that the highest response to DNCB was elicited by amla treated group followed by levamisole, Vitamin E, bhang, *Carbo animalis* and polyzyme treated groups.

In general thickened skin, induration and scab formation were found which was more pronounced in control (Gr. IX), levamisole treated group (Gr. I) and amla treated group (Gr. V). Histologically, the changes in the abdominal skin, was of delayed type hypersensitivity which were characterized by oedema marked infiltration of dermis with mononuclear cells, heterophils and monocytes.

4.6.2CBH response by broiler chicken to PHA-P

The injection of PHA-P is presented in table-14. The perusal of table revealed that a significant increase in skin thickness was only found at 24 hours post challenge among different treatment groups (gr. I to VI). Further the result showed that there was increase in CBH response, as evidenced by skin

Table - 14

Effect of different drugs/agents on CBH reaction to PHA- P in chickens.

Group & Treatment	Mean* ± SE of CBH response after PHA -P (Tuberculin) challenge (mm)			Mean ± SE of skin (hours post challenge) after PHA-P challenge (mm)					
	12 Hours	24 Hours	48 Hours	12 Hours		24 Hours		48 Hours	
I Levamisole	0.53^{NS} ± 0.041	0.70^{c} ± 0.081	0.64^{NS} ± 0.013	1.720 ± 0.148	1.190 ± 0.099	2.119 ± 0.121	1.419 ± 0.186	1.971 ± 0.022	1.331 ± 0.091
II Polyzyme	0.44^{NS} ± 0.033	0.59^{bc} ± 0.019	0.46^{NS} ± 0.062	1.442 ± 0.634	1.002 ± 0.129	1.961 ± 0.590	1.371 ± 0.291	1.671 0.121	1.211 0.212
III Vitamin E	0.46^{NS} ± 0.019	0.63^{bc} ± 0.512	0.54^{NS} ± 0.036	1.653 ± 0.431	1.193 ± 0.222	2.048 ± 0.066	1.418 ± 0.422	1.853 ± 0.081	1.313 ± 0.054
IV Bhang	0.51^{NS} ± 0.121	0.66^{bc} ± 0.098	0.58^{NS} ± 0.120	1.710 ± 0.510	1.200 ± 0.520	2.09 ± 0.098	1.43 ± 0.062	1.876 ± 0.069	1.296 ± 0.035
V Amla	0.60^{NS} ± 0.090	0.74^{c} ± 030	0.68^{NS} ± 0.009	1.814 ± 0.321	1.214 ± 221	2.191 ± 0.098	1.451 ± 0.147	2.059 ± 0.012	1.379 ± 0.122
VI Carbo animalis	0.50^{NS} ± 0.066	0.69^{bc} ± 0.059	0.58^{NS} ± 0.036	1.706 ± 0.122	1.126 ± 0.310	2.034 ± 0.163	1.364 ± 0.069	1.868 ± 0.122	1.298 ± 0.088
VII No Medic-ation	0.33^{NS} ± 0.052	0.48^{ab} ± 0.069	0.35^{NS} ± 0.011	1.310 ± 0.321	0.980 ± 0.069	1.661 ± 0.012	1.181 ± 0.055	1.359 ± 0.031	1.009 ± 0.011
VIII No Medic-ation	0.26^{NS} ± 0.066	0.36^{a} ± 0.035	0.29^{NS} ± 0.038	1.183 ± 0.631	0.923 ± 0.211	1.139 ± 0.322	1.031 ± 0.162	1.278 ± 0.042	0.988 ± 0.009
IX No. Med./ No. Vac.	0.43^{NS} ± 0.051	0.69^{c} ± 0.034	0.58^{NS} ± 0.066	1.830 ± 0.063	1.400 ± 0.111	2.234 ± 0.009	1.544 ± 0.059	1.988 ± 0.034	1.408 ± 0.029

CBH – Cutaneous Basophilic Hypersensitivity.

* Means of five observation

Means with common superscript (a,b,c) do not differ significantly ($P<0.05$)

thickness, due to different drugs agents treatment (Gr. I to VI) in comparison to the group which did not received any treatment (Gr. VII). When compared with the control group which did not received either treatment or IBD vaccine, the IBD vaccinated and untreated group (Gr. VII) showed low CBH response (Table-3). In general, the effect of different drugs/agents showed that the highest CBH response was achieved by amla treated group followed by levamisole, *carbo animalis*, bhang, Vitamin E and polyzyme treated groups.

In general erythrema followed by pallor was observed accompanied by thickening of skin. The histological changes present in the abdominal skin were characterized by oedema, diffuse infiltration of the dermis with polymorphonuclear granulocytes and mononuclear cells. There was a marked thickening and hyperkeratinization of stratum corneum.

4.6.3Cutaneous Basophilic sensitivity reaction of broiler chickens to PPD (tuberculin).

The results of CBH to PPD are shown in table-15. The perusal of table reveals that a maximum responsiveness of the chickens was observed at 24 hour post injection. Furthermore, a significant increment was recorded at 24 hours post injection among different groups (I-IX). Compared with the group which did not received either drugs/agents or IBD vaccine (Gr. IX) with the group which did

Table - 15

Effect of different drugs/agents on CBH reaction to PPD (Tuberculin) in chickens.

Group & Treatment	Mean ± SE of CBH response after PPD (Tuberculin) challenge (mm)			Mean ± SE of skin thickness (hours post challenge) after DNCB challenge (mm)					
	12 Hours	24 Hours	48 Hours	12 Hours		24 Hours		48 Hours	
I Levamisole	0.36^{NS} ± 0.041	0.53^{cd} ± 0.012	0.41^{NS} ± 0.032	1.498 ± 0.512	1.138 ± 0.411	1.908 ± 0.432	1.378 ± 0.300	1.724 ± 0.034	1.314 ± 0.122
II Polyzyme	0.26^{NS} ± 0.011	0.43^{bc} ± 0.060	0.30^{NS} ± 0.070	1.260 ± 0.021	1.000 ± 0.120	1.730 ± 0.035	1.300 ± 0.077	1.696 ± 0.071	1.266 ± 0.033
III Vitamin E	0.32^{NS} ± 0.031	0.50^{cd} ± 0.211	0.39^{NS} ± 0.009	1.462 ± 0.121	1.142 ± 0.089	1.881 ± 0.921	1.381 ± 0.123	1.689 ± 0.066	1.299 ± 0.51
IV Bhang	0.35^{NS} ± 0.045	0.46^{bc} ± 0.038	0.38^{NS} ± 0.066	1.548 ± 0.161	1.198 ± 0.612	1.781 ± 0.054	1.321 ± 0.362	1.626 ± 0.121	1.246 ± 0.034
V Amla	0.49^{NS} ± 0.032	0.63^{d} ± 0.055	0.58^{NS} ± 0.036	1.693 ± 0.312	1.203 ± 0.444	2.030 ± 0.300	1.400 ± 0.062	1.891 ± 0.033	1.311 ± 0.120
VI Carbo animalis	0.30^{NS} ± 0.033	0.49^{bcd} ± 0.069	0.39^{NS} ± 0.057	1.366 ± 0.319	1.066 ± 0.078	1.731 ± 0.351	1.241 ± 0.066	1.590 ± 0.012	1.200 ± 0.031
VII No Medic-ation	0.20^{NS} ± 0.0216	0.34^{ab} ± 0.023	0.31^{NS} ± 0.044	1.100 ± 0.011	0.90 ± 0.031	1.433 ± 0.211	1.093 ± 0.126	1.313 ± 0.121	1.003 ± 0.111
VIII No Medic-ation	0.16^{NS} ± 0.032	0.22^{a} ± 0.077	0.19^{NS} ± 0.012	1.052 ± 0.055	.892 ± 0.073	1.221 ± 0.019	1.001 ± 0.034	1.150 ± 0.069	.960 ± 0.032
IX No. Med. No. Vac.	0.30^{NS} ± 0.007	0.52^{cd} ± 0.012	0.39^{NS} ± 0.030	1.622 ± 0.069	1.322 ± 0.054	1.882 ± 0.110	1.362 ± 0.121	1.712 ± 0.088	1.322 ± 0.124

CBH – Cutaneous Basophilic Hypersensivity

* Mean of five observation

Means with atleast one common superscript (a,b,c,d) did not differed significantly

received only IBD vaccine and no treatment (Gr. VII). A immuno suppressive effect was founds evidenced by decreased skin thickness. On further perusal an increment in CBH response was found in case of the groups which received drugs/agents along with IBD vaccine (gr.I-VI), in comparison with the group which received IBD vaccine and not any treatment (gr. VII).

In general maximum CBH response was revealed by amla treated group followed by levamisole, Vit –E, *carbo animalis*, bhang and polyzyme treated group.

Histopathological examination of the section of PPD injected skin revealed a pronounced infiltration of mononuclear cells. Infiltration of mononuclear cells.

4.7 Challenge study

The result of challenge study revealed no clinical sings in the treatment groups. No mortality was recorded in vaccinated and treated group (gr. I-VI).. Highest mortality was recorded in group which received field virus instead of vaccine strain (gr. VIII) followed by IBD unvaccinated (gr. IX) and IBD vaccinated and untreated group (gr. VII) (table 17).

IBDV antigen was demonstrated in bursa of Fabricius by AGPT seven days after challenge in unvaccinated and untreated group (gr. IX) and group which received field virus (gr. VIII).

Table - 16

Mean[#] ± S.E. of bursal lesion score in different treatment groups of broiler chickens sacrificed on 7 day post challenge.

Group & Treatment	Inter-Follicular oedema	Follicular Changes					Epithelial Changes			Interstitial fibrosis	Cellular Infiltration	Total lesion score
		Lymphoid necrosis	Lymphoid depletion	Reticulo epithelial hyperplasia	Vacuolar degeneration	Follicular cyst	Hyperplasia	Epithelial invagination	Cyst formation	Vacuolation			
I Levamisole	0.2^{a} ± 0.245	0.8^{a} ± 0.200	0.8^{ab} ± 0.200	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.00 ± 0	0.200^{a} ± 0.245	0.00	0.0 ± 0	0.00 ± 0.0	0.0 ± 0.0	0.18^{a} ± 0.75
II Polyzyme	0.4^{a} ± 0.200	1.2^{a} ± 0.200	1.0^{ab} ± 0.0	0.0 ±0	0.0 ± 0	0.0 ± 0	0.80^{ab} ± 0.200	0.800^{bc} ± 0.200	0.00	0.0 ± 0	0.200^{a} ± 0.245	0.200^{a} ± 0.200	0.42^{a} ± 0.71
III Vitamin E	0.0 ± 0	1.2^{a} ± 0.200	0.8^{ab} ± 0.200	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.00 ± 0	0.400^{ab} ± 0.245	0.00	0.0 ± 0	0.00 ± 0.00	0.00 ± 0	0.25^{a} ± 0.85
IV Bhang	0.0 ± 0	1.0^{a} ± 0.000	0.6^{a} ± 0.245	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.00 ± 0	0.200^{a} ± 0.200	0.00	0.0 ± 0	0.00 ± 0.0	0.0 ± 0	0.20^{a} ± 0.81
V Amla	0.0 ± 0	0.8^{a} ± 0.200	0.6^{a} ± 0.245	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.00 ± 0	0.200^{a} ± 0.200	0.00	0.0 ± 0	0.00 ± 0	0.0 ± 0	0.16^{a} ± 0.64
VI *Carbo animalis*	0.0 ± 0	1.0^{ab} ± 0.316	0.8^{ab} ± 0.200	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.20^{a} ± 0.200	0.400^{a} ± 0.245	0.00	0.0 ± 0	0.200^{a} ± 0.245	0.0 ± 0.0	0.26^{a} ± 0.66
VII No Medication	0.6^{a} ± 0.316	1.4^{b} ± 0.245	1.2^{bc} ± 0.200	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.4^{a} ± 0.245	1.20^{cd} ± 0.200	0.200^{a} ± 0.245	0.0 ± 0	0.40^{a} ± 0.245	0.40^{a} ± 0.245	0.516^{a} ± 0.83
VIII * No Medication	1.2^{b} ± 0.200	1.6^{b} ± 0.245	1.6^{c} ± 0.245	1.8^{a} ± 0.200	1.6^{a} ± 0.245	1.2^{a} ± 0.200	1.200^{b} ± 0.200	1.60^{cd} ± 0.245	1.20^{b} ± 0.200	0.600^{a} ± 0.245	0.4^{b} ± 0.245	1.2^{b} ± 0.02	1.366^{b} ± 0.405
IX No. Med./ No. Vac.	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0

* Received file virus instead of vaccine strain.

\# Means of five observation

Means with atleast one common superscript (a,b,c,d) did not differ significantly ($P<0.05$)

The bursa : body weight (B:BW) ratio on seven day post challenge (42 day of age) showed a lower B:BW ratio in case of IBD unvaccinated and untreated group (gr. IX) in comparison to group VII, which received IBD vaccination and no treatment. Among the treated groups, highest B:BW ratio was recorded in bhang treated group followed by amla, levamisole, Vitamin-E, *Carbo animalis* and polyzyme treated group (table 17).

Histopathological examination of bursa (bursa score) of birds challenged with IBDV showed mild damage as evidenced by lesion score (table-16). The highest lesion score was recorded for group followed by group VII. The group VIII, which received field virus instead of vaccine strain showed damage to greater degree. Among the treatment group the lowest lesion score was recorded by amla treated group followed by levamisole, bhang, Vitamin-E, *carbo-animalis* and polyzyme treated group.

Table - 17.

Effect of different drugs / agents on bursa weight , body weight and bursa : body weight ratio of IBD vaccinated Chicken 7 day post challenge (32 days post IBD vaccination)

Group	Treatment	Age at vaccination (days)			Bursa weight (g)	Body weight (g)	Bursa : body Weight ratio	(No. of sample Positive for Virus/ No. of Sample taken)	No. of mortality / No. of birds taken
		IBDV (IV95 strain)	RDV (F- strain)	RDV (LaSota strain)					
I	Levamisole	10	5	25	$1.703^{f} \pm 0.008$	$1535^{c} \pm 64.43$	$1.109^{c} \pm 0.012$	0/5	0/12
II	Polyzyme	10	5	25	$1.612^{e} \pm 0.019$	$1612^{cf} \pm 23.21$	$1.00^{b} \pm 0.019$	0/5	0/12
III	Vitamin-E	10	5	25	$1.821^{g} \pm 0.013$	$1687^{f} \pm 31.26$	$1.079^{c} \pm 0.031$	0/5	0/12
IV	Bhang	10	5	25	$1.831^{g} \pm 0.012$	$1620^{f} \pm 6.29$	$1.130^{c} \pm 0.011$	0/5	0/12
V	Amla	10	5	25	$1.616^{e} \pm 0.033$	$1451^{d} \pm 19.99$	$1.113^{c} \pm 0.009$	0/5	0/12
VI	*Carbo animalis*	10	5	25	$1.270^{d} \pm 0.062$	$1268^{c} \pm 24.67$	$1.001^{bc} \pm 0.017$	0/5	0/12
VII	-	10	5	25	$1.012^{c} \pm 0.031$	$1148^{b} \pm 22.22$	$0.881^{ab} \pm 0.011$	0/5	1/12
VIII	-	10*	5	25	$0.722^{a} \pm 0.110$	$1012^{a} \pm 16.49$	$0.713^{a} \pm 0.021$	4/5	7/12
IX	-	-	5	25	$0.894^{b} \pm 0.051$	$1297^{c} \pm 12.57$	$0.689^{a} \pm 0.016$	3/5	6/12

* Received field virus instead of vaccine virus.

Means with atleast one common superscript (a,b,c,d,e.f,g) did not differed significantly ($P<0.05$)

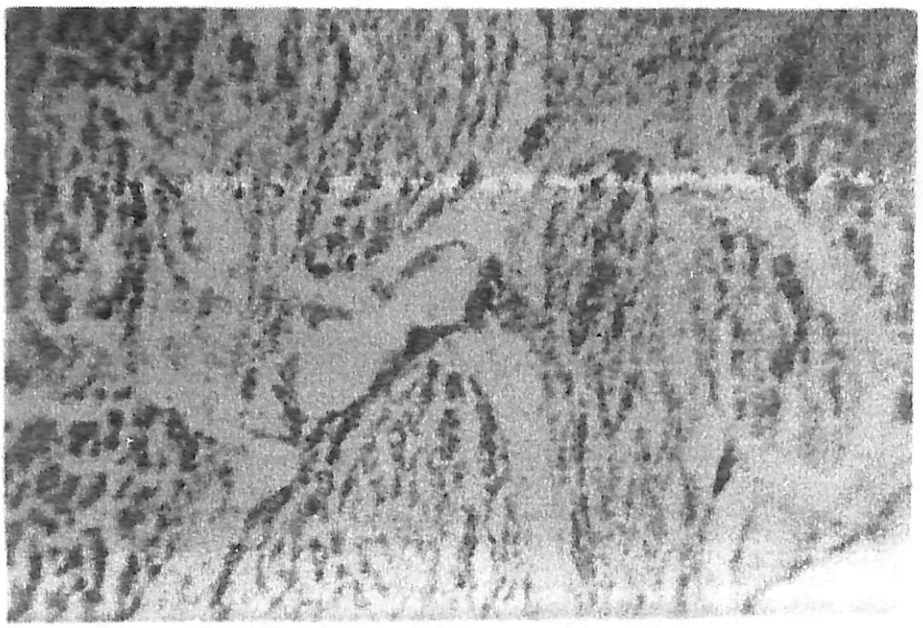


Fig. 1 Microphotograph of bursa of Fabricius showing depletion and necrosis of lymphocytes.

(H & E x 150)

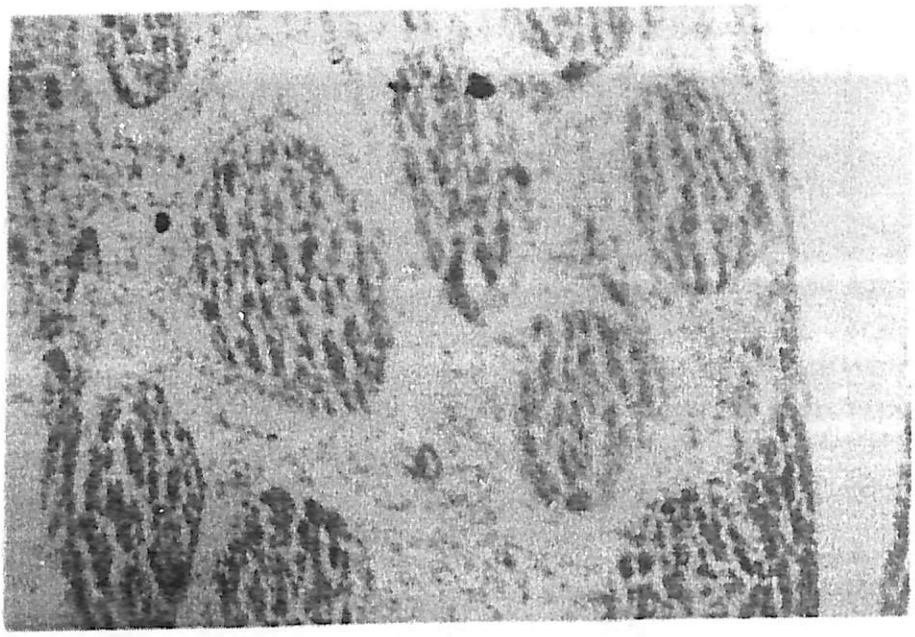


Fig. 2 Microphotograph of bursa of Fabricius showing marked oedema In interfollicular space.

(H & E x 150)

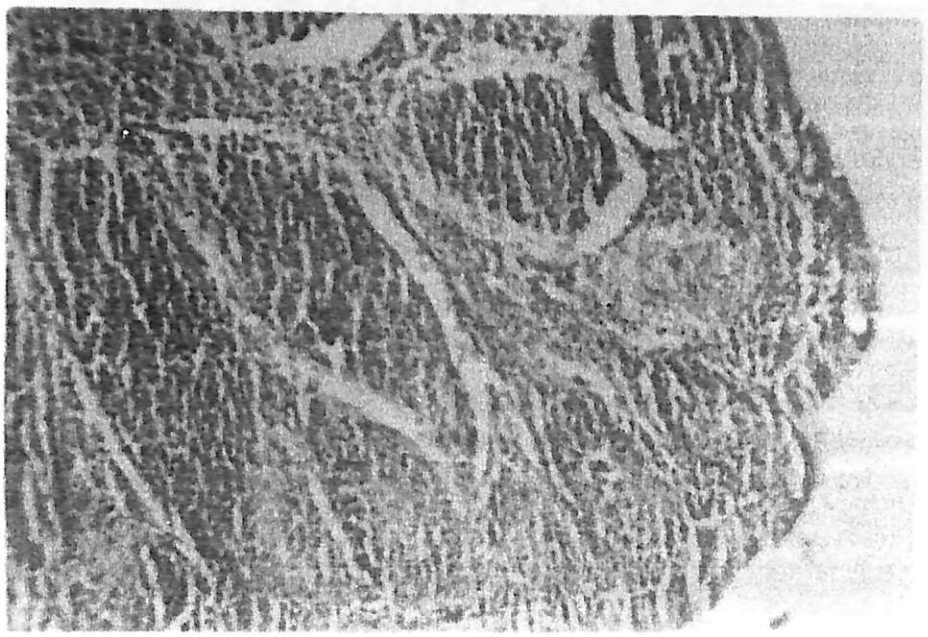


Fig. 3 Microphotograph of bursa of Fabricius showing vacuolation of Plical epithelium.

(H & E x 150)

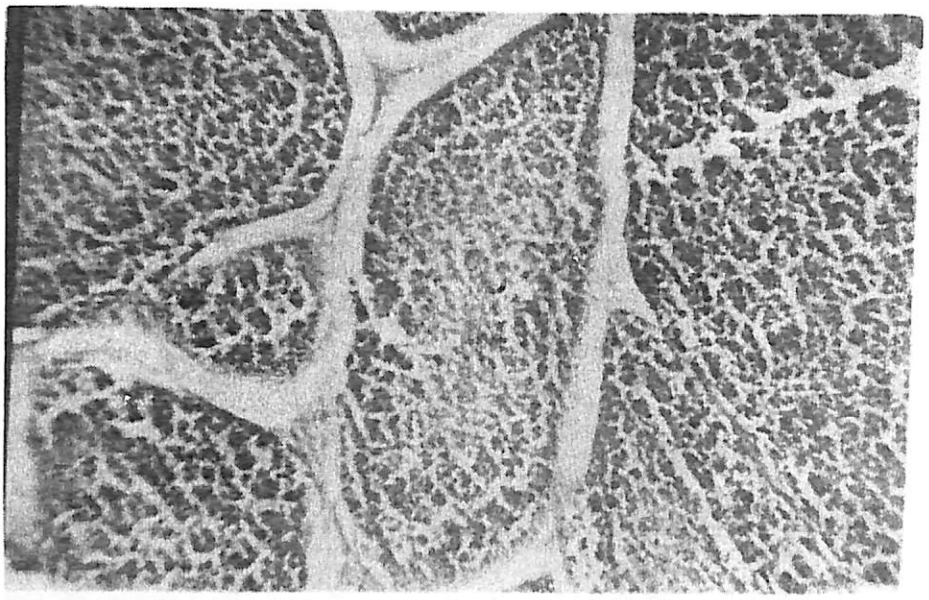


Fig. 4 Microphotograph of bursa of Fabricius showing with Accumulation of oedematous fluid.

(H & E x 150)

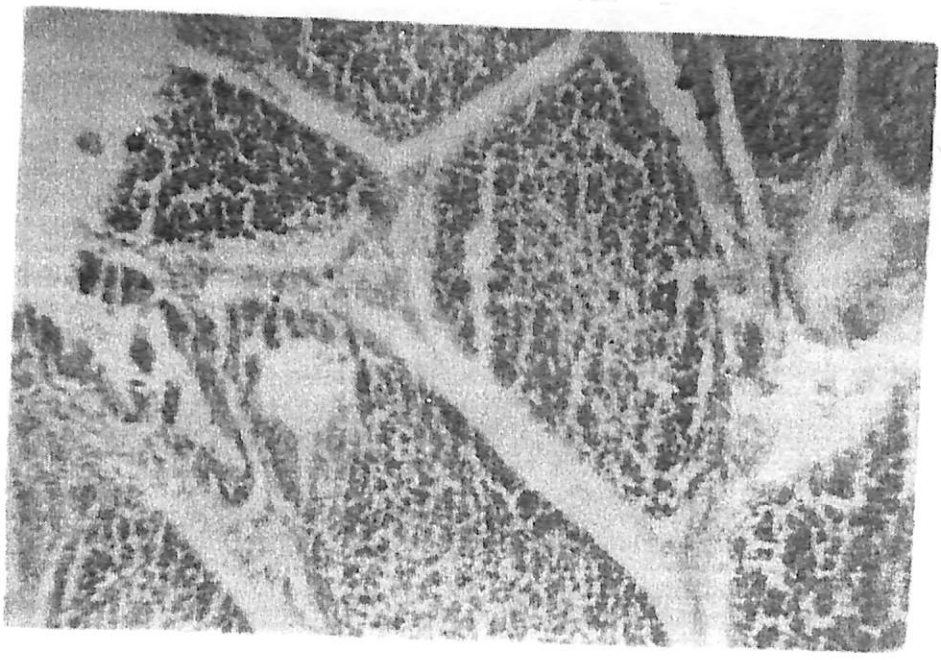


Fig. 5 Microphotograph of bursa of Fabricius showing necrosis Of lymphocytes degeneration

(H & E x 150)

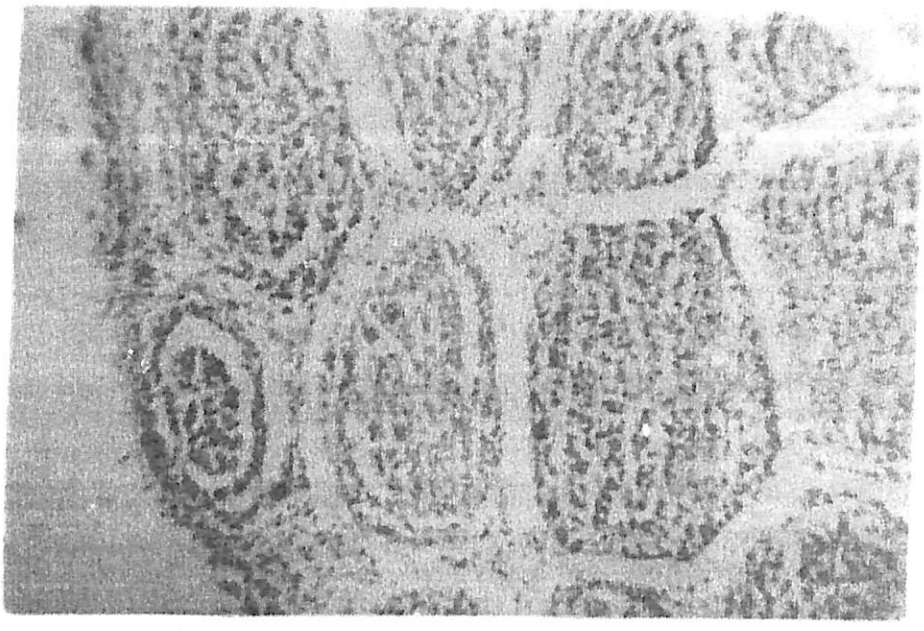


Fig. 6 Microphotograph of bursa of Fabricius showing with Accumulation of oedematous fluid.

(H & E x 150)

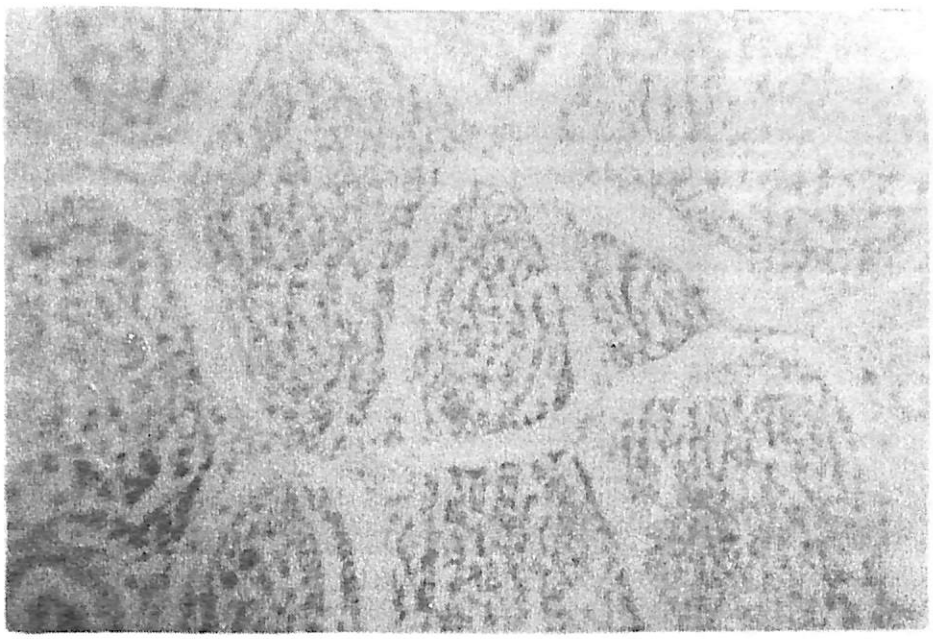


Fig. 7 Microphotograph of bursa of Fabricius showing depletion and Necrosis of lymphocytes.

(H & E x 150)

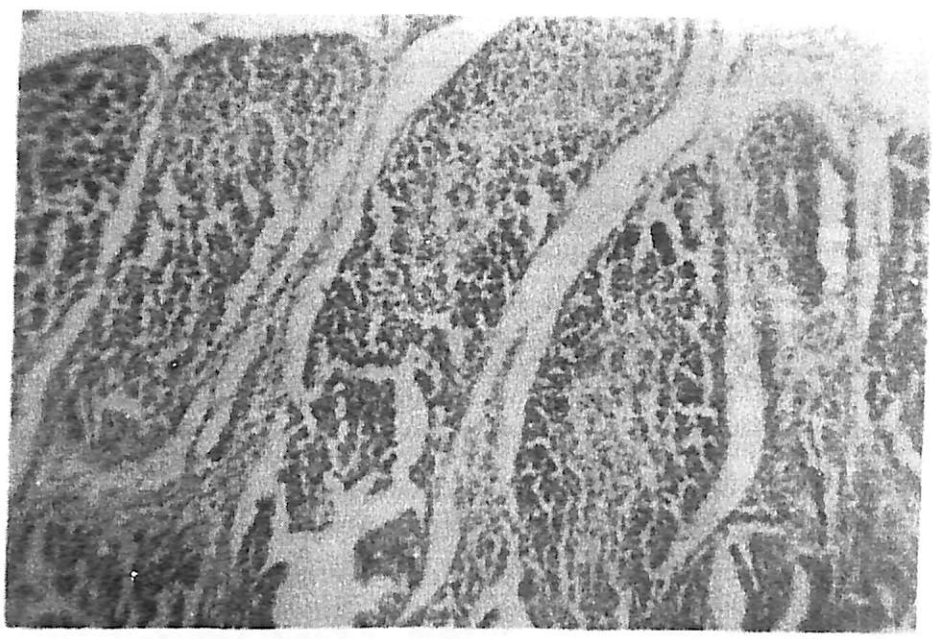


Fig. 8 Microphotograph of bursa showing degenerative changes in the bursal follicle .

(H & E x 150)

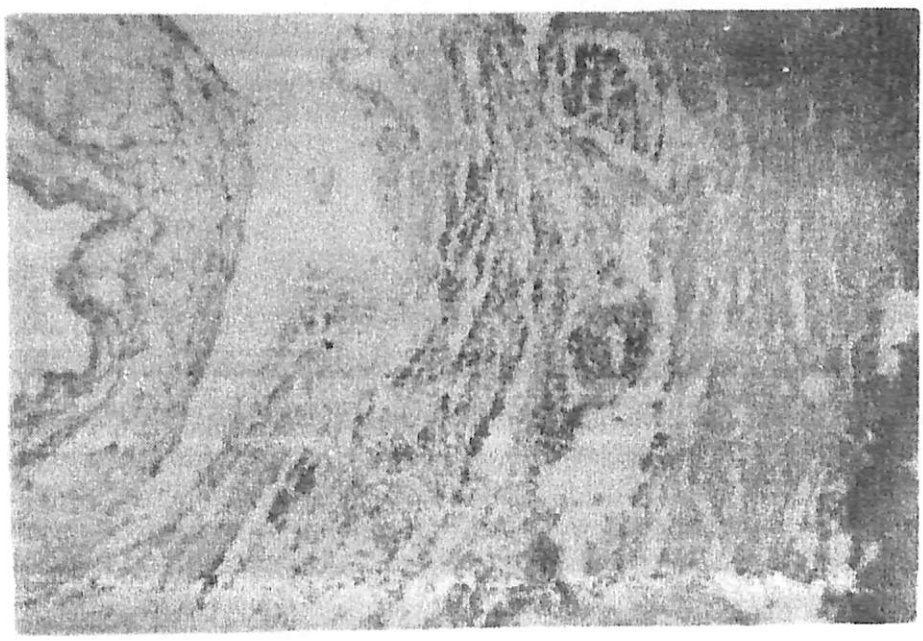


Fig. 9 Microphotograph of abdominal skin after 48 hours of injection of PHA-P showing infiltration of mononuclear cells.

(H & E x 150)

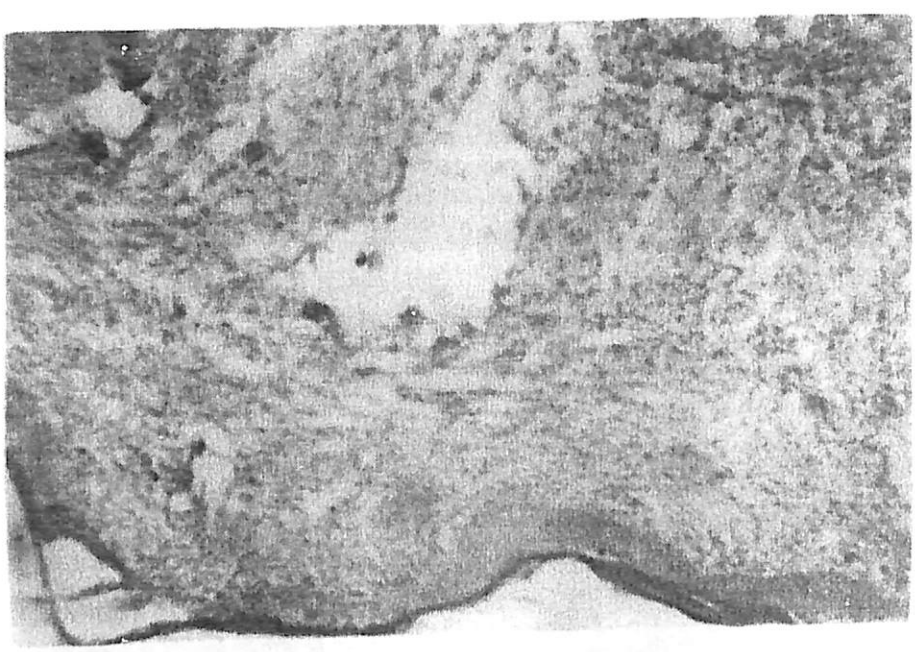


Fig. 10 Microphotograph of abdominal skin showing oedema at 24 hrs. Post challenge.

(H & E x 150)

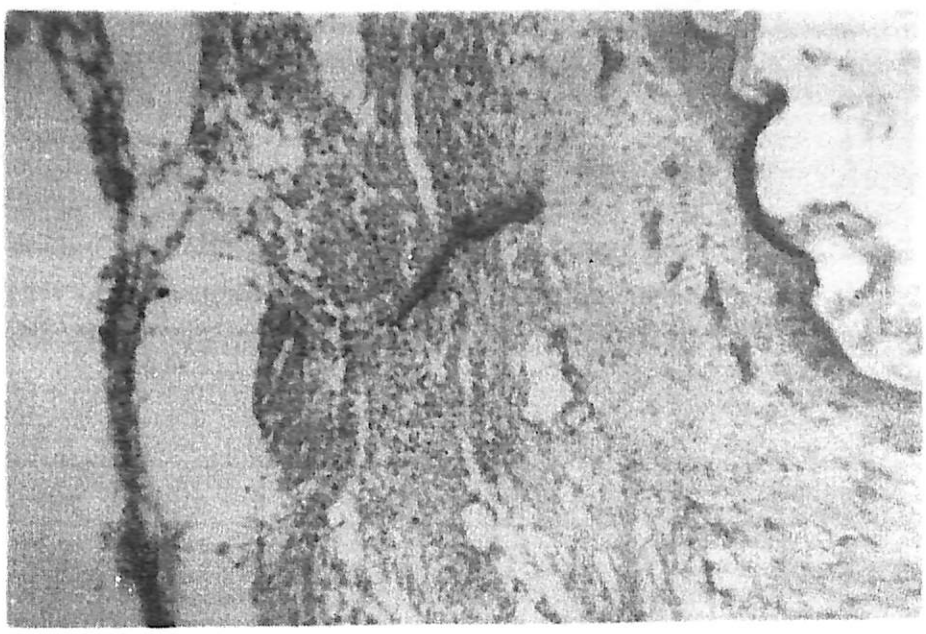


Fig. 11 Microphotograph of abdominal skin showing infiltration of Heterophils .

(H & E x 150)

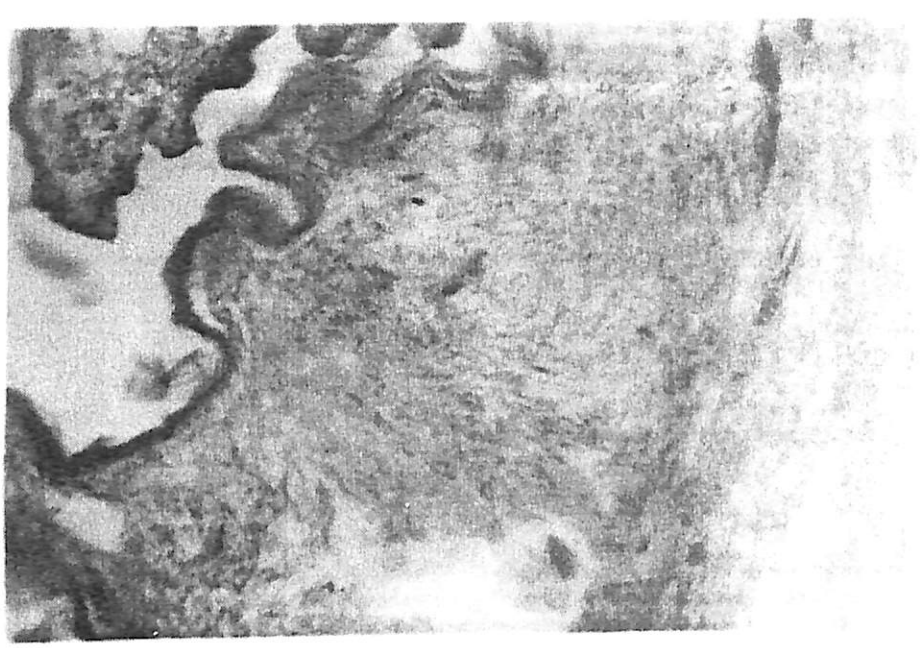


Fig. 12 Microphotograph of abdominal skin showing mild degeneration with Infiltration of polymorpho nuclear cells.

(H & E x 150)

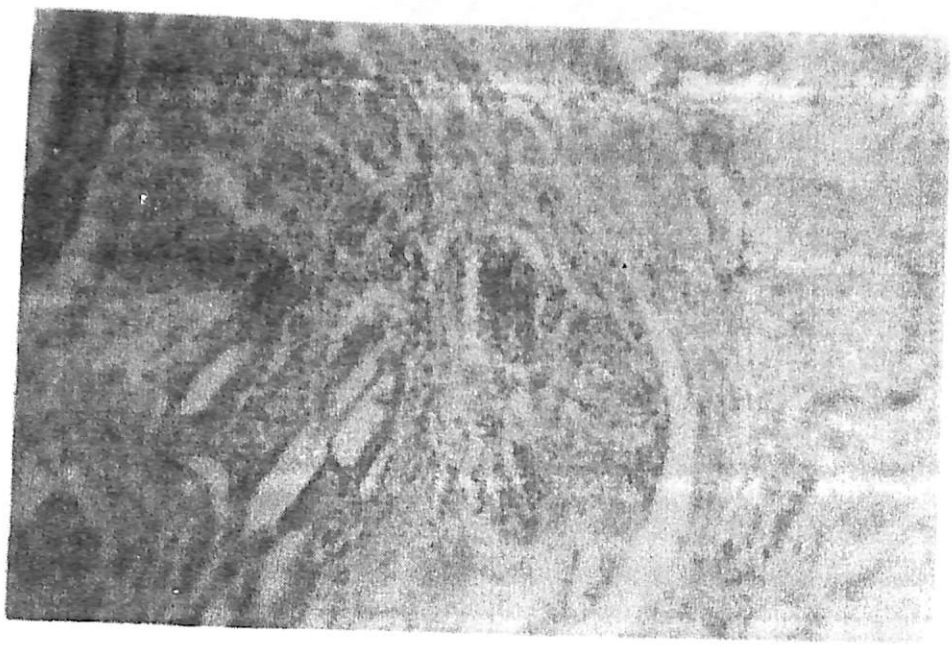


Fig. 13 Microphotograph of abdominal showing diffuse infiltration of Monocytes.

(H & E x 150)

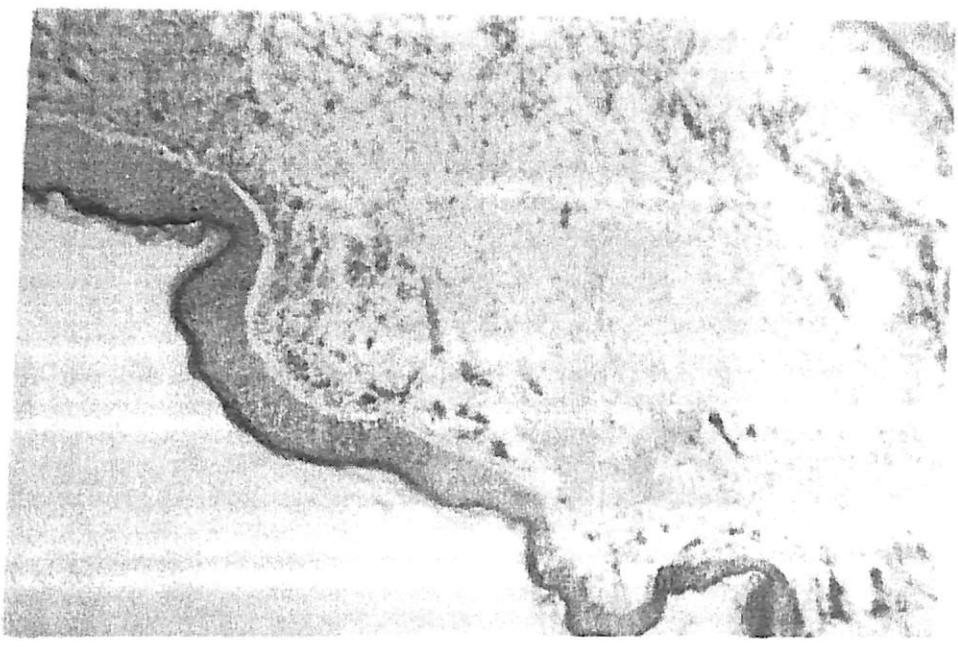


Fig. 14 Microphotograph of abdominal skin showing hyperkeratosis of epithelium.

(H & E x 150)

CHAPTER V

DISCUSSION

Infectious bursal disease (IBD) is an acute highly contagious immunosuppressive viral disease of young chicken which causes great economic losses directly through death and indirectly through damage of immune system. Since the formal documentation of disease by Cosgrove (1962) in broiler, the disease was reported from all poultry producing pockets including India (Mohanty *et al.*, 1971) and from Bihar (Singh *et al*; 1994b; Sinha, 1997). Initially, the disease was caused by classical virus of serotype type I IBDV, producing mild clinical or subclinical infection resulting in poor growth and immuno suppression with low mortality. To combat the disease, effective vaccination was practiced using mild or intermediate strain vaccine. Around 1989 a new form of IBD struck the poultry which complicated the scenario owing to heavy mortality even in flocks which had been vaccinated with mild or intermediate strain vaccine. The virus identified, as very virulent strain, was able to break the maternal antibody barrier causing mortality as high as 70-100%in susceptible flock. A number of measures were tried by the owners of different farm with a view to minimize the economic losses as well as to check the propagation of virus in the surroundings. These included repeated vaccination with same intermediate strain IBD vaccine in the face of outbreaks as well as administration of antibiotics, electrolyte, superdietary vitamin A, Vitamin-E, as well as known immunomodulators like Zeetress, levamisole etc. Although, these treatments were not performed under controlled condition but there were indications of reduction in number of death and improvement in body weight gain ,however, the outbreak due to vvIBD continued unabated. To control the infection by very virulent strain IBD virus a more invasive vaccine (commonly called intermediate plus or invasive strain or moderate hot strain),

capable of stimulating immunity in presence of maternal antibody, was introduced. The vaccine invoked desired immune response, however, it caused mild immunosuppression and residual pathogenicity. The study conducted in this department further confirmed the residual pathogenicity and invasiveness inherent in this vaccine (Kumar, 1998). This study also limelighted the immunosuppressive effect of this new vaccine strain. It is in this backdrop of the above study as well as taking into account the earlier reports the residual pathogenecity of the vaccine strain, it was felt necessary to look for some alternative approach which could efficiently counter the complications that accompany this vaccination to make this vaccine more acceptable to the poultry farmers. The efficacy of certain immunopotentiating drugs/agents in controlling the after effects of newly introduced vaccine.

The immune responses to IBD virus vaccine (IV 95 strain) in broiler chicken at different intervals post vaccination in different treatment groups are shown in table 6. The perusal of this table revealed that the seroconversion occurred by seven day post IBD vaccination. A number of workers have reported seroconversion between 3-7 days post infection/vaccination (Ley *et al.*, 1983; Dash *et al.*, 1991; Thangavelu *et al.*, 1993; Kumar, 1998). On the other hand several workers have also reported seroconversion by 14 day post vaccination (dpv) when the birds were carrying maternal antibody (Hirai *et al.*, 1972; Ley *et al.*, 1983; Zorman Rojs *et al.*, 1996; Kumar, 1998). However, in the present study the birds were negative for the presence of Mab at the time of vaccination. Besides, the vaccination in present case was carried out at 10 days of age. Both Mab level and age factor are considered to affect the immune response to IBD vaccine (Gordon and Jordan, 1982;

Goddard *et al*; 1994; Tsai *et al.*, 1995; Kumar *et al*; 2000). The present finding of seroconversion to IBD vaccine by seven dpv may be explained on above grounds as the vaccination in the present study was conducted on 10 day of age. Further, the seroconversion which was first detected at 7 dpv showed increasing trend till the last day of observation (35 dpv) in drug treated group except polyzyme treated group where the increase in antibody titre continued only by 28 dpv. In case of vaccinated but untreated group (group VII) and group which received field virus (Group VIII) the increase in antibody titre continued only by 28 dpv. The result also demonstrated that the antibody levels in different drug treated groups were invariably higher post IBD vaccination than the titres recorded in vaccinated but untreated group (Gr. VII). This clearly suggested that all the six drugs/agents employed in this study were effective in enhancing the immune response to IBD vaccine. A number of workers have reported applications of various agents/drugs in IBD infected/vaccinated birds as antistress, adaptogenic and immunopotentiator in order to combat the immunosuppressive effect of virus as well as to harness the potentials of vaccines to a maximum level (McIlroy *et al.*, 1993; Singh and Dhawedkar, 1993; El-Zanty, 1994; Panda and Rao, 1994; Skalan *et al.*, 1994; Franchini *et al.*, 1995; Rao *et al.*, 1995; Szigeti *et al.*, 1998; Sadekar *et al.*, 1998a; Sadekar, 1998b; Kolte *et al.*, 1999; Saravanabava *et al.*, 1999; Gromov, 1999; Sofei and Bucur, 1999; Wu *et al.*, 2000; Amakye et al., 2000; Mohanty *et al*, 2000. The present findings are in agreement with the observation made by earlier workers.

During the present investigation, the AGPT was routinely employed for detection of antibodies and its titre against

IBDV. Several workers have reported the application of AGPT for demonstration of antibodies and its level against IBDV (Schneider and Haass, 1969; Hirai et al., 1974 Wyeth and Cullen, 1976; Wilke *et al.*, 1978; Wood *et al.*, 1979). Though a number of workers found merit in ELISA and SNT over AGPT (Nicholas et al., 1985; Solono *et al.*, 1986; Dash *et al.*, 1991; Synder *et al.*, 1992; Desh Pande and Muniyappa, 1996; Mangla gowri *et al.*, 1996), there is general consensus that AGPT is a specific, easy to perform and highly reproducible (Wilke *et al.*, 1978; Dash *et al.*, 1991; Thangavelu, *et al.*, 1993). In the present study also this test was found to be easily reproducible and easy to perform and hence used routinely. In general upto three precipitin lines were discernible when reference antigen and hyperimmune serum were employed. However, only one to two precipitin lines were seen between the central well containing reference antigen and peripheral well containing test serum samples. Whereas, the first precipitin lines appeared between 16 to 30 hours depending on variation in room temperature and was closer to the antigen well, second precipitin line generally appeared between 30-48 hours, and was midway between antigen and antiserum wells. The third precipitation line when detected appeared between 48 to 72 hours and was closer to antiserum wells. A number of workers reported similar pattern of precipitin lines in AGPT employing IBD antigen and antibody (Faragher, 1971; Hirai *et al.*, 1974; Wilke *et al.*, 1978; Takase *et al.*, 1993). However variation in precipitin lines with IBD antigens and antibody system have also been reported (Takase *et al.*, 1993). A number of factors determine the pattern of precipitation line antibody concentration of antigen and antibody (Wood *et al.*, 1979; Mohanty *et al.*, 1981). Hence, some variations in pattern of precipitin lines are possible.

It may be mentioned that IBD virus has suppressive effect on immune responses to various vaccines whereas normally the response to IBD vaccine itself remain unaffected. Therefore, the use of such immunopotentiating agent for enhancing the response to IBD vaccine may not seem proper. However, in such a scenario where the birds are continuously exposed to several other immunosuppressive agents, infectious or otherwise including one most commonly encountered, the aflatoxin and also where the nutritional requirements of the birds are not provides to the optimum level, it would not be proper to expect getting normal immune responses to vaccines including IBD vaccines. Under such circumstances the relevance of immunopotentiating agent similar to one used in the present study cannot be denied. Besides, there are also reports that factors like stress, climatic change and several other unknown or known factors, can adversely affect the immune response to vaccines (Saran and Sharma, 1996). Accordingly it has become common practice to use some agents of proven immunopotentiating effects as an adjunct to vaccination programme, so that the immune responses of the vaccine expresses the fullest level. The relatively better immune responses recorded in different treatment groups (group I-VI) for the different intervals may be due to antistress, adaptogenic and restorative effects of drugs/agents used (Table – 6).

Levamisole is a broad spectrum anthelmintic commonly used in veterinary and human medicine. In addition, it has been widely used as immunopotentiating agent in human, livestock and poultry to enhance the vaccinal responses (Panigrahy *et al.*, 1979; Hogarth-Scott *et al.*, 1980; Panda and Rao, 1994;

Shadaksharappa *et al.*, 1998; Kalita and Dutta, 1999; Mohanty *et al.*, 2000). In the present study levamisole treated birds (group I) exhibited comparatively higher antibody titre to IBD vaccine at all intervals post IBD vaccination when compared with other groups (group II, VII & VIII) for the corresponding periods. Several workers have reported the application of levamisole in IBD vaccinated chicken and also reported enhancement of immune response in treated groups (Shadaksharappa, 1998; Mohanty *et al.*, 2000) which support the present finding. It may be mentioned that in most of the cases levamisole is used in livestock and poultry as an immunomodulator to enhance immune responses to different vaccines in immuno-compromised animals. IBD virus is a proven immunosuppressant which immuno compromises the birds to immune response to several vaccines including RD vaccine and the drug like levamisole is being used in such cases to enhance antibody level (Singh and Dhawedkar, 1994a). However, application of this drug for enhancement of antibody titre to IBD vaccine itself is a new approach and need consideration whether it is at all necessary. This is so because there appears to be dearth of information on failure of immune response to IBD vaccines to an unprotective level. However, such a situation cannot be denied when the birds are immunosuppressed prior to receiving IBD vaccine. Immunosuppression due to aftatoxin and other mycotoxin is widespread and well documented (Allan *et al.*, 1972; Thaxton *et al.*, 1974). There are also reports that immune response to IBD vaccine may be adversely affected in birds which are already immunocompromised due to aflatoxin (Giambrone *et al.*, 1978; Kumari, 2002). Besides, there are several other immunosuppressive agents such as nitrated proteins formed due to nitration of amino acid during stress condition (Durhams,

2002) to which the birds may be exposed leading to immunosuppression. In all such conditions, the response to IBD vaccine may not be proper when the use of levamisole or similar immunomodulatory agents may have to be considered along with this vaccine. In any case the purpose to use levamisole in the present investigation was to counter the after effects of IV 95 strain vaccine which is known to have relatively more residual pathogenicity and immunosuppressive effect. It is in this process that its effects on immune response to IBD was also examined and the drug was found to be immunostimulatory in this regard too. The result obtained is suggestive of the additional benefits of levamisole to which the poultry farmers, who may be using, have to be appraised for better acceptability of this drug. The present finding may also be considered encouraging even in a situation when IBD vaccine could have produced optimum antibody level by itself but after administration of levamisole the titre is enhanced further. It is hoped that such enhancement of antibody level due to levamisole may be helpful in providing better resistance in vaccinated birds so much so that such birds can with stand exposure to even high multiplicity of infection when there are outbreaks of the disease in the surrounding localities. Further, any enhancement in the antibody level over and above the optimal vaccinal response will certainly allow the persistence of protective antibody level for longer period and thus immunity of longer duration which is always welcomed. Accordingly, this finding may have for reaching consequences and hence needs to be highlighted.

Cereals or their by product used in poultry feed consist of high fibre (choct, 1996) and anti nutritive factos such as NSP (cellulose, arabinoxylans and mixed ß – glucans). Causing

sticky dropping and reduced nutrient utilization and growth rate, especially in young poultry (Hesselman & Jeroch 1983). Insoluble fibre tends to increase transit time and to form an insulating coat on the digestible nutrients, thus reducing nutrient supply whereas soluble fibres slow down the transit rate, but their gelling ion exchange and absorbing characteristics retard digestion and absorption (Krogdahl, 1986).

Enzyme supplementation in diet could be used to break down antinutritional substances found in feed raw materials, thus augmenting the digestive capacity of the birds, improving availability of feed nutrients such as starch, protein, lipid, minerals and vitamins in upper part of the gastrointestinal tract thereby improving feed conversion ratio (FCR) of birds (Nasi, 1989; Choct *et al.*, 1996) reported that enzyme supplementation largely eliminated the fermentation in small intestine, reducing the physiological stress in the intestine and hence improving the well being of the birds.

In the present study, polyzyme which comprises various digestive enzymes, was incorporated in the diet to evaluate the influence of different production parameters in broiler chicken (Table – 12) as well as its effects on immunological response to IBD vaccine (Table – 6). The result indicated higher QAGPT titre to IBD vaccine in the group which received polyzme (Gr. II) when compared with the titre in the IBD vaccinated but untreated group (Gr. VII) for the corresponding interval (Table – 6). The enhancement of immune response to IBD vaccine in polyzyme treated group (Gr. II) appears to be quite natural since any effort to improve feed utilization as well as for the fullest exploitation of nutrient content of the feed stuffs should have encouraging effect on the immune system of the

birds which could be finally expressed in terms of better immune response to a vaccine like IV 95 strain vaccine as employed in the present study. Rajmane *et al.* (1994) also reported stimulating effect of polyzyme on immune system of birds which further supports the present finding.

The vit. E used in this study is a form of synthetic a - tocopherol is dl-a-tocopherol. Which is now rather generally employed in clinical work, as a standard in either biological or physico-chemical assay, and as a source of the vitamin in experimental animal work (West *et al.*, 1996). Moreover, the use of vitamin E as adjuvant along with vaccines are an age old practice and is well documented (Tengerdy *et al.*, 1983; McKercher, 1986; Kumar, 1987; Tengerdy *et al.*, 1991a; Tengerdy *et al.*, 1991b; Hogen *et al.*, 1993; Franchini *et al.*, 1995, Rawat *et al.*, 2002)

In the present study dl-a-tocopherol acetate, was used to examine the countering the immunosuppressive effects of IV 95 IBD virus vaccine by way of enhancement of immune responses to RD vaccine, its role in cell mediated immunity by cutanaeous basophilic hypersensitivity reaction (CBH) as well as the effect on certain growth parameters due to its administration, which will be discussed afterwards several workers have reported the usefulness of vitamin E in potentiation of immune responses to viral vaccines (Franchini *et al.*, 1995, Shadaksharappa *et al.*, 1998). The investigators have even come up with a hypothesis on mechanism of immunostimulation by vitamin E (Tengerdy, 1975; Tengerdy and Brown, 1977; Tengerdy *et al.* 1990; Tizzard, 1996; Das *et al.* 1996). According to these workers, this vitamin acts as an antioxidant which prevents free radicals (Peroxides and Super Oxides)

release and combat some of the chemical reaction is cells that result in nitrated protein formation during the disease or vaccinal challenge from the damaged cellular and inter cellular structures which also includes lymphocytec cells of immune system. The vitamin E is stored in the lipoprotein fraction of cell membrane and acts as efficient scavanger of free radical. The toxicity of free radical to cell is mainly because of their attack on unsaturated fatty acid component of membrane lipid thus, damaging the membrane structure. On the other hand the nitrated protein formed during stress causes several undesirable effects, including slow growth and lower immune response (Durham, 2002). Recently vitamin E along with selenium have been found to be responsible for erythrocyte membrane integrity. Since both erythrocyte and lymphoid cell originate from the same stem cells, vitamin E may be associated with membrane fluidity of lymphoid cells, thus affecting immune response mechanism as well. Further vitamin E stimulates IgG synthesis. Interestingly the level of vitamin E to be included in the diet for above purpose should be ten to thirty times the recommended dietary level. Reports are also available having revealation that action of vitamin E is dose dependent and the immunomodulatory effect can be best appreciated when it is given in the amount several times higher than the dietary requirement i.e. 48 IU/Kg feed. (Tengerdy *et al.*, 1977; McIlroy *et al.*, 1993). In addition vitamin E increases phagocytosis probably by regulating the biosynthesis of prostaglandin, and their effect on functional activity and proliferative capacity of immune system cells such as B and T lymphocytes macrophages and polymorphonuclear dendritic and plasma cells (Franchini *et al.*, 1995). Again the E type prostaglandin is known to effect immune response (Likoff *et al.*, 1978) and supplementation of vitamin E reduces the

prostaglandin level in immunopoeitic organs and simultaneously improve antibody response. Therefore, it is obvious that whereas vitamin E has two folds action, first by way of its antioxidant property, thus preventing the release of free radicals and the second by inhibiting the production of prostaglandin E, thereby enhancing humoral immune response.

In the present study efforts were made to study the influence of this drug on immune response to IBD vaccine (IV95 strain). The result demonstrated that it has potentiating effect both on humoral immune and cell mediated immune response to IBD vaccine as evident from higher antibody titres in group III than the titres in group VII which received IBD vaccine but not any drug treatment overall the period post vaccination.

Similar studies conducted in this department using E-care-Se, a combination of vitamin E and selenium (Hindustani, 2000) and Charak-E-Sel, a combination of vitamin E, Selenium and Biotin also shown significantly high titres when given in IBD vaccinated (IV95 strain) chicken in comparison to their unvaccinated counterparts.

Ayurvedic medicines are largely based upon herbal and herbomineral preparations and specific diagnostic and therapeutic principles (Patwardhan and Hooper, 1992). Modulation of immune responses to alleviate the diseases, using natural ingredients, chiefly herbs, has been of interest for many years and the concept of "*Rasayna Chikitsa*" (herbal product therapy) in Ayurveda is based on related principles (*Charak Samhita*, 1949). The herbs or their product are

endowed with multiple property like delayed ageing, antistress, adaptogenic, rejuvenator and immuno modulating effects.

Several herbal / natural products have been documented for their immunomodulating properties growth promotion and enhancement of disease resistance. Of these, some of them are studied scientifically. (Sadekar *et al.*, 1998a; Sadekar *et al.*, 1998b; Thatte *et al.*, 1986; Kolte *et al.*, 1999; Suresh and Vasudevan; 1994; Ziauddin, 1996; Koul *et al.*, 1990; Godhwani *et al.*, 1988; Kuttan, G, 1996).

Bhang and Amla were the two other natural herbs/agents used in this study. Bhang (*Cannabis sativa*) is an indigenous plant belonging to family *Cannabinaceae*, naturalized in the Sub-Himalyan tract and abundant in waste lands from Punjab eastwards to Bengal and Bihar and extending southwards to Deccan. It is largely used for improving digestion, appetite and also to derive more energy, specially by rural people. Some workers reports it as a good appetizer, stomachic, antidiarrhoeal and effective nervine tonic (Dastur, 1977; Kirtikar and Basu, 1935), analgesic, sedative and anodyne (Chopra *et al.*, 1956). The biological activity of Bhang is due to phenolic and alcoholic compounds such as cannabinol, pseudo cannabinol and cannabinin (Chopra *et al*; 1956). Egyptian variety contains cannabidiol, Cannabol and Cannabinol. Pure cannabinol has no hashish activity; physiologically active fraction is a reddish brown oil. Cannin, a crystalline compound, when administered to a dog in dose of 0.1 mg/kg produces in co-ordination movement after 2 hrs. which persists for 4 hours. Alcoholic extracts of American Cannabis vary considerably in their relative activities.

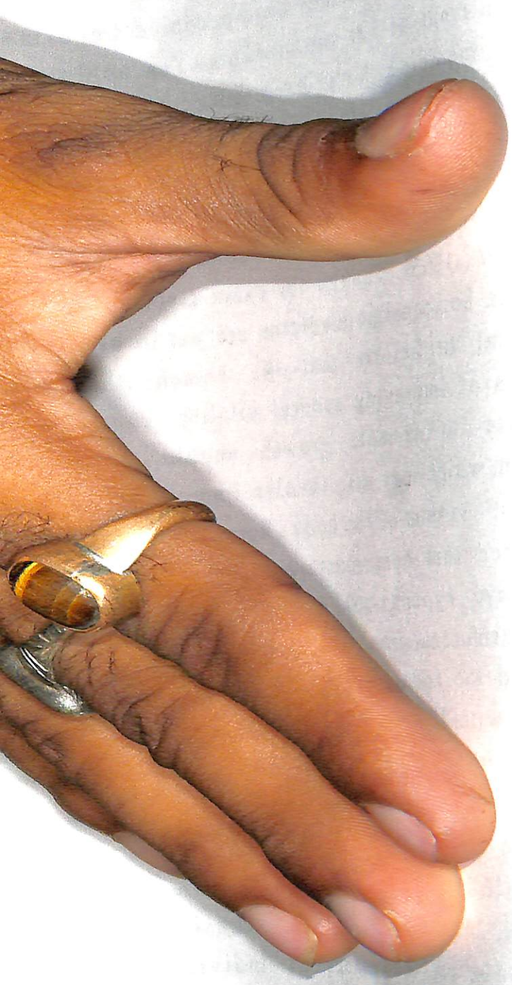
The present study has been undertaken to investigate the effect of *Cannabis sativa* dry leaves powder on immune responses in birds vaccinated with moderate hot strain IBD vaccine. This study clearly indicated that Bhang leaves feeding at lower dose (40 mg/bird) produced enhancing effect on antibody titres to moderate hot strain IBD virus over all the periods post IBD vaccination. When compared with antibody titre to group VII (Table – 6). The comparison of immune response exhibited by different drugs/agents used in this study clearly demonstrated that Bhang treated group showed the highest antibody titre post IBD vaccination (Table – 6).

Phyllanthus emblica (amla); Synonymously called *Emblica officinalis*, belongs to family *Euphorbiaceae*. Fruit is useful in haemorrhage, diarrhoea, dysentery, dyspepsia, anuria, constipation etc. It is reported that the fruit of amla has got antibacterial property against *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Agrobacterium lumefaciens* and *Mycobacterium* tuberculosis (Dhar *et al.*, 1968). Amla has pronounced anti-viral properties (Antarkar, *et al*; 1980; Babbar *et al*; 1982; Singh, 1983; Handa *et al*; 1986; Suresh and Tripathi, 1987). The antiviral activity detected in plants appear to be carried out by protein molecules resembling inter feron (Dhar *et al.*; 1968; Gupta *et al.*, 1985). It has been found that amla enhance natural killer (NK) Cell activity and antibody dependent cellular cytotoxicity and antibody dependent cellular cytotoxicity (ADCC) in mice (Suresh and Vasudevan 1994). A number of workers advocated the use of *Phyllanthus emblica* as an immunomodulator (Suresh and Vasudevan, 1994; Jamkhedkar, 1996; Chauhan, 2002). Additionally the fruit is rich natural source of Vitamin-C (Chopra *et al.*, 1956; Suresh and Vasudevan, 1994; Vijay

Kumar and Vasudevan, 1978). Besides, there are several reports regarding the immunostimulatory activity of ascorbic acid (Vitamin-C) through its influence on various immune parameters (Siegel and Mortan, 1977; Anderson, 1981; Brin, 1981; Shadaksharappa *et al.*, 1998; Wu *et al.*, 2000; Amakye, 2000) and also through proliferation of T-lymphocytes, lymphokine production, increase in antibody production (Chauhan, 2002), interferon inducing capacity (Siegel and Mortan 1977; Anderson, 1981) as well as marked increased level of cytolytic activity of killer cells (Brin, 1981). Reports are also available regarding its property to counteract the adverse effect of stress in poultry – both broiler and layers (Seokand and Singh, 1996; Dahir, 1996). Vitamin-C is also considered to be general antioxidant (Murray *et al.*, 1996; khopde *et al.*, 2001). It stabilizes vitamin A and enhance its intestinal absorption and its hepaic accumulation. The usefulness of this Vitamin has also been established in enhancement of immune response to IBD vaccine (Shadakshrappa et al., 1998; Wu et al., 2000; Amakye et al., 2000). Several workers have also reported supplementation of vitamin-C in poultry feed or through drinking water to replace the severe loss of this vitamin that is likely to occur during stress. Since, any vaccination exerts some sorts of stress it would be logical to expect higher immune response when vitamin-C supplementation is done. It was with this intention that effect of amla on immune response to IBD vaccine, due to its vitamin-C content and antiviral property, was studied and the result revealed its immunopotentiating effect on immune response to IBD vaccine as evident from antibody titres recorded at different intervals post IBD vaccination when compared with titres for corresponding intervals of the control group (Table – 6). The present findings is in agreement with

the findings observed earlier (Shadaksharappa *et al.*, 1998; Wu *et al.*, 2000; Amakye *et al.*, 2000).

Homeopathic medicines have occupied important position in the treatment of various ailments. The application of this group of medicine have been advocated to cure various conditions in poultry (Patra, 1983; Bera, 1983; Jagtap *et al.*, 1993; Samarth *et al.*, 2002). Presently some homeopathic drug have been employed to combat the adverse effect of vaccines, improve growth condition and counter stresses (Patra, 1983; Jagtap *et al.*, 1993). Though the exact mechanism of action of most of the homeopathic medicine are yet to be established, it was logical to believe that the homeopathic drugs with indications of improving general vitality, countering stresses and ability to promote growth should work through a mechanism which may also involve immunological component and defense system of the body. Recenty Samarth *et al.*, 2002 has reported that homoeopathic drugs probably act through hypothalamic hypophyseal axis thereby regulating hunger, thirst, thermoregulation, and metabolism. It was in this background the homoeopathic medicines were considered for use along with IV95 IBD vaccine. Initial study on effect of homoeopathic drug combination consisting of *Thuja occidentalis Carbo Vegetabilis and Carbo animlis* on immune response to IBD vaccine as well as immune response to RD vaccine in IBD vaccinated birds provided encouraging results (Hindustani, 2000). After this it was considered worthwhile to conduct study on the effect of individual component of homoeopathic drug combination used earlier. It was in this context that one of the component of the drug combination namely "*Carbo animalis*" was used in order to study its effect on immune response to IBD vaccine as well as imuune



response to RD vaccine in IBD vaccinated chickens. The precipitating antibody titres in *carbo animalis* treated group (Group VI) clearly demonstrated a higher antibody levels at all intervals post IBD vaccination when compared with the values for the corresponding intervals of group VII (Control). *Carbo animalis* also showed high HI titre to RD vaccine as evident from the values recorded for group VI (Table-7) when compared with the HI titres for the corresponding interval of group VII overall the intervals post IBD vaccination. Therefore, it is obvious that *Carbo animalis*, component of earlier used homeopathic drug combination consisting of *Thuja occidentalis, Carbo Vegetabilis* and *Carbo animalis* has immunopotentiating effect on immune response to IBD vaccine as well as immune response to RD vaccine in birds immunocompromised by vaccine strain of IBD virus. The present finding is quite significant because it has been possible to bring improvement in antibody titre in both the cases which were by and large comparable to the titre obtained when the combination of *Thuja oc*; *Carbo. Veg.* and *Carbo. anim.* had been used (Hindustani, 2000).

On overall consideration, the present finding with respect to use of carbo animalis for enhancing antibody levels after IBD vaccination as well as after RD vaccination in IBD vaccinated chickens is sufficiently suggest that *carbo animalis* alone or in combination of three homoeopathic drugs used earlier can be used as immunomodulating agent (Hindustani, 2000). Because this is going to be cost effective and it will also avoid the unnecessary use of the other two drug components of combination. It would be also possible to hold this observation until such time we conduct study with respect

to the other two components of the combination namely *Thuja occidentalis* and *carbo vegetabilis*.

Immunosuppressive effect of IBD virus is well documented (Faragher *et al.*, 1974; Ajinkya *et al.*, 1980; Nakamura *et al.*, 1992; Praveen *et al.*, 1995; Christopher *et al.*, 1997). However, variations in degree of immuno suppression have also been reported by a number of workers from time to time (Thornton and Pattison, 1975; Giambrone and Clay, 1986a; Mazariegos *et al.*, 1990). A number of factors such as strain of virus age of birds at infection as well as size of inoculum have been attributed for variation in degree of immunosuppression (Edwards et al., 1982; Ezeokoli *et al.*, 1990; Mazariegos *et al.*, 1990; Mahesh and Muniyappa, 1996; Bekhit, 1997). Control of IBD by prophylaxis is a common practice worldwide. Contrary to this, there are numerous reports that vaccine strain itself produced some degree of immunosuppression (Montgomery *et al.*, 1986; Ezeokoli *et al.*, 1990; Mazariegos *et al.*, 1990; Saif, 1991; Das *et al.*, 1996; Thangavelu *et al.*, 1998) Kumar (2000) also reported that IBD vaccine had lowering effect on HI titre to RDV. In the present study IV95 strain IBD vaccine showed lowering effect of HI titre to RDV (F strain and LaSota strain) Vaccine when compared with the corresponding value of the control group (group IX) over all the intervals till the termination of experiment (Table – 7).

The recently introduced moderate hot strain IBD vaccine marketed in the name of invasive intermediate strain (IV95), Inter plus or Bursine plus etc. are pronounced to have relatively more invasiveness and residual pathogenicity as well. Consequently such strains have more efficacy for

protection and immunosuppressive effect of higher magnitude than the conventional vaccine (Kowenhoven and VandenBos. 1994; Coletti *et al.*, 1994; Surashe, 1996; Khaliel *et al.*, 1998; Kumar, 1998; Kumar, 2000. In a consolable situation where even the vaccine strain can have marked immunosuppressive effect as apparent from significantly lower level of antibody titre to RD vaccines in IBD vaccinated birds of group VII when compared with titres in group IX (Table – 7), it would not be out of way to suggest the use of drugs/agents concomitantly with IBD vaccine in order that the aftereffect of vaccine is prevented or at least minimized without in anyway affecting the response to IBD vaccine. In the present study all the six drugs/agents employed showed immune enhancing effect on antibody response to RD vaccine in IBD vaccinated chickens. Interestingly these agents also showed potentiating effect on response to IBD vaccine itself which have been discussed above in this eontext. The comparison of antibody titres to RD vaccine in treatment groups (gr. I-VI) revealed that the antibody levels differed. Significantly among themselves by and large the titres were higher than that recorded in vaccinated group which did not receive any drug (gr. VII). Further, the comparison of antibody titres of treatment groups with the titres observed in none IBD vaccinated untreated control group (gr. IX) showed that whereas all the six drugs/agents had different degree of immunopotentiating effect on response to RD vaccination, none of the treatment group exhibited antibody level comparable to group IX except the group which has been treated with bhang (gr. IV) where a non-significant, albeit higher HI titre was recorded. In other words only bhang was able to bring improvement in antibody level to RD vaccine at par, with levels of antibody shown by none IBD vaccinated control birds (group IX) while other

immunopotentiating agents were not efficacious in improving the antibody level to RD vaccine at par with the control group (group IX) (Table 7). A number of workers have reported that in general the immunomodulators have enhancing effect on immune response to vaccines in immunocompromised animals but in most of the cases they failed to bring the antibody level comparable to one observed in immunocompetent animals (Singh *et al.*, 1993; Sadekar *et al.*, 1998a, b; Sarabanabava *et al.*, 1999; Kalita and Dutta, 1999; Saravanabavaet *al.*, 2001). Which supports the present findings.

In the present study altogether six drugs were evaluated for their immunopotentiating effect on response to RD vaccine in birds receiving moderate hot strain IBD vaccine (IV95 strain). The bhang showed the best immune enhancing effect on HI antibody titre to RD vaccine followed by levamisole and amla, *Carbo animalis* and Vitamin-E, and polyzyme treated groups (table 7). As the titres in the different treatment groups did not differ significantly but differed significantly except bhang treated group (gr. IV) and polyzyme treated group (Gr. II) and also that titres were higher than protective level ($\log_2 4$), all the six drugs could be accepted as acceptable subject to the cost effectiveness. duration of treatment and ease of availability. However, the use of the homeopathic drugs to control immunosuppressive effect of IBD virus is rarely reported and therefore, it would be advisable to undertake further study to suggest its mechanism of action. In the mean time the present study is clearly indicative of the fact that the above noted drugs have immune enhancing effects and therefore, may be advised to poultry farmers for use in chickens to be vaccinated with IBD vaccine especially intermediate plus strain of IBD vaccine or IV95 strain vaccine.

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Pathological changes and lesion score proved a very sound criteria for determining the virulence of IBD virus (Muskett *et al.*, 1979; Naqi *et al.*, 1980; Mazariegos *et al.*, 1990). The same criteria has -also been extensively used to assess the residual pathogencity of vaccine strain of IBD virus(Mazariegos *et al.*, 1990; Khaliel *et al.* 1998; Jeurissen *et al.* 1998). The microscopic changes noted in the bursa of vaccinated chickens were characteristic of IBD virus (Winterfield and Thacker, 1978; Winterfield *et al.*, 1980; Ezeokoli *et al.*, 1990). In the present study lymphoid necrosis and depletion in the bursa of Fabricius constituted the predominant lesions. Several workers have recorded both necrosis and depletion in the bursa of Fabricius as the main lesions in IBD infected/vaccinated birds which corroborates the present findings. (Del Bono *et al.*, 1968; Cheville, 1967; Thornton and Pattison, 1975; Ajinkya *et al.*,1980; Ley *et al.*, 1983; Edwards *et al.*, 1982; Lukert and Hitchner, 1984; Ezeckoli *et* al., 1990; Jhala *et al.*, 1990; Khafagy *et al.*,1991; Yamaguchi et al., 1996). The other changes recorded during this study such as inter follicular oedema, epithelial invagination al., 1995; Panigrahy et al., 1986; Del Bono et al., 1968; Edward et al.,1982; Lukert and Hitchner, 1984). Where as the histological changes recorded in the bursa of Fabricius were clearly suggestive of the possession of residual pathogenicity in the Intermediate plus IBD vaccine virus (Coletti *et al.*, 1994) observed . Thronton and Pattison, (1975) also studied nine IBD vaccines obtained from seven different sources and found that each of the vaccines invariably produced bursal damage of varying degree as evidenced by histopathological changes but none of them produced clinical disease. They also opined that the variations in protection levels observed with different

vaccines did not depends on degree of damage to the bursa. Hence the sort of histopathological lesions recorded in bursa of vaccinated chickens which was not accompanied by clinical disease may not be taken as criteria for non-acceptance/acceptance of the vaccine unless it proves so in challenge study.

The bursa: body weight ratio provides one of the important criteria for determining the residual pathogenicity and immunosuppressive effect of IBD virus by a number of workers (Giambrone and Clay, 1986a; Ezeokoli *et al.*, 1990; Mazariegos *et al.*, 1990; Christopher *et al.*, 1994; Hussain and Saif, 1996; Jackson, 1996, Okoye, 1996; Makesh *et al.*, 1998; Sivaseelan and Balachandran, 1999). The B.BW ratios were invariably higher in groups which received IBD vaccine (gr. I-VII) when compared with the value in group IX which did not received IBD vaccine. However, an overall consideration of the findings recorded in the present study B:BW ratio alone can not be taken as a criteria for determining the residual pathogenecity of vaccine virus. Therefore present study also different three parameter were considered for determining the degree of pathogenecity of the vaccine strain employed.

Several workers have studied the body weight gain and feed conversion ratio in order to find out the overall response of IBD vaccine in chicken (Tengerdy, 1975; McIlroy *et al*; 1993; Rao et al., 1995; Kwon., 2000). In the present study also the birds which had received only IBD vaccine but not any drug (group VII) exhibited lower body weight gain and higher FCR when compared with non IBD vaccinated non-treated control group (gr. IX). The result further suggested immunosuppressive properties inherent in the vaccine strain

employed in this study. The present finding is in consonance with the earlier observation made by a number of workers that IBD vaccine virus are also having some degree of immunosuppressive characteristic as evidenced by lower immune response to RD vaccine, poor body weight gain, feed conversion ratio and enhanced mortality. (Mahesh and Muniyappa, 1996). In this study percent mortality in IBD vaccinated but untreated group (gr. VII) was 7.5 whereas it was 5.0 percent in control group (group IX). However, application of different drugs (group I-VI) helped by improving body weight gain, FCR as well as mortality when compared with the corresponding valued of group VII which received only IBD vaccine and no other treatment (Table – 12). Out of the six drugs amla showed the best results in terms of body weight gain, FCR as well as mortality (Table-12) whereas polyzyme treated group could bring the lowest degree of improvement in respect of above parameters when compared with other treated groups. A more remarkable findings observed after administration of different drugs/agents (gr. I-VI) were the improvement in body weight gain and FCR over and above the corresponding values in unvaccinated and untreated control group (gr. IX) except the bhang treated group where the highest body weight gain was accompanied by higher FCR in comparison to control (gr. IX).

The present study suggest that there is scope of further improvement in immune response to IBD vaccine. The study also revealed that the vaccine virus has residual pathogenicity and immunosuppressive effect as evidenced by immune response to RD vaccine body weight gain, FCR and percent mortality. The result pertaining to above parameter in the drug treated groups (gr. I-VI) showed encouraging results.

The cell mediated immunity in the chickens depends on the activity of thymus dependent lymphocytes. The experiments conducted to study cell mediated immune response of chickens infected with IBD virus gave conflicting results. Several workers reported CMI response in IBD infected birds (Sivanandan and Maheswaran, 1981; Lukert and Hitchner, 1984; Singh, 1987; Rao *et al.*, 1985; Vegad, 1996).

As stated earlier that Invasive intermediate strain of IBD vaccine has inherent property to suppress immunity albeit to lower degree. In the present study also the Invasive intermediate vaccine (IV95) also produced suppressive effect on the thymus dependent cellular response in IBD vaccinated chickens as measured by cutaneous basophilic hypersensitivity to DNCB, PHA and PPD (tuberculin). Relatively lower degree of monoculear cells infiltration in IBD vaccinated chickens as evidenced from skin section from IBD vaccinated chickens than those observed in IBD unvaccinated and untreated groups. Treatment with different drugs/agents brought about improvement in cell mediated immunity as evidenced from enhanced CBH response. Further, on comparison among treatment groups (gr. I to VI) amla showed best CBH response to mitogen (DNCB, PHA and PPD) whereas the lowest CBH response was elicited by polyzyme treated group.

Similar improvement in cell mediated immune response in IBD infected birds were also reported using different agents (Rao, *et al.*, 1985; Sadekar, 1998a; Sadekar, 1998b; Kolte *et al.*, 1999).

Bursa:Body weight ratio (B:BW), bursal lesion scoring and demonstation of IBDV antigen by AGPT are important parameters to evaluate the results of challenge study (Ismail and Saif, 1991; Hassan and Saif, 1995; Makesh et al., 1998). In the present study also B:BW ratio, AGPT and lesion score were taken as parameters to evaluate the immunosuppressive effect and efficacy of protection of IV95 vaccine against IBD infection in chicken. The B:BW ratio was lower in IBD unvaccinated chicken (gr., IX) which indicated a bursal damage. The IBD vaccine, group (gr. I-VII) were thus, protected against IBDV. Among the treatment groups (gr. I-VI) bhang and amla treated group had the highest B:BW ratio which indicated, better protection against IBDV. *In toto*, the six drugs used for the study were helpful enough to enhance immune response so as to protect the bird against infection.

The lesion score indicated that the highest score was recorded for IBD unvaccinated and untreated group (gr. IX) in comparison to Group VII which is indicative of the susceptibility towards IBD infection. Further the IBD vaccinated and treated groups (gr. I-VI) has significantly lower lesion score. The least lesion score was recorded for amla treated group followed by levamisole, bhang Vitamin-E, carbo animalis and polyzyme treated group. All the six drugsemployed here helped in reducing the lesion score because of enhanced immunity.

The absence of IBDV antigen in IBD vaccinated group may be due to neutralization of the challenge virus by the increased level of antibodies. Further all the six drugs employed here enhanced immunity and sustained the challenge study as evidenced by high B:BW ratio, low bursal lesion

score, absence of IBDV antigen and absence of mortality are indicative enough that all the drugs used here has got immune enhancing property.

CHAPTER VI

SUMMARY

vvIBD has been reported from all poultry producing pockets of Bihar (Singh, 2001). To combat the menace of this disease moderate hot strain (invasive strain) has been incorporated in the regimen of prophylaxis. However, it is reported that the vaccine brings out immunosuppression and residual pathogenicity. Earlier studies conducted in this department also confirms the above observation. Therefore, to counteract the aftereffect of moderate hot strain vaccine, quest for certain drugs/agents was felt necessary to ameliorate the immunosuppressive effects of vaccine strain of IBD virus. With this view the present study was planned.

A total of 450-day-old broiler chickens were procured and divided into nine groups, each group consisting of 50 chicks. The birds were vaccinated for RD at 5 and 25 day of age employing F and LaSota strain RD vaccine, respectively. The birds were also vaccinated on 10-day of age against IBD by "moderate hot strain" IBD vaccine except the group VIII which received field virus instead of IBD vaccine and group IX which received PBS instead. First six groups were treated with levamisole,polyzyme, Vitamin E, bhang, amla and *Carbo animalis*, respectively. Blood samples were collected at weekly interval post IBD vaccination; serum was separated and processed to determine QAGPT for IBD vaccination and HI titre for RD vaccination.Five birds from each group were sacrificed 96 hours post IBD vaccination and Bursa: Body weight ratio was determined. 5 birds from each group were selected separately on day-14 of age for DNCB, PHA-P and PPD (tuberculin) to determine cutaneous basophilic hypersensitivity reaction. The birds were sensitized using

respective mitogen. On 14-day of sensitization; birds were challenged using same mitogen and swelling of abdominal skin was measured as on 12,24 and 48 hours post challenge.

The mean QAGPT was highest in bhang treated group followed by amla, *Carbo animalis*, Vitamin E, levamisole and lowest in polyzyme treated groups. The results revealed that all the six drugs/agents employed in the study showed immune enhancing effect. The precipitating antibody titre in different treatment groups showed increasing trend till last day of observation(35 dpv)except group II and group VII which showed increase only at 28 dpv.

In the present study IV95 strain IBD vaccine demonstrated immunosuppressive effect on HI titer to RD vaccine. Further, all the six drugs employed showed immunopotentiating effect on antibody response to RD vaccine in IBD vaccinated chicken. The HI titre recorded in bhang treated group produced higher titre followed by amla, levamisole, *Carbo animalis*, Vitamin E and polyzyme treated groups.

The bursa: body weight (B:BW), ratios were invariably higher in different drug treated groups when compared with value of IBD vaccinated but untreated groups and IBD unvaccinated and untreated group. Similar trend in B:BW ratio was observed in case of birds sacrificed at the termination of experiment except the values were markedly lower than values recorded at 96 hrs. post IBD vaccination.

Possession of residual pathogenicity by IV95 strain was further validated by production of typical lesion in the bursa of Fabricius. The mean lesion score was higher in birds which received only IBD vaccine but no treatment (gr. VII). The different drug given in groups I to VI helped in reduction of bursal lesion score.Among the treatment groups lowest lesion was detected in amla treated group followed by bhang, *carbo animalis*, vitamin E, levamisole and polyzyme treated group. No changes were appreciable in the control group (gr. IX) which neither received IBD vaccine nor any medication.

The body weight gain was lowest in case of IBD vaccinated untreated group (gr. VII) followed by control group (gr. IX) whereas, the values were invariably higher in case of different treatment groups (gr. I to VI) when compared with group VII. Further the body weight gain was highest in bhang treated group followed by amla. Vitamin E, levamisole, *Carbo animalis* and polyzyme treated groups.

The feed conversion ratio was highest in bhang treated group followed by control group (gr. VII) and was lowest in levamisole treated group of chicken. The general trend was suggestive of poor ratio in IBD vaccinated but untreated group. However, it was better in chickens receiving various treatment groups(gr.I-VI). Among the treatment groups lowest FCR was recorded in levamisole treated group and it was poorest in bhang treated group. However, the ratios in various treatment groups were

better when compared with the values even in control group.

The CBH response using DNCB, PHA-P and PPD (tuberculin) reflected, suppressive effect on cell mediated immunity. However, drug treatment ameliorated the adverse effect of IV95 vaccine. Among the treatment group highest CBH was elicited by amla treated group followed by bhang, levamisole, *Carbo animalis*, Vitamin E and polyzyme treated group which are evidenced by increased skin thickness.

IV95 strain IBD vaccine was sufficiently protective against virulent IBD virus which was used for challenge study. The drug treated groups were competent in overcoming the immuno suppressive effect of the vaccine strain as evidenced by low bursal lesion score, better bursa: body weight ratio. This further suggests the immuno potentiating property of the drugs/agents employed in this study.

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CHAPTER VII

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