EVALUATION OF CERTAIN IMMUNOMODULATORY AGENTS IN COUNTERING IMMUNOSUPPRESSIVE EFFECTS OF A VACCINE STRAIN OF INFECTIOUS BURSAL DISEASE VIRUS IN CHICKEN

THESIS

SUBMITTED TO THE

RAJENDRA AGRICULTURAL UNIVERSITY

(FACULTY OF VETERINARY SCIENCE)

In partial fulfilment of the requirements

FOR THE DEGREE OF

Master of Veterinary Science

IN

VETERINARY MICROBIOLOGY

By

Rekha Teresa Kujur

(Registration No. M/Vety. Micro./27/1998-99)

Department of Veterinary Microbiology

BIHAR VETERINARY COLLEGE

PATNA, BIHAR (INDIA)

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2001


Dedicated to
My
PARENTS

Department of Veterinary Microbiology

Bihar Veterinary College, Patna – 800014.
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CERTIFICATE – I

This is to certify that the thesis entitled "*EVALUATION OF CERTAIN IMMUNOMODULATORY AGENTS IN COUNTERING IMMUNOSUPPRESSIVE EFFECTS OF A VACCINE STRAIN OF INFECTIOUS BURSAL DISEASE VIRUS IN CHICKEN*" submitted in partial fulfilment of the requirements for the **Degree of Master of Veterinary Science (Veterinary Microbiology) of the faculty of Post-graduate studies, Rajendra Agricultural University, Pusa, Bihar** is the record of bonafide research carried out by **Dr. Rekha Teresa Kujur** 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 Advisory Committee of **DR. REKHA TERESA KUJUR**, a candidate for the degree of **Master of Veterinary Science** with **Major in Veterinary Microbiology,** have gone through the manuscript of the thesis and agree that the thesis entitled "***EVALUATION OF CERTAIN IMMUNOMODULATORY AGENTS IN COUNTERING IMMUNOSUPPRESSIVE EFFECTS OF A VACCINE STRAIN OF INFECTIOUS BURSAL DISEASE VIRUS IN CHICKEN***" may be submitted by **Dr. Rekha Teresa Kujur** in partial fulfilment of the requirement for the degree.

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LIST OF ABBREVIATIONS

Ab	Anti body
AGPT	Agar gel precipitation test
ANOVA	Analysis of variance
BF	Bursa of Fabricius
^{0}C	Degree Centigrade
CS	Course Spray
Co.	Company
Dr.	Doctor
dpv	Days post-IBD vaccination
D.F.	Degree of Freedom
D.W.	Drinking Water
ELISA	Enzyme Linked Immuno Sorbent Assay
EDTA	Ethylene diamine tetraacetic acid
edn.	edition
Fig.	Figure
FCR	Feed Conversion ratio
g	Grams
gr.	Groups
H & E	Haemotoxyline and Eosin
Hb	Haemoglobin
HA	Haemagglutination
HI	Haemagglutination inhibition
Homoeo. dr.	Homoeopathic 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
LDH	Lactate dehydrogenase
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
PI	Post infection / post inoculation
PCV	Packed Cell Volume
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
SGOT	Serum Glutaimic oxalic transaminase
SRBC	Sheep red blood cells
TLC	Total Leucocyte count
Thuja oc.	Thuja occidentalis
Uv	Unaccinated
v	vaccinated
vvIBD	Very virulent infectious bursal disease
WLH	White Leghorn
Wt.	Weight
W/V	Weight by volume
%	percent

CHAPTER - I

INTRODUCTION

INTRODUCTION

Infectious bursal disease (IBD) has emerged as one of the most important poultry disease in recent past because of its wide spread occurrence and its ability to cause heavy morbidity and mortality resulting in substantial financial loss to farmers. It is highly contagious and immunosuppressive viral disease of young chickens with the greatest incidence in chickens of 3-6 weeks of age. IBD was first reported in 1962 by Cosgrove in United States of America from a place named Gumboro near Delware. The term infectious bursal disease (IBD) was proposed by Hitchner (1970). On the basis of pathognomonic lesions in the bursa of Fabricius. In India, IBD was reported for the first time by Mohanty *et al.* (1971) from U.P. and subsequently from different parts of the country by different workers (Chetty, 1975; Rao *et al.*, 1979; Kumar *et al.*, 1984; Panisup and Verma, 1986; Sharma *et al.*, 1993; Sah *et al.*, 1995; Survashe, 1996 and Thangavelu *et al.*, 1998) and the disease has now become endemic in the country.

Infectious bursal disease causes destruction of lymphocytes in the bursa of Fabricius and other lymphoid organs. The virus produces direct and indirect effects in susceptible chickens. The direct effect is manifested in the form of the death of affected chickens, retardation in growth rate and weight loss. Indirectly, IBD

causes impairment of immune system of the infected birds leading to lowered immune response to vaccination against several important infections like Ranikhet disease Marek's disease and infectious bronchitis.

The conventional IBD vaccine including mild strain (Lukert type) IBD vaccine used in day old chicks, Intermediate strain (Georgia type) IBD vaccine which is given in 14-18 day old chicks and inactivated IBD vaccine which is largely used in breeder flocks as a booster vaccine, are not sufficiently effective in controlling the newly emerged pathotype of classical Serotype-I IBD virus which causes high morbidity and mortality and is commonly referred to as very virulent infectious bursal disease (vvIBD). This new form of IBD was first reported in 1987 form Netherland (Box, 1989) and subsequently from different parts of European and Asian countries such as England (Chettle *et al.*, 1989), Belgium (Vandenberg *et al.*, 1991), Germany (Oppling *et al.*, 1991), (Brown *et al.*, 1994), Japan (Tsukamoto *et al.*, 1992), Botswana (Wibberly, 1994) and Egypt (Khafagy *et al.*, 1991; Bekhit, 1997) etc. In India, this disease was for the first time reported from the state of Bihar (Singh *et al.*, 1994) in 1991 and subsequently recorded in other parts of the country including Southern States (Sulochana *et al.*, 1991, Sah *et al.*, 1995, Survashe, 1996). The clinical picture of the vvIBD is marked by sudden onset, whitish diarrhoea,

splashing haemorrhages in the skeletal muscles, oedema and haemorrhages of bursa of Fabricius, high morbidity and mortality ranging between 40-70% in layers and 20-30% in broilers. The mortality may go up to 80% or above in complicated cases, especially when concomitant infection of inclusion body hepatitis or RD virus infection is present.

vvIBD outbreaks are being reported even in vaccinated lots. Besides birds with passive immunity are also susceptible to vvIBD as this virus strain has ability to cross maternal antibody barrier of birds with passive immunity and cause the disease. This has led to emergence of many disease which were under control and against which we had developed the so called potent vaccines. Recently introduced intermediate plus strain or Invasive intermediate strain or moderate hot strain IBD vaccine appears to be the only vaccine virus that can confer effective protection against the newly introduced vvIBD. This vaccine strain has been shown to have ability to penetrate high level of M_{Ab} and incite immune response but are also reported to produce varying degree of immunosuppression. (Kowenhoven and Bos 1994, Khaliel *et al.*, 1998), (Kim *et al.*, 1999), (Mazariegos *et al.*, 1990), (Tsukamoto *et al.*, 1995). To make this vaccine more meaningful and also to combat its adverse effects in terms of residual pathogenicity and immunosuppressive effects, the use of certain immunostimulatory agents are being felt very seriously.

Immunomodulators are drugs that directly modify a specific immune function or have a net positive or negative effect on the activity of the immune system. Depending on the dose range, one and the same agent is able to exert immunostimulatory as well as immunosuppressive effects, many a times a general term immunomodulators is used.

Now-a-days a number of immunostimulating agents are available but their efficacy have not been conclusively tried in case of IBD. Some works in this direction have been reported specially with respect to use of Levamisole (Singh and Dhawedkar, 1993; Singh *et al.*, 1988), *Azadirachta indica*, a neemoil (Upadhyay *et al.*, 1992), Royal jelly, a secretion of nurse bee (Al-Mufarrej *et al.*, 1997), Vitamin E (Franchini, 1995; Tengerdy, 1975), Levamisole, vitamin E and vitamin C (Shadaksharappa *et al.*, 1998), Vitamin A (Skalan *et al.*, 1994), *Azadirachta indica*, a Neem leaves (Sadekar *et al.*, 1998b), *Ocimum sanctum*, a Tulsi leaves (Sadekar *et al.*, 1998a), Thymus extract (Abdel – Fattah *et al.*, 1999), *Ficus racemosa*, a leaf gall of Gular (Kolte el al., 1999), Thymic hormones and Zinc (Barbour *et al.*, 1998), Tuftsin, a tetrapeptide (Sarvanabava *et al.*, 1999), Stressroak (Pradhan, 1995; Ather, 1996) and IMMU – 21 (Chatterjee, 1994a), *Mycobacterium phlei* (Kishore *et al.*, 1998) etc. in controlling immunosuppressive effects of IBD. Whereas the earlier works were limited to potentiate the immune response of conventional IBD

vaccines and to counter its side effects but no work has been reported on use of immune enhancing agents to counteract the adverse effect of newly introduced intermediate plus IBD vaccine. Considering the usefullness of similar type of agents or drugs to combat the immunosuppressive and residual pathogenecity of newly introduced Intermediate plus IBD vaccines, the present study was proposed with the following objectives :-

1. To study the immunosuppressive effect of Intermediate plus strain of IBD vaccine in chicken.
2. To study the efficacy of selected immunomodulatory agents incountering the immunosuppressive effect of above vaccine in chicken.
3. To study the certain blood parameters and blood bio-chemical profiles of IBD vaccinated chickens treated with drug/agents or combination of agents.

CHAPTER - II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Cosgrove (1962) was the first to report this disease as 'avian nephrosis' in young broilers of 3-4 weeks age. He recognised the first outbreak of IBD in united state from a place named Gumboro near Delaware. From its geographical origin the disease is also known as 'Gumboro disease'.

IBD is characterized by clinical signs like depression, anorexia, retarded growth, white and watery diarrhoea and ruffled feathers. Gross lesions include changes like edematous and haemorrhagic bursa with atrophy in later stages, petechial haemorrhages in skeletal muscles and at the juncture of proventriculus and gizzard. Spleen, kidney and thymus are the other organs involved (Lukert and Hitchner, 1984).

IBD is now reported from all major poultry producing areas world wide (Okoye, 1984). In India, the disease was first reported from Uttar Pradesh by (Mohanty *et al.*, 1971). 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; 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.

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 secretary 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 quantitative agar gel precipitation test (QAGPT) was used to measure the maternal antibody level in chicks form IBDV infected parents (Wyeth and Cullen, 1976). Wood *et al.* (1979) standardized 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 9 products from 7 different sources intended for use as vaccines against infectious bursal disease of chickens. No vaccine caused clinical disease after administration to chicks at 7 days of age, but one cause significant impairment of weight gain, and when given to one 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 and reduction in size of this organ. 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.

Winter field 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, where as AGPT positive chickens were always protected.

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

Edward *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 old. 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 ten bursa examined.

The serological study of the IBD vaccinated group given *Brucella abortus* strain 19 at 7 and 14 days old developed mean titre which were significantly depressed with those of the relevant controls. Some degree of immunosuppression also observed when the birds were inoculated with *Brucella abortus* S19 on 21 and 28 days post vaccination. On 35 days post vaccination immunosuppression became non-significant while on 42 days post vaccination titre to *Brucella* was higher but not significantly differ.

Mc Ferran *et al.* (1982) conducted field studies with an inactivated vaccine against infectious bursal disease. Vaccination using an inactivated IBD 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 old specific pathogen free chickens inoculated with IBDV. A detectable precipitin antibody response occurred between 3 and 5 days post inoculation. Evaluation of pooled serum samples obtained from infectious bursal disease virus infected chicken revealed 58% decrease in potassium concentration, 63% decrease in cholesterol concentration at day 3 PI, from the pre inoculation value. LDH values increased 52% on day 3 PI and then decreased 45% by day 7 PI. SGOT increased 59% (day 3 PI) above the pre inoculated value. Electrophoratic profile of equal volume pools of pre inoculation and infected group sera showed 67% decrease in albumin and 50% increase in alpha-1 globulin, 53% increase in gamma globulin and 52% decrease in albumin : globulin ratio on day 3 PI in comparison to pre inoculated samples. Individual serum samples analyzed for uric acid concentration indicated that several IBDV infected chickens have serum uric acid concentration above the normal comparison range.

Histopatholgic 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. Non specific 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 follicle. The lymphocytes were soon replaced by hetrophils, debris and reticulo epithelial 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 (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 the intermediate IBV 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 the experiment he concluded that in IBV 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 cholesterol 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 bursa of Fabricius were characterised by massive cystic degeneration of follicle with depletion of lymphocytes, interfollicular oedema and fibrosis and infiltration by heterophils and macrophages. No lesions were present in the bursa of Fabricius of control chicken.

Ezeckoli *et al.* (1990) evaluated the affect 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 heterologous vaccine or wild type IBD virus. The Bursa Fabricius was examined for histopatholgoical 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.

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 5 days observation period. All the birds showed significant enlargement of

bursa of Fabricius at 48 hours PI and atrophy at 5 days PI. Histophathologically there were necrosis and depletion of lymphocytes in bursal follicles at 48 hours PI. Bursal lesion became severe at 5 days PI, where bursal follicles were found atrophied with regression on size. The corticomedullary epithelium showed formation of cyst severe proliferation of fibrous connective tissue was observed in the interfollicular space. 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 specific 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. The result reveals that these vaccine 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.

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

Kumar and Rao (1991) studied the haematological and blood biochemical changes in experimental infectious bursal disease virus infected chickens. Experimentally infected birds developed leukocytosis, heterophilia, lymphocytopenia, decreased hemoglobin value and packed cell volume, prolonged clotting time and prothrombin time, raised cholesterol, creatinine and lactate dehydrogenase and decreased glucose, uric acid, urea and acid and alkaline phosphatase concentration.

Nakamura *et al.* (1992) compared the immunosuppressive effect of highly virulent infectious bursal disease virus on vaccination against New castle 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 IBDV 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 isolate from such outbreaks was investigated in SPF chickens. Infected birds developed diarrhoea within 24 hours of infection and showed depression, trembling, ruffled feathers and were prostate. The 5 isolates caused 30-70% 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 surviving 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 M_{Ab} 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 56 hours later and their bursa 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.* (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 commercial 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 (48 IU/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 without 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. 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.

Singh *et al.* (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 of 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.* (1994) investigated the immuno-modulatory 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 experimental animals, it may be due to its decreasing effect on circulating level of corticosteroids under the basal level. Increase in soluble immune complex in the serum of the experimental animals also indicated immunopotentiating action of IMMU-21.

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 material antibody levels at the time of vaccination.

Kouwenhoven *et al.* (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 Netherlands 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, LZ228E and Bursa plus 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 is slightly more virulent than the other two 'hot' vaccines. They also observed that 'hot' vaccines were slightly more pathogenic than the Intermediate vaccines.

Pande *et al.* (1994) studied the immunomodulatory effect of Zeetress in chicken vaccinated against ND (F strain) virus. Zeetress was administered at the rate of 5g/1000 chicks through the drinking water for first 10 consecutive days and thereafter at the rate of 10g/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 intraconjuntival inoculation at one day old. Two groups of IBD infected birds and two 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 (Ecare Se), at the rate of 25 mg/bird orally in drinking water on alternate days from one day old through out the experiment, was given to 2 groups of birds. 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. Bursa : body wt. index lower than 0.85 were considered to have bursal atrophy. Antibody titres were detected only in response to *B. abortus* stimulation. The immunosuppressive effect of the IBD virus was indicated by the fact that the IBD infected untreated (with 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. 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%. Three virus isolates were recovered from the affected tissue. Majority of acute IBD outbreaks followed revaccination with RD vaccine.

Kembi *et al.* (1995) compared the effect of three different routes of administration 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 chicks (layer) were divided into 8 equal groups, which included 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 mediated 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 atrophied as a result of live IBD vaccine were partially protected / spared 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 was present in viral vaccine lacking bacterial antigens.

Kouwenhoven and 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 chickens vaccinated with Gumboro. 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 immunosuppresive 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 manufactures 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 day and their half-life was 3.6 and varied among breeds under field conditions. 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.

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 embayonated 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 infected chicken were similar to those in classical strains infected chickens. Cross-Virus neutralization analysis showed antigenic diversity between the cell culture adapted HV-IBDV strains and classical strains. 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 IBD 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 after 7 day post vaccination. The increase of antibody was found 14 days after vaccinations. No clinical sings of IBD were seen.

Al–Mufarrej *et al.* (1997) studied the immunostimulating effects of Royal jelly (Secretion of nurse bee) in chickens when immunized with sheep red blood cells and reimmunized 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 moralities (56%). He suggested that the present outbreaks of IBD were attributed to very virulent IBDV belonging to standard Serotype 1. 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 of Ranikhet disease at the field level. They statistically

analysed the seroepidemiological data of Ranikhet disease and infectious bursal disease, before (during 1991-92) ad after (during 1993-94) the outbreak of very virulent form of IBD (vvIBD) in Tamilnadu. During 1993-94 the half life 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 $T_{½}$ of RD vaccinal antibodies was 1.85 days. They observed 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 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 1kg of eggs was 2.23, 2.23, 2.36 and 2.20 Kg. There were 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 tires or in the histological finding in the spleen, bursa of Fabricii, thymus or ileum.

Panda *et al.* (1997) investigated the significance of Ashwagandha (*Withania somnifera*) root extract in the thyroid

function of cockerel and they found that its root extract (20 mg/day/bird for 30 days) increased serum thyroxins (T_4) concentration 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% protection, while IBDV strain 494 and IBDV strain 194 gave 88% and 76% 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 an mortality in day old chicks.

Vervelde *et al.* (1997) characterized leucocytic changes and determined tropism of infectious bursal disease virus following infection of newborn and 3 week old chickens. In the bursa of both age groups rapid depletion of B lymphocytes and an influx of $CD4^+$ TCR-$\alpha\beta_1^+$ and $CD8^+$ TCR - $\alpha\beta_1^+$ cell was detected within 4 days after inoculation. From 8 days after inoculation and onwards 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 might be due to immunological factors, cytokine release, or blood factor.

Prabhakaran *et al.* (1997) studied haemogram and serum chemistry in six weeks old white leghorn layer chickens on third day post IBD outbreak, birds were concurrently infected with *E. coli* and coccidia species. The result showed very low increase of haemoglobin concentration, decrease in erythrocyte value and increase in lymphocyte percentage. Analysis of serum protein value showed an increase in serum globulin, proportionate decrease in albumin, increase in creatinine value, enhancement of alkaline phosphatase activity increase of serum cholesterol and a significant decrease in A/G ratio.

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 strain) on 8 commercial farms in Solvenia. 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 folcks with the intermediate strain at 8 day of age, IBD was diagnosed in one flock. IBD was diagnosed in 6 of 8 flocks after administration of intermediate vaccine strains on 15 and 22 days of age with

mortality of 2.5%. It was concluded that neither vaccine can fully protect broiler against very virulent IBD virus strains.

Al-Afaleq (1998) studied the biochemical and hormonal changes associated with experimental infection of chicks with infectious bursal disease virus. Twenty, 2 week-old chicks was inoculated with infectious bursal disease virus through the oculonasal route. 12 similar birds were kept as uninfected control. All birds were bled 3, 6, 10 and 16 days after inoculation. Infected birds had clinical signs of IBD. These chicks seroconverted and had significantly decreased total protein, lipid and decreased albumin to globulin ratio. There was a significant increase in the concentration of corticosterone and thyroxin but not in the levels of tri-iodothyronine in the blood serum of infected birds.

Kumar *et al.* (1998) studied the influence of immunostimulation with *Mycobacterium phlei* (ISMP) and bone marrow culture supernatent (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 *et al.* (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). Moderate transient bursal atrophy was seen one week after immunization with the intermediate plus vaccine. 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. 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 particularly when given after the intermediate vaccine. Both immunosuppressive and immunological effects of the vaccine were transient and within safe limit.

Shadaksharappa *et al.* (1998) evaluated the imunomodulatory 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,

vitamin c treated and levamisole treated and vaccinated groups than vaccinated control group. The mean antibody titres showed appreciable increase when combinedly 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 usefullness 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 significant high titres 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.* (1998 b) 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 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 imunosupprressed 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 thereafter. It resulted in a 38-226% increase in GMT after parental 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 pomona.*

Thangavelu (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 dayold chicks. One field isolate (N35/93) was found to be more pathogenic and immunosuppressive than the other (N45/92) while non 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 thymulin and $ZnCl_2$, second group-received thymopoietin and Zn Cl_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 thymulinthymopoietin $ZnCl_2$ 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, triiodothyronine (T_3), Thyroxine (T_4) 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% 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 groups.

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 SPF 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 days postimmunization. Initially, the virus caused extensive necrosis of bursal B lymphocytes 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. Both IBDV-VAC and IM – IBDV caused suppression of the primary antibody response to antigens.

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 immunosupressed 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 tratment group. These observations were clearly indicative of the fact that all the tested plant preparations have specific immuno

stimulatory 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) planned an experiment to assess 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 Tiuftsin as compared to the birds vaccinated without tuftsin both in the immunosuppressed and immunocompetant birds. 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. It was concluded that tuftsin produced significant reversing effect of immunosuppression caused by IBDV infection and significant immune enhancement in immunocompetamt birds irrespective of the schedule of vaccination and type of vaccine virus used.

Van den Berg, (2000) Reported emergence of new IBDV variants and sequelae to IBD virus infection like immunosuppression and vaccinal failures have been found to cause increased number of

outbreaks and the disease has even been recorded in IBD vaccinated flocks resulting into heavy morbidity and mortality losses. He observed that the virus is extremely lymphocidal with an affinity for immature B-cells resulting in bursal atrophy approximately four days post infection.

Cardoso *et al.* (2000) observed that the initial vaccine developed for controlling IBD though helped is bringing down the mortalities due to IBD in chicks but also caused severe immunosuppression and in some cases even frank clinical disease resembling the natural infection. So, this promoted the development of attenuated and killed oil emulsified vaccine.

Yehuda *et al.* (2000) studied that inactivated vaccines might prove helpful if they can induce higher antibody levels in breeders, which will than be passively transmitted to the offspring and protect them during their entire growing period. Transfer of Antibodies elicited by baculovirus – derived VP_2 of vvIBD virus strains to progeny of commercial breeder chickens.

CHAPTER - III

MATERIALS AND METHODS

MATERIALS AND METHODS

MATERIALS

Chicks :

Three to four weeks old apparently healthy broiler chicks unvaccinated against IBD and free from antibodies to IBD, were routinely used for propagation of virus as well as production of antigen. The chicks were obtained from private poultry farms located in Patna.

Antigen :

Poona stain of IBD virus being maintained in this department in the form of 50% bursal homogenate was used as a reference antigen throughout this study.

Antiserum :

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

Vaccines :

F-strain RDV vaccine :

A commercially available F-Strain vaccine, manufactured by Indovax Pvt. Ltd. Siswala, Hariyana, India was used for vaccination of 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.

IV 95 IBD vaccine :

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

Chicken red blood cells :

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

Drugs / Agents :

IMMU-21

A commercially available polyherbal formulation of (Indian herbs research and supply co. Pvt. Ltd. Saharanpur, India) contained the extracts of natural plants such a *Withania somnifera* and O*cimum sanctum* as major constituents was used.

Charak-E-Sel

A commercially available combination of vitamin E, selenium and biotin water soluble powder manufactured by Charak animals health care ever green Industrial estate, Shakti Mills Lane. Mumbai, India was used.

Neem (Azadirachta indica)

Dried Neem leave powder was used during this study

Tulsi (Ocimum sanctum)

Dried Tulsi leave powder was used in this study.

Homoeopathic Medicine

Thuja occidentalis 200 (B & T original) were given simultaneously during this experiment.

BUFFERS

i) For agar gel precipitation test (Aziz, 1985)

(a) Solution A :

$Na_2 HPO_4.2H_2O$	1.4 gm
Double distilled water	100 ml

(b) Solution B :

$NaH_2 Po_4$	1.4 gm
Double distilled water	100 ml

Composition of the agar gel :

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

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

ii) Phosphate buffer saline (Aziz, 1985)

Nacl	2.0 gm
Kcl	0.05 gm
$Na_2Hpo_4. 2H_2O$	0.14 gm
$KH_2 Po_4$	0.05 gm
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 refrigerator temperature till used. This buffer was used for reconstitution and preparation of antigen as well as preparation of red blood cells suspension.

METHODS

Preparation of antigen :

Poona strain of IBDV in the form of 10 % bursal suspension in PBS, was inoculated into 3 to 4 weeks old broiler chicks at the rate of 0.2 ml of suspension per chicks by intraocular route. The chicks were sacrificed 48 hours post inoculation and the bursa were collected aseptically and homogenized in a sterile motar using glass wool as an abrasive.

The homogenate was diluted 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 were 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). Then 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.

Production of Hyperimmune Serum :

Hyperimmune serum against IBDV was raised in 20 weeks old 6 apparently healthy chickens. Each bird was given Georgia

strain of IBDV through occulo-nasal route at weekly intervals. 2 weeks after the 4th 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.

Collection of serum samples from chicken :

2 to 3 ml blood was taken from the wing vein of each birds with the help of 5ml sterilized disposable syringe using 24 or 26 gauze needles. The blood drawn was immediately transferred into sterilized test tube, which there after kept in a slanting position and the blood was allowed to clot. After 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). The serum samples were stored at 0^0c until processed.

Collection of blood samples from chicken for Haematological study :

2 to 2.5 ml of blood drawn as described above was immediately transferred into clean and sterilized vials of 5ml capacity. 1 % solution of EDTA 0.1 ml per ml of blood was kept at 4^0c until further use.

Chicken red blood cell suspension :

Two adult chickens were used as donor of blood. 1 to 1.5 ml of blood was collected from each bird in an anticoagulant, disodium salt of ethylene diamine tetra acetic acid (EDTA) at the rate

of 1 mg per ml of blood. Supernatant fluid was removed after centrifugation at 500 rpm for 10 minutes. The packed cells were washed three times with PBS. Finally, 0.8 percent RBC suspension was made in PBS and stored at refrigerator temperature. This RBC suspension was used only for four day after preparation and thereafter the fresh RBC suspension was prepared.

Assessment of Immune Response following IBD vaccination in chicken after administration of different drugs/agents :

(i) Agar gel precipitation test :

The test was done following the method of Hirai *et al.*, (1972) with some modification. The glass microscopic slide (75 mm x 25 mm) were precoated 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 a performed glass pipette and allowed to solidify. After setting the slides were kept at 4^0c for over night to facilitate punching of gels. A hexagonal well pattern consisting of a central well and five peripheral well 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 4 wells were used for test sera. The slides were incubated in humidified chamber at room temperature and observed daily for three days.

(ii) Quantitative agar gel precipitation test (QAGPT) :

The level of precipitating antibody was determined as per the method of Cullen and 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 the peripheral well contained two fold dilution of sera. The volume of reagents put in each well was 0.2 ml. Three replicate for each test were carried out simultaneously. The reciprocal of the highest dilution of serum which gave precipitating line was taken as the test of the serum. The mean antibody titre of the positive samples were calculated according to Villagas and Purchase (1980).

(iii) Haemagglutination (HA) Test :

The HA test was performed in perspex plate to prepare 4 HA units of RD virus as described by Beard (1980). Taking 0.5 ml of virus material two fold serial dilution 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 induced. 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 bottom 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.

(iv) Haemagglutination inhibition (HT) Test :

The HI test was performed in U-shape bottomed microtitration plate as per the method suggested by Beard (1980). 4 HA units of virus antigen (Charan *et al.*, 1981) and 0.8% chicken RBC suspension were used in this test. Using 0.25 ml of serum sample tow fold serial dilution were made in PBS. To each serum dilution 0.25 ml (4HA 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 and RBCs and incubated at room temperature for 40 minutes. The HI antibody titre was taken so the reciprocal of the highest dilution of serum showing complete inhibition of agglutination of RBCs.

"Evaluation of certain immunomodulatory agents in countering immunosuppressive effects of a vaccine strain of infectious bursal disease virus in chicken".

This study was carried out as per the experimental design detailed below.

EXPERIMENTAL DESIGN :

Day old chick, numbering 175 chicks were obtained from central poultry farm, Patna and randomly divided into seven equal groups of 25 chicks each. All the chicks in the different groups were housed under identical feeding and managemental condition for 42

days. Birds in all the seven groups were given F-strain RDV vaccine intraocularly at the dose rate of 0.05 ml per bird at zero day of age, while all the birds from group I to VI received IBD vaccine (IV 95 strain) intraocularly at 14 days of age. The treatments that the birds received groupwise are shown in table 1.

From each group of chickens pre IBD vaccinated blood and serum samples were collected on 13 days of age and post IBD vaccinated blood and serum samples were collected on 21, 28, 35 and 42 days of age. Blood samples collected were evaluated for heamatological changes including total leucocyte count (TLC), haemoglobin percent (Hb%) and packed cell volume (PCV). Serum samples were evaluated for determination of antibody titres to IBD vaccine and RD F-Strain vaccine as well as estimation of serum protein and serum electrolytes level. Five birds from each group were sacrificed 96 hours post IBD vaccination and Bursa: body weight ratio was determined. Portions of bursa, kidney, liver and spleen were collected in 10% formalin saline for histopathological examination. Body weight gain and feed conversion ratio (FCR) were determined at 42 day of age.

Protein Estimation :

Total serum protein, albumin and globulin were determined by biuret method as described by Reinhold (1953), with some modifications.

Serum electrolyte estimation :

Serum sodium and potassium were evaluated by flame photometry as per Oser (1979).

Haemoglobin estimation :

It was determined by Sahli's Haemoglobinometer method as described by Jain (1986)

Packed cell volume :

PCV was calculated by employing wintrobe haematocrit tube as per method described by Jain (1986). The percentage value of PCV uses calculated as follows

$$PCV(\%) = \frac{\text{Height of red blood cells (mm)}}{\text{Total height of column (mm)}} \times 100$$

Total leucocyte count :

It was determined by Haemocytometer which contains one counting chamber (Slide and Cover), one RBC pipette and one WBC pipette, as described by Natt & Herick (1952).

Body weight gain and feed conversion ratio :

The body wt. gains of chickens were recorded by substracting the live weight of chicks at day 2 from live weight of chicken at day 42. Feed conversion ratio per bird at 42 days of age was determined as follows :

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

Bursa : Body weight (B:BW) ratio :

This was calculated as follows :

$$B : BW = \frac{\text{Bursa weight (gm)}}{\text{Body weight (gm)}} \times 1000$$

Histopathology :

The tissue samples for histopathological examination were processed in acetone benzene (Lillie and Fullmer 1976), wax (melting point 62°C) and embedded in paraffin. Five-micron thick sections were cut and stained by haematoxylin and eosin (Drury and Wallington, 1980).

Scoring of bursal lesions :

The scoring system suggested by Wenterfield 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, Inter follicular oedma, epithelial changes, Interstitial fibrosis and cellular infiltration.

Statistical analysis :

The mean and standard errors of the values obtained were determined. The percent values mortality were transformed into Arc sin inverse data. Analysis of variance (Anova) was performed wherever necessary as per Snedecor and Cochran (1967).

TABLE – 1 : EXPERIMENTAL DESIGN

Group.	No. of chicks	Treatment route & dosage	Period of treatment	Age at IV95 strain IBD vaccination (day) (route)	Age at RDV (F-Strain) Vaccination (day) (route)	No. of chicks Sacrificed 96 hrs. Post IBD vaccination	OBSEVATION PLANNED.
I	25	IMMU - 21 (D.W) **(12 mg/25 birds)**	Day 5 to 24	14 (i.o)	Zero (i.o)	5	1. Collection of pre IBD vaccinated blood samples on 13 day of age for determination of MDA level of IBD, Abtitre of RDV, normal biochemical status and normal haematological status of chicks. 2. Collection of blood samples at 7, 14 21 and 28th days post IBD vaccination. i) For determination of Ab titre to IBDV by QAGPT. ii) For determination of Ab titre to RDV (Fstrain) by HI test. iii) For estimation of serum total protein, albumin, globulin and serum Na, K level. iv) For determination of TLC, PCV and Hb. 3. Collection of BF, Spleen, Kidney & liver in 10% formalin saline for its histopathological examination by H & E Staining method. 4. Body wt. gain and FCR recorded on termination of experiment. 5. Determination of bursa : body wt. ratio on 96 hrs. Post IBD vaccination.
II	25	Charak-E-Sel. (D.W.) **(2.5/25 birds)**	Day 5 to 24	14 (i.o)	Zero (i.o)	5	
III	25	Neem leave powder **(150 mg/bird)**	Day 5 to 24	14 (i.o)	Zero (i.o)	5	
IV	25	Tulsi leave powder **(250 mg/bird)**	Day 5 to 24	14 (i.o)	Zero (i.o)	5	
V	25	Thuja oc. (D.W.) **4ml/25 birds.**	Weekly, Starting from day 7 of age	14 (i.o)	Zero (i.o)	5	
VI	25	Untreated	0	14 (i.o)	Zero (i.o)	5	
VII	25	Untreated **(control)**	0	Unvaccinated	Zero (i.o)	5	

CHAPTER - IV

RESULTS

RESULTS

Effect of selected drugs/agents on immune responses to IBDV (IV 95 strain) in chickens :

The immune responses to IBD virus Vaccine (IV95) in different drug treated groups of chicken are presented in table-2. The perusal of the table-2 demonstrated the occurrence of seroconversion by 14 days post IBD vaccination in group of birds that received IBD vaccine (i.e. gr. I - VI). The perusal of this table also revealed progressive rise in precipitating antibody titres till the last day of observation (i.e. 28 days post IBD vaccination) in all the vaccinated groups. Further the comparison of QAGPT titres between different groups (gr. I - VI) clearly demonstrated higher titres in groups which received drugs/agents (gr. I - V) than the titres observed in groups VI which received only IBD vaccine but no drug treatment over all the intervals post IBD vaccination (table – 2 & Fig. 14a, b). Again comparison of QAGPT titres between different drug treated groups (gr. I to V) revealed significantly lower titre in IMMU – 21 and Charak – E – sel treated groups (gr. I and II) than the titres recorded in Neem, Tulsi and Thuja oc treated groups (gr. III - V) by 21 and 28 days post IBD vaccination. However, comparison of precipitating antibody titres of group III - V (Neem, Tulsi and Thuja oc) treated groups suggested highest precipitating antibody level in Neem treated group on 21st and 28th day post IBD vaccination when compared with the titres of Tulsi and Thuja oc treated groups for the corresponding period. No antibody could be detected in the last group (gr. VII) which did not receive IBD vaccine.

TABLE 2. EFFECT OF DRUGS/AGENTS ON IMMUNE RESPONSES TO A VACCINE STRAIN OF IBD VIRUS IN CHICKENS.

Group	Age at IBD Vaccination (Days)	Treatment	Mean ± SE of QAGPT titre (log_2) to IBDV Vaccine				
			Pre IBD Vaccinated	Days Post IBD Vaccination			
				7	14	21	28
			- ve	- ve	1.80^{abc} ± 0.200 (5)	2.80^{bc} ± 0.200 (5)	3.80^{bc} ± 0.200 (5)
II	14	Charak-E-Sel	- ve	- ve	1.40^{ab} ± 0.245 (5)	2.20^{ab} ± 0.200 (5)	3.20^{b} ± 0.200 (5)
III	14	Neem	- ve	- ve	2.00^{bc} ± 0.00 (5)	3.80^{d} ± 0.200 (5)	4.80^{d} ± 0.200 (5)
IV	14	Tulsi	- ve	- ve	2.20^{bc} ± 0.200 (5)	3.60^{d} ± 0.245 (5)	4.40^{cd} ± 0.400 (5)
V	14	Thuja oc.	- ve	- ve	1.60^{abc} ± 0.246 (5)	3.20^{cd} ± 0.377 (5)	4.20^{cd} ± 0.200 (5)
VI	14	Untreated	- ve	- ve	1.20^{a} ± 0.200 (5)	2.00^{a} ± 0.346 (5)	2.20^{a} ± 0.374 (5)
VII	Unvaccinated	Untreated	- ve	- ve	- ve	- ve	- ve

- **Figures in parentheses indicate number of observations.**
- **Mean bearing common superscripts (a, b, c, d) in individual column did not differ significantly (P<0.05).**

Fig.14 (a) Histogram showing effect of agents on Ab titre to IBD vaccine in Chickens

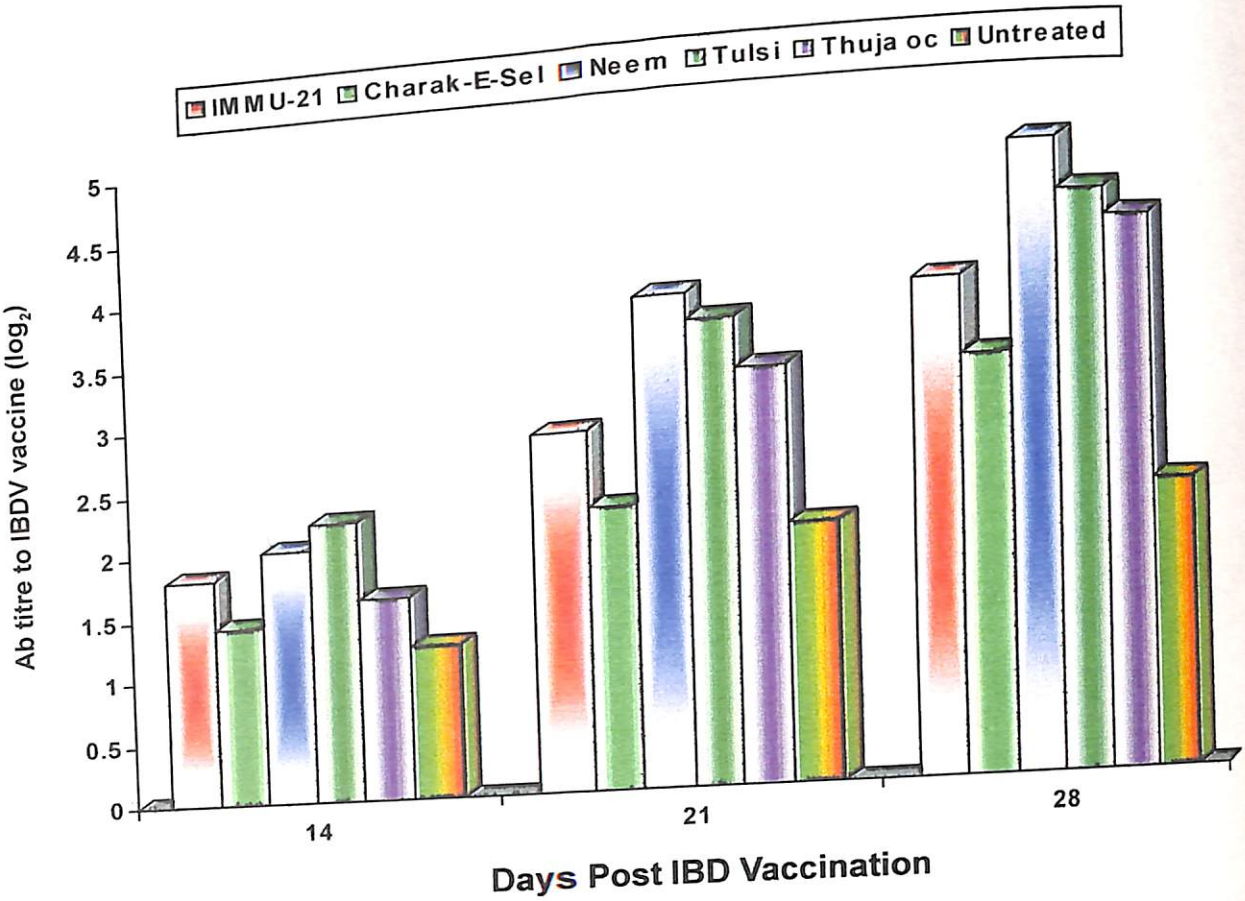

Fig.14 (b) Line graph showing effect of agents on Ab titre to IBD vaccine in Chickens

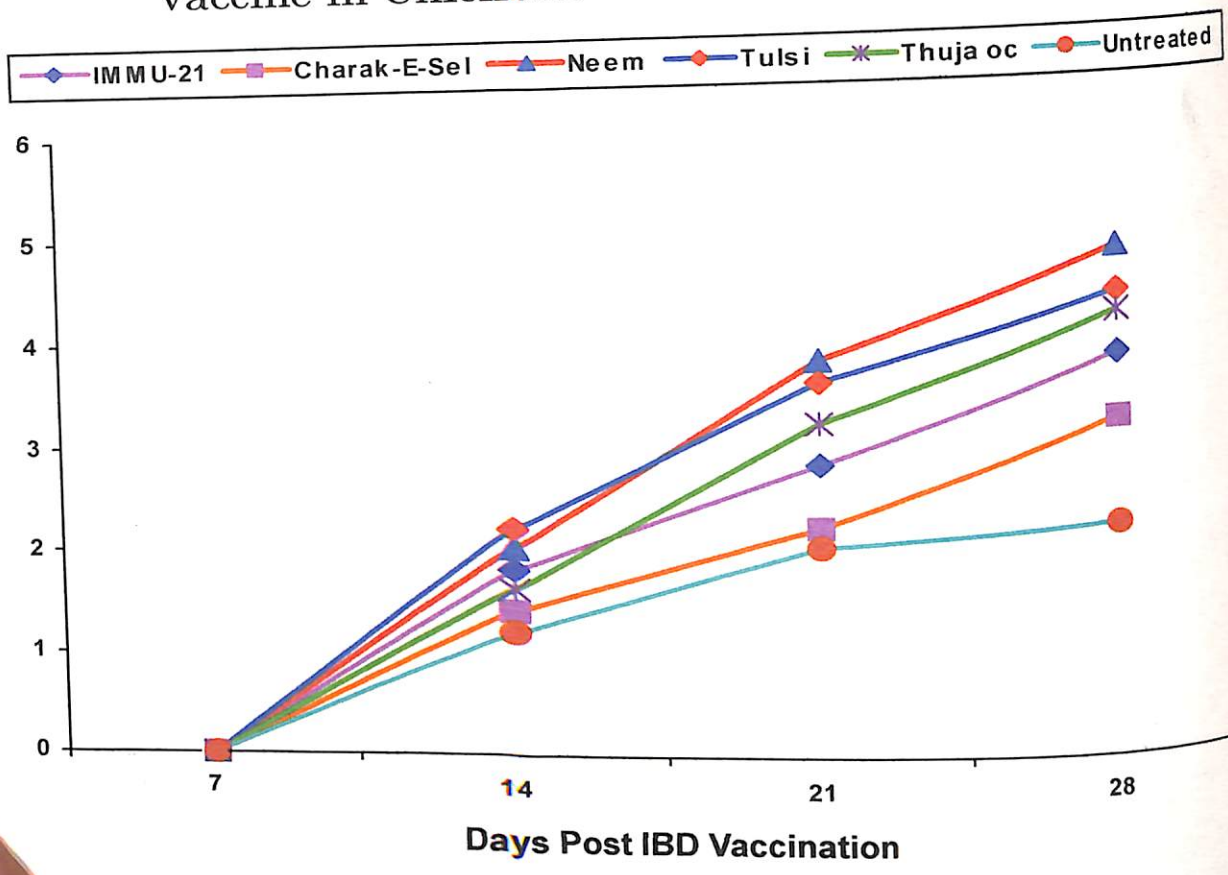

TABLE 2. EFFECT OF DRUGS/AGENTS ON IMMUNE RESPONSES TO A VACCINE STRAIN OF IBD VIRUS IN CHICKENS.

Group	Age at IBD Vaccination (Days)	Treatment	Mean ± SE of QAGPT titre (log_2) to IBDV Vaccine				
			Pre IBD Vaccinated	Days Post IBD Vaccination			
				7	14	21	28
			- ve	- ve	$1.80^{abc} \pm 0.200$ (5)	$2.80^{bc} \pm 0.200$ (5)	$3.80^{bc} \pm 0.200$ (5)
II	14	Charak-E-Sel	- ve	- ve	$1.40^{ab} \pm 0.245$ (5)	$2.20^{ab} \pm 0.200$ (5)	$3.20^{b} \pm 0.200$ (5)
III	14	Neem	- ve	- ve	$2.00^{bc} \pm 0.00$ (5)	$3.80^{d} \pm 0.200$ (5)	$4.80^{d} \pm 0.200$ (5)
IV	14	Tulsi	- ve	- ve	$2.20^{bc} \pm 0.200$ (5)	$3.60^{d} \pm 0.245$ (5)	$4.40^{cd} \pm 0.400$ (5)
V	14	Thuja oc.	- ve	- ve	$1.60^{abc} \pm 0.246$ (5)	$3.20^{cd} \pm 0.377$ (5)	$4.20^{cd} \pm 0.200$ (5)
VI	14	Untreated	- ve	- ve	$1.20^{a} \pm 0.200$ (5)	$2.00^{a} \pm 0.346$ (5)	$2.20^{a} \pm 0.374$ (5)
VII	Unvaccinated	Untreated	- ve	- ve	- ve	- ve	- ve

- **Figures in parentheses indicate number of observations.**
- **Mean bearing common superscripts (a, b, c, d) in individual column did not differ significantly ($P<0.05$).**

Effect of selected agents/drugs on immune responses to RDV (F strain) in IBD Vaccinated Chickens :

Immune response to RD virus in IBD vaccinated chickens are presented in table – 3. The perusal of the table showed that the IBD vaccine strain (IV 95) virus had immunosuppressive effect as evident from lower HI titres in IBD vaccinated but untreated group of birds (gr. VI) over all the periods post IBD vaccination when compared with the HI titres for the corresponding intervals in birds which neither received IBD vaccine nor any drug treatment (gr. VII). The result also revealed marked immunosuppressive effect of IBD virus on 14 and 28 day post IBD vaccination as antibody titres to RDV in vaccinated but untreated group (gr. VI) was significantly lower than the corresponding values in unvaccinated and untreated control group (gr. VII) (Table 3 and Fig. 15a, b).

Further, it was also noted that different drug treatments resulted in enhancement of HI titre over all the intervals post IBD vaccination except at 7 day post IBD vaccination in IMMU – 21 treated group (table 3 & Fig. 15 a, b) when compared with the HI titters for the corresponding intervals of birds in group VI which received only IBD vaccine but no drug treatment. Again comparison of HI titre of birds in different drug treatment groups (gr. I - V) with the HI titres of IBD unvaccinated and untreated control birds (gr. VII) for the corresponding period clearly demonstrated that none of the drugs has been successful in bringing enhancement of HI titre comparable to the titres in group VII. It was also observed that the rise in HI titre continued by 14 day post IBD vaccination and thereafter the titres started declining.

TABLE 3. IMMUNE RESPONSES TO RDV (F-STRAIN) VACCINE IN IBD VACCINATED CHICKEN AFTER ADMINISTRATION OF DIFFERENT AGENTS.

Group	Age at RDV (F – Strain) vaccination (days)	Age at IBD vaccination (Days)	Treatment	Mean ± SE of HI antibody titre (log_2) to RDV vaccine				
				Pre IBD vaccinated	Days Post IBD Vaccination			
					7	14	21	28
I	0	14	IMMU-21	$3.60^{a} \pm 0.245$ (5)	$3.80^{a} \pm 0.200$ (5)	$6.40^{abc} \pm 0.245$ (5)	$4.60^{a} \pm 0.245$ (5)	$4.20^{abc} \pm 0.489$ (5)
II	0	14	Charak-E-sel	$4.00^{a} \pm 0.00$ (5)	$4.20^{a} \pm 0.489$ (5)	$6.00^{ab} \pm 0.00$ (5)	$4.60^{a} \pm 0.245$ (5)	$3.80^{ab} \pm 0.200$ (5)
III	0	14	Neem	$3.80^{a} \pm 0.200$ (5)	$4.60^{a} \pm 0.244$ (5)	$6.80^{c} \pm 0.200$ (5)	$5.40^{a} \pm 400$ (5)	$4.80^{ab} \pm 0.200$ (5)
IV	0	14	Tulsi	$4.20^{a} \pm 0.200$ (5)	$4.60^{a} \pm 0.244$ (5)	$6.60^{bc} \pm 0.245$ (5)	$5.20^{a} \pm 0.374$ (5)	$4.60^{bc} \pm 0.245$ (5)
V	0	14	Thuja oc.	$4.00^{a} \pm 0.00$ (5)	$4.20^{a} \pm 0.489$ (5)	$6.60^{bc} \pm 0.245$ (5)	$4.80^{a} \pm 0.200$ (5)	$4.40^{bc} \pm 0.400$ (5)
VI	0	14	Untreated	$3.60^{a} \pm 0.245$ (5)	$4.00^{a} \pm 0.00$ (5)	$5.80^{a} \pm 0.200$ (5)	$4.20^{a} \pm 0.489$ (5)	$3.20^{a} \pm 0.200$ (5)
VII	0	Unvaccinated	untreated	$3.80^{a} \pm 0.200$ (5)	$4.80^{a} \pm 0.200$ (5)	$6.80^{c} \pm 0.200$ (5)	$5.60^{a} \pm 0.245$ (5)	$5.00^{c} \pm 0.547$ (5)

- Figures in parentheses indicate number of observations.
- Mean bearing common superscripts (a, b, c) in individual column did not differ significantly ($P<0.05$).

Effects of selected agents/drugs on gross and Histopathology in IBDV vaccinated chickens :

The IBD specific clinical symptoms were absent in different IBD vaccinated group (gr. I – VI) as well as IBD unvaccinated control group (gr. VII) throughout the experimental period. However, some deaths were recorded in different groups. The perusal of the table 11 clearly depicted lower mortality rates in IBD vaccinated groups which received different drug treatment (gr. I – V) than the mortality rate recorded in the group which received only IBD vaccination. Interestingly the same mortality rate of 18.44% was also recorded in the control group which neither received IBD vaccine nor any drug treatment.

The study on effect of vaccine strain (IV 95) of IBD virus on bursae of Fabricius in experimentally infected chickens sacrificed 96 hours post IBD vaccination demonstrated production of bursal lesion typical of IBD virus. The changes were marked by lymphoid depletion and necrosis, interfollicular oedema, epithelial invegination, interstitial fibrosis, cellular infiltration and vacuolar degeneration in few cases.

The perusal of this table also revealed that though the total lesion score was below one, the virus produced considerable effect on lymphoid cells of the bursal follicles as evident from changes such as lymphoid depletion and necrosis. The overall lesion score

TABLE 4. MEAN ± S.E. OF BURSAL LESION SCORES IN DIFFERENT GROUPS OF CHICKENS SACRIFICED 96 HOURS POST IBDV (IV 95 STRAIN) VACCINATION.

Group & Treatment	Follicular Changes						Epithelial Changes				Interstitial fibrosis	cellular infiltration	Total lesion score.
	Inter follicul- oedema	Lymphoid necrosis	Lymphoid depletion	Reticuloepit - helial hyperplasia	Vacuol - ar degene - ration	Follicu - lar cyst	Hyper plasia	Epithelial invaginati - on	Cyst formati - on	Vacuo- lation			
I IMMU-21	1.4[a] ± 0.40 (5)	1.8[a] ± 0.58 (5)	1.0[a] ± 0.32 (5)	0.0 (5)	0.2[a] ± 0.20 (5)	0.0 (5)	0.0 (5)	1.0[a] ± 0.32 (5)	0.0 (5)	0.0 (5)	0.4[a] ± 0.20 (5)	0.0 (5)	0.48[a] ± 0.083 (60)
II Charak-E-Sel	1.0[a] ± 0.00 (5)	2.0[a] ± 0.54 (5)	1.0[a] ± 0.32 (5)	0.0 (5)	0.2[a] ± 0.20 (5)	0.0 (5)	0.0 (5)	1.2[a] ± 0.20 (5)	0.0 (5)	0.2[a] ±0.20 (5)	1.4[a] ± 0.40 (5)	0.0 (5)	0.58[a] ± 0.090 (60)
III Neem	1.4[a] ± 0.40 (5)	1.6[a] ± 0.40 (5)	1.4[a] ± 0.40 (5)	0.2[a] ± 0.20 (5)	0.0 (5)	0.0 (5)	0.2[a] ± 0.20 (5)	0.8[a] ± 0.37 (5)	0.0 (5)	0.0 (5)	0.0 (5)	0.0 (5)	0.46[a] ± 0.086 (60)
IV Tulsi	1.0[a] ± 0.44 (5)	1.4[a] ± 0.40 (5)	1.6[a] ± 0.40 (5)	0.0 (5)	0.4[a] ± 0.24 (5)	0.0 (5)	0.0 (5)	0.8[a] ± 0.20 (5)	0.0 (5)	0.2[a] ± 0.20 (5)	0.0 (5)	0.0 (5)	0.46[a] ± 0.081 (60)
V Thuja oc.	1.2[a] ± 0.37 (5)	2.0[a] ± 0.40 (5)	1.2[a] ± 0.37 (5)	0.0 (5)	0.0 (5)	0.0 (5)	0.0 (5)	1.0[a] ± 0.63 (5)	0.0 (5)	0.2[a] ± 0.20 (5)	0.4[a] ± 0.24 (5)	0.2[a] ±0.20 (5)	0.46[a] ± 0.072 (60)
VI Untreated (V)	0.8[a] ± 0.20 (5)	2.0[a] ± 0.31 (5)	1.4[a] ± 0.24 (5)	0.0 (5)	0.0 (5)	0.0 (5)	0.0 (5)	1.6[a] ± 0.60 (5)	0.4[a] ± 0.40 (5)	0.0 (5)	0.6[a] ± 0.24 (5)	0.2[a] ± 0.20 (5)	0.58[a] ± 0.092 (60)
VII Untreated (UV)	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

- Figures in parentheses indicate number of observations.
- Mean bearing common superscript (a) in individual column did not differ significantly ($P<0.05$).

suggested that the present vaccine strain still retained residual pathogenicity (Fig 1 to 13). Further comparison of the total lesion score between different treatment groups revealed highest lesion score in IBD vaccinated but untreated group (gr. VI) as well as Charak – E – sel treated group. However, the lesion scores in IMMU – 21, Neem, Tulsi and Thuja treated groups were numerically lower than the corresponding value is group VI (IBD vaccinated but untreated). However, the analysis of lesion scores between different treatment groups (gr. I - VI) failed to demonstrate the effect of drug treatments in lowering the lesion scores. (Table – 4).

Effects of agents/drugs on biochemical profile in IBDV (IV 95) vaccinated chickens :

The effect of IBD vaccine virus on serum total protein values in chickens after administration of different immunomodulating agents are presented in table 5. The perusal of the table revealed that there was reduction in serum total protein value in vaccinated group (gr. VI) when compared with the values for the corresponding intervals post IBD vaccination in birds which neither received IBD vaccine nor any drug treatment (gr. VII). In the drug treated group improvement in total serum protein value were noticeable at all the intervals post IBD vaccination except in case of Thuja oc treated group (gr. V) when compared with the corresponding value in the vaccinated group (gr. VI). Further some significant

enhancement in total serum protein level were also noticeable in IMMU-21, Tulsi and Neem treated group (gr. I, III and IV) at 14 and 28 day post IBD vaccination when compared with the corresponding value in the vaccinated group (gr. VI) (table 5 & fig. 16).

Mean ± SE of serum albumin values are shown in table-5. The perusal of the table showed rising trends in serum albumin value of control group (gr. VII) with increase in age. On the other hand the birds which received IBD vaccine (gr. I-VI) demonstrated decline in serum albumin value on 7 and 14 day post IBD vaccination when compared with their pre-vaccinated value irrespective of the type of drugs used except in case of Neem treated group (gr. III) (Fig. 17). However, none of the values were found to be different significantly. Nevertheless albumin levels were invariably lower in vaccinated group (gr. VI) than its corresponding value in unvaccinated and untreated control group (gr. VII) over all the intervals post IBD vaccination.

The mean ± SE of serum globulin values are depicted in table–5. In general the serum globulin values did not differ significantly at any intervals post IBD vaccination except on 14 day when the value was significantly higher in group IV than its corresponding values in rest of the groups but for group I. Further it was also observed that serum globulin values in the control group (gr. VII) were lower than the corresponding value of the vaccinated group

(gr. VI) and also of different drug treated groups over all the intervals post IBD vaccination except in case of Charak-E-sel treated and Thuja oc treated groups (gr. II and V) on 21 days post IBD vaccination (fig. 18 a.b.).

Table-6 shows the mean ± S.E. values of serum sodium level and serum potassium level at different interval post IBD vaccination. On observation it was found that the serum sodium value did not differ significantly in any of the groups over any interval post IBD vaccination.

However, effect of IBD virus on potassium level revealed some significant differences only by 7 and 14 days post IBD vaccination. Whereas, the level of the potassium in birds that received IBD vaccine alone (gr. VI) were significantly lower ($p<0.01$) than the value of potassium level in untreated unvaccinated group (gr. VIII). The potassium levels were also significantly ($p<0.01$) higher in IMMU-21, Charak-E-sel and Tulsi treated group (gr. I, II & IV) post 7 and 14 days post IBD vaccination when compared with its corresponding values in vaccinated alone group (gr. VI). Further comparison of values for potassium level of vaccinated untreated group (gr. VI) with the values of drug treated groups (gr. I - V) in general demonstrated higher potassium value in drug treated groups at all interval post IBD vaccination except at 28 day post IBD vaccination in Thuja oc treated group (gr. V) (fig. 19).

TABLE 5. SERUM TOTAL PROTEIN, SERUM ALBUMIN & SERUM GLOBULIN VALUES (G/100ML) IN DIFFERENT GROUPS OF CHICKENS.

Group	Age at IBD Vaccination (Days)	Treatment	Mean ± S.E of serum Total protein.				
			Pre IBD Vaccinated	Days Post IBD Vaccination			
				7	14	21	28
I	14	IMMU-21	$3.21^{a} \pm 0.012$ (5)	$3.65^{a} \pm 0.067$ (5)	$3.92^{b} \pm 0.067$ (5)	$4.14^{a} \pm 0.159$ (5)	$4.30^{c} \pm 0.120$ (5)
II	14	Charak-E-Sel	$3.30^{a} \pm 0.134$ (5)	$3.43^{a} \pm 0.069$ (5)	3.65^{ab} 0.066 (5)	$3.71^{a} \pm 0.139$ (5)	$3.98^{abc} \pm 0.108$ (5)
III	14	Neem	$3.27^{a} \pm 0.087$ (5)	$3.43^{a} \pm 0.069$ (5)	$3.82^{ab} \pm 0.086$ (5)	$3.98^{a} \pm 0.108$ (5)	$4.25^{c} \pm 0.109$ (5)
IV	14	Tulsi	$3.27^{a} \pm 0.122$ (5)	$3.38^{a} \pm 0.110$ (5)	$3.87^{b} \pm 0.101$ (5)	$4.03^{a} \pm 0.199$ (5)	$4.14^{bc} \pm 0.101$ (5)
V	14	Thuja oc.	$3.43^{a} \pm 0.069$ (5)	$3.32^{a} \pm 0.103$ (5)	$3.54^{a} \pm 0.087$ (5)	$3.60^{a} \pm 0.102$ (5)	$3.76^{a} \pm 0.101$ (5)
VI	14	Untreated	$3.32^{a} \pm 0.103$ (5)	$3.27^{a} \pm 0.087$ (5)	$3.54^{a} \pm 0.122$ (5)	$3.65^{a} \pm 0.267$ (5)	$3.82^{ab} \pm 0.120$ (5)
VII	Unvaccinated	Untreated	$3.38^{a} \pm 0.063$ (5)	$3.54^{a} \pm 0.122$ (5)	$3.76^{ab} \pm 0.200$ (5)	$4.09^{a} \pm 0.120$ (5)	$4.14^{bc} \pm 0.158$ (5)

Group	Age at IBD Vaccination (Days)	Treatment	Mean ± S.E of serum Albumin				
			Pre IBD Vaccinated	Days Post IBD Vaccination			
				7	14	21	28
I	14	IMMU-21	$2.13^{a} \pm 0.199$ (5)	$1.96^{a} \pm 0.057$ (5)	$2.07^{a} \pm 0.110$ (5)	$2.18^{a} \pm 0.087$ (5)	$2.23^{a} \pm 0.134$ (5)
II	14	Charak-E-Sel	$2.07^{a} \pm 0.069$ (5)	$1.91^{a} \pm 0.170$ (5)	$1.96^{a} \pm 0.199$ (5)	$2.13^{a} \pm 0.199$ (5)	$2.18^{a} \pm 0.148$ (5)
III	14	Neem	$2.07^{a} \pm 0.069$ (5)	$1.85^{a} \pm 0.054$ (5)	$2.12^{a} \pm 0.057$(5)	$2.29^{a} \pm 0.109$ (5)	$2.34^{a} \pm 0.110$ (5)
IV	14	Tulsi	$2.12^{a} \pm 0.057$ (5)	$1.74^{a} \pm 0.067$ (5)	$1.85^{a} \pm 0.134$ (5)	$2.23^{a} \pm 0.134$ (5)	$2.34^{a} \pm 0.110$ (5)
V	14	Thuja oc.	$1.96^{a} \pm 0.199$ (5)	$1.86^{a} \pm 0.132$ (5)	$1.91^{a} \pm 0.170$ (5)	$2.02^{a} \pm 0.162$ (5)	$2.13^{a} \pm 0.199$ (5)
VI	14	Untreated	$2.02^{a} \pm 0.110$ (5)	$1.80^{a} \pm 0.063$ (5)	$1.90^{a} \pm 0.149$ (5)	$2.07^{a} \pm 0.162$ (5)	$2.12^{a} \pm 0.050$ (5)
VII	Unvaccinated	Untreated	$2.01^{a} \pm 0.069$ (5)	$2.12^{a} \pm 0.057$ (5)	$2.23^{a} \pm 0.102$ (5)	$2.34^{a} \pm 0.067$ (5)	$2.50^{a} \pm 0.101$ (5)

Group	Age at IBD Vaccination (Days)	Treatment	Mean ± S.E of serum Globulin.				
			Pre IBD Vaccinated	Days Post IBD Vaccination			
				7	14	21	28
I	14	IMMU-21	$1.09^{a} \pm 0.121$ (5)	$1.70^{a} \pm 0.545$ (5)	$1.86^{bc} \pm 0.055$ (5)	$1.97^{a} \pm 0.159$ (5)	$2.08^{a} \pm 0.069$ (5)
II	14	Charak-E-Sel	$1.42^{a} \pm 0.103$ (5)	$1.53^{a} \pm 0.137$ (5)	$1.69^{ab} \pm 0.013$ (5)	$1.58^{a} \pm 0.100$ (5)	$1.80^{a} \pm 0.109$ (5)
III	14	Neem	$1.20^{a} \pm 0.156$ (5)	$1.59^{a} \pm 0.055$ (5)	$1.70^{ab} \pm 0.053$ (5)	$1.70^{a} \pm 0.053$ (5)	$1.91^{a} \pm 0.122$ (5)
IV	14	Tulsi	$1.16^{a} \pm 0.102$ (5)	$1.65^{a} \pm 0.087$ (5)	$2.02^{c} \pm 0.068$ (5)	$1.81^{a} \pm 0.139$ (5)	$1.80^{a} \pm 0.067$ (5)
V	14	Thuja oc.	$1.47^{a} \pm 0.161$ (5)	$1.47^{a} \pm 0.064$ (5)	$1.64^{ab} \pm 0.120$ (5)	$1.58^{a} \pm 0.100$ (5)	$1.64^{a} \pm 0.120$ (5)
VI	14	Untreated	$1.26^{a} \pm 0.066$ (5)	$1.48^{a} \pm 0.067$ (5)	$1.64^{ab} \pm 0.086$ (5)	$1.75^{a} \pm 0.067$ (5)	$1.70^{a} \pm 0.100$ (5)
VII	Unvaccinated	Untreated	$1.37^{a} \pm 0.000$ (5)	$1.42^{a} \pm 0.102$ (5)	$1.53^{a} \pm 0.131$ (5)	$1.64^{a} \pm 0.122$ (5)	$1.64^{a} \pm 0.087$ (5)

- Figures in parentheses indicate number of observations.
- Mean bearing common superscripts (a, b, c) in individual column did not differ significantly ($P<0.05$).

Fig. 16. Histogram showing total serum protein level in different groups of chickens pre & post IBD vaccination.

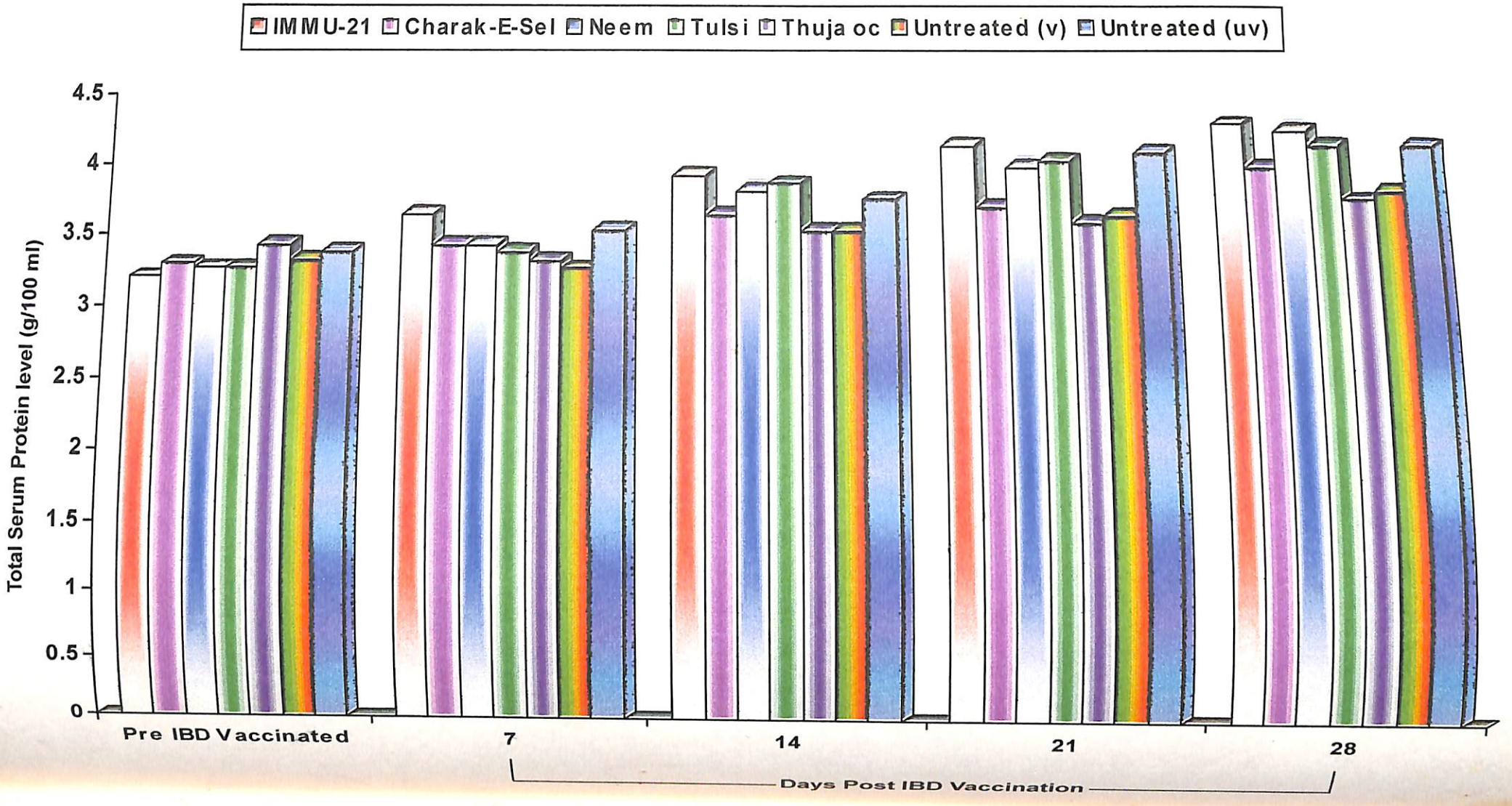

TABLE 5. SERUM TOTAL PROTEIN, SERUM ALBUMIN & SERUM GLOBULIN VALUES (G/100ML) IN DIFFERENT GROUPS OF CHICKENS.

Group	Age at IBD Vaccination (Days)	Treatment	Mean ± S.E of serum Total protein.				
			Pre IBD Vaccinated	Days Post IBD Vaccination			
				7	14	21	28
I	14	IMMU-21	$3.21^{a} \pm 0.012$ (5)	$3.65^{a} \pm 0.067$ (5)	$3.92^{b} \pm 0.067$ (5)	$4.14^{a} \pm 0.159$ (5)	$4.30^{c} \pm 0.120$ (5)
II	14	Charak-E-Sel	$3.30^{a} \pm 0.134$ (5)	$3.43^{a} \pm 0.069$ (5)	3.65^{ab} 0.066 (5)	$3.71^{a} \pm 0.139$ (5)	$3.98^{abc} \pm 0.108$ (5)
III	14	Neem	$3.27^{a} \pm 0.087$ (5)	$3.43^{a} \pm 0.069$ (5)	$3.82^{ab} \pm 0.086$ (5)	$3.98^{a} \pm 0.108$ (5)	$4.25^{c} \pm 0.109$ (5)
IV	14	Tulsi	$3.27^{a} \pm 0.122$ (5)	$3.38^{a} \pm 0.110$ (5)	$3.87^{b} \pm 0.101$ (5)	$4.03^{a} \pm 0.199$ (5)	$4.14^{bc} \pm 0.101$ (5)
V	14	Thuja oc.	$3.43^{a} \pm 0.069$ (5)	$3.32^{a} \pm 0.103$ (5)	$3.54^{a} \pm 0.087$ (5)	$3.60^{a} \pm 0.102$ (5)	$3.76^{a} \pm 0.101$ (5)
VI	14	Untreated	$3.32^{a} \pm 0.103$ (5)	$3.27^{a} \pm 0.087$ (5)	$3.54^{a} \pm 0.122$ (5)	$3.65^{a} \pm 0.267$ (5)	$3.82^{ab} \pm 0.120$ (5)
VII	Unvaccinated	Untreated	$3.38^{a} \pm 0.063$ (5)	$3.54^{a} \pm 0.122$ (5)	$3.76^{ab} \pm 0.200$ (5)	$4.09^{a} \pm 0.120$ (5)	$4.14^{bc} \pm 0.158$ (5)

Group	Age at IBD Vaccination (Days)	Treatment	Mean ± S.E of serum Albumin				
			Pre IBD Vaccinated	Days Post IBD Vaccination			
				7	14	21	28
I	14	IMMU-21	$2.13^{a} \pm 0.199$ (5)	$1.96^{a} \pm 0.057$ (5)	$2.07^{a} \pm 0.110$ (5)	$2.18^{a} \pm 0.087$ (5)	$2.23^{a} \pm 0.134$ (5)
II	14	Charak-E-Sel	$2.07^{a} \pm 0.069$ (5)	$1.91^{a} \pm 0.170$ (5)	$1.96^{a} \pm 0.199$ (5)	$2.13^{a} \pm 0.199$ (5)	$2.18^{a} \pm 0.148$ (5)
III	14	Neem	$2.07^{a} \pm 0.069$ (5)	$1.85^{a} \pm 0.054$ (5)	$2.12^{a} \pm 0.057$(5)	$2.29^{a} \pm 0.109$ (5)	$2.34^{a} \pm 0.110$ (5)
IV	14	Tulsi	$2.12^{a} \pm 0.057$ (5)	$1.74^{a} \pm 0.067$ (5)	$1.85^{a} \pm 0.134$ (5)	$2.23^{a} \pm 0.134$ (5)	$2.34^{a} \pm 0.110$ (5)
V	14	Thuja oc.	$1.96^{a} \pm 0.199$ (5)	$1.86^{a} \pm 0.132$ (5)	$1.91^{a} \pm 0.170$ (5)	$2.02^{a} \pm 0.162$ (5)	$2.13^{a} \pm 0.199$ (5)
VI	14	Untreated	$2.02^{a} \pm 0.110$ (5)	$1.80^{a} \pm 0.063$ (5)	$1.90^{a} \pm 0.149$ (5)	$2.07^{a} \pm 0.162$ (5)	$2.12^{a} \pm 0.050$ (5)
VII	Unvaccinated	Untreated	$2.01^{a} \pm 0.069$ (5)	$2.12^{a} \pm 0.057$ (5)	$2.23^{a} \pm 0.102$ (5)	$2.34^{a} \pm 0.067$ (5)	$2.50^{a} \pm 0.101$ (5)

Group	Age at IBD Vaccination (Days)	Treatment	Mean ± S.E of serum Globulin.				
			Pre IBD Vaccinated	Days Post IBD Vaccination			
				7	14	21	28
I	14	IMMU-21	$1.09^{a} \pm 0.121$ (5)	$1.70^{a} \pm 0.545$ (5)	$1.86^{bc} \pm 0.055$ (5)	$1.97^{a} \pm 0.159$ (5)	$2.08^{a} \pm 0.069$ (5)
II	14	Charak-E-Sel	$1.42^{a} \pm 0.103$ (5)	$1.53^{a} \pm 0.137$ (5)	$1.69^{ab} \pm 0.013$ (5)	$1.58^{a} \pm 0.100$ (5)	$1.80^{a} \pm 0.109$ (5)
III	14	Neem	$1.20^{a} \pm 0.156$ (5)	$1.59^{a} \pm 0.055$ (5)	$1.70^{ab} \pm 0.053$ (5)	$1.70^{a} \pm 0.053$ (5)	$1.91^{a} \pm 0.122$ (5)
IV	14	Tulsi	$1.16^{a} \pm 0.102$ (5)	$1.65^{a} \pm 0.087$ (5)	$2.02^{c} \pm 0.068$ (5)	$1.81^{a} \pm 0.139$ (5)	$1.80^{a} \pm 0.067$ (5)
V	14	Thuja oc.	$1.47^{a} \pm 0.161$ (5)	$1.47^{a} \pm 0.064$ (5)	$1.64^{ab} \pm 0.120$ (5)	$1.58^{a} \pm 0.100$ (5)	$1.64^{a} \pm 0.120$ (5)
VI	14	Untreated	$1.26^{a} \pm 0.066$ (5)	$1.48^{a} \pm 0.067$ (5)	$1.64^{ab} \pm 0.086$ (5)	$1.75^{a} \pm 0.067$ (5)	$1.70^{a} \pm 0.100$ (5)
VII	Unvaccinated	Untreated	$1.37^{a} \pm 0.000$ (5)	$1.42^{a} \pm 0.102$ (5)	$1.53^{a} \pm 0.131$ (5)	$1.64^{a} \pm 0.122$ (5)	$1.64^{a} \pm 0.087$ (5)

- Figures in parentheses indicate number of observations.
- Mean bearing common superscripts (a, b, c) in individual column did not differ significantly ($P<0.05$).

Fig. 17. Histogram showing serum albumin level pre and post IBD vaccination in different groups of chickens.

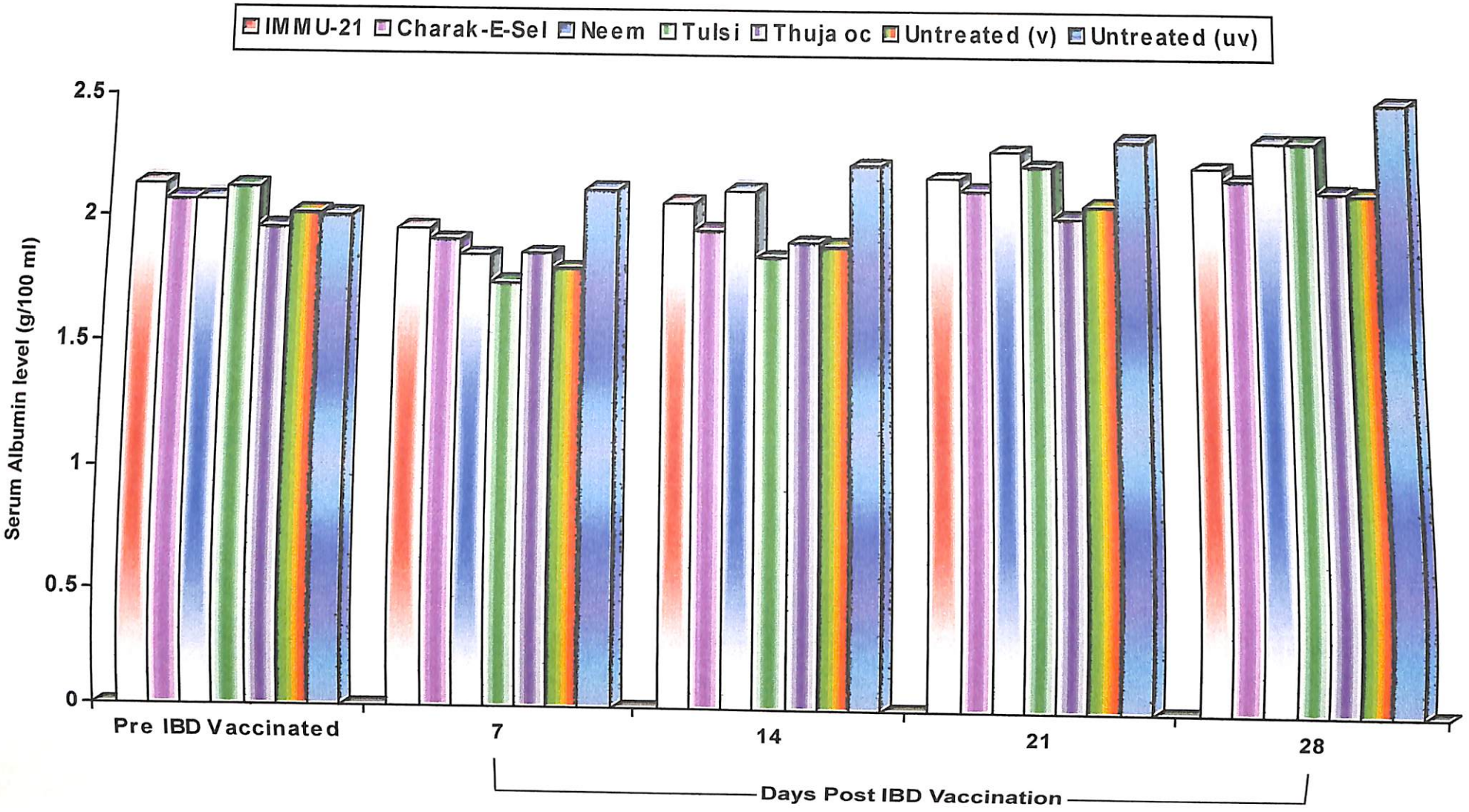

Fig.18 (a) Histogram showing serum globulin level in different groups pre & post IBD vaccination.

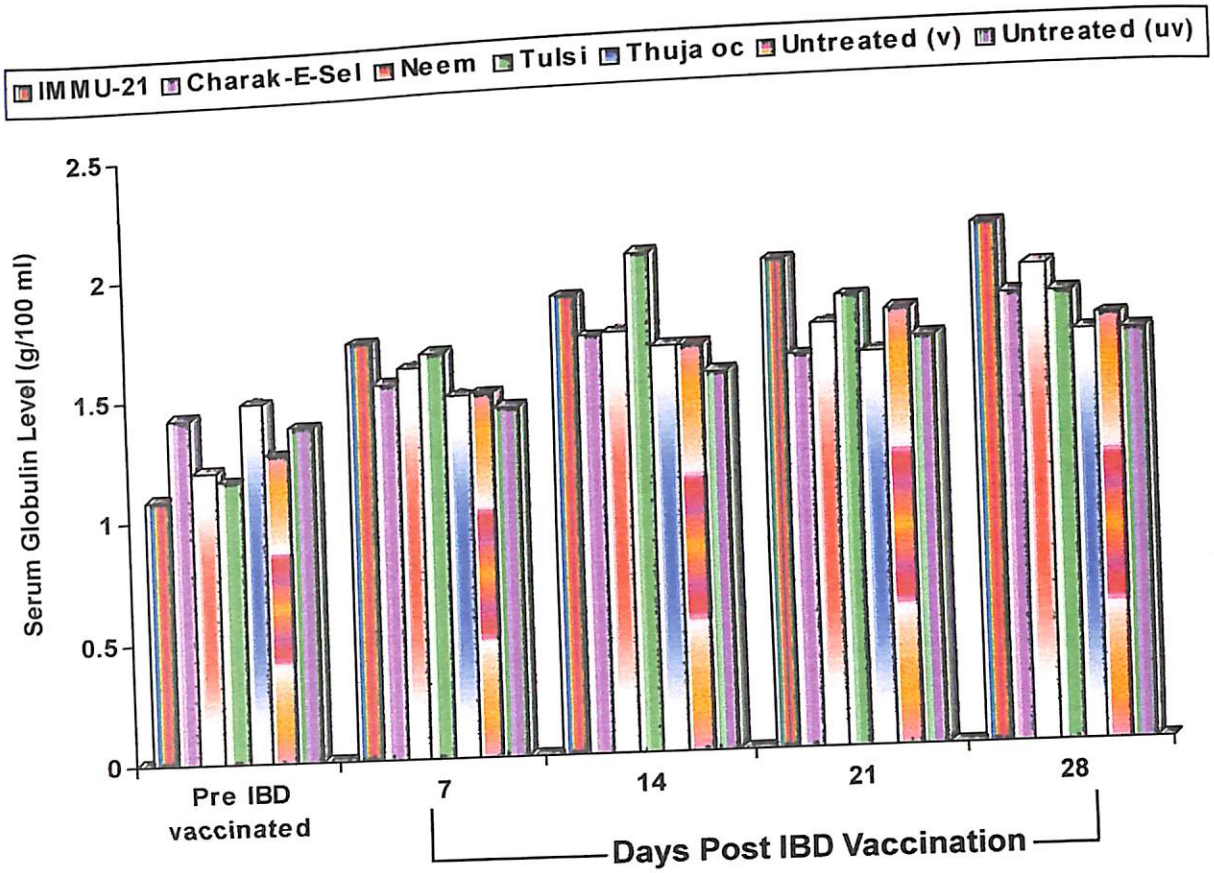

Fig.18 (b) Line graph showing serum globulin level in different groups pre & post IBD vaccination.

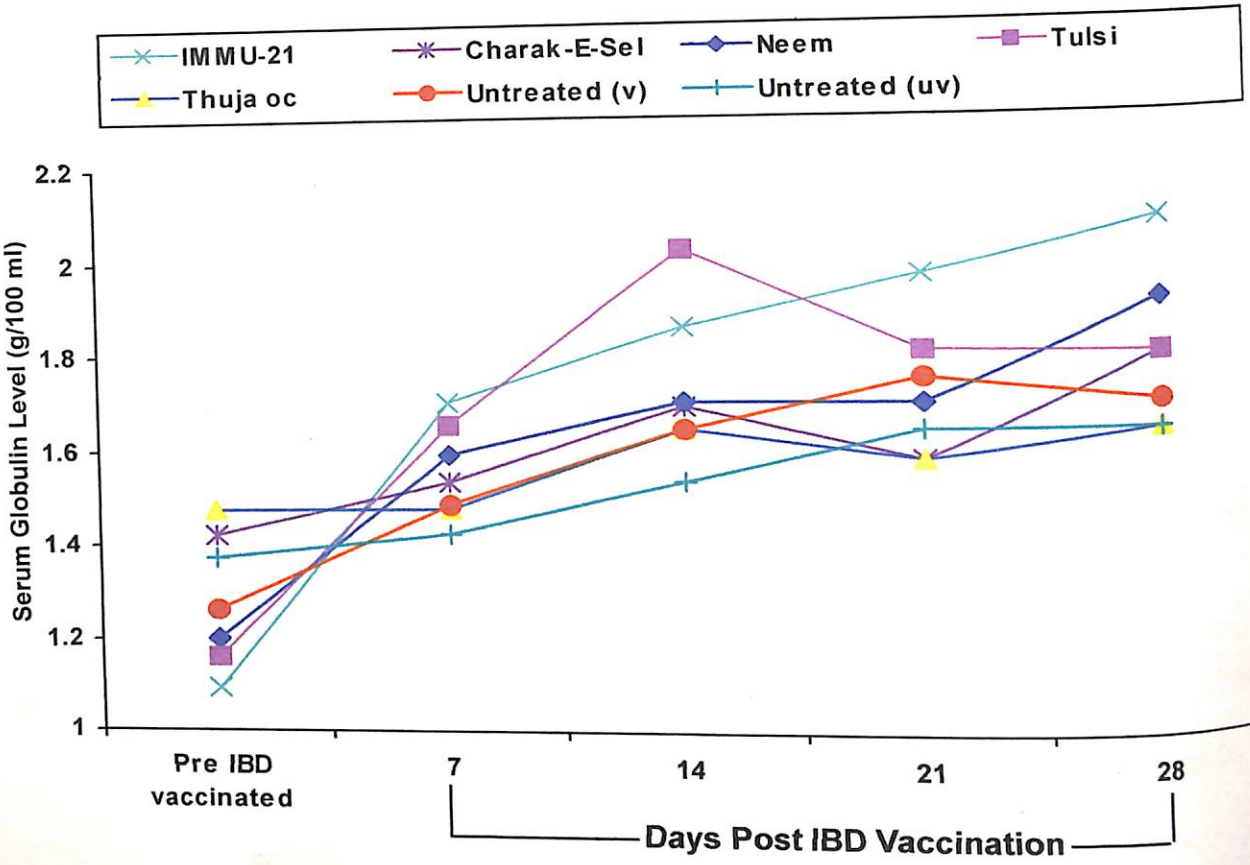

Fig. 19. Histogram showing Serum potassium level pre & post IBD vaccination in different groups of Chickens.

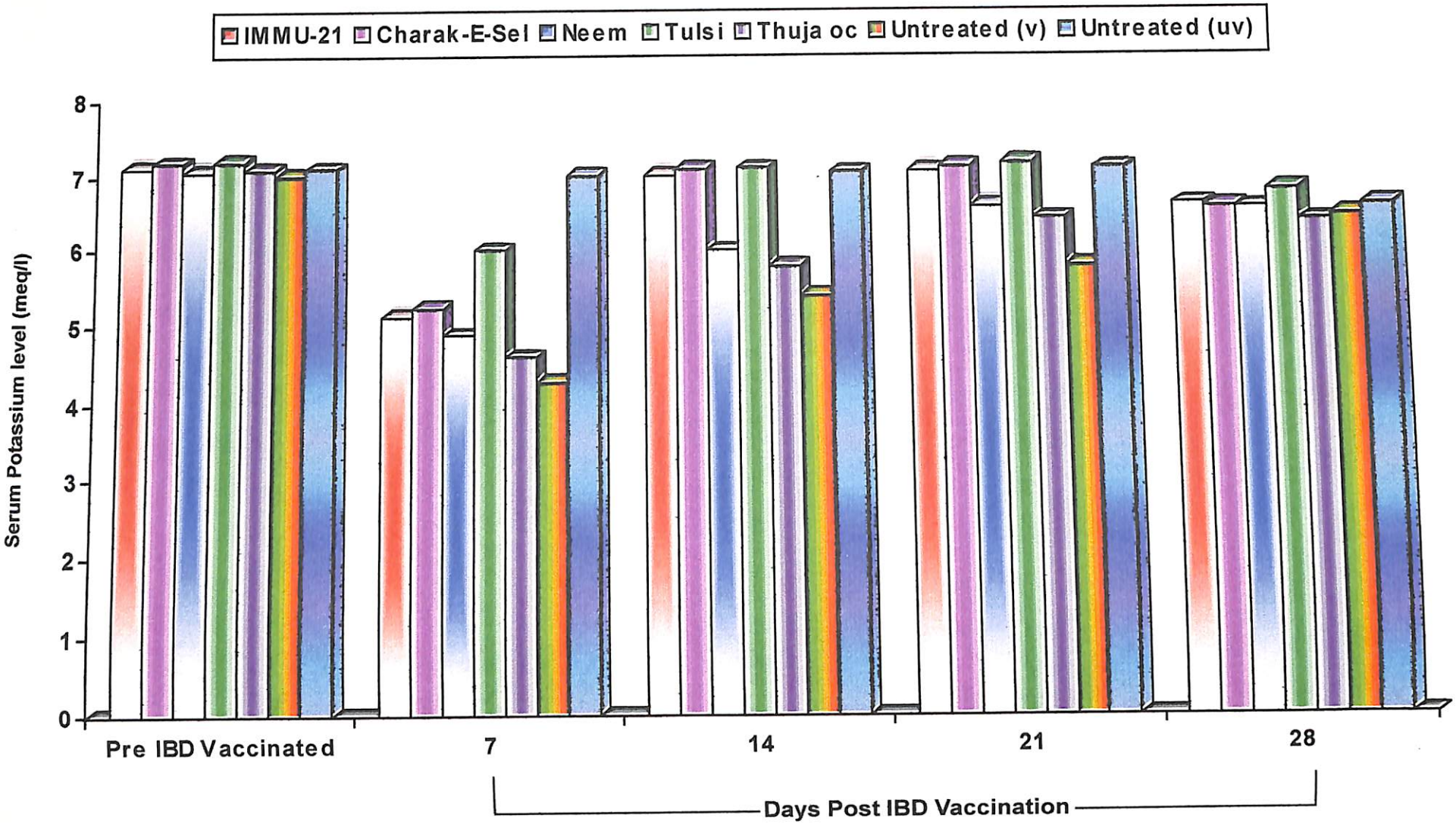

TABLE – 6. SERUM SODIUM AND POTASSIUM LEVELS (meq/l) IN DIFFERENT GROUPS OF CHICKENS.

Group	Age at IBD vaccination (days)	Treatment	Mean ± SE of Serum Sodium Level					Mean ± Mean SE of Serum potassium Level				
			Pre IBD Vaccinated	Day Post IBD Vaccination				Pre IBD Vaccinated	Day Post IBD Vaccination			
				7	14	21	28		7	14	21	28
I	14	IMMU-21	147.8^{a} ± 2.009 (5)	150.8^{a} ± 0.860 (5)	154.2^{a} ± 1.655 (5)	164.2^{a} ± 0.583 (5)	165.2^{a} ± 1.240 (5)	7.12^{a} ± 0.685 (5)	5.12^{b} ± 0.425 (5)	7.00^{b} ± 0.308 (5)	7.06^{a} ± 0.514 (5)	6.62^{a} ± 0.115 (5)
II	14	Charak- E – Sel	148-4^{a} ± 2. 014 (5)	151.6^{a} ± 1.077 (5)	153.8^{a} ± 1.240 (5)	164.00^{a} ± 0.707 (5)	165.4^{a} ± 1.208 (5)	7.18^{a} ± 0.645 (5)	5.24^{b} ± 0.335 (5)	7.08^{b} ± 0.399 (5)	7.12^{a} ± 0.321 (5)	6.58^{a} ± 0.168 (5)
III	14	Neem	147.2^{a} ± 1.714 (5)	150.6^{a} ± 1.077 (5)	153.4^{a} ± 1.568 (5)	163.4^{a} ± 0.812 (5)	163.8^{a} ± 0.860 (5)	7.06^{a} ± 0.620 (5)	4.88^{ab} ± 0.188 (5)	6.00^{a} ± 0.184 (5)	6.58^{a} ± 0.208 (5)	6.56^{a} ± 0.150 (5)
IV	14	Tulsi	150.2^{a} ± 0.969 (5)	151.00^{a} ± 1.870 (5)	153.6^{a} ± 1.363 (5)	163.0^{a} ± 1.643 (5)	165.00^{a} ± 0.707 (5)	7.20^{a} ± 0.568 (5)	6.00^{c} ± 0.170 (5)	7.10^{b} ± 0.268 (5)	7.16^{a} ± 0.285 (5)	6.80^{a} ± 0 .234 (5)
V	14	Thuja oc.	149.6^{a} ± 2.561 (5)	151.2^{a} ± 0.583 (5)	154.00^{a} ± 1.732 (5)	163.2^{a} ± 1.428 (5)	164.8^{a} ± 0.800 (5)	7.08^{a} ± 0.570 (5)	4.60^{ab} ± 0.122 (5)	5.80^{a} ± 0.273 (5)	6.42^{a} ± 0.215 (5)	6.40^{a} ± 0.277 (5)
VI	14	Untreated	150.00^{a} ± 1.224 (5)	151.4^{a} ± 1.630 (5)	153.00^{a} ± 1.224 (5)	158.8^{a} ± 1.881 (5)	164.00^{a} ± 1.881 (5)	7.00^{a} ± 0.433 (5)	4.28^{a} ± 0.058 (5)	5.40^{a} ± 0.230 (5)	5.80^{a} ± 0.234 (5)	6.46^{a} ± 0.211 (5)
VII	Unvaccinated	Untreated	149.00^{a} ± 2.167 (5)	150.4^{a} ± 1.029 (5)	152.8^{a} ± 1.392 (5)	159.80^{a} ± 2.416 (5)	163.00^{a} ± 1.224 (5)	7.10^{a} ± 0.424 (5)	7.00^{d} ± 0.298 (5)	7.04^{b} ± 0.315 (5)	7.10^{a} ± 0.412 (5)	6.60^{a} ± 0.181 (5)

- Figures in parentheses indicate number of observations.
- Mean bearing common superscripts (a, b, c, d) in individual column did not differ significantly ($P<0.05$).

Effects of selected drugs/agents on haematological profile of IBD (IV95) vaccinated chickens :

The mean ± SE of Hb values have been presented in table 7. The values did not differ significantly in any of the groups at any intervals post IBD vaccination.

The effect of IBD vaccine on PCV values are depicted in table 8. The PCV values of the group which received only IBD vaccination alone (gr. VI) were found to be lower than the corresponding values in the control group (gr. VII) over all the periods post IBD vaccination. But none of the values differed significantly. However, the PCV values in the drug treated groups (gr. I - V) were within the physiological range and they also did not differ significantly from each other at any intervals post IBD vaccinations when compared with the control values (gr. VII).

Mean ± S.E. of TLC value are depicted in table-9. In the group which received only IBD vaccination (gr. VI) the TLC values were significantly lower than the corresponding values in the control group (gr. VII) over all the periods post IBD vaccination. Further, the TLC values in all the treatment groups (gr. I to V) on 7 and 14 days post IBD vaccination was significantly higher than the corresponding values of vaccinated group (gr. VI) which did not receive any drug treatment. The IMMU-21 and Charak-E-sel treated group (gr. I and II) showed significantly higher TLC values than corresponding values in other drug treated groups (gr. III, IV and V) on 21 days post IBD vaccination. However, the TLC values in the drug treated groups (gr. I – V) showed variations but all these values were within the physiological range.

TABLE 7. HAEMOGLOBIN LEVEL (gm %) IN DIFFERENT GROUPS OF CHICKENS

Group	Age at IBD Vaccination	Treatment	Mean ± SE of Hb level				
			Pre IBD Vaccinated	Post IBD Vaccination Days			
				7	14	21	28
I	14	IMMU-21	10.26^{a} ± 0.186 (5)	10.12^{a} ± 0.165 (5)	11.28^{a} ± 0.193 (5)	10.86^{a} ± 0.143 (5)	10.68^{a} ± 0.159 (5)
II	14	Charak-E-Sel	10.28^{a} ± 0.208 (5)	10.32^{a} ± 0.215 (5)	10.84^{a} ± 0.156 (5)	11.20^{a} ± 0.158 (5)	11.38^{a} ± 0.222 (5)
III	14	Neem	10.34^{a} ± 0.215 (5)	10.24^{a} ± 0.186 (5)	11.36^{a} ± 0.218 (5)	11.18^{a} ± 0.203 (5)	11.34^{a} ± 0.215 (5)
IV	14	Tulsi	10.22^{a} ± 0.171 (5)	10.18^{a} ± 0.177 (5)	11.30^{a} ±0.194 (5)	11.26^{a} ± 0.186 (5)	10.88^{a} ± 0.165 (5)
V	14	Thuja oc.	10.30^{a} ± 0.194 (5)	10.28^{a} ± 0.193 (5)	10.98^{a} ± 0.185 (5)	11.14^{a} ± 0.196 (5)	11.30^{a} ± 0.194 (5)
VI	14	Untreated	10.20^{a} ± 0.158 (5)	10.16^{a} ± 0.215 (5)	10.88^{a} ± 0.086 (5)	11.22^{a} ± 0.171 (5)	11.26^{a} ± 0.174 (5)
VII	Unvaccinated	Untreated	10.32^{a} ± 0.198 (5)	10.34^{a} ± 0.067 (5)	10.96^{a} ± 0.267 (5)	11.06^{a} ± 0.102 (5)	11.28^{a} ± 0.188 (5)

- **Figures in parentheses indicate number of observations.**
- **Mean bearing common superscripts (a) in individual column did not differ significantly ($P<0.05$).**

TABLE 8. PACKED CELL VOLUME (%) IN DIFFERENT GROUP OF CHICKENS.

Group	Age at IBD Vaccination	Treatment	Mean ± SE of packed cell volume				
			Pre IBD Vaccinated	Days Post IBD Vaccination			
				7	14	21	28
I	14	IMMU-21	33.45^{a} ± 0.374 (5)	33.70^{a} ± 0.458 (5)	33.33^{a} ± 0.306 (5)	33.08^{a} ± 0.306 (5)	33.33^{a} ± 0.233 (5)
II	14	Charak-E-Sel	33.45^{a} ± 0.317 (5)	33.57^{a}± 0.422 (5)	33.58^{a} ± 0.151(5)	32.83^{a} ± 0.154(5)	32.32^{a} ± 0.252 (5)
III	14	Neem	33.95^{a}± 0.232 (5)	34.07^{a}± 0.151(5)	33.82^{a}± 0.375 (5)	33.32^{a}± 0.458 (5)	32.95^{a}± 0.375(5)
IV	14	Tulsi	33.82^{a}± 0.341 (5)	33.95^{a}± 0.411 (5)	34.07^{a}±0.151 (5)	33.57^{a}± 0.422 (5)	33.07^{a}± 0.460(5)
V	14	Thuja oc.	33.33^{a}± 0.306 (5)	33.45^{a}± 0.318 (5)	33.45^{a}± 0.317 (5)	32.70^{a}± 0.235 (5)	32.45^{a}± 0.238 (5)
VI	14	Untreated	33.82^{a}± 0.484 (5)	33.82^{a}± 0.341 (5)	33.45^{a}± 0.374(5)	33.20^{a}± 0.394 (5)	32.58^{a}± 0.281(5)
VII	Unvaccinated	Untreated	33.82^{a}± 0.521 (5)	33.95^{a}± 0.231(5)	34.20^{a}± 0.151 (5)	33.82^{a}± 0.341(5)	33.20^{a}± 0.523 (5)

- Figures in parentheses indicate number of observations.
- Mean bearing common superscripts (a) in individual column did not differ significantly ($P<0.05$).

TABLE 9. TOTAL LEUCOCYTE COUNT (THOUSAND/μl) IN DIFFERENT GROUPS OF CHICKENS.

Group	Age at IBD Vaccination (Days)	Treatment	Mean ± SE total leucocyte count				
			Pre IBD Vaccinated	Days Post IBD Vaccination			
				7	14	21	28
I	14	IMMU-21	$18.70^{a} \pm 0.194$ (5)	$19.80^{cd} \pm 0.228$ (5)	$20.00^{c} \pm 0.437$ (5)	$21.00^{b} \pm 0.368$(5)	$22.30^{b} \pm 0.141$ (5)
II	14	Charak-E-Sel	$18.80^{a} \pm 0.454$ (5)	$19.50^{bcd} \pm 0.248$(5)	$20.00^{c} \pm 0.284$ (5)	$21.10^{b} \pm 0.187$ (5)	$22.00^{ab} \pm 0.161$(5)
III	14	Neem	$18.60^{a} \pm 0.230$(5)	$18.90^{b} \pm 0.433$(5)	$19.00^{b} \pm 0.262$(5)	$20.00^{a} \pm 0.308$(5)	$22.24^{b} \pm 0.153$(5)
IV	14	Tulsi	$18.90^{a} \pm 0.433$(5)	$19.00^{bc} \pm 0.288$(5)	$19.60^{bc} \pm 0.230$(5)	$20.10^{a} \pm 0.447$(5)	$21.90^{ab} \pm 0.083$(5)
V	14	Thuja oc.	$19.00^{a} \pm 0.386$ (5)	$19.10^{bcd} \pm 0.130$ (5)	$19.80^{bc} \pm 0.356$ (5)	$20.00^{a} \pm 0.291$ (5)	$22.00^{ab} \pm 0.130$(5)
VI	14	Untreated	$19.10^{a} \pm 0.202$ (5)	$16.80^{a} \pm 0.366$ (5)	$17.30^{a} \pm 0.336$ (5)	$19.50^{a} \pm 0.284$ (5)	$21.60^{a} \pm 0.151$ (5)
VII	Unvaccinated	Untreated	$19.20^{a} \pm 0.343$ (5)	$19.90^{d} \pm 0.173$ (5)	$20.00^{c} \pm 0.291$ (5)	$21.20^{b} \pm 0.158$ (5)	$22.20^{b} \pm 0.192$ (5)

- **Figures in parentheses indicate number of observations.**
- **Mean bearing common superscripts (a, b, c, d) in individual column did not differ significantly ($P<0.05$).**

Fig. 20. Histogram showing Total leucocyte count pre & post IBD vaccination in different groups of chickens.

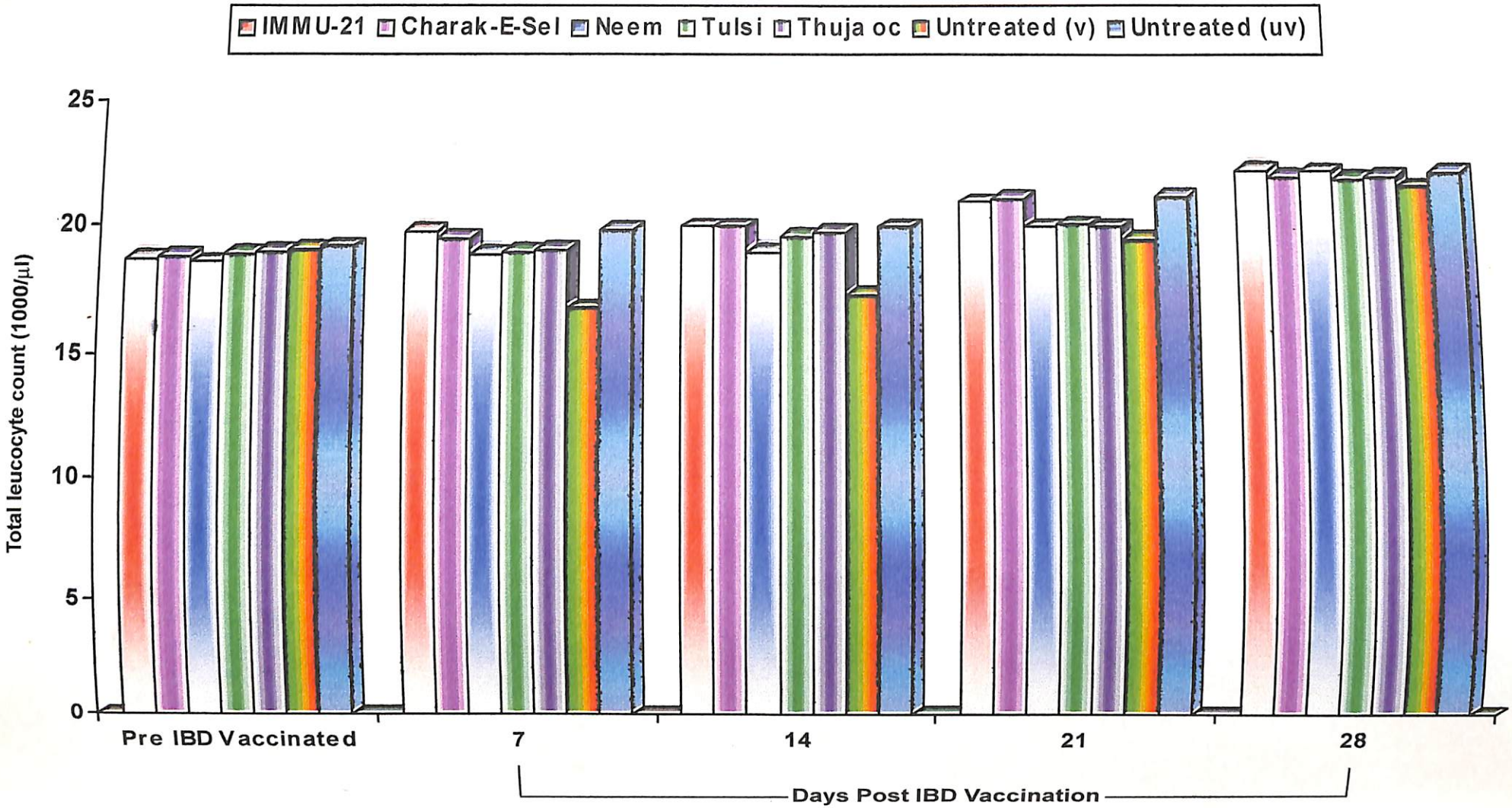

TABLE – 11. DATA OF PERFORMANCE OF CHICKENS IN DIFFERENT TREATMENT GROUPS.

Group	Age at IBD Vaccination (days)	Treatment	Mean ± S.E of Initial body wt; final body wt., Body wt. gain & FCR				Mortality (%)
			Initial body wt. on day –1 (gm)	Final body wt. on day – 42 (gm)	Body wt. gain on day 42 (gm)	Feed conversion ratio	
I	14	IMMU – 21	$42.40^{a} \pm 0.598$ (30)	$1097.38^{cd} \pm 28.267$ (21)	$1054.48^{cd} \pm 28.628$ (21)	$2.10^{a} \pm 0.060$ (21)	9.98
II	14	Charak-E-Sel	$43.17^{a} \pm 0.744$ (30)	$1097.38^{cd} \pm 28.267$ (21)	$1053.24^{bcd} \pm 28.637$ (21)	$2.22^{a} \pm 0.075$ (21)	9.98
III	14	Neem	$43.10^{a} \pm 0.578$ (30)	$1056.67^{bc} \pm 14.913$ (21)	$1013.67^{bc} \pm 15.116$ (21)	$2.21^{a} \pm 0.046$ (21)	0
IV	14	Tulsi	$43.27^{a} \pm 0.830$ (30)	$1131.19^{de} \pm 20.029$ (21)	$1087.86^{d} \pm 20.500$ (21)	$2.28^{a} \pm 0.047$ (21)	0
V	14	Thuja oc	$42.43^{a} \pm 0.572$ (30)	$1034.29^{b} \pm 17.896$ (21)	$992.29^{ab} \pm 18.139$ (21)	$2.12^{a} \pm 0.059$ (21)	14.18
VI	14	Untreated	$42.45^{a} \pm 0.511$ (30)	$954.34^{a} \pm 14.516$ (21)	$916.95^{a} \pm 17.965$ (21)	$2.29^{a} \pm 0.055$ (21)	18.44
VII	Unvaccinated	Untreated	$45.43^{a} \pm 0.183$ (30)	$1158.81^{e} \pm 22.667$ (21)	$1110.14^{d} \pm 22.566$ (21)	$2.25^{a} \pm 0.184$ (21)	18.44

- **Figures in parentheses indicate number of observations.**
- **Mean bearing common superscript (a, b, c, d, e) in individual column did not differ significantly ($P<0.05$).**

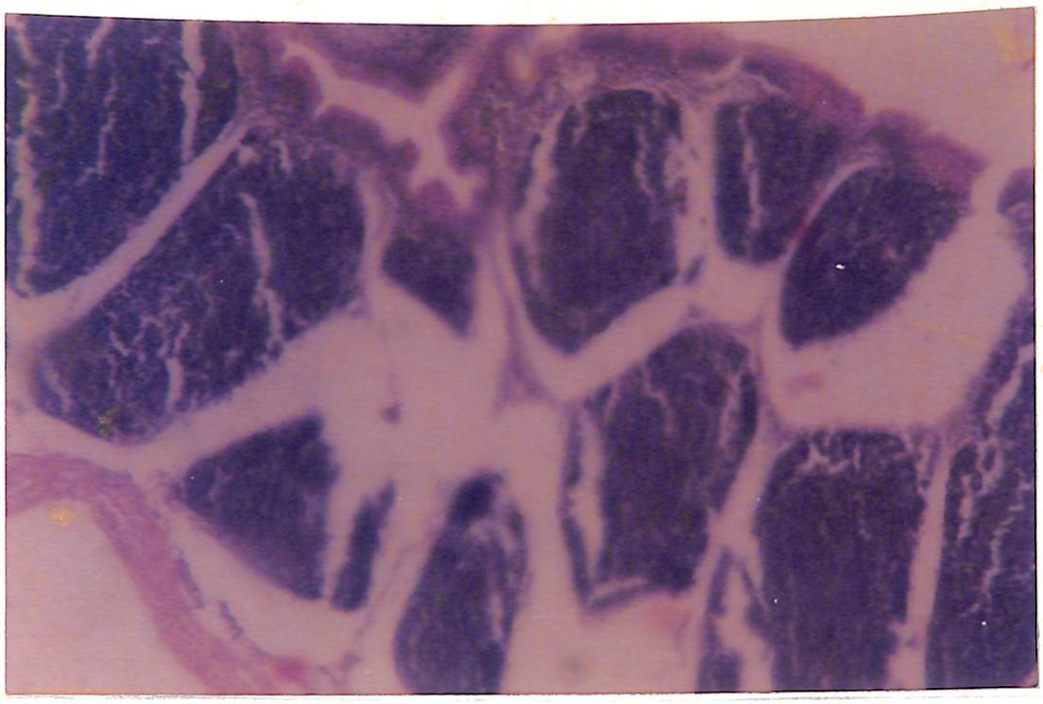

Fig. 1 Microphotograph of bursa of Fabricius of chick sacrificed 96 hrs. Post IBD vaccination showing aggregation of degenerated and necrosed cell leaving vaccant follicular space (H & E x 100)

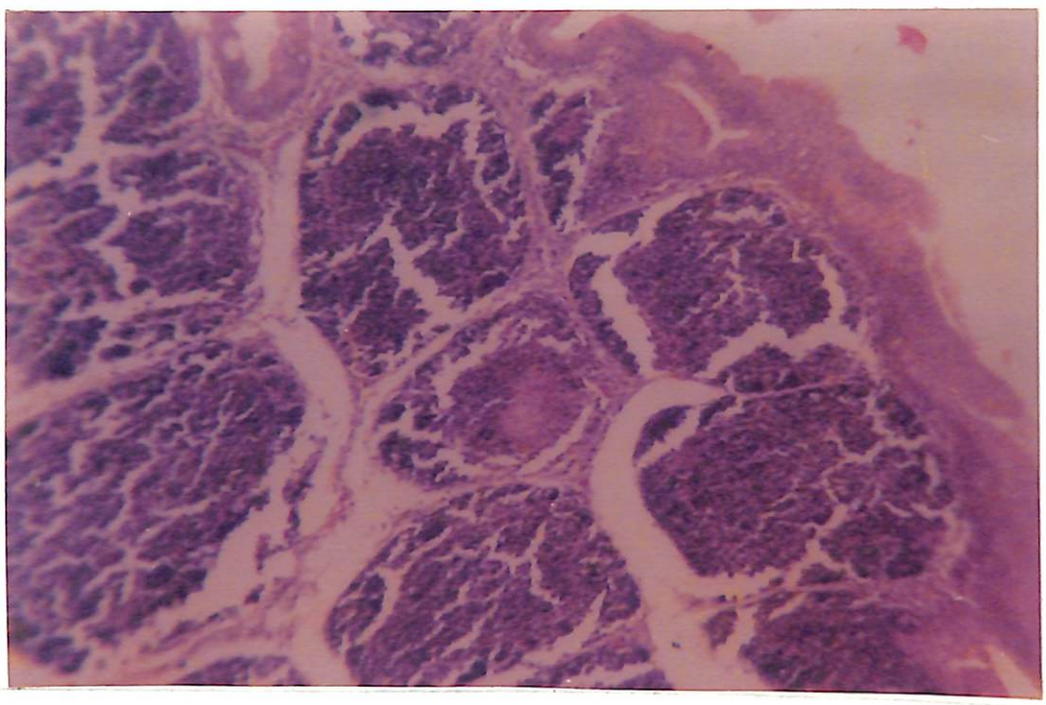

Fig. 2 Microphotograph of bursa of Fabricius of chick sacrificed 96 hrs. Post IBD vaccination showing marked degeneration of lymphoid cells as evidenced by presence of homogenous mass in the bursal follicle (H & E x 100).

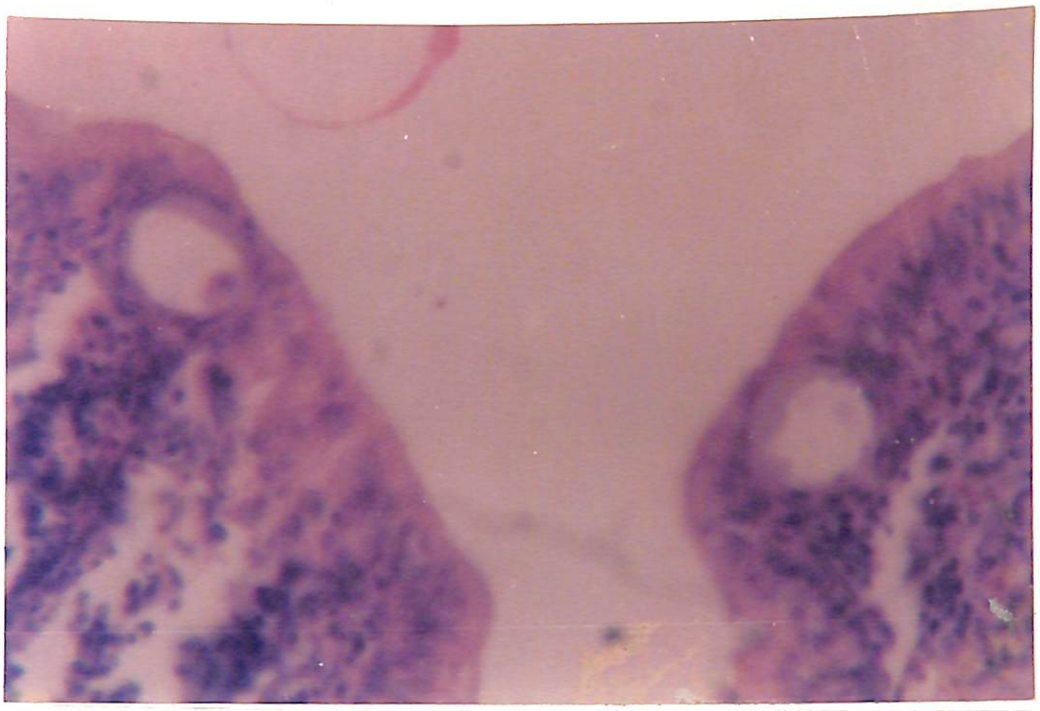

Fig. 3 Microphotograph of bursa of Fabricius of chick sacrificed 96 hrs. Post IBD vaccination showing presence of plical cyst.
(H & E x 400).

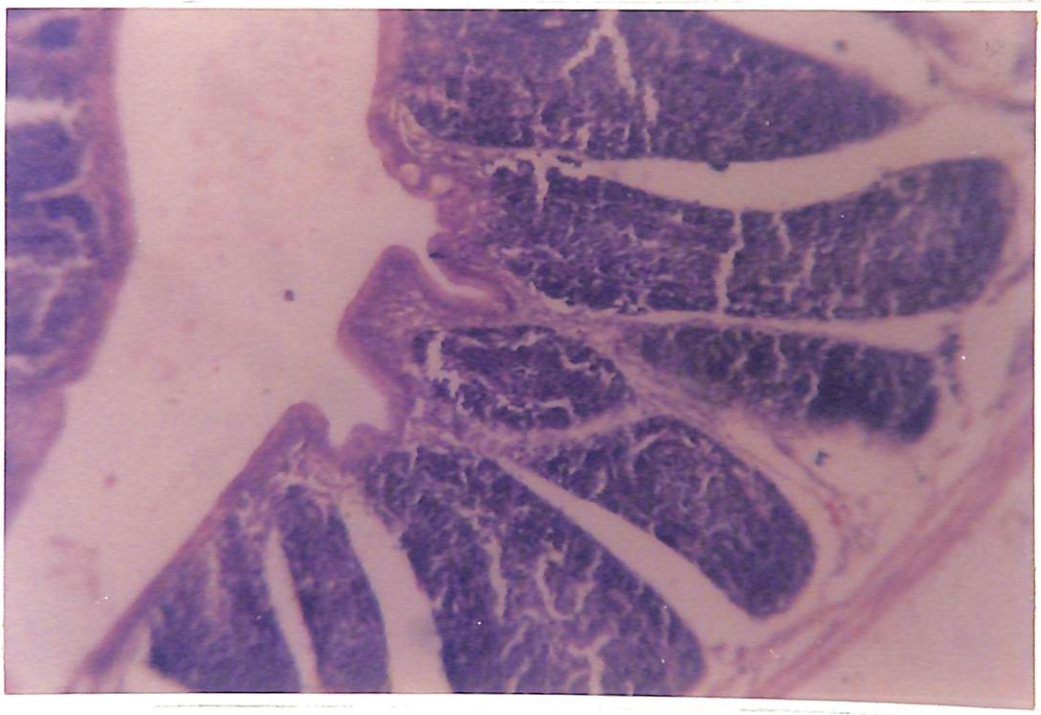

Fig. 4 Microphotograph of bursa of Fabricius of chick sacrificed 96 hrs. Post IBD vaccination showing marked invagination and vacuolar degeneration of plical epithelium (H & E x 100).

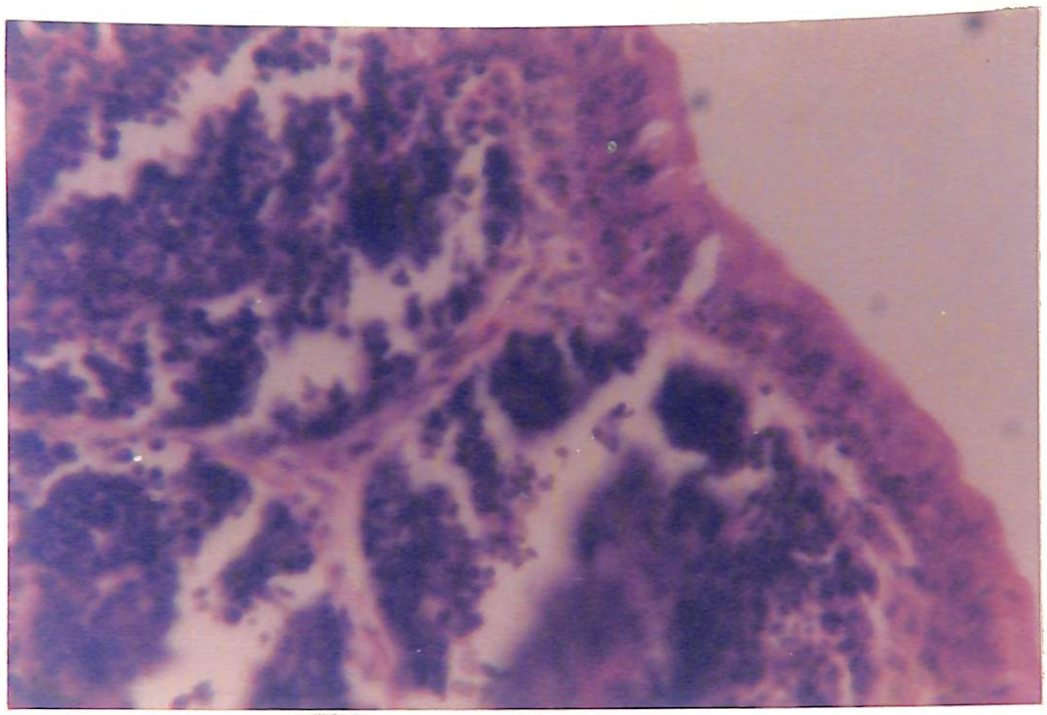

Fig. 5 Microphotograph of bursa of Fabricius of chick sacrificed 96 hrs. Post IBD vaccination showing degeneration and depletion of lymphoid cells in the bursal follicle (H & E x 400).

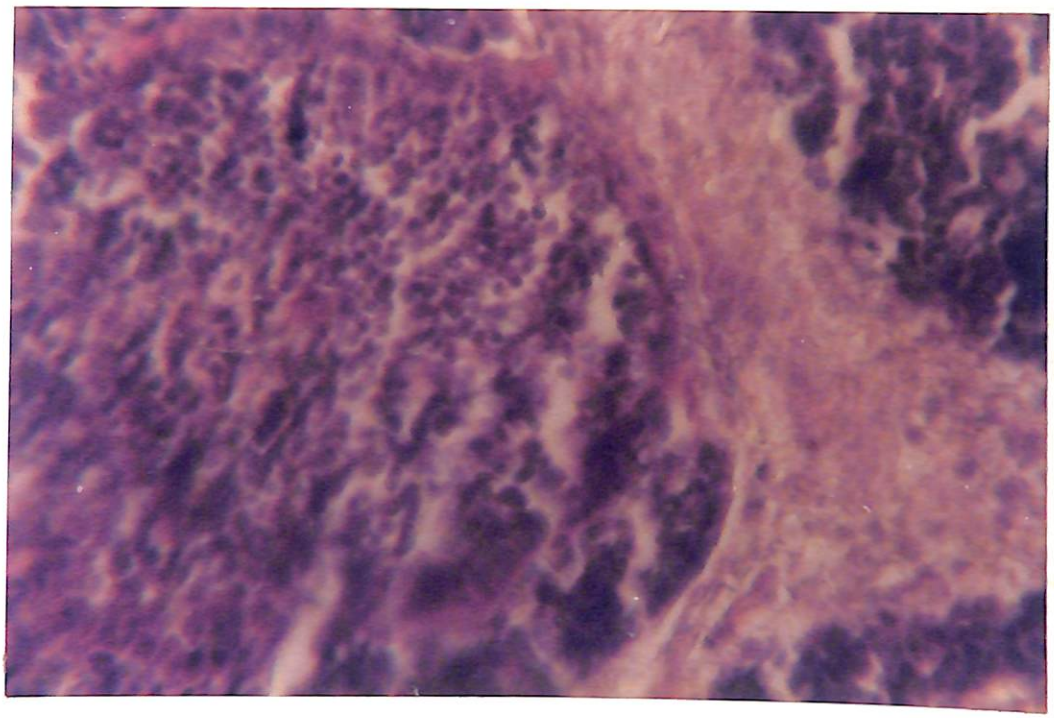

Fig. 6 Microphotograph of bursa of Fabricius of chick sacrificed 96 hrs. Post IBD vaccination showing extensive fibrosis of interfollicular space and marked degeneration of follicular lymphoid cells (H & E x 400).

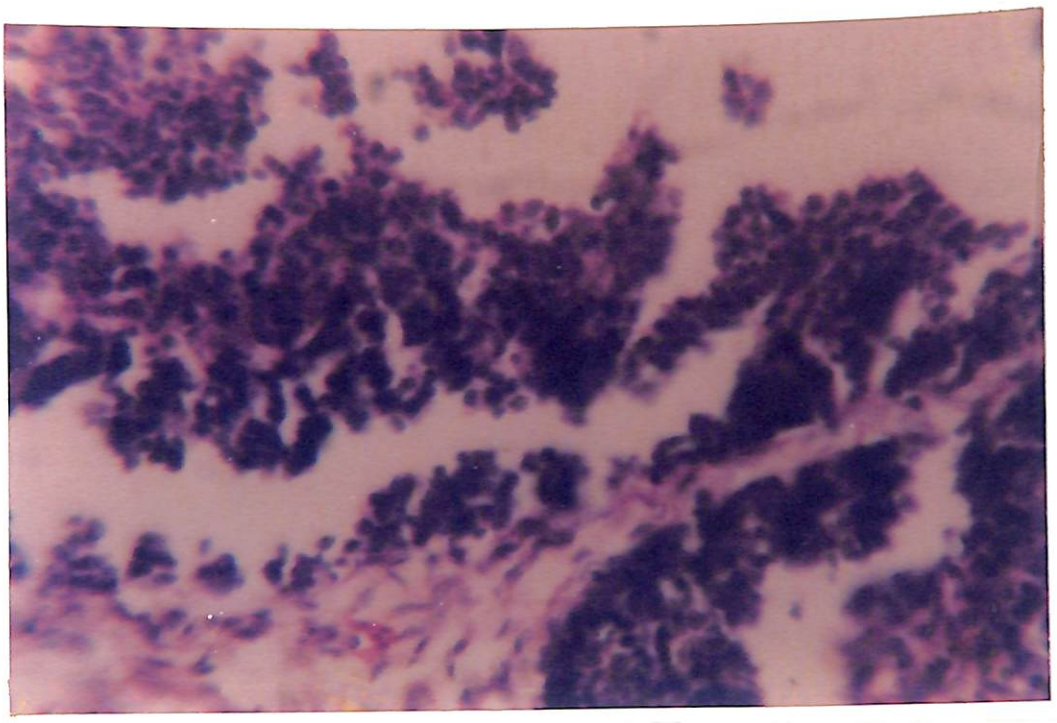

Fig. 7 Microphotograph of bursa of Fabricius of chick sacrificed 96 hrs. Post IBD vaccination showing marked loss of lymphoid cells and aggregation of degenerative cells with pyknotic nuclei in the bursal follicle (H & E x 400).

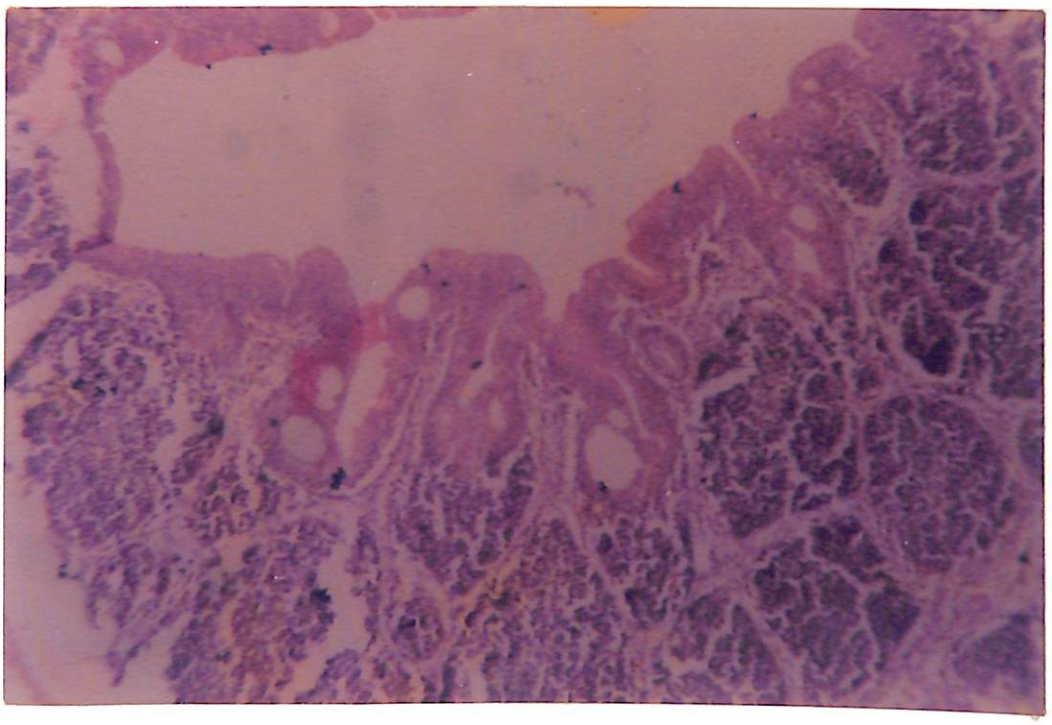

Fig. 8 Microphotograph of bursa of Fabricius of chick sacrificed 96 hrs. Post IBD vaccination showing marked invagination and cystic degeneration of plical epithelium (H & E x 100).

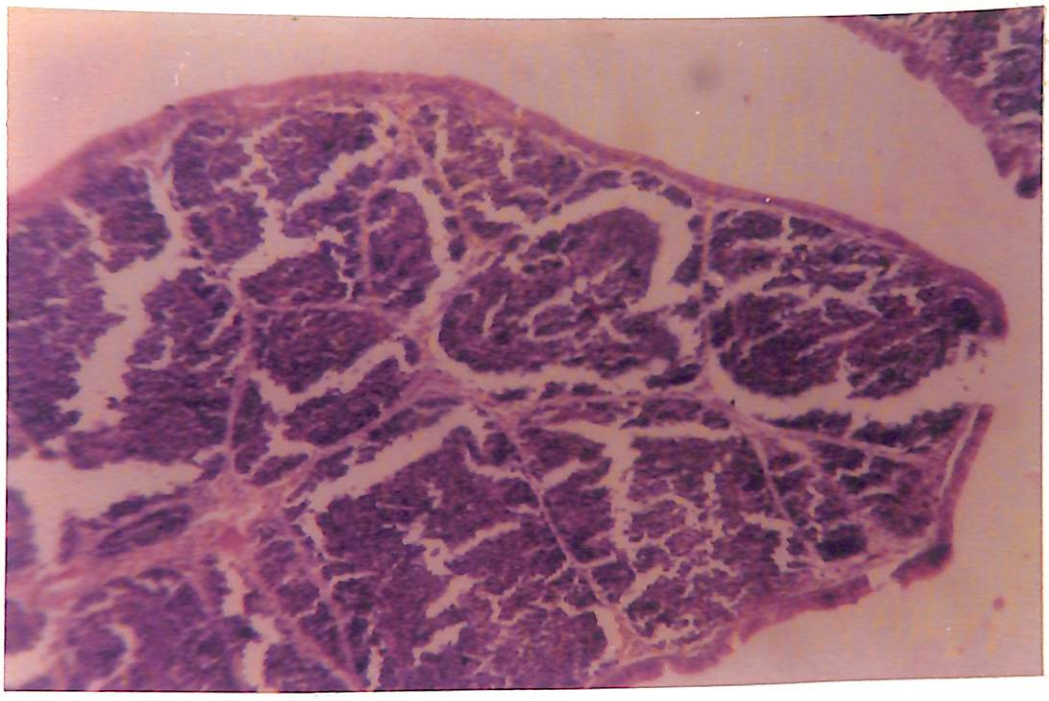

Fig. 9 Microphotograph of bursa of Fabricius of chick sacrificed 96 hrs. Post IBD vaccination showing degeneration and loss of lymphoid in the follicle (H & E x 100).

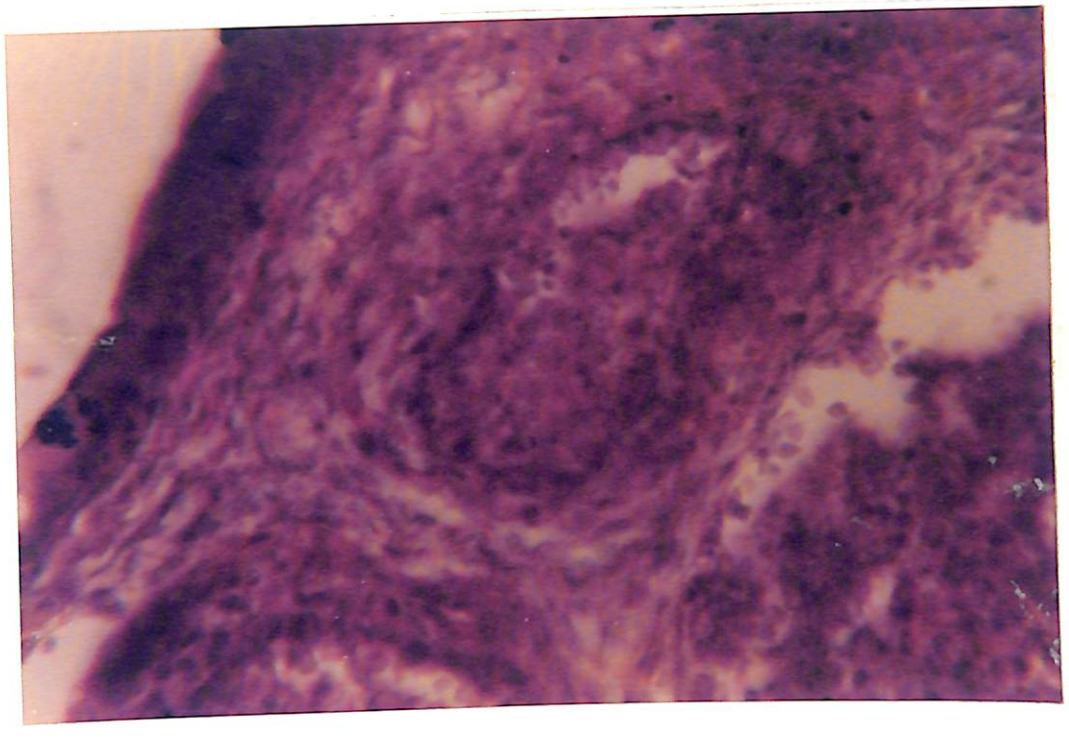

Fig. 10 Microphotograph of bursa of Fabricius of chick sacrificed 96 hrs. Post IBD vaccination showing extensive fibrosis replacing the normal lymphocytic cell population of follicle (H & E x 400).

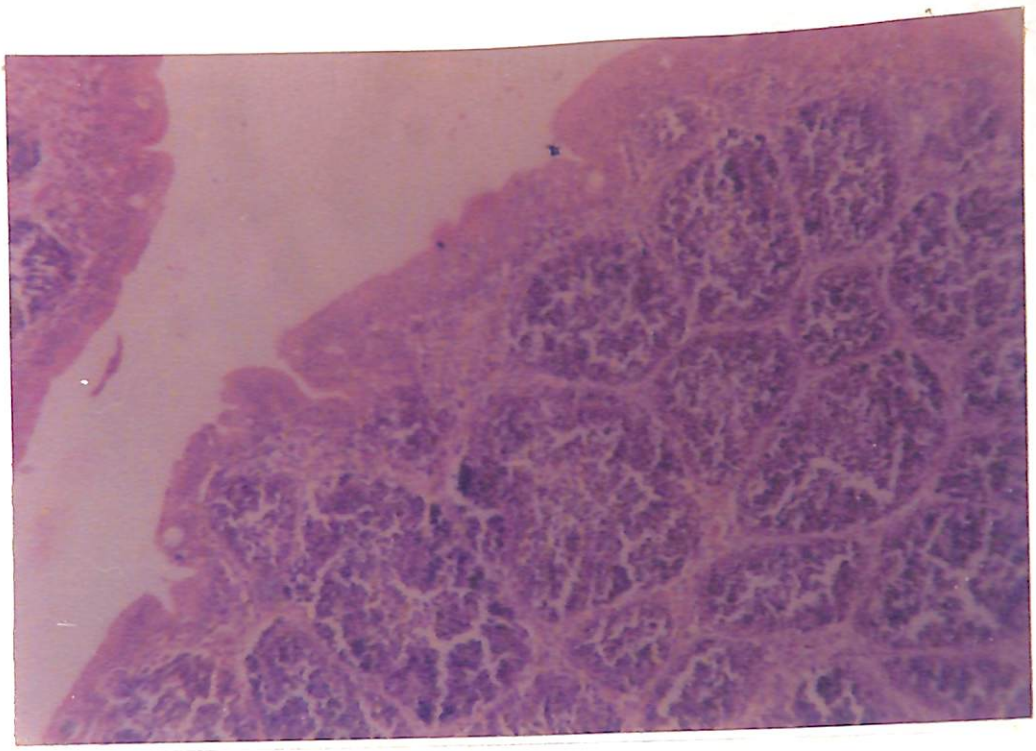

Fig. 11 Microphotograph of bursa of Fabricius of chick sacrificed 96 hrs. Post IBD vaccination showing marked invagination of plical epithelium (H & E x 100).

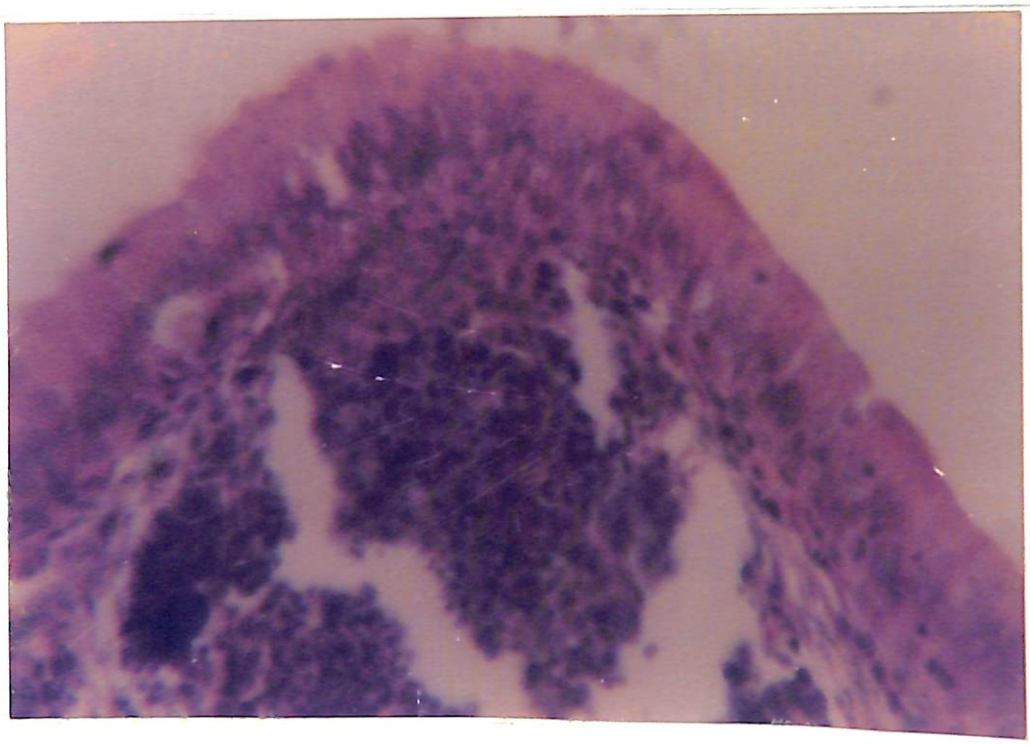

Fig. 12 Microphotograph of bursa of Fabricius of chick sacrificed 96 hrs. Post IBD vaccination showing marked necrosis and depletion of lymphoid cells of the follicle (H & E x 400).

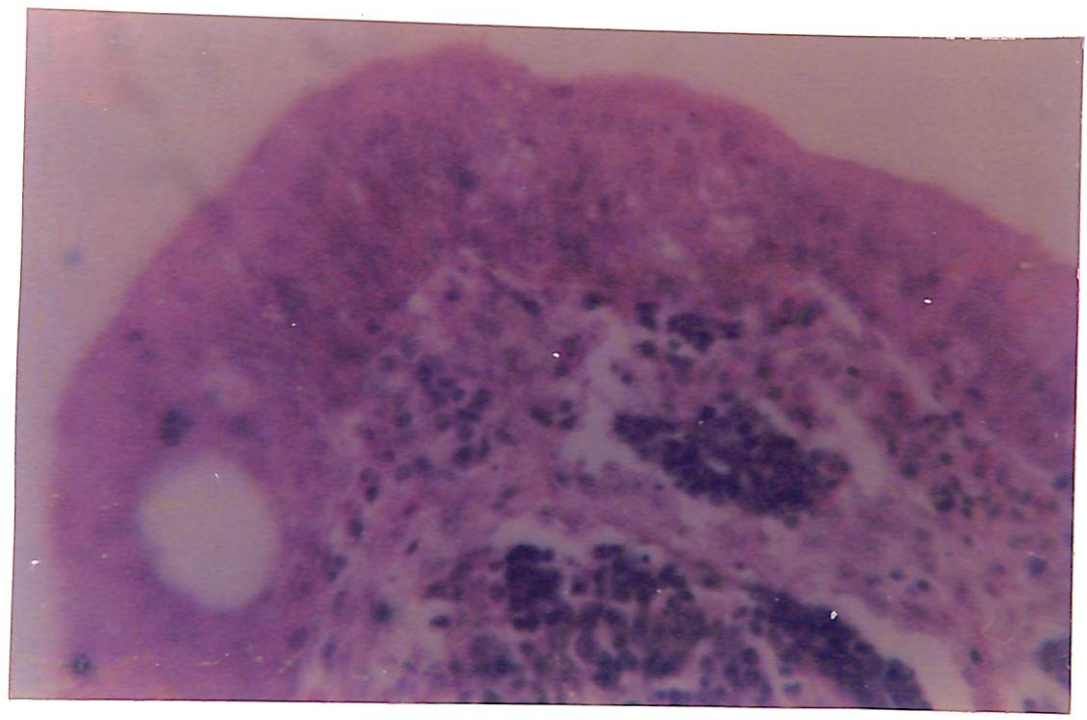

Fig. 13 Microphotograph of bursa of Fabricius of chick sacrificed 96 hrs. Post IBD vaccination showing marked thickening of plical epithelium and presence of plical cyst (H & E x 400).

CHAPTER - V

DISCUSSION

DISCUSSION

Infectious bursal disease (IBD) is a highly contagious immunosuppressive viral disease of young chickens which causes significant economic losses to poultry industry worldwide. Since 1991-92 a new form of IBD, also called very virulent infectious bursal disease (vvIBD) is being reported from different parts of the country including the state of Bihar (Singh *et al.* 1994, Sinha 1997). This new form of IBD causes massive immunosuppression in infected flocks and mortality as high as 80% and above. The disease mostly occurred in the age group of 3-8 weeks and in some cases adult birds of 12 weeks of age were also effected. vvIBD outbreaks have also been reported from farms which has received intermediate strain IBD vaccine at two weeks of age. A number of measures were tried by the owners of different farms with a view to minimise the economic losses as well as to stop the perpetuation of virus in the surroundings. These measures mainly included repeat vaccination with the same intermediate strain IBD vaccine as well as administration of antibiotics or electrolytes, vitamin A, vitamin E as well as some known immunomodulators like Zeetress, Levamisole etc. Though these treatments were not performed under controlled condition but there were indications of reduction in number of death and improvement in body wt. gain. However, the outbreaks due to vvIBD continued unabated until such time a new vaccine called invasive intermediate strain or

intermediate plus or Hot strain vaccine was introduced by the end of the year 1996. Though the new vaccine is being used strictly as per the manufacturer's direction, it is also not foolproof because a number of complications are being reported on such farms which have used this vaccine. The occurrence of such complications has been correlated with the state of immunosuppressions which may be the consequence of administration of this new vaccine which is reported to posses relatively more residual pathogenicity as well as invasiveness as compared to conventional IBD vaccines. 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 the light of the above study as well as taking into account the earlier reports of residual pathogenicity of the vaccine strain it was felt necessary to look for some alternative approach which could successfully counter the aftereffects that follows this vaccination and therefore, makes this vaccine more acceptable by the farmers. It is in this backdrop that the present study has been planned to evaluate the efficacy of certain immunopotentiating agents/drugs in controlling the aftereffects of newly introduced vaccine.

The antibody levels at various intervals post IBD vaccination are shown in table - 2. Perusal of this table revealed that the antibody titre to IBD vaccine has rising tendency right from the

time when first seroconversion was detected i.e. by 14 days post IBD vaccination till the last days of observation on 28 days post vaccination (Fig. 14a). One of the observations recorded in the present study was detection of seroconversion on 14 days post vaccination but not on 7 dpv. A number of workers have reported seroconversion to IBD virus on 14 days post Vaccination (Hirai *et al.*, 1972; Ley *et al.*, 1983; Zorman Rojs *et al.*, 1996; Kumar, 1998). On the contrary there are several reports that the seroconversion to IBD virus occurred as early as 3-7 days post infection (Dash *et al.*, 1991; Thangavelu *et al.*, 1993; Ley *et al.*, 1983). However, in the later case the birds employed for the study were free from maternal antibody and of higher age group (21 days old in one trial, 35 days old in another trial). There are number of reports that both maternal antibody and age factor can effect the onset of immune response, (Winterfield and Thancker, 1978; Naqi *et al.*, 1982; Gordon and Jordon, 1982; Mazorieges *et al.*, 1990; Wyeth and Chettle, 1990; Goddard *et al.*, 1994; Tsai *et al.*, 1995; Kumar 1997). Besides, factors like strain of virus (MacFerran *et al.*, 1980) type of birds (Winterfield and Thacker, 1978) route of vaccinations, (Sharma, 1985; Kembi *et al.*, 1995) and nutritional status (Marsh *et al.*, 1982) of the birds may also affect the immune response. Further, the progressive rise in the antibody titre to IBD vaccine was recorded till the last days of observation on 28 days post vaccination. Dash et al., (1991) observed a rising trend of antibody level upto 6 weeks post infection after which

it started declining. Kumar (1998) and Hindustani (2000) also reported progressive rise in antibody titre which continued till last days of observation (on 28 days post vaccination). Thus the present observation may be considered in consonance with the observation recorded by other workers cited above.

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). In the present study also this test was found to be easily reproducible and easy to perform and hence used routinely. In general, up to three precipitations lines were discernable 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 precipitation line appeared between 20 to 30 hours depending on variation in room temperature and was closer to the antigen well, the second precipitation line generally appeared between 30 to 46 hours, and was midway between antigen and antiserum wells. The third precipitation line when detected appeared between 46 to 70 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, variations in pattern of precipitin lines with IBD antigen and antibody system have also been reported (Takease *et al.*, 1993). A number of factors determine the pattern of precipitation line including concentration of antigen and antibody (Wood *et al.*, 1979; Mohanty *et al.*, 1981). Hence, some variations in pattern of precipitin lines are possible.

In recent past a number of workers have reported the application of various agents/drugs in IBD infected/vaccinated as antistress, adaptogenic and immunopotentiator in order to combat the immunosuppressive effect of the virus as well as to harness the potentials of the vaccines to a maximum level (Mc Ilroy *et al.*, 1993; Singh *et al.*, 1993; El- Zanty K, 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; Saravanabave *et al.* 1999; Hindustani, 2000). In the present study all the five agents/ drugs employed were effective in enhancing the immune response to IBD vaccine as evident from antibody titres recorded over all the intervals, when compared with corresponding values of IBD vaccinated but untreated group (gr. VI). It may be mentioned that IBD virus has suppressive effect on immune response to various vaccines, whereas normally the response to IBD virus itself remain unaffected. Therefore, the use of such immunopotentiatng

agent for enhancing the response to IBD vaccine may not sound 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 requirement of the birds are not met to the optimal level, it would not be proper to expect getting normal immune response to vaccines including IBD vaccines. Under such circumstances the relevance of immunopotentiating agent similar to one used in present study can not be denied, besides there are also report that factors like stress, climatic changes and several other known or unknown factors can adversely affect the immune response to vaccines (Saran and Sharma, 1996). Accordingly, it has became common practice to use some agents of proven immunopotentiating effect as an adjunct to vaccination programme so that the immune response of the vaccine expresses in fullest level. The relatively better immune responses recorded in different treatment groups (gr. I - V) over all the intervals may be due to antistress, adaptogenic and restorative effects of the agents used. Further the use of adjuvant along with vaccines is an age old practice and the adjuvant effect of one of the agent namely vitamin E (gr. II) 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). In the present study Charak -E-sel, (a combination of vitamin E, Selenium and Biotin) was used mainly to examine its role in

countering the immnosuppressive effects of IV95 IBD virus vaccine by way of enhancement of immune response to RD virus vaccine as well as its role on certain growth parameters to 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 et al., 1977, Dash 1980; Tengerdy, 1990; Tizzaird, 1996). According to these workers, this Vitamin E acts as antioxidant and prevents the free radicals (Peroxides and superoxides) released during disease or vaccinal challenge from the damaged cellular and intercellular structures which also includes lymphocytic cells of immune system. Whereas vitamin E is stored in the lipoprotein fraction of cell membrane, selenium forms an integral component of enzyme glutathione peroxidase, which is present in cytosol of all cells. The toxicity of free radical to cells is mainly because they attack unsaturated fatty acid component of membrane lipid, thus, damaging membrane structure. Vitamin E is the cell membrane and selenium containing enzyme in the cytosol, glutathione peroxidase form vital part of biological antioxidant system in the cells. Vitamin E being the component of cell membrane acts as an efficient scavenger of free radicals and selenium in coordination with glutathione peroxidase present in cytosol convert free radicals to inert substances rendering them harmless. Recently

both vitamin E and selenium have been found to be responsible for erythrocyte membrance integrity, since both erythrocytes and lymphoid cell originate from common stem cells, vitamin E and selenium may be associated with membrane fluidity of lymphoid cells, thus affecting immune response mechanism as well. Further vitamin E stimulate IgG synthesis and selenium promotes the increased synthesis of IgM antibody. Interestingly the level of vitamin E and selenium to be included in the diet for above purpose should be ten to thirty times the recommended dietary level. There are reports that the action of vitamin E is dose dependent and the inmunomodulatory 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; Mc Ilory *et al.*, 1993). In addition vitamin E increases humoral immunity in chicken, turkey and mammal and also 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 polymorphonucleated dendritic and plasma cells (Franchini *et al.*, 1995). Again the E type prostaglandin is known to effect immune response (Likoff *et al*, 1978) and suplementation of vitamin E reduces the prostaglandin level in immunopoietic organs and simultaneously improve antibody responses. 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 inmmune response. On other hand selenium as a constituent of cytosolic engzme, glutathione peroxidase renders the preformed free radicals inert, thus making it harmless.

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 on humoral immune response to IBD vaccine as evident from higher antibody titres in group II than the titres recorded in group VI which received IBD vaccine but not any drug treatment over all the periods post vaccination. The comparison of antibody titres between the different drug treated groups (gr. I - V) showed lowest antibody titre in charak-E-sel treated group (table - 2, Fig 14 a & b). Sheffy and Schultz (1979) and Colnago *et al*, (1984) reported synergistic action of vit. E and Selenium in their experiment whereas Reffett *et al*, (1988) and Larsen *et al*., (1981) did not.

The added importance of charak - E sel is also due to its Biotin component which has significant role in enhancement of body wt., reduction of mortality rate and improved immunity (Annon, 2001). Besides Biotin enhances the antistress activity of vit E and selenium combined which is also suggestive of the synergistic action of biotin in Charak-E-Sel. It was with this intention that this drug combination was used to counteract the aftereffects of moderate hot

strain IBD vaccine (IV95) in chicken. It may be mentioned that vit. E & selenium combination marketed as E- care - se (VET care, DIVN. of TETRAGON chemie Ltd. Yelahanka Newtown, Banglore, India) has been used in similar situation and was found to be effective as immunopotentiator, growth promoter, antistress and adaptogenic. However, charak-E-sel which is supposed to produce more beneficial effect than any drug combination which consist of vit E & selenium by virtue of the presence of Biotin showed immune enhancing effect on response to IBD vaccine (table - 2), helped in reduction of immunosuppressive effect as evident from HI titre to RD vaccine (table- 3) as well as improved body wt gain and feed conversion ratio (table - 11) when compared with the corresponding control. Further, the bursa : body wt. ratio recorded in charak-E-Sel treated chickens (gr. II) were comparable to the value of the ratio in unvaccinated control birds (gr. VII), which is suggestive of the positive role of this drugs in bringing the size of bursa to normalcy. It is also suggestive of the fact that this drug has been able to check the damaging effect of the vaccine virus on lymphoid cells of the bursa of Fabricius which is also reflected in terms of relatively lower mortality in this group of bird (table - 11). On over all consideration charak - E - Sel may be considered to have encouraging role as immnopotantiator, growth promotor and enhanced body resistance in terms of reduced mortality.

The role of Herbal preparation in immnomodulation, growth promotion and enhancement of resistance to diseases has been well documented (Chaterjee, 1994a; Vijay Kumar, 1994; Agrawal, 1994, Mandal *et al.*, 1992; Rao *et al.*, 1995) IMMU - 21 is polyherbal formulation of Indian Herbs, Saharanpur, which contains the extracts of natural plants such as *ocimum sanctum* (Tulsi) and *Withania Sommifera* (Ashwathgandha) as a major constituent. Different study on IMMU - 21 showed that it has antistress, adaptogenic, immunomodulatory and antioxidant potential, by way of adaptogenic and antistress effect it helps the individual to cope better during stressfull situation, retard the ageing process and give feeling of well being (Chatterjee, 1994b; Bhattacharya and Ghosal 1994). Interestingly each of the ingredients has shown immunomodulatory effect when used separately (Sadeker *et al*, 1998a; Thatte *et al.*, 1986; Kolte *et al.*, 1999). The work conducted so far have revealed that their mechanism of action is mainly by enhancing humoral immune response, macrophage activity, phagocytosis, as well as T- cell mediated immunity. It is claimed to be an antistress and immunomodulator, which increase body resistance by modulating hypothalumopitutory- adrenal Axis (Agrwal, 1994) and attenuates the augmented levels of circulatory corticosteroids due to oxidative stress which is commonly observed and is responsible for immunosupression. In the present study this drug produced enhancing effect on antibody titre to hot strain IBD virus at different

intervals post IBD vaccination, when compared to antibody titre of group VI (Table - 2). The present finding corroborates the observation of the earlier workers.

Neem and Tulsi are other natural herbal drugs/agents used in this study. Neem (*Azadiracta indica*) is a indigenous tree and is attributed to have many medicinal properties. Immunopotentiating effects of this plant in term of enhanced humoral antibody response have been observed (by Sen *et al* 1992, Sadekar *et al* 1998b; Upadhyay *et al* 1992). The present investigation has been undertaken to investigate the effect of (*Azadiracta indica*) Neem dry leaves powder on humoral and cell mediated immune response in birds vaccinated with hot strain IBD vaccine. This study clearly indicate neem leaves feeding produced enhancing effect on antibody titres to hot strain IBD virus in different intervals post IBD vaccination when compared to antibody titres of gr. VI (Table - 2). The present finding corroborates the observation of the earlier workers that neem has immunopotentiating response to IBD vaccine virus (Sadekar *et al* 1998b). The comparison of the over all immune response exhibited by different drugs/agents used in this study clearly demostrated that neem showed the best immunoenhancing effect and showed the highest level of antibody titre on 21 and 28 days post IBD vacciniton (Table - 2 and figure 14a,b).

Ocimum sanctum (Tulsi) has been attributed to possess adaptogenic (antistress) and immunopotentiating properties (Bhargava and Singh 1981; Godhwani *et al*, (1988). The earlier study by Godhwani *et al.*, (1988) and Medirapta et al., (1988) have indicated cellular immunogenic response to *Ocimum sanctum* in albino rates as represented by E-rosette formation and lymphocytosis. In goats of either sex, Kotle (1993) has reported that *Ocimum sanctum* stimulated cell mediated immunity response in these animals as indicated by lymphocytosis and profound DNCB contact skin hypersensitivity reaction.

The number of workers have suggested the use of *Ocimum sanctum* (Tulsi) as an immunomodulator in chicken immunocompromised by IBD virus (Kolte *et al* 1999, Sadekar 1998a). Earlier the work conducted in this department on Zeetress, having *ocimum sanctum* (Tulsi) as the main constituent showed that it has immunopotentiating response to IBD vaccine virus (Hindustani 2000). In the present study Tulsi also produced enhancing effect on antibody titers to hot strain IBD virus at different interval post IBD vaccination when compared to antibody titre of group VI (Table-2). The comparison of the overall immune response exhibited by different herbal agents/drugs used in this study clearly demonstrated that Neem showed highest antibody titre followed by Tulsi and IMMU - 21 on 21 and 28 days post IBD vaccination. However, Tulsi produced the higher antibody titre at 14 days post vaccination when compared with

the antibody titre in the other groups reciving different agents on the same interval. There are reports that the level of antibody may not be true representative of individual resistance to infection/challenge. (Tengerdy 1975, Tengerdy *et al.*, 1977).

Due to cost effectiveness, easy availability in combating a number of livestock and poultry diseases with least side effect and drug resistance can merit, Neem, Tulsi & IMMU-21 selection as an immunomodulator for use along with IBD vaccines which incorporates virus strain with relatively more residual pathogenicity such as one used in the present study to control the outbreaks of vvIBD.

Homeopathic medicines have occupied important position in the treatment of various aliments. The application of this group of medicine have been advocated to cure various conditions in poultry (Patra, 1983; Bera, 1983; Jagtap *et al*, 1993). Presently some homoeopathic drugs have also 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 defence system of body. It was

in this background that homoeopathic medicines were considered for use along with IV95 IBD vaccine. Initial study on effect of Homeopathic drug combination consisting of Thuja occidentalis, carbo vagetabilis, corboanaemalis 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 Homeopathic drug combination used earlier. It was in this context that one of the component of the drug combination namely "**Thuja occidentalis**" was used in order to study its effect on immune response to IBD vaccine as well as immune response to RD vaccine in IBD vaccinated chickens. The precipitating antibody titres in group V (Thuja oc) clearly demonstrated higher antibody levels at all intervals post IBD vaccination when compared with the values for the corresponding intervals of gr. VI. Thuja oc. also showed higher HI titre to RD vaccine as evident from the values recorded for group V (Table - 3 & fig - 15 a,b) when compared with the HI titres for the corresponding intervals of gr. VI overall the intervals post IBD vaccination. Therefore, it is obvious that the Thuja oc. component of earlier used Homeopathic drug combination consisting of Thuja occidentalis,carbovagetabilis,carboanaemalis 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, carbovagetabiles & corboanaemalis had been used (Hindustani 2000).

On over all consideration the present finding with respect to use of thuja oc. for enhancing antibody levels after IBD vaccination as well as after RD vaccination in IBD vaccinated chickens is sufficiently suggestive that Thuja oc alone can be advocated for use by poultry farmers instead of suggesting the combination of three homoeopathic drugs used ealier (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 carbovagetabilis and carbo anaemalis.

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, variation in degree of immunosuppression have been reported by a number of workers from time to time (Thornton and Pattison, 1975; Giamborne 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 reported to be responsible for variation in degree of immunosuppression (Edward *et al.*, 1982; Ezeokoli *et al.*, 1990; Mazariegos *et al*; 1990; Mahesh and Muniyappa 1996; Bekhit, 1997). Control of IBD by vaccination is in practice worldwide. However, there are number of reports that even IBD vaccine produced some degree of immunosuppression (Montogomery *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 IV 95 strain IBD vaccine showed lowering effect on HI titre to RDV (F strain) vaccine when compared with the corresponding value of the control group (gr. VII) over all the interval till the termination of experiment (Table -3 and Fig. 15a,b).

The recently introduced hot strain IBD vaccine marketed in the name of invasive intermediate strain (IV 95), Inter plus or Bursine plus etc are known to have relatively more residual pathogenicity and invasiveness and consequently such strains are more prone for production of immunosuppressive effect of higher magnitude than the conventional vaccine (Kowenhoven and Bos, 1994; Coletti *et al.*, 1994; Survashe, 1996; Khaliel *et al.*, 1998; kumar, 1998; Kumar, 2000). In a 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 VI when compared with titres in group VII (table -3), it would not be out of way to suggest the use of agents/drugs in conjugation with IBD vaccine in order that the aftereffect of vaccine is prevented or at least minimized without in any way affecting the response to IBD vaccine. In the present study all the five drugs/agents employed showed immune enhancing effect on antibody response to RD vaccine in IBD vaccinated chicks. Interestingly these agents also showed potentiating effect on response to IBD vaccine itself which have been discussed above in this text. The comparison of antibody titres to RD vaccine in treatment groups (gr. I - V) revealed that the antibody levels did not differ significantly among themselves, by and large the titres were higher than that recorded in vaccinated group which did not receive any drug (gr. VI). Further, the comparison of antibody titres of treatment groups with the titres observed in none IBD vaccinated untreated control group (gr. VII) showed that whereas all the five drugs/agents had different degree of immunopotentiating effect on response to RD vaccination, none of the treatment group exhibited antibody titres which could have been comparable to the titres in group VII at any interval post IBD vaccination. In other words none of the immunopotentiating agents was able to bring improvement in antibody level to RD vaccine at par with the levels of antibody shown by none IBD vaccinated control birds. (gr. VII). 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; Saravanvave *et al.*, 1999) which support the present findings.

In the present study altogether five drugs were evaluated for their immunopotentiating effect on response to RD vaccine in birds receiving hot strain IBD vaccine (IV 95 strain). The Neem showed the best immune enhancing effect on HI antibody titre to RD vaccine followed by Tulsi, Thuja occidentalis as well as IMMU-21 and Charak-E-sel treated groups. As the titres in the different treatment groups did not differ significantly among themselves and also that the titres were always higher than the protective level(2^4), all the five drugs should be 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 chicks to be vaccinated with IBD vaccine especially intermediate plus strain of IBD vaccine or IV 95 strain vaccine.

Pathological changes and lesion score proved a very sound criteria for determining the virulence of IBD virus (Naqi *et al.*, 1980, Mazariegos *et al.*, 1990). The same criteria have also been

extensively used to assess the residual pathogenicity 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 of lymphoid cells 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. (Thornton and Pattison, 1975; Ajinkya *et al.*, 1980; Ley *et al.*, 1983; Edward *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 interfollicular oedema, epithelial invagination, vacuolation in plical cells, epithelial hyperplasia, cellular infiltration etc. have also been reported by one or other worker from time to time (Rao *et al.*, 1995; Panigrahy *et al.*, 1986; Edward *et al.*, 1982; Lukert and Hitchner, 1984). Whereas, the histopathological changes recorded in the bursa of Fabricius were clearly suggestive of the possession of residual pathogenicity in the IBD vaccine virus (IV 95 strain). In none of the groups the clinical signs and symptoms typical of IBD virus could be 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 histopathologocal changes but none of them

produced clinical disease. Hence, the sort of histopathological lesions recorded in bursa of vaccinated chicks 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. Histopahtological examination of section of liver, kidney and spleen did not reveal any changes. A number of workers reported mild changes in non-bursal organs in IBD infected birds (Aziz, 1985; Singh, 1987; Sah *et al.*, 1995). However, Ley *et al* (1983) reported that the changes observed in non-bursal organs were nonspecific.

Further the total lesion score was found to be higher in IBD vaccinated but untreated group (gr. VI) than the lesion scores recorded in any of the treatment groups except group II (Charak-E-sel treated). Though none of the value differed signficantly from each other (table - 4). Thus it appears that the different drugs employed was helpful in bringing reduction in lesion score except Charak-E-sel treated group (gr. II). Earlier study conducted in this department also suggested role of selected drugs in reducing the lesion scores in IBD vaccinated birds (Hindustani, 2000). Any reduction in lesion score due to application of a particular drug/agent should be considered in the light of the overall effect of these drug on other parameters such as Immune response to RD vaccine, body weight gain ad FCR value. The positive response in respect of immune response to RD vaccine has already been discussed in this text. Similar response on body weight gain and FCR value (to be described later) sufficiently confirms the

present finding. However, further study employing drugs having similar effects will reveal more facts about the usefulness of such agents in a larger way. It is also possible that Challenge study should be helpful in determining the reduction in residual pathogenicity of vaccine virus.

Total serum protein concentration is convenient indicator of hydration (Ley *et al.*, 1983). A number of workers have reported decrease in total serum protein in IBD infected chickens (El-Batrawi *et al.*, 1993; Singh *et al.*, 1995; Al-afaleq, 1998). In the present study also reduction in total serum protein value was noticeable in the IBD vaccinated group (gr. VI) when compared with the values in the unvaccinated control group (gr. VII). Cheville (1967) and Panigrahy *et al.* (1986) observed that the decline in total serum protein values in IBD infected chicks may be due to inflammatory exudation of serum albumin into the bursa of Fabricius and its subsequent excretion in the faeces which will finally lead to reduction in total serum protein values. However, this needs further confirmation since some workers have also reported, no change in serum total protein after IBD infection (Ley *et al.*, 1983). Nevertheless, there appears to be dearth of literature in respect of IBD vaccine virus on total serum protein. However, as the vaccine strain used in this study still possesses some residual pathogenicity and therefore it is but logical to assume that the sort of reduction in protein level observed during this study may be the consequence of this vaccine virus. It is also likely that the

reduction in serum protein recorded in this study may be result of interaction between IBD vaccine virus and aflatoxin contained in feed given to the birds in this experiment. Chang, *et al.*, (1982) also reported potentiation of reducing effect of IBD virus on serum protein due to aflatoxin. Since the screening of feeds supplied to experimental chicks in this study could not be undertaken it would be premature to derive any conclusion unless experiment is planned to demonstrate the combined effect of aflatoxin and IBD virus.

The total protein values in different drugs/agents treated groups were found to be higher at all intervals post IBD vaccination when compared with the corresponding value in vaccinated group (gr. VI) except in case of Thuja oc. treated group (gr. V) at 14, 21 and 28 days intervals (Table - 5 and Fig. 16). As all these drugs have shown immunopotentiating effect as evident from enhancement in antibody titre to IBD and RD vaccine (Table - 2 and 3), the improvement in total serum protein values in the drug treated groups described above is quite understandable and gives further support to our earlier finding on immunopotentiating effect of this drug. A number of workers have reported decrease in serum albumin but increase in globulin values after IBD virus infection (Cheville, 1967; Ley *et al.*, 1983; Panigrahy *et al.*, 1986). In the present study also similar trends were observed in respect of serum albumin and globulin value (Table - 5 and Fig. 17, 18 a, b) in the IBD vaccinated but untreated group (gr. VI) as compared to control group (gr. VII).

Administration of different drugs/agents helped in bringing enhancement in albumin level except in Tulsi treated group (gr. IV) at 7 and 14 days post IBD vaccination and Thuja oc. treated group (gr. V) at 21 days post IBD vaccination when compared with its corresponding values in the vaccinated but untreated group (gr. VI). Further, the administration of different drugs failed to bring significant improvement in globulin over and above the values obtained in vaccinated but untreated group (gr. VI) at different intervals post IBD vaccination except in case of Tulsi treated group (gr. IV) at 14 days post IBD vaccination. (Table - 5 and Fig. 18 a, b). However, as the vaccination specifically potentiates the gamma-globulin fraction (Tizzard, 1996; Ananthanarayan and Jayaram Panikar, 1999), any conclusion could be possible only when different fractions of globulin are studied.

In the present study there was decline in potassium level in the birds which received IBD vaccine (gr. VI) when compared to control group (gr. VII). Ley *et al.* (1983) and El-Batrawi *et al.*, (1993) also reported decline in potassium value in IBD infection. However, another group of workers failed to detect any decline in potassium level (Panigrahy *et al.*, 1986) after IBD infection. In a situation where conflicting reports are available on the level of potassium a number of factors such as strain of the virus, size of inoculum, breed of birds, and feed given to the birds etc. would be required to be ascertained before arriving at a definite conclusion. As regards the levels of

sodium no significant difference were noted in any of the groups when compared to control group (gr. VII). Similar observation have also been made by several workers (Panigrahy, 1986; Ley *et al.*, 1983). The administration of different drugs/agents were helpful in cutting short the lowering effect of vaccine virus on potassium level. The significant decline in potassium level continued till the 14 days of observation in vaccinated group (gr. VI) whereas, in the drug treated group the significant lowering effect was visible only upto 7 days post IBD vaccination except in case of Neem and Thuja oc. treated groups (gr. III and V) in which case the lowering effect could be observed till 14 days post IBD vaccination when compared with unvaccinated control birds (gr. VII). The significance of this result will have to determined in the light of the result obtained in respect of the immunostimulating agent which are under study in this laboratory.

A number of workers have reported the alteration in haematological values due to IBD virus in chickens (Panigrahy *et al.*, 1986; Kumar and Rao, 1991; Singh and Dhawedkar 1994a). In a number of cases such reports are conflicting. Both haemoglobin level and packed cell volume value have been reported to decrease in IBD infected chicken (Kumar and Rao 1991; Cho and Edger 1972; Panigrahy *et al.*, 1986). On the contrary, both Hb and PCV values did not differed significantly in vaccinated group (gr. VI) when compared with the control group (gr. VII). However, it is difficult to attach much significance in the variation of the result with respect to Hb

and PCV values as very scanty reports are available so far. Further, there are few reports of lowered Hb value in IBD infected birds which has been attributed to haemorrhages in the musculature of infected chickens (Panigrahy *et al.*, 1986). It has to be seen in the light of the present finding whether such haemorrhage as noticeable in IBD infected chicks produced only transitory effect on lowering of Hb value. It is also possible that degree of fall of Hb value may be linked with degree of haemorrhage. However, it may be mentioned that apparent haemorrhage was not observed in any of the birds sacrificed 96 hours post IBD vaccination. Though all these values were within the physiological range (Goodwin *et al.*, 1991) but it needs further study before some conclusion are drawn in respect of role of PCV values in disease resistance. Further, Chang and Hamilton (1982) also observed that packed cell volume and haemoglobin value were decreased by aflatoxin but not by the IBD infection. But it has to be seen whether the same affect is produced when the birds are simultaneously exposed to both aflatoxin and IBD virus.

In the present study there was decline in total WBC count in the birds which received IBD vaccine (gr. VI) when compared to control group (gr. VII). Hudson *et al.* (1975); Singh *et al.* (1994) and Kumar and Rao (1991) also reported decline in total WBC count in IBDV infection. It may be mentioned that lymphocytes constitute approximately 80% of WBC (Lucas and Jamroz, 1961) and also majority of the circulating lymphocytes have been found to be T cells

(Hudson *et al.*, 1975; Tizzard 1996). Therefore, it is likely that decrease in total WBC count in IBDV infected chickens may be related to decrease in circulating T cell numbers. However, another group of workers failed to detect any significant difference in total WBC count(Montogomery *et al.*, 1986) of IBDV infected chicks from that of uninfected control.

Further, the total WBC count in birds receiving drugs/agents (gr. I - 0V) were found to be higher at all intervals post IBD vaccination than the corresponding value of birds receiving only IBD vaccine (gr. VI) (Table -9). Therefore, there are reasons to conclude that the different drug treatments have been able to bring improvement in total WBC count, which may have ultimate bearing in the enhancement of resistance to diseases. Limited works conducted on usefulness of certain drugs/drug combination as immunomodulator have suggested that Vit. E, Zeetress as well as combination of Vit. E and Selenium play role in increased resistance to diseases in chickens by enhancing the proliferating ability of lymphocytes (Marsh *et al.*, 1982, Rao *et al.*, 1995; Chatterjee, 1994b), which also support the present observation.

The bursa : body wt. 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; Mazaricgos *et al*, 1990). The B : B ratios were invariably higher in groups which

received IBD vaccine (gr. I - VI) except group II (Charak-E-sel treated group) when compared with the value in group VII which did not receive IBD vaccine. In the present study B : B ratio of Charak-E-Sel treated group was quite closer to the value recorded in unvaccinated control group (gr. VII). However, on overall consideration of the findings recorded in the present study B : B ratio alone can not be taken as a criteria for determining the residual pathogenicity of vaccine virus.

A number of workers studied the body weight gain and feed conversion ratios to ascertain the efficacy of live IBD vaccine in chicken (Thornton *et al*; 1975; Henry *et al.*, 1980; Wyeth *et al.*, 1981; Kouwenhoven and Bos, 1996; Abdel-Fattah *et al.*, 1999). In the present study the vaccinated but untreated group (gr. VI) exhibited lower body weight gain and higher FCR values (table -11) when compared with non IBD vaccinated untreated control group (gr. VII). Henry *et al.* (1980), Thornton *et al.*, (1975) and Abdel-Fattah *et al.* (1999) also recorded similar results while studying the effects of conventional IBD vaccines in chickens. The findings further suggested immunosuppressive properties inherent in the vaccine strain employed in this study. This provides additional support to the observations made by a number of workers that IBD vaccine virus are also having some degree of immunosuppressive effect as evidenced by lowered immune response to RD vaccine, poor body wt. gain and feed conversion ratio (Mahesh and Muniyappa, 1996). Interestingly,

application of different drugs (gr. I-V) have been successful in improving body wt. gain, FCR as well as mortality when compared with the corresponding values of group VI which received only IBD vaccine and no other treatment (table - 11). The body wt. gain recorded in group VI which received only IBD vaccine but no drug treatment was significantly lower than the value recorded in group VII which neither received IBD vaccine nor any drug treatment. This clearly suggested immunosuppressive property inherent in vaccine virus. Application of different drugs (gr. I - V) were helpful in bringing improvement in body weight gain (Table - 11). Intrestingly, the values of body wt. gain were significantly higher in different drug treated group (except gr. V), when compared with its value in group VI.

Since body weight gain is very important criteria on which economy of the farm rests, the present finding is quite encouraging. Truly speaking the drugs/agents employed in the present study has created a ray of hope for the poultry farmers who are using such moderate hot strain vaccine like one used in the present study. This is more so because these drugs have also shown encouraging results with respect to immune response to IBD vaccine as well as immune response to RD vaccine in IBD vaccinated birds. Further, the FCR value was found to be highest in IBD vaccinated but untreated group (table - 11). The different drug treatments were successful in lowering the FCR value which is specially marked in

IMMU-21 treated group followed by Thuja occidentalis, Neem and Charak-E-sel. Interestingly, the application of drugs has been successful in lowering the FCR value even below the value obtained in normal birds (gr. VII) as evident from the value in group I, II, III and V. On overall consideration the drugs employed in this study may be considered important as they have brought improvement in body weight gain, FCR value as well as HI titre to RD vaccine and precipitating titre to IBD vaccine virus.

CHAPTER - VI

SUMMARY

SUMMARY

The new form of IBD, also called very virulent IBD (vvIBD) is widely prevalent in the state of Bihar (Singh, 2000). To control this dreaded form of IBD moderate "hot strain" of IBD virus is used as vaccine. Such vaccines are marketed in the name of Intermediate plus, IV 95 (invasive strain) or Interplus IBD vaccines. It is reported that such vaccines possess residual pathogenicity and immunosuppressive properties. Earlier studies conducted in the department in respect of such moderate "hot strain" vaccines have further confirmed the above observation (Kumar, 1998). Therefore, it was considered necessary to look for some drugs/agents which could act as immunomodulator and counter the aftereffect which can follow vaccination with above mentioned vaccine and hence, this study was planned.

Day old chick, numbering 175 chicks were procured from Central Poultry Farm, Patna and randomly divided into seven equal groups of 25 chicks each. Birds in all the seven groups were given F-strain RDV vaccine intraocularly at zero day of age, while birds from group I-VI received IBD vaccine (IV 95 strain) intraocularly at 14 days of age. Further, the birds of group I-V were given IMMU – 21, Charak-E-Sel, Neem, Tulsi and Thuja occidentalis respectively. The birds of group VI remained untreated and served as vaccinated

control whereas, the birds of group VII neither received any drugs nor IBD vaccine and served as control group. Post IBD vaccinated blood and serum samples were collected at weekly intervals from each group of chickens. Blood samples collected were evaluated for haematological and serum biochemical profiles. Serum samples were evaluated for determination of antibody titres to IBD vaccine by QAGPT and RD (F-strain) vaccine by HI test. Five birds from each group were sacrificed 96 hours post IBD vaccination for histopathological examination of bursa of Fabricius, Kidney, liver and spleen and for Bursa : Body weight ratio. Body weight gain and FCR were determined at the end of experiment (on 42 day of age).

All the five drugs employed in this study produced higher QAGPT titres which ranged between 3.20 ± 0.200 to 4.80 ± 0.200 than the titre (2.20 ± 0.374) recorded in the IBD vaccinated but untreated group of birds (gr. VI). However, the highest level of precipitating antibody was recorded in Neem treated group (gr. III) followed by Tulsi, Thuja oc, IMMU – 21 and Charak – E – Sel treated groups. Interestingly, the titres in treatment groups (gr. I-V) on 28 day post IBD vaccination even differed significantly from only IBD vaccinated group (gr. VI). The result clearly depicted that whereas IV95 strain IBD vaccine led to progressive rise in antibody titre, all the five drugs employed in this study have potentiating effect on immune response to the vaccination.

The IBD vaccine used in this study showed immunosuppressive effect on response to RD vaccine (F strain) as evident from lowered HI antibody titre recorded in IBD vaccinated group of birds (gr. VI) when compared with the titres shown by none IBD vaccinated control birds (gr. VII). Further, the above noted five drugs (gr. I-V) also showed improvement in HI antibody titres to RD vaccine over and above the titre recorded in group VI which received only IBD vaccine but no any drug treatment. The HI titre in the drug treated group ranged between 3.80 ± 0.200 to 4.80 ± 0.200 and it was highest in Neem treated group followed by Tulsi, Thuja oc, IMMU–21 and Charak – E – Sel treated groups.

Further characterization of IV95 strain vaccine virus revealed the possession of residual pathogenicity as characterised by production of typical lesions in the bursa of Fabricius which included lymphoid depletion and necrosis, interfollicular oedema, epithelial invagination, interstitial fibrosis, cellular infiltration and vacuolar degeneration. The application of above noted drugs except Charak-E-Sel helped in reduction of total lesion score when compared with the value of the lesion score in IBD vaccinated but untreated group (gr. VI). The vaccinated birds (gr. VI) also demonstrated higher Bursa : Body wt. ratio than the value of the ratio in group of birds which neither received IBD vaccine nor any drug treatment (gr. VII). However, application of drug (gr. I-V) failed to demonstrate any significant effect on B : B ratio.

Further study revealed the lowering effect of vaccine strain of IBD virus on Body wt. gain and poor FCR (2.29 ± 0.055). Interestingly, administration of any of the five drugs namely IMMU-21, Charak-E-Sel, Neem, Tulsi and Thuja oc in IBD vaccinated birds (gr. I-V) helped in bringing improvement in FCR as evident from the lower FCR value which ranged between 2.10 ± 0.060 to 2.28 ± 0.047 than the corresponding value in the vaccinated but untreated group (gr. VI). Within the treatment groups lowest FCR was visible in IMMU-21 treated group followed by Thuja oc treated group. However, the ratios in different treatment groups except in Tulsi treated group were better when compared with the values even in control group (gr. VII). Besides, these drugs also demonstrated higher Body wt. gain in IBD vaccinated group (gr. I-V) than the value of the Body wt. gain in the group of birds which received only IBD vaccine but no any drug treatment (gr. VI). The comparison of the Body wt. gain shown by different drug treated group revealed the highest gain in Tulsi treated group followed by IMMU – 21 and Charak-E-Sel treated groups.

The results of the study on serum biochemical profile in respect of total protein, albumin, globulin, sodium and potassium in general were not suggestive of the effect of the vaccine virus and consequently the role of any of the drugs in vaccinated group of birds. However, a further study is suggested employing other moderate hot IBD vaccine available in the market before arriving at a final conclusion.

The haematological profile showed that the haemoglobin and PCV value did not differ significantly in any of the groups (gr. I-VII) at any intervals post IBD vaccination. The PCV values in vaccinated but untreated group (gr. VI) were lower than the corresponding value in none IBD vaccinated control group (gr. VII). The PCV values of different drugs treated group did not show any conclusive trend. Since all these values were within the physiological range nothing could be inferred at this stage.

Further, the study also revealed that the TLC value in the IBD vaccinated but untreated group (gr. VI) were significantly lower than the corresponding value in the none IBD vaccinated control group (gr. VII) till termination of the experiment. The values of TLC in birds receiving drugs/agents (gr. I-V) were suggestive of the fact that although the values did not differ significantly among themselves on 28 day post IBD vaccination, they were invariably higher than the corresponding value of IBD vaccinated but untreated group (gr. VI).

Finally the present study suggested the presence of residual pathogenicity and immunosuppressive effect of the vaccine strain of IBD virus (IV95) as employed in this study. Further, the five drugs included is this study such as IMMU – 21, Charak-E-Sel, Neem, Tulsi and Thuja oc proved to have enhancing effect on immune response to IBD vaccine as well as on response to RD vaccine in birds

immunocompromised by moderate hot strain vaccine (IV95) which is widely used to control vvIBD scenario is this state. Comparative evaluation of the above five drugs in respect of immunopotentiating effect on response to IBD vaccine as well as RD vaccine in IBD vaccinated birds revealed Neem leaves powder as the best agent followed by Tulsi, Thuja oc, IMMU – 21 and Charak-E-Sel. Further, all these drugs also showed improvement in FCR when compared with the value recorded in group of birds which received only IBD vaccine but no any drug. However, the lowest FCR was observed in IMMU – 21 treated group followed by Thuja oc., Neem, Charak-E-Sel and Tulsi treated groups. Therefore, on overall consideration it may be concluded that all the five drugs employed in this study had immunopotentiating effect and hence, may be advised for use by poultry farmers along with such moderate hot strain vaccine like one used in this study (IV95) depending upon local availability and cost factor. Further, the homoeopathy drug, Thuja occidentalis may also be given preference because of cost effectiveness and frequency of administration (given on weekly interval). It is also possible to hold this view till such time similar studies incorporating more and more drugs/agents having some degree of immunostimulating potentials are studied and their comparative efficacy are determined.

CHAPTER - VII

BIBLIOGRAPHY

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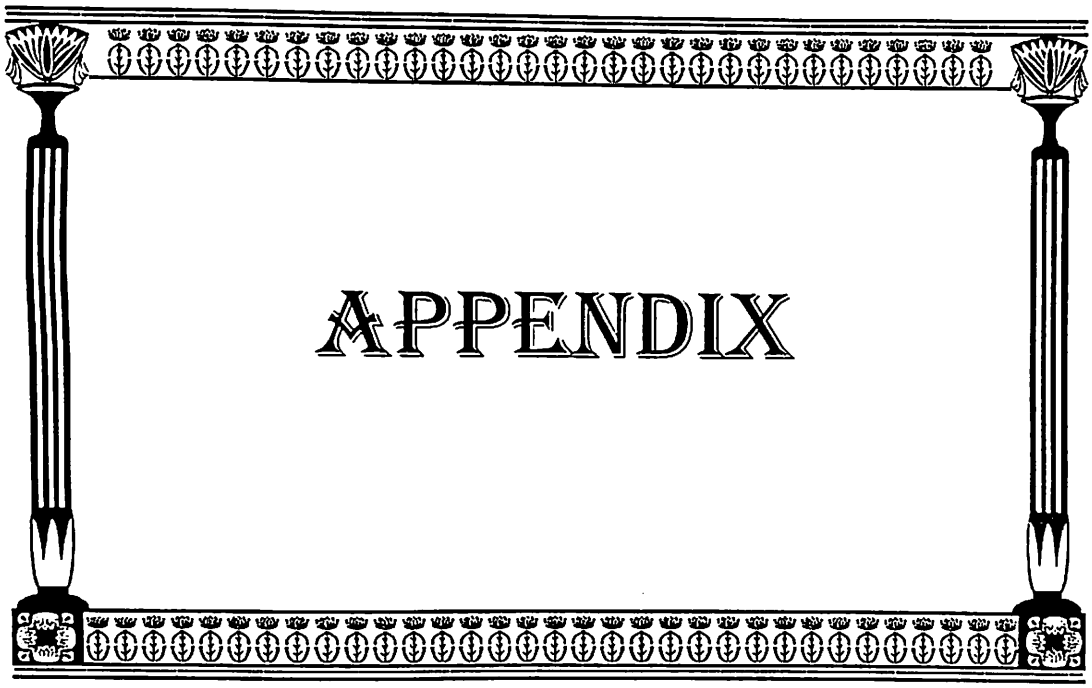
APPENDIX

APPENDIX - I

I. Analysis of variance showing effect of different treatment groups (dpv) on the Ab titre against IBDV in chickens.

Sources of Variation	D.F.	Days Post IBD Vaccination					
		14		21		28	
		M. S	F	M. S	F	M. S	F
Between treatment groups	5	0.7	3.5*	2.69	7.69**	4.44	11.68**
Error	24	0.2	-	0.35	-	0.38	-
Total	29	-	-	-	-	-	-

*** Indicates significance at (P < 0.05), ** Indicates significance at (P < 0.01)**

II. Analysis of variance showing effect of treatment groups (pre & dpv) on Ab titre against RDV (F-strain) in chickens.

Sources of Variation	D.F	Pre IBD vaccinated		Days Post IBD Vaccination							
				7		14		21		28	
		M. S	F	M. S	F	M. S	F	M. S	F	M. S	F
Between treatment groups	6	0.247	$1.45^{N.S.}$	0.657	$1.35^{N.S.}$	0.762	3.56**	1.257	$2.31^{N.S.}$	1.923	3.06*
Error	28	0.171	-	0.485	-	0.214	-	0.542	-	0.628	-
Total	34	-	-	-	-	-	-	-	-	-	-

*** Indicates significance at (P < 0.05), ** Indicates significance at (P < 0.01), NS indicates non-significance.**

APPENDIX - II

III. Analysis of variance showing effect of treatment groups on different bursal lesion score in chicken sacrificed 96 hrs. post IBD vaccination.

Sources of variation	Follicular changes									
		Interfollicular oedema		Lymphoid depletion		Lymploid Necrosis			Vacuolar degeneration	
	D.F	M. S	F.	M. S	F	M. S	F	DF	M. S	F
Between treatment	5	0.294	0.50^{NS}	0.294	0.49^{NS}	0.294	0.29^{NS}	2	0.065	0.28^{NS}
Within treatment	24	0.583	-	0.6	-	1.016	-	12	0.233	-
Total	29	-	-	-	-	-	-	14	-	-

Sources of variation		Epithelial changes		Interstitial fibrosis			Total Lesion Score		
		Epithelial invagination							
	D. F	M. S	F.	DF	M. S	F	DF	M. S	F
Between treatment	5	0.454	0.50^{NS}	3	1.133	2.67^{NS}	6	0.095	0.91^{NS}
Within treatment	24	0.9	-	16	0.425	-	413	0.104	-
Total	29	-	-	19	-	-	419	-	-

NS indicates non-significance.

APPENDIX - III

IV. Analysis of variance showing effect of treatment groups (Pre & dpv) on the serum total protein value in chickens.

Sources of variation	D.F.	Pre IBD vaccinated		Days Post IBD Vaccination							
				7		14		21		28	
		M.S.	F	M.S.	F	M.S.	F	M.S.	F	M.S.	F
Between treatment	6	0.048	0.941^{NS}	0.102	1.47^{NS}	0.165	2.70*	0.255	1.88^{NS}	0.219	3.26*
Within treatment	28	0.051	-	0.069	-	0.061	-	0.135	-	0.067	-
Total	34	-	-	-	-	-	-	-	-	-	-

* Indicates significance at (P <0.05), NS indicates non-significance.

V. Analysis of variance showing effect of treatment groups (Pre & dpv) on TLC In Chickens

Sources of variation	D.F.	Pre IBD vaccinated		Days Post IBD Vaccination							
				7		14		21		28	
		M.S.	F	M.S.	F	M.S.	F	M.S.	F	M.S.	F
Between treatment groups	6	0.233	0.411^{NS}	5.466	13.52**	4.873	9.49**	2.257	5.70**	0.291	2.66*
Within treatment	28	0.567	-	0.404	-	0.513	-	0.395	-	0.109	-
Total	34	-	-	-	-	-	-	-	-	-	-

* Indicates significance at (P < 0.05), ** Indicates significance at (P < 0.01), NS indicates non-significance.

APPENDIX - IV

VI. Analysis of variance showing effect of treatment groups (Pre & dpv) on the serum albumin value in chickens.

Sources of variation	D.F.	Pre IBD vaccinated		Days Post IBD Vaccination							
				7		14		21		28	
		M.S.	F	M.S.	F	M.S.	F	M.S.	F	M.S.	F
Between treatment groups	6	0.016	0.21^{NS}	0.078	1.71^{NS}	0.095	0.98^{NS}	0.079	0.82^{NS}	0.096	0.15^{NS}
Within treatment	28	0.077	-	0.045	-	0.096	-	0.095	-	0.083	-
Total	34	-	-	-	-	-	-	-	-	-	-

VII. Analysis of variance showing effect of treatment groups (Pre & dpv) on the serum globulin value in chickens.

Sources of variation	D.F.	Pre IBD vaccinated		Days Post IBD Vaccination							
				7		14		21		28	
		M.S.	F	M.S.	F	M.S.	F	M.S.	F	M.S.	F
Between treatment groups	6	0.103	2.06^{NS}	0.049	1.33^{NS}	0.135	2.73^{NS}	0.095	1.53^{NS}	0.126	2.33^{NS}
Within treatment	28	0.050	-	0.037	-	0.049	-	0.062	-	0.054	-
Total	34	-	-	-	-	-	-	-	-	-	-

NS indicates non-significance.

APPENDIX - V

VIII. Analysis of variance showing effect of treatment groups (Pre & dpv) on Hb in chickens.

Sources of variation	D.F.	Pre IBD vaccinated		Days Post IBD Vaccination							
				7		14		21		28	
		M.S.	F	M.S.	F	M.S.	F	M.S.	F	M.S.	F
Between treatment groups	6	0.013	0.07^{NS}	0.035	0.21^{NS}	0.240	1.28^{NS}	0.253	0.14^{NS}	0.361	2.00^{NS}
Within treatment	28	0.183	-	0.163	-	0.186	-	1.694	-	0.180	-
Total	34	-	-	-	-	-	-	-	-	-	-

IX. Analysis of variance showing effect of treatment groups (Pre & dpv) on PCV in chickens.

Sources of variation	D.F.	Pre IBD vaccinated		Days Post IBD Vaccination							
				7		14		21		28	
		M.S.	F	M.S.	F	M.S.	F	M.S.	F	M.S.	F
Between treatment groups	6	0.659	0.42^{NS}	0.250	0.41^{NS}	0.567	1.65^{NS}	0.786	1.32^{NS}	0.770	1.22^{NS}
Within treatment	28	1.570	-	0.608	-	0.342	-	0.595	-	0.629	-
Total	34	-	-	-	-	-	-	-	-	-	-

NS indicates non- significance.

APPENDIX - VI

X. Analysis of variance showing effect of treatment groups (Pre & dpv) on serum sodium level in chickens.

Sources of variation	D.F.	Pre IBD vaccinated		Days Post IBD Vaccination							
				7		14		21		28	
		M.S.	F	M.S.	F	M.S.	F	M.S.	F	M.S.	F
Between treatment groups	6	6.457	0.36^{NS}	0.933	0.12^{NS}	1.314	0.12^{NS}	22.914	2.04^{NS}	3.876	0.78^{NS}
Within treatment	28	17.671	-	7.585	-	10.742	-	11.192	-	4.914	-
Total	34	-	-	-	-	-	-	-	-	-	-

XI. Analysis of variance showing effect of treatment groups (Pre & dpv) on serum potassium level in chickens.

Sources of variation	D.F.	Pre IBD vaccinated		Days Post IBD Vaccination							
				7		14		21		28	
		M.S.	F	M.S.	F	M.S.	F	M.S.	F	M.S.	F
Between treatment	6	0.023	0.01^{NS}	4.268	12.85**	2.655	6.32**	1.303	2.38^{NS}	0.081	0.41^{NS}
Within treatment	28	1.633	-	0.332	-	0.42	-	0.546	-	0.195	-
Total	34	-	-	-	-	-	-	-	-	-	-

** Indicates significance at ($P < 0.01$); NS indicates non-significance.

APPENDIX - VII

XII. Analysis of variance showing effect of treatment groups on initial Body wt., final Body wt., weight gain and feed conversion ratio.

Sources of variation	D.F	Initial Weight		D.F.	Body wt. at 42 days		Body wt. gain at 42 days		D.F.	Feed conversion ratio	
		M.S.	F		M.S.	F	M.S.	F		M.S.	F
Between treatment groups	6	33.599	2.09^{NS}	6	97027.8	9.90**	88650.8	8.56**	6	0.028	1.22^{NS}
Within treatment	203	16.050	-	140	9799.17	-	10348.2	-	14	0.023	-
Total	209	-	-	146	-	-	-	-	20	-	-

** Indicates significance at (P < 0.01), NS indicates non-significance.

XIII. Analysis of variance showing effect of treatment groups on Bursa : Body wt. ratio at 96 hours Post IBD vaccination

Sources of variation	D.F.	Bursa : Body wt. ratio	
		M.S.	F
Between treatment groups	6	0.3811	2.80*
Within treatment	28	0.1359	-
Total	34	-	-

* Indicates significance at (P < 0.05).