# Pathological studies on immune complex vaccine of infectious bursal disease in broiler chicks

**[1]Bhumika K. Kapadiya, [2]B. P. Joshi, [3]D. J. Ghodasara, [4]C. J. Dave, [5]R. K. Prajapati**

[1]Former M.V.Sc Scholar, [2]Retired Professor, [3]Professor and Head, [4]Assistant Professor, [5]Former M.V.Sc Scholar

[1]Department of Veterinary Pathology

[1]College of veterinary science and animal husbandry, Anand agricultural university, Anand, Gujarat -388 001, India

*Abstract* -**The present study was carried out to know the protective efficacy of immune complex vaccine in comparison to commercially used intermediate plus vaccine in broiler chicks by experimental challenge with field IBD virus. Chicks were divided into six different groups as shown in experimental protocol. Experimental study relating to vaccination with two different IBD vaccines and subsequent challenge with IBD virus showed clinical signs only in group II which was challenged with field IBD virus on day twenty eight. There were no any appreciable gross lesions observed in BF in control and experimental group on first and second week of sacrifice. On third and fourth week of age, only smallness of the size of bursa of Fabricius was observed in few birds belonging to immune complex as well as intermediate plus vaccinated group. One week after challenge i.e. seventh day of post infection on day thirty five, there were no gross lesions observed in bursa of Fabricius in control group I, whereas positive control group II showed atrophy of bursa of Fabricius in almost all the birds. Among vaccinated groups there was atrophy of bursa of Fabricius observed both in unchallenged and challenged groups of both the vaccines but the extent of distribution was more with immune complex vaccinated groups compared to intermediate plus vaccinated groups. The microscopic lesions appeared during the present study indicated that both the vaccines i.e. immune complex vaccine given on day one as well as intermediate plus vaccine given on day fourteen could induce bursal lesions of variable degrees of bursal score on each interval post vaccinal sacrifice till day forty two of age. The bursal lesions gradually become more pronounced as the age of bird advanced. Though the bursal lesions were appeared with both the vaccines, the severity and extent of distribution of bursal lesions was more with immune complex vaccine compared to intermediate plus vaccine. Subsequent upon challenge with bursal homogenate prepared from field Infectious bursal disease outbreaks on day twenty eight, the bursal lesions were seen both in vaccinated challenged and unchallenged birds both on day seven and day fourteen of post infection. The lesions were more pronounced in vaccinated and challenged group of birds compared to that of vaccinated and unchallenged group of birds on day seven of post infection as compared to day fourteen of post infection with both the vaccines. Bursa of Fabricius to body weight ratio among different experimental groups revealed significant decrease in immune complex vaccinated group III as compared to the control group I from twenty one days onwards. On day thirty five there was significant decrease in ratio between the control group I and positive control group II as well as control group I and experimental groups III, IV, V and VI. The bursa of Fabricius to body weight ratio was lowest in positive control group II challenged with field IBD virus. Among the vaccinated groups, the ratio was lower in challenged groups compared to unchallenged groups with both the vaccines. On the forty second day of experiment there was significant decrease in bursa of Fabricius to body weight ratio in positive control group II and also in experimental groups III, IV, V and VI compared to the control group I. The overall nature of both the vaccines revealed that immune complex vaccine given on day one could result in more bursal damage than intermediate plus vaccine given on day fourteen as compared by bursa of Fabricius to body weight ratio and gross and microscopic lesions. The representative tissue samples of bursa of Fabricius were collected from the birds belonged to group II, IV and VI on thirty fifth day of sacrifice for confirmation of IBD virus**.

*Key words* : **Immune complex vaccine, Intermediate plus vaccine, Bursa of Fabricius, Atrophy**

## I. INTRODUCTION

Infectious Bursal Disease (IBD) is an acute highly contagious globally occurring viral poultry disease. It is the major health and production constrain of young chicken. The causal agent belongs genus *Avibirnavirus* of family *Birnaviridae*. Infectious bursal disease (IBD), initially reported as Gumboro disease, is an acute, highly contagious viral infection of young chickens first described by Cosgrove (1962)1, who found that B lymphocytes are the primary target cells. The main clinical signs include watery diarrhoea, depression, ruffled feathers, anorexia, trembling, prostration and death after two to three days of clinical signs onset2. The major post-mortem lesions may include dehydration of the muscles with numerous ecchymotic hemorrhages, swelling and discoloration of the kidneys, with urates in the tubules, inflammation, edema and bursal hemorrhages or atrophy3. An effective IBD control programme is very much required to minimize losses due to this disease. Recently there has been addition of new vaccine called immune complex IBD vaccine to control the infectious bursal disease in the field. Such vaccines are claimed to have properties to protect the birds against IBD regardless of maternal antibody levels. As the maternal antibody levels goes down, the live vaccine virus releases and maintain the antibody level and thus protects the bird from IBDV infection. The present study was undertaken to know the protective efficacy of immune complex vaccine in comparison to commercially used intermediate plus

vaccine in broiler chicks by experimental challenge with field IBD virus by pathological studies like clinical signs and mortality, gross and histopathological lesions and bursa of Fabricius to body weight ratio (BF : BW).

## II. MATERIALS AND METHODS

**Experimental birds**

The study was conducted on a total of 192, day-old cobb-400 broiler chicks which were procured from Shakti Hatcheries Pvt. Ltd., Sarsa, Anand, Gujarat and maintained under standard managemental conditions.

**Ethical Approval**

The experiment procedures were reviewed and approved by the Institutional Animal Ethics Committee (IAEC).

**Housing and Management**

Broiler chicks were housed as per the standards provided by IAEC in the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Anand. Environmental temperature and lighting regimes were applied according to the guidelines for management of cobb-400 broiler chicks.

**Feeding**

All the birds were given a balanced broiler pre-starter feed for first 7 days followed by broiler starter feed for subsequent 28 days and broiler finisher feed till end of the experiment. The feed was offered ad libitum and water was freely made available at all times throughout the study.

**Experimental Bursal Homogenate**

Bursal homogenates prepared from field outbreaks of IBD formed the source of inoculum for the experimental study. Bursa of Fabricius collected from field outbreaks of IBD were confirmed positive by RT-PCR. The bursa samples obtained from the natural outbreaks of IBD were collected and washed three times with sterile calcium-magnesium free phosphate buffer saline (CMF-PBS) containing kanamycin (100 *µg/ml)* and homogenized into 50 per cent suspension (w/v) using PBS. After three cycles of freezing (-20°C) and thawing (37°C) the material was spun at the speed of 10,000g for 30 minutes at 4°C and resultant supernatant was stored at -20ºC after adding 1000 IU of penicillin and one mg of streptomycin per ml of supernatant and was used as source of inoculum for *in vivo* experimental transmission of IBD. The obtained supernatant was injected into two, three weeks old broiler chickens at the rate of two equal doses of 0.05 ml each by intraocular and oral route. After 72 hours birds were sacrificed and bursa of Fabricius collected and were confirmed positive by RT-PCR (Fig. 1). These IBDV positive bursal samples were processed and supernatant was obtained by above described method. On 28$^{th}$ day, the obtained supernatant was injected in sixteen birds which belonged to groups II, IV and VI as described in experimental protocol (Table 1) at the rate of two equal doses of 0.05 ml each by intraocular and oral route.

**Experimental Design**

Chicks were divided into six different groups as shown in experimental protocol (Table 1). Group I was considered as control without any treatment, whereas group II was considered as positive control with experimental challenge of field IBD virus on 28$^{th}$ day of age. The group III and IV were given immune complex IBD vaccine at day old age at hatchery level (0.2 ml S/C). Among these groups III and IV, only group IV was experimentally challenged with field IBD virus on 28$^{th}$ day. Similarly groups V and VI were given intermediate plus IBD vaccine at 14$^{th}$ day by eye drop method. Among these groups V and VI, only group VI was experimentally challenged with field IBD virus on 28$^{th}$ day. All the groups were vaccinated with LaSota strain of ND vaccine on 7$^{th}$ and 21$^{st}$ days of age by eye drop method. All the groups were monitored for any abnormal behavioural signs and mortality without any intervention up to 42 days of age. Eight birds were sacrificed from each group for observation of set parameters i.e. gross and histopathological examination as well as weekly bursa of Fabricius to body weight ratio (BF : BW).

**Clinical signs and mortality**

All the birds from each group (Group I to Group VI) were observed daily for any abnormal physical or behavioural changes and mortality throughout the period of experiment**.**

**Table.1. Experimental Protocol**

All the Groups were vaccinated with LaSota vaccine on 7th and 21st day by eye drop method (one drop in eye)

| Group No. | No. of broiler Chicks | IBD Immune complex vaccine (0.2 ml S/C on day one at hatchery ) | IBD Intermediate plus vaccine (eye drop on 14th day) | Challenge with IBD field virus (on 28th day 0.05 ml I/O and Per Os ) | Day of sacrifice |
|---|---|---|---|---|---|
| I (Control) | 48 | × | × | × | 7, 14, 21, 28, 35, 42 |
| II(Positive) | 16 | × | × | √ | Surviving birds on day 42 |
| III | 48 | √ | × | × | 7, 14, 21, 28, 35, 42 |
| IV | 16 | √ | × | √ | 35, 42 |
| V | 48 | × | √ | × | 7, 14, 21, 28, 35, 42 |
| VI | 16 | × | √ | √ | 35, 42 |

**Gross and histopathological lesions in bursa of Fabricius (Bursal score) in different groups at each sacrifice**

Detailed postmortem examination was carried out from broiler chicks sacrificed at each weekly interval belonging to all different groups and gross lesions were recorded. Special emphasis was given on gross lesions suggestive of infectious bursal disease. Tissue pieces of bursa of Fabricius were collected in 10 % Neutral Buffered Formalin from different groups at each sacrifice during the experiment and was processed by paraffin embedding methods. Sections of 5 to 6 micron thickness were cut and stained by haematoxylin and eosin. The bursal lesions were scored as per scoring system suggested by Winterfield and Thacker (1978)[4]. Bursal lesion score was done on a scale of 0 (none), 1 (minimal), 2 (mild), 3 (moderate), 4 (marked) and 5 (severe) by criteria suggested by them.

**Weekly bursa of Fabricius to body weight ratio (BF: BW)**

The body weights of chicks as well as weight of bursa of Fabricius were recorded at every week interval. BF: BW was determined for each bird by dividing the weight of bursa of Fabricius over the body weight, during each weekly interval sacrifice.

**Confirmation of infectious bursal disease virus by RT-PCR**

The representative tissue samples of bursa of Fabricius were collected from the birds belonged to group II, group IV and group VI on 35th day of sacrifice for confirmation of IBD virus. The samples were submitted to Hester Biosciences limited, Meda-Adraj, Gujarat for detection of IBD virus by RT-PCR.

**Statistical analysis**

The relevant data pertaining to observations made during experimental studies of IBD were subjected to test of significant and analysis of variance as per standard methods[5].

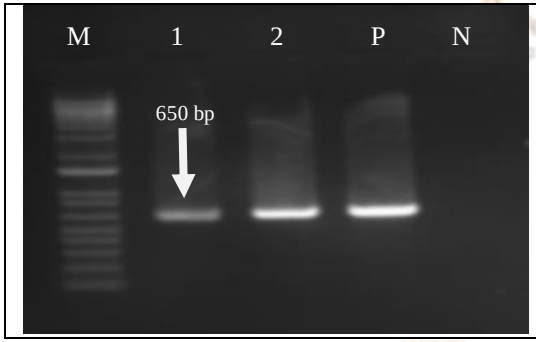
## III. RESULTS

### Clinical Signs and Mortality

All the birds of control group I, were appeared normal and did not reveal any clinical signs throughout the period of experimental study. From remaining groups II to VI, the clinical signs were observed only in group II which was challenged with field IBD virus on day 28. Clinical symptoms like dullness, depression, stunted growth, dehydration, reluctance to move, ruffled feathers, closed eyes and decreased feed intake were observed (Fig. 2) in few of the birds on 4 day PI i.e. on 32$^{nd}$ day and remained till the end of the experiment. Rest of the other vaccinated groups (group III, IV, V and VI) did not show any behaviour alteration throughout the period of experiment.

### Gross lesions

There were no any appreciable gross lesions observed in BF in control and experimental group on day 7 and 14 i.e. on first and second week of sacrifice (Fig. 3). On day 21 and 28, only smallness of the size of BF was observed in few birds belonging to immune complex vaccinated as well as intermediate plus vaccinated group (Fig. 4). One week after challenge i.e. 7 day PI on day 35 (fifth week of age), there were no gross lesions observed in BF in control group I, whereas positive control group II showed atrophy of BF in almost all the birds (Fig. 5). On subsequent sacrifice i.e. 14 day PI on day 42 (sixth week of age), control group I did not show any gross lesions in bursa of Fabricius, whereas positive control group II showed atrophy of bursa of fabricius in two to three birds. Group IV showed atrophy of bursa in two to three of the birds, whereas group III showed atrophy of bursa in one to two birds. Similarly group VI showed atrophy of bursa of Fabricius in two to three birds, whereas group V showed atrophy of bursa of Fabricius in one to two birds (Fig. 6). The bursal atrophy was gradually reduced in vaccinated groups of both the vaccines and their extent of distribution was less compared to day 35 of the experiment.



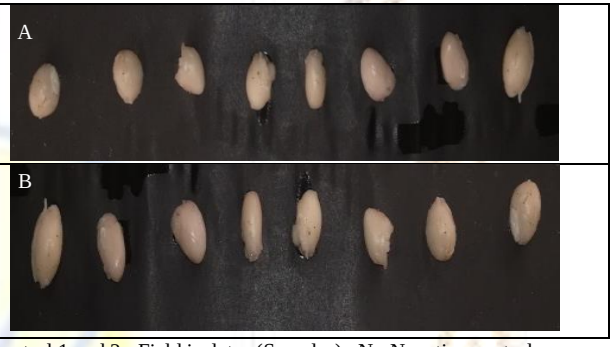




Fig.1. Agarose gel image showing amplified product of IBD virus (Approximately 650 bp).M- Ladder- 1 kb plus DNA ladder, P-IBD Positive control,1 and 2 : Field isolates (Samples), N : Negative control.
Fig.2.Photograph of a bird from group II showing dullness, depression, closed eyes and ruffled feathers on 4 day PI (32$^{nd}$ day) of the experiment.
Fig.3.Photograph showing BF at 14$^{th}$ day of age from control group I (A) and experimental (immune complex vaccinated) group III (B).Note: No apparent variation in the size is noticed.


Fig.4. Photograph showing BF at 28$^{th}$ day of age from control group I (A) and experimental group III (B) and V(C). Note: Marked atrophy of BF in group III (B) compared to group I (A) and V (E).

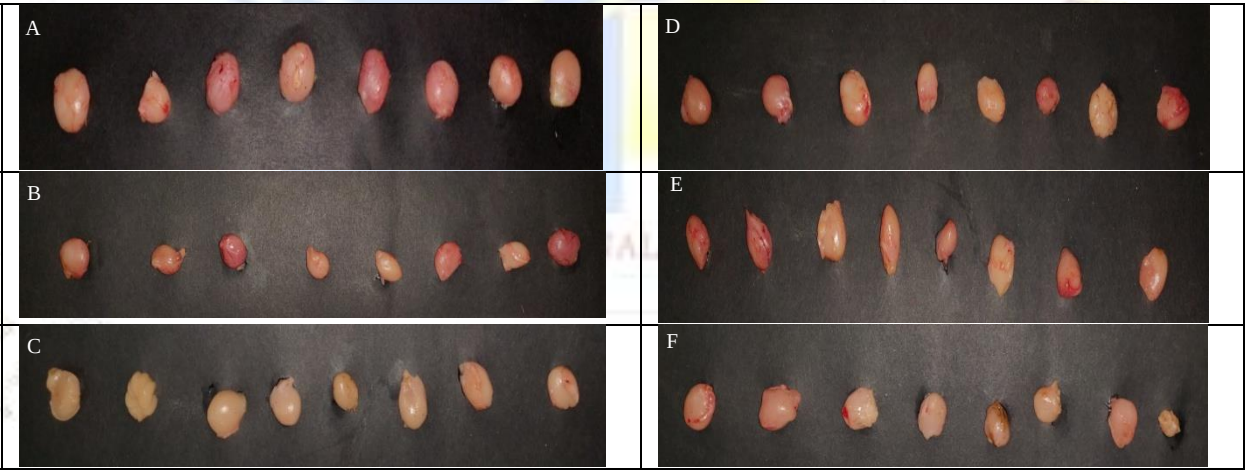



Fig.5. Photograph showing BF at 35$^{th}$ day of age (7 day PI) from group I (A) to VI (F). Note: Marked atrophy of BF in group II (B) in comparison with other experimental groups.

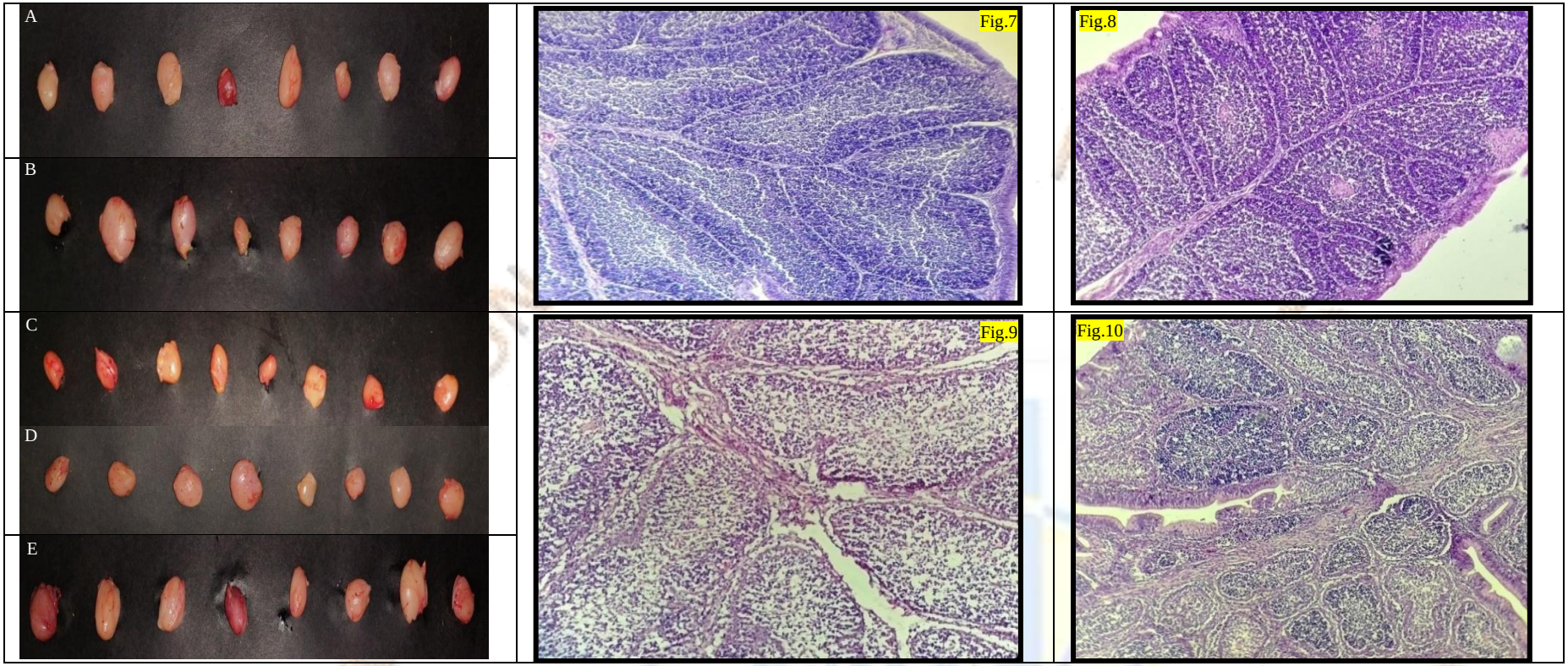



Fig.6. Photograph showing BF at 42nd day of age (14 day PI) from group II (A) to VI (E). Note: marked atrophy of BF in group II (A) III (B), IV(C) and VI (E).













Fig .7. Section of bursa of Fabricius from control group I showing normal parenchyma on day 14th of experiment (Bursal lesion score '0') (H & E stain, 120X). Fig 8. Section of bursa of Fabricius from immune complex vaccinated group III showing mild depletion of lymphocytes and focal necrosis in isolated follicles on day 7th of experiment (Bursal lesion score '1')(H & E stain, 120X).Fig.9. Section of from group III showing mild to moderate generalized depletion of lymphocytes on day 14th of experiment (Bursal lesion score '2') (H & E stain, 240X).Fig.10. Section of BF from group III showing mild to moderate generalized depletion of lymphocytes, hyperplasia and hypertrophy of corticomedullary epithelium and increase interfollicular connective tissue on day 21st of experiment (Bursal lesion score '3') (H & E stain, 120X).

**Histopathological lesions**

The extent to which histological changes occurred in the BF at different interval before and after inoculation with bursal homogenates during the study has been summarized in Table 2. There were no any appreciable lesions in control group I and bursal parenchyma appeared normal (Fig. 7) both on day 7 as well as on day 14 of the experiment. On day 7 and 14 bursal lesions of experimental group III were comparable with grade 0, 1 and 2 and characterized by mild to moderate depletion of lymphocytes and mild necrosis in isolated follicles (Fig. 8) as well as moderate generalized lymphocyte depletion or isolated follicles with severe depletion (Fig. 9). The BF lesions were comparable with grade 0, 2, 3 and 4 (Fig. 10) on day 21 in group III whereas on day 28 the BF lesions were comparable with grade 0, 1, 3 and 4 (Fig. 12). The BF lesions were comparable with grade 0, 1, 2 and 4 (Fig. 11) on day 21 in group V whereas on day 28 i.e. at fourth week of sacrifice BF lesions were comparable with grade 0, 1, 2, 3 and 4 (Fig. 13). On day 35 i.e. 7 day PI there were no microscopic lesions in bursa of Fabricius in control group I whereas positive control group II challenged with field IBDV showed BF lesions comparable with grade 3 and 4 (Fig. 14 and 15). The group III birds showed microscopic lesions comparable with grade 0, 1 and 4 (Fig. 16) whereas group IV birds showed bursal lesions comparable with grade 0, 1, 3 and 4 (Fig. 17). The group V birds showed bursal lesions comparable with grade 1, 2 and 4 (Fig. 18) whereas group VI birds showed bursal lesions comparable with grade 1, 3 and 4 (Fig. 19). On subsequent sacrifice i.e. 14 day PI on day 42 (sixth week of sacrifice), there were similar experimental groups as on day 42. The control group I did not show any microscopic lesions in bursa of Fabricius whereas positive control group II showed bursal lesions comparable with grade 1, 2 and 4 (Fig. 20). Group IV showed BF lesions comparable with grade 1, 2 and 4 (Fig. 22) whereas group III showed lesions comparable with grade 1, 3 and 4 (Fig. 21). Similarly group VI showed BF lesions comparable with grade 0, 2 and 3 (Fig. 24) whereas group V showed microscopic lesions comparable with grade 0, 1 and 2 (Fig. 23).

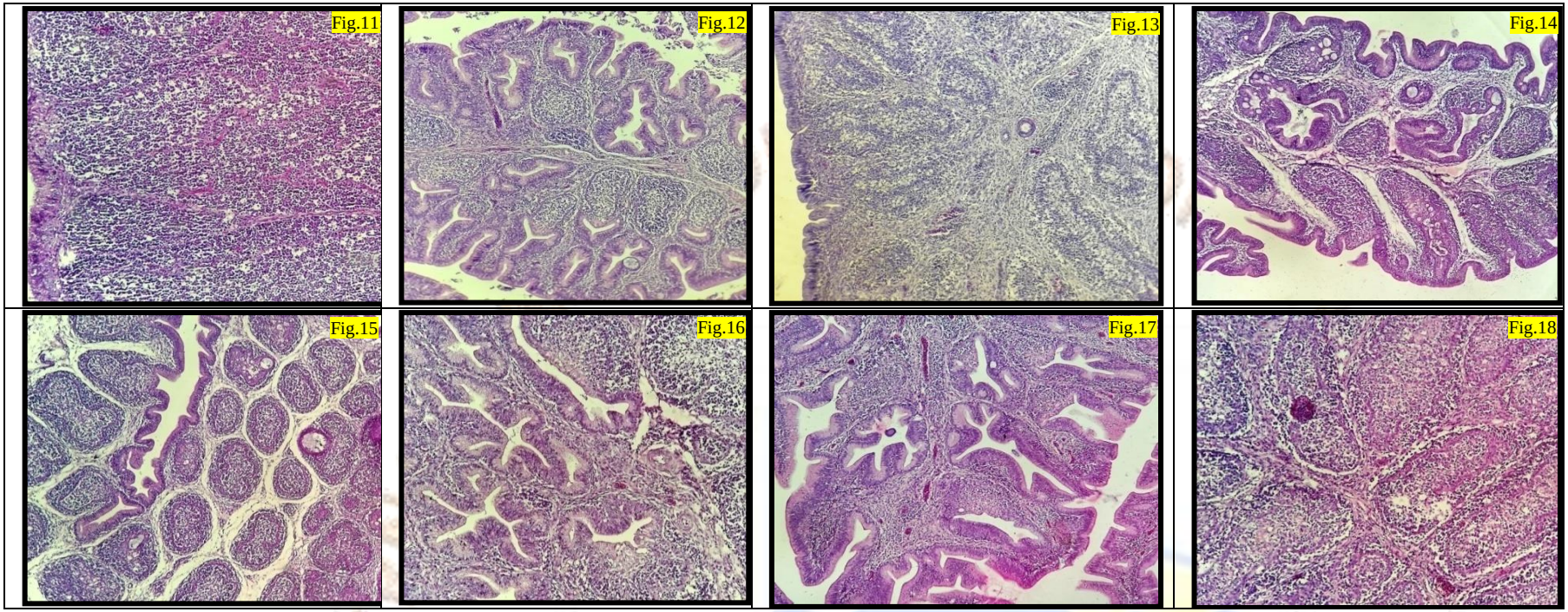
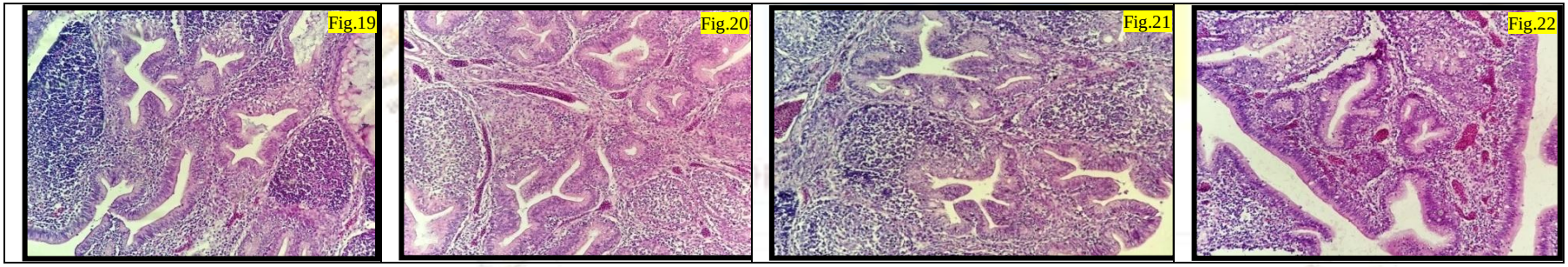
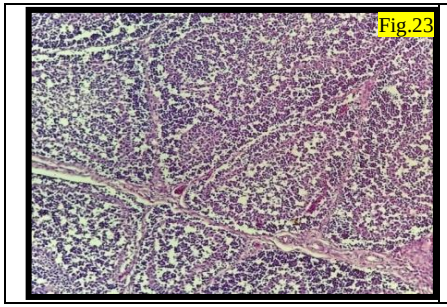
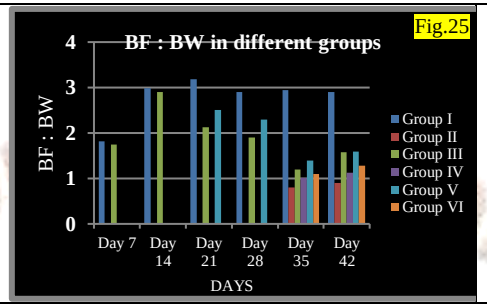



Fig.11. Section of BF from group V showing mild to moderate generalized depletion of lymphocytes, on day 21st of experiment (Bursal lesion score '2') (H & E stain, 240X). Fig.12. Section of BF from group III showing mild to moderate generalized depletion of lymphocytes, hyperplasia and hypertrophy of cortico-medullary epithelium, invagination of mucosal lining epithelium giving glandular appearance in the mucosa as well as in follicles and increase interfollicular connective tissue on day 28th of experiment (Bursal lesion score '4') (H & E stain, 120X).Fig.13. Section of BF from group V showing moderate generalized depletion of lymphocytes, hyperplasia and hypertrophy of corticomedullary epithelium and increase interfollicular connective tissue on day 28th of experiment (Bursal lesion score '3') (H & E stain, 120X).Fig.14. Section of BF from group II showing mild to moderate generalized depletion of lymphocytes, invagination of mucosal lining epithelium giving glandular appearance in the mucosa as well as in follicles, increase interfollicular connective tissue, cystic spaces in the follicles on day 35th of experiment (Bursal lesion score '4') (H & E stain, 120X).Fig.15. Section of BF from group II showing moderate generalized depletion of lymphocytes, infiltration of inflammatory cells, increase interfollicular connective tissue, cystic spaces in the follicles on day 35th of experiment (Bursal lesion score '4') (H & E stain, 120X).Fig.16. Section of BF from group III showing lesions comparable with bursal lesion score '4' (H & E stain, 240X).Fig.17. Section of BF from group IV showing lesions comparable with bursal lesion score '4' (H & E stain, 120X).Fig.18. Section of BF from group V showing lesions comparable with bursal lesion score '3' (H & E stain, 240X).









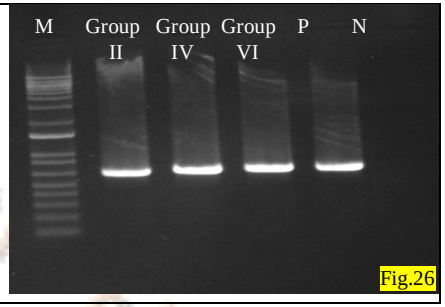




Fig.19. Section of BF from VI showing lesions comparable with bursal lesion score '4' (H & E stain, 240X).Fig.20. Section of BF from group II showing lesions comparable with bursal lesion score '4' (H & E stain, 240X). Fig.21. Section of BF from group III showing lesions comparable with bursal lesion score '4' (H & E stain, 240X).Fig.22. Section of BF from group IV showing lesions comparable with bursal lesion score '4' (H & E stain, 240X).Fig.23. Section of BF from group V showing lesions comparable with bursal lesion score '2' (H & E stain, 240X). Fig.24. Section of BF from group VI showing lesions comparable with bursal lesion score '2' (H & E stain, 240X).Fig.25. Graph showing BF: BW in different experimental groups at different weekly intervals. Fig.26. Agarose gel image showing amplified product of IBD virus (Approximately 650 bp) in experimental study. M: 1 kb plus DNA ladder; GP VI: test sample +ve; GP II: test sample +ve; P: IBD Positive control; GP IV: test sample +ve; N: Negative control.

**Table 2. Histopathological bursal lesion score in different experimental groups at different weekly interval.**

| Days / Groups | 7th day | | | | | | 14th day | | | | | | 21st day | | | | | | 28th day | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Bursal lesion score*** | **0** | **1** | **2** | **3** | **4** | **5** | **0** | **1** | **2** | **3** | **4** | **5** | **0** | **1** | **2** | **3** | **4** | **5** | **0** | **1** | **2** | **3** | **4** | **5** |
| **Control (I)** | 8 | - | - | - | - | - | 8 | - | - | - | - | - | 8 | - | - | - | - | - | 8 | - | - | - | - | - |
| **Immune complex vaccine (III)** | 2 | 4 | 2 | - | - | - | 2 | 3 | 3 | - | - | - | 2 | - | 3 | 1 | 2 | - | 2 | 3 | - | 2 | 1 | - |
| **Intermediate plus vaccine (V)** | - | - | - | - | - | - | - | - | - | - | - | - | 3 | 1 | 3 | - | 1 | - | 2 | 2 | 2 | 1 | 1 | - |

| Days / Groups | 35th day (Unchallenged group) | | | | | | 42nd day (Unchallenged group) | | | | | | Days / Groups | 35th day (Challenged group) | | | | | | 42nd day (Challenged group) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Bursal lesion score*** | **0** | **1** | **2** | **3** | **4** | **5** | **0** | **1** | **2** | **3** | **4** | **5** | **Bursal lesion score*** | **0** | **1** | **2** | **3** | **4** | **5** | **0** | **1** | **2** | **3** | **4** | **5** |
| **Group I** | 8 | - | - | - | - | - | 8 | - | - | - | - | - | **Group II** | - | - | - | 3 | 5 | - | - | 2 | 2 | - | 4 | - |
| **Group III** | 1 | 5 | - | - | 2 | - | - | 4 | - | 2 | 2 | - | **Group IV** | 1 | 2 | - | 3 | 2 | - | - | 2 | 4 | - | 2 | - |
| **Group V** | - | 5 | 2 | - | 1 | - | 2 | 4 | 2 | - | - | - | **Group VI** | - | 4 | - | 3 | 1 | - | 4 | - | 2 | 2 | - | - |

Bursal lesions score:

0: No change
1: Mild necrosis in isolated follicles
2: Moderate generalized lymphocyte depletion or isolated follicles with severe depletion
3: Hyperplasia and hypertrophy of cortico-medullary epithelium giving adenomatoid appearance, over 50 percent of follicles with lymphoid depletion
4:Vacuolation in the mucosal lining epithelium, invagination of mucosal lining epithelium giving glandular appearance in the mucosa as well as in the follicles, increase in interfollicular connective tissue, cystic spaces in the follicles with corrugated epithelium.
5: Loss of all follicular architecture with fibroplasias.

**Bursa of Fabricius to Body Weight Ratio (BF: BW)**

BF/BW ratio is one of the most important parameter to evaluate residual pathogenicity of IBDV vaccines[6]. Data relating to BF: BW of different experimental groups at each weekly interval have been given in Table 3 and also depicted in Fig. 25. BF: BW among differential experimental groups revealed significant decrease ($P < 0.05$) in group III as compared to the control group I from 21 days onwards. There was no significant difference between experimental groups V as compared to the control group I on 21$^{st}$ day of age. On day 28 there was significant ($P < 0.05$) decrease in BF: BW of experimental groups III and V as compared to the control group I. On day 35, i.e. on 7 day PI there was significant ($P < 0.05$) decrease between the control group I and positive control group II as well as control group I and experimental groups III, IV, V and VI. The BF: BW was lowest in positive control group II challenged with field IBD virus. Among the vaccinated groups, the BF: BW was lower in challenged groups (group IV and VI) compared to unchallenged groups (group III and V) with both the vaccines. On the 42$^{nd}$ day of experiment i.e. on 14$^{th}$ day of PI, there was significant ($P < 0.05$) decrease in BF: BW in positive control group II and also in experimental groups III, IV, V and VI compared to the control group I. Among the vaccinated groups, the BF: BW was lower in challenged groups (group IV and VI) compared to unchallenged groups (group III and V) with both the vaccines. There was improvement in BF: BW on day 42 among different experimental groups as compared to day 35 of the experiment.

**Confirmation of infectious bursal disease virus by RT-PCR**

The bursa of Fabricius tissues of experimental birds from group II, IV and VI were tested along with the positive control for the presence of *IBD* virus by RT-PCR. The expected amplicon product of IBD virus of size 650 bp were detected in ethidium bromide stained agarose gel in comparison with positive control and molecular marker (DNA ladder). All the three samples of experimental groups II, IV and VI were found positive for *IBDV* (Fig.26).

## IV. DISCUSSION

Infectious bursal disease (IBD), a highly contagious and immunosuppressive viral disease of chickens, was first recognized in 1962 as "Avian Nephrosis" because of prominent lesion in kidney. The disease is also known as 'Gumboro' disease because of an outbreak in Gumboro area of southern Delaware, USA. Winterfield and Hitchner (1962)[7] were the first to isolate the etiological agent. The etiological agent *Infectious bursal disease virus* (IBDV) is a small, non-enveloped virus, that is a member of the genus *Avibirnavirus* of the family Birnaviridae (OIE 2008; Kasanga *et al.,* 2013)[8,9]. The present study describes the protective efficacy of immune complex vaccine in comparison to commercially used intermediate plus vaccine in broiler chicks by experimental challenge with field IBD virus. Clinical symptoms of infectious bursal disease were observed in few of the birds on 4 day PI in positive control group II. Clinical symptoms like dullness, depression, ruffled feathers, decrease feed and water consumption in all birds in IBDV challenged group were observed. These findings were in consistent with results obtained by Prajapati (1999)[10], Coletti *et al.* (2001)[11], Otsyina *et al.* (2009)[12], Zhai *et al.* (2014)[13], Prandini*et al.* (2016)[14], Dey*et al.* (2017)[15], Dačić *et al.* (2018)[16] and Sedeik*et al.* (2019)[17]. Contrary to the present study yellowish or whitish diarrhoea in most of the affected birds were observed by them. During the present study no mortality was observed in any of experimental groups during the period of experiment. The similar findings were also reported by Prajapati (1979)[18], Ray *et al.* (1985)[19], Shukla (1993)[20], Inoue *et al.* (1994)[21] and Jaikumar *et al.* (1994)[22] during their experimental study on IBD in poultry. Contrary to the present findings Prajapati (1999)[10], Otsyina *et al.* (2009)[12], Prandini*et al.* (2016)[14], Dey*et al.* (2017)[15], Dačić *et al.* (2018)[16] and Sedeik*et al.* (2019)[17] reported variable mortality per cent ranging from 5 to 100 per cent during their study on experimental infection of IBD in broilers / layers.

Contrary to the present findings Coletti *et al.* (2001)[11] reported bursal lesions such as edema in different degrees and hemorrhages on the mucosal surface of bursa of Fabricius in unvaccinated challenged groups and similar to present findings they observed reduction in bursal size on third day of PI in vaccinated challenged groups. Similarly Rautenschlein *et al.* (2005)[23] observed gelatination and hemorrhages of the bursa of Fabricius, in vaccinated and unvaccinated challenged birds at 7 days of PI during their study. Contrary to the present findings Lone *et al.* (2012)[24] reported marked hemorrhagic lesions on pectoral, breast and thigh muscles with atrophied bursa and enlarged spleen on 4$^{th}$ to 7$^{th}$ day of post-challenge in control group however, no lesions were observed in bursa and spleen in vaccinated groups during their study. In consistent with the present findings Camilotti *et al.* (2016)[25] reported atrophy of bursa of Fabricius at 10 day PI during their study. Contrary to the present findings Zahid *et al.* (2017)[28] observed mild changes in bursa of Fabricius with swollen bursal fold and mild hemorrhages in birds of immune complex vaccinated unchallenged group and similar to present finding they also observed atrophy of BF in birds of IBD vaccinated unchallenged group during their study. In contrast to the present findings Preeti *et al.* (2018)[26] reported severe hemorrhages on breast and thigh muscles, bursal changes i.e. edematous, enlarged and severely hemorrhagic and similar to present finding they observed atrophy of BF. Similarly Yilmaz *et al.* (2019)[27] reported distinct edema, hemorrhage, enlargement and remarkable atrophy in some bursae of Fabricius during their field outbreak study on IBD infection in broilers. Gross lesions appeared during the present study when compared to the reports of earlier workers, it could be stated that the atrophy of bursa of Fabricius observed during the present study might be after initial development of lesions i.e. peribursal edema and enlargement. As the sacrifice interval was of seven days, the gross lesions appeared only in the form of smallness/atrophy of bursa of Fabricius.

In consistent with the present microscopic lesions Coletti *et al.* (2001)[11] observed lymphocyte depletion and necrosis from day 1 of PI and later on atrophy of follicles, cysts and increase of cellular connective tissue stroma in vaccinated challenged groups. They also observed edema, lymphocyte necrosis, and heterophil infiltration in vaccinated challenged and vaccinated unchallenged groups during their study. Similar to present findings Rautenschlein *et al.* (2005)[23] during their study observed histopathology lesions only in intermediate plus vaccinated group, such as lymphoid cell depletion at 14 days post vaccination (PV) and bursa lesions of a score 4 (76%–100% of follicles showing cellular depletion) at 21 days post vaccination (PV) whereas no bursa lesions were observed in birds of the no vaccinated control group as well as birds vaccinated groups. They also observed bursa lesions of a score 3 (51%–75% of follicles showing cellular depletion) in all challenged birds from the no vaccinated group as well as from vaccinated groups during their study. In consistent with the present findings Zahid *et al.* (2017)[28]reported BF lesions such as mild lymphocyte depletion and epithelial necrosis at 14$^{th}$ day of post-vaccination, moderate lymphoid depletion with proliferating fibrous tissue at 21 day of post-vaccination, epithelial cyst formation, moderate to severe lymphoid depletion with infoldings of epithelium and marked fibroplasias at 28$^{th}$ day of post-vaccination in birds of all vaccinated groups whereas no histological lesions observed in control unvaccinated birds at 21day of age during their experimental study. Similarly, Yilmaz *et al.* (2019)[27] reported different sizes of vacuolizations prominent in the plicae of lamina epithelialis in the bursa of Fabricius, invaginations in lamina epithelialis and correspondingly gland like structures formed both in the follicles and lamina epithelialis, vacuolizations and necrosis in the medullar areas and in some of them infiltration of endothelial macrophages, diffuse vacuolizations, an excess formation of fibrous connective tissue and follicular atrophy in bursa of Fabricius of IBDV infected chickens during their field outbreak study on IBD infection in broilers. The microscopic lesions appeared during the present study indicated that both the vaccines i.e. immune complex vaccine given on day 1 as well as intermediate plus vaccine given on day 14 could induce bursal lesions of variable degrees of bursal score on each interval post vaccinal sacrifice till day 42 of age. Similar to present findings Prajapati (1999) reported significant ($p < 0.05$) reduction in BF : BW of vaccinated group as compared to the control group on 17$^{th}$ day of age i.e. three day post vaccination, on day 34 i.e. six days after boostering with IBD vaccine there was a significant ($p < 0.05$) reduction in vaccinated groups as compared to control group. They also observed highest BF: BW in control unchallenged group compare to control challenged and vaccinated groups at 9$^{th}$ day PI during their experimental study. Similarly control group did not show any reduction of the bursa/body-weight ratio reported by Rautenschlein *et al.* (2005)[23]during their experimental study. They also reported significant ($p < 0.05$) reduction in the bursa/body-weight ratio at seven day of PI in intermediate plus vaccinated group. In agreement with present study significantly less mean bursal weight of the unvaccinated challenged control group birds than those of the vaccinated challenged birds during experimental study on IBD infection in chickens reported by Otsyina *et al.* (2009)[12]. They also reported lower mean BF: BW in birds vaccinated with the intermediate plus vaccine and challenged with IBDV compared to birds vaccinated with live intermediate vaccines and challenged with IBDV on 15$^{th}$ day PI during their study. In consistent with the present findings Jackwood *et al.* (2011)[29] and Kurukulasuriya *et al.* (2017)[30] also reported mean B/BW ratios in the challenged groups significantly ($p < 0.05$) lower than those noninoculated control birds during their experimental study. Similar to the present study the ratio of BF/BW in unvaccinated was numerically higher than that in vaccinated challenged group, no detectable bursal atrophy was found in unvaccinated group, significant ($p < 0.01$) bursal atrophy in all chickens in the nonvaccinated but challenged group indicating massive IBDV infection reported by Zhai *et al.* (2014)[13] during their experimental study. Similarly, Dey *et al.* (2017)[15] and Azzam *et al.* (2019)[31] reported significantly ($p < 0.01$) higher BF / BW ratios in the vaccinated groups and normal uninfected control group than the control challenged group during their experimental study on IBD infection in chickens.

## V. CONCLUSIONS

FOLLOWING CONCLUSIONS WERE DRAWN FROM THE PRESENT STUDY:

Experimental challenge with field IBD virus showed mild clinical signs like dullness, depression, stunted growth, dehydration, reluctance to move, ruffled feathers, closed eyes and decreased feed intake only in group challenged with field IBD virus. Among the vaccinated groups i.e. immune complex vaccinated or intermediate plus vaccinated groups, none of groups showed mortality after challenge with field IBD virus on 28$^{th}$ day. Even there appeared no mortality in any of the experimental groups, vaccinated with either immune complex or intermediate plus IBD vaccine and subsequently challenged with IBD virus. The atrophy of bursa of Fabricius observed during the present study might be after initial development of lesions i.e. peribursal edema and enlargement. As the sacrifice interval was of seven days, the gross lesions appeared only in the form of smallness /atrophy of bursa of Fabricius. Even at 14$^{th}$ day PI, there was gradual reduction in the number of birds showing atrophy of bursa of Fabricius. This might be due to regeneration of bursa of Fabricius. The present gross finding only of the nature of atrophy of bursa of Fabricius and absence of mortality during the experimental period also suggested that experimental reproduction of field IBDV was mild in nature. The microscopic lesions appeared during the present study indicated that both the vaccines i.e. immune complex vaccine given on day one as well as intermediate plus vaccine given on day 14 could induce bursal lesions of variable degrees of bursal score on each interval post vaccinal sacrifice till day 42 of age. The BF: BW observed in vaccinated challenged and unchallenged groups also suggested that vaccinated challenged groups induce more damaging bursal lesions as compared to unchallenged vaccinated groups with both the vaccines. It also indicated that among both the vaccines, immune complex vaccine cause more gradual bursal damage as compared to intermediate plus vaccine. The field IBD virus alone cause more severe damage to bursa of Fabricius as evident by BF: BW as well as gross and microscopic lesions. Experimental challenge with field IBD virus showed mild clinical signs like dullness, depression, stunted growth, Molecular detection of *IBDV* confirmed by RT-PCR from representative tissue samples of bursa of Fabricius collected from the birds belonged to group II, IV and VI. The overall nature of both the vaccines revealed that immune complex vaccine given on day one could result in more bursal damage than intermediate plus vaccine given on day 14 as revealed by gross and microscopic lesions and BF: BW.

**REFERENCES**


1.Cosgrove, A. S. (1962). An apparently new disease of chickens: avian nephrosis. *Avian diseases*, 6(3), 385-389.
2.Chansiripornchai, N., & Sasipreeyajan, J. (2005). The efficacy of intermediate and intermediate-plus infectious bursal disease vaccine for the prevention of infectious bursal disease in broilers. *The Thai Journal of Veterinary Medicine*, *35*(4), 91-96.
3.Chansiripornchai, N., & Sasipreeyajan, J. (2009). Comparison of the efficacy of the immune complex and conventionally live vaccine in broilers against infectious bursal disease infection. *Thai J. Vet. Med*, 39(2), 115-120.
4.Winterfield, R. W., & Thacker, H. L. (1978). Immune response and pathogenicity of different strains of infectious bursal disease virus applied as vaccines. *Avian Diseases*, 721-731.
5.Snedecor, G. W., & Cochran, W. G. (1980). Statistical method 7th Ed. *The Iowa state university press, Ames, Iowa, USA*, 39-63.
6.Bolis, D. A., Paganini, F. J., Simon, V. A., Zuanaze, M. F., Scanavini Neto, H., Correa, A. R. A., & Ito, N. M. K. (2003). Gumboro disease: evaluation of serological and anatomopathological responses in vaccinated broiler chickens challenged with very virulent virus strain. *Brazilian Journal of Poultry Science*, 5(2), 137-146.
7.Winterfield, R. W., & Hitchner, S. B. (1962). Etiology of an infectious nephritis-nephrosis syndrome of chickens. *American Journal of Veterinary Research*, 23, 1273-1279.
8.OIE, (2008). Manual of diagnostic tests & vaccines for terrestrial animals 5th ed., Chapter 2.3.12.Infectious bursal disease (Gumboro disease).
9.Kasanga, C. J., Yamaguchi, T., Munang'andu, H. M., Ohya, K., & Fukushi, H. (2013). Molecular epidemiology of infectious bursal disease virus in Zambia. *Journal of the South African Veterinary Association*, 84(1), 1-4.
10.Prajapati, M. G. (1999). Immunopathological studies on commercial intermediate plus infectious bursal disease vaccine in chicken. M.V.Sc. Thesis, Dept. *Vet. Patho.* Gujarat Agricultural University, Anand.
11.Coletti, M., Del Rossi, E., Franciosini, M. P., Passamonti, F., Tacconi, G., & Marini, C. (2001). Efficacy and safety of an infectious bursal disease virus intermediate vaccine in ovo. *Avian diseases*, 1036-1043.
12.Otsyina, H. R., Amakye-Anim, J., & Aning, K. G. (2009). Protective efficacy of commercial live vaccines against very virulent infectious bursal disease virus (vvIBDV) in Ghana. *Journal of Veterinary Medicine and Animal Health*, 1(2).
13.Zhai, L., Wang, Y., Yu, J., & Hu, S. (2014). Enhanced immune responses of chickens to oral vaccination against infectious bursal disease by ginseng stem-leaf saponins. *Poultry science*, 93(10), 2473-2481.
14.Prandini, F., Simon, B., Jung, A., Pöppel, M., Lemiere, S., & Rautenschlein, S. (2016). Comparison of infectious bursal disease live vaccines and a HVT-IBD vector vaccine and their effects on the immune system of commercial layer pullets. *Avian Pathology*, 45(1), 114-125.
15.Dey, S., Chellappa, M., Pathak, D., Gaikwad, S., Yadav, K., Ramakrishnan, S., & Vakharia, V. (2017). Newcastle Disease Virus Vectored Bivalent Vaccine against Virulent Infectious Bursal Disease and Newcastle Disease of Chickens. *Vaccines*, 5(4), 31.
16.Dačić, M., Resanovic, R., Rasic, Z., Valcic, M., Milovanovic, A., & Velhner, M. (2018). Efficacy of recombinant VAXXITEK HVT-IBDv vaccine against very virulent Infectious bursal disease virus (vvIBDv) challenge in layer chicks: A pilot study. *Journal of the Hellenic Veterinary Medical Society*, 69(1), 823-830.
17.Sedeik, M. E., El-shall, N. A., Awad, A. M., Abd El-Hack, M. E., Alowaimer, A. N., & Swelum, A. A. (2019). Comparative Evaluation of HVT-IBD Vector, Immune Complex, and Live IBD Vaccines against vvIBDV in Commercial Broiler Chickens with High Maternally Derived Antibodies. *Animals*, 9(3), 72.
18.Prajapati, K. S. (1979). Infectious bursal disease (Gumboro) in chicken. M.V.Sc. Thesis, Dept. *Vet. Patho.* Bombay veterinary College, Maharashtra.
19.Ray, D.K., Bhowmik, M.K., & Sarkar, P. (1985). Effect of experimental infection of Infectious bursal disease virus in White Leghorn chickens. *Indian J.Poult.Sci.* 20(4), 249-254.
20.Shukla, R.N. (1993). Pathological studies on lymphoid organs in poultry with special reference to infectious bursal disease. M.V.Sc. thesis, submitted to Gujarat Agricultural University, Anand Campus, Anand.
21.Inoue, M., Fukuda, M., & Miyano, K. (1994). Thymic lesions in chickens infected with Infectious bursal disease virus. *Avian Dis.* 38(4), 839-846.
22.Jaikumar, D., Mukhopadhyay, H.K., Reddy, Y.K., Dorairajan, N., & Koteeswaran, A. (1994). Infectious bursal disease in commercial White Leghorn cockerels. *Indian Vet. J.* 71(12), 1168-1170.
23.Rautenschlein, S., Kraemer, C. H., Vanmarcke, J., & Montiel, E. (2005). Protective efficacy of intermediate and intermediate plus infectious bursal disease virus (IBDV) vaccines against very virulent IBDV in commercial broilers. *Avian diseases*, 49(2), 231-237.
24.Lone, N. A., Rehmani, S. F., Khan, T. A., & Kazmi, S. U. (2012). Efficacy of live attenuated and inactivated oil emulsion infectious bursal disease virus vaccines in broiler chicks. *Pakistan Veterinary Journal*, 32(4), 539-542.
25.Camilotti, E., Moraes, L. B. D., Furian, T. Q., Borges, K. A., Moraes, H. L. D. S., & Salle, C. T. P. (2016). Infectious bursal disease: Pathogenicity and immunogenicity of vaccines. *Brazilian Journal of Poultry Science*, 18(2), 303-308.

26.Preeti, S. K., Bai, L., Ramkaran, N. J., & Gupta, R. (2018). Epidemiological studies on infectious bursal disease in broiler chickens in Haryana state during 2012-2015.*The pharma journal*, 7(4), 282-285.
27.Yilmaz, A., Turan, N., Bayraktar, E., Gurel, A., Cizmecigil, U. Y., Aydin, O. & Cakan, B. (2019). Phylogeny and evolution of infectious bursal disease virus circulating in Turkish broiler flocks. *Poultry science*.
28.Zahid, B., Aslam, A., Chaudhry, Z. I., & Akhtar, R. (2017). Biochemical and Histopathological Changes in Immune and Non Immune Broilers after Inoculation of Field Infectious Bursal Disease Virus. *Pakistan Journal of Zoology*, 49(4).
29.Jackwood, D. J., Sommer-Wagner, S. E., Crossley, B. M., Stoute, S. T., Woolcock, P. R., & Charlton, B. R. (2011). Identification and pathogenicity of a natural reassortant between a very virulent serotype 1 infectious bursal disease virus (IBDV) and a serotype 2 IBDV. *Virology*, 420(2), 98-105.
30.Kurukulasuriya, S., Ahmed, K. A., Ojkic, D., Gunawardana, T., Goonewardene, K., Gupta, A., & Gomis, S. (2017). Modified live infectious bursal disease virus (IBDV) vaccine delays infection of neonatal broiler chickens with variant IBDV compared to turkey herpesvirus (HVT)-IBDV vectored vaccine. *Vaccine*, 35(6), 882-888.
31.Azzam, M. M., Jiang, S. Q., Li, L., & Jiang, Z. Y. (2019). Effect of Soybean Isoflavones on Growth Performance, Immune Function, and Viral Protein 5 mRNA Expression in Broiler Chickens Challenged with Infectious Bursal Disease Virus. *Animals*, 9(5), 247.