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# Winterfield 2512 G-61 Strain of IBDV Vaccine (CEVAC® IBD L) Showed Reduced Pathogenicity in Commercial Chickens

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**A B S T R A C T**

A live, freeze-dried commercial vaccine prepared from the "Winterfield 2512 G-61 strain" of infectious bursal disease virus (IBDV) was tested for it's pathogenicity in commercial chickens. A total of 200 unvaccinated Cobb-500 chicks obtained commercial sources, raised in relative isolation from day old chick (DOC) were used in the experiment. A total of 3 chicks were randomly collected from the experimental flock on day 11 ($D_{11}$), $D_{13}$, $D_{15}$, $D_{17}$, $D_{20}$ $D_{23}$ and $D_{26}$. The vaccine was administered through drinking water at $D_{11}$ and the booster was also given on $D_{17}$ through drinking water. All the sampled birds were euthanized for necropsy. The visible gross morbid lesions, bursa-body weight ratios and histomorphology including bursal lesion scores were recorded following necropsy and histopatholog. An infected flock was included in this study for comparison. Data of bursa-bodyweight ratios in relation to bursal lesion scores was analyzed statistically. No detectable gross lesions were found during necropsy and bursa-body weight ratios were 2.75±0.60, 2.71±0.39, 2.44±0.42, 3.39±0.13, 2.58±0.55, 2.15±0.16, 2.41±0.28 and 2.45±0.09 on $D_{11}$, $D_{13}$, $D_{15}$, $D_{17}$, $D_{20}$, $D_{23}$ and $D_{26}$, respectively. Histopathological lesions were characterized as varying degrees of lymphoid depletion, and the bursal lesion scores were 0.67±0.33, 0.67±0.33, 2.00±0.58, 0.67±0.33, 1.0±0.00, 0.67±0.33 and 0.33±0.33 on $D_{11}$, $D_{13}$, $D_{15}$, $D_{17}$, $D_{20}$ $D_{23}$ and $D_{26}$, respectively. The vaccine virus showed reduced levels of pathogenicity in broiler chickens. No disease outbreaks were noted in the vaccinated group, but significant changes were found in the naturally infected flock.

**Keywords:** Winterfield 2512 G-61 strain, IBDV vaccine, pathogenicity



## Introduction

Gumboro disease (Infectious bursal disease/IBD) is a highly contagious viral disease of young chickens causing severe bursal lesions followed by immunosuppression (Lukert and Saif, 2003). IBD virus (IBDV), belonging to the genus *Birnavirus* (sub-genus *Avibirnavirus*, family Birnaviridae), is the causative agent of IBD. There are two distinct serotypes of IBDV: serotype1 and serotype 2. Both serotypes can infect chickens and turkeys, but clinical disease is recognized only in chickens (Yamaguchi *et al.* 1996). Serotype 1 has four pathotypes: classical virulent, attenuated strains, very virulent and antigenic variant (Van den Berg, 2000; Lukert and Saif, 2003), and related immunosuppression (Faragher *et al*., 1974). IBDV is exclusively a lymphotrophic virus targeting and destroying the growing B lymphocytes bearing cell-surface IgM (Hirai *et al.,* 1979), and thereby developing a severe morphological alteration of Bursa of Febricius (BF) and producing a profound immunosuppression (Raue *et al.,* 2004). There is no alternative to vaccination in the prevention of IBD, although clinical outbreaks in vaccinated flocks are also reported (Raue *et al.,* 2004). In order to control IBD with live virus vaccine, it is critical to vaccinate commercial chickens that have maternal antibodies. Live vaccines have the ability to overcome the maternal antibodies at certain levels, and vaccination during low maternal antibody titres result in better immune response than when administered in the presence of high maternal antibody titres (Giasuddin *et al*., 2003).

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Neutralization of vaccine virus by pre-existing antibodies is considered to be one of the major factors causing vaccination failure. To overcome this problem, vaccine containing higher residual pathogenicity was developed to withstand the neutralizing effects of maternal antibodies. The antigenic variation among viruses may also cause vaccination failure, particularly when antigenic structures between field and vaccine strains differ widely (Van den Berg, 2000). No vaccine based on vvIBDV is yet commercially available. The immunogenicity of viruses may differ between strains. The intermediate vaccine strain produce moderate to severe bursal lesions as previously reported by others (Abdel-Alim and Saif, 2001). The better protection observed with more virulent strains of IBDV is attributed to increased antigenic stimulation based on higher and longer replication in lymphoid tissues. The time of vaccination, type of the vaccine, maternally derived antibodies in the progeny chicks and pathogenicity of IBDV field challenge are the important factors determining the efficacy of the vaccination (Lukert and Saif, 2003). The present study has been carried out to determine the degree of pathogenicity of the Winterfield 2512 G-61 strain of IBDV used in CEVAC® IBD L vaccine in commercial chickens.

## Materials and Methods

**Experimental chickens, research area and research period**

A total of 500 unvaccinated Cobb-500 Day Old Chicks (DOC) were selected from a commercial poultry farm in Saidpur, Nilphamari, Bangaldesh. Poultry rearing and vaccination was

done at the source farm. Laboratory work was conducted at the Department of Pathology and Parasitology, Hajee Danesh Science and Technology University (HSTU), Bangladesh. The duration of the research work was six months from September 2009 to February 2010.

**Vaccine and Vaccination**
The Winterfield 2512 G-61 strain of IBDV, a live freeze-dried form of the isolate, was obtained directly from the ACI, Bangladesh Ltd., veterinary product seller and stored at 4ºC until used. The virus was propagated in embryonated chicken eggs obtained from specific-pathogen-free (SPF) flocks.

The vaccine was administered in drinking water to the broiler chicks from 10 to 18 days of age, depending on the level of maternally derived antibodies (MDA) present. The dose of vaccine, route of administration and other instructions were strictly followed as per manufacturer and the factors related to vaccine breaks were avoided. Serological levels of MDA, however, could not be confirmed due to laboratory limitation before vaccination of the experimental chickens of the present study.

**Management of chickens**
The birds were reared in relative isolation. The room was thoroughly cleaned by sweeping and then washing with tap water using hose pipe connected with a tap. The room was disinfected with a household phenolic disinfectant (Phenyl) and fumigated before placing the DOC. Relatively optimum temperature in the brooder house was maintained using electric bulbs in required number and at required distances. Rice husk was the litter material which was placed at a 2-3 inch depth and was replaced as needed following wetting either by faeces, water or by both. For the first week white paper was placed in the brooder which was replaced regularly. Feeding and watering was *ad libitum*. For the first two days birds were maintained on *suji* (a coarse flour of wheat) followed by commercial broiler starter and grower feed. In addition, electrolytes and vitamins were given in water from time to time until selling. Entry to the house was restricted. Disinfectant foot baths were compulsory during entry and exit.

**Sampling and experimental design**
The birds were collected from the flock, and brought to the Department of Pathology and Parasitology for the necessary laboratory examination as per as experimental design outlined in Table 1.

**Necropsy and bursa-body weight (B/BW) ratio**
Necropsy was performed at the Pathology Laboratory of HSTU, Dinajpur as per standard procedure (Charlton, 2000). Each bird was weighed before euthanasia, and the visible gross morphological lesions were recorded. The Bursa of Fabricius was weighed and the average bursa-body weight ratio was determined as per the Tanimura *et al.*, (1995) formula:

$$\text{B/BW ratio} = \frac{\text{Bursal weight in grams}}{\text{Live body weight of individual bird in grams}} \times 100$$

Where B= Bursa and BW= Body weight.
The bursae of the naturally infected birds were collected and compared with the vaccinated flocks based on age of the experimental groups.

**Histopathological study and scoring of bursal lesions**
During necropsy, bursa of Fabricius was collected and preserved in 10% formalin for histopathological studies. The fixed tissue samples were further processed, embedded with paraffin, sectioned and stained with haematoxylin and eosin (H & E) as per standard method (Luna, 1968). The slides were studied under a light microscope at various magnifications. The bursal lesions were recorded and scored on the basis of the following criteria (Raue *et al.*2004): apparently normal lymphoid follicles (score 0), mild lymphoid depletion (score 1), moderate lymphoid depletion (score 2), severe lymphoid depletion (score 3) and atrophy of follicles with or without cystic spaces (score 4).

**Statistical analysis of bursal lesion scoring**
The raw data were recoded, entered and sorted using the MS Excel. The experimental data were calculated by the SPSS (Statistical Package for Social Sciences) (Version 11.5) for analysis. Mean differences were done by F test, t test and chi square test.

## Results

**Clinical examination of the experimental flocks**
There was no visible clinical manifestation in birds of the vaccinated flock. Birds typically affected with IBD showed varying degrees of appetite loss, reluctance to move, drowsiness, severe depression, whitish diarrhoea and death.

**Gross pathological examination**
Gross lesions of the vaccinated flock were indistinct but the affected flock showed swollen, oedematous, haemorrhagic and atrophied bursae. Varying degrees of haemorrhages were found in the thigh and breast muscles, and at the junction between the gizzard and proventriculus.

**Bursa-body weight ratios**
The bursa-body weight ratios for the vaccinated flock were 2.75±0.60, 2.71±0.39, 2.44±0.42, 3.39±0.13, 2.58±0.55, 2.15±0.16 and 2.41±0.28 on $D_{11}$, $D_{13}$, $D_{15}$, $D_{17}$, $D_{20}$, $D_{23}$ and $D_{26}$ respectively that differed significantly. The unvaccinated infected flock's values on D-23 were 2.453±0.09 (Table 2).

**Histopathology and bursal lesion scores**
Histopathological features of the bursa of Fabricius are presented in Figure 1. Most bursal follicles were apparently normal and characterized as having unifom cellular concentrations in the follicles. Mild depletion of lymphoid cells were also found in some follicles in the same examined bird. Moderate depletion of lymphoid cells was found in few bursal follicles. Severe lymphoid depletion of lymphoid cells was also found in fewer follicles. Follicular atrophy without the development of follicular cysts was also observed, but this histopathological characteristic was more prevalent in the flocks showing typical outbreak of Gumboro.

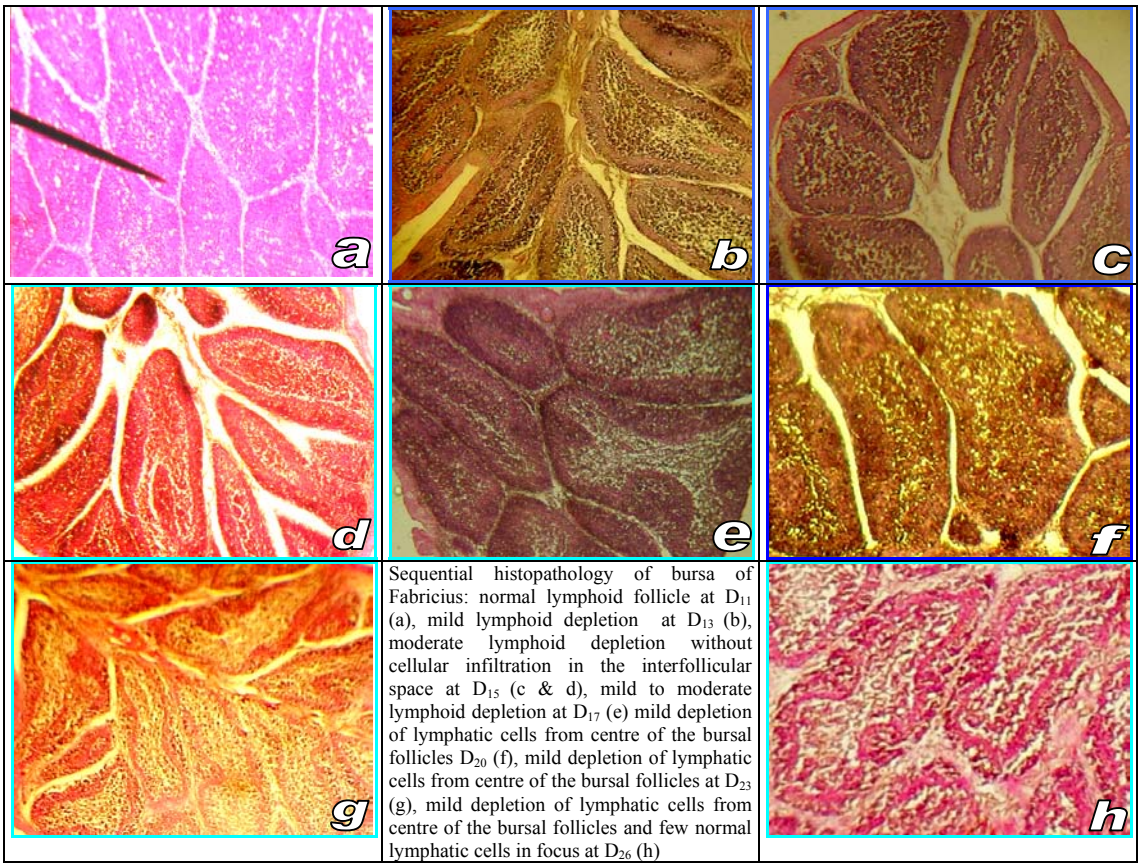
Bursal lesion scores for the vaccinated birds were 0.67±0.33, 0.67±0.33, 2.00±0.58, 0.67±0.33, 1.00±0.00, 0.67±0.33 and 0.33±0.33 and on $D_{11}$, $D_{13}$, $D_{15}$, $D_{17}$, $D_{20}$, $D_{23}$ and $D_{26}$ respectively, and these differed significantly ($P<0.01$). The score for the naturally infected birds was 3.33±0 on $D_{23}$ (Table 3).

## Discussion

Pathogenicity of the Gumboro vaccine (CEVAC® IBD L) used in this study, was evaluated in commercial broiler chickens and showed relatively reduced pathogenicity in the broiler chickens under farm condition.

The present study investigated the performance of the IBD vaccine after experimental inoculation of commercial broiler chickens by evaluating parameters such as clinical signs, gross morphological lesions, bursa-body weight ratios and histopathological lesions including bursal lesion scores.

Deviation from recommended vaccine regimen in vaccination programs involving live vaccines may result in vaccine failures and even disease outbreaks originating from the vaccine strain(s). Manufacturer's instructions were strictly followed in this study. No vaccination related breaks were observed in this study. Maternally derived antibodies (MDA) are detected in the first few days of a chickens life and last for variable periods of time ranging from 7-14 days (Giasuddin *et al.,* 2003). Existing maternal antibodies are important factors in causing inactivation of the vaccine virus resulting vaccination failure (Lukert and Saif, 2003). The experimental flock in the present study, however, was vaccinated on $D_{11}$, and boosted on $D_{17}$ without determining the MDA level and sampling was done following




Sequential histopathology of bursa of Fabricius: normal lymphoid follicle at $D_{11}$ (a), mild lymphoid depletion at $D_{13}$ (b), moderate lymphoid depletion without cellular infiltration in the interfollicular space at $D_{15}$ (c & d), mild to moderate lymphoid depletion at $D_{17}$ (e) mild depletion of lymphatic cells from centre of the bursal follicles $D_{20}$ (f), mild depletion of lymphatic cells from centre of the bursal follicles at $D_{23}$ (g), mild depletion of lymphatic cells from centre of the bursal follicles and few normal lymphatic cells in focus at $D_{26}$ (h)

**Fig. 1. Histopathological features of the bursa of Fabricius**

**Table 1. Sampling occasion at vaccination**

| Sampling occasion | Vaccination Status | No. of birds for Necropsy | Parameters studied |
|---|---|---|---|
| $D_{11}$* | - | 3 | Clinical signs and |
| $D_{13}$ | 2 (DPV) | 3 | symptoms Gross morbid |
| $D_{15}$ | 4 (DPV) | 3 | lesions Bursa-body weight |
| $D_{17}$** | 6 (DPV) | 3 | ratios Histopathology |
| | | | Bursa lesion score |
| $D_{20}$ | 3 (DPB) | 3 | Clinical signs and |
| $D_{23}$ | 6 DPB | 3 | symptoms Gross morbid |
| $D_{26}$ | 9 DPB | 3 | lesions Bursa-body weight |
| $D_{23}$ | - | 3 | ratios Histopathology |
| | | | Bursa lesion score |

DPV =Days post vaccination, DPB = Days post boosting,
$D_{11}$* = Primary vaccination, $D_{17}$** = Boosting

**Table 2. Statistical analysis of bursa-body weight ratios of chickens at different sampling occasions**

| Statistical analysis | Sampling occasion (Day) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $D_{11}$ | $D_{13}$ | $D_{15}$ | $D_{17}$ | $D_{20}$ | $D_{23}$ | $D_{26}$ | $D_{23}$* |
| Mean±SE | 2.75 ± 0.60 | 2.71 ± 0.39 | 2.44± 0.42 | 3.39 ± 0.13 | 2.58 ± 0.55 | 2.15 ± 0.16 | 2.41 ± 0.28 | 2.45 ± 0.09 |
| P Value | 0.0044 | 0.0256 | 0.2144 | 0.0129 | 0.0051 | 0.0041 | 0.0036 | 0.1024 |
| Level of sig. | ** | * | NS | ** | ** | ** | ** | NS |

$D_{23}$* =Affected flock, NS = Not Significant (P>0.05),
** = Significant (P<0.01) * = Significant (P<0.05)

**Table 3. Statistical analysis of bursal lesion scores of chickens at different sampling occasions**

| Sampling occasion | No. of sampled birds | Bursal lesion score | Mean±SE |
|---|---|---|---|
| $D_{11}$ | 3 | 0,1,1 | 0.67±0.33 |
| $D_{13}$ | 3 | 1,2,1 | 0.67±0.33 |
| $D_{15}$ | 3 | 1,2, 3 | 2.00±0.58 |
| $D_{17}$ | 3 | 0,1,1 | 0.67±0.33 |
| $D_{20}$ | 3 | 2,1,1 | 1.00±0.00 |
| $D_{23}$ | 3 | 1,2,1 | 0.67±0.33 |
| $D_{26}$ | 3 | 1,2,1 | 0.33±0.33 |
| $D_{23}$* | 3 | 3, 3, 4 | 3.33±0.33 |
| P Value | | | 0.0003 |
| Level of significance | | | ** |

** = Significant (P<0.01), $D_{23}$* = Affected flock

primary and booster vaccinations as indicated in Table 1.

Gumboro disease is a highly fatal disease where the morbidity rate is close to 100% and the mortality rate is variable but may be up to 80% (Chowdhury, *et al.,* 1996; Hoque, *et al.,* 2001). Nonetheless there was no apparent morbidity recorded in the present study and the mortality rate was zero following vaccination. This finding agrees with that of other researchers (Babiker, *et al.,* 2004).

The clinical manifestations of typical Gumboro disease are high fever, off feed, reluctance to move, depression, drowsiness, watery diarrhoea and vent picking (Van den Berg 2000). None of these signs were recorded in the vaccinated flock of the present study as similarly described by many authors (Babiker, *et al.,* 2004). It should be noted that vaccinated flocks may also

show different clinical signs characteristic of the disease which will certainly determine vaccination failure caused by a variety of factors (Eterradossi, 2001).

The routine necropsy was done following primary vaccination and boosting as per as experimental design (Table 1). There was no relevant gross morbid lesion recorded during the course of necropsy among the vaccinated birds. Hemorrhages in the skeletal muscles, and junction between the proventriculus and gizzard plus varying degrees of bursal lesions and enteritis are common gross pathological lesions observed in both vaccinated and unvaccinated flocks where vaccination failures or IBD outbreaks (Hoque, *et al*., 2001)

Bursa-body weight ratio is the vital factor in determining the pathogenicity of the respective IBDV and there is a proportional relationship between bursa - body weight ratio and the pathogenicity of the respective virus (Mazariegos *et al*., 1990). The bursa-body weight ratios were 2.75±0.60, 2.71±0.39, 2.44±0.42, 3.39±0.13, 2.58±0.55, 2.15±0.16, 2.41±0.28 and 2.45±0.09 on $D_{11}$, $D_{13}$, $D_{15}$, $D_{17}$, $D_{20}$, $D_{23}$ and $D_{26}$ respectively (Table 2).

The bursa of unprotected/unvaccinated birds with virulent virus histopathologically shows mild to severe lymphoid depletion, follicular atrophy, cystic formation of follicles and bursal hemorrhages (Hoque *et al*., 2001). The extent of lesions produced in the bursa of fabricius is also proportionally related to the degree of pathogenicity of the infecting virus. In the present study the bursal lesions of the vaccinated flock were histopathologically characterized as either normal follicles, with or without mild to moderate lymphoid depletion, and without follicular atrophy or the development of cystic follicles. There was no indication of follicular regeneration either. However, the histopathological lesions observed in the present study did not imply vaccine failure, as it is normal for such lesions to result from vaccination with live virus, as observed by other workers who previously characterized different bursal lesions produced by other vaccine strains (Raue *et al.,* 2004). Bursal lesion scores were 0.67±0.33, 0.67±0.33, 2.00±0.58, 0.67±0.33, 1.00±0.00, 0.67±0.33, 0.33±0.33 and 3.33±0 on $D_{11}$, $D_{13}$, $D_{15}$, $D_{17}$, $D_{20}$, $D_{23}$ and $D_{26}$ respectively, with statistical significance ($P<0.01$) (Table 3). Relatively minimal lesion scores were observed for all sampling occasions. These results agree with those of other investigators (Raue *et al.,* 2004; Hoque *et al*., 2001).

This experiment was set, conducted and completed under environmental conditions similar to those under which the vaccine may be applied and vaccination failures are common. However, from the above facts and findings it was concluded that the live freeze-dried Winterfield 2512 G-61 strain used in the vaccine "CEVAC® IBD L, ACI, Bangladesh Limited" showed reduced pathogenicity and could potentially prevent IBD outbreaks in healthy flocks without the development of clinical and gross pathological signs.

Nevertheless for the IBDV strain used in the study to be considered as a vaccine candidate, serological evaluation (antibody measurement) and challenge with very virulent strain of IBDV (vvIBDV) following vaccination is a prerequisite.

## Conclusion

This study revealed that administration of CEVAC® IBD L; a live freeze-dried vaccine produced by ACI, Bangladesh Limited from the "Winterfield 2512 G-61 strain" of infectious bursal disease virus was effective for protection against IBDV in chickens and the vaccine virus showed the reduced level of pathogenicity in broiler chickens. No detectable gross lesions were found during necropsy and bursa-body weight ratios determinations. Histopathological lesions were characterized as varying degrees of lymphoid depletion, and the bursal lesions scores. No outbreaks were noted in the vaccinated flock.

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