

# Comparative Immunogenicity Evaluation of Two Infectious Bursal Disease Vaccines Commonly Used in Broiler Chickens in Ethiopia

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**Purpose:** Infectious bursal disease (IBD) is one of the most endemic diseases of commercial poultry in Ethiopia. Vaccination is used as the major means of IBD prevention and control. A study was conducted to compare the immunogenicity of two commercially available IBD vaccines in broiler chicken with maternally derived antibody (MDA).

**Methods:** A total of 270 day-one-old chicks were randomly assigned to three groups, group 1 vaccinated with product A vaccine at the age of 7 and 19 days and group 2 with product B vaccine on day 15 and 22 while group 3 were kept as control. Six chickens were also randomly selected and bled on day 1 for differential leukocyte count (DLC) and determination of MDA. Representative chickens from each group were bled at 24th and 42nd days of age for antibody titration using the indirect ELISA test. DLC scores were determined in the 1st and 24th days.

**Results:** The result revealed highly significant differences ( $P = 0.001$ ) between group 1 and group 2 in DLC at 24th days of age. Antibody titers against IBD were differed significantly ( $P = 0.02$ ) at 24th and 42nd days of age in broilers vaccinated with product A and product B vaccines.

**Conclusion:** Both vaccines have induced an adequate immunological response at the end of the experiment; however, product A has shown significantly higher antibody titers against the IBDV and DLC than product B.

**Keywords:** antibody titer, broiler chicken, IBD vaccines, immunogenicity evaluation

## Introduction

The poultry industry is one of the most productive agricultural sectors in Ethiopia, which serves as a source of major protein supplement for the growing population of the country. However, disease outbreaks are a major constraint of this industry.<sup>1,2</sup>

Infectious bursal disease (IBD), also known as Gumboro, is one of the most important contagious viral diseases of immature chickens globally.<sup>3</sup> The affected chickens have reduced antibody response to vaccinations, strong post-vaccine reactions, and increased susceptibility to concurrent or secondary infections.<sup>4</sup> The disease was reported in Ethiopia for the first time on farms at Bishoftu in 2002 with a mortality rate as high as 50% in the affected broilers and layers.<sup>5</sup> The most economic losses associated with IBD emanated from its immunosuppressive effect that leads to poor vaccination response, secondary bacterial, viral, protozoan infections, poor performance, and treatment expenses.<sup>3,6</sup>

This disease is caused by the IBD virus (IBDV) that belongs to the genus *Avibirnavirus* of the family *Birnaviridae* with a bi-segmented double-standard (ds) RNA genome.<sup>7-10</sup> IBDV is highly stable and resistant to many physical and chemical agents and prone to extreme antigenic variability with increased virulence.<sup>11</sup> The clinical signs of infected chickens relate to the age of the chickens and the virulence and the strain of the virus.<sup>12</sup> The disease can also infect other poult including turkeys.<sup>9</sup> Chickens are more susceptible at 3 to 6 weeks of age and get infected via the oro-faecal route

in a direct mode of transmission since the virus is shed in high amounts in faeces from 48 hours up to 2 weeks post-infection.<sup>13</sup>

There are two distinct serotypes of IBDV. Strains belonging to serotype 1 are pathogenic causing disease in young chickens<sup>3</sup> and comprise several pathotypes, such as classical virulent, antigenic, and very virulent strains. Strains in serotype 2 are apathogenic for chickens.<sup>6</sup> The classical strain of IBDV belonging to serotype 1 shows the ability to cause clinical disease in infected chickens.<sup>14,15</sup> In Ethiopia, two genogroups of IBDVs (genogroups 1 and 3), which antigenically represent classically virulent and very virulent, respectively, are circulating. Moreover, a recent genogrouping analysis conducted by Bari (2021) showed very virulent strains into new sub genogrouping 3. The study confirmed the evidence of Ethiopian isolates in known genogroups and the need of assessing the effectiveness of the available vaccines.<sup>16</sup>

The IBDV, like most poultry viruses, has no specific known treatment. Hence, proper prevention and control strategies need to be implemented on the poultry farms that include strong biosecurity and vaccination at the appropriate time.<sup>17</sup> However, extensive usage of live vaccines results in the evolution of new strains.<sup>3</sup> Though a recent study conducted in Ethiopia evaluated the immunogenicity and efficacy of Vero cell-adapted IBDV LC-75 vaccine strain,<sup>18</sup> there is no published study that compared the immunogenicity of IBD vaccines available in the local Ethiopian market. Moreover, despite the regular vaccination schedule, there are still frequent reports of IBDV outbreaks in Ethiopia, specifically in Bishoftu town, which could be either due to vaccine ineffectiveness or the circulation of a new sub-group in the poultry farms. Therefore, the current study was conducted to determine the level of protection of two IBD vaccination programs in commercial broiler chickens by evaluating the immunogenicity of two commonly used vaccines; one was locally produced and the other was imported from abroad.

## Materials and Methods

### Study Area and Population

The experimental study was carried out in poultry houses of the College of Veterinary Medicine and Agriculture, Bishoftu, Ethiopia from February to April 2020. Bishoftu town is found at about 47 km to the southeast of Addis Ababa and the town where most commercial poultry farms are located.<sup>19</sup>

A total of 276 broilers of Cobb 500 breeds were purchased for this experiment from Alema Farms PLC, a private commercial broiler farm located at Bishoftu town, Ethiopia. These broilers were randomly and equally allocated in various experimental groups and were maintained throughout the experiment in separate isolation units under suitable conditions for vaccine immunogenicity tests.

### Housing and Management of Experimental Chickens

In this study, broilers were kept in a floor housing system. The house, feeder, and water utensils were thoroughly cleaned and disinfected before stocking the broilers. The utensils were also cleaned twice daily to avoid contamination. Thus, the broilers were kept under strict pathogen-free conditions through repeated cleaning and disinfection. They were fed ad libitum on a commercial broiler starter, grower, and finisher diet based on their ages throughout the experiment. A continuous heating program with 200-watt bulbs was suspended at the head height of the broilers. The room temperature was monitored and recorded. The heat was adjusted based on the age of chickens from the suspended lump.

### Study Design

The experimental design employed was a stratified randomized controlled trial in the form of follow-up. From a total of 276 one-day-old chickens, six of them were randomly selected and bled on day 1 for differential cell counting and determination of MDA. The rest 270 were randomly assigned to three groups: group 1, group 2, and group 3 with 90 chickens per group and each group with three replicates of 30 chickens per replicate. Group 1 was vaccinated with the recommended dose and as per the schedule of product A vaccine (CEVAC<sup>®</sup> IBD L) at 7th and 19th days of age; while group 2 were vaccinated with the recommended dose and schedule of product B vaccine at 15th and 22nd days of age. The third group was used as a control.

## Study Methods

### Vaccination of Chicks

Two types of commercial IBDV vaccines, purchased from the market, were used in this study. The product A vaccine (CEVAC<sup>®</sup> IBD L, the live freeze-dried vaccine of Winterfield 2512 strain produced by Ceva Sante Animale, France) was purchased from an importer (Alema PLC, Bishoftu, Ethiopia) whereas the product B vaccine (Live freeze-dried vaccine of IBDV intermediate standard strain produced by the National Veterinary Institute [NVI], Ethiopia) was purchased directly from the manufacturer. They were live vaccines and administered to chicken as per the manufacturer's directions and given orally by drinking water.

### Sample Collection and Serological Test

On a day old of age, six broilers were randomly selected, and blood collection was performed by incising the jugular vein of broilers and pouring the blood directly into a plain vacutainer tube. At 24th and 42nd days of age, 3 mL of blood samples were collected from ten representative broilers for each group using a disposable 3 mL syringe with a 22-gauge needle from the wing vein. Blood samples were centrifuged at 2000 rpm for 10 min for separation of sera and sera samples were harvested into cryovials and labelled. Samples were kept at  $-20^{\circ}\text{C}$  until laboratory analysis was conducted at the NVI.

### Differential Leukocyte Count (DLC)

For DLC, thin blood smears were prepared from the blood samples collected at 1st and 24th days of age. The smears were stained by the Wright-Giemsa stain. A stained smear was examined to determine the percentage of each type of leukocyte present. Each white cell was recorded on a differential cell counter until 100 white cells were counted.

### Enzyme-Linked Immunosorbent Assay (ELISA) to Determine Antibodies Against IBD Vaccine

Antibodies against the IBD vaccine were measured in serum samples that were collected at the age of one day, 24 days (17 days post-primary immunization and 5 days post-second immunization for product A vaccine) and (9 days post-primary immunization and 2 days post-second immunization for product B vaccine) and 42 days. The mean antibody titer in the serum samples was analyzed to compare the mean titer after IBD vaccinations with the baseline titer and to compare the mean titer among the experimental groups.

According to the instruction of the kit manufacturer (ID.vet, France), the indirect enzyme-linked immunosorbent assay (ELISA) diagnostic kit was used to detect the antibodies of the chicken directed against the IBD vaccine. Briefly, five hundred-fold (1:500) dilutions were used. About 245  $\mu\text{L}$  of dilution buffer 14 was added to each well of the microplate. Then, 5  $\mu\text{L}$  of negative control was added to wells A1 and B1 and 5  $\mu\text{L}$  of positive control was added to wells C1 and D1. After that 5  $\mu\text{L}$  of each sample to be tested was added to the remaining wells to make prediluted samples. Then, 90  $\mu\text{L}$  of dilution buffer 14 and 10  $\mu\text{L}$  of the pre-diluted samples were dispersed into the appropriate 96-well plate coated with IBDV viral antigen and the plate was covered and incubated at room temperature for 30 minutes. The plate was washed 3 times with 300  $\mu\text{L}$  of the wash solution 1x at the end of the incubation period followed by the addition of horseradish peroxidase conjugate into each well. The plate was incubated at room temperature for 30 min and washed 3 times again before adding 10  $\mu\text{L}$  of the substrate solution to each test well which was then incubated for 15 min at a dark place at room temperature. Finally, 100  $\mu\text{L}$  of stop solution was added to each well to stop the reaction and the absorbance was read at 650 nm.

## Statistical Analysis

The data collected in this study were stored in MS excel and analyzed. The mean antibody titer, standard deviation, and coefficient of variation (CV, %) have been calculated by using Microsoft offices excel. Furthermore, STATA version 13 was used to analyze the data obtained from the study. Specifically, One-way ANOVA was performed to compare the mean antibody titer of experimental groups. The differences were considered statistically significant at the level of  $p \leq 0.05$ . The CV (%) is interpreted as <30% excellent, 30–50% good, 51–80% fair, and >90% poor response to vaccine. A comparison of differential leukocyte count (DLC) values among different groups were compared by using linear regression.

**Table 1** Antibodies Against IBDV at 24 and 42 Days Old (Mean  $\pm$  Standard Deviation)

Group	Products of Vaccine	Antibody Titration at Different Days	
		Day 24 (Mean $\pm$ SD)	Day 42 (Mean $\pm$ SD)
Group 1*	Product A	2475 $\pm$ 148.49	2026 $\pm$ 291.51
Group 2**	Product B	1250 $\pm$ 353.55	1520 $\pm$ 419.11
Control***	Non-vaccinated	0.00	0.00

**Notes:** \*Experimental group vaccinated with vaccine product A; \*\*Experimental group vaccinated with vaccine product B; \*\*\*Non-vaccinated group.

**Abbreviation:** SD, standard deviation.

## Ethics Statement

All animal experiment activities, such as the collection of sera from chickens and immune protection tests of commercial vaccines, were carried out according to protocols approved by the Institutional Review Board of Addis Ababa University College of Veterinary Medicine and Agriculture (VM/ERC/01/13/12/2020) and performed following approved poultry care guidelines<sup>20</sup> and the Revised Guideline for Animal Research Ethics of the Addis Ababa University.<sup>21</sup>

## Results

### Antibody Measurement

Maternal antibody titers from the serum samples of chicks showed a wide variation. It ranges from 5040 to 11,300 and the average MDA of 1-day-old chickens was  $6570 \pm 2368.48$ . Antibody titers against IBDV in broilers vaccinated with product A and product B vaccines showed significant differences between ( $p < 0.05$ ) group 1 and group 2 on day 24. There is no antibody detected at this age in the control group. IBDV antibodies in broilers vaccinated with product A and product B vaccines were significantly different ( $p < 0.05$ ) at age 42 days. The coefficient of variation (CV) of group one vaccinated with product A vaccine and group two vaccinated with product B vaccine was 14.39% and 27.57%, respectively, at 42nd days of age. This CV result of both groups indicates an excellent response to vaccination ( $CV < 30\%$ ). As the CV of group 1 was less than the CV of group 2, broilers vaccinated with product A vaccine expressed more response to vaccination (Table 1).

### Differential Leukocyte Count (DLC)

An experimental trial for comparing the immunogenicity of vaccines in broilers was conducted to evaluate the haematological changes. DLC was recorded from six broilers of a day-old age and in all experimental groups on 24th days of age from ten representative broilers for each group and a comparison of these values among different groups using linear regression was summarized (Table 2).

The mean total DLC of heterophils, eosinophils, basophils, monocytes and lymphocytes were recorded and analyzed at the age of 24 days in all experimental groups. The statistical analysis of lymphocytes showed a highly significant ( $p < 0.01$ ) increase in group 1 and group 2 in comparison to the control group (Table 2). The mean percentage of basophil,

**Table 2** The Linear Regression Analysis of Differential Leukocyte Count Values in Different Groups with Different Days of Age

Group	Age (Day)	Heterophils (%) Mean $\pm$ SD	Eosinophils (%) Mean $\pm$ SD	Basophils (%) Mean $\pm$ SD	Monocytes (%) Mean $\pm$ SD	Lymphocytes (%) Mean $\pm$ SD
Baseline	01	41.5 $\pm$ 1.87	2.67 $\pm$ 0.81	1.0 $\pm$ 0.00	3.67 $\pm$ 0.81	51.17 $\pm$ 2.48
Group 1*	24	15.9 $\pm$ 1.66	4.9 $\pm$ 0.99	1.3 $\pm$ 0.48	7.4 $\pm$ 1.35	70.5 $\pm$ 2.22
Group 2**	24	19.1 $\pm$ 2.47	7.2 $\pm$ 1.99	1.2 $\pm$ 0.63	8.3 $\pm$ 2.41	64.2 $\pm$ 3.39
Control***	24	28.5 $\pm$ 2.07	9.9 $\pm$ 1.37	1.2 $\pm$ 0.63	6.5 $\pm$ 1.58	53.9 $\pm$ 1.97

**Notes:** \*Experimental group vaccinated with vaccine product A; \*\*Experimental group vaccinated with vaccine product B; \*\*\*Non-vaccinated group.

**Abbreviation:** SD, standard deviation.

eosinophil and monocyte in group 1, group 2 and group 3 revealed a significant increase ( $p < 0.05$ ), but heterophil values revealed a significant decrease ( $p < 0.05$ ) in group 1, group 2 and group 3 on 24 days of age (Table 2).

A highly significant increase in the mean lymphocyte count was observed with vaccinated groups in comparison with the control group. This indicates the response of the immune system of broilers to a vaccine.

## Discussion

The timing of broiler vaccination depends on the level of MDA present in the chicks.<sup>4</sup> The efficacy of the IBD vaccination is related to the level of MDA in the chickens. The MDA of chickens can hinder the virus in vaccines infected to the target cells and also reduce the ability of viruses in vaccines to stimulate the chicken's immune system.<sup>22,23</sup> Vaccination against IBD in the presence of MDA is not recommended as high levels of MDA at the time of vaccination will neutralize the vaccine virus.<sup>4,22,24</sup> This may be the case for some vaccination failures.

In the current study, the baseline antibody titer which was identified from serum sampled from day-old chickens before vaccination was found to be protective. This is because the antibody that comes from the mother is protective until the age day 18.<sup>22</sup> The MDA prevents IBDV infection in chickens until the age of 4 weeks.<sup>25</sup> Kreider et al, 1991 divided the ELISA titer of the MDA of chickens into 3 levels: the low level ( $<3000$ ), intermediate level (3000–5000), and high level ( $>6000$ ).<sup>26</sup> In our experiment, the average MDA of day-old chickens was  $6570 \pm 2368$ , which was considered high level in the previous study.

The decay pattern of IBDV-specific MDA proved to be more complex, as it depends largely on initial antibody levels, which may vary from chicken to chicken, making it difficult to predict the optimal time for vaccination.<sup>27</sup> The previous study revealed a 73.6% maternal antibody transfer rate.<sup>28</sup> Researchers confirmed that the level of MDA of chicks was below the level of protection between 15–20 days<sup>29</sup> and 15 days<sup>30</sup> and 4–7 days<sup>31</sup> after hatching. However, the use of different types of vaccine and vaccination schedules affects the protection of MDA.<sup>29</sup>

The lower baseline antibody titer clearly shows that the level of MDA was minimal. The lower mean antibody titer following the primary vaccination could be due to the younger age of chicken and the low antibody level at baseline. In younger chickens, the immune function is not well developed.<sup>32</sup> After the booster vaccination was given, chicken in both treatment groups had a significantly higher mean antibody titer. However, the mean antibody titer was shown to be different among the two treatment groups and the difference observed was statistically significant. At the age of 24 and 42 days, broilers vaccinated with product A vaccine at 7th and 19th days and with product B vaccine at 15th and 22nd days showed significant differences in IBDV antibody titers ( $p < 0.05$ ).

It has been clearly shown that in this study the antibody titer of both groups differs significantly. Furthermore, it has been observed that two doses, one at day 7 and the other at day 19 of their age of broilers, vaccinated with product A vaccine induce protective antibodies against IBDV. Although the product A vaccine is also sensitive to neutralization by MDA, this vaccine may be administered at 7 days of age to protect a chick that may not have a sufficient level of MDA.

Antibody titration of product A vaccine in which the booster dose is given after 12 days of initial dose showed significant differences compared to product B vaccine, which is boosted after 7 days of the initial dose. This result is in line with the study that revealed the booster dose was required after 10–20 days of the initial dose.<sup>33</sup> The initial dose is required for the priming of the vaccine while the booster is required for maximum protection against the antigen. The lack of booster dose results in low antibody titers, resulting in vaccine failure. Furthermore, all chicks were found healthy, and no mortality and morbidity were seen for all groups.

In this study, the DLC was found to differ significantly among the treatment groups. Leukocytes have been known to involve in defending animals against various infections. Lymphocytes and monocytes are particularly important cells in defending the body systems against viral infections.<sup>32</sup> The increasing counts of lymphocytes and monocytes in response to vaccination are good evidence for improvement in the immune response of chicken as a result of vaccination. Lymphocytes were known to play a central role in regulating immune response, antibody production, and in effecting cell-mediated immune response<sup>34</sup> while monocytes were known for their role in presenting antigens to lymphocytes and for their final effector function in clearing intracellular infections.<sup>35</sup> Therefore, the higher lymphocytes and monocytes

count in chicken vaccinated with product A and product B vaccines are in line with the observation of higher antibody titer and higher weights of lymphoid organs.<sup>36</sup>

A highly significant increase in the mean lymphocyte count was observed with vaccinated groups in comparison with control the group. This indicates the response of the immune system of broilers to vaccines. The higher the lymphocyte number, the more the vaccine was immunogenic. From this result, the product A vaccine was more immunogenic than the product B vaccine, as the mean lymphocyte of group 1 was greater than group 2.

Evaluation of IBDV-vaccine efficacy is difficult in commercial broilers.<sup>15</sup> In Ethiopia, although IBD outbreaks are reported from both broilers and layers farms, efficacy testing is not a well-explored area of science, and therefore still much has to be done to protect the chicken from a disease outbreak. The present study was the first of its kind in Ethiopia and was conducted in experimental settings to compare the immunogenicity of available and commonly used IBD vaccines. The majority of poultry veterinarians and chicken producers in Bishoftu town claimed for good protection of the chicken flock from IBD while using product A (product of Ceva Sante Animale, France). Our findings may provide valuable information to researchers, poultry veterinarians and producers in devising the time of administration of the vaccines for the prevention of Gumboro disease in Ethiopia.

There are two major limitations in this study that could be addressed in future research. First, the study focused only on the immunological evaluation of two IBDV vaccines available in the Ethiopian market and commonly used in broilers were used. Second, the efficacy of the two vaccines was not evaluated using a challenge test. It was majorly due to the ethical issues associated biosecurity level of the experimental chicken houses as well as lack of access to a well-known virulent strain of the virus. Hence, detailed studies on efficacy testing by challenging the chickens with virulent strains of the virus would suggest the overall protection level of the vaccines.

## Conclusion

The results of the present study indicated that both vaccines induce an adequate positive immunological response at the end of the experiment. Moreover, broilers vaccinated with imported product A vaccine (Ceva Sante, France) showed a better immune response to the IBDV vaccination program than locally produced product B, suggesting the need for a booster dose after 10–20 days of the initial dose. However, a detailed scientific investigation on the vaccination schedule should be done as the vaccination schedule was followed as per the manufacturer's recommendations. Further detailed similar studies in layers should also be conducted.

## Abbreviations

CV, coefficient of variation; DLC, differential leukocyte count; ELISA, enzyme-linked immunosorbent assay; IBD, infectious bursal disease; IBDV, infectious bursal disease virus; MDA, maternally derived antibody; NVI, National Veterinary Institute.

## Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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## Disclosure

The authors declare no conflicts of interest for this work. The funder had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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