

## A field study on the evaluation of application of MB-1® live attenuated vaccine in broilers

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### ABSTRACT

The research aimed to evaluate the application of MB-1 vaccine at the hatchery to prevent Gumboro disease in commercial broilers. One hundred serum samples were randomly collected from commercial white broilers to examine infectious bursal disease virus (IBDV) and Newcastle disease virus (NDV) antibody titers. The surveyed chickens exhibited an average maternally derived antibody (MDA) level of  $3,258 \pm 1,551$  (titers). At 36 and 42 days of age, IBDV antibody titers exceeded the protection level, with very high uniformity:  $4,217 \pm 911$  and  $4,413 \pm 1,153$  (titers), respectively. In addition, the humoral immune response to the ND vaccine is good with antibody titers of  $3,180 \pm 2,103$  at 42 days of age. IBDV of MB-1 vaccine was detected in bursa from 15 days of age and persisted until the last sample was taken at 36 days of age. The bursa lesion scores (BLS) were at normal ranges, gradually increasing from 1 to 2.8 between 18 and 36 days of age. The bursa index (BI) was highest when the chicken was at 15 days old (0.23%) and decreased to the lowest (0.06%) when the chicken was at 36 days old. In summary, the use of MB-1 vaccine in the hatchery has shown early localization of the MB-1 vaccine virus in the bursa, provided a strong immune response to IBDV without affecting the humoral immune response to the ND killed vaccine, and was safe for the bursa when applied to commercial broilers at the hatchery.

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### 1. Introduction

Infectious bursal disease (IBD) is an acute infectious disease that is highly contagious and causes great economic loss to the poultry industry. Every year, the broiler industry loses

3.9 thousand tons of meat due to the effects of variant IBDV strains (Zachar et al., 2016). The causative agent is the infectious bursal disease virus, a virus belonging to the Birnaviridae family and non-envelope (Dobos et al., 1979; Muller et al., 1979). Initially, IBDV multiplies in

macrophages in the lymph nodes, then the virus attacks the lymph nodes in the bursal, leading to the bursal becoming inflamed, and shrunk, causing immunodeficiency, and therefore making chickens susceptible to secondary infections (Dey et al., 2019).

Vaccination is the best way to control IBDV. Many new vaccine technologies have been researched and brought to the market to increase the effectiveness of IBD prevention including IBD hatchery vaccines (live attenuated, immune complex, and recombinant vaccines (Muller et al., 2012), and on-farm vaccines (live attenuated and inactivated IBD vaccines) (de Wit et al., 2021). Live vaccines have been proven to be a powerful tool in controlling Gumboro disease (Muller et al., 1979; van den Berg et al., 2000; Eterradossi & Saif, 2013). However, the problem encountered when using live vaccines is the interference of MDA (Dey et al., 2019).

In particular, an application of hatchery vaccine is easier to implement, increases standardization, and ensures animal welfare compared to forms of vaccination at the farm (Franzo et al. 2020). According to Phibro Animal Health, MB-1 vaccine contained a naked live Gumboro disease virus with the M.B. strain adapted for in-ovo or subcutaneous injection at the hatchery. The mode of action of MB-1 vaccine is maternal antibodies dependent. According to Wein et al. (2023), the ability of MB-1 virus vaccine to survive within macrophages ensures its survival during chicks with effective MDA protection. After MB-1 vaccination, the vaccine virus was carried by skin resident macrophages and then migrated via the bloodstream to invade the spleen. In addition, MB-1 vaccine virus infects and survives splenic macrophages. This mode of action allows MB-1 vaccine virus to spread, invade, and replicate in Bursa. It provides

a clear explanation of the ability of the MB-1 virus vaccine to survive in maternally immune chicks and replicate properly to allow efficient, early, and continuous immunization against Gumboro disease.

The vaccine virus release and replication in the bursa is individually adjusted to the MDA level and MB-1 vaccine has been proven safe when applied to chicks. The MB-1 vaccine ensures that chicks have been vaccinated at the right time and induced full protection against a vvIBDV for commercial broilers (de Wit et al., 2021). According to Ray et al. (2021), a hatchery vaccine named MB-1 was safety, early onset of immunity, and applied convenience in a hatchery.

Within the past five years, Gumboro field studies have been conducted by Ashash et al. (2019) in Latin, Asia, and Asia. The US, Africa, and Israel have proven to believe that the IBDV M.B. strain from the IBD hatchery vaccine named MB-1 vaccine also gave a good immune response and did not affect productivity indicators. In India, Ray et al. (2021) suggested that the use of live attenuated IBDV vaccine M.B. strain on chicks in the hatchery is effective even when the chickens have high levels of MDA. At the same time, the study also showed that when using the M.B. strain vaccine, chickens at the hatchery had lower bursal lesion scores and a high productivity index compared to using an immune complex vaccine. de Wit et al. (2021) demonstrated that the use of M.B. vaccine strain in commercial broiler hatcheries, it has been successful and has full protection against vvIBDV (very virulent infectious bursal disease virus) strains at 22 and 36 days of age. In Vietnam, the MB-1 application of the hatchery Gumboro vaccine showed that the virus was located early in the bursa, and IBDV antibodies formed and developed quickly and evenly; in particular, it

did not affect the humoral immune response of chickens to Newcastle disease vaccine when applied to Luong Phuong broilers (Nguyen et al., 2022a), Isa Brown layers (Nguyen et al., 2022b) and Ac chicken (Nguyen et al., 2023).

This study aims to evaluate the effectiveness of the immune response of commercial white broilers to the MB-1 vaccine. This is a breed that often has very high levels of MDA (thanks to the work of making inactivated Gumboro vaccine for breeders). Therefore, we proposed improvements and changes in IBD vaccination methods in white commercial broilers to contribute to the development of the overall health of poultry.

**Table 1.** Vaccine program

| Day of age | Vaccine               | Brand          |
|------------|-----------------------|----------------|
| 1          | MB-1/ - 0.1 mL        | Phibro         |
|            | ND-killed - SC/0.1 mL | MSD            |
|            | ND-IB live/Spray      | Zoetis         |
| 10 - 15    | ND-IB-H9-killed       | No information |
| 22         | ND-IB live/DW         | MSD            |

## 2.2. Gumboro and Newcastle antibody titers

Chickens were sampled to measure IBDV antibody titers to evaluate development of IBD immune response after MB-1 vaccination. One of the concerns when applying live attenuated IBD vaccines is the risk of immunodeficiency meaning that live virus vaccine affects to humoral immune response to other vaccine antigens, such as ND-killed vaccine. So that was the reason why we measured NDV antibody titers in this study. We randomly selected 20 chickens to take wing vein blood at five-time points at 1, 28, 32, 36 and 42 days after MB-1 vaccination. Blood samples were allowed to clot naturally, then stored at a temperature of 2°C - 8°C on farm and then it was sent to the laboratory to test. A total of 100

## 2. Materials and Methods

### 2.1. Experimental design

The study was conducted with a total of 15,000 one-day-old Ross 308 breed chicks vaccinated with the Gumboro MB-1 vaccine and ND-killed vaccine subcutaneously in the same hatchery. Other vaccines on the farm were applied according to the farm's vaccination program (Table 1). All chickens are raised in cold houses in Binh Duong province from August to October in 2023. Farm management followed the instructions of the Emivest breeding company with a good uniform about feeding, drinking water, temperature, humidity, and ventilation.

samples were taken for ELISA testing according to the IDEXX's protocol instructions of IDEXX IBD Ab test and IDEXX ND Ab test.

### 2.3. Bursal index (BI)

The BI value was calculated to determine the relative increase or decrease in weight of the bursa to the chicken's body weight. In the experiment, 6 chickens were randomly selected at 15, 18, 21, 28 and 36 days of age to weigh, dissect and cut the bursa according to the method described by Cazaban et al. (2015). A total of 30 bursa samples were taken for the survey. The BI ratio was calculated according to the following formula:

$$BI = \text{Bursa weight (g)} \times 100 / \text{body weight (g)}$$
 (Olesen et al., 2018).

#### 2.4. Infectious Bursal Disease identification by RT-PCR and Nucleotide sequence analysis

The bursa samples from the assigned birds were smeared directly into circles of FTA cards on 15, 18, 21, 24 and 36 days of age. Total 6 bursas were smeared each FTA cards per time. They were sent to Clinic for Poultry and Fish Medicine, Department for Farm Animals and Public Health in Veterinarian (Vetmeduni Vienna) for PCR amplification and amplicon sequence analysis of pooled samples. RNA extraction of all samples was performed using QIAamp cadon Pathogen Kit (Qiagen, Hilden, Germany). Initially, all RNA samples were investigated by the RT-PCR for the presence of IBDV RNA, using primers and probes for the VP3 gene in the A segment of the IBDV genome (Escaffre et al., 2010 family Birnaviridae, genus Avibirnavirus; Ashash et al., 2019).

IBDV-positive samples were further subjected in-house RT-PCR to amplify 750 bp region of the VP2 gene. Positive PCR products were excised from the gel and purified with the MinElute Gel Extraction Kit (Qiagen, Valencia, CA). The purified PCR products were submitted for Sanger sequencing using the BigDye Terminator v3.1 Cycle sequencing kit (ThermoFisher Scientific, Waltham, MA) and run on a 3500 Genetic Analyzer (ThermoFisher Scientific). Raw sequence data were analyzed with the Lasergene/EditSeq application, and sequence consensus construction was produced with the

SeqMan application (DNASTAR, 2006). The IBDV strain differentiation analyses utilized the GenBank database and were conducted with the Molecular Evolutionary Genetic Analysis (MEGA) software (Kumar et al., 2004).

#### 2.5. Bursal lesion score (BLS)

The bursa from the assigned birds was removed at 18, 21, 28 and 36 days of age. A total of 24 bursa tissues were fixed in 10% neutral buffered formalin. All samples were sent to the Vet Hospital of Nong Lam University. Based on a level of affected bursa, Muskett et al. (1979) scored in the range of 0 to 5: (0) normal bursa; (1) 1 - 25% of the follicles show lymphoid depletion; (2) 26 - 50% of the follicles show nearly complete lymphoid depletion; (3) 51 - 75% of the follicles show lymphoid depletion; affected follicles show necrosis and severe influx of heterophils is detected; (4) 76 - 100% of the follicles show nearly complete lymphoid depletion, hyperplasia and cyst structure are detected; (5) 100% follicles show nearly complete lymphoid depletion; complete loss of follicular structure, thickened and folded epithelium, and fibrosis of bursal tissue. The average bursal lesion score was calculated by dividing the total of bursal lesion score by the number of bursa samples (Olesen et al., 2018) the histological bursal lesion score (HBLS). A summary of the research parameters is presented in Table 2.

**Table 2.** Research parameters

| Parameters          | Day of samples (days of age) | Number of samples/times | Total samples |
|---------------------|------------------------------|-------------------------|---------------|
| IBDV/NDV ELISA      | 1, 28, 32, 36, 42            | 20                      | 100           |
| Busa Index          | 15, 18, 21, 28, 36           | 6                       | 30            |
| RT-PCR              | 15, 18, 21, 24, 36           | 6                       | 30            |
| Bursal lesion score | 18, 21, 28, 36               | 6                       | 24            |

## 2.6. Statistical analysis

The data were processed and calculated by using Microsoft Excel 2019. The data were presented as averages of the parameters.

## 3. Results and Discussion

### 3.1. RT-PCR and sequencing

The RT-PCR and gene sequencing were used to determine the presence of vaccine virus in the chicken's bursa. At the age of 15 and 18 days old, RT-PCR and sequencing results from the bursa showed positive results for IBDV and distinguished the M.B. vaccine virus strain.

In the following sampling times, when the chickens were at 21, 24 and 36 days old, all FTA cards showed positive IBDV results and identified 100% of the virus as belonging to the M.B. strain of vaccine (Table 3). Furthermore, if it was a virus strain different from the vaccine strain, it would have been present a few days later, but the strain identification results only showed the presence of the M.B. vaccine strain, not the presence of the environmental strain. Thus, from 15 days of age, the vaccine virus had located in the bursa and could stimulate B lymphocytes to produce antibodies against IBDV. According to Ashash et al. (2019), from 33% to 100% of the genome of M.B. strain was detected in the bursa of 24-day-old chickens and by 28 days of age, this rate increased from 83% to 100%. Therefore, this study showed that the vaccine virus located in the bursa earlier, when the chickens were 15 days old.

In another experiment on MB-1 vaccine, Ashash et al. (2019) detected 33% - 100% of the MB-1 vaccine virus genome in all bursa samples of chickens vaccinated with MB-1 at the time

of sampling at 24 days of age. In addition, in an experiment comparing the effectiveness of two chicken flocks using MB-1 vaccine with immune complex 228E (Icx), Ray et al. (2021) showed that vaccine virus 228E was detected as early as 17 days of age after Icx injection but disappeared at 21 days of age and recorded that MB-1 virus appeared at 24 days of age and at later times on the animals. Ray et al. (2021) also proved that MB-1 strain's ability to spread and occupy space in the bursa was very good and stronger than 228E, so it can replace 228E when the distance between the house using MB-1 vaccine is too close to the house using 228E vaccine. On the other hand, MB-1 contained free-living viral particles but was not completely neutralized by MDA (Ashash et al., 2019) and its replication in the bursa was not detected before 21 days of age (depends on MDA), which might allow the full differentiation and maturation of bursa-derived lymphocytes (Ray et al., 2021).

**Table 3.** RT-PCR and sequencing

| Days of age | RT-PCR   | Sequencing  |
|-------------|----------|-------------|
| 15          | Positive | M.B. Strain |
| 18          | Positive | M.B. Strain |
| 21          | Positive | M.B. Strain |
| 24          | Positive | M.B. Strain |
| 36          | Positive | M.B. Strain |

### 3.2. Bursal index and Bursal lesion scores

Bursal index and Bursal lesion scores are used as indicators of body health and immune response. One of the clinically based features to know the localization of vaccine virus is that the bursa size becomes smaller (Etteradossi & Saif, 2013). The cause may be that when the IBD virus replicates in B lymphocytes that are in the differentiation stage and lasts about 7 to 10 days, it may reduce the number of B lymphocytes in

the cortex and medulla causing the reduction in bursa size (Sharma et al., 2000). At 15 and 21 days old, BI was at a high value of 0.17 - 0.20, respectively, then it gradually decreased to 0.13 at 28 days old. The lowest BI (0.06) was found at 36 days old (Table 4). The BI values gradually decreased from 18 to 36 days of age, indicating normal development and maturation of the bursa, consistent with the physiological changes of the bursa as the chicks grew up. The images of bursal lesion score are shown in Figure 1.

**Table 4.** Bursa index (BI) and bursal lesion scores (BLS)

| Days of age | BI   | BLS  |
|-------------|------|------|
| 15          | 0.17 |      |
| 18          | 0.20 | 1.50 |
| 21          | 0.17 | 1.00 |
| 28          | 0.13 | 2.17 |
| 36          | 0.06 | 2.80 |



**Figure 1.** Bursal lesion scores range from 0 to 5 based on the level of affected bursa.

Bursal lesion scores increased from 1.0 when the chicken was 18 days old and increased to 2.8 when the chicken was 36 days old. The cause may be that IBDV replicated in B lymphocytes that were in the differentiation stage and survive for about 7 to 10 days. The virus may have reduced the number of B lymphocytes in the cortex and medulla, causing the bursa to decrease in size (Sharma et al., 2000). According to Ashash et al. (2019), the BLS - after MB-1 vaccination were lower than those reported for other vaccines, suggesting a milder impact on the bursa. According to Mazariegos et al. (1990), a BLS result greater than or equal to 4 in chickens vaccinated with intermediate-virulence IBD vaccine is considered above the standard of vaccination and indicates exposure of the chickens to the virus in the field. The results of this experiment showed that BLS had gradually increased since the time the vaccine virus was located and was at an average level and was all less than 4 points. In an experiment in India, it had been shown that chickens using MB-1 had lower BLS than immune complex vaccines and live vaccines of the M.B strain. normally (Ray et al., 2021). Because the experimental conditions did not extend the sampling time, it was not possible to monitor at later stages to assess recovery via BLS. However, the recovery ability of the bursa through BLS was recorded and reported on Luong Phuong broilers (Nguyen et al., 2022a), Isa Brown layers (Nguyen et al., 2022b).

The effectiveness of the MB-1 vaccine in this study were consistent with previous studies conducted in different regions. Some field studies on MB-1 in Latin America, Africa, and Israel had been shown to be safe and effective in commercial broiler chickens having varying MDA (Ashash et al., 2019). According to Ashash et al. (2019) the mode of action of MB-1, they have found that

MDA mediates the timing of replication, and it is related to the MDA titers of the individual bird.. In Vietnam, the trial of using MB-1 for one-day-old Isa Brown chicks at the hatchery provided the early localization of vaccine virus (21 days old), rapid and uniform development active IBD antibodies; it also did not affect to the immune response of ND vaccination and was safe for the bursa (Nguyen et al., 2022b). In another trial in Luong Phuong broiler, MB-1 vaccination also provided good results for immunity response (Nguyen et al., 2022a). According to Nguyen et al. (2023), Ac chicken had IBD titers at 28 days of age so high with the average titer of 6218. The positive results observed in the immune response and production performance of broilers vaccinated with MB-1 highlight its potential for improving the control and prevention of IBD.

### **3.3. Humoral immune response**

#### **3.3.1. IBD humoral response**

Maternal derived antibodies are key to protecting chicks against IBD during the first weeks. The MDA helps B lymphocytes have time to increase in number, mature and can protect chicks for 2 - 3 weeks of age (de Wit et al., 2001). At 1 day old, the average MDA of the surveyed chickens reached  $3,258 \pm 1,551$  (titer). According to Kreider et al. (1991), Antibody titers are divided into 3 levels: low level ( $< 3,000$ ), medium level ( $3,000 - 5,000$ ) and high level ( $> 5,000$ ). Thus, the MDA of the surveyed chickens was at an average level and had a good coefficient of variation CV% of 47.61% (Legese et al., 2022). After vaccination with MB-1 vaccine, the time of vaccine virus release to the Bursa will depend on the level of MDA of each chicken. In chicks with low MDA titers the vaccine virus will replicate earlier in comparison to chicks with higher MDA titers, thus onset of immunity

and onset of antibody titer will also be earlier in chicks with low MDA titers (Wein et al., 2023). Therefore, this mode of operation ensures that each chicken is vaccinated at the right time (Ray et al., 2021) and shortens the immune gap when MDA falls below the protective level and active immunity has not yet increased to level of protection (Le Gros et al., 2009). The results of the study showed that the IBD virus was localized from 15 days old and 100% of the M.B. was detected through genetic sequencing at 21 days of age and there was absolutely no existence of field virus in samples taken throughout the sampling process. Therefore, environmental viruses do not have favorable conditions to attack the bursa since the time of MB-1 localization, helping chickens to be partially protected by this positioning mechanism. Furthermore, vaccine virus strain M.B. had the ability to respond quickly and strongly (Quach et al., 2018) so it could shorten the immune gap susceptible to IBDV (Berg et al., 2000).

IBDV antibody titers are an important part of Gumboro's protection mechanism. The average antibody results by age are shown in Table 5 and Figure 2. At 28 days old, the average IBDV antibody titers reached  $238 \pm 660$  (titers), with a very high coefficient of variation of 277.21%. MDA at this time decreased sharply, this showed that the IBDV of MB-1 vaccine had multiplied in the bursa and stimulated active B lymphocytes to create antibodies. The humoral immune response to the MB-1 vaccine was very strong, and elevated IBDV antibody titers was observed at 32, 36 and 42 days of age. At 32 days of age, the average IBDV antibody titers had increased and reached an antibody level of  $1,854 \pm 1,144$  (titer), the coefficient of variation CV% was at a fairly

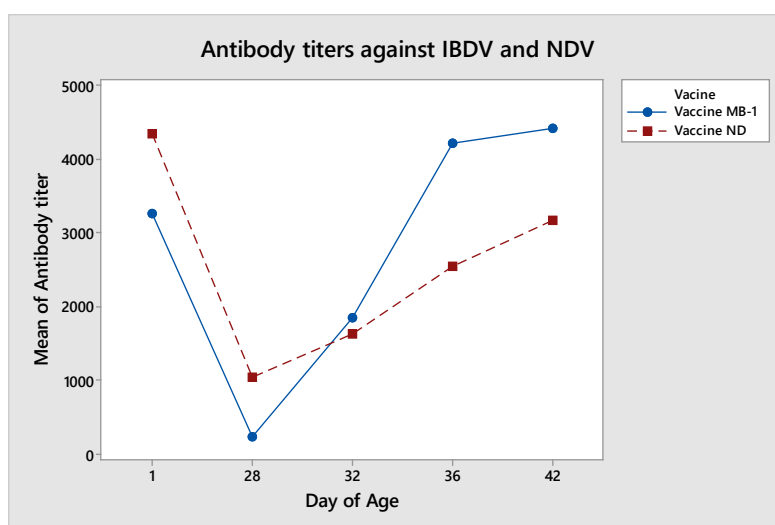
high level of 61.70%. Similarly, at 36 and 42 days old stages, an average IBDV antibody titers had  $4,217 \pm 911$  and  $4,413 \pm 1,153$  (titer), respectively. According to IDEXX, the average baseline for IBD resistance is recommended at 1,000 (titer). Thus, at 36 and 42 days of age, IBDV antibody titers reached a level above the protection level, with very high IBDV antibody titer uniformity with CV% at 21.6% and 26.13%, respectively. Thanks to the uniformity of the immune response between each individual in the herd, it will help minimize the possibility of spreading IBDV virus into the environment (Quach et al., 2018), further supporting the immune response process when using MB vaccine.

### 3.3.2. NDV antibodies titers

According to van den Berg et al. (2000) when using vaccines to prevent IBD, one of the concerns is the risk of the virus negatively affecting B lymphocytes, which are responsible for humoral immune response of poultry (ND-killed used frequently for broiler chickens). Therefore, the study conducted a NDV antibody titers when ND-killed vaccination to evaluate the humoral immune response of chickens. Chickens have a good immune response to the NDV antibody titers vaccine that gradually increases at the stage of chickens from 28 to 42 days of age, more uniformly at 42 days of age (Table 5, Figure 2). At 32 days old, the chicken's NDV antibody titers were  $1,622 \pm 926$  (titer) with a CV of 57.06% and increased at later days of age. At 36 and 42 days old, NDV antibody titers were  $2,550 \pm 2,338$  and  $3,180 \pm 2,103$  (titer). Thus, chickens still respond humorally to inactivated ND vaccine when injected on the same day as MB-1 vaccine.

**Table 5.** Infectious bursal disease virus (IBDV) and Newcastle disease virus (NDV) antibody titers

| Date sample | Statistical parameters | IBDV antibody titers | NDV antibody titers |
|-------------|------------------------|----------------------|---------------------|
| 1           | $\pm$ SD               | $3.258 \pm 1.551$    | $4.348 \pm 1.719$   |
|             | CV %                   | 47.61                | 39.54               |
| 28          | $\pm$ SD               | $238 \pm 660$        | $1,046 \pm 999$     |
|             | CV %                   | 277.21               | 95.47               |
| 32          | $\pm$ SD               | $1,854 \pm 1.144$    | $1,622 \pm 926$     |
|             | CV %                   | 61.7                 | 57.06               |
| 36          | $\pm$ SD               | $4,217 \pm 911$      | $2,550 \pm 2.338$   |
|             | CV %                   | 21.6                 | 91.69               |
| 42          | $\pm$ SD               | $4,413 \pm 1,153$    | $3,180 \pm 2,103$   |
|             | CV %                   | 26.13                | 66.14               |

**Figure 2.** Antibody titers against infectious bursal disease virus (IBDV) and Newcastle disease virus (NDV).

#### 4. Conclusions

The application of the MB-1 vaccine at the hatchery for commercial broilers resulted in early localization of the vaccine virus in the bursa, rapid and uniform development of active IBD antibodies. The MB-1 vaccine did not negatively affect the immune response to Newcastle disease vaccination and was safe for the bursa (a good

BI and BLS) when applied to one-day-old chicks. These findings support the use of the MB-1 vaccine as an effective strategy for improving Gumboro disease.

#### Conflict of interest

The authors have no conflicts of interest to declare.

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