EFFICACY OF LIVE ATTENUATED MB-1 VACCINE AGAINST INFECTIOUS BURSAL DISEASE IN ISA BROWN AT FIELD TRIAL

Nguyen K. T. Oanh, Nguyen. M. Ho and Quach T Anh *

Faculty of Animal Science and Veterinary Medicine, Nong Lam University,

Ho Chi Minh City, Vietnam

Email: anh.quachtuyet@hcmuaf.edu.vn

ABSTRACT

The objectives of this study were to evaluate the safety and effectiveness of the attenuated infectious bursal disease (IBD) vaccine named MB-1 in the prevention of Gumboro in layer chickens. Over 50,000 one-day-old Isa Brown chicks were injected with MB-1 vaccine together with commercial Marek's Disease vaccine type 1 and 3 by subcutaneous injection at the hatchery. Elisa test kits were used to detect the antibody titer of IBD and Newcastle Disease (ND) at 1, 14, 18, 21, 24, 28, 32, 35, 42, and 56d. Bursa to body weight ratio (BI), bursal smears on Flinders Technology Associates (FTA) cards for PCR, and bursa lesion scores will be taken from 6 birds at 21, 28, 35, and 42d. The average level of maternal derived antibodies against IBD were 3876. The PCR result showed that MB-1 virus vaccine was located early in the Bursa from 21 and presented til 42d without the presence of environmental viruses or other strains of vaccine viruses. The IBD antibody level rapidly dropped from 1 to 18d; then this level started to rise and reach at 3330 on 28d. Bursa lesion score and BI of chickens receiving the MB-1 vaccine were in the normal range and showed signs of recovery at 32d. The average ND antibody titer was 1411 at 24d and reached a high level at 56d and very uniform with good CV (39.38%) which proved that MB-1 did not interfere with the humoral immune response of other diseases, specifically ND. In summary, the application of MB-1 vaccine for one-day-old layer chicks at the hatchery would provide with early localization of the vaccine virus in the Bursa and rapid and uniform development of active IBD antibodies. The MB-1 vaccine did not affect the immune response of chicks to the ND vaccination and was safe for the Bursa.

Keywords: Antibody titer, attenuated IBD vaccine, Gumboro, localization, MB-1

1. Introduction

For many years, the emergence of antigenic variants and very virulent strains of infectious bursal disease virus (vvIBDV) seriously affect the livestock industry. So it is essential to control IBDV at an early age. But there are a lot of problems with vaccination practices including the timing of vaccination and safety of IBDV vaccines that made the control of IBDV by vaccination more challenging (Eterradossi & Saif, 2013). Too early application may lead to neutralization of the vaccine by maternal derived antibodies (MDA); on the contrary, the birds are faced with delaying the onset of immunity leading to a window of susceptibility when MDA decreases to below protective levels and active immunity has not increased to the level of protection if vaccination is too late (Dey et al., 2019). According to the degree of attenuation, live IBD vaccines are classified as mild, intermediate, intermediate plus and hot IBD vaccines (Müller et al., 2003; Rautenschlein et al., 2005). Mild vaccines have exhibited poor efficacy in the presence of MDA even at low levels. Alternatively, vaccines of higher pathogenicity (intermediate, intermediate plus) may have better efficacy and breakthrough high levels of MDA but they can induce bursa lesions with subsequent immunosuppression leading to other infections (Dey et al., 2019). A mixture of the IBD intermediate plus strain with IBD antibody vaccine can cause a delayed immune response in

vaccinated chicks and may be not prevent from the field IBD virus (Gelb et al., 2016; Ray et al., 2021). Infectious bursal disease vaccines can inject In-ovo or one-day old chick (DOC) at the hatchery which were developed to solve these problems mentioned above.

For that reason, a new generation of hatchery vaccine was developed with the advantage of their overcoming the interference with MDA, safety, early onset of immunity, and applied convenience in a hatchery named MB-1 vaccine (Ray et al., 2021). Vaccine MB-1 contained a naked live virus with a special mechanism to adjust to varying MDAs of individual chicks were ensured of having vaccinated at the right time and induced full protection against a vvIBDV challenge for commercial broilers between 22 and 36d (De -Wit et al., 2021). A series of four independent commercial broiler field trials in four different countries during the years 2016 and 2017 measured the relative safety, IBD immune parameters and production performances of MB-1 versus current IBDV attenuated live and immune complex vaccines in a variety of commercial broiler systems (Ray et al., 2021). These findings supported that the M.B. strain was not only the first conventional live vaccine that could use In-ovo and DOC application but also produced a good immune response, a safe and viable solution against infection with IBDV.

In Viet Nam, there is no published report on this MB-1 vaccine to inject **for layer breed.** Hence, this experiment was conducted to evaluate the efficacy and safety of MB-1 vaccinated Isa Brown layer in the field trial.

2. Materials and methods

2.1. Experiment design

The study was carried out on 50,000 one-day-old Isa Brown chicks from December 2020 to February 2021 in Tien Giang province, Viet Nam. MB-1 vaccine is an infectious bursal disease live attenuated virus vaccine, originated from the M.B. strain, adapted for in-ovo or subcutaneous (SC) injection at the hatchery. Other vaccines in the experiment were applied according to the farm's vaccination programme (Table 1). Feeding, drinking water, ventilation and farming management were according to the standards of this **layer farm.**

Table 1. Layer vaccination program

Age (days)	Vaccines	Applications
1	Marek MB-1	0.2mL/bird by SC injection
1	ND + IB live	Coarse spray
10	IB variant	Eye drop
18	ND + IB live	Eye drop
18	ND killed	0.25mL/bird by SC injection
21	Fowl Pox	Wing web puncture
28	AI killed	0.5 mL/bird by SC injection
35	ILT	Nose Drop
42	Coryza	0.25mL/bird by SC injection
42	ND+IB live	Drinking water
42	ND killed	0.25mL/bird by SC injection

2.2. Serology

On days 1, 14, 18, 21, 24, 28, 32, 35, 42, and 56 of age, more than 20 blood samples per time were taken, let clotting naturally, then stored at 2 – 8^0C and sent to the An Phu Tien laboratory for evaluation the IBD ELISA titers in **layer** vaccinated with MB-1 and evaluation the immunosuppressive effect in MB-1® vaccinated **layer** to induce humoral immune respond by testing the ND antibody titers (IDEXX, USA) according to the manufacturer's instructions.

2.3. Bursa to body weight ratio (BBWR)

At 21, 24, 28, 32, 35, and 42 days of age, six birds were euthanatized and sampled in a method described by Cazaban et al. (2015). Total samples were 36 in this trial, each Bursa was weighed before processing and the BBWR was calculated to determine the relative mass increase or decrease of the Bursa. The ratio was established as follows: weight of Bursa (g) * 100/ body weight (g) (Olesen et al., 2018).

2.4. Infectious Bursal Disease identification by reverse transcription PCR (RT-PCR) and Sequencing

At 21, 24, 28, 32, 35, and 42 days of age after doing BBWR from 6 birds every times, total samples was 36, Bursa were smeared directly on Flinders Technology Associates (FTA) cards. One Bursa was smeared per each card circle. RNA extraction of all samples was performed using QIAamp cador Pathogen Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Then detection of the nucleic acid of infectious bursal disease virus (IBDV) by real-time and conventional PCR based on VP2 gene with sequencing in samples from birds (Pos./neg. cut-off value Cq < 40.0) (Ashash et al., 2019).

2.5. Bursal lesion score (BLS)

The Bursa were removed and weighed at 21, 24, 28, 32, 35, and 42 days of age. 36 bursa tissues were fixed in 10% neutral buffered formalin and all samples were sent to the Nong Lam University lab in Ho Chi Minh City. The samples were done with paraffin embedding procedures and were stained with hematoxylin and eosin (H&E). The Bursa of the trial were microscopically evaluated by the same certified pathologist and scored in the range of 0 for a normal bursa to 5 for a severely affected bursa based on Muskett et al. (1979).

0 = No lesion, normal bursa.

1 = 1 – 25% of the follicles show lymphoid depletion (i.e. less than 50% depletion in 1 affected follicle) influx of heterophils in lesions.

2 = 26 – 50% of the follicles show nearly complete lymphoid depletion (i.e more than 75% depletion in 1 affected follicle), affected follicles show necrosis and severe influx of heterophils may be detected.

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; affected follicles show necrosis and severe influx of heterophils is detected.

5 = 100% follicles show nearly complete lymphoid depletion; complete loss of follicular structure, thickened and folded epithelium, fibrosis of bursal tissue.

The average bursa lesion score was calculated by dividing the total of bursal lesion score by the number of analysis Bursa (Olesen et al., 2018).

2.6. Statistical analysis

The data were processed and calculated by using Microsoft Excel 2010. The data were presented as averages of the parameters.

3. Results

3.1. Serology

The results of the IBDV Elisa Idexx of the trial were summarized in Table 2. Before MB-1 vaccination, the average titer of one day old chicks was 3,876 then the IBD antibody titer levels rapidly dropped from 1 to 18d, were only 203 at 18 days of age. It could be maternal antibodies decreased. After 18d, IBD antibody titer started to rise. At 24 days of age, mean IBD titers was 1730 with 10/22 samples had IBD antibody titers above 1000 (Idexx, 2019). At 28d, the induction of an active immune response were higher than 24d with 100% samples had titers above 1000 Elisa Idexx. At 32, 35, 42, 56 days of age, an active immune response was still developed and was maintained in good uniformity.

Table 2. IBD ELISA titers average and CV%

Day of Age	Mean	CV(%)	SEM	N
1	3,876	55.1	338	40
14	730	92.7	144	22
18	203	77.8	34	22
21	619	165.7	219	22
24	1,730	108.3	415	22
28	3,330	27.7	206	23
32	3,680	27.7	213	23
35	4,023	36.2	297	24
42	5,091	28.7	299	24
56	5,890	35.8	472	20

Table 3. ND ELISA titers average and CV%

Day of Age	Mean	CV%	SEM	N
1	7084	47.90	536	40
14	1207	74.79	192	22
18	352	94.36	71	22
21	575	77.07	95	22
24	1411	197.56	594	22
28	5581	74.00	880	22
32	6497	79.13	1096	22
35	8504	43.93	835	20
42	9101	51.91	1056	20
56	7822	39.38	689	20

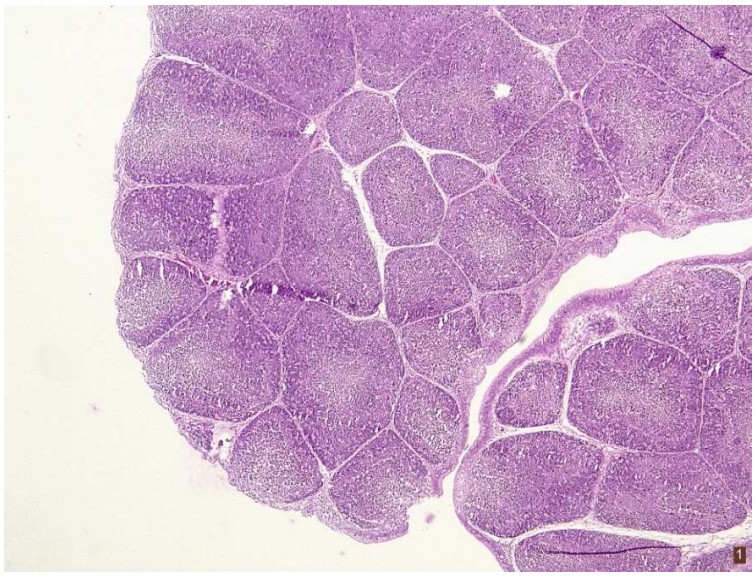
The results in Table 3 showed that layers vaccinated with MB-1 had high and uniform Newcastle titers after MB-1 vaccination. The ND antibody titer gradually increased from day 21 and rated as average level following Idexx Standard. The average of titers was 5581 at 28d and reached 7822 titers at 56d. Therefore, MB-1 vaccine did not affect the humoral immune respond to result in immunosuppression in vaccinated birds (injected ND killed vaccine at 18d).

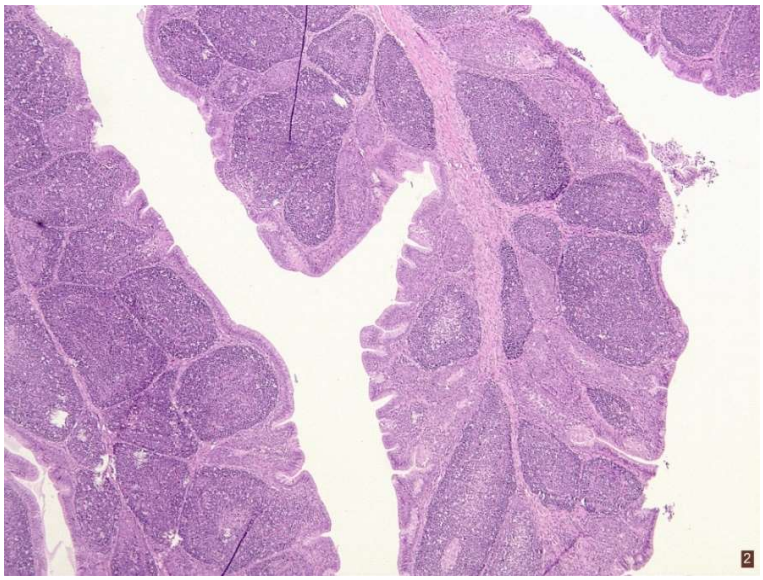
3.2. Bursa to bodyweight ratio (BBWR), IBDV RT-PCR/sequencing and bursa lesion scores

The mean BBWR scores at times were summarized in Table 4. The results ranged from 0.10 - 0.27 with good uniformity. Especially, it had started recovery very early from 32d.

MB-1 vaccine virus strain appeared at 21d and was the only IBD virus strain detected until 35d without the presence of environmental viruses or other strains of vaccine viruses.

At 42d, RT – PCR was positive for IBDV, but cDNA was too low for successful sequencing. The mean BLS scores were normal with signal recovery and ranged from 1.33 - 3.33 at the stage of 21 - 63d (Figure 1).





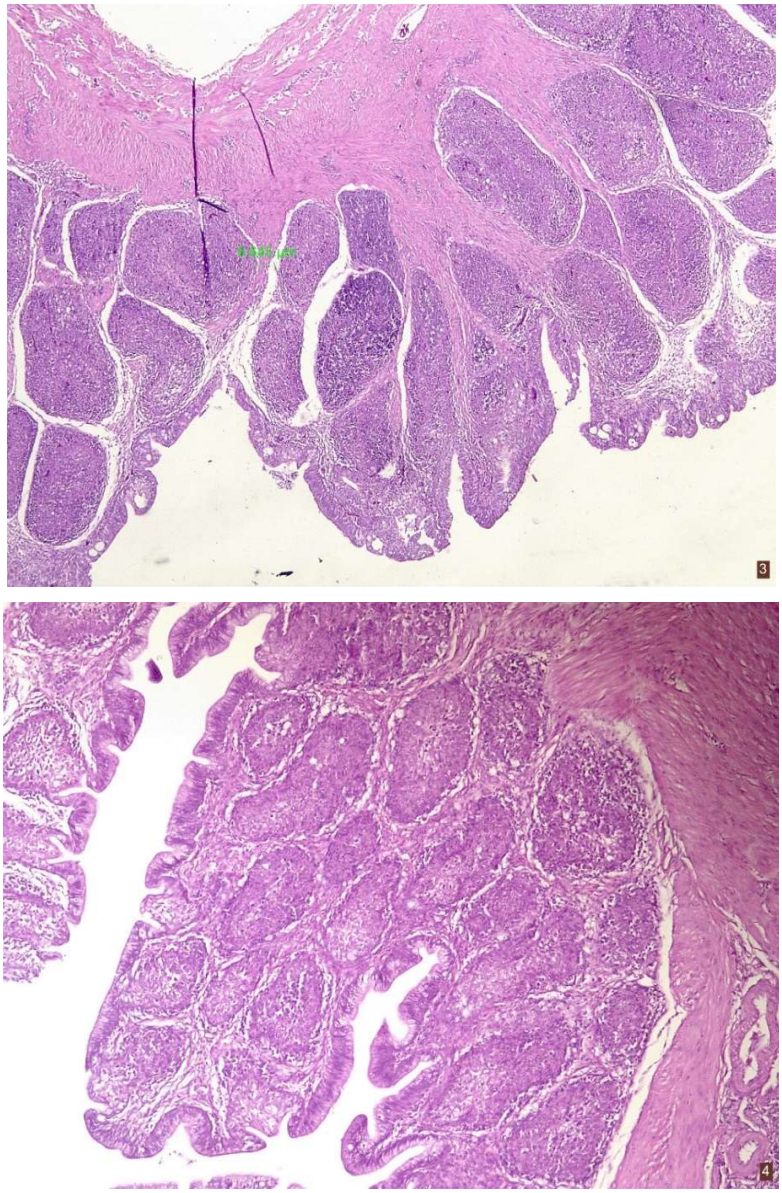


Figure 1. Lesion scores for the bursa ranging from 0 for normal bursae to +4 for severely affected bursae.

4. Discussions

Maternal derived antibodies influence the efficacy of IBD vaccination program. The interference of MDA makes it difficult to choose the right time to apply live attenuated vaccines to chicks against Gumboro disease (Boudaoud et al., 2016). In this study, MDA was 3876 titers (Table 2) and they decreased during the first few weeks of young chicken. The half-life of MDA for egg-laying hens is 5.5 days (De Wit & Deventer, 2001), it takes 14 - 17d to reduce MDA below 800 IDEXX ELISA (the minimum MDA breakthrough of the M.B. virus strain) for about 70% of the flocks. And this is the optimum time to apply attenuated vaccine without neutralizing 70% chicken of these flocks by MDA. When using the MB-1 vaccine, we do not need to know the MDA levels of flocks because MB-1 has been recently developed for hatchery applications that can release vaccine virus to vary MDA of the individual bird (Lazarus et al., 2008; Ray et al., 2021). It helps to ensurechicks vaccinated at the right time and no need of revaccination at the farm.

In bursa where most B lymphocytes are in the actively dividing stage in young chicks and it is also the target organ for viral replication as the sterm cells and peripheral B cells do not support this (Dey et al., 2019). The rate of replication of the vaccine virus in B lymphocytes in Bursa plays a very important role in the protection of IBD (Dey et al., 2019). IBDV can be detected from 14 to 28 days post-vaccination in the Bursa (Iván et al., 2005). In the experiment, the vaccine virus was detected early at 21 days of age in the first sample and persisted until 35 days of age with 100 percent of M.B. strain without environment or other vaccine virus strain (Table 4). After IBD viral replication, cell-mediated immunity responses are considered equally important in the field challenges with rapid infiltration of T cells into the Bursa and upregulation of cell-mediated immunity-related genes infection following very virulent IBD (Dey et al., 2019). Stimulation of specific CD8+ cell-mediated immunity contributes to the response against IBDV (Ingrao et al., 2017) in the early stages following vaccination, despite the detection of low levels of antibodies (Gelb et al., 2016). In addition, the absence of environmental virus strains and only the presence of MB-1 strain through RT - PCR and gene sequencing at 21, 24, 28, 32, and 35d showed that the MB-1 vaccine virus had the ability to locate strongly and did not replace by the environmental virus strain, so since the time of localization of MB-1, the chicks have been partially protected by this immune response.

The Bursa of infected birds presents with inflammation, hemorrhage, or atrophy, depending on the infection period (Chansiripornchai and Sasipreeyajan, 2009). Because VP2 and VP5 proteins of the virus induce apoptosis in B cells and other lymphoid cells (Qin and Zheng, 2017). Histological damage to the BF as a result of vaccination has been demonstrated by others (Cursiefen et al., 1979; Muskett et al., 1979; Winterfield & Thacker, 1978). At 21 days of age, the Bursa has a BBWR of 0.27 and a BLS of 3.00 (Table 4). After that, BBWR tends to decrease until 35d and increase to 56d, BLS increased from 21d and started to decrease in the period 32 – 63d showing signs of recovery. Overall safety indicators related to BBWR and BLS are in the low range without exceeding the level of risk (Mazariegos et al., 1990). Ray et al. (2021) showed numerically lower scores in the MB-1 groups, the Icx vaccine, and the conventional live vaccine, the M.B. group, suggesting lesser stress on birds and quicker recovery.

The induction of humoral immunity is correlated with the IBDV replication while conventional live IBDV vaccine, inflicts transient bursal damage increasing the bursal lesion scores in broiler chickens (Rautenschlein et al., 2005). Based on the results showing the MB-1 vaccine virus was detected in Bursa at 21d (the first day of sampling for IBDV PCR) and the development of IBD antibodies from 21d (619 titers), perhaps the MB-1 vaccine was localized in the Bursa 1 week earlier (De Wit et al., 2021) in those chicks with low MDA. After that, IBD titers gradually increased from 21d to 56d, reaching 1730 titers at 24d and continued to increase rapidly to 3330 titers with CV only 27.7% at 28d (Table 2). The findings of IBDV virus vaccine in birds at 21 days of age and a good CV(27.7%) will minimize the pressure of IBDV field strains in poultry flocks (Quach et al., 2018).

Destruction of B cells and macrophages, and their functions contribute to IBDV-induced immunosuppression (Sharma et al., 2000). This might have been caused by some virus-neutralizing factor (VNF) still binding to the virus in the vaccine resulting in the active immunity not working properly (Chansiripornchai & Sasipreeyajan, 2009). According to Van den Berg et al. (2000), one of the concerns when applying live attenuated IBD vaccines is the risk of immunodeficiency, meaning that the vaccine virus affect the ability to respond to humoral immunity to other vaccine antigens, such as the ND killed vaccine. In this study, ND antibody titers reached at 5581 at 28d, and kept the high antibody level of 7822 at 56d (Table 3). It has been shown that MB-1 does not affect the humoral immune response of the ND killed vaccine.

Conclusions

MB-1 was proven to be safe for in-ovo or one day old subcutaneous administration with the correct balance to stimulate an early onset of immunity. The IBD antibody titer develops rapidly, the early and long-term localization of the vaccine virus confers protection against IBD even in the absence of antibodies. Furthermore, the data demonstrate the lack of adverse effects and does not affect the immune response to ND killed vaccine. Therefore, the MB-1 vaccine is highly efficacious and safe for layers birds.

Conflict of Interest Declaration

We guarantee that the article is done by the author's team and there are no conflicts between the authors.

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