Assessment of the immunity gap of two vaccination programs against Gumboro disease in Luong Phuong chickens

Lan T. H. Huynh, Truc L. T. Nguyen, Ho M. Nguyen, & Anh T. Quach*
Faculty of Animal Science and Veterinary Medicine, Nong Lam University, Ho Chi Minh City, Vietnam

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***Corresponding author**

Quach Tuyet Anh
Email:
anh.quachtuyet@hcmuaf.edu.vn

ABSTRACT

Maternal-derived antibody (MDA) is the priority protection against environmental Infectious Bursal Disease Virus (IBDV) in the first weeks. The passive immunity decreases, but the active immunity is not enough to protect chicks, so shortening the high-risk period is crucial to IBD control. The objective of this study was to evaluate the immunity gap between 2 vaccination programs against infectious bursal disease (IBD) in Luong Phuong chickens. A total of 34,600 chicks were administered by subcutaneous injection of IBD vaccine at 0.1 mL/dose at the hatchery. At 12 days old, 18,000 chicks were vaccinated with the M.B strain vaccine and 16,600 chicks were vaccinated with the 228E strain vaccine by drinking water. The IBD and Newcastle disease (ND) antibody evaluations were based on the Enzyme-linked immunosorbent assay (ELISA) technique. Parameters were recorded until slaughter including body weight, average daily gain, feed conversion rate, and mortality. The IBD MDA at 1 day old was medium and uniform (3809 and 45.3%), which could protect against IBD virus from 1 to 2 weeks old. At 28 days old, the IBD antibody titer of the MB vaccine was higher than that of the 228E vaccine, various proportions of samples in the M.B group exceeding 1,000 titers (40% vs. 0%), and it was a statistically significant difference (1,133 vs. 161) ($P < 0.01$). Besides, the M.B vaccine created a faster and stronger immune response than the 228E vaccine, shortening the immune gap and protecting chicks earlier. The humoral immune response to the ND vaccine was good, with no difference between 2 groups, which proved that the M.B virus did not cause immunosuppression. The production parameters of chickens between the 2 groups were the same. In summary, the M.B vaccine made a short immune gap and did not cause immunodeficiency in chickens.

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1. Introduce

The poultry industry is facing many serious challenges, including Gumboro disease caused by Infectious Bursal Disease Virus (IBDV) which is the aetiological agent of an acute, highly contagious, and immunosuppressive disease particularly affecting chicks of 3 to 6 weeks of age. This infection transmits via the fecal-oral route and two serotypes of IBDV are identified: serotype 1 is pathogenic, while serotype 2 is non-pathogenic. Serotype 1 is classified into classical, intermediate, and very virulent strains (Jayasundara et al., 2017). Following oral infection, IBDV enters the bloodstream, replicates in the macrophages of the gut-associated tissues and lymphoid cells before it attains the bursa of fabricius (BF) (Xu et al., 2024). In the fully susceptible chicken flocks, the clinical disease includes dullness, depression, ruffled feathers, anorexia, whitish loose diarrhoea and severe dehydration (Islam & Samad, 2004). The chickens less than 3 weeks of age do not clearly exhibit clinical signs (Dahshan & Hussien, 2011). Recovery from disease and subclinical infection causes immunosuppression, principally directs towards the B lymphocytes, influences cell-mediated immunity, and leads to vaccination failures (Ingrao et al., 2013). Control of infectious bursal disease (IBD) depends on poultry health management, especially appropriate immunization schedules and maintenance of good hygienic conditions in farms (Farooq et al., 2003). However, IBDV infection is mainly controlled by live attenuated virus vaccines which are classified into a mild, intermediate, intermediate plus, or hot based on their residual virulence (Courtillon et al., 2022). The parent stocks are administered with an emulsion oil vaccine to boost an immune response and Maternal-derived antibody (MDA) in unvaccinated chickens persists up to 3 weeks old and completely decays by 4 to 5 weeks of age (Ahmed & Akhter, 2003). Moreover, the interference of MDA becomes a serious problem at the proper time of vaccination against IBD with a live vaccine (Berg, 2000). When the young chickens are vaccinated with attenuated vaccines too early that may lead to the neutralization of vaccine by MDA, and otherwise, the chicken flocks are poorly protected if applied too late due to the low level of MDA and the active immunity is not enough to prevent the field challenges (Dey et al., 2019), so shortening the high-risk period is crucial to IBD control.

Recently, the M.B strain vaccine has been used quite commonly in chicken farms and walks through the MDA levels of 800 Enzyme-linked immunosorbent assay (ELISA) Idexx while intermediate and intermediate plus vaccines break through the levels of MDA titers are 125 and 500, respectively (De Wit, 2001). Besides, the 228E strain vaccine is capable of walking through the MDA levels of 500 ELISA Idexx (De Wit, 2001). Intermediate and intermediate plus vaccines create better protection than mild vaccines, but they can cause severe bursal lesions and induce corresponding levels of immunosuppression (Rautenschlein et al., 2005). Therefore, the level of live attenuated vaccine influences the humoral immune response, and especially the M.B strain breaks through a higher MDA level. The objectives of this study were to compare the immunity gap of 2 vaccination programs and simultaneously to check whether the M.B vaccine caused immunodeficiency like other hot strain vaccines.

2. Materials and Methods

2.1. Experimental design

The study was carried out on a total of 34,600 Luong Phuong chickens, which were kept in 2 broiler houses of one commercial operation farm with the same management procedures from November 2023 to January 2024 in Binh

Duong Province, Vietnam. All day-one chickens (DOC) of the experiment were bought from the same breeder company and therefore, they were assumed to have the same MDA. All of them were administered by subcutaneous injection (SC) with an IBD immune complex vaccine dose of 0.1 mL at the hatchery. At 12 days old, house 1, 16,600 chicks, were vaccinated with the live attenuated 228E strain vaccine containing at least 10^2 embryo infective dose of 50% per dose by drinking water that was used in this farm for a long time and was suitable for the epidemical condition. Hence, house 1 was used as the control group. Besides, house 2, 18,000 chicks, were vaccinated with the M.B strain vaccine containing at least $10^{2.5}$ - 10^3 embryo infective dose of 50% per dose by drinking water at the same time. Other vaccines in the study were applied according to the below immunization schedule (Table 1).

Table 1. Immunization schedule of the current study

228E group (house 1)		M.B group (house 2)		Application
Age (days)	Vaccine	Age (days)	Vaccine	
1 (hatchery)	ND killed	1 (hatchery)	ND killed	SC with a dose of 0.1 mL/chick
	IBD		IBD	SC with a dose of 0.1 mL/chick
	IB + ND		IB + ND	Spray
7	IB + ND	10	IB + ND	Drop eye
12	228E strain	12	M.B strain	Drinking water
14	AI	15	AI	SC with a dose of 0.3 mL/chick
21	IB + ND	28	IB + ND	Drinking water
35	IB + ND	35	ND	Drinking water

2.2. Serology

Blood samples from 20 chicks were randomly collected for the determination of the IBD and ND MDA at 1 day old. After the second IBD vaccination, at 21, 24, 28, and 34 days of age for the determination of IBD antibodies and at 21, 28, and 42 days of age for the determination of ND antibodies (Figure 1). Randomly selected 15 chicks per house were taken vein blood samples at 21 days old and they were taken the leg mark ring to follow the individual antibody chicks. Then, these chicks were raised with the same environmental conditions as others in the house and continued to record their level of antibodies at other times. All blood samples were let clot naturally, were stored 2 - 8^{o}C, and were sent to the An Phu Tien laboratory in Dong Nai Province, Vietnam. They were centrifuged at 3,000 rpm for 5 minutes to extract serum. Two types of commercial enzyme-linked immunosorbent assay kits (Idexx, Maine, USA) were used as described by the manufacturer to detect the antibodies against IBD and ND in chicken serum. As a result, the serum sample with an S/P ratio ≤ 0.2 (titer ≤ 396) is negative and an S/P ratio > 0.2 (titer > 396) is positive.

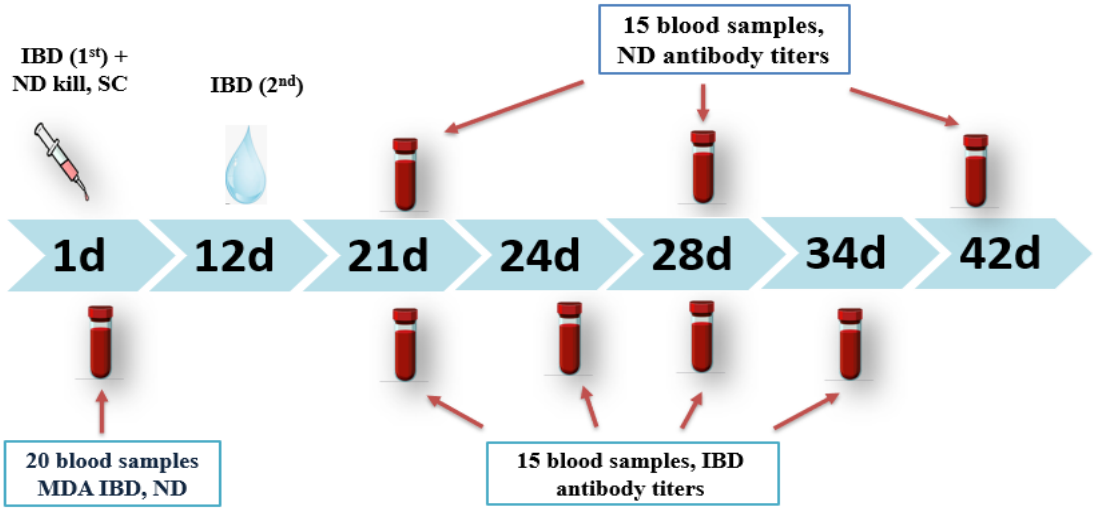

Figure 1. The experimental design per house.

2.3. Performance

The survey was conducted to compare the performance indicators of the broiler chicken flocks when they were vaccinated with two immunization schedules. The performance parameters were followed until slaughter, including body weight, average daily gain, feed conversion ratio, and mortality. The total chicken flock weight was only recorded at the time of sale and the amount of feed was monitored throughout the implementation period. The productivity norms were calculated according to the formula.

Average body weight (kg/chicken) = total weight of chickens/total number of chickens

Average daily gain (g/day) = (total of final weight - total of beginning weight)/total number of survival chicken days)

Feed conversion rate = total amount of consumed feed/total weight of chickens

Mortality (%) = total of dead chickens/total of beginning chickens *100

2.4. Statistical analysis

The data were collected and analyzed by Microsoft Excel 2016 and Minitab 16 software. Using a one-way ANOVA model and the T-test to compare the average level of antibodies between 2 groups. The differences were considered statistically significant with $P < 0.05$. The coefficient variation CV (%) is interpreted as < 30% excellent, 30 - 50% good, 51 - 80% fair, and > 90% poor response to vaccine.

3. Results and Discussion

3.1. Maternally derived IBD antibodies

The MDA is key to protecting chicks against virulent field IBDV strains during the first weeks of age. According to Kreider et al. (1991), the MDA is divided into 3 levels: low level (< 3,000), medium level (3,000 - 5,000) and high level (> 6,000). Collecting randomly 20 serum samples at 1 day old to determine their IBD MDA titers based on the ELISA technique. The titers ranged from 841 to 7,039 and the average titer was medium and uniform (3,809 and 45.3%). The

chicks of 2 houses were bought from the same breeder company. The half-life time of MDA is 3.8 days for Luong Phuong chickens (De Wit, 2001), so these titers can protect the young chickens against field viruses from 1 to 2 weeks old. The MDA can potentially neutralize the vaccine if done on very younger progeny chickens (Ahmed & Akhter, 2003). The interference of MDA is a major problem for the best time to vaccinate, serological monitoring is necessary to evaluate the level of MDA and decide the appropriate timing for vaccination (Berg, 2000). According to the Deventer formula to determine the age of vaccination application (De Wit, 2001). The M.B. strain was able to break through the MDA level of 800 ELISA Idexx and therefore a suitable time could be vaccinated at 13 days old. On the other hand, the available vaccination procedure of the farm was applied by the 228E vaccine at 12 days old, which was considered a standard program, and suitable for epidemical conditions. Hence, the second vaccination against IBD used for 2 programs was at 12 days of age.

3.2. IBD antibodies post second vaccination

Table 2. Infectious bursal disease (IBD) antibody titers

Age	M.B group			228E group			*P*
	Mean titer	CV (%)	N	Mean titer	CV (%)	N	
IBD 21 days old	186	55.7	15	168	83.4	14	0.696
IBD 24 (25) days old	146	41.9	14	117	80.4	14	0.328
IBD 28 days old	1,133[a]	88.4	15	161[b]	97.1	14	0.001
IBD 34 days old	2,215	41.1	15	1,991	56.6	15	0.554

[a- b]Mean values of traits in rows, marked with different letters, differ statistically significantly between groups (P < 0.01).

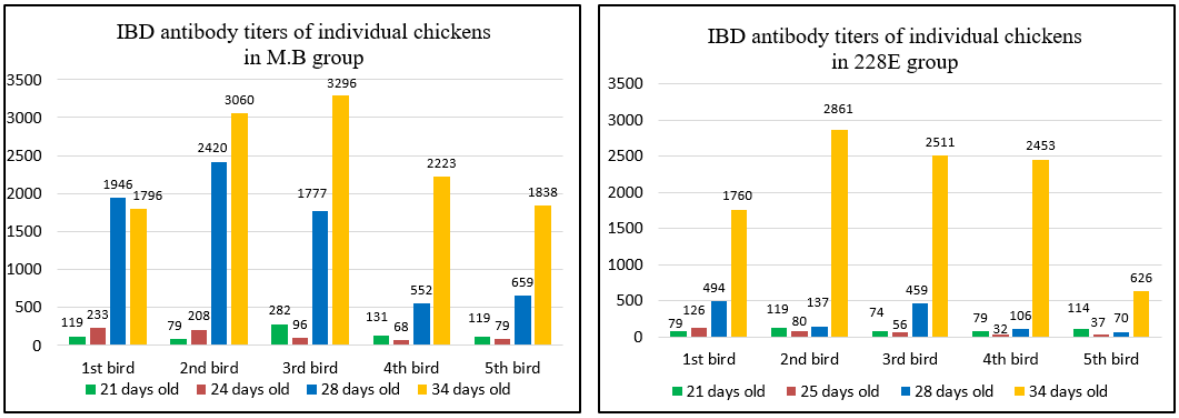

Figure 2. Infectious bursal disease (IBD) antibody titers of individual chickens.

At 21 days old, blood samples of 15 chicks per house were randomly taken and simultaneously were made the leg mark ring to evaluate the change of individual antibody chicks and could skip antibody titers of abnormal samples in the following times (Figure 2). Circulating ELISA IBD antibodies ranged from 52 to 414 in the M.B group (mean titer: 186, CV: 55.7%) and from 52 to 590 in the 228E group (mean titer: 168, CV: 83.4%). The MDA titers of both groups decreased greatly. There was no statistically significant difference between 2 groups ($P > 0.05$) (Table 2).

At 24 days old, circulating IBD antibodies ranged from 68 to 233 in the M.B group (mean titer: 146, CV: 41.9%). The passive immunity continued to reduce and the active antibodies began to create (28.6% samples) with good uniformity in the M.B group. Samples of the control group were collected at 25 days old, delaying one day due to a few objective reasons. At 25 days old, IBD antibodies in the 228E group ranged from 32 to 402 (mean titer: 117, CV: 80.4%). The difference was not statistically significant between 2 groups ($P > 0.05$) (Table 2). The same as the M.B group, the passive immunity continued to reduce greatly in the 228E group and the active antibodies also began to create (14.3% samples). Although the 228E group was one more day compared to the M.B group, but the percentage of active antibodies of samples in this group was still smaller (14.3% versus 28.6%).

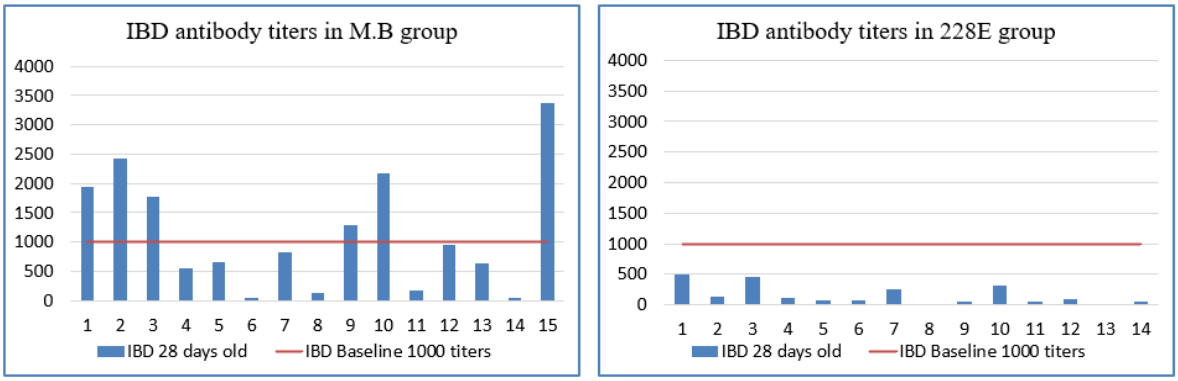

Figure 3. Infectious bursal disease (IBD) antibody titers at 28 days old of 2 vaccination programs.

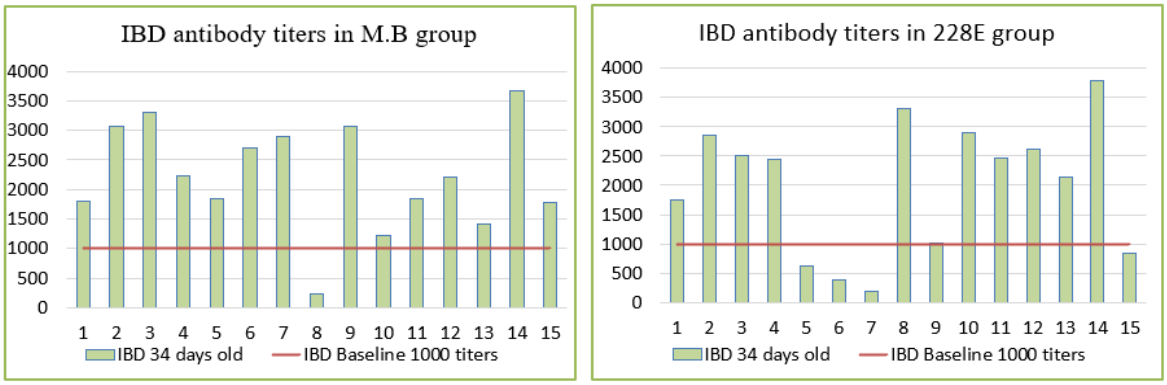

Figure 4. Infectious bursal disease (IBD) antibody titers at 34 days old of 2 vaccination programs.

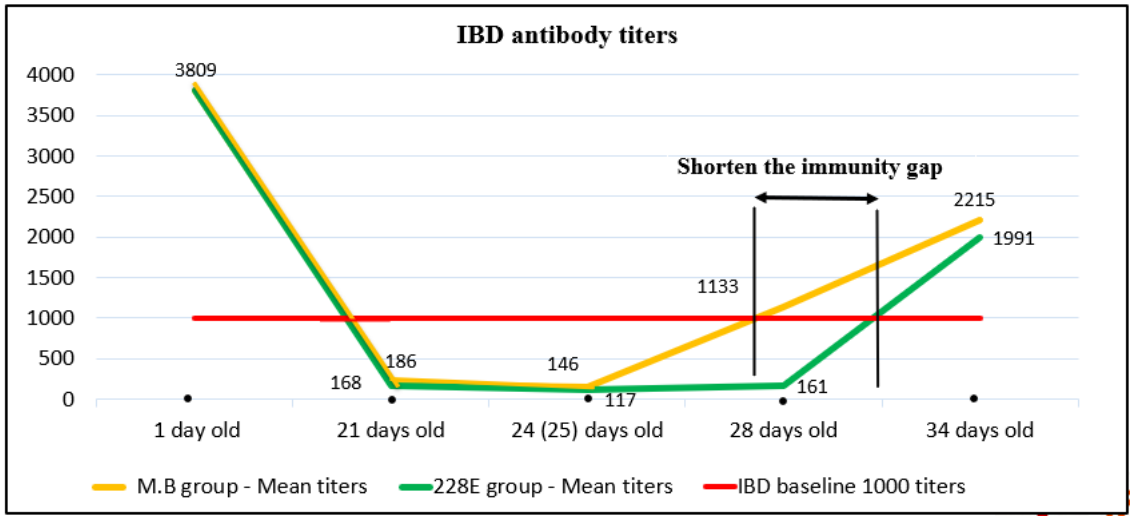

Figure 5. The average level of infectious bursal disease (IBD) antibody titers of 2 vaccination programs.

At 28 days old, IBD antibody titers ranged from 41 to 3,362 in the M.B group (mean titer: 1,133, CV: 88.4%) and from 18 to 494 in the 228E group (mean titer: 161, CV: 97.1%) (Figure 3). The active immunity in the M.B group was higher and faster than the active immunity in 228E group had recently appeared, the statistically significant difference was between 2 groups ($P < 0.01$) (Table 2). According to the Idexx recommendation, the protective antibody titers against IBD were 1,000 for the broiler chickens. The proportions of samples exceeding 1,000 titers in the M.B and the 228E vaccines were 40% and 0%, respectively. According to Smith (2019), herd immunity occurs when a sufficient proportion of the group has immunity against contagion of the pathogen to susceptible animals and even helps to maintain the stable existence of the pathogen in a population instead of completely removing field viruses in the farm. The target organ of IBDV is the bursal Fabricius at its maximum development and the acute disease is directly related to the susceptible B lymphocyte cells, so the most sensitive age is from 3 to 6 weeks old (Berg, 2000). In addition, young chickens are the most susceptible to IBDV around 30 to 35 days old (Ahmed & Akhter, 2003). Therefore, the administered M.B vaccine chickens were better protected and concurrently the herd immunity was able to reduce partially the risk of field challenges for the susceptible chickens. Furthermore, the average level of IBD antibody titers in M.B group started to overcome 1,000 titers ELISA Idexx at 27 days old, which meant the active immunity could be enough to protect chickens during the sensitive period.

At 34 days old, IBD antibodies ranged from 238 to 3,661 in the M.B group (mean titer: 2,215, CV: 41.1%), from 179 to 3,788 in the 228E group (mean titer: 1,991, CV: 56.6%) (Figure 4). The result showed that the active immunity of 2 groups was increased. There was no significant difference ($P > 0.05$). The ratios of samples exceeding 1,000 titers in the M.B and 228E groups were 93.3% and 73.3% respectively, which proved that the ability of community immunity in vaccinated M.B vaccine chickens was better to suffer from the environmental challenges.

Besides, the average level of IBD antibody titers in the 228E group started to overcome 1,000 titers at about 30 days old. As a result, the vaccination program using the M.B strain affected shortening the window of susceptibility no protection about 3 days when compared between 2 vaccination programs (Figure 5).

Another strategy to control IBD is based on a uniform active immune response post-vaccination in all individuals of the flock, environmental viruses have no chance to attach, or replicate in any chickens, and therefore, a type of optimal vaccine will generate better uniformity (CV is lower) (Nguyen et al., 2018). The result revealed that the uniformity of both groups was clearly improved respectively from 28 days to 34 days of age: the M.B group was 88.4% and 41.1% and the 228E group was 97.1% and 56.6%. Additionally, the value of CV immunity response in the M.B group was lower than the 228E group at 4 time points (Figure 6). This result indicated the better ability of individual protection of vaccination program using the M.B strain and reduced the infectious risk to other chickens in the flock.

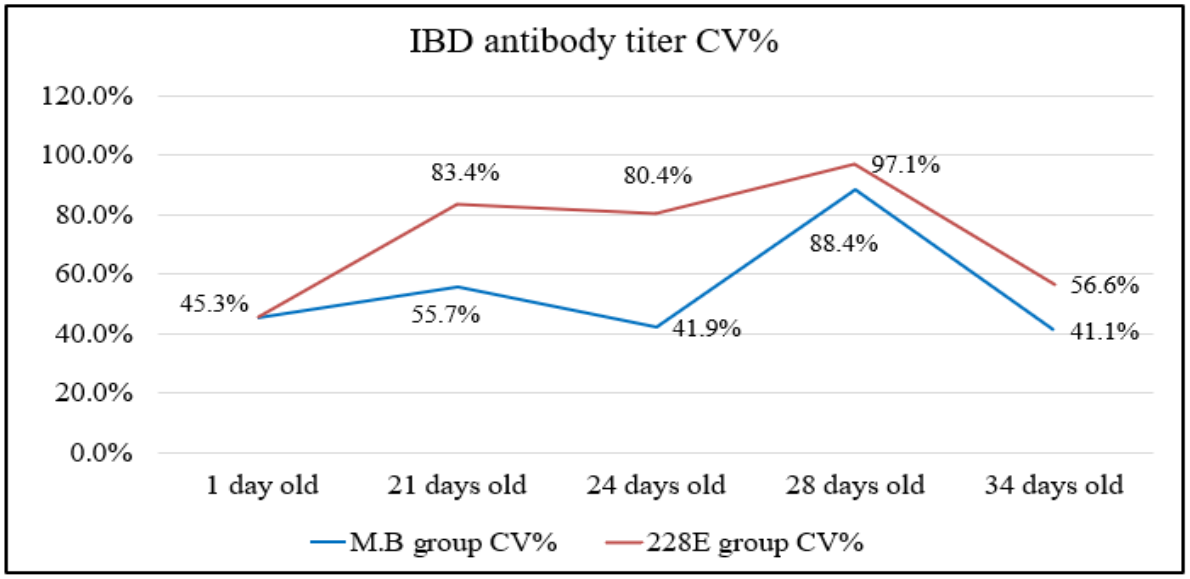

Figure 6. The uniformity of infectious bursal disease (IBD) antibody titers of 2 vaccination programs.

3.3. Humoral immune response to inactivated ND vaccine

Table 3. Newcastle disease (ND) antibody titers

Age	M.B group			228E group			*P*
	Mean titer	CV (%)	N	Mean titer	CV (%)	N	
ND 21 days old	444	183.2	15	292	116.6	15	0.509
ND 28 days old	1,199	64.4	15	1,129	102.4	15	0.848
ND 42 days old	3,029	68.5	15	1,885	50.0	15	0.062

The intermediate plus or hot live attenuated IBD vaccine can be used in complex epidemics, but these vaccines can cause B cell depletion in the bursa and immunosuppression (Courtillon et al., 2022). Immunosuppressive chicken flock is a significant concern because of losing the ability of pathogen resistance and failing other vaccination programs. In this study, both groups were assessed the humoral immune response to other antigens, such as the inactivated ND vaccine. According to the IDL (2015), the protective antibody titers against ND are divided into 3 levels: low level (1,000 - 5,000), medium level (7,000 - 12,000) and high level (16,000 - 25,000). At 1 day old, the average ND antibody titers were low and uniform (3,701 and 41.7%). All the chicks were bought from a breeder company, so the same MDA against ND. At 21, 28, and 42 days old, ND antibody titers were not statistically different between 2 groups ($P > 0.05$) (Table 3). Therefore, the M.B strain virus did not affect the ability of the humoral immune response when compared to a standard immunization schedule on the farm (Figure 7). According to Lazarus et al. (2008), a dosage of the M.B strain in the range of 10^2 to 10^4 embryo infective dose of 50% is safe and protective for commercial chicks. Another research took place in commercial broiler chickens to evaluate the efficacy of M.B, LIBDV, and Winterfield 2512 strain vaccines against IBD, this study proved that the M.B strain vaccine did not cause immunosuppression (Nguyen et al., 2018). According to Nguyen et al. (2022), the M.B strain virus was located early in the BF to field virus competition, but it did not cause serious damage, showed recovery signs, and did not affect immunodeficiency.

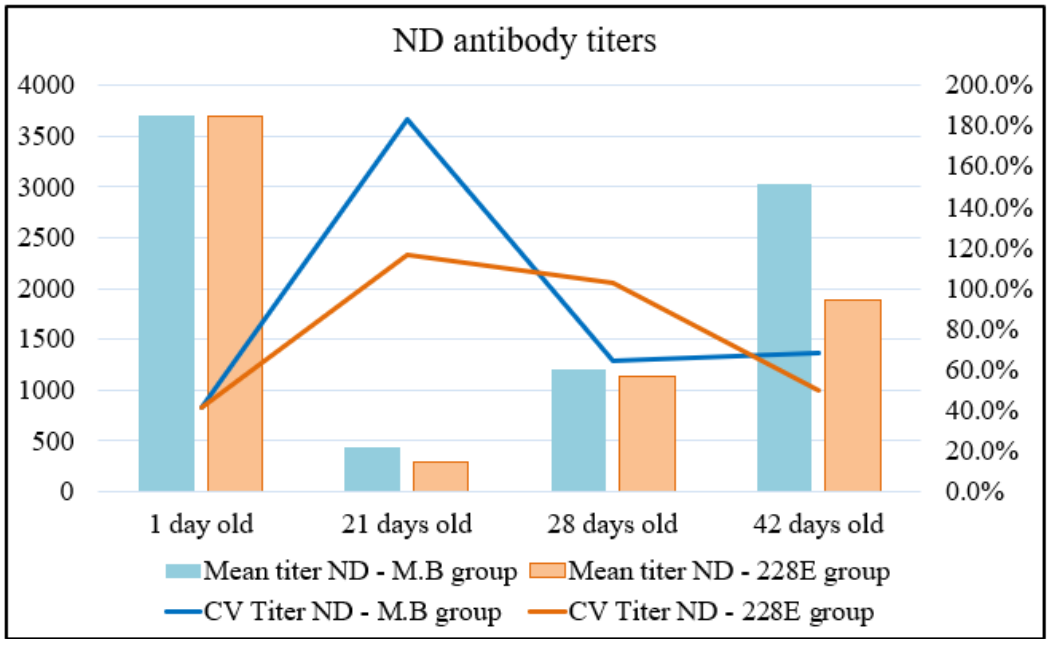

Figure 7. Newcastle disease (ND) antibody titers.

3.4. Production performance

The broiler chicken performance can be evaluated during the rearing phase, control of IBD-induced immunosuppression results in a decrease in lesions caused by bacterial secondary infections and a decrease in carcass condemnation at slaughterhouses (Lemiere, 2013). During the trial period, a total of chickens were recorded with no IBD and ND symptoms. An optimal vaccine does not only stimulate a good immune response, but also the productivity reaches the target. The body weight and feed intake were recorded for the entire trial to assess the growth performance at market slaughter age. Overall, the production parameters of 2 groups were fairly good and no significant difference (Table 4). A part of the experimental chickens should be challenged with the field virus and recorded the productivity data to obviously evaluate the difference between 2 vaccination programs, but the limitations of this study do not allow this to be done.

Table 4. Broiler performance

No.	Parameters	M.B group	228E group
1	Growing period (days)	55	54
2	Mortality (%)	3.63	4.07
3	Average body weight (kg)	1.491	1.494
4	Feed conversion ratio	2.316	2.318
5	Average daily gain (g/day)	26.42	27.06

4. Conclusions

In summary, the M.B strain vaccine generated the immune response against IBD earlier than the 228E strain and shortened the high-risk period in the susceptible chicken flock. In addition, the M.B strain virus did not cause immunodeficiency through the ability of humoral immune response with the inactivated ND vaccine and did not affect negatively the production performance. These findings support using the M.B strain vaccine as an effective strategy for preventing Gumboro disease in Luong Phuong chickens.

Conflict of interest

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

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