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Immuno-protective effect of commercial IBD vaccines against emerging novel variant infectious bursal disease virus in specific-pathogen-free chickens

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ABSTRACT

Importance: Infectious bursal disease (IBD) is an important viral poultry disease that vaccination can control.
Objective: This study examined the immune protection of immune-complex (Vaccine A) and attenuated live (Vaccine B) IBD vaccines in specific-pathogen-free (SPF) chickens against a novel Malaysian variant IBD virus (vaIBDV) challenge.
Methods: One-day-old (n =75) SPF chickens were divided randomly into the following three groups of 25 chicks each: Control, Vaccine A, and Vaccine B groups. The vaIBDV strain, UPM1432/2019, was used for the challenge at 21 and 28days post-vaccination (dpv). Five birds from unchallenged and challenged groups were sacrificed seven days post-challenge, and blood, bursa, spleen, and cloacal swabs were collected. The IBD antibodies (Abs), lymphoid lesions, and viral load were determined.
Results: The UPM1432/2019 virus induced bursal damage in vaccinated SPF chickens despite Ab titers. The mean Ab titers of the Vaccine A challenged group were significantly lower ($p < 0.002$) than in the unchallenged group at 28 dpv. The bursal indices of the vaccinated unchallenged groups did not differ significantly from those of the vaccinated challenged groups ($p = 0.94$). Microscopically, the bursae of the challenged groups showed significant atrophy. The bursal lesion score was higher ($p < 0.05$) in the control and Vaccine B challenged groups than the Vaccine A challenged group. The challenged group had a higher viral load than the vaccinated groups ($p < 0.001$).
Conclusions and Relevance: Neither vaccine fully protected against a vaIBDV challenge, highlighting the limitations of current vaccines and the need for further research.

Keywords: Infectious bursal disease virus; novel; variant IBDV; vaccines; chickens

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Conflict of Interest
The authors declare no conflicts of interest.

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INTRODUCTION

The infectious bursal disease (IBD), caused by the infectious bursal disease virus (IBDV), is a highly contagious disease of chickens characterized by severe damage to the bursa of Fabricius (BF) that induces immunosuppression. Vaccination is a crucial method for controlling IBD [1,2], but the emergence of variant IBDV (vaIBDV) [3] and very virulent IBDV (vvIBDV) [4] challenges the effectiveness of current vaccines. The incidence of IBD cases in vaccinated poultry flocks is often reported in the field, possibly due to a viral mutation resulting in increased virulence or reduced neutralization by classical antibodies or inefficiency of the currently available IBD vaccines. Different live IBD vaccine strains provide valuable protection efficacy and lymphoid tissue damage [5-8].

Interestingly, because of severe bursal damage induced by conventional live vaccines, new-generation IBD vaccines have been developed to improve the efficacy of live attenuated vaccines even in the presence of high maternally derived antibodies. An immune-complex (Icx) vaccine is one such vaccine that produces adequate protection with minimal bursal and spleen damage [9,10]. The vaccine was reported to be safe for administration at day-old chicks [11]. Several studies have reported the efficacy of various IBD vaccines against the vvIBDV strain [1,8,10,12].

Jackwood [13] highlighted recent advances in IBD vaccines that enhance control of IBD in endemic vvIBDV areas. Despite the protective effects of these vaccines, the Malaysian poultry industry faces new challenges in controlling IBD in vaccinated flocks. The authors reported the emergence of novel vaIBDV, whose pathogenicity is seen mainly as bursal atrophy and lesions without mortality in specific-pathogen-free (SPF) chickens [14,15]. Surprisingly, this new vaIBDV strain is similar to the Chinese novel vaIBDV, which could escape neutralizing Ab-induced by classical vaccine strains, inducing immunosuppression [16]. The present study determined the immune-protective effects of two commercial IBD vaccines against emerging novel Malaysian vaIBDV strains.

METHODS

Ethics statement

The Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM) has approved the animal trials with the reference number UPM/IACUC/AUP-R023/2019.

Chickens

Eight-day-old embryonated SPF chicken eggs (single-comb White Leghorn) were purchased from Malaysian Vaccine Pharmaceuticals (MVP) and incubated at 37°C in the hatchery facility of Laboratory of Vaccine and Biomolecules, Institute of Bioscience, UPM. Eighty, one-day-old SPF chickens were transferred to the animal room facility of the Faculty of Veterinary Medicine, UPM. Water and commercial feed were provided to the chickens *ad libitum*.

Vaccines

This study used two vaccine types often used against IBD in Malaysia: the immune-complex classical IBDV strain vaccine (Vaccine A; Ceva Sante Animale, Hungary) and live attenuated vvIBDV strain vaccine (Vaccine B; MVP Sdn Bhd, Malaysia).

Challenge virus

The vaIBDV strain, UPM1432/2019 (accession numbers MT505343 and MT505344), whose pathogenicity was previously described [15], was used for the challenge.

The median embryo infectious dose (EID_{50}) of the virus was determined using the method described by Reed and Muench [17].

Experimental design

One-day-old (n = 75) SPF chickens were used in the study. The birds were divided randomly into three groups of 25 chicks each. The group 1 birds served as the control. The group 2 and 3 birds at one day old were vaccinated with vaccines A and B, respectively. The birds were challenged independently with 1 mL of vaIBDV (virus titer of $10^{5.4}$ EID_{50}/mL) orally at 21 and 28 days post-vaccination.

At 21 days of age before the first challenge, five chickens in all three groups were sacrificed, and the blood, bursa, and spleen were collected. The birds were examined for gross lesions of the disease. The bursa and spleen weights were recorded, and the bursa-to-body weight (BBW) and spleen-to-body weight (SBW) ratios were calculated. In addition, the bursal body weight index (BBIX) was calculated. Each bursa and spleen sample was divided into two portions. One part was immediately placed into a tube containing RNALater (Ambion, Austin, Texas, USA) for RNA extraction (viral load). The second part was fixed in 10% neutral buffered formalin for the histopathological examination. In addition, swabs were collected from the sampled birds before euthanasia to determine the extent of virus shedding, and the swab samples were placed in PBS and stored at −20°C until use. At the same time, another five chickens from all three groups were transferred to a different room, and the challenge virus was administered orally at $10^{5.4}$ EID_{50}/bird.

At 28 days of age before the second challenge, five unchallenged birds from all three groups and the previously challenged ones were sacrificed, and the sampling methods, challenge, and sample processing were carried out as described for the challenge at 21 dpv.

Determination of antibody titer

The anti-IBDV levels were determined from the serum samples using a commercial ELISA kit (BioChek BV, Netherlands) according to the manufacturer's instructions.

Determination of bursa and spleen indices

The BBW or SBW ratios and BBIX were calculated as described earlier [18]. The formulae are shown below:

$$\text{BBW or SBW Ratios} = \frac{Weight\ of\ Bursa/Spleen\ (gram)}{Bird's\ Body\ Weight\ (gram)} \times 1{,}000$$

$$\text{BBIX} = \frac{BBW\ of\ Infected\ Birds}{BBW\ of\ Control\ Birds}$$

Chickens with BBIX < 0.7 were considered atrophied [19].

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Histopathology

The histopathological tissues were processed using the standard histological procedure. The lesions were scored based on the extent of tissue damage, and the bursal lesion scores (BLS) of 0–5 were used to describe the relative degree of severity [20].

Detection of viral RNA using quantitative reverse transcription polymerase chain reaction (RT-qPCR)

Primer design and optimization for RT-qPCR

The primer blast tool was used to design specific primer pairs and a probe that only amplifies a fragment of VP2 of the challenge virus, UPM1432/2019. Three primer sets were designed and optimized (**Supplementary Table 1**) using gradient RT-qPCR. The vaccines and the challenge virus were used for optimization. The primers were synthesized by Microgen (Korea).

Viral RNA extraction and real-time RT-qPCR

The total viral RNA from the control and infected bursae, spleens, and cloacal swabs, vaccines, and challenged viruses were extracted using the innuPREP Virus RNA Kit (Analytik Jena AG, Germany) according to the manufacturer's instruction.

One-step RT-qPCR reactions were set up using a SensiFAST probe No-ROX kit (Meridian Bioscience, UK). The reaction was conducted according to the manufacturer's instructions. Briefly, 10 µL of 2x RT-PCR buffer mix, 0.8 µL each of forward and reverse primers (final concentration: 400 nM), 0.2 µL of the probe (final concentration: 100 nM), 0.2 µL of reverse transcriptase, 0.4 µL of RNase inhibitors, 3 µL of RNA template (denatured at 92°C for 3 min), and finally 4.6 µL of nuclease-free water to top up to a 20 µL reaction volume were used. BioRad thermal cycler (CFX 96 BioRad, USA) was used under the following conditions: reverse transcription at 45°C for 10 min, polymerase activation at 95°C for 2 min, and 40 amplification cycles of denaturation at 95°C for 5 sec and annealing/extension at 60°C for 20 sec.

Viral copy number determination

The viral load was determined based on the Avogadro's number approximation, 6.023×10^{23} molecules/mol. The formula below was used to calculate the viral copy number (VCN).

$$\text{Copy Number} = \frac{Viral\ Concentration\ \left(\frac{g}{\mu L}\right) \times 6.023 \times 10^{23}}{660 \times Amplicon\ Size}$$

Statistical analysis

Statistical software: SPSS version 23 (IBM, USA) and GraphPad Prism version 9 were used for data analysis. The data obtained from the study were reduced to the mean and standard deviation. A student *t*-test, one-way and two-way ANOVA with Tukey's post hoc test was conducted to test the difference between groups. The Kruskal–Wallis test was carried out for BLS; p values < 0.05 were considered significant.

RESULTS

Clinical signs, mortality, and gross pathology

No apparent clinical signs or gross lesions were observed in the unchallenged control group, but bursal atrophy was observed in all the challenged groups.

Table 1. Mean antibodies, body weight, bursal and spleen indices, and bursal lesion scores at 21 days following vaccination of SPF chickens with different IBD vaccines

Groups	Antibodies	Body weight (g)	Bursal/body weight	Spleen/body weight	Bursal body index	Bursal lesion scores
Control	ND	74.2 ± 09.58	2.33 ± 0.61[a]	0.77 ± 0.25	1.00	0.40 ± 0.55[a]
Vaccine A	8,639 ± 1,451	73.6 ± 09.10	1.23 ± 0.27[b]	0.84 ± 0.10	0.53	3.60 ± 0.55[b]
Vaccine B	7,709 ± 2,577	67.2 ± 11.82	0.80 ± 0.48[b]	0.86 ± 0.16	0.34	4.80 ± 0.44[c]

Values are presented as mean ± SD.
SPF, specific-pathogen-free; IBD, infectious bursal disease; ND, no detectable antibodies.
[a,b,c]Significant differences between rows ($p < 0.05$).

Antibody response to vaccines

Anti-IBDV was detected at 21 dpv in both vaccinated groups (**Table 1**). The Ab titers of the vaccinated groups were similar ($p = 0.51$), but the vaccine A group showed a higher titer. The vaccines elicited high Ab titers.

Body weight and bursal and spleen indices post-vaccination

Table 1 lists the body weight, BBW, SBW, BBIX, and BLS of the SPF chickens at 21 dpv. The mean BBW for the control group was significantly higher ($p = 0.001$) than vaccinated groups. The mean BBIX of 0.53 and 0.34 were recorded for the vaccine A and B groups, respectively. The mean BLS for the vaccine B group was the highest ($p < 0.05$) compared to the vaccine A and control groups (**Table 1**).

Antibody response post-challenge

The anti-IBDV was detected at 28- and 35-dpv in the vaccinated and control challenged groups (**Fig. 1**). At 28 dpv, after the first challenge, the Ab response in the challenged control chickens was significantly higher ($p < 0.001$) compared to the unchallenged control. The Ab titer in the vaccine A challenged group was significantly lower ($p = 0.002$) than the vaccine A unchallenged group. In contrast, there was no significant difference ($p = 0.84$) between the Ab titers of the challenged and unchallenged vaccine B groups (**Fig. 1A**). The mean Ab titer for the vaccine B challenged group was significantly higher ($p = 0.001$) than that of the vaccine A challenged group (**Fig. 1A**).

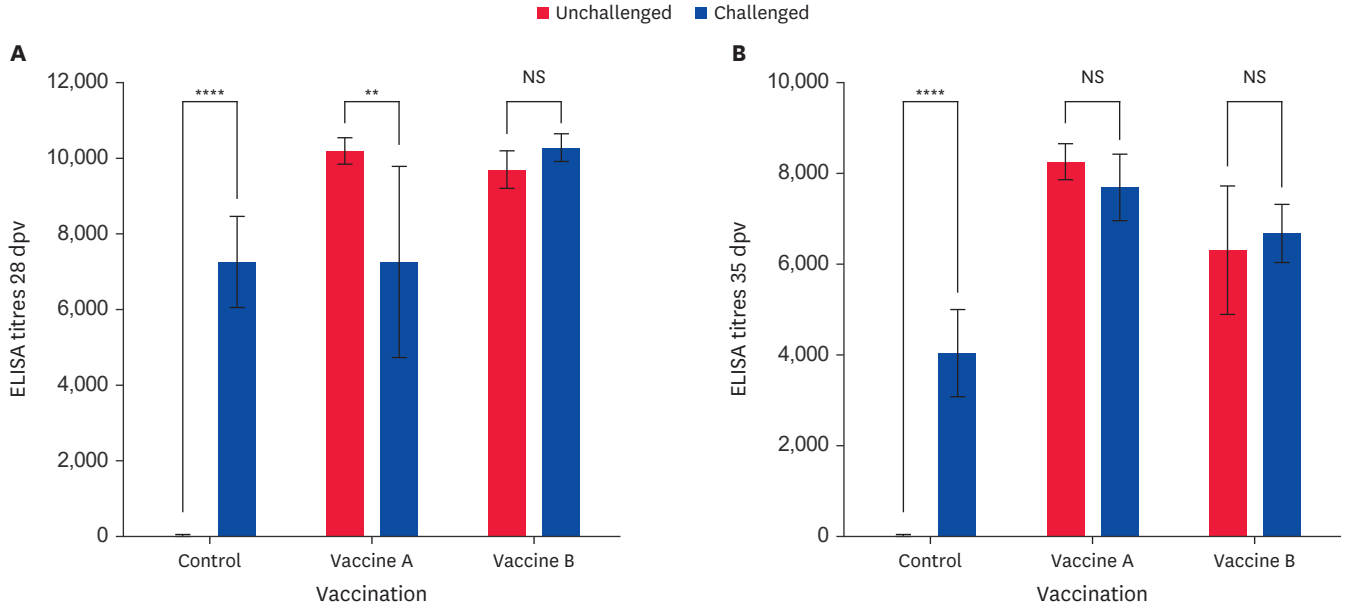

Fig. 1. Mean antibody titer at seven days following challenge of infectious bursal disease vaccinated (28 and 35 dpv) and non-vaccinated specific-pathogen-free chickens.
ELISA, enzyme-linked immunosorbent assay; NS, not significant.
$p > 0.05$ (NS), $^{**}p < 0.001$, $^{****}p < 0.0001$.

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Table 2. Mean bursal and spleen indices and mean bursal lesion scores at seven days following challenge of IBD vaccinated (28 and 35 dpv) and non-vaccinated SPF chickens

DPV	Groups	Bursal indices ± SD		Spleen indices ± SD		Bursal lesion score ± SD	
		Unchallenged	Challenged	Unchallenged	Challenged	Unchallenged	Challenged
28 dpv	Control	$3.10 \pm 0.46^{a,x}$	$1.60 \pm 0.34^{a,y}$	$1.14 \pm 0.21^{a,x}$	$1.76 \pm 0.23^{a,y}$	$0.60 \pm 0.55^{b,x}$	$4.80 \pm 0.45^{a,y}$
	Vaccine A	$1.20 \pm 0.42^{b,x}$	$1.20 \pm 0.29^{a,b,x}$	$0.87 \pm 0.24^{a,x}$	$0.96 \pm 0.22^{b,x}$	$2.80 \pm 0.45^{a,b,x}$	$4.00 \pm 1.00^{b,y}$
	Vaccine B	$0.85 \pm 0.09^{b,x}$	$0.74 \pm 0.21^{b,x}$	$0.77 \pm 0.27^{a,x}$	$1.09 \pm 0.25^{b,x}$	$5.00 \pm 0.00^{a,x}$	$5.00 \pm 0.00^{a,x}$
35 dpv	Control	$3.08 \pm 0.77^{a,x}$	$1.46 \pm 0.14^{a,y}$	$0.87 \pm 0.14^{a,x}$	$0.90 \pm 0.57^{a,x}$	$0.60 \pm 0.55^{b,x}$	4.60 ± 0.55^{y}
	Vaccine A	$1.36 \pm 0.25^{b,x}$	$1.10 \pm 0.28^{a,b,x}$	$0.84 \pm 0.16^{a,x}$	$1.00 \pm 0.40^{a,x}$	$3.00 \pm 1.22^{a,x}$	4.00 ± 1.00^{x}
	Vaccine B	$0.73 \pm 0.12^{c,x}$	$0.81 \pm 0.20^{b,x}$	$0.89 \pm 0.18^{a,x}$	$1.12 \pm 0.17^{a,x}$	$4.80 \pm 0.45^{a,x}$	5.00 ± 0.00^{x}

IBD, infectious bursal disease; SPF, specific-pathogen-free.
^{x,y}Significant differences within rows of values of the same sampling tissue ($p < 0.05$).
^{a,b,c}Significant differences within columns of values of the same sampling age ($p < 0.05$).

After the second challenge (35 dpv), the mean Ab titers for the challenged control chickens were significantly higher ($p < 0.001$) than the unchallenged control group (**Fig. 1B**). The Ab titers were similar in the unchallenged and challenged birds in the vaccine groups (**Fig. 1B**).

Bursal and spleen indices post-challenge

After the first challenge (28 dpv), a significant interaction between vaccination and challenge ($p < 0.001$) was observed on the mean BBW (**Table 2**). The BBW between vaccinated unchallenged and challenged groups did not differ significantly ($p = 0.94$). On the other hand, there was a significant decrease ($p < 0.001$) in the mean BBW in the control group after the challenge. In addition, the mean BBW of the challenged control group was significantly higher ($p = 0.004$) than that of the vaccine B challenged group.

An analysis of SBW revealed significant effects on IBD vaccination ($p < 0.001$) and challenge ($p < 0.001$) (**Table 2**). The mean SBW for the challenged control group was significantly higher ($p < 0.001$) than the vaccinated challenged groups. No significant increase in SBW was observed after the challenge in vaccine groups (**Table 2**).

Following the second challenge (35 dpv), a significant interaction was noted between vaccination and challenge ($p < 0.001$) on the BBW (**Table 2**). The BBW of the unchallenged vaccine groups was significantly lower than that of the unchallenged control group ($p < 0.001$). In addition, the unchallenged vaccine A group had a higher mean BBW than the unchallenged vaccine B group ($p = 0.031$). After the challenge, no significant decrease ($p = 0.44$) in BBW was observed between the vaccine groups (**Table 2**).

An analysis of SBW showed no significant interaction between the effects of vaccination and challenge ($p = 0.75$). No significant increase in SBW was observed after the challenge in all the groups (**Table 2**).

Histopathology of bursa and spleen post-vaccination

Fig. 2 shows the histopathology of the bursa and spleen of SPF chickens at 21 dpv. The BF and spleen of the control group were normal (**Fig. 2A and B**). In the vaccine A group, however, mild atrophy and degeneration of lymphoid follicles were observed (**Fig. 2C**). The spleen had multiple areas of necrosis with eosinophilic debris around the reticular cells (**Fig. 2D**). Similarly, in the vaccine B group, the lymphoid follicles were severely atrophied with moderate inflammatory cell infiltration (**Fig. 2E**). In addition, the spleen showed hyalinization of capillaries sheaths (**Fig. 2F**).

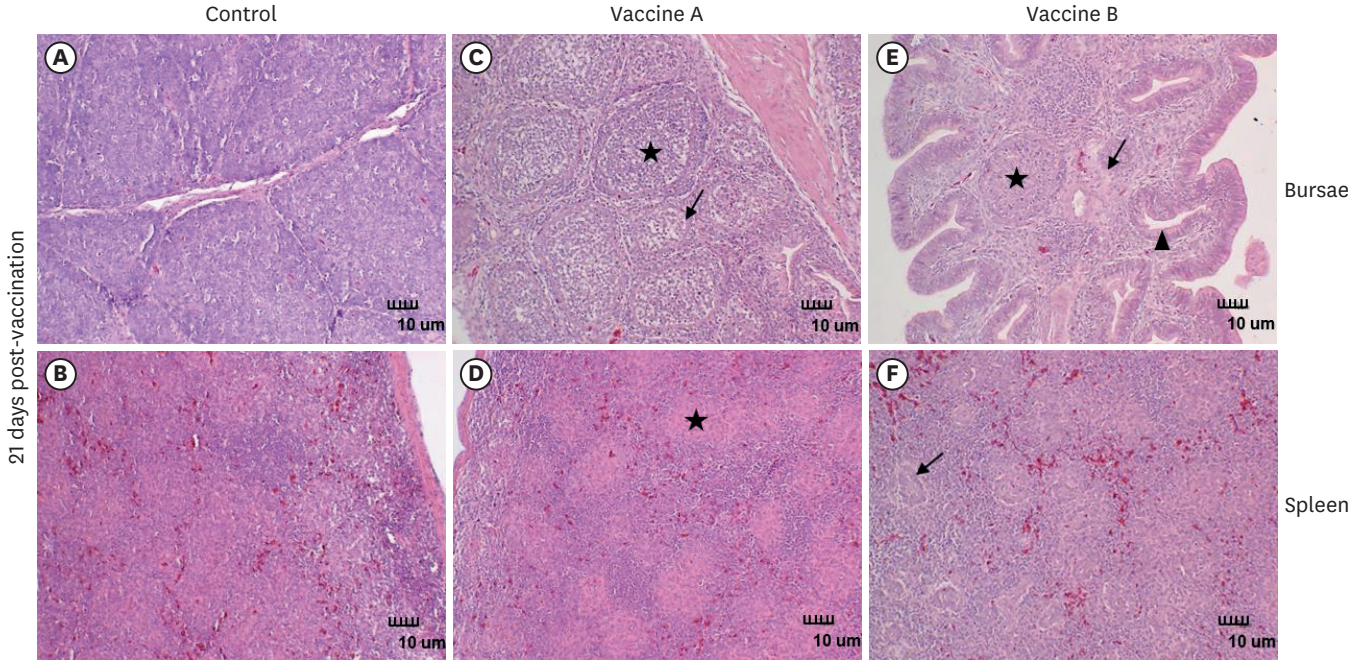

Fig. 2. Histopathology of bursa and spleen of specific-pathogen-free chickens at 21 days post-infectious bursal disease vaccination; Control (A, bursa and B, spleen); Vaccine A (C, bursa and D, spleen); Vaccine B (E, bursa and F, spleen). (A) the bursal follicles were intact, and (B) the spleen was normal. (C) bursa showing mild atrophy (arrow), the degeneration of lymphoid follicles, and lymphocyte depletion (star) of the bursa of Fabricius (lesion score of 3). (D) spleen, showing an increased zone of white pulp and multiple areas of necrosis with eosinophilic debris (star). (E) bursa, showing severe atrophy of lymphoid follicles (arrow), the presence of ductal structures (arrowhead), moderate inflammatory cell infiltrations, and marked lymphocyte depletion (star) (lesion score of 5). (F) spleen, showing hyalinization of capillaries sheaths (arrow). (A-F) Hematoxylin and eosin stains, scale bar = 10 μm.

Histopathology of bursa post-challenge

The BF of the SPF chickens at 28 and 35 dpv (7 dpc) are presented (**Fig. 3**). **Table 2** lists the mean BLS. After the first challenge, the BF was normal for the control unchallenged groups (**Fig. 3A**). On the other hand, the control challenged group of birds showed severe atrophy of lymphoid follicles, cyst formation, and thickened interfollicular tissue with cellular infiltration (**Fig. 3B**), and the highest mean BLS was recorded (**Table 2**). The vaccine A unchallenged group showed mild atrophy of lymphoid follicles and lymphocyte depletion of the BF (**Fig. 3C**), and the bursae had a mean BLS of 2.8 ± 0.45. On the other hand, the vaccine A challenged group exhibited severe atrophy of lymphoid follicles with fibrous connective tissues and moderate inflammatory cell infiltrates. (**Fig. 3D**) with a mean BLS of 4 ± 1.00. The vaccine B-unchallenged group presented severe atrophy of lymphoid follicles, cellular infiltration, and marked lymphocyte depletion (**Fig. 3E**). The bursal tissues from the vaccine B-challenged group showed similar lesions to those birds in the unchallenged group with the highest BLS (**Fig. 3F**).

Similarly, after the second challenge, the bursal tissues from the unchallenged control groups appeared normal (**Fig. 3G**). The control-challenged group showed severe bursal damage with large cystic formations containing edema fluids and cellular debris (**Fig. 3H**). The mean BLS of 4.6 ± 0.55 was recorded (**Table 2**). Mild atrophy of the lymphoid follicles and lymphocyte reduction of the BF were observed in the vaccine A-unchallenged group with a mean BLS of 3 ± 1.22 (**Fig. 3I**). The vaccine A-challenged group showed severe atrophy of the lymphoid follicles, cellular infiltration, and hyperplastic fibrous connective tissues (**Fig. 3J**). The bursa had an increased mean BLS. The vaccine B-unchallenged group exhibited severe atrophy of the lymphoid follicles, epithelial vacuolation, and marked lymphocyte depletion (**Fig. 3K**).

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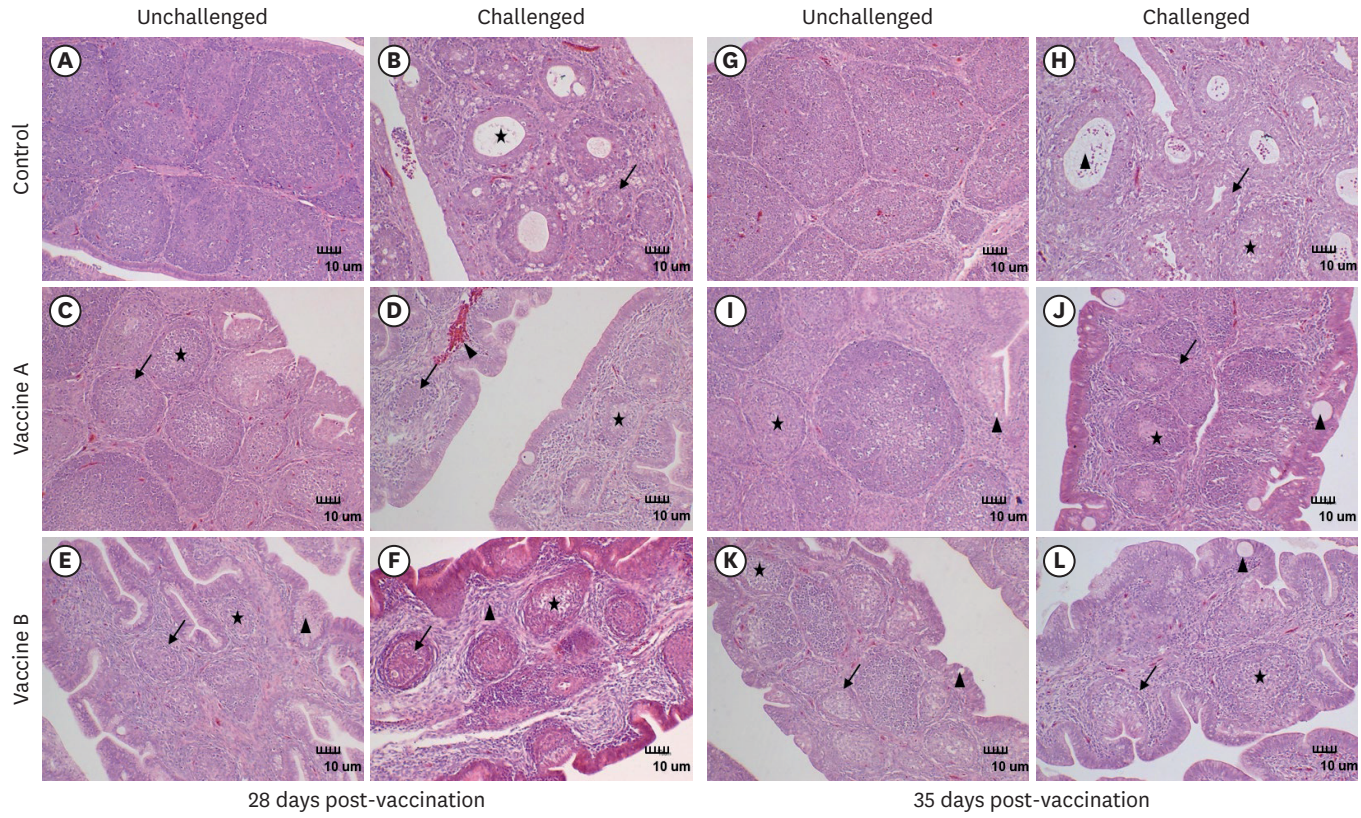

Fig. 3. BF of specific-pathogen-free chickens at 28 and 35 days post-vaccination, unchallenged groups; (A and G) Control, (C and I) Vaccine A, and (E and K) Vaccine B. Seven days post-challenged (vaIBDV $EID_{50} = 10^{5.4}$); (B and H) Control, (D and J) Vaccine A, and (F and L) Vaccine B. (A) The bursal follicles were intact. (B) there was severe atrophy of lymphoid follicles (arrow), the formation of a cyst in the medulla with edema fluid, and marked lymphocyte depletion (star) (lesion score of 5). (C) Mild atrophy of lymphoid follicles (arrow) and lymphocyte depletion of BF (star) (lesion score of 3). (D) Severe atrophy of lymphoid follicles (arrow), hemorrhages (arrowhead), and marked lymphocyte depletion (star) (lesion score of 4). (E) Severe atrophy of lymphoid follicles (arrow), presence of ductal structures (arrowhead), epithelial vacuolation, and marked lymphocyte depletion (star) (lesion score of 5). (F) Severe atrophy of lymphoid follicles (arrow), presence of ductal structures, degeneration of the medullary areas, marked fibrous connective tissues (arrowhead), and marked lymphocyte depletion (star) (lesion score of 3). (G) Intact bursal follicles. (H) Severe atrophy of lymphoid follicles (arrow), formation of some cystic cavities in the medulla with edema fluid (arrowhead), necrotic debris, and marked lymphocyte depletion (star) (lesion score of 5). (I) Degeneration, small cyst formation (arrowhead), and mild lymphocyte depletion (star) of the BF (lesion score of 3). (J) Severe atrophy of the lymphoid follicles (arrow), small cyst formation (arrowhead), epithelial vacuolation, and marked lymphocyte depletion (star) (lesion score of 4). (K) Severe atrophy of lymphoid follicles (arrow), epithelial vacuolation (arrowhead), and marked lymphocyte depletion (star) (lesion score of 5). (L) Severe atrophy of lymphoid follicles (arrow), epithelial vacuolation (arrowhead), marked fibrous connective tissues, and marked lymphocyte depletion (star) (lesion score of 5). (A-L) Hematoxylin and eosin stains, scale bar = 10 μm.
BF, bursa of Fabricius.

In addition, the bursal tissues from the vaccine B-challenged group revealed similar severe lesions (**Fig. 3L**) with the highest mean BLS. The BLS was similar in the unchallenged and challenged vaccine B groups.

Primers optimization

The primer pair and probe sequences of F3 'TGCCATCACTAGTCTCAGCG,' R3 'CGCAGTCCCATCAAAGCCTA,' and Probe3 'TCCAAAACCTTGTACTGGGTGCCA' flanking 834–939 of the challenge virus were highly specific (**Supplementary Fig. 1**).

VCN

IBDV RNA loads in lymphoid organs and cloacal swabs

The viral loads were determined in the bursa and spleen of all the challenged birds, and cloacal viral shedding was observed in the control-challenged birds (**Table 3**). After the first challenge (28 dpv), the bursal and spleen viral loads detected for the control challenged

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Table 3. Mean bursal, spleen, and cloacal swab virus copy number of the challenged virus in 28- and 35 days post-vaccination of SPF chickens

Groups	Mean virus copy number ± SD (log_{10})					
	VCN at 28 dpv			VCN at 35 dpv		
	Bursa	Spleen	Swab	Bursa	Spleen	Swab
Control	$11.09 \pm 0.15^{a,x}$	$10.19 \pm 0.13^{a,y}$	8.49 ± 0.10^{z}	$10.82 \pm 0.13^{a,x}$	$10.55 \pm 0.06^{a,y}$	8.05 ± 0.13^{z}
Vaccine A	$8.28 \pm 0.06^{b,x}$	$8.17 \pm 0.13^{b,x}$	ND	$8.12 \pm 0.02^{b,x}$	$7.79 \pm 0.14^{b,y}$	ND
Vaccine B	$7.84 \pm 0.10^{c,x}$	$7.37 \pm 0.17^{c,y}$	ND	$8.17 \pm 0.05^{b,x}$	$7.70 \pm 0.07^{b,y}$	ND

SPF, specific-pathogen-free; ND, no detectable virus.
[a,b,c]Significant differences within columns ($p < 0.05$).
[x,y,z]Significant differences within rows of values of the same sampling period ($p < 0.05$).

group were significantly higher ($p < 0.001$) than that detected for the vaccine A group. The lowest VCN was observed in the vaccine B group, which was significantly lower ($p = 0.003$) than the viral load for the vaccine A group (**Table 3**).

Similarly, after the second challenge (35 dpv), the detected bursal and spleen viral load was significantly higher ($p < 0.001$) in the control group than in the vaccinated groups (**Table 3**). The mean VCN values of the vaccinated groups were similar ($p > 0.72$). The viral RNA for the vaccine A group was similar ($p > 0.51$) to that of the vaccine B group (**Table 3**).

For the tissues and swab samples in both challenged periods, the viral loads were significantly higher in the bursa than in the spleen and lowest ($p < 0.001$) in the swab (**Table 3**).

DISCUSSION

This study evaluated the protective efficacy of two commercial IBD vaccines against a novel Malaysian vaIBDV strain in SPF chickens. The Ab titer, gross lesions, bursal and spleen indices, histopathological changes, lymphoid tissues, and cloacal swab viral load were evaluated to assess the effectiveness of these vaccines.

Although both vaccines elicited Ab responses, they did not fully protect the chickens from bursal damage or viral replication after the challenge. Although the vaccines alone induced similar atrophy to that observed in a field virus [21-24], vaccine B, a live attenuated vvIBDV vaccine, caused more bursal damage than the immune-complex vaccine (vaccine A). These findings support previous research indicating that live attenuated IBD vaccines can cause varying degrees of bursal destruction [25]. In contrast, some studies reported no bursal damage with live attenuated variant vaccine strain [26].

Vaccine A is less invasive than vaccine B, as indicated by the BLS and body weight gain. This result is consistent with the finding that the Icx vaccine produced less bursal damage than live IBD vaccines [10]. In contrast, Camilotti et al. [6] reported that the Icx IBD vaccine induced more severe bursal damage than the intermediate IBD vaccine. Although vaccine B is a live attenuated vvIBDV vaccine, it appears to have caused reduced feed intake, affecting the body weight gain after vaccination. In addition, splenomegaly is not expected to be observed at 21 dpv because the effects of the vaccine viruses on the spleen might have subsided [27]. Another study reported that adequate spleen development in different IBD vaccines is accomplished, resulting in no significant increase in spleen index [1].

Vaccines may have offered partial protection as significant bursal atrophy was not observed after the challenge in the vaccinated groups. Nevertheless, the challenge virus could induce

bursal damage in both vaccinated groups, possibly because of the inadequate protection from the available vaccine-induced immune response. The effect was unsurprising because some amino acid (aa) changes occurred in the VP2 region, which is critical for antigenicity [26,28-30]. This finding is consistent with a report that novel Chinese vaIBDV induced severe bursal damage in IBD-vaccinated chickens [16]. The antigenic variations often seen within the variable domain of IBDV contribute to the ability of a variant strain to escape the host immune response, leading to viral replication and bursal damage [29,30]. A previous study reported that a single aa mutation T222A or S254N of a vaIBDV escaped the parental variant vaccine-induced immune response [26]. In addition, Jiang et al. [31] reported that D279N substitution reduces the neutralizing ability of anti-vvIBDV against a novel vaIBDV.

Furthermore, although the challenge virus could replicate in the vaccinated group, both vaccines could reduce the extent of virus replication in the lymphoid tissues as the viral load detected in the control challenged group was significantly higher than in the vaccinated groups. Hence, the immune response induced by the vaccines could react with the challenge virus through non-specific or cellular immunity. Yeh et al. [32] reported this type of protection, where cell-mediated immune response protected IBDV challenge without Abs. The vaccine-induced Ab may be non-neutralizing because of aa mutations of the challenge virus at the neutralizing epitopes, 222T, 318D, and 323A [30]. Therefore, the Abs may not thoroughly neutralize the challenge virus, as reported elsewhere [16,25].

Surprisingly, in both phases of the experiments, the authors observed a significant increase in BLS after the challenge of birds vaccinated with vaccine A, and the detected challenge virus RNA was lower than in the control challenge bursa. Therefore, the authors concluded that the bursa of the vaccinated birds was not fully protected against the vaIBDV. On the other hand, the vaccine B group of birds showed no increase in BLS in the experiment in the presence of challenge virus RNA. The observation could be explained by the few regenerating lymphocytes that the challenge virus could replicate in the bursae and the lower viral RNA detection, particularly after the first challenge. Alternatively, the bursal damage with reduced B cells may have contributed to the lower viral RNA detection in the vaccinated groups. This observation is supported by the argument that vaccinated birds may be protected against an IBDV challenge due to a lack of target cells for the challenge virus because of vaccine virus-induced bursectomy [8,32]. The inability to differentiate between vaccine and challenge virus-induced damage is supported by Prandini et al. [21], who could not histologically distinguish vaccine and challenge virus-induced bursal lesions.

Interestingly, viral shedding was detected only in unvaccinated challenged birds of both challenges, suggesting that the vaccines might induced cell-mediated immunity where macrophage-induced functions play a significant role via the interferon pathway or dendritic function [33-35].

In conclusion, taking together the bursal indices, bursal lesion scores, and viral load in bursal tissue, neither of the tested vaccines fully protected against Malaysian vaIBDV challenge under the experimental conditions despite the high Ab titer production. Vaccine B induced more bursal damage than vaccine A. Hence, assessing bursal damage protection in birds vaccinated with vaccine B was challenging due to the vaccine virus-induced lesions. These findings highlight the limitation of current vaccines against emerging vaIBDV strains, emphasizing the need for further research to develop more effective vaccines.

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SUPPLEMENTARY MATERIALS

Supplementary Table 1

Primer set for the optimization and amplification of the UPM1432/2019 strain

Supplementary Fig. 1

Primer optimization and specificity for the challenge virus, UPM1432/2019, using quantitative reverse transcription polymerase chain reaction.

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