Original Article

Efficacy of immune complex infectious bursal disease vaccines against very virulent infectious bursal disease virus challenge in commercial layer chicken

Nataya Charoenvisal[1] Phornphisut Areeraksakul[1] Choew Kong Mah[2]

Nida Sirikobkul[3] Jiroj Sasipreeyajan[1*]

Abstract

The vaccination program for controlling Infectious bursal disease (IBD) was complicated due to high maternal antibody in day old chick. Therefore, our study was performed in young layer chickens with high maternal antibody as commonly seen in our country. The objective of this study was to determine the protective efficacy of immune complex IBD vaccines against very virulent infectious bursal disease virus (vvIBDV) challenge in commercial layer chickens. As immune complex vaccine A was recommended in layers by the company, the first aim of the study was to compare immune complex vaccine A (group 1) and B (group 3). The result showed a similar survival rate but group 1 had better body weight gain than group 3. Moreover, the negative control group had better body weight than group 3 but not significantly different. The serology profiles of group 1 and 3 were not significantly different. Bursa size, bursa to body weight ratios and bursal histopathological lesion scores of group 1 and 3 were not significantly different as well. The second aim was to compared immune complex vaccine A (vaccination one time at 1-day-old (group 1)) with immune complex vaccine A (vaccination at 1-day-old) and booster with live IBD vaccine at 25-days-old (group 2). The chickens which received a booster vaccine had a better survival rate, body weight gain and milder bursal lesion scores after receiving vvIBDV. In conclusion, vaccine A showed better weight gained in layers, while, the immune complex vaccine with a booster of live vaccine showed more promise on IBD protection.

Keywords: layers, immune complex IBD vaccine, very virulent IBD virus, pathogenicity, efficacy

[1]*Avian Health Research Unit, Department of Veterinary Medicine, Faculty of Veterinary Science, Chulalongkorn University, Henri-Dunant Street, Bangkok 10330, Thailand*

[2]*Zoetis International Operations Outcomes Research, 15/F, Sunyoung Center, 398 Jiangsu Road, Shanghai, P. R. China 200050*

[3]*Zoetis (Thailand), Co. Ltd., Bangkok, Thailand*

***Correspondence**: Jiroj.s@chula.ac.th* (J. Sasipreeyajan)

Received January 7, 2022

Accepted August 18, 2022

https://doi.org/10.14456/tjvm.2022.68

Introduction

Infectious bursal disease (IBD) or Gumboro disease is one of the major impact diseases in the poultry industry worldwide. The etiology of the disease is infectious bursal disease virus (IBDV), which is a non-enveloped, double-stranded RNA virus. The virus is highly contagious among chicken flocks. Mostly, it attacks chickens between 3 - 6 weeks-old. Broilers, breeders, and layers can be sick due to this virus but layers are the most susceptible. The virus' target organ is bursa of Fabicius, and the important threat is that the virus attacks the immune system of young chickens. The immunosuppressive conditions induced by the virus cause inefficacy of vaccination and also secondary infection by other pathogens (van den Berg, 2000, van den Berg *et al*., 2000, Eterradossi and Saif, 2020).

At present, IBDV is classified into 7 genogroups. The first 3 genogroups are well known and are circulating in many countries; genogroup 1 is predominantly classic IBDV, genogroup 2 is predominantly variant IBDV and genogroup 3 is predominantly very virulent IBDV (vvIBDV). Other genogroups are circulating in 1 or 2 countries; genogroup 4 contains strain dIBDV circulating in the United Arab Emirates, genogroup 5 circulating in Mexico, genogroup 6 circulating in Saudi Arabia and Italy and genogroup 7 circulating in Australia (V877 strain) and Russia (Michel and Jackwood, 2017). In Thailand, classic IBDV (genogroup 1) and vvIBDV (genogroup 3) have been reported (Jackwood and Sommer-Wagner, 2007). The other variants have not been reported in Thailand yet.

Several types of IBD vaccines have been developed. Most of the commercial live viral vaccines use classic IBDV strains (including Winterfield 2512 strain) as seed viruses and classify the pathogenicity of the vaccine as "mild", "intermediate" and "intermediate-plus or hot" vaccines. The intermediate-plus live vaccine has a strong ability to break through the maternally-derived antibody (MDA). The mild type vaccine breaks poorly through the MDA. The inactivated vaccine induces high antibody titer but takes more time than live vaccine to induce detectable titer. Moreover, it does not induce mucosal immunity. As a result, the inactivated vaccine is usually used in breeders to induce and pass the high level of antibody to their offspring as MDA. The virus-vector vaccine and immune complex vaccine were invented to solve the problem of MDA interference. An immune complex vaccine consists of IBD virus vaccine covered with IBDV-specific antibody (hyperimmune neutralizing serum). After the immune complex vaccine enters the chick body, it is trapped by follicular dendritic cells, macrophages and B cells, then, localized in the germinal center of chick's spleen or bursa (Jeurissen at al., 1998, Corley *et al*., 2002). Theoretically, the virus vaccine does not activate the immune system until the covering antibody fully diminishes in 14 days after vaccination (Muller *et al*., 2012, Ingrao *et al*., 2013).

The immune complex vaccines have been able to protect chickens against IBDV infection. Although, the antibody titer induced by the immune complex vaccine is not as high as the intermediate-plus vaccine or recombinant vaccine, the mild bursal lesion proves that the modified live virus replicates in the bursa (Abou El-Fetouh, 2020).

This study determined the protective efficacy of immune complex IBD vaccine in commercial layer pullets. The results compared the efficacy of 2 different strains of immune complex vaccine. In addition, a comparison was drawn between the efficacy of immune complex vaccine alone and immune complex vaccine that was followed by a booster vaccination with live IBD vaccine.

Materials and Methods

Animals: One hundred and fifty-eight (158) commercial layer chicks were moved from the hatchery to the animal facility at 1-day-old. Chicks were free from common chicken pathogens, screening by 1) the breeder flock did not detect any unhealthy condition and passed routine health check, 2) at hatchery this flock of chickens did not have Salmonellosis or Mycoplasmosis. Chickens were divided into 5 groups and housed in ABSL-2 facility at Chulalongkorn University Laboratory Animal Center (CULAC), Bangkok, Thailand. Chickens were monitored daily. Standard commercial feed for commercial layer pullets and water were provided *ad libitum*. Guidelines and legislative regulations on the use of animals for scientific purposes of Chulalongkorn University were followed as is certified in permission No. 1873013.

Vaccines and virus: Three types of commercial IBD vaccines were used in this study. Vaccine A is an immune complex IBD vaccine, each dose contains IBDV (strain V877) at least $10^{1.3}$ EID_{50} conjugated with hyperimmune antibodies against IBDV. Vaccine B is an immune complex IBD vaccine, each dose contains IBDV (strain Winterfield 2512 G-61) at least 0.1 CID_{50} conjugated with hyperimmune antibodies against IBDV. Route of administration of both vaccines was subcutaneous injection and was administered at 1-day-old, 0.1 ml per bird. Vaccine C was a lyophilized live IBD vaccine, each dose contains IBDV (strain V877) at least $10^{2.2}$ EID_{50}. The live vaccine was orally administered at 25-days-old. The challenge virus in this study was a Thai field isolate vvIBDV (genogroup 3), which is 95.77 percent of similarity compared to Thailand01 TH1 isolate (accession number DQ916245) by partial part (400 base-pairs) of VP2 gene (Jackwood and Sommer-Wagner, 2007). The virus was propagated in 9-day-old specific pathogen free chicken embryos on the chorioallantoic membrane in year 2018. The propagated virus was titrated using the reed-müench method and it was identified by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) that there was no Newcastle disease virus and Avian influenza virus contamination. Each chicken received the challenge virus approximately 4×10^5 EID_{50} by oral route (Wu *et al*., 2000; Chansiripornchai and Sasipreeyajan, 2009).

Experimental designs: One day old, 158 commercial layer chicks were divided into 5 groups. There were 30, 39, 30, 39 and 20 chickens in group 1, 2, 3, 4 and 5, respectively. Chickens in group 1 received vaccine A at 1-day-old. Chickens in group 2 received vaccine A at 1-day-old and a booster vaccine at 25-days-old. Chickens in group 3 received vaccine B at 1-day-old. Groups 4 and 5 were the non-vaccinated control groups. The vvIBDV was orally challenged in chickens in groups 1, 2, 3 and 4 individually at 35-day-old, with 20 chickens of each group. Chickens in group 4 were the non-vaccinated, challenged (positive control) group. Chickens in group 5 were the non-vaccinated, unchallenged (negative control) group. Mortality was recorded daily after vvIBDV challenge for 10 day-post-inoculation (DPI). All chickens were weighed at 1, 35 and 45-day-old. All surviving chickens were humanely euthanized at 45-days-old (10 DPI). Blood samples were collected at 1, 21, 25, 35 and 45-day-old. At 25-days-old, 10 chickens in group 2 and 4 were used for bursa collection. Then, at 35-days-old, 9 chickens in group 2 and 4, and 10 chickens in group 1 and 3 were used for bursa collection. At 45-days-old, all surviving chickens were used for bursa collection. The number of animals in vaccination, virus challenge, blood collection and bursa collection is shown in Table 1. The reason that the number of chickens in group 2 and 4 (39 chickens per group) is more than chickens in group 1 and 3 (30 chickens per group) is because 10 chickens in group 2 and 4 were used to examine the bursa size, bursa to body weight ratios and bursal histopathological lesion scores at 25-days-old. The reason that only bursa of group 2 chickens was compared with the group 4 chickens (control group) is because at 25-days-old group 1 and 2 had similar vaccinations and vaccine B which was administered to group 3 had been studied in a previous publication (Chansiripornchai and Sasipreeyajan, 2009).

Table 1 Number of animals in vaccination, virus challenge, blood collection and bursa collection

Group	No. of chick	Vaccination		IBDV challenge	Blood collection					Euthanasia and bursa collection		
		1 D	25 D	35 D	1 D	21 D	25 D	35 D	45 D[A]	25 D	35 D	45 D[A]
1	30	30 (Vac A)	0	20	5	20	20	20	17	ND	10	17
2	39	39 (Vac A)	30 (Vac C)	20	5			20	20	10	9	20
3	30	30 (Vac B)	0	20	5	20	20	20	17	ND	10	17
4	39	0	0	20	5	20	20	20	13	10	9	13
5	20	0	0	20	5				20	ND	ND	20

Note:
D = -day-old, ND = not determined
[A]All surviving chickens underwent blood collection and bursa collection

Serological evaluation: Blood samples were randomly collected at 1-day-old to determine maternally-derived antibody (MDA) against IBDV, 5 chickens per group. Then, 20 chickens per group were bled as described in the experimental designs. Antibodies against IBDV were determined by 2 commercial ELISA test kits (Synbiotics-IBD Classic, Synbiotics Corp., USA and IDEXX, IDEXX Laboratories, USA). The results were compared.

Examination of bursa of Fabricius: Gross lesions of the bursae were examined. The size of bursae was measured by Bursameter™ (provided by Zoetis (Thailand), Co. Ltd.). Then, bursae were weighed and bursa to body weight (B/BW) ratios (B/BW ratio = bursa weight (gm.) x 1,000 / body weight (gm.)) were calculated according to Kim *et al*. (1999). Bursae were fixed in 10% neutral buffered formalin, sectioned, and stained with H&E. Histopathological lesion scores (HLS) of bursae were evaluated (Muskett *et al*., 1979). The HLS protocol was score 0 for no damage, score 1 for mild necrosis in isolated follicles, score 2 for moderate generalized lymphoid depletion or isolated follicles with severe depletion, score 3 for over 50% of follicles with severe lymphocyte depletion, score 4 for the outline of follicles remaining with lymphocytes and increase in connective tissue, cysts and thickening corrugated epithelium, and score 5 for loss of all follicular architecture with fibroplasia.

Statistical analysis: Body weight, B/BW ratios, and antibody titers were analyzed and compared between groups using ANOVA and the least significant difference (LSD) test. Histopathological lesion scores of bursae were compared between groups using the Kruskal-Wallis test. Mortality rate was compared using the Chi-square test. Differences between groups were considered significant at $p<0.05$.

Results

Mortality rate: Chickens showed clinical signs of depression, anorexia and ruffled feathers (Fig. 1). Mortality rate was recorded for 10 DPI. There was no mortality observed in chickens in groups 2 (booster group) and 5 (the non-vaccinated, unchallenged control group). Groups 1 and 3 were 15% (3/20) mortality, while the non-vaccinated, challenged group (group 4) showed 35% (7/20) mortality (Table 2). The result indicated group 4 (non-vaccinated, challenged) had the highest mortality rate. Followed by group 1 (vaccine A) and group 2 (vaccine B), which showed a similar mortality rate.

Body weight: Body weight was recorded at 1, 35 and 45-day-old (Table 3). From 35 to 45-days-old, the average body weight gain of the non-vaccinated, unchallenged control group (group 5) was

185.00±17.01 grams. The average body weight gain of group 2 was 174.50±11.46 grams, which was not significantly different from group 5. The average body weight gain of group 1 was 124.35±41.28 grams which was significantly lower than those of groups 2 and 5. While the average body weight gain of groups 3 and 4 was 78.24±48.89 and 84.62±78.81 grams, respectively and significantly lower than those of groups 1, 2 and 5. The result showed that average body weight gain of group 2 (booster group) and group 5 (non-vaccinated, unchallenged control group) was higher than group 1 (vac A group). Then group 1 was higher than group 3 (vac B group) and 4 (non-vaccinated, challenged group).

Figure 1 Chickens in group 4 (non-vaccinated, challenged group) showed clinical signs of depression, anorexia and ruffled feathers.

Table 2 Mortality rate and survival rate

Group	Vaccine		Virus inoculation (day-old)	Mortality		Survival rate	
	1-day-old	25-day-old		Number^{A}	Percent	Number^{B}	Percent
1	Vaccine A	-	35	3/20	$15^{a,b}$	17/20	$85^{a,b}$
2	Vaccine A	Vaccine C	35	0/20	0^{a}	20/20	100^{a}
3	Vaccine B	-	35	3/20	$15^{a,b}$	17/20	$85^{a,b}$
4	-	-	35	7/20	35^{b}	13/20	65^{b}
5	-	-	-	0/20	0^{a}	20/20	100^{a}

Note:
^{A}Number of dead chickens / total chickens in the group
^{B}Number of surviving chickens / total chickens in the group
^{a,b}The different superscript in each column means statistically significant difference (p<0.05).

Table 3 Body weight and body weight gain

Group	Body weight (gm/bird)			Body weight gain^{C} (35 to 45-days-old)
	1-day-old	35-days-old (0 DPI)	45-days-old (10 DPI)	
1	41.17±2.21^{A} (30)^{B}	447±26.38 (20)	569±45.98^{a} (17)	124.35±41.28^{a} (17)
2	40.67±2.36 (29)	437±24.30 (20)	612±31.50^{b} (20)	174.50±11.46^{b} (20)
3	41.40±2.19 (30)	447±26.58 (20)	521±54.71^{c} (17)	78.24±48.89^{c} (17)
4	41.55±2.34 (29)	438±28.63 (20)	532±89.43^{c} (13)	84.62±78.81^{c} (13)
5	41.25±2.38 (20)	437±26.58 (20)	622±38.06^{b} (20)	185.00±17.01^{b} (20)

Note:
^{A}Mean ± standard deviation (SD) ^{B}Number of chickens in the group ^{C}Gm/bird
^{a,b,c}The different superscript in each column means statistically significant difference (p < 0.05).

Antibody titers against IBDV: The antibody titers against IBDV determined by Synbiotics and IDEXX ELISA test kits were in the same direction (Table 4). Chickens in all groups had high antibody titer at 1-day-old. The antibody titer of the non-vaccinated, unchallenged control group continuously declined until the end of the study. The non-vaccinated, challenged group showed high antibody titer at 10 DPI. Groups 1 (vac A group) and 3 (vac B group) also showed the same direction with the non-vaccinated, challenged group. However, group 2 (booster group) was different. At 25-days-old, determined by Synbiotics kit, only 10 out of 20 birds showed antibody titer higher than the cut-off value with a mean antibody titer of 416±456.36. By IDEXX kit, only 2 out of 20 birds showed measurable antibody titer with a mean antibody titer of 261±160.20. At 35-days-old or 10 days after booster with the live vaccine, mean antibody titer significantly increased to 3,203±2,054.92 (18 positive samples of 20 samples tested) determined by Synbiotics kit and 4,196±2,390.61 (19 positive samples of 20 samples tested) determined by IDEXX kit.

The result indicated that chickens in group 2 (booster group) had high antibody titer at 35-days-old because they responded to the live booster vaccine. But other groups had high titer only at 45-days-old, responding to the vvIBDV challenged.

Table 4 Antibody titers against IBDV measured by Synbiotics and IDEXX ELISA test kits

<table>
<tr><th rowspan="2">Group</th><th colspan="5">IBDV antibody titers (Synbiotics)</th></tr>
<tr><th>1-day-old</th><th>21-days-old</th><th>25-days-old</th><th>35-days-old</th><th>45-days-old</th></tr>
<tr><td>1</td><td>3,813±1,315.78^{A} (5/5)^{B}</td><td rowspan="2">807±821.45^{a} (12/20)^{a,b}</td><td rowspan="2">416±456.36 (10/20)</td><td>67±206.30^{a} (2/20)^{a}</td><td>6,566±1,279.09^{a} (17/17)^{a}</td></tr>
<tr><td>2</td><td>4,604±1,808.70 (5/5)</td><td>3,203±2,054.92^{b} (18/20)^{b}</td><td>6,198±1,552.18^{a,b} (20/20)^{a}</td></tr>
<tr><td>3</td><td>4,651±2,310.11 (5/5)</td><td>735±539.56^{a} (15/20)^{a}</td><td>323±436.89 (8/20)</td><td>31±139.31^{a} (1/20)^{a}</td><td>5,296±1,246.94^{c} (17/17)^{a}</td></tr>
<tr><td>4</td><td>3,755±2,349.11 (5/5)</td><td rowspan="2">336±444.94^{b} (8/20)^{b}</td><td rowspan="2">232±439.46 (5/20)</td><td rowspan="2">0±0^{a} (0/20)^{a}</td><td>5,030±1,184.40^{c} (13/13)^{a}</td></tr>
<tr><td>5</td><td>3,947±1,083.11 (5/5)</td><td>0±0^{d} (0/20)^{b}</td></tr>
<tr><td colspan="6">IBDV antibody titers (IDEXX)</td></tr>
<tr><td>1</td><td>4,848±1,930.62^{A} (5/5)^{B}</td><td rowspan="2">507±336.32^{a,b} (10/20)</td><td rowspan="2">261±160.20^{a} (2/20)^{a}</td><td>124±84.66^{a} (0/20)^{a}</td><td>6,730±1,365.84^{a} (17/17)^{a}</td></tr>
<tr><td>2</td><td>4,759±1,960.82 (5/5)</td><td>4,196±2,390.61^{b} (19/20)^{b}</td><td>4,930±1,264.61^{b} (20/20)^{a}</td></tr>
<tr><td>3</td><td>4,059±1,995.63 (5/5)</td><td>631±454.76^{b} (13/20)</td><td>439±384.33^{b} (10/20)^{b}</td><td>188±228.64^{a} (2/20)^{a}</td><td>6,377±1,229.29^{a} (17/17)^{a}</td></tr>
<tr><td>4</td><td>3,164±2,193.05 (5/5)</td><td rowspan="2">397±210.30^{a} (7/20)</td><td rowspan="2">304±172.16^{a,b} (5/20)^{a,b}</td><td rowspan="2">134±105.64^{a} (1/20)^{a}</td><td>6,227±1,178.40^{a} (13/13)^{a}</td></tr>
<tr><td>5</td><td>5,110±1,855.17 (5/5)</td><td>51±95.39^{c} (1/20)^{b}</td></tr>
</table>

Note:
^{A} Mean ± standard deviation (SD) ^{B} Number of positive samples / Total samples tested
^{a,b,c,d} The different superscript in each column means statistically significant difference ($p < 0.05$).

Examination of bursa of Fabricius: During necropsy, IBDV specific lesions were observed and bursa were collected. The results of bursa size measured by Bursameter™, bursa to body weight (B/BW) ratios and histopathological lesion scores (HLS) of bursae were rather in the same way (Table 5). Measuring by Bursameter™, bursae of groups 1, 3 and 4 were significantly bigger than those of group 2 at 35-day-old (Fig. 2). After vvIBDV inoculation, necropsy was performed on dead chickens and bursa lesions were observed (Fig.3). At 45-days-old (10 DPI), bursae of groups 1, 2, 3 and 4 were not significantly different in size but significantly smaller than those of the non-vaccinated, unchallenged control group (group 5) (Fig.4). The bursa to body weight ratios of group 2 was significantly lower than that of groups 1, 3 and 4 at 35-days-old. At 10 DPI, B/BW ratios of chickens in groups 1, 2, 3 and 4 were not significantly different but were significantly different from those of group 5. The average HLS of bursae of chickens in group 2 was 2.89±0.78 at 35-days-old, while other groups showed a score of 0 on the same day. At 10 DPI, the results of HLS of groups 1, 3 and 4 were not significantly different. While the score of group 2 was significantly lower than that of groups 1, 3, and 4. The score of group 5 showed a score of 0, which means no lesion was present in the bursae. Two bursae from group 4 (non-vaccinated, challenged group) were collected and performed RT-PCR and sequencing confirmed that it was the same virus as the challenge virus.

The result indicated that bursa of chicken in group 2 (booster group) were mildly to moderately atrophied since 35-days-old and responded to live vaccine booster. Then, at 45-days-old, bursae of group 2 were not as responsive to vvIBDV as group 1, 3 and 4.

Table 5 Size of bursae measured by Bursameter™, B/BW ratios, and HLS

Group	Size of bursae			B/BW ratios			HLS		
	25 D	35 D (0 DPI)	45 D (10 DPI)	25 D	35 D (0 DPI)	45 D (10 DPI)	25 D	35 D (0 DPI)	45 D (10 DPI)
1	ND	7.90±0.57[a] (10)	4.53±0.51[a] (17)	ND	6.18±1.53[a] (10)	1.56±0.31[a] (17)	ND	0±0[a] (10)	3.93±0.92[a] (14)
2	5.70±0.48[A] (10)[B]	5.22±0.44[b] (9)	4.45±0.51[a] (20)	5.46±0.72[A] (10)[B]	2.43±0.50[b] (9)	1.33±0.19[a] (20)	0±0[A] (10)[B]	2.89±0.78[b] (9)	1.93±0.59[b] (15)
3	ND	7.50±0.53[a,c] (10)	4.35±0.49[a] (17)	ND	5.54±1.29[a] (10)	1.73±0.26[a] (17)	ND	0±0[a] (10)	3.80±1.21[a] (15)
4	5.60±0.52 (10)	7.11±0.33[c] (9)	4.38±0.51[a] (13)	5.87±1.13 (10)	6.0±0.77[a] (9)	1.74±0.34[a] (13)	0±0 (10)	0±0[a] (9)	4.31±0.75[a] (13)
5	ND	ND	7.00±0.65[b] (20)	ND	ND	5.83±1.61[b] (20)	ND	ND	0±0[c] (20)

Note:
D = -day-old, ND = not determined
[A] Mean ± standard deviation (SD) [B] Number of bursae examined
[a,b,c] The different superscript in each column means statistically significant difference (< 0.05).

Figure 2 This picture shows formalin fixed bursae from chickens in group 1-4 at 35-days-old. Bursae of groups 1, 3 and 4 were significantly bigger than those of group 2

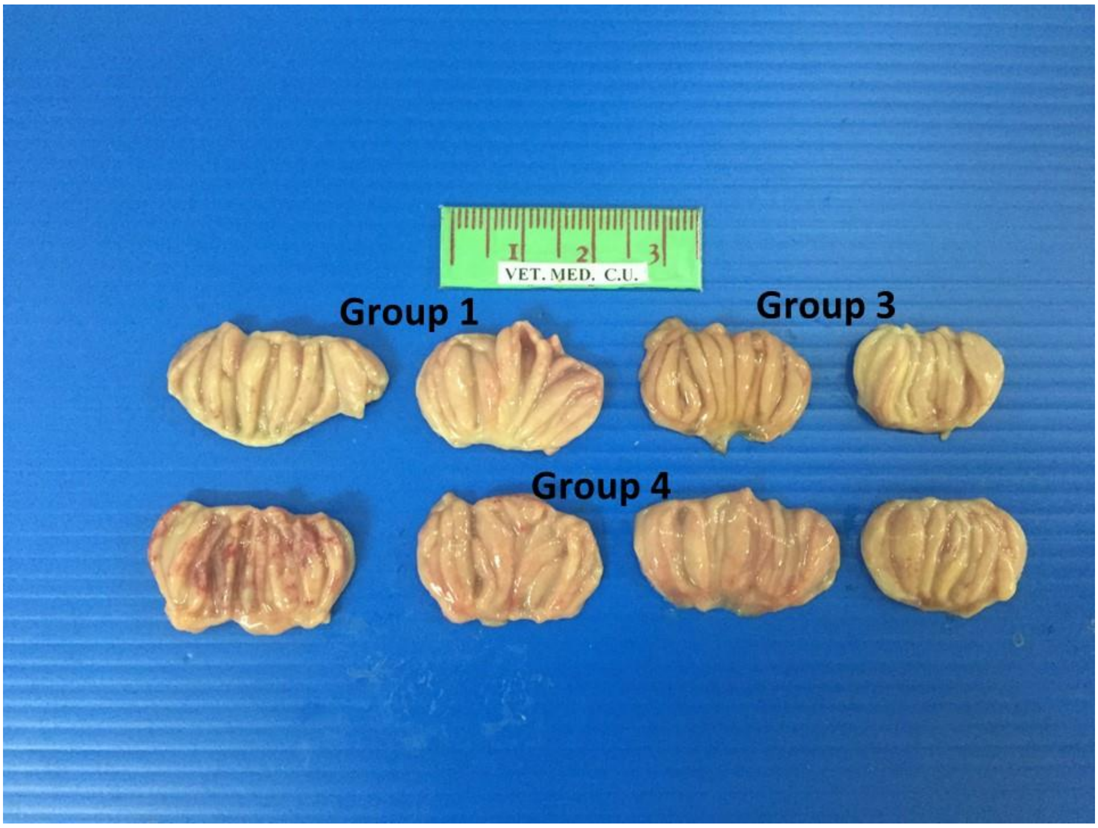

Figure 3 This picture shows bursa of dead chickens after vvIBDV challenge. Bursal hemorrhage was observed in some bursa.

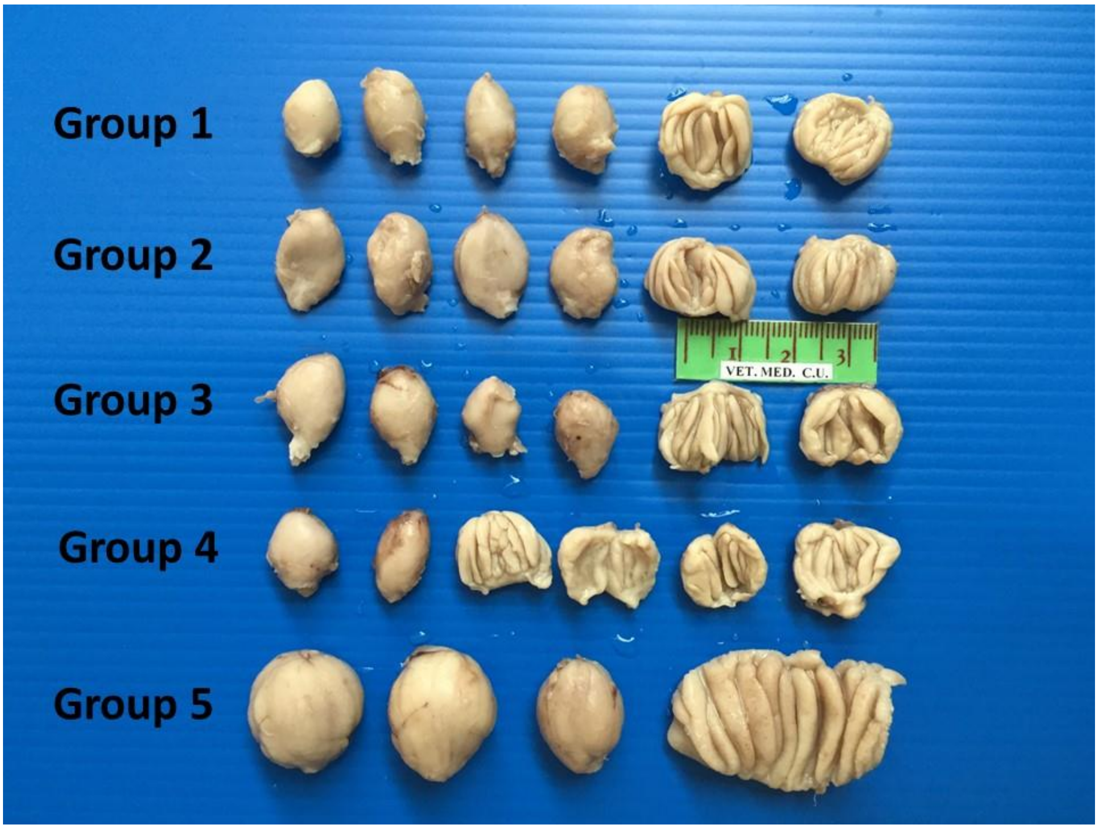

Figure 4 This picture shows formalin fixed bursae from chickens in group 1-5 at 45-days-old (10 DPI). Bursae of chickens in groups 1, 2, 3 and 4 were not significantly different in size but significantly smaller than those of the non-vaccinated, unchallenged control group (group 5)

Discussion

MDA is one of the major challenges in planning an IBDV vaccination program for long. As a result, recombinant and immune complex vaccines have been developed. But most studies on immune complex vaccine have been done in SPF and broiler chickens (Haddad *et al*., 1997, Ivan *et al*., 2005, Hassanzadeh *et al*., 2006, Camilotti *et al*., 2016, Cazaban *et al*., 2018, Muniz *et al*., 2018, Arshad *et al*., 2019, Sedeik *et al*., 2019, Abou El-Fetouh *et al*., 2020). Thus, this study is the first study to investigate the efficacy of immune-complex vaccine in layer chickens.

The study shows that chickens in group 2 which were vaccinated with immune complex vaccine A at 1-day-old and a booster with the live vaccine at 25-days-old had the best protection against vvIBDV. The results of mortality rate and body weight gain were significantly better than other challenged groups and not significantly different from the non-vaccinated, unchallenged control group. The antibody titers at the challenge day were significantly higher than the groups that did not receive the booster vaccine. As a result, the immunity of chickens in group 2 was able to protect all of them against the challenge virus and there were no chickens in this group that died due to vvIBDV. Moreover, at 10 DPI, bursa size and B/BW ratios of chickens in group 2 were not significantly different from other challenged groups. However, group 2 was the only group that showed bursal lesion scores before IBDV challenge due to live virus vaccine, resulting in significantly milder bursal lesion scores after IBDV challenge.

In Thailand, either broiler or layer farms in IBDV endemic areas usually perform at least 2 times vaccination of IBDV vaccine. Due to high IBDV, MDA in day old chicks is preferred. However, high MDA may neutralize live vaccine. So, a vaccine booster is necessary and usually done when the MDA declines. In this study, we administered a live vaccine booster to group 2 chickens at 25-days-old because in the past 5 years IBDV field challenge generally has been occurring at 5-6 weeks-old. As a result, the vaccine booster was proposed to move forward to 25-day-old. Moreover, the strain of virus vaccine is one of the widely discussed topics. In field practice, strain V877 has been recommended for use in layers because the live vaccine strain of Winterfield 2512 is possibly too strong. As layers are more susceptible to IBDV than broilers, layers may develop mild pathogenic lesions and immunosuppression from strong intermediate-plus vaccine. So live attenuated strain V877 (Vaccine C) was chosen for a booster vaccine.

To be practical in comparing the serological results of this study with field practice, 2 commercial ELISA test kits were used. The reason is that Synbiotics was marketed in Thailand before, so it was commonly used by farms in Thailand. However, IDEXX is commonly used worldwide. Comparing groups 1 and 3 that only received different immune complex vaccines at 1-day-old, the mortality rate was no different. However, group 1 showed better weight gain than group 3. As group 3 chickens showed the lowest average body weight gain, this may be the result of vaccine B being too strong for layers. The antibody titers at the challenge day were low and not significantly different from each other. The antibody titers at 10 DPI for both groups were high and could be the result of virus infection. Although, at 10 DPI, antibody titer of chickens in group 1 determined by Synbiotics ELISA test kit were significantly higher than those of group 3, it cannot be concluded that the titer of both groups were not significantly different when measured by IDEXX kit. The bursa size, B/BW ratios and the HLS of the bursae were not significantly different between groups 1 and 3 and also did not significantly differ from the non-vaccinated, challenged group.

However, earlier studies showed that immune complex vaccine alone was able to induce adequate antibody (Ivan *et al*., 2005, Cazaban *et al*., 2018, Muniz *et al*., 2018, Arshad *et al*., 2019) and were able to protect the chickens against IBDV challenged (Haddad *et al*., 1997, Hassanzadeh *et al*., 2006, Camilotti *et al*., 2016, Sedeik *et al*., 2019, Abou El-Fetouh *et al*., 2020). Other studies in SPF chickens showed seroconversion as early as 14 days after vaccination (Ivan *et al*., 2005, Muniz *et al*., 2018, Abou El-Fetouh *et al*., 2020). While in broilers with MDA, the seroconversion showed on day 21 to 28 post-vaccination, depending on the MDA level (Ivan et la., 2005, Cazaban *et al*., 2018, Muniz *et al*., 2018, Abou El-Fetouh *et al*., 2020). The differences between SPF and commercial broiler chickens were the MDA. The IBD virus vaccine of immune complex vaccine did not activate host immune response until the MDA declined to the lowest level (Ivan *et al*., 2005, Hassanzadeh *et al*., 2006). As a result, chickens with high MDA titer showed a slow immune response to the immune complex IBD vaccine (Muniz *et al*., 2018). Therefore, our study was performed in layers in which the half-life of MDA declined more slowly than in broilers (de Wit, 2001). Our study started with high MDA at one day old. At 35-days-old, chickens vaccinated with the immune complex vaccines alone, still showed similar antibody titer levels to the non-vaccinated control group which indicates that the vaccinated chickens did not develop active immunity against the immune complex IBD vaccine. In contrast, chickens that received the booster vaccination with the live vaccine showed high antibody titer at 35-days-old. The mentioned serological response indicates that the intermediate-plus vaccine may able to break through the high MDA and induce the acquired immune response. But the immune complex vaccine alone has to wait for the diminishing of MDA.

The examination of the bursae including, the size of the bursae, B/BW ratios and the HLS of the bursae was pointed out in the same way as the antibody titer. Many studies concluded that immune complex vaccines were able to be the cause of bursal atrophy with mild lesions such as mild follicular depletion (Jeurissen *et al*., 1998, Camilotti *et al*., 2016, Arshad *et al*., 2019). The lesions were due to the replication of virus vaccine in the bursae. After replication of the virus in the bursa, an active immune response was induced (Sedeik *et al*., 2019). In our study, the size of the bursae, B/BW ratios and the HLS of the bursae of the immune complex vaccination groups at 35-days-old were no different from the non-vaccination control group.

The results of this study showed that chickens which received either immune complex vaccine had a better survival rate than the non-vaccinated group. Nevertheless, the booster of the live vaccine was also important in the farms located in the vvIBDV endemic area. The IBD vaccination program of immune complex vaccine at 1-day-old will provide up to 100% of protection if the chickens receive the booster vaccine at the appropriate time. Moreover, the booster of the live vaccine was able to help prevent damage of the bursa due to vvIBDV infection. In low IBDV incidence areas, single vaccination with live IBDV vaccine may provide adequate immunity but the appropriate time is uncertain. So, the booster vaccination was considered to make sure that all chickens had enough immunity. Meanwhile, chicken flocks with low MDA in the low IBDV incidence area receiving only immune complex vaccine in ovo or at 1-day-old, were enough to protected the chickens from IBDV infection (Camilotti *et al*., 2016, Haddad *et al*., 1997).

However, it should be borne in mind that the best vaccine and vaccination program should be applied with strict biosecurity. Proper cleaning and disinfection of chicken houses helps reduce the number of viral loads and also provides more time for chicken immunity to be mature (Ingrao *et al*., 2013). As a result, farm biosecurity is also important and cannot be ignored.

Acknowledgements

This study was financially supported by Zoetis and the Avian Health Research Unit of the Ratchadaphiseksomphot Endowment Fund, Chulalongkorn University.

References

Abou El-Fetouh, M.S., Hafez, M.H., El-Attar, E.R., and El-Agamy, M.E. 2020. Immune-Complex infectious bursal disease virus versus live attenuated vaccines to protect SPF chicken against very virulent virus challenge. J World Poult Res. 10(4): 556-564.

Arshad, R.W., Aslam, A., Imran, M.S., Asharal, K. and Akhtar, R. 2019. Comparative evaluation of live vector, immune complex and intermediate plus vaccines of infectious bursal disease in broiler chicken. Pakistan J Zool. 51(5): 1837-1841.

Cazaban, C., Swart, W.B.F., Rietema, R.M.W., de Wit, J.J., Palya, Y. and Garyin, Y. 2018. Field assessment of an immune-complex infectious bursal disease vaccine in chicks born to non-hyperimmunized broiler breeders. J Vet Sci Ani Husb. 6(3): 304-313.

Camilotti, E., Moraes, L.B., Furian, T.Q., Borges, K.A., Moraes, H.L.S. and Salle, C.T.P. 2016. Infectious bursal disease: pathogenicity and immunogenicity of vaccines. Braz J Poult Sci. 18(2): 303-308.

Chansiripornchai, N. and Sasipreeyajan, J., 2009. Comparison of the efficacy of the immune complex and conventionally live vaccine in broilers against infectious bursal disease infection. Thai J Vet Med. 39(2): 115-120.

Corley, M.M., Giambrone, J.J. and Dormitorio, T.V. 2002. Evaluation of the immune response and detection of Infectious bursal disease viruses by Reverse Transcriptase-Polymerase Chain Reaction and Enzymed-Linked Immunosorbent Assay after In ovo vaccination of commercial broilers. Avian Dis. 46: 803–809.

de Wit, S. 2001. Gumboro Disease: estimation of optimal time of vaccination by the deventer formula. In: Annual Report and Proceedings of COST Action 839: Immunosuppressive Viral Diseases in Poultry. 170-178.

Eterradossi, N. and Saif, Y.M. 2020. Infectious bursal disease. In: Diseases of Poultry. 14th edition. Editor in chief D.E. Swayne. Wiley and Sons, Inc. USA. 257-283.

Haddad, E.E., Whitfill. C.E., Avakian, A.P., Ricks, C.A., Andrews, P.D., Thoma, J.A. and Wakenell., P.S. 1997. Efficacy of a novel infectious bursal disease virus immune complex vaccine in broiler chickens. Avian Dis. 41: 882-889.

Hassanzadeh, M., Bozorgmehri, F. and Tooluo, A. 2006. Evaluation of the immunogenicity of immune complex infectious bursal disease vaccine delivered in ovo to embryonated eggs or subcutaneously to day-old chickens. Inter J Poult Sci. 5(1): 70-74.

Ingrao, F., Lambrecht, R.F. and van den Berg, T. 2013. Infectious bursal disease: a complex host-pathogen interaction. Dev Comp Immunol. 41: 429-438.

Ivan, J., Velhner, M., Ursu, K., German, P., Mato, T., Dren, C.N. and Meszaros, J. 2005. Delayed vaccine virus replication in chickens vaccinated subcutaneously with an immune complex infectious bursal disease vaccine: quantification of vaccine virus by real-time polymerase chain reaction. The Canadian J Vet Res. 69: 135-142.

Jackwood. D.J. and Sommer-Wagner, S. 2007. Genetic characteristics of infectious bursal disease viruses from four continents. Virology, 365: 369-375.

Jackwood, D.J., Sommer-Wagner S.E., Stoute, S.T., Woolcock, P.R., Crossley, B.M., Hietala, S.K. and Charlton, B.R. 2009. Characteristics of a Very Virulent Infectious Bursal Disease Virus from California. Avian Dis. 53(4): 592–600.

Jeurissen, S.H.M., Janse, E.M., Lehrbach, P.R., Haddad, E.E., Avakian, A. and Whitfill, C.E. 1998. The working mechanism of an immune complex vaccine that protects chickens against infectious bursal disease. Immunol. 95: 494-500.

Kim I.J., Gagic, M. and Sharma, J.M. 1999. Recovery of Antibody-Producing Ability and Lymphocyte Repopulation of Bursal Follicles in Chickens Exposed to Infectious Bursal Disease Virus. Avian Dis. 43:401-413.

Muller, H., Mundt, E., Eterradossi, N. and Islam, M.R. 2012. Current status of vaccines against infectious bursal disease. Avian Path. 41(2): 133-139.

Muniz, E.C., Resende, M.S., Silva, A.F.A., Verdi, R., Di Fabio, J. and Bordin, E.L. 2018. Histopathology and serology reaction to an immune complex infectious bursal disease vaccine (V877 strain) in SPF and commercial birds. ARS Vet J SP. 34(2): 69-76.

Muskett, J.C., Hopkins, I.G., Edwards, K.R. and Thornton, D.H. 1979. Comparison of two infectious bursal disease vaccine strains: efficacy and potential hazards in susceptible and maternally immune birds. Vet Rec. 104:332–334.

Sedeik, M.E., El-shall, N.A., Awad, A.M., Abd El-Hack, M.E., Alowaimer, A.N. and Swelum, A.A. 2019. Comparative evaluation of HVT-IBD vector, immune complex, and live IBD vaccines against vvIBDV in commercial broiler chickens with high maternally derived antibodies. Animals. 72(9): 1-13.

Van den Berg, T.P. 2000. Acute infectious bursal disease in poultry: a review. Avian Path. 29: 175-194.

Van den Berg, T.P., Eterradossi, N., Toquin, D. and Meulemans, G. 2000. Infectious bursal disease (Gumboro disease) (review article). Rev Sci Tech Off Inn Epiz. 19(2): 527-543.

Wu, C.C., Dorairajan, T. and Lin, T.L. 2000. Effect of ascorbic acid supplement on immune response of chickens vaccinated and challenged with infectious bursal disease virus. Vet Immunol Immunopathol. 74(1-2): 145-152.