

## Efficacy of Infectious Bursal Disease Vaccine in Broiler Chickens Receiving Different Vaccination Programs

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### *Abstract*

The objective of this study was to evaluate the efficacy of 3 infectious bursal disease vaccination programs in commercial broiler chickens. One hundred and forty, 1-day-old female broiler chickens were divided into 4 groups of 35 chickens each. Groups 1-3 were vaccinated with infectious bursal disease (IBD) vaccine via oral administration at 1, 1 and 16, and 16-day-old, respectively. Group 4 served as a positive control group. Body weight, sera collection, bursa: body weight (B:BW) ratios and histopathological lesion scores (HLS) were evaluated at 16, 30 and 40-day-old. All the chickens in groups 1 to 4 were challenged with IBD virus at 30-day-old. The survival rate of groups 1-4 were 95.83, 100, 100 and 95.83 percent, respectively. Body weight, B:BW ratios and HLS of each group at 16, 30 and 40-day-old were not significantly different ( $p>0.05$ ). At 7 and 16-day-old, the average antibody levels of the 1-day-old vaccinated group were significantly lower than the non-vaccinated at 1-day-old group ( $p<0.05$ ). At 30 and 40-day-old, there was no significant difference in antibodies against IBD virus in all the groups ( $p>0.05$ ). In conclusion, the vaccination at 1-day-old, 1 and 16-day-old and 16-day-old did not cause adverse effects to body weight, B:BW ratios and HLS.

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**Keywords:** broiler chickens, Infectious bursal disease, vaccination program

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## บทคัดย่อ

### ประสิทธิภาพของวัคซีนป้องกันโรคเบอร์ซาอักเสบดีดื้อในไก่เนื้อที่ได้รับโปรแกรมวัคซีนต่างกัน

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วัตถุประสงค์ของการศึกษาเพื่อประเมินประสิทธิภาพของ 3 โปรแกรมวัคซีนป้องกันโรคเบอร์ซาอักเสบดีดื้อในไก่เนื้อ ไก่เนื้อเพศเมียอายุ 1 วัน จำนวน 140 ตัว แบ่งออกเป็น 4 กลุ่มๆละ 35 ตัว โดยกลุ่มทดลองที่ 1-3 ได้รับวัคซีนป้องกันโรคเบอร์ซาอักเสบดีดื้อ ด้วยวิธีการหยอดปาก ที่อายุ 1 วัน ที่อายุ 1 และ 16 วัน และที่อายุ 16 วัน ตามลำดับ ไก่ทดลองกลุ่มที่ 4 เป็นกลุ่มควบคุม ไม่ได้รับวัคซีน ทำการชั่งน้ำหนัก เก็บตัวอย่างซีรัม วัดอัตราส่วนระหว่างน้ำหนักต่อมเบอร์ซาและน้ำหนักตัวไก่ และให้คะแนนรอยโรคทางจุลพยาธิวิทยา เมื่อไก่อายุ 16, 30 และ 40 วัน ไก่ทุกตัวได้รับการป้อนเชื้อพิษ ที่อายุ 30 วัน ไก่กลุ่ม 1-4 มีอัตราการรอดชีวิต ร้อยละ 95.83, 100, 100 และ 95.83 ตามลำดับ ในขณะที่ไม่พบความแตกต่างของน้ำหนักตัวไก่ อัตราส่วนระหว่างน้ำหนักต่อมเบอร์ซาและน้ำหนักตัวไก่ และการให้คะแนนรอยโรคทางจุลพยาธิวิทยาของไก่ทุกกลุ่ม เมื่อไก่อายุ 16, 30 และ 40 วัน ( $p>0.05$ ) ที่อายุ 7 และ 16 วัน ระดับแอนติบอดีเฉลี่ยของไก่ที่ได้รับวัคซีนที่อายุ 1 วัน มีระดับแอนติบอดีต่ำกว่าของไก่ในกลุ่มที่ไม่ได้รับวัคซีนที่อายุ 1 วัน ( $p<0.05$ ) ที่อายุ 30 และ 40 วัน ระดับแอนติบอดีต่อไวรัสเบอร์ซาอักเสบดีดื้อของไก่ทดลองทั้ง 4 กลุ่มไม่มีความแตกต่างกัน ( $p>0.05$ ) สรุป การให้วัคซีนป้องกันโรคเบอร์ซาอักเสบดีดื้อในไก่เนื้อที่อายุ 1 วัน อายุ 1 และ 16 วัน หรือ อายุ 16 วัน ไม่ส่งผลเสียต่อน้ำหนักตัวไก่ อัตราส่วนระหว่างน้ำหนักต่อมเบอร์ซาและน้ำหนักตัวไก่ และคะแนนรอยโรคทางจุลพยาธิวิทยา

**คำสำคัญ:** ไก่เนื้อ โรคเบอร์ซาอักเสบดีดื้อ โปรแกรมวัคซีน

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## Introduction

Infectious bursal disease (IBD) or Gumboro disease is caused by a virus of the Birnaviridae family. The virus has double-stranded RNA. The disease can be found in chickens and other avian species such as ducks, turkeys and ostriches. Only infected chickens show clinical signs but no clinical signs had been found in other infected avian species (Wyeth, 2000). The clinical signs of infected chickens relate to the age of the chickens and the virulence and the strain of virus. The classical strain of IBD virus (IBDV) belonging to serotype 1, shows the ability to cause clinical disease in infected chickens (Rautenschlein et al., 2003). The clinical signs are depression, reluctance to move, ruffled feathers, loss of appetite, white watery diarrhea and death 2-3 days after the clinical disease has been diagnosed (Chansiripornchai and Sasipreeyajan, 2005). The major symptoms of clinical diseased chickens are dehydration, petechial hemorrhage at the thigh and breast muscles or the juncture between the proventriculus and the ventriculus. Bursa of Fabricius of infected chickens reveals inflammation, edema, hemorrhage or atrophy depending on the course of the infection (Chansiripornchai and Sasipreeyajan, 2009; Sharma et al., 2000). The infection caused by the variant strain of serotype 1 reveals non clinical disease (Elankumaran et al., 2002).

Important methods of IBD prevention and control in the poultry industry are disinfection, biosecurity and vaccination at the appropriate time (Chansiripornchai and Sasipreeyajan, 2009). The commercial vaccines can be divided into 3 groups depending on the virulence of the virus origin; mild, intermediate and intermediate-plus or hot vaccines. The viruses in mild and intermediate vaccine are frequently destroyed by maternal derived antibodies (MDA) in the chicks. Mild and intermediate vaccines will be used in the chicks that have a low level of MDA (Tsukamoto et al., 1995) and the vaccinated chicks should have a booster dose in next 2 weeks to get the optimum antibody protection against IBD infection. On the other hand the intermediate-plus or hot vaccines are suitable for the high MDA chickens (Haddad et al., 1997) because the virus in vaccines can break through the MDA and stimulate the active antibodies against the IBDV in vaccines (Chansiripornchai and Wanasawaeng, 2009). Currently, many IBD vaccines have been developed such as an immune complex vaccine to lessen the neutralizing effect between IBDV and MDA (Chansiripornchai and Sasipreeyajan, 2009; Haddad et al., 1997) or a genetically modified vaccine of the VP2 gene of viral RNA (Chang et al., 2002). The MDA have an affect on the suitable time of the IBD vaccination,

due to the neutralization between the virus in the vaccine and MDA. The objective of this study was to evaluate the efficacy of 3 IBD vaccination programs in commercial broiler chickens.

### Materials and Methods

**Chickens:** One hundred and forty, 1-day-old female Arbor Acres broiler chickens were obtained from a commercial hatchery (Krungthai, Chonburi, Thailand). The chickens were fed *ad libitum* (Betagro, Bangkok, Thailand). At the onset of the experiment (16-day-old), there was no statistical significant difference in the average weight of the experimental groups ( $p>0.05$ ). The guidelines and legislative regulations on the use of animals for scientific purposes of Chulalongkorn University, Bangkok, Thailand were followed as is certified in permission no. 44/2537.

**Vaccines:** The commercial vaccines, Univac-BD® and Bursa Vac® were intermediate and intermediate plus vaccines, respectively, belonging to the classical type of Moulthrop strain. The vaccine was applied according to the manufacture's recommendation.

**Experimental designs:** One hundred and forty, 1-day-old female broiler chickens were divided into 4 groups of 35 each. In group 1, the chickens were vaccinated with IBDV vaccine of the classical type 1, Moulthrop strain (Univac-BD®, Intervet-Schering Plough Animal Health, Nebraska, USA) at 1-day-old by subcutaneous injection. In group 2, the chickens were vaccinated with IBDV vaccine (Univac-BD®) by subcutaneous injection at 1-day-old and also the chickens were vaccinated with IBDV vaccine of the classical type 1, Moulthrop strain (Bursa Vac®, Intervet-Schering Plough Animal Health, Nebraska, USA) by the oral route at 16-day-old. In group 3, the chickens were vaccinated with IBDV vaccine of the classical type 1, Maltrop strain (Bursa Vac®) by the oral route at 16-day-old. Group 4 served as a positive control group and the chickens were not vaccinated but were challenged with IBDV. Sera were collected at 1 day of age for maternal antibody determination. The broilers were weighed and sera were collected at 7, 16, 30 and 40-day-old. The collected sera were tested for IBD antibodies by ELISA (Synbiotics, USA). Feed intakes were measured for the calculation of the feed

conversion ratio (FCR). At 30-day-old, all the chickens were challenged orally with IBDV-CU-1, a local strain of very virulent IBDV at a concentration of  $4 \times 10^5$  embryo infective dose (EID<sub>50</sub>/bird) for 300 µl/dose (Chansiripornchai and Sasipreeyajan, 2009). Following these vaccinations and challenges, all the chickens were observed for any adverse clinical symptoms (morbidity) and mortality for 10 days.

**Bursa: Body weight ratios and bursal scoring:** Bursa of Fabricius was collected at 16, 30 and 40-day-old for histopathology lesion scoring (HLS) and calculation of the bursa/body weight (B:BW) ratios for 10 chickens in each group. The B:BW ratios were calculated by the bursa of Fabricius weight (g)/ body weight (g)  $\times 1000$ . Bursal histopathology scoring was performed according to Muskett et al., 1979 following: 0: no damage, 1: mild necrosis in isolated follicles, 2: moderate generalized lymphocyte depletion or isolated follicles with severe depletion, 3: over 50% of follicles with severe lymphocyte depletion, 4: outline of follicles only remaining with few lymphocytes and an increase in connective tissue, cysts and thickened corrugated epithelium, 5: loss of all follicular architecture with fibroplasia.

**Statistical analysis:** FCR and antibody titers were analyzed and compared using Duncan's multiple range test. HLS was calculated using Kruskal Wallis test. Differences between groups were considered significant at  $p<0.05$ . Statistical analysis was calculated by SPSS for Window.

### Results

**Antibody titers against IBDV:** Antibody titers against IBDV were measured at 7 and 16-day-old. There were significantly different antibody titers ( $p<0.05$ ) between the non vaccinated chickens at 1 day old and the vaccinated chickens at 1-day-old (Table 1). Before challenge (30-day-old), only one chicken in group 1 showed antibody titer against IBDV but there was no significant difference between groups ( $p>0.05$ ) (Table 3). Ten days after challenge (40-day-old), all groups of chickens showed rising antibody titers with no significant difference ( $p>0.05$ ) (Table 4).

**Table 1** Antibodies against IBDV at 7 and 16-day-old (Mean $\pm$ standard deviation)

Group	Antibodies against IBDV	
	7-day-old	16-day-old
Non vaccinated at 1-day-old	4,209 $\pm$ 1,262 <sup>a</sup> (20/20)*	1,200 $\pm$ 717 <sup>a</sup> (19/20) <sup>a</sup>
Vaccinated at 1-day-old	3,157 $\pm$ 1,228 <sup>b</sup> (19/20)	621 $\pm$ 664 <sup>b</sup> (11/20) <sup>b</sup>

Different superscripts mean statistically significant difference ( $p<0.05$ )

Number of positive/ Total number tested

**Bursal: body weight ratios and histopathological lesion scores:** At 16-day-old, there was no significant difference in the B:BW ratios and HLS between the non vaccinated chickens at 1-day-old and vaccinated chickens at 1-day-old ( $p>0.05$ ) (Table 2). Before (30-

day-old) and after challenge (40-day-old), there was no significant difference in the B:BW ratios and HLS among all groups ( $p>0.05$ ) (Table 3 and 4). Anyhow, at 30-day-old, all groups of chickens revealed higher

B:BW ratios and lower HLS compared to those of 10 days after challenge. After challenge, the lymphocytes in the bursal follicles were severely decreased by more than 50% and there was more connective tissue and cyst formation on the epithelium of bursal

follicles. There was no significant difference in the mortality rate among all groups ( $p>0.05$ ). One chicken in groups 1 and 4 died; a 95.8% survival rate. By contrast in groups 2 and 3, no chickens died; a 100% survival rate.

**Table 2** Pathological changed of Bursa of Fabricius at 16-day-old (Mean±standard deviation)

Group	Pathological changed at 16-day-old	
	Bursa:Body weight ratio	Histopathological lesion score
Non vaccinated at 1-day-old	2.0923±0.627 <sup>a</sup>	0.9±0.32
Vaccinated at 1-day-old	2.2051±0.453 <sup>a</sup>	0.9±0.32

Different superscripts mean statistically significant difference ( $p<0.05$ )

**Table 3** Body weight (gram/bird), B:BW ratios, HLS and antibodies against IBDV at 30-day-old (Mean±standard deviation)

Group	Body weight	B:BW	HLS	Antibodies against IBDV
1	1,056.18±117.48 <sup>a</sup>	2.74±0.55 <sup>a</sup>	0.1±0.32 <sup>a</sup>	37±165 (1/20)*
2	1,092.94±98.98 <sup>a</sup>	2.35±0.50 <sup>a</sup>	0.2±0.42 <sup>a</sup>	0 (0/20)
3	1,073.82±109.32 <sup>a</sup>	2.13±0.61 <sup>a</sup>	0.3±0.48 <sup>a</sup>	0(0/20)
4	1,034.41±106.58 <sup>a</sup>	2.16±0.56 <sup>a</sup>	0.5±0.53 <sup>a</sup>	0(0/20)

Different superscripts mean statistically significant difference ( $p<0.05$ )

\*Number of positive/Total number tested

**Table 4** Body weight (gram/bird), B:BW ratios, HLS and antibodies against IBDV at 40-day-old (Mean± standard deviation)

Group	Body weight	B:BW	HLS	Antibodies against IBDV
1	1,363.33±208.05 <sup>a</sup>	0.73±0.18 <sup>a</sup>	4.50±0.69 <sup>a</sup>	3,479±1,107 <sup>a</sup> (10/10)*
2	1,443.04±108.48 <sup>a</sup>	0.82±0.18 <sup>a</sup>	4.50±0.61 <sup>a</sup>	3,309±537 <sup>a</sup> (10/10)
3	1,445.22±108.20 <sup>a</sup>	0.81±0.21 <sup>a</sup>	4.25±0.85 <sup>a</sup>	2,576±711 <sup>a</sup> (10/10)
4	1,276.96±100.92 <sup>a</sup>	0.78±0.16 <sup>a</sup>	4.35±0.75 <sup>a</sup>	3,289±770 <sup>a</sup> (10/10)

Different superscripts mean statistically significant difference ( $p<0.05$ )

\*Number of positive/Total number tested

## Discussion

The efficacy of IBD vaccination program was related to the level of MDA in the chickens. The MDA of chickens can impede the virus in vaccine infected to the target cells and also reduce the ability of virus in vaccine to stimulate the chicken's immune system (Chansiripornchai and Wanasawaeng, 2009; Naqi et al., 1983). Anyhow, the MDA is of benefit to IBDV infection in chickens at the age of 1-4 weeks (Al-Natour et al., 2004). Kreider et al. (1991) divided the ELISA titer of the MDA of 1-day-old chickens into 3 level; the low level (<3,000), intermediate level (3,000-5,000) and high level (>6,000). In our experiment, the average MDA of 1-day-old chickens was 6,740±1,282 which was categorized in the high level of MDA. The formula of determining the appropriate age of chickens for IBD vaccination was the mean square root titer of MDA at 1-day-old – the mean square root of target titer/2.82 (Kouwenhoven and van den Bos, 1995). For intermediate plus vaccine, an MDA equal to 334 is the suitable time for vaccination. According to the formula, the appropriate date of intermediate plus vaccination in the experiment is 22-day-old. Therefore, vaccination at 1 or 16-day-old may be

affected by MDA resulting in the partial neutralization of the vaccine virus by MDA before the vaccine virus can stimulate chickens' immunity against IBDV infection. At 30-day-old, no antibody titers against IBDV were detected in groups 2, 3 and 4 and also a low level of antibody against IBDV was detected in group 1. These results may have been caused by the early application of vaccines. At 30-day-old, the antibody against IBDV of chickens vaccinated at 1-day-old was low and did not differ from the control group. Komine et al. (1995) and Knoblich et al. (2000) reported that subcutaneous vaccination of IBDV vaccine at 1-day-old, would not accelerate the reduction of antibodies and antibodies would not be detected at 28-day-old. Also, the antibodies of these chickens would be higher and longer than the antibodies of non vaccinated chickens. Moreover, the detection of antibodies against IBDV after challenge revealed that subcutaneous injection at 1-day-old would stimulate the active immunity of chickens (Haddad et al., 1997).

No difference of B:BW ratios of all groups of chickens at 16 and 30-day-old indicated that subcutaneous vaccination at 1-day-old and oral vaccination at 16-day-old did not effect the

development of bursa of Fabricius. Anyhow, at 40-day-old (10 days after vaccination), the bursa of all groups of chickens was reduced and histopathological damage, indicated that the experimental chickens were susceptible to the challenge virus. The results accorded with the no detection of antibodies against IBDV at 30-day-old (before challenge). Also, at 30-day-old, no active immunity had been developed due to the effect of the intermediate or high level of MDA (Winterfield and Thacker, 1978; Tanimura et al., 1995). After challenge, there was no significant difference ( $p>0.05$ ) in B:BW ratios and HLS of all groups because there was no difference in antibody titers before challenge. The challenge virus resulted in the high level of HLS and also the bursal follicles were destroyed in all groups of vaccination and non vaccination. Anyhow, the survival rate of chickens in each group was more than 95%. The heavy breed chickens reveal lower mortality than the light breed chickens (Bumstead et al., 1993; Chansiripornchai and Wanasawaeng, 2009).

From the experiment, the efficacy of IBDV vaccine in broilers was related to the level of MDA against IBDV at the vaccination date. Vaccination at 1-day-old, 1 and 16-day-old and 16-day-old of chickens that have the ELISA titers of MDA of more than 6,000 at 1-day-old may not be effective enough to elicit the antibodies at 30-day-old. More research should be performed. Further study in low or intermediate MDA chickens should be done. Experiments on virus protection from challenge and the availability of the virus in infected organs are required for evaluation of the efficiency of a vaccination program.

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