Original Article

Comparison of the Efficacy of the Immune Complex and Conventionally Live Vaccine in Broilers against Infectious Bursal Disease Infection

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Abstract

One hundred and thirty two, one day old broiler chickens were divided into 4 groups. Group 1 (1 day old) was subcutaneously vaccinated with immune complex vaccine into the nape of the neck. Group 2 and 3 (14 days old) were vaccinated with different commercial products, intermediate-plus strains of live attenuated infectious bursal disease (IBD) vaccine I and II, respectively, via the oral route. Group 4 acted as the positive control. Chickens were bled at days 1, 14, 28 and 38 for evaluating IBD virus antibody titers by ELISA. The body weight, FCR, bursa to body weight ratios and bursal lesion scores at days 14, 28 and 38 were compared. All groups were challenged with local strain of IBD virus at 28 days old. The study revealed that the antibody titers of group 3 were significantly higher ($p<0.05$) than those of the other groups at day 28. At 38 days old, the body weight of group 2 was significantly lower ($p<0.05$) than that of group 3. The bursal lesion scores of group 3 were significantly lower ($p<0.05$) than those of other groups. In conclusion, the immune complex vaccine was shown to be safe for 1 day old vaccination.

Keywords : broiler chickens, immune complex vaccine, infectious bursal disease, intermediate plus vaccine

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บทคัดย่อ

การเปรียบเทียบประสิทธิภาพของวัคซีนอิมมูนคอมเพล็กซ์และวัคซีนชนิดเชื้อเป็นในไก่เนื้อในการป้องโรคเบอร์ซาอักเสบติดต่อ

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ไก่เนื้อคละเพศอายุ 1 วัน จำนวน 132 ตัว แบ่งออกเป็น 4 กลุ่ม กลุ่มที่ 1 ได้รับวัคซีนอิมมูนคอมเพล็กซ์ เมื่ออายุ 1 วัน ด้วยวิธีฉีดเข้าใต้ผิวหนังบริเวณคอ กลุ่มที่ 2 และ 3 ได้รับวัคซีนเชื้อเป็นชนิดรุนแรงปานกลางพิเศษชนิดที่ 1 และ 2 ตามลำดับโดยการหยอดปากเมื่ออายุ 14 วัน กลุ่มที่ 4 เป็นกลุ่มควบคุมที่ไม่ได้รับวัคซีน ทำการเจาะเลือดไก่เพื่อตรวจหาระดับของแอนติบอดีต่อไวรัสเบอร์ซาอักเสบติดต่อเมื่ออายุ 1 14 28 และ 38 วัน ทำการเปรียบเทียบน้ำหนักตัว อัตราแลกเนื้อ ค่าดัชนีต่อมเบอร์ซา และค่าคะแนนรอยโรคทางจุลพยาธิวิทยาเมื่อไก่อายุ 14 28 และ 38 วัน ในวันที่ 28 ของการทดลองทำการฉีดเชื้อพิษให้กับไก่ทุกกลุ่ม ผลการทดลองแสดงให้เห็นว่าที่อายุ 28 วัน ระดับแอนติบอดีของไก่ในกลุ่มที่ 3 สูงกว่าไก่กลุ่มอื่นอย่างมีนัยสำคัญ ($p<0.05$) ที่อายุ 38 วัน น้ำหนักของไก่กลุ่มที่ 2 ต่ำกว่าไก่กลุ่มที่ 3 อย่างมีนัยสำคัญ ($p<0.05$) และค่าคะแนนรอยโรคทางจุลพยาธิวิทยาของไก่กลุ่มที่ 3 ต่ำกว่าไก่กลุ่มอื่นอย่างมีนัยสำคัญ ($p<0.05$) จากการทดลองนี้สรุปว่า วัคซีนอิมมูนคอมเพล็กซ์มีความปลอดภัยในการป้องกันโรคเมื่อนำมาใช้ในลูกไก่อายุ 1 วัน

คำสำคัญ : ไก่เนื้อ วัคซีนอิมมูนคอมเพล็กซ์ โรคเบอร์ซาอักเสบติดต่อ คซีนอินเตอร์ชนิดรุนแรงปานกลางพิเศษ

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Introduction

Infectious bursal disease (IBD) is an important disease that causes damage to the poultry industry. The first disease outbreak occurred in 1957 in Gumboro, Delaware, USA (Cosgrove, 1962). The disease is caused by the infectious bursal disease virus (IBDV), an Avibirnavirus. It has been classified as part of the Birnaviridae family. The virus is resistant to acid conditions (pH 3), ether and chloroform, resulting in the difficulty in eradicating the virus from infected farms (Murphy et al., 1999). The virus can be divided into 3 strains, according to their virulence; 1) the classical strain 2) the variant strain and 3) the very virulent strain. Virus mutation will lead to a new strain that can cause more virulence. VP2 variable domain (vVP2) is the point of most mutation of the virus (van den Berg, 2000). The virus targets the B-cells of bursa of Fabricius. Infected chickens of less than 3 weeks old will show no clinical signs but a severe immuno-suppressive condition will have occurred. Clinical signs can be seen when chickens are infected at the age of 3-6 weeks by IBDV (Lukert and Saif, 1997; Chansiripornchai and Sasipreeyajan, 2005; Sasipreeyajan et al., 2007). Infected chickens reveal depression, ruffled feathers, anorexia, white watery diarrhea, dehydration and death. Necropsied findings show pale carcasses, hemorrhaging at the pectoral and thigh muscles and the juncture of the proventriculus and the gizzard, swollen kidneys and urate to be found in the kidneys (Chansiripornchai and Sasipreeyajan, 2005). Around 3-4 days after infection, swollen, gelatinous, yellowish exudates covering the bursa are found. By day 8 of infection, the bursa will decrease in size and the bursal weight will be reduced by one-third of the normal weight (Cheville, 1967). Layer chickens display severe clinical signs and a higher mortality rate than broiler chickens (Chansiripornchai and Sasipreeyajan, 2005). The mortality rate in layers is 1-50% as opposed to 3% in broilers (Müller et al., 2003). Infectious bursal disease can

be prevented by the vaccination of breeders and broilers. Killed vaccine is frequently used in breeders and is expected to convey on immunity and protect offspring at the age of 1-3 weeks old. Live vaccine is frequently used in broilers to stimulate active immunity. The major problem of IBD vaccination in broilers is neutralization between the virus in the vaccines and the maternally derived antibody (MDA) against IBD resulting in no virus from vaccine being able to stimulate immunity against IBDV. This problem can be overcome by antibody detection in chicks in order to find an appropriate time for vaccination (van den Berg and Meulemans, 1991). Furthermore, more virulent viruses are always occurring so new vaccines have to be developed to tackle the virulent strains. The virus-antibody complex concept was developed to overcome the neutralizing antibody effect between the vaccine virus and the MDA. In the virus-antibody complex, vaccine virus binds to the virus neutralizing factor (VNF). Therefore, the virus-antibody complex will not be neutralized by MDA and also the virulent effect of vaccine virus to the chickens is reduced (Haddad et al., 1997). Progressively, the antibodies are metabolized, as well as VNF and the vaccine virus will release and synchronize with the decrease of MDA. When the vaccine virus is stronger than the VNF-MDA action, the virus will start replication and vaccination. The virus in vaccine will be protected and will not be damaged by MDA against IBD and other diseases (Negash et al., 2004). The objective of this study was to compare the efficacy of immune complex and conventionally live IBD vaccines administered at 1 and 14 days, respectively.

Materials and Methods

Experimental animals: Unvaccinated 1 day old ROSS-308 broiler chicks, obtained from a commercial hatchery were used for the vaccine efficacy studies. The chicks were maintained in isolation units. They were fed *ad lib* on commercial poultry feed (Betagro, Bangkok, Thailand). Chickens were identified individually by numbered leg tags. 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. 04/2005.

Experimental designs: Chicks were divided into 4 groups. In group 1 the thirty-six broilers were vaccinated subcutaneously at the nape of the neck with immune complex vaccine (IBDV strain W2512 and IBDV antibodies) 0.1 ml/dose at 1 day old according to the manufacturers' recommendation. In group 2 the thirty-two broilers were vaccinated orally with live IBD vaccine 30 μl/dose, intermediate plus I (strain W2512) at 14 days old. In group 3 the thirty-two broilers were vaccinated orally with live IBD vaccine 30 μl/dose intermediate plus II (strain W2512) at 14 days old. Each dose of vaccine contains approximately 10^2 EID_{50} of IBDV. Group 4 was the unvaccinated control group in which the thirty two broilers received no vaccine. The broilers were weighed and sera were collected at 1, 14, 28 and 38 days 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 28 days old, all the chickens were challenged orally with IBDV-CU-1, a local strain of IBDV which has been proven to be very virulent IBDV (Sasipreeyajan, 2004) at the concentration of $4x10^5$ embryo infective dose (EID_{50})/bird for 300 μl/dose (Wu et al., 2000; Chansiripornchai and Sasipreeyajan, 2005). 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 scoring: Bursa of Fabricius was collected at 14 and 28 days old for histopathology scoring and calculation of the bursa/body weight ratios (B/BW ratios). At 14 days old, representative bursa of Fabricius were collected from groups 1 and groups 2-4; a total of 6 of each. At 28 days old, 6 bursas were collected from each group of 1-4. The B/BW ratios were calculated by the bursa of Fabricius weight (g)/ body weight (g) x 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 were analyzed and compared between groups using Duncan's multiple range test. Morbidity and mortality were calculated using Chi-square values. Differences between groups were considered significant at $p<0.05$. Statistical analysis was calculated by SPSS for Window version 9.0.

Results

The average body weights of the chickens in groups 1, 2, 3 and 4 at 1, 14, 28 and 38 days old were shown in table 1. No significant difference were found ($p>0.05$) in the average body weight of each group at 1, 14 and 28 days old. At 38 days old (10 days after challenge), the average body weight of chickens in group 2 was significantly less than that of chickens in group 3 ($p<0.05$). The FCR of the chickens between 1-14 and 14-28 days old in groups 1, 2, 3 and 4 was shown in table 1. The FCR of chickens during 28-38 days old is shown in table 2. The B/BW ratios at 14 days old of the chickens in groups 1 and 2-4 were 1.84±0.34 and 2.01±0.40, respectively. The B/BW ratios at 28 days old of the chickens in groups 1, 2, 3 and 4 were 2.03±0.31, 2.16±0.48, 2.59±0.37 and 2.14±0.39, respectively. At 14 and 28 days old, no significant difference in the B/BW ratios was found ($p>0.05$). The B/BW ratios at 38 days old are shown in table 2. The bursal scoring at 14 days old for chickens in groups 1 and 2-4 was 0.00±0.00 and 0.17±0.41, respectively. At 28 days old, the bursal scoring of the chickens in groups 1, 2, 3 and 4 was 0.17±0.41, 0.17±0.41, 0±0 and 0±0, respectively. There was no significant difference in bursal scoring at 14 and 28 days old. At 38 days old, the bursal scoring of the chickens in groups 1, 2, 3 and 4 is shown in table 2. The chickens in group 3 showed a significantly lower bursal score than others groups ($p<0.05$). The antibody titers tested by ELISA are shown in table 3. At 28 days old, the antibody titers of the chickens in group 3 were significantly higher than the other groups ($p<0.01$). After challenge (28-38 days old), the mortality rate was shown in table 2. No statistical difference was found in the mortality rate.

Discussion

MDA has an influence on the IBD vaccination program. Haddad et al. (1997) divided the MDA at 1 day old into 3 groups; the low (<3000) intermediate (3000-5000) and high (>5000) group. In this experiment, 10% of the antibody belonged to the low group, 55% of the antibody belonged to the intermediate group and 35% of the antibody belonged to the high group. The average antibody level at 1 day old was 4579±1529. At 14 days old, no significant difference in body weight, FCR, B/BW ratios, bursal histopathology scores and antibody level was found among group 1 and other groups, meaning that the vaccine in group 1 had no effect on growth rate, lesions and MDA. In this experiment, the vaccine in group 1 was also saved for vaccination at 1 day old, contrary to Hair-Bejo et al. (2004) where using the hot strain of vaccine in 1 day old broilers affected body weight, bursa and MDA. At 28 days old, the antibody level in group 3 was significantly higher than the other groups ($p<0.05$). The antibody titer of the broilers in group 1 was 69±215. This might have been caused by some virus neutralizing factor (VNF) still binding to the virus in the vaccine resulting in the active immunity not working properly. The antibody titer of broilers in group 2 was 255±323 because the antibody was produced by the vaccination at 14 days old. At 28 days old, no antibody titer was detected in group 4 (the unvaccinated control group). The MDA was gradually reduced from 1 to 28 days old so no antibody titer was detected at 28 days old according to Skeeles et al. (1979) where the half life of MDA was 3-5

days. According to the results of antibody titers against IBD at 28 days old, the potency of vaccines could be arranged. The vaccine that vaccinated in group 3 was more potent than the vaccine in group 2 and the vaccine used in group 1 (immune complex vaccine) was the least potent. Between 28-38 days old, broilers revealed clinical signs 2 days after challenge and died 3 days after challenge. However, no significant difference was found in the mortality rate of any group. The number of antibodies of the broilers in groups 1-3 was higher

Table 1 Average body weight (mean±SD) of chickens at 1, 14, 28 and 38 days old and FCR of chickens during 1-14 and 14-28 days old

Group	Average body weight (gram)				FCR	
	1 day old	14 days old	28 days old	38 days old	1-14 days old	14-28 days old
1	49.17±0.72	411.25±23.00	1194.58±151.46	1700.00±97.88[a,b]	1.32	1.65
2	49.17±2.60	401.88±31.03	1215.21±128.77	1578.50±156.75[a]	1.33	1.57
3	47.92±0.72	408.75±20.86	1250.00±75.74	1714.13±97.38[b]	1.26	1.55
4	49.17±0.72	401.25±23.04	1193.75±93.42	1625.00±181.99[a,b]	1.28	1.59

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

Table 2 FCR (28-38 days old), average B/BW ratios (mean±SD), bursal scores (mean±SD) and mortality rate at 38 days old (10 days post challenge)

Group	FCR	Average B/BW ratios	Average bursal scores	Mortality rate
1	2.55	0.60±0.15[a,b]	4.16±1.12[a]	2/23*
2	3.00	0.65±0.12[a]	4.58±0.84[a]	4/24
3	2.60	0.52±0.12[b]	2.74±1.48[b]	0/23*
4	2.60	0.63±0.14[a,b]	4.21±0.92[a]	2/24

Different superscripts mean statistically significant difference ($p<0.05$).
*one chicken of each group was culled before the challenge.

Table 3 average antibody titer (mean±SD) and numbers of sera collection (positive titer/serum samples) of chickens at 1, 14, 28 and 38 days old

Day / Group	1 day old	14 days old	28 days old	38 days old
1	4579±1529* (20/20)***	785±655[d] (14/20)	69±215[a] (2/20)	2569±735[c] (10/10)
2		725±577[d]** (14/20)	255±323[a] (8/20)	2976±1260[c] (10/10)
3			917±948[b] (13/20)	2362±1137[c] (9/10)
4			0±0[a] (0/20)	1918±875[c] (1/10)

Different superscripts mean statistically significant difference ($p<0.05$).
*average antibody titer of chickens in groups 1-4,
**average antibody titer of chickens in groups 2-4,
***numbers of sera collection (positive titer/serum samples)

than the antibodies of chickens in group 4 due to the vaccination effect. The B/BW ratios of the chickens in group 3 was significantly less than that of chickens in group 2 ($p<0.05$). The body weight of the chickens in group 3 was higher than that of the chickens in group 2 resulting in lower B/BW ratios of the chickens in group 3 compared to the B/BW ratios of chickens in group 2. The bursal histology scores of the chickens in group 3 were significantly lower than the other groups ($p<0.05$) revealing that the vaccine in group 3 tended to have greater efficacy in protecting IBD infection than the vaccine in group 2. In conclusion, the immune complex vaccine is safe for the vaccination of 1 day old chicks but the vaccine could not protect the damage of bursa of Fabricius. Moreover, immune complex vaccine that is vaccinated *in ovo* or at 1 day old can reduce the stress from a normal vaccination program at between 10-14 days old.

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