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การตอบสนองทางภูมิคุ้มกันชนิดสารน้ำต่อเชื้อไวรัสต่อมเบอร์ซ่าอักเสบติดต่อในไก่เนื้อที่มีภูมิคุ้มกันจากแม่ในระดับสูงภายหลังการได้รับวัคซีนชนิดไวรัสเชื้อเป็น ชนิดสารประกอบเชิงซ้อนทางอิมมูน และชนิดดีเอ็นเอสายผสม

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บทคัดย่อ: การศึกษาการตอบสนองของภูมิคุ้มกันชนิดสารน้ำต่อเชื้อไวรัสต่อมเบอร์ซ่าอักเสบติดต่อในไก่เนื้อที่มีภูมิคุ้มกันจากแม่ในระดับสูงลูกไก่อายุ 1 วันจำนวน 1,200 ตัวถูกแบ่งออกเป็น 4 กลุ่มๆ ละ 300 ตัว กลุ่มที่ 1 เป็นกลุ่มควบคุมที่ไม่ได้รับวัคซีนไวรัสต่อมเบอร์ซ่าอักเสบติดต่อกลุ่มที่ 2 เป็นกลุ่มที่ได้รับวัคซีนชนิดเชื้อเป็นสายพันธุ์ Winterfield 2512 โดยการผสมน้ำดื่มเมื่ออายุ 2 สัปดาห์ กลุ่มที่ 3 และ 4 ได้รับวัคซีนชนิดสารประกอบเชิงซ้อนทางอิมมูน (Live immune complex vaccine) และชนิดดีเอ็นเอสายผสม (recombinant vaccine) ตามลำดับโดยการฉีดเข้าใต้ผิวหนังเมื่อไก่อายุ 1 วัน ทำการเจาะเก็บเลือดไก่ก่อนและหลังการทำวัคซีนทุกสัปดาห์เพื่อตรวจหาระดับภูมิคุ้มกันต่อเชื้อไวรัสต่อมเบอร์ซ่าอักเสบติดต่อโดยใช้วิธี enzyme-linked immunosorbent assay (ELISA) ผลการทดสอบพบว่าระดับภูมิคุ้มกันหลังจากได้รับวัคซีน 1 สัปดาห์ ในกลุ่มที่ใช้วัคซีนชนิดสารประกอบเชิงซ้อนทางอิมมูนและวัคซีนชนิดดีเอ็นเอสายผสมมีระดับการตอบสนองทางภูมิคุ้มกันสูงกว่ากลุ่มที่ไม่ได้รับวัคซีนอย่างมีนัยสำคัญทางสถิติ ($P<0.05$) ในขณะที่กลุ่มที่ได้รับวัคซีนชนิดเชื้อเป็นสายพันธุ์ Winterfield 2512 ภายหลังที่ไก่ได้รับวัคซีน 3 สัปดาห์ แต่ระดับการตอบสนองทางภูมิคุ้มกันไม่มีความแตกต่างทางสถิติเมื่อเปรียบเทียบกับกลุ่มที่ได้รับวัคซีนทั้งสารประกอบเชิงซ้อนทางอิมมูนและวัคซีนชนิดดีเอ็นเอสายผสมที่อายุ 5 สัปดาห์ (5 สัปดาห์หลังได้รับวัคซีน) นอกจากนี้อัตราส่วนของต่อมเบอร์ซ่าต่อน้ำหนักตัวไก่ที่อายุ 5 สัปดาห์ พบว่ามีค่าเท่ากับ1.30±0.49, 1.35±0.72, 1.32±0.54 และ 1.64±0.44 สำหรับกลุ่มที่ 1-4 ตามลำดับ ซึ่งอัตราส่วนดังกล่าวไม่มีความแตกต่างทางสถิติซึ่งแปลผลที่ได้ว่าวัคซีนต่อมเบอร์ซ่าอักเสบติดต่อทั้ง 3 ชนิดไม่มีผลกระทบต่อการพัฒนาต่อมเบอร์ซ่าในไก่การศึกษาครั้งนี้สรุปได้ว่าวัคซีนต่อมเบอร์ซ่าอักเสบติดต่อทั้ง 3 ชนิดมีการตอบสนองต่อการสร้างภูมิคุ้มกันชนิดสารน้ำได้อย่างมีนัยสำคัญทางสถิติ รวมทั้งไม่มีผลรบกวนต่อการพัฒนาต่อมเบอร์ซ่าในไก่ที่มีภูมิคุ้มกันจากแม่ในระดับสูง

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Humoral Immune Response against Infectious Bursal Disease Using Live Attenuated, Live Immune Complex and Recombinant Vaccines in Broilers with High Maternal Derived Antibody Level

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Abstract: The aim of present study was to evaluate the immunological response of 3 difference Infectious bursal disease (IBD) vaccines in broiler chickens, even with high maternal derived antibody level. One thousand and two hundreds1-day-old broilers were divided into 4 groups. The chickens in the first group were unvaccinated with any IBD vaccine. The chickens in the second were vaccinated by the use of live attenuated vaccine Winterfield 2512 strain in drinking water at 2-week-old. The chickens in the third and fourth groups were vaccinated with live immune complex and recombinant vaccines by subcutaneous injection at 1-day-old, respectively. Chicken blood samples were collected at 1-day-old chick, once a week after vaccination and weekly until slaughtering. Subsequently, IBD antibody titer levels were measured by using enzyme-linked immunosorbent assay (ELISA) method. The chickens were vaccinated with both live immune complex-, as well as recombinant-vaccinated showed statistically significant high immune response compared with those in the unvaccinated group. However, all 5-week-old vaccinated chickens showed no difference of IBD antibody titer level. In addition, Bursa of Fabricius to body weight (B:BW) ratio at 5-week-old, were showed to be 1.30±0.49, 1.35±0.72 1.32±0.54 and 1.64±0.44 for the first to fourth group respectively, meaning that this parameter did not interfere with the Bursa of Fabricius developing after IBD vaccination. The present study indicated that all tested types of IBD vaccine generated significant responsiveness in term of humoral immunity and did not impair the Bursa of Fabricius development, even in the presence of high level of maternal derived antibody in broilers.

Keywords: Infectious bursal disease, Live attenuated vaccine, Live immune complex vaccine, Recombinant vaccine

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Introduction

Gumboro disease or Infectious bursal disease (IBD), is a serious problem for the poultry industry, due to significant economic losses resulting from high mortality and immunosuppression, especially in young chickens. However, IBD virus is very resistant under environmental conditions, and as a result, the pathogens still persist in chicken houses. Therefore, controlling and prevention of this disease depend mainly on vaccination (Al-Natour *et al*., 2004).

Currently, the commercial vaccines can be classified into 3 groups, depending on the virulence of virus, namely mild, intermediate and intermediate-plus. The main problem of mild and intermediate vaccines concerns vanishing maternal derived antibodies (MDA) in young chicks, so these vaccines should be applied when MDA level became low (Tsukamoto *et al*., 1995) while re-vaccination in next 2 weeks is needed for optimal antibody protection against IBD infection. Nevertheless, several researchers reported that the intermediate-plus vaccine could be applied even during high MDA chickens (Haddad *et al*., 1997), because the vaccine virus could break through the MDA, and also stimulate the active immunity and enhance antibodies against the IBDV (Chansiripornchai and Wanasawaeng, 2009).

Nowadays, there are several technologies for IBD vaccines development, such as an immune complex vaccine to lessen the neutralizing effect between IBDV and MDA (Chansiripornchai and Sasipreeyajan, 2009; Haddad *et al*., 1997), including a genetically modified vaccine, especially regarding the VP2 gene of viral RNA (Chang *et al*., 2002) namely recombinant vaccine.

The objective of the present study was to evaluate the immunological response of difference 3 IBD vaccine types: live attenuated, live immune complex and recombinant vaccine, in commercial broiler chickens even when possessing high maternal derived antibody.

Materials and Methods

Chickens and feeding

One thousand and two hundred, 1-day-old mixed breed (Cobb 500 and Ross 308) of broiler chickens, were obtained from a commercial hatchery (Saraburi, Thailand). The chickens were fed *ad libitum* (Betagro, Bangkok, Thailand).

Vaccines

The commercial IBD vaccines representative for a live attenuated Winterfield 2512 strain, a live immune complex, and a recombinant vaccine which is turkey Herpesvirus expressing the VP2 gene of the IBDV, were used for the present study.

Experimental designs

One thousand and two hundred broilers, were divided into 4 groups of 300 each. The first group was not vaccinated with any IBD vaccine, being a control group. The second group was vaccinated with live attenuated vaccine by drinking water at 14-day-old. The third and fourth group, were vaccinated with live immune complex and recombinant vaccine by subcutaneous injection at 1-day-old, respectively. Thirty broilers in each group, were randomly selected for weighting and serum collection at 0, 1, 2, 3, 4 and 5-week-old. The collected sera were tested for IBD antibodies measuring using enzyme-linked immunosorbent assay (ELISA) test kit by ProFLOK® PLUS (Synbiotics Corp, USA) and then antibody titers were express as $\log_{10}$.

Bursa:bodyweight ratios

In each group, chicken and Bursa of Fabricius were weighted at 5-week-old for calculation of the bursa/body weight (B:BW) ratios for 10 chickens. The B:BW ratios were calculated by the Bursa of Fabricius weight(g) × 1,000 / body weight (g).

Statistical analysis

All data were entered into a spreadsheet program (Excel 2000; Microsoft Corporation) and transferred to Program R software. Data of different parameters were analyzed using analysis of variance (ANOVA) as repeated measurement with Turkey HSD's multiple range test. The difference between parameters was regarded as significant when the p value was less than 0.05.

Results

Table 1showed the mean and standard deviation of IBD antibody titers in the present study. At 1-day-old, the antibody titers of all chicken before dividing and vaccination, were detected at the high level of 4.23±0.05 $\log_{10}$. This result indicated that chickens received the passive immunity from breeding hens. Since 3-week-old, all 3 vaccinated groups, possessed statistically significantly (P<0.05) higher antibody level than the unvaccinated group while no difference was found among all of vaccinated group (Table 1).

Table 2 shows the body weight, Bursa of Fabricius weight and Bursa to body weight (B:BW) ratios at 5-week-old. All of vaccinated groups showed no difference in body weight and B:BW ratio, including in comparison with unvaccinated chickens.

Summary and Discussion

Generally, the MDA is of benefit against IBDV infection in chickens, especially during the first month of age (Al- Natour *et al*., 2004). The ELISA titer of the MDA was divided into 3

Table 1. Mean ± SD of IBD antibody titer (as log_{10}) in unvaccinated and vaccinated chickens

	Chicken age					
Group	0-wk	1-wk	2-wk	3-wk	4-wk	5-wk
Unvaccinated	4.23±0.05	4.23±0.03^{a}	4.16±0.08^{ab}	3.21±0.33^{b}	2.86±0.44^{c}	2.67±0.68^{b}
Live attenuated	4.23±0.05	4.23±0.03^{a}	4.16±0.08^{ab}	3.76±0.33^{a}	3.57±0.30^{a}	3.67±0.25^{a}
Live immune complex	4.23±0.05	4.17±0.05^{b}	4.10±0.18^{b}	3.69±0.69^{a}	3.28±0.58^{b}	3.70±0.35^{a}
Recombinant	4.23±0.05	4.17±0.09^{b}	4.20±0.05^{a}	3.90±0.24^{a}	3.64±0.26^{a}	3.88±0.20^{a}

^{a,b,c} Values within the same column with different superscripts mean statistically significant difference (P<0.05)

Table 2. Mean ± SD of chicken body weight, Bursa of Fabricius weight and Bursa to body weight (B:BW) ratio at 5-week old

Group	Body weight (g)	Bursa weight (g)	B:BW ratio
Unvaccinated	1,480±0.17^{b}	1.90±0.70^{b}	1.30±0.49
Live attenuated	1,980±0.20^{a}	2.70±1.40^{ab}	1.35±0.72
Live immune complex	2,050±0.20^{a}	2.72±1.14^{ab}	1.32±0.54
Recombinant	2,000±0.17^{a}	3.24±0.91^{a}	1.64±0.44

^{a,b} Values within the same column with different superscripts mean statistically significant difference (P<0.05)

level; the low level (<3,000 or <3.48 log_{10}), intermediate level (3,000- 5,000 or 3.48-3.78 log_{10}) and high level (>6,000 or >3.78 log_{10}) (Kreider *et al*. 1991). The present study shows the average MDA of all 1-day-old chickens as 4.23±0.05 log_{10}, which was categorized within the high level of MDA. At 5-week-old, live attenuated, live immune complex and recombinant vaccine group, showed ELISA titer as 3.67±0.25, 3.70±0.35 and 3.88±0.20, respectively, indicating intermediate, intermediate and high level, respectively. However, the unvaccinated group, with 2.67±0.68 log_{10} indicated the negativity of IBD antibody (<3.48 log_{10}) (Table 1). These results indicate that all vaccinated groups showed statistically significant high immune responsiveness, compared with unvaccinated group, however each vaccinated group was not significant difference from the other two.

Chansiripornchai and Wanasawaeng (2009) reported that the IBD-Blen® (Merial, USA) vaccine produced from Winterfield 2512 IBD virus strain, which is a commercial live attenuated IBD vaccines belong to intermediate-plus or hot vaccines group.

These strains should not be recommended to vaccinate younger than 10-day-old of chickens. (Chansiripornchai and Wanasawaeng, 2009; Haddad *et al*., 1997). The major problem of this virus group, is the interference with MDA, including possible neutralization of the vaccine virus. Thus the timing of IBD vaccine administration is crucial (Saif, 1998; van den Berg, 2000; Müller *et al*., 2003). The present study also showed significant humoral antibody response against IBD 1-week-post vaccination with live attenuated Winterfield 2512 strain by drinking water at 14-day-old.

The present study also applied IBD vaccine using live immune complex in a single administration by subcutaneous route at 1-day-old chicks. This vaccine contains the Winterfield 2512 strain of IBD live virus in complex with IBD antibodies. The humoral immunity in the present study showed significant titer 1-week post vaccination. Several researches such as Herczeg *et al*. (2011) reported that Cevac®Transmune IBD vaccine (CEVA, Hungary), which is live attenuated immune complex vaccine, could be fully protective against IBD, in term of mortality prevention, less severe clinical disease and weight loss, including reduced acute lesions of Bursa of Fabricius due to very virulent IBDV infection; it also provides homogenous flock protection in the presence of highly varying maternally derived antibody levels (Herczeg *et al*., 2011).

The final group, was vaccinated by recombinant vaccine, that is turkey Herpesvirus (HVT) expressing the VP2 gene of the IBDV. This recombinant vaccine could not only prevent IBD, but also protected against Marek's disease at the same time, after applied by the subcutaneous route at 1-day-old (Rashid *et al*., 2013). The present study also indicated that humoral immunity could be induced by vaccination at 1-day-old with recombinant vaccine.

In addition, Bursa of Fabricius to body weight (B:BW) ratios were shown to be 1.30±0.49, 1.35±0.72 1.32±0.54 and 1.64±0.44 for the first to fourth groups respectively, interpreted that among all groups in the present study, there were no significant differences, meaning that this parameter did not effect or interfere with the development of Bursa of Fabricius after IBD vaccination, compared with unvaccinated group.

Conclusion

The humoral immune reaction against IBD of all vaccinated broiler chickens, subsequent to use live attenuated, live immune complex and recombinant vaccine, was significant responsiveness with no difference for ratio of Bursa to body indicated that all vaccines did not interfere with the

Bursa of Fabricius development after vaccination. Thus, all types of IBD vaccine tested in the current study generated significant responsiveness in terms of humoral immunity and did not impair the Bursa of Fabricius development, even in the presence of high level of maternal derived antibody in broilers.

Conflict of interest

There is no conflict of interest.

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References

Al-Natour, M.Q., Ward, L.A., Saif, Y.M., Stewart-Brown, B. and Keck, L.D. 2004. Effect of Different Levels of Maternally Derived Antibodies on Protection Against Infectious Bursal Disease Virus. *Avian dis.*, 48: 177-182.

Chang, H.C., Lin, T.L. and Wu, C.C. 2002. DNA-mediated vaccination against infectious bursal disease in chickens. *J. Vaccine*. 20(3-4): 328-335.

Chansiripornchai, N. and Wanasawaeng, W. 2009. Avian immune system. In: Avian Immune System and Serum Interpretation. Chansiripornchai, N. (ed), Trionasarn Co. Ltd., Bangkok. 27-74.

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(4): 882-889.

Herczeg, J., Nagy, M., Makranszki L., Balla, E., Kustos, K., Foucauld J.D., Thevenon, J. and Alva, B. 2011. Laboratory efficacy testing of subcutaneously administered Cevac®Transmune vaccine in broiler chickens. Proceedings of the XVII Congress of the World Veterinary Poultry Association, August 14-18, Cancun, Mexico.

Müller, H., Islam, M.R. and Raue R. 2003. Research on infectious bursal disease--the past, the present and the future. *Vet. Microbiol.* 97(1-2):153-165.

Kreider, D.L., Skeeles, J.K., Parsley, M., Newberry, L.A. and Story, J.D. 1991. Variability in a commercially available enzyme-linked immunosorbent assay system: I. Assay variability. *Avian Dis*. 35(2): 276-287.

Rashid, M.H., Luo, H., Akhter, J.,Islam, M.T., Islam, M.R., Rahman, M.M., Cao, Y. and Xue, C. 2013. Protective effect of Vaxxitek HVT+IBD vaccine against

Infectious Bursal Disease in broiler chickens. *Progress. Agric.* 24(1&2): 69–78.

Saif, Y.M. 1998. Infectious bursal disease and hemorrhagic enteritis. *Poult. Sci.* 77(8):1186-9.

Tsukamoto, K., Tanimura, N., Kakita, S., Ota, K., Mase, M., Imai, K. and Hihara, H. 1995. Efficacy of three live vaccines against highly virulent infectious bursal disease virus in chickens with or without maternal antibodies. *Avian Dis*. 39(2): 218-229.

van den Berg, TP. 2000. Infectious bursal disease in poultry: a review. *Avian Pathol*. 29: 175-194.