Original Article

Efficacy of Different Vaccination Programs against Velogenic Newcastle Disease Virus Challenged in 28-day-old Broiler Chickens

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Abstract

In the present study, the efficacy of different vaccination programs against a velogenic Newcastle disease virus (vNDV) challenged in 28-day-old broiler chickens was examined. The chickens were divided into 5 groups, 20 chickens in each. Group 1 did not receive any vaccine and served as the non-vaccinated control group. Groups 2-5 were vaccinated with different vaccination programs against vNDV. At 1-day-old, the chickens in all the vaccinated groups were vaccinated with live Newcastle disease (ND) vaccine, Ulster 2C strain, followed by different inactivated vaccines including vaccine A in groups 2 and 4 and vaccine B in groups 3 and 5. At 7-day-old, revaccination was performed in the vaccinated groups including live vaccine, Ulster 2C strain, in groups 2 and 3 and live vaccine, B1 strain, in groups 4 and 5. Finally, the chickens in every group were challenged with vNDV at 28-day-old. Results showed that NDV HI titer at the challenge inoculation time of all the vaccinated groups was significantly higher ($p < 0.05$) than that of the non-vaccinated group. At 14 days post-inoculation, mortality rate of the vaccinated groups was significantly lower ($p < 0.05$) than that of the non-vaccinated group. Body weights of all the vaccinated groups were significantly ($p < 0.05$) higher than those of the non-vaccinated group. The results indicate that all the vaccination programs used in this study could alleviate body weight loss and protect the chickens from lethal infection with vNDV.

Keywords: chickens, vaccine, Newcastle disease virus, efficacy

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บทคัดย่อ

ประสิทธิภาพของโปรแกรมวัคซีนที่แตกต่างกันต่อการป้องกันการติดเชื้อไวรัสนิวคาสเซิลสายพันธุ์รุนแรงในไก่เนื้ออายุ 28 วัน

ธวัชชัย โพธิ์เฮือง [1] นิดา สิริกอบกุล [2] จิโรจ ศศิปรียจันทร์ [3]*

การศึกษาครั้งนี้เป็นการทดสอบประสิทธิภาพของโปรแกรมวัคซีนที่แตกต่างกันในการป้องกันเชื้อไวรัสนิวคาสเซิลสายพันธุ์รุนแรงในไก่เนื้ออายุ 28 วัน โดยแบ่งไก่ทดลองออกเป็น 5 กลุ่มๆ ละ 20 ตัว กลุ่มที่ 1 เป็นกลุ่มควบคุมที่ไม่ได้รับวัคซีน ส่วนกลุ่มที่ 2-5 ให้วัคซีนป้องกันเชื้อไวรัสนิวคาสเซิลด้วยโปรแกรมที่แตกต่างกัน กล่าวคือ เมื่อไก่อายุ 1 วัน ให้วัคซีนเชื้อเป็นสายพันธุ์ Ulster 2C ในไก่ทุกตัว ร่วมกับการให้วัคซีนเชื้อตายชนิด A ในไก่กลุ่มที่ 2 และ 4 และวัคซีนเชื้อตายชนิด B ในไก่กลุ่มที่ 3 และ 5 เมื่อไก่อายุ 7 วัน ให้วัคซีนซ้ำ โดยให้วัคซีนเชื้อเป็นสายพันธุ์ Ulster 2C ในกลุ่มที่ 2 และ 3 และให้วัคซีนเชื้อเป็นสายพันธุ์ B1 ในไก่กลุ่มที่ 4 และ 5 เมื่อไก่อายุ 28 วัน ทำการให้เชื้อพิษทับ จากการศึกษาพบว่า NDV HI titer ในวันที่ไก่ได้รับเชื้อพิษทับของไก่กลุ่มที่ได้รับวัคซีนมีระดับสูงกว่าไก่ที่ไม่ได้รับวัคซีนอย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) ภายหลังจากไก่ได้รับเชื้อพิษทับ 14 วัน พบว่าอัตราการตายของไก่ที่ได้รับวัคซีนทุกกลุ่มต่ำกว่าไก่ที่ไม่ได้รับวัคซีนอย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) น้ำหนักตัวของไก่ที่ได้รับวัคซีนทุกกลุ่มมากกว่าไก่ที่ไม่ได้รับวัคซีนอย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) ผลการทดลองแสดงให้เห็นว่าโปรแกรมวัคซีนที่ใช้ในการศึกษาครั้งนี้ทุกโปรแกรมสามารถลดการสูญเสียน้ำหนักของไก่และป้องกันการตายจากการติดเชื้อไวรัสนิวคาสเซิลสายพันธุ์รุนแรงได้

คำสำคัญ: ไก่ วัคซีน ไวรัสนิวคาสเซิล ประสิทธิภาพ

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Introduction

Newcastle disease virus (NDV), classified as member of the genus *Avulavirus* in the family Paramyxoviridae, is one of the most important infectious agents encountered in poultry industry worldwide due to the high mortality and production loss associated with the disease (Mayo, 2002). Major disease problems in many countries of the world, including Asian countries, are caused by velogenic NDV (vNDV) (Awan et al., 1994). Virulent NDV affects nervous, respiratory and gastrointestinal systems (Brown et al., 1999; Alexander, 2003). Clinical signs are characterized by listlessness, respiratory distress and weakness, followed later by prostration and death. Infected chickens may appear suddenly with high mortality occurring in the absence of other clinical signs. Morbidity and mortality rates of infected chickens vary from 1-100% (Alexander, 2003).

Maternally-derived antibodies (MDA) in young chicks are primary means of antigen-specific protection against many pathogens in the field (Hamal et al., 2006) but they may be at risk to infection when under protective level of MDA titer occurs. Previously, we found that MDA in 14- and 21-day-old chickens at level of 3.85$\pm$1.04 and 1.55$\pm$0.94, respectively, could not protect the chickens against challenge with vNDV. However, our vaccination programs designed for that experiment could induce protective efficacy against infection in the 14- and 21-day-old chickens (Sasipreeyajan et al., 2012). Therefore, chickens at 28-day-old which have MDA lower than those of 14- and 21-day-old are stated to be at higher risk of vNDV infection. Moreover, the risk will be increased if there are no effective methods of infectious disease prevention and control.

Due to serious economic loss caused by ND, effective methods of control are needed urgently. Prevention and control are through the use of live attenuated or killed vaccine and many of the vaccination programs have been used in commercial chicken flocks (Khalifeh et al., 2009). To achieve reasonable protection against NDV, vaccination programs against ND have been designed to stimulate not only systemic but also mucosal immune responses (Takada and Kida, 1996). Inactivated vaccines have been used for inducing mainly systemic immunity (Rauwa et al., 2009). Live vaccines prepared

from lentogenic strains such as LaSota, Clone 30 and VG/GA are widely used because they provide high efficacy of protection through the induction of both systemic and local immunity (Seal et al., 2000; Rauwa et al., 2009). However, undesirable vaccine reactions may occur, especially after the application of live LaSota strain (Mast et al., 2005). To avoid vaccine reactions, mild ND vaccine, Hitchner B1 strain and avirulent ND vaccine, Ulster 2C strain, are commercially available (van Eck et al., 1991). Vaccination programs and procedures differ between countries and even between farms in the same country, depending on local circumstances (Rehmani, 1996). Therefore, it is necessary, in all countries, to compare the efficacy of vaccines that are available and to ascertain efficient methods of application. In the current study, the objective was to determine the protection afforded by different vaccination programs against challenge with vNDV in 28-day-old broiler chickens.

Materials and Methods

***Chickens*:** One hundred, one-day-old female broiler chickens (Cobb 500) were moved from a commercial hatchery (the Krungthai hatchery, a subsidiary company of GFPT, Chonburi province) to the University. The chickens were housed in the experimental animal facility at the Livestock Hospital at the Faculty of Veterinary Science, Chulalongkorn University, Nakhornpathom, Thailand. The chickens were divided into 5 groups of 20 chickens each. Groups 2, 3, 4 and 5 were vaccinated with different regimes of vaccination program against NDV as shown in Table 1. Group 1 served as the non-vaccinated control group, which did not receive any NDV vaccine. Feed and water were provided *ad libitum*. Guidelines and legislative regulations on the use of animals for scientific purposes of Chulalongkorn University, Bangkok, Thailand were followed as certified in permission No. 12310005.

***Vaccines*:** Four commercial vaccines were used in this study. Inactivated ND vaccine A (Chick i N-K, Fort Dodge, Campinas, Brazil) was given at 1-day-old by subcutaneous (S/Q) injection at the base of the skull (0.1 ml/bird, each 0.1 ml of the vaccine containing at least $10^{7.0}$ EID_{50} of LaSota strain NDV). Inactivated ND vaccine B (Poulvac® i N LaSota, Fort Dodge, Campinas, Brazil) was given at 1-day-old by S/Q injection at the base of the skull (0.1 ml/bird, each 0.5 ml of the vaccine containing at least $10^{8.0}$ EID_{50} of LaSota strain NDV). Live ND, Ulster 2C strain (Poulvac® NDW, Fort Dodge, Campinas, Brazil) was given intra-nasally (I/N) at 1- and 7-day-old (1 dose/bird, each dose of the vaccine containing at least $10^{6.5}$ EID_{50} of Ulster 2C strain NDV). Live ND, B1 strain (Fort Dodge Animal Health, Fort Dodge, USA) was given I/N at 7-day-old (1 dose/bird, each dose of the vaccine containing at least $10^{6.3}$ EID_{50} of B1 strain NDV).

***Challenge study*:** Chickens from every group were challenged with vNDV at 28-day-old. Each chicken received approximately 10^{6} EID_{50} of vNDV by oral drops (Chansiripornchai and Sasipreeyajan, 2006). Clinical signs and mortality were observed for 14 days post-inoculation (DPI). Dead chickens were necropsied and gross lesions were observed. To confirm the infection in dead chickens, they were necropsied and gross lesions of typical ND were recorded. Tracheas were collected for detection of NDV and typing as velogenic strain by using one-step RT-PCR followed by restriction endonuclease analysis (Creelan et al., 2002).

***Body weight*:** All the chicken were weighed individually at 28-day-old before challenge and at 42-day-old which was 14 DPI.

***Serological evaluations*:** Thirty blood samples were randomly collected from all groups at 1-day-old. Ten, 10 and 20 blood samples/group were collected at 7-, 14- and 21-day-old. Before the challenge at 28-day-old, 20 blood samples from each group were collected. After challenge at 42-day-old (14 DPI), the remaining chickens in each group were bled. Sera were collected and tested for NDV antibody titers by the haemagglutination-inhibition (HI) test, micro method (Allan and Gough, 1974).

***Statistical analysis*:** Body weights and antibody titers were analyzed and compared between groups using ANOVA and least significant difference (LSD) tests. Mortality was compared using Chi-square test. Significance was tested at a probability level of 0.05.

Results

***Body weights*:** Before the challenge inoculation at 28 days old, the body weights of the vaccinated group 4 were the lowest and significantly different ($p < 0.05$) from the others. At 42-day-old (14 DPI), the body weights of all the vaccinated groups were higher ($p < 0.05$) than those of the non-vaccinated control group. Among the vaccinated groups, there was no significant difference ($p > 0.05$) in their body weights at 14 DPI (Table 2).

***Mortality rate*:** After the challenge inoculation, the mortality rate of the vaccinated groups observed at 14

Table 1 Vaccination programs and age of challenge.

Group	1-day-old		7-day-old	28-day-old
	Live vaccine	Inactivated vaccine	Live vaccine	NDV challenge
1	-	-	-	20
2	Ulster 2C strain - I/N[A]	A 0.1 ml/bird - S/Q[B]	Ulster 2C strain - I/N	20
3	Ulster 2C strain - I/N	B 0.1 ml/bird - S/Q	Ulster 2C strain - I/N	20
4	Ulster 2C strain - I/N	A 0.1 ml/bird - S/Q	B1 strain - I/N	20
5	Ulster 2C strain - I/N	B 0.1 ml/bird - S/Q	B1 strain - I/N	20

[A] I/N : intra-nasally; [B] S/Q : subcutaneously

DPI was significantly lower ($p < 0.05$) than that of the non-vaccinated control group. Among the vaccinated groups, the mortality rate was not significantly different ($p > 0.05$) (Table 2). Dead chickens were necropsied and gross lesions of typical ND were observed including conjunctivitis, hemorrhage and congestion in the tracheal mucosa, hemorrhage in the mucosa of the proventriculus, hemorrhage and necrosis in intestinal lymphoid aggregates, enlargement and necrosis of cecal tonsils and hemorrhage of the coronary fat. All of the tracheal samples collected from them were positive for the detection of NDV using one-step RT-PCR followed by restriction endonuclease analysis (data not shown).

***NDV HI titers*:** MDA at 1-day-old was 7.27±0.94. It declined to 1.65±0.81 at 28-day-old. At 14-day-old, the NDV HI titer of group 5 was the highest (5.10±0.99) and it was significantly different ($p < 0.05$) from the others. Before the challenge inoculation, the NDV HI titers of the vaccinated groups at 7-, 21- and 28-day-old were not significantly different ($p > 0.05$) from each other. For the non-vaccinated control group, the NDV HI titer was not significantly different ($p > 0.05$) from the vaccinated groups at 7-, 14- and 21-day-old, but at the time of inoculation (28-day-old), the NDV HI titers of the non-vaccinated control group were significantly lower ($p < 0.05$) than those of the others (Table 3).

Discussion

Previously, we observed a similar rate of protection after vNDV challenge in chickens which were vaccinated with live and, simultaneously, with inactivated vaccines at 1-day-old and revaccinated with live B1 or LaSota strains at 7-day-old (Sasipreeyajan et al., 2012). However, LaSota vaccine is reported to cause damage in the tracheal epithelium of vaccinated chickens. This predisposes them to secondary infections (Mast et al., 2005). Therefore, we decided to use a mild ND vaccine, B1 strain and the avirulent ND vaccine, Ulster 2C strain at the revaccination time. The challenge experiment demonstrated that all of the vaccination programs could alleviate weight loss and the mortality rate of the infected chickens, consistent with observations by other investigators who found that the body weight of the vaccinated and challenged chickens was significantly higher than that of the non-vaccinated and challenged chickens (Ezema et al., 2009;

Table 2 Body weights before and after vNDV challenge (28- and 42-day-old), mortality rate and percentage of protection at 14 DPI

Group	Body weight (gm/bird)		Mortality		Percent of protection
	28-day-old (0 DPI)	42-day-old (14 DPI)	Number	Percent	
1	1,334.0 ± 75.98[A,a] (n=20)[B]	1,386.7 ± 476.90[a] (n=3)	17/20[C,a]	85	15
2	1,326.5 ± 109.51[a] (n=20)	1,981.1 ± 146.81[b] (n=19)	1/20[b]	5	95
3	1,321.5 ± 86.59[a,b] (n=20)	1,894.7 ± 296.95[b] (n=19)	1/20[b]	5	95
4	1,276.0 ± 59.95[b] (n=20)	1,802.5 ± 401.31[b] (n=20)	0/20[b]	0	100
5	1,320.0 ± 68.06[a,b] (n=20)	1,907.4 ± 434.27[b] (n=19)	1/20[b]	5	95

[a,b,c] The different superscript in each column means statistically significant difference ($p < 0.05$).
[A] Mean±standard deviation (SD).
[B] Number of chickens in the group.
[C] Number of dead chickens / total chickens in the group.

Table 3 Mean HI antibody titers (log_2) before and after vNDV challenge

Group	HI titers (Log_2)					
	1-day-old	7-day-old	14-day-old	21-day-old	28-day-old	42-day-old
1	7.27 ± 0.94[A] (n=30)[B,C]	5.90 ± 0.57[a] (n=10)	4.30 ± 0.82[a,b] (n=10)	2.35 ± 1.09[a] (n=20)	1.65 ± 0.81[a] (n=20)	10.00 ± 1.0[a,b] (n=3)
2	-	5.60 ± 0.97[a] (n=10)	3.60 ± 0.70[a] (n=10)	3.60 ± 1.19[a] (n=20)	3.70 ± 2.11[b] (n=20)	9.26 ± 1.41[a,b] (n=19)
3	-	5.50 ± 1.27[a] (n=10)	3.60 ± 0.97[a] (n=10)	3.75 ± 0.85[a] (n=20)	3.45 ± 1.10[b] (n=20)	8.95 ± 1.39[a,b] (n=19)
4	-	5.00 ± 0.82[a] (n=10)	3.80 ± 0.79[a] (n=10)	3.25 ± 1.16[a] (n=20)	3.30 ± 1.17[b] (n=20)	8.85 ± 1.09[a] (n=20)
5	-	5.80 ± 1.14[a] (n=10)	5.10 ± 0.99[b] (n=10)	3.60 ± 1.14[a] (n=20)	3.60 ± 1.19[b] (n=20)	10.47 ± 1.65[b] (n=19)

[a,b] The different superscript in each column means statistically significant difference ($p < 0.05$).
[A] Mean±standard deviation (SD).
[B] Random sampling from all chickens.
[C] Number of chickens tested.

Sasipreeyajan et al., 2012). The mortality rate of the vaccinated chickens was lower than that of the non-vaccinated chickens. This was consistent with other authors who have studied the efficacy of different vaccination programs against vNDV (Chansiripornchai and Sasipreeyajan, 2005; Chansiripornchai and Sasipreeyajan, 2006; Sasipreeyajan et al., 2012). Based on the results of alleviation in weight loss and mortality rate of vaccinated and challenged chickens, the protective efficacy of vaccination programs against challenge with vNDV was indicated.

The protection against challenge with vNDV occurring in this study may be due to the combined advantageous effects of using both live attenuated and inactivated vaccines and revaccination with live vaccine. This protection phenomenon is similar to the recovery of other researchers who found a high level of protection against challenge with vNDV in chickens vaccinated with live and inactivated vaccines (Chansiripornchai and Sasipreeyajan, 2005; Chansiripornchai and Sasipreeyajan, 2006; Sasipreeyajan et al., 2012). In vaccinated chickens, local IgA and IgM on the mucosal surface of the respiratory tracts plays a critical role as a primary barrier against viral infection, resulting in the protection of the chickens from subsequent systemic infection (Takada and Kida, 1996). Live vaccines could induce local antibody responses such as IgA production in the Harderian gland (Russell and Koch, 1993) along with lacrimal IgM following intraocular inoculation with NDV (Russell, 1993). Humoral immune response in the live vaccinated chickens was detected at 7 DPV, which was earlier than in the chickens vaccinated with inactivated vaccine, but at 28 DPV, lower titers than those observed in the group receiving inactivated vaccine were detected. However, live vaccine could induce cell-mediated immune response to vNDV superior to that received from the inactivated vaccine (Lambrecht et al., 2004). A single subcutaneous vaccination with inactivated vaccine was not effective in inhibiting infection of vNDV on mucosal surfaces but it could inhibit tissue infection mechanisms of the virus which contribute to protection from lethal systemic infection (Takada and Kida, 1996). Al-Garib et al. (2003) found that at 14, 21 and 28 days post-vaccination (DPV), serum IgM response was higher in birds that had been systemically immunized with inactivated vaccine than in the chickens vaccinated with live NDV vaccine. However, IgG was delayed in chickens that had been immunized with inactivated vaccine compared to the chickens immunized with live vaccine.

All of the vaccination programs used in this study provided a high level of protection against challenge with vNDV at 21 days post-revaccination. It may be that the vaccinated chickens had sufficient protective immunity at those times. Al-Garib et al. (2003) reported that the HI titers in the serum of chickens inoculated with live vaccine reached a plateau level at 14 DPV. IgG in the serum reached a plateau level at 14 DPV. Rauwa et al. (2009) found that live vaccines induced NDV-specific HI and IgG antibodies from 7 and 14 DPV, respectively. They could still be detected until 35 DPV. Systemic NDV specific IgA was first observed at 7 DPV and showed a peak value at 14 DPV. After that, it decreased but could still be detected until 35 DPV. NDV specific cell-mediated immunity after vaccination with live vaccines was observed from 7 to 35 DPV. Duodenal NDV specific IgA was detected from 14 to 35 DPV. Al-Garib et al. (2003) also reported that after vaccination with inactivated vaccine, NDV HI titer was detected from 7 DPV onwards. In serum, IgM was firstly detected at 14 DPV and remained at a high level until 28 DPV. IgG and low levels of IgA response were detected from 14 DPV onwards.

At the challenge inoculation time, although the NDV HI titer of the non-vaccinated control group remained (1.65 ± 0.81), the mortality rate of the chickens in this group was the highest. This indicates that MDA at this level could not provide protection against challenge with vNDV. On the other hand, the NDV HI titer observed in the vaccinated chickens was composed of both MDA and an active immune response induced by vaccines. Therefore, the level was higher than that of the non-vaccinated control group and it gave significantly better protection than that of the non-vaccinated control chickens. These suggest that MDA may not be sufficient in the protection of offspring and a major concern is the necessity for vaccination at an early age in order to get the highest protective level against infection with vNDV. Herein, we found that the lowest NDV HI titer of 3.30 ± 1.17 showed a protection of 100%. This was consistent with the report of Hamid et al. (1991), who observed no clinical signs in the immunized chickens with an HI antibody of 3 $\log_2$ and above but the chickens with lower HI titres ($\leq 3 \log_2$) showed depression and anorexia. The protection observed in the vaccinated chickens with low HI titres in their serum may be due to the effect of cell-mediated immune response and local immunity in the respiratory and intestinal tracts induced by vaccines (van Eck et al., 1991; Takada and Kida, 1996; Lambrecht et al., 2004; Rauwa et al., 2009).

In conclusion, the protection against vNDV was observed in all of the vaccination programs used in this work. Therefore, simultaneous vaccination with live Ulster 2C strain intra-nasally, inactivated LaSota strain subcutaneously injected into the base of the skull at 1 day old, and a booster vaccination with live Ulster 2C or B1 strain intra-nasally at 7-day-old was effective against the challenge with vNDV at 28-day-old.

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