*Original Article*

# Efficacy of Different Vaccination Programs against Newcastle Disease Virus Challenge in Broiler Chickens

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***Abstract***

The present study was designed to determine the protection afforded by different vaccination programs against the virulent Newcastle disease virus (vNDV). The chickens were divided into 7 groups, 40 chickens in each. Group 1 did not receive any vaccine and served as a negative control group. Groups 2-7 were vaccinated with different vaccination programs against vNDV. At 1 day old, the chickens in all the vaccinated groups were inoculated with live attenuated vaccine, Ulster strain, followed by different inactivated vaccines including 0.1 ml/chicken of inactivated vaccine A in groups 2 and 4, 0.1 ml/chicken of inactivated vaccine B in groups 3 and 6, 0.25 ml/chicken of inactivated vaccine A in group 5 and 0.25 ml/chicken of inactivated vaccine B in group 7. At 7 days old, revaccination was performed in the vaccinated groups with live attenuated vaccines, B1 strain in groups 2 and 3 and LaSota strain in groups 4-7. The chickens in each group were challenged with vNDV at 14 and 21 days old, 20 chickens at a time. The results at 14 days post-infection (DPI) showed that the mortality rate of the vaccinated groups was significantly lower ($p< 0.05$) than that of the non-vaccinated group. Body weights were not significantly different between the non-vaccinated group and the vaccinated groups which were challenged at 14 days old ($p> 0.05$). For the groups which were challenged at 21 days old, the body weights of the vaccinated groups were higher than that of the non-vaccinated group, but only the body weights of vaccinated groups 2, 4 and 6 were significantly different ($p<0.05$) from the non-vaccinated group. It is indicated that all of the vaccination programs used in this study could alleviate body weight loss and protect the chickens from lethal infection with vNDV.

**Keywords:** chickens, Newcastle disease virus, vaccine

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# บทคัดย่อ

## ประสิทธิภาพของโปรแกรมวัคซีนที่แตกต่างกันต่อการป้องกันเชื้อไวรัสนิวคาสเซิลในไก่เนื้อ

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การศึกษาครั้งนี้เป็นการประเมินประสิทธิภาพของโปรแกรมวัคซีนที่แตกต่างกันในการป้องกันเชื้อไวรัสนิวคาสเซิลสายพันธุ์รุนแรงในไก่เนื้อ โดยแบ่งไก่ทดลองออกเป็น 7 กลุ่มๆ ละ 40 ตัว กลุ่มที่ 1 เป็นกลุ่มควบคุมผลลบซึ่งไม่ได้รับวัคซีน ส่วนกลุ่มที่ 2-7 ให้วัคซีนป้องกันเชื้อไวรัสนิวคาสเซิลด้วยโปรแกรมที่แตกต่างกัน กล่าวคือ เมื่อไก่อายุ 1 วัน ให้วัคซีนเชื้อเป็นสายพันธุ์ Ulster 2C ในไก่ทุกตัวของกลุ่มที่ได้รับวัคซีน ร่วมกับการให้วัคซีนเชื้อตายชนิด A ขนาด 0.1 มล.ต่อตัวในไก่กลุ่มที่ 2 และ 4 และขนาด 0.25 มล.ต่อตัวในไก่กลุ่มที่ 5 และวัคซีนเชื้อตายชนิด B ขนาด 0.1 มล.ต่อตัวในไก่กลุ่มที่ 3 และ 6 และขนาด 0.25 มล.ต่อตัวในไก่กลุ่มที่ 7 เมื่อไก่อายุ 7 วัน ให้วัคซีนซ้ำ โดยให้วัคซีนเชื้อเป็นสายพันธุ์ B1 ในกลุ่มที่ 2 และ 3 และให้วัคซีนเชื้อเป็นสายพันธุ์ LaSota ในไก่กลุ่มที่ 4-7 เมื่อไก่อายุได้ 14 วัน ทำการให้เชื้อพิษทับแก่ไก่จำนวน 20 ตัวของแต่ละกลุ่ม ส่วนไก่ที่เหลืออีก 20 ตัวของแต่ละกลุ่ม ให้เชื้อพิษทับเมื่อไก่อายุ 21 วัน ผลการศึกษาพบว่า 14 วัน หลังจากไก่ได้รับเชื้อพิษทับ อัตราการตายของไก่ที่ได้รับวัคซีนทุกกลุ่มมีค่าต่ำกว่าไก่ที่ไม่ได้รับวัคซีนอย่างมีนัยสำคัญทางสถิติ ($p< 0.05$) ไก่ที่ได้รับเชื้อพิษทับเมื่ออายุ 14 วัน มีน้ำหนักตัวมากกว่าไก่ที่ไม่ได้รับวัคซีน แต่ไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($p> 0.05$) ส่วนไก่ที่ได้รับเชื้อพิษทับเมื่ออายุ 21 วัน พบว่าไก่กลุ่มที่ได้รับวัคซีนแต่ละกลุ่มมีน้ำหนักตัวมากกว่าไก่ที่ไม่ได้รับวัคซีน ซึ่งไก่กลุ่มที่ได้รับวัคซีน กลุ่มที่ 2, 4 และ 6 เป็นกลุ่มที่มีน้ำหนักตัวมากกว่าไก่ที่ไม่ได้รับวัคซีนอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) ผลการทดลองทั้งหมด แสดงให้เห็นว่าโปรแกรมวัคซีนที่ใช้ในการศึกษาครั้งนี้ทุกโปรแกรมสามารถลดการสูญเสียน้ำหนักของไก่และป้องกันการตายจากการติดเชื้อไวรัสนิวคาสเซิลสายพันธุ์รุนแรงได้

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## *Introduction*

Newcastle disease (ND) is one of the most important infectious viral diseases and results in a significant economic loss to commercial chicken industries in many countries of the world. The causative agent, Newcastle disease virus (NDV), is an enveloped RNA virus, belonging to the genus *Avulavirus* in the family Paramyxoviridae (Mayo, 2002). Strains of NDV have been classified into five pathotypes including viscerotropic velogenic, neurotropic velogenic, mesogenic, lentogenic and asymptomatic enteric (Office International des Epizooties, 2008). The disease caused by virulent NDV (vNDV) is a major disease problem in the poultry of Africa and Asia (Awan et al. 1994). It affects the nervous, respiratory and gastrointestinal systems (Brown et al., 1999; Alexander, 2003). The disease is 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).

Control of ND by vaccination is a routine in commercial chicken flocks in many countries. Inactivated vaccines have been used for inducing mainly systemic immunity (Rauwa et al., 2009). Live attenuated vaccines prepared from lentogenic strains such as Hitchner B1, 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). Many of the vaccination programs have been used in commercial chicken flocks to achieve reasonable protection against NDV (Khalifeh et al., 2009). The 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 vNDV.

## *Materials and Methods*

***Chickens:*** Two hundred and eighty female broiler chickens (Cobb 500) were moved from a commercial hatchery (the Krungthai hatchery, a subsidiary company of GFPT, Chonburi province) to the university at one day of age. The chickens were housed in the experimental animal facility at the Livestock Hospital at the Faculty of Veterinary Science, Chulalongkorn University, Nakhornpathom, Thailand. The guidelines and legislative regulations on the use of animals for scientific purposes of Chulalongkorn University, Bangkok, Thailand were certified in permission No. 12310005. The chickens were divided into 7 groups with 40 chickens each. Groups 2-7 were vaccinated with different regimes of vaccination program against NDV as shown in Table 1. Group 1 did not receive any NDV vaccine and served as a negative control group. Feed and water were provided *ad libitum*.

***Vaccines:*** Five 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 or 0.25 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 or 0.25 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 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 days old (1 dose/bird, each dose of the vaccine containing at least $10^{6.3}$ $EID_{50}$ of B1 strain NDV). Live ND, LaSota strain (Fort Dodge Animal Health, Fort Dodge, USA) was given I/N at 7 days old (1 dose/bird, each dose of the vaccine containing at least $10^{6.3}$ $EID_{50}$ of LaSota strain NDV).

***Challenge study:*** Chickens from each group were challenged with vNDV (CU-2 strain, ICPI=1.86) at 14 and 21 days old, 20 chickens/group at a time. Each chicken received approximately $10^6$ $EID_{50}$ of vNDV by oral drop (Chansiripornchai and Sasipreeyajan, 2006). Clinical signs and mortality were observed for 14 days post-infection (DPI). To confirm the infection in dead chickens, they were necropsied and the 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:*** Each chicken in all groups was weighed at 14 and 21 days old before challenge and at 28 and 35 days old which was 14 DPI.

***Serological evaluations:*** Blood samples were randomly sampled from all groups at 1 and 7 days old, 30 samples at a time, to determine NDV maternally-derived antibodies (MDA). Before challenge at 14 and 21 days old, 20 blood samples from each group were collected. After challenge at 28 and 35 days old (14 DPI), the remaining chickens in each group were bled. Sera were collected and tested for NDV antibody titers by the hemagglutination-inhibition (HI) test, micro method (Allan and Gough, 1974).

***Statistical analysis:*** Body weights and NDV HI titers were analyzed and compared between groups using ANOVA and Duncan's multiple range test. Mortality after vNDV challenge was analyzed using Chi-square values. Differences between groups were considered significant at $p< 0.05$.

## *Results*

***Body weights:*** In comparison among the groups which were challenged at 14 days old (Table 2), the body weights of the chickens before challenge were not significantly different ($p> 0.05$). At 28 days old (14 DPI), the body weights among the vaccinated groups and between the vaccinated groups and non-vaccinated control group were not significantly different ($p> 0.05$). However, the body weights of the vaccinated groups were slightly higher than those of the non-vaccinated control group.

**Table 1** Vaccination programs and age of challenge.

| Group | Vaccination | | NDV challenge | |
|---|---|---|---|---|
| | 1 day old | 7 days old | 14 days old | 21 days old |
| 1 | Non-vaccinated control | - | 20 | 20 |
| 2 | Live Ulster 2C strain - I/N[A]<br>Inactivated vaccine A 0.1 ml/bird - S/Q[B] | Live B1 strain - I/N | 20 | 20 |
| 3 | Live Ulster 2C strain - I/N<br>Inactivated vaccine B 0.1 ml/bird - S/Q | Live B1 strain - I/N | 20 | 20 |
| 4 | Live Ulster 2C strain - I/N<br>Inactivated vaccine A 0.1 ml/bird - S/Q | Live LaSota strain - I/N | 20 | 20 |
| 5 | Live Ulster 2C strain - I/N<br>Inactivated vaccine A 0.25 ml/bird - S/Q | Live LaSota strain - I/N | 20 | 20 |
| 6 | Live Ulster 2C strain - I/N<br>Inactivated vaccine B 0.1 ml/bird - S/Q | Live LaSota strain - I/N | 20 | 20 |
| 7 | Live Ulster 2C strain - I/N<br>Inactivated vaccine B 0.25 ml/bird - S/Q | Live LaSota strain - I/N | 20 | 20 |

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

In comparison among the groups which were challenged at 21 days old (Table 3), the body weights of the non-vaccinated control group was higher ($p< 0.05$) than those of the vaccinated groups 3 and 5. However, the body weights of all the vaccinated groups were not significantly different ($p> 0.05$) from each other. At 35 days old (14 DPI), the body weights of the vaccinated groups 2, 4 and 6 were significantly higher ($p< 0.05$) than those of the non-vaccinated control group. The body weights of the vaccinated groups 3, 5 and 7 were also higher than those of the non-vaccinated control group but they were not significantly different ($p> 0.05$) from each other. Among the vaccinated groups, there was no significant difference ($p> 0.05$) in their body weights at 14 DPI.

***Mortality rate:*** The mortality rate of the vaccinated groups which were challenged at both 14 and 21 days old was significantly lower ($p< 0.05$) than that of the non-vaccinated control group. Among the vaccinated groups, the mortality rate compared at 14 DPI was not significantly different ($p> 0.05$) (Table 2 and 3). Dead chickens were necropsied and gross lesions of typical ND were observed. Gross lesions were including conjunctivitis, hemorrhage and congestion in the tracheal mucosa, hemorrhage in the mucosa of the proventriculus, hemorrhage and necrosis in intestinal lymphoid aggregates, enlarged and necrosis of cecal tonsils and hemorrhage of the coronary fat. All of the tracheal samples were collected for detection of NDV by using one-step RT-PCR followed by restriction endonuclease analysis were positive.

**Table 2** Body weights and NDV HI titers ($log_2$) before and after vNDV challenge (14 and 28 days old), mortality rate and percentage of protection at 14 DPI.

| Group | Body weight (gm/bird) | | NDV HI titer ($Log_2$) | | Mortality | Protection (%) |
|---|---|---|---|---|---|---|
| | 14 days old (0 DPI) | 28 days old (14 DPI) | 14 days old (0 DPI) | 28 days old (14 DPI) | | |
| 1 | 446.0 ± 16.0[A,a] (n=20)[B] | 1,070.0 ± 67.8[a] (n=5) | 3.85 ± 1.04[b] (n=20) | 7.60 ± 1.67[a] (n=5) | 15/20[C,a] | 25 |
| 2 | 443.0 ± 14.9[a] (n=20) | 1,125.6 ± 194.9[a] (n=18) | 3.20 ± 1.20[a,b] (n=20) | 7.72 ± 1.41[a] (n=18) | 2/20[b] | 90 |
| 3 | 440.5 ± 13.6[a] (n=20) | 1,139.4 ± 194.6[a] (n=17) | 3.10 ± 1.02[a] (n=20) | 7.76 ± 1.75[a] (n=17) | 3/20[b] | 85 |
| 4 | 442.0 ± 15.8[a] (n=20) | 1,131.3 ± 250.9[a] (n=15) | 3.60 ± 0.94[a,b] (n=20) | 7.47 ± 1.41[a] (n=15) | 5/20[b] | 75 |
| 5 | 442.0 ± 7.7[a] (n=20) | 1,128.9 ± 194.1[a] (n=18) | 4.60 ± 1.27[c] (n=20) | 7.94 ± 1.47[a] (n=18) | 2/20[b] | 90 |
| 6 | 443.5 ± 12.3[a] (n=20) | 1,136.3 ± 205.1[a] (n=19) | 3.30 ± 0.73[a,b] (n=20) | 5.89 ± 2.02[b] (n=19) | 1/20[b] | 95 |
| 7 | 445.0 ± 11.5[a] (n=20) | 1,131.2 ± 151.9[a] (n=17) | 3.60 ± 0.82[a,b] (n=20) | 5.76 ± 2.59[b] (n=17) | 3/20[b] | 85 |

[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** Body weights and NDV HI titers ($log_2$) before and after vNDV challenge (21 and 35 days old), mortality rate and percentage of protection at 14 DPI.

| Group | Body weight (gm/bird) | | NDV-HI titer ($Log_2$) | | Mortality | Protection (%) |
|---|---|---|---|---|---|---|
| | 21 days old (0 DPI) | 35 days old (14 DPI) | 21 days old (0 DPI) | 35 days old (14 DPI) | | |
| 1 | 865.0 ± 34.7[A,b] (n=20)[B] | 1,430.0 ± 28.3[a] (n=2) | 1.55 ± 0.94[a] (n=20)[B] | 8.50 ± 2.12[a] (n=2) | 18/20[C,a] | 10 |
| 2 | 837.5 ± 51.7[ab] (n=20) | 1,679.4 ± 126.5[b] (n=18) | 3.10 ± 1.12[c] (n=20) | 9.56 ± 1.69[a] (n=18) | 2/20[b] | 90 |
| 3 | 820.5 ± 63.3[a] (n=20) | 1,668.0 ± 250.6[a,b] (n=20) | 2.10 ± 1.33[a,b] (n=20) | 10.10 ± 1.68[a] (n=20) | 0/20[b] | 100 |
| 4 | 830.5 ± 46.5[ab] (n=20) | 1,692.8 ± 169.2[b] (n=18) | 2.95 ± 1.50[c] (n=20) | 9.83 ± 1.65[a] (n=18) | 2/20[b] | 90 |
| 5 | 819.0 ± 45.3[a] (n=20) | 1,628.9 ± 213.4[a,b] (n=19) | 3.05 ± 1.05[c] (n=20) | 9.16 ± 1.92[a] (n=19) | 1/20[b] | 95 |
| 6 | 844.0 ± 61.1[ab] (n=20) | 1,689.5 ± 271.0[b] (n=19) | 2.75 ± 1.12[b,c] (n=20) | 8.95 ± 1.90[a] (n=19) | 1/20[b] | 95 |
| 7 | 841.5 ± 52.4[ab] (n=20) | 1,624.4 ± 307.1[a,b] (n=18) | 2.55 ± 1.15[b,c] (n=20) | 9.00 ± 1.46[a] (n=18) | 2/20[b] | 90 |

[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.

***NDV HI titers:*** MDA at 1 day old was 7.27±1.17. It declined to 5.47±1.14, 3.85±1.04 and 1.55±0.94 at 7, 14 and 21 days old, respectively. Among the groups which were challenged at 14 days old, before challenge inoculation the NDV HI titer of group 5 was the highest and it was significantly different ($p< 0.05$) from the others. NDV HI titers of the vaccinated groups, excluding group 5, were not significantly different ($p> 0.05$) from each other. For the non-vaccinated control group, NDV HI titer was not significantly different ($p> 0.05$) from the vaccinated groups 2, 4, 6 and 7. At 14 DPI, NDV HI titers of groups 6 and 7 were significantly lower ($p< 0.05$) than those of the others.

In comparison of the groups which were challenged at 21 days old, before challenge inoculation the NDV HI titers of the vaccinated groups 2, 4, 5, 6 and 7 were not significantly different ($p> 0.05$) from each other. Group 3 had an NDV HI titer significantly lower ($p< 0.05$) than that of the vaccinated groups 2, 4 and 5. No significant difference was found between NDV HI titer of the vaccinated group 3 and the non-vaccinated control group. At 14 DPI, the NDV HI titers of all of the experimental groups were not significantly different ($p> 0.05$) from each other.

## *Discussion*

NDV infection can be controlled by vaccination programs designed to stimulate not only systemic but also mucosal immune responses (Takada and Kida, 1996). Therefore, the combination of both live attenuated and inactivated vaccines was used in this study. The results showed that all of the vaccination programs could alleviate weight loss and the mortality rate of the infected chickens. This indicated protection against challenge with vNDV. The findings on the alleviation of weight loss in vaccinated and challenged chickens were similar to the report of Ezema et al. (2009) who found that the body weight of the vaccinated chickens was significantly higher ($p< 0.05$) than that of the non-vaccinated control at 12-20 DPI. Protection against the challenged virus found in all of the vaccination programs used in this work was similar to the study of Chansiripornchai and Sasipreeyajan (2005) who observed protection against challenge with vNDV in chickens vaccinated with live B1 strain vaccine and inactivated oil adjuvant vaccine at 1 day old and revaccinated at 14 days old with different live attenuated vaccines including B1, LaSota and Ulster 2C. We also observed that chickens that received a revaccination with B1 or LaSota strains at 7 days old did not have a different protection rate after vNDV challenge. However, LaSota vaccine is reported to cause damage in the tracheal epithelium, resulting in cuboidal epithelium and a lack of mature goblet cells and cilia in the tracheal epithelium of the vaccinated chickens. This predisposes them to secondary infections because they cannot trap particles such as pathogenic bacteria in mucus and the mucus-associated particles cannot be cleared by ciliary movement (Mast et al., 2005). Therefore, we suggest that revaccination with mild ND vaccine, B1 strain, should be used to avoid the adverse effects of LaSota strain vaccine.

The protection phenomenon in this study may be due to the combined advantageous effects of using both live attenuated and inactivated vaccines and revaccination with live attenuated vaccine. Previously, Takada and Kida (1996) reported that a single subcutaneous vaccination of inactivated vaccine could induce IgG antibody response in serum, but not in the nasal wash. HI titers of 1:4-1:128 and neutralizing activity at the titers of 1:160 were observed. They found that only 4 of 10 chickens were protected against challenge with vNDV. The virus could be detected in the tissues and the sinuses of some of the chickens, but the virus titers were lower than those of the control chickens. Moreover, the virus was not detected in the blood samples of the single subcutaneous vaccinated chickens until the end of the study. This indicates that serum IgG antibodies may contribute to protection from lethal systemic infection, but they are not effective in inhibiting infection on the mucosal surfaces. Al-Garib et al. (2003) found that a single subcutaneous vaccination with commercial inactivated vaccine could induce both of IgM and IgG antibody responses in the serum of vaccinated chickens. At 14, 21 and 28 days post-vaccination (DPV), the serum IgM response was higher in the birds that had been systemically immunized with inactivated vaccine than in the chickens vaccinated with live attenuated NDV vaccine. However, the IgG was delayed in chickens that were immunized with the inactivated vaccine compared to the chickens immunized with live vaccine. Consistent with the study of Lambrecht et al. (2004), they observed that a humoral response was detected as early as 7 DPV with live attenuated vaccine, but it was detected at 14 DPV with inactivated vaccine. At 28 DPV, live vaccine elicited lower titers than those observed in the group receiving inactivated vaccine. It was reported that local IgA and IgM, rather than IgG antibodies, on the mucosal surface of the respiratory tracts of vaccinated chickens play a critical role as a primary barrier against viral infection on the mucosal surface, resulting in protection of the chickens from subsequent systemic infection (Takada and Kida, 1996). High levels of IgA, IgM and IgG antibodies were observed in vaccination with live attenuated vaccines (Russell and Ezeifka, 1995). They also induced 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). Moreover, live attenuated vaccine could induce cell-mediated responses to vNDV superior to those achieved with inactivated vaccine. Lambrecht et al. (2004) showed that the ChIFN-γ, released from CMI, was detected earlier in chickens vaccinated with live attenuated vaccine than those vaccinated with inactivated vaccine. Therefore, the protection against challenge with vNDV in our work occurred due to the combined advantageous effects of live attenuated and inactivated vaccines.

In this work, protection against challenge with vNDV was observed at both 7 and 14 days post-revaccination. It may be that the vaccinated chickens had sufficient protective immunity at these times. Al-Garib et al. (2003) demonstrated that the HI titers in the serum of the chickens inoculated with live attenuated vaccine were firstly detected at 7 DPV and reached a plateau level at 14 DPV. In serum, IgM was produced early from 4 DPV and reached a plateau level at 7 DPV. IgG was detected at 7 DPV and reached a plateau level at 14 DPV. The highest level of NDV-specific IgA response was detected between 7 and 14 DPV. In trachea, IgM response was first detected at 4 DPV and peaked at 7 DPV. IgG was detected the same day as those in serum. IgA response was detected at 4 DPV and reached plateau level at 7 DPV. These are similar to the report of Rauwa et al. (2009), who found that live attenuated vaccines induced NDV-specific HI and IgG antibodies from 7 and 14 DPV, respectively. Systemic NDV specific IgA was first observed at 7 DPV and showed a peak value at 14 DPV. It could induce a measurable level of NDV-specific CMI within 7 DPV. Al-Garib et al. (2003) also reported that after vaccination with inactivated vaccine, HI response was recorded at 14 DPV. In serum, IgM was firstly detected at 14 DPV and remained at a high level until 28 DPV. IgG and a low IgA response were detected from 14 DPV onward. In trachea, IgM and IgG responses were detected from 7 and 14 DPV, respectively, but IgA response was not observed. Based on this fact, the early protection after vaccination was mainly due to immunization with live attenuated vaccine and the protection at 14 DPV was the result of immunity induced by both type of vaccines.

The dose-response was one of the factors that influenced the efficacy of NDV vaccines. This finding had been reported by Mass et al. 1999 who compared the dose-response effects of three inactivated ND vaccines. The results showed that chickens vaccinated with different doses of 1/12.5, 1/25, 1/50, 1/100, 1/200 and 1/400 had a similar dose-response curve on serologic response and clinical protection for the three vaccines. When the vaccine dose was lowered, a decrease in antibody titers and clinical protection was observed. However, the dose response effect was not found in this study. Our results showed that mortality rate, NDV HI titers and body weights of the chickens vaccinated with 0.1 ml and 0.25 ml were not significantly different ($p > 0.05$). These results may be due to the fact that different amount of inactivated virus such as immunogenic glycoprotein present in the vaccines between 0.1 and 0.25 ml were in a range that could induce sufficient protective immunity. In the study of Mass et al. 1999, the dose-response effect was found when a vaccine was initially diluted at 1/12.5, but in our work a vaccine was only diluted at 1/2.5 (from 0.25 ml to 0.1 ml). Moreover, in our study, we vaccinated with live attenuated simultaneously with inactivated vaccines and boosted with live attenuated vaccine.

In the groups which were challenged at 14 days old, although NDV HI titers at the inoculated time of the non-vaccinated control group were not significantly different ($p > 0.05$) from the others except for group 4, the mortality of the non-vaccinated control group was the highest and was significantly different from the others. This indicates that MDA, which is passive immunity, could not provide protection against the challenged virus as well as active immunity. On the other hand, the chickens of the vaccinated groups had both MDA and immune response induced by vaccines that gave significantly better protection than those of the non-vaccinated control chickens. This finding suggests that MDA may not be sufficient in the protection of offspring and a major concern is the necessity of vaccination at an early age in order to get the highest protective level against infection with vNDV. In the groups which were challenged at 21 days old, while MDA declined and had low titers as observed in the non-vaccinated control group, the NDV HI titers of the vaccinated groups only slightly declined and the level was significantly higher ($p < 0.05$) than that of the control group. This was the active immunity stimulated by the vaccines. Our work found that the lowest NDV HI titer of 2.10 showed a protection of 100%. It was the lower level of NDV HI titer that could protect against vNDV challenge compared to those reported by 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 (≤3 $\log_2$) showed depression and anorexia. The protection observed in vaccinated chickens with low serum titers may have been due to the effect of local immunity that was produced within upper respiratory and intestinal tracts after vaccination (van Eck et al., 1991; Takada and Kida, 1996; Rauwa et al., 2009). In this work, we observed that the NDV HI titers of the vaccinated groups 6 and 7 at 28 days old (14 DPI) was significantly lower ($p < 0.05$) than the other vaccinated groups and non-vaccinated control group. The results of low NDV HI titers found in those 2 vaccinated groups whereas high NDV HI titer found in non-vaccinated control group were similar to the observation of Ezema et al. (2009). They reported that at 20 DPI, the vaccinated group had NDV HI titer of 5.2 $\log_2$ whereas the non-vaccinated group had an NDV HI titer of 10 $\log_2$. In the case of lower NDV HI titers found in vaccinated groups 6 and 7 compared to those of other vaccinated groups, this might be due to the differences in the vaccination programs of each group.

In conclusion, all of the vaccination programs were able to provide a good protection against challenge with vNDV. The protection was observed in the chickens either infected at 7 and 14 days post-revaccination. The dose response effect was not found when vaccinated with 0.1 and 0.25 ml of inactivated vaccines. Moreover, revaccination with B1 or LaSota strains at 7 days old did not make a difference to the protection rate. However, it is necessary to evaluate the efficacy of vaccination programs that are available and to ascertain the most efficient method of vaccine administration practice in the field situation.

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