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Original Article

Efficacy of different vaccination programs of recombinant HVT-NDV vaccine against genotype VII NDV Challenge in broiler chickens

Nataya Charoenvisal[1] Bubpa Supannamoke[2] Rik Koopman[3] Jiroj Sasipreeyajan[1*]

Abstract

This study determined the efficacy of recombinant HVT-NDV (rHVT-NDV) vaccine simultaneously vaccinated with live vaccine at 1 day old and a booster vaccination with live vaccine at 10 days old against Asiatic, Genotype VII NDV in broiler chickens. The chickens were divided into 5 groups. The chickens in groups 1, 2 and 3 were vaccinated with rHVT-NDV vaccine and different live vaccines at 1 day old, and then finally received a booster vaccination at 10 days old. The chickens in groups 4 and 5 served as the positive and negative control groups, respectively. The chickens in groups 1, 2, 3 and 4 were challenged with NDV at 14, 21, 28 and 35 days old, 20 chickens of each group at a time. Clinical signs and mortality were observed for 14 days after each time of challenge. Blood samples were collected at 1 and 10 days old, on the virus inoculation day and 14 days post-inoculation, to determine NDV antibodies. Swab samples were collected from the chickens challenged with the virus at 35 days old to detect NDV RNA by real-time PCR. The results reveal that the protection rate of the vaccinated chickens, which were challenged at 14 days old was 70-95%, while the protection rate of all vaccinated groups increased to 90-100% when they were challenged at 21, 28 or 35 days old. The vaccinated groups had a lower rate of virus shedding when compared to the challenged control group. It is concluded that all of the vaccination programs of rHVT-NDV vaccine in this study could be used to reduce economic loss due to infection by Genotype VII NDV.

Keywords: chicken, recombinant HVT-NDV vaccine, efficacy, Newcastle disease virus

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Introduction

Newcastle disease (ND) is one of the major diseases affecting the poultry industry worldwide, and both broilers and layers of all ages are susceptible. This disease is caused by the Newcastle disease virus (NDV), also known as avian paramyxovirus type 1. It is a member of genus Avulavirus, family Paramyxoviridae. NDV is a non-enveloped, non-segmented, negative-sense RNA virus, which consists of 6 genes, which encode 6 structural proteins, including Nucleoprotein (NP), Phosphoprotein (P), Matrix protein (M), Fusion protein (F), Hemagglutinin-neuraminidase (HN) and Large protein (L). Importantly, F and HN are the surface glycoproteins, which bind to the host cell and initiate infection, and also induce host neutralizing antibodies. Moreover, the F protein cleavage site determines the virulence of NDV (Dortmans et al., 2011; Suarez, 2013; Ganar et al., 2014).

NDV has been roughly classified into 4 pathotypes depending on the virulence of the virus. The first pathotype is avirulent NDV, which causes no disease. Secondly, is the lentogenic NDV, which has low virulence and causes low mortality, but may affect egg production. Thirdly, there is the mesogenic NDV, which causes moderate virulence, and may raise mortality up to 50%. The last pathotype is velogenic NDV, which has high virulence and causes severe disease with high mortality. The latter pathotype is further subdivided into neurotrophic velogenic and viscerotropic velogenic NDV (OIE, 2009). The strains of NDV have been divided into 2 classes according to the nucleotide sequence of F and L genes (Umali et al., 2013). Class I has low virulence and is typically isolated from wild birds and Class II, which has been further divided into 16 genotypes (Kapczynski et al., 2013). Genotype I is low virulence, except for one velogenic NDV that caused an outbreak in Australia. Genotype II is a low virulent NDV, which has been used as a seed virus for vaccine production, such as B1, LaSota, Clone 30 and VG/GA strains. Genotypes III - IV are mostly mesogenic NDVs, while, genotype V - IX and XI - XVI are velogenic NDVs and genotype X is the low virulence virus (Diel et al., 2012b; Kapczynski et al., 2013; Suarez, 2013). Moreover, from the year 2000 onward, genotype VII NDV has been causing big outbreaks in many countries in Asia including countries in East Asia, China, Taiwan, Korea, Japan, Vietnam and Cambodia (Jeon et al., 2008; Miller et al., 2010; Choi et al., 2013; Umali et al., 2013; Choi et al., 2014; Ganar et al., 2014). The virus has been spreading to Africa, some countries in Europe, North America (where it is called exotic NDV) and South America has also been reported (Kapczynski and King, 2005; Susta et al., 2011; Diel et al., 2012a).

Although, many strains of NDV circulate in each part of the world, all of them are one serotype (Kapczynski et al., 2013). As a result, vaccination is a common way of protecting chickens from NDV. Various types of NDV vaccine are commercially available such as live attenuated, inactivated or killed, recombinant Pox-NDV and the recombinant herpes virus of turkey-NDV (rHVT-NDV) vaccines. Live vaccine mostly contains lentogenic NDV because regulations of the European Union have decreed that it must have an intracerebral pathogenicity index (ICPI) of not more than 0.4 (Suarez, 2013). The most common seed viruses for live vaccine production are LaSota, Clone 30, B1 and Ulster 2C strains. Live vaccine is efficacious in inducing IgA and local immunity with high levels of IgA, IgY and IgM (Takada and Hida, 1996; Seal et al., 2000; Suarez, 2013). However, maternally-derived antibody (MDA) is able to neutralize live vaccine (Suarez, 2013). Killed vaccines are also produced from those lentogenic ND viruses and some of the mesogenic NDV. The killed vaccines induce high and long-lasting neutralizing antibodies in the circulation but induce low local immunity (Takada and Hida, 1996; Kapczynski et al., 2013; Suarez, 2013). Recombinant vaccines are generally available nowadays. The F or HN glycoprotein genes of NDV were inserted into the virus vector of Poxvirus or serotype 3 Marek's disease virus (Herpesvirus of turkey or HVT). The chicken's immune system is able to produce neutralizing antibodies against F and HN glycoprotein of NDV and Poxvirus or Marek's disease virus (Seal et al., 2000). The recombinant HVT - NDV (rHVT-NDV) vaccine used in this study is HVT virus inserted with the F gene of genotype II NDV. The objective of this study was to determine the efficacy of rHVT-NDV vaccine simultaneously vaccinated with live NDV vaccine at 1 day old with a booster vaccination with live vaccine at 10 days old against challenge with Asiatic, Genotype VII NDV in broiler chickens.

Materials and Methods

Animal: Four hundred, female, broiler chickens (Ross 308) were brought from the commercial hatchery to the experimental animal facility at the Livestock Hospital, Faculty of Veterinary Science, Chulalongkorn University, Nakhon Pathom, Thailand. Commercial feed for broiler chickens and water were provided *ad libitum*. The guidelines and legislative regulations on the use of animals for scientific purposes of Chulalongkorn University were followed as certified in the permission No. 16310113.

Vaccines and virus: Vaccines used in this study include recombinant HVT- NDV (rHVT- NDV) vaccine (Innofusion® ND-SB, Intervet, Inc., USA) and 5 live attenuated vaccines, which were C2, NDV vaccine (Nobilis® ND C2, Intervet International B.V., The Netherlands), Clone 30, NDV vaccine (Nobilis® Clone 30, Intervet International B.V., The Netherlands), combined MA5, IBV + Clone 30, NDV vaccine (Nobilis® MA5 + Clone 30, Intervet International B.V., The Netherlands), MA5, IBV vaccine (Nobilis® IB MA5, Intervet International B.V., The Netherlands) and 4-91, IBV vaccine (Nobilis® IB 4-91, Intervet International B.V., The Netherlands). One dose of the recombinant vaccine contained at least 1,810 plaque forming units (PFU) of rHVT-NDV. One dose of live C2 and Clone 30 NDV vaccines contained at least $10^{5.5}$ 50% embryo infective dose (EID_{50}) and $10^{6.0}$ EID_{50} of virus, respectively. One dose of live Ma5 and 4-91 IBV vaccines contained at least $10^{3.0}$ EID_{50} and $10^{3.6}$ EID_{50} of virus, respectively. One dose of a combined

MA5+Clone 30 vaccine contained at least $10^{3.0}$ EID_{50} of IBV and $10^{6.0}$ EID_{50} of NDV, respectively. All the vaccines were provided by Intervet (Thailand) Ltd. The challenge virus was Asiatic, Genotype VII NDV (CU-2 strain, ICPI=1.86), which contained approximately 10^5 EID_{50}/0.1 ml.

Experimental design: As soon as the chickens arrived at the experimental site, they were randomly divided into 5 groups with 80 chickens in each group. Chickens in groups 1-3 were subcutaneously vaccinated with rHVT-NDV vaccine at 1 day old, 1 dose or 0.2 ml/bird. Then, the chickens in group 1 were vaccinated intra-ocularly with C2 NDV vaccine, group 2 were vaccinated intra-ocularly with combined MA5, IBV + Clone 30, NDV vaccine, and group 3 were vaccinated intra-ocularly with MA5 strain and 4-91 strain IBV vaccines. At 10 days old, the chickens in groups 1 and 2 received the same booster vaccination with combined MA5, IBV + Clone 30, NDV vaccine by the drinking water route. The chickens in group 3 received a booster vaccination with Clone 30 NDV vaccine by the drinking water route. To perform a booster vaccination by the drinking water route, the volume of water intake per chicken was measured 1 day before the vaccination day. Firstly, water was taken out of the cage for 2 hours. Then, 1 liter of water was placed in the cage for 45 minutes. After 1 hour, the average water intake per chicken was calculated by subtracting the remaining water from 2 liters and dividing by the number of chicken in one group (80 chickens). On the vaccination day, water was taken out from the cage for 2 hours as on the previous day. Doses of the vaccine were added into a specific volume of water which was determined from the previous day. Then, the chickens were allowed to consume water containing vaccine for around 45 minutes. Water intake had been continuously monitored in order to ensure that each chicken consumed the exact volume of water which contained one dose of the vaccine. The chickens in groups 4 and 5 were the positive and negative control groups, respectively. Next, each chicken in groups 1, 2, 3 and 4 was orally challenged at 14, 21, 28 and 35 days old, 20 chickens/group at a time, 0.1 ml/bird (Table 1). Clinical signs and mortality were observed for 14 days after each time of challenge. Dead chickens were necropsied and any gross lesions were observed. Body weights were measured on the inoculation day and 14 days post inoculation (DPI).

Table 1 Vaccination program and age of challenge

Group	Vaccination program		Number of chickens challenged with NDV			
	1 day old	Booster at 10 days old	14 D	21 D	28 D	35 D
1	C2 (I/O) rHVT-NDV (S/Q)	MA5+Clone 30 (DW)	20	20	20	20
2	MA5+Clone 30 (I/O) rHVT-NDV (S/Q)	MA5+Clone 30 (DW)	20	20	20	20
3	MA5 (I/O) & 4/91 (I/O) rHVT-NDV (S/Q)	Clone 30 (DW)	20	20	20	20
4	Non-vaccinated control	-	20	20	20	20
5	Non-vaccinated control	-	-	-	-	-

NDV = Newcastle disease virus, D = day old
C2 = C2 NDV vaccine, rHVT-NDV = recombinant Herpes virus of turkey-Newcastle disease virus vaccine, MA5+Clone 30 = combined MA5 IBV and Clone 30 NDV vaccine, MA5 & 4/91 = MA5 IBV and 4/91 IBV vaccines
S/Q = subcutaneous injection, I/O = intra-ocular route, DW = drinking water route

Serological test and real-time polymerase chain reaction (PCR): Five blood samples per group were randomly collected at 1 day old. After that, blood samples were collected at 10 days old, on the inoculation day (0 DPI) and 14 DPI. Sera were collected and tested for NDV antibodies by the haemagglutination-inhibition (HI) test.

The virus shedding of the 35-day-old inoculated chickens was determined by collecting 10 oropharyngeal swabs of 10 chickens and 10 cloacal swabs of another 10 chickens from each treatment group at 2, 4, 7, 10 and 14 DPI. The RNA from each swab sample in viral transporting medium was extracted using QIAamp® Viral RNA Mini kit (Qiagen, USA), followed by converting the RNA samples to cDNA using Thermo Scientific Revert Aid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA). Real-time PCR was performed using Kapa Probe fast qPCR kit (Kapa Biosystems, USA) and Rotor-Gene™ Q real-time PCR machine (Qiagen, USA). Primers and fluorescence probe were specific to NDV's Matrix gene. The real-time PCR machine revealed results in graphs, Ct value, and calculation of the concentration in copies number/microliter. The R2 value of the tests were $\geq$ 0.95 and the threshold was set above the no template control sample (NTC) (around 0.029).

Statistical analysis: Body weights and antibody titers were analyzed and compared between groups using ANOVA and least significant difference (LSD) test using SPSS software version 22. The percentage of mortality was calculated using Chi-square values. Differences between groups were considered significant at $p<0.05$. The normal distribution was tested using SPSS software version 22.

Results

Mortality rate: The mortality of chickens in group 1 was 25 and 10 percent, when they received the challenged virus at 14 and 21 days old, respectively. But there was no mortality when they received the virus at 28 and 35 days old. The mortality of chickens in group 2 was 30, 10, 5 and 10 percent, when they received the challenge virus at 14, 21, 28 and 35 days old, respectively. The mortality of chickens in group 3

was 5 and 10 percent, when they received the challenge virus at 14 and 28 days old, respectively. But there was no mortality when they received the virus at 21 and 35 days old. The mortality of chickens in group 4, which was the non-vaccinated challenged control group, was 95, 95, 100 and 100 percent, when they received the challenge virus at 14, 21, 28 and 35 days old, respectively. There was no mortality of chickens in group 5, which was the non- vaccinated and unchallenged control group (Table 2).

Table 2 Mortality rate after NDV inoculation

Group	NDV challenge at							
	14 days old		21 days old		28 days old		35 days old	
	Number[A]	Percent	Number[A]	Percent	Number[A]	Percent	Number[A]	Percent
1	5/20	25[a,b]	2/20	10[a]	0/20	0[a]	0/20	0[a]
2	6/20	30[a]	2/20	10[a]	1/20	5[a]	2/20	10[a]
3	1/20	5[b,c]	0/20	0[a]	2/20	10[a]	0/20	0[a]
4	19/20	95[d]	19/20	95[b]	20/20	100[b]	20/20	100[b]
5	0/20	0[c]	0/20	0[a]	0/20	0[a]	0/20	0[a]

DPI = day post inoculation, D = day old

[A] Number of dead chickens / Total chickens in the group

[a,b,c,d] Different superscript (a, b, c, d) in each group of the same column means statistically significant difference ($p<0.05$).

Body weight: The body weight was measured before challenge (0 DPI) and at 14 PDI. The mean body weight of each measuring day was similar between each treatment group. Except in group 4 of the 14-day-old inoculated date where there was only one chicken left weighing 575 grams, while the mean body weight of the chickens in the other groups at 28 days old (14 DPI) was between 1,204 ± 202.47 to 1,290 ± 230.50 grams. The mean body weight of 21 days old inoculated chickens was not different between each group (ranging from 810 ± 32.48 to 812 ± 31.48 at 0 DPI and from 1,505 to 1.681 ± 291.65 at 14 DPI). The mean body weight of 28 days old inoculated chickens was also no different between each group (ranging from 1,201 ± 55.02 to 1,206 ± 56.51 at 0 DPI and from 2,312 ± 73.86 to 2,371 ± 369.10 at 14 DPI). The mean body weight of 35 days old inoculated chickens was also similar between each group (ranging from 1,638 ± 101.87 to 1,643 ± 128.66 at 0 DPI and from 2,392 ± 525.00 to 2,572 ± 225.93 at 14 DPI) (Table 3).

Table 3 Mean body weight on the challenge day (0 DPI) and 14 DPI

Group	NDV challenge at							
	14 days old		21 days old		28 days old		35 days old	
	0 DPI (14 D)	14 DPI (28 D)	0 DPI (21 D)	14 DPI (35 D)	0 DPI (28 D)	14 DPI (42 D)	0 DPI (35 D)	14 DPI (49 D)
1	456 ± 27.62[A] (20)[B]	1,204 ± 202.47 (15)	811 ± 49.63 (20)	1,681 ± 291.65 (18)	1,206 ± 56.51 (20)	2,339 ± 238.50 (20)	1,641 ± 104.94 (20)	2,392 ± 525.00 (20)
2	456 ± 26.39 (20)	1,290 ± 230.50 (14)	811 ± 30.50 (20)	1,640 ± 301.51 (18)	1,204 ± 88.55 (20)	2,328 ± 178.00 (19)	1,643 ± 128.66 (20)	2,572 ± 225.93 (18)
3	456 ± 29.55 (20)	1,271 ± 136.52 (19)	811 ± 577.94 (20)	1,660 ± 228.47 (20)	1,205 ± 78.25 (20)	2,371 ± 369.10 (18)	1,638 ± 101.87 (20)	2,466 ± 261.71 (20)
4	456 ± 30.01 (20)	575 (1)	810 ± 32.81 (20)	1,505 (1)	1,205 ± 68.58 (20)	-[C]	1,641 ± 105.18 (20)	-[C]
5	456 ± 23.67 (20)	1,230 ± 73.77 (20)	812 ± 31.48 (20)	1,632 ± 135.98 (20)	1,201 ± 55.02 (20)	2,312 ± 73.86 (20)	1,643 ± 112.77 (20)	2,421 ± 161.82 (20)

DPI = day post inoculation, D = day old

[A] All data of body weight in this table is presented as mean body weight (gm/bird) ± standard deviation (SD).

[B] Number in parentheses under each body weight means number of chickens in the group.

[C] All chickens died due to ND before 14 DPI.

Serological results: Chickens had high maternally-derived antibodies (MDA) at 1 day old (7.00±1.00 to 7.80±0.84 log_2), but this slowly declined to 3.90±0.88 to 4.50±0.71 log_2, when the chickens were 10 days old, and continuously declined to 2.40±0.50 to 3.00±0.97 log_2 when the chickens were 14 days old. In chickens which had not received the vaccine (groups 4 and 5), the antibody titers slowly declined to 1.45±0.69 to 1.55±0.83 log_2 at 21 days old. The antibody titers of chickens in groups 1, 2 and 3 before virus inoculation were significantly higher than those of groups 4 and 5 at 21, 28 and 35 days old. In all challenged chickens (groups 1-4), antibody titers at 14 DPI highly increased, compared to 0 DPI. In contrast, an increase of antibody titers in the negative control group (group 5) was not detected (Table 4).

Viral detection by real-time PCR: Virus shedding was determined by collecting 10 oropharyngeal swabs from 10 chickens and 10 cloacal swabs from another 10 chickens. The swabs were collected from 35-day-old NDV inoculated chickens (groups 1, 2, 3 and 4) at 2, 4,

7, 10 and 14 DPI. The results reveal that at 2 DPI, group 1 had the highest number of chickens (11 birds) shed the virus, groups 2 and 4 had 8 positive birds, and group 3 had 5 positive birds. At 4 DPI, the number of birds that shed the virus was 5 birds, which was equaled in groups 1, 2 and 3, while group 4 had 14 birds shed the virus. At 7 DPI, 6, 4 and 2 birds of groups 1, 2 and 3 shed the virus, respectively. All chickens in group 4 were dead before 7 DPI. At 10 DPI, 6, 1, and 1 bird of groups 1, 2 and 3 shed the virus. At 14 DPI, 4 birds in group 1 and 1 bird in group 2 shed the virus, while there was no shedding found in group 3 birds. Total swab samples of group 1 at 2, 4, 7, 10 and 14 DPI were 100 samples, of which 32 were positive (32 percent). Fifty samples were oropharyngeal swabs, of which 14 were positive (28 percent) and another 50 samples were cloacal swabs, of which 18 were positive (36 percent). The total swab samples of group 2 at 2, 4, 7, 10 and 14 DPI were 94 samples, of which 19 were positive (20.21 percent). Forty-seven samples were oropharyngeal swabs, of which 5 were positive (10.64 percent) and another 47 samples were cloacal swabs, of which 14 were positive (29.79 percent). The total swab samples of group 3 at 2, 4, 7, 10 and 14 DPI were 100 samples, of which 13 were positive (13 percent). Fifty samples were oropharyngeal swabs, of which 8 were positive (16 percent) and another 50 samples were cloacal swabs, of which 5 were positive (10 percent). Total swab samples of group 4 at 2 and 4 DPI were 40 samples, of which 22 were positive (55 percent). Twenty samples were oropharyngeal swabs, of which 11 were positive (55 percent) and another 40 samples were cloacal swabs, of which 11 were positive (55 percent) (Table 5).

Table 4 Antibody titers against NDV measured by the HI test

Group	ND-HI titer (log_2)^{A}									
	Maternally-derived antibodies		NDV challenge at 14 days old		NDV challenge at 21 days old		NDV challenge at 28 days old		NDV challenge at 35 days old	
	0 D	10 D	0 DPI (14 D)	14 DPI (28 D)	0 DPI (21 D)	14 DPI (35 D)	0 DPI (28 D)	14 DPI (42 D)	0 DPI (35 D)	14 DPI (49 D)
1	7.80 ± 0.84 (5)^{B}	3.90 ± 0.88 (10)	2.65 ± 0.99^{a,b} (20)	7.53 ± 2.00^{b} (15)	2.35 ± 0.81^{b} (20)	10.78 ± 1.40^{a} (18)	2.90 ± 1.83^{a} (20)	10 ± 2.20^{b} (20)	3.60 ± 0.68^{a} (20)	11.20 ± 1.40^{a,b} (20)
2	7.80 ± 0.45 (5)	4.50 ± 0.71 (10)	3.00 ± 0.97^{a} (20)	8.21 ± 2.22^{a,b} (14)	2.05 ± 0.76^{b,d} (20)	9.59 ± 1.87^{b} (18)	2.10 ± 1.21^{b} (20)	11.63 ± 1.80^{a} (19)	3.85 ± 0.88^{a} (20)	10.50 ± 1.47^{b} (18)
3	7.20 ± 1.10 (5)	4.20 ± 0.92 (10)	2.40 ± 0.50^{b} (20)	6.05 ± 1.65^{c} (19)	4.85 ± 1.23^{c} (20)	8.45 ± 2.06^{c} (20)	3.95 ± 1.61^{c} (20)	11.83 ± 1.10^{a} (18)	4.55 ± 0.89^{b} (20)	11.30 ± 1.13^{a,b} (20)
4	7.00 ± 1.00 (5)	4.20 ± 1.03 (10)	2.60 ± 0.88^{a,b} (20)	9.00 (1)	1.45 ± 0.69^{a} (20)	8.00 (1)	1.00 ± 0.00^{d} (20)	-^{C}	1.00 ± 0.00^{c} (20)	-^{C}
5	7.20 ± 0.45 (5)	4.20 ± 0.79 (10)	2.60 ± 0.99^{a,b} (20)	1.10 ± 0.31^{d} (20)	1.55 ± 0.83^{a} (20)	1.00 ± 0.00^{d} (20)	1.00 ± 0.00^{d} (20)	1.00 ± 0.00^{c} (20)	1.00 ± 0.00^{c} (20)	1.00 ± 0.00^{c} (20)

DPI = day post inoculation, D = day old

^{A} All data of HI titers in this table is presented as mean ± standard deviation (SD).

^{B} Number in parentheses under each HI titer means number of samples tested.

^{C} All chickens died due to ND before 14 DPI.

^{a,b,c,d} Different superscript (a, b, c, d) in each group of the same column means statistically significant difference ($p<0.05$).

Table 5 Number of NDV shedding chickens via oral and cloacal routes at 2, 4, 7, 10 and 14 DPI of 35 days old inoculated chickens that were positive for real-time PCR specific for NDV M gene

Group	Number of chickens that were NDV positive by real-time PCR^{A}														
	2 DPI			4 DPI			7 DPI			10 DPI			14 DPI		
	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T
1	4 (10^{B})	7 (10)	11 (20)	2 (10)	3 (10)	5 (20)	2 (10)	4 (10)	6 (20)	2 (10)	4 (10)	6 (20)	4 (10)	0 (10)	4 (20)
2	1 (10)	7 (10)	8 (20)	2 (10)	3 (10)	5 (20)	1 (9)	3 (9)	4 (18)	0 (9)	1 (9)	1 (18)	1 (9)	0 (9)	1 (18)
3	4 (10)	1 (10)	5 (20)	1 (10)	4 (10)	5 (20)	2 (10)	0 (10)	2 (20)	1 (10)	0 (10)	1 (20)	0 (10)	0 (10)	0 (20)
4	3 (10)	5 (10)	8 (20)	8 (10)	6 (10)	14 (20)	(-)^{C}	(-)^{C}	(-)^{C}	(-)^{C}	(-)^{C}	(-)^{C}	(-)^{C}	(-)^{C}	(-)^{C}

DPI = day post inoculation, O = oropharyngeal swab, C = cloacal swab, T = total oropharyngeal and cloacal swabs

^{A} All data in this table is number of samples that were NDV positive.

^{B} Number in parentheses means number of chickens examined.

^{C} All group 4 chickens died before 7 DPI.

Discussion

Genotype VII NDV have been reported in South East Asia countries, such as Cambodia and Vietnam, which are neighboring countries of Thailand (Choi et al., 2013; Choi et al., 2014). Based on the geographical location of these outbreaks, Thailand is at great risk, so effective preventive measures are highly recommended. Besides a strict policy of animal trading and biosecurity, an efficacious vaccination strategy is one solution that can protect chickens from this emerging virus. This study determined the efficacy of recombinant HVT-NDV vaccine simultaneously vaccinated with live vaccine at 1 day old, followed by a booster vaccination with live vaccine at 10 days old, against challenge with Asiatic, Genotype VII NDV in broiler chickens.

NDV vaccines are able to protect the animal from morbidity and mortality, but chickens are still susceptible to the virus and are able to shed the virus. However, the amount of virus shedding depends on the host's immunity, the virulence of the virus, the viral load, the type of vaccine, the route of vaccination and the duration between vaccination and challenge (Miller et al., 2013). In this study, the chickens in the vaccinated groups had a significantly lower mortality rate than the non-vaccinated challenged control group.

The MDA were as high as 7.00±1.00 to 8.40±0.55 log_2 at 1 day old and declined to very low level (1.45±0.69 to 1.55±0.83 log_2) at 21 days old. Some of the vaccinated groups had a lower rate of protection against early NDV challenge at 14 days old. This might be due to the decrease of MDA to a level, which could not protect the chickens, while the antibody induced by vaccination was still insufficient to protect them against the challenge virus. As a result, there was a high risk of NDV infection during 2 - 3 week of age, when the chickens were still susceptible to the virus, especially when the biosecurity level was not good.

Interestingly, on the challenge date at 14 days old, chickens in group 3 had the lowest antibody titers but the lowest mortality rate. The low antibody titers at 14 days old represented the remains of passive antibody or MDA, since the immune response induced by the recombinant vaccine was the antibody against the F protein of NDV, which could not be detected by the HI test. Moreover, the chickens' immune system might not yet respond to the booster vaccination of live vaccine at 10 days old. Therefore, the specific antibody against the F protein of NDV, might play an important role against NDV challenge. Unfortunately, the serum was not tested by ELISA specific to F protein. Therefore, the level of antibody against the NDV F protein was not determined.

On the challenge date at 21, 28 and 35 days old, the HI antibody titers against NDV of the vaccinated groups were significantly higher than those of the non-vaccinated control groups. Among the vaccinated groups, the chickens in group 3 had the highest antibody titers. This result might be due to the difference of vaccination program for chickens in group 3 which were not vaccinated with live NDV vaccine at 1 day old but were vaccinated with live NDV vaccine at 10 days old. Therefore, the interference of IBV vaccine on the antibody response of NDV vaccine was not affected. The interference between IBV and NDV was previously studied by other researchers. Live combined ND-IB vaccine did not have any effect on IBV antibody level but had an effect on NDV antibody level. This occurred due to both IBV and NDV primarily infecting the same target cells which were respiratory epithelial cells and replicate in the cytoplasm. Moreover, IBV vaccine can decrease Harderian gland activity and result in a lower immune response to NDV, which cause lower NDV antibody titer as chickens in groups 1 and 2 of our study (Montgomery et al., 1997; Cardoso et al., 2005). However, all vaccinated groups had high rate of protection against the challenge virus. All surviving chickens at 14 DPI had significantly higher NDV titers when compared to those of 0 DPI, which were due to NDV challenge.

The non-vaccinated, challenged control group had 95 percent mortality when challenged at 14 and 21 days old, and 100 percent mortality when were challenged at 28 and 35 days old. That is to say that, the MDA of the non-vaccinated chickens diminished before the chickens were 28 days old. There are several pieces of research that have monitored MDA levels against NDV. Rahman et al. (2002) reported that MDA HI titers of up to 16 (4 log_2) failed to protect the chickens against virulent NDV, but if the HI titers were 32 (5 log_2) and above, the birds were able to resist NDV infection. Jalil et al. (2009) summarized that MDA titers of 128 (7 log_2) or above are adequate to protect chickens from virulent NDV challenge. In our study, the birds in group 4 had antibody titers of 2.6 ± 0.88 (log_2) at 14 days old, which was one of the challenge days. Birds in group 4 that were challenged on that day had 95% mortality. At 21 days old, the birds in group 4 had 1.45 log_2 antibody titers and also had 95% mortality. In other words, MDA titer around 2-3 log_2 is not sufficient to protect birds from virulent NDV strains. On the other hand, chickens in the vaccinated groups had antibody titers around 2.05 - 4.85 log_2 and exhibited significantly milder clinical signs and a lower mortality rate. For example, chickens in group 3, challenged at 14 days old, had antibody titer of 2.40 ± 0.50 log_2, which was lower than that of group 4. But the mortality rate of group 3 was only 5%. This result could be due to the protective efficacy of the specific antibody induced by the inserted F protein of NDV in the recombinant vaccine. Although, NDV has 16 genotypes all of them are the same serotype. So the F gene inserted in this HVT-NDV vaccine, which was from genotype II NDV, was able to stimulate neutralizing antibody that protected the chickens from genotype VII NDV challenged. However, it was unable to be detected by the HI test as previously mentioned. Moreover, local immunity and cell-mediated immunity (CMI), induced by live vaccine also were not detected by the HI test, and could be additional immunity to support the high protection rate of those vaccinated chickens with low HI titers. In contrast, MDA is a passive immunity, where the antibodies can be directly transferred to the newborns and it is in the blood circulation only.

NDV shedding of the 35-day-old challenged chickens was detected by real-time PCR. There was no significant relation between the number of virus copies and duration of viral shedding, either in the vaccinated

or the non-vaccinated groups (data is not shown). Virus shedding was detected as early as 2 DPI in both vaccinated and non-vaccinated birds. At 4 DPI, the numbers of oropharyngeal swabs that were NDV positive were as high as 80% in the non-vaccinated chickens, compared to only 10-20% in the vaccinated chickens. While the results of cloacal swabs at 4 DPI, were 60% NDV was positive in the non-vaccinated groups, and 30-40% positive in the vaccinated groups. The number of chickens that were NDV positive in the vaccinated chickens declined at 7 DPI, while all of the non-vaccinated chickens died before 7 DPI. Chickens in groups 1 and 2 continued to shed the virus until 14 DPI, while chickens in group 3 shed the virus only until 10 DPI. Comparing groups 1-3, the least virus shedding group was group 3 which was 13 percent (13 positive samples from 100 samples tested). There were no differences between NDV positive results detected from oropharyngeal and cloacal swabs.

Likewise, at 35 days old, before challenge, the chickens in group 3 had the highest mean antibody titer levels, followed by group 2 and group 1. As mentioned earlier, vaccination could protect the chickens from morbidity and mortality, but did not protect them from infection and virus shedding (Miller et al., 2013). However, vaccination programs used in this study were able to decrease virus shedding. Hence, excellent biosecurity will further assist reducing the risks of having pathogens in farms and will aid controlling unwanted infections.

In conclusion, chickens which received either live C2, NDV or MA5, IBV + Clone 30, NDV vaccines simultaneously vaccinated with recombinant HVT-NDV vaccine at 1 day old and further received a booster vaccination with live combined MA5, IBV + Clone 30, NDV vaccine at 10 days old, and chickens which received recombinant HVT-NDV at 1 day old and were simultaneously vaccinated with live MA5, IBV and live 4-91, IBV vaccines and received a booster vaccination with live Clone 30, NDV vaccine at 10 days old, all had a high protection rate against high challenge level of Asiatic, Genotype VII NDV at 14, 21, 28 and 35 days old.

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บทคัดย่อ

ประสิทธิภาพของวัคซีนรีคอมบิแนนท์เฮชวีทีเอ็นดีวีในโปรแกรมวัคซีนที่แตกต่างกันต่อการป้องกันเชื้อไวรัสนิวคาสเซิลจีโนไทป์ 7 ในไก่เนื้อ

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การศึกษาครั้งนี้เป็นการทดสอบประสิทธิภาพของวัคซีนรีคอมบิแนนท์เฮชวีทีเอ็นดีวี โดยให้พร้อมกับวัคซีนเชื้อเป็นเมื่อไก่อายุ 1 วัน และให้วัคซีนเชื้อเป็นซ้ำเมื่อไก่อายุ 10 วัน จากนั้นให้ไวรัสนิวคาสเซิล จีโนไทป์ 7 ในไก่เนื้อ โดยแบ่งไก่เป็น 5 กลุ่ม ไก่ในกลุ่ม 1, 2 และ 3 ได้รับวัคซีนรีคอมบิแนนท์เฮชวีทีเอ็นดีวี และวัคซีนเชื้อเป็นต่างชนิดกันเมื่ออายุ 1 วัน และได้รับวัคซีนเชื้อเป็นซ้ำเมื่ออายุ 10 วัน ไก่ในกลุ่ม 4 และ 5 เป็นกลุ่มควบคุมบวกและกลุ่มควบคุมลบตามลำดับ ไก่ในกลุ่ม 1, 2, 3 และ 4 ได้รับไวรัสนิวคาสเซิล เมื่ออายุ 14, 21, 28 และ 35 วัน จำนวน 20 ตัว/กลุ่ม/ครั้ง สังเกตอาการป่วยและอัตราการตายเป็นเวลา 14 วัน ภายหลังไก่รับไวรัสแต่ละครั้ง และเก็บเลือดเพื่อตรวจแอนติบอดีต่อไวรัสนิวคาสเซิลเมื่อไก่อายุ 1 วัน 10 วัน วันที่ไก่ได้รับไวรัส และ 14 วันภายหลังไก่ได้รับไวรัส นอกจากนี้ยังได้เก็บตัวอย่างจากไก่ที่ได้รับไวรัสเมื่ออายุ 35 วัน เพื่อตรวจหาสารพันธุกรรมของไวรัส ผลการทดลองพบว่า ไก่ที่ได้รับวัคซีนและได้รับไวรัสที่อายุ 14 วัน มีอัตราการรอดชีวิต 70-95% ส่วนกลุ่มที่ได้รับวัคซีนและไวรัสที่อายุ 21, 28 หรือ 35 วัน มีอัตราการรอดชีวิตเพิ่มขึ้นเป็น 90-100% นอกจากนี้ ยังพบว่า กลุ่มที่ได้รับวัคซีน มีจำนวนไก่ที่ปล่อยไวรัสออกจากร่างกายน้อยกว่า กลุ่มที่ไม่ได้รับวัคซีน ผลการทดลองนี้สรุปได้ว่า โปรแกรมวัคซีนที่ประกอบด้วยวัคซีนรีคอมบิแนนท์เฮชวีทีเอ็นดีวี ทั้ง 3 โปรแกรม สามารถลดอัตราการสูญเสียจากไวรัสนิวคาสเซิลจีโนไทป์ 7 ได้

คำสำคัญ: ไก่ วัคซีนรีคอมบิแนนท์เฮชวีทีเอ็นดีวี ประสิทธิภาพ ไวรัสนิวคาสเซิล

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