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# Protective Efficacy of Live LaSota Strain Newcastle Disease Virus Vaccine in Layer-type Chickens

Jiroj Sasipreeyajan<sup>1\*</sup> Phornphisut Areeraksakul<sup>1</sup> Somkid Khanda<sup>1,2</sup>

## *Abstract*

The purpose of this study was to evaluate the protective efficacy of live LaSota strain Newcastle disease virus (NDV) vaccine administered with different routes against a NDV challenge in layer-type chickens. Fifty male layer-type chickens were divided into 3 groups. There were 20 chickens/group for the vaccinated groups (Groups 1 and 2) and 10 chickens for the non-vaccinated control group (Group 3). Chickens in Groups 1 and 2 received the vaccine individually at 4 weeks old by eye drop and oral drop, respectively. All chickens received NDV challenge individually by oral drop at 7 weeks old or 3 weeks post-vaccination (PV). At 3 weeks PV, the results reveal that the haemagglutination-inhibition (HI) titer of the vaccinated Group 1 was significantly higher than that of the vaccinated Group 2 ( $p < 0.05$ ) and they were significantly higher than that of the non-vaccinated Group 3 ( $p < 0.05$ ). The protection rate of the vaccinated Groups 1 and 2 were 100 and 60 percent, respectively. There was no protection in the non-vaccinated Group 3. The results of this study indicate that single vaccination with live LaSota strain NDV vaccine is adequate in protection against a NDV challenge in layer-type chickens if the chickens receive the appropriate route of vaccine administration.

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**Keywords:** chicken, live LaSota strain vaccine, route, Newcastle disease virus, efficacy

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## Introduction

Newcastle disease (ND) is a major problem faced by the poultry industry worldwide as it can cause 100% mortality in non-vaccinated chickens. ND is caused by the Newcastle disease virus (NDV), an enveloped RNA virus, belonging to genus *Avulavirus* in the family Paramyxoviridae. Strains of NDV have been classified into 5 pathotypes: viscerotropic velogenic, neurotropic velogenic, mesogenic, lentogenic and asymptomatic enteric pathotypes. ND caused by virulent NDV (vNDV) is a notable disease problem in the Middle East, Africa and Asia, and the spread of vNDV has been recorded between countries. The routes of infection are inhalation of viral aerosol or ingestion of viral contaminated feed and water. Viral infection affects the respiratory, nervous and gastrointestinal systems. ND is characterized by listlessness, respiratory distress and weakness, followed by head or muscular tremors, torticollis and paralysis, approximately 5 days after infection (Ahmad et al., 2007; Kapczynski et al., 2013; Miller and Koch, 2013; Okwor et al., 2013).

Presently, ND is controlled by good management practices, biosecurity and good hygiene in conjunction with vaccination. Vaccination is routinely practiced in commercial farms in many countries (Miller and Koch, 2013; Ganar et al., 2014). Many lentogenic and asymptomatic enteric pathotype NDVs have been used as live vaccine, including B1, LaSota, VG/GA, Ulster 2C and Queensland V4 (Villegas, 1998; Glisson, 2013). LaSota strain NDV is a very low virulent virus (Gallili and Ben-Nathan, 1998), commonly used as a live vaccine as it replicates in the respiratory system (Al-Garib et al., 2003).

Live lentogenic vaccines can be administered by the intraocular route, nasal route, through drinking water and by spray (Abbas et al., 2006). Immune response after vaccination depends on the route of administration. Mass application of live vaccine by drinking water is often practiced due to its convenience, less cost and less time consumed compared to individual vaccination. While intraocular administration is highly effective and induces better immune response, it is a time consuming method (Collett, 2013; Miller and Koch, 2013).

Administration methods and vaccination programs may be different depending on local disease situations (Rehmani, 1996). Therefore, each country needs to evaluate the efficacy of available vaccines and ascertain the most efficient application methods. One study reported only 53% population protection after chickens received a live vaccine in drinking water compared to 93% population protection in chickens which received the vaccine by the intraocular route (Degefa et al., 2004). The much lower protection rate of chickens that received the vaccine through drinking water was probably due to the inability to control the amount of water consumed per bird (Glisson, 2013). This leads to the hypothesis, if each chicken receives the right amount of vaccine orally, they will receive the same level of protection as chickens which receive the vaccine intraocularly. The objective of this study was to evaluate the protective efficacy of a live LaSota strain NDV vaccine given at 4 weeks old and with different

routes of administration, ocular and oral drop, against a NDV challenge in layer-type chickens. In this study, an oral drop replaced the drinking water method to ensure that each chicken received the right amount of vaccine orally.

## Materials and Methods

**Chickens:** Fifty male layer-type chickens (Babcock B380) were brought from a commercial hatchery in Chachoengsao province to Chulalongkorn 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, Nakhon Pathom, Thailand. The chickens were divided in 3 groups at 4 weeks old. There were 20 chickens/group for the vaccinated groups (Groups 1 and 2) and 10 chickens for the non-vaccinated control group (Group 3). Chickens in Groups 1 and 2 received NDV vaccine individually at 4 weeks old by eye drop and oral drop, respectively. Commercial feed for layer pullet and water were provided *ad libitum*. The guidelines and legislative regulations on the use of animals for scientific purposes of Chulalongkorn University, Bangkok, Thailand were followed as is certified in permission No. 13310057.

**Vaccines:** Live LaSota strain NDV vaccine (New Vac-LS®, Fort Dodge, Brazil) was obtained from Zoetis (Thailand) Limited. Each chicken received 1 dose of the vaccine containing at least  $10^{6.2}$  EID<sub>50</sub> of LaSota strain NDV.

**Challenge study:** At 7 weeks old (3 weeks PV), all chickens in Groups 1, 2 and 3 were individually challenged with virulent NDV (CU-2 strain, ICPI=1.86). Each chicken received approximately  $10^6$  EID<sub>50</sub> of virulent NDV by oral drop (Sasipreeyajan et al., 2012). Clinical signs and mortality were observed for 2 weeks post-inoculation (PI). Typical gross lesions of ND were confirmed from post-mortem examination, and NDV was identified and typed as a virulent strain by using one-step RT-PCR followed by restriction endonuclease analysis (Creelan et al., 2002).

**Body weight:** Individual chicken in Groups 1, 2 and 3 were weighed before vaccination, before NDV challenge (3 weeks PV) and 2 weeks PI at 4, 7 and 9 weeks old, respectively. Average body weight of each group was calculated and compared.

**Serological evaluations:** Individual chicken in Groups 1, 2 and 3 was bled before vaccination, before NDV challenge (3 weeks PV) and 2 weeks PI at 4, 7 and 9 weeks old, respectively. Sera were collected and tested for NDV antibodies by the haemagglutination inhibition (HI) test, micro method (Allan and Gough, 1974). Average NDV titer of each group was calculated and compared.

**Statistical analysis:** Body weight and NDV HI titers were analyzed and compared between groups using ANOVA and the least significant difference (LSD) test. Mortality and protection rate after NDV challenge was

analyzed using Chi-square values. Differences between groups were considered significant at  $p < 0.05$ .

## Results

**Body weight:** Chickens in Group 2 started at lower body weight than those of Groups 1 and 3 at 4 weeks old, but they had the same health status as chickens in Groups 1 and 3. At 9 weeks old (2 weeks PI), body weight of chickens in Group 2 was slightly lower than that of Group 1 but they were not significantly different ( $p > 0.05$ ) (Table 1).

**NDV HI titers:** At 4 weeks old, before chickens in Groups 1 and 2 received the vaccine, antibodies against NDV of chickens in Groups 1, 2 and 3 were at the same level,  $\leq 1.0 \log_2$ . At 7 weeks old (3 weeks PV), antibodies of chickens in Group 1 increased to  $3.65 \pm 1.39 \log_2$  which was significantly higher than that of Group 2, which was  $1.80 \pm 1.74 \log_2$ . HI titer of the non-vaccinated control Group 3 was  $\leq 1.0 \log_2$  which was the same as at 4 weeks old. Antibodies of chickens in Groups 1 and 2 increased to  $12.43 \pm 1.43 \log_2$  and  $11.92 \pm 1.16 \log_2$ , respectively at 9 weeks old (2 weeks PI) due to the NDV challenge (Table 2).

**Table 1** Average body weight of each group at different ages

Group	Body weight (gm/bird)		
	4 weeks old	7 weeks old	9 weeks old
1	423±23.53 <sup>A,a</sup> (20) <sup>B</sup>	907±54.47 <sup>a</sup> (20)	1,150±101.8 <sup>a</sup> (20)
2	380±37.80 <sup>b</sup> (20)	838±86.87 <sup>b</sup> (20)	1,063±274.86 <sup>a</sup> (12)
3	423±25.41 <sup>a</sup> (10)	913±83.61 <sup>a</sup> (10)	- <sup>C</sup>

<sup>A</sup> Mean  $\pm$  standard deviation (SD)

<sup>B</sup> Number of chickens in the group

<sup>C</sup> All chickens died due to ND before 9 weeks old.

<sup>a,b</sup> The different superscript in each column means statistically significant difference ( $p < 0.05$ ).

**Table 2** Mean HI titers before and after NDV challenge

Group	Mean HI titers ( $\log_2$ )		
	4 weeks old	7 weeks old	9 weeks old
1	$\leq 1.0 \pm 0^A$ (20) <sup>B</sup>	3.65±1.39 <sup>a</sup> (20)	12.43±1.43 <sup>a</sup> (20)
2	$\leq 1.0 \pm 0$ (20)	1.80±1.74 <sup>b</sup> (20)	11.92±1.16 <sup>a</sup> (12)
3	$\leq 1.0 \pm 0$ (10)	$\leq 1.0 \pm 0^c$ (10)	- <sup>C</sup>

<sup>A</sup> Mean  $\pm$  standard deviation (SD)

<sup>B</sup> Number of samples tested

<sup>C</sup> All chickens died due to ND before 9 weeks old.

<sup>a,b,c</sup> The different superscript in each column means statistically significant difference ( $p < 0.05$ ).

**Table 3** Mortality and protection rate after NDV challenge

Group	Vaccine		Mortality (2 wk PI)		Protection (2 wk PI)	
	Age (wk)	Route	Number	Percent	Number	Percent
1	4	eye drop	0/20 <sup>A,a</sup>	0	20/20 <sup>B,a</sup>	100
2	4	oral drop	8/20 <sup>b</sup>	40	12/20 <sup>b</sup>	60
3	4	non-vaccinated	10/10 <sup>c</sup>	100	0/10 <sup>c</sup>	0

<sup>A</sup> Number of dead chickens / total chickens in the group

<sup>B</sup> Number of survival chickens / total chickens in the group

<sup>a,b,c</sup> The different superscript in each column means statistically significant difference ( $p < 0.05$ ).

**Protection rate:** At 2 weeks PI, there was 100% mortality in the non-vaccinated control group (Group 3). There were 0% (0/20) and 40% (8/20) mortality or 100% (20/20) and 60% (12/20) protection in vaccinated Groups 1 and 2, respectively. Protection rate of each vaccinated group was significantly higher than that of the non-vaccinated group. The protection rate of chickens which received the vaccine by eye drop (Group 1) was higher than that of chickens that received the vaccine by oral drop (Group 2) (Table 3). Typical gross lesions of ND were observed from post-mortem examination, including conjunctivitis; hemorrhages and congestion in the tracheal mucosa; hemorrhages in the mucosa of the proventriculus;

hemorrhages and necrosis in intestinal lymphoid aggregates; enlargement, hemorrhages and necrosis of cecal tonsils and hemorrhages on visceral organs. Pooled samples of trachea and cecal tonsil collected from dead chickens were positive for the detection of virulent NDV using one-step RT-PCR followed by restriction endonuclease analysis (Creelan et al., 2002).

## Discussion

The purpose of this study was to evaluate the protective efficacy of live LaSota strain NDV vaccine administered with different routes against a NDV challenge in layer-type chickens. The results reveal that eye drop vaccination (Group 1) induced a higher level

of HI titer and protection compared to oral drop (Group 2), which is similar to an earlier report in which the authors compared similar routes of administration of C2 strain NDV vaccine (Sasipreeyajan and Chansiripornchai, 2007). Instead of comparing eye drop and oral drop routes, Rehmani (1996) compared the vaccination methods of LaSota strain NDV vaccine between the eye drop and drinking water method in 12 days old chickens. Chickens which received the vaccine by eye drop also had a higher HI titer and protection rate compared to those chickens which received the vaccine by drinking water method. Similar results were also reported by Degefa et al. (2004).

In the current study, the eye drop vaccinated chickens in Group 1 at 3 weeks PV had an HI titer of  $3.65 \pm 1.39 \log_2$  compared to  $1.80 \pm 1.74 \log_2$  of the oral drop vaccinated chickens in Group 2. The results were related directly to route of vaccination. With vaccination by eye drop, the vaccine virus has a chance to stimulate lymphoid cells in Harderian glands, which are located at the median side of the eyeballs (Payne, 1994), to produce local antibody responses such as IgA and lacrimal IgM (Russell, 1993; Russell and Koch, 1993; Salam et al., 2003). However, with vaccination by oral drop, the vaccine virus might have a chance to pass down to the gastrointestinal tract and might be destroyed by gastric secretion (Tizard, 2013). Moreover, LaSota strain NDV vaccine has a high affinity for cells of the respiratory system, making it incompatible with oral route administration (Irena et al., 2008). Therefore, a lower HI antibody titer is anticipated in chickens that received the vaccine by oral drop or drinking water route. The protective efficacy of chickens in Group 1 that received the vaccine by eye drop in this study was 100%, which are contrary to an earlier report of only 67% (Sasipreeyajan, 2005). The results compared between each study could be different, probably due to some differences in the details of each study such as the amount or virulence of the challenge virus.

In conclusion, the results of this study show that live LaSota strain NDV vaccine administered to chickens via the eye drop route induced a higher antibodies response and better protection against the NDV challenge than did administration of the same vaccine to chickens by oral drop. Therefore, appropriate route of vaccine administration should be seriously considered by the practitioner in order to get suitable protective results.

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## บทคัดย่อ

### ประสิทธิภาพในการป้องกันโรคของวัคซีนนิวคาสเซิลสายพันธุ์ลาโซตาชนิดเชื้อเป็นในไก่ไข่

จิโรจ ศศิปริยจันทร์<sup>1\*</sup> พรพิสุทธิ์ อารีรักษากุล<sup>1</sup> สมคิด ขานดา<sup>1,2</sup>

วัตถุประสงค์ของการทดลองครั้งนี้ เพื่อประเมินประสิทธิภาพในการป้องกันโรคของวัคซีนนิวคาสเซิลชนิดเชื้อเป็นสายพันธุ์ลาโซตาในไก่ไข่ที่ได้รับวัคซีนด้วยวิธีที่ต่างกัน โดยทดลองในไก่ไข่เพศผู้ จำนวน 50 ตัว แบ่งไก่ออกเป็น 3 กลุ่ม กลุ่มที่ 1 และ 2 กลุ่มละ 20 ตัว ไก่แต่ละตัวได้รับวัคซีน เมื่อไก่อายุ 4 สัปดาห์ ด้วยวิธีหยอดตาและหยอดปาก ตามลำดับ ไก่กลุ่มที่ 3 จำนวน 10 ตัว เป็นกลุ่มควบคุม ไม่ได้รับวัคซีนใดๆ ไก่ทั้งหมดได้รับเชื้อพิษหับ ด้วยการหยอดปาก เมื่อไก่อายุ 7 สัปดาห์ หรือภายหลังไก่ได้รับวัคซีน 3 สัปดาห์ ผลการทดลองพบว่า ภายหลังไก่ได้รับวัคซีน 3 สัปดาห์ แอนติบอดีต่อวัคซีนนิวคาสเซิล ของไก่กลุ่มที่ 1 มีค่าสูงกว่าไก่กลุ่มที่ 2 อย่างมีนัยสำคัญ ( $p < 0.05$ ) ขณะที่แอนติบอดีของไก่กลุ่มที่ 1 และ 2 สูงกว่าแอนติบอดีของไก่กลุ่มที่ 3 อย่างมีนัยสำคัญ ( $p < 0.05$ ) ความต้านทานโรครายหลังไก่ได้รับเชื้อพิษหับ พบว่าไก่ที่ได้รับวัคซีน กลุ่มที่ 1 และ 2 มีความต้านทานโรคร้อยละ 100 และ 60 ตามลำดับ ขณะที่ไก่กลุ่มที่ไม่ได้รับวัคซีน (กลุ่มที่ 3) ไม่มีความต้านทานโรค (อัตราการตายร้อยละ 100) ผลของการศึกษาในครั้งนี้ บ่งชี้ว่า ไก่ได้รับวัคซีนชนิดเชื้อเป็นสายพันธุ์ลาโซตา ครั้งเดียว เพียงพอต่อการป้องกันโรคนิวคาสเซิลที่เกิดจากเชื้อชนิดรุนแรง ถ้าไก่ได้รับวัคซีนด้วยวิธีที่เหมาะสม

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**คำสำคัญ:** ไก่ วัคซีนชนิดเชื้อเป็นสายพันธุ์ลาโซตา วิธีให้วัคซีน วัคซีนนิวคาสเซิล ประสิทธิภาพ

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