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Efficacy of Live H120 Strain against Variant Genotype Infectious Bronchitis Virus Isolated in Thailand in Broiler Chickens

Tawatchai Pohuang¹ Jiroj Sasipreeyajan^{2*}

Abstract

This study investigated the level of protection generated by vaccination with a live attenuated infectious bronchitis virus (IBV) vaccine, H120 strain against variant genotype IBV isolated in Thailand. Sixty 1-day-old female broiler chickens were randomly divided into 4 groups of 15 chickens each. The chickens in Group 1 were vaccinated at 1 day old, whereas the chickens in Group 2 were vaccinated at 7 days old. Groups 3 and 4 did not receive IBV vaccine and served as positive and negative control groups, respectively. At 21 days of age, the chickens in Groups 1-3 were individually challenged with $10^{4.8}$ EID₅₀ of variant genotype IBV (isolate THA90151). Protection was evaluated at 7 days post-inoculation. Results revealed that the clinical signs, histopathological lesion score of the trachea, ciliostasis score and infection rate of the tracheas and kidneys of the vaccinated chickens were lower than those of the non-vaccinated, challenged control chickens. These results suggest that vaccination with live IBV vaccine, H120 strain can induce a certain level of protection for broiler chickens challenged with a variant IBV strain isolated in Thailand.

Keywords: chicken, efficacy, H120 strain, infectious bronchitis virus, vaccine

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Introduction

Infectious bronchitis (IB) is one of the most notable respiratory diseases in chickens caused by the infectious bronchitis virus (IBV). It causes considerable economic losses to the poultry industry worldwide. In addition to being a respiratory disease, affecting all ages and growth, it is one of the major causes of loss of egg production and poor eggshell quality (Gough et al., 1992). Some IBV strains have a predilection for the kidney and are associated with acute nephritis and urolithiasis causing a high mortality of infected chickens (Ziegler et al., 2002; Liu and Kong, 2004). Furthermore, the disease process is often complicated by secondary bacterial infections resulting in an even higher morbidity and mortality as well as an increasing condemnation rate at processing plants (Ziegler et al., 2002). Currently, one of the major problems of IBV is the frequent emergence of new variant strains (Yu et al., 2001). Different serotypes have been reported worldwide and new variant genotypes continue to be recognized. The existence of many genotypes of IBV that do not confer cross-protection against each other has been observed (Gelb et al., 1991; Jia et al., 1995; Liu and Kong 2004; Kulkarni and Resurreccion, 2010; Mahmood et al., 2011). The emergence of new genotypes is of great concern to poultry producers.

Determination of the strain or genotype of field isolates is important not only for the study of emerging viruses and virus evolution, but also for the selection of an appropriate vaccine against future outbreaks of IBV. In a recent survey of IBV in commercial chicken flocks in Thailand, it was found that outbreaks were caused by two distinct IBV strains, a new variant which was unique to Thailand and QX-like IBV. For the new variant IBV, the nucleotide sequence identities were 85% and amino acid sequence identities were 84% in common with IBVs published in the GenBank database (Pohuang et al., 2009a). Subsequently, it was found that the new variant strain in Thailand was a recombinant virus which emerged from parent THA001 isolated in Thailand in 1998 and Thai QX-like IBV (Pohuang et al., 2011). The pathogenicity of these IBVs was confirmed by inoculation into experimental chickens. The virus had an effect on chicken growth during the acute phase of infection. Moreover, it could cause disease in chickens with maternally-derived antibodies (Pohuang and Sasipreeyajan, 2012). In Thailand, where commercial chickens can have a great economic impact, a preventive program is crucial to control the spread of these genotypes of IBV.

To prevent economic loss due to IBV infection, many strains of vaccine have been routinely administered. There are different vaccination programs to achieve reasonable protection against IB infection; however, complete protection is provided only when vaccine and infectious virus are a homologous strain or serotype (Al-Tarcha and Sadoon, 1991; Liu et al., 2009). To date, many serotypes and variant IBV strains have been isolated worldwide (Gelb et al., 1991; Gough et al., 1992; Liu and Kong, 2004). Therefore, it is impossible to provide homologous vaccines for the entire variant field strains of IBV. It has been reported that the use of a

combination of two live attenuated vaccines could be effective against heterologous IBV strains (Cook et al., 2001; Martin et al., 2007). However, the use of multiple strains of live vaccine may be the cause of emerging variant viruses by recombination between vaccine viruses and field strains (Farsang et al., 2002). Although vaccination with one heterologous strain does not provide complete protection against IBV infection, partial protection against damage caused by IBV variants can be achieved. Live vaccines based on the Massachusetts serotype such as H120 strain are widely used because of their high efficacy and availability. The H120 strain vaccine was effective against the Australian T strain (Darbyshire, 1985). After challenged by 1171 and 1449 IBV strains, the clinical signs of chickens vaccinated with the H120 strain were lower than those of non-vaccinated chickens (Wang et al., 1996). Therefore, the objective of this study was to determine if the live attenuated IB vaccine, H120 strain could provide adequate protection for broiler chickens against a new variant genotype IBV isolated in Thailand.

Materials and Methods

Virus: A variant genotype IBV, isolate THA90151 (Accession number GQ503617) used as a challenge virus, was isolated, identified and characterized as previously described (Pohuang et al., 2009a). The stock virus, which is the fifth passage, was inoculated into the allantoic cavity of 10-day-old embryonated chicken eggs, and the allantoic fluid was harvested after 96 hr. Determination of virus titer in the allantoic fluid was performed by the 10-fold serial dilution method. Each dilution was inoculated into the allantoic cavity of 10-day-old embryonated chicken eggs, 5 eggs per dilution with 100 μ l per egg. The embryos were examined for IBV lesions (stunting, curled or urate retention in the mesonephrons) at 7 days (Ziegler et al., 2002). The control embryonated eggs inoculated with phosphate buffered saline (PBS) pH 7.4 had no lesions during the entire observation period. The titer expressed as embryo infectious dose 50% per 100 μ l (EID₅₀/100 μ l) was calculated according to the method of Reed and Muench (1938).

Experimental design: Sixty 1-day-old female broiler chickens (Cobb 500) were randomly divided into 4 groups of 15 chickens each and housed in separate experimental rooms. Feed and water were supplied *ad libitum*. A live attenuated IBV vaccine strain H120 (Poulvac® IB H-120, Fort Dodge Saude Animal Ltda, Campinas-SP-Brazil) was administered to the chickens via eye drop at 1 day of age in the chickens of Group 1 and at 7 days of age in the chickens of Group 2. Group 3 was a positive control group that received only the challenge virus at 3 weeks of age. Group 4 served as a negative control group that did not receive any IBV vaccine nor challenge virus. At 21 days of age, the chickens in Groups 1-3 were inoculated with 100 μ l of isolate THA90151 via eye drop. The chickens received approximately 10^{4.8} EID₅₀ of the challenge virus per bird. All study procedures and animal care activities were conducted under authorization by the Institutional Animal Care and Use Committee in

accordance with university regulations and policies governing the care and use of laboratory animals, approval number 13310082, issued by the Faculty of Veterinary Science, Chulalongkorn University.

Clinical signs and body weight gain: After the challenge, a clinical sign of tracheal rale was daily observed for 7 days. The chickens were weighed before the IBV inoculation and at 7 dpi.

Ciliary activity inhibition: Seven days after the challenge, level of protection of the trachea was examined using a ciliostasis test on 10 tracheal ring explants per chicken. Ten chickens per group were humanely killed and the tracheas were carefully removed. Immediately after the removal, the trachea was stored in PBS. Ten 1 to 2 mm sections of tracheal rings were prepared from each trachea. The level of ciliostasis was determined by low-power microscopy and scored as follows: 0 = all cilia beating, 1 = 75% beating, 2 = 50% beating, 3 = 25% beating, and 4 = none beating (100% ciliostasis). The protection against the IBV challenge was determined and calculated as described by Cubillos et al. (1991); Cavanagh et al. (1997) and Cook et al. (1999).

Virus detection: Of all chickens in each group, 15 of them, the caudal part of each trachea and the cranial part of each left kidney were removed and placed in sterile plastic bags separately. Ten percent w/v suspension was prepared and centrifuged at $1,800 \times g$ for 10 min. Supernatant was then collected for RNA extraction. RNA was extracted from 200 μ l of the supernatant using a Viral Nucleic Acid Extraction Kit (Real Biotech, Taiwan) according to the manufacturer's instructions. The extracted RNA was used in a reverse transcriptase-polymerase chain reaction (RT-PCR) using the one-step RT-PCR system (AccessQuick™ RT-PCR System, Promega, USA) with the specific primers as described by Sasipreeyajan et al. (2012). Briefly, the one-step RT-PCR profiles involved 45 min of RT reaction at 48 °C, heating at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at 54 °C for 30 sec and polymerization at 72 °C for 30 sec with a final elongation step of 10 min at 72 °C. A fragment of about 437 base pairs was determined by electrophoresis on 1.2% agarose gel. It was stained with ethidium bromide (0.5 μ g/ml) and visualized by using an ultraviolet transilluminator.

Histopathological examination: Of all chickens in each group, 15 of them, the cranial part of each trachea was removed and placed in 10% neutral buffered formalin. Tissues were embedded in paraffin, sectioned, stained with haematoxylin and eosin. Tracheal lesion scores according to Ratanasethakul et al. (1999) were evaluated as follows: 0 = no lesions, 1 = epithelial deciliation and desquamation with minimal lymphoid infiltration in lamina propria and submucosa, 2 = generalized epithelial deciliation and hyperplasia with moderate lymphoid infiltration in lamina propria and submucosa, and 3 = generalized epithelial deciliation and hyperplasia with heavy lymphoid infiltration in lamina propria and submucosa.

Detection of IBV antibody titer: At 1 day of age, blood samples were randomly collected from 20 chickens. At 7 days of age, blood samples were randomly collected from 10 chickens of Groups 2 and 3. At 21 and 28 days of age, blood samples were collected from all chickens in each group. The serum samples were kept at -20 °C. IBV antibody titer was determined using a commercial enzyme-linked immunosorbent assay (ELISA) test kit (BioChek, Holland).

Statistical Analysis: A comparison of the antibody titers among the experimental groups was performed using analysis of variance followed by the least significant difference (LSD) test. The Chi-square test was used to test differences in clinical signs and IBV detection among groups. The histopathological lesion score was analyzed using the Kruskal-Wallis test, and the Wilcoxon test was used for pair-wise comparison between groups. $p < 0.05$ was interpreted as a statistically significant result.

Results

Clinical signs and body weight gain: In the vaccinated groups, the number of chickens which showed clinical signs of tracheal rale were significantly lower ($p < 0.05$) than that of the positive control group. None of the chickens in any group died during the experiment. None of the chickens in the negative control group showed clinical signs of infection. At 21-28 days of age, the body weight gain of chickens in the vaccinated Group 2 and the negative control group (Group 4) was higher than that of the vaccinated Group 1 and the positive control group (Group 3) (Table 1).

Virus detection: In this study, the primer set for RT-PCR could detect a new variant genotype but it could not detect H120 strain. The challenge virus detection in the tracheas and kidneys in the chickens of Groups 1 and 2 was significantly lower ($p < 0.05$) than that of the positive control group (Group 3) (Table 1). No virus was detected in the tracheas and kidneys of the negative control group (Group 4).

Histopathological examination: At 7 dpi, microscopic evaluation of the tracheas was performed. Lesions observed in the tracheas included loss of cilia from epithelial cells, desquamation of epithelial cells and thickening of the mucosa due to lymphoid infiltration in lamina propria and submucosa. The histopathological lesion score in the tracheas of the vaccinated Group 2 was significantly lower than that of the non-vaccinated Group 3 ($p < 0.05$) (Table 1).

Ciliary activity inhibition: Inhibition of ciliary activity of the tracheal epithelium was evaluated at 7 days post-inoculation. Percentage of protection was calculated. The mean ciliostasis score of the vaccinated chickens was lower than that of the non-vaccinated chickens. The percentage of protection of the chickens vaccinated with H120 vaccine at 7 days of age was significantly higher than that of the non-vaccinated chickens ($p < 0.05$). The percentage of protection of the chickens that received H120 vaccine at 1 day of age was lower than that of the chickens that received H120 vaccine at

7 days of age, but they were not significantly different ($p>0.05$) as shown in Table 1.

IBV antibody titer: A comparison of the average antibody titers among the experimental groups is shown in Table 2. At 1 day of age, the average IBV antibody titer of the experimental chickens was $2,880\pm 1,536$. At 7 days, the average IBV antibody titer was not significantly different ($p>0.05$) among the groups. At 21 days, the average IBV antibody titers of Groups 1 and 2 were significantly higher ($p<0.05$) than that of Group 3, but not significantly different ($p>0.05$) from Group 4. No significant difference was found

between Groups 3 and 4. At 28 days, the average IBV antibody titers of Groups 1 and 2 were significantly higher ($p<0.05$) than those of Groups 3 and 4. No significant difference was found between Groups 3 and 4. The titers of Groups 1 and 2 at 28 days increased significantly from 21 days due to anamnestic response after they received IBV vaccine as the first stimulation. The titer of Group 3 at 28 days slightly increased due to short duration after the first stimulation by IBV challenged at 21 days of age. In contrast, the titer of Group 4 at 28 days declined to 12 ± 22.32 due to natural decline of the maternal derived antibodies.

Table 1 Body weight gain, clinical sign, IBV detection, tracheal lesion score and ciliostasis score of experimental chickens

Group	Body weight gain Gm/bird/day 21-28 days of age	Clinical sign (%)	IBV detection ^B		Tracheal histopathological lesion score ^C	Protection of the trachea	
			Trachea	Kidney		Ciliostasis score ^E	Protection ^F
1	76	6/15 ^A (40) ^a	7/15 ^a	2/15 ^a	1.87±1.25 ^{D,a}	3.02±1.34 ^a	2/10(20) ^{a,b}
2	84	2/15(13.3) ^{a,b}	5/15 ^a	3/15 ^a	0.87±1.06 ^b	2.40±1.29 ^a	5/10(50) ^a
3	75	12/15(80) ^c	15/15 ^b	11/15 ^b	2.47±0.74 ^a	4.00±0 ^b	0/10(0) ^b
4	87	0/15(0) ^b	0/15 ^a	0/15 ^a	0.27±0.59 ^b	0.50±0.36 ^c	10/10(100) ^c

^{a, b, c} Significantly different ($p<0.05$) in the same column

^A Number of chickens showing clinical sign of tracheal rale/ total chickens in the group

^B Number of positive IBV detection/ total chickens examined

^C Sum of tracheal lesion score/ total chickens examined (10 chickens/group)

^D Mean±SD

^E Mean ciliostasis score of 10 tracheas examined in each group

^F Number of tracheas evaluated as protected/ total tracheas examined (percentage of protection)

Table 2 Comparison of average antibody titer levels among experimental groups

Group	IBV antibody titer			
	1 day old	7 days old	21 days old	28 days old
1		-	146±131.10 ^a (n=15)	1,372±876.57 ^a (n=15)
2	2,880±1536 ^A (n=20) ^B	908±429.28 ^a (n=10)	148±145.35 ^a (n=15)	1,154±627.97 ^a (n=15)
3		760±381.85 ^a (n=10)	64±54.46 ^b (n=15)	116±143.08 ^b (n=15)
4		-	88±76.79 ^{a,b} (n=15)	12± 22.32 ^b (n=15)

^{a, b} Significantly different ($p<0.05$) in the same column

^A Mean±SD

^B Number of chickens examined

Discussion

This study's results demonstrate the improvement in protection against a challenge virus with variant genotype IBV isolated in Thailand which can be achieved by using a single dose of live attenuated vaccine, H120 strain. The clinical signs, tracheal histopathological lesion score and ciliostasis score of the vaccinated chickens were lower when compared to those of the infected chickens that were not vaccinated. These results indicate that the H120

vaccine used in this study can induce a certain level of protection against the challenge virus. However, in this study, complete protection was not observed. The results are consistent with a report by Albassam et al. (1986) which showed that chickens that received the H120 strain vaccine at 10 days of age and were infected 4 weeks later with Gray, Holte, Australian T and Italian strains did not show tracheal lesions. Wang et al. (1996) evaluated the efficacy of the H120 strain vaccine against 1171 and 1449 IBV strains. They found that only 1 out of 10 of the vaccinated chickens challenged with

the 1171 strain and none of the vaccinated chickens challenged with the 1449 strain showed clinical signs of infection. A better level of protection achieved by a single dose of vaccination was also reported by Cook et al. (1999). They showed that the mean ciliostasis score of the chickens which received a single dose of H120 vaccine at 1 day of age followed by a challenge with three IBV strains (Italy 710, Italy 2149 and B1648) was lower than the score of the non-vaccinated chickens. Similarly, in a previous research by this study's researchers, it was found that the vaccination of H120 vaccine at 7 days of age could decrease mortality rate and alleviate weight loss caused by a nephropathogenic IBV isolated in Thailand (Pohuang et al., 2009c).

In this report, the lower number of chickens in the vaccinated groups showing clinical signs of infection was related to the number of infected chickens found in the same groups. Darbyshire (1985) reported that the H120 strain vaccine could reduce the amount of the Australian T strain of IBV 30,000 times within 4 days of infection. The H120 vaccine given at 1 day of age did not afford complete protection against pathogenic CK/CH/LDL/97I strain. However, the number of vaccinated chickens that showed clinical signs (4/10) was lower than that of the non-vaccinated chickens (10/10). The re-isolation of the virus from tracheas and kidneys of the vaccinated chickens at 5 days post-challenge also decreased (Liu et al., 2009). Live vaccines have the ability to induce mucosal immunity that inhibits invasion and propagation of the virus in the tracheal mucosa. Immunity induced by the heterologous vaccine strains cannot inhibit the invasion of some infectious agents to the organs resulting in morbidity that is less severe (Pensaert and Lambrechts, 1994). Nakamura et al. (1991) demonstrated that IgM, IgA and IgG in the trachea of chickens that were resistant to the disease were more often found when compared to susceptible chickens. Thompson et al. (1997) reported the mucosal immunity of IBV infected chickens and found that 70% of the samples contained IgA, 52% of the samples contained IgG and 56% of the samples contained IgM. Furthermore, the induction of cellular immunity was also found after the use of live vaccine that can inhibit virus attack as well. The study of Pei et al. (2003) showed that transferring CD8+ T cells isolated from IBV infected chickens to 6-day-old chicks could protect the experimental chicks against the challenge strain. The antibody titer at the day of inoculation also plays a role in the inhibition of the invasion of the challenge virus to visceral organs which results in a decrease in morbidity in the vaccinated chickens. Hence, the average antibody titers at the day of challenge inoculation of the vaccinated groups were higher than those of the positive control group. The clinical signs and infection rate of the vaccinated chickens were lower than those of the challenge control group. A previous study showed that humoral immunity played an important role against viremia of IBV. These phenomena were important in the protection of non-respiratory tissues, reducing tissue damage by the challenge virus (Terregino et al., 2008; Lui et al., 2009).

The results of this study show that the H120 vaccine given alone at 1 day of age had protection of

20% against the challenge virus, whereas that given at 7 days showed protection of 50%. Moreover, at 7 days after the challenge, the body weight gain of the chickens vaccinated at 7 days was higher than that of the chickens vaccinated at 1 day of age. These indicated higher efficacy of protection when the vaccine was applied at 7 days of age. A factor that might play a role in this observation is the difference in level of maternal derived antibodies at the vaccination date. At 1 day of age, the maternal derived antibodies of the chickens was higher than those of the chickens at 7 days of age. These results suggest that the immune response of young chickens are interfered by the maternal derived antibodies. However, there are many reports which indicate that protection can be provided after vaccination with live attenuated IBV vaccine in maternal derived antibody chickens. For example, a report of Al-Taracha and Sadoon (1991) showed that chickens with maternal derived antibodies could be protected against a challenge with IBV strain M 41 by vaccination via intranasal-eye drop at 1 day of age. Another example is the findings of Pensaert and Lambrechts (1994) which indicated that vaccination of 1-day-old chicks having maternal derived antibodies could induce protection against a challenge strain, but the protection was lower than for chicks without maternal derived antibodies.

In conclusion, vaccination with live attenuated vaccine, H120 strain can induce a certain level of protection in broiler chickens, decrease tracheal lesions and decrease the number of infected chickens from new variant IBV strain isolated in Thailand. However, there is a need for further study to evaluate an appropriate vaccination protocol to achieve a higher protection rate against this genotype of IBV challenge.

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บทคัดย่อ

ประสิทธิภาพของวัคซีนเชื้อเป็นสายพันธุ์ H120 ต่อการป้องกันเชื้อไวรัสหลอดลมอักเสบติดต่อสายพันธุ์แวนเรียนท์ที่แยกได้ในประเทศไทยในไก่เนื้อ

ธวัชชัย โพธิ์เฮือง¹ จิโรจ ศศิปรีชญานันท์*

การศึกษานี้เป็นการประเมินผลการให้วัคซีนเชื้อเป็นสายพันธุ์ H120 ต่อการป้องกันเชื้อไวรัสหลอดลมอักเสบติดต่อสายพันธุ์แวนเรียนท์ที่แยกได้ในประเทศไทย โดยทดลองในไก่เนื้อเพศเมีย จำนวน 60 ตัว แบ่งไก่ออกเป็น 4 กลุ่มๆ ละ 15 ตัว ไก่กลุ่มที่ 1 ได้รับวัคซีนเมื่อไก่อายุ 1 วัน ไก่กลุ่มที่ 2 ได้รับวัคซีนเมื่อไก่อายุ 7 วัน ในขณะที่ไก่กลุ่มที่ 3 และ 4 ไม่ได้รับวัคซีนและเป็นกลุ่มควบคุมผลบวกและผลลบตามลำดับ เมื่อไก่อายุ 21 วัน ไก่แต่ละตัวในกลุ่มที่ 1-3 ได้รับเชื้อพิษหับซึ่งเป็นเชื้อไวรัสหลอดลมอักเสบติดต่อสายพันธุ์แวนเรียนท์ (isolate THA90151) ที่แยกได้ในประเทศไทย ขนาด $10^{4.8}$ EID₅₀/ตัว ประเมินผลการป้องกันโรคภายหลังจากไก่ได้รับเชื้อพิษหับเป็นเวลา 7 วัน การศึกษาพบว่า ไก่ที่ได้รับเชื้อพิษหับทั้ง 3 กลุ่ม จำนวนไก่ที่แสดงอาการป่วย รอยโรคทางจุลพยาธิวิทยาที่ท่อลม ค่าการยับยั้งการทำงานของเซลล์ที่ท่อลม และการติดเชื้อในท่อลมและไต ของไก่กลุ่มที่ได้รับวัคซีน (กลุ่มที่ 1 และ 2) มีค่าต่ำกว่าไก่กลุ่มที่ไม่ได้รับวัคซีน (กลุ่มที่ 3) แสดงให้เห็นว่าการให้วัคซีนเชื้อเป็นสายพันธุ์ H120 สามารถป้องกันโรคในไก่เนื้อที่มีต้นเหตุจากไวรัสหลอดลมอักเสบติดต่อสายพันธุ์แวนเรียนท์ที่แยกได้ในประเทศไทยได้บางส่วน

คำสำคัญ: ไก่ ประสิทธิภาพ สายพันธุ์ H120 ไวรัสหลอดลมอักเสบติดต่อ วัคซีน

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