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Original Article

Efficacy of different vaccination programs of live 4/91 strain against Thai QX-like infectious bronchitis virus in broiler chickens

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Abstract

Thai QX-like infectious bronchitis virus (IBV) has caused problems in commercial chicken farms as well as small farms in many parts of Thailand. This study investigated the level of protection generated by vaccination with different IBV vaccination programs, 4/91 strain against Thai QX-like IBV. One hundred 1 day old female broiler chickens were randomly divided into 5 groups of 20 chickens each. The chickens in Group 1 were vaccinated with Ma5 strain IBV vaccine at 1 day old and with 4/91 strain IBV vaccine at 14 days old. The chickens in Group 2 were vaccinated with Ma5 and simultaneously with 4/91 strain IBV vaccine at 1 day old. The chickens in Group 3 were vaccinated with a combined Newcastle disease virus, C2 strain and IBV, B48 strain vaccine at 1 day old and with 4/91 strain IBV vaccine at 14 days old. The chickens in Groups 4 and 5 did not receive IBV vaccine and served as positive and negative control groups, respectively. At 28 days of age, the chickens in Groups 1-4 were individually challenged with $10^{4.2}$ EID_{50} of Thai QX-like IBV (isolate THA80151). Protection was evaluated at 7 days post-inoculation (DPI). Results revealed that the clinical sign and histopathological lesion score of the tracheas of the vaccinated chickens were significantly ($p<0.05$) lower than those of the non-vaccinated, challenged control chickens. These results indicate that improvement in protection was achieved against the challenge with Thai QX-like IBV by the vaccination programs used in this study. However, complete protection was not observed.

Keywords: broiler chicken, live attenuated vaccine, 4/91 strain, Thai QX-like infectious bronchitis virus, efficacy

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Introduction

Infectious bronchitis (IB) is a worldwide respiratory disease of chickens caused by the infectious bronchitis virus (IBV), a member of the genus *Coronavirus* in the family *Coronaviridae* (Cavanagh et al., 1986). It causes considerable economic losses to commercial chicken farms by causing respiratory disease, affecting growth and decreasing egg production and eggshell quality (Gough et al., 1992). IBV infection is commonly followed by secondary bacterial infection. Therefore, it is thought that infection with IBV could also predispose chickens to bacterial infection, resulting in complicated morbidity and increased mortality (Shimazaki et al., 2008). So far, one of the major problems of IBV is the frequent emergence of new variant strains (Yu et al., 2001). More than 50 different serotypes of IBV have been identified, and new variants continue to emerge (Bochkov et al., 2006). QX IBV was described for the first time in 1996 in China (Wang et al., 1998), after which the prevalence of the so-called QX-like IBV has become the field strain predominant in poultry farms in many countries of Europe (Bochkov et al., 2006), Africa (Toffan et al., 2011) and Asia (Pohuang et al., 2009; Mo et al., 2013). The circulation of QX-like IBV in Thailand has also been reported (Pohuang et al., 2009). A phylogenetic analysis on IBV strains isolated from broiler farms in Thailand between 2008 and 2009 showed that it was genetically related to QX IBV. Furthermore, a recombinant analysis revealed that it was a recombinant strain which emerged from parent QX IBV and another strain of Chinese IBV (Pohuang et al., 2011). The virulence of the virus was confirmed by inoculation into experimental chickens. The virus could cause respiratory and kidney lesions as well as affect growth rate of infected chickens (Pohuang and Sasipreeyajan, 2012).

Various strains of live vaccine have been used to control clinical disease associated with IBV infections worldwide, but new variant strains continue to emerge and cause clinical disease and production problems in vaccinated flocks (Shimazaki et al., 2008). For successful protection of chickens against infection, it is essential to identify prevalent genotypes in the region, determine the cross-protective potential of available vaccines and optimize strategic vaccination programs (Chhabra et al., 2015). In general, complete protection is provided only when vaccine and infectious virus are a homologous strain or serotype (Liu et al., 2009). Sometimes, improvement in protection might be achieved by using different IB vaccination (Fabio et al., 2000; Terregino et al., 2008; Pohuang and Sasipreyajan, 2015). 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). Vaccination programs based on live attenuated vaccine such as Connecticut, H120, Ma5, M41 and Armidale A3 have been implemented for many years to control IB in Thailand (Antarasena et al., 1990). However, most of the recent IBV field isolates recovered from commercial chickens in Thailand have been classified into the QX-like IBV (Pohuang et al., 2011). This fact suggests that the vaccination programs are not effective to control QX-like IBV infection. A vaccine containing 4/91 strain was approved to be used in many countries such as Japan (Shimazaki et al., 2008). The 4/91 vaccine used alone and, particularly, in a combined vaccination program provided excellent protection against challenge with D207, Arkansas, FB3 (Cook et al., 1999) and a nephropathogenic strain B1648 (Cook et al., 2001). Therefore, this study was carried out to examine the protection induced by different vaccination programs with a live vaccine, the 4/91 strain, against Thai QX-like IBV.

Materials and Methods

***Virus*:** A Thai QX-like IBV isolate THA80151 used for this challenge was isolated from a broiler farm in Thailand. It was identified and characterized by using reverse transcriptase-polymerase chain reaction (RT-PCR) and sequencing of S1 gene (Pohuang et al., 2009). The nucleotide sequence of S1 gene of the isolate THA80151 was deposited in GenBank by accession number GQ503616. The fifth passage of stock virus was inoculated into the allantoic cavity of 10-day-old embryonated chicken eggs, and the allantoic fluid was harvested at 96 hr later. Determination of the virus titer in the allantoic fluid was performed by the 10-fold serial dilution method as previously described (Sasipreeyajan et al., 2012). The virus titer expressed as embryo infectious dose 50% per 100 µl (EID_{50}/100 µl) was calculated according to the method of Reed and Muench (1938).

***Vaccine*:** Three commercial live attenuated IBV vaccines were received directly from the vaccine's supplier in Thailand: Ma 5 IBV vaccine (Nobilis® IB Ma 5, Intervet International B.V., Boxmeer, Holland), 4/91 IBV vaccine (Nobilis® IB 4/91, Intervet International B.V., Boxmeer, Holland) and a combined Newcastle disease virus, C2 strain and IBV, B48 strain vaccine (Nobilis® ND+IB C2M, Intervet International B.V., Boxmeer, Holland). One dose of each vaccine (Ma5, 4/91 and combined vaccines) contained approximately $10^{3.0}$ EID_{50}, $10^{3.6}$ EID_{50} and $10^{2.8}$ EID_{50} of IBV, respectively. Each vaccine was administered individually by eye drop, one dose/bird.

Experimental design: One hundred female broiler chickens (Cobb 500) were moved from a commercial hatchery (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 of the Faculty of Veterinary Science, Chulalongkorn University, Nakhon Pathom, Thailand. 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. 13310031. The chickens were randomly allocated into 5 groups of 20 chickens in each group. Groups 1-3 were vaccinated using different vaccination program regimes against IBV (Table 1). Groups 4 and 5 did not receive IBV vaccine and served as positive and negative control groups, respectively. They were housed in separate experimental rooms.

Commercial feed for broiler chickens and water were provided *ad libitum*.

***Challenge study*:** All chickens of each group except the chickens in Group 5 (negative control) were challenged at 28 days old. Each chicken received approximately $10^{4.2}$ EID_{50} of the Thai QX-like IBV isolate THA80151, by eye drop. Each chicken of all groups was weighed at 28 days before challenge and 35 days, which was 7 days post-inoculation (DPI). Clinical signs of tracheal rale were observed for 7 days.

***Serological evaluation*:** Thirty blood samples were randomly collected at 1 day old. All chickens of all groups were bled at 14 and 21 days. Blood samples were collected before and after IBV challenge at 28 and 35 days, respectively. Sera were collected and tested for IBV antibodies by ELISA (BioChek, Holland). Serum-to-positive ratios (S/P-ratios) and individual serum titers were calculated according to the manufacturer's instructions. Average titer of each group of the same age was compared.

Table 1 Vaccination programs and age of challenge

Group	Number of chicken	Vaccination[A]		IBV challenge[B]
		1 day old	14 days old	
1	20	Ma5	4/91	28 days old
2	20	Ma5, 4/91	-	28 days old
3	20	C2M	4/91	28 days old
4	20	-	-	28 days old
5	20	-	-	-

[A] Each chicken received vaccine by eye drop route, one dose/bird
[B] Each chicken received IBV challenge by eye drop route, approximately $10^{4.2}$ EID_{50} of Thai QX-like IBV/bird

***Histopathological lesion score evaluation*:** At 35 days (7 DPI), the cranial part of the trachea of each chicken was collected and placed in 10% neutral buffered formalin, sectioned, stained with hematoxylin and eosin, and evaluated for histopathological lesion score by the method of Ratanasethakul et al. (1999). Briefly, lesions of the tracheas were scored as follows: 0 = No lesion, 1 = Epithelial deciliation and desquamation with minimal lymphoid infiltration in the lamina propria and submucosa, 2 = Generalized epithelial deciliation and hyperplasia with moderate lymphoid infiltration in the lamina propria and submucosa, and 3 = Generalized epithelial deciliation and hyperplasia with heavy lymphoid infiltration in the lamina propria and submucosa.

***Statistical analysis*:** Comparison of mean body weight and antibody titers among the experimental groups was performed by the analysis of variance (ANOVA) and least significant difference (LSD) tests. Percentage of chickens showing clinical signs of tracheal rale after IBV challenge was calculated by using the Chi-square values. The histopathological lesion score was analyzed using the Kruskal-Wallis test and the Wilcoxon test was used for pair-wise comparison between the treatment groups. Differences between groups were considered significant at $p<0.05$.

Results

Clinical sign and body weight: In the vaccinated groups, the number of chickens that showed clinical signs of tracheal rale was significantly lower ($p<0.05$) than that of the positive control group. None of the chickens in the negative control group showed clinical signs of infection. At 35 days of age, the body weight of chickens in the vaccinated Group 1 was significantly higher ($p<0.05$) than that of the vaccinated Group 3 and the positive control group (Table 2). The body weight of chickens in the vaccinated Groups 2 and 3 was not significantly different ($p>0.05$) from that of the positive control group.

***Serological evaluation*:** The comparison of the average IBV antibody titers among the experimental groups is shown in Table 3. At 1 day of age, the average IBV antibody titers were 5,471±2,489.9. At 14 days, the average titers were not significantly different ($p>0.05$) among the groups. At 21 days, the average titers of Groups 1 and 3 were significantly higher ($p<0.05$) than those of the others. No significant difference ($p>0.05$) was found between Groups 1 and 3, between Groups 2 and 4, and between Groups 4 and 5. At 28 days, the average titers of Groups 1 and 3 were significantly higher ($p<0.05$) than those of Groups 2 and 4. No significant difference ($p>0.05$) was found between Groups 1 and 3, and between Groups 2 and 4. At 35 days, the average titers of Groups 1 and 3 were significantly higher than those of the other groups ($p<0.05$).

Histopathological examination: At 7 DPI, the histopathological lesions in the tracheas showed varying degrees of loss of cilia from epithelial cells, desquamation of epithelial cells and thickening of the mucosa due to lymphoid infiltration in lamina propria and submucosa. Marked histopathological lesion scores in the tracheas occurred in the non-vaccinated Group 4 with a mean score of 2.55±0.8, which was significantly higher than those of all the vaccinated groups ($p<0.05$) (Table 2). Among the vaccinated groups, the lowest histopathological lesion score in the tracheas was found in Group 2, and it was significantly different ($p<0.05$) compared to the others.

Discussion

In terms of developing strategies to control IBV infection, the protectotype concept should be considered. It has a more practical relevance for control

strategies than knowing a serotype of field strain IBVs (Cook et al., 1999). The present results demonstrate the protection of broiler chickens against a challenge virus with Thai QX-like IBV by different programs of a live attenuated vaccine, the 4/91 strain. The clinical sign and tracheal histopathological lesion score of the vaccinated chickens were lower when compared to those of the infected non-vaccinated chickens. These results indicate that the improvement in protection was achieved against the challenge with Thai QX-like IBV by the vaccination programs used in this experiment. This might be that the vaccine could reduce and prevent the replication of QX-like IBV in the respiratory tract. As a consequence, the lesions induced by the virus in the trachea decreased. However, complete protection was not observed. It is known that the best protection against IBV infection is achieved by a vaccine containing the homologous strain, and a low level or no protection is observed when vaccination with vaccines that contain heterologous strains is given (Gelb et al., 1997; Liu et al., 2009). However, partial protection may be provided after vaccination with live attenuated vaccines (Wang et al., 1996; Liu et al., 2009). Furthermore, using a combined vaccination program incorporating different live attenuated vaccine strains provides good protection against heterologous strain (Cook et al., 2001; Martin et al., 2007; Sasipreeyajan et al., 2012). The partial protection is an important aspect for controlling clinical disease associated with new strains or variant strains of IBV infection. This is the case in this study. The results are consistent with those of Terregino et al. (2008), who evaluated the efficacy of vaccination protocol with Ma5 at 1 day old followed by 4/91 at 14 days old in commercial chicks. After a challenge with QX-like IBV variant strain isolated in Italy, some of the vaccinated chicks showed very mild conjunctivitis that resolved within 48 hr. They found that 6 out of 12 of the vaccinated broiler chicks were IBV positive in the trachea and 1 out of 12 of the vaccinated broiler chicks were IBV positive in the oviduct. As reported by Cook et al. (1999), the improvement in protection against heterologous IB serotypes was found after application of the Ma5 vaccine at 1 day old followed by the 4/91 vaccine at 14 days old. The vaccination program showed excellent protection against the challenge with D207, Arkansas, FB3 and each of the Brazilian isolates, except D1466. However, for D1466, 80% of the chickens were scored as protected, indicating that some protection was achieved. The results are also consistent with a previous study which found clinical protection and decrease in tracheal histopathological lesions against the challenge with QX-like IBV in chickens vaccinated with the H120 vaccine at 1 day old followed by the 4/91 vaccine at 14 days old (Pohuang et al., 2014).

Table 2 Body weight, clinical sign of tracheal rale and histopathological lesion score of the tracheas of experimental chickens

Group	Body weight (gm/bird)		Tracheal rale		Histopathological lesion score
	28 days old	35 days old	Number	Percent	
1	1,420±97.7[A]	1,847±95.7[a]	0/20[B,a]	0	1.45±0.8[A,a]
2	1,429±105.0	1,799±151.8[a,b]	0/20[a]	0	1.00±0.9[b]
3	1,397±71.4	1,768±90.7[b]	6/20[b]	30	1.60±0.8[a]
4	1,399±48.3	1,762±130.4[b]	13/20[c]	65	2.55±0.8[c]
5	NDC	ND	0/20[a]	0	0.10±0.3[d]

[A] Mean±standard deviation (SD)
[B] Number of chickens showing clinical sign of tracheal rale/Total number of chickens in the group
[C] Not done
[a,b,c,d] Different superscript letters in each column mean statistically significant difference ($p < 0.05$).

Table 3 Antibodies against IBV before and after IBV challenge

Group	Mean ELISA titer ± SD				
	1 day old	14 days old	21 days old	28 days old	35 days old
1	5,471±2,489.9[A] (30)[B,C]	642±212.2 (20)	1,165±753.7[a] (20)	2,774±2,396.1[a] (20)	3,265±1,774.4[a] (20)
2		666±249.6 (20)	536±331.8[b] (20)	611±416.6[b] (20)	2,002±1,179.3[b] (20)
3		599±208.4 (20)	1,237±846.7[a] (20)	2,827±1,847.9[a] (20)	2,907±1,499.9[a] (20)
4		639±281.4 (10)	160±142.3[b,c] (10)	292±130.6[b] (20)	407±370.1[c] (20)
5		554±160.9 (10)	51±63.2[c] (10)	-[D]	105±161.8[c] (20)

[A] Mean±standard deviation (SD)
[B] Thirty blood samples were randomly collected from all chickens before starting the trial at 1 day old.
[C] Number of samples tested
[D] Not done
[a,b,c] Different superscript letters in each column mean statistically significant difference ($p<0.05$).

The average IBV antibody titers of Groups 1 and 3 at 21 and 28 days increased significantly compared to that of Group 2 due to an anamnestic response after they received the booster IBV vaccine at 14 days old. In contrast, the titers of Groups 4 and 5 gradually decreased with time due to natural decline of maternally-derived antibodies (Hamel et al., 2006). On the day of challenge inoculation, the average antibody titers of Groups 1 and 3 were significantly higher ($p<0.05$) than that of Group 2, but the lesion in the tracheas at the time of evaluation was higher. These antibodies might not be the primary protection mechanism against IBV infection. Humoral immunity to IBV plays a direct role against viremia of the viruses; therefore, it is important in the protection of non-respiratory tissues (Terregino et al., 2008; Lui et al., 2009). It is possible that the mucosal immunity induced by live vaccine prohibits invasion and propagation of the virus in the tracheal mucosa. Nakamura et al. (1991) found IgM, IgA and IgG against IBV in the trachea more often in chickens that were resistant to the disease compared to susceptible chickens. Collisson et al. (2000) reported that the development of cell-mediated immune response (CMI) correlated with effective virus clearance, reduction in clinical signs, and resolution of lesions in chickens which were experimentally challenged with IBV. Kotani et al. (2000) showed that cytotoxic T-lymphocytes at the tracheal mucosa were proposed to be involved in the clearance of IBV from the tracheal mucosa in an early phase of the infection.

In this study, the significantly ($p<0.05$) higher tracheal histopathological lesions in Groups 1 and 3 than in Group 2 might be lesions that occurred by the revaccination with 4/91 vaccine at 14 days old combined with the lesions from the challenge virus. Although a lack of virulence of the vaccine strain was found after attenuation, the live vaccine was still able to replicate in the tracheas, which induced mild histological lesions. Histopathological lesions in the trachea induced by vaccine strain occur during an experimental period from 4 to 14 days post-inoculation (Lee et al., 2010). However, it is likely that some damage to the tracheal mucosa is necessary for the development of local immunity against IBV (Jackwood et al., 2003). For the result of significantly ($p<0.05$) higher tracheal rale in Group 3 than in the other vaccinated groups, this might be due to the vaccination with combined Newcastle disease virus, IBV vaccine and booster vaccination with 4/91 vaccine, which could damage the tracheal epithelium more than those of the other vaccinated groups. Therefore, the significance of this relation should be further determined.

In conclusion, all of the vaccination programs used in this study provided partial protection against Thai QX-like IBV. The vaccination program with live vaccine Ma5 strain combined or followed by 4/91 strain performed better than the program of C2M followed by 4/91. Furthermore, it will be important to determine whether this suggested vaccination program may provide improvement in protection against Thai QX-like IBV in field situation.

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บทคัดย่อ

ประสิทธิภาพของวัคซีนเชื้อเป็นสายพันธุ์ 4/91 โปรแกรมต่าง ๆ ต่อการป้องกันเชื้อไวรัสหลอดลมอักเสบติดต่อสายพันธุ์ Thai QX-like ในไก่เนื้อ

ธวัชชัย โพธิ์เฮือง[1] ศราวุธ ธนเสถียร[2] จิโรจ ศศิปรียจันทร์[3*]

เชื้อไวรัสหลอดลมอักเสบติดต่อสายพันธุ์ Thai QX-like เป็นเชื้อที่ทำให้เกิดปัญหาต่ออุตสาหกรรมการเลี้ยงไก่ รวมถึงผู้เลี้ยงไก่รายย่อย ในประเทศไทย การศึกษาครั้งนี้ทำการศึกษาผลของการให้วัคซีนเชื้อเป็นสายพันธุ์ 4/91 โปรแกรมต่าง ๆ ต่อการป้องกันเชื้อไวรัสหลอดลมอักเสบติดต่อสายพันธุ์ Thai QX-like โดยทดลองในไก่เนื้อเพศเมียจำนวน 100 ตัว แบ่งไก่ออกเป็น 5 กลุ่ม ๆ ละ 20 ตัว ไก่กลุ่มที่ 1 ได้รับวัคซีนสายพันธุ์ Ma5 เมื่ออายุ 1 วัน และได้รับวัคซีนสายพันธุ์ 4/91 เมื่ออายุ 14 วัน ไก่กลุ่มที่ 2 ได้รับวัคซีนเชื้อเป็นสายพันธุ์ Ma5 และ 4/91 เมื่ออายุ 1 วัน ไก่กลุ่มที่ 3 ได้รับวัคซีนรวมนิวคาสเซิลสายพันธุ์ C2 กับวัคซีนหลอดลมอักเสบติดต่อสายพันธุ์ B48 เมื่ออายุ 1 วัน และได้รับวัคซีนสายพันธุ์ 4/91 เมื่ออายุ 14 วัน ในขณะที่ไก่กลุ่มที่ 4 และ 5 ไม่ได้รับวัคซีนและเป็นกลุ่มควบคุมผลบวกและผลลบ ตามลำดับ เมื่อไก่อายุ 28 วัน ไก่แต่ละตัวในกลุ่มที่ 1-4 ได้รับเชื้อพิษทับซึ่งเป็นเชื้อไวรัสหลอดลมอักเสบติดต่อสายพันธุ์ Thai QX-like (isolate THA80151) ขนาด $10^{4.2}$ EID_{50} ทำการประเมินผลการป้องกันโรคภายหลังจากไก่ได้รับเชื้อพิษทับเป็นเวลา 7 วัน การศึกษาพบว่าอาการป่วยและรอยโรคทางจุลพยาธิวิทยาที่ท่อลมของไก่ทุกกลุ่มที่ได้รับวัคซีนมีค่าต่ำกว่าในไก่กลุ่มที่ไม่ได้รับวัคซีนแต่ได้รับเชื้ออย่างมีนัยสำคัญทางสถิติ ($p<0.05$) แสดงให้เห็นว่าโปรแกรมวัคซีนที่ใช้ในการศึกษาครั้งนี้สามารถให้การป้องกันโรคที่เกิดจากเชื้อไวรัสหลอดลมอักเสบติดต่อสายพันธุ์ Thai QX-like อย่างไรก็ตาม ไม่พบการป้องกันโรคแบบสมบูรณ์ในไก่ที่ได้รับวัคซีน

คำสำคัญ: ไก่เนื้อ วัคซีนเชื้อเป็น สายพันธุ์ 4/91 ไวรัสหลอดลมอักเสบติดต่อสายพันธุ์ Thai QX-like ประสิทธิภาพ

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