Original Article

Efficacy of Different Vaccination Programs against Thai QX-like Infectious Bronchitis Virus

Jiroj Sasipreeyajan[1*] Tawatchai Pohuang[2] Nida Sirikobkul[3]

Abstract

The present study was designed to evaluate the protection induced by different vaccination programs against Thai QX-like infectious bronchitis virus (IBV). The chickens were divided into 5 groups, 16 chickens in each. Groups 1-4 were vaccinated with different regimes of vaccination program against IBV. Groups 5 did not receive IBV vaccine and served as a positive control group. At 1 day old, the chickens in groups 2 and 3 were vaccinated with QX vaccine whereas the chickens in group 4 were vaccinated with H120 vaccine. At 14 days old, revaccination was performed in group 2 with QX vaccine whereas groups 3 and 4 were vaccinated with a combined vaccine, Newcastle disease virus strain B1 combined with IB strain Massachusetts (Mass) and Connecticut (Conn) (B1/Mass/Conn). The chickens in group 1 were only vaccinated with QX vaccine at 7 days old. At 28 days old, the chickens in groups 1-5 were individually challenged with 10^4 EID_{50} of Thai QX-like IBV (isolate THA80151). The protection was evaluated at 7 days post-inoculation. The results showed that the clinical signs of all vaccinated groups were lower (p<0.05) than those of the positive control group. Virus detection in the tracheas and the histopathological lesion score of the tracheas and kidneys of the vaccinated groups were not significantly different (p>0.05) from those of the positive control group. The body weights of the vaccinated groups excluding group 3 were not significantly different (p>0.05) from that of the positive control group. It is suggested that all of the vaccination programs used in this study offered clinical protection against Thai QX-like IBV, but they did not provide sufficient protection against virus infection and histopathological lesions in the tracheas and kidneys.

Keywords: chickens, infectious bronchitis virus, vaccine

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บทคัดย่อ

ประสิทธิภาพของโปรแกรมวัคซีนแบบต่างๆ ต่อการป้องกันเชื้อไวรัสหลอดลมอักเสบติดต่อสายพันธุ์ QX-like ที่แยกได้ในประเทศไทย

จิโรจ ศศิปรียจันทร์[1] ธวัชชัย โพธิ์เฮือง[2] นิดา สิริกอบกุล[3]*

การศึกษาครั้งนี้เป็นการประเมินผลการป้องกันเชื้อไวรัสหลอดลมอักเสบติดต่อสายพันธุ์ QX-like ที่แยกได้ในประเทศไทยด้วยการให้โปรแกรมวัคซีนที่แตกต่างกัน โดยแบ่งไก่ทดลองออกเป็น 5 กลุ่มๆ ละ 16 ตัว กลุ่มที่ 1-4 ได้รับโปรแกรมวัคซีนแตกต่างกัน ส่วนกลุ่มที่ 5 เป็นกลุ่มควบคุมผลบวก เมื่อไก่อายุได้ 1 วัน ไก่กลุ่มที่ 2 และ 3 จะได้รับวัคซีนสายพันธุ์ QX ส่วนไก่กลุ่มที่ 4 ได้รับวัคซีนสายพันธุ์ H120 หลังจากนั้นเมื่อไก่อายุ 14 วัน ไก่กลุ่มที่ 2 ได้รับวัคซีนสายพันธุ์ QX ในขณะที่ไก่กลุ่มที่ 3 และ 4 ได้รับวัคซีนรวมที่ประกอบด้วยไวรัสนิวคาสเซิลสายพันธุ์ B1 รวมกับไวรัสหลอดลมอักเสบติดต่อสายพันธุ์แมสซาชูเซตส์และคอนเนคติคัท (B1/Mass/Conn) สำหรับกลุ่มที่ 1 ได้รับวัคซีนสายพันธุ์ QX ที่อายุ 7 วันเพียงครั้งเดียว เมื่อไก่อายุ 28 วันไก่แต่ละตัวในกลุ่มที่ 1-5 ได้รับเชื้อพิษทับซึ่งเป็นเชื้อไวรัสหลอดลมอักเสบติดต่อสายพันธุ์ QX-like ที่แยกได้ในประเทศไทยขนาด 10^4 EID_{50} ทำการประเมินผลการป้องกันโรคภายหลังจากให้เชื้อพิษทับเป็นเวลา 7 วัน ผลการศึกษาพบว่าไก่ทุกกลุ่มที่ได้รับวัคซีนมีจำนวนไก่ที่แสดงอาการป่วยน้อยกว่ากลุ่มควบคุมผลบวกอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) แต่การป้องกันการติดเชื้อที่ท่อลมและรอยโรคทางจุลพยาธิวิทยาที่ท่อลมและไตไม่แตกต่างอย่างมีนัยสำคัญทางสถิติกับกลุ่มควบคุมผลบวก ($p>0.05$) ส่วนน้ำหนักตัวไก่นั้นพบว่าไก่กลุ่มที่ได้รับวัคซีนยกเว้นกลุ่มที่ 3 มีน้ำหนักตัวไม่แตกต่างอย่างมีนัยสำคัญทางสถิติกับกลุ่มควบผลบวก ($p>0.05$) แสดงให้เห็นว่าโปรแกรมวัคซีนที่ใช้ในการศึกษาครั้งนี้ทุกโปรแกรมสามารถลดอาการป่วยจากการติดเชื้อไวรัสสายพันธุ์ QX-like ที่แยกได้ในประเทศ แต่ไม่สามารถป้องกันการติดเชื้อและการทำให้เกิดรอยโรคทางจุลพยาธิวิทยาที่ท่อลมและไต

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Introduction

Infectious bronchitis virus (IBV), a member of the coronavirus, is a highly infectious virus which causes respiratory disease in chickens. It is a major cause of respiratory problems in broilers and of poor egg production in breeders and layers worldwide (De Wit et al., 2010). Some strains of IBV can cause acute nephritis and urolithiasis associated with a high mortality rate in infected chickens (Ziegler et al., 2002; Liu and Kong, 2004). In addition, the infected chickens become susceptible to secondary bacterial infections resulting in even higher morbidity and mortality (Ziegler et al., 2002). Prevention and control of the disease are through the use of many types of vaccines. In spite of the intensive use of vaccines, outbreaks of IB frequently occur in the field in many countries (Gelb et al., 1991; Gough et al., 1992; Liu and Kong, 2004). This situation may be due to the emergence of new variant serotypes of IBV (Gelb et al., 1991; Gough et al., 1992; Jia et al., 1995; Liu and Kong, 2004; Pohuang et al., 2009b). Consequently, these emergences are of great concern to poultry producers.

In the case of IB, complete protection is provided by vaccination with a homologous strain; however, partial protection may be provided after vaccination with a live attenuated heterologous strain (Wang et al., 1996; Liu et al., 2009). Especially, use of a combined vaccination program incorporating different live attenuated vaccine strains provides good partial protection against heterologous strain (Cook et al., 1999; Martin et al., 2007). Although many strains of vaccine are commercially available at present, it is unknown whether the currently used vaccines offer enough cross-protective capability against the IBV strain present in the field in Thailand.

Since the outbreak of IB in Thailand was initially reported between 1953 and 1954 (Chindavanig, 1962), IB has continued to be an economically important disease in the Thai poultry industry and has been found all over the country (Upatoom et al., 1983, Antarasena et al., 1990). Recently, we characterized the Thai IBVs isolated

during 2008-2009 by an analysis of complete S1 genes. The results showed that QX-like IBV was the most prevalent of IBV strains isolated in the current outbreak in Thailand (Pohuang et al., 2009[a]). QXIBV was first described and identified in China in 1996 and, after that, outbreaks of the so-called QX-like IBV genotype were reported to be the most prominent genotypes in many countries (Beato et al., 2005; Bochkov et al., 2006; Gough et al., 2008). Interestingly, we found that the Thai QX-like IBV was a recombinant virus that emerged from QXIBV and another strain of Chinese IBV (Pohuang et al., 2011). Hence, the objective of the present study was to examine the protection afforded by different vaccination programs against Thai QX-like IBV.

Materials and Methods

Virus: Thai QX-like IBV (isolate THA80151) was isolated from commercial chicken flocks in Thailand, 2008. The virus was previously identified by reverse transcriptase-polymerase chain reaction (RT-PCR), followed by sequencing of S1 gene (Pohuang et al., 2009[a]). The accession number deposited in GenBank database of the THA80151 isolate was GQ503616. This virus was used as the challenge IBV strain. The stock virus was propagated and titrated by inoculation into 9-11 days old embryonated chicken eggs. Virus concentration was determined by the method of Reed and Muench (1938). After that, allantoic fluid was harvested and kept at -70^{0}C until use.

Comparison of S1 gene: The S1 gene sequences of Thai QX-like IBV and vaccine strains used in this study were obtained from the GenBank database. The accession numbers of the S1 genes of IBV strains were: THA80151 (GQ503616), H120 (FJ888351), M41 (AY561711), Connecticut (L18990) and QX-like vaccine strain L-1148 (DQ431199). The multiple sequence alignments and determination of the nucleotide and amino acid identities were performed using BioEdit version 7.0.5.2 (Hall, 1999).

Experimental design: One hundred and nine, female Cobb 500 broiler chickens were moved from a commercial hatchery to the university at 1 day old. Each group of chickens was kept in a separate room of the experimental animal's facility at the Livestock Hospital, Faculty of Veterinary Science, Chulalongkorn University, Nakhornpathom, Thailand. The chickens were divided into 5 groups, 16 chickens in each. Groups 1-4 were vaccinated with different regimes of a vaccination program against IBV (Table 1). Group 5 did not receive IBV vaccine and served as the positive control group. The extra 29 birds were bled at 1 day old. Feed 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. 11310011.

Vaccines: Three commercial live attenuated IBV vaccines were received directly from the vaccine's distributor in Thailand, which were QX IBV vaccine (Poulvac® IB QX, Fort Dodge Animal Health, Weesp, Holland), H120 IBV vaccine (Poulvac® IB H120, Fort Dodge Animal Health, Weesp, Holland) and a combined Newcastle disease virus, B1 strain and IBV, Massachusetts (Mass) and Connecticut (Conn) strains (B1/Mass/Conn) vaccine (Fort Dodge Animal Health, Fort Dodge, USA). One dose of each vaccine (QX, H120 and combined vaccines) contained approximately $10^{4.2}$ EID_{50}, $10^{3.5}$ EID_{50} and $10^{3.8}$+$10^{3.8}$ EID_{50} of IBV, respectively. Each vaccine was administered individually by eye drops at one dose/bird.

Challenge study: All the chickens in each group were challenged at 28 days old. Each chicken received approximately 10^4 EID_{50} of Thai QX-like IBV by eye drops. Clinical signs of tracheal rales were observed for 7 day post-inoculation (dpi). Each chicken was weighed before the challenge inoculation and at 7 dpi.

Virus detection: The tracheas and kidneys were separately collected from individual chickens for virus detection. The samples were prepared as 10% w/v suspensions in PBS and centrifuged at 1,800xg for 10 min. The supernatants were then collected for RNA extraction using a viral nucleic acid extraction kit (Real Biotech, Taiwan) following the manufacturer's instructions. The extracted RNA was subjected to RT-PCR which was performed using one-step RT-PCR (AccessQuick™ RT-PCR System, Promega, USA) following the manufacturer's instructions. The primer sets used in this study were newly designed to differentiate between the challenge virus and vaccine strains. The primer sequences are available upon request. The one-step RT-PCR was performed by RT reaction at 48^{0}C for 45 min, heating at 94^{0}C for 5 min and 35 cycles of denaturation at 94^{0}C for 30 sec, annealing at 56^{0}C for 30 sec and polymerization at 72^{0}C for 30 sec with a final elongation step at 72^{0}C for 10 min. The amplified RT-PCR product, 144-bp fragment, was analyzed by electrophoresis on 1.2% agarose gel, followed by its staining with ethidium bromide (0.5 µg/ml) and then it was visualized using an ultraviolet transilluminator.

Table 1 Vaccination programs and age of challenge.

Test group	Number of chicken	Vaccination			IBV challenge
		1-day-old	7-day-old	14-day-old	
1	16	-	QX	-	28-day
2	16	QX	-	QX	28-day
3	16	QX	-	B1/Mass/Conn	28-day
4	16	H120	-	B1/Mass/Conn	28-day
5	16	-	-	-	28-day

Histopathological lesion score: At 35 days old (7 dpi), all birds of each group were euthanized and necropsied. The cranial part of the trachea and the cranial part of the left kidney of each bird were taken and fixed in 10% neutral buffered formalin for histopathology. The histopathological lesion scores of the tracheas and kidneys were examined and evaluated following the method of Ratanasethakul et. al. (1999). Briefly, lesions in the tracheas were evaluated as follows: 0: no lesions, 1: epithelial deciliation and desquamation with minimal lymphoid infiltration in the lamina propia and submucosa, 2: generalized epithelial deciliation and hyperplasia with moderate lymphoid infiltration in the lamina propia and submucosa, 3: generalized epithelial deciliation and hyperplasia with heavy lymphoid infiltration in the lamina propia and submucosa. Lesions in the kidneys were evaluated as follows: 0: no lesions, 1: few small areas of lymphoid infiltration in the interstitial tissue, 2: severe small areas of lymphoid infiltration in the interstitial tissue, 3: diffuse lymphoid infiltration in the interstitial tissue.

Serological evaluation: Twenty nine blood samples were randomly collected at 1 day old. Ten and 16 blood samples of each group at 7 and 14 days old were collected. Blood samples were collected before and after IBV challenge at 28 and 35 days old, respectively. Sera were collected and tested for IBV antibodies by ELISA (BioChek®, Holland).

Statistical Analysis: Body weight and antibody titers were analyzed and compared between groups using ANOVA and Duncan's multiple range test. The histopathological lesion scores of the tracheas and kidneys were compared between groups using the Kruskal-Wallis test and the Wilcoxon test was used for pair-wise comparison between treatment groups. The percentage of chickens showing clinical signs of tracheal rales after IBV challenge was calculated using Chi-square values. Differences between groups were considered significant at $p<0.05$.

Results

Comparison of the S1 gene: The nucleotide and deduced amino acid sequences of the S1 gene obtained from GenBank were compared (Table 2). Thai QX-like IBV (isolate THA80151) had a high nucleotide and amino acid identity (93.1% and 89.2%, respectively) with the QX vaccine strain. However, when compared with other vaccine strains, it shared 71.5-72.8% of nucleotide identity and 50.3-50.6% of amino acid identity.

Clinical sign and mortality: After IBV challenge, there was no mortality. The clinical signs of tracheal rales were observed. There were 1 to 3 birds of each vaccinated group which exhibited clinical signs of tracheal rales compared to 12 birds in the positive control group (group 5) (Table 4).

Body weight: The body weights of chickens before IBV challenge at 28 days old were not significantly different ($p>0.05$). At 35 days old which was 7 dpi, the body weight of chickens in groups 2, 3 and 4 which had received 2 times the vaccination was better than that of the positive control group (group 5). A significant difference ($p<0.05$) in body weight was found between group 3 and the positive control group, but the other vaccinated groups were not significantly different ($p>0.05$) from the positive control group (Table 2).

Virus detection: At 35 days old (7 dpi), the challenge virus, Thai QX-like IBV, was detected in the tracheas and kidneys of each chicken of all groups. The detection rate of the virus in the kidneys was lower than in the trachea. The detection rate of the virus in the vaccinated groups was lower than that of the positive control group (group 5), but it was not significantly different ($p>0.05$) (Table 4).

Histopathological lesion score: The histopathological lesion scores of the tracheas of all vaccinated groups and the positive control group (group 5) at 35 days old (7 dpi) were not significantly different ($p>0.05$). The histopathological lesion scores of the kidneys in each group were not significantly different ($p>0.05$) (Table 4).

Serological evaluation: Maternally-derived antibodies against IBV at 1 day old were 1,949.6±1,099.9. They declined to 409.9-797.0 at 7 days old and they were very low at 14 days old. Antibodies against IBV vaccine were detected at 28 days old. The chickens in group 3 (receiving QX and B1/Mass/Conn vaccines) had the highest titer level. Antibodies against IBV in each group were increased at 35 days old due to IBV challenge (Fig 1).

Discussion

In this study, the efficacy of different IBV vaccination programs was evaluated after the challenge with Thai QX-like IBV, isolate THA80151. Four parameters were used for the evaluation protection including clinical protection based on tracheal rales because of the trachea being the primary site of infection, protection against weight loss as an important economical parameter, virological protection and protection against microscopic lesions in the tracheas and kidneys as the site of IBV replication. The results of clinical protection showed that respiratory signs (tracheal rales) in chickens vaccinated with all of the IBV vaccination programs

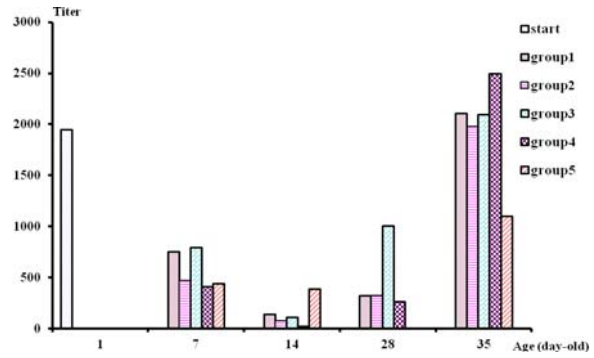

Figure 1 IBV antibody titers of the experimental chickens

were significantly lower (p<0.05) than that of the non-vaccinated chickens, indicating that clinical protection had been achieved. The reasons for the clinical protection against the challenge strain might be that live attenuated vaccines induced local immunity in the upper respiratory tract which prohibited the invasion of the challenge virus in the tracheal mucosa. Nakamura et al. (1991) found IgM, IgA and IgG in the trachea more often in chickens that were resistant to the challenge compared to susceptible chickens. Thompson et al. (1997) examined the mucosal immunity of IBV infected chickens and found that 70% of the samples contained IgA, 52% and 56% of the samples contained IgG and IgM, respectively. Moreover, live vaccines can also induce cellular immunity, which can prohibit virus attack as well. Pei et al. (2003) found that transfer of CD8+ T cell isolated between 3 and 6 weeks after infection caused by IBV to 6 days old chicks can protect the chicks from infection.

Clinical protection against Thai QX-like IBV was conferred following all of the vaccination programs used in the study, but the vaccination programs could not provide sufficient protection against histopathological lesions in the tracheas and kidneys after challenge with Thai QX-like IBV. For protection against virus infection, the challenge virus could be detected in the tracheas of the vaccinated group as with the positive control group, but virus detection in the kidneys was lower than that of the positive control group. Based on these results it might be concluded that the local immunity induced by live vaccines could reduce the replication of the challenge virus and low amounts of the virus were found in the infected tissues. Moreover, it could reduce the spreading of the virus from primary site of replication to other organs. Consistent with a study of Darbyshire (1985) who reported that chickens vaccinated with the H120 strain could reduce the amount of Australian T strain 30,000 times within 4 days post-infection. Pensaert and Lambrechts (1994) demonstrated that vaccination at 1 day old either with the homologous strain, H120 or combined H120 and D274 vaccines reduced the duration of virus replication in the tracheas of the chickens inoculated with the B1648 strain at 4 weeks old. Liu et al. (2009) showed that clinical protection against pathogenic CK/CH/LDL/97I was conferred following vaccination with the heterologous vaccine strains CK/CH/LDT3/03 P120 and J9, but the respiratory

Table 2 Nucleotide and amino acid identities of S1 gene.

	Amino acid identity (%)				
	M41	**H120**	**Connecticut**	**QX-like vaccine**	**THA80151**
M41		92.8	83.0	50.7	50.3
H120	97.5		83.4	50.1	50.2
Connecticut	95.5	94.6		51.6	50.6
QX-like vaccine	72.3	71.4	71.5		89.2
THA80151	72.8	71.8	71.5	93.1	
	Nucleotide identity (%)				

Table 3 Body weight before and after IBV challenge (28 and 35 days old) and clinical sign after IBV challenge.

Test group	**Body weight (gm/bird)**		**Clinical sign (tracheal rale)**	
	28 days old	**35 days old**	**Number**	**Percent**
1	1,409.4±41[A]	1762.5±92[a]	2/16[B,a]	12.50
2	1,407.5±48	1780.0±46[a]	1/16[a]	6.25
3	1391.3±77	1852.5±93[b]	2/16[a]	12.50
4	1405.6±81	1821.9±112[a,b]	3/16[a]	18.75
5	1400.0±60	1763.1±61[a]	12/16[b]	75.00

[A]Mean±standard deviation (SD).
[B]Number of chickens with clinical signs of tracheal rales / total chickens in the group.
[a,b]The different superscript in each column means a statistically significant difference (p<0.05).

Table 4 Histopathological lesion score and IBV detection in the tracheas and kidneys at 7 dpi (35 days old).

Test group	**Histopathological lesion score**		**IBV detection**		
	Trachea	**Kidney**	**Trachea**	**Kidney**	**Chicken[B]**
1	2.28±0.77[A]	0.25±0.51	9/16[C,a]	1/16[a]	10/16[a]
2	2.50±0.62	0.78±1.21	12/16[a,b]	4/16[a,b]	12/16[a,b]
3	2.22±0.94	0.38±0.49	13/16[a,b]	9/16[b,c]	14/16[a,b]
4	2.28±0.73	0.25±0.44	12/16[a,b]	4/16[a,b]	12/16[a,b]
5	2.34±0.75	0.31±0.47	16/16[b]	11/16[c]	16/16[b]

[A]Mean±standard deviation (SD).
[B]IBV was detected either in the tracheas or the kidneys or in both tissues of each chicken.
[C]Number of positive samples / total samples tested.
[a,b,c]The different superscript in each column means a statistically significant difference (p<0.05).

protection conferred by the heterologous vaccine strains was <50%, as determined by virus isolation from the tracheas. Subsequently, from the reduction of the amount of virus in infected chickens it can be deduced that the low amounts of the virus might not be enough to induce clinical signs of infection and this might protect chickens vaccinated with some vaccination programs in this study against weight loss. This notion is supported by a study of Ignjatovic et al. (2003). They found that the 2 weeks old, W breed chickens inoculated with $2x10^2$ CD_{50} of N1/62 strain did not show any clinical signs, but clinical signs were observed in 70% of the chickens inoculated with $2x10^4$ CD_{50}. Similar to the results observed in 4 weeks old, S breed chickens, the 45 and 75% mortality rates were observed when inoculated with $2x10^2$ CD_{50} and $2x10^4$ CD_{50}, respectively.

In an experimental study, vaccination with QX vaccine alone provided a low clinical sign of respiratory disease, but it could not prevent the effect of the disease on body weight gain. In addition, the challenge virus could be detected in vaccinated chickens and the histopathological lesions were not different from those in non-vaccinated chickens. These results suggest that QX vaccine can not protect against infection with Thai QX-like strain but it provides a clinical sign protection. Although in most cases IBV strains with high S1 similarity induce a high degree of cross-protection against the challenge strain, it has been reported that sometimes, IBV strains induce low cross-protection against challenge with high S1 similarity strain. In a related study, Gelb et al. (2005) observed that chickens vaccinated with DE/072/92 had 55% of protection against challenge with 93% S1 similarity strain, DE/406/99. They also found that H120 strain induced 58% of protection against challenge with 96% S1 similarity strain, Is/385/97. However, when revaccination with different serotypes was performed, the protection was improved. Similarly with a previous report by Cook et al. (1999), they found that when the H120 vaccine was applied at 1 day old and followed by the antigenically different 4/91 vaccine at 14 days old, improved protection against challenge with the heterologous strain was observed as compared with either a single dose of H120 vaccine or with H120 vaccine followed by Ma5 vaccine which was the same serotype.

Commercial chickens with maternally-derived antibodies were used in this experiment because we expected that this was the same situation as that in field conditions. The IBV antibody titers of the chickens gradually declined by 14 days old. This observation was consistent with the report on the changes in maternal antibody titers (Al-Tarcha et al., 1991; Hamel et al., 2006). The maternally-derived antibodies transferred from hens to their chicks are estimated to be 30%. The levels of anti-IBV antibodies in the chicks serum detected by ELISA kit are at their highest in 3 days old and decrease substantially in 7 and 14 days old, respectively (Hamel et al., 2006). Before challenge inoculation at 28 days old, the seroconversion was stronger in the chickens vaccinated with QX vaccine followed by B1/Mass/Conn than the others. Although the serological titer was the highest, it did not induce a different IBV protection level of clinical protection, virological protection and protection of microscopic lesion in the tracheas and kidneys compared with other vaccination programs. IBV antibodies response combined with the results of clinical sign, virus detection and microscopic evaluation of the tracheas and kidneys in this study support other research studies that suggest that no correlation was found with serum antibody titers and the degree of IBV protection (Pensaert and Lambrechts, 1994; Alvarado et al., 2003; Martin et al., 2007).

In conclusion, all of the vaccination programs used in this study offered good clinical protection against Thai QX-like IBV, but the vaccination programs did not provide sufficient protection against virus infection and histopathological lesions in the tracheas and kidneys.

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