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Original Article

The Effectiveness of Vaccination with Two Live Infectious Bronchitis Vaccine Strains against QX-Like Infectious Bronchitis Virus Isolated in Thailand

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Abstract

The purpose of this study was to evaluate the level of protection generated by vaccination with 2 live attenuated vaccine strains against QX-like infectious bronchitis virus (IBV) isolated in Thailand. Eighty-four 1-day-old broiler chickens were randomly allocated into 6 groups with 14 chickens in each group. Groups 1-4 were vaccinated with different vaccination programs against IBV. Groups 5 and 6 did not receive any IBV vaccine and served as positive and negative control groups, respectively. At 1 day old, the chickens in groups 1 and 4 were vaccinated with H120 vaccine whereas the chickens in groups 2 and 3 were vaccinated with 4/91 vaccine. At 14 days old, revaccination was done in groups 1 and 2 with H120 vaccine whereas groups 3 and 4 were vaccinated with 4/91 vaccine. At 28 days old, the chickens in groups 1-5 were individually challenged with $10^{4.17}$ EID_{50} of QX-like IBV (isolate THA80151). The protection level was evaluated at 7 days post-inoculation. Results showed that the body weight of the vaccinated chickens was higher than the infected but non-vaccinated chickens ($p<0.05$). Furthermore, the morbidity rate and tracheal histopathological lesion score of the vaccinated chickens were lower than that of the infected chickens that were not vaccinated ($p<0.05$), although the infection rate of the tracheas was similar. These results suggested that the two live attenuated vaccines administered at an interval of 2 weeks used in this study could induce clinical protection and decrease tracheal histopathological lesions, but could not protect against the infection of a challenge strain.

Keywords: chicken, effectiveness, QX-like infectious bronchitis virus, vaccine

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Introduction

Infectious bronchitis virus (IBV) is known to be a causative agent of a highly infectious upper respiratory tract disease in chickens which causes considerable economic loss to the poultry industry worldwide. In hens, respiratory distress, a decrease in egg production and poor eggshell quality have been reported (Gough et al., 1992). Some IBV strains can infect the kidneys and are associated with acute nephritis and urolithiasis causing a high mortality rate of infected chickens (Ziegler et al., 2002; Liu and Kong, 2004). Continuous emergence of new variant IBV strains has been found in many countries (Gelb et al., 1991; Gough et al., 1992; Jia et al., 1995; Liu and Kong, 2004; Pohuang et al., 2009b) and these are thought to be generated by mutations or recombinations of its genome (Jia et al., 1995). The existence of new variant strains causes vaccine failure and is of great concern for both the poultry industry and vaccine manufacturers.

A new variant strain called QX Infectious bronchitis virus (QXIBV) was identified 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 one of the most predominant field strains of IBV in poultry farms in many countries (Beato et al., 2005; Bochkov et al., 2006; Gough et al., 2008). Although all QX-like IBV isolated from different countries are genetically closely related, pathological changes reported at the time of disease outbreaks have shown considerable variation (Benyeda et al., 2009). Recently, the molecular characterization of IBV isolates from commercial broiler flocks in Thailand showed that the outbreaks were caused by two distinct IBV strains, a new variant which was unique to Thailand and QX-like IBV (Pohuang et al., 2009a). Subsequently, we have found that QX-like IBV isolated in Thailand is a recombinant strain and it has emerged from parent QXIBV and another strain of Chinese IBV (Pohuang et al., 2011).

To prevent economic loss associated with IBV infection, IB vaccines are routinely administered. It has been reported that complete protection is provided when vaccine and infectious virus are of as homologous strain or serotype (Al-Tarcha and Sadoon, 1991). To date, many variant IBV strains have been detected (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. Although vaccination with one heterologous strain does not protect chickens from IBV infection, sometimes broad protection against damage caused by IBV variants can be achieved by using a combined vaccination program incorporating different live attenuated vaccine strains. Cook et al. (2001) reported that vaccination using a combination of Ma5 and 4/91 vaccine strains could provide good protection for the kidneys against heterologous nephropathogenic IBV. Martin et al. (2007) demonstrated that chickens vaccinated with Holland combined with Arkansas strains had protection against challenge with isolate CA99, which has been typed to be different from vaccine serotypes. Hence, the objective of this work was to determine if two times vaccination with live attenuated IB vaccines, belonging to different serotypes, would provide adequate protection against QX-like IBV isolated in Thailand.

Materials and Methods

Virus: The challenge strain, QX-like IBV (isolate THA80151), was isolated, identified and characterized as previously described (Pohuang et al., 2009a). The stock virus, which is the fifth passage, was propagated by inoculation of 10 days old embryonated chicken eggs via the allantoic cavity. At 96 h after inoculation, the allantoic fluid was harvested and determination of the virus concentration was performed by the 10 folds serial dilution method. Each dilution was inoculated into the allantoic cavity of 5 embryonated chicken eggs with 100 µl per egg. Seven days after the inoculation, the embryos were examined for IBV lesions (stunting, curled toes or urates in the mesonephrons) (Ziegler et al., 2002). The control embryonated eggs did not have these lesions during the same period. An embryo infectious dose of 50% (EID_{50}) /100 µl was calculated according to the method of Reed and Muench (1938).

Experimental design: Eighty-four 1 day old female broiler chickens (Cobb 500) were randomly allocated into 6 groups with 14 chickens in each group. They were housed in separated experimental rooms. Feed and water were provided *ad libitum*. An infectious bronchitis vaccine was administered to the chickens via eye drops at 1 and 14 days old. Two strains of vaccine including 4/91 (Intervet international B.V., Boxmeer, Holland) and H120 (Intervet international B.V., Boxmeer, Holland) provided by Intervet, Thailand were used for the vaccination. All groups were treated with the following regimes shown in Table 1. At 28 days old, the chickens in groups 1-5 were inoculated with 100 µl of isolate THA80151 via eye drops. The concentration of the challenge virus was approximately $10^{4.17}$ EID_{50}. This experiment was approved 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 0931008, issued by the Faculty of Veterinary Science, Chulalongkorn University.

Clinical signs and body weight gain: Clinical signs included gasping, coughing, sneezing, or tracheal rales and mortality were observed daily for 7 day post-infection (dpi). All chickens were weighed before the challenge inoculation and at 7 dpi.

Virus detection: In each group, pooled caudal parts of the tracheas or of the left kidneys were placed in sterile plastic bags. Ten percent (w/v) suspensions were prepared and centrifuged at 1,800 × g for 10 min. Supernatants were then collected for RNA extraction. RNA was isolated 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).

The primer sequences were FOR216 (AAGGACGTCTATAATCAAAG) and RE769 (AGTACCATTAACAAAATAAGC). The one-step RT-PCR was conducted by 45 min of RT reaction at 48 ˚C, heating at 94 ˚C for 5 min and 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. The RT-PCR product was determined by electrophoresis on 1.2% agarose gel. The fragments were stained with ethidium bromide (0.5 µg/ml) and then the 437-bp fragment was visualized using an ultraviolet transilluminator.

Histopatholological lesion score evaluation: The cranial part of the tracheas were placed in 10% neutral buffered formalin, sectioned, stained with haematoxylin and eosin, and evaluated for histopathological lesion scores by the method of Ratanasethakul et al. (1999). Briefly, lesions of the tracheas were scored 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.

Detection of IBV antibody titer: At 1 day old, blood samples were randomly collected from 20 chickens. At 7 days old, blood samples were randomly collected from 10 chickens in groups 1, 3 and 6. At 14 and 21 days old, blood samples were randomly collected from 10 chickens in each group, except group 6. Then, blood samples were randomly collected from 10 chickens in each group for every week of age. The serum samples were kept at -20 ^{o}C. IBV antibody titer was determined using commercial enzyme-linked immunosorbent assay (ELISA) test kit (BioChek, Reeuwijk, Holland).

Statistical analysis: A comparison of average body weights and antibody titers among the experimental groups was performed using analysis of variance (ANOVA) and followed by the least significant difference (LSD) test. Morbidity rate among the groups was compared by Chi-square test. Histological lesion scores were analyzed using the Kruskal-Wallis test and the Wilcoxon test was used for pair-wise comparison between the treatment groups. A $p<0.05$ was interpreted as a statistically significant different result.

Results

Clinical signs and body weights: In the infected groups, some chickens showed tracheal rales, coughing and gasping at 2 dpi. Decreased feed consumption and ruffled feathers were observed. In the vaccinated groups, the number of chickens which showed the clinical signs was significantly lower ($p<0.05$) than that of the positive control group (Table 1). Dead chickens were not found in the entire group until the end of the experiment. There were neither clinical signs nor deaths in the negative control group. At 7 dpi, the body weights of chickens in all the infected groups were significantly lower ($p<0.05$) than those of the negative control group. However, the body weights of chickens in the vaccinated groups were significantly higher ($p<0.05$) than those of the positive control group (Table 1).

Virus detection: RT-PCR was performed using a new primer set that could detect the isolate THA80151 but could not detect vaccine strains H120 and 4/91. IBV was 100% detected in the tracheas of groups 1, 3 and 5 and 76.8% detected in the tracheas of groups 2 and 4 (Table 1). IBV was not detected in the tracheas of the negative control group.

Histopathological lesion score evaluation: At 7 dpi, the tracheas of the vaccinated chickens and the positive control chickens had histopathological lesions including loss of cilia from the epithelial cells, desquamation of epithelial cells and thickening of the mucosa due to lymphoid infiltration in the lamina propria and submucosa. No prominent histopathological lesions were observed in the negative control chickens. The average histopathological lesion scores of the tracheas of the

Table 1 An average body weight, morbidity, IBV detection and tracheal lesion score of experimental chickens

Group	Vaccination	Body weight (g)[A]		Morbidity (%)	IBV detection (%)	Tracheal lesion score[D]
	1, 14 days old	28 days old (0 dpi)	35 days old (7 dpi)			
1	H120, H120	1,347.1±51.5	1,842.9±126.0^{b}	3/14[B,b] (21.4)	14/14[C,a] (100)	2.00^{b}
2	4/91, H120	1,334.3±65.4	1,854.3±103.0^{b}	2/14^{b} (14.3)	11/14^{a} (78.6)	1.50^{b}
3	4/91, 4/91	1,366.4±57.5	1,862.1±91.1^{b}	3/14^{b} (21.4)	14/14^{a} (100)	1.93^{b}
4	H120, 4/91	1,352.9±61.3	1,835.7±83.9^{b}	2/14^{b} (14.3)	11/14^{a} (78.6)	1.57^{b}
5	positive control	1,332.9±47.0	1,742.9±183.2^{c}	14/14^{a} (100)	14/14^{a} (100)	2.86^{a}
6	negative control	1,330.0±65.5	1,947.1±101.5^{a}	0/14^{b} (0)	0/14^{b} (0)	0.00^{c}

[a, b, c] The different superscripts in each column mean statistically significant difference ($p<0.05$).
[A] Mean ± standard deviation (SD)
[B] Number of chickens with clinical signs / Total chickens examined
[C] Number of positive IBV detected / Total chickens examined
[D] Sum of tracheal lesion scores / Total chickens examined

vaccinated chickens were significantly lower (p<0.05) than those of the non-vaccinated chickens (Table 1).

IBV antibody titer: The average antibody titers compared among the experimental groups are shown in Table 2. At 1 day old, the average IBV antibody titer of the experimental chickens was 5,783±2,743. At 7 days old, the average IBV antibody titers were not significantly different (p>0.05) among the groups. At 14 days old, a significantly difference in the average IBV antibody titers (p<0.05) was only found between groups 3 and 5. At 21 days old, the average IBV antibody titers were not significantly different (p>0.05) among the groups. At 28 days old, the average titer of group 4 was significantly different (p<0.05) from groups 1, 3, 5 and 6. The average titer of group 2 was not significantly different (p>0.05) from groups 1 and 4 but significantly different (p<0.05) from the others. The average titer of group 1 was not significantly different (p>0.05) from groups 2, 3, 5 and 6. At 35 days old, the average titers of groups 1 and 4 were significantly different (p<0.05) from the others. The average titer of group 3 was not significantly different (p>0.05) from group 2 but significantly different from the others. The average titer of group 5 was not significantly (p>0.05) different from groups 2 and 6 but significantly different (p<0.05) from the others.

Discussion

The results of this study showed that the body weights of the vaccinated chickens were higher than those of the infected but non-vaccinated chickens. Furthermore, the morbidity rate and tracheal histopathological lesion scores of the vaccinated chickens were lower than those of the infected chickens that were not vaccinated, although the number of infected chickens was not significantly different. Interestingly, clinical protection was also found in the chickens vaccinated 2 times with the same IBV vaccine strains; however, they appeared to have a slightly lower protection than those vaccinated 2 times with the heterologous strains. These results suggest that the live attenuated vaccines used in this study could induce clinical protection when administered at an interval of 2 weeks but could not protect against the infection of a challenge strain. The results are consistent with other studies that show the level of protection of the heterologous challenge test. Martin et al. (2007) reported that a vaccination protocol consisting of vaccination with Mass/Conn at hatching followed by Hol/Ark at 18 days old could reduce disease associated with IBV isolate CA99, which was typed into serotype CA99 as distinct from Ark, Mass, and Conn serotypes. Terregino et al. (2008) described a vaccination program using Ma5 at 1 day old and 4/91 at 14 days old. This could protect the chickens from infection with the QX-like IBV variant strain isolated in Italy. They found that no clinical signs attributable to IBV infection were observed in the vaccinated chickens and the number of IBV positive tracheal samples from the vaccinated SPF and vaccinated commercial broilers was lower than the infected non-vaccinated chickens.

The antibody titers in chicken sera collected before the challenge inoculation date gradually declined with time, which is consistent with a report on the changes in maternal antibody titers (Al-Tarcha and Sadoon, 1991). Hens transmit maternal antibodies to their offspring by depositing antibodies in the eggs. The percentage of IgY transfer from hen plasma to their chicks is estimated to be 30 percent. The levels of anti-IBV antibody in the chicks serum detected by ELISA were highest at 3 days old and decreased substantially by 7 and 14 days old, respectively (Hamel et al., 2006). Although the immune responses of young chickens are

Table 2 The average antibody titer levels comparing among the experimental groups

Group	IBV antibody titer					
	1 day old	7 days old	14 days old	21 days old	28 days old	35 days old
1	5,783±2,743[A] (n=20)[B,C]	2,144±1,465 (n=10)	86.3±181[a,b] (n=10)	150±368 (n=10)	482±437[b,c] (n=10)	4,113±919.8[a] (n=10)
2	-	-	41.5±85.5[a,b] (n=10)	55.7±89.8 (n=10)	862±762[a,b] (n=10)	1,167±649[bc] (n=10)
3	-	1,667±1,073 (n=10)	25.9±66.8[b] (n=10)	49.5±123 (n=10)	395±314[c] (n=10)	1,396±681[b] (n=10)
4	-	-	196±541[a,b] (n=10)	91±176 (n=10)	1,202±704[a] (n=10)	2,932±1,484[a] (n=10)
5	-	-	344±388[a] (n=10)	79.2±184 (n=10)	73.9±80.7[c] (n=10)	560±584[c] (n=10)
6	-	1,516±766 (n=10)	-	-	146.4±99.6[c] (n=10)	140±180[c] (n=10)

[a,b] The different superscripts in each column mean statistically significant difference (p<0.05).
[A] Mean ± standard deviation (SD).
[B] Random sampling from all chickens.
[C] Number of chickens tested.

interfered with by maternal derived antibodies, protection can be provided after vaccination. Al-Tarcha and Sadoon (1991) showed that chickens having maternal derived antibodies and receiving vaccine via intranasal-eye drop at 1 day old were able to gain protection against intra-tracheal challenge with IBV strain M 41. Pensaert and Lambrechts (1994) showed that vaccination of 1 day old chicks with maternal derived antibodies induced protection against a challenge strain but the protection was lower than that in chicks without maternal derived antibodies.

On the day of challenge inoculation, the average antibody titers of the vaccinated groups appeared to be higher than the positive control group but the infection rate at the time of evaluation was high. These antibodies might not be the primary protection mechanism against IBV infection. Humoral antibodies might play a role in reducing the tissue damaged by the challenge virus. It has been shown that humoral immunity to IBV plays a direct role against viremia of the viruses; therefore, it is important in protection of non-respiratory tissues (Terregino et al., 2008; Lui et al., 2009). However, complete protection did not occur in this study because the vaccine strains and the challenge virus were heterologous. The antibody titers induced by vaccines might occur from the group specific epitopes located on the envelope of the virus (Parr and Collisson, 1993) and they induce partial protection against a challenge virus. Antibody titers of all challenge inoculation groups at 35 days old were increased when compared to the titer of each group at 28 days old, which was due to IBV infection.

In conclusion, vaccination with two live attenuated vaccine strains used in this study administered at an interval of 2 weeks could induce clinical protection, alleviate body weight loss and produce a low score of tracheal histopathological lesions, but it could not protect against infection by the challenge strain.

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บทคัดย่อ

ประสิทธิภาพของวัคซีนหลอดลมอักเสบติดต่อชนิดเชื้อเป็น 2 สายพันธุ์ต่อการป้องกันเชื้อสายพันธุ์ QX-like ที่แยกได้ในประเทศไทย

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การศึกษาครั้งนี้เป็นการประเมินผลของวัคซีนเชื้อเป็น 2 สายพันธุ์ต่อการป้องกันเชื้อไวรัสหลอดลมอักเสบติดต่อสายพันธุ์ QX-like ที่แยกได้ในประเทศไทย การทดลองในไก่เนื้อ จำนวน 84 ตัว โดย แบ่งไก่ออกเป็น 6 กลุ่มๆ ละ 14 ตัว กลุ่มที่ 1-4 ได้รับโปรแกรมวัคซีนแตกต่างกัน ส่วนกลุ่มที่ 5 และ 6 เป็นกลุ่มควบคุมผลบวกและผลลบตามลำดับ เมื่อไก่อายุ 1 วัน ไก่กลุ่มที่ 1 และ 4 ได้รับวัคซีนสายพันธุ์ H120 ส่วนไก่กลุ่มที่ 2 และ 3 ได้รับวัคซีนสายพันธุ์ 4/91 หลังจากนั้นเมื่อไก่อายุ 14 วัน ไก่กลุ่มที่ 1 และ 2 ได้รับวัคซีนสายพันธุ์ H120 ในขณะที่ไก่กลุ่มที่ 3 และ 4 ได้รับวัคซีนสายพันธุ์ 4/91 เมื่อไก่อายุ 28 วัน ไก่แต่ละตัวในกลุ่มที่ 1-5 ได้รับเชื้อพิษทับซึ่งเป็นเชื้อไวรัสหลอดลมอักเสบติดต่อสายพันธุ์ QX-like ที่แยกได้ในประเทศไทยขนาด $10^{4.17}$ EID_{50} ทำการประเมินผลการป้องกันโรคภายหลังจากไก่ได้รับเชื้อพิษทับเป็นเวลา 7 วัน ผลการศึกษาพบว่าไก่ทุกกลุ่มที่ได้รับวัคซีนมีน้ำหนักตัวมากกว่าไก่กลุ่มที่ไม่ได้รับวัคซีนแต่ได้รับเชื้ออย่างมีนัยสำคัญทางสถิติ ($p<0.05$) รวมทั้งอัตราการป่วยและรอยโรคทางจุลพยาธิวิทยาที่ท่อลมในไก่กลุ่มที่ได้รับวัคซีนต่ำกว่าไก่กลุ่มที่ไม่ได้รับวัคซีนแต่ได้รับเชื้ออย่างมีนัยสำคัญทางสถิติ ($p<0.05$) แต่อัตราการติดเชื้อไม่แตกต่างกัน แสดงให้เห็นว่าการให้วัคซีนเชื้อเป็น 2 ครั้งห่างกัน 2 สัปดาห์ที่ใช้ในการศึกษาครั้งนี้สามารถลดอาการป่วยและการเกิดรอยโรคทางจุลพยาธิวิทยาที่ท่อลมจากการติดเชื้อไวรัสสายพันธุ์ QX-like ที่แยกได้ในประเทศ แต่ไม่สามารถป้องกันการติดเชื้อได้

คำสำคัญ: ไก่ ประสิทธิภาพ ไวรัสหลอดลมอักเสบติดต่อสายพันธุ์ QX-like วัคซีน

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