

6-1-2014

Efficacy of Live Infectious Bronchitis Vaccine Programs against Infection by QX-Like Strain of Infectious Bronchitis Virus

Ekasingh Sarueng

Wisanu Wanasawaeng

Jiroj Sasipreeyajan

Niwat Chansiripornchai

Follow this and additional works at: <https://digital.car.chula.ac.th/tjvm>



Part of the [Veterinary Medicine Commons](#)

Recommended Citation

Sarueng, Ekasingh; Wanasawaeng, Wisanu; Sasipreeyajan, Jiroj; and Chansiripornchai, Niwat (2014) "Efficacy of Live Infectious Bronchitis Vaccine Programs against Infection by QX-Like Strain of Infectious Bronchitis Virus," *The Thai Journal of Veterinary Medicine*: Vol. 44: Iss. 2, Article 3. Available at: <https://digital.car.chula.ac.th/tjvm/vol44/iss2/3>

This Article is brought to you for free and open access by the Chulalongkorn Journal Online (CUJO) at Chula Digital Collections. It has been accepted for inclusion in The Thai Journal of Veterinary Medicine by an authorized editor of Chula Digital Collections. For more information, please contact ChulaDC@car.chula.ac.th.

Efficacy of Live Infectious Bronchitis Vaccine Programs against Infection by QX-Like Strain of Infectious Bronchitis Virus

Ekasingh Sarueng¹ Wisanu Wanasawaeng² Jiroj Sasipreeyajan¹ Niwat Chansiripornchai^{1*}

Abstract

The aim of this study was to evaluate the efficacy of live infectious bronchitis (IB) vaccine programs against infection by a QX-like strain of the IB virus isolated in Thailand. The experimental groups were divided into 5 groups. Group 1 broilers were administered with Poulvac IB QX-like at 1 day old. Group 2 broilers were administered with Poulvac IB QX-like at 1 and 14 days old. Group 3 broilers were administered with Nobilis IB H120 and Poulvac IB QX-like at 1 and 14 days old, respectively. Group 4 broilers were administered with Nobilis IB H120 and Nobilis IB 4-91 at 1 and 14 days old, respectively. Group 5 served as a positive control group. All groups were challenged with IBV THA80151 at a concentration of $10^{5.62}$ ELD₅₀/0.1 ml. Tracheal rales, mortality rate, body weight, ciliostasis, histopathological lesions, immunological responses and virus isolations were monitored. Although different vaccination programs were used in this study, all the vaccinated groups demonstrated protection against damage after the challenge with live IB QX-like virus. The vaccines were able to decrease the tracheal rales, ciliary activity and histological lesion scores in the vaccinated groups compared with the positive control group but the vaccination programs could not prevent infection because IB QX-like virus was still re-isolated from some infected organs. The booster vaccine with the heterologous strain was able to increase the cross protection of IBV QX-like challenge.

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

¹Avian Health Research Unit, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand

²Department of Pathology, Faculty of Veterinary Medicine, Mahanakorn University of Technology, Bangkok 10530, Thailand

*Correspondence: cniwat@chula.ac.th

Introduction

Infectious bronchitis (IB) is caused by Coronavirus which is a single strand RNA virus. The virus infects the respiratory tract, reproductive tract and the kidneys (Cavanagh and Naqi, 2003). Severity of the respiratory disease resulting from infection by IB virus (IBV) is increased when there is co-infection with other infectious agents such as *E. coli*, *Mycoplasma gallisepticum* and/or *Mycoplasma synoviae* occurs. Manifestation of infected chickens with IBV, which is a highly contagious disease, causes potential economic loss of performance through viral respiratory disease. It causes loss of egg production and poor egg quality and has a high mortality rate with some strains linked to nephritis (Cook et al., 2012). Outbreaks of IBV have been repeatedly found worldwide. During the early 1980s, Dutch variant strains (D1466 and D274) of IBV were found in the Netherlands (Terregino et al., 2008). Later in England, an outbreak of the 793B (4-91 or CR88) strain was found during the early 1990s. An outbreak of the Italian strain (IT-02) was reported at the turn of the century and, currently, an outbreak of the IB QX-like (Terregino et al., 2008). An IBV QX strain outbreak was reported in China in September 1996, where lesions were found with a swelling of the stomach in the city of Qingdao (YuDong et al., 1998). Association of the virus with either proventriculitis or severe kidney damage has been found in IBV vaccinated chickens in various provinces in China (Yu et al., 2001; Liu and Kong, 2004). In Europe, the first report of IB QX-like caused a high incidence of false layers and was detected in the kidneys and oviducts of specific challenged pathogen free and commercial chickens in the Netherlands between 2003 and 2004 (Terregino et al., 2008). Then it spread to Russia and was reported in European countries including the Netherlands, Germany, France and Belgium (Cook et al., 2012). It is likely that IB QX infection in the first week of its life may affect the last stage of post embryo development of the oviduct. It might cause an obstruction of the posterior part of the oviduct because of inflammation and subsequent strictures in the walls of the cloaca or the entry of the oviduct, resulting in fluid accumulation and dilatation of the oviduct which have been observed in experiments on QX-like (Benyeda et al., 2009).

In Thailand, Pohuang and co-workers (2011) reported the detection of IBV in Thailand and classified them into three groups; TH1, TH2 and Massachusetts strains. The TH1 strain was found to have been present in Thailand for a long time but TH2 virus was a new strain (QX-like virus) with genetic similarities to the QX strain virus which have had outbreaks in China (Pohuang et al., 2011).

The S1 spike gene of IBV is able to generate antigenic variants caused by the mutation or, sometimes, recombination of the S1 spike gene (Gelb et al., 2005; Worthington et al., 2008). Two major and very important mechanisms in IBV evolution are recombination and mutation (Mardani et al., 2010). The IBV variant strain is created by mutation processes including deletion and insertion of the nucleotides within the IBV genome. Moreover, evolution by

genetic recombination has also been reported (Jia et al., 1995). The heterologous IBV vaccine against the virus shows a little cross protection (Liu et al., 2009) that tends to decrease as the degree of amino acid identity between the spike glycoprotein S1 subunit of the two IBV strains decreases (Lee et al., 2010). The vaccine strains of IBV used in Thailand consist of H120, Mass, Mass and Connecticut, MA5 and 4-91, which have differences in the serotypes or genotypes of the QX-like strain that might result in vaccine protection failure (Pensert and Lamberhts, 1994). The IBV 4-91 is the only variant strain of vaccine which is available on the market and no QX-like vaccine strain is registered in Thailand. Using a vaccine with one homologous strain does not protect chickens. Vaccination programs incorporated with Ma5 and 4-91 vaccine strains could protect the kidneys against heterologous strain of IBV (Cook et al., 2001). Vaccination with two or more different live attenuated IBV vaccines confers broad protection against many important heterologous serotypes of IBV infection (protectotype concept) (Terregino et al., 2008; Mahgoub et al., 2010; de Wit et al., 2011; Cook et al., 2012; Lim et al., 2012). The aim of this study was to evaluate the efficacy of vaccination programs of IBV vaccines against IBV QX-like strain infection.

Materials and Methods

Infectious bronchitis virus: IBV strains were isolated in Thailand during the year 2008 from those previously identified by Pohuang et al. (2009). THA80151 (QX-like) strain virus was selected and used for this experiment as a challenge strain. The virus was propagated and titrated by inoculation into 9-11 days old embryonated chicken eggs. The concentration of IBV was calculated using the ELD₅₀ method of Reed and Munch (Reed and Munch, 1938).

Experimental designs: Two hundred and twenty, 1-day-old female Arbor Acres broiler chickens were kept in the experimental animal facility at the Livestock Hospital, Faculty of Veterinary Science, Chulalongkorn University, Nakorn Pathom, Thailand. Randomly, twenty chicks were collected for blood sampling and then euthanized. The chickens were randomly divided into 5 groups of 40 chicks each. In each group, the chicks were randomly divided into 5 subgroups, each containing 8 chicks. The chickens in groups 1-4 were vaccinated with different IBV vaccine strains (Table 1). The chickens in group 5 served as positive controls and were not given IBV vaccine but were challenged with the IBV QX-like strain (Table 1). At 28 days old, the chickens in groups 1-5 were challenged with the IBV QX-like strain. The chickens were fed *ad libitum* before and during the experiment. The guidelines and legislative regulations of Chulalongkorn University, Bangkok, Thailand, on the use of animals for scientific purposes were followed as certified in permission no. 12310013.

Vaccines: The vaccines used in this trial were IBV QX-like vaccine strain-1148 (DQ431199) (Poulvac®IB QX, Fort Dodge Animal Health, Weesp, Holland)

containing approximately 10^3 - 10^5 EID₅₀, IB H120 (Nobilis IB H120, Intervet, Holland) containing approximately 10^3 EID₅₀ and IB MA5 (Nobilis IB 4-91, Intervet, Holland) containing approximately $10^{3.6}$ EID₅₀, respectively (Table 1).

Challenge study: At 28, 35 and 42 days old, the chickens in each group were weighed. All groups were challenged with IBV THA80151 (QX-like strain). At 28 days old, each chicken was challenged via ocular route inoculation with 100 µl of IBV virus at a dose of $10^{5.62}$ ELD₅₀ per 0.1 ml. After the challenge, all the chickens were observed daily for clinical signs and tracheal rales, for 7 days continuously and each chicken was weighed at 7 days post challenge.

Serological assay: Twenty, one day old chicks were randomly selected for blood collection and then euthanized. Every week, blood was randomly collected from 20 chickens per group via a wing vein from 1 to 6 weeks old. Blood samples were tested for antibodies by ELISA test kit (BioChek, Reeuwijk, Holland).

Ciliostasis scores: At 7 days post challenge, 5 chickens per group were humanely killed and labelled. The tracheas were removed immediately and immersed in Minimum Essential Medium Eagle/Eagle's Balance Salts (MEM/EBSS) (HyClone[®], Utah, USA). Ciliostasis scoring was performed and evaluated by low power microscopy (Cook et al., 1999). The trachea was divided into 3 parts (proximal, middle and distal) and was cut into 10 rings (3 from the proximal, 4 from the middle and 3 from the distal section) with a thickness of less than 2 mm/piece. Then, each piece of trachea was placed into a 24-well plate with MEM/EBSS. The ten rings were examined by light microscope and each ring was scored on a scale of 0-4 as follows; 0 (all cilia beating), 1 (75% of cilia beating), 2 (50% of cilia beating), 3 (25% of cilia beating) and 4 (no cilia beating). The maximum total score was 40 points (4x10) for each trachea. Each chicken that had the protection of IBV was recorded if the ciliostasis score of the trachea was less than 20. The percentage of protection was calculated by the formula the numbers of chickens in a group having a ciliostasis score for the trachea of less than 20/total number of chickens in group. The percentage protection score was calculated by (1-mean ciliostasis score for vaccinated challenge group/mean ciliostasis score for corresponding non vaccinated challenge group) x 100. The higher the score, the better

the level of protection provided by that vaccination program (Cook et al., 1999).

Histopathological examination: At 3, 5, 7 and 14 days post challenge, 5 chickens per group were humanely killed. Necropsy sampling tissues from the trachea, lungs and kidneys were fixed in 10% buffered formalin and taken for histological examination. The histopathological lesion scores followed Chen et al. (1996). The examination and evaluation was by a scoring range of 0-3 (0: no change, 1: mild, 2: moderate, 3: severe). The trachea was observed for epithelial deciliation, epithelial degeneration, decrease in mucous glands, heterophil infiltration, epithelial hyperplasia, lymphoid infiltration and edema. The kidneys were observed for epithelial degeneration, ductotubular dilation, lymphoid infiltration, epithelial regeneration, lymphoid nodules and fibroblastic proliferation. The lungs were observed for lymphoid infiltration and heterophil infiltration.

Virus detection: At 4, 5 and 6 days post challenge, samples of the trachea, lungs, kidneys and duodenum of each chicken in all groups were collected for IBV detection by reverse-transcription polymerase chain reaction (RT-PCR). These samples were prepared by adding to 10% Phosphate Buffered Saline (PBS) and homogenizing and then centrifuging at 1,800xg for 10 min. Supernatant was collected for RNA extraction using a viral nucleic acid and extraction kit (HiYield[™] Viral Nucleic Acid Extraction Kit, RBC Bioscience Corp., New Taipei city, Taiwan). Primers were designed based on differentiation between the IBV QX-like THA80151 and the IBV QX-like virus strain L1148, IB H120 strain and IB 4-91 strain from the vaccine. The forward and reverse primer sequences were F1547- TAATGAAACTGGTTCAGCC and

Table 1 Vaccination programs of IB against IBV QX-like strain (THA80151) challenge

Groups	Vaccination (days)		Challenges (days)
	1	14	28
1	QX-like	-	+
2	QX-like	QX-like	+
3	H120	QX-like	+
4	H120	4-91	+
5	-	-	+

Table 2 Tracheal rale presence at 5 days after challenge and body weight (mean±SD) at 28, 35 and 42 days old

Groups	Tracheal rale presence (30 chickens/group)	Body weight (grams/bird)		
		28 days	35 days	42 days
1	1 ^a	1504.29±81.61	1936.00±78.50	2380.67±138.85
2	2 ^a	1535.71±82.76	1945.00±111.76	2394.67±146.67
3	4 ^{a,b}	1510.00±60.97	1921.50±241.03	2334.67±188.52
4	2 ^a	1508.86±67.12	1936.50±100.17	2363.33±189.50
5	9 ^b	1503.46±73.67	1874.00±117.98	2295.33±185.85

a,b different superscripts in each column mean a statistically significant difference ($p < 0.05$).

Table 3 Antibody titers against IBV (mean±SD) at 7, 14, 21, 28, 35 and 42 days old

Groups	Mean ELISA Titer					
	7 days	14 days	21 days	28 days	35 days	42 days
1	901.60±158.41	240.00±159.56 ^a	37.33±63.31 ^a	83.27±94.53 ^a	2754.20±1785.88 ^a	2269.90±1023.21 ^a
2	656.73±378.83	134.67±132.97 ^a	97.80±193.98 ^a	420.53±614.37 ^a	4170.33±2654.94 ^b	4501.00±2235.75 ^b
3	916.07±441.76	237.40±174.83 ^a	200.93±186.63 ^a	1089.13±71-.75 ^b	7643.33±2427.30 ^c	6432.70±2986.75 ^b
4	622.13±283.50	186.93±106.19 ^a	205.87±227.61 ^a	2509.53±1192.07 ^c	3387.47±1435.37 ^{ab}	4431.30±322.76 ^b
5	890.93±717.94	501.47±607.87 ^b	692.93±865.49 ^b	546.27±784.80 ^{ab}	3963.85±677.17 ^a	1022.50±337.12 ^a

a,b different superscripts in each column mean a statistically significant difference ($p < 0.05$)

R1691-GCGGTACTATTGCTTAATAA, respectively (Pohuang et al., 2012). One-step RT-PCR was performed by RT reaction at 48 °C for 45 min, heating at 94 °C for 5 min and with 35 cycles of degeneration at 94 °C for 30 sec, annealing at 56 °C for 30 sec and polymerization at 72 °C for 10 min. The amplified RT-PCR product of 1440 basepair fragments was analyzed by electrophoresis on 1.2% agarose gel and followed by staining with ethidium bromide (0.5 µg/ml) and was visualized using an ultraviolet transilluminator (Sasipreeyajan et al., 2012).

Statistical Analysis: A comparison between the experimental groups of body weight and antibody titer response was done by ANOVA and Duncan's multiple range test. Tracheal rale, ciliostasis score, histopathological score and virus detection were compared between the groups by Kruskal-Wallis test. Statistical significance was considered at $p < 0.05$

Results

Clinical signs, Mortality and Body Weight: After challenge with IBV THA80151, the clinical signs and mortality were observed. No mortality was found throughout the experiment. The tracheal rales in the vaccinated groups were significantly less than the positive group ($p < 0.05$) except for group 3 (Table 2). The body weight of the chickens in each group at 28, 35 and 42 days old showed no significant difference. However, the body weights of the chickens in the positive control groups at 35 and 42 days old (7 and 14 days after the challenge, respectively) were 1874.00±117.98 and 2295±185.85, respectively which were lower than all the vaccinated groups (Table 2).

IBV antibody titers: At 1 day old, the average of maternally derived antibodies (MDA) against the IBV in chicks was equal to 4871±1801.36 and declined to 890.93±717.94 and 501.47±607.87 at 7 and 14 day old, respectively. While the antibody titers against the IBV in chickens in the vaccinated groups declined until 21 days old (Table 3), at 28 days old, the antibody titers against IBV in chickens in the vaccinated groups 1-4 (83.27±94.53, 420.53±614.37, 1089.13±71.75 and 2509.53±1192.07, respectively) increased. Chickens in

group 4 that were vaccinated with IB H120 and Nobilis IB 4-91 at 1 and 14 days old, respectively, revealed the highest antibody titers ($p < 0.05$). At 35 days old, the chickens in group 3 that were vaccinated with IB H120 and Poulvac IB QX at 1 and 14 days old, respectively, revealed the highest antibody titers (7643±2427.30) ($p < 0.05$). At 42 days old, the antibody titers of the chickens in groups 2, 3 and 4 were 4501±2235, 6432±2986.75 and 4431±322.12, respectively, revealing significantly better antibody titers ($p < 0.05$) than those of the chickens in groups 1 and 5, which were 2269.90±1023.21 and 1022.5±337.12, respectively (Table 3)

Ciliostasis score: The ciliostasis test of the trachea at 7 days post challenge (35 days old) with IBV THA80151 was evaluated in 10 tracheal rings per bird. The results revealed that the mean ciliostasis scores of chickens in the vaccinated groups 1, 2, 3 and 4 were 7.8, 3.2, 7.6 and 0.0, respectively which were significantly lower than that of the positive control group (30.4) ($p < 0.05$) (Table 4). The chickens in group 4 vaccinated with IB H120 and Nobilis IB 4-91 at 1 and 14 days old, respectively, revealed the lowest mean ciliostasis lesion scores and showed the highest protection scores (100). The ciliostasis scores in all the vaccinated groups revealed 100% protection while the positive control revealed 20% protection, meaning that the four vaccination programs could protect the chickens from the challenge with THA80151 (IB QX-like virus) (Table 4).

Table 4 Mean ciliostasis score, % protection and protection score at 7 days after challenge with IBV QX-Like virus (THA80151)

Groups	Mean ciliostasis score	% Protection	Protection score
1	7.80 ^a	100	74.34
2	3.20 ^{a,b}	100	89.47
3	7.60 ^a	100	75.00
4	0.00 ^b	100	100
5	30.4 ^c	20	0.00

a,b different superscripts in each column mean a statistically significant difference ($p < 0.05$)

Table 5 Mean histological score of trachea, lungs and kidneys at 3, 5, 7 and 14 days post challenge with IBV THA80151 (IB QX-like virus)

Groups	Mean histopathological scores											
	3 days post challenge			5 days post challenge			7 days post challenge			14 days post challenge		
	Trachea	Lung	Kidney	Trachea	Lung	Kidney	Trachea	Lung	Kidney	Trachea	Lung	Kidney
1	1.7	1.0	0.04	2.2	0.7	0.13	1.8	0.2	0.33	1.1	0.2	0.00
2	2.0	0.0	0.00	2.1	0.3	0.13	1.5	0.0	0.46	1.0	0.0	0.42
3	1.2	0.5	0.08	1.6	0.3	0.04	1.7	0.5	0.58	0.9	0.7	0.54
4	1.0	0.0	0.04	0.9	0.0	0.13	0.8	0.5	0.13	0.2	0.0	0.29
5	2.0	0.0	0.08	1.7	0.2	0.13	1.4	0.3	0.63	1.5	0.3	0.58

Table 6 IBV detection by reverse transcriptase PCR at 4, 5, 6 days post challenge

Groups	IBV detection											
	4 days post challenge				5 days post challenge				6 days post challenge			
	Trachea	Lung	Kidney	Intestine	Trachea	Lung	Kidney	Intestine	Trachea	Lung	Kidney	Intestine
1	1/3	1/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3	0/3	0/3	0/3
2	2/3	0/3	0/3	0/3	0/3	1/3	0/3	0/3	0/3	0/3	0/3	0/3
3	1/3	0/3	1/3	0/3	0/3	0/3	0/3	0/3	1/3	1/3	0/3	0/3
4	0/3	0/3	2/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
5	3/3	1/3	0/3	0/3	1/3	0/3	0/3	1/3	1/3	0/3	0/3	0/3

Histopathological lesion scores for trachea, lungs and kidneys: At 3, 5, 7 and 14 days post challenge, the histopathological lesion scores for chickens in each group and on each day revealed no significant difference. By 14 days post challenge, the histopathological lesion scores declined, resulting in a better response to IBV infection. The chickens in group 4 had the lowest histopathological lesion scores of the trachea (0.2), lungs (0.0) and kidneys (0.29). At 5 days post challenge, the histopathological scores for the trachea in groups 1 and 2 were 2.2 and 2.1, respectively, which were higher than those of groups 3 (1.6) and 4 (0.9). At 7 days post challenge, the histopathological scores for the kidneys in all the vaccinated groups 1, 2, 3 and 4 were 1.33, 0.46, 0.58 and 0.13, respectively, lower than that of the positive control group (0.63) (Table 5).

IBV detection in trachea, lungs, kidneys and small intestine: RT-PCR was used to detect IBV THA80151. No significant difference was found for IBV detection in each organ for each day. The trachea was the organ that was found to have the highest rate of IBV detection. At 4 days post challenge (32 days old), no IBV was detected in the intestine. At 5 days post challenge, the number of IBV detections reduced in almost all ages and groups. IBV was only detected in the positive control group. At 6 days post challenge, IBV was found only in the trachea and lungs (Table 6).

Discussion

The experiment aimed to demonstrate the efficacy of IB vaccination programs against IBV QX-like strain challenge by evaluating tracheal rales, body weight, histopathology, virus detection and ciliostasis scores. Tracheal rales could be detected both in the vaccinated and positive control groups at 3-7 days post challenge but no mortality rate was found. The chickens in the vaccinated groups revealed significantly fewer tracheal rales than the chickens in the positive control group ($p < 0.05$). The results revealed that re-vaccination with different IBV serotype vaccines could improve protection against heterologous IBV serotype challenge. Vaccination with IBV live-attenuated vaccine can stimulate local, systemic and cell-mediated immunity (Bijlenga et al., 2004) to protect tracheal mucosa from damage after IBV THA80151 challenge. A booster vaccination with a heterologous strain can reduce clinical signs after challenge but chickens that are raised in commercial farms may experience a higher mortality rate due to overcrowded population, improper environment or bacterial co-infection. The body weights of the vaccinated groups and the positive control group were not different. This may be caused by the low density of chickens and also the good arrangement of the

experimental rooms in accordance with animal welfare regulations. The ciliostasis scores of the vaccinated groups demonstrated better protection against IBV THA80151 than the positive control group ($p < 0.05$). As for ciliostasis score evaluation, all the vaccinated groups significantly revealed 100% protection ($p < 0.05$) against IBV THA80151 compared to 20% protection in the positive control group (Table 4). Ciliary activity revealed that the chickens in the vaccinated groups, whether by homologous or booster heterologous vaccine virus, could be protected against the IBV THA80151 challenge. The protectotype concept can explain the efficacy of the vaccination through boosting with a heterologous vaccine strain that can protect the chickens from IBV challenge in a similar way to the homologous vaccine strain (Cook et al., 1999; Terregino et al., 2008; Mahgoub et al., 2010; De Wit et al., 2011; Lim et al., 2012).

The histopathological scores of the trachea, lungs and kidneys showed no significant difference between the vaccinated and the positive control groups. The IBV THA 80151 more severely damaged the trachea than the lungs and kidneys. At 14 days post challenge, the chickens might recovered from the infection because of the reduction in the histopathological lesion scores of the trachea, lungs and kidneys. This may be caused by the chickens having elicited local and cell mediated immunity against IBV infection (Cavanagh, 2003). Vaccination will reduce infection and the clinical signs of IBV challenge. Cytotoxic T-cells play a role in IBV challenge after vaccination. The IB vaccine can decrease early infection and clinical signs by the cytotoxic T-cells. Cytotoxic T-cell activity was shown to be a major histocompatibility complex and lysis was mediated by CD8+CD4- cells (Seo et al., 1997). This mechanism might help to reduce the histopathological lesion scores at 14 days post challenge. The chickens in group 4 had the lowest histological scores compared to the other vaccination programs. This might be caused by the higher antibody titers against IBV at 28 days old and also the low mean ciliostasis score, resulting in better protection against tracheal damage after challenge. For the vaccinated groups, the IBV antibody titers at 7, 14 and 21 days old were lower than those of the positive control group. Due to neutralization between the MDA titers and the IBV virus in the vaccine (Cavanagh and Gelb, 2008), the antibody titers of the vaccinated groups increased 2 weeks after the vaccination (28 days old). According to Hamal et al. (2006), the MAD titers from the positive control group were lowest at 14 days old. At 28 days old, the chickens in group 4 had the highest antibody titers which might be caused by the IBV 4-91 vaccine strain having more virulence than the other vaccine strain. The chickens in groups 3 had the highest antibody titers at 35 days old. The chickens in this group received Nobilis IB H120 and Poulvac IB QX-like at 1 and 14 days old, respectively, and were then challenged with IBV QX-like virus at 28 days old. This means that the chickens received heterologous strain vaccine and a booster with homologous strain vaccine and then challenged with homologous IBV QX-like virus. According to the protectotype concept, this might promote this group of chickens to achieve better antibody response at 35 days

old. The results of RT-PCR detection showed that the trachea was the organ that revealed higher numbers of RT-PCR detection. It agrees with De wit (2000) that the respiratory tract has the highest concentration of IBV during the first 3 to 5 days post-infection (p.i.) and that after this period, the virus titers drop rapidly in the second week p.i., to below detection level. Similarly, Sasipreeyajan et al. (2012), also found higher numbers of IBV re-isolation from tracheas. For the IBV detection by RT-PCR, the tracheas were suitable organs to detect the IBV re-isolation. None of the vaccination programs in this experiment could prevent the infection by IBV THA80151 because the IBV THA80151 could be re-isolated after the challenge, but all the vaccination programs in this experiment could decrease tracheal rales, ciliary activity and histological lesion scores in the vaccinated groups compared to the positive control group. Tracheal rales and the ciliostasis scores were the appropriate techniques for evaluating the efficacy of vaccine and judging whether a booster vaccination with a homologous or heterologous strain of IBV vaccines could protect chickens from showing clinical signs after challenge.

Acknowledgements

This work was financially supported by the 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund) and the Graduate School of the Faculty of Veterinary Science, Chulalongkorn University. We would like to thank staff from Department of Veterinary Medicine, Faculty of Veterinary Science, Chulalongkorn University, BETAGRO, Zoetis (Thailand) Limited, MSD (Thailand) Limited, Advance Pharma Co.,LTD. and BioChek (Thailand) for their support.

References

- Benyeda Z, Mato T, Suveges T, Szabo E, Kardi V, Abonyi-Toth Z, Rusvai M and Palya V 2009. Comparison of the pathogenicity of QX-like, M41 and 793/B infectious bronchitis strains from different pathological conditions. *Avian Pathol.* 38(6): 449-456.
- Bijlenga G, Cook JKA, Gelb J and de Wit JJ 2004. Development and use of the H strain of avian infectious bronchitis virus from the Netherlands as a vaccine: a review. *Avian Pathol.* 33(6): 550-557.
- Cavanagh D 2003. Severe acute respiratory syndrome vaccine development: experiences of vaccination against avian infectious bronchitis coronavirus. *Avian Pathol.* 32(6): 567-582.
- Cavanagh D and Gelb J Jr 2008. Infectious Bronchitis. In: *Diseases of poultry* 12th ed. YM Saif, LK Nolan, AM Fadly, JR Glisson, LR McDougald, DE Swayne (eds.) Iowa State University Press: Blackwell Publishing Professional. 117-135.
- Chen BY, Host S, Nunoya T and Itakura C 1996. Histopathology and immunohistochemistry of renal lesions due to infectious bronchitis virus in chicks. *Avian Pathol.* 25(2): 269-283.

- Cook JKA, Chesher J, Baxendale W, Greenwood N, Huggins MB and Orbell SJ 2001. Protection of chickens against renal damage caused by a nephropathogenic infectious bronchitis virus. *Avian Pathol.* 30(4): 423-426.
- Cook JKA, Jackwood M and Jones RC 2012. The long view: 40 years of infectious bronchitis research. *Avian Pathol.* 41(3): 239-250.
- Cook JKA, Orbell SJ, Woods MA and Huggins MB 1999. Breadth of protection of the respiratory tract provided by different live-attenuated infectious bronchitis vaccines against challenge with infectious bronchitis viruses of heterologous serotypes. *Avian Pathol.* 28 (5): 477-485.
- De Wit JJ 2000. Detection of infectious bronchitis virus. *Avian Pathol.* 29(2): 71-93.
- De Wit JJ, Cook JKA and Van der Heijden HMJ 2011. Infectious bronchitis virus variants: A review of the history, current situation and control measures. *Avian Pathol.* 40(3): 223-235.
- Gelb J, Weisman Y, Ladman BS and Meir R 2005. S1 gene characteristics and efficacy of vaccination against infectious bronchitis virus field isolates from the United State and Israel (1996-2000). *Avian Pathol.* 34(3): 194-203.
- Hamal KR, Burgess SC, Pevzner LY and Erf GF 2006. Maternal antibody transfer from dams to their egg yolks, egg whites, and chicks in meat lines of chickens. *Poul Sci.* 85: 1364-1372.
- Jia W, Karaca K, Parrish CR and Naqi SA 1995. A novel variant of avian infectious bronchitis Virus resulting from recombination among three different strains. *Arch Virol.* 140: 259-271
- Lee HJ, Youn HN, Kwon JH, Lee YJ, Kim JH, Lee JB, Park SY, Choi IS and Song CS 2010. Characterization of a novel live attenuated infectious bronchitis virus vaccine candidate derived from a Korean nephropathogenic strain. *Vaccine.* 28(16): 2887-2894.
- Lim TH, Kim JH, Jang JH, Lee DH, Park JK, Youn HN, Lee JB, Park SY, Choi IS and Song CS 2012. Live attenuated nephropathogenic infectious virus vaccine provide broad cross protection against new variant strains. *Poul Sci.* 91(1): 89-94.
- Liu S and Kong X 2004. A new genotype of nephropathogenic infectious bronchitis virus circulating in vaccinated and non-vaccinated flocks in China. *Avian Pathol.* 33(3): 321-327.
- Liu S, Zhang X, Wang Y, Li C, Liu Q, Han Z, Zhang Q, Kong X and Tong G 2009. Evaluation of the protection conferred by commercial vaccines and attenuated heterologous isolates in China against the CK/CH/LDL/97I strain of infectious bronchitis coronavirus. *The Vet J.* 179(1): 130-136.
- Mahgoub KM, Bassiouni AA, Manal AA and Rabie AN 2010. The prevalence of infectious bronchitis (IB) outbreaks in some chicken farms. III, cross protection of vaccinated chickens versus field IB virus. *J. Am. Sci.* 6(9): 94-108.
- Mardani K, Noormohammadi MH, Ignjatovic J and Browning GF 2010. Naturally occurring recombination between distant strains of infectious bronchitis virus. *Arch Virol.* 155(10): 1581-1586.
- Pohuang T, Chansiripornchai N, Tawatsin A and Sasipreeyajan J 2009. Detection and molecular characterization of infectious bronchitis virus isolated from recent outbreaks in broiler flocks in Thailand. *J Vet Sci.* 10(3): 219-223.
- Pohuang T, Chansiripornchai N, Tawatsin A and Sasipreeyajan J 2011. Sequence analysis of S1 genes of infectious bronchitis virus isolated in Thailand during 2008-2009: identification of natural recombination in the field isolates. *Virus Genes.* 43(2): 254-260.
- Prasaert M and Lambrechts C 1994. Vaccination of chickens against a Belgian nephropathogenic strain of infectious bronchitis virus B1648 using attenuated homologous strains. *Avian Pathol.* 23(4): 631-641.
- Reed LJ and Muench H 1938. A simple method for estimating fifty percent endpoints. *Am J Hyg.* 27: 493.
- Sasipreeyajan J, Pohuang T and Sirikubkul N 2012. Efficacy of different vaccination programs against Thai QX-like infectious bronchitis virus. *Thai J Vet Med.* 42(1): 73-79
- Seo SH and Collosson EW 1997. Specific cytotoxic T lymphocytes are involved in vivo clearance of infectious bronchitis virus. *J. Virol.* 71(7): 5173-5177.
- Terregino C, Toffan A, Beato MS, Nardi RD, Vescellari M, Meini A, Ortali G, Mancin M and Capua I 2008. Pathogenicity of a QX strain of infectious bronchitis virus in specific pathogen free and commercial broiler chickens, and evaluation of protection induced by a vaccination programme based on the Ma5 and 4-91 serotype. *Avian Pathol.* 37(5): 487-493.
- Worthington KJ, Currie RJ and Jones RC 2008. A reverse transcriptase-polymerase chain reaction survey of infectious bronchitis virus genotypes in Western Europe from 2002 to 2006. *Avian Pathol.* 37(3): 247-257.
- Yu L, Jiang Y, Low S, Wang Z, Nam SJ, Liu W and Kwang J 2001. Characterization of three infectious bronchitis virus isolates from China associated with proventriculus in vaccinated chickens. *Avian Dis.* 45: 416-424.
- Yu Dong W, YongLin W, ZiChun Z, GenChe F, Yihai J, Xiang L, Jiang D and Shushuang W 1998. Isolation and identification of glandular stomach type IBV (QX IBV) in chicken. *Chinese J Ani Quarantine.* 15(1): 1-3.

บทคัดย่อ

ประสิทธิภาพของโปรแกรมวัคซีนหลอดลมอักเสบติดต่อเชื้อเป็นต่อการติดเชื้อไวรัสหลอดลมอักเสบติดต่อสายพันธุ์คล้ายคิวเอ็กซ์

เอกสิงห์ สาเรือง¹ วิษณุ วรรณแสง² จิโรจ ศศิปรียจันทร์¹ นิวัตร จันท์ศิริพรชัย^{1*}

วัตถุประสงค์ของการศึกษาเพื่อประเมินประสิทธิภาพของโปรแกรมวัคซีนโรคหลอดลมอักเสบติดต่อชนิดเชื้อเป็นต่อการติดเชื้อไวรัสโรคหลอดลมอักเสบติดต่อสายพันธุ์ QX-like ที่แยกได้ในประเทศไทย กลุ่มทดลองแบ่งเป็น 5 กลุ่ม กลุ่ม 1 ไก่เนื้อได้รับวัคซีน Poulvac IB QX-like ที่อายุ 1 วัน กลุ่ม 2 ไก่เนื้อได้รับวัคซีน Poulvac IB QX-like ที่อายุ 1 และ 14 วัน กลุ่ม 3 ไก่เนื้อได้รับวัคซีน Nobilis IB H120 และ Poulvac IB QX-like ที่อายุ 1 และ 14 วัน ตามลำดับ กลุ่ม 4 ไก่เนื้อได้รับวัคซีน Nobilis IB H120 และ Nobilis IB 4-91 ที่อายุ 1 และ 14 วัน ตามลำดับ กลุ่ม 5 เป็นกลุ่มควบคุมบวกรักษา ไก่ทุกกลุ่มได้รับเชื้อ IBV THA80151 ที่ความเข้มข้น $10^{5.62}$ ELD₅₀/0.1 ml ทำการตรวจติดตามผลจากเสียงกรนในท่อลม อัตราการตาย น้ำหนักตัว การพับปีกของซีเลีย รอยโรคทางจุลพยาธิวิทยา การตอบสนองของภูมิคุ้มกัน และการแยกเชื้อไวรัส ในการศึกษาครั้งนี้โปรแกรมวัคซีนมีความแตกต่างกัน แต่พบว่าไก่ทุกกลุ่มที่ได้รับวัคซีนสามารถป้องกันความเสียหายภายหลังการได้รับไวรัส IB QX-like วัคซีนสามารถลดความรุนแรงจากเสียงกรนในท่อลม ระดับคะแนนการพับปีกของซีเลีย และรอยโรคทางจุลพยาธิวิทยาในกลุ่มที่ได้รับวัคซีน เปรียบเทียบกับกลุ่มที่ไม่ได้รับวัคซีน อย่างไรก็ตามโปรแกรมวัคซีนไม่สามารถป้องกันการติดเชื้อ เพราะยังสามารถแยกไวรัส IB QX-like ได้จากบางอวัยวะที่ติดเชื้อ การให้วัคซีนซ้ำด้วยเชื้อไวรัสหลอดลมอักเสบติดต่อที่มีสายพันธุ์แตกต่างจากครั้งแรกสามารถเพิ่มการปกป้องข้ามไปยังเชื้อไวรัสหลอดลมอักเสบ QX-like

คำสำคัญ: ไก่ ประสิทธิภาพ ไวรัสหลอดลมอักเสบติดต่อ ไวรัสคล้ายคิวเอ็กซ์ วัคซีน

¹หน่วยปฏิบัติการวิจัยสุขภาพสัตว์ปีก คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพฯ 10330

²ภาควิชาพยาธิวิทยา คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเทคโนโลยีมหานคร กรุงเทพฯ 10530

*ผู้รับผิดชอบบทความ E-mail: cniwat@chula.ac.th