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The Pathogenesis of a New Variant Genotype and QX-like Infectious Bronchitis Virus Isolated from Chickens in Thailand

Tawatchai Pohuang

Jiroj Sasipreeyajan

Original Article

The Pathogenesis of a New Variant Genotype and QX-like Infectious Bronchitis Virus Isolated from Chickens in Thailand

Tawatchai Pohuang[1] Jiroj Sasipreeyajan[2*]

Abstract

This study was designed to investigate the pathogenesis of infectious bronchitis virus (IBV), a new variant genotype and QX-like IBV, isolated in Thailand. Sixty, 28-day-old, female broiler chickens were divided into 3 groups of 20 chickens each (groups 1-3) and sixty, 1-day-old, female broiler chickens were divided into 3 groups of 20 chickens each (groups 4-6). The chickens in groups 2 and 5 were inoculated with QX-like IBV (isolate THA80151) whereas the chickens in groups 3 and 6 were inoculated with a new variant genotype IBV (isolate THA90151). The chickens in groups 1 and 4 served as negative control groups. Clinical signs and mortality rates were observed for 14 days post-inoculation (dpi). The chickens were weighed at 0, 7 and 14 dpi. At 7 and 14 dpi, five chickens per group were humanely killed. The tracheas and kidneys were collected for IBV detection and histopathological lesion score evaluation. The results revealed that at 7 dpi, the average body weight of the chickens in groups 2 and 6 was significantly lower than that of the negative control groups (groups 1 and 4, respectively) (p<0.05). Groups 2 and 5 had one dead chicken in each (5% per group). As for the histopathological lesion score evaluation, lesion scores for the trachea were not significantly different among infected groups (p>0.05) at 7 dpi but the score for group 5 was significantly higher than that for group 2 at 14 dpi (p<0.05). As for the kidneys, there was no significant difference in the lesion score among the infected groups at both 7 and 14 dpi (p>0.05). This study indicates that both of the Thai IBV isolates have an affinity to cause respiratory and kidney lesions and an effect on growth rate was found over 7 dpi.

Keywords: chickens, infectious bronchitis virus, pathogenesis

[1] *Department of Veterinary Medicine, Faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen 40002, Thailand*
[2] *Avian Health Research Unit, Department of Veterinary Medicine, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand*
***Corresponding author: E-mail:** jiroj.s@chula.ac.th*

บทคัดย่อ

ความสามารถในการก่อโรคของเชื้อไวรัสหลอดลมอักเสบติดต่อสายพันธุ์แวเรียนชนิดใหม่และสายพันธุ์ QX-like ที่แยกได้จากไก่ในประเทศไทย

ธวัชชัย โพธิ์เฮือง[1] จิโรจ ศศิปรียจันทร์[2]*

การทดลองนี้ศึกษาความสามารถในการก่อโรคของเชื้อไวรัสหลอดลมอักเสบติดต่อสายพันธุ์แวเรียนชนิดใหม่และสายพันธุ์ QX-like ที่แยกได้ในประเทศไทย โดยทำการทดลองในไก่เนื้อเพศเมีย อายุ 28 วัน จำนวน 60 ตัว แบ่งออกเป็น 3 กลุ่มๆ ละ 20 ตัว (กลุ่มที่ 1-3) และไก่อายุ 1 วัน จำนวน 60 ตัว แบ่งออกเป็น 3 กลุ่มๆ ละ 20 ตัว (กลุ่มที่ 4-6) ไก่กลุ่มที่ 2 และ 5 ได้รับเชื้อไวรัสหลอดลมอักเสบติดต่อสายพันธุ์ QX-like (isolate THA80151) ไก่กลุ่มที่ 3 และ 6 ได้รับเชื้อสายพันธุ์แวเรียนชนิดใหม่ (isolate THA90151) ส่วนไก่กลุ่มที่ 1 และ 4 เป็นกลุ่มควบคุมที่ไม่ได้รับเชื้อไวรัส สังเกตอาการป่วยและอัตราการตายเป็นเวลา 2 สัปดาห์ ชั่งน้ำหนักไก่ภายหลังจากได้รับเชื้อ 0 7 และ 14 วัน ทำการฆ่าไก่จำนวน 5 ตัว/กลุ่ม/ครั้ง และเก็บตัวอย่างท่อลมและไตภายหลังจากได้รับเชื้อ 7 และ 14 วัน เพื่อตรวจหาการติดเชื้อและประเมินคะแนนรอยโรคทางจุลพยาธิวิทยา ผลการทดลองพบว่าภายหลังไก่ได้รับเชื้อ 7 วันไก่กลุ่มที่ 2 และ 6 มีน้ำหนักตัวเฉลี่ยต่ำกว่ากลุ่มควบคุม (กลุ่ม 1 และ 4 ตามลำดับ) อย่างมีนัยสำคัญ ($p<0.05$) ส่วนน้ำหนักตัวเฉลี่ยของกลุ่มที่ได้รับเชื้อทุกกลุ่มภายหลังจากได้รับเชื้อ 14 วันไม่แตกต่างจากกลุ่มควบคุมอย่างมีนัยสำคัญ ($p>0.05$) พบการตายของไก่กลุ่มที่ 2 และ 5 กลุ่มละ 1 ตัว คิดเป็นอัตราการตายร้อยละ 5 ในแต่ละกลุ่ม สำหรับคะแนนรอยโรคทางจุลพยาธิวิทยาของท่อลมภายหลังจากไก่ได้รับเชื้อ 7 วันในไก่ที่ได้รับเชื้อทุกกลุ่มไม่แตกต่างกันอย่างมีนัยสำคัญ ($p>0.05$) แต่ภายหลังจากได้รับเชื้อ 14 วัน กลุ่มที่ 5 มีคะแนนรอยโรคที่ท่อลมสูงกว่ากลุ่มที่ 2 อย่างมีนัยสำคัญ ($p<0.05$) ส่วนคะแนนรอยโรคที่ไตในกลุ่มที่ได้รับเชื้อไม่แตกต่างกันอย่างมีนัยสำคัญ ($p>0.05$) ทั้งที่ 7 และ 14 วันภายหลังได้รับเชื้อ การทดลองนี้แสดงให้เห็นว่าเชื้อไวรัสทั้ง 2 สายพันธุ์สามารถทำให้เกิดการติดเชื้อและทำให้เกิดรอยโรคได้ทั้งท่อลมและไต และทำให้ไก่เจริญเติบโตช้าลงในช่วง 7 วันภายหลังได้รับเชื้อ

คำสำคัญ: ไก่ เชื้อไวรัสหลอดลมอักเสบติดต่อ ความสามารถในการก่อโรค

[1] *ภาควิชาอายุรศาสตร์ คณะสัตวแพทยศาสตร์ มหาวิทยาลัยขอนแก่น อ.เมือง จ.ขอนแก่น 40002*

[2] *หน่วยปฏิบัติการวิจัยสุขภาพสัตว์ปีก ภาควิชาอายุรศาสตร์ คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ปทุมวัน กรุงเทพฯ 10330*

***ผู้รับผิดชอบบทความ E-mail:** jiroj.s@chula.ac.th*

Introduction

The infectious bronchitis virus (IBV), a group 3 coronavirus, is 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 and a decrease in egg production have been reported (Gough et al., 1992). Some strains of IBV can cause acute nephritis and urolithiasis associated with the high mortality of infected chickens (Ziegler et al., 2002; Liu and Kong, 2004). Moreover, the disease is a predisposing factor for secondary bacterial infection resulting in an even higher morbidity and mortality rate (Ziegler et al., 2002). One of the major problems of IBV is the frequent emergence of new variant serotypes. Different serotypes have been reported worldwide and new variant strains continue to be recognized (Gelb et al., 1991; Gough et al., 1992; Jia et al., 1995; Liu and Kong, 2004; Pohuang et al., 2009[b]). The diversity of IBV strains is thought to be generated by nucleotide point mutations, insertions, deletions or a recombination of its genome (Jia et al., 1995). The new serotypes or variant strains of IBV are often reported to cause disease in chickens although they have been vaccinated (Gelb et al., 1991; Gough et al., 1992; Liu and Kong, 2004). Therefore, these emergences are of great concern to poultry producers.

Infectious bronchitis (IB) outbreaks have been reported in Thailand (Upatoom et al., 1983; Antarasena et al., 1990; Pohuang et al., 2009[a]). Recently, the molecular characterization of IBV isolates from commercial broiler flocks showed that they were caused by two distinct IBV strains, a new variant which was unique to Thailand and QX-like IBV (Pohuang et al., 2009[a]). In 1996, QXIBV was first described and identified in China, after which the prevalence of the so-called QX-like IBV genotype has been reported and it became one of the most

prominent genotypes 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 closely genetically related, pathological changes reported at the times of disease outbreaks have shown considerable variation (Benyeda et al., 2009). In the case of the Thai QX-like IBV, we characterized them by analysis of complete S1 genes and found that they were a recombinant virus that had emerged from QXIBV and another strain of Chinese IBV (Pohuang, et al., 2011). Hence, the present study was designed to investigate the pathogenesis of a new variant genotype and QX-like IBV isolated in Thailand.

Materials and Methods

Virus: Two different IBV genotypes, the new variant genotype (isolate THA90151) and QX-like IBV (isolate THA80151), were isolated from commercial chicken flocks in Thailand, 2008. The viruses had previously been identified by reverse transcriptase-polymerase chain reaction (RT-PCR) which was followed by sequencing of the S1 gene (Pohuang et al., 2009[a]). Accession numbers were deposited in the GenBank database of isolate THA90151 and isolate THA80151 were EU925649 and FJ156076, respectively. The stock viruses were propagated by inoculation in 9-11-day-old embryonated chicken eggs via the allantoic cavity. At 96 hours after inoculation, the allantoic fluid was harvested and kept at -70^{0}C.

Virus titration: Determination of the virus concentration in the stock solution was done by the 10 fold serial dilution method. Five, 10-day-old embryonated chicken eggs were inoculated in the allantoic cavity with 100 µl per egg of each tenfold serial dilution of the virus and kept at 37^{0}C. Seven days after inoculation, the embryos were examined for IBV lesions (stunting, curled toes or urates in the mesonephrons) (Ziegler et al., 2002). The control embryonated eggs had none of these lesions over the same period. The embryo infectious dose of 50% (EID_{50}/100 µl) was calculated according to the Reed and Muench (1938) method.

Experimental design: Sixty, 28-day-old female Cobb 500 were weighed and then divided into 3 groups of 20 chickens each (groups 1, 2 and 3), and sixty, 1-day-old female Cobb 500 were also weighed and then divided into 3 groups of 20 chickens each (groups 4, 5 and 6). Each group of chickens was kept in a separate experimental room. Feed and water were provided *ad libitum*. The chickens in groups 1 and 4 served as negative control groups. After grouping, the chickens in groups 2 and 5 were individually inoculated with 100 µl ($10^{4.5}$ EID_{50}) of isolate THA80151 via eye-drop. The chickens in groups 3 and 6 were individually inoculated with 100 µl ($10^{4.6}$ EID_{50}) of isolate THA90151 via an eye-drop. The negative control groups were individually inoculated with 100 µl of phosphate-buffered saline (PBS) via an eye-drop. At 7 and 14 days post-inoculation (dpi), five chickens of each group were randomly and humanely killed. The cranial parts of the tracheas and of the left kidneys were collected for histopathological lesion score evaluation and the caudal parts of the trachea and of the left kidney were collected for virus detection. The guidelines and legislative regulations on the use of animals for scientific purposes of Chulalongkorn University, Bangkok, Thailand were followed as certified in permission No. 0931021.

Clinical signs and body weight: Clinical signs which included gasping, coughing, sneezing, depression and ruffled feathers were observed for 14 dpi. Dead chickens were necropsied and gross pathologic lesions were examined. The tracheas and kidneys were collected for detection of IBV. Each chicken was weighed before the challenge inoculation and at 7 and 14 dpi

Virus detection: The tracheas and kidneys were separately collected for virus detection. In each group, the pooled caudal parts of the tracheas or of the left kidneys were placed in sterile plastic bags. The samples were prepared as 10% w/v suspensions in PBS and centrifuged at 1,800 x g 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 were FOR2 (5'-CAG TGT TTG TCA CAC ATT GT -3') and RE2 (5'-CCA TCT GAA AAA TTG CCA GT-3'). The one-step RT-PCR was conducted 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 54^{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, 400-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.

Histopathological lesion score evaluation: The cranial part of the tracheas and of the left kidneys were placed in 10% neutral buffered formalin, sectioned, stained with hematoxylin and eosin, and evaluated for their histopathological lesion score by the method of Ratanasethakul et al. (1999).

Briefly, lesions of 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 of 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.

IBV antibody response: Blood samples were randomly collected from 10 chickens in each group before the challenge inoculation and at 7 and 14 dpi.

Table 1 Mean body weight and mortality rate of infected chickens.

Group	Inoculation	Body weight			Mortality rate	
		0 dpi	7 dpi	14 dpi	Number	Percent
1	PBS at 28 days old	1,122±215.35[A]	1,490 ±117[a]	1,834 ±195	0/20[B]	0
2	THA80151 at 28 days old	1,122 ±214.25	1,385 ±174[b]	1,821 ±97.5	1/20	5
3	THA90151 at 28 days old	1,122 ±215.12	1,460 ±187[a,b]	1,845 ±99.1	0/20	0
4	PBS at 1 day old	44 ±0.81	155 ±8.27[c]	315 ±31.5	0/20	0
5	THA80151 at 1 day old	44 ±0.76	149 ±19.9[c,d]	301 ±57.1	1/20	5
6	THA90151 at 1 day old	44 ±0.93	144 ±18[d]	325 ±46.1	0/20	0

[A] mean±sd (gm/bird), [B] Number of dead chickens/total chickens in the group.
[a,b,c,d] The different superscript in each column means a statistically significant difference (p<0.05).

Table 2 Histopathological lesion score evaluation of the tracheas and kidneys of infected chickens.

	Group	Tracheal lesion score [A]				Average [B]	Kidney lesion score [A]				Average [B]
		0	1	2	3		0	1	2	3	
7 dpi	1	0/5 (0)	0/5 (0)	0/5 (0)	0/5 (0)	0[a]	0/5 (0)	0/5 (0)	0/5 (0)	0/5 (0)	0[a]
	2	0/5 (0)	2/5 (40)	0/5 (0)	3/5 (60)	2.2[b,c]	4/5 (80)	1/5 (20)	0/5 (0)	0/5 (0)	0.2[a,b]
	3	0/5 (0)	2/5 (40)	2/5 (40)	1/5 (20)	1.8[b,c]	4/5 (80)	1/5 (20)	0/5 (0)	0/5 (0)	0.2[a,b]
	4	0/5 (0)	0/5 (0)	0/5 (0)	0/5 (0)	0[a]	0/5 (0)	0/5 (0)	0/5 (0)	0/5 (0)	0[a]
	5	0/5 (0)	1/5 (20)	1/5 (20)	3/5 (60)	2.4[b,c]	1/5 (20)	2/5 (40)	1/5 (20)	1/5 (20)	1.4[b]
	6	0/5 (0)	2/5 (40)	2/5 (40)	1/5 (20)	1.8[b,c]	3/5 (60)	1/5 (20)	0/5 (0)	1/5 (20)	0.8[b]
14 dpi	1	0/5 (0)	0/5 (0)	0/5 (0)	0/5 (0)	0[a]	0/5 (0)	0/5 (0)	0/5 (0)	0/5 (0)	0[a]
	2	0/5 (0)	3/5 (60)	2/5 (40)	0/5 (0)	1.4[b]	3/5 (60)	1/5 (20)	0/5 (0)	1/5 (20)	0.8[a,b]
	3	0/5 (0)	2/5 (40)	2/5 (40)	1/5 (20)	1.8[b,c]	2/5 (40)	2/5 (40)	0/5 (0)	1/5 (20)	1[a,b]
	4	0/5 (0)	0/5 (0)	0/5 (0)	0/5 (0)	0[a]	0/5 (0)	0/5 (0)	0/5 (0)	0/5 (0)	0[a]
	5	0/5 (0)	0/5 (0)	2/5 (40)	3/5 (60)	2.6[c]	2/5 (40)	2/5 (40)	0/5 (0)	1/5 (20)	1[a,b]
	6	0/5 (0)	2/5 (40)	1/5 (20)	2/5 (40)	2.0[b,c]	2/5 (40)	1/5 (20)	0/5 (0)	2/5 (40)	1.4[b]

[a,b,c] The different superscript in each column means a statistically significant difference (p<0.05).
[A] Number of chickens with tracheal or kidney lesions / total samples examined (percentage of lesions in parentheses).
[B] Sum of tracheal or kidney lesion score/total samples examined

The serum samples were kept at -20^{0}C. IBV antibody titers were determined by commercial an Enzyme Linked Immunosorbent Assay (ELISA) test kit (BioChek, Holland).

Data Analysis: A comparison of the mean body weight and antibody titers among the experimental groups was performed by analysis of variance (ANOVA) and least significant difference (LSD) test. The mortality rate was compared by Chi-square test. The histopathological lesion score was analyzed using a Kruskal-Wallis test and a Wilcoxon test was used for pair-wise comparison between the treatment groups. Differences between groups were considered significant at p<0.05.

Results

Clinical signs and mortality rate: Some chickens in both of the infected groups exhibited tracheal rales, coughing and gasping at 2 dpi. Decreased feed consumption and ruffled feathers were observed. Dead chickens were found in group 2 (one chicken; 5%) and in group 5 (one chicken; 5%), but not in groups 3 and 6 (Table 1). There were neither clinical signs nor death in the negative control groups.

Body weight of chickens: To demonstrate the effects of different virus isolates on chicken body weight, individual chickens were weighed weekly. At 7 dpi, the average body weight of the chickens in groups 2 and 6 was significantly lower than that of the negative control groups (p<0.05), but there was no difference between groups 3 and 5 and the negative control groups (Table 1). At 14 dpi, the average body weight of chickens in both of the infected and the negative control groups was not significantly different (p>0.05) (Table 1).

Virus detection: Detection of IBV was performed by RT-PCR. At 7 and 14 dpi, IBV was detected in the tracheas and kidneys of all infected groups. IBV was also detected in the collected tissue from the dead chickens in groups 2 and 5. In the negative control groups, IBV was not found until the end of the experiment at 14 dpi.

Histopathological lesion score evaluation: The histopathological lesion score of the tracheas and kidneys was evaluated at 7 and 14 dpi. The tracheas of chickens infected with both IBV genotypes showed desquamation of the epithelial cells with varying degrees of lymphoid infiltration in the lamina propia and submucosa. In the kidneys of the infected chickens, varying degrees of lymphoid infiltration were observed. Lesions could be seen over the entire study period in the tracheas and kidneys of the infected chickens. The tracheal histopathological lesion score was not significantly different among the infected groups (p>0.05) at 7dpi, but the score of group 5 was significantly higher than that of group 2 at 14 dpi (p<0.05). As for the histopathological lesion score of the kidneys, no significant difference was found among the infected groups at both 7 and 14 dpi

(p>0.05) (Table 2). There was no evidence of a histopathological lesion score of the tracheas and kidneys of the negative control chickens.

IBV antibody response: In the groups of chickens inoculated at 1 day old, the average IBV antibody titers before inoculation were not significantly different (p>0.05). At 7-14 dpi, the titers of the inoculated groups (groups 5 and 6) decreased in the same pattern as the negative control (group 4).

In the groups of chickens inoculated at 28 days old, the average IBV antibody titers before inoculation were not significantly different (p>0.05) among the experimental groups but the titers of the inoculated groups increased and were distinctly elevated above the negative control group (group 1) at 7-14 dpi. At 7 dpi, the average titers of group 2 (3,505.1±3,007.2) and group 3 (2,395.3±1,223.6) were significantly different (p<0.05) from group 1 (485.2±300.6). At 14 dpi, the average titers of group 2 (5,595.2±2,661.5) and group 3 (6,290.6±2,457.2) were significantly higher (p<0.05) than that of group 1 (853.8±571.8).

Discussion

The outbreak of IB in Thailand was initially reported between 1953-1954 (Chindavanig, 1962) and since then IB has continued to be an economically important disease in the Thai poultry industry and can be found all over the country (Upatoom et al., 1983, Antarasena et al., 1990). However, the genotypes of all IBVs isolated from these outbreaks were not characterized. In 2009, we characterized an IBV isolated in Thailand in 1998 by analysis of the HVR of S1 genes. The results showed that the isolate was different from the IBV in other countries and it was unique to Thailand (Pohuang et al, 2009[b]). Recently, we characterized the Thai IBVs isolated during 2008-2009 by analysis of complete S1 genes. The results showed that the Thai IBVs were divided into three distinct groups, unique to Thailand (new variant genotype), QX-like IBV and Massachusetts type. Interestingly, the recombination events were found in the new variant genotype and QX-like IBV (Pohuang et al, 2010). The pathogenesis of these new viruses was considered to be inexplicable because they were a new emergent genotype. Therefore, this study was undertaken to investigate their pathogenesis.

The average body weight at 7 dpi of the chickens infected with QX-like IBV at 28 days old and of the chickens infected with the new variant genotype at 1 day old were significantly lower than those of the non-infected chickens. At the same time, although significant differences were not observed in other infected groups, the average body weight was lower than that of the non-infected chickens. Consistent with previous work, the low weight gain associated with IBV infection was reported by many authors (Grgić et al., 2008; Pohuang et al., 2009[b]). By the end of the experiment, the differences in the average body weight among chickens inoculated with different isolates were compensated. These indicated that both of the viruses had an effect on the growth of chickens during the acute phase of infection.

In this investigation, chickens infected with both IBV genotypes showed respiratory signs including tracheal rales, coughing and gasping. The virus was detected in the tracheas by specific RT-PCR; moreover, the relevant histopathological lesions were detected in the tracheas of infected chickens. This indicated that both of the IBV genotypes had an affinity for the respiratory tract. The average histopathological lesion score of the tracheas in group 5 was significantly higher than that in group 2. In this case, the chickens in group 5 were younger than the chickens in group 2. This suggests that younger chickens are more susceptible to Thai QX-like IBV infection. However, this occurrence was not found between groups 3 and 6 which had been inoculated with the new variant genotype. Based on previous reports, although most of the authors found a greater severity of IBV infection in young chickens than in adults (Animas et al. 1994; Ignjatovic et al. 2003), an inverse age resistance to IBV infection had been reported by Macdonald et al. (1980). The possibility was that the strains of IBV have wide and variable genotypes in tissue tropisms and the clinical manifestations of the disease can be diverse (Albassam et al., 1986; Chandra, 1987).

In addition to causing respiratory disease, the QXIBV reported in China was associated predominantly with various forms of kidney pathology (Liu and Kong, 2004). In this study, the gross lesions of the kidneys were only observed in dead chickens that had been inoculated with the QX-like isolate. For the humanely killed chickens at 7 and 14 dpi, we could not find gross pathologic lesions such as pale and swollen kidneys or urate deposited in the kidneys of all infected groups at the time of necropsy. However, when the histopathological lesion score of the kidneys was evaluated, the lesion score was observed with varying severity. In this case, it seemed to be that our QX-like isolate did not induce severe gross lesions of kidneys as had been reported in the Chinese QX isolate (Liu and Kong, 2004). The observations herein agreed with the study of Benyeda et al. (2009) who reported that clinical signs and pathological changes induced by QX-like IBV strains differ in some aspects from the infection of the original QX isolate. Five QX-like IBV strains isolated from different countries (China, France, Slovakia, Greece and Hungary) had differences in their pathogenicity under experimental condition. The mildest lesions were caused by the Greek isolate whereas the most severe lesions in the investigated organs were found in chickens infected with the Chinese QX isolate. A possibly closer relationship with the original QX isolate might be the reason for this occurrence.

Commercial chickens with maternally-derived antibodies were used in the experiment because we expected that this was the same situation as that of field conditions. The antibody titers of the chickens in groups 4, 5 and 6 gradually declined over time, which is consistent with the report on the changes in maternal antibody titers (Al-Taracha et al., 1991). Hens transmit maternal antibodies to their offspring by depositing the antibodies in the eggs. The

percentage of IgY transfer from hen plasma to their chicks is estimated to be 30%. The levels of anti-IBV antibody in the chicks serum detected by ELISA kit were highest in 3 days old and decreased substantially in 7 and 14 days old, respectively (Hamel et al., 2006). Interestingly, the respiratory signs, histopathological lesion score and positive result of IBV detection were found in chickens with maternally-derived antibodies infected with both IBV genotypes. This indicates that maternally-derived antibodies in our experimental chickens could not protect them from the infection with either of the IBV genotypes. After inoculation with both of the IBV genotypes, the chickens inoculated at 1 day old did not seroconvert within 7 dpi while chickens inoculated at 28 days old did. This is similar to our previous report that 2 days old chickens inoculated with IBV isolate THA001 did not seroconvert within 7 dpi; however, the IBV titers of the IBV inoculated chickens were higher than those of the unchallenged control chickens at 14 dpi (Pohuang et al., 2009[b]). A possible explanation could be that the immunocytes of these chickens were still immature, hence, they did not respond sufficiently to the infection (Animas et al., 1994).

This study indicates that the new variant genotype and QX-like IBV isolated in Thailand have an affinity to cause respiratory and kidney lesions. The viruses have an effect on chicken growth during the acute phase of infection. Moreover, they can cause disease in chickens with maternally-derived antibodies. These findings will be of great concern to commercial chicken farms. Therefore, further study should aim to determine if the available commercial IB vaccines provide sufficient protection against these Thai IBV isolates.

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