Original Article

Effect of a rapid heat treatment on Thai QX-like infectious bronchitis virus and evaluation of protection induced by a heat-treated virus

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Abstract

The purpose of this study was to evaluate the effect of a rapid heat-treatment method on Thai QX-like infectious bronchitis virus (IBV) and the efficacy on protection from the virus after heat treatment. QX-like IBV isolate THA46 was incubated at 56 ^{o}C. The incubated virus was collected every 5 min for up to 1 h and then they were inoculated into embryonated chicken eggs. At 6 days post-inoculation, allantoic fluid was harvested from the embryonated eggs which were inoculated with virus of the longest heat-treatment time that induced lesions in the embryos. That allantoic fluid was used in a subsequent round of heat treatment followed by inoculation into embryonated eggs. The procedure was repeated eight times. The longest heat-treatment time at 56 ^{o}C that induced lesions in the embryos for Thai QX-like IBV varied from 30 to 60 min. S1 and N genes of the virus after each heat-treatment time were sequenced and none of the nucleotide changes were found. The efficacy of the heat-treated virus was investigated by immunizing chicks at 1 and 14 days of age and challenging with the pathogenic isolate THA46 at 28 days of age. The results showed that virus detection in the tracheas, the gross lesion score of the tracheas and the histopathologic lesion score of the tracheas and kidneys of the chickens immunized with heat-treated virus were significantly lower (p<0.05) than those of the positive control group. The study demonstrates that a rapid heat-treatment method has no effect on the S1 and N gene of IBV isolate used in this work. A heat-treated Thai QX-like IBV provided a good protection against challenge with pathogenic Thai QX-like IBV.

Keywords: chicken, heat treatment, infectious bronchitis virus, protection

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Introduction

Infectious bronchitis (IB) caused by infectious bronchitis virus (IBV), a member of the family *Coronaviridae*, is a highly contagious disease of chickens with a worldwide distribution (Cavanagh and Gelb, 2008). The disease causes economic loss to poultry production due to decreased growth rate, increased feed conversion ratios (Ziegler et al., 2002), decreased egg production and poor eggshell quality (Cavanagh and Gelb, 2008). High mortality occurs in infected chickens as a result of acute nephritis and urolithiasis caused by nephropathogenic strains (Ziegler et al., 2002, Liu and Kong, 2004). The disease is often complicated by secondary bacterial infections that cause the increased mortality of IBV infected chickens (Ziegler et al., 2002). Moreover, the emergence of IBV variant strains that do not confer cross-protection against each other has been increasing and has complicated disease control worldwide (Liu and Kong, 2004; Kulkarni and Resurreccion, 2010; Mahmood et al., 2011).

The QX strain of IBV was first identified in chickens from China in 1996 (Wang et al., 1998). Subsequently, there have been increasing numbers of IB outbreaks around the world which have nucleotide sequence similarities to QX strain with the so-called QX-like IBV (Valastro et al., 2010). QX-like IBV has been introduced to Thailand and it was first identified in chickens with respiratory disease in January, 2008 (Pohuang et al., 2009). The phylogenetic tree shows that it has formed a new cluster in the field. Moreover, QX-like IBV in Thailand is a recombinant virus that emerged from QXIBV and another strain of Chinese IBV (Pohuang et al., 2011). To prevent economic loss due to IBV infection, intensive vaccination efforts have been routinely administered. However, in the case of IB, complete protection is provided only when vaccine and infectious virus are of a homologous strain or serotype (Al-Tarcha and Sadoon, 1991; Liu et al., 2009). Therefore, the development of a vaccine from a local strain is necessary for IBV control in Thailand.

Live attenuated IB vaccines have been used worldwide because vaccination with inactivated virus is less effective than that with live viruses. However, producing a live IBV vaccine requires lengthy strategies that are time, cost, and labor intensive (Huang and Wang, 2006). Continuous passage and attenuation of IBV in the chicken embryos is a common method for the development of attenuated vaccines. The number of passages required for attenuation can vary from 70-110 and can require a year or more for completion (Huang and Wang, 2006; Liu et al., 2009). Moreover, not all IBV strains stimulate immunity after many serial passages in embryonated chicken eggs (Liu et al., 2009). Recently, a rapid heat-treatment method was described by Jackwood et al. (2010). The method was used to fast track the attenuation process of a new serotype IBV, GA08 strain. The attenuation process took a shorter time than required to attenuate IBV by serial passage in embryonated chicken eggs without heat treatment. In the present study, we evaluated the effect of a rapid heat- treatment method on QX-like infectious bronchitis virus (IBV) isolated in Thailand and the efficacy on protection after treatment. The results of this work might provide information for further development of an attenuated Thai QX-like IBV vaccine.

Materials and Methods

Virus: QX-like IBV, isolate THA46 (titer $10^{6.5}$ median embryo infectious dose [EID_{50}] /ml) was used as a starting virus for rapid heat treatment. It was isolated from commercial chicken farms in Thailand which had experienced respiratory diseases in 2010. The virus was identified by reverse transcriptase-polymerase chain reaction (RT-PCR), followed by sequencing of the S1 gene (Pohuang et al., 2009).

Heat treatment: A rapid heat-treatment method was performed as described by Jackwood et al. (2010). Briefly, the virus', fourth passage THA46 isolate (THA46 pass 4) was incubated at 56 ^{o}C. One ml of incubated virus was collected every 5 min for up to 1 h and inoculated (0.1 ml) into allantoic cavity of 10 day old embryonated chicken eggs. The embryos were examined for IBV lesions of curling and stunting embryos at 6 days post inoculation. The allantoic fluid was harvested from embryonated chicken eggs which were inoculated with the virus of the longest heat-treatment time that induced lesions. A subsequent round of heat treatment followed by inoculation into embryonated chicken eggs was done using that allantoic fluid. The procedure was repeated eight times (passage 5-12). Allantoic fluid harvested from the last heat- treatment round was subsequently passaged for four additional times in 10 days old embryonated chicken eggs without heat treatment (passage 13-16). The virus at passage 16 (four initial passages, plus eight heat-treatment passages and plus four additional passages) designated THA46H8P4 being titrated by inoculating 10-fold serial dilutions of the virus into 10 day old embryonated chicken eggs. Virus concentration was determined by the method of Reed and Muench (1938).

Molecular characterization: Molecular characterization was done using ten viruses including THA46 pass 4, THA46H8P4 and eight heat-treated viruses from each heat-treatment passage (passage number and heat-treatment time as shown in Table1). Viral RNA was extracted from 200 µl of allantoic fluid containing IBV using Viral Nucleic Acid Extraction Kit (Real Biotech, Taiwan) according to the manufacturer's instructions. S1 and N genes were RT-PCR amplified from the extracted RNA. The primers F23 (GCCAGTTGTTAATTTGAAAAC) and R89 (TAATAACCACTCTGAGCTGT) were used to amplify a fragment of 988 base pairs covering hypervariable region I and II of S1 gene (Pohuang et al., 2011). The primers FN1 (ATT CCA AGG GAA AAC TTG TG) and RN2 (TCA TTC ATC TTG TCA TCA CC) were used to amplify a fragment of 830 base pairs of N gene. RT-PCR was performed using one-step RT-PCR system (AccessQuick™ RT-PCR System, Promega, USA). The cycling conditions were 45 min of RT reaction at 48 ˚C, heating at 94 ˚C for 5 min, followed by 35 cycles of 94 ˚C for 30 sec, 58 ˚C for 30 sec, 72 ˚C for 30 sec. A single final extension step was performed at

72 °C for 10 min. RT-PCR products were purified using the NucleoSpin® Extract II (Macherey-Nagel, Germany) following the manufacturer's protocol. Nucleotide sequencing was performed in both of the forward and the reverse directions by a commercial service (First Base, Selangor, Malaysia). Sequences were compared using BioEdit version 7.0.5.2 (Hall, 1999).

Table 1 The longest heat-treatment (56 °C) time that induced lesions in the embryos.

Passage number following heat treatment	Heat-treatment time (min)
5[A]	60[B]
6	55
7	55
8	35
9	60
10	60
11	30
12	55

[A]Passages 1 to 4 in embryonated chicken eggs were conducted prior to heat treatment. Passage 5, 6, 7, 8, 9, 10, 11 and 12 were four initial passages plus heat-treatment passages 1, 2, 3, 4, 5, 6, 7 and 8, respectively.
[B]The longest heat-treatment (56 °C) time that caused lesions in embryos at 6 days post inoculation.

Experimental design: The viruses used for immunization were a heat-treated IBV (THA46H8P4) and a commercial live attenuated vaccine, H120 strain (Bioral® Merial, Lyon, France). In order to establish the similarity between vaccine, H120 strain and the challenge virus, the S1 gene of virus vaccine was amplified, sequenced and analyzed as described above. The efficacy on protection was performed in commercial chickens. Eighty, 1 day old commercial chickens were randomly divided into four groups of 20 chickens each and housed in separate rooms. The chickens in the first group were immunized by eye-drop with a live attenuated IBV vaccine, Bioral® H120 at 1 and 14 days of age. The second group was immunized intraocularly with 10^4 EID_{50}/bird of THA46H8P4 at 1 and 14 days of age. The third and fourth groups were not vaccinated and served as positive and negative controls, respectively. The first, second and third groups of chickens were challenged intraocularly with 10^4 EID_{50}/ bird of pathogenic virus, THA46 pass 4 at 35 days of age. At 7 days post challenge, all chickens were humanely killed and examined post mortem. All study procedures and animal care activities were conducted in accordance with Khon Kaen University guidelines for the care and use of laboratory animals, approval number AEKKU 60/2554.

Gross lesion examination: The upper parts of the tracheas (approximately 1 inch distal from the larynx) and cranial right kidneys were collected for gross lesion scoring. The lesions were scored for each trachea on a scale from 0 to 3 with 0= no lesions; 1= slight increase of mucin; 2= large increase of mucin; 3= large increase of mucin and mucosal congestion (Wang and Huang, 2000). Lesions in the kidneys were scored as follows: 0= no lesions; 1= swelling and urate visible under stereomicroscopy; 2= swelling with urate; 3= swelling with large amount of urate (Wang and Huang, 2000).

Ciliostasis test: The middle parts of the trachea from 10 chickens per group were randomly selected and placed in minimum essential medium (MEM). Immediately after the trachea was stored in MEM, 5 tracheal rings were carefully cut by hand. The level of beating of the cillia was determined by low-power microscopy and scored as follows: 0= no beating (100% ciliostasis); 1= >0-25% beating; 2= >25-50% beating; 3= >50-75% beating; and 4= >75-100% beating (De Wit et al., 2010). The protection in an individual chick was recorded if the ciliostasis score was more than 10. The protection against challenge was determined and calculated as described by De Wit et al. (2010).

Histopathological examination: The lower parts of the trachea and the cranial left kidneys were collected and fixed in 10% buffered formalin. Tissues were embedded into paraffin and sectioned for haematoxylin and eosin staining. The lesions were scored for each trachea from 0 to 3 with 0 = no lesions, 1 = epithelial deciliation and desquamation with minimal lymphoid infiltration in lamina propria and submucosa, 2 = generalized epithelial deciliation and hyperplasia with moderate lymphoid infiltration in lamina propria and submucosa, 3 = generalized epithelial deciliation and hyperplasia heavy lymphoid infiltration in lamina propria and submucosa (Ratanasethakul et al., 1999). Kidney lesions were scored from 0 to 3 as follows: 0= No lesions, 1= A few small areas of lymphoid infiltration in the interstitial tissue, 2= Severe small areas of lymphoid infiltration in the interstitial tissue and 3= diffuse lymphoid infiltration in the interstitial tissue (Ratanasethakul et al., 1999).

Virus detection: After a tracheal gross lesion scoring, a tracheal swab was performed at the upper part of trachea. The swabs were placed into 1 ml of phosphate-buffered saline pH 7.4 (PBS). Viral RNA was extracted from 200 µl of PBS of the tracheal swab using a Viral Nucleic Acid Extraction Kit (Real Biotech, Taiwan) according to the manufacturer's instructions. RT-PCR was performed using one-step RT-PCR system (AccessQuick™ RT-PCR System, Promega, USA) with the specific primers for QX-like IBV as described by Sasipreeyajan et al. (2012). The one-step RT-PCR was conducted by 45 min of RT reaction at 48 ˚C, heating at 94 ˚C for 5 min and followed by 35 cycles of 94 ˚C for 30 sec, 54 ˚C for 30 sec, 72 ˚C for 30 sec. A final extension step was performed at 72 ˚C for 10 min. RT-PCR products were stained with ethidium bromide (0.5µg/ml). They were determined by electrophoresis on 1.2% agarose gel and visualized using an ultraviolet transilluminator.

IBV antibody titer: At 1 day old, blood samples were randomly collected from 20 chickens. At 7 and 14 days old, blood samples were randomly collected from 10 chickens in groups 1, 2 and 4. At 21 and 28 days old, blood samples were randomly collected from 10 chickens in groups 1, 2 and 3. Then, blood samples were randomly collected from 10 chickens in each group at 35 and 42 days old. The serum samples were kept at -20 ºC. IBV antibody titer was determined using a commercial enzyme-linked immunosorbent assay (ELISA) test kit (BioChek, Reeuwijk, Holland).

Data analysis: A comparison of the antibody titers among the experimental groups was performed using analysis of variance (ANOVA), followed by Duncan's Multiple Range test. Chi-square test was used to test the differences in IBV detection among groups. The histological lesion scores were analyzed using Kruskal-Wallis test and the Wilcoxon test was used for pair-wise comparison between groups. A $p<0.05$ was interpreted as a statistically significant result.

Results

Heat-treatment time: As shown in Table 1, the time of heat treatment at 56 ºC that caused curling and stunting embryos ranged from 30 to 60 min and did not appear to show a relationship with the passage number of virus.

Molecular characterization: The nucleotide sequences of S1 and N gene of heat-treated IBV were determined and compared with those of the virus before heat treatment. The results showed no genetic change was observed in either the S1 or N gene of all heat-treated virus. The S1 gene of the vaccine strain H120 was also determined. The results show that the vaccine strain had nucleotide identity of 73.29% and amino acid identity of 51.25% with the challenge virus.

Gross and histopathological examination: As shown in Table 2, in group 1 and 2, the gross lesion scores of the tracheas were significantly lower ($p<0.05$) than control group 3 but the kidney lesion scores were not significantly different ($p>0.05$) from group 3. The histopathological lesion scores of the tracheas of groups 1 and 2 were significantly lower ($p<0.05$) than those of group 3 but they were significantly higher ($p<0.05$) than those of group 4.

Table 2 Gross lesion score, histopathological lesion score, IBV detection and protection score at 7 days post challenge of the experimental chickens

Group	Gross lesion score[A]		Histopathological[A] lesion score		IBV detection[C] (%)	Protection score		
	Trachea	Kidney	Trachea	Kidney		Ciliary activity[D]	Individual[E] (%)	Percentage[F]
1	0.2±0.4[Ba]	0[B]	1.2±1.1[Bb]	2.0±1.3[Bc]	16/20[b] (80)	139	7/10 (70)	69.5
2	0[a]	0	0.8±0.7[b]	1.3±1.0[b]	4/20[a] (20)	184	10/10 (100)	92
3	1.1±0.5[b]	0.1±0.3	2.8±0.6[c]	2.8±0.7[d]	20/20[b] (100)	0	0/10 (0)	0
4	0[a]	0	0.1±0.3[a]	0.4±0.5[a]	0/20[a] (0)	200	10/10 (100)	100

[a,b, c,d]statistical difference ($p<0.05$) in the same column.
[A]Sum of lesion score/ total chickens examined.
[B]Mean ± standard deviation (SD).
[C]Number of positive IBV detection/total chickens examined.
[D]Sum of the score of ciliary activity in each group.
[E]Number of chick with the ciliostasis score ≥ 10/ total chickens examined
[F][(total of the individual scores)/(number of individuals X 20)]X100

***Ciliostasis test and percentage of protection*:** After the first time of immunization at 1 day old, clinical signs of sneezing were observed in some of the chickens of group 2. No clinical signs were observed in the chickens in the other groups. After challenge of pathogenic virus at 35 days old, clinical signs were observed in some of the chickens of group 1 and in all of the chickens of group 3. No clinical signs were observed in the chickens of group 2 and 4. The level of beating of the cillia and a percentage of protection for each group was calculated and is shown in Table 2. In this study, a better level of protection achieved when a higher score was found. In group1, the result for individual protection was 70% and the protection score

was 69.5%, indicating that some protection had been achieved. In group2, the cilia beating was high and 100% of the chickens were recorded for an individual protection. The protection score was 92%, indicating that good protection had been achieved.

Virus detection: The results of the virus detection are presented in Table 2. In group 3, challenge virus was detected by RT-PCR from the tracheas of all birds. In the vaccinated group 1, the numbers of infected chickens were not significantly different (p>0.05) from the control group 3. In the immunized group 2, the numbers of infected chickens were significantly lower (p<0.05) than the control group 3 and vaccinated group 1. The virus was only detected in the trachea of 4 of 20 birds at 7 days post challenge.

IBV-ELISA titer: The results of IBV-ELISA titers are summarized in Table 3. At 1 day old, the average IBV antibody titer of experimental chickens was 3,691±1,489. At 7 and 14 days old, the average IBV antibody titer was not significantly different (p>0.05) among the groups. At 21, 28 and 35 days old, the average IBV antibody titers of group 1 and 2 were significantly higher (p<0.05) than those of group 3 and 4. At 42 days old, the average IBV antibody titer was significantly different (p<0.05) among the groups of experimental chickens.

Table 3 The average antibody titer levels compared among the experimental groups

Group	IBV-ELISA titer						
	1 day old	7 days old	14 days old	21 days old	28 days old	35 days old	42 days old
1	3691±1489 [A] (20)[B, C]	2109±1007 (10)	742±257 (10)	706±411[b] (10)	799±240[b] (10)	983±607[b] (10)	7790±2007[d] (10)
2		1729±965 (10)	858±695 (10)	844±603[b] (10)	670±312[b] (10)	1175±791[b] (10)	1127±652[b] (10)
3		ND[D]	ND	172±158[a] (10)	151±61[a] (10)	39±28[a] (10)	3301±1426[c] (10)
4		1964±932 (10)	614±402 (10)	ND	ND	48±43[a] (10)	66±24[a] (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.
[D] not done

Discussion

QX-like IBV has been one of the major types of IBV circulating in Thailand in recent years (Pohuang et al., 2009; Pohuang et al., 2011). Experimental infections and field results have shown that available commercial vaccines provide partial protection against QX-like IBV isolated in Thailand (Sasipreeyajan et al., 2012). In order to find the best protection, we tried to develop a live attenuated vaccine candidate from Thai QX-like IBV. However, producing a live attenuated vaccine requires lengthy strategies that are time, cost and labor intensive. Then, a rapid attenuation process was selected. A rapid heat-treatment method was previously reported to shorten the time to attenuate GA08 strain of IBV by exposure of the virus to 56 ^{o}C followed by propagation in embryonated chicken eggs eight times (pass 5 to pass 12) (Jackwood et al., 2010). The results of this study showed that the longest heat-treatment time at 56 ^{o}C that induced lesions in the embryos varied from 30 to 60 min. Similarly, the longest 56 ^{o}C incubation time that did not completely inactivate IBV strain GA08 varied from 15 to 55 min (Jackwood et al., 2010). It is assumed that heat treatment played a role in inactivation of IBV. IBV is considered heat labile and it can be inactivated at 56 ^{o}C for 15 min (Cavanagh and Gelb, 2008).The mechanism of action of heat inactivation for IBV is thought to be through disruption of the virus structure (Jackwood et al., 2010).

This study's results demonstrate that good protection can be provided by a homologous strain between immunization and challenge virus. Consistent with previous work, the best protection against challenge with pathogenic IBV was achieved by a vaccine containing the homologous strain (Liu et al., 2009; Feng et al.; 2015). Liu et al. (2009) showed that complete protection against challenge with IBV strain CK/CH/LDL/97I in China was observed in the chickens which were immunized with passage 115 of CK/CH/LDL/97I virus. We found that the antibody response after challenge in chickens immunized with a heat-treated virus was not increased whereas the response in chickens immunized with H120 vaccine was sharply increased. The result might be that immunity induced by a heat-treated virus was more effective on virus neutralization than that of immunity induced by H120 vaccine. When the pathogenic virus was inhibited, the chicken was not infected or a low level of infection occurred. This mechanism might be a cause of no increment of antibody titer post- challenge in the chickens immunized with heat-treat virus in our

work. According to Feng et al. (2015), the chickens immunized with passage 90 of YX10 IBV had 100% clinical protection against virulent YX10 virus and 6 out of 12 chickens had ELISA antibody negative at 14 days post-challenge. In contrast, the chickens immunized with H120 strain vaccine had 25% clinical protection and none of experimental chickens had ELISA antibody negative (100% positive) at 14 days post-challenge. However, antibody titer was not shown in that work.

Live vaccines based on the Massachusetts serotype such as H120 strain are widely used. An improvement in protection against a heterologous challenge can be achieved in H120 vaccinated chickens (Darbyshire, 1985; Wang et al., 1996; Liu et al., 2009). In this study, although virus detection from a tracheal swab of H120 vaccinated chickens was no different from those of the infected chickens that were not vaccinated, the tracheal gross lesion score and histopathologic lesion score of trachea and kidney were lower than non-vaccinated chickens. This data suggests that H120 vaccine, given at 1 and 14 days of age provided partial protection against challenge with QX-like IBV. The H120 strain vaccine could reduce the amount of the challenge virus (Darbyshire, 1985). Live vaccines induce mucosal immunity such as IgA (Nakamura et al., 1991) and cellular immunity (Pei et al., 2003) that inhibits invasion and replication of the virus in the tracheal mucosa. The antibody titer at the day of challenge partially inhibited invasion by the infectious virus into visceral organs. This phenomenon could reduce tissue damage resulting in less severe of morbidity in the vaccinated chickens (Pensaert and Lambrechts, 1994; Terregino et al., 2008; Lui et al., 2009). However, some of challenge virus was able to penetrate to other tissues and replication. Therefore, a high number of infected chickens and an increase in antibody response after infection were found at 7 dpi.

On the basis of clinical signs in chickens, Jackwood et al., (2010) reported that no clinical signs were observed after immunization with the GA08 heat-treated virus. In this work, QX-like heat-treated IBV still induced mild clinical signs in some chickens after immunization. The result suggests that a rapid heat-treatment method may not provide a complete attenuation for Thai QX-like IBV. The factors that had an effect on the mechanism of heat-treatment attenuation included the strain of viruses (Jackwood et al., 2010) and the number of serial passage of virus in embryonated chicken eggs (Huang and Wang, 2006; Liu et al., 2009). S1 subunit is known to contain several epitopes that induce virus neutralizing antibodies (Kingham et al., 2000). The N protein contains the regions that induce a T-cell response and protection (Lai and Holmes, 2001). Therefore, in this study it follows that for heat-treated Thai QX-like IBV to induce protection against challenge virus, the S1 and N gene of the heat-treated virus ought to be relatively similar to the virus before heat treatment. Based on the comparison of nucleotide sequences in the S1 and N gene, no point mutation was found in either S1 or N gene of high numbers of heat-treated virus. This finding indicates that rapid heat treatment and passage in embryonated chicken eggs did not result in genetic changes of Thai QX-like IBV and the heat-treated Thai QX-like IBV had the ability to induce neutralizing antibodies against a pathogenic virus which was shown by the efficacy studies in chickens.

In this study, the QX-like heat-treated IBV which was used for immunization had the ability to induce lesions in embryonated chicken eggs. This indicated that heat-treated IBV had a replication capacity in the embryo. The immunogenicity was maintained as shown in the immunization and challenge test. The replication capacity in the embryo and immunogenicity after the attenuation process are important for the seed virus vaccine (Feng et al.; 2015). Generally, the attenuated IBV vaccine strains are produced by multiple passages of field strain in embryonated chicken eggs. During this process, the virus becomes more efficient at replication and more pathogenic to the embryo, with concomitant attenuation in chickens (Liu et al., 2009; Zhao et al., 2014). Sometimes, after several passages in embryonated chicken eggs these resulted a loss of their immunogenicity and they were not suitable to use as a vaccine strain (Feng et al.; 2015). Thus, the seed virus selected for vaccine candidate should maintain its ability to replicate and immunogenicity after the attenuation process. Although Thai QX-like heat-treated IBV could provide good protection against disease in the immunization and challenge test, other criteria such as safety, pathogenicity and virulence reversion remain unclear. We state that these criteria need to be tested before use as a vaccine candidate.

In conclusion, a rapid heat-treatment method has no effect on genetic changes in the S1 and N gene of Thai QX-like IBV. The heat-treated virus provides good protection against disease. However, there is a need for further study to evaluate the desirable level of heat-treatment attenuation of Thai QX-like IBV and the safety of the heat-treated virus for possible future use as live attenuated vaccine candidate.

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