Genetic variation and molecular epidemiology of infectious bronchitis virus in southern Thailand

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Abstract

The emergence of new variant strains of avian infectious bronchitis virus (IBV) affects range of symptoms and causes global economic losses in the poultry industry. Here we investigated genetic features of the recent 24 IBV isolates from native, broiler and layer chickens in the south of Thailand between 2008 and 2013. Analysis of the S1 glycoprotein genes revealed that the Thai IBV isolates comprised four distinct genetic groups. Seven IBV variants isolated in 2008 and 2009 were clustered as Thailand THA001 group I. Group II, forming a larger phylogenetic branch, was classified as QX-like and contained 15 variants recovered from 2009-2013. The isolates from group I and group II were genetically distinct from vaccine strains commercially used in Thailand. Pathology varied from respiratory symptoms to intestinal and kidney diseases. Moreover, a single isolate in 2012 from a broiler without any specific clinical outcome, categorized into group III, belonged to Mass-type vaccine strain. Group IV, a virus isolated in 2013 from a layer, was identified to be 4/91. Interestingly, 15 out of 24 isolates in group I and group II were from native chickens associated with a range of pathology. They were raised as village chickens and two of them were domesticated (bantam rooster) without history of vaccination. Our results indicate that the variant lineages flow in the south of Thailand, and are endemic to that area. Potentially native chickens are the main host of IBV and likely able to spread the virus through the chicken population within the area. Housing systems, animal biosecurity and proper vaccination are ultimately required for IBV control.

Keywords: Infectious bronchitis virus, S1 glycoprotein, Phylogenetic analysis, Southern Thailand

Introduction

Infectious bronchitis (IB), caused by gammacoronavirus avian infectious bronchitis virus (IBV), is among the major highly contagious diseases of the poultry industry. IB is frequently involved in upper respiratory disease, nephritis and enteritis. In layers, poor fertility and low egg production commonly occur. Recently, disease appears to extensively affect other tissues e.g. proventriculus (reviewed in de Wit et al., 2011; Cook et al., 2012). Moreover, severity of IB is often caused by super infection and other notable bacterial co-infection (Bakaletz, 1995).

The structural glycoprotein spike (S) of IBV, forming large club-shaped projections, is a dimer or trimer (Cavanagh, 2007). The S protein is proteolytically highly glycosylated class I viral fusion protein (Bosch et al., 2003) and is processed into two non-covalently bound polypeptides S1 and S2 (Stern and Sefton, 1982). Function of the S1 protein is to attach host cell receptor molecules and composes of epitopes and determinants for virus neutralizing antibodies, protective immunity, hemagglutination-inhibiting antibodies and serotype specificity. Thus, S1 gene analysis has been widely used to differentiate IBV genotypes and serotypes (Ignjatovic and Galli, 1994; Ladman et al., 2006; Cavanagh, 2007). It is well known that there are two major forces that drive coronavirus evolution i.e. mutation due to high error rates of the viral RNA polymerase, and recombination. Genetic changes in terms of mutation by nucleotide deletion and insertion in the hypervariable region of the S1 glycoprotein would generate new strains or genotypes of IBV worldwide (Jia et al., 1995; Yu et al., 2001; Jackwood et al., 2012).

The first IBV outbreak in Thailand was reported between 1953 and 1954 (Chindavanig, 1962). Later on, despite several available commercial vaccines, e.g. M41, H120, Ma5, Connecticut, 4/91 and in-house DLD vaccine, which have been widely introduced for years, IBV outbreaks are still continuing and seem to be endemic. The relationship of Thai isolates and vaccine strains as well as foreign variants has recently been studied. It has been shown that common Thai IBV circulation in central region could be clustered as i). an indigenous Thailand THA001 IBV, ii). a unique group IBV, iii). a QX-like IBV and iv). a Massachusetts strain (Pohuang et al., 2009a; Pohuang et al., 2011). In southern Thailand, IBV has been reported and the disease was mainly associated with respiratory distress and kidney disease (Antarasena et al., 1990; Antarasena et al., 2008).

In this study we aim at investigating the IBV situation by collecting the confirmed IBV field isolates from different bird types isolated from different regions in southern Thailand from 2008-2013, differentiating them by sequence analysis and determining their relationships with the vaccine and the reference strains.

Materials and methods

Viruses

Twenty four samples of IBV isolated from different regions in the south of Thailand between 2008 and 2013 were used in this study. The case histories of local isolates were listed in Table 1. Virus isolation and propagation were achieved by inoculating 0.2 ml of 10% (w/v) pooled homogenized suspected tissues into the allantoic cavity of 9-to-11-day old antibody-free embryonated chicken eggs (ECE) which came from healthy flocks screened for key poultry diseases (Guy, 2008). After 48-72 hr inoculation, allantoic fluid and chorioallantoic membrane (CAM) were harvested. Three-to-five blind passages were accomplished to identify the causative virus.

Indirect immunofluorescence

Infected CAMs were used for detecting virus antigens by fluorescent antibody test (FAT). Briefly, 5-µm frozen tissue sections were acetone-fixed for 10 min, followed by incubating slides with nucleocapsid protein-specific mouse monoclonal antibody (1:100 dilution, clone Ch/IBV 48.4; Prionics, The Netherlands) at 37°C for 30 min. After three washing with PBS (pH 7.2-7.4), antibody binding was detected by incubating slides with FITC-conjugated rabbit anti-mouse immunoglobulin (Dako, Denmark) at 37°C for 30 min. Next, the sections were washed in a similar manner and examined by epifluorescence microscopy (Nikon, Tokyo) at 100-200X magnification.

Viral RNA extraction, RT-PCR amplification (diagnostic and phylogenetic)

The allantoic fluids harvested from inoculated ECE possessing IBV positive on CAM by FAT were kept at -80°C until use. Viral genomic RNA was extracted from allantoic fluid using viral RNA extraction kit (Omega bio-tek, USA). A single-step RT-PCR was performed by a commercial kit (Qiagen, USA) according to the manufacturer's protocol to amplify cDNA fragments of the 3'UTR. This gene targets the predicted products of 298 bp to confirm all IBV strains (UTR 1^- 5′-GCTCTAACTCTATACTAGCCTAT-3′ and UTR 2^+ 5′-AAGGAAGATAGGCATGTAGCTT-3′) (Adzhar et al., 1996). In brief, amplification of specific IBV gene was accomplished using the reverse transcription (RT) mixture in a final volume of 50 µl containing 5x QIAGEN® OneStep RT-PCR Buffer, 5x Q-solution, 10 mM dNTP, 2µl QIAGEN® OneStep Enzyme Mix, 10 mM of each forward and reverse primers, and 10 µl of RNA template (200 ng). The mixture was subjected to RT at 50°C for 30 min and heating at 95°C for 15 min. PCR was subsequently achieved by 35 cycles of denaturation at 95°C, 30 sec, annealing at 50°C, 30 sec, extension at 72°C, 60 sec. and a final extension at 72°C, 10 min. The PCR products were analyzed by electrophoresis on 1.5% agarose gel, staining with SYBR® Safe DNA gel stain (Invitrogen) and visualized by an ultraviolet transilluminator.

When indicated, the viral RNA extracts were phylogenetically characterized by using specific S1 glycoprotein gene primer set: XCE 1^+ 5′-CACTGGTAATTTTTCAGATGG-3′ and XCE 3^- 5′-CAGATTGCTTACAACCACC-3′, producing partial S1 gene fragment with an amplicon size of 385 bp (Adzhar et al., 1996; Cavanagh et al., 2002). RT-PCR was done according to the same manner as mentioned above. The amplified RT-PCR products were purified using QIAquick PCR Purification kit (Qiagen, USA). The purified products were submitted for sequencing by commercial service (SolGent ASSA service, Korea).

Sequencing and phylogenetic analysis

The obtained partial S1 nucleotide and deduced amino acid sequences from Thai IBV isolates were assembled, and aligned using a BLAST search via the National Center of Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST/) for screening the relevant isolates. Reference IBV vaccine strains commonly used in Thailand and published foreign IBV variants were also included in the alignment and phylogenetic construction. The multiple sequence alignments and determination of the nucleotide and amino acid identities were performed using Clustal W in the Bioedit software version 7.2.5.0 (www.mbio.ncsu.edu). Aligned sequences were used for phylogenetic analysis using the neighbor-joining method available in the MEGA software version 6.0 (Tamura et al., 2013). The bootstrap values were determined from 1000 replicates of the original data to assign confidence levels to branches.

GenBank accession numbers

The GenBank accession numbers for S1 nucleotide sequences of the reference IBV strains were shown in Table 2.

Results

Village chickens are among the main host of IBV infection displaying varied clinical symptoms

We routinely performed virus isolation and identification from necropsied birds submitted to the laboratory. IBV was causative agent detected by FAT using mAb against nucleocapsid protein on CAM of infected ECE. Subsequently, RT-PCR was achieved to confirm the target gene of IBV. Here we showed that the field IBV isolates disseminated in different geography mostly in central part of southern region. Between 2008 and 2013, the highest prevalence was found in 2010. Fifteen out of 24 positive for IBV were from native chickens. They were raised in different compartments including free-range backyard, housing (confinement), fighting game, and multi-purpose. The clinical signs from these birds were non-specific, compared to broilers and layers, exhibiting mild to moderate illness associated with respiratory, enteric and kidney diseases. They might generally confer to other respiratory and consistent diseases. In addition, symptoms from some cases, however, were unclear, only experiencing depression with low morbidity and mortality. Co-infection or secondary bacterial infection was not found in any diseased birds (Table 1).

Phylogenetic relationships among field IBV isolates can be clustered into four groups undergoing endemic in the south of Thailand

Phylogenetic analysis of 24-purified PCR products revealed that the local isolates could be clustered into four groups. Group I comprised seven isolates correlating to the previously published

THA001. Group II, hosting a larger branch, consisted of 15 isolates. This group belonged to QX-like correlating to the previous isolate reported by Pohuang et al. (2011) while other two single isolate group III and group IV had a closer relationship with Massachusetts-type vaccine and 4/91 strain, respectively (Figure 1). Geographically, the group I viruses were isolated in 2008-2009 disseminating in the southernmost areas and southwestern coast. The isolates in group II were found in 2009-2013, distributing mostly in the central part of the southern region and ranging from the west to the east coast. The single isolate of group III and group IV could be classified in 2012 and 2013, respectively (Figure 2). This indicates that IBV infection is endemic and regularly circulated within this region. Moreover, occasional co-circulation was present in some areas.

The nucleotide and amino acid identity was then identified within and between phylogenetic groups. The isolates in group I were highly related at the nucleotide and amino acid level of 92.2-98% and 89.2-97.5% identity, respectively. The isolates of QX-like group II shared 89.3-97.7% nucleotide identity and 85-95.8% amino acid identity to each other. According to genetic relationship order, despite not sharing the same group, the isolates of group I yielded high identity values close to a Mass-type group III (79.1-84.4% nucleotide identity and 79.3-85.1% amino acid identity). Group II possessed <82.5% nucleotide identity and <80.5% amino acid identity with group IV (4/91). For other comparisons between group I & group II, group I & group IV, and group II & group III, they showed low similarity, less than 77% nucleotide and 75-77% amino acid identities (Table 3).

Finally, we determined phylogenetic relationships of the local isolates with vaccine strains commercially used in Thailand and with other geographic isolates accessible from GenBank. For this, we found that only isolates B/Sk3589/55 and L/Nk3251/56 fell within Mass-type (89.2% amino acid similarity to Ma5 vaccine) and 4/91 group (89.5% amino acid similarity), respectively. None of the other isolates could be grouped with neighboring variants except that they were clustered into two distinct groups already identified previously in the central area of Thailand (Pohuang et al., 2009a; Pohuang et al., 2011). This indicates that the field IBV isolates are commonly disseminating through the country.

Discussion

IBV remains a significant pathogen of commercial broilers, layers and also village or free range birds of not only vaccinated but also unvaccinated flocks. Reasons behind this are due to the flock management system, mismatched vaccination and emerging as well as re-emerging new variant strains. Extensive antigenic variation, caused by genetic mutation in the hypervariable region of S1 glycoprotein, is a feature of IBV and new variants (Jackwood et al., 2012). Moreover, S1 protein is associated with virus-neutralizing epitopes thereby inducing a strain-specific neutralizing antibody (Hofstad, 1981; Cavanagh, 2005; de Wit et al., 2011). In Thailand, commercially available IBV vaccines are mostly based on Mass-type (M41, Ma5, H120 etc.) and variant 4/91 strain basically applied in intensive and alternative poultry farming. Aside from commercial hybrid broilers and layers, limited numbers of backyard chickens, raised for food in most villages in remote areas, are vaccinated. This is, at least in part, why IBVs are still present. Better understanding genetic diversity of the S1 gene of IBV from diverse geography is essential for effective control and prevention of IBV. Selection of matched or combined proper vaccines is an ultimate goal in order to minimize economic loss by this pathogen. For instance, Pohuang et al. (2009b) studied efficacy of available IBV vaccines; Ma5, H120 and in-house vaccine; practically used in Thailand against nephropathogenic Thai IBV. The study demonstrated a successful reduction of mortality rate albeit chickens could exhibit clinical symptoms after vaccination.

IBV has been reported to circulate in southern Thailand (Antarasena et al. 1990). Positive IBV field samples have been therefore continually collected as seed viruses for decades. Antarasena et al. (2008) classified the field isolates into two distinct phylogenetic groups co-circulating in the southern area in 2007. The study demonstrated that group I viruses were associated with respiratory distress closely related (but not sharing the same group) to the nephropathogenic Chinese SH3 strain. Group II, classified as nephropathogenic Thai variant, had a closer relationship to proventriculitis-associated Chinese LDL/97I/97, but yielded a lower degree of amino acid identity. Since then we extended our study in order to gain more data on evolution and epidemiology of the local 24-isolates from 2008-2013. They were genetically conformed into four groups. Major IBV isolates found currently are group I THA001 and group II QX-like, which share the corresponding clusters to previously reported outbreaks in 2007 (Antarasena et al., 2008). A major difference is that pathology and virus tropism become widely ranged not just associated with respiratory disease. When comparing degree of nucleotide and amino acid identity of the recent isolates group I and group II with the reference foreign IBV variants, group I viruses have a relationship with a nephropathogenic Chinese SH3, but share less than 84% amino acid similarity and chickens experience varied pathology. For QX-like group II, the viruses shared not more than 90% amino acid identity with a novel Chinese QX-IBV. Pathology of chickens affected with QX-like IBV infection differs from a previously reported prominent proventriculitis-, severe nephritis- and drop in egg production-associated QX strain (Yudong et al., 1998). In addition to this study, only a single isolate from group III and group IV appeared to be vaccine-derived strains, Ma5 and 4/91, respectively. A Mass-type-derived virus, B/Sk3589/55, was isolated from a 30-day-old broiler exhibiting depression and without vaccination. We consider that infection might be multifactorial. An assumption is that the virus transmitted via neighbor backyard chickens or free range birds next to the farm. The isolate L/Nk3251/56 was recovered from a 240-day-old layer with history of vaccination, showing more specific findings associated with respiratory, enteric disease and low egg production. This indicates that the isolated IBV might be a field challenge which comes from mutation or evolution events of vaccine strains allowing birds to accommodate viruses during vaccination over time.

In central Thailand, which has the highest population of commercial chickens, Pohuang et al. (2009a) demonstrated that two IBV groups were identified and circulated in broilers. Group I THA001 was distinct from IBV isolated in other countries whereas group II was associated with kidney disease from Chinese IBV. Later, Pohuang et al. (2011) reported three IBV groups circulating in central Thailand, isolated from commercial poultry, and associated with respiratory signs including a unique group, a QX-like group and a Massachusetts group. Subsequently, Pohuang and Sasipreeyajan (2012) studied pathogenicity of Thai new variant and QX-like strains, illustrating that both of them were able to induce tracheitis, nephritis and retard growth after 7 days of infection. For our study, the current southern variants, a group I THA001, a QX-like group II and a Mass-type group III share the corresponding groups as reported in central Thailand (Pohuang et al., 2009a; Pohuang et al., 2011). Taken together, it seems like at least four-to-five IBV clusters are currently circulating in Thailand, partly in the central and southern regions. Infection remains endemic possibly due to geographic pattern of animal migration and mechanical transmission. Further study on molecular epidemiology of IBVs in the whole country should be carried out to reveal the whole spectrum of classified IBVs and their origin. It also must be emphasized that the complete S1 gene analysis needs to be clarified to study detailed bioinformatics and recombination events.

Focusing on affected birds, native chickens are considered to be major hosts of IBV since they are raised for various purposes. Vaccination programs in village chickens are likely omitted unless they are pet birds. These birds show a variety of clinical outcomes compared to more specific appearances in broilers and layers. On one hand, it is probably due to susceptibility of native chickens and how they experience any pathogens in the environment, thus allowing them being capable of resisting to pathogens (Otsuki et al., 1990; Nakamura et al., 1991). Furthermore, village birds can get exposed to and spread not only IBV but also other pathogens that exist in their environment. In rural areas, the housing system as free range/backyard, however, is still practical. When infected, birds that are carriers associated with subclinical or without illness can facilitate transmission via aerosol-fecal route, enabling dissemination of viruses to commercial sectors and vice versa via animals, humans, transportation, and materials.

In summary, isolation and typing of the IBV field isolates is necessary, not only for the study of virus evolution and epidemiology, but also for efficacy of modified vaccination programs (Cavanagh et al., 2005). Diversity of IBVs appears to occur and circulate within Thailand. Diseases are associated with a range of clinical manifestations probably due to different types/breeds of birds. Besides, whether different sequences within spike protein among IBV strains are responsible for virus tropism and pathogenicity, they remain to be elucidated. (Callison et al., 2001; Promkuntod et al., 2014). Finally, control and prevention strategy in particular native birds is very important in order to decrease any risk of possible IBV infection. Hence, the following actions should be potentially taken including arranging a compartment system and biosecurity, providing vaccines relevant to the IBV strains into the areas of such outbreak or regular updating an appropriate vaccination program within the affected area.

Acknowledgement

This work was supported by Department of Livestock Development, Ministry of Agriculture and Cooperatives, Thailand (Project 85/2013). The authors would like to acknowledge director of Veterinary Research and Development Center (Southern Region) for research support and staff members of Virology laboratory for excellent technical assistance. We thank Anna Schmitt Nagelbach for proofreading and grammar editing.

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Table 1. IBV strains isolated from flocks in different areas of southern provinces, Thailand

Isolate designation	Accession number	Year of isolation	Geographic origin	Province	Chicken type	Age	IBV vaccination	Clinical signs**
N/Nr755/51	KM583999	2008	Southernmost	Narathiwat	Native*	>1 y	No	D, E
N/Nr1007/51	KM584001	2008	Southernmost	Narathiwat	Native	>180 d	No	D
N/Tr3228/51	KM584004	2008	West coast	Trang	Native	>180 d	No	D, E
B/St3601/51	KM584008	2008	West coast	Satun	Broiler	21 d	No	D, R, E
B/Pn312/52	KM583997	2009	Southernmost	Pattani	Broiler	38 d	No	D
B/Pn610/52	KM583998	2009	Southernmost	Pattani	Broiler	25d	No	D
N/Pn833/52	KM584000	2009	Southernmost	Pattani	Native	90 d	No	D
N/Pl5864/52	KM584016	2009	East coast	Patalung	Native	30 d	No	D, K
N/Yl8320/52	KM584020	2009	Southernmost	Yala	Native	90 d	No	D
B/Kb1916/53	KM584002	2010	West coast	Krabi	Broiler	35 d	No	R
N/Rn3213/53	KM584003	2010	West coast	Ranong	Native	>180 d	No	D, K
N/Sk3512/53	KM584006	2010	East coast	Songkhla	Native	60 d	No	D, E
N/Nk3751/53	KM584009	2010	East coast	Nakhon si thammarat	Native	120 d	No	D
B/Kb3874/53	KM584010	2010	West coast	Krabi	Broiler	28 d	Yes	D, R, E
N/Nk4764/53	KM584012	2010	East coast	Nakhon si thammarat	Native	>180 d	No	D
N/Nk4969/53	KM584013	2010	East coast	Nakhon si thammarat	Native	72 d	No	D
N/Png5143/53	KM584014	2010	West coast	Pang-nga	Native	1 y	No	D
N/Pn5353/53	KM584015	2010	Southernmost	Pattani	Native	60 d	No	E
B/Sk3589/55	KM584007	2012	East coast	Songkhla	Broiler	30 d	No	D
N/Tr4270/55	KM584011	2012	West coast	Trang	Native	90 d	No	D, R, E
L/Nk3251/56	KM584005	2013	East coast	Nakhon si thammarat	Layer	240 d	Yes	D, R, E, RP
L/Nk5933/56	KM584017	2013	East coast	Nakhon si thammarat	Layer	21 d	Yes	D, R
B/Pl7393/56	KM584019	2013	East coast	Patalung	Broiler	38 d	No	D, R
N/Kb6108/56	KM584018	2013	West coast	Krabi	Native	>180 d	No	D

* Native chickens include game fowl and bantam rooster

** Clinical signs; D: depression, R: respiratory, E: enteric, K: kidney, RP: reproductive (egg drop)

Table 2. Infectious bronchitis virus isolates used in sequence comparison and phylogenetic analysis

Strain/Isolate	Tropism	Accession number
M41	Respiratory	M21883
Beaudette	Respiratory	AF151954
4/91	Respiratory	AF093794
Ark99	Respiratory	AF094814
ArkDPI	Respiratory	AF006624
Connecticut	Respiratory	L18990
Gray	Nephropathogenic	L14069
H52	Respiratory	AF352315
H120	Respiratory	M21970
Florida	Respiratory	AF027512
Ma5	Nephropathogenic	AY561713
Australian T	Nephropathogenic	AY775779
China CK/CH/Fujian/Putian3/0910	Nephropathogenic	GU938410
China CK/CH/Guangxi/Luchuan2/0910	Nephropathogenic	GU938404
China Jilin	Nephropathogenic	AY839144
China JX/99/01	Nephropathogenic	AF210735
China Chicken/China/LDL97I/97	Proventriculus	DQ068701
China LS2	Nephropathogenic	AY278246
China CK/CH/LTJ/95I	Nephropathogenic	DQ167151
China LX4	Nephropathogenic	AY338732
China QXIBV	Proventriculus	AF193423
China SH3	Nephropathogenic	DQ069317
China Tx94a	Nephropathogenic	AB274268
Japan JP8443	Respiratory/enteric	AY296745
Malaysia MH5365/95	Nephropathogenic	EU086600
Taiwan A1211	Resp./nephropath./reproductive	AF250006
Taiwan 3385/06	Respiratory	GQ229247
Taiwan Tw97-4	Respiratory/nephropathogenic	AY296742
Thailand THA001	Respiratory	GQ906705
Thailand THA80151	Respiratory	GQ503616

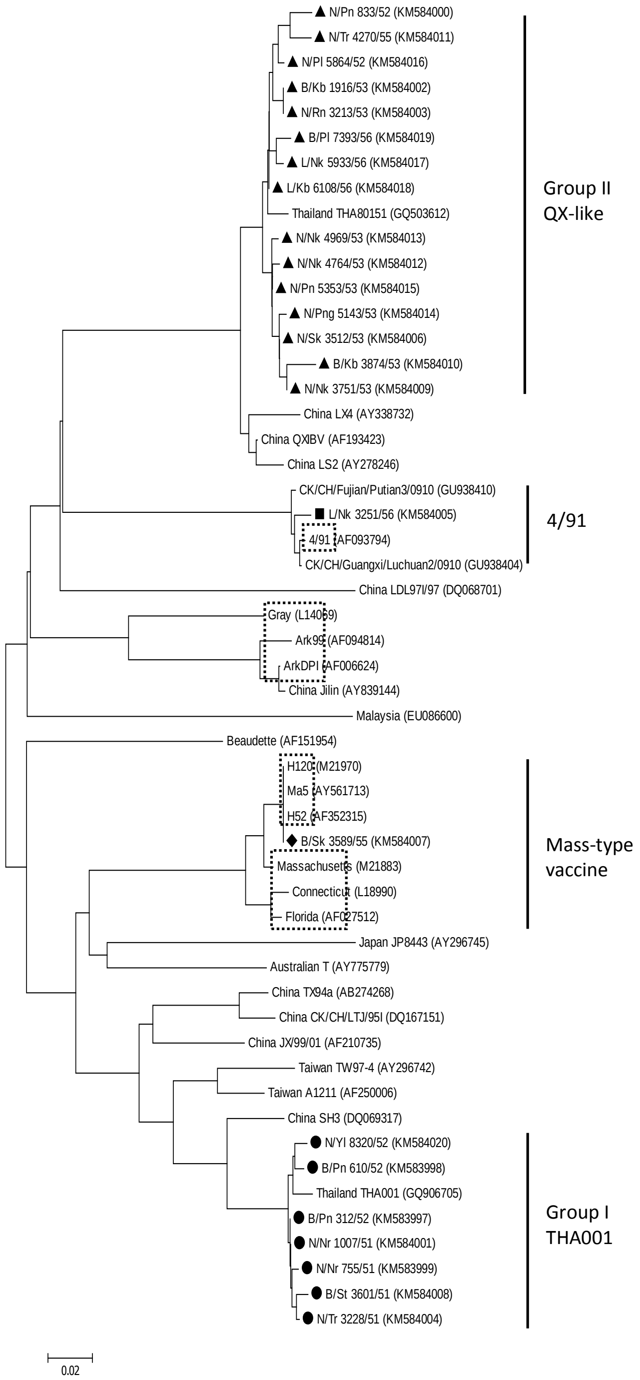

Figure 1. Phylogenetic relationship among vaccine and global IBV strains. Analysis was based on partial S1 nucleotide sequences determined using MEGA 6.0 with the Clustal W method. The isolates sequenced in this study are illustrated with black triangles, black squares, black diamonds and black circles. Open dash squares indicate the IBV vaccine strains. Numbers in the parentheses indicate accession number of the isolates and the reference strains.

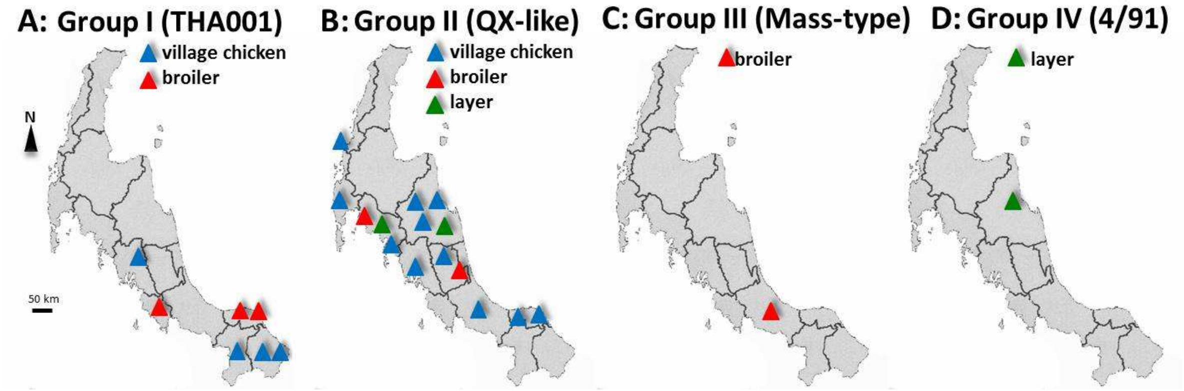

Figure 2. Geographic distribution of IBV field isolates in southern Thailand between 2008 and 2013. (A) Group I, the THA001 isolates were recovered between 2008 and 2009. (B) Group II, QX-like field isolates circulating from 2009 to 2012, (C) Group III Mass-type isolated in 2012, and (D) Group IV 4/91 clustered in 2013.

Table 3. Comparison of the nucleotide and amino acid sequences from the S1 gene of 24 Thai IBV isolates (2008-2013)

Nucleotide identity (%)

IBV isolates	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1 B/Kb 1916/53		75	89.3	74.8	75.5	76.4	71.9	91.3	97.2	97.2	75.6	76.4	96.3	88.3	96.3	96.9	98.3	95.5	73.7	96.3	95.5	95.5	79.5	94.4
2 N/Yl 8320/52	73.5		69.2	97.2	97.2	84	92.2	70.9	75.3	75.6	96.3	97.2	76.1	68.8	75.9	75.9	75.3	75.4	93	74.5	75	75	75.9	75.4
3 B/Kb 3874/53	85.7	67.7		69.3	69.7	70	69.3	95.3	89.6	89.6	69.2	69.5	89.1	89.7	89.1	89.6	89.1	90.5	69.6	87.7	88.7	90.6	75.4	87.8
4 B/Pn 312/52	75.2	95	70.2		96.6	84.4	93.3	70.9	75.1	75.9	97.2	97.4	76.2	69.1	76.2	76.2	74.8	75.6	94.1	74.3	75.1	75.1	75.9	75.9
5 B/Pn 610/52	74.3	94.2	68.5	95		83.5	92.5	71.3	75.9	76.4	96.3	96.6	76.4	69	76.2	76.4	75.3	75.4	93.9	75	75.5	75.5	76.3	75.6
6 B/Sk 3589/55	71	83.4	64.4	85.1	83.4		79.1	71.4	76.1	76.7	83.6	83.2	77	69.7	77	76.7	76.4	75.9	79.8	75	75.6	76.1	73.1	76.2
7 B/St 3601/51	72.7	89.2	70.2	93.3	89.2	79.3		69.6	72	72.5	93.5	93.3	72.8	68.8	72.6	72.5	71.7	72.3	95.2	71.6	72.7	72.9	75.4	71.9
8 N/Nk 3751/53	88.2	70.2	93.1	72.7	71.9	66.9	72.7		91.6	91.6	70.9	71.1	90.7	90.4	91.3	91.9	91	92.7	70.1	89.3	90.7	92.6	76.8	89.5
9 N/Nk 4764/53	94.9	74.3	86.5	76	75.2	71.9	73.5	89		97.7	76.1	76.7	96.6	86.9	96.9	97.4	97.2	96.1	73.8	94.4	95.5	94.9	78.8	94.4
10 N/Nk 4969/53	94.9	74.3	86.5	76	75.2	71.9	73.5	89	96.6		76.7	77.2	96.1	86.9	96.6	97.7	96.6	96.1	74.3	94.9	94.7	94.7	78.8	94.1
11 N/Nr 755/51	75.2	93.3	70.2	97.5	93.3	83.4	93.3	72.7	76	76		98	76.7	68.5	76.7	76.4	75.9	75.4	95	74.5	75.3	75.6	76.1	75.7
12 N/Nr 1007/51	76.6	91.7	72.5	95.8	92.5	81.8	91.7	73.3	77.5	77.5	95		76.7	68.9	77.3	77.2	76.4	76.2	94.1	75.9	75.3	75	76.7	75.4
13 N/Pl 5864/52	92.5	74.3	85	76	76	71.9	73.5	89.1	94.1	94.1	76	77.5		88.3	96.1	96.6	97.2	95	74.1	94.1	96.1	96.3	78.7	95.5
14 N/Pn 833/52	86.5	68.3	83.6	69.4	68.5	64.4	71	86.3	84.8	84.8	70	70	85.8		87	86.7	88	86.7	67.7	88	87.7	89.2	72.4	87
14 N/Pn 5353/53	94.9	74.3	86.5	76	75.2	71.9	75.2	90.7	95.7	94.9	76	76.6	93.3	85.7		97.7	95.8	97.2	74.6	93.9	93.9	94.1	78.8	94.1
16 N/Png5143/53	94.9	75.2	87.3	76.8	76	72.7	74.3	89.9	95.7	95.7	76.8	78.3	93.3	84.8	94.9		96.3	96.6	74.3	94.7	94.9	94.9	79.1	94.1
17 N/Rn 3213/53	96.6	75.2	84.8	76.8	75.2	71.9	73.5	88.2	94.9	93.2	76	76.6	94.1	87.2	94.1	94.1		95.2	73.5	95.2	94.9	96	79.3	94.4
18 N/Sk 3512/53	93.3	74.3	86.6	76	75.2	71.9	74.3	90	94.1	93.3	76	78.3	95	85	95	94.1	93.3		74.1	93	93.6	94.1	78.3	94.7
19 N/Tr 3228/51	75.2	90.9	70.2	95	91.7	80.9	93.3	72.7	75.2	74.3	95	93.3	75.2	68.5	76	76	75.2	75.2		73.7	72.9	73.4	75.6	73
20 N/Tr 4270/55	93.2	72.7	85.7	74.3	73.5	68.5	71.9	85.7	90.7	91.5	74.3	76.6	90	84.8	90.7	91.5	91.5	90	73.5		92.7	93	77.3	91.9
21 B/Pl7393/56	91.6	74.3	85.8	76.8	76	72.7	74.3	88.3	92.5	92.5	76	77.5	94.1	85.8	92.5	92.5	92.5	92.5	73.5	90		97.1	78.9	96.3
22 N/Kb 6108/56	92.5	75.2	86.6	77.6	76.8	73.5	75.2	89.1	93.3	93.3	76.8	79.1	95	86.6	93.3	93.3	93.3	94.1	74.3	90.8	97.5		80.5	95.8
23 L/Nk 3251/56	80	72.7	75	73.5	73.5	67.7	74.3	78.3	80.8	80	73.5	75	81.6	75.8	81.6	81.6	80	83.3	72.7	77.5	80.8	82.5		77.9
24 L/Nk 5933/56	91.7	76	85.9	78.5	77.6	74.3	74.3	87.6	92.5	92.5	77.6	77.6	94.2	84.2	92.5	92.5	92.5	92.5	75.2	90	95.8	95.8	79.3	

Amino acid identity (%)

ความผันแปรทางพันธุกรรมและระบาดวิทยาโมเลกุลของเชื้อไวรัสหลอดลมอักเสบติดต่อในไก่ในภาคใต้ของประเทศไทย

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บทคัดย่อ

เชื้อไวรัสหลอดลมอักเสบติดต่อในไก่ (avian infectious bronchitis virus, IBV) มีความแปรปรวนทางพันธุกรรมในส่วนของโปรตีนโครงสร้างส่วนไกลโคโปรตีน spike (S1) ทำให้เกิดสายพันธุ์ใหม่ๆขึ้นตลอดเวลา ซึ่งสร้างปัญหาให้กับอุตสาหกรรมการเลี้ยงไก่ทั่วโลก ทำการวิเคราะห์ลำดับนิวคลีโอไทด์ในส่วน S1 ยีน ของเชื้อ IBV ที่แยกได้จากไก่พื้นเมือง ไก่กระทง และไก่ไข่ที่เลี้ยงในภาคใต้ของประเทศไทยในช่วงปี พ.ศ. ๒๕๕๑ ถึงปี พ.ศ. ๒๕๕๖ จำนวน ๒๔ ตัวอย่าง พบว่าเชื้อ IBV ที่แยกได้ในภาคใต้สามารถจัดกลุ่มได้เป็น ๔ กลุ่มสายพันธุ์ คือกลุ่มที่ ๑ เป็นกลุ่มที่เกิดเฉพาะในประเทศไทยเรียกว่าสายพันธุ์ THA001 มีจำนวน ๗ ตัวอย่าง แยกได้ในระหว่างปี พ.ศ. ๒๕๕๑ และ พ.ศ. ๒๕๕๒ กลุ่มที่ ๒ จัดอยู่ในกลุ่ม QX-like มีจำนวน ๑๕ ตัวอย่าง แยกได้ตั้งแต่ปี พ.ศ. ๒๕๕๒ ถึง พ.ศ. ๒๕๕๖ เชื้อ IBV ทั้งสองกลุ่มสายพันธุ์มีความแตกต่างทางพันธุกรรมจากสายพันธุ์วัคซีนที่นิยมใช้ในประเทศไทย ส่วนกลุ่มสายพันธุ์ที่ ๓ และกลุ่มสายพันธุ์ที่ ๔ แยกได้จากไก่กระทงและไก่ไข่อย่างละหนึ่งรายซึ่งจัดอยู่ในกลุ่มวัคซีน Massachusetts และสายพันธุ์ ๔/๙๑ ตามลำดับ จากการตรวจแยกเชื้อทั้งหมด ๒๔ ตัวอย่าง พบว่าสามารถแยกเชื้อ IBV ได้จากไก่พื้นเมืองจำนวน ๑๕ ตัวอย่าง ไก่เหล่านี้มักไม่ค่อยแสดงอาการของโรคหลอดลมอักเสบติดต่อที่ชัดเจน อีกทั้งไก่พื้นเมืองมีรูปแบบการเลี้ยงที่แตกต่างกันทั้งแบบปล่อยหลังบ้านและเลี้ยงเพื่อความสวยงามและไม่พบประวัติการทำวัคซีนป้องกันโรคหลอดลมอักเสบติดต่อ จึงมีโอกาสรับเชื้อและแพร่กระจายเชื้อ IBV ได้ง่าย จากการศึกษานี้พบว่าเชื้อ IBV มีการกระจายอยู่ในภาคใต้ของประเทศไทยและพบการเกิดโรคอยู่เสมอ การจัดการรูปแบบการเลี้ยงที่เหมาะสมโดยเฉพาะในไก่พื้นเมือง รวมทั้งการคัดเลือกวัคซีนที่เหมาะสมกับสายพันธุ์ของเชื้อ IBV ที่แยกได้จะทำให้การควบคุมโรคมีประสิทธิภาพมากขึ้น

คำสำคัญ: เชื้อไวรัสหลอดลมอักเสบติดต่อในไก่, ไกลโคโปรตีน spike (S1), phylogenetic analysis, ภาคใต้ของประเทศไทย