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Review article

A review of molecular characterization of infectious bursal disease virus circulating in Thailand according to the newly unified genotypic classification scheme

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Abstract

Infectious bursal disease virus (IBDV) is one of the leading pathogens affecting the Thai poultry industry. However, there remains little information concerning the genetic characteristics of Thai IBDVs available to predict the current field strains of IBDV. This review attempted to summarize the genotypes of IBDV in Thailand based on the newly unified genotypic classification scheme. Phylogenetic analysis was performed by using the nucleotide sequences of Thai IBDV published in the GenBank database. The results demonstrated that two genogroups of segment A, including A1 (classical) and A3 (very virulent), and two genogroups of segment B, including B1 (classical-like) and B3 (early Australian-like), were circulating in Thailand. A combination analysis of both segments A and B suggested that two genotypes of IBDV could be found in Thailand, including genotype A1B1 and A3B3. At present, no variant of IBDV has been reported in Thailand. However, it has been found in several countries that share borders with Thailand. Consequently, the molecular surveillance of IBDV should be performed continuously.

Keywords: Genotype, Infectious bursal disease virus, Phylogenetic analysis, Thailand

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INTRODUCTION

Infectious bursal disease (IBD) is an acute, highly contagious viral disease that threatens poultry production worldwide. It is one of the most significant causes of economic losses in the industry due to high mortality rates, poor feed conversion, and an increased incidence of secondary infections (Sharma et al., 2000). One of the major problems of IBD that threatens chickens is immunosuppression. IBD involves the destruction of B cells in lymphoid organs, resulting in immunosuppression of infected flocks (Lian et al., 2022) as well as increased susceptibility to other infections and diseases (Delmas et al., 2019). In addition, the severe immunosuppressive effect can lead to the failure of other vaccines used by poultry farms (Sparkman et al., 2017).

Infectious bursal disease virus (IBDV) is a member of the family *Birnaviridae* and genus *Avibirnavirus*. Two distinct serotypes of IBDV have been identified by cross-neutralization assays, serotypes 1 and 2, but only serotype 1 is pathogenic to chickens. The genome of IBDV is composed of two linear double-stranded RNA genomic segments (A and B) (Delmas et al., 2019). Segment A is approximately 3.2 kb long and encodes two partially overlapping open reading frames (ORFs). The larger ORF encodes a precursor polyprotein, preVP2-VP4-VP3, that is subsequently processed into mature proteins VP2, VP3, and VP4 (Mundt et al., 1995). VP2 is the major structural protein that contains virus-neutralizing epitopes. VP3 plays a role in virus replication and induces non-neutralizing antibodies. VP4 co-translationally cleaves a precursor polyprotein, preVP2-VP4-VP3, to generate mature proteins (Sanchez and Rodriguez, 1999). The smaller ORF encodes for the non-structural protein, VP5, which is associated with the release of the virus from infected cells (Méndez et al., 2017). Segment B is approximately 2.8 kb long and encodes for the RNA-dependent RNA polymerase (RdRp), VP1, which plays a role in viral replication and transcription (von Einem et al., 2004).

While extensive vaccination strategies have been implemented to control outbreaks of IBD in Thailand, the occurrence of the disease remains commonly found in the country (Charoenvisal, 2021; Junnu and Pohuang, 2023). This highlights the need to characterize the true nature of IBDV in Thailand and establish suitable strategies for vaccination and control. However, information concerning the genetic characteristics of Thai IBDVs available that can predict the current field strains of IBDV in Thailand remains limited. Therefore, this review attempts to summarize the main features of IBDVs, including the genotypes, as well as antigenic and genetic diversity of IBDVs in Thailand.

RECENT SCHEME FOR IBDV GENOTYPE CLASSIFICATION

Traditionally, serotype 1 IBDVs have been classified into 4 different levels of pathogenicity, including classical, very virulent, variant, and attenuated IBDV. However, the traditional descriptive classification method does not adequately describe the IBDV found worldwide (Michel and Jackwood, 2017). Further, it cannot classify the novel variant strains occurring from genetic mutations due to their pathogenicity often being modified (Jackwood et al., 2011). In the last decade, a combination of phylogenetic analysis and sequence similarity of the VP2 gene has been widely used to cluster IBDV isolates into different genotypes. Michel and Jackwood (2017) proposed a clustering system by using the nucleotide sequences of the hypervariable region (HVR) in the VP2 of segment A of serotype 1 only and classified IBDV into seven genogroups (1–7). According to this classification scheme, the traditional pathotypes of classical, variant, and very virulent IBDV are clustered into genogroups G1, G2, and G3, respectively. Other IBDVs, which have genetics distinct from the three major genogroups, are classified separately into

genogroups G4, G5, G6, and G7. However, the virulence of IBDV has been reported to be associated with both genome segments A and B (Jackwood et al., 2011). Segments A and B of IBDV frequently co-evolve, and reassortant viruses have been identified. The pathogenicity of reassortant IBDV is often different from the parental strains. Therefore, the molecular characterization of IBDVs based on both genome segments is highly recommended (WOAH, 2024).

Recently, the classification of IBDVs based on the analysis of both genome segments A and B was proposed. Islam et al. (2021) selected a 366-bp region of segment A (nt 785–1150, aa 219–340) of serotype 1 and categorized it into eight genogroups including A1 (classical), A2 (US antigenic variant), A3 (very virulent), A4 (dIBDV), A5 (atypical Mexican), A6 (atypical Italian), A7 (early Australian) and A8 (Australian variant). Segment B (508 bp region, nt 328–835, aa 73–241) was classified into five genogroups designated as B1 (classical-like), B2 (very virulent-like), B3 (early Australian-like), B4 (Polish & Tanzanian), and B5 (Nigerian). Wang et al. (2021) proposed a similar scheme to classify IBDV genogroups by analysis of 558-bp of segment A (nt 547–1104, aa 183–368), containing the hypervariable region (HVR) of VP2 (aa 206–350), and a 1252-bp of segment B (nt 300–1551, aa 100–517), encompassing the B marker (aa 110–252) of VP1. In this scheme, serotype I of IBDV was classified into eight genogroups of segment A (A1–A8), including A1 (classical), A2 (variant strain), A3 (very virulent), A4 (distinct IBDV (dIBDV) strains isolated in South America), A5 (IBDVs isolated in Mexico), A6 (distinctive IBDV identified in Italy), A7 (Australian strains) and A8 (attenuated strains). Additionally, genogroup A2 was further divided into four lineages of A2a, A2b, A2c, and A2d. Segment B was divided into four genogroups (B1–B4). The classical, attenuated strains, early variant strains, and Algerian reassortant strains were included in genogroup B1. Very virulent strains, HLJ0504-like strains, and transitional-lineage strains in Poland and Finland were classified into genogroups B2, B3, and B4, respectively. Subsequently, Gao et al. (2023) proposed a modified classification scheme due to some controversy regarding the classification of attenuated strains. Gao et al. (2023) use a 391-bp of segment A (nt 631–1021, aa 211–340) and a 528-bp of segment B (nt 217–744, aa 73–248) for phylogenetic analysis of IBDV. In this system, the analysis largely agrees with the classification schemes mentioned by Islam et al. (2021) and Wang et al. (2021), but separates attenuated strains into genogroup A9.

Overall, the new genotyping system has proved to be practical for evaluating the genetic evolution of IBDV. It can identify the novel variant strains occurring from genetic reassortment and recombination events. However, the genotype classification scheme for IBDV should continue to be improved as more information on molecular epidemiology becomes available.

IBDV GENOTYPES IN COUNTRIES NEIGHBORING THAILAND

Recent situations of IBDV outbreaks in the countries neighboring Thailand are important to disease prevention and control strategies because of the possibility of the disease spreading or transferring across close borders. In 2017, atypical IBD causing subclinical symptoms in chickens was a novel threat in China. This newly emerging IBD was caused by a novel variant of IBDV belonging to genotype A2dB1. After that, it became widespread, and sequence numbers have shown a rapid growth trend in China (Zhang et al., 2022). A study of IBDV molecular epidemiology in China from 2019 to 2020 revealed that almost all very virulent IBDVs detected in the country belonged to genotype A3B3. Out of the 86 very virulent IBDV strains in this report, genotypes A3B2 and A3B3 accounted for 34.9% (30/86) and 65.1% (56/86), respectively (Jiang et al., 2021). In December 2020, a naturally reassortant and recombinant IBDV (designated GXB02) was isolated from 20-day-old local Chinese meat-type chickens affected with bursal hemorrhage

and/or atrophy but without mortality. Molecular characterization suggested that its segment A might originate from the attenuated IBDV strain with two recombinant events, and segment B might derive from the classical strains (Feng et al., 2021), indicating that segment reassortment among circulating strains and recombination events have an important role in the evolution of IBDV. According to the new classification scheme by Islam et al. (2021) and Wang et al. (2021), GXB02 is clustered into genotype A1B1.

In Southeast Asia, very virulent IBDV strains are the most prevalent in this region. A report of IBDVs in the Mekong Delta of Vietnam during 2015-2018 showed that all field IBDVs were very virulent strains based on the molecular characterization of the partial VP2 gene (Ngo et al., 2024). However, the viruses in this report are not classified by the newly unified genotypic classification scheme. Another report in Vietnam by Le et al. (2023) found that three A-genotypes, A1, A3, and A7, and two B-genotypes, B1 and B3, were identified among the Vietnamese IBDV isolates. The A3 genotype predominated in Vietnam from 1987 to 2021, and it remained the dominant IBDV genotype from 2016–2021. A recent report of Malaysian IBDV isolated from IBD vaccinated commercial flocks collected between 2017–2019 by Aliyu et al. (2021) showed that there were three genogroups of segment A based on the classification scheme of Michel and Jackwood (2017), including the vaccine strain (genogroup 1), variant strains (genogroup 2), and very virulent strains (genogroup 3) (Aliyu et al., 2021). In segment B, the variant strain was highly identical to variant E and SHG19, whereas very virulent strains were highly similar to previous very virulent IBDV in Malaysia and the UK661 strain. According to the classification system mentioned by Islam et al. (2021) and Wang et al. (2021), Malaysian IBDVs comprise three A-genotypes, A1, A2, and A3, whereas two B-genotypes, B1 and B2, are found. This report is interested in the variant of IBDV circulating in Southeast Asia. Therefore, the possibility of transboundary transmission in this area should be of concern.

IBDV GENOTYPES CIRCULATING IN THAILAND

There have been few publications concerning the genetic characteristics of Thai IBDV. Perhaps only three reports of molecular characterization have been published in international journals. The presence of IBD was first reported in Thailand in 1973 (Suwatanaviroj, 1973). The molecular genotyping of IBDV in Thailand was first described by Jackwood and Sommer-Wagner (2007). The nucleotide sequence of positions 737 to 1479, encompassing the HVR of VP2, was determined for each virus. Among the 8 IBDV isolates in 1997–2001 in this study, 7 isolates were assigned to the very virulent genogroup, and 1 isolate was classified as a non-vvIBDV strain. After that, Charoenvisal (2021) collected the bursa of Fabricius from 16 chicken farms in the eastern and central parts of Thailand between 2017 and 2019 for the detection of IBDVs. The results showed that, of the 12 farms with a positive test, samples from six farms were classical IBDV positive, and samples from the other six farms were very virulent IBDV positive. In that study, IBDV was classified based on the scheme of Michel and Jackwood (2017). The results showed that at least two genogroups of IBDV, including genogroup 1 and genogroup 3, were circulating in Thailand. Recently, we characterized IBDVs isolated in Thailand during 2011–2015 by analysis of both genome segments A and B. The virus was characterized by using the newly unified schemes described by Islam et al. (2021) and Wang et al. (2021). The results demonstrated that there were two groups of IBDV circulating in Thailand. The first group was genotype A3B3 (HLJ0504-like vvIBDV), and the second group was genotype A1B1 (classical virulent IBDV). Interestingly, it was also found that genotype A1B1 had a recombination event in its segment A (Junnu and Pohuang, 2023).

At present, there are only 20 nucleotide sequences of Thai IBDV deposited in the GenBank database, including partial sequences of segment A (11), complete coding sequences of segment A (4), and complete coding sequences of segment

B (5) (Table 1). According to the newly unified genotypic classification schemes of IBDV, the nucleotide sequences of Thai IBDV in the GenBank database were used for the phylogenetic analysis in this report. The phylogenetic tree of a 366-bp region of segment A (nt 785–1150) was generated using a maximum likelihood (ML) model in MEGA software version 11 with bootstrapping 1000 replicates (Tamura et al., 2021). The results showed two genogroups of segment A, including A1 (classical) and A3 (very virulent) (Figure 1). For segment B, only the sequences reported in our previous study are deposited in the GenBank database. Therefore, the tree of segment B was not constructed again in this study. Our previous report showed that two genogroups of segment B, including B1 (classical-like) and B3 (early Australian-like), were found in Thailand. The combination analysis of both segments A and B revealed that two genotypes of IBDV were circulating in Thailand, including genotype A1B1 and A3B3 (Junnu and Pohuang, 2023). Until now, no other variant of IBDVs has been reported in Thailand. Therefore, further research is required to update the genetic characteristics of the IBDV circulating in Thailand.

Table 1 Segments A and B of Thai IBDV published in GenBank database and genotype-specific amino acids of segment A.

Genotype	Strain	Collection date	Accession number	Base pairs	genotype-specific amino acids				
					222	253	256	294	299
Segment A									
A1	Thailand97TH4	1997	DQ916252	658	P	Q	V	I	N
A3	TH1	2001	DQ916245	663	A	Q	I	I	S
A3	TH2	2001	DQ916246	663	A	Q	I	I	S
A3	TH3	2001	DQ916247	663	A	Q	I	I	S
A3	TH4	2001	DQ916248	658	A	Q	I	I	S
A3	TH5	2001	DQ916249	663	A	Q	I	I	S
A3	TH6	2001	DQ916250	663	A	Q	I	I	S
A3	TH7	2001	DQ916251	663	A	Q	I	I	S
A3	SK53	2010	KJ198843	3150	A	Q	I	I	S
A1	KK54	2011	KJ198844	3150	P	Q	V	I	N
A3	KC58	2015	ON737860	3073	A	Q	I	I	S
A3	THCU07	2017	MZ614612	3260	A	Q	I	I	S
A3	Thai 4	2019	MW248905	402	A	Q	I	I	S
A3	M.B.	2019	MW248904	402	A	Q	I	I	S
A1	V217	2019	MW248903	402	P	Q	V	I	N
Segment B									
B3	SK53	2010	KJ198845	2726					
B1	KK54	2011	KJ198846	2726					
B1	CB57	2014	ON692921	2640					
B3	SR58	2015	ON692922	2640					
B3	KC58	2015	ON692923	2640					

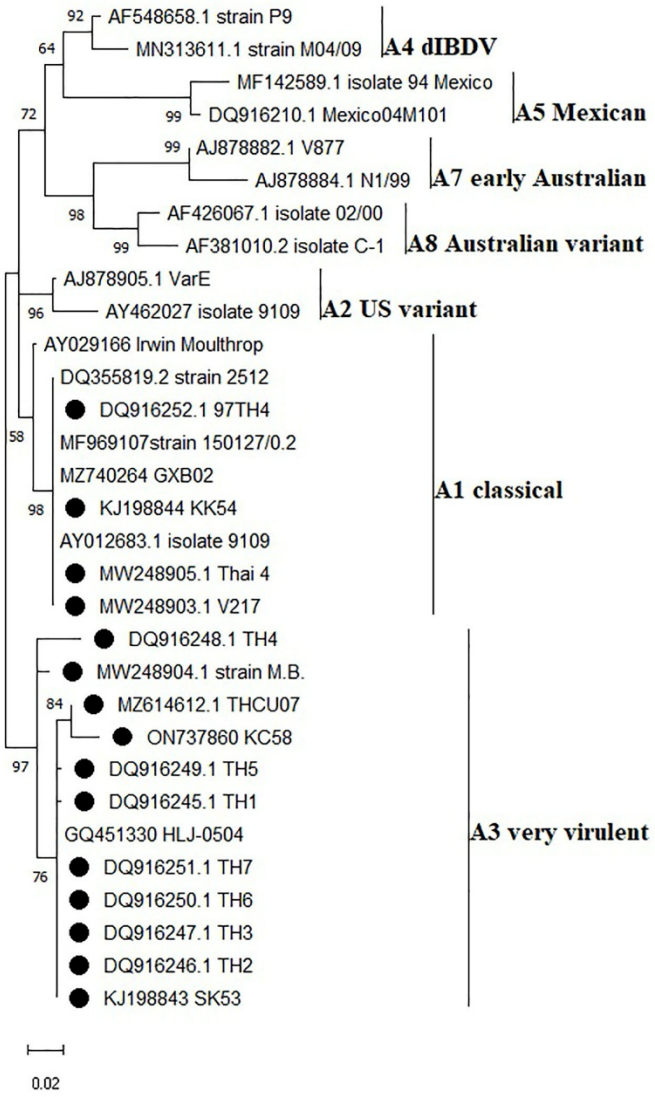

Figure 1 Phylogenetic analysis of the nucleotide sequences of segment A (nt 785–1150) of the representative strains of IBDV in Thailand (filled black circle).

PUTATIVE VIRULENCE DETERMINANTS AND IMMUNODOMINANT EPITOPES

The VP2 protein is considered to be the major host-protective antigen, containing immunodominant epitopes responsible for inducing neutralizing antibodies against IBDV. The hypervariable region of VP2, located in amino acid residues 206 to 350, comprises a conformational neutralizing domain, which is the major immunodominant epitope (Bayliss et al., 1990). Two major hydrophilic domains are contained in the hypervariable region, including major hydrophilic peak A (amino acid residues 212–224) formed in loop P_{BC} and peak B (amino acid residues 312–324) formed in loop P_{HI}. Two additional minor hydrophobic domains, peak A (amino acid residues 248–254) formed in loop $P_{DE,}$ and peak B formed in loop P_{FG} (amino acid residues 279–290) formed in loop P_{HI} are identified within major hydrophilic domains. Antigenic variation of IBDV mainly occurs in the two hydrophilic regions (Vakharia et al., 1994). It has been reported that a single

mutation in the major hydrophilic regions can significantly affect the neutralizing epitope, resulting in the ineffectiveness of available IBD vaccines (Jackwood and Sommer-Wagner, 2011).

Due to the incompleteness and difference in the length of Thai IBDV sequences, the VP2 HVR amino acid residues 214–350 were compared in this study. As shown in Figure 2, the differences between Thai IBDV genogroups A1 and A3 were found at residues 217, 222, 242, 256, 270, and 299. A prominent difference was observed in major hydrophilic peak A, loop $P_{BC,}$ whereas major hydrophilic peak A, minor hydrophobic peak A, and minor hydrophobic peak B appeared to be similar among Thai IBDVs. Analysis of the VP2 HVR amino acid sequences representing the putative virulence determinants of Thai IBDV revealed that genogroup A1 had amino acids 222 (P), 253(Q), 256 (V), 294 (I), and 299 (N) defined for classical virulent IBDV. Genogroup A3 had signature amino acids, 222 (A), 253(Q), 256 (I), 294 (I), and 299 (S), described for very virulent IBDV.

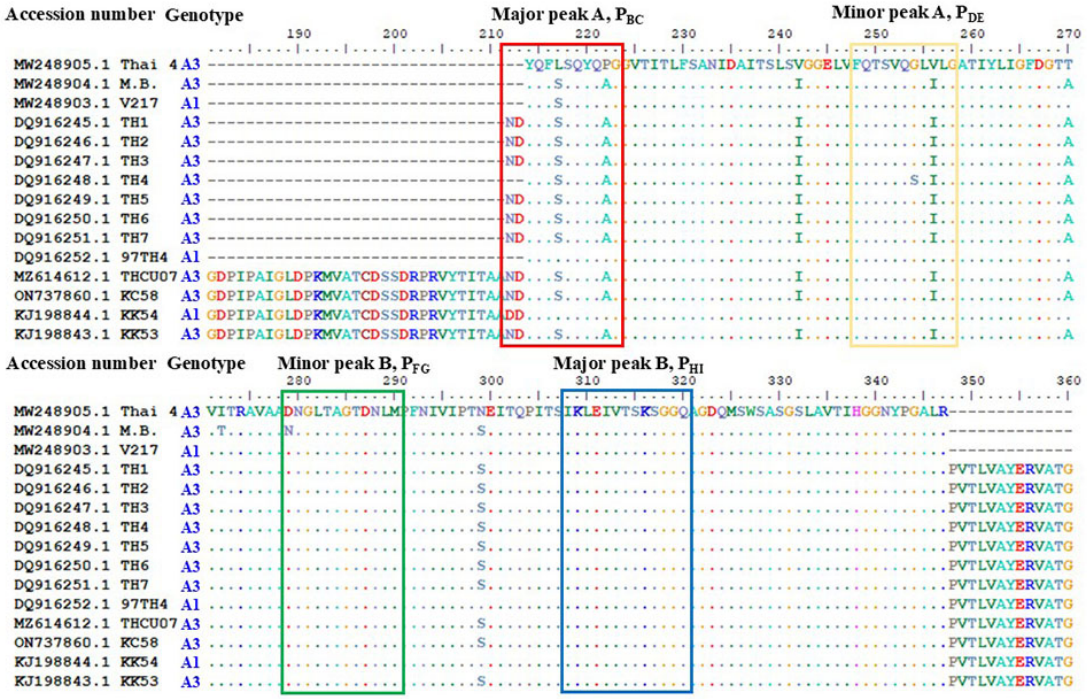

Figure 2 Deduced amino acid sequences of the VP2 hypervariable region of Thai IBDVs from amino acid position 212 to 350. Major hydrophilic peaks and minor hydrophobic peaks are shown in boxes.

CONTROL OF THAI IBD THROUGH VACCINATION

Outbreaks of IBD have primarily been controlled by vaccination. Several types of vaccines are commercially available in Thailand, including attenuated live, inactivated, and immune complex vaccines (Charoenvisal, et al., 2022). Although the efficacy of vaccinations against Thai IBD has been published, few reports have provided information concerning the genetic characterization of a challenging virus. Two commercial live intermediate-plus vaccines of strain W2512 were tested against the Thai vvIBDV isolate CU-1. The protective effects on alleviating body weight loss and lesion score in the bursa of Fabricious were different between them (Chansiripornchai and Sasipreeyajan, 2009). Another study of live vaccine belonging to the classical type of the Moulthrop strain showed that the protection

against challenge with isolate CU-1 appeared to be better than non-vaccinated chickens, though it was not significantly different (Sarachai et al., 2010). However, the genotype of isolate CU-1 in both studies has not been characterized yet. Due to the antigenic divergence among IBDV strains, the antigenic mismatch between the field strains and commercial vaccines may play a role in the efficacy of the vaccine.

Recently, the immune complex IBD vaccine was tested against Thai vvIBDV, which showed 95.77% partial VP2 gene similarity to genotype A3. The results showed that commercial layer chickens vaccinated with an immune complex IBD vaccine had a better survival rate than non-vaccinated chickens. Importantly, 100% protection was found when using vaccination with an immune complex vaccine at 1-day-old, followed by receiving the live vaccine at the appropriate time (Charoenvisal et al., 2022). While the use of vaccines has previously been shown to be effective in controlling Thai IBD, there remains a limitation in the genetic characteristics of the IBDV strains currently in Thailand. Moreover, the emergence of new variant IBDVs in countries neighboring Thailand should be considered when choosing an appropriate vaccine in the future.

CONCLUSION

This review demonstrates that two genogroups of segment A, including A1 and A3, and two genogroups of segment B, including B1 and B3, are circulating in Thailand. A combination analysis of both segments A and B suggests that two genotypes of IBDV can be found in Thailand, including genotypes A1B1 and A3B3. No other variant IBDVs have been reported in Thailand. However, it has been found in several countries that share borders with Thailand or are in the same region. The possibility of the disease spreading or transferring between close borders remains, demonstrating that the molecular surveillance of IBDV should be performed continuously.

AUTHOR CONTRIBUTIONS

Tawatchai Pohuang: Conceptualization (lead); Writing-original draft (lead); Writing-review and editing (equal).
Sucheeva Junnu: Conceptualization (supporting); Writing-original draft (supporting); Writing-review and editing (equal).

CONFLICT OF INTEREST

The authors have declared that there exists no conflict among the authors of this article.

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