Research Article

Molecular Characterization of the Circulating Avian Infectious Bronchitis Viruses in the Mekong Delta Provinces in Vietnam

Nguyen Phuc Khanh[1]*, Tran Ngoc Bich[1], Nguyen Thi Cam Loan[2], Chau Thi Huyen Trang[1], Nguyen Thanh Lam[1], Pham Quynh Yen Thanh[1], Nguyen Thi Thuy Hang[3]

[1]Faculty of Veterinary medicine, College of Agriculture, Can Tho University, Campus II, 3/2 Street, Ninh Kieu District, Can Tho, Vietnam; [2]Faculty of Economy, Agriculture and Processing technology, Vinh Long College, Vinh Long, Vietnam; [3]Faculty of Applied biological sciences, Vinh Long University of Technology education, Vinh Long, Vietnam.

Abstract | Avian infectious bronchitis (IB) is an acute respiratory disease affecting the poultry industry worldwide. The S1 sequences of infectious bronchitis virus (IBV) were used to characterize the genetic diversity of the virus in broiler chickens in Vietnam from 2022 to 2023. Phylogenetic and pairwise sequence analyses classified the detected IBV strains into two groups. Group 1 (IBV IBV-VN-CTU-ST1 and IBV-VN-CTU-VL1) was clustered to lineage GI-1 (Genotype Massachusetts) and shared 97.05 – 98.83% and 94.95 – 97.89% nucleotide and amino acid similarities, respectively. In addition, amino acid sequences of S1 region in both strains differ 33 positions with IBV vaccine strain H120 (KF188436). Meanwhile, IBV detected strains in Group 2 (IBV-VN-CTU-ST2, IBV-VN-CTU-VL2, and IBV-VN-CTU-VL3) were clustered to lineage GI-13 (genotype 4/91) and shared 97.80 – 98.56% nucleotide similarity and 96.42 – 97.89% amino acid similarity. Substitutions were identified at 34 of 547 amino acid positions compared to the 4/91 strain. Moreover, the amino acid sequences of the detected strains in the GI-1 and GI-13 lineages contained HVR1 and HVR-2 regions compared to the vaccine strain. This study illustrates the coexistence of the IBV lineages GI-1 and GI-13 in Vinh Long and Soc Trang provinces (The Mekong Delta provinces), Vietnam.

Keywords | Chicken, Infectious bronchitis virus, S1, Vietnam

Received | March 12, 2025; **Accepted** | April 04, 2025; **Published** | May 17, 2025
***Correspondence** | Nguyen Phuc Khanh, Faculty of Veterinary medicine, College of Agriculture, Can Tho University, Campus II, 3/2 Street, Ninh Kieu District, Can Tho, Vietnam; **Email:** npkhanh@ctu.edu.vn
Citation | Khanh NP, Bich TN, Loan NTC, Trang CTH, Lam NT, Thanh PQY, Hang NTT (2025). Molecular characterization of the circulating avian infectious bronchitis viruses in the mekong delta provinces in Vietnam. Adv. Anim. Vet. Sci. 13(6): 1255-1262.
DOI | https://dx.doi.org/10.17582/journal.aavs/2025/13.6.1255.1262
ISSN (Online) | 2307-8316; **ISSN (Print)** | 2309-3331

INTRODUCTION

Infectious bronchitis virus (IBV) belongs to genus *Gammacoronavirus,* family *Coronaviridae.* It prominently causes infectious bronchitis (IB) in chickens, a serious infectious disease affecting the respiratory, renal, and reproductive systems (Abbas *et al.*, 2021). Weight loss in broilers and poor egg production and quality in laying hens result from IBV infection, which is a negative economic impact of IB (Cavanagh, 2007; Zhong *et al.*, 2016; Zhang *et al.*, 2020; Bo *et al.*, 2022). The primary host of IBVs is chickens (Gallus gallus); however, enteritis in turkeys and renal, and respiratory lesions in pheasants caused by IBV have been described by De Wit (2000) and Cavanagh et al. (2002).

The occurrence of nephropathogenic IBVs and/or secondary bacterial or viral infections might result these losses (Wu *et al*., 2024). Besides, molecular characterization of IBV were reported worldwide such as in Malaysia (Bhuiyan *et al*., 2023), China (Wu *et al*., 2022; Wu *et al*., 2024), Ecuador (Loor-Giler *et al*., 2024), Iraq (Sherzad Raoof *et al*., 2021), Bangladesh (Bhuiyan *et al*., 2019), Costa Rica (Villalobos-Agüero *et al*., 2022), and Europe (Lisowska *et al*., 2017).

The IBV genome is a single-stranded, positive-sense RNA virus approximately 27.6 kb in length. The genome has at least 10 open reading frames (ORFs), which encode numerous proteins essential for virus replication and viral structure. Four structural proteins are encoded from the spike (S), small envelope (E), membrane (M), and nucleocapsid (N) genes (Cavanagh, 2007). The S protein is essential for virus attachment and entry into host cells and contains a virus-neutralizing epitope (Wickramasinghe *et al*., 2014). The S glycoprotein is divided into two subunits such as S1 and S2. S1, located at the N-terminal region of the S protein, is in charge of binding to host cell receptors, while S2 contributes to the membrane fusion of IBV (Wickramasinghe *et al*., 2011). Currently, genotyping based on the S1 region is the most popular within IBV classification (Lisowska *et al*., 2017). It comprised 6 genotypes (GI to GVI) with 32 lineages (Valastro *et al*., 2016). Furthermore, IBV shows antigenic and genetic diversity, leading to the creation of new genotypes, lineages, and variants (Ma *et al*., 2019; Molenaar *et al*., 2020; Bo *et al*., 2022). Normally, a difference of amino acids in the S1 region by 20 to 50% or 10 to 15 amino acids can cause the emergence of diverse IBV serotypes (Jiang *et al*., 2017).

In Vietnam, very few studies had been carried out in the Mekong Delta in southern Vietnam to assess the prevalence and molecular characterization of IBVs. Therefore, the study aims to detect and analyze the molecular characterization of circulating IBV strains in some provinces in the Mekong Delta River, Vietnam.

MATERIALS AND METHODS

Ethical Approval

The proposal of this study was allowed by the Institutional Animal Care and Use Committee of Can Tho University, Vietnam.

Samples Collection

A total of 249 mixed samples (tracheas, kidneys, and lungs) were collected from poultry farms in Vinh Long and Soc Trang provinces in the Mekong Delta River (Figure 5) that reported clinical IBV outbreaks from 2022 to 2023. The collected samples were stored in ice bins on the way to the laboratory; and stored in deep freeze (-70°C).

IBV Screening using RT-PCR

RNA extraction: Total RNA from mixed samples was isolated using a kit TopPURE® Tissue Viral Extraction Kit (ABT, Vietnam) according to the manufacturer's instruction.

cDNA synthesis: cDNA synthesis was performed by using the SensiFAST™ Kit (Bioline, UK) as follows: 10µl total reaction volume consist of 3,5µl of RNase-free water, 4µl of isolated RNA sample, 0,5µl of reverse transcriptase primer, and 2µl of 5x TransAmp Buffer. Then, the mixture was incubated at 25°C for 10 minutes followed by heating at 42°C for 15 minutes, 85°C for 5 minutes, and put on ice for at least 1 minute. Then, cDNA was immediately used for PCR or stored in a deep freezer (-30°C) for further use.

Polymerase chain reaction (PCR): The amplification reaction partial N gene was performed by the MyTaq™ DNA Polymerase Kit (Bioline, UK) based on the manufacturer's instruction. The forward primer (5′-TTTTGGTGATGACAAGATGAA-3′) and reverse primer (5′-CGCATTGTTCCTCTCCTC-3′) targeted an amplicon of 403 bp (Feng *et al*., 2012). PCR reaction was performed as follows: initial denaturation at 95°C for 3min, 35 cycles of degeneration, annealing, and extension (95°C for 15s, 60°C for 15s, 72°C for 30s, respectively), 1 cycle of final extension at 72°C for 3min, and hold at 4°C. Then, PCR products were detected on 1,5% agarose gel electrophoresis and visualized by subsequent UV transillumination.

Reverse transcription – polymerase chain reaction (RT-PCR): The S1 gene was amplified by using RT-PCR. The forward primer (GG(T/C) GC(T/G) TAT GC(A/G) GT(A/T) G(T/A)N AA) and reverse primer (CCA TTT A(A/G)A TA(T/A/G/C) AC(A/G) GAT GT) for targeting the S1 gene were designed using NCBI Primer3 (available on the website: https://www.ncbi.nlm.nih.gov/tools/primer-blast/) and BLAST tools (available on the website: https://blast.ncbi.nlm.nih.gov). Then, primer specificity was evaluated by aligning these primers with IBV reference strains using MEGA 6 software.

Sequencing and Phylogenetic Analysis

PCR products were purified using Mega quick-spin™ (Intro Biotechnology, Korea). Following, they were sequenced by using Sanger sequencing. The genetic relationship of S1 sequence between IBV detected and reference strains retrieved from GenBank NCBI (Table 2) was conducted by using MEGA 6 and BioEdit. Sequences of S1 gene were analyzed by using BioEdit sequence alignment editor. Phylogenetic tree of S1 sequences were constructed with MEGA 6 using the maximum likelihood method with 1,000 bootstrap replicates based on the Tamura-Nei parameter model.

Table 1: Nucleotide and amino acid similarities (percent of identity) of S1 gene between detected IBV strains and IBV reference strains from different genotypes.

Amino acid (%)																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1		77.05	76.63	95.37	77.26	59.58	59.58	77.68	76.42	96.00	76.84	58.74	79.58	95.58	65.05	53.05	94.95	55.58
2	78.59		97.68	78.95	96.63	61.68	61.47	80.42	97.05	78.74	97.26	58.53	81.68	78.74	66.95	52.00	78.53	52.63
3	78.45	98.28		78.53	97.26	61.47	61.26	79.37	96.42	78.32	96.63	58.11	80.63	78.32	66.95	52.21	78.11	52.63
4	97.32	79.27	79.14		78.95	60.84	60.84	79.58	78.32	97.89	78.74	60.00	81.68	97.68	66.53	54.74	96.84	57.26
5	78.38	98.35	98.42	79.14		61.26	61.05	80.21	97.47	78.95	97.89	57.68	81.47	78.74	67.37	52.42	78.74	53.05
6	66.09	66.51	66.58	66.58	66.23		99.16	60.42	61.26	60.84	61.47	68.63	63.37	60.63	59.58	52.63	60.63	52.63
7	66.37	66.44	66.51	66.85	66.16	98.76		60.42	61.05	60.84	61.26	68.63	63.16	60.63	59.37	52.42	60.63	52.42
8	78.52	80.30	79.62	78.86	79.82	65.20	65.48		79.79	79.16	80.00	58.74	79.37	78.74	66.32	53.47	78.74	55.58
9	78.59	97.80	97.87	79.20	98.42	66.09	66.03	79.75		78.11	99.58	58.11	81.68	78.11	67.58	51.79	77.89	52.63
10	97.39	79.20	78.93	98.83	79.07	66.23	66.51	78.59	79.14		78.53	59.79	81.47	99.37	66.32	54.74	98.95	57.47
11	78.65	97.87	97.94	79.27	98.56	66.16	66.09	79.75	99.86	79.20		58.32	82.11	78.53	67.79	51.79	78.32	52.84
12	65.13	64.72	64.65	65.55	64.52	69.05	68.98	63.76	64.72	65.55	64.79		60.00	59.79	60.42	51.79	59.58	52.84
13	79.00	80.85	80.58	79.20	80.16	66.92	66.99	77.90	80.37	79.48	80.51	64.86		81.26	69.47	55.37	80.84	56.21
14	97.32	79.34	79.07	98.76	79.20	66.37	66.64	78.45	79.27	99.79	79.34	65.55	79.41		66.32	54.74	98.74	57.26
15	68.29	69.46	68.91	68.43	69.18	65.41	65.34	67.60	69.11	68.50	69.11	64.38	68.57	68.50		52.00	66.53	52.42
16	62.18	61.22	61.02	62.25	60.54	59.64	59.78	61.84	60.88	62.59	60.88	59.16	61.98	62.53	59.30		54.95	53.05
17	97.05	79.27	79.00	98.49	79.14	66.23	66.51	78.45	79.20	99.66	79.27	65.41	79.34	99.59	68.57	62.59		57.26
18	61.84	60.67	60.26	62.59	60.12	59.23	59.23	61.63	60.26	62.05	60.33	58.54	60.47	62.11	59.09	63.90	62.05	
Nucleotide (%)																		

(1) IBV-VN-CTU-VL1; **(2)** IBV-VN-CTU-VL2; **(3)** IBV-VN-CTU-VL3; **(4)** IBV-VN-CTU-ST1; **(5)** IBV-VN-CTU-ST2; **(6)** I0221/17_(MK217372); **(7)** VNUA11_(KY992865); **(8)** gammaCoV/Ck/Italy/I2022/13_(KP780179); **(9)** 4/91_vaccine_(KF377577); **(10)** H120_(KF188436); **(11)** 4/91(UK)_(JN192154); **(12)** N1/08_(JN176213); **(13)** ck/CH/LDL/091022_(HM194640); **(14)** H120_(FJ807652); **(15)** N5/03_(DQ059619); **(16)** PA/1220/98_(AY789942); **(17)** H52_(AF352315); **(18)** 98-07484_(AF288467).

RESULTS AND DISCUSSION

Infectious bronchitis (IB) is a serious disease affecting the global chicken meat and egg production**.** In Northern Vietnam, Roan *et al*. (2023) successfully analyzed the whole genome of IBV. However, no studies about the S1 region have been reported in the Southern region. The S1 glycoprotein contains neutralizing epitopes capable of generating neutralizing antibodies and is associated with serological evolutionary processes, phenotypic changes, and genetic variety. In the present study, the result revealed that S1 region of IBV strains exhibited varying nucleotide lengths, encompassing a range of mutations. It has been reported that serological differences in IBV associated with variants in the hypervariable region (HVR) of the S1 region, and there was a partial genotypic division based on the HVR (Cavanagh *et al*., 1988; Moore *et al*., 1997; Cavanagh, 2007).

In this study, the presence of IBVs has been confirmed by RT-PCR targeting the partial N gene. The nucleotide sequences of the S1 region from 5 detected IBV strains and 36 reference strains were used to determine the genetic relationship between these IBV strains. The phylogenetic analysis employing the Maximum Likelihood method showed that the 5 detected IBV strains from the Soc Trang and Vinh Long provinces were grouped as genotype I. Among those detected IBV strains, two IBV strains in Group 1 (IBV-VN-CTU-ST1 and IBV-VN-CTU-VL1) clustered to the same group with H120 (KF188436), H120 (FJ807652), and H52 (AF352315) belonging to lineage GI-1, and shared 97.05% – 98.83% nucleotide similarity and 94.95% – 97.89% amino acid similarity (Figure 1 and Table 1). Meanwhile, three IBV strains in Group 2(IBV-VN-CTU-ST2, IBV-VN-CTU-VL2, and IBV-VN-CTU-VL3) formed a cluster with the 4/91 vaccine strains (KF377577) and 4/91 (UK) (JN192154) belonging to lineage GI-13, and shared a high nucleotide similarity (97.80% – 98.56%) and amino acid similarity (96.42% – 97.89%) (Figure 1 and Table 1). On the other hand, the nucleotide and amino acid similarities between the detected IBV strains and those strains in other lineages including GII-1 [PA/1220/98 (AY789942)], GIII-1 [N1/08 (JN176213)], GIV-1 [98-07484 (AF288467)],

GV-1 [N5/03 (DQ059619)], and GVI-1 [VNUA11 (KY992865) and I0221/17 (MK217372)] were relatively low with 60.26% – 69.46% and 52.00% – 67.37%, respectively (Table 1). Based on the results, the IBV strains circulating in commercial chicken farms were classified into two lineages: GI-1 and GI-13. They were the two lineages commonly present worldwide, alongside lineages GI-16 and GI-19 (Valastro *et al*., 2016).

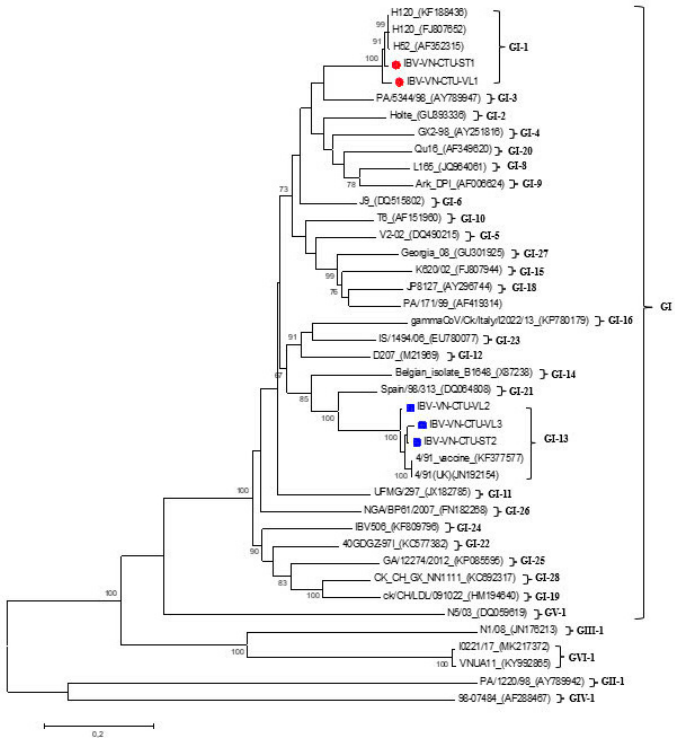

Figure 1: Phylogenetic relationships of the detected IBV strains and published IBV strains based on S1 nucleotide sequences determined using MEGA 6 with the Clustal W method.

The results of this study were in line with a previous report by Valastro *et al*. (2016), which showed the nucleotide and amino acid sequence similarity within the identical genotypes of 87% and 86%, respectively. Therefore, there was a circulation of IBV-VN-CTU-ST1 and IBV-VN-CTU-VL1 belonging to the GI-1 lineage; and IBV-VN-CTU-ST2, IBV-VN-CTU-VL2, and IBV-VN-CTU-VL3 belong to the GI-13 lineage. These five strains isolated from chickens exhibiting respiratory and renal symptoms were characterized. The prevalence of these symptoms has been supported by reports from Bhuiyan *et al*. (2019), Lian *et al*. (2021) and Khue *et al*. (2023).

The S1 region comprises a receptor-binding domain (RBD) (positions 19 – 272); along with 3 hypervariable regions: HVR-1 (positions 38 – 67), HVR-2 (positions 91 – 141), and HVR-3 (positions 274 – 387) (Valastro *et al*., 2016; Zhang *et al*., 2020). In addition, just a change of three amino acids within the RBD can alter cell tropism. Besides, these changes that occur within the HVR regions can impact the generation of neutralizing antibodies (Leyson *et al*., 2016). In the present study, there was a distinct differentiation in amino acid sequences of the S1 region among five detected IBV sequences (Figure 2). In particular, two strains in Group 1 (IBV-VN-CTU-VL1 and IBV-VN-CTU-ST1) showed a loss of one amino acid at position 12 and a few substitution mutations compared to other strains in Group 2 (IBV-VN-CTU-ST2, IBV-VN-CTU-VL2, and IBV-VN-CTU-VL3). In addition, IBV strains in Group 2 shared a high similarity among themselves and showed considerable differences from IBVs in Group 1 (Figure 2). This result suggests that IBV-VN-CTU-ST2, IBV-VN-CTU-VL2, and IBV-VN-CTU-VL3 might have genetic similarities. Similarly, IBV-VN-CTU-VL1 and IBV-VN-CTU-ST1 are genetically related. Besides, IBV strains in Group 1 and Group 2 exhibited genetic similarity to the GI-1 and GI-13 lineages, respectively.

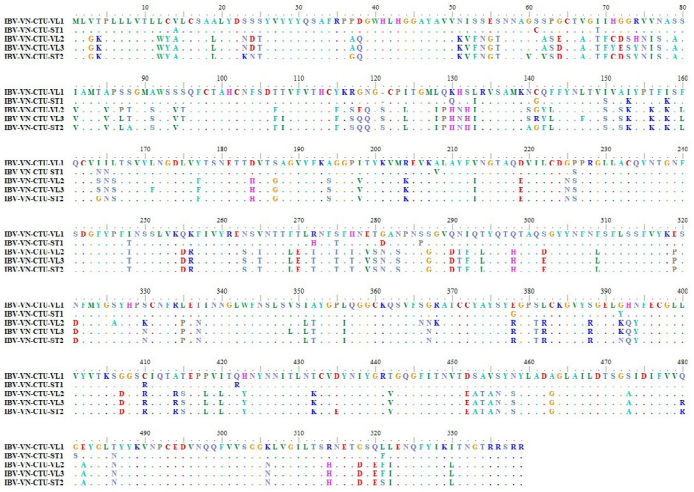

Figure 2: Amino acid sequence analysis of the S1 region between detected IBV strains.

Amino acid sequences of the S1 region between IBV strains in Group 1, Group 2, and vaccine strains [H120 (KF188436) and 4/91(JN192154)] differ in 33 and 34 positions, respectively. This can be caused by the use of live attenuated vaccines, leading to the virus's conditional persistence in the environment. In addition, the insertion and substitution mutations at amino acid positions in the HVR-1 and HVR-2 regions were recorded in the detected strains. These mutations might lead to changes in antigenic structure, resulting in antibody evasion and decreased neutralizing antibody efficacy. In addition, these changes can affect the ability of the virus to stimulate the immune response, allowing it to adapt to immune pressure from the host and vaccines, thereby increasing the likelihood of new variant emergence. According to Smati *et al*., (2002), the IBV outbreaks in vaccinated chickens arise commonly, possibly due to inappropriate vaccination or quick emergence of new IBV variants.

Table 2: GenBank accession numbers of detected and IBV reference strains.

No.	Accession No.	Strain	Genotype	Country	Year
1	PV102041	IBV-VN-CTU-VL1	-	Vietnam	2022
2	PV102042	IBV-VN-CTU-VL2	-	Vietnam	2023
3	PV102043	IBV-VN-CTU-VL3	-	Vietnam	2023
4	PV102044	IBV-VN-CTU-ST1	-	Vietnam	2023
5	PV102045	IBV-VN-CTU-ST2	-	Vietnam	2023
6	AF352315	H52	GI-1	China	2005
7	FJ807652	H120	GI-1	China	2009
8	KF188436	H120	GI-1	India	2015
9	GU393336	Holte	GI-2	USA	2011
10	AY789947	PA/5344/98	GI-3	USA	2016
11	AY251816	GX2-98	GI-4	China	2016
12	DQ490215	V2-02	GI-5	Australia	2008
13	DQ515802	J9	GI-6	China	2016
14	JQ964061	L165	GI-8	USA	2013
15	AF006624	Ark_DPI	GI-9	USA	2016
16	AF151960	T6	GI-10	New Zealand	2016
17	JX182785	UFMG/297	GI-11	Brazil	2012
18	M21969	D207	GI-12	Netherlands	1993
19	JN192154	4/91(UK)	GI-13	UK	2016
20	KF377577	4/91	GI-13	China	2013
21	X87238	Belgian_isolate_B1648	GI-14	UK	1996
22	FJ807944	K620/02	GI-15	Korea	2016
23	KP780179	gammaCoV/Ck/Italy/I2022/13	GI-16	Italy	2015
24	AF419314	PA/171/99	GI-17	USA	2016
25	AY296744	JP8127	GI-18	Taiwan	2016
26	HM194640	ck/CH/LDL/091022	GI-19	China	2016
27	AF349620	Qu16	GI-20	Canada	2016
28	DQ064808	Spain/98/313	GI-21	Spain	2008
29	KC577382	40GDGZ-97I	GI-22	China	2013
30	EU780077	IS/1494/06	GI-23	Israel	2016
31	KF809796	IBV506	GI-24	India	2014
32	KP085595	GA/12274/2012	GI-25	USA	2016
33	FN182268	NGA/BP61/2007	GI-26	USA	2016
34	GU301925	Georgia_08	GI-27	USA	2016
35	KC692317	CK_CH_GX_NN1111	GI-28	China	2015
36	AY789942	PA/1220/98	GII-1	USA	2016
37	JN176213	N1/08	GIII-1	Australia	2012
38	AF288467	98-07484	GIV-1	USA	2016
39	DQ059619	N5/03	GV-1	Australia	2016
40	KY992865	VNUA11	GVI-1	Vietnam	2018
41	MK217372	I0221/17	GVI-1	China	2019

Moreover, the five detected IBV sequences exhibited numerous substitution and insertion mutations compared to the VNUA11 (KY992865) (Figure 3 and Figure 4). Although the VNUA11 (KY992865) strain was isolated in Vietnam, the similarities in nucleotide and amino acid of S1 sequences between this strain and the detected strains were very low. One possible explanation for this discrepancy is that the VNUA11 strain was obtained from lay-

ing hens, different geographical region (Hai Phong), and pathological conditions. Cavanagh (2003) revealed that a 5% difference in the S1 gene sequence would result in poor cross-protection, which would certainly increase the possibility of IBV infection, even in vaccinated chickens. Furthermore, vaccination practices are associated with the evolution of the virus (Franzo *et al*., 2019). Two main aspects explain the genetic variability of IBV. Firstly, high mutation (substitution, deletion, and insertion) rates cause the errors of the RNA-dependent RNA polymerase (RDRP) during viral genome replication (Mahmood *et al*., 2011; Feng *et al*., 2017). Secondly, template switching mechanism in IBV replication might lead to a high incidence of genetic recombination (Cavanagh *et al*., 2005). This generates genetic variations of IBVs that contribute to their evolution.

Figure 3: Amino acid sequence analysis of the S1 region between IBV-VN-CTU-ST1, IBV-VN-CTU-VL1 and IBV vaccine strain.

Figure 4: Amino acid sequence analysis of the S1 region between IBV-VN-CTU-ST2, IBV-VN-CTU-VL2, IBV-VN-CTU-VL3 and IBV vaccine strain.

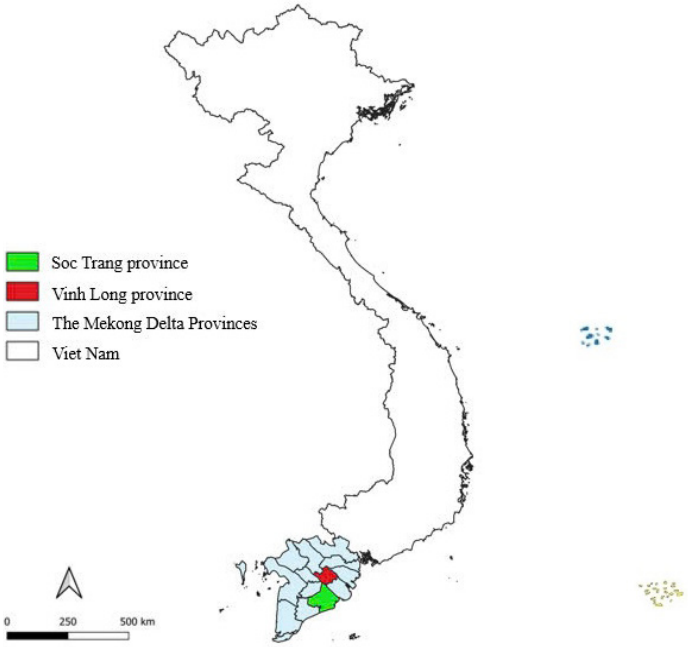

Figure 5: The Mekong Delta map shows the areas where samples were collected. Samples were collected from 2 provinces consisting of Soc Trang (9.6025° N, 105.9739° E) and Vinh Long (10.2396° N, 105.9572° E).

CONCLUSIONS AND RECOMMENDATIONS

The five detected strains identified in this study consisting of IBV-VN-CTU-ST1 and IBV-VN-CTU-VL1 belong to the GI-1 lineage (Massachusetts). Meanwhile, IBV-VN-CTU-ST2, IBV-VN-CTU-VL2, and IBV-VN-CTU-VL3 belong to the GI-13 lineage (4/91); and Lineages GI-1 and GI-13 are recognized as common pathogenic lineages in Vinh Long and Soc Trang provinces (The Mekong Delta provinces).

ACKNOWLEDGMENTS

This study was incompletely supported by a fund in part by the Can Tho University Improvement Project VN14-P6, supported by a Japanese ODA loan.

NOVELTY STATEMENT

The novelty in the present study is the identification of circulation of the IBV lineages GI-1 (Massachusetts) and GI-13 (4/91) in some provinces in the Mekong Delta, Vietnam. This contributes to further studies on vaccine development for the control of IBV in Vietnam.

AUTHOR'S CONTRIBUTIONS

Nguyen Phuc Khanh, Tran Ngoc Bich, and Nguyen Thi

Cam Loan contributed to the study design; Pham Huynh Yen Thanh, Nguyen Thi Thuy Hang conducted the data collection and analysis. Nguyen Phuc Khanh, Chau Thi Huyen Trang, Nguyen Thanh Lam, and Pham Huynh Yen Thanh prepared the manuscript. All authors have read and approved the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

Abbas G, Zhang Y, Sun X, Chen H, Ren Y, Wang X, Ahmad MZ, Huang X, Li G (2021). Molecular Characterization of Infectious Bronchitis Virus Strain HH06 Isolated in a Poultry Farm in Northeastern China. Front. Vet. Sci., 8:794228. https://doi.org/10.3389/fvets.2021.794228

Bhuiyan MSA, Sarker S, Amin Z, Rodrigues KF, Bakar A, Saallah S, Md Shaarani S, Siddiquee S (2023). Seroprevalence and molecular characterisation of infectious bronchitis virus (IBV) in broiler farms in Sabah, Malaysia. Vet. Med. Sci., 10(2). https://doi.org/10.1002/vms3.1153

Bhuiyan ZA, Ali MZ, Moula MM, Giasuddin M, Khan ZUM (2019). Prevalence and molecular characterization of infectious bronchitis virus isolated from chicken in Bangladesh. Vet. World, 12(6): 909-915. https://doi.org/10.14202/vetworld.2019.909-915

Bo Z, Chen S, Zhang C, Guo M, Cao Y, Zhang X, Wu Y (2022). Pathogenicity evaluation of GVI-1 lineage infectious bronchitis virus and its long-term effects on reproductive system development in SPF hens. Front. Microbiol., 13:1049287. https://doi.org/10.3389/fmicb.2022.1049287

Cavanagh D, Davis PJ, Mockett AP (1988). Amino acids within hypervariable region 1 of avian coronavirus IBV (Massachusetts serotype) spike glycoprotein are associated with neutralization epitopes. Virus Res., 11(2): 141–150. https://doi.org/10.1016/0168-1702(88)90039-1

Cavanagh D, Mawditt K, Welchman DdeB, Britton P, Gough RE (2002). Coronaviruses from pheasants (*Phasianus colchicus*) are genetically closely related to coronaviruses of domestic fowl (infectious bronchitis virus) and turkeys. Avian Pathol., 31(1): 81–93. https://doi.org/10.1080/03079450120106651

Cavanagh D (2003). Severe acute respiratory syndrome vaccine development: Experiences of vaccination against avian infectious bronchitis coronavirus. Avian Pathol., 32(6): 567–582. https://doi.org/10.1080/0307945031000162198

Cavanagh D, Picault JP, Gough RE, Hess M, Mawditt K, Britton P (2005). Variation in the spike protein of the 793/B type of infectious bronchitis virus, in the field and during alternate passage in chickens and embryonated eggs. Avian Pathol., 34(1): 20–25. https://doi.org/10.1080/03079450400025414

Cavanagh D (2007). Coronavirus avian infectious bronchitis virus. Vet. Res., 38(2): 281–297. https://doi.org/10.1051/vetres:2006055

De Wit JJ (2000). Detection of infectious bronchitis virus. Avian Pathol., 29(2): 71-93. https://doi.org/10.1080/03079450094108

Feng J, Hu Y, Ma Z, Yu Q, Zhao J, Liu X, Zhang G (2012). Virulent avian infectious bronchitis virus, People's Republic of China. Emerg. Infect. Dis., 18(12): 1994-2001. https://doi.org/10.3201/eid1812.120552

Feng K, Wang F, Xue Y, Zhou Q, Chen F, Bi Y, Xie Q (2017). Epidemiology and characterization of avian infectious bronchitis virus strains circulating in southern China during the period from 2013–2015. Sci. Rep., 7(1):6576. https://doi.org/10.1038/s41598-017-06987-2

Franzo G, Legnardi M, Tucciarone CM, Drigo M, Martini M, Cecchinato M (2019). Evolution of infectious bronchitis virus in the field after homologous vaccination introduction. Vet. Res., 50(1):92. https://doi.org/10.1186/s13567-019-0713-4

Jiang L, Zhao W, Han Z, Chen Y, Zhao Y, Sun J, Li H, Shao Y, Liu L, Liu S (2017). Genome characterization, antigenicity and pathogenicity of a novel infectious bronchitis virus type isolated from south China. Infect. Genet. Evol., 54: 437–446. https://doi.org/10.1016/j.meegid.2017.08.006

Khue NT, Duc LM, Hien NTT, Huong DTT (2023). Whole genome sequencing analysis of avian infectious bronchitis virus isolated in Hung Yen province in 2021. Vietnam J. Biotechnol., 21(4): 645-654. https://doi.org/10.15625/1811-4989/19002

Leyson CLM, Jordan BJ, Jackwood MW (2016). Insights from molecular structure predictions of the infectious bronchitis virus S1 spike glycoprotein. Infect. Genet. Evol., 46: 124–129.

Lian J, Wang Z, Xu Z, Chen T, Shao G, Zhang X, Qin J, Xie Q, Lin W (2021). Distribution and molecular characterization of avian infectious bronchitis virus in southern China. Poult. Sci., 100(7):101169. https://doi.org/10.1016/j.psj.2021.101169

Lisowska A, Sajewicz-Krukowska J, Fusaro A, Pikula A, Domanska-Blicharz K (2017). First characterization of a Middle-East GI-23 lineage (Var2-like) of infectious bronchitis virus in Europe. Virus Res., 242: 43–48. https://doi.org/10.1016/j.virusres.2017.09.010

Loor-Giler A, Muslin C, Santander-Parra S, Coello D, De la Torre D, Abad H, Nuñez L (2024). Simultaneous detection of infectious bronchitis virus and avian metapneumovirus genotypes A, B, and C by multiplex RT-qPCR assay in chicken tracheal samples in Ecuador. Front. Vet. Sci., 11:1387172. https://doi.org/10.3389/fvets.2024.1387172

Ma T, Xu L, Ren M, Shen J, Han Z, Sun J, Zhao Y, Liu S (2019). Novel genotype of infectious bronchitis virus isolated in China. Vet. Microbiol., 230: 178–186. https://doi.org/10.1016/j.vetmic.2019.01.020

Mahmood ZH, Sleman RR, Uthman AU (2011). Isolation and molecular characterization of Sul/01/09 avian infectious bronchitis virus, indicates the emergence of a new genotype in the Middle East. Vet. Microbiol., 150(1-2): 21–27. https://doi.org/10.1016/j.vetmic.2010.12.015

Molenaar RJ, Dijkman R, de Wit JJ (2020). Characterization of infectious bronchitis virus D181, a new serotype (GII-2). Avian Pathol., 49(3): 243–250. https://doi.org/10.1080/03079457.2020.1713987

Moore KM, Jackwood MW, Hilt DA (1997). Identification of amino acids involved in a serotype and neutralization specific epitope within the s1 subunit of avian infectious bronchitis virus. Arch. Virol., 142(11): 2249–2256.

Roan DT, Khue NT, Minh Duc L, Thu Hien NT, Thanh Hoa L, Hue LT, Kim Xuyen LT, Thanh Huong DT (2023). Whole genome sequencing analysis of avian infectious bronchitis virus isolated in Hung Yen province in 2021. Vietnam J. Biotechnol., 21(4): 645–654. https://doi.

org/10.15625/1811-4989/19002

Sherzad Raoof H, Baba Sheikh MO, Sulaiman RR (2021). Detection and molecular characterization of infectious bronchitis virus from recent outbreaks in broiler flocks in Sulaimani Governorate. Vet. Res. Forum, 12(1): 117-120. https://doi.org/10.30466/vrf.2018.94774.2281

Smati R, Silim A, Guertin C, Henrichon M, Marandi M, Arella M, Mersouki A (2002). Molecular characterization of three new avian infectious bronchitis virus (IBV) strains isolated in Quebec. Virus Genes, 25: 85–93. https://doi.org/10.1023/a:1020178326531

Valastro V, Holmes EC, Britton P, Fusaro A, Jackwood MW, Cattoli G, Monne I (2016). S1 gene-based phylogeny of infectious bronchitis virus: An attempt to harmonize virus classification. Infect. Genet. Evol., 39: 349-364. https://doi.org/10.1016/j.meegid.2016.02.015

Villalobos-Agüero RA, León B, Zamora-Sanabria R, Karkashian-Córdoba J (2022). Molecular characterization of the S1 gene in GI-17 and GI-13 type isolates of avian infectious bronchitis virus (IBV) in Costa Rica, from 2016 to 2019. Virus Dis., 33(1): 84-95. https://doi.org/10.1007/s13337-022-00762-2

Wickramasinghe IN, van Beurden SJ, Weerts EA, Verheije MH (2014). The avian coronavirus spike protein. Virus Res., 194: 37–48. https://doi.org/10.1016/j.virusres.2014.10.009

Wickramasinghe IN, de Vries RP, Gröne A, de Haan CA, Verheije MH (2011). Binding of avian coronavirus spike proteins to host factors reflects virus tropism and pathogenicity. Virol. J., 85(17): 8903–8912. https://doi.org/10.1128/JVI.05112-11

Wu Q, Xu M, Wei D, Zhang X, Li D, Mei M (2024). Pathogenicity and molecular characterization of a GI-19 infectious bronchitis virus isolated from East China. Front. Vet. Sci., 11:1431172. https://doi.org/10.3389/fvets.2024.1431172

Wu Z, Fang H, Xu Z, Lian J, Xie Z, Wang Z, Qin J, Huang B, Feng K, Zhang X, Lin W, Li H, Chen W, Xie Q (2022). Molecular Characterization Analysis of Prevalent Infectious Bronchitis Virus and Pathogenicity Assessment of Recombination Strain in China. Front. vet. sci., 9:842179. https://doi.org/10.3389/fvets.2022.842179

Zhang X, Deng T, Lu J, Zhao P, Chen L, Qian M, Guo Y, Qiao H, Xu Y, Wang Y, Li X, Zhang G, Wang Z, Bian C (2020). Molecular characterization of variant infectious bronchitis virus in China, 2019: Implications for control programmes. Transbound. Emerg. Dis., 67(3): 1349–1355. https://doi.org/10.1111/tbed.13477

Zhong Q, Hu YX, Jin JH, Zhao Y, Zhao J, Zhang GZ (2016). Pathogenicity of virulent infectious bronchitis virus isolate YN on hen ovary and oviduct. Vet. Microbiol., 193: 100–105. https://doi.org/10.1016/j.vetmic.2016.08.017