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Research article

Epidemiological and molecular characteristics of Infectious bursal disease virus naturally infected in the broiler flocks in the Mekong Delta of Vietnam during 2015 and 2018

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Abstract

Infectious bursal disease is a highly contagious and economically devastating disease in the poultry industry worldwide caused by Infectious bursal disease virus (IBDV). However, the data on epidemiological and molecular characteristics of the IBDV outbreak in broiler flocks in the Mekong Delta of Vietnam is unclear. Herein, the epidemiological data of IBDV-positive flocks over a period of 2015-2018 were recorded and the hypervariable region of the VP2 gene of IBDV was amplified to analyze the local phylogeny. The current investigation showed that the overall morbidity and mortality rates of IBDV-positive flocks were 45% and 4.81%, respectively. The IBDV-positive birds occurred clinical signs and macroscopic findings involved with the very virulent (vv) IBDV outbreak. Epidemiological results revealed that IBDV was frequently infected in broiler flocks at 12-42 days, and birds belonging to Tau Vang and Binh Dinh breeds were more sensitive to IBDV. Also, the morbidity rate of IBDV was dramatically decreased in the open farming system. Flocks with complete vaccination significantly dropped morbidity in comparison with other groups. Regarding phylogenetic analysis, all identified IBDV sequences clustered in the same branch of vv phenotype and closely homology with prior strains circulated in Vietnam and other countries. These findings first indicated the epidemiological characteristics of the IBDV-positive broiler flocks in the Mekong Delta and highlighted the IBDV strain circulating in this region.

Keywords: Broiler, Epidemiology, Gumboro disease, Mekong Delta region, Phylogenetic analysis.

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INTRODUCTION

Infectious bursal disease (IBD) or also namely Gumboro is an immunodeficiency disorder disease of chicken caused by the IBD virus (IBDV) (Lukert and Saif, 1997; Pikuła et al., 2020; Feng et al., 2022). The IBDV is a double-stranded RNA virus belonging to the Birnaviridae family and possesses two main segments (A and B) in a genome. Segment A is approximately 3.2 kb in size and contains two main overlapping open reading frames (ORFs), which encode proteins VP2 (outer capsid), VP3 (inner capsid), VP4 (protease), and VP5 (nonstructural protein). Segment B is approximately 2.9 kb in size and contains ORF that encodes a 90–kDa protein called VP1. Protein VP1 has a significant role in genome replication and transcription, as well as viral virulence (Lukert and Saif, 1997). Protein VP2 has been recognized as a very important structural protein that is responsible for the antigenic variation of IBDV and is widely studied in epidemiological and molecular analyses globally (To et al., 1999; Alfonso-Morales et al., 2013; Lupini et al., 2016; de Fraga et al., 2019; Ali Khan et al., 2019; Le et al., 2019; Pikuła et al., 2020; Thai et al., 2021; Feng et al., 2022; Omer and Khalafalla, 2022).

IBDV is presently classified into two serotypes including I and II, however, only serotype I is pathogenic to chickens (Lukert and Saif, 1997; de Fraga et al., 2019; Feng et al., 2022). In serotype I, subdividing into classic, variant, very virulent (vv), and attenuated phenotypes was widely agreed with previous studies (Feng et al., 2022). Since the first outbreak of vvIBDV in Europe in 1980s, the circulation of vvIBDV strains was previously documented until the present (Chettle et al., 1989; To et al., 1999; Alfonso-Morales et al., 2013; Lupini et al., 2016; de Fraga et al., 2019; Ali Khan et al., 2019; Le et al., 2019; Pikuła et al., 2020; Thai et al., 2021; Feng et al., 2022). In Vietnam, outbreaks of vvIBDV in clinically suspected chickens were recorded in earlier publications since 1987 (To et al., 1999; Le et al., 2019). Besides the prevalence of vvIBDV strain, classical strain, variant strain, and vaccine-like subclade were additionally documented in Vietnam (To et al., 1999; Le et al., 2019).

At present, IBD is still a global disease in the poultry industry. The circulation of IBDV was continuously reported in several geographical regions worldwide (Silva et al., 2013; Aliyu et al., 2016; Lupini et al., 2016; de Fraga et al., 2019; Le et al., 2019; Pikuła et al., 2020; Touzani et al., 2020; Thai et al., 2021; Wang et al., 2021; Bedasa et al., 2022; Feng et al., 2022). In Vietnam, prior studies on the change in genetic characterization of IBDV are scarce. A few reports phylogenetically characterized the IBDV strains from clinically infected birds (To et al., 1999; Le et al., 2019). However, there was a lack of epidemiological analysis of the infected flocks. Consequently, studies on the epidemiological and genetic status of IBDV circulating in Vietnam are needed due to the lack of information in this field. The present study aimed to characterize the epidemiology and phylogenetics of IBDV naturally infected in broiler flocks reared in six Mekong Delta provinces. The findings of this study may contribute to the diversity of basic knowledge regarding IBD and control strategies for this disease in the future.

MATERIALS AND METHODS

Broiler herd and farm characterization

This study was performed in the local broiler households and broiler farms in the Mekong Delta which is the huge and traditional broiler production region of Vietnam. In 2019, the Mekong Delta contributed approximately 17.15% of the poultry population of the whole country (Birhanu et al., 2021). The local household size was 50–180 birds and the broiler farm size was 2,000–5,000 birds. In our investigation, there were four main broiler breeds were reared including one cross or "Noi Lai" (Noi x Binh Dinh) and three indigenous (Tau Vang, Binh Dinh, Luong Phuong). Most of the broiler flocks were origin from commercial reproduction farms in other regions. One day old chickens were bought and reared in the local broiler households and broiler farms. Vaccination for Marek's, Newcastle, Gumboro, Infectious laryngotracheitis, Fowl pox, and avian influenza (H5N1) disease was carried out as a general vaccination program in this region.

For the farming system, the birds were reared under three main kinds including open, semi–open, and closed systems. The open system is a traditional farming system in this region, the birds were freedom of movement in the backyard and utilized the natural feed source in the fruit trees gardens or available feed from households. For closed systems, the birds were completely captivated in the pen and raised according to the process with commercial feed by workers. The semi–open system is a combination of the open and closed system, the birds were reared in the pen, but can freedom of movement in the backyard, and the commercial feed was supplied by households or workers.

Sample collection

Over the period of 2015–2018, a total of 131 broiler flocks with clinically suspected IBDV reared in six provinces of the Mekong Delta were investigated. Firstly, 4-6 clinically suspected IBDV birds in individual flocks were checked by rapid IBDV Ag Test (Green Age Ltd., Vietnam) via fecal sample (Figure 1). The birds were then gross examined and performed postmortem. Following, the bursa of Fabricius of all birds in the same flock was pooled to be one sample. Overall, 10 pooled samples were recruited for viral extraction. The distribution of investigated broiler flocks and performed RT–PCR samples in each province was shown in Figure 2. Prior to the postmortem, all the information on clinical signs, morbidity and mortality, and epidemiology of the investigated flocks was carefully recorded in the structured questionnaire. The gross examination was fully performed in all selected samples. The specimens were sampled under the control of the Sub–Department of Animal Health in the collected provinces. The tissue samples were stored at –80°C until extracted RNA.

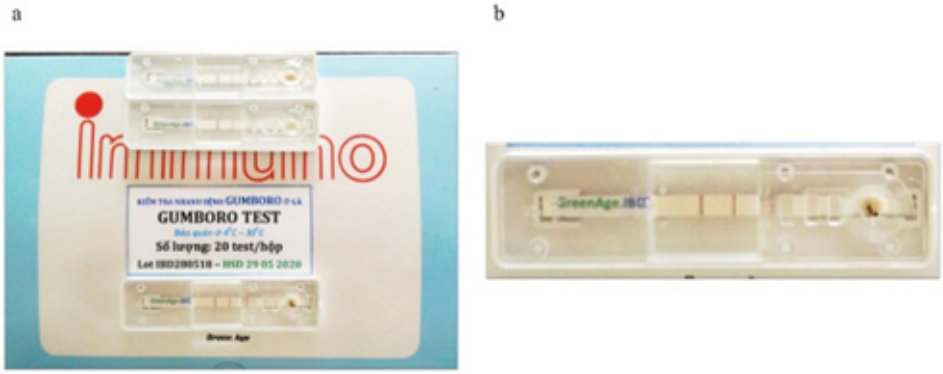

Figure 1 The rapid IBDV Ag Test (Green Age Ltd., Vietnam) using in this study (a). The positive result was shown in the rapid test (b).

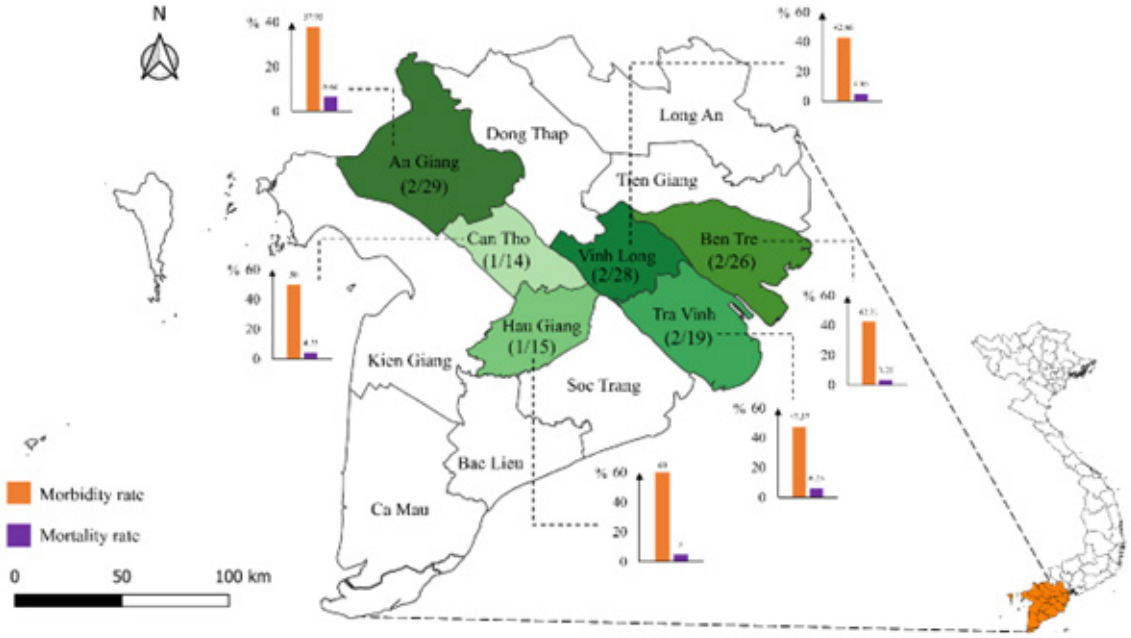

Figure 2 The geographical location of survey provinces, number of samples, morbidity, and mortality rate of IBDV–positive broiler flock within each province in the Mekong Delta of Vietnam. The survey provinces were highlighted in different colors on the map. In which, the letters described the name of provinces and the number in the parentheses described the total performed RT–PCR samples per the number of broiler flocks with clinically suspected IBDV. The map was constructed by the QGIS software package version 3.22.6.

Viral extraction

Total RNA was extracted from the bursal supernatant of 10 samples according to the manufacturer guideline of RNeasy Mini Kit (Qiagen, Germany). Following, the Maxima Reverse Transcriptase Kit (Thermo Fisher Scientific, USA) was utilized to synthesize complementary DNA (cDNA). All products were stored at -20°C until use for the next steps.

Reverse transcriptase polymerase chain reaction (RT–PCR)

In this study, the primer pair was manually designed to amplify the hypervariable region of the VP2 gene (HVR–VP2) of IBDV; forward primer GVF (5'–CAAACGATCGCAGCGATGACAAACCTGCAAGAT–3') and reverse primer GVR (5'–GGCTTCAAAGACATAATTCGGGCC–3'). The RT–PCR product was estimated to be a size of 474 bp fragment (Figure 3) from nucleotide 785 to 1,259 of the HVR–VP2. Briefly, RT–PCR was conducted in a 50 µL final volume containing 25 µL of Dream Taq PCR Mastermix (Thermo Fisher Scientific, USA), 1 µL of each primer (10 pmol/µL), 5 µL of template DNA, and 18 µL of nuclease–free water. The amplification followed the thermal cycling conditions: Initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 1 min, 65°C for 1 min, and 72°C for 2 min, and final extension at 72°C for 10 min. The amplified product was then electrophoresed in 2% agarose gel, stained in Ethidium Bromide, and visualized in an ultraviolet cabinet. Finally, the positive product was purified using the QIAquick PCR Purification Kit (Qiagen, Germany) and sequenced via the Sanger method in a commercial company (Macrogen, South Korea).

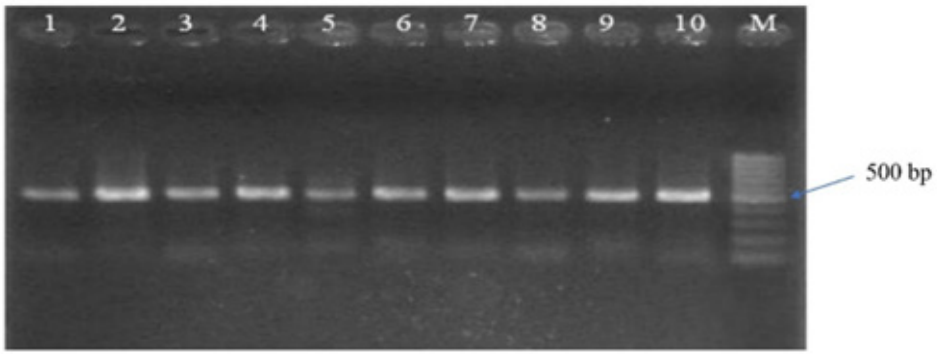

Figure 3 The PCR gel electrophoresis image of the HVR–VP2 of IBDV. Only the positive IBDV bands were shown at 474 bp. M represented the 1,000 bp DNA marker (Bioline, United Kingdom).

Phylogenetic analysis of the HVR–VP2 of IBDV

The nucleotide sequences of IBDV were manually assembled and aligned using the BioEdit software package version 7.2.5 (Hall, 1999). The HVR–VP2 was confirmed by the BLAST tool of the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/blast). The phylogenetic tree was constructed via the maximum likelihood method with 1,000 bootstrap replications in molecular evolutionary genetics analysis (MEGA) software package version 7.0.26 (Kumar et al., 2016). In the present study, the reference sequences were chosen from prior studies in Vietnam and the world (Le et al., 2019; Feng et al., 2022). To compare with vaccine strains, the commercial vaccine strains against IBDV in the Mekong Delta were analyzed based on the information from the questionnaire, the accession numbers of field vaccine strains were then retrieved from available data on GenBank. The field vaccine strains circulating in other regions of Vietnam were additionally chosen for this study (Le et al., 2019). Summary information of the reference IBDV strains was presented in Supplementary Table 1.

Data analysis

The recorded data from the questionnaire was initially calculated using Microsoft Excel software package version 2016 and exported in SAS software package version 9 for Windows (SAS Institute, USA), using the Chi–square test. A *p*–value less than 0.05 is considered statistically significant.

RESULTS

The morbidity and mortality rate of IBDV–positive broiler flock

In the current study, the overall prevalence of IBDV infection in the Mekong Delta was 45%, meanwhile for the highest rate of IBDV infection, it was Hau Giang province (60%) and for the lowest was An Giang province (37.93%). In the investigated broiler flocks, the mortality rate was documented. The results revealed that the overall mortality rate of IBDV–infected flocks in the Mekong Delta was 4.81%. Interestingly, An Giang province was the lowest morbidity rate of IBDV (37.93%), however, the mortality rate was the highest (6.64%). Besides, the lowest mortality rate was Ben Tre province (3.25%). The distribution of the morbidity and mortality rates in individual provinces was represented in Figure 2.

Clinical signs and macroscopic findings of IBDV-positive broiler flock

In the IBDV–positive flock, the affected birds frequently displayed signs of anorexia, ruffled feathers, water consumption, depression, and white and watery diarrhea (Figure 4a). Regarding macroscopic examination, hemorrhages in skeletal muscles (thigh and pectorals muscles) and bursa of Fabricius were commonly found, meanwhile, hemorrhages in the junction between the proventriculus and the gizzard were infrequently occurred and swollen in the spleen/kidney as well as bursal atrophy were uncommonly observed (Figure 4b).

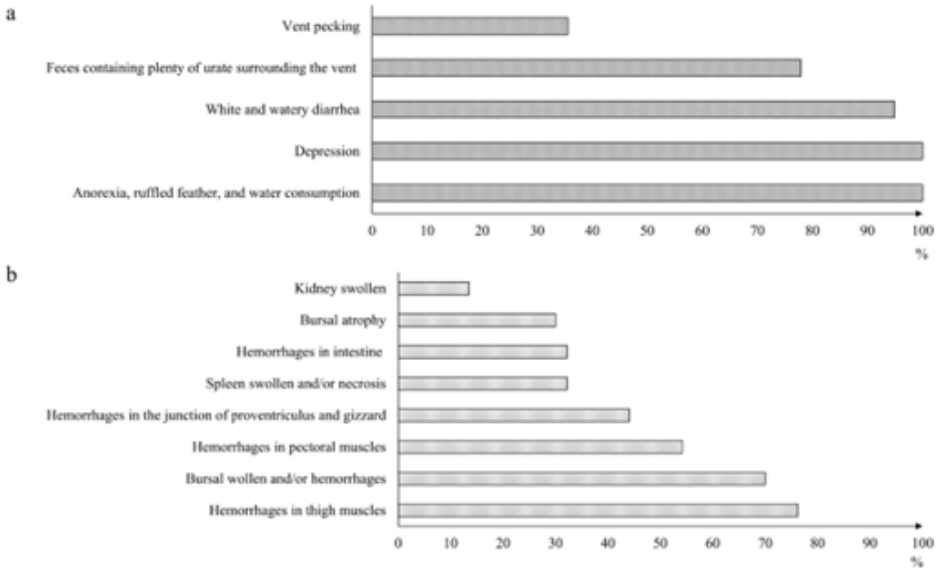

Figure 4 Clinical signs (a) and macroscopic findings (b) of IBDV-positive broiler flocks.

Epidemiological characteristics of IBDV-positive broiler flock

The epidemiological information of the IBDV–positive flocks was investigated and recorded in the structured questionnaire according to the positive results of the rapid IBDV Ag Test (Green Age Ltd., Vietnam) combined with clinical signs and macroscopic findings. Regarding age, the broiler flocks over 42 days old were lower affected by IBDV than other groups of age ($p < 0.01$). For breed, we noticed that the IBDV–positive rate of broiler flocks belonging to Tau Vang and Binh Dinh breeds was statistically higher ($p < 0.01$) than that in Luong Phuong and cross breeds. There was dramatically difference ($p < 0.01$) among broiler flocks reared in the open system, semi–open, and closed system. Regarding vaccination, the IBDV–positive rate of broiler flocks with complete vaccination was significantly lower ($p < 0.01$) than those with incomplete vaccination and not used vaccine (Table 1).

Table 1 The distribution of IBD in different ages, breeds, farming systems, and vaccination status.

Category	Total flock	Number of IBDV-positive flock	*p*-value
Age			
12–21 days old	43	20(46.51)	
22–42 days old	54	31(57.41)	0.01
Over 42 days old	34	8(23.53)	
Breed			
Cross	52	15(28.85)	
Tau Vang (Indigenous)	19	13(68.42)	0.01
Binh Dinh (Indigenous)	31	17(54.84)	
Luong Phuong (Indigenous)	29	14(48.28)	
Farming system			
Open	50	14(28)	
Semi-open	60	33(55)	0.01
Close	21	12(57.14)	
Vaccination status			
Not used vaccine	36	24(66.67)	
Incomplete vaccination	42	22(52.38)	0.01
Complete vaccination	53	13(24.53)	

Molecular analysis of the HVR-VP2 of IBDV

Herein, a 474 bp fragment located in the HVR-VP2 region of IBDV was amplified by RT-PCR from 10 selected broiler flocks in six provinces of the Mekong Delta, Vietnam. Subsequent to sequencing, a total of 10 IBDV sequences were recruited and analyzed for molecular characteristics. The phylogenetic analysis according to the nucleotide sequences of the HVR-VP2 region demonstrated that all field IBDV sequences isolated in the Mekong Delta were arranged into a narrow cluster with the IBDV strains circulating in China, South Korea, Japan, Pakistan, Israel, United Kingdom, Netherlands, and prior Vietnamese strains, belonging to a vv phenotype (Figure 5).

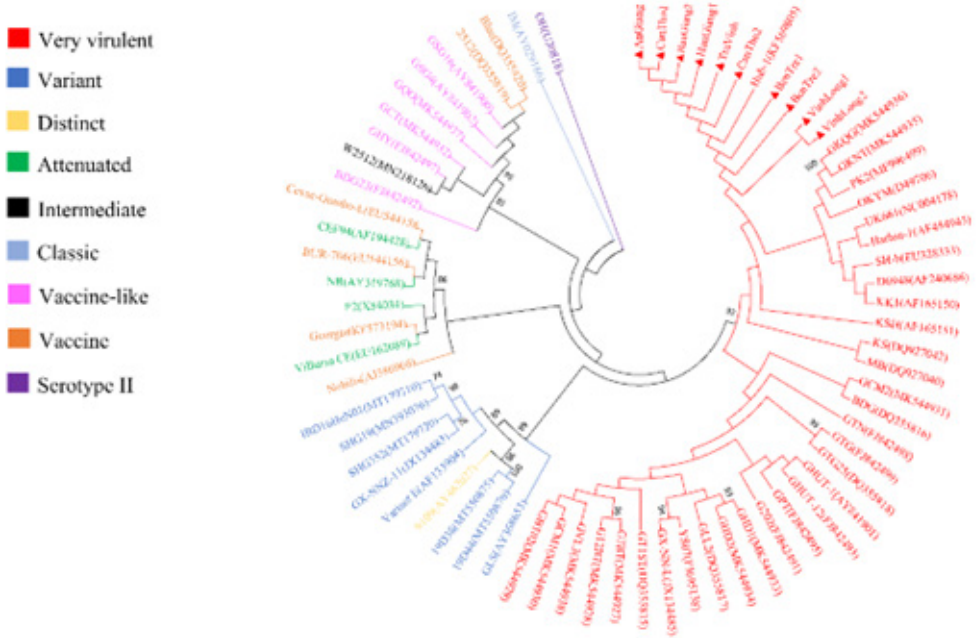

Figure 5 Phylogenetic tree analyzing the HVR–VP2 nucleotide sequences of IBDV in this study and other reference IBDV strains, constructed by the maximum likelihood method in the MEGA software package with 1,000 bootstrap replications. The red triangles represent the IBDV strains detected among survey provinces in the Mekong Delta over a period from 2015 to 2018. The color of the branches represents the phenotype of the IBDV strains. Only bootstrap values above 70% were presented in the phylogenetic tree

Regarding sequence identity analysis, the field IBDV sequences shared a close relationship to a vv phenotype, a similarity up to 99.99% and 100% for nucleotide and amino acids, respectively. In comparison to other Vietnamese strains, the nucleotide and amino acid sequence identities were 99.97% and 99.98%, respectively. Notably, the high identity with the vaccine and vaccine–like strains were observed in this work, sharing 99.98% and 99.96% identities at nucleotide and 99.98% and 99.97% at amino acid, respectively (Table 2). For amino acid sequence alignment, the amino acid sequences isolated in the Mekong Delta were closely related to the HVR–VP2 region of vvIBDV strains on GenBank at five hydrophilic peaks and heptapeptide domains (Figure 6).

Table 2 Comparison of nucleotide and amino acid sequence identities in this study and other reference IBDV strains.

Phenotype	(%) identity with IBDV sequences in this study	
	Nucleotide	Amino acid
Very virulent	99.88–99.99	99.93–100
Variant	99.84–99.98	99.86–99.99
Distinct	99.85–99.95	99.88–99.97
Attenuated	99.88–99.97	99.88–99.98
Intermediate	99.89–99.95	99.87–99.96
Classic	99.90–99.93	99.90–99.98
Vaccine–like	99.88–99.96	99.88–99.97
Vaccine	99.87–99.98	99.90–99.98
Serotype II	99.54–99.62	99.55–99.62
Other Vietnamese strains	99.93–99.97	99.93–99.98

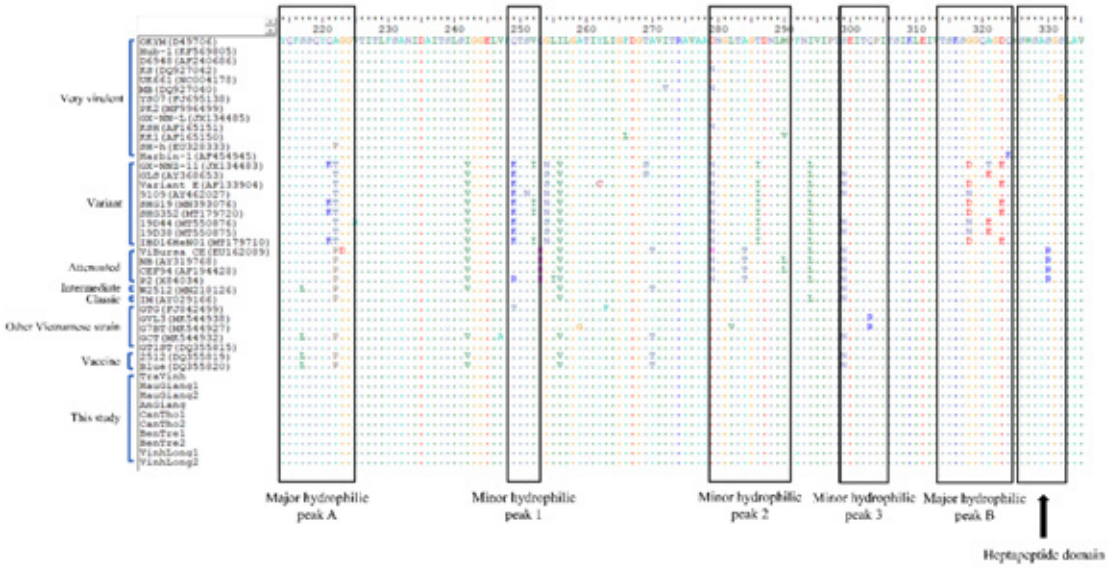

Figure 6 Alignment of the deduced amino acid sequences in the HVR-VP2 region of IBDV in the present study and other reference IBDV strains. Dots represent the identical residues in sequences and letters represent the differences

DISCUSSION

At present, IBD is still circulating and seriously affecting the poultry industry of Vietnam and worldwide (Silva et al., 2013; Zachar et al., 2016; de Fraga et al., 2019; Le et al., 2019; Pikuła et al., 2020; Touzani et al., 2020; Bedasa et al., 2022; Feng et al., 2022). Further studies on epidemiological and genetic characterization of IBDV are still suggested for better understanding and may contribute to the control strategies of this disease globally. The current study amplified the HVR–VP2 region of IBDV isolated from the bursal samples of clinically suspected broiler flocks within six provinces of the Mekong Delta, Vietnam. The findings of this study first revealed the epidemiological characteristics of infected broiler flocks and highlighted the genotype subgroup of IBDV circulating in the Mekong Delta of Vietnam.

Herein, data on morbidity and mortality as well as clinical signs and macroscopic findings of IBDV–positive flocks were documented. The findings showed that the overall morbidity and mortality rates of IBDV–positive flocks were 45% and 4.81%, respectively. However, our observations were lower than the results of the prior studies that revealed the morbidity and mortality rates of vvIBDV flocks ranged from 40–90% and 9–76%, respectively (Aliyu et al., 2016; Omer and Khalafalla, 2022). In fact, the morbidity and mortality rates of IBDV–positive flocks were associated with the epidemiology status, serotypes of the virus, co–infection status, host immunity status, vaccine handling, treatment, etc (Mutinda et al., 2014; Aliyu et al., 2016; Wang et al., 2021; Feng et al., 2022), therefore, it was frequently different among studies in other geographical regions. Besides, the clinical signs and macroscopic results of this study revealed that the IBDV-positive flocks occurred with the typical characteristics of vvIBDV outbreaks as same as prior studies worldwide. Indeed,

earlier reports demonstrated that birds infected with vvIBDV showed signs of depression, ruffled feathers, and white and watery diarrhea and macroscopic examination of hemorrhages in skeletal muscles, bursa of Fabricius, and the junction between the proventriculus and the gizzard (Aliyu et al., 2016; Le et al., 2019; Touzani et al., 2020).

Due to the differences in the nature of IBDV outbreaks, the present study partly focused on the epidemiology status of the IBDV naturally infected broiler flocks. IBD has been widely known as an immunodeficiency disorder disease of young birds (Müller et al., 2012). According to this work, birds from 12–42 days old were more sensitive to IBDV than those over 42 days old. This observation agreed with previous studies, the age of vvIBDV–infected birds ranged from 15 to 38 days (Aliyu et al., 2016; Lupini et al., 2016; Touzani et al., 2020; Feng et al., 2022). Regarding breed, Tau Vang and Binh Dinh breeds were a higher prevalence of IBDV than Luong Phuong and cross breeds. It is the first report that showed a difference in morbidity of IBDV among indigenous broiler breeds reared in the Mekong Delta of Vietnam. Further studies are suggested to clearly indicate the correlation between Vietnamese indigenous broiler breeds and the morbidity of IBDV. Interestingly, birds reared in an open system showed a significantly lower IBDV–infected rate than those in other farming systems. This could be related to the free contact between host and pathogen in closed and semi–open systems, birds were reared in a high population density and free contact with other IBDV–suspected birds and their droppings. Similarly, current scientific evidence demonstrated that the faeco–oral route was recommended as the main route of vvIBDV transmission in the battery cage brooding system rather than the aerosol route (Aliyu et al., 2016). In addition, IBDV infection was dramatically dropped in a group with complete vaccination compared with other groups. Indeed, vaccination is one of the most important strategies to control IBD in the poultry industry worldwide (Müller et al., 2012; Mutinda et al., 2014). However, there were several factors correlated to the vaccination failures in IBDV outbreaks such as poor vaccine storage and failed vaccine handling practices of households (Mutinda et al., 2014). Therefore, IBDV outbreak in complete vaccination flocks was occasionally found. Altogether, our findings imply that the IBDV–positive flocks in the Mekong Delta were closely related to the characteristics of vvIBDV outbreaks.

In the current work, all field IBDV sequences were classified as a vv phenotype via phylogenetic analysis according to the VP2 nucleotide sequences. This result was concordant with the previous studies on the circulation of vvIBDV in Vietnam (To et al., 1999; Le et al., 2019). VP2 protein encoded from the VP2 gene has been generally accepted as a key structural protein that involves the virulence, antigenic determinants, and tropism of IBDV (Alfonso-Morales et al., 2013; Touzani et al., 2020; Feng et al., 2022). Prior reports in Vietnam also utilized the VP2 gene to analyze the genetic characteristics of field IBDV sequences (To et al., 1999; Le et al., 2019). Interestingly, all field IBDV sequences were grouped in a narrow branch corresponding to the vvIBDV strain of Vietnam, China, South Korea, Japan, Pakistan, Israel, United Kingdom, and Netherlands. In comparison to earlier studies in Vietnam, 10 IBDV sequences of this study formed an individual branch with classical and variant strains and vaccine-like subclade (To et al., 1999; Le et al., 2019). The

transmission route of IBDV globally is understudied, it was a hypothesis that the globalized migration of avian species could link to the vvIBDV outbreak in several geographical regions (Alfonso-Morales et al., 2013). On the other hand, IBDV tended to cluster in the same geographic subdivision (Alfonso-Morales et al., 2013), therefore, there was a similarity of IBDV strains among neighboring countries. For further studies, the transmission route of the virus among geographical regions is suggested to indicate the origin of IBDV circulating in Vietnam. Also, alignment of the deduced amino acid sequences revealed that all field sequences shared high similarity to reference vvIBDV strains at five hydrophilic peaks and heptapeptide domains of the HVR–VP2 region. According to the literature, the vvIBDV strain could be predicted via the changes in seven special amino acid sites located in the hydrophilic peaks and heptapeptide domains of the HVR–VP2 region including P222A, V242I, V256I, T270A, N279D, L294I, and N299S (Silva et al., 2013; Feng et al., 2022). Taken together, our findings imply that the IBDV outbreak in the Mekong Delta over 2015–2018 performed the epidemiological and molecular characteristics of the vvIBDV outbreak.

The current study encountered unavoidable limitations. First, there was a limitation in sample size, only 10 samples were recruited from the IBDV–positive samples via rapid IBDV Ag Test and submitted to RT–PCR to amplify the HVR–VP2 gene. Further studies should perform a larger sample size and amplify the full–length genome of IBDV to clearly indicate the molecular characteristics of IBDV. Second, the recombinant between field IBDV sequences and field vaccine strains were not supported in the current study. Further studies on naturally recombinant between field IBDV strains and field vaccine strains in broad geographical regions are needed to clearly understand the viral pathogenicity circulating in Vietnam. Last, this study was limited to the investigated area, the study was performed in the Mekong Delta region of Vietnam, therefore, our results could not display the prevalence of IBDV and their characteristics in the whole country. This limitation should be noted in further investigations.

CONCLUSIONS

The current study first revealed the epidemiological characteristics of the IBDV–positive broiler flocks in the Mekong Delta of Vietnam and highlighted the IBDV strain circulating in this region which was a very virulent strain. Studies on the natural recombinant of the virus should be further recommended for a sufficient understanding of IBDV circulating in this region and the whole country.

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AUTHOR CONTRIBUTIONS

B.N.T. and K.P.N. conceptualized and designed the study. C.N.P., D.T.A.T., T.M.V., and B.N.T. collected field samples and performed molecular experiments. C.N.P., D.T.A.T., T.M.V., K.P.N., and T.Q.L. analyzed and interpreted data. C.N.P., K.P.N., T.Q.L., and B.N.T. wrote the first draft and revised the manuscript. We declare that the submitted manuscript was approved by all authors.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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