Characterization and pathogenicity of infectious bursal disease virus in Southern China

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ABSTRACT Infectious bursal disease virus (**IBDV**) is a widespread pathogen that induces immunosuppression in 3 to 6-wk-old chickens, casuing great threaten to the poultry industry worldwide. Previously, the very virulent IBDV (**vvIBDV**) was mainly prevalent in China. In recent years, the novel variant IBDV (**nvIBDV**) occurred in China. In this study, we isolated 30 IBDV strains of IBDV from vaccinated chicken flocks in 8 provinces of southern China. Among these isolates, vvIBDV group (13/30) and nvIBDV group (17/30) were identified according to the genome sequencing and phylogenic analysis. Moreover, HB2021-5 and GD2021-17 have pathologic characteristics with severe bursal lesions, as evidenced by necrosis, depletion of lymphocytes, and follicle atrophy. Our findings provide an important reference for understanding the epidemiological status and the evolution of IBDV.

Key words: IBDV, epidemiology, phylogenetic analysis, pathogenicity

INTRODUCTION

Infectious bursal disease (**IBD**), which is caused by IBD virus (**IBDV**), is an acute and highly contagious disease in chickens (Brown and Skinner, 1996; Sharma et al., 2000; Jackwood, 2017). IBDV mainly targets B lymphocytes in the bursa of Fabricius (**BF**), resulting in severe immunosuppression (Berg, 2000; Kurukulsuriya et al., 2016) and enhancing susceptibility to other pathogens in 3 to 6-wk-old chickens (Kibenge et al., 1988).

IBDV is a non-enveloped virus, belonging to the *Avibirnavirus* genus of the *Birnaviridae* family. Viral genome is composed of 2 segments (A and B) of double-stranded RNA (Müller et al., 1979). Segment A consists of 2 overlapping open reading frames (**ORF**), encoding viral non-structural proteins VP5 and polyprotein VP2-VP4-VP3 (PP), which are cleaved into VP2, VP3 and VP4 by autoproteolysis (Birghan et al., 2000; Luque et al., 2009a; Raja et al., 2016). VP2 is the major structural protein, participating in viral entry, cell tropism, virulence, and antigenic variation (Jackwood et al.,

2008; Qi et al., 2016; Wu et al., 2020). VP3 is a multifunctional structural protein, involving viral life cycle and viral assembly (Luque et al., 2009b). VP4 is a viral protease protein, contributing to the self-processing of the polyprotein (Nouën et al., 2006). Segment B contains only one ORF that encodes protein VP1, which plays an important role in genome transcription, genetic evolution, and virulence (von Einem et al., 2004; Wang et al., 2021a).

Two distinct serotypes (1 and 2) of IBDV have been identified previously (Jackwood et al., 1982). Serotype 1, which has been demonstrated to be pathogenic in chickens, can be further divided into 4 phenotypes, including the classical IBDV (**clIBDV**), vvIBDV, attenuated IBDV (**atIBDV**) and variant IBDV (**varIBDV**) (Jackwood, 2017). Serotype II was non-pathogenic to chickens (Jackwood, 2017). The classical strain was first described in the United States in 1957 (Lasher and Davis, 1997) while the vvIBDV and varIBDV emerged in the United States and Europe in the 1980s (Jackwood and Saif, 1987; Chettle et al., 1989; de Wit et al., 2018). IBDV has been circulating in China since 1979. Although commercial vaccines against IBDV have been widely used in poultry industry, sporadic outbreaks of IBD are still reported in China (Feng et al., 2021). Due to the live vaccines promoting a genetic variation of field circulating viruses and the rapid genetic evolution of the hyper variable region of *vp*2 gene, the genome

and pathological type of IBDV evolving in recent years (Wang et al., 2021b).

In 2017, nvIBDV was identified from suspected subclinical infections of IBDV (Fan et al., 2019; Xu et al., 2020). The nvIBDV evolutionarily belonged to the varIBDV because of the same typical residues (222 T, 249 K, 286I, and 318D), but showed an obvious difference in amino acid sequence identity compared to the American variants of IBDV. Since 2017, nvIBDV has been widespread across the country. The Chinese nvIBDV is significantly different from the early American varIBDV (Fan et al., 2020). It has been reported that the nvIBDV directly damages the immune organs, leading to severe and prolonged immunosuppression in young chickens, but does not clinical morbidity and death (Fan et al., 2020). Subsequently, the classical vaccines have been proved to be ineffective in controlling the nvIBDV. In recent 2 yr, the nvIBDV pandemic remains serious. Given the recent epidemic of IBDV in southern China, determining the epidemiology and genetic characteristics is important for the prevention and control of IBDV in China and the world.

In this study, we focused on the prevalence and pathogenicity of the IBDV in southern China. Surveillance to be conducted to identify IBDV strains isolated from commercial chicken farms. Phylogenetic analysis of IBDV isolates was performed to investigate the prevalence. Furthermore, the representative strains of vvIBDV HB2021-5 and nvIBDV GD2021-17 were full-genome sequenced and the molecular characteristics were analyzed. Subsequently, a pathogenicity experiment of HB2021-5 and GD2021-17 was performed. The results of our findings provided a basis for the epidemiology and evolution of IBDV in southern China.

MATERIALS AND METHODS

Clinical Samples Collection and RT-PCR Detection

In 2021, a total of 96 clinical samples were collected from the immunized chicken flocks in 8 provinces (Guangdong, Hubei, Jiangsu, Guangxi, Guizhou, Anhui, Yunnan, and Jiangxi) of China. Samples were frozen and thawed 3 times, suspended in phosphate buffered saline (**PBS**) containing 200 U/mL penicillin and 200 μg/mL streptomycin, and then centrifuged at $5,000 \times g$ for 5 min (Lian et al., 2022). RNA was extracted from samples using Viral DNA/RNA Miniprep Kit (Axygen, Hangzhou, China) according to the manufacturer's instructions. Specific primer pairs (VP2-F: 5'-CCTAA-GAACGGTTGGAAT-3', VP2-R: 5'-TACTCTCTA-CACACACAC-3') based on the conserved region of $vp2$ gene were designed. The reverse transcription-polymerase chain reaction (**RT-PCR**) was performed to verify IBDV. RT-PCR was performed using the PrimeScript One Step RT-PCR Kit (TaKaRa, Dalian, China) at 95°C for 5 min followed by 35 cycles at 95°C for 30 s, 55°C for 30 s, 72°C for 1 min, and final extension at 72°C for 10 min. The RT-PCR products were analyzed by electrophoresis on 1.0% agarose gel and then observed by an ultraviolet transilluminator. Each positive segment was sequenced by Shanghai Sang-gong Biological Engineering Technology & Services Co., Ltd (Shanghai,

Table 1. IBDV strains used in this study.

Strains	Phenotype	GeneBank accession No.		Origin
		Segment A	Segment B	
GLS	Variant	AY368653	AY368654	USA
Variant E	Variant	AF133904	AF133905	USA
9109	Variant	AY462027	AY459321	USA
SHG19	novel variant	MN393076	MN393077	China
SHG352	novel variant	MT179720	MT179722	China
SHG358	novel variant	MT179721	MT179723	China
BD/399	very virulent	AF362776	AF362770	Bangladesh
D6948	very virulent	AF240686	AF240687	Netherlands
Gx	very virulent	AY444873	AY705393	China
Harbin-1	very virulent	EF517528	EF517529	China
HLJ-0504	very virulent	GQ451330	GQ451331	China
OKYM	very virulent	NC-004178	NC-004179	Japan
UK661	very virulent	X92760	X92761	Europe
02015.1	very virulent	AJ879932	AJ880090	France
Cu-1wt	Classical	AF362747	AF362748	Germany
Faragher 52/70	Classical	HG974565	HG974566	UK
W2512	intermediate-plus	MN218126	MN218127	USA
B87	attenuated	DQ906921	DQ906922	China
CEF94	attenuated	AF194428	AF194429	Netherlands
CT	attenuated	AJ310185	AJ310186	France
Cu-1	attenuated	X16107	AF362775	Germany
D78	attenuated	AF499929	AF499930	USA
Gt	attenuated	DQ403248	DQ403249	China
HZ2	attenuated	AF321054	AF493979	China
P2	attenuated	X84034	X84035	Germany
OH	SerotypeⅡ	U30818	U30819	Canada
23/82	SerotypeⅡ	AF362773	AF362774	Germany

Abbreviation: IBDV, infectious bursal disease virus.

China). The virus isolation and follow-up experiments were carried out in biosafety level 2 (**BSL-2**) biocontainment laboratory.

Virus Isolation

The supernatants of the IBDV-positive samples were inoculated into 10-day-old SPF embryonated chicken eggs via the chorioallantoic membrane (**CAM**) route. The inoculated embryos were incubated at 37°C. After a 5-d observation, the CAM homogenates and the allantoic fluid were harvested and confirmed viral existence using an RT-PCR assay.

Genome Sequencing

To evaluate the molecular characterization of IBDV isolates, the complete genome of vvIBDV isolates HB2021-5 and nvIBDV isolates GD2021-17 were sequenced. cDNA was reverse transcribed from the viral RNA with HiScript III 1st Strand cDNA Synthesis Kit (Vazyme, Nanjing, China). The full-length segment A

and segment B were amplified using polymerase chain reaction (**PCR**) using the specific primers as previously described (Lu et al., 2015a). A total of 50 μL was used for PCR amplification, including 25 μL of PrimeSTAR Max Premix (TaKaRa), 1 μL of cDNA, mixed with 10 μmoL of each primer and 22 μL of ddH$_2$O (35 cycles at 98°C for 10 s, 55°C for 5 s and 72°C for 20 s). The PCR product was purified using a Universal DNA Purification Kit (TIANGEN, Beijing, China), cloned into the T/A cloning vector pMD19-T (TaKaRa). The PCR products were ligated into the pMD19-T vector (TaKaRa), and then sequenced by Shanghai Sang-gong Biological Engineering Technology & Services Co., Ltd (Shanghai, China).

Phylogenetic and Amino Acid Analyses

The nucleotide sequences of the *vp2* gene of all the IBDV isolates were aligned by Clustal W from the MEGA program (version 7.0.14) and analyzed with 25 reference IBDV strains published in GenBank using MegAlign program (DNASTAR, Madison, WI). The information of the reference IBDV strains are listed in
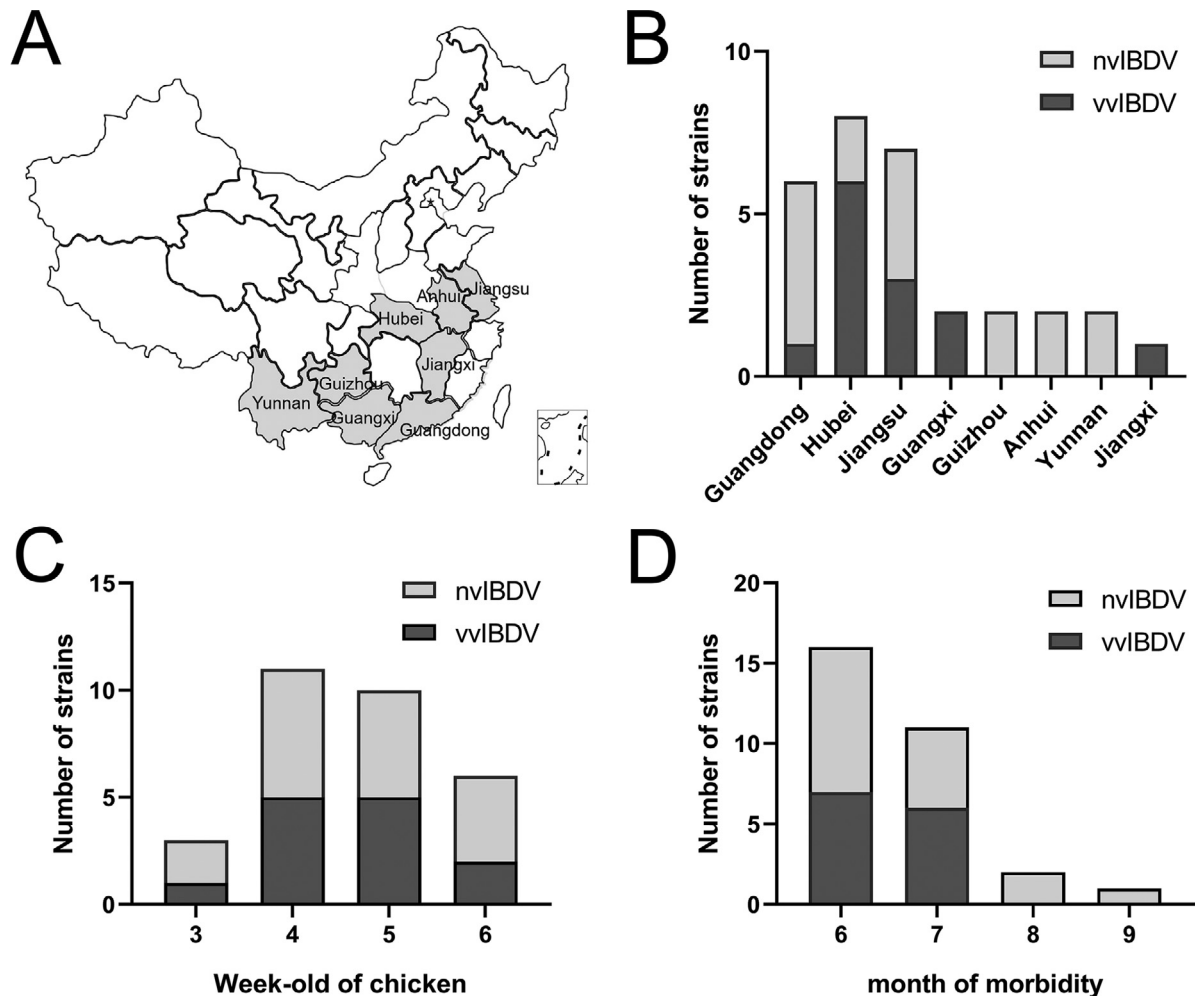

Figure 1. The provinces surveyed during the period from 2021 are indicated by shading (A). The map is created by ArcGIS 9.1 software. The percentage of IBDV strains isolated from each province is shown by genotype (B). The number of strains isolated from different ages of chickens (C). The number of strains was isolated from different months of infected flocks (D). Abbreviation: IBDV, infectious bursal disease virus.

Table 1. Phylogenetic analysis of the nucleotide sequences of *vp2* gene was performed with the neighbor-joining method using MEGA program. The bootstrap values were determined from 1,000 replicates of the original data.

The nucleotide and amino acid sequences corresponding to *vp5* gene, *polyprotein*, and *vp1* gene of HB2021-5 and GD2021-17 have aligned with the reference strains and phylogenetic analyzed use the same method as previously described.

Pathogenicity Experiment

To evaluate the virulence of the vvIBDV and nvIBDV field isolates, an animal experiment was performed using the representative vvIBDV strain HB2021-5 and nvIBDV strain GD2021-17. A total of fifteen 21-day-old SPF chickens were randomly divided into 3 groups. Chickens in the first group (n = 5) and the second group (n = 5) were infected with HB2021-5 and GD2021-17, respectively, via the oral routes. Chickens in the third group (n = 5) were inoculated with phosphate buffered saline (**PBS**) as a negative control. Clinical symptoms were recorded daily. At 4-d post inoculation (**d p.i.**), all the chickens were euthanized for necropsy and examination of pathological changes. The bursa and body weights of all the chickens were determined, and the bursa: body weight index (**BBIX**) was calculated along with the standard deviation [BBIX = (bursa: body weight ratios) / (bursa: body weight ratios in the negative group)]. The mean values and standard deviations of the data obtained from chicken samples were calculated. Bursae with a BBIX less than 0.7 were considered atrophy (Lucio and Hitchner, 1979). The affected tissues (BF) were fixed in 10% neutral-buffered formalin, routinely processed, embedded in paraffin, sectioned (4-μm thick), and stained with hematoxylin and eosin (H&E) according to standard protocols. Pathological changes were examined by light microscopy.

Statistical Analyses

All data in this study were processed using GraphPad Prism 8.0. A one-way ANOVA was employed to evaluate the significance of the differences among the different groups. Differences with $P < 0.05$ were considered significant.

RESULTS

Clinical Characteristics of IBDV Isolates From Southern China

An epidemiological investigation was performed in immunized chicken flocks of yellow-feathered broilers to monitor IBDV epidemic in southern China in 2021. A total of 97 diseased samples were collected from vaccinated flocks, which were associated with the typical clinical symptoms of IBDV. All the samples were detected using RT-PCR assay. As a result, thirty IBDV strains (30/97, 30.93%) were identified and isolated using SPF chicken embryonated eggs, originating from Guangdong (n = 6), Hubei (n = 8), Jiangsu (n = 7), Guangxi (n = 2), Guizhou (n = 2), Anhui (n = 2), Yunnan (n = 2), and Jiangxi (n = 1; Figures 1A and 1B). The variant field strains account for 56.7% (17/30) of isolates and the rest were vvIBDV strains (13/30). All the IBDV isolates were mainly isolated from 3 to 6-wk-old chickens from June to September (Figure s1C and 1D). The sequences of *vp2* gene of all the isolates were submitted to GenBank under accession numbers OL790263-OL790292 (Table 2).

Phylogenetic Analysis of the IBDV Strains

To assess the genetic relatedness among the IBDV strains, a phylogenetic tree was constructed based on the nucleotide sequences of *vp2* gene (Figure 2A). Consistent with previous study (Tomas et al., 2020), 7 distinct clusters were identified in this study, including vvIBDV (vv), varIBDV (Var), nvIBDV (nVar), clIBDV (Cl), atIBDV (At), intermediate-plus IBDV (Int) and serotype Ⅱ strains (Figure 2B). Seventeen field isolates were clustered with nvIBDV strain SHG19 with a high degree of nucleotide similarity (94.9−99.2%). Thirteen isolates were located at the evolutionary branches of vvIBDV strains, including BD/3 99, D6948, Gx, Harbin-1, HLJ-0504, OKYM, UK661 and 02015.1. These vvIBDV isolates possessed 95.2 to 99.5% nucleotide identity with the Chinese reference strains (Gx, Harbin-

Table 2. IBDV strains isolated in this study.

Strain	Phenotype	Days	Accession No.	Origin
GD2021-1	very virulent	38	OL790263	broiler
GD2021-3	novel variant	32	OL790264	broiler
GD2021-5	novel variant	27	OL790265	broiler
GD2021-10	novel variant	34	OL790266	broiler
GD2021-14	novel variant	30	OL790267	broiler
GD2021-17	novel variant	22	OL790268	broiler
HB2021-2	novel variant	21	OL790269	broiler
HB2021-3	novel variant	42	OL790270	broiler
HB2021-4	very virulent	27	OL790271	broiler
HB2021-5	very virulent	31	OL790272	broiler
HB2021-6	very virulent	33	OL790273	broiler
HB2021-7	very virulent	22	OL790274	broiler
HB2021-8	very virulent	27	OL790275	broiler
HB2021-9	very virulent	31	OL790276	broiler
JS2021-1	novel variant	45	OL790277	broiler
JS2021-2	novel variant	19	OL790278	broiler
JS2021-3	novel variant	19	OL790279	broiler
JS2021-4	very virulent	26	OL790280	broiler
JS2021-6	very virulent	24	OL790281	broiler
JS2021-7	very virulent	38	OL790282	broiler
JS2021-8	novel variant	30	OL790283	broiler
GX2021-2	very virulent	30	OL790284	broiler
GX2021-4	very virulent	18	OL790285	broiler
GZ2021-1	novel variant	26	OL790286	broiler
GZ2021-2	novel variant	25	OL790287	broiler
AH2021-1	novel variant	27	OL790288	broiler
AH2021-2	novel variant	39	OL790289	broiler
YN2021-1	novel variant	28	OL790290	broiler
YN2021-2	novel variant	28	OL790291	broiler
JX2021-1	very virulent	30	OL790292	broiler

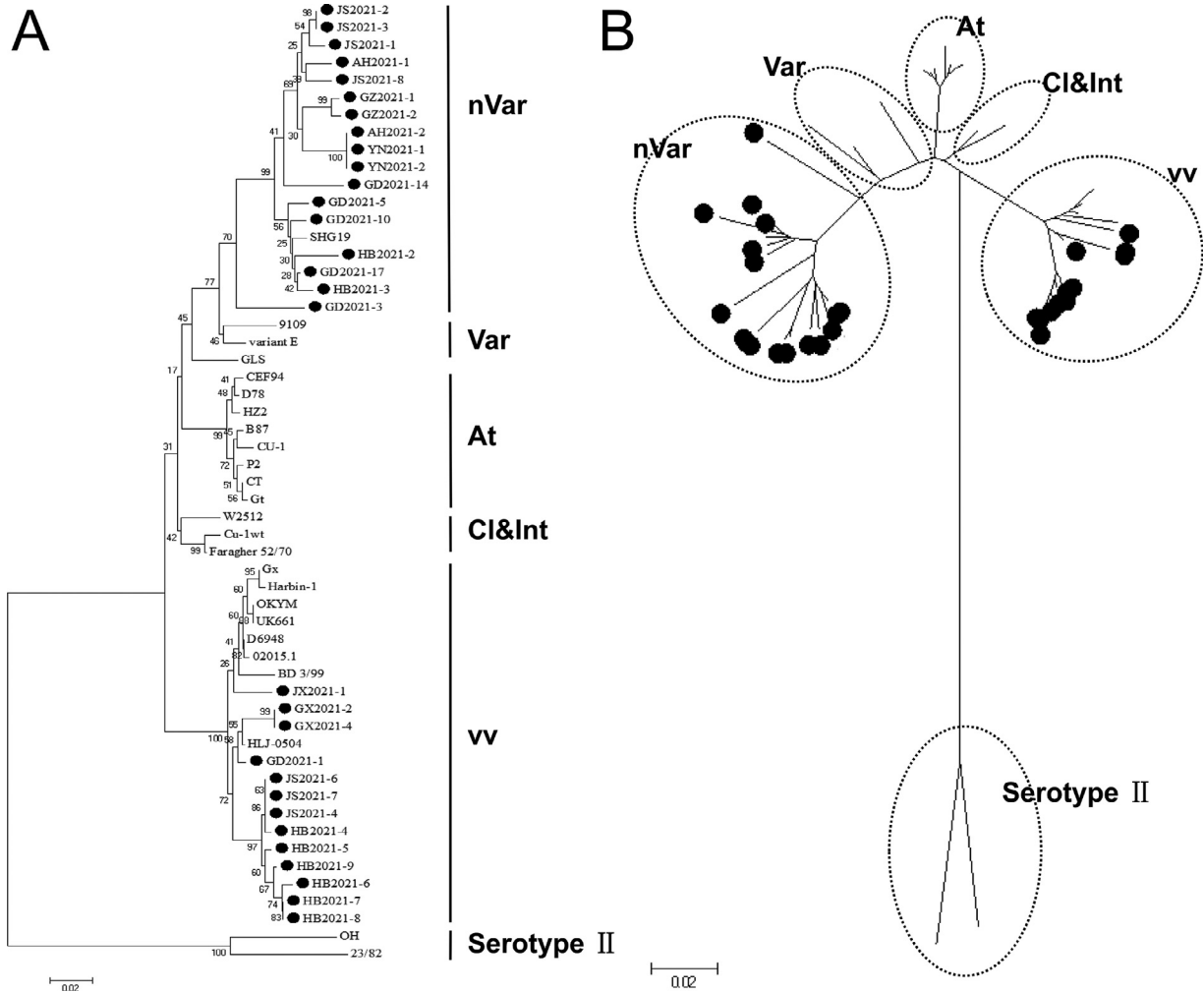Abbreviation: IBDV, infectious bursal disease virus.

Figure 2. Phylogenetic tree analysis of the nucleotide sequence of *vp2* gene (A&B). The trees were generated by the neighbor-joining method using MEGA7. The isolates detected in this study are highlighted with a solid circle.

1, and HLJ-0504). Classical strains and attenuated strains clustered into 2 independent genetic groups. The serotype II strains (OH, 23/82), which were evolutionarily distant from all the field isolates and the other reference strains, are locating in different evolutionary branches. Taken together, nvIBDV strains and vvIBDV strains were the major epidemic strains in southern China.

Phylogenetic Analysis and Molecular Characterization of IBDV Strain HB2021-5 and GD2021-17

To illustrate the molecular characteristics of IBDV isolates further, the representative strains of vvIBDV HB2021-5 and nvIBDV GD2021-17, were full-length sequenced. The phylogenetic trees were constructed based on HB2021-5, GD2021-17, and 26 reference strains. The genome sequences of HB2021-5 were submitted to GenBank and the accession numbers assigned for segment A and segment B were OM937740 and OM937741. The accession numbers assigned for segment A and segment B of GD2021-17 were OL828536 and OL828537.

The phylogenetic tree of *vp5* gene showed that HB2021-5 was clustered in a mixed clade and more closely related to the vvIBDV HLJ-0504 that emerged in China. GD2021-17 was different from early America varIBDV and was clustered together with the representative nvIBDV, including SHG19, SHG352, and SHG358 (Figure 3A). Phylogenetic analysis based on the PP showed that all serotype I strains were divided into 3 clades. The first clade contained the atIBDV, clIBDV, and varIBDV. The second clade consisted of the vvIBDVs. The third clade contained nvIBDV. HB2021-5 was clustered with vvIBDV while GD2021-17 was located in the third clade (Figure 3B). The nucleotide sequences of *vp1* gene of HB2021-5 and GD2021-17 were also closely related to the vvIBDV strain HLJ-0504 and nvIBDV strains, respectively (Figure 3C).

To understand the amino acid characteristics further, the alignment of each protein of HB2021-5 and GD2021-17 was performed (Figure 3D). The deduced amino acid sequences of VP5 exhibited a high conservatism for the HB2021-5 or GD2021-17, in which none of the mutation site was shown. Three amino acid differences (222V, 642R, and 744L) were observed in PP of HB2021-5 compared to vvIBDV reference strains. While only one specific mutation (875I) was observed in PP of GD2021-17
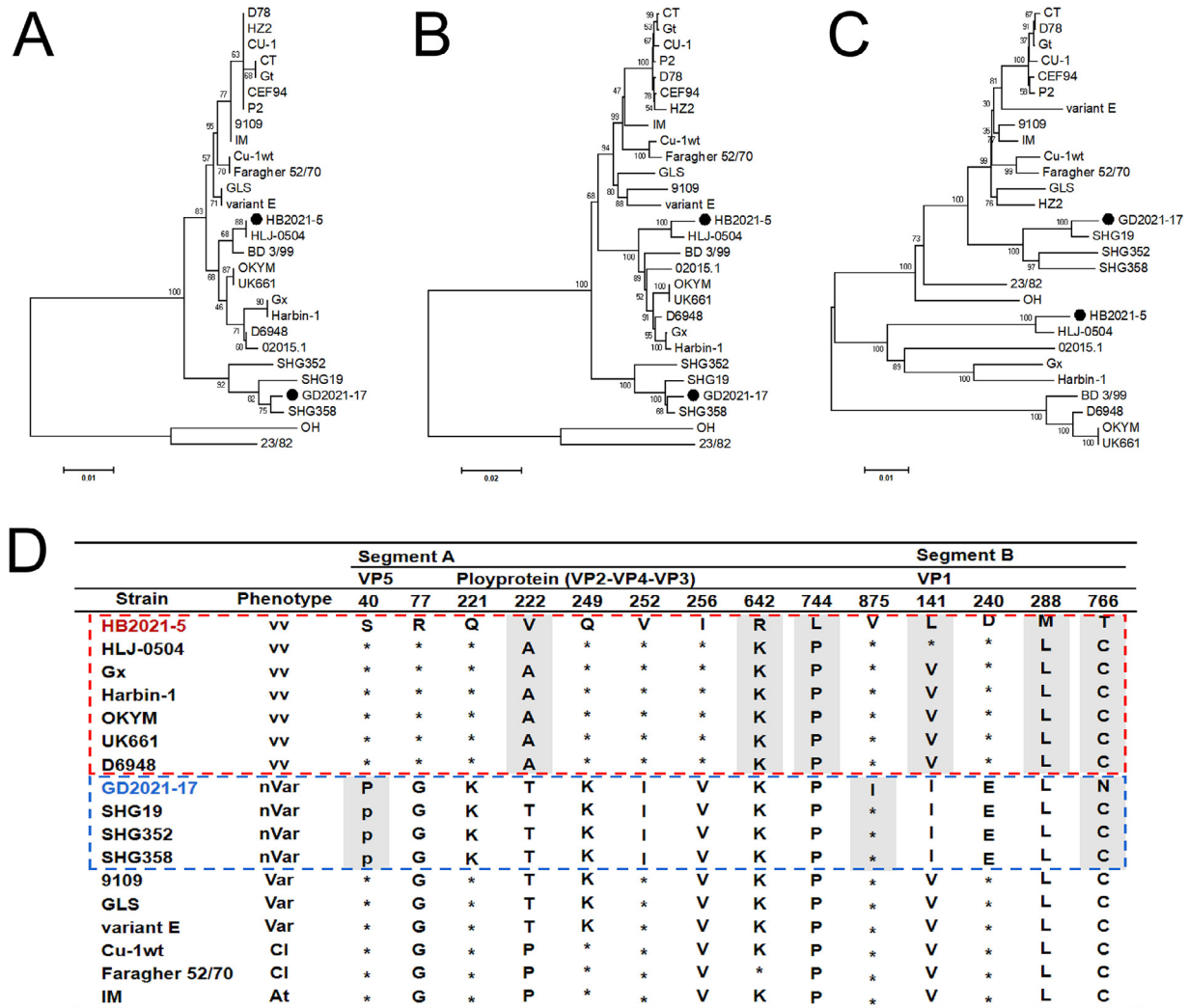
| | | Segment A | | | | | | | | | | Segment B | | | |
| | | VP5 | | Ployprotein (VP2-VP4-VP3) | | | | | | | | VP1 | | | |
Strain	Phenotype	40	77	221	222	249	252	256	642	744	875	141	240	288	766
HB2021-5	vv	S	R	Q	V	Q	V	I	R	L	V	L	D	M	T
HLJ-0504	vv	*	*	*	A	*	*	*	K	P	*	*	*	L	C
Gx	vv	*	*	*	A	*	*	*	K	P	*	V	*	L	C
Harbin-1	vv	*	*	*	A	*	*	*	K	P	*	V	*	L	C
OKYM	vv	*	*	*	A	*	*	*	K	P	*	V	*	L	C
UK661	vv	*	*	*	A	*	*	*	K	P	*	V	*	L	C
D6948	vv	*	*	*	A	*	*	*	K	P	*	V	*	L	C
GD2021-17	nVar	P	G	K	T	K	I	V	K	P	I	I	E	L	N
SHG19	nVar	p	G	K	T	K	I	V	K	P	*	I	E	L	C
SHG352	nVar	p	G	K	T	K	I	V	K	P	*	I	E	L	C
SHG358	nVar	p	G	K	T	K	I	V	K	P	*	I	E	L	C
9109	Var	*	G	*	T	K	*	V	K	P	*	V	*	L	C
GLS	Var	*	G	*	T	K	*	V	K	P	*	V	*	L	C
variant E	Var	*	G	*	T	K	*	V	K	P	*	V	*	L	C
Cu-1wt	Cl	*	G	*	P	*	*	V	K	P	*	V	*	L	C
Faragher 52/70	Cl	*	G	*	P	*	*	V	*	P	*	V	*	L	C
IM	At	*	G	*	P	*	*	V	K	P	*	V	*	L	C

Figure 3. The phylogenetic tree represented the nucleotide sequences of VP5 (A), polyprotein (PP) (B), VP1 (C) of HB2021-5 and GD2021-17. The GD2021-17 and GD2021-17 were highlighted with a solid circle. The trees were generated by the neighbor-joining method using MEGA7 software. The differences of the aa residues among the vvIBDV (vv), nvIBDV (nVar), attenuated (At), variant (Var), classical (Cl), the representative isolates HB2021-5 and GD2021-17 in VP5, polyprotein (PP) and VP1 (D).

compared to nvIBDV reference strains. Compared to the other vvIBDVs, 3 distinct conserved amino acid residues (141 L, 288 M and 766 T) were observed in VP1. Only one distinct conserved amino acid residue (766 N) was observed in VP1 of GD2021-17 compared to nvIBDV reference strains.

All these data indicated that only minor evolution occurred in vvIBDV strain HB2021-5 or nvIBDV strain GD2021-17 and most mutations are synonymous.

Pathogenicity of vvIBDV Strain HB2021-5 and nvIBDV Strain GD2021-17

The pathogenicity experiment of vvIBDV strain HB2021-5 and nvIBDV strain GD2021-17 to 21-day-old SPF chickens was performed. Typical clinical symptoms of IBD including ruffled feathers, depression, decreased appetite and drinking, and reluctance to move were observed in some birds of the groups challenged with HB2021-5 and GD2021-17. The mean body weight was significantly reduced in birds challenged with HB2021-5 compared to the blank controls ($P < 0.01$). Surprisingly, there was no significant difference in body weight between the GD2021-17 groups and PBS control groups (Figure 4A). At necropsy, the bursa was atrophied (BBIX < 0.7) in all challenged birds significantly (Figure 4B). Compared to the chickens in the control group, the mortality rates in the groups challenged with HB2021-5 and GD2021-17 were 60% (3/5) and 0% (0/5), respectively. And HB2021-5-infected chickens began to die at 36 hpi (Figure 4C). Slight and severe bursal atrophy were induced by HB2021-5 and GD2021-17 at 4 d p. i. Typical pathological bursa atrophy was observed in all the birds challenged with HB2021-5 and GD2021-17. Interestingly, bursa haemohemorrhag was only observed in the birds challenged with HB2021-5 (Figure 4D).

Three randomly selected bursal tissues from all groups were subjected to HE staining. Microscopic lesions were observed in the bursa tissues of all the challenged birds. Interestingly, the most severe lesions were observed in the HB2021-5 groups with follicle atrophy
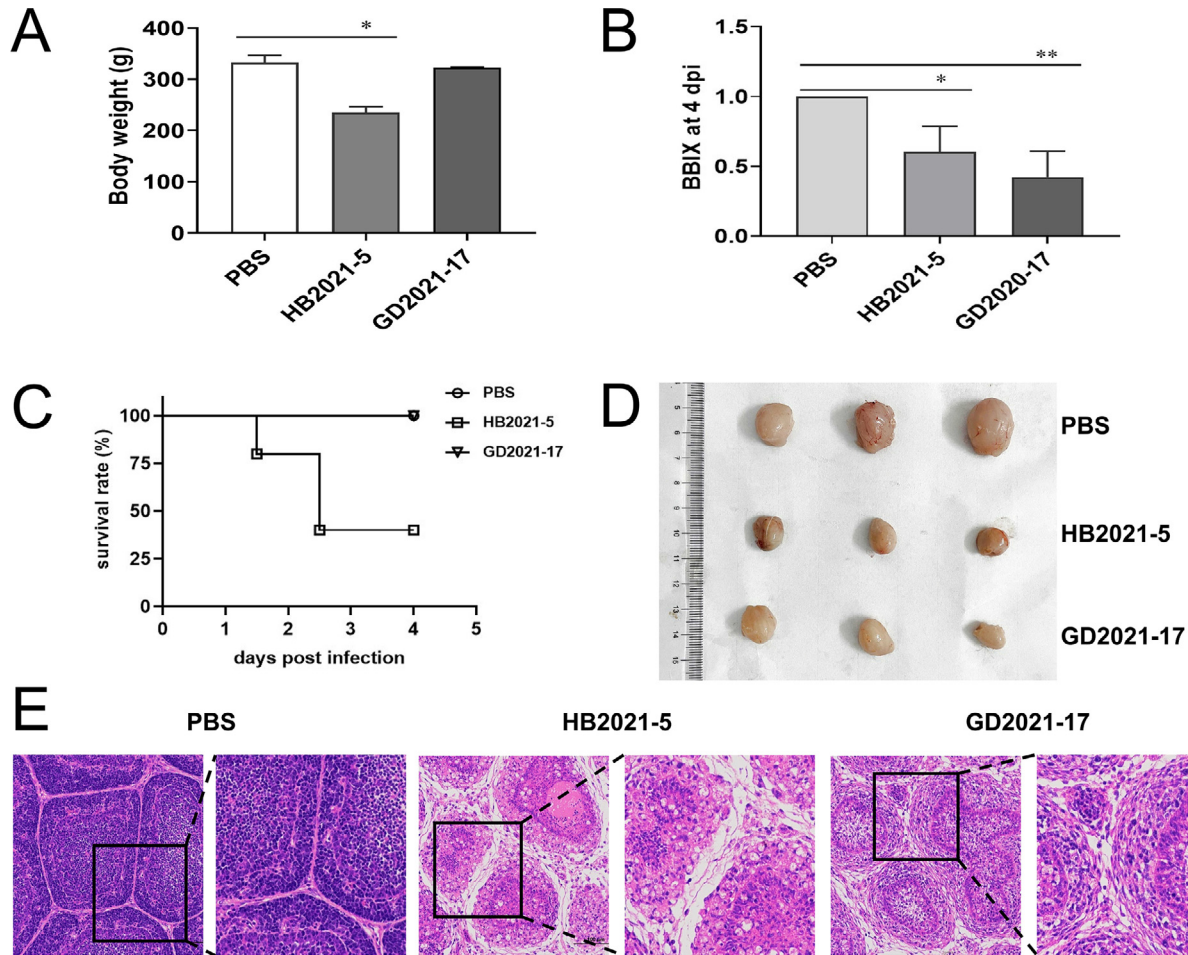
Figure 4. Pathogenic analysis of HB2021-5 and GD2021-17 in SPF chickens. (A) Body weights. (B) The bursa/body-weight index (BBIX) ratio. (C) Survival rates for each group. (D) The BF images of the challenged and the control groups at 4 dpi. (E) Histopathological changes of BF of birds from different groups at 4 dpi (200 ×).

and loss of the outline of follicular architecture, being replaced by the proliferated connective tissue and reticular cells. And then the GD2021-17 groups with half of the follicles have atrophy or depletion and vacuoles in the germinal center of the BF (Figure 4E). These data confirmed that HB2021-5 and GD2021-17 showed high pathogenicity in the chicken.

DISCUSSION

IBD is one of the most economically destructive immunosuppressive viral diseases in poultry and spreading worldwide. Following the first emergence of classical IBDV in the United States in 1957, the clIBDV, vvIBDV, and varIBDV emerged successively around the world. Since the first report of IBDV in China, IBD induced by various phenotypes of IBDV caused direct economic losses due to high morbidity and mortality rates and has posed severe damage to the poultry industry (He et al., 2014; Lu et al., 2015b; Wang et al., 2019). In 2017, the Chinese nvIBDV was isolated and identified from a number of suspected subclinical infections of IBDV (Fan et al., 2019; Fan et al., 2020). Since then, nvIBDV has been widespread across the country and

changed the prevalence of IBDV in southern China. In addition, the emergence of the naturally recombinant infectious bursal disease virus in China has been reported frequently (Wang et al., 2021a; Feng et al., 2021).

In this study, we identified 30 IBDV strains, including 13 vvIBDV and 17 nvIBDV, which were isolated from 8 provinces in south China. It shows that the IBDV is still circulating in southern China. The infected flocks were observed predominately in 3 to 6-wk-old chickens and rooted from June to September. It shows that the IBDV mainly infects young chickens in summer.

IBDV is dependent on epitopes or specific molecular signatures on the *vp2* gene (Letzel et al., 2007) that are commonly used for the identification and molecular characterization of IBDV (Letzel et al., 2007). A sequence analysis based on the representative fragment of *vp2* showed that all 30 strains formed 2 branches with the vvIBDV and nvIBDV strains, respectively. The nvIBDV isolates shared a high degree of nucleotide (94.9 −99.2%) similarity to the SHG19 while the vvIBDV isolates shared 95.2 to 99.5% nucleotide identity to HLJ-0504 from China, respectively. The homology analysis suggested the internal evolution of IBDV isolates. The result illustrated that the epidemic trend of IBDV in southern China in 2021 emerged as a new situation and

universality. This is consistent with the previous reports (Feng et al., 2021; Lian et al., 2022).

Though the phylogenetic and comparative analyses revealed the highly homologous of vvIBDV and nvIBDV isolates, respectively, further research on the molecular characteristic of the representative strains of vvIBDV and nvIBDV isolates is worthy and necessary (Feng et al., 2021; Wang et al., 2021b). The phylogenetic trees were constructed based on the sequences of *vp5* gene, *PP* gene and *vp1* gene all showed that HB2021-5 and GD2021-17 were also closely related to the vvIBDV HLJ-0504 and nvIBDV SHG19, respectively. Besides, 6 amino acid residue differences of HB2021-5 located in PP (222V, 642R, and 744L) and VP1 (141 L, 288 M and 766 T) compared to the other vvIBDV. Similarly, compared to nvIBDV reference strains, only 2 amino acid residue differences located in PP (875I) and VP1 (766 N) were also found in GD2021-17. These results showed that HB2021-5 and GD2021-17 had high homology with the strains spreading in China and the mutated amino residues located in the conserved region.

To evaluate the pathogenicity, the HB2021-5 and GD2021-17 were selected to infect 21-day-old chickens in our study (Feng et al., 2021). Compared to the blank controls, the mean body weight was reduced in chicken challenged with HB2021-5 significantly but GD2021-17, which was different from the previous report on nvIBDV (Fan et al., 2020; Feng et al., 2021; Wang et al., 2021b; Lian et al., 2022). For IBDV, the degree of damage to the bursa has been extensively used as a good index of virulence. The bursa was atrophied (BBIX < 0.7) in all the challenged birds significantly. This is consistent with the previous reports (Wang et al., 2021b; Lian et al., 2022). Furthermore, the histopathological changes in the bursa were detected in detail. Also, HB2021-5 had more significant histopathological changes compared to GD2021-17, including follicle atrophy and loss of the outline of follicular architecture.

In conclusion, we tracked the complex epidemiology and pathogenicity of the IBDV in southern China. Surveillance was conducted to identify IBDV strains isolated from commercial chicken farms. IBDV strains were isolated and phylogenetic analysis was performed to investigate the prevalence. Furthermore, the representative strains of vvIBDV HB2021-5 and nvIBDV GD2021-17 were sequenced and the molecular characteristics were analyzed. Subsequently, a pathogenicity experiment of HB2021-5 and GD2021-17 was performed. The results of our findings provided a basis for the epidemiology and evolution of IBDV in southern China.

Ethical approval and consent to participate: The animal experiments were approved by the Animal Care Committee of the South China Agricultural University, Guangzhou, China (approval ID: SYXK-2019-0136). All study procedures and animal care activities were conducted by the national and institutional guidelines for the care and use of laboratory animals. The birds were maintained in isolators with negative pressure and food and water were provided ad libitum.

DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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