Antigenic and Molecular Characterization of Infectious Bursal Disease Virus in China From Layer Chicken Flocks*

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Abstract Five field isolates of infectious bursal disease virus (IBDV) were isolated from bursae of sick young chickens by inoculating chicken embryo fibroblasts (CEF) while the morphological and physiochemical properities of these isolates were detected. Subtype of the virus isolates were analysed by cross virus neutralization. Virulence of IBDV isolates was determined with specific pathogen-free (SPF) chicken inoculation. Nucleotide sequence of genomic segment A of the isolate NB was further sequenced and analysed. All bursal suspensions produced cytopathic effects (CPE) in CEF after 2~14 passages. The physiochemical and morphological properties of IBDV isolates were in accordance with that of typical IBDV. The three CEF-adapted virus isolates JD1, JD2 and NB were divided into 3 subtypes of serotype Ⅰ IBDV except IBDV isolates HZ1 and HZ2 belonged to classical IBDV. In a pathogenicity experiment, the clinical signs and bursal atrophy resembling those of field outbreaks were produced and microscopic bursa lesions revealed that there was degeneration, necrosis and disappearance of lymphocytes in the medullary area of bursal follicles. Sequence data demonstrated that genomic A-segment of the isolate NB was composed of 3 259 nucleotides, encoding VP5 of 145 amino acid residues and the polyprotein (VP243) of 1 012 amino acid residues. Compared with serotype Ⅰ IBDV strains from GenBank, within serotype Ⅰ IBDV strains, the isolate NB has the highest identity to the polyprotein of the isolate JD1 (99.5%), VP2 of the isolates JD1, CEF94 and D78 (99.8%), VP3 of the isolate JD1 (99.2%), VP4 of the isolate JD1 (100%) and VP5 of the isolates JD1, HZ2, P2, CEF94, CT, Cu-1 and D78 (99.3%). In the VP2-predicted amino acid sequence of the isolate NB, amino acid residues at positions 253, 280 and 284 were consistent with and other published variant and classical IBDV strains, and different from very virulent IBDV. These results indicated that antigenic epitopes of IBDV are conformational and subtype forming of IBDV isolates originated from classical IBDV attenuated vaccine strains.

Key words infectious bursal disease virus, physiochemical characteristics, subtype, genomic A-segment

Infectious bursal disease virus (IBDV) is a member of the family Birnaviridae, which is the causative agent of infectious bursal disease (IBD) and results in a highly contagious and immunosuppressive disease of great economic importance to the poultry industry. IBDV genome consists of two segments (A and B) of double-stranded RNA molecules incorporated into a non-enveloped icosahedral virion 60 nm in diameter[1]. Genome of IBDV encodes five viral polypeptides, designated VP1~5. VP2~5 are encoded by the large segment A, and VP1 is encoded by the smaller segment B[2]. VP2 and VP3 are the major capsid proteins, making up 51% and 40% of the virion, respectively. The VP2 is the major host-protective immunogen, with epitopes that elicit neutralizing antibodies that determine serotype and strain specificity. The VP3 has a group-specific antigen, while the VP5 plays a role in virus pathogenesis[3]. Furthermore, Lombardo *et al.*[4] and Yao *et al.*[5] have proved that VP5 is a cytolytic protein that accumulates within the plasma membrane of infected cells and promotes the egress of the viral progeny.

In comparison with the antigenic relationships of IBD viruses isolated from chickens, turkeys and ducks, McFerran *et al.*[6] found that there are at least two serotypes of IBDV. In similar studies on IBD viruses isolated in the United States, Jackwood *et al.*[7] also demonstrated the existence of two serotype, which they designated serotype Ⅰ and serotype Ⅱ. Neutralizing antibodies to serotype Ⅰ and serotype Ⅱ were detected in 77% and 47%, respectively, of 75 Ohio commercial chicken layer flock[8]. Serotype Ⅰ viruses is most prevalent in chickens and serotype Ⅱ is most prevalent in turkeys, although cross-species infection may occur. Field studies have shown that certain chicken flocks may be infected with virus isolates serologically related but not identical to

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serotype Ⅰ IBDV, when vaccinated with classical serotype Ⅰ IBDV vaccine[9]. Rosenberger and Cloud[10] isolated four serotype Ⅰ IBDV that also exhibited antigenic differences from serotype I IBDV reference strains. Jackwood and Saif [11] found that there were six subtypes among the thirteen serotype Ⅰ strains tested. Restriction fragment length polymorphism (RFLP) has been used to form six different molecular groups of IBDV[12,13]. Nucleotide sequencing of RT-PCR products is widely used for further characterization of IBDV strains [14~18]. Therefore, the pathogenic serotype Ⅰ IBDV isolates are subdivided into classical, antigenic-variant, and very virulent isolates. Variant strains of IBDV were found to have single amino acid changes in a specific region of the VP2 protein (the hypervariable region, HVR) from the classical strains, causing immunosuppression but not clinical disease[19]. Since 1988, in Europe there were reports describing IBDV isolates that had an enhanced virulence [very virulent IBDV (vvIBDV)] while having the same antigenic structure as classical isolates[20]. Amino acid differences between viral proteins of vvIBDV and classical IBDV isolates were found scattered throughout all viral proteins, although most of them were found in the hypervariable region of VP2[21]. It is currently unknown whether all or only a few of these amino acid mutations contribute to the enhanced virulence of the vvIBDV isolates.

Since 1988, many clinical IBD cases have been reported in China and IBD has become a familiar infectious disease in chicken farms. Unfortunately, although the control measures of IBD were widely vaccination of live attenuated IBD vaccine (classical virus strain) such as B87 and D78 strain in China, chicken flocks vaccinated with live attenuated IBD vaccine usually fail to present full protection from field virulent IBDV challenge. A recent investigation indicated that there was still prevalence of IBD in Zhejiang, China. We postulated that there might be antigenically several different subtypes among serotype Ⅰ IBDV strains involved in the outbreaks. The objectives of present study were to isolate the field IBDV isolates and detect their antigenic relatedness. The symptom and pathological changes similar to the field outbreaks were reproduced in specific-pathogen-free (SPF) chickens and the virus isolate was molecularly characterized.

1 Materials and methods

1.1 Materials

1.1.1 Chicken embryos and chickens. SPF white leghorn embryonated chicken eggs and chickens were purchased from Beijing Merial Vital Laboratory Animal Technology Co., Ltd., Beijing, China. Embryonated eggs were used for isolation of field isolates and re-isolation attempt of IBV from bursal tissues of the experimental infected chickens. SPF chickens were used for pathogenicity experiment.

1.1.2 Vaccine viruses. Commercial chicken embryo fibroblast adapted strain IBDV B87——a very common vaccine IBDV strain in China, was obtained from Hangzhou Jianliang Veterinary Bioproducts co., Ltd., Hangzhou, Zhejiang, China.

1.2 Methods

1.2.1 Cell culture and isolation of viruses. Chicken embryo fibroblast (CEF) was prepared using 9 to 11 days old embryos from SPF chicken embryonated eggs. The bursas clinically diagnosed as IBD were homogenized with a mortar and pestle in sterile normal saline (25% *w*/*v*), designated respectively as HZ1, HZ2, JD1, JD2, and NB according to their sources. The suspension was subjected to three cycles of freezing and thawing. This was followed by centrifugation at 7 800 *g*, 4℃ for 20 min. The suspension was filtered through 0.22 μm membrane (Acrodisc® Syringe Filters, Pall Life Sciences, New York, USA) and infused into CEF monolayer in a dose of 0.2 ml per 25 cm^2 flask for virus isolation.

1.2.2 Virus purification and electron microscopic examination. CEF-adapted virus isolates were frozen and thawed three times and centrifuged at 7 800 *g*, 4℃ for 30 min. Supernatants were collected and subjected to further centrifugation at 120 000 *g*, 4℃ for 3 h. Precipitates were then resuspended in the ultra-purified water. Virus samples were negatively stained with 2% sodium phosphotungstate and observed under the transmission electron microscope.

1.2.3 Physical and chemical characteristics. CEF-adapted virus isolates were exposed to 20% ether, acidic (pH 3.0) and alkaline (pH 12) conditions, heat (56℃), repeated cycles of freezing and thawing, respectively. A volume of 0.2 ml of such treated virus isolates was inoculated into flask containing monolayer of CEF cells. Both cytopathic effects (CPE) and $TCID_{50}$ were determined on the fifth day of incubation.

1.2.4 Antiserum. Eighty-day-old SPF chickens were inoculated intramuscularly with 0.5 ml of the inactivated virus suspensions in complete Freund's adjuvant. The chickens received boosting injection (1 ml per chicken) 15 days later and were bled 20 days thereafter for examination of serum antibody responses to the viruses. Antiserum to IBDV B87 strain was purchased from China Institute of Veterinary Drug Control, Beijing, China.

1.2.5 Detection of $TCID_{50}$ and cross virus neutralization test. CEF-adapted IBDV isolates HZ1, HZ2, JD1 and NB at the 15th passage and isolate JD2 at the 13th passage were sequentially diluted with MEM medium and infused respectively into the thin monolayers of CEF cells for detecting their $TCID_{50}$. $TCID_{50}$ of the virus isolate was calculated by Reed-Muench method. Two hundred $TCID_{50}$ of each virus isolate were mixed with the same volume of reference antiserum against IBDV and incubated for 1 h at 37℃. Then virus-serum mixtures were inoculated into the monolayers of CEF cells and CPE were examined on the fifth day of incubation. Antibody titres were determined to homologous and heterologous IBDV strains. To facilitate interpretation of the virus neutralization (VN) results, the formula of Archetti and Horsfall[22] was employed to express the antigenic relatedness of viruses within a serotype.

1.2.6 Experimental inoculation. 28 day-old SPF leghorn chicks were divided into 6 groups, with 5 chicks per group. Each group was inoculated intraocularly with a dose of 0.1 ml (50 000 $TCID_{50}$) of the viral suspension containing the CEF-adapted virus isolates HZ1 and HZ2 at 3rd passage, JD1 at 15th passage, JD2 at 11th passage and NB at 7th passage, respectively. The inoculated chicks were slayed with CO_2 in a box 72h post-inoculation for examination of body weight and bursa lesions. Bursa-body index (BBIX) was defined as the division of bursa-to-body ratio of infected chickens by bursa-to-body ratio of negative control chickens. Bursal histological lesions were examined with Hematoxylin-Eosin staining.

1.2.7 Amplification and analysis of genomic segment A for the virus isolate. Primers for amplification of A-segment of the virus isolate were designed with reference to the nucleotide sequence of IBDV-P2

Table 1 GenBank accession numbers of genomic segment A of IBDV used in this study

Strain	Accession Number	Serotype	Virulences	Countries or areas isolated	Year
TASIK	AF322444	Ⅰ	VV	Indonesia	1994
HK46	AF092943	Ⅰ	VV	Hong Kong, China	1994
KS	L42284	Ⅰ	VV	Israeli	1995
OKYM	D49706	Ⅰ	VV	Japan	1991
UK661	X92760	Ⅰ	VV	United Kingdom	1989
SH95	AY134874	Ⅰ	VV	China	
T09	AY099456	Ⅰ	VV	Nigeria	1998
D78	AF499929	Ⅰ	VV	Nigeria	1995
UPM94/273	AF527039	Ⅰ	VV	Malaysia	1994
Harbin-1	AF454945	Ⅰ	VV	China	
002/73	X03993	Ⅰ	CV	Australia	1972
52/70	D00869	Ⅰ	CV	United Kingdom	1976
STC	D00499	Ⅰ	CV	USA	1975
IM	AY029166	Ⅰ	CV	USA	
GLS	M97346	Ⅰ	AV	USA	1987
E	AF133904	Ⅰ	AV	USA	1994
XJ-9	M97346	Ⅰ	AV	USA	
Cu1-M	AF362771	Ⅰ	A	Germany	1994
Cu-1	X16107	Ⅰ	A	Germany	1977
P2	X84034	Ⅰ	A	Germany	1973
PBG98	D00868	Ⅰ	A	United Kingdom	1989
Edgar	AY462026	Ⅰ	A	USA	
JD1	AF321055	Ⅰ	AV	China	1997
HZ2	AF321054	Ⅰ	CV	China	1997
CEF94	AF194428	Ⅰ	A	Netherlands	1973
Soroa	AF140705	Ⅰ	A	Cuba	1997
OH	U30818	Ⅱ		Canada	1991
23/82	AF362773	Ⅱ		Germany	1985

CV: classical virulent; VV: very virulent; A: attenuated; AV: antigenic variant.

strain (GenBank Accession No.X84034) with a few modifications. A-forward (5' GGAATTCGGATACGA TCGGTCTGACCCCGG 3') and A-reverse [5' GCGC GCGGCCGC GGGGACCCGCGAACGGAT 3' (for PCR) or GGGGACCCGCGAACGG (for RT)] primers flanked a 3.3-kilobase sequence containing the whole A-segment gene. The genomic RNA of the isolate NB was extracted as described by Zhou *et al*[23]. RT-PCR was carried out with some modification as described by Zhou *et al*[24]. Briefly, Full-length complementary DNA (cDNA) of A-segments of IBDV was synthesized using SuperScript Ⅱ RNase H-Reverse Transcriptase (Invitrogen). The cDNA template was amplified using One Shot LA PCR™ Mix (TaKaRa, Cat. No. DRR04). After denaturing the template DNA for 5 min at 94℃, PCR consisted of 30 cycles of denaturation at 94℃ for 15 s, primer annealing at 61℃ for 15 s, and primer extension at 68℃ for 3 min, with a final single extension step at 72℃ for 10 min. The PCR products were analyzed on a 1% agarose gel. The amplified PCR product containing the entire genomic segment A were purified and ligated with pCI-neo vector using DNA Ligase Kit Ver.2 (TaKaRa, Cat. No. C610-00) at 16℃ for 1 h. Ligation products were transformed into the competent *E.coli* Top 10 strain (Invitrogen). Five clones were sequenced in an automated DNA sequencer (ABI prism 377; ABI Porkin-Elmer Corporastion, Foster city, Ca, USA). The alignment and phylogenetic analysis of the deduced amino acid sequences of A segment gene were performed with the Jotun Hein algorithm and Clustal method by DNAStar (DNASTAR, Madison, Wisc., USA). GenBank accession numbers of IBDV strains used in this study were shown in Table 1.

2 Results

2.1 Virus isolation and physiochemical properties

The virus isolates HZ1 and HZ2 produced CPE in CEF at the 2nd passage while the isolates NB, JD2 and JD1 formed CPE at the 6th, 10th and 14th passage respectively. The CPEs were evident with the virus isolates HZ1, HZ2, JD1 and JD2 24 h post-inoculation. For the isolate NB, however, CPEs were observed 48 h post-inoculation. Characteristics of CPE for CEF monolayer infected with the inoculum were that the infected cells become round, increasing light refrangibility. The virions were hexagonal, measuring. In electron microscopy, the size of the virus isolate was 55~60 nm in diameter and the virions were hexagonal (Figure 1).

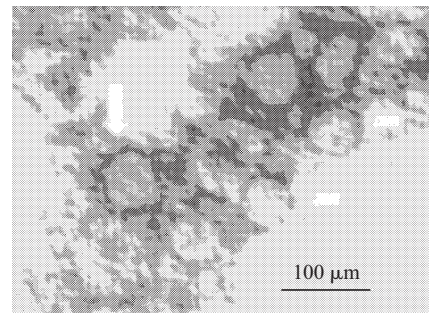

Fig. 1 Electron micrograph of virion of isolate NB
Virions were negatively stained with 2% sodium phosphotungstate

CEF-adapted IBDV isolates HZ1, HZ2, JD1, JD2, and NB were able to infect the monolayer of CEF after treatment with 20% ether for 20 min and acid for 3 h at room temperature, exposure to 56℃ and repeated cycles of freezing and thawing. IBDV isolates HZ1, HZ2, JD1, JD2, and NB exposed to alkaline lost their infectivity to CEF monolayer (Table 2).

Table 2 Infectivity of CEF-adapted IBDV isolates on chicken embryo fibroblasts after physical and chemical treatments

Virus isolate	56℃		Frozen-thawing		pH value		20% ether	Virus control
	90 min	120 min	5 times	10 times	3	12		
HZ1	$10^{4.83*}$	$10^{5.37}$	$10^{5.83}$	$10^{5.67}$	$10^{5.83}$	0	$10^{5.83}$	$10^{5.50}$
HZ2	$10^{5.50}$	$10^{5.32}$	$10^{5.63}$	$10^{5.38}$	$10^{4.83}$	0	$10^{5.00}$	$10^{5.50}$
JD1	$10^{4.32}$	$10^{5.34}$	$10^{5.83}$	$10^{5.62}$	$10^{5.83}$	0	$10^{5.67}$	$10^{5.50}$
JD2	$10^{4.00}$	$10^{4.83}$	$10^{5.50}$	$10^{5.62}$	$10^{5.83}$	0	$10^{4.83}$	$10^{4.83}$
NB	$10^{3.17}$	$10^{3.83}$	$10^{3.83}$	$10^{3.83}$	$10^{3.83}$	0	$10^{3.83}$	$10^{3.83}$

* indicates $TCID_{50}$.

2.2 Serosubtype of the virus isolates

The Data in Table 3 shows the antigenic relatedness of 6 IBDV strains. The first subtype included the vaccine virus strain B87, the isolates HZ1 and HZ2. These three virus strains had an average *R* value of 0.87. The field isolate JD1 exhibited a mean *R* value of 0.71 against the first subtype. The mean *R* value for the isolate JD2 was 0.68 against the first

subtype and 0.58 against the isolate JD1. The isolate NB had average *R* values of 0.61 against the first subtype, 0.61 against JD1 and 0.50 against JD2.

Table 3 Antigenic relatedness expressed as *R* values of infectious bursal disease vaccine virus strains and field virus isolates

Virus strain	HZ1	HZ2	B87	JD1	JD2	NB
HZ1	1.00					
HZ2	0.87	1.00				
B87	0.87	0.87	1.00			
JD1	0.62	0.76	0.76	1.00		
JD2	0.66	0.66	0.72	0.58	1.00	
NB	0.71	0.66	0.47	0.61	0.50	1.00

2.3 Pathogenicity to chicken

Chicken artificially inoculated with CEF-adapted IBDV isolates HZ1, HZ2, JD1, JD2, and NB exhibited the clinical signs as ruffled feather and depression 48 h post inoculation, and presented bursal atrophy (Figure 2) and a decrease in bursa-body index (Table 4) 72 h post inoculation at autopsy. In double immunodiffusion test, antiserum to IBDV B87 strain appeared respectively white precipitate lines with the supernatant of the bursal suspension from the chickens infected with IBDV isolates HZ1, HZ2, JD1, JD2 and NB. Microscopic bursa lesions revealed that there was degeneration, necrosis and disappearance of lymphocytes in the medullary area of bursal follicles, and there was a fibroplasia in interfollicular connective tissue (Figure 3).

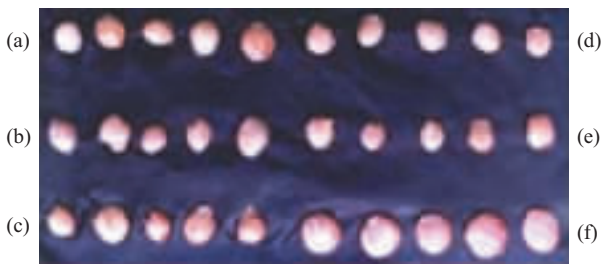

Fig. 2 Bursal gross lesion 72 h postinoculation
(a) the isolate NB; (b) the virus isolate JD1; (c) the virus isolate JD2; (d) the virus isolate HZ1; (e) the virus isolate HZ2; (f) control.

Table 4 Bursa-body indices of chickens experimentally infected by the field virus isolates

Virus isolate	HZ1	HZ2	JD1	JD2	NB
Bursa-body index	0.67	0.55	0.62	0.82	0.89

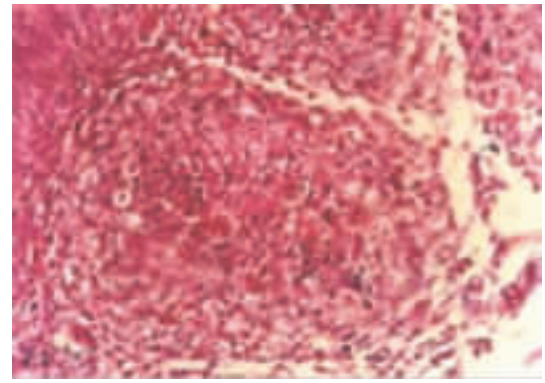

Fig.3 The degeneration, necrosis and disappearance of lymphocytes in the medullary area of bursal follicles 72 h postinoculation

2.4 Molecular characterization of genomic segment A

We efficiently generated full-length cDNA of genomic segment A of IBDV isolate NB by long and accurate PCR (LA-PCR) in a single step (Figure not shown). The sequence identified, genomic segment A was composed of 3 259 nucleotides (GenBank accession number: AY319768), containing two separate and partially overlapping ORFs, encoding VP5 and the polyprotein (VP243). The predicted amino acid sequences of the polyprotein and VP5 consist of 1 012 and 145 amino acid residuels in length respectively, consistent with the length of other IBDV strains. The deduced amino acid sequence of encoding proteins for genomic segment A of IBDV isolate NB was compared with serotype Ⅰ IBDV strains from GenBank, the polyprotein revealed 95.7% to 99.7% identities, VP2 shared 93.4% to 99.8% identities, VP3 appeared 94.2% to 99.2% homologies, VP4 exhibited 96.3% to 100% and VP5 indicated 94.5% to 99.3% homologies. Compared with serotype Ⅱ IBDV strains OH and 23/82, the isolate NB was 90.0% to 90.9% homologies to the polyprotein, 87.9% to 89.1% homologies to VP2, 93.0% to 93.8% homologies to VP3, 91.4% homology to VP4 and 78.1% to 80.8% identities to VP5. Within serotype Ⅰ IBDV strains, the isolate NB has the highest identity to the polyprotein of the isolate JD1 (99.5%), VP2 of the isolates JD1, CEF94 and D78 (99.8%), VP3 of the isolate JD1 (99.2%), VP4 of the isolate JD1 (100%) and VP5 of the isolates JD1, HZ2, P2, CEF94, CT, Cu-1 and D78 (99.3%) when the predicted amino acid sequences of the encoding protein of segment A were compared with other published IBDV strains in GenBank. Amino acid analysis also showed that amino

acid residues in the VP2-predicted amino acid sequence of NB and other published variant and classical IBDV strains are respectively histidine at position 253, asparagines at position 280 and threonine at position 284 while very virulent IBDV and classical IBDV 52/70 strain originated from Europe are respectively glutamine at position 253, aspartic acid at position 280 and alanine at position 284 (Figure 4). Meanwhile, homological comparisons indicated that amino acid residue at position 137 is arginine in the VP5-predicted amino acid sequences of NB and other published variant and classical IBDV strains while that of very virulent IBDV strain is tryptophan. As shown in Figure 5, in addition, phylogenetic analysis showed that the predicted amino acid sequence of polyprotein of the isolate NB exhibited the close genetic relationship with other classical and variant IBDV strains in serotype Ⅰ of IBDV.

	250	260	270	280	290
NB	FQT	SVHGLVLGAT	IYLIGFDGTA	VITRAVAANN	GLTTGTDNLL
JD1	...	.....	.....	.....	.....
HZ2	...	.....	.....	.....	.....
D78	...	.....	.....	.....	.....
CEF94	...	.....	.....	.....	.....
Cu-1 M	...	.....	.....T	.....	.....M
CT	.R.	.....	.....T	.....	.....M
PBG98	.R.	.....	.....T	.....T	.....M
Bursine-2	...	.....	.....T	.....	.....M
TS	...	.....	.....T	.....	.....D.M
HN3	...	.....	.....T	.....	.....M
UK661	...	..Q..I....	.....	.....D.	...A.....M
OKYM	...	..Q..I....	.....	.....D.	...A.....M
HK46	...	..Q..I....	.....	.....D.	...A.....M
HK96	...	..Q..I....	.....	.....D.	...A.....M
D11-2	...	..Q..T....	.....	...T....D.	...A.....M
52/70	...	..Q.....	.....	.....D.	...A.....M
Miss	...	..QS.....	V.....AT	.....D.	...A.....M
Ga	.K.	..QS.....	.....Q	.....	...A.....M
E	.K.	..QS.....	.C.....	.....	...A.....M
GLS	.K.	...S.....	.....S.	.....	.....M
GZ902	.K.	...S.....	.....	.....	.....M
Var A	.K.	...S.....	.....	.....	.....M

Fig. 4 Homological comparisons based on the VP2-predicted amino acid sequence of the virus isolate NB

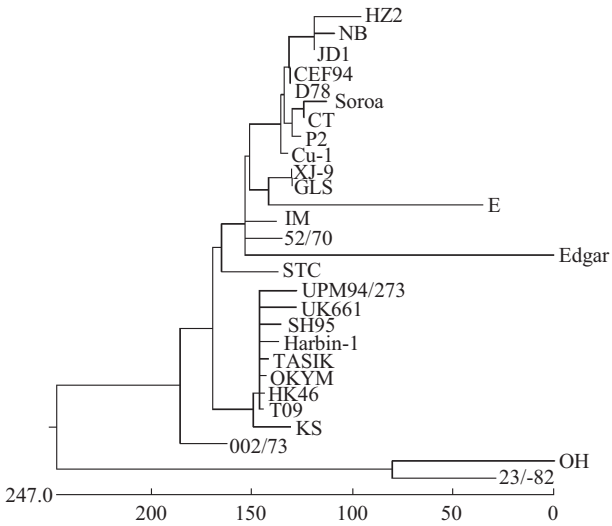

Fig.5 Phylogenetic tree constructed on the basis of the predicted amino acid sequence of the full-length VP2-VP4-VP3 polyprotein of the virus isolate NB

3 Discussion

The virus isolates HZ1, HZ2, JD1, JD2 and NB recovered from bursae of the IBD-vaccinated younger layer flocks showed similar characteristics of IBDV such as size in diameter, morphology and physiochemical properties. After cell-adapted IBDV isolates were passaged in chickens in succession, there were obvious lesions in bursae from the infected chickens with gradual decrease in the bursa-body index (Table 4). The gross and microscopic lesions of the infected chickens with gradual decrease in the bursa-body index (Figure 2 and Figure 3) were the same as those infected with IBDV [25,26]. RT-PCR products of genomic segment A of the virus isolate were obtained using specific primer of IBDV, and its nucleotide sequence also showed high identity with the published certain IBDV isolates. These results indicated that the characteristics of the virus isolates were consistent with that of known IBDV strains as well as the causative agent of IBD for the field outbreaks.

Isolation of IBDV from diseased chickens is generally difficult and involves a time-consuming process: bursal materials →chicken embryo →bursal cells from chicken embryo→kidney cells from chicken embryo→chicken embryo fibroblasts[27,28]. In this study,

bursal suspensions were directly and blindly passaged in CEF monolayers. The virus isolates grew in CEF and formed CPE. However, their adaptability in CEF varied among different field isolates. The virus isolates HZ1 and HZ2 were more readily adapted in CEF than the isolates JD1, JD2 and NB and formed CPE at the 2nd passage, while isolates NB, JD2 and JD1 induced CPE at the 6th, 10th and 14th passage respectively. Whether differences in their adaptability in CEF were related to variation of the viruses was unknown.

In this study, 5 field isolates and 1 commercial classical vaccine strain were classified into 4 subtypes according to their antigenic relatedness (Table 3). Cross virus neutralization analysis reveals the close antigenic relationship within the virus isolates HZ1, HZ2 and B87 belonged to the classical IBDV strain. However, compared with the virus isolates HZ1, HZ2 and B87, the virus isolates JD1, JD2, and NB belong to the different subtypes, respectively, indicating that the virus isolates JD1, JD2, and NB has lower antigenic relationship with the classical strains HZ1, HZ2, and B87. Thus, the virus isolates in the present study have the properties of subtype IBDV and agrees with several earlier reports[6, 10, 11, 29~31].

In analysis of the predicted amino acid sequence of the ORF-encoding proteins for genomic segment A of IBDV strains, the polyprotein encoded by ORFs of the different IBDV strains revealed higher identity between the different subtypes within serotype Ⅰ IBDV strains (95.7% to 99.7%), while the isolate NB is the highest identity with the isolate JD1 including VP2, VP3, VP4 and VP5 proteins. Furthermore, amino acid residues in the VP2 protein of NB and other published variant and classical IBDV strains are respectively histidine at position 253, asparagines at position 280 and threonine at position 284 while very virulent IBDV are respectively glutamine at position 253, aspartic acid at position 280 and alanine at position 284(Figure 4). Moreover, in the VP5 protein of NB and other published variant and classical IBDV strains, amino acid residue at position 137 is arginine while that of very virulent IBDV strain is tryptophan. In addition, the predicted amino acid sequence of polyprotein of the isolate NB exhibited the close genetic relationship with other classical and variant IBDV strains in serotype Ⅰ of IBDV in phylogenetic analysis. These molecular characteristics of NB isolate implied that subtype forming of IBDVs originated from classical IBDV attenuated vaccine strains and antigenic variation of IBDVs is a gradual change. We also postulated that antigenic epitopes of IBDV are conformational dependences according to cross virus neutralization and molecular characteristics of NB isolate. Many works demonstrated that the VP2 epitopes were recognized as conformation dependent [32~36]. Similarly, amino acid residues at positions 222 (A), 256 (I) and 294(I) have been found to be unique to vvIBDV stains in VP2 protein[15, 37, 38]. In VP2 protein, the serine rich heptapeptide SWSASGS located immediately downstream of the second hydrophilic region was believed to involve the virulence of IBDV. All vvIBDV, classical virulent and variant strains possess four serine residues within this region.

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传染性法氏囊病毒的抗原及分子特征 *

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摘要 用鸡胚成纤维细胞对来自野外的 5 个传染性法氏囊病毒株 (IBDV-JD1、JD2、NB、HZ1、HZ2) 进行分离，测定理化特性、致病性，同时进行血清亚型测定及 A 片段基因组的克隆分析. 试验所用 5 个法氏囊组织悬液在鸡胚成纤维细胞盲传 2~14 代后适应细胞并产生细胞病变. 细胞适应的 IBDV 毒株的理化和形态特征与经典传染性法氏囊病毒株一致. 除 IBDV-HZ1、HZ2 属经典 IBDV 血清型外，IBDV-JD1、JD2 和 NB 毒株分属不同的血清亚型. 人工感染实验结果显示，分离的 IBDV 毒株产生与野外病例相似的临床症状和病变，出现法氏囊滤泡髓质的淋巴细胞变性、坏死和消失. 基因组序列分析显示，IBDV-NB毒株 A 片段由 3 264 个核苷酸组成，编码由 145 个氨基酸残基组成的 VP5 和由 1 012 个氨基酸残基组成的多聚蛋白. 与来自 GenBank 的 IBDV Ⅰ型毒株比较，NB 毒株 A 片段编码的多聚蛋白与 JD1 毒株的同源性最高，达 99.5%，VP2 与 JD1、CEF94、D78 的同源性为 99.8%，VP3 与 JD1 的同源性为 99.2%，VP4 与 JD1 的同源性为 100%，VP5 与 JD1，HZ2，P2，CEF94，CT，Cu-1 和 D78 毒株的同源性为 99.3%. NB 毒株 VP2 蛋白的第 253、280、284 位氨基酸残基与 IBDV 变异毒株和经典毒株一致，但不同于 IBDV 超强毒株. 这些结果暗示 IBDV 的抗原表位是构象依赖性表位，IBDV 血清亚型的形成与 IBDV 弱毒疫苗病毒株密切相关.

关键词 传染性法氏囊病毒，理化特性，血清亚型，基因组 A 片段

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