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Characteristics of very virulent infectious bursal disease viruses isolated from Chinese broiler chickens (2012–2013)

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ABSTRACT

The objective of this study was to characterize the infectious bursal disease viruses (IBDVs) circulating in broiler chicken farms in China between 2012 and 2013. The VP2 gene sequences of nine newly isolated IBDVs, obtained using reverse transcriptase polymerase chain reaction, were determined and compared with worldwide reference isolates, which have been previously well characterized. Phylogenetic analysis revealed that the nine broiler IBDV isolates are closely related to very virulent IBDV (vvIBDV) strains. Analysis of the predicted amino acid sequences of VP2 from the nine vvIBDVs isolated from the broilers revealed that they share 99.2 to 100% sequence similarity. Additionally, amino acids A222, I242, I256, I294 and S299 of VP2 that are conserved among previously characterized vvIBDV strains are also encoded by the nine isolates. This study confirms the circulation of vvIBDVs in Chinese broiler chicken farms experienced slow evolution and was relatively stable in China.

1. Introduction

Infectious bursal disease virus (IBDV), a member of the genus *Avibirnavirus* in the family *Birnaviridae*, is the causative agent of an acute and highly contagious immunosuppressive disease, which can cause anorexia, depression, ruffled feathers, diarrhea, prostration and death in chickens (Sharma et al., 2000). Two serotypes of IBDV, serotype 1 and 2, have been differentiated by a virus neutralization test. However, only serotype 1 strains are pathogenic to chickens. Serotype 1 strains can be further classified into four groups: classical virulent strains, antigenic variant strains, very virulent (vv) strains, and attenuated strains (McFerran et al., 1980). vvIBDVs were first reported in Europe in 1989 (Chettle et al., 1989; Van den Berg et al., 1991), these viruses can severely affect the poultry industry by causing up to 70% mortality in chicken flocks. Despite being antigenically very similar to the classical strains (Marel et al., 1990), they show a marked increase in virulence, thus continuing to pose a serious threat to the commercial poultry industry by reducing the food utilization rate and inducing immunosuppression in the birds, which leads to secondary viral and bacterial infections (Saif, 1991).

The IBDV genome is a bisegmented double-stranded RNA consisting of two segments, named segment A and B, which are encapsidated within a single-shelled icosahedral particle with a diameter of 65 to 70 nm. Segment B encodes the VP1 protein, the viral RNA-dependent RNA polymerase (Ursula et al., 2004), whereas segment A encodes VP5 and a protein precursor that separates into VP2, VP3 and VP4. VP2 has been identified as the principal structural and virulence protein, and the viral capsid comprises trimeric VP2. Moreover, the conformational dependent immunodominant epitopes located in a hypervariable region of VP2 (vVP2) are responsible for eliciting type-specific neutralizing antibodies and protective antibodies against IBDV (Brandt et al., 2001; Van den Berg et al., 1996). The vVP2 sequence contains two major hydrophilic peaks (211D-224G, 314T-324Q), three minor hydrophilic peaks (Vakharia et al., 1994), and a serine-rich seven-peptide region SWSASGS (326S–332S). Although vVP2 displays the greatest amount of amino acid sequence variation among pathogenic serotype 1 strains (Bayliss et al., 1990), some parts of the serotype 1 genome also contain relatively conserved regions, which helps in identification of virus types (Hoque et al., 2001). Thus, the molecular characteristics of the VP2 region can provide clues about the evolution and antigenic variation in IBDVs.

Research has also confirmed that VP2 contains amino acid sequences important for tissue tropism (Qi et al., 2009). Field isolates of vvIBDV cannot reproduce in cells, while passaged attenuated virus can propagate in cells and cause cytopathic

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Table 1
Descriptions of the IBDV strains isolated in this study.

Strain	Geographic location	Year	Age (d)[a]	Vaccinated[b]	GenBank accession no.
BJ-2012-1	Beijing, China	2012	27	Yes	KJ624999
BJ-2012-2	Beijing, China	2012	38	Yes	KJ625000
BJ-2012-3	Beijing, China	2012	32	Yes	KJ625001
HLJ-2012-1	Heilongjiang, China	2012	36	Yes	KJ625004
HLJ-2012-2	Heilongjiang, China	2012	31	Yes	KJ625005
HLJ-2012-3	Heilongjiang, China	2012	29	Yes	KJ625006
HB-2012-1	Hubei, China	2012	39	Yes	KJ625003
SD-2013-1	Shandong, China	2013	35	Yes	KJ625007
GD-2013-1	Guangdong, China	2013	35	Yes	KJ625002

[a] Vaccinated: denotes whether the isolate came from a vaccinated chicken.
[b] Age: denotes the age of the chicken from which the isolate was obtained.

effects (Brandt et al., 2001). Yamaguchi compared the polyprotein sequence of the attenuated OKYMT strain with the very virulent strain OKYM strain and found five areas of divergence; one of them was located in VP3 and four of them were located in VP2 (Yamaguchi et al., 1996). Further analysis has shown that tissue tropism is determined by amino acid positions 253, 279 and 284 of VP2 (Brandt et al., 2001).

Nowadays, vaccines are widely used to control IBDV infection in the poultry industry. However, serious outbreaks of IBD still occur in vaccinated chicken flocks. It is suggested this is because some antigenic variant strains bypass the protection conferred by maternal antibodies and commercially available vaccines (Jackwood et al., 2001; Sreedevi et al., 2007). Some variation in amino acids at specific sites in the virus can lead to immune escape. For example, Del-E-222, which is identical to Del-E with the exception of alanine (A) at position 222, and Del-E-254 where asparagine (D) displaces threonine (T) at position 254, can break though the neutralizing immunity provided by Del-E and cause typical macroscopic lesions characteristic of IBDV infection (Jackwood and Sommer-Wagner, 2011). The same authors also found evidence of recombination between the classic and variant amino acid sequence markers, which has not been identified previously in any IBDV strains (Jackwood, 2012). All these situations would lead to immune failure and it is possible that a new vaccine may be required.

IBDV was first reported in China in the 1980s. Since then, IBDV infections that cause high mortality and immune suppression have become prevalent, thus causing great economic losses to the Chinese poultry industry (Chen et al., 2012; He et al., 2012). Several prevalent vvIBDV strains and some amino acid variations in such strains have been reported in previous studies (Li et al., 2009; Liu et al., 2012; Yuwen et al., 2008). In addition, some recombination between vvIBDV strains has been reported in China (Chen et al., 2012; Gao et al., 2007). The aim of the present study was to characterize IBDV strains circulating in broiler chicken farms in China during 2012 and 2013, to further our knowledge on the prevalence of different types of strains of the virus.

2. Materials and methods

2.1. Viruses

Nine IBDV field isolates were obtained from diseased broiler flocks in different provinces between 2012 and 2013. The descriptions of the IBDV strains isolated in this study are listed in Table 1. All the strains were propagated in 10-day specific-pathogen-free chicken embryos via the chorioallantoic membrane route, and the allantoic fluid that contained infectious viruses was harvested as a source of stock viruses and stored at −20 °C until further analysis.

2.2. Viral RNA extraction

A 250 μl volume of homogenized allantoic fluid was extracted with 750 μl of TRIZOL (Invitrogen, Carlsbad, CA, USA), thoroughly mixed with 200 μl of chloroform, and incubated at room temperature for 5 min. The mixture was subjected to centrifugation at 15,000 × *g* for 15 min at 4 °C. Viral RNA in the supernatant was precipitated with cold isometric isopropanol and collected using centrifugation at 12,000 × *g* for 10 min at 4 °C. The supernatants were discarded and the sediments were rinsed twice using 1 ml of 75% ethanol in DEPC-treated water. After discarding the supernatants, the RNA precipitate was air-dried on a super-clean bench for 5–10 min. The precipitate was dissolved in 9 μl of DEPC and 1 μl of RNAsin (Takara, Osaka, Japan). Thereafter, the viral RNA was reverse transcribed into cDNA or stored at −80 °C.

2.3. RT-PCR

Reverse transcription reactions were conducted as follows: four microliters of the extracted RNA and 1 μl of random primers (500 μg/ml, Promega, Madison, WI, USA) was incubated in a 70 °C water bath for 5 min, immediately cooled in an ice-bath for 2 min, and then mixed with 4 μl M-MLV RT 5× Buffer, 2 μl dNTPs (2.5 mM, Takara, Osaka, Japan), 1 μl RNAsin (50 U/μl, Takara), 0.5 μl M-MLV (10 U/μl, Promega) and 7.5 μl DEPC. The mixture was incubated at 37 °C for 1 h and 95 °C for 5 min.

The 20 μl PCR reaction mixture contained 4 μl cDNA, 5 μl ddH_2O, 10 μl 2× EasyTaq PCR SuperMix (Transgen, Beijing, China) and 0.5 μl of each primer specific for a 1478 bp (86–1582) region of the VP2 gene (forward: 5′-TGCTATCATTGATGGTTA-3′, reverse: 5′-AGGCCCGAATTATGTCTT-3′). PCR comprised 95 °C for 5 min, 30 cycles at 94 °C for 45 s, 48 °C for 45 s, and 72 °C for 90 s, and a final extension at 72 °C for 10 min.

2.4. Nucleotide sequencing

PCR products were electrophoresed on 1% agarose gels (voltage of 150 V for 20 min). VP2 gene-specific products were purified from agarose gels using EasyPure Quick Gel Extraction Kit (TransGen) according to the manufacturer's instructions. The purified products were cloned into a pEasy-T1 Cloning Vector (TransGen), and sequenced by SunBiotech Co., Ltd. (Beijing, China). The DNA sequences were assembled using DNAStar software (Version 7.0, Madison, WI, USA).

2.5. Sequence and phylogenetic analysis

The nucleotide sequences and deduced amino acid sequences were edited using EditSeq (DNA Star). The sequences were aligned using Clustal W in the MegAlign software (DNA Star). Phylogenetic trees were conducted using the neighbor-joining