Virus Research 203 (2015) 92–95

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Short communication

Naturally occurring reassortant infectious bursal disease virus in northern China

Zhen Lu[a], Lizhou Zhang[a], Nian Wang[a], Yuming Chen[a], Li Gao[a], Yongqiang Wang[a], Honglei Gao[a], Yulong Gao[a], Kai Li[a], Xiaole Qi[a,*], Xiaomei Wang[a,b]

[a] Division of Avian Infectious Diseases, State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, the Chinese Academy of Agricultural Sciences, Harbin 150001, PR China
[b] Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Disease and Zoonoses, Yangzhou 225009, PR China

ARTICLE INFO

Article history:
Received 31 January 2015
Received in revised form 27 March 2015
Accepted 1 April 2015
Available online 11 April 2015

Keywords:
Infectious bursal disease virus (IBDV)
Reassortant
Genome
Evolution

ABSTRACT

Infectious bursal disease virus (IBDV) is a bi-segmented, double-stranded RNA virus that belongs to the genus *Avibirnavirus* of the family of *Birnaviridae*. The co-evolution of genome segments is a major evolutionary feature for IBDV. However, in recent years, some strains exhibited markedly different genetic relationships for segments A and B. In this study, we firstly isolated a new type of reassortment IBDV strain named IBD13HeB01 from northern China. The full-length genomes of segments A and B were cloned and identified. Sequence analysis revealed that IBD13HeB01 was a segment-reassortment strain, whose segment A was derived from very virulent strain and segment B from attenuated IBDV. In addition, the virulence of IBD13HeB01 strain was evaluated using SPF chickens. This study is not only beneficial for further understanding of the viral evolution but also suggests the potential risk of application of the live vaccines of IBDV.

Infectious bursal disease virus (IBDV), is the causative agent of a highly contagious immunosuppressive disease in young chickens, which causes considerable economic losses to the poultry industry worldwide (Sharma et al., 2000). The viral genome consists of two segments of double-stranded RNA (A and B), which are approximately 3200 and 2800 nucleotides (nt) long, respectively. Segment A contains two partially overlapping open reading frames (ORFs), ORF1 and ORF2. The smaller ORF encodes the non-structural protein VP5, which is non-essential for viral replication (Lombardo et al., 2000). The larger ORF encodes a polyprotein (NH2-VP2-VP4-VP3-COOH), which is cleaved by autoproteolysis to produce VP2, VP3, and VP4 (Da Costa et al., 2002; Luque et al., 2009). Segment B contains only one ORF, encoding VP1, which is an RNA-dependent RNA polymerase (RdRp) responsible for viral genome replication and RNA synthesis (von Einem et al., 2004).

There are two serotypes of IBDV, I and II. Serotype II IBDVs are avirulent to turkeys and chickens (Ismail et al., 1988). Serotype I IBDVs are pathogenic to chickens and can be further classified into four subtypes, very virulent, classical, antigenic variant, and artificially attenuated strains (Müller et al., 2003). Since it was firstly discovered in the USA in 1961 (Cosgrove, 1962), numerous IBDV isolates have been continually reported in chickens from different parts of the world. The first reported Chinese IBDV strain was isolated from Beijing in 1982 (Zhou et al., 1982), and then very virulent IBDV (vvIBDV) strains have spread widely in China (He et al., 2012). To control IBD, the attenuated IBDV (atIBDV) strains were selectively used as vaccines to immunize chickens. For viruses with multi-segments genome, segment-reassortment is a relatively common evolution case. Recently, a few proofs of IBDV reassortments have been reported in different parts of the world (Chen et al., 2012; Jackwood et al., 2011; Le Nouën et al., 2006). Of late, three reassortant IBDV strains with attenuated segment A and very virulent B, ZJ2000 (Wei et al., 2006), TL2004 (Wei et al., 2008), and HN04 (Cui et al., 2013), have been described in southern and central China. In this report, a new natural reassortant IBDV was isolated from Hebei province of northern China.

In January 2013, severe gross lesions in the bursa of Fabricius were observed in a few sick chickens in 25-day-old commercial flocks in Hebei province. The IBDV strain designated as IBD13HeB01 was isolated from chicken bursas with clinical signs of IBD as described previously (Yuwen et al., 2008). Total viral RNA was extracted from pathologic bursa samples using Purelink™ RNA

* Corresponding author at: Harbin Veterinary Research Institute, 427 Maduan Street, Harbin 150001, PR China. Tel.: +86 18946066078; fax: +86 451 51997166.
E-mail addresses: luzhen9122@163.com (Z. Lu), zhanglizhou88@126.com (L. Zhang), w-nian@foxmail.com (N. Wang), cym27@sina.cn (Y. Chen), gaoli0820@163.com (L. Gao), yqw-19@yahoo.cn (Y. Wang), ghl@hvri.ac.cn (H. Gao), ylg@hvri.ac.cn (Y. Gao), likaihvri@163.com (K. Li), qxl@hvri.ac.cn (X. Qi), xmw@hvri.ac.cn (X. Wang).

http://dx.doi.org/10.1016/j.virusres.2015.04.003
0168-1702/

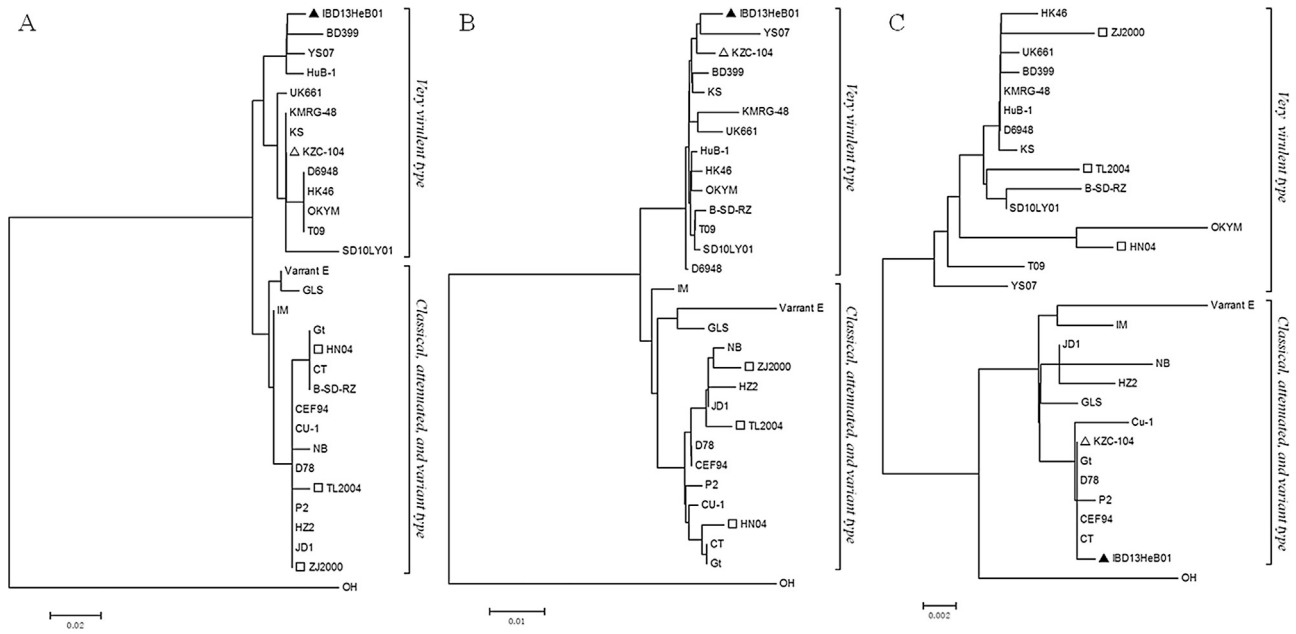

Fig. 1. Phylogenetic tree analysis of amino acid sequence of VP5 (A), polyprotein (B), and VP1 (C). Trees were generated by the neighbor-joining method using MEGA 6 with 1000 replications. The reassortant virus IBD13HeB01 isolated in this study is highlighted with solid triangle. Other reported reassortant viruses are marked with hollow triangle or square. The GenBank accession numbers of virus strains used in this study are as follows: BD399 (AF362776, AF362770), B-SD-RZ (GQ166972, GQ166971), D6948 (AF240686, AF240687), HK46 (AF092943, AF092944), HuB-1 (KF569805, GQ449693), KMRG-48 (AB368970, AB368971), KS (DQ927042, DQ927043), OKYM (D49706, D49707), SD10LY01 (KF569803, KF569804), UK661 (NC-004178, NC-004179), YS07 (FJ695138, FJ695139), GLS (AY368653, AY368654), Variant E (AF133904, AF133905), IM (AY029166, AY029165), CT (AJ310185, AJ310186), CU-1 (X16107, AF362775), D78 (AF499929, AF499930), Gt (DQ403248, DQ403249), HZ2 (AF321054, AF493979), JD1 (AF321055, AY103464), NB (AY319768, AY654284), P2 (X84034, X84035), KZC-104 (AB368968, AB368969), HN04 (KC109816, KC109815), TL2004 (DQ088175, DQ118374), ZJ2000 (AF321056, DQ166818), and OH (U30818, U30819).

Mini kit (Invitrogen, Carlsbad, CA, USA). The first-strand cDNA was synthesized by M-MLV Reverse Transcriptase (Invitrogen Life Technologies, Carlsbad, CA, USA) from the viral RNA with random primer pd(N)9. After that, two fragments of the genome segment A or B were obtained by polymerase chain reaction (PCR) using the specific primers, which were designed according to the sequences of full-length segment A or B of the vvIBDV HuB-1 strain (GenBank accession numbers KF569805 and GQ449693). The PCR products of segments A and B were cloned into pMD18-T Vector (Takara Bio INC., Japan), respectively. To identify the genome sequence, at least three independent positive clones presented for each fragment were sequenced. Sequencing results showed that the segment A of IBD13HeB01 strain contained 3260 nucleotides, including 5′-non-coding-region (NCR, 96 nt), two partially overlapping ORFs (3073 nt), and 3′-NCR (91 nt). Segment B consisted of 5′-NCR (111 nt), VP1 coding region (2637 nt), and 3′-NCR (79 nt). The genome sequence of IBD13HeB01 was submitted to GenBank with accession number (A, KP676467; B, KP676468).

Using the Basic Local Alignment Search Tool (BLAST), we found that the full-length segment A sequence of IBD13HeB01 was highly homologous (approximately 97–99%) to that of very virulent strains, whereas the nucleotide sequence of segment B showed more than 95% identity with the attenuated strains. Therefore, we chose some representative IBDV strains for further analysis. The phylogenetic trees were constructed from the aligned amino acid sequences of 30 representative strains obtained from GenBank using the Neighbor-Joining method in MEGA 6 software (Tamura et al., 2013). The reliability of the phylogenetic trees was established by bootstrap analysis with 1000 replications. Phylogenetic analysis based on the amino acid sequences of VP5 and polyprotein in segment A of the 30 IBDV strains showed that all the IBDV strains, except OH strain (serotype II), were distinctly divided into two major groups (Fig. 1A and B). In both trees, IBD13HeB01 was clustered with the vvIBDV strains. Interestingly, the phylogenetic tree constructed based on the deduced amino acid sequences of VP1 from segment B also revealed three distinct groups, but the IBD13HeB01 was clustered with the attenuated strains (Fig. 1C).

Multiple sequence alignments of nucleotides and the deduced amino acids were performed using Clustal X program (Larkin et al., 2007). Sequence distances derived from the multiple alignment of the amino acid sequences revealed that the polyprotein of IBD13HeB01 shared more than 99% identity with those of very virulent strains: D6948, HK-46, OKYM, BD399, HuB-1, SD10LY01, KS, and T09. Sixteen amino acid residues in VP2 (222A, 242I, 253Q, 256I, 279D, 284A, 299S, 330S, 451L), VP4 (541I, 680Y, 685N, 715S, 751D), and VP3 (981P, 1005A), which are usually conserved in vvIBDV strains, were also found in IBD13HeB01 (Fig. 2). Besides, one amino acid substitution in VP3 at position 778 (V to A) was unique to IBD13HeB01 strain (data not shown). In addition, although the deletion of four extra amino acid residues (MLSL) at the N-terminus existed, three conserved amino acid residues in VP5 of vvIBDV at positions 49R, 78F, and 129P were observed in IBD13HeB01 (Fig. 2). The deduced amino acid sequence of VP5 of IBD13HeB01 was closely related to the vvIBDV strains: BD399, KMRG-48, KS, HuB-1, and YS07, with sequence identity ranging from 98.0% to 98.6%. Taken together, segment A of IBD13HeB01 possessed of the characteristics of vvIBDV, which suggested that the segment A of IBD13HeB01 was most likely derived from vvIBDV. Whereas, the homology analysis revealed that VP1 of IBD13HeB01 was most similar to that of European attenuated strain D78 with an identity of 99.9%. As was shown in Fig. 2, 15 characteristic amino acid residues for attenuated IBDV (4I, 61V, 145N, 146E, 147G, 242D, 287T, 390L, 508R, 511R, 546L, 562S, 646G, 687S, and 695K) were also conserved in IBD13HeB01. Those findings suggested that the source of segment B of IBD13HeB01 could be the attenuated vaccine strain. Based on the phylogenetic analysis and the comparison of deduced