Arch Virol (2016) 161:3589–3592
DOI 10.1007/s00705-016-3055-9

ANNOTATED SEQUENCE RECORD

Genomic characterization of three bovine viral diarrhea virus isolates from cattle

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Received: 20 July 2016 / Accepted: 4 September 2016 / Published online: 9 September 2016

Abstract Three strains of the bovine viral diarrhea virus (BVDV) were isolated from cattle in Beijing, China. To investigate their genomic features, we sequenced and characterized the complete genome of each of the isolates. Each of the three virus genomes is about 12,220 bp in length, containing a 5′ untranslated region (UTR), one open reading frame (ORF) encoding a 3897-amino-acid polypeptide, and a 3′ UTR. The nucleotide sequence of the three isolates were 99.0 % identical to each and other shared nucleotide sequence identities of 73.4 % to 98.3 % with other BVDV-1 strains, about 70.0 % with BVDV-2 strains, about 67.0 % with BVDV-3, and less than 67.0 % with other pestiviruses. Phylogenetic analysis of the full-length genome, 3′ UTR, and the N^{pro} gene demonstrated that the three viruses were BVDV-1 isolates. This is the first report of complete genome sequences of BVDV 1d isolates from China and might have implications for vaccine development.

Bovine viral diarrhea virus (BVDV) is a member of the genus *Pestivirus* of the family *Flaviviridae*, together with the classical swine fever virus (CSFV) and sheep border disease virus (BDV). Based on antigenic and nucleotide sequence differences, two genotypes, categorized as BVDV-1 or BVDV-2, have been discriminated in the past years. So far, 21 subgenotypes (BVDV-1a to BVDV-1u) have been reported worldwide [1–4]. In addition to BVDV-1 and BVDV-2, genetically distinct isolates including Th/04_KhonKaen virus, D32/00-'HoBi', and CHKaHo/cont may be members of a third putative species and are referred to as "HoBi-like", "BVDV-3", or "atypical pestiviruses" [5, 6]. The BVDV genome comprises a positive single-strand RNA (ssRNA) molecule of approximately 12.3 kilobases (kb) in length, with one open reading frame (ORF) flanked by 5′ and 3′-untranslated regions (UTR). The BVDV ORF encodes a polyprotein that is cleaved into four structural proteins (C, E^{rns}, E1, and E2) and seven non-structural proteins (N^{pro}, p7, NS2-3, NS4A, NS4B, NS5A, and NS5B). In addition, based on their ability to cause a cytopathic effect, BVDV strains are further categorized as cytopathogenic (CP) or noncytopathogenic (NCP). In the past years, BVDV 1b, 1m, and 1q have commonly been detected in Chinese cattle and pigs [7–11]. However, the prevalence of BVDV-1d in cattle in China has rarely been reported [12]. This subtype is also occasionally detected in yaks [13]. In the current study, we determined the full-length genome sequences of three BVDV isolates and analyzed the specific genomic information and phylogenetic relationship to other recently reported novel and representative pestiviruses.

Three BVDV strains (BJ1201, BJ1305, and BJ1308) were isolated from cows between 2012 and 2013 in Beijing, China, and stored in our laboratory. These three viruses were isolated from serum samples as described in a previous report [14]. Further historical and epidemiological details about these isolates are shown in Table S1. The three isolates were propagated in Madin-Darby bovine

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Electronic supplementary material The online version of this article (doi:10.1007/s00705-016-3055-9) contains supplementary material, which is available to authorized users.

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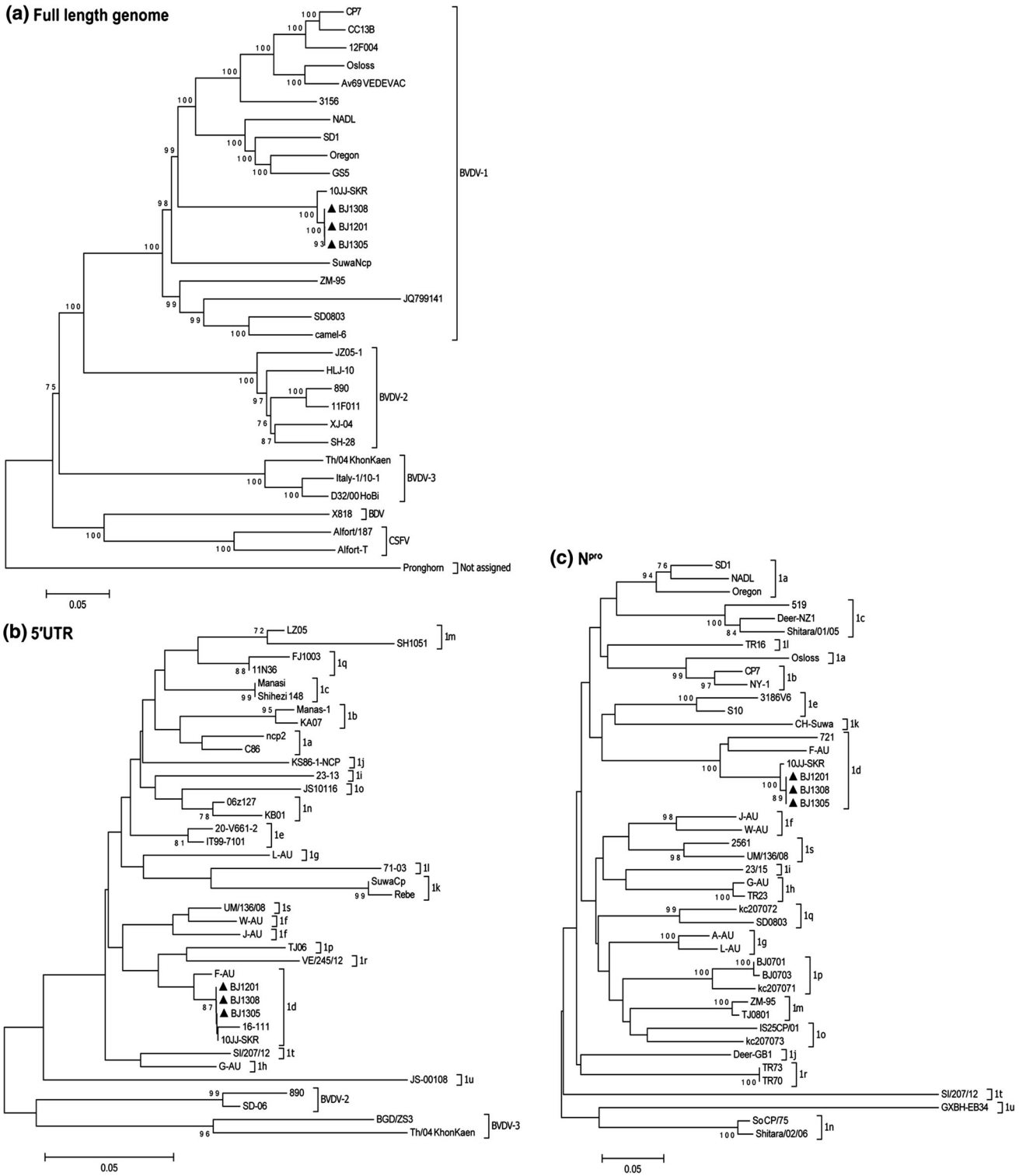

Fig. 1 Phylogenetic analysis based on the nucleotide sequences of (a) the full-length genome, (b) the 5′-UTR, and (c) the N^{pro} gene

kidney (MDBK) cells, and no cytopathic effect was observed. Viral RNA was then extracted from the cellular fraction using a Quick-RNA Kit (Aidlab Biotechnologies Co., Ltd, China), following the manufacturer's instructions. The three complete genome sequences have been deposited in the GenBank database under accession numbers KT943518, KT951840, and KT951841. Sequence alignments and identity analysis were performed using the

Table 1 Nucleotide sequence identity (%) of the coding sequences of the BJ1305 virus to those of other isolates of BVDV-1 and BVDV-2

Strain	N^{pro}	C	E^{rns}	E1	E2	P7	NS2	NS3	NS4A	NS4B	NS5A	NS5B
10JJ-SKR	98.8	99	98.4	97.6	98.4	98.1	98.6	99.0	99.5	98.1	98.5	99.2
890	69.8	68.1	72.1	69.7	66.6	62.9	58.5#	77.1	72.4	74.1	68.0	71.0
CP7	80.4	79.9	81.1	81.0	74.7	73.8	78.6#	83.6	83.3	82.1	77.4	82.8
JQ799141	75.0	73.5	71.4	76.2	69.0	54.3	67.4	77.8	78.1	75.8	76.6	77.4
JZ05-1	70.2	72.5	71.7	70.3	67.7	59.0	57.3	75.5	76.6	71.0	68.4	71.4
NADL	80.0	78.9	81.1	81.0	76.7	74.3	66.0	83.1	82.3	82.4	78.4	81.8
Osloss	80.0	81.4	79.4	81.2	74.4	72.9	77.6	83.2	82.8	81.9	78.2	82.2
SH-28	69.4	65.2	71.1	70.9	65.7	61.9	58.5	77.5	75.0	75.2	67.9	70.6
VEDEVAC	81.3	82.8	79.6	81.5	73.5	72.9	77.6#	82.8	80.7	82.9	77.8	82.8
ZM-95	78.6	80.4	76.7#	80.3	74.7	76.2	78.0	83.7	84.4	81.3	75.3	80.5

Sequences with an insertion in the E^{rns} or NS2 gene were omitted

MegAlign program (DNASTAR) and NCBI BLAST (BLAST: Basic Local Alignment Search Tool) online.

To investigate the evolutionary relationship of BVDV isolates, phylogenetic analysis was performed using Molecular Evolutionary Genetics Analysis (MEGA) software, version 6.06 [15]. Three genome sequences were aligned with reference strains retrieved from GenBank, including BVDV, CSFV, and other representative strains. Phylogenetic trees were reconstructed using MEGA software, based on the full-length genome, 5′-UTR, and N^{pro} gene. The bootstrap values were obtained for 1000 replicates using the neighbor-joining algorithm (NJ), and evolutionary distances were determined using the Kimura two-parameter model. Based on BLAST analysis and known characteristics of putative post-transcriptional processing sites of other BVDVs [7, 8], the positions of the coding genes and UTRs in the genome of the three isolates were determined (Table S2).

A phylogenetic tree constructed based on full-length genome sequences revealed that the isolates BJ1201, BJ1305, and BJ1308 clustered with previous BVDV-1 isolates (Fig. 1a). The nucleotide sequence identity among the three isolates was 99.0 %, and the deduced polypeptide sequences of BJ1201 and BJ1308 were 100 % identical. The three genome sequences shared nucleotide sequence identities of 73.4 % to 98.3 % with the other BVDV-1 strains, about 70.0 % with BVDV-2 strains, about 67.0 % with BVDV-3, about 66.3 % with CSFV strains, 66.3 % with the BDV strain X818, and 60.2 % with the pronghorn strain. Furthermore, the three isolates clustered with Korean strain 10JJ-SKR (Fig. 1a), which belongs to BVDV-1d subgenotype [16]. Twelve genes of BJ1305 were compared with those of seven BVDV-1 strains and three BVDV-2 strains (Table 1). The sequences of BVDV-2 viruses had a higher degree of divergence from those of BJ1305 than from those of the BVDV-1 strains. In the coding sequences, the highest degree of shared identity was observed between the BJ1305 and 10JJ-SKR strains. The full-length genome sequence of 10JJ-SKR was 98.3 % identical to those of the three isolates. However, the 3′UTR gene of BJ1305 was significantly different from that of 10JJ-SKR. The 3′UTR sequences of BJ1305 and 10JJ-SKR were 12.1 % identical. In a comparison of the coding sequences of BVDV-1 and BVDV-2, the ZM-95 virus was found to have a six-nucleotide insertion in the E^{rns} gene, and 890, CP7 and VEDEVAC were found to have insertions of 228, 27 and 45 nucleotides, respectively, in the NS2 gene (Table 1). These results show that the BJ1305 virus differs genetically from other BVDV-1 strains.

To confirm the subtype assignment based on the full-length genome sequence and to compare our isolates to other reference strains, phylogenetic trees based on 5′-UTR and N^{pro} gene were reconstructed (Fig. 1b, c). For this analysis, sequences from members of 21 genetic subgroups of BVDV-1 from different regions of the world were used [1, 3, 7, 8, 17]. The two phylogenetic trees showed that isolates BJ1201, BJ1305, and BJ1308 clustered in the same phylogenetic branches as in the phylogenetic tree based on the full-length genome (Fig. 1b, c). The 5′-UTRs of the 16-111 and F-AU viruses had 96.7 % and 97.5 % sequence identity, respectively, to those of the three isolates in the present study. The N^{pro} the isolates of 721 and F-AU had 89.7 % and 89.9 % sequence identity to those of the three isolates. In a previous study, Weng et al. reported the 5′-UTR and N^{pro} sequences of 18 BVDV isolates, including BJ1201, BJ1305, BJ1308 [14], but the results differed from those of this study. First, the samples for virus isolation in this study were different from those used in the previous study (Table S1). Second, there were distinct differences in the nucleotide sequence of these isolates, as shown in Table S3. Finally, the subgenotype assignment for BJ1305 and BJ1308 in our study was different from that reported by Weng et al. (BJ1305-1m, BJ1308-1a).

In conclusion, we sequenced the complete genomes and analyzed the phylogenies of isolates BJ1201, BJ1305, and BJ1308 and obtained detailed genomic information about these three strains. Phylogenetic and sequence analysis based on the full-length genome, 5′-UTR, and N^{pro} gene showed that these viruses are strains of the BVDV-1d subgenotype. This is the first report of the genomic sequences of BVDV 1d viruses in China.

Acknowledgement This work was supported by grants from the Beijing Dairy Industry Innovation Team of China.

Compliance with ethical standards

Conflict of interest The authors declare no competing interests. This article does not contain any studies with human participants or animals performed by any of the authors.

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