Complete Genome Sequence of Noncytopathic Bovine Viral Diarrhea Virus 1 Contaminating a High-Passage RK-13 Cell Line

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Bovine viral diarrhea virus (BVDV) is a nonenveloped, positive-sense, single-stranded RNA virus with a genome size of approximately 12.3 to 12.5 kb. The virus is a member of the genus *Pestivirus* in the family *Flaviviridae*, which also includes border disease virus and classical swine fever virus (1, 2). Two major genotypes of BVDV are recognized (types 1 [BVDV-1] and 2 [BVDV-2]). BVDV-1 strains are genetically subdivided into at least 17 subtypes (a to q) and BVDV-2 strains into four subtypes (a to d) (3–6). In addition, two distinct biotypes within each genotype have been identified: cytopathic viruses (cpBVDV) that cause cytopathic effects in cultured cells and noncytopathic viruses (ncpBVDV) that do not cause cytopathic effects in cultured cells (7–10). It was previously reported that a number of cell lines (e.g., cattle, sheep, goat, deer, bison, rabbit, and domestic cat origins), including the RK-13 cell line (CCL-37; American Type Culture Collection [ATCC], Manassas, VA) are persistently infected with ncpBVDV, resulting from the use of BVDV-contaminated fetal bovine serum in cell culture medium (11–15). Many laboratories use the RK-13 cell line from the ATCC or its derivatives for research and laboratory confirmation of various viral agents. Our laboratory has been using high-passage RK-13 cells (P399-409; HP-RK-13 [KY]) for routine laboratory diagnostic investigation for >50 years (16). In this study, we determined the complete genome sequence of ncpBVDV present in the HP-RK-13 [KY] cells (P404) using next-generation sequencing (NGS) technology on an Illumina MiSeq platform, according to previously established procedures (17). The sequences were mapped to all known BVDVs, and mapped read sets were used for *de novo* assembly using ABySS version 1.3.7 (BC Cancer Agency, Vancouver, Canada) and Geneious 7.0.6 software (Biomatters Ltd., Auckland, New Zealand).

The complete genome of ncpBVDV contaminating the HP-RK-13 [KY] cell line (ncpBVDV HP-RK-13 [KY] strain) is composed of 12,271 nucleotides (nt) and contains a 5′ untranslated region (UTR) (386 nt), a single open reading frame (ORF) (11,697 nt [nt 387 to 12083]), and a 3′ UTR (188 nt). The single ORF encodes a 3,098-amino acid polyprotein, which is predicted to be cleaved into 12 proteins. The ncpBVDV HP-RK-13 [KY] strain had 85.2% to 99.7% identity with 11 strains of BVDV-1b and 68.6% to 70.9% identity with eight strains of BVDV-2 at the whole-genome level. Interestingly, the ncpBVDV HP-RK-13 [KY] strain is very closely related to the recently described ncpBVDV present in the HP-RK-13 cells reported from Japan (RK13/E strain [GenBank accession no. JX419397.1]; 12,064 nt, 99.7% identity) (18). The ncpBVDV HP-RK-13 [KY] strain had several nucleotide insertions and deletions compared to several of the other BVDV-1b strains. However, the specific insertion(s) and/or deletion(s) that are responsible for the establishment of persistent infection in the HP-RK-13 [KY] cell line have not been determined. Phylogenetic analysis of the complete genome sequence of the virus ncpBVDV HP-RK-13 [KY] established that it is of the BVDV-1b genotype.

**Nucleotide sequence accession number.** The complete genomic sequence of ncpBVDV KY-HP-RK-13 strain has been submitted to GenBank under accession no. KT355592.

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