Differentiation of bovine herpesvirus1 subtypes based on UL0.5 gene sequencing

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Abstract Infectious bovine rhinotracheitis/infectious pustular vulvovaginitis is one of the high economic importance diseases of cattle and caused by bovine herpesvirus1 (BoHV1). Based on the restriction endonuclease fingerprinting of viral DNA, the BoHV1 can be divided into three subtypes viz., BoHV1.1, 1.2a, and 1.2b. Since this method requires a pure viral DNA, it is time-consuming and labour intense. In the current study, the UL0.5 gene based PCR sequencing has been used for the subtyping of BoHV1. Out of five isolates, four had BoHV1-like signatures and one isolate had BoHV1.2-like signatures. Further, these viruses phylogenetically clustered under the respective subtypes. These results indicate that the UL 0.5 gene based PCR sequencing could be used as an alternate method of subtyping of BoHV1.

Keywords Bovine herpesvirus1 · Subtyping · BoHV1.1 · BoHV1.2 · UL0.5 gene

Bovine herpesvirus1 (BoHV1) is responsible for causing infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis/Infectious balanoposthitis (IPV/IBP) in cattle. The virus belongs to the genus *Varicellovirus*, subfamily *Alphaherpesvirinae* of the family *Herpesviridae*. BoHV1 primarily affects cattle but other Artiodactyla (e.g. goats, sheep, water buffaloes, camelids)

may also be infected. The virus is transmitted through inhalation of respiratory droplets and natural service or artificial insemination using infected bulls or semen from them [10].

Based on the restriction endonuclease (RE) fingerprinting of pure viral DNA, the BoHV1 is divided into three subtypes (some author referred as types) viz., BoHV1.1, BoHV1.2a, and BoHV1.2b [1, 9, 11]. Ackermann and Engels [1] classified the virus as per the clustering pattern using four RE. Recently, whole genome sequencing followed by the single nucleotide polymorphisms (SNPs) matching pattern has been used for the differentiation of the subtypes of BoHV1 from field and vaccine strains [5, 6]. In one study, pyrosequencing assay has been used for the typing of alphaherpesviruses by targeting US8 gene [7]. In our previous study, markers present in the gC gene were used for the subtyping of BoHV1 [8]. In the present study, UL0.5 gene was targeted for the differentiation of BoHV1 subtypes by PCR followed by sequencing.

A total of five BoHV1 isolates viz., BoHV1/cattle/India/UP/216/1975(BoHV1.1)—KX688217; BoHV1/cattle/India/UP/215/1975(BoHV1.1)—KX688218; BoHV1/cattle/India/NDDB/2291/1975(BoHV1.1)—KX688219; BoHV1/cattle/India/NDDB/2778/1975(BoHV1.1)—KX688220; BoHV1/cattle/India/UP/257/1975(BoHV1.2)—KX688221 was propagated in Madin-Darby bovine kidney (MDBK) cells and DNA was extracted using GeneJET Viral DNA/RNA Purification Kit (Thermo Scientific, USA) as per the manufacturer's instructions. The PCR was carried out in a total volume of 50 µL which consists of 0.25 µL (1.25 U) of OneTaq[®] Hot Start DNA Polymerase (NEB, UK), 10 µL of 5X GC Reaction Buffer, 10 µL of High GC Enhancer, 1 µL of dNTPs (0.2 mM final conc.), 1 µL each of forward and reverse primers (0.2 µM final conc.), 1 µL template (~ 0.5 µg total DNA), and the final volume was adjusted

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with nuclease free water. The thermal profile comprised of initial denaturation at 94 °C for 5 min followed by 30 cycles of amplification – 94 °C for 30 s; 62 °C for 30 s; 68 °C for 60 s, and a final extension at 68 °C for 10 min. The PCR amplicons were purified with GeneJET Gel Extraction Kit (Thermo Fisher, USA) and commercially sequenced by Sanger's method.

The genetic analyses of UL0.5 gene were carried out using MEGA6.0 and Jalview2.9.0b2 programs. For the analysis, in addition to the five isolates used in the current study, reference sequences available in the GenBank were also included. The genetic analysis of UL0.5 gene of reference sequences revealed the existence of five BoHV1.1 and BoHV1.2 differentiating markers at the nucleotide

level and all leads to the nonsynonymous amino acid changes (Table 1). All the five isolates analyzed in the present study had an ORF length of 264 bp with four of them showed BoHV1.1-like signatures and one isolate showed BoHV1.2-like signatures.

The phylogenetic analysis was carried out in MEGA6 using Neighbor-Joining method with Kimura 2 parameter model and the bootstrap value of 1000. The BoHV1.1 and BoHV1.2 sequences formed two separate clades wherein four Indian isolates viz., BoHV1.1-215, BoHV1.1-216, BoHV1.1-2778, and BoHV1.1-2291 clustered under the BoHV1.1 clade whereas one isolate—BoHV1.2-257 clustered under BoHV1.2 clade (Fig. 1). Our report reveals the existence of BoHV1.1 as well as the BoHV1.2 in India. Similarly, presence of BoHV1.1 and 1.2 were reported from West Bengal and Karnatka [2, 13].

Classifying either by RE analysis of pure viral DNA or whole genome sequencing is time-consuming and labour intensive. Further, these methods cannot be used for the subtyping of the virus directly from the clinical samples. In the present study, UL0.5 gene was targeted for the differentiation of BoHV1.1 and BoHV1.2 subtypes. The UL0.5 gene is only present in BoHV1 and even the most closely related BoHV5 lacks a homologue of UL0.5 [3, 4, 12]. Therefore, UL0.5 gene can also be targeted for the specific detection of BoHV1. Our study indicates that the UL0.5 gene-based sequencing will be useful in typing of BoHV1.

Table 1 Unique nucleotide and amino acid signatures observed in the UL0.5 gene of BoHV1.1 and BoHV1.2 sequences

Position	Nucleotide		Position	Amino acid	
	BoHV1.1	BoHV1.2		BoHV1.1	BoHV1.2
14	T	C	5	L	P
70	A	G	24	S	G
116	T	C	39	L	P
244	C	T	82	R	C
250	A	G	84	I	V

Fig. 1 Phylogenetic analysis of BoHV1 based on UL0.5 gene sequences. The Indian BoHV1 isolates are indicated in bold letters

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