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Genetic characterization of bovine herpesvirus 1 (BoHV-1) isolates from India

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The bovine herpesvirus 1 (BoHV-1), an important pathogen of cattle, has worldwide distribution. BoHV-1 is subdivided in 2 distinct yet closely related subtypes, viz. BoHV-1.1 and BoHV-1.2 (Miller *et al.* 1991, Roizman *et al.* 1992). Engels *et al.* (1992) also proposed that such subtypes may be associated to distinct manifestations of disease in cattle. Metzler *et al*. (1985) classified subtype 2 into 2a and 2b. Subtypes 1 and 2a were isolated from aborted foetuses, whereas the less virulent subtype 2b is yet to be associated with abortion (Edwards *et al.* 1991). Infection with BoHV-1 also occurred in wild ruminants and swine (Radostits *et al.* 2000). Bovine herpesvirus-1 infection was first reported in India in 1976 (Mehrotra *et al.*1976) and since then various workers have reported its widespread prevalence (Deka *et al.* 2005, Trangadia *et al.* 2010). Analysis at PD_ADMAS, indicated an apparent positivity of 36% for IBR (Patil *et al.* unpublished results). Different types and subtypes of bovine herpesvirus 1 are associated to different clinical conditions of cattle, hence differentiation of type/subtype becomes essential for understanding the pathogenesis and epidemiology of BoHV infections. Besides, the genus *Varicellovirus* includes a cluster of viruses that are antigenically and genetically related to bovine herpesvirus 1 (BoHV-1), viz. BoHV-5, BuHV-1, caprine herpesvirus 1 (CpHV-1), cervid herpesviruses 1 (CvHV-1) and 2 (CvHV-2) and elk herpesvirus 1 (ElkHV-1). Many authors have used phylogenetic analysis based on gC (Esteves *et al.* 2008) gB, gE (Thiry *et al.* 2007), gD (Afonso *et al.* 2007) to differentiate BoHV-1 and other alphaherpes viruses. Keeping these facts in mind, the present study was undertaken to generate baseline information about Indian BoHV-1 subtypes and also to increase our understanding about the genetic relatedness of Indian BoHV-1 with other bovine alpha herpesviruses (BoHV-1.1, 1.2 and 5).

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Two bovine herpesvirus isolates, viz. PD_ADMAS-1 (isolated from nasal secretions from cattle with respiratory disease) and PD_ADMAS-2 (isolated from semen from a bull having reproductive infection, Ooty, Tamil Nadu) were retrieved from liquid nitrogen and passed once in Madin darby bovine kidney (MDBK) cells. Total DNA was extracted from the supernatant of the infected MDBK cells using kit as per the manufacturer's instructions. Extracted DNA was subjected to PCR using the previously published primer pairs (IBR443F: 5' -tcgaargccgagtacctgcg–3', nt positions 56051-56070 of AJ004801 and IBR443R: 5'-ccagtcccaggcraccgtcac-3' nt positions 56494–56474 of AJ004801) specific for gB genomic region (Ros and Belak, 1999). Briefly, the reaction was carried out in 25 µl volume. To 50 ng (5 µl) of the total DNA extracted from the supernatant, following reagents were added– 2.5 µl of 10X PCR buffer, 2.5 µl (2.5 mM) of $MgCl_2$, 1.0µl (10mM) of dNTP mix, 1.0µl (10U/µl) of *Taq* DNA polymerase enzyme, 1.0µl (25 pmol) each of forward and reverse primers. Final volume was adjusted with nuclease free water. The PCR amplification was carried out with the initial denaturation at 95°C for 5 min and 35 cycles each of denaturation at 94°C for 50s, annealing at 58°C for 50s and extension at 72°C for 50s. One cycle of final extension was carried at 72°C for 5 min. The PCR amplified products were visualized on 2% agarose gel and purified using a kit. So obtained PCR products were sequenced at both strands from a commercial service provider. For the phylogenetic analysis, in addition to the 2 nucleotide (nt) sequences generated in this study, following nucleotide sequences retrieved from the NCBI genbank were also included. The details of the isolates and genbank accession numbers are given in Fig. 1. Briefly, the nt sequences were aligned using Genedoc (Nicholas and Nicholas 1997) and edited manually. Neighbour joining tree was constructed using 1000 bootstrap resamples with the programme MEGA 4.1 (Tamura *et al.* 2007). The evolutionary distances were computed using the Tamura-Nei method (Tamura and Nei 1993). The rate variation among sites was modeled with a gamma distribution (shape

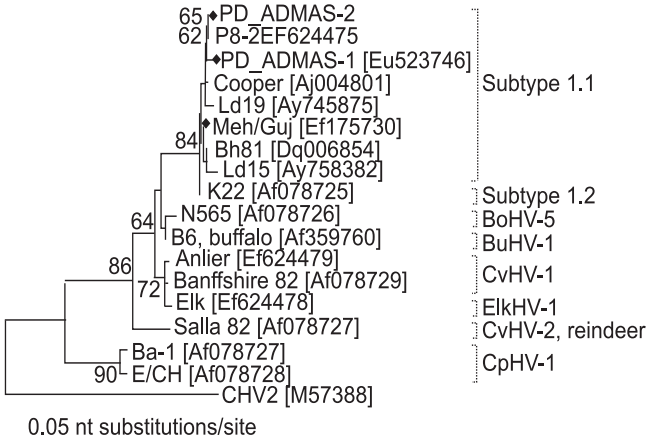

Fig. 1. Phylogenetic tree based on the analysis of 400 nucleotides within gB gene. The tree is outgrouped to the corresponding sequence of the CHV-2 (GenBank Acc. No. M57388) and constructed using the neighbour-joining method. Numbers at the node represent the bootstrap values after 1000 replicates. Genbank accession numbers are given in the parenthesis. ♦ indicates Indian isolates.

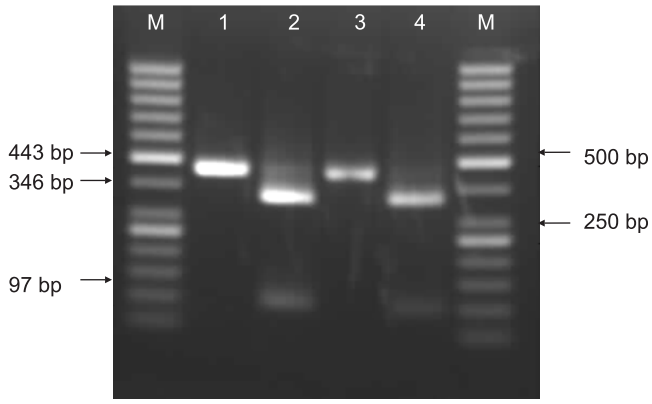

Fig. 2. Lane M: 50bp ladder; lanes 1, 3: PCR amplified gB regions of PD_ADMAS isolates, 1 & 2 respectively; lanes 2, 4 *Dde* I digested above PCR products.

parameter = 0.64). The differences in the composition bias among sequences were considered in evolutionary comparisons (Tamura and Kumar 2002). All positions containing alignment gaps and missing data were eliminated only in pair-wise sequence comparisons (pair-wise deletion option).

In this study, BoHV-1 isolates previously recovered from 2 animals showing different clinical symptoms, i.e., respiratory disease and balanoposthitis were partially nucleotide sequenced at the gB gene. Before nucleotide sequencing, the identity of these PCR products (443 bp) was confirmed by digestion with restriction enzyme, *Dde* I, which yielded 2 fragments of size 346bp and 97bp (Fig. 2). The aim of the present study was to generate baseline information about Indian BoHV-1 subtypes and also to increase our understanding about the genetic relatedness of Indian BoHV-1 with other bovine alphaherpesviruses (BoHV-1.1, 1.2 and 5) based on the analysis of the region of the gB gene. Although a product length of 443 nts was generated by PCR, only 400 nts corresponding to position from 56086 to 56485of Cooper strain (AJ004801) was aligned and used for nt sequence analysis due to non availability of that stretch of sequence from many other types/subtypes. Aligned nt sequences revealed high degrees of identity in all alphaherpesviruses compared in the study. The nucleotide sequence identity among the Indian isolates varied from 99–99.5%, so was their identity with Cooper strain. The nucleotide sequence homology among the isolates of subtype 1.1 was 97.8 to 100%. In the aligned region (data not shown), the Indian strain PD_ADMAS-2 differed from the reference Cooper strain by one nucleotide (position 56483, A→T) while PD_ADMAS-1 in addition to the above position (A→C) also differed at position, 56485 (C→T). Similar to the nucleotide sequence aligned, amino acid sequences revealed high degree of identity in all the BoHV-1 isolates used in the study.

The phylogenetic tree (Fig. 1) allowed the grouping of viruses according to their respective types or subtypes (Metzler *et al.* 1986, Miller *et al.* 1991); it revealed that the strains of BoHV-1 were grouped in 2 different nodes, one related to BoHV-1.1 and another to BoHV-1.2. Our isolate, PD_ADMAS-2 shares the node with Brazilian strain, P8-2 indicating a close genetic relationship. Although not very significantly different, another isolate, PD_ADMAS-1 is branching out from this node. It can be seen that both the above nodes have reasonably good bootstrap support. The third Indian isolate, Meh/Guj is placed in a different node along with the strains, BH81 & LD15. Since this node lacks good bootstrap support, it could be dangerous to attach any significance to this grouping. Taken together, the phylogenetic analysis indicates that both the PD_ADMAS isolates belong to subtype 1.1 and so is the Gujarat isolate, Meh/Guj. In addition, the study did not reveal geographic region based grouping of the BoHV-1 isolates.

Although innumerable serological studies have suggested the high incidences of BoHV-1 infection in cattle and buffaloes in India, literature on isolation and characterization of the virus strains is scanty. The present work is a step in the latter direction. Nucleotide sequence analysis of 2 BoHV-1 isolates in the present study indicated that both these along with an isolate from Gujarat belong to subtype 1.1, despite being recovered from animals showing different clinical manifestations. Though epidemiological data strongly suggested that BoHV-1.1 and -1.2 strains differ in the induction of clinical disease, the basis for such differences in the relationship of host/parasite remains unclear (Rijsewijk *et al.*1999, Spilki *et al.*2004). Further, both the subtypes are shown to infect respiratory and genital tract of cattle and this appears to be the case with our isolates. Finally, the data generated in present study has provided some useful information about prevalence of BoHV-1 subtypes in India and future work should focus on the virus isolations and

characterization in large number to aid the epidemiological studies and also to take up the control programmes.

SUMMARY

Two bovine herpesvirus-1 (BoHV-1) isolates previously recovered from 2 animals showing different clinical symptoms, i.e. respiratory disease and balanoposthitis, were partially nucleotide sequenced at the gB gene. Aligned nucleotide sequences revealed high degree of identity among all the alphaherpesviruses compared in the study. The nucleotide sequence identity among the Indian isolates varied from 99–99.5% and so was their identity with reference Cooper strain. The phylogenetic analysis indicated that both the isolates belonged to subtype 1.1 despite being isolated from animals with different clinical manifestations.

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