RESTRICTION ENDONUCLEASE ANALYSIS OF DNA FROM INDIAN ISOLATES OF BOVINE HERPESVIRUS 1

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Summary. – The genomic variation of three isolates of bovine herpesvirus 1 (BHV-1) originating from different geographical regions and displaying different clinical symptoms were studied by restriction analysis using four different restriction endonucleases. *Eco*RI displayed an uniform restriction pattern for all the isolates and *Hind*III showed marginal variation among the isolates. *Bst*EII and *Pst*I displayed unique restriction patterns, based on which the isolates could be grouped into two subgroups of BHV-1.1. *Bst*EII appears to be the enzyme of choice for differentiating BHV-1.1.

Key words: bovine herpesvirus 1; Indian isolates; DNA restriction pattern

Introduction

BHV-1, otherwise known as infectious bovine rhinotracheitis virus (BRV), has been associated with a variety of clinical syndromes. These are primarily respiratory, but may also include genital infections (infectious pustular vulvo-vaginitis, IPV), conjunctivitis, abortions, encephalitis and generalized systemic infections (Gibbs and Rweyemamu, 1977). The virus has a worldwide distribution. In India, the virus was first isolated in 1977 (Mehrotra, 1977). Subsequently, numerous virus isolations have been made in different parts of the country from animals with different clinical manifestations (Harishankar *et al.,* 1986; Mehrotra *et al.,* 1994; Misra and Mishra, 1987; Mohankumar *et al.,* 1994; Rao and Char, 1991; Singh *et al.,* 1989; Suribabu and Mallick, 1983), which apparently indicates that BHV-1 is an emerging bovine pathogen in India.

The differentiation of BHV-1 isolates using restriction analysis of the genome is now possible. Accordingly, BHV-1 strains can be grouped into 3 genotypes, namely BHV-1.1 (IBR-like), BHV-1.2 (IPV-like) and BHV-1.3 (neuropathogenic). Genotyping of the viruses is a prerequisite for solution of diagnostic, epidemiological and vaccination problems.

Among the Indian BHV-1 isolates, so far, a respiratory isolate referred to as No. 216 has been studied extensively using restriction analysis and subtyped as BHV-1.1 (Kataria and Rai, 1992). Here, we report restriction patterns of two BHV-1 respiratory isolates and one abortion isolate with *Hind*III, *Eco*RI, *Bst*EII and *Pst*I.

Materials and Methods

Virus isolates. The BHV-1 respiratory isolates, IBRV Hassan and 216/IBR-II, were obtained from Veterinary College, Bangalore, and Indian Veterinary Research Institute, Izatnagar, respectively. A BHV-1 abortion isolate, IBRH/167 VS, was obtained from College of Veterinary Sciences, Tirupati. A German BHV-1 respiratory isolate (Straub and Mawhinney, 1988), obtained from Tamilnadu Veterinary and Animal Sciences University, Madras, was used as a reference strain. The BHV-1 isolates were propagated in Madin-Darby bovine kidney (MDBK) cells using Eagle's Minimum Essential Medium (MEM) by a standard procedure. Confluent monolayers in roller bottles were infected with plaque purified BHV-1 isolates at a multiplicity of infection of 0.01 – 0.1 $TCID_{50}$/cell and the virus containing medium was harvested after 2-3 days when the cytopathic effect (CPE) reached 80 – 100% of the cells.

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Abbreviations: BHV-1 = bovine herpesvirus 1; CPE = cytopathic effect; IBRV = infectious bovine rhinotraecheitis virus; IPV = infectious pustular vulvo-vaganitis; MDBK = Madin-Darby bovine kidney; MEM = Eagle's Minimum Essential Medium;

Virus purification and DNA extraction. Virus was purified by a modification of the methods described by Collins *et al.* (1984) and Edwards *et al.* (1990). The virus was precipitated with PEG 6,000 and NaCl, collected by centrifugation and dissolved in the Tris-NaCl-EDTA (TNE) buffer. After clarification by centrifugation at 5,000 x g, the virus was pelleted at 80,000 x g through 30% (w/v) sucrose cushion and purified by 20 – 60% sucrose density gradient centrifugation. DNA from purified virions was extracted according to Simard *et al.* (1990) with minor modifications. Briefly, virus was incubated with DNase and RNase (final concentration 50 µg/ml each) in the presence of 10 mmol/l $MgCl_2$ at 37°C for 1 hr. Then DNase was inhibited by adding 0.02 volume of 0.5 mol/l EDTA. The mixture was further incubated at 50°C for 2 hrs after adding sodium dodecyl sulphate (SDS) (final concentration 1%) and proteinase K (final concentration 250 µg/ml). Viral DNA released from ruptured virions was extracted thrice with phenol-chloroform-isoamyl alcohol (25:24:1) and twice with chloroform-isoamyl alcohol (24:1), and ethanol-precipitated. The precipitated DNA was washed with 70% ethanol, vacuum dried and dissolved in Tris-EDTA (TE) buffer pH 8.0 (Sambrook *et al.*, 1989).

Restriction endonuclease digestion and agarose gel electrophoresis. One µg of viral DNA in 25 µl of appropriate buffer was digested with 20 U of given restriction endonuclease (Boehringer Mannheim) according to the manufacturer's protocol. Digested DNA was analyzed by electrophoresis through 0.7% agarose gel in Tris-borate-EDTA (TBE) buffer at 40 V for 20 hrs. Lambda phage DNA digested with *Hind*III was used as molecular size marker. In general, standard procedures were employed (Sambrook *et al.*, 1989).

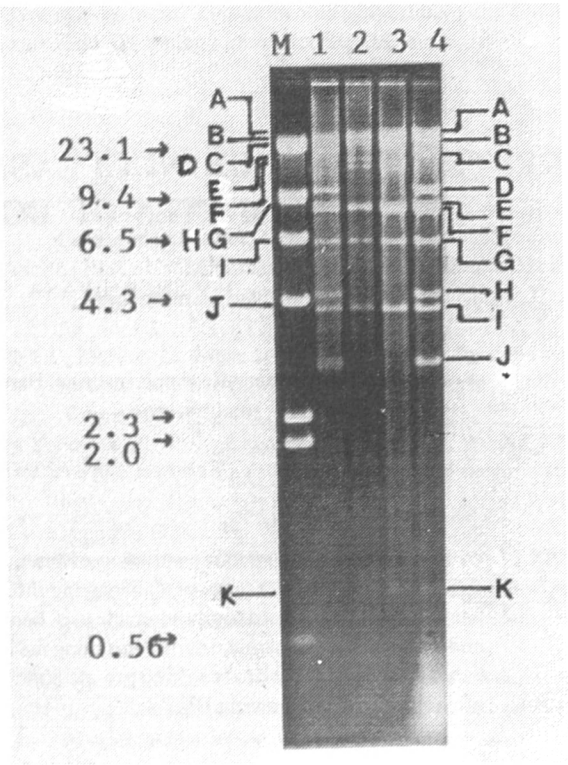

Fig. 1
***Bst*EII restriction endonuclease patterns of DNA from BHV-1 isolates**
Reference strain (lane 1), IBRV Hassan isolate (lane 2), 216/IBR-II isolate (lane 3), IBR/H 167 VS isolate (lane 4). Size markers in kbp (lane M). Letters on the left side correspond to fragments in lanes 2 and 3, while those on the right side to lanes 1 and 4.

Results and Discussion

Restriction analysis

The *Eco*RI digestion of DNA from all the BHV-1 isolates under study revealed an uniform pattern of 7 fragments, ranging in size from 53 to 3.2 kb, and was found to be in exact correlation with the pattern reported for respiratory isolates of BHV-1 (Misra *et al.*, 1983). The *Hind*III pattern, corresponded to 5 fragments ranging in size from 21 to 0.4 kb in all the isolate, (data not shown) was in conformity with the type 1 pattern of Metzler *et al.* (1985) which was reported to be characteristic for the respiratory isolates of BHV-1 (Edwards *et al.*, 1991; Engels *et al.*, 1986, 1987; Mayfield *et al.*, 1983; Simard *et al.*, 1990; Seal *et al.*, 1985). These results were also consistent with those reported for an Indian respiratory isolate (Gupta and Rai, 1993; Kataria and Rai, 1992).

The *Bst*EII patterns are shown in Fig. 1. This enzyme demonstrated an unambiguous variation among the isolates. The reference strain and IBRH/167 VS had 3 additional fragments (D, H and J), the equivalents of which were absent in IBRV Hassan and 216/IBR-II (Fig. 1, lanes 2 and 3). The fragments E and F of the latter isolates, however, did not have equivalent fragments in the former isolates. The intensity of the *Bst*EII fragments of 216/IBR-II and IBRV Hassan also exhibited a variation. The bands of the fragments C, D, E and F of these isolates which should have had a stronger intensity than that of lighter fragments (G and H) due to their greater size, were in fact faint probably because of their presence in half molar concentrations. Judging from the intensity of the bands in the ethidium bromide-stained gel, it appears that none of the fragments was in half molar concentration with respect to the reference and IBRH/167 VS isolates. This, however, needs confirmation by radiolabelling studies which would determine the molarity of each fragment (Seal *et al.*, 1985). The fragments E and F of the reference and IBRH/167 VS isolates, even though they co-migrated, could be resolved into 2 distinct bands. The equivalent fragments from the other isolates (G and H, Fig. 1, lanes 2 and 3) were not clearly resolvable into 2 distinct bands but apparently merged into one band of markedly increased intensity. The digestion of BHV-1 DNA from different isolates with *Bst*EII resulted 11 fragments in contrast to 10 reported (Edwards *et*

al., 1990; Engels *et al.*, 1986, 1987) for BHV-1.1 and BHV-1.2 isolates by other authors (Edwards *et al.*, 1990; Engels *et al.*, 1986, 1987). The 11th fragment designated K of 0.77 kb formed an extra band was consistently identified in our study. The pattern of the 216/IBR-II and IBRV Hassan isolates correspond to that reported for the Los Angeles strain, a prototype BHV-1.1 (Simard *et al.*, 1991). The IBRH/167 VS isolate displayed a different pattern. Based on the *Bst*EII profile, the existence of 2 subgroups within BHV-1.1 has been reported (Magyar *et al.*, 1993). The correspondence of our *Bst*EII groups to the subgroups of Magyar *et al.* (1993) could not, however, be confirmed.

The *Pst*I digestion resulted in a complex electrophoretic pattern with more than 35 fragments (Fig. 2), out of which fragments of up to 2 kb were considered for comparative purposes as they were clearly delineated. These fragments were grouped into 5 clusters, the number of band in each cluster varying from 2 to 5. The reference strain, however, showed a heavy band A of approximately 8.2 kb (Fig. 2, lane 1) which differentiated the virus from the 216/IBR-II and IBRV Hassan isolates. The clusterwise analysis of the fragment pattern also showed a variation among the reference and two Indian respiratory isolates. The first cluster (I) of the reference strain displayed 4 well separated bands, whereas the other two showed 5 bands each. The clusters II and III were doublets in all the three isolates without any variation in the mobility. The cluster IV also displayed minor variation among the isolates. While the reference strain and 216/IBR-II isolate showed 4 well separated bands, the IBRV Hassan isolate displayed 2 doublets with reduced mobility. The cluster V was uniform in all the isolates. Although the digestion was incomplete, the IBRV/167 VS isolate (Fig. 2, lanes 4 and 7) displayed a pattern comparable with that of the reference strain and other reported patterns (Whetstone *et al.*, 1986, 1989). The *Pst*I patterns of the Indian respiratory isolates were distinct from the reported patterns.

In summary, the restriction analysis of 3 Indian isolates of BHV-1 using *Eco*RI and *Hind*III endonucleases clearly demonstrated that these isolates belong to the BHV-1.1 (IBR-like) subtype. The isolates, however, could be differentiated from each other using *Bst*EII and *Pst*I enzymes. It is proposed that *Bst*EII may be the enzyme of choice for virus strain identification. This, along with *Pst*I may be useful molecular epidemiological tools for assessing the emergence of BHV-1 within the country and its dissemination in different geographical regions.

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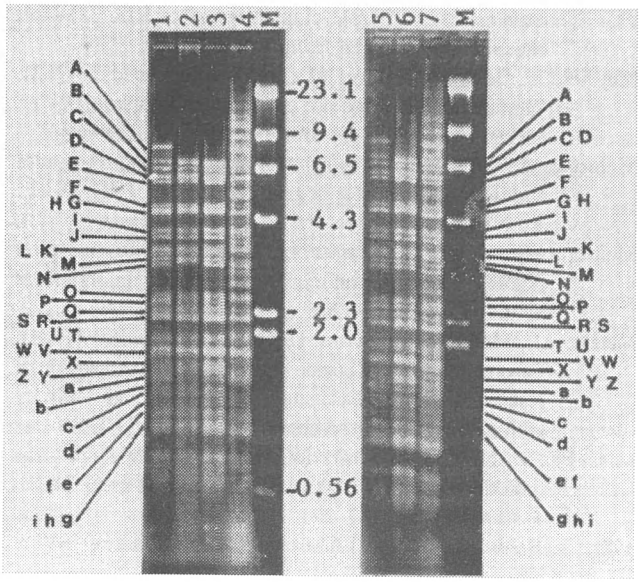

Fig. 2
***Pst*I restriction endonuclease patterns of DNA from BHV-1 isolates**
Reference strain (lanes 1, 5), IBRV Hassan isolate (lane 2), 216/IBR-II isolate (lanes 3, 6), IBR/H 167 VS isolate (lanes 4, 7). Size markers as in Fig. 1.

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