Differentiation of Indian Isolates of Equine Herpes Virus (EHV-1) by Restriction Endonuclease Digestion

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The genome of five local isolates of EHV-1 (Hisar-90-7, Jind-96, Tohana-96-2, Delhi-98 and Raj-98) and one reference strain 592 was digested with different restriction endonucleases (RE). On electrophoresis of digested DNA in 0.8% agarose gel, Raj-98 could be differentiated from other viral isolates using BamHI and KpnI. Reference strain (592) differed from other viruses in RE profile of HindIII (one band of ~3500 bp lacking) and XbaI (2 bands of ~4800 and ~2900 bp missing). This study indicates that at least two genetically variant isolates of EHV-1 are circulating amongst the equines of northern India.

Keywords: EHV-1, RE analysis, restriction endonucleases, genetic variation

Equine herpes virus-1 (EHV-1) is associated with manifestations like abortion (Jain et al, 1976; Singh et al, 1991, 1999), stillbirth and foal mortality (Jain & Ram, 1980), neurological disorders (Batra et al, 1982; Shankar & Yadav, 1986) and respiratory syndromes (Tewari et al, 1992). Although EHV-1 has been isolated from different outbreaks by various workers in India, only a few attempts have been made to study the antigenic (Singh et al, 2001) and genetic variations (Yadav, 1993) employing monoclonal antibodies (Mabs) and restriction endonuclease (RE). Outbreaks of EHV-1 infection are reported despite routine vaccination with commercial vaccine (Burki et al, 1990). The possible emergence of genetic and antigenic variant strains of EHV-1 circulating world wide in equines may be a potential obstacle to vaccination-induced immunity (Allen et al, 1983a).

Restriction endonuclease (RE) analysis of DNA of various EHV-1 isolates is a useful tool for detecting genetic variations. Many workers have examined the DNA profile generated by digested EHV-1 DNA with RE enzymes (Sabine et al, 1981; Studdert et al, 1981; Allen et al, 1983a; Allen & Bryans, 1986). However, there are few reports from India on RE analysis of indigenous EHV-1 isolates. The comparative RE mapping of Indian EHV-1 isolates with exotic strains were done earlier (Uppal et al, 1991; Singh et al, 1994). In the present study, variations in EHV-1 isolates from different states of North India were compared by RE analysis of their DNA.

The designation and history of Indian EHV-1 isolates included in the present study is given in Table 1. Reference EHV-1 UK strain 592 was also included for comparing the RE analysis. Equine embryonic dermis (EED) cell line was used for cultivation of virus. EED cells were grown in Eagle's autoclavable minimum essential medium (MEM, Gibco BRL, UK) supplemented with 10% fetal bovine serum (FBS), 100 µg/ml streptomycin and 100 IU penicillin G/ml and inoculated with early passage of each virus. Maintenance medium consisted of MEM with 2% FBS.

Each viral isolate was inoculated in EED cell monolayers and incubated till more than 80% cells were showing typical cytopathic effects (CPE). After three cycles of freezing and thawing, the virus harvest was obtained by centrifuging at 4200 g for 20 min at 4°C. The virus from supernatant was pelleted at 60,540 g for 3 hrs in Beckman L57 centrifuge and the pellet thus obtained after centrifugation was suspended in 1.0 ml of TNE buffer (0.01 M Tris-HCl, 0.15 M NaCl, 0.001 M EDTA, pH 7.4) and purified by rate zonal centrifugation in 25-50% sucrose gradient at 44,000 rpm for 3 hrs. The virus-containing band was collected and TNE buffer was added to give 20 ml volume and repelleted at 60,540 g for 3 hrs. The virus pellet was finally suspended in 1.0 ml of TNE and stored at -20°C.

The purified viruses were treated at 37°C with DNase1 and RNase enzymes at final concentration of 40 µg/ml for 30 min to eliminate cellular DNA, followed by RNase A 40 µg/ml for 30 min, proteinase K (250 µg/ml) for 15 min and sodium dodecyl sulphate (0.5% final concentration) for 1 hr as per Manniatis et al (1989). DNA was extracted twice with phenol-chloroform-
isoamylalcohol mixture (25:24:1) and once with chloroform-isoamylalcohol (24:1). DNA was precipitated by 0.3 M sodium acetate, pH 5.2 and 2.5 volume ethanol and keeping overnight at -20°C. DNA was pelleted at 10,000 g for 15 min, washed once with 70% alcohol and resuspended in TE buffer (0.01 M Tris-HCl, 0.001 M EDTA, pH 7.4). The optical density at 260 and 280 nm was taken in Beckman DU-6 spectrophotometer to check the purity and concentration of DNA.

Restriction enzymes and their respective buffers (EcoRI, BamHI, HindIII, BglII, XbaI and KpnI) were procured from Gibco BRL. DNA of each virus isolate was digested to completion with each enzyme separately according to manufacturer’s recommendations at 37°C overnight. The digested samples were resolved in 0.8% agarose gel containing ethidium bromide (0.5 μg/ml) in TAE buffer (0.04 M Tris-acetate, 0.001 M EDTA) at 130 volt for 3 hrs. Gels were visualized under UV transilluminator and photographed.

Five Indian EHV-I isolates (4, aborted mare and 1, foal mortality) included in this study originated from Haryana, Delhi and Rajasthan between 1990 and 1998 (Table I). The restriction patterns of viral isolates in the present study with different enzymes corresponded well with the profiles already documented for characteristic EHV-1 isolates (Studdert et al, 1984, 1992; Crandell et al, 1988). The present study is important in light of demonstration of differences in the RE profile within the Indian abortigenic EHV-1 isolates using 6 different restriction enzymes. This may be important while selecting the viral strain for pathogenicity/immunogenicity study of this disease.

RE analysis of herpes viruses serves as a valuable method for unambiguous differentiation from one another (Buchman et al, 1978). Fingerprinting of EHV-1 DNA has been used to trace the spread of virus strain in equine population (Studdert et al, 1992; Allen et al, 1983a). EHV-1 isolates were subdivided into 16 genetic strains by five restriction enzymes (Allen et al, 1983a) despite uniformity in EHV-1 RE profiles (Studdert 1983).

EcoRI and Bgl II digestion revealed no difference in the EHV-1 isolates in this study (Fig. 1) except that EcoRI digestion detected minor differences in the mobility of one fragment of ~8500 bp in case of Raj-98 isolate (Fig. 1, lane 5). Such mobility difference in the corresponding fragments of different isolates has been reported to occur mostly in restriction fragments containing inverted repeat sequences of the viral DNA, due to insertion or deletion of small number of DNA base pairs (Henry et al, 1981; Allen et al, 1983a). Bgl III digested DNA of different EHV-1 isolates yielded identical profile among all the isolates (Fig. 1, lanes 8-14).

The RE profile of DNA of different EHV-1 isolates with BamHI and HindIII is shown in Fig. 2. Of the various BamHI profiles reported, IP and IB were.

![Fig. 1—EcoRI and BglIII cleavage profile of DNA of different EHV-1 isolates. DNAs from purified Hisar-90-7, Tohana-96-2, Jind-96, Delhi-98, Raj-98 and 592 isolates after restriction endonuclease digestion were resolved in 1% agarose gels in lanes 1-6 (EcoRI) and lanes 8-13 (BglIII), respectively. Lambda DNA/HindIII digest run as molecular weight marker in lane 14 resolved 6 bands of 23130, 9416, 6557, 4361, 2322 and 2027 bp. Arrow (→) to the left of lane 5 shows mobility difference in the corresponding band of Raj-98 with EcoRI.](image-url)

<table>
<thead>
<tr>
<th>Virus designation</th>
<th>Place</th>
<th>Origin of EHV-1 isolate</th>
<th>Year of isolation</th>
<th>Passage level in EED cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hisar-90-7</td>
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<td>Abortion</td>
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<td>3</td>
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<tr>
<td>Tohana-96-2</td>
<td>Tohana, Haryana</td>
<td>Abortion</td>
<td>1996</td>
<td>2</td>
</tr>
<tr>
<td>Jind-96</td>
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<td>Foal mortality</td>
<td>1996</td>
<td>2</td>
</tr>
<tr>
<td>Delhi-98</td>
<td>Delhi, Delhi</td>
<td>Abortion</td>
<td>1998</td>
<td>3</td>
</tr>
<tr>
<td>Raj-98</td>
<td>Bikaner, Rajasthan</td>
<td>Abortion</td>
<td>1998</td>
<td>3</td>
</tr>
</tbody>
</table>
Fig. 2—BamHI and HindIII cleavage profile of DNA of different EHV-1 isolates. DNAs from purified Hisar-90-7, Tohana-96-2, Jind-96, Delhi-98, Raj-98 and 592 isolates after restriction endonuclease digestion were resolved in 1% agarose gels in lanes 1-6 (BamHI) and lanes 8-13 (HindIII), respectively. Lambda DNA/HindIII digest run as molecular weight marker in lane 14 resolved 6 bands of 23130, 9416, 6557, 4361, 2322 and 2027 bp. Arrow (→) to the left of lane 5 shows an additional band in Raj-98 isolate on BamHI digestion and in lane 13 shows loss of band in Delhi-98 on HindIII digestion.

Fig. 3—XbaI and KpnI cleavage profile of DNA of different EHV-1 isolates. DNAs from purified Hisar-90-7, Tohana-96-2, Jind-96, Delhi-98, Raj-98 and 592 isolates after restriction endonuclease digestion were resolved in 1% agarose gels in lanes 1-6 (XbaI) and lanes 8-13 (KpnI), respectively. Lambda DNA/HindIII digest run as molecular weight marker in lane 14 resolved 6 bands of 23130, 9416, 6557, 4361, 2322 and 2027 bp. Arrow (→) to the left of lane 6 show differences in 592 with XbaI and in lanes 8 and 12 show loss of band in Hisar-90-7 and Raj-98 on KpnI digestion.

seen in nearly 80% of EHV-1 outbreaks (Allen et al., 1983b; Allen & Bryans, 1986; Studdert et al., 1984, 1992). BamHI profile of all the Indian isolates in the present study was of IP type. BamHI digestion yielded one extra band of ~4361 bp in Raj-98 (Fig. 2, lane 5). Hind III profile revealed loss of band in Delhi-98 in lane 13. However, reference strain 592 differed from other viruses as one fragment of ~3500 bp was absent in it.

Hisar-90-7 differed from other isolates in XbaI profile as it lacked fragment ~2900 bp (Fig. 3, lane 1). Reference strain 592 was variable in XbaI profile as two bands (~4800 and ~2900 bp) were absent and two additional bands (~2700 and ~2500 bp) were present (Fig. 3, lane 6). The KpnI digestion revealed identical profile in all isolates except Hisar-90-7 and Raj-98 in which band (~3500 bp) was absent (Fig. 3, lanes 8, 12). Overall XbaI and KpnI restriction profiles of EHV-1 isolates in this study corresponded well with the published patterns (Whalley et al., 1981).

Antigenic variations among Indian EHV-1 isolates were reported earlier using Mabs (Singh et al., 2001). The findings of the present study indicate that more than one genetically different isolates of EHV-1, on the basis of RE profiles, are circulating in northern India. Hisar-90-7 and Raj-98 differed from each other and from other Indian isolates in restriction profiles. Reference strain 592 differed from the Indian isolates in RE profiles of HindIII and XbaI. This study will help in selecting the suitable Indian variant strains for incorporating in EHV-1 vaccine being developed at this centre for prophylactic immunization of Indian horses as outbreaks of EHV-1 are being reported from different equine populations despite the use of commercial vaccine (Mumford & Bates, 1984; Burki et al., 1990). Further, the selected different EHV-1 strains on the basis of RE profiles in present study may help in pathogenicity study about the different clinical manifestations observed in horses with this viral infection.

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References