Genomic Heterogeneity of Equine Betaherpesviruses

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SUMMARY

The genomes of 51 isolates of slowly cytopathic equine herpesviruses were examined by digestion with restriction endonucleases. Forty-seven of the isolates showed considerable fragment pattern heterogeneity although common fragments were evident, especially when any two isolates were compared or when they were digested with SalI. Fifteen of the 47 viruses, selected for their diverse fragment patterns, showed a high degree of homology in Southern blot hybridization. In contrast, four viruses, representing three epidemiologically distinct isolations, shared few, if any, comigrating fragments with the 47 equine herpesvirus 2 (EHV-2) isolates, although they shared comigrating fragments with each other. These four viruses showed reduced homology to a representative EHV-2 isolate by Southern blot hybridization under stringent conditions. Although not sharply delineated from EHV-2, these four viruses grew very slowly and had low yields in vitro, and preliminary data suggested they had a significantly smaller genome than EHV-2 (148 ± 12 kb compared to 190 kb). These four viruses may be prototypic of a novel equine betaherpesvirus.

Equine herpesvirus 2 (EHV-2; equine cytomegalovirus; slowly cytopathic equine herpesvirus), provisionally included in the betaherpesviruses (Roizman, 1982), is among the most common viral infections of the horse, but its role in disease remains uncertain. It has been isolated from the leukocytes of 89% of apparently normal horses (Kemeny & Pearson, 1970; Roeder & Scott, 1975) and has also been frequently isolated from the upper respiratory tract and conjunctiva, as well as the vagina, mammary gland, bone marrow and kidney (Plummer & Waterson, 1963; Kono & Kobayashi, 1964; Karpas, 1966; Turner et al., 1970; Studdert, 1971; Harden et al., 1974). Limited experimental infections have produced mild rhinitis and conjunctivitis (Gleeson & Studdert, 1977) and chronic follicular pharyngitis (Blakeslee et al., 1975), although roles in poor performance (Studdert, 1974) and in immunosuppression (Palfi et al., 1978; Belak et al., 1980; Sugiura et al., 1983; Jolly et al., 1986) have also been suggested.

Previous studies have demonstrated considerable antigenic and biological diversity among EHV-2 isolates (Turner & Studdert, 1970; Plummer et al., 1973; Harden et al., 1974; Wilks & Studdert, 1974; Mumford & Thomson, 1978). The aim of this study was to compare the genomes of a collection of 51 viruses provisionally considered to be EHV-2 and to estimate the degree of homology between some of these by Southern blot hybridization.

Fifty-one slowly cytopathic equine herpesviruses, which were passaged only in equine foetal kidney (EFK) cells, were compared by restriction endonuclease analysis and plaque diameters. Details of 19 of these isolates, which were studied in more detail, are listed in Table 1. Viral stocks were diluted, inoculated onto confluent EFK cell monolayers and overlaid with methyl cellulose medium as described by Studdert & Blackney (1979). After 14 days incubation the overlay was removed and the monolayer stained with 1% crystal violet in 10% formalin. The diameter of well isolated plaques was measured. DNA was extracted from mock-infected and virus-infected EFK cells, digested to completion with restriction endonucleases, separated by...
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**Table 1. Slowly cytopathic equine herpesviruses: origins and plaque diameters**

<table>
<thead>
<tr>
<th>No.</th>
<th>Isolate</th>
<th>Place</th>
<th>Year</th>
<th>Site*</th>
<th>Passage</th>
<th>Plaque size (mm ± S.D.)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32B</td>
<td>Bundoora, Vic.</td>
<td>1967</td>
<td>N</td>
<td>10</td>
<td>2.2 ± 0.7</td>
<td>Turner &amp; Studdert (1970)</td>
</tr>
<tr>
<td>2</td>
<td>34</td>
<td>Bundoora, Vic.</td>
<td>1967</td>
<td>N</td>
<td>10</td>
<td>1.2 ± 0.3</td>
<td>Turner &amp; Studdert (1970)</td>
</tr>
<tr>
<td>3</td>
<td>76</td>
<td>Woodend, Vic.</td>
<td>1967</td>
<td>N</td>
<td>8</td>
<td>2.4 ± 0.4</td>
<td>Turner &amp; Studdert (1970)</td>
</tr>
<tr>
<td>4</td>
<td>86</td>
<td>Mordialloc, Vic.</td>
<td>1967</td>
<td>N</td>
<td>17</td>
<td>2.4 ± 0.5</td>
<td>Studdert et al. (1970)</td>
</tr>
<tr>
<td>5</td>
<td>2-16V</td>
<td>Parkville, Vic.</td>
<td>1967</td>
<td>V</td>
<td>5</td>
<td>2.5 ± 0.6</td>
<td>Turner et al. (1970)</td>
</tr>
<tr>
<td>6</td>
<td>1-141N</td>
<td>Parkville, Vic.</td>
<td>1967</td>
<td>N</td>
<td>9</td>
<td>2.5 ± 0.6</td>
<td>Turner et al. (1970)</td>
</tr>
<tr>
<td>7</td>
<td>F1Eye</td>
<td>Frankston, Vic.</td>
<td>1969</td>
<td>E</td>
<td>6</td>
<td>3.7 ± 0.6</td>
<td>Studdert (1971)</td>
</tr>
<tr>
<td>8</td>
<td>5FNas</td>
<td>Frankston, Vic.</td>
<td>1969</td>
<td>N</td>
<td>6</td>
<td>2.3 ± 0.5</td>
<td>Studdert (1971)</td>
</tr>
<tr>
<td>9</td>
<td>F4N30</td>
<td>Laverton, Vic.</td>
<td>1972</td>
<td>N</td>
<td>4</td>
<td>1.5 ± 0.4</td>
<td>Wilks &amp; Studdert (1974)</td>
</tr>
<tr>
<td>10</td>
<td>F2N60</td>
<td>Laverton, Vic.</td>
<td>1972</td>
<td>N</td>
<td>2</td>
<td>2.5 ± 0.5</td>
<td>Wilks &amp; Studdert (1974)</td>
</tr>
<tr>
<td>11</td>
<td>F4N150</td>
<td>Laverton, Vic.</td>
<td>1972</td>
<td>N</td>
<td>2</td>
<td>2.0 ± 0.5</td>
<td>Wilks &amp; Studdert (1974)</td>
</tr>
<tr>
<td>12</td>
<td>F7B150</td>
<td>Laverton, Vic.</td>
<td>1972</td>
<td>B</td>
<td>3</td>
<td>2.7 ± 0.4</td>
<td>Wilks &amp; Studdert (1974)</td>
</tr>
<tr>
<td>13</td>
<td>NZ8</td>
<td>New Zealand</td>
<td>1978</td>
<td>N</td>
<td>3</td>
<td>2.4 ± 0.4</td>
<td>G. W. Horner, unpublished</td>
</tr>
<tr>
<td>14</td>
<td>P2</td>
<td>Bacchus Marsh, Vic.</td>
<td>1982</td>
<td>P</td>
<td>4</td>
<td>2.2 ± 0.3</td>
<td>Unpublished</td>
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<td>15</td>
<td>T2</td>
<td>Cobbitty, N.S.W.</td>
<td>1983</td>
<td>L</td>
<td>6</td>
<td>2.4 ± 0.5</td>
<td>Unpublished</td>
</tr>
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<td>16</td>
<td>2-141N</td>
<td>Parkville, Vic.</td>
<td>1967</td>
<td>N</td>
<td>4</td>
<td>2.2 ± 0.5</td>
<td>Turner et al. (1970)</td>
</tr>
<tr>
<td>17</td>
<td>3-141N</td>
<td>Parkville, Vic.</td>
<td>1967</td>
<td>N</td>
<td>5</td>
<td>2.0 ± 0.5</td>
<td>Turner et al. (1970)</td>
</tr>
<tr>
<td>18</td>
<td>M2BO</td>
<td>Laverton, Vic.</td>
<td>1972</td>
<td>B</td>
<td>4</td>
<td>1.7 ± 0.6</td>
<td>Wilks &amp; Studdert (1974)</td>
</tr>
<tr>
<td>19</td>
<td>253-72</td>
<td>England</td>
<td>1972</td>
<td>N</td>
<td>4</td>
<td>2.0 ± 0.5</td>
<td>Unpublished</td>
</tr>
</tbody>
</table>

* N, nasal; V, vaginal; E, eye; B, buffy coat; P, pharynx; L, lung.

electrophoresis through submerged 0.5% agarose gels, stained with ethidium bromide and photographed (Studdert, 1983). DNA from representative strains of EHV-1 (438/77; Studdert, 1983), -3 (M2; Kamada & Studdert, 1983) and -4 (405/76; Studdert et al., 1970) were also included. Each sample contained 0.25 to 1.0 μg of viral DNA. After photography, DNA was transferred and fixed to nitrocellulose (Maniatis et al., 1982). DNA from EHV-2 strain 86 was extracted from the cytoplasm of infected cells (Pignatti et al., 1979) and radiolabelled by either nick translation in the presence of [α-35S]dGTP (Maniatis et al., 1982) or DNA synthesis primed by random hexanucleotides in the presence of [α-35S]dCTP (Feinberg & Vogelstein, 1983) to a specific activity of 10^8 to 5 × 10^8 d.p.m./μg. The Southern blots were hybridized to 0-2 × 10^7 to 10^7 d.p.m. of radiolabelled DNA (1 M-Na+, 68 °C) for 4 h, then washed under highly stringent conditions (16.5 mM-Na+, 68 °C) for 2 h, dried and autoradiographed at − 70 °C for 3 to 30 days (Maniatis et al., 1982). Such conditions were calculated to allow 10% base pair mismatch.

The means and standard deviations of the plaque diameters of 19 representative isolates are listed in Table 1. All 51 isolates were distinguishable from the equine alphaherpesviruses (Studdert, 1974; Studdert & Blackney, 1979; Kamada & Studdert, 1983), by their slow growth which was reflected in the small diameter of plaques at 14 days. Although there was considerable variation in plaque diameter among the isolates, no subgrouping was apparent.

There was extensive heterogeneity in the restriction endonuclease patterns and two different groups were discerned. One group comprised 47 of the 51 isolates and 15 representative isolates selected because of their diverse restriction patterns are shown in Fig. 1. Although very few fragments were common to all 15 isolates in BamHI, EcoRI and HindIII digests, comparison of any two isolates revealed more comigrating fragments and all 15 shared similar SalI patterns. Fourteen passages of EHV-2 strain 86 in EFK cell cultures produced no changes in restriction patterns (data not shown). Southern blot hybridization of these isolates to the genomic DNA of EHV-2 strain 86 demonstrated that all 15 shared a high degree of homology throughout the genome, but had no homology to the equine alphaherpesviruses (Fig. 2).

The second group, comprising four isolates (2-141N, 3-141N, M2BO and 253-72; viruses 16, 17, 18 and 19 in Table 1), which shared restriction endonuclease patterns only with each other, are compared to four representatives (viruses 6, 5, 11 and 13 in Table 1) of the other 47 isolates (Fig. 3). These isolates, representing three epidemiologically distinct isolations (Table 1) had similar patterns in all digests, but shared no comigrating fragments with the other 47 isolates.
Fig. 1. Restriction endonuclease fragment patterns of slowly cytopathic equine herpesviruses listed 1 to 15 in Table 1. Representative patterns of EHV-1, -3 and -4, and uninfected cells (lane M) are also shown. HindIII-cut λ DNA is shown as a kilobase marker. (a) BamHI, (b) HindIII, (c) EcoRI, (d) SalI.

Fig. 2. Southern blot analysis of BamHI patterns shown in Fig. 1. The probe was genomic DNA from isolate 86 which is present in lane 4.
Fig. 3. The distinctly different restriction endonuclease patterns of four isolates, each compared to four representative EHV-2 (isolates typical of the other 47) isolates. Lanes are numbered as listed in Table 1. (a) BamHI, (b) HindIII, (c) EcoRI, (d) SalI.

The degree of hybridization of the DNA of EHV-2 strain 86 to three of these four isolates (viruses 16, 17 and 18 in Table 1) was much lower than to four representatives (viruses 1, 4, 12 and 8 in Table 1) of the other 47 EHV-2 isolates (Fig. 4).

Restriction endonuclease patterns and DNA–DNA filter hybridization demonstrated two distinct groups of equine betaherpesviruses with limited, but detectable, base sequence homology throughout the genome. The first group, representing 47 of the 51 viruses, had similar
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Fig. 4. BamHI restriction endonuclease patterns (a) of seven isolates of slowly cytopathic equine herpesviruses compared to a Southern blot (b) derived from this gel. The blot was probed with EHV-2 strain 86 DNA and demonstrates lower homology of isolates 16, 17 and 18 with EHV-2 isolates. Lane numbers are as listed in Table 1.

restriction endonuclease patterns to the EHV-2 type strain, LK, which has been characterized as having a genome of about 190 kilobase pairs (Wharton et al., 1981), while fragment summation of representatives of the second group suggested a significantly smaller genome (148 ± 12 kilobase pairs). Previous studies have determined the G + C content of both genomes to be 57 moles % and have shown them to be distinct in cross-neutralization tests (Plummer et al., 1973). The limited degree of homology between the two groups of equine betaherpesviruses is reminiscent of that between two equine alphaherpesviruses, EHV-1 and EHV-4 (Allen & Turtinen, 1982), suggesting that the equine betaherpesviruses may have diverged at a similar time to these equine alphaherpesviruses.

Although the 47 EHV-2 isolates showed a high degree of homology in hybridization studies (Fig. 2), restriction endonuclease patterns demonstrate very considerable intratypic heterogeneity among these viruses, which contrasts sharply with the homogeneity of any given type of alphaherpesvirus. The genomic heterogeneity of the 47 EHV-2 isolates appears greater than that reported for any other reasonably large collection of herpesviruses. Such diversity, reflecting that observed in both biological and antigenic characteristics, is approached only by human cytomegalovirus (Huang et al., 1980), although preliminary investigations suggest tupaia herpesvirus may also exhibit considerable genomic variation (Koch et al., 1985). That three betaherpesviruses exhibit such variation, in contrast to alphaherpesviruses, indicates that this may be a general feature of the betaherpesviruses, and appears greater than could be explained only by larger genomic size as suggested by Huang et al. (1980), especially in the case of the equine betaherpesviruses which have the smallest genomes thus far recorded for any betaherpesviruses. The apparent homogeneity of the second group of equine betaherpesviruses
reported in these studies may be a consequence of small sample size and sampling bias introduced by the difficulty of isolation due to the generally lower titres and slower progress of cytopathology in vitro (Turner & Studdert, 1970; Wilks & Studdert, 1974).

Two different types of betaherpesvirus have yet to be identified unambiguously in any other single species; however, the significance of the two types of equine betaherpesvirus and the heterogeneity observed among EHV-2 in the pathogenesis of infection by these viruses remains to be fully assessed. Multiple infection by genomically distinct EHV-2, sometimes concurrently, has been demonstrated (Browning & Studdert, 1987), but the genomic location of the intratypic and intertypic differences recognized for equine betaherpesviruses, as well as the significance of the differences in vivo, remains to be defined.

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REFERENCES


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