

# Diversity of genomic electropherotypes of naturally occurring equine herpesvirus 1 isolates in Argentina

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## Abstract

The genomes of 10 equine herpesvirus 1 (EHV-1) strains isolated in Argentina from 1979 to 1991, and a Japanese HH1 reference strain were compared by restriction endonuclease analysis. Two restriction enzymes, *Bam*HI and *Bgl*III, were used and analysis of the electropherotypes did not show significant differences among isolates obtained from horses with different clinical signs. This suggests that the EHV-1 isolates studied, which circulated in Argentina for more than 10 years, belong to a single genotype.

## Key words

- Argentina
- Electropherotypes
- Equine herpesvirus

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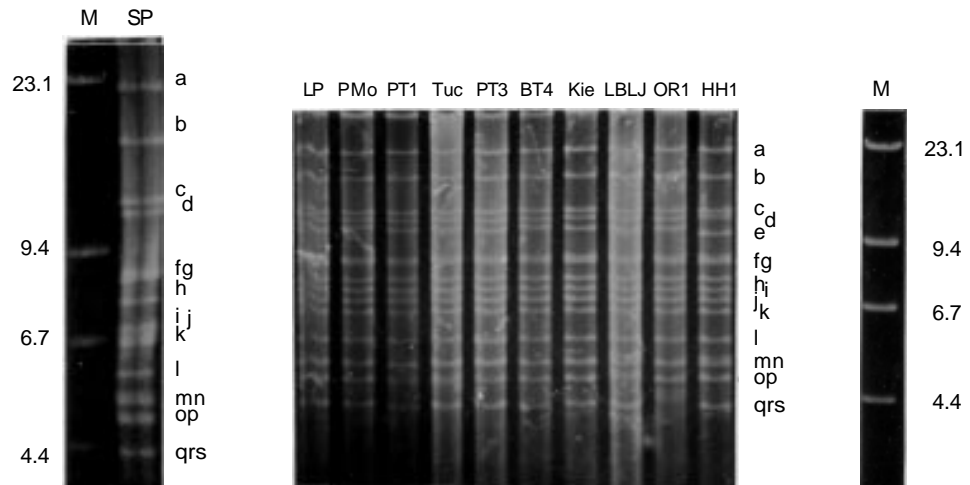
Equine herpesviruses 1 and 4 (EHV-1 and EHV-4) and asinine herpesvirus 3 are related alphaherpesviruses infecting members of the family equidae (1). EHV-1 (equine abortion virus) has long been causally implicated in the occurrence of abortion, respiratory disease, neonatal deaths and neurological disorders in horses. Infection by these viruses is a serious economic problem in the horse industry worldwide, especially on breeding farms (2,3). In attempts to prevent EHV-1 infection, both inactivated and attenuated vaccines have been used in several countries (4). Only the use of inactivated vaccines has been permitted in Argentina since 1982 and no earlier information about EHV vaccines is available. The first isolation of EHV-1 in Argentina occurred in 1979 (5). Since then, 14 viral isolates have been obtained, mainly from aborted fetuses, al-

though one of them (LP isolate) was obtained from a horse with respiratory symptoms (6), one from a horse with neonatal disease (OR1) and one (LBLJ) was recovered from an aborted fetus on a farm where the horses showed neurological clinical signs.

It has not been possible to establish any relationship between clinical signs in horses and genomic characteristics of EHV-1 (7,8). It is generally understood that a single EHV-1 genotype induces different clinical signs depending on the immune condition of the host animals. At least two electropherotypes of EHV-1 (1P and 1B) have been detected (2). The purpose of the present investigation was to detect possible differences in genomic types among EHV-1 strains isolated in Argentina by restriction endonuclease (RE) analysis (9-13).

A total of ten EHV-1 isolates from our

Figure 1 - Restriction fragment obtained with *Bam*HI of 10 EHV-1 strains isolated in Argentina from 1979 to 1991. M: Molecular weight marker fragments ( $\lambda$ DNA *Hind*III digested) are reported as kilobases. Fragment nomenclature is shown on the right according to the report for the HH1 strain (4).



laboratory were used. Isolates SP and LP were obtained by primary tissue culture of equine fetal kidney (EFK), and the other isolates (PMo, PT1, Tuc, PT3, BT4, Kie, LBLJ, and OR1) were obtained from RK13 cells. The SP isolate was passed through different non-equine cells 62 times, while the LP and PMo isolates were passed through RK13 cells 18 times. The Japanese strain HH1 was used as the reference strain (14). All viral isolates were plaque purified using EFK cells. Cells were grown in Eagle's minimum essential medium (Nissui, Tokyo) containing 10% heat-inactivated fetal calf serum. For RE analysis, total DNA from EFK cells infected with each EHV-1 isolate was used. The total DNA was prepared as described by Echeverría et al. (15), and 1.5  $\mu$ g of DNA from each virus was digested with *Bam*HI and *Bgl*II and separated by electrophoresis in 0.7% agarose gel in Tris-acetate buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) at 20 V for 16 h, stained with ethidium bromide (1  $\mu$ g/ml) and visualized with a UV illuminator.

Figure 1 shows the RE cleavage patterns with *Bam*HI. The SP isolate can be differentiated from the other Argentinian isolates and from the Japanese reference strain HH1 by the mobility of the group of fragments "h", "i", "j" and "k", and by the lack of

fragment "e". The mobility of fragment "e" differed between the Japanese HH1 strain and the isolates from Argentina. The LBLJ isolate has fragments of the same mobility as the HH1 "m" and "n" fragments, and shows slight differences in the mobility of the group of fragments "o" and "p" with respect to the other isolates. Fragments "q", "r", and "s" show decreased mobility in the OR1 isolate.

The *Bgl*II RE patterns (Figure 2) showed that the SP isolate was distinguishable from the others by the group of fragments "b", "c" and "d". BT4, Kie and OR1 isolates showed slightly different mobilities for fragments "k", "l" and "m", while the same fragments in the HH1 strain migrated faster than in the other strains. In addition, the PT3 isolate is also different, since a band is clearly visible between the "j" and "k" fragments.

No difference in electropherotype was observed between DNA obtained directly from seed stock and DNA extracted from plaque purified virus. This is in contrast to the data reported by Allen et al. (16). Only the SP isolate showed a restriction pattern alteration consistent with a loss of specific cleavage sites as shown in Figure 1. This difference may be due, in part, to the effects of the high passage level of this isolate through heterologous cell cultures (17). Some viral isolates showed small mobility shifts of

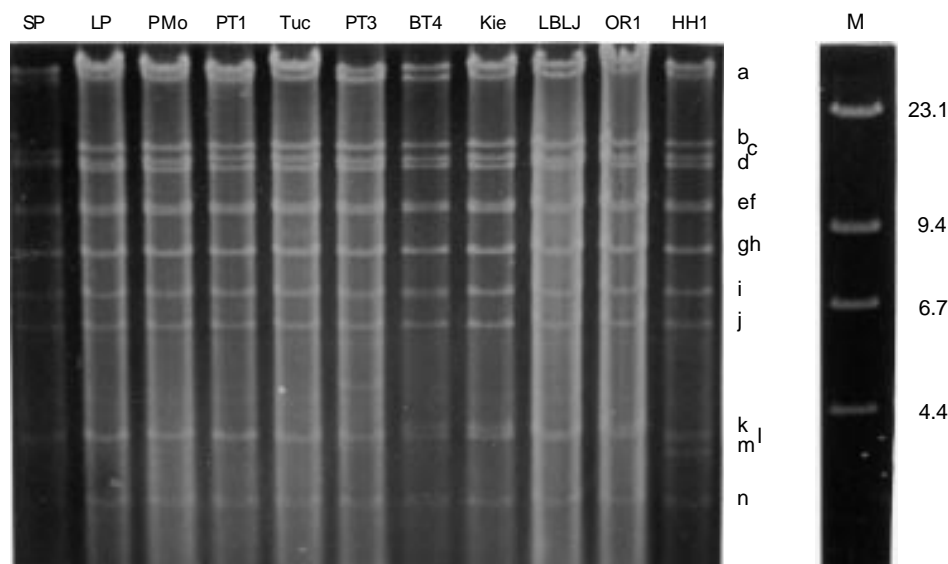


Figure 2 - Restriction fragment obtained with *Bgl*II from 10 EHV-1 strains isolated in Argentina from 1979 to 1991. M: Molecular weight marker fragments ( $\lambda$ DNA *Hind*III digested) are reported as kilobases. Fragment nomenclature is shown on the right according to the report for the HH1 strain (4).

the DNA fragments, but the differences among them were generally not significant.

In this study, we were not able to find significant differences in RE fragment patterns among isolates obtained from horses with different clinical signs, suggesting that the viral strains belong to a single genotype. The *Bam*HI pattern seemed to be related to that of EHV-1 type 1P reported by Allen et al. (9), and none resembled the 1B variant. We did not detect genomic fragments derived from recombination between attenuated vaccines and wild type virus, in contrast to the data reported by Palfi and Christensen (8) and Meyer et al. (17,18). Similar to the results of Kirisawa et al. (4), no variability in the *Bam*HI "g" fragment of Argentinian EHV-1 isolates was detected, indicating that this fragment is highly stable. Thus, the different clinical signs produced by the investigated viruses might be due to the different host immune response and they should not be

related to the virus genotype.

This is the first molecular epidemiological investigation of Argentinian isolates of EHV-1. The reason why the DNA genomes of these field isolates show minor electropherotype differences is yet to be determined. It also remains to be shown whether there is a relationship between these alterations and variations in the antigenicity and pathogenicity of EHV-1 isolates.

Our results were obtained with a small number of EHV-1 isolates which were studied by restriction digestion with only two enzymes. Therefore, they may not accurately account for the genetic variability that these viruses may undergo. However, the lack of significant differences in restriction patterns over a period of approximately one decade among most of the EHV-1 isolates suggests that this virus has not shown a significant amount of genetic diversity in Argentina recently.

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