

Detection of equine herpesvirus 1 genome 1B in Argentina

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Summary

To determine the genomic variation of equine herpesviruses (EHVs) isolated in Argentina between 1979 and the first half of 2004, DNA sequences from all 69 strains isolated were analysed. Sixty strains were recovered from aborted fetuses, one from leucocyte-rich plasma from a horse with respiratory signs and eight from cases of neonatal disease. The DNA was extracted from rabbit kidney epithelial (RK13) cells infected with each strain and digested with three restriction endonucleases (*Bam*HI, *Bgl*II and *Kpn*I). Two strains could be differentiated using *Bam*HI restriction and were assigned to the EHV-1 1B prototype group. Only one of these two strains was typed EHV-1 1B with *Bgl*II. DNA digestion with *Kpn*I was ineffective. The results obtained in this study demonstrate that the EHV-1 1B genome has been present in Argentina since at least 1996. The finding of two strains with this electropherotype suggests that there is genomic heterogeneity among Argentinian isolates.

Keywords

1B genome – Argentina – Equine herpesvirus 1 – First detection – Restriction endonuclease analysis.

Equine herpesvirus 1 (EHV-1) is a major cause of epidemic abortion, neonatal mortality, respiratory disease and neurological disorders in horses. Infection with this virus is a serious economic problem in the equine industry worldwide, especially on breeding farms (3, 4). The severity of disease resulting from EHV-1 infection is influenced by a number of factors, including the age and physical condition of the host; whether the infection is primary, secondary or a reactivation of latent virus; the immune status of the host and the virulence of the strain involved. Studies conducted by Nugent *et al.* (15) indicate that alteration of a single amino acid in the DNA polymerase enzyme is strongly associated with neurological versus non-neurological disease.

Genomic fingerprints are commonly used to detect DNA sequence variation among EHVs. Current epidemiological studies show both similarities and variations among strains (13). At least two genotypes of EHV-1 (1P and 1B) have been detected (1). The 1P genotype was the main electropherotype recovered in the United States of America (USA) prior to 1981; following this, the incidence of abortion associated with EHV-1 1B began to increase (2). In Ontario (Canada) all isolates obtained before 1987 had the 1P electropherotype; between 1987 and 1988 the predominant electropherotype became 1B but then shifted back to 1P in 1989 (13). Similar studies have been reported in Australia, and the results have revealed that the 1P type was the most common cause of EHV-1 abortion

there (18). In Japan, it was initially reported that all of the EHV-1 isolates were homogeneous and were identified as EHV-1 1P (9). However, Matsumara (unpublished data) has recently isolated EHV-1 1B from aborted fetuses.

In Argentina, antibodies against EHV-1 were first detected in 1965 (10) and the most recent serological study using the virus neutralisation test showed that 80% of horses were seropositive (Galosi *et al.*, 2000, unpublished data). It must be noted that the serum neutralisation (SN) test does not discriminate between EHV-1 and EHV-4. The only specific test able to differentiate between antibodies to the two viruses is an enzyme-linked immunosorbent assay based on glycoprotein G (Svanova Biotech, Uppsala, Sweden).

The first isolate from an equine fetus aborted due to EHV-1 was reported in 1979 (5) and EHV-related neurological signs in adult horses were described in 1984 (14). An EHV-1 strain was isolated from leucocyte-rich plasma from a horse with respiratory symptoms in 1985 (6). Since then, several viral isolates have been obtained from aborted fetuses and cases of neonatal disease.

Different vaccines have been used to prevent EHV-1 infections (3). Only inactivated vaccines have been used in Argentina since 1982 and information about other EHV vaccines used earlier is unavailable. Abortion due to EHV-1 occurs sporadically or endemically even in vaccinated mares. The continuous traffic of horses from abroad and the occurrence of epizootic abortion outbreaks on several farms suggest that new strains of EHV-1 could be circulating throughout the country. The purpose of the present work was to detect possible genomic variation among EHV-1 strains infecting Argentinian horses using restriction endonuclease (RE) analysis. This study reports the first detection of the EHV-1 1B electropherotype in Argentina.

In total, 69 strains isolated between 1979 and 2004 from different geographic zones were analysed. Ten strains were included to confirm previous results (7). Sixty strains were recovered from aborted fetuses, one from leucocyte-rich plasma from a horse with respiratory signs, and eight from cases of neonatal disease. Sample processing and virus isolation were carried out by standard methods. All strains were isolated in equine fetal kidney cell cultures or in rabbit kidney epithelial (RK13) cells. The EHV-1 isolates were typed by immunofluorescence testing with specific monoclonal or polyclonal antibodies and passaged no more than ten times in RK13 cells before DNA extraction. The Japanese HH1 strain was used as the reference strain. The RE analysis was carried out as described by Galosi *et*

al. (7). Infected RK13 cells were treated with proteinase K, and total DNA was extracted with phenol and phenol-chloroform-isoamylalcohol, precipitated with cold absolute alcohol and, finally, cleaved with *Bam*HI, *Bgl*II or *Kpn*I. The restriction patterns were obtained by 0.5% agarose gel electrophoresis at 20V for 16 h in Tris-acetate buffer (40 mM Tris-acetate, 1 mM ethylenediamine tetra-acetic acid (EDTA), pH 8.0). Bands were visualised by ultraviolet transillumination after staining with ethidium bromide. The resultant band patterns were compared with the results published by other authors (2, 13).

When electropherotypes of all strains were analysed, 67 exhibited identical *Bam*HI restriction patterns to the EHV-1 1P genotype. Only small differences in the mobility of variable fragments *Bam*HI 'e', 'o-p' and 'q-r-s' could be observed in some strains. The first strain isolated in Argentina (designated 1/79) (8) was confirmed to show differences in the mobility of fragments *Bam*HI 'h-i-j-k' and a lack of fragment *Bam*HI 'e'. In contrast to the results obtained by other authors (11, 12, 17), *Bam*HI restriction did not detect any strain with genomic fragments derived from recombination between attenuated vaccines and wild-type virus.

In this work, two Argentinian strains (designated 1/96 and 3/01) were typed 1B with *Bam*HI. Studdert *et al.* (18), in similar studies with strains isolated in Australia, could differentiate EHV-1 1B from 1P with *Bgl*II. In this work only one strain (3/01) was typed to 1B with *Bgl*II (13). Furthermore, *Kpn*I was not able to differentiate genomes 1B and 1P.

Genome 1B was detected in the USA in the 1970s (2) and in Canada, possibly because of its geographic proximity to the USA, in 1987 (13). It is possible that the EHV-1 detected in Japan could have come from the USA because the first EHV-1 1B in that country was isolated from a horse imported from Kentucky, USA (16). In this investigation it was not possible to establish a foreign origin or geographical relationship between the two Argentinian 1B genome strains.

The results presented here demonstrate that the EHV-1 1B genome has been present in Argentina since at least 1996. The finding of two strains with this electropherotype suggests that there is genomic heterogeneity among EHV-1 isolates in Argentina and confirms previous results from isolates analysed before 1996, which showed similar electropherotypes (7). Despite the continuous traffic of horses from countries where EHV-1 1B is common, the epidemiology of this virus in Argentina has not altered significantly. This report describes the first study to analyse all EHV-1 strains isolated in Argentina in the last 25 years.

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Détection du génome de l'herpèsvirus 1 équin 1B en Argentine

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Résumé

Afin de déterminer la variation génomique des herpèsvirus équins isolés en Argentine entre 1979 et le premier semestre 2004, une analyse des séquences d'ADN a été réalisée sur les 69 souches isolées pendant cette période. Soixante d'entre elles provenaient de fœtus avortés, une autre du plasma riche en leucocytes d'un cheval présentant des troubles respiratoires et les huit dernières de poulains victimes d'affections néonatales. L'ADN extrait de cellules épithéliales de rein de lapin (RK 13) infectées avec chacune de ces souches a été digéré par trois endonucléases de restriction (*Bam*HI, *Bgl*II et *Kpn*I). Les fragments de restriction *Bam*HI ont permis de différencier deux des souches et de les caractériser comme appartenant au groupe prototype 1B de l'herpèsvirus équin 1. Cette caractérisation a été confirmée, mais sur l'une des deux souches seulement, en utilisant *Bgl*II. La digestion de l'ADN avec *Kpn*I n'a pas été concluante. Les résultats de cette étude montrent que le génome de l'herpèsvirus 1 1B était présent en Argentine depuis 1996 au moins. Le fait que deux souches présentaient ce profil électrophorétique semble indiquer que les isolats argentins présentent des génomes hétérogènes.

Mots-clés

Analyse par endonucléase de restriction – Argentine – Génome 1B – Herpèsvirus équin 1 – Première détection.



Detección en Argentina del genoma 1B del herpesvirus equino 1

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Resumen

Los autores describen un estudio encaminado a determinar la variación genómica de los herpesvirus equinos (EHV) aislados en Argentina desde 1979 hasta la primera mitad de 2004, para lo cual se analizó la secuencia de ADN de las 69 cepas aisladas. De ellas, sesenta provenían de fetos abortados, una del plasma rico en leucocitos de un caballo con síntomas respiratorios y ocho de otros tantos casos de enfermedad neonatal. El ADN, obtenido a partir de células

epiteliales de conejo (RK13) infectadas con cada una de las cepas, se sometió a digestión con tres endonucleasas de restricción (*Bam*HI, *Bgl*II y *Kpn*I). Los fragmentos de restricción correspondientes a *Bam*HI permitían distinguir entre dos cepas, asignadas al grupo prototípico EHV-1 1B. La tipificación con *Bgl*II sólo confirmó el resultado en una de esas dos cepas. La digestión del ADN con *Kpn*I resultó infructuosa. Los resultados de ese estudio demuestran que el genoma del EHV-1 1B está presente en Argentina por lo menos desde 1996. El descubrimiento de dos cepas con este electroferotipo es señal de que existe heterogeneidad genómica entre las cepas aisladas en Argentina.

Palabras clave

Análisis por endonucleasas de restricción – Argentina – Genoma 1B – Herpesvirus equino 1 – Primera detección.



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