Equine herpesvirus 1 (EHV-1): characterisation of a viral strain isolated from equine plasma in Argentina

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Summary: Strain "LP-3" of equine herpesvirus 1 (EHV-1), isolated from plasma of horses with clinical signs of rhinopneumonitis, was characterised by cultural, physicochemical, electron microscopic, serological and immunohistochemical studies. This was the first isolation from Argentine horses with respiratory disease. Another strain, SP-1, was isolated in the same laboratory from an aborted fetus in 1980.

KEYWORDS: Argentina - Equine herpesvirus - Horse diseases - Respiratory diseases - Viral diseases.

INTRODUCTION

Infection of horses by equine herpesvirus 1 (EHV-1) is a leading cause of illness and death in the horse population and a source of serious economic loss to the horse breeding industry. EHV-1 infections may result in epizootics of respiratory tract disease, abortion and a paralytic disorder of the central nervous system (2, 3).

There are two genetically and antigenically distinct herpesviruses, designated as EHV-1 and EHV-4, with different patterns of epizootiology, pathogenesis and clinical disease (1, 5).

EHV-1 was first isolated in Argentina from an aborted fetus in 1980 (strain SP-1) (4). In 1985 an outbreak of respiratory disease affected horses on a breeding farm. On that occasion a herpesvirus was isolated from plasma rich in leukocytes of horses that showed respiratory signs.

This paper describes the characterisation of the newly-isolated strain "LP-3".

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MATERIALS AND METHODS

Samples

Samples were obtained from 28 horses with clinical rhinopneumonitis. Nasal swabs were transported in Minimum Essential Medium (MEM) with 5% fetal calf serum (FCS), then centrifuged at 3000 rpm at 4°C for 10 minutes, and finally stored at −70°C.

Blood was collected in 0.2% EDTA and kept at room temperature for 60 minutes. A plasma fraction rich in leukocytes was prepared and stored at −70°C.

Faeces were resuspended in MEM with 5% FCS and antibiotics, centrifuged at 3000 rpm at 4°C for 10 minutes and filtrated.

Cell cultures

Primary fetal equine kidney monolayer (FEK), fetal bovine kidney monolayers (FBK) and the cell lines VERO, BHK and MA-104 were inoculated with material obtained from the three types of samples. The inoculated cell lines were incubated for 7 days at 37°C. Nasopharyngeal samples were inoculated in amniotic and allantoic cavities of embryonated hens’ eggs. Three passages were carried out before considering that a sample was negative. Infected monolayers were fixed in Carnoy for routine staining or cold acetone for immunocytochemical methods. The neutralisation test (NT) was conducted by the fixed virus-variable serum method. Equine arteritis virus (EAV), equine adenovirus (EAdV) and EHV-1 antisera were used in the test. EAV and EHV-1 antisera were provided by Dr J.T. Bryans (Kentucky, USA), and EAdV antisera by Dr Y. Ando (Tochigi, Japan).

Immunofluorescent and immunoperoxidase techniques

The indirect immunofluorescent test (IF) was performed on infected tissue cultures. Fluorescein isothiocyanate (FITC) labelled anti-equine immunoglobulin (Cappel Lab., USA) was employed. The peroxidase-antiperoxidase (PAP) technique was performed as previously described (6), with the primary antiserum from horses diluted 1:100, 1:200, 1:400 and 1:800. All samples were stained in duplicate. Infected cultures with normal rabbit serum were used as controls.

Electron microscopy

Cell cultures were examined 24 hours after viral inoculation, when a cytopathic effect (CPE) was slightly evident. The samples were fixed in buffered glutaraldehyde and then treated with osmium tetroxide.

Physicochemical characterisation

The material obtained in FBK monolayers was analysed by the following methods:

a) Filtration, performed in Millipore filters of 50 nm, 100 nm, 225 nm and 450 nm.

b) Sensitivity to ether and chloroform. Viral particles were treated with 20% ethyl ether for 18 hours at 4°C, and with 5% chloroform for 10 minutes at room temperature.

c) Sensitivity to heat. The virus was exposed to 56°C for 15 and 30 minutes.
d) Sensitivity to trypsin. The isolate was treated with trypsin 1% and 0.2% at 37°C for 60 minutes.

e) Sensitivity to pH. The material was treated at pH 3 at 4°C for 18 hours and then inoculated.

All samples were titrated before and after the above-described treatments. Tests were repeated at least twice. Controls were carried out in parallel in all cases.

RESULTS

No CPE was apparent in cell cultures inoculated with samples from faeces and nasopharyngeal swabs. No haemagglutinating agent was recovered from embryonated eggs after three passages.

A FEK monolayer inoculated with plasma rich in leukocytes showed a clear CPE in the second passage after three days. The CPE increased after another passage. Intranuclear inclusion bodies were evident in the haematoxylin-eosin stained monolayers. CPE was absent from the other cell lines and no haemagglutinating agent was recovered from embryonated eggs. The virus was subsequently adapted to VERO, RK-13 and FBK cells.

Both IF and PAP methods were positive for EHV-1 (Fig. 1). NT was specific against EHV-1 antiserum when using horse and rabbit EHV-1 antisera.

FIG. 1

Peroxidase-antiperoxidase (PAP) positive cells around areas of cytopathic effect. FBK cell culture, 400×
Electron microscopy revealed viral particles in the nucleus and in the cytoplasm. Their ultrastructure agreed with previous descriptions of EHV-1 (Fig. 2). Cell membrane budding was also observed.

**FIG. 2**

Electron micrograph of FBK cell culture inoculated with EHV-1. Viral particles are seen in the cytoplasm, 50,000 ×

The isolated virus proved to possess an envelope, and to be sensitive to ether, chloroform, pH 3, temperature and trypsin. It passed through the 450 nm and 225 nm Millipore filters, and was retained by those of 100 and 50 nm.

**DISCUSSION**

There is little information on the epidemiology of EHV infections of horses in Argentina. Infection of horses with both respiratory and abortigenic strains is common in all areas of the country.

Several attempts at virus isolation and characterisation of EHV strains have been made. One strain has been isolated from an aborted fetus and another from nervous tissue (4, 9).

The presence of EHV was suspected from the epidemiological features of the present outbreak. Nevertheless, because of the possibility of the occurrence of other viral diseases, samples from faeces, plasma and nasal swabs were collected and inoculated in different cell lines and embryonated hens’ eggs.

Only fetal equine kidney cells inoculated with the plasma fraction rich in leukocytes showed CPE.

Scott *et al.* (10) considered that EHV-1 exists in the infected mononuclear cells in noninfective or subvirion forms. However, we isolated EHV-1 from plasma rich
in leukocytes. Plasma or leukocytes might have been the source of virus. It is possible that many virions had become adsorbed to leukocytes membranes, being released after a freeze-thaw cycle. The possibility of isolation from the plasma remains, although several authors consider that it is difficult (2, 7, 10).

A CPE was clear at the third day of the second passage on FEK cells. This may have been a consequence of the low titre of virus in the inoculated samples. Later the virus became adapted to VERO, RK-13 and FBK cells.

The observed cultural properties, physicochemical characteristics and electron microscopic studies were those of a herpesvirus (8).

Immunological techniques (PAP, IF and NT) using specific rabbit and horse EHV-1 antisera showed that the isolated virus was EHV-1. Isolation of the virus from plasma rich in leukocytes, the host cell range, and the time at which CPE was observed, suggest that the isolated virus is EHV-1 rather than EHV-4.

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Résumé : La souche «LP-3» de l’herpèsvirus équin 1 (EHV-1), isolée du plasma de chevaux présentant des signes cliniques de rhinopneumonie, a été caractérisée en l’étudiant par mise en culture, par microscopie électronique et du point de vue de ses propriétés physico-chimiques, sérologiques et immuno-histochimiques. Il s’agit du premier isolement de ce virus à partir de chevaux argentins atteints de maladie respiratoire. Une autre souche, SP-1, avait été isolée au même laboratoire en 1980 à partir d’un avorton.


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Resumen: Una cepa de herpesvirus equino 1 (EHV-1) fue aislada a partir de plasma de equinos que presentaron signos clínicos de rinoneumonitis. El virus fue caracterizado mediante su comportamiento en cultivos celulares, microscopía electrónica y por sus características fisicoquímicas, serológicas e inmunohistoquímicas. La cepa fue denominada LP-3 y constituye el primer aislamiento realizado en Argentina a partir de equinos con enfermedad respiratoria. Otra cepa, denominada SP-1, fue aislada en el laboratorio en 1980 a partir de un feto abortado.

REFERENCES


