# NOTES

## Isolation of Equine Herpesvirus Type 1 from a Horse with an Acute Paralytic Disease

J. Thorsen and P. B. Little*

ABSTRACT

**A Standardbred mare became paralyzed shortly after showing signs of an upper respiratory infection. The mare was euthanized and equine herpesvirus type 1 was isolated from the brain and spinal cord.**

RÉSUMÉ

**Une jument "Standardbred" devint paralysée peu de temps après avoir manifesté des signes d'une infection des voies respiratoires supérieures. Les auteurs procédèrent à l'euthanasie de la bête et isolèrent le virus herpès type 1 équin de son cerveau et de sa moelle épinière.**

Neurological disturbances and paralysis are observed frequently in horses in Canada concurrently with epizootics of respiratory disease due to equine herpesvirus type 1 (EHV 1) or after individual cases of rhinopneumonitis. This report concerns the isolation of a strain of EHV 1 from a five year old Standardbred mare which became paralyzed three days after developing a cough and pyrexia.

In December 1973, a tetraparetic horse was received at the Ontario Veterinary College, Case No. 35076. The horse was one of three which had undergone respiratory infection on the same farm. One of the other two horses had shown some signs of nervous involvement but had recovered. The horse which arrived at the College was in lateral recumbency. The time elapsed was nine days since the onset of illness with cough and fever. Prior to euthanasia a serum sample was collected from the horse.

At autopsy, sections of lumbar, thoracic and cervical spinal cord as well as brain were quick frozen and stored at −70°C. Three weeks later 10% w/v suspensions of the specimens were prepared in phosphate buffered saline and were inoculated into primary horse kidney cell cultures which were incubated at 37°C in a stationary position.

Antisera were prepared in rabbits against the Kentucky D strain of EHV 1 and against the virus isolated. The viruses were compared by reciprocal plaque reduction tests in RK 13 cell cultures in plastic trays

*Department of Veterinary Microbiology and Immuology and Department of Pathology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada.

Submitted October 18, 1974.

with 16 mm wells[1].

The neutralizing antibody titre against EHV 1 of the serum sample collected at the time of autopsy was also determined by a plaque reduction test.

Sections of cervical and thoracic spinal cord, four microns thick, were cut on a Lab Tek cryostat and mounted on glass slides. Sections were air-dried, fixed in acetone, rinsed in phosphate buffer saline and stained. An indirect staining method was used with either normal rabbit serum, rabbit antiserum against EHV 1 or rabbit antiserum against equine globulin as the first stain. This was followed by staining with a fluorescein conjugated goat globulin anti-rabbit globulin stain. All sera were absorbed with horse brain powder prior to use in the test.

Tissues for histopathological examination were fixed in unbuffered formalin and were stained with hematoxylin and eosin.

In two of three cell cultures inoculated with cervical cord and brain and one of three tubes inoculated with thoracic cord material cytopathic effects consisting of rounding and clustering of cells and formation of giant cells with eosinophilic intranuclear inclusions were visible by the fourth day postinoculation. On subculture the effects were visible by the day after inoculation.

The cell controls remained normal and cell cultures inoculated with other specimens of nervous tissue also remained normal.

An antiserum prepared in rabbits against the virus isolate, designated V 35076, had a 50% plaque reduction titre of 1:850 against the homologous virus and of 1:410 against EHV 1. A rabbit antiserum prepared against EHV 1 had a 50% plaque reduction titre of 1:400 against EHV 1 and of 1:320 against V 35076. The serum sample from the horse had a titre of 1:300 against EHV 1.

No localization of viral antigen or equine globulin could be detected by examination of sections stained by fluorescent antibody.

On histological examination lesions in the spinal cord and brain were those of a disseminated necrotizing myeloencephalitis with multiple small focal areas of necrosis, hemorrhage and fibrinoid vascular changes.

Although there are several reports in the literature of the association of equine herpesvirus type 1 with paralytic syndromes in horses (1, 2, 3, 4, 5, 6, 7), only one successful isolation of the virus from the nervous tissue of affected horses has been reported (7). It has been suggested that the difficulty in isolation may be due to binding of the virus with antibody at the time of preparation of the tissue for inoculation (4). No special precautions were taken in the present case to obviate this possibility but the spinal cord sections were removed with the dura mater intact to minimize contamination with blood and specimens for virus isolation were taken from the middle of these sections after carefully removing the dura mater. Collection of tissues at the time the animal was acutely affected clinically and immediate freezing of the tissue in a dry ice-acetone bath may have led to the successful isolation.

[1]Linbro Chemical Co. Inc., New Haven, Connecticut.

## ACKNOWLEDGMENTS

This investigation was supported in part by a grant from a group of horsemen's associations, administered by the Ontario Harness Horsemen's Association and in part by the Ontario Ministry of Agriculture and Food. The case was submitted by Dr. Merle Bauman, a veterinary practitioner at Elmira, Ontario. We are grateful to N. Weninger for able technical assistance.

### REFERENCES

1. **BITSCH, V. and A. DAM.** Nervous disturbances in horses in relation to infection with equine rhinopneumonitis virus. Acta. vet. scand. **12**: 134-136. 1971.
2. **COLLINS, J. D.** Virus abortion outbreak in Ireland. Vet. Rec. **91**: 129. 1972.
3. **DALSGAARD, H.** Enzootic paresis in horses as a consequence of outbreaks of rhinopneumonitis (virus abortion). Medlemsbl. danske Dyrlaegeforen. **53**: 71-76. 1970.
4. **JACKSON, T. and J. W. KENDRICK.** Paralysis of horses associated with equine herpesvirus type 1 infection. J. Am. vet. med. Ass. **158**: 1351-1357. 1971.
5. **MANNINGER, R.** Studies on infectious abortion in mares due to a filterable virus. Acta vet. hung. **1**: 62-72. 1949.
6. **PETZOLDT, K., U. LUTTMANN, J. POHLENZ and U. TEICHERT.** Virologische Untersuchungen des Zentralnervensystems von Pferdefeten und Befunde bei zentralnervös erkrankten Stuten nach Aborten durch das Equine Herpesvirus 1. Schweizer Arch. Tierheilk. **114**: 129-139. 1972.
7. **SAXEGAARD, F.** Isolation and identification of equine rhinopneumonitis virus (equine abortion virus) from cases of abortion and paralysis. Nord. VetMed. **18**: 504-512. 1966.