J Vet Diagn Invest 3:137–143 (1991)

Experimental equine herpesvirus-1 infection in llamas (*Lama glama*)

James A. House, Douglas A. Gregg, Juan Lubroth,
Edward J. Dubovi, A. Torres

Abstract. Three llamas (*Lama glama*) were experimentally infected intranasally with an isolate of equine herpesvirus-1 (EHV-1) from the brain of an alpaca that had experienced severe neurologic signs. Two of the 3 llamas developed severe neurologic disorders following inoculation; 1 died, and 1 was euthanized in a moribund state. The third llama showed only mild neurologic signs. The euthanized llama had preexisting antibodies to EHV-1, and the remaining 2 llamas were seronegative (virus neutralization titer <6) at the time of inoculation. One of the seronegative llamas died acutely without production of detectable antibodies, and the other developed antibodies typical of a primary immune response. The EHV-1 virus was recovered only from a sample of the thalamus of the llama that died acutely. Histopathologic lesions were observed in the brain and retina of the dead and euthanized animals. This study verifies the pathogenic potential of EHV-1 for llamas.

Equine herpesvirus-1 (EHV-1) infection causes a respiratory disease (rhinopneumonitis) that is most frequently seen in foals and yearlings.[3,15,22] In pregnant mares, EHV-1 is a major cause of abortion storms in late gestation. In addition, neurologic disturbances associated with an encephalomyelitis may occur in mature animals following infection with EHV-1. Although neurologic disorders are not common, they can be an important sequela. Clinical signs associated with the neurologic form of disease initially reflect lower motor neuron defects, including an initial dysuria, constipation, perineal analgesia, protrusion of the penis, locomotor dysfunction varying from mild ataxia to severe paralysis, and possible tetraplegia. When the cortex or brainstem is involved, encephalitic signs, including convulsions, may occur.[3,4,10,12,19,22,26]

Equine herpesvirus-1 has a worldwide distribution. Of the 9 countries in North and Central America, it occurs in Canada, the United States, Guatemala, Costa Rica, and Panama. In South America, EHV-1 has been confirmed only in Brazil, Chile, and Peru.[17] Although EHV-1 is considered a disease of equids, it has been isolated from other species, including aborted bovine fetuses,[4,6] Grevy's zebras experiencing abortion and perinatal foal mortality,[27] an aborted onager fetus,[14] and an antelope showing signs of malignant catarrhal fever.[4] Infection with EHV-1 has also been associated with myelitis in a zebra.[14]

In 1984, an epizootic of total blindness of unknown origin affected 23 animals in a herd of 100 alpacas and llamas at a game farm in New York state.[20] Antibodies against EHV-1 were found in the 21 serum samples from affected animals. Serum samples from healthy cohorts revealed EHV-1 neutralizing antibodies (A. Torres, unpublished data). The ocular lesions described in affected llamas and alpacas included retinal detachment, optic disc necrosis, and hemorrhage.[20] Blindness was often central as well as peripheral, with necrosis of the optic nerve and lateral geniculate nucleus. The period from the onset of fever to blindness was 2–4 weeks. Some of the animals with a more severe case of generalized encephalitis died. A herpesvirus, serologically indistinguishable from EHV-1, was isolated from the brain or ocular tissues of 4 alpacas and 1 llama.[20]

In Chile and Peru where EHV-1 infections are known to occur in equids,[17] neither blindness of unknown etiology or infection with EHV-1 has been documented in camelids.[21] The purpose of this study was to determine the susceptibility of the llama to an EHV-1 virus isolated from the outbreak in New York state.

Materials and methods

Animals. Three healthy male llamas, born in the USA, numbered 1, 2, and 3, and aged 3, 2, and 6 yr, respectively, were housed in an animal isolation room at the Foreign Animal Disease Diagnostic Laboratory (FADDL) on Plum

From the US Department of Agriculture, Animal and Plant Health Inspection Service, Science and Technology, National Veterinary Services Laboratories, Foreign Animal Disease Diagnostic Laboratory, PO Box 848, Greenport, NY 11944 (House, Gregg), the Mexico–United States Commission for the Prevention of Foot-and-Mouth Disease and Other Exotic Animal Diseases, USDA, APHIS, IS, AGAFT, "AMEMB, Mexico City," PO Box 3087, Laredo, TX 78044 (Lubroth), the Diagnostic Laboratory, New York State College of Veterinary Medicine, Cornell University, Ithaca, NY 14850 (Dubovi), and SmithKline Beecham Animal Health, Norden Laboratories, 601 W Cornhuskers Highway, Lincoln, NE 68521 (Torres).

Received for publication August 24, 1990.

Table 1. Results of serologic tests on sera from llamas exposed to equine herpesvirus-1 (alpaca isolate 259306).

Animal	DPI*	VN titer†	IFA‡
Llama 1	0	56	+
	7	450	+
	8	180	+
Llama 2	0	5	−
	7	5	−
Llama 3	0	<4	−
	7	<4	−
	10	<4	−
	14	6	+
	20	6	+
	32	45	+

* = Days postinfection.

† Virus neutralization titer expressed as the reciprocal of dilution equivalent to 50% endpoint determined by the Spearman–Karber method; titer of ≥6 is considered positive.

‡ IFA = indirect fluorescent antibody test. + = specific fluorescence at a 1:20 serum dilution; − = no specific fluorescence at a 1:20 serum dilution.

Island, New York. The laboratory provides greater than Biosafety Level 3 containment.

Virus. An isolate of EHV-1 from the brain of an alpaca, designated as EHV-1 isolate 259306,[20] was passaged twice in fetal equine kidney cells and once in a simian virus 40-transformed equine ovary (EO) cell line. The titer of the virus was $10^{5.8}$ $TCID_{50}$/ml.

Animal inoculation and observations. The llamas were exposed to virus by spraying approximately 1 ml of cell culture fluid of isolate 259306 into each nostril and tilting the head upward during administration. Animals were observed daily and clinical signs and rectal temperatures were recorded. Animals were euthanized at 32 days postinoculation (DPI) or when moribund.

Sample collection. Ocular and nasal swabs were collected from 3 to 12 DPI. Swabs were placed in Eagle's minimum essential medium (EMEM) and stored at −70 C. Tissue samples including brain, adrenal gland, kidney, liver, spinal cord, lung, spleen, and vitreous humor were collected aseptically at necropsy and stored at −70 C. Blood samples were collected for virus isolation and serologic testing (Table 1).

Virus isolation. Swabs and 10% tissue suspensions made in EMEM were centrifuged at 900 × *g* for 10 min. All samples for virus isolation were inoculated onto confluent monolayers of EO cells between the 23rd and 27th passage level. The supernatant was adsorbed for 1 hr at 37 C, followed by 3 washes with EMEM. The cell cultures were maintained with EMEM containing nonessential amino acids, 5% fetal bovine serum (FBS), and 300 μg of gentamicin and 2.5 μg of amphotericin-B per ml. Most samples were passaged by scraping the cells from the flask and inoculating them into freshly prepared monolayers of EO cell cultures; a few cell cultures were frozen at −70 C until additional cell cultures were available for subpassage. Inoculated cell cultures were observed daily and passaged 2–3 times at 5–7-day intervals before a final determination was made.

Samples of nasal and ocular swabs, kidney, adrenal gland, brain, lung, spleen, and spinal cord were inoculated onto confluent monolayers of equine dermis (ED) and embryonic equine kidney (EEK) cell cultures.[a] Fresh tissue samples including nasal mucosa, spleen, thalamus, thoracic spinal cord, and vitreous humor from llama 3, collected at 32 DPI, were minced and placed on confluent monolayers of ED cell cultures to determine if virus could be isolated by cocultivation.

Virus neutralization (VN) test. A strain of EHV-1 isolated from an aborted equine fetus was used at about the 25th passage level in EEK or ED cell cultures. Approximately 30 $TCID_{50}$ of virus was used in a microtiter test. After 6 days incubation at 37 C in a 5% CO_2 humidified atmosphere, the cells were fixed and stained with a solution of 0.15% crystal violet in 2% glutaraldehyde and read microscopically.

Indirect fluorescent antibody (IFA) test. The virus used for the VN test was diluted and inoculated onto monolayers of ED cell cultures on 8-chamber slides.[b] A dilution of virus yielding about 15 infected cells per 200× field was used to prepare the slides for the IFA test. After 2 days of incubation at 37 C in a 5% CO_2 humidified atmosphere, the medium was decanted, and the monolayers were rinsed and fixed in 60% acetone and 40% methanol at 4 C for 10 min. Llama serum was diluted at 1:20 and applied to the cell sheet for 30 min at 25 C. After washing, the slides were stained with a fluorescein isothiocyanate-conjugated rabbit anti-camelid gamma globulin antiserum. This antiserum was produced by multiple injections of rabbits with gamma globulin fractions of llama, alpaca, and camel sera prepared by ammonium sulfate precipitation. After 30 min at 25 C, the slides were rinsed in phosphate-buffered saline (PBS), pH 7.4, rinsed in water, and dried. The cell sheet was examined for the presence of specific fluorescence under UV incident illumination on a fluorescence microscope.[c]

Tissue fixation. Both eyes from each animal were fixed in Bouin's fixative for 48 hr, rinsed in water, and dehydrated in graded ethanol. All other tissues for hematoxylin and eosin (HE) and immunohistochemical staining were fixed in 10% formalin, processed, and paraffin embedded. Selected tissue sections were cut at 3 μm and placed on 0.1% poly-L-lysine[d] (>300,000 MW)-coated slides, dried for 2–4 hr at 45 C, and deparaffinized.

Immunohistochemistry. For avidin–biotin complex–alkaline phosphate (ABC–AP) immunostaining, sections were rehydrated briefly in PBS then treated with 0.1% trypsin[e] (prepared in 0.01 M Tris buffer pH 7.6 with 0.1% $CaCl_2$ and NaCl) for 30 min at 37 C. Enzyme digestion was stopped by washing in 2% glycine for 10 min then in PBS for 10 min. Sections were blocked with 2% normal goat serum for 30 min at 37 C and drained. Primary anti-EHV-1 antibody[a] (1:250 in PBS) was applied, and the slides were incubated overnight at 4 C. After washing, the secondary biotinylated anti-rabbit conjugate[f] was applied, and the slides were incubated for 1 hr at 37 C. ABC reagent[f] was prepared 30 min before using and applied for 1 hr at 37 C. Nitroblue tetrazolium substrate indicator[f] was applied, and the slides were incubated in the dark for 45 min. Sections were counterstained with 1% nuclear fast red and read microscopically.

Electron microscopy. For electron microscopy, tissue was selected from paraffin-embedded blocks where intranuclear inclusion bodies (INIBs) were found by HE staining. These

samples were deparaffinized, rehydrated, fixed in 4% osmium tetroxide, dehydrated in acetone, and embedded in epoxy resin.[8]

Results

Clinical observations

Llama 1. At 5 DPI, saliva was observed dripping from the mouth. The animal was febrile at 5 and 8 DPI (39 C and 39.5 C, respectively). At 7 DPI, the animal was depressed and apprehensive, moved slowly, and expelled froth from the mouth. At 8 DPI, severe neurologic signs, including head pressing (Fig. 1), opisthotonos, blindness, crossed or splayed forelimbs, staggering, ear fasciculations, head tremors, and oblivion to voices were noted. The animal was euthanized at 8 DPI.

Llama 2. Slight depression was noted at 5 DPI. A frothy oral discharge with a foul odor was present at 6 DPI, and the animal was hyperexcitable and apprehensive. At 7 DPI, there was a frothy nasal discharge, and the nasal septum was hyperemic and contained a necrotic epithelial focus (5 × 8 cm) on the right aspect. The palpebral conjunctiva and nictitating membranes of both eyes were congested. The animal continued to be hyperexcitable, paced the room continuously in a counterclockwise direction, and had head tremors. Rectal temperature was elevated at 6 (40 C), 7 (42 C), and 8 (39.5 C) DPI, and the llama died at 8 DPI.

Llama 3. This animal did not show remarkable nervous signs during the course of infection except a suggestion of decreased visual acuity beyond 8 DPI. Congestion of the oral, nasal, and ocular membranes was observed 5–9 DPI. Between 6 and 8 DPI, a mild depression and elevated temperature (maximum of 39.2 C at 7 DPI) were noted. Except for the diminished visual acuity, the animal remained clinically normal 10–32 DPI, when the clinical study was terminated.

Virus isolation studies

All samples tested were negative for virus isolation except for 1 sample of the thalamus of llama 2. Herpesvirus-like foci (rounded cells in grape-like clusters) appeared in EO cell cultures 1 day after inoculation, and the cytopathic effect (CPE) progressed to 100% by 3 DPI. A duplicate sample was tested in EEK and ED cell cultures with the same result. The virus was identified as EHV-1 by VN.

IFA and VN Studies

Llama 1 was seropositive at 0 DPI and developed an anamnestic response after inoculation with EHV-1. Llamas 2 and 3 were seronegative (VN titer <6) at 0 DPI. Llama 3 seroconverted in a pattern typical of a primary immune response. There was a good correlation between the VN and IFA tests (see Table 1).

Figure 1. Llama 1 8 days postinoculation with EHV-1 showing head pressing, apparent blindness, and ataxia.

Histopathology

Gross lesions, except for a necrotic hyperemic focus (5 × 8 cm) in the nasal septal mucosa of llama 2, were not observed in any of the llamas at necropsy. Histologically, llamas 1 and 2 were similar, and lesions were limited to the central nervous system. Brain lesions were most severe in the anterior cerebrum where there was marked cerebral edema of the Virchow–Robin spaces and perineuronal tissue. The edema was multifocal, sometimes sparing the cerebral cortex on 1 side of a sulcus, while being severe in the opposing cortical grey matter (Fig. 2). In severely affected foci, there was scattered hemorrhage accompanying small arteriole and capillary endothelial cell degeneration, including rounding, pyknosis, and occasional INIBs. Shrunken, angular, and degenerate neurons with a slight increase in the number of satellite cells could be found in the molecular layer where edema was most severe. In some of the neurons and in a few glial cells, INIBs could be found. Similar scattered neuronal changes were present

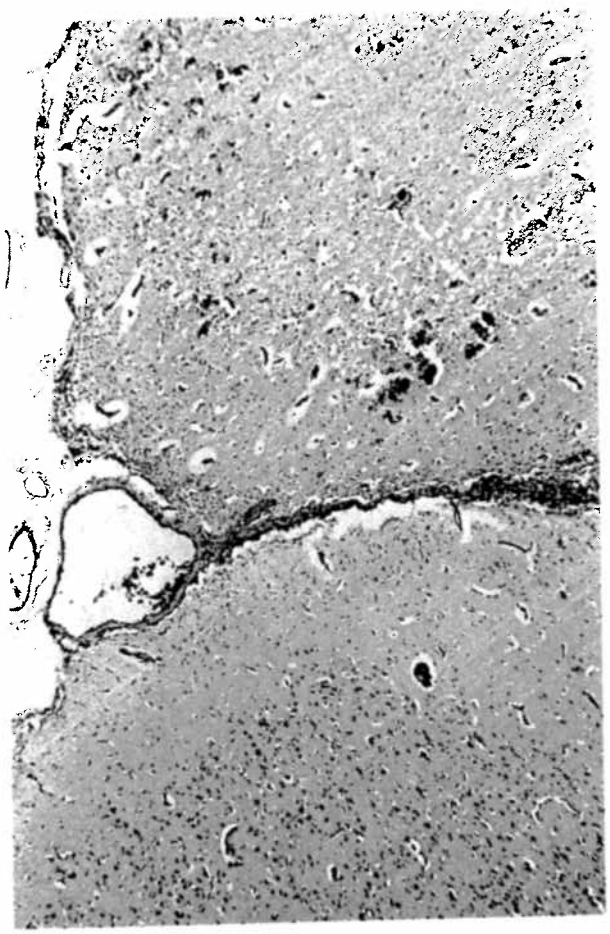

Figure 2. Llama 1; anterior cerebrum with focal hemorrhage and edema of the molecular layer and nonsuppurative meningitis. Note the difference in severity on either side of the sulcus. HE stain.

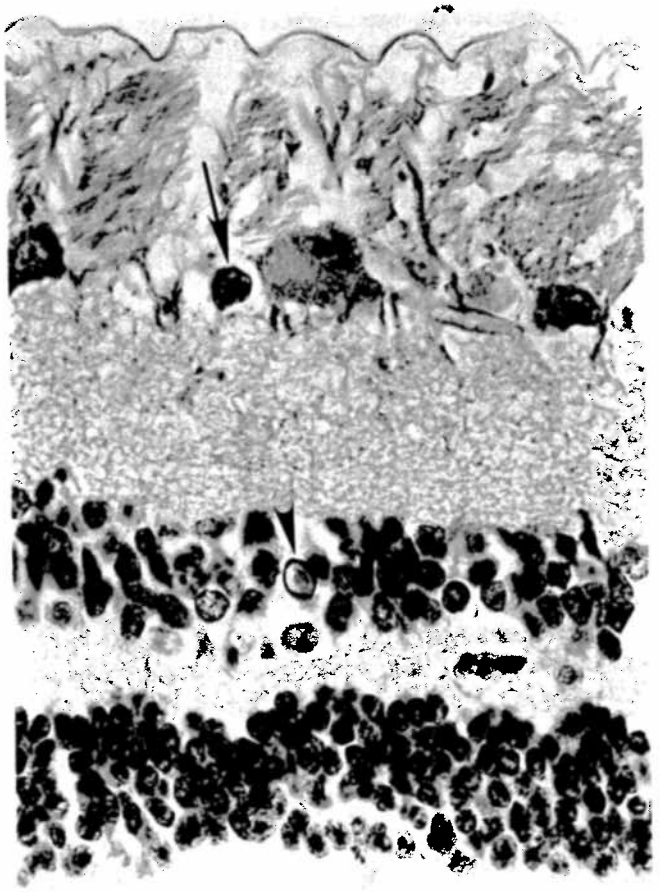

Figure 3. Llama 1; optic retina showing a moderate edema of the ganglion layer (top) with mild degeneration of neurons and 1 intranuclear inclusion body (arrow). Another inclusion is present in the inner nuclear layer (arrowhead). HE stain.

in the hippocampus and in the caudate, hypothalamic, and thalamic nuclei. Neuronal changes were mild or absent caudal to the level of the thalamus. Most white matter of the corona radiata and optic nerve had moderate edema, giving it a "moth-eaten" appearance. Larger vessels in affected foci and most of the meninges were surrounded by several layers of lymphocytes. No significant lesions were found in the spinal cord.

In 1 eye of llama 1, 1 quadrant of the retina had marked edema, especially in the ganglionic layer. In this region, there were multiple small to medium arterioles and a few capillaries with endothelial degeneration, consisting of oval and rounded nuclei with marginated chromatin and central deep magenta INIBs filling the nucleus. A few neurons in the ganglion layer had chromatolysis, nuclear swelling, and distinct well-demarcated INIBs (Fig. 3). In the subjacent inner nuclear layer, a few neuronal nuclei had similar changes and INIBs. The optic nerve was moderately edematous with a mild infiltration of gitter cells that rarely contained INIBs. No significant inflammatory infiltrate was present in the eye.

Immunohistopathology

Intense immunostaining was observed in the cytoplasm of angular and degenerating neurons located within focal areas of the cerebral cortex having the most severe edema and hemorrhage (Fig. 4). This staining corresponded to areas where INIBs were found in serial sections stained by HE. Adjacent, less edematous areas of cortex in the same gyrus did not immunostain.

Electron microscopy

Sections with numerous inclusion bodies and specific immunostaining were reembedded for EM. Intranuclear viral inclusion bodies were found with characteristic herpesvirus particles within endothelial cells and neurons. Glial cells were often found with granular inclusion bodies, but virions were not found in these cells.

Discussion

The isolation of EHV-1 from horses showing nervous signs is difficult and highly variable.[3,10,19,22,26] The

isolation of EHV-1 from llamas and alpacas showing nervous signs, blindness, and acute death[20] has expanded the known host range for the virus. The present study experimentally confirmed the ability of EHV-1 to cause severe neurologic signs and death in llamas.

The difficulty in isolating EHV-1 from infected animals was demonstrated by the failure to isolate virus from all but 1 of the samples tested in this study. Virus was not recovered from llama 1, which was euthanized in a moribund state, nor from llama 3, which showed minimal clinical signs of infection. Llama 1 was the only animal that had neutralizing antibodies and positive IFA reaction prior to exposure to the virus, possibly accounting for the failure to isolate virus as has been previously noted.[10] Also, immunohistopathology from llama 1 revealed that the infected foci in the brain were widely scattered, and a focus of viral replication may have been inadvertently missed during sample collection. Because there was rhinitis early after infection, it is difficult to explain the failure to reisolate virus from all nasal swabs. Perhaps transient or abortive infection occurs in llama respiratory epithelium.

The role of antibodies in protection against EHV-1 infection is uncertain. Llama 1 had a VN titer of 56 at the time of inoculation with EHV-1. This llama developed grave clinical signs and mounted a significant anamnestic response (VN titer 450) to EHV-1. It is difficult to explain the presence of specific antibodies to EHV-1 in llama 1, because the prevalence of antibodies in llamas is quite low (E. Dubovi, unpublished data). Six horses with VN antibodies to EHV-1 were experimentally infected with EHV-1; these horses developed severe neurologic signs and demonstrated an anamnestic response to EHV-1.[10] Low levels of antibodies to EHV-1 failed to protect horses against equine respiratory disease in New Zealand.[9]

The histopathologic lesions observed in llamas inoculated with EHV-1 are not typical of those seen in foals or horses. Lesions in the llama brain included a severe multifocal acute vasculitis with acute cerebral and retinal edema and a moderate multifocal acute meningoencephalitis with INIBs. The eye lesion was a moderate acute multifocal necrotizing retinitis with INIBs. The lesions in foals usually occur in multiple organs and consist of focal necrosis with inclusion bodies. The brain is often not involved. The lesions in adult horses may be primarily pulmonary, or as in cases involving neurologic disorders, the spinal cord may have a vascular distribution of hemorrhage, with inclusion bodies seldom seen. The severity of EHV-1 infection in llamas is similar to herpesvirus encephalitides caused by herpesviruses infecting an unnatural host, such as simian herpesvirus B in humans[18] or herpes simplex (HSV) in the gibbon[23] and in mice.[2,7] Herpesvirus infections in foreign hosts often result in a fulminating lethal encephalitis in which neuronal INIBs are present.

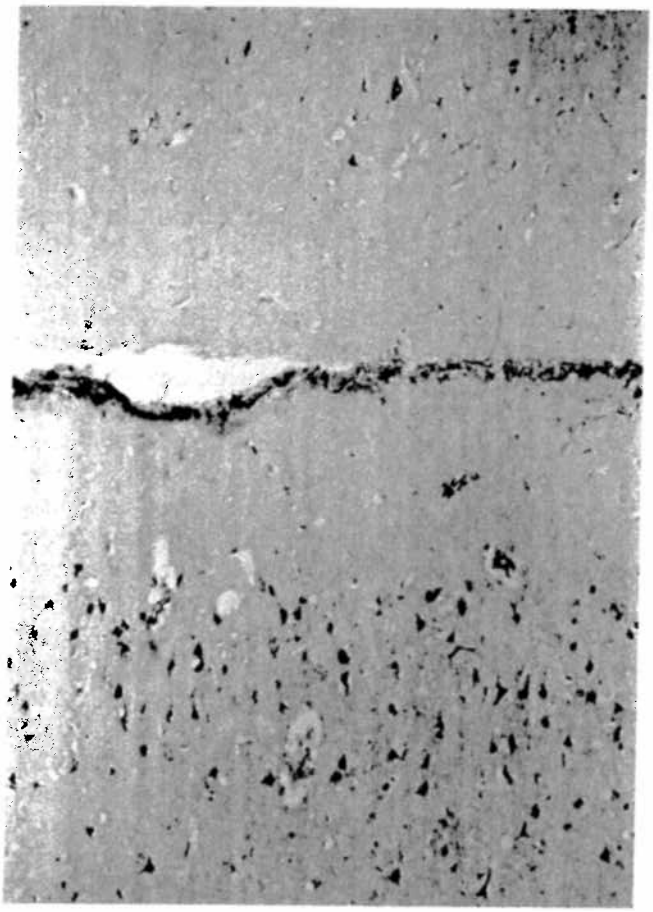

Figure 4 Llama 1; midcerebrum paraffin section immunostained by ABC–alkaline phosphatase method showing dark staining shrunken neurons with perivascular and perineuronal edema on 1 side of the sulcus and only a few neurons immunostained in the opposing grey matter.

The typical ocular lesion of HSV in humans or infectious bovine rhinotracheitis in cattle is a keratoconjunctivitis that does not spread or ascend to the retina and brain. A severe necrotizing retinitis and encephalitis, as in the llamas of this study, have been seen with HSV in human infants[5,8] and in experimental infections with HSV-1 in mice.[2] These naive hosts were exposed in the eye or nasal mucosal surfaces. The virus probably spreads via the cranial nerves to the brain to cause an encephalitis.[11] However, the route of infection to the retina is uncertain and often debated. In humans and mice, several reports support viral spread via the optic nerve,[1,13] although other reports indicate hematogenous spread to the retina in a human infant.[5] Direct histopathologic or electron microscopic evidence of spread is rarely seen. In llama 1, INIBs were found in focal areas in the retinal vascular endothelial cells, with adjacent INIBs in the ganglionic and inner nuclear layer. These foci of retinal infection centering around

and involving the endothelium of blood vessels suggest a hematogenous spread and are nearly identical to those seen in the retina of a human infant infected with HSV.[5]

In the initial field outbreak in alpacas,[19] there were ophthalmoscopic findings of a retinitis with a vascular pattern and hemorrhage, but the blindness in these alpacas was due to both peripheral retinitis and central optic tract necrosis. Because of the chronicity of the lesions in the alpacas, the pathogenesis of the retinal lesion could not be ascertained by histopathology. In these cases, there was severe necrosis of the retina, optic nerve, lateral geniculate nuclei, and all parts of the optic pathway (D. A. Gregg, unpublished data). Although these lesions were found in most field cases, they were not reproduced experimentally. Our experimental infection may have been so acute as to cause a severe vasculitis and encephalitis with cerebral edema and death before the optic tract lesion had a chance to develop. Because herpesviruses usually travel centripetally up cranial nerves (the olfactory nerve pathway to the frontal lobe of the cerebral cortex), intranasal inoculation with a relatively large dose of virus may have resulted in more severe anterior cerebral involvement than seen in the field cases. This possibility is supported by findings in human fetuses of an olfactory pathway of infection with HSV-1[16] and more recent reports of olfactory cerebral cortex infections in mice[25] and rabbits[24] following intranasal inoculation of HSV-1. The retinal infection may be secondary, following the olfactory tract infection and an early viremia. Such a hematogenous route to the retina seems likely based on the common occurrence of INIBs in endothelial cells in the vessels of the cerebrum and in the vessels and ganglion layer of the retina.

Acknowledgements

We thank Marjory E. Llewellyn and Christopher DeMaula for their excellent technical assistance, the Animal Care Staff of the FADDL for their excellent assistance and care of the llamas, Dr. Carol House for editorial comments during manuscript preparation, and Mr. Stephen E. Perlman, librarian, for his help with literature searches.

Sources and manufacturers

a. National Veterinary Services Laboratories (S. Wessman), Ames, IA.
b. Miles Laboratories, Elkhart, IL.
c. E. Leitz, Wettler, Germany.
d. Sigma Chemical Co., St. Louis, MO.
e. Grand Island Biologicals, Grand Island, NY.
f. Vector Laboratories, Burlingame, CA.
g. Fullam Laboratories, Latham, NY.

References

1. Anderson JR, Field HJ: 1983, The distribution of herpes simplex type I antigen in the mouse central nervous system after different routes of inoculation. J Neurol Sci 60:181–195.
2. Anderson JR, Field HJ: 1984, An animal model of ocular her pes: keratitis, retinitis and cataract in the mouse. Br J Exp Path 65:283–297.
3. Bitsch V, Dam A: 1971, Nervous disturbances in horses i relation to infection with equine rhinopneumonitis virus. Act Vet Scand 12:134–136.
4. Chowdhury SI, Kubin G, Ludwig H: 1986, Equine herpesvir type 1 (EHV-1) induced abortions and paralysis in a Lipizzan stud: a contribution to the classification of equine herpesviruse Arch Virol 90:273–288.
5. Cibis GW, Flynn JT, Davis EB: 1978, Herpes simplex retiniti Arch Ophthalmol 96:299–302.
6. Crandell RA, Ichimura H, Kit S: 1988, Isolation and compa ative restriction endonuclease DNA fingerprinting of equi herpesvirus-1 from cattle. Am J Vet Res 49:1807–1813.
7. Field HJ, Anderson JR, Wildy P: 1982, Atypical patterns neural infection produced in mice by drug resistant strains herpes simplex virus. J Gen Virol 59:91–99.
8. Greer CH: 1980, Bilateral necrotising retinitis complicati fatal encephalitis probably due to herpes simplex type Ophthalmologia 180:146–150.
9. Horner GW: 1981, Serological relationships between abor facient and respiratory strains of equine herpesvirus type 1 New Zealand. NZ Vet J 29:7–8.
10. Jackson T, Kendrick JW: 1971, Paralysis of horses associat with equine herpesvirus 1 infection. J Am Vet Med Assoc 1 1351–1357.
11. Knotts FB, Cook ML, Stevens JG: 1974, Pathogenesis of h petic encephalitis in mice after ophthalmic inoculation. J Inf Dis 130:16–26.
12. Mahew IG, MacKay RJ: 1982, The nervous system: spi infection and inflammation. *In:* Equine medicine and surge ed. Mansmann RA, McAllister ES, Pratt PW, pp. 1228–12 American Veterinary Publications, Santa Barbara, CA.
13. Minckler DS, McLean EB, Shaw CM, et al.: 1976, Herpes vi hominis encephalitis and retinitis. Arch Ophthalmol 94:89–
14. Montali RJ, Allen GP, Bryans JT, et al.: 1988, Equine herp virus type 1 abortion in an onager and suspected herpesvi myelitis in a zebra. J Am Vet Med Assoc 187:1248–1249.
15. O'Callighan DJ, Gentry GA, Randall CC: 1983, The equ herpesvirus. *In:* The herpesvirus II, ed. Roizman R, pp. 2 318. Plenum Press, New York, NY.
16. Ojeda VJ: 1980, Fetal herpes simplex encephalitis with d onstration of virus in the olfactory pathway. Pathology 12:
17. Pan American Health Organization: 1983, Diagnosis of Ani Health in the Americas, Washington, DC, USA. Scientific P lication No. 452, pp. 61–71.
18. Pierce EC, Pierce JD, Hull RN: 1985, B virus: its current nificance. Description and diagnosis of a fatal human infect Am J Hyg 68:242–250.
19. Pursell AR, Sangster LT, Bryars TD, et al.: 1979, Neurol disease induced by equine herpesvirus 1. J Am Vet Med A 175:473–474.
20. Rebhun WC, Jenkins DH, Riss RC, et al.: 1988, An epizo of blindness and encephalitis associated with a herpesviru distinguishable from equine herpesvirus 1 in a herd of alp and llamas. J Am Vet Med Assoc 192:953–956.
21. Rivera H, Madwell BR, Ameghina E: 1987, Serological su of viral antibodies in the Peruvian alpaca (*Llama pacos*). J Vet Res 48:189–191.
22. Saxegaard F: 1966, Isolation and identification of equine rh pneumonitis virus (equine abortion virus) from cases of a tion and paralyses. Nord Veterinaermed 18:504–512.
23. Smith PC, Yuill TM, Buchanan RD, et al.: 1969, The gi (*Hylobates lar*); a new primate host for herpesvirus homir A natural epizootic in a laboratory colony. J Infect Dis 292–297.

24. Stroop WG, McKendall RR, Battles EM, et al.: 1990, Spread of herpes simplex virus type 1 in the central nervous system during experimentally reactivated encephalitis. Microb Pathog 8:119–134.
25. Stroop WG, Rock DL, Fraser NW: 1984, Localization of herpes simplex virus in the trigeminal and olfactory system of the mouse central nervous system during acute and latent infections by *in situ* hybridization. Lab Invest 51:27–38.
26. Thorsen J, Little PB: 1975, Isolation of equine herpesvirus type 1 from a horse with an acute paralytic disease. Can J Comp Med 39:358–359.
27. Wolff PL, Meehan JP, Basgall EJ, et al.: 1986, Abortion and perinatal foal mortality associated with equine herpesvirus type 1 in a herd of Grevy's zebra. J Am Vet Med Assoc 189:1185–1186.