Encephalitis in a rabbit caused by human herpesvirus-1

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Case Description—An 8-month-old sexually intact male rabbit was examined because of a 2-day history of anorexia, epiphora of the left eye, bruxism, hypersalivation, and ataxia.

Clinical Findings—Physical examination of the rabbit revealed bilateral conjunctivitis, hypersalivation, and severe signs of CNS dysfunction such as incoordination, intermittent myoclonic seizures, and opisthotonus. Results of hematologic and serum biochemical analyses revealed only lymphopenia, a relative monocytosis, and an increase in serum activity of creatine phosphokinase and serum concentration of total protein. Serum antibodies against *Encephalitozoon cuniculi* and *Toxoplasma gondii* were not detected.

Treatment and Outcome—Despite IV administration of crystalloid fluids and treatment with antimicrobials, vitamin B complex, nutritional support, and prednisolone, the condition of the rabbit deteriorated; it was euthanized 7 days after admission. Histologic evaluation of brain tissue revealed lesions characteristic of severe, diffuse, nonsuppurative meningoencephalitis and a few large, eosinophilic, intranuclear inclusion bodies in neurons and glial cells. The DNA of human herpesvirus-1 was detected in the nuclei of glial cells, lymphocytes, and neurons by means of in situ hybridization. The rabbit's owner, who reported having had a severe labial and facial herpesvirus infection 5 days before the onset of clinical signs in the rabbit, was suspected to be the origin of infection for the rabbit.

Clinical Relevance—Human herpesvirus-1 may be transmissible from humans to rabbits, and infection with this virus should be considered as a differential diagnosis in rabbits with CNS signs of disease. (*J Am Vet Med Assoc* 2009;235:66–69)

An 8-month-old, 2.4-kg (5.3-lb), sexually intact male rabbit (*Oryctolagus cuniculus*) was evaluated for a 2-day history of anorexia, epiphora of the left eye, bruxism, hypersalivation, and ataxia. The rabbit lived indoors and had no contact with other rabbits since it was weaned at 8 weeks of age. The owner reported having had a severe labial and facial herpesvirus infection 5 days before the onset of clinical signs in the rabbit. While infected, the owner had regular intensive nose-to-nose and mouth-to-nose contact with the rabbit. The clinical signs that the owner detected in the rabbit were anorexia with mild epiphora of the left eye. Ataxia was observed 2 days afterward.

When admitted to the hospital (day 1), the rabbit was normothermic (rectal temperature, 38.9°C [102.0°F]), with a heart rate of 300 beats/min (reference range, 130 to 325 beats/min) and a respiratory rate of 60 breaths/min (reference range, 32 to 60 breaths/min). The rabbit had marked hypersalivation. Examination of the eyes revealed bilateral prolapse of the nictitating membranes, marked bilateral conjunctivitis, and mild keratitis in the left eye (**Figure 1**). Neurologic examination revealed incoordination, intermittent myoclonic seizures, and opisthotonus. Results of a CBC indicated relative neutrophilia (proportion of neutrophils, 79% [reference range, 40% to 70%]), relative and absolute lymphopenia (proportion of lymphocytes, 10% [reference range, 20% to 80%]; granulocyte-to-lymphocyte ratio, 0.64 [reference range, 2 to 20]), and relative monocytosis (propor-

Abbreviation

HHV-1 Human herpesvirus-1

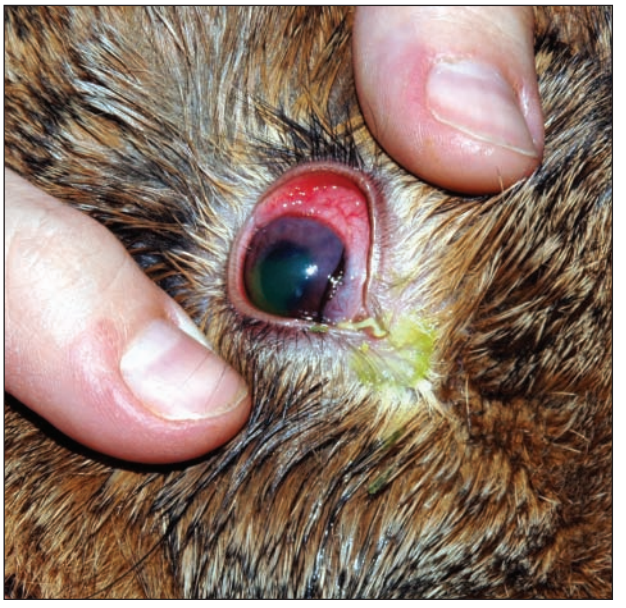

Figure 1—Photograph of acute conjunctivitis in a pet rabbit with an HHV-1 infection.

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tion of monocytes, 10% [reference range, 0% to 4%]). Biochemical abnormalities included an increase in serum activity of creatine phosphokinase (5,908 U/L [reference range, 50 to 250 U/L]) and serum concentration of total protein (76 g/L [reference range, 49 to 71 g/L]). Results of serologic and indirect fluorescent antibody tests for infection with *Encephalitozoon cuniculi* and *Toxoplasma gondii* were negative. The owner refused additional diagnostic tests such as computed tomographic evaluation or examination of a CSF sample.

The rabbit was treated with enrofloxacin (10 mg/kg [4.5 mg/lb], SC, q 24 h); trimethoprim (8 mg/kg [3.6 mg/lb])-sulfamethoxazole (40 mg/kg [18.1 mg/lb]), PO, q 12 h; vitamin B complex (1 mL/kg [0.45 mL/lb], SC, q 24 h); and IV administration of a crystalloid solution (50 mL/kg [23 mL/lb], SC, q 12 h). Nutritional support was provided via syringe feeding. Despite the increase of the body temperature from 39.7°C (103.4°F) on day 2 to 40.3°C (104.5°F) on day 4, the neurologic status of the rabbit improved slightly for 2 days, and myoclonic seizures and hypersalivation ceased. Nevertheless, the rabbit remained uncoordinated and lethargic. On day 6, the neurologic signs worsened, and the respiratory rate increased to 160 breaths/min. The rabbit was treated with prednisolone (1 mg/kg, SC, q 12 h). On day 7 after admission, the rabbit became recumbent and stuporous. A nasal stomach tube was placed because of dysphagia. Later that day, the rabbit developed permanently spastic forelimbs. Because of the poor prognosis, it was euthanized with pentobarbitone (100 mg/kg [45 mg/lb], IV) and submitted for necropsy 7 days after admittance.

Necropsy revealed mild, focal, erosive keratitis of the right eye. The right kidney had evidence of focal, chronic infarction. No macroscopic abnormalities were detected in the brain, spinal cord, or musculoskeletal system. Histologic examination of neural tissue revealed severe, diffuse, nonsuppurative meningoencephalitis with severe, acute, multifocal neuronal degeneration and necrosis and degeneration in the cerebrum, cerebellum, and brainstem. A few large, eosinophilic, intranuclear inclusion bodies were multifocally distributed in neurons and glial cells of the cerebrum (**Figure 2**). In addition to keratitis, the rabbit also had mild, focal, nonsuppurative, anterior uveitis in the right eye. Giemsa and periodic acid–Schiff staining of sections of brain tissue failed to reveal the presence of *E cuniculi* and *T gondii*.

In situ hybridization was performed to detect HHV-1 DNA in sections of brain tissue by use of a digoxigenin-labeled 408–base pair probe of the HHV-1 genome hybridized[1] within the highly conserved UL33 open-reading frame. Brain tissue of a rabbit without neurologic signs of disease and without histologic lesions was used as negative control sample. The UL33 gene was amplified from virion DNA of HHV-1 strain KOS[a] via PCR assay by use of primers HUL33_F (5′–GGGGCGAAGTTGCCATG–3′; nucleotides 69,147 to 69,163) and HUL33-R (5′–TCAGCCCCGCAGAATC–3′; complementary to nucleotides 69,538 to 69,554). The process revealed HHV-1 DNA in the nuclei of glial cells, lymphocytes, and neurons.

To confirm the presence of HHV-1 in the CNS, tissue from 6 brain regions was analyzed via PCR assay with primers specific for the UL33 gene, which encodes a subunit of the protein complex required for cleavage and packaging of viral DNA into capsids.[2] For this procedure, total DNA was extracted from paraffin-embedded brain sections obtained from the rabbit and a control rabbit by use of a commercial kit.[b] For detection of the HHV-1 genome, oligonucleotide primers[c] HUL33-NF (5′–AGCGAACTTTACGGGACAC–3′; nucleotides 69,189 to 69,207 of GenBank accession No. X14112) and HUL33-NR (5′–AAGACAACCTCCAGCTCGG–3′; reverse of nucleotides 69,318 to 69,336) were deduced from the published DNA sequence.[3] As a positive control sample, DNA from an uninfected rabbit was mixed with 20 fg to 200 pg of DNA prepared from gradient-purified HHV-1 particles. The PCR products were isolated from gel slices by use of a commercial extraction kit,[d] and their DNA sequence was determined with amplification primers HUL33-NF and HUL33-NR, a sequencing kit,[e] and a genetic analyzer.[f]

The expected 148–base pair amplification product representative of HHV-1 was detected in 4 specimens of brain tissue from the diseased rabbit but not in specimens from the uninfected control rabbit (**Figure 3**).

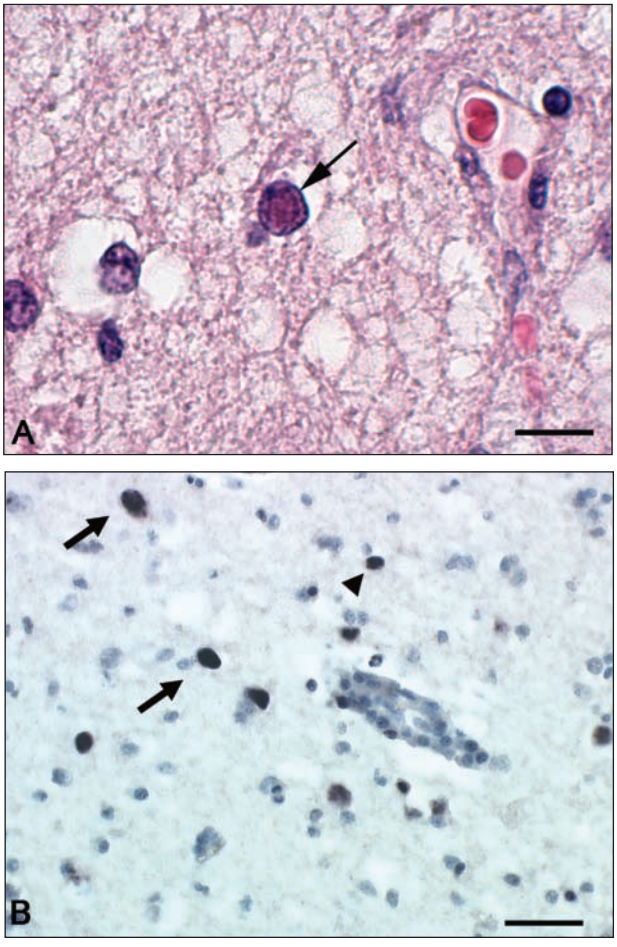

Figure 2—Photomicrographs of specimens of brain tissue from a rabbit infected with HHV-1. A—Multifocal, large, amphophilic intranuclear inclusion bodies (arrow) were evident within glial cells and neurons. H&E stain; bar = 20 μm. B—In situ hybridization with a digoxigenin-labeled 408–base pair probe of the HHV-1 genome revealed intranuclear HHV-1–specific DNA in neurons (arrows) and glial cells (arrowhead). Bar = 50 μm.

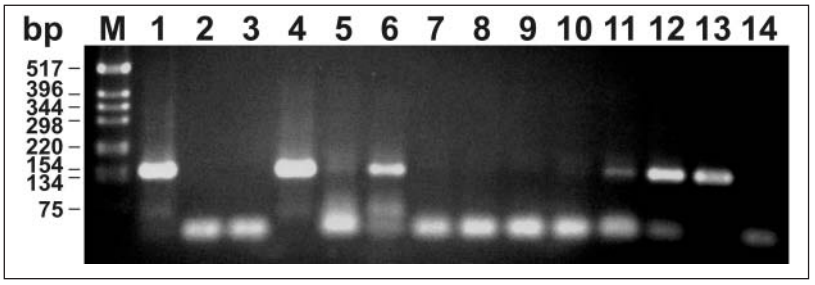

Figure 3—Results of PCR assay for detection of HHV-1 DNA in brain tissue of a rabbit suspected of having HHV-1 encephalitis. Samples of cellular DNA (approx 50 ng each) prepared from various brain sections of the infected rabbit (lanes 1 to 6) and a control rabbit (lanes 7 to 13) were used for PCR amplification with primers specific for the HHV-1 UL33 gene. To determine PCR sensitivity, control samples were supplemented with 20 fg (lane 9), 200 fg (lane 10), 2 pg (lane 11), 20 pg (lane 12), or 200 pg (lane 13) of purified HHV-1 DNA, and a reaction without any template DNA served as negative control reaction (lane 14). A prominent band at 148 base pairs (bp) indicates HHV-1 infection. M = Molecular-weight marker.

The specificity of the obtained products was verified via Southern blot hybridization with the plasmid-cloned UL33 gene of HHV-1. Furthermore, comparison of the DNA sequence of the PCR products from the rabbit of the present report with the published genome sequence of HHV-1[3] revealed 100% homology. The intensity of 3 of the positive PCR signals was similar to or even higher than that obtained with 20 pg of HHV-1 virion DNA in 50 ng of cellular DNA, which corresponded to approximately 15 virus genomes/cell. This indicated a productive, lytic HHV-1 infection of the brain. The clinical, necroscopic, and in situ hybridization findings therefore led to the diagnosis of severe HHV-1–associated encephalitis in the rabbit.

Discussion

Human herpesvirus-1 (herpes simplex virus) belongs to the family of alphaherpesviruses. Similar to most alphaherpesviruses, it is strongly neurotropic and is able to establish latent infections in neuronal cells. The primary hosts of HHV-1 are humans, and the seroprevalence of infection is reportedly 80%.[4] In humans, infection with the virus causes labial, oral, and occasionally ocular lesions.[5] Encephalitis in humans is rare and is the result of an ascending infection via the olfactory, optical, or trigeminal nerves.[6–8] Other HHV-1–susceptible species include rats, rabbits, mice, and chinchillas.[9–11] Rabbits are particularly susceptible and are used to investigate the pathogenesis of HHV-1 encephalitis in humans.[12] In rabbits, the virus is exclusively neurotropic and infection is almost always fatal.

Reports[13,14] of natural herpesvirus infections of pet rabbits are rare. In contrast to the circumstances in those reports, clinical data and a comprehensive history of the affected rabbit and owner were available for the present report. The first clinical signs were epiphora and conjunctivitis of both eyes, and these signs have also been reported for rabbits with experimentally induced herpesvirus infection.[15] This is important because epiphora and conjunctivitis are usually not evident in rabbits with other neurologic diseases such as head trauma, brain abscess or tumor, infection with *E cuniculi* or *T gondi*i, migration of *Baylisascaris* spp larvae, uremia, hepatoencephalic syndrome, arteriosclerosis, and intoxication.[16,17] Detection of severe conjunctivitis and keratitis might therefore be useful for the clinical diagnosis of herpesvirus infection in rabbits with signs of CNS impairment. Lymphopenia, relative heterophilia, and monocytosis in rabbits may be directly caused by acute viral disease.[18] In the rabbit of the present report, the increase in serum activity of creatinine kinase probably resulted from rhabdomyolysis attributable to myoclonic seizures and trauma.

The source of the HHV-1 infection in the rabbit was most likely human. This supposition was based on the history of close (including oral-to-oral) and exclusive contact of the rabbit with the owner, who had a concurrent, acute HHV-1 infection. However, human-to-rabbit (anthropozoonotic) transmission was not verified via genetic sequence analysis of viral isolates obtained from the owner and rabbit, so the actual source of infection remains unknown. Reports of human-to-animal transmission of disease are generally less common than those of animal-to-human transmission. Nevertheless, veterinarians should be aware that reverse zoonoses exist. For rabbits in close contact with humans, an HHV-1 infection should therefore be included as differential diagnosis for causes of encephalitis. In addition, veterinarians should discourage owners with HHV-1 infections from closely contacting rabbits, particularly their mucous membranes, while infected to protect their pet from a potentially fatal disease. Although the exact route of infection in the rabbit of the present report was unclear, the oral-to-oral contact of the rabbit with the infected owner as well as the conjunctivitis and uveitis in the rabbit suggested an ocular or oronasal infection.

When rabbits are experimentally infected with HHV-1, the distribution of the virus and the associated lesions vary according to the route of infection. In such rabbits, corneal, intraocular, and oronasal inoculation with HHV-1 results in distinct distribution patterns in the brain.[12,19,20] Given the high susceptibility of rabbits to alphaherpesviruses, the high prevalence of alphaherpesvirus infections in humans, and the potential for close contact of pet rabbits with their owners, the relatively low prevalence of HHV-1 infection in pet rabbits is surprising. One reason may be that such infections in rabbits may be underdetected because rabbits with equivocal neurologic signs are rarely submitted for postmortem examination to determine the cause.[13] On the other hand, in rabbits that are submitted for necropsy, HHV-1 infections might also be histologically underdiagnosed when a low number of intranuclear inclusion bodies exist. Furthermore, clinical signs of encephalitis may be confused with those of *E cuniculi* infection, which is more common than HHV-1 infection in rabbits.

a. Provided by P. G. Spear, Chicago, Ill.
b. DNeasy, Qiagen, Valencia, Calif.
c. MWG operon, Eurofins Scientific Inc, Memphis, Tenn.
d. Qiaquick gel extraction kit, Qiagen, Valencia, Calif.
e. BigDye Terminator v1.1 Cycle Sequencing Kit, Applied Biosystems, Foster City, Calif.
f. 3110 Genetic Analyzer, Applied Biosystems, Foster City, Calif.

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