

Letters to the Editor

Editor:

Nonhuman primates might be highly susceptible to cross-species infectivity by human α -herpesviruses such as herpes simplex virus (HSV). In a recent issue, Mätz-Rensing et al. reported the occurrence of a severe acute stomatitis related to human HSV-1 infection in a group of eight common marmosets (*Callithrix jacchus*).⁵ We report here the occurrence of a similar HSV-1 outbreak, characterized by severe ulcerative gingivostomatitis, encephalitis, and rapidly fatal outcome in a family of six zoo-housed marmosets of the subspecies *Callithrix geoffroyi* at Doué La Fontaine, France.

In August 2000, a 5-year-old male marmoset (animal No. 1) was found to be affected with lethargy, hypothermia, and anisocoria and died a few hours later. On the same day, two 3-year-old animals of the same group, a female (animal No. 2) and a male (animal No. 3), had high fever and ulcerative lesions of cheeks, lips, and oral mucosa. Treatment including local application of chlorhexidine and systemic administration of dexamethasone resulted in apyrexia and improvement of cutaneous and mucosal lesions. However, the female died 2 weeks later with symptoms of encephalitis. During the same period, a 5-month-old male (animal No. 4) and a 5-month-old female (animal No. 5), her offspring developed similar symptoms. One of them died rapidly, the other was euthanatized. Serologic analysis performed on the serum collected from animal Nos. 1 and 4 shortly after the onset of symptoms failed to detect specific antibodies to α -herpesviruses. In September 2000, 1 month after the beginning of the epidemic, only two animals were surviving. Although both were treated with aciclovir (30 mg/kg/day), one of them (animal No. 6) died 5 days later. Despite its clinical recovery, the animal No. 3 was euthanatized in November 2000.

Tissue samples from brain, lung, myocardium, liver, spleen, kidney, gut, lips, and tongue were collected from animal Nos. 4 and 5, fixed in formol and stained with hematoxylin and eosin. Histology revealed ulcerative and necrotizing lesions involving lips and tongue. In the margins of mucosal ulcers, epithelial cells contained intranuclear amphiphilic inclusions. Brain meningeal and cerebral vessels were congestive and surrounded by perivascular cuffings consisting of lymphocytes and mononuclear cells. Foci of neuronal necrosis were associated with inflammatory infiltrates. Rare neuronal cells contained intranuclear amphiphilic inclusions. Pulmonary lesions consisted of mild edema associated with inflammatory infiltrates in the interstitium.

Examination of liver sections only showed minimal portal infiltration by lymphocytes and macrophages. Other organs including spleen were normal. Immunohistochemistry performed on paraffin-embedded tissue sections using a rabbit antibody directed against glycoproteins of HSV-1 and HSV-2 envelope (DAKO, Glostrup, Denmark) demonstrated strong positive staining in brain neuronal cells, in lips, and tongue epithelial cells as well as in mononuclear cells of pulmonary infiltrates. The presence of intranuclear inclusions in epithelial and neuronal cells and the positivity of immunohistochemistry with anti-HSV antibody were highly suggestive of disseminated herpesvirus infection.

Deoxyribonucleic acid (DNA) was extracted from cryopreserved tissue samples collected at necropsy from animal No. 4 using proteinase K digestion, phenol-chloroform extraction, and ethanol precipitation. Nested-polymerase chain reaction (PCR) analysis with primers targeted to the highly conserved DNA polymerase sequence of herpesvirus was performed according to a previously published method.⁴ Water and PCR mix were used as negative controls and DNA from Epstein-Barr virus-infected BC-1 cells as positive control. A fragment of the predicted size (236 base pairs ; bp) was amplified in specimens collected from brain, tongue, muscle, and skin. The same fragment was also detected in samples from gut, lung, and liver, although with a weaker signal. Nucleotide sequence analysis of this 236-bp fragment showed a 100% sequence identity with the DNA polymerase gene of HSV-1.

In humans, the clinical course of HSV-1 infection is usually benign, although severe or disseminated forms can occur in neonates and immunocompromised hosts. High susceptibility of nonhuman primates to human HSV has been established in several case reports. Localized benign HSV infections have been reported in chimpanzees, whereas systemic infections with fatal outcome have been described in gorillas, white-handed gibbons, white-faced saki monkeys, owl monkeys, and common marmosets.^{1-3,5-10} Severe gingivostomatitis, characterized by vesicular and ulcerative mucocutaneous lesions, and meningoencephalitis are the most prominent symptoms. Disseminated infections with necrotic lesions of digestive tract, lungs, liver, and adrenal glands have also been reported.^{1,7,9} Histologic findings consisted of necrosis, inflammatory infiltrates, and intranuclear inclusions. Antibodies used for immunohistochemistry might cross-react with several α -herpesviruses from both human and non-human primates and thus fail to discriminate between HSV-1 and HSV-2. In our cases, molecular analysis on the basis of PCR and sequencing provided the evidence for HSV-1 infection in the affected tissues and excluded the involvement of other herpesviruses of animal origin. Negative results of serologic analysis in two animals of our colony might be explained by short delays between the onset of symptoms and the collection of sera. Such negative serologic results have been reported previously.^{9,10}

To our knowledge, HSV-1 infection has never been reported previously in *C. geoffroyi*. However, the origin of HSV-1 infection was unknown. The first step might be the contamination of one animal by a HSV-1-infected human either through indirect contact with a park visitor (probably through food) or through closer contact especially with an-

imal handlers. Rapid spreading of HSV-1 through the colony strongly suggested a subsequent animal-to-animal transmission that might be facilitated by the high susceptibility of monkeys to HSV-1 and the presence of virus in mucosal secretions of infected animals. The high mortality rate of such infections is a solid argument to justify prophylactic strategies. The simplest one would consist of recommending to strictly avoiding contact of captive marmosets with HSV-1-infected humans.

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References

- 1 Heldstab A, Rüedi D, Sonnabend W, Deinhardt F: Spontaneous generalized Herpesvirus hominis infection of a lowland gorilla (*Gorilla gorilla gorilla*). *J Med Primatol* **10**:129-135, 1981
- 2 Huemer HP, Larcher C, Czedik-Eysenberg T, Nowotny

- N, Reifinger M: Fatal infection of a pet monkey with human herpesvirus. *Emerg Infect Dis* **8**:639–642, 2002
- 3 Juan-Sallés C, Ramos-Vara JA, Prats N, Solé-Nicolaas J, Segalés J, Marco AJ: Spontaneous herpes simplex virus infection in common marmosets (*Callithrix jacchus*). *J Vet Diagn Invest* **9**:341–345, 1997
- 4 Lacoste V, Mauclere P, Dubreuil G, Lewis J, Georges-Courbot MC, Gessain A: A novel γ 2-herpesvirus of the Rhadinovirus 2 lineage in chimpanzees. *Genome Res* **11**:1511–1519, 2001
- 5 Mätz-Rensing K, Jentsch KD, Rensing S, Langenhuyzen S, Verschoor E, Niphuis H, Kaup FJ: Fatal Herpes simplex infection in a group of common marmosets (*Callithrix jacchus*). *Vet Pathol* **40**:405–411, 2003
- 6 McClure HM, Swenson RB, Kalter SS, Lester TL: Natural genital *Herpesvirus hominis* infection in chimpanzees (*Pan troglodytes* and *Pan paniscus*). *Lab Anim Sci* **30**:895–901, 1980
- 7 Melendez LV, España C, Hunt RD, Daniel MD, Garcia FG: Natural herpes simplex infection in the owl monkey (*Aotus trivirgatus*). *Lab Anim Care* **19**:38–45, 1969
- 8 Ramsay E, Stair EL, Castro AE, Marks MI: Fatal *Herpesvirus hominis* encephalitis in a white-handed gibbon. *J Am Vet Med Assoc* **181**:1429–1430, 1982
- 9 Schrenzel MD, Osborn KG, Shima A, Klieforth RB, Maalouf GA: Naturally occurring fatal herpes simplex virus 1 infection in a family of white-faced saki monkeys (*Pithecia pithecia pithecia*). *J Med Primatol* **32**:7–14, 2003
- 10 Smith PC, Yuill TM, Buchanan RD, Stanton JS, Chai-cumpa V: The gibbon (*Hylobates lar*); a new primate host for *Herpesvirus hominis*. A natural epizootic in a laboratory colony. *J Infect Dis* **120**:292–297, 1969