Naturally transmitted herpesvirus papio-2 infection in a black and white colobus monkey

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Case Description—A 6.5-year-old female eastern black and white colobus monkey (Colobus guereza) was evaluated after acute onset of ataxia and inappetence.

Clinical Findings—The monkey was ataxic and lethargic, but no other abnormalities were detected via physical examination, radiography, or clinicopathologic analyses. During the next 2 days, the monkey’s clinical condition deteriorated, and its WBC count decreased dramatically. Cytologic examination of a CSF sample revealed marked lymphohistiocytic inflammation.

Treatment and Outcome—Despite supportive care, the monkey became apneic; after 20 hours of mechanical ventilation, fatal cardiac arrest occurred. At necropsy, numerous petechiae were detected within the white matter tracts of the brain; microscopic lesions of multifocal necrosis and hemorrhage with intranuclear inclusions identified in the brain and adrenal glands were consistent with an acute herpesvirus infection. A specific diagnosis of herpesvirus papio-2 (HVP-2) infection was made on the basis of results of serologic testing; PCR assay of tissue specimens; live virus isolation from the lungs; and immunohistochemical identification of the virus within brain, spinal cord, and adrenal gland lesions. Via phylogenetic tree analysis, the colobus HVP-2 isolate was grouped with neuroinvasive strains of the virus. The virus was most likely transmitted to the colobus monkey through toys shared with a nearby colony of baboons (the natural host of HVP-2).

Clinical Relevance—To the authors’ knowledge, the first reported case of naturally transmitted HVP-2 in a nonhost species. Infection with HVP-2 should be a differential diagnosis for acute encephalopathy in primate monkeys and humans, particularly following exposure to baboons. (J Am Vet Med Assoc 2007;231:1878–1883)

An approximately 6.5-year-old 11.2-kg (24.6-lb) female eastern black and white colobus monkey (Colobus guereza) was evaluated at the North Carolina Zoological Park’s veterinary hospital after an acute onset of ataxia and inappetence. This monkey was born at the North Carolina Zoological Park and was housed with its sire and a 16-year-old female colobus monkey. The colobus monkey exhibit at the North Carolina Zoological Park was in close proximity to 2 other exhibits, one housing a large group of hamadyrayas baboons (Papio hamadyraya) and the other housing a pair of Debrazzas’s monkeys (Cercopithecus neglectus). No other primates had signs of illness at the time of evaluation of the colobus monkey. Various other mammal, bird, and reptile species were also housed in the same building as the colobus monkey.

At the initial examination, the monkey was obviously ataxic and was unable to climb and jump normally on the climbing features in the exhibit and holding areas. Head bobbing and swaying from side to side were also noticeable. The monkey was lethargic, and its responsiveness to stimuli was decreased from that expected in a clinically normal monkey. A limited physical examination was performed after the monkey was placed in a squeeze cage, and no abnormalities (except the observed behavioral changes) were detected. Anesthesia was induced with medetomidine (0.013 mg/kg [0.006 mg/lb]) and ketamine (1.8 mg/kg [0.82 mg/lb]) via IM injection; the monkey was intubated, and anesthesia was maintained with isoflurane. The action of medetomidine was reversed with atipamezole (0.065 mg/kg [0.03 mg/lb]) immediately following intubation. A thorough physical examination was performed, but again, no abnormalities were identified. Ventrodorsal and lateral radiographic views of the thorax and abdomen were considered unremarkable, as were ventrodorsal, lateral, and oblique radiographic views of the skull. Results of serum biochemical analyses and a CBC were within reference limits. The PCV was 37% (reference range, 32% to 40%), and the WBC count was 9.8 × 10³ cells/µL (reference range, 4.7 × 10³ cells/µL to 10.4 × 10³ cells/µL); the differential assessment revealed 59% neutrophils, 35% lymphocytes, and 6% monocytes. The monkey recovered
Formalin-fixed tissues were routinely processed for paraffin embedding, sectioning, and H&E staining. Throughout examined sections of the cerebrum and brainstem, there were small areas of hemorrhage (< 1 mm) that effaced the nervous tissue and also scattered foci of necrosis-associated karyolysis, karyorrhexis, and cell debris. Similar to the gross findings, these lesions were observed primarily in the white matter tracts. In both the white and gray matter of the brainstem, there was also multifocal expansion of the Virchow-Robin spaces around blood vessels by small to moderate numbers of monocytes, lymphocytes, and plasma cells and occasional areas of hemorrhage. Individual neuronal necrosis associated with neurophagia and the formation of intranuclear inclusion bodies was frequently identified in tissues adjacent to the areas of perivascular cuffing. These intranuclear inclusion bodies (7 to 8 µm in diameter) varied from eosinophilic inclusions surrounded by a clear halo to glassy amphiphilic inclusions that were often peripheral to the nuclear chromatin (Figure 2). The meninges contained multifocal accumulations of small numbers of macrophages, lymphocytes, and plasma cells. Scattered, small areas of hemorrhage were also present in the meninges overlying the cerebrum.

Similar areas of inflammation and necrosis were present in the gray matter of the thoracolumbar portion of the spinal cord. Within the adrenal glands, multifocal, coalescing areas of acute hemorrhage and smaller, multifocal areas of necrosis were evident. Numerous cells with intranuclear inclusions similar to those detected within the brain were associated with these areas of necrosis (Figure 3). Multifocal hemorrhages were also detected within the spleen, and 2 small foci of hemorrhage and necrosis were present in the liver. Severe, acute, diffuse lymphoplasmacytic myositis was identified in the stomach wall. Chronic lesions that were considered unrelated to the terminal illness included focal, moderate, diffuse lymphoplasmacytic enteritis and multifocal, mild glomerulonephritis. No lesions were present in examined tissue sections of bone marrow, eyes, colon, urinary bladder, and lungs.

The acute lesions of multifocal hemorrhage and necrosis with intranuclear inclusions in multiple organs were strongly suggestive of a herpesvirus infection. To
Adjacent to the vessel, cells with intranuclear inclusions (arrowheads) and scattered karyorrhectic debris (arrows) are visible. H&E stain; bar = 20 µm.

identify the specific etiology, frozen tissue samples from the spleen, a kidney, liver, a lung, heart, and a lymph node as well as formalin-fixed brain tissues were sent to the National B Virus Resource Center, Georgia State University, Atlanta, Ga, for analysis. By use of a real-time TaqMan PCR assay, tissue samples were assessed for presence of HSV types 1 and 2, B virus (Cercopithecine herpesvirus 1), and HVP-2 (Cercopithecine herpesvirus 16) DNA.1 All samples were negative for B virus, HSV-1, and HSV-2, but 6 of the 7 samples were positive for HVP-2 (no viral DNA was detected in the lymph node). Live virus was also isolated from the lung sample via cell culture; results of a PCR assay and western blot analysis confirmed the virus to be HVP-2. A 1.1-kilobase DNA fragment previously used for the phylogenetic analysis of HVP-2 strains was amplified from the colobus monkey HVP-2 isolate by a PCR procedure; the fragment was designated C1490 and sequenced. Because this fragment contained both coding regions (the complete gl gene and partial gD and gG genes) and highly variable guanine-cytosine-rich noncoding regions, which are difficult to align properly, only the gl (US5) gene sequence that encodes a protein associated with protection from apoptosis was used for the phylogenetic tree construction.2 The C1490 isolate was grouped by gl-based phylogenetic tree analysis with the neuroinvasive strains of HVP-2, which were separated into the distinct clade in agreement with previously published results.3 (Figure 4).

Concentrations of antibody against the HVP-2 C1490 isolate in serum samples obtained from the colobus monkey 2 days prior to death and on the day that cardiac arrest occurred, as well as in a baseline sample obtained 2 months prior to illness, were evaluated via an ELISA and western blot analysis. None of the 3 samples had detectable concentrations of C1490 isolate–specific IgG. However, results of the ELISA indicated that the serum concentration of anti–HVP-2 C1490 IgM antibody had increased by 33% after development of clinical signs, which was strongly indicative of an acute primary infection. The serum samples were also assessed via ELISA for several nonhuman primate alphaherpesviruses; results for IgG antibodies against B virus, SA8 (Cercopithece herpesvirus 2), and HVP-2 lab strain X2980 were negative.4 In addition, the serum sample obtained prior to death was evaluated for presence of HVP-2 DNA via PCR assay. Results of that assay were positive; it was estimated that there were approximately 1,000 viral genomes/1 mL of serum.

A direct link between HVP-2 and the histologic lesions was identified immunohistochemically by use of pooled sera from anti–HVP-2 antibody-positive baboons (at the National B Virus Resource Center, Atlanta, Ga) on formalin-fixed, paraffin-embedded tissue sections. Sera from baboons that were negative for anti–HVP-2 antibody were also used to confirm specificity of immunohistochemical staining. In examined sections of brain, spinal cord, and adrenal glands obtained from the colobus monkey, HVP-2–positive cells were primarily located within histologic lesions. Immunohistochemical analysis of sections of spleen, liver, kidneys, mesenteric lymph node, skeletal muscle (diaphragm), eyes, urinary bladder, small intestine, heart, and lungs did not reveal any substantial areas of viral antigen localization; HVP-2–positive cells were only occasionally detected in some tissues.

Figure 2—Photomicrograph of a section of the brain of the colobus monkey infected with HVP-2. Notice the prominent perivascular cuffing by mononuclear cells. H&E stain; bar = 50 µm.

Figure 3—Photomicrograph of a section of an adrenal gland from the colobus monkey infected with HVP-2. Throughout the section, necrosis, hemorrhage, and numerous intranuclear inclusions (arrowheads) can be seen. H&E stain; bar = 20 µm.

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monkey lomyelitis resulting from infection with B virus, which is carried by macaques.\textsuperscript{16,17} Fatal HSV-1 infections have been reported in several monkey species, as well as in tree shrews, lemurs, rabbits, and even a pygmy African hedgehog.\textsuperscript{18–20} Differences in neurovirulence among these viruses have also been highlighted by findings derived from experimental infections of mice. In 1 study,\textsuperscript{21} high doses of HSV resulted in neurologic signs in only a few of the exposed mice, and no mice exposed to SA8 developed clinical signs of disease. In contrast, both B virus and HVP-2 induced CNS disease in experimentally infected mice; in those animals, the virus ascended through the peripheral nervous system from a variety of inoculation sites into the CNS.\textsuperscript{22,23} Interestingly, HVP-2 strains could be subdivided into 2 groups on the basis of their behavior within the CNS of experimentally infected mice. Nonvirulent strains (designated HVP-2ap), which apparently cannot effectively replicate at sites of inoculation, spread to the CNS, whereas virulent strains (designated HVP-2nv) cause severe CNS lesions similar to those described in humans with B virus infection. Phylogenetic analysis was able to separate HVP-2 strains into separate clades, corresponding to the observed in vivo behavior of the virus in experimentally infected mice.\textsuperscript{3}

To the authors’ knowledge, this report is the first in which a case of natural infection of a nonbaboon with HVP-2 is described. An initial diagnosis of herpesvirus infection was made on the basis of the characteristic histologic lesions, in particular the multifocal necrosis associated with formation of intranuclear inclusions. However, although the distribution of the lesions within the cerebrum, cerebellum, and brainstem explained the clinical signs of incoordination and eventual respiratory depression, a specific etiologic diagnosis could not be determined solely on the basis of histopathologic findings because those were not sufficiently specific to allow differentiation among species of herpesvirus. In the monkey of this report, etiologic diagnosis of HVP-2 infection required isolation of live virus from a frozen lung sample and its identification as HVP-2 via PCR assay and sequence analysis, as well as detection of HVP-2 DNA in multiple formalin-fixed tissues (spleen, kidneys, liver, lungs, and heart) by use of real-time PCR analysis. Immunohistochemical detection of HVP-2 viral antigens within the histologic lesions further confirmed the link between the HVP-2 viral infection and disease in the colobus monkey. The increased anti–HVP-2 IgM antibody titers after clinical signs developed and the absence of a detectable IgG response were clearly indicative of an acute, primary infection. Finally, negative results of other PCR procedures and lack of detectable IgG against HSV 1 and 2, B virus, and SA8 ruled out infection with another closely related alphaherpesvirus.

Phylogenetic analysis of the colobus strain of HVP-2 (designated CI490) grouped this isolate with the neurovirulent HVP-2 strains.\textsuperscript{3} This finding was consistent with the clinical signs and histologic lesions in the monkey of this report, which were strikingly similar to those that develop in mice infected with HVP-2nv. In those mice, infection is associated with severe CNS lesions, adrenal gland necrosis with prominent inclusion body formation, and multifocal necrosis and in-
The ability of HVP-2 to be naturally transmitted from baboons to other species has clear management implications. Ideally, monkeys should be housed separately from other primates, with as much distance between groups as possible. Toys should not be shared between groups of animals, even if cleaned before transfer. Also, the genetic and pathologic similarities between HVP-2 and B virus raise the possibility of potential HVP-2 infections in humans, particularly given the high percentage of carriers in captive baboon populations. Herpesvirus papio-2 infection should be considered as a differential diagnosis following development of neurologic signs in any human or other animal that has been exposed to baboons or material with which baboons have been in contact. In general, zoonoctic herpesvirus infections have a high mortality rate, but timely supportive treatment and administration of antiviral drugs, such as acyclovir or ganciclovir, may slow progression of the disease and prevent development of fatal encephalitis.

As highlighted by the infection of the colobus monkey of this report, HVP-2 can be transmitted naturally from baboons to a nonhost species and result in development of fatal encephalitis. Given the extreme neurovirulence of HVP-2 in nonhost species, HVP-2 should be considered a potential zoonotic risk, and contact between baboons and other animals, especially other primate monkeys and humans, should be monitored closely.

References

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Selected abstract for JAVMA readers from the American Journal of Veterinary Research

Effects of epidural administration of dexmedetomidine on the minimum alveolar concentration of isoflurane in dogs
Daniela Campagnol et al

Objective—To evaluate the effects of epidural administration of 3 doses of dexmedetomidine on isoflurane minimum alveolar concentration (MAC) and characterize changes in bispectral index (BIS) induced by nociceptive stimulation used for MAC determination in dogs.

Animals—6 adult dogs.

Procedures—Isoflurane-anesthetized dogs received physiologic saline (0.9% NaCl) solution (control treatment) or dexmedetomidine (1.5 [DEX1.5], 3.0 [DEX3], or 6.0 [DEX6] µg/kg) epidurally in a crossover study. Isoflurane MAC (determined by use of electrical nociceptive stimulation of the hind limb) was targeted to be accomplished at 2 and 4.5 hours. Changes in BIS attributable to nociceptive stimulation and cardiopulmonary data were recorded at each MAC determination.

Results—With the control treatment, mean ± SD MAC values did not change over time (1.57 ± 0.23% and 1.55 ± 0.25% at 2 and 4.5 hours, respectively). Compared with the control treatment, MAC was significantly lower at 2 hours (13% reduction) but not at 4.5 hours (7% reduction) in DEX1.5-treated dogs and significantly lower at 2 hours (29% reduction) and 4.5 hours (13% reduction) in DEX3-treated dogs. The DEX6 treatment yielded the greatest MAC reduction (31% and 22% at 2 and 4.5 hours, respectively). During all treatments, noxious stimulation increased BIS; but changes in BIS were correlated with increases in electromyographic activity.

Conclusions and Clinical Relevance—In dogs, epidural administration of dexmedetomidine resulted in dose-dependent decreases in isoflurane MAC and that effect decreased over time. Changes in BIS during MAC determinations may not represent increased awareness because of the possible interference of electromyographic activity. (Am J Vet Res 2007;68:1308–1318)