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, this is the first reported case of natural transmission of HVP-2 to 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)

Abbreviations

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<td>HSV</td>
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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 exhibited ataxia at the North Carolina Zoological Park was in close proximity to 2 other exhibits, one housing a large group of hamadyras baboons (Papio hamadyras) and the other housing a pair of Debrassæ’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 $\times$ 10^3 cells/µL (reference range, 4.7 $\times$ 10^3 cells/µL to 10.4 $\times$ 10^3 cells/µL); the differential assessment revealed 59% neutrophils, 35% lymphocytes, and 6% monocytes. The monkey recovered...
from anesthesia without incident and was returned to the exhibit holding area for observation.

By the following morning, the monkey was obtunded and tachypneic. Anesthesia was induced via inhalation of isoflurane (delivered by use of a face mask), and the monkey was then intubated. Following induction of anesthesia, the monkey became apneic and required positive-pressure ventilation; this was first provided manually and later by use of a mechanical ventilator. Physical examination was repeated and revealed no apparent external abnormalities. A sample of CSF was collected via lumbar puncture and submitted for analysis. Another blood sample was collected for a CBC, serum biochemical analyses, serologic testing, and assessment of lead and zinc concentrations. Because of the possibility of intoxication, gastric lavage and administration of activated charcoal were performed. No abnormal material was detected within the stomach contents. Results of the CBC indicated that the PCV was 38% and that the WBC count was 6.7 × 10³ cells/µL; the differential assessment revealed 43% neutrophils, 1% band neutrophils, 46% lymphocytes, and 8% monocytes. Treatment was initiated with lactated Ringer’s solution (2 to 10 mL/h, IV), a combination of penicillin G benzathine and penicillin G procaine (36,000 U/kg [16,364 U/lb], IV, q 8 h), cefazidine (18 mg/kg [8.2 mg/lb], IV, q 12 h), dexamethasone (1 mg/kg [0.45 mg/lb], IV, q 12 h), and calcium EDTA (22 mg/kg [10 mg/lb], SC, q 8 h).

The monkey did not regain the ability to breathe spontaneously and required continuous positive-pressure ventilation via a mechanical ventilator for approximately 20 hours prior to death. Periodically, the monkey partially regained consciousness and attempted to dislodge the endotracheal tube but remained apneic. To maintain ventilation, it was necessary to keep the monkey sedated with ketamine (via 15- to 20-mg boluses administered IM hourly).

On the third morning, the monkey appeared to be breathing spontaneously and was allowed to regain consciousness. However, shortly after extubation, the monkey became apneic, went into cardiac arrest, and died despite attempts at resuscitation. Analysis of blood collected prior to death revealed severe leukopenia; the WBC count was 880 cells/µL, and only lymphocytes were present. Results of the analysis of a CSF sample collected after death indicated marked lymphocytic to lymphohistiocytic inflammation (WBC count, 90 cells/µL; RBC count, 441 cells/µL; and protein concentration, 547.6 mg/dL). Assessments of blood for lead and zinc (performed at Rollins Diagnostic Laboratory, Raleigh, NC) yielded negative results. Immediately following death (the cause of which was presumed to be acute viral infection), the cadaver was submitted for a complete postmortem examination.

At necropsy, gross lesions were confined to the brain. There was marked congestion of the cerebral blood vessels. Numerous petechiae were scattered throughout the white matter of the cerebral cortex (Figure 1), and occasional petechial hemorrhages were detected in the brainstem white matter. The entire brain and representative sections of visceral organs and spinal cord were preserved via freezing (at −80°C) and also via immersion in neutral-buffered 10% formalin.

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 myocarditis 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
of the 7 samples were positive for HVP-2 (no viral DNA samples were negative for B virus, HSV-1, and HSV-2, but 1), and HVP-2 (Cercopithecin herpesvirus 16) DNA. HSV types 1 and 2, B virus (Cercopithecine herpesvirus 2), and HVP-2 lab strain X20980 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, kidney, liver, mesenteric lymph node, skeletal muscle (diaphragm), eye, 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.

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.4 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 g1 gene and partial gD and gG genes) and highly variable guanine-cytosine-rich noncoding regions, which are difficult to align properly, only the g1 (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 g1-based phylogenetic tree analysis with the neuroinvasive strains of HVP-2, which were separated into the distinct clade in agreement with previously published results5 (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 (Cercopithecine herpesvirus 2), and HVP-2 lab strain X20980 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.

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. Adjacent to the vessel, cells with intranuclear inclusions (arrowheads) and scattered karyorhectic debris (arrows) are visible. 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.

Figure 4—Photomicrograph of a section of a lymph node from a colobus monkey infected with HVP-2. Numerous perivascular cuffs and intranuclear inclusions (arrowheads) are visible. H&E stain; bar = 50 μm.
monkey JAVMA, lomyelitis resulting from infection with B virus, which and other primates have been attributed to encepha respectively), and numerous deaths among humans natural hosts (African green monkeys and baboons, and HVP-2 were thought to cause disease only in their nonhost species varies markedly. Simian agent 8 reported virulence of the primate alphaherpesviruses lesions within the sensory ganglia, from which the virus can be reactivated and shed through saliva or from the genital mucosa. Rarely, HVP-2 can cause fatal necrotizing pneumonia in neonatal baboons, much like HSV in human neonates.

In contrast to infections in natural host species, the reported virulence of the primate alphaherpesviruses in nonhost species varies markedly. Simian agent 8 and HVP-2 were thought to cause disease only in their natural hosts (African green monkeys and baboons, respectively), and numerous deaths among humans and other primates have been attributed to encephalomyelitis resulting from infection with B virus, which is carried by macaques. 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. Differences in neurovirulence among these viruses have also been highlighted by findings derived from experimental infections of mice. In 1 study, 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. 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.

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 C1490) grouped this isolate with the neurovirulent HVP-2 strains. 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 in-
flammation within the gastrointestinal tract and liver; the disease rapidly progresses and death results.\textsuperscript{21} The clinical signs and histologic lesions in the colobus monkey were also markedly similar to those described in humans infected with B virus.\textsuperscript{20,24}

The most likely source of the HVP-2 C1490 infection in the colobus monkey of this report was the large baboon colony within the zoological park. Results of ELISA screening of sera from those baboons indicated that 13 of 19 (68\%) were positive for anti–HVP-2 IgG antibody, which is a somewhat lower rate than that reported for other institutions, but several juvenile monkeys (which are more likely to shed the virus) were present in the colony at the time of transmission.\textsuperscript{12} Although there was no direct contact between the groups of baboons and colobus monkeys, and the display exhibits occupy separate locations, the off-exhibit facilities for the colobus monkeys and baboons are located at an angle on either side of an open hallway, approximately 2.5 m (99 inches) apart at the closest point. The off-exhibit enclosures are made of galvanized and stainless-steel mesh with openings (2 \times 2-inch openings on the baboon side and 1 \times 1.5-inch openings on the colobus monkey side). Additionally, the 2 groups shared enrichment toys that were cleaned between uses with an ammonium chloride product.\textsuperscript{b} The colobus monkey of this report was observed sucking and chewing on these toys, some of which were hollow with holes extending into the interior. Because alphaherpesviruses are spread easily through saliva and pulmonary lesions that might indicate aerosolized spread of the virus were not detected in the monkey, we suspect that ingestion of HVP-2 from saliva-contaminated toys was the most probable route of transmission.

In 1981, fatal herpesvirus infection in a black and white colobus monkey and 2 Patas monkeys was reported.\textsuperscript{25} The viral isolates from that outbreak could not be conclusively determined to be either B virus or HSV via cell culture of tissue samples or serum neutralization. This ambiguity emphasizes the difficulty of differentiating between antigenically similar alphaherpesviruses on the basis of serologic findings alone, and the more specific technique of PCR assay determination was not widely available at that time. According to the report,\textsuperscript{25} baboons and rhesus macaques were housed in cages adjacent to the colobus monkeys. The lesions in the brain and stomach of the affected colobus monkey were identical to those observed in the monkey of this report. Comparable lesions were also present in the 2 affected Patas monkeys, which were housed in the same building as but not adjacent to the baboons. Herpesvirus papio-2 had not been described at the time of that outbreak, but given the similarities in findings between those cases and the colobus monkey of this report, one could speculate that those earlier deaths might represent an unrecognized outbreak of HVP-2 infection.

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-2nv 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, zoonotic 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.\textsuperscript{20}

As highlighted by the infection of the colobus monkey of this report, HVP-2nv can be transmitted naturally from baboons to a nonhost species and result in development of fatal encephalitis. Given the extreme neurovirulence of HVP-2nv 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.

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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)