Amoebic meningoencephalitis and disseminated infection caused by *Balamuthia mandrillaris* in a Western lowland gorilla (*Gorilla gorilla gorilla*)

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CASE DESCRIPTION
A 22-year-old male gorilla (*Gorilla gorilla gorilla*) housed in a zoo was evaluated for signs of lethargy, head-holding, and cervical stiffness followed by development of neurologic abnormalities including signs of depression, lip droop, and tremors.

CLINICAL FINDINGS
Physical examination under general anesthesia revealed a tooth root abscess and suboptimal body condition. A CBC and serum biochemical analysis revealed mild anemia, neutrophilia and eosinopenia consistent with a stress leukogram, and signs consistent with dehydration. Subsequent CSF analysis revealed lymphocytic pleocytosis and markedly increased total protein concentration.

TREATMENT AND OUTCOME
Despite treatment with antimicrobials, steroids, and additional supportive care measures, the gorilla's condition progressed to an obtunded mentation with grand mal seizures over the course of 10 days. Therefore, the animal was euthanized and necropsy was performed. Multifocal areas of malacia and hemorrhage were scattered throughout the brain; on histologic examination, these areas consisted of necrosis and hemorrhage associated with mixed inflammation, vascular necrosis, and intralesional amoebic trophozoites. Tan foci were also present in the kidneys and pancreas. Immunohistochemical testing positively labeled free-living amoebae within the brain, kidneys, eyes, pancreas, heart, and pulmonary capillaries. Subsequent PCR assay of CSF and frozen kidney samples identified the organism as *Balamuthia mandrillaris,* confirming a diagnosis of amoebic meningoencephalitis.

CLINICAL RELEVANCE
Infection with *B mandrillaris* has been reported to account for 2.8% of captive gorilla deaths in North America over the past 19 years. Clinicians working with gorillas should have a high index of suspicion for this diagnosis when evaluating and treating animals with signs of centrally localized neurologic disease. (*J Am Vet Med Assoc* 2016;248:315–321)

A 22-year-old male Western lowland gorilla (*Gorilla gorilla gorilla*) that had resided at the North Carolina Zoo for 5.5 years was examined in August 2013 for signs of lethargy, head-holding, decreased appetite, and cervical stiffness. The gorilla was housed with 3 adult female and 2 juvenile male conspecifics in a large outdoor exhibit with a shallow concrete pool and indoor holding area. The 2-month period prior to examination had been notable for unusually high rainfall in the region. The animal had a single episode of vomiting and lethargy 1 month previously and had lost body condition as well as 8% body weight (184 kg [405 lb] in June 2013 to 168 kg [370 lb] in August 2013). The weight loss was of major concern and had been monitored closely, with frequent assessment of possible contributory husbandry and behavioral factors including dietary intake, activity level, and social group dynamics. No husbandry, behavioral, or social causes for the weight loss were identified. The gorilla was initially treated empirically with an NSAID (ibuprofen [4.5 mg/kg {2 mg/lb}, PO, q 8 h for 4 days]), nasal decongestant (pseudoephedrine [0.7 mg/kg {0.3 mg/lb}, PO, q 12 h for 3 days]), and opioid analgesic (tramadol [0.25 mg/kg {0.11 mg/lb}, PO, q 8 h for 3 days]), but showed no improvement over the course of 4 days. Principal preliminary differential diagnoses included minor head trauma, dental disease, otitis, cervical pain, or CNS disease of viral, bacterial, neoplastic, or vascular origin.

Examination under general anesthesia on day 5 revealed a tooth root abscess and suboptimal body condition. When compared with International Species Information System values for captive gorillas,[1] a CBC performed at this examination revealed mild, microcytic, normochromic anemia as well as mild neutrophilia and eosinopenia consistent with a stress leukogram. A concurrently performed serum biochemical analysis indicated mild hyperphosphatemia and hyperalbuminemia likely related to dehydration. The affected teeth were removed, and treatment and supportive care including crystalloid fluid therapy, opioid

analgesia (buprenorphine [0.002 mg/kg {0.001 mg/lb}, IM or PO, q 8 h for 2 doses followed by tramadol as previously prescribed]), continued NSAID administration (meloxicam [0.2 mg/kg {0.09 mg/lb}, SC, once followed by ibuprofen as previously prescribed]), and administration of antimicrobials (clindamycin [5 mg/kg {2.3 mg/lb}, IV, once during the procedure followed by amoxicillin–clavulanic acid at 4 mg/kg {1.8 mg/lb}, PO, q 12 h]); however, there was no sustained clinical improvement.

By day 7, the gorilla's condition had declined rapidly, and the gorilla had become progressively more depressed, developed a lower lip droop with subtle tremors, and begun exhibiting mild ataxia that was challenging to characterize because of the animal's depressed state and minimal activity. Determination of progressive CNS involvement was difficult because of confounding factors including postoperative oral pain, sedation related to analgesic treatment, presumed generalized weakness due to the animal's debilitated state, and potential complications from the dental procedure. Because of this, the animal was again anesthetized on day 8 for reevaluation, CSF collection, and additional parenterally administered treatments consisting of IV crystalloid fluid therapy, an NSAID (meloxicam [SC, once]), pain medication (buprenorphine [SC, once]), and antimicrobials (clindamycin [IV, once] and tulathromycin [2.5 mg/kg {1.14 mg/lb}, SC, once]). Analysis of a CSF sample indicated a lymphocytic pleocytosis with a markedly elevated protein concentration (938.0 mg/dL; laboratory-established human reference range, 12 to 60 mg/dL), confirming disease of the CNS. Refined differential diagnoses included viral encephalitis, protozoal infection, fungal disease, autoimmune disease, and neoplasia.

The animal's clinical signs continued to worsen on day 9 with progression to an obtunded mentation, development of grand mal seizures, systemic hypertension attributed to cerebral edema, muscle tremors associated with voluntary movements, and anisocoria with partial retinal detachment. Continuous IV fluid therapy and additional treatment including an antimicrobial (enrofloxacin [4.5 mg/kg, SC, q 24 h]), a corticosteroid (dexamethasone [0.8 mg/kg {0.36 mg/lb}, IM or IV, q 12 h]), a gastroprotectant (ranitidine [0.3 mg/kg {0.14 mg/lb}, IM or IV, q 12 h]), cytosine arabinoside (10 mg/m^2, IV, q 4 h), and a benzodiazepine (midazolam [0.1 mg/kg {0.045 mg/lb}, IM, as needed]) to control seizures were administered. The animal did not respond to any of these empirical treatments or supportive care efforts; therefore, euthanasia was performed on day 10 because of the grave prognosis given the severity of the animal's neurologic disease.

Results of serologic and PCR assay testing of blood samples obtained antemortem were negative for Eastern equine encephalitis,[a] Western equine encephalitis,[a] West Nile virus,[b] *Borrelia burgdorferi*,[c] *Ehrlichia* spp,[d] and several additional tick-borne diseases. Necropsy revealed mild diffuse cerebral edema with slight flattening of the gyri and variably thickened and dull meninges. Numerous 5- to 20-mm-diameter foci of malacia and hemorrhage affecting approximately 10% of the gray matter of the cerebrum, brainstem, and cerebellum were visible on both the external surface and cut sections of the brain (Figure 1). The kidneys were bilaterally enlarged with numerous firm, well-demarcated, white, 1- to 5-mm-diameter, bulging foci scattered throughout the cortices. The pancreas was diffusely moderately edematous, and the majority of the head of the pancreas was thickened. Numerous 2- to 5-mm-diameter firm white foci were evident within the parenchyma of the body and tail of the pancreas. A draining tract containing purulent exudate extended from the site of the recent tooth extraction to the zygomatic arch. Additional gross necropsy findings included numerous shallow erosions and ulcerations of the gastric mucosa and serous atrophy of retrobulbar fat. Representative tissue samples were collected and fixed in neutral-buffered 10% formalin solution and stored at room temperature for 7 days prior to histopathologic evaluation. The brain was fixed largely intact for 48 hours prior to sectioning.

Formalin-fixed tissues were trimmed and embedded in paraffin blocks. Sections measuring 5 μm thick were prepared and routinely stained with H&E and Giemsa stains. Histologically, the brain contained numerous, variably sized, well-demarcated foci of liquefactive necrosis associated with moderate numbers of frequently degenerative neutrophils and vacuolated macrophages (ie, Gitter cells; Figure 2). These lesions centered on the gray matter of the cerebrum and brainstem with similar foci scattered randomly within the cerebellar folia. Intralesional arterioles and smaller vessels were diffusely necrotic with leakage of fluid, fibrin, and hemorrhage. Intravascular thrombi were commonly observed. Around the margin of the lesions, there were myriad 15- to 25-μm-
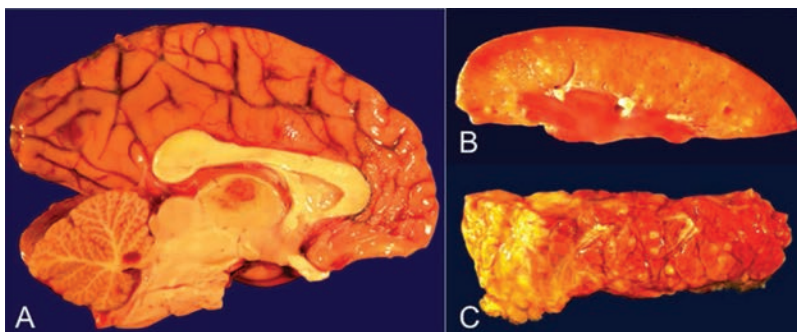

Figure 1—Gross appearance of pathological lesions found in a 22-year-old male Western lowland gorilla (*Gorilla gorilla gorilla*) with amoebic meningoencephalitis and disseminated infection caused by *Balamuthia mandrillaris*. A—Well-demarcated foci of hemorrhage and malacia are present within the cerebrum, brainstem, and cerebellum. B—Numerous slightly bulging white foci are evident within the renal cortex. C—The head of the pancreas is thickened and pale with smaller white foci scattered throughout the remaining parenchyma. Individual pancreatic lobules are separated by edema.

diameter amoebic trophozoites characterized by foamy eosinophilic cytoplasm and a prominent 5-μm-diameter, spherical, basophilic nuclei. Binucleation of the organisms was occasionally noted. Amoebae were frequently present within the walls and along the endothelial surface of necrotic vessels. Smaller numbers of trophozoites were observed within the central necrotic areas, the parenchyma, and the vessels of the intact adjacent neuropil. A wide band of the adjacent neuropil exhibited marked gliosis with increased numbers of reactive astrocytes and microglia. Prominent expansion of the Virchow-Robin space by lymphoplasmacytic and histiocytic perivascular cuffs was also noted. The overlying meninges and choroid plexus were multifocally expanded by mild to moderate lymphoplasmacytic and histiocytic infiltrates. Minimal, multifocal lymphoplasmacytic and histiocytic meningitis was noted within the examined sections of spinal cord taken from the cervical region and cauda equina.

Within the left eye, approximately 30% of the retina and choroid was infiltrated and disrupted by a mixed population of neutrophils, lymphocytes, and macrophages admixed with hemorrhage and large conglomerations of amoebic trophozoites. The affected retina was segmentally detached, and arterioles within the inflammatory region were frequently necrotic. A neutrophilic focus with intralesional trophozoites was observed between the retinal pigmented epithelium and choroid of the right eye.

Multifocal to regionally extensive areas of the pancreas were replaced by extensive pyogranulomatous inflammation and fibrosis, which frequently contained central areas of lytic necrosis (Figure 2). Adjacent acini were disorganized with individualization of cells, loss of zymogen granules, and increased interstitial fibrosis. Vascular necrosis and hemorrhage were less commonly observed than in the brain and eye. Amoebic trophozoites were few and concentrated within the central necrotic foci.

The renal cortex and, more rarely, the medulla contained similar multifocal areas of necrosis and pyogranulomatous inflammation associated with lymphoplasmacytic and histiocytic interstitial nephritis of the adjacent parenchyma (Figure 2). Individual and clustered amoebic trophozoites and fewer cysts were also observed within affected tubules, glomeruli, and interstitium **(Figure 3)**. The
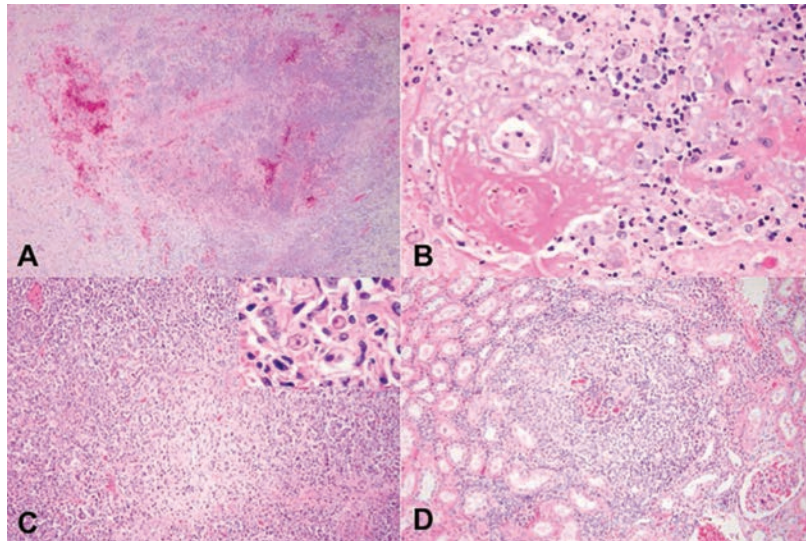

Figure 2—Photomicrographs of lesions associated with disseminated *B mandrillaris* infection in the brain, kidney, and pancreas of the gorilla in Figure 1. A—Locally extensive region of cerebral parenchyma effaced by inflammation and hemorrhage with a medium-caliber necrotic vessel visible within the center of the lesion. H&E stain. B—Higher magnification of the lesion depicted in panel A with transmural infiltration of a small vessel by amoebic trophozoites. H&E stain. C—Pancreatic parenchyma distorted by pyogranulomatous inflammation and fibrosis. Insert depicts amoebic trophozoites admixed with individualized, pleomorphic pancreatic acinar cells. H&E stain. D—A necrotic glomerulus surrounded by a wide band of lymphoplasmacytic and histiocytic interstitial nephritis. H&E stain.
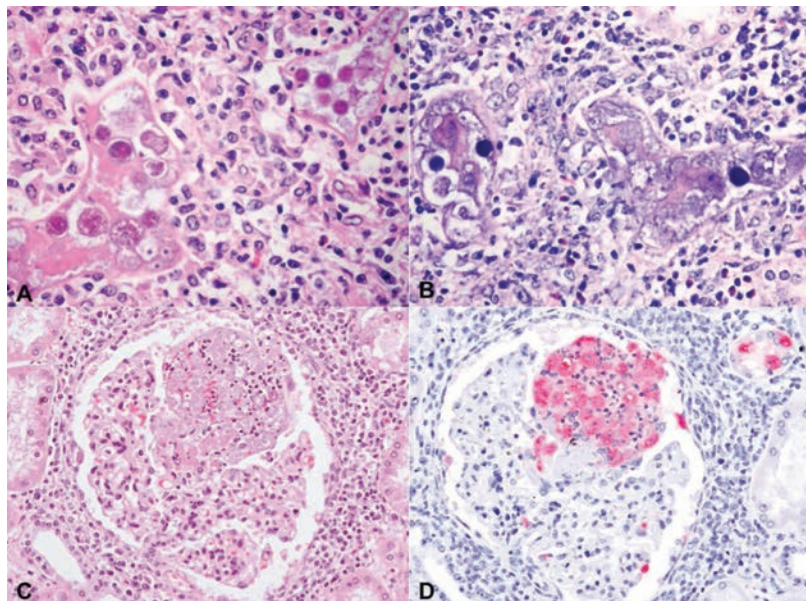

Figure 3—Photomicrographs demonstrating the histopathologic appearance of *B mandrillaris* organisms and results of immunohistochemical staining for free-living amoebae in renal tissues from the gorilla in Figure 1. A—A group of necrotic tubules within an area of nephritis contains a range of amoebic stages including pale basophilic irregular trophozoites, smaller circular cysts with amphophilic walls, and numerous intermediate forms characterized by variably sized eosinophilic intracellular bodies. H&E stain. B—Amoebic trophozoites within renal tubules are moderately amphophilic to basophilic with increased delineation of the nucleus and foamy cytoplasm. Cysts are uniformly basophilic. Giemsa stain. C—A glomerular segment is largely replaced by aggregates of amoebic trophozoites, which fill and expand the capillary profiles. H&E stain. D—Aggregates of amoebic trophozoites within a serial section of the glomerulus depicted in panel C are highlighted by immunohistochemical staining for free-living amoebae. Alkaline-phosphatase fast red chromogen with hematoxylin counterstain.

cysts were smaller (12 to 20 μm in diameter) and characterized by a thin, basophilic, undulating outer wall; a smooth, 1- to 2-μm-wide amphophilic inner wall; and variable numbers of circular, eosinophilic, intracellular bodies. Occasionally, small groups of necrotic tubules demarcated only by a residual basement membrane were packed with amoebae. Many of these amoebae had formed or were forming cysts. Vascular necrosis and hemorrhage were rare. Moderate, diffuse fibrosing cardiomyopathy was noted as an unrelated lesion.

Giemsa staining highlighted the presence of amoebae within the brain, pancreas, eye, and kidney. The trophozoites were moderately amphophilic to basophilic with increased delineation of the nucleus, while cysts were uniformly deeply basophilic. A single ameobic cyst was identified by Giemsa stain within an inflammatory focus between the retinal pigmented epithelium and choroid of the left eye. Organisms were also stained with periodic acid–Schiff stain but not with Grocott-Gomori methenamine silver stain.

Immunohistochemical testing of selected tissues for identification of pathogenic free-living amoeba consisting of rabbit polyclonal antibodies against *Naegleria fowleri*, *Acanthamoeba culbertsoni*, and *Balamuthia mandrillaris* was performed by the CDC Infectious Diseases Pathology Branch. Positively stained amoebic organisms were observed within sections of brain and within the interstitium, renal tubules, and glomeruli of the kidney (Figure 3). Focally dense areas of positively stained amoebic organisms were present within multiple large areas of necrosis and fibrosis of pancreatic tissue. Positively staining organisms were also evident in areas of inflammatory infiltrate subjacent to the retinal pigmented epithelium and rarely within the retina of 1 eye. In the lung, small numbers of intracapillary trophozoites were observed; however, there were no associated lesions. A single amoebic organism was identified within a focal area of lymphohistiocytic myocarditis. Liver, spleen, and oral mucosa from the site of tooth extraction yielded negative results for all tests. Results of immunohistochemical testing for *Acanthamoeba healyi*, which cross-reacts with other *Acanthamoeba* spp, were negative for sections of brain and kidney.

Real-time PCR assay testing of frozen kidney and CSF samples for *Acanthamoeba* spp, *N fowleri*, and *B mandrillaris* was performed by the CDC Parasitic Diseases Branch by means of previously described techniques.[2] Results were positive for *B mandrillaris*, confirming the diagnosis of *B mandrillaris* amoebic encephalitis with disseminated infection in this gorilla.

Discussion

Balamuthia mandrillaris is a free-living pathogenic amoeba that causes fatal meningoencephalitis in humans and many species of animals. Since its initial isolation in 1986 from a mandrill at the San Diego Zoo Wild Animal Park, there have been over 150 human cases worldwide, and infection with *B mandrillaris* is considered an emerging disease of increasing importance.[3-6] Infections have been reported globally with human cases documented predominantly in the southern United States and South America.[6,7] Humans and Old World nonhuman primates are the species most commonly affected, and the disease has been documented in orangutans, gibbons, colobus monkeys, mandrills, and gorillas.[8-13] It is particularly important to note that amoebic meningoencephalitis is not limited to primates, having been diagnosed in dogs, horses, and sheep,[14-17] and is quite possibly underreported in domestic species.

Studbook records and data from the Gorilla Species Survival Plan[e] indicate that 6 of the 564 captive gorillas maintained in North America over the past 19 years (from 1994 through 2013) have died of infection with *B mandrillaris*.[18,e] This accounts for 2.8% of all gorilla deaths in North America over this period of time. *Balamuthia mandrillaris* is 1 of 4 known free-living pathogenic amoebae that infect humans and other mammals. Although infection with *B mandrillaris* is considered rare, it is associated with a high mortality rate of approximately 90% in humans.[6,7] Infection is thought to occur via the respiratory tract or by contact with nonintact skin. Subsequent spread may occur hematogenously to the brain and other organs or via olfactory nerves.[19] The exact mechanism by which the organism traverses the blood-brain barrier is not fully understood, and immunocompetent as well as immunocompromised individuals are susceptible to infection.[7,20] In both humans and nonhuman primates, development of the disease may occur rapidly over the course of days in acute cases or over the span of months and, rarely, years in individuals exhibiting a more chronic progression.[3,11,21]

Disease in the gorilla of this report progressed rapidly with severe neurologic deterioration occurring within 10 days of initial signs. The total duration of clinical signs reported in 4 other gorilla cases of *B mandrillaris* infection ranged from 19 days to 3 months with vague clinical signs, such as lethargy, weakness, anorexia, vomiting, and headache, reported for 1 to 2 months before rapid clinical deterioration in 2 of these animals.[8,10,11,13] Clinical signs associated with infection in humans include fever, headache, cervical stiffness, lethargy, nausea, cranial nerve dysfunction, seizures, and altered mentation.[4,6] Infections can initially manifest as cutaneous plaques or as central neurologic deficits and then typically progress to fatal encephalitis. The majority of human cases involve the CNS and skin; however, disseminated infection has been reported in immunocompromised individuals.[22] In human patients with fatal encephalitis, amoebae have been less commonly reported in other tissues, including liver, kidney, and lung.[22-25] *Balamuthia mandrillaris* has also been transmitted in humans by transplantation of solid organs including the kidney, kidney-pancreas, and liver from donors who died of CNS disease.[24,25] The occurrence of such cases involving organs other than the CNS and skin is likely underreported because of the decreasing frequency of full autopsies in human medicine and often limited diagnostic familiarity with free-living amoebic infections.[26]

The gorilla described in the present report had disseminated infection with organisms found in the kidneys, pancreas, eyes, heart, and lungs, with infection confirmed by means of immunohistochemical staining and PCR assay. Severe disseminated infection has also been reported in 2 other gorillas.[11,13] The necrotic foci associated with mixed inflammation in tissues of the CNS and other organs during histopathologic evaluation suggested an acute process. However, fibrosis within the pancreas was indicative of a more chronic lesion. As such, we suggest that this animal may have had a prior unrelated episode of pancreatitis or pancreatic injury resulting in fibrosis prior to B mandrillaris infection. However, it is also possible that infection of the pancreas occurred early in the course of disease, and the development of fulminant clinical signs occurred later with dissemination of the organism to other tissues. Early pancreatic involvement could explain the episode of lethargy and vomiting that transpired 1 month prior to evaluation as well as the animal's decline in overall condition and loss of body weight.

Free-living pathogenic amoebae are thought to be ubiquitous in the environment, and although difficult to isolate, B mandrillaris has been identified in soil, dust, and water.[27-29] The gorilla of this report, along with 5 conspecifics, was housed in a large natural outdoor exhibit with a small circulating cement pool and an indoor holding facility. The 2 months prior to this animal's evaluation were unusually wet with monthly precipitation levels greater than double the regional averages for the previous 5 years. Accumulation of standing water within portions of the outdoor exhibit was observed following heavy rainfall. Alterations to the exhibit were made to provide additional drainage; however, exposure to standing water occurred intermittently for several weeks before modifications to the enclosure were complete. We considered that the unusually high rainfall and standing water within the exhibit during the months immediately prior to this gorilla's examination may have played a role in the exposure or transmission of the B mandrillaris organism in this case. The gorilla described in the present report had been previously seronegative for simian and human immunodeficiency viruses in 2005 and had no prior evidence of an immunocompromised status. At the time of writing, no other gorillas from the same exhibit had developed signs of disease.

Infection with this organism is thought to occur by exposure through nonintact skin or inhalation, but experimental infection by exposure through the gastrointestinal tract has been demonstrated in mice.[30] Subsequent spread of the organism to the brain and other organs may occur hematogenously or via olfactory nerves, which has occurred following intranasal exposures in mice.[20,31] The route of exposure in this case was unknown. Although no obvious external skin lesions were identified during examinations or at necropsy, small abrasions and minor breaks in the skin are a common occurrence for gorillas. With sufficient healing, especially in chronic cases, evidence of such exposures would be difficult to detect. Some human cases of amoebic encephalitis have been associated with lesions of the hard palate and oral cavity.[3,4] Although it is unlikely that the tooth root abscess reported in this case was caused by infection with B mandrillaris, the abscess may have served as a portal of entry for the organism. Initial exposure to the organism via the respiratory or gastrointestinal tract from ingestion of soil-contaminated food or standing water from the exhibit was also a possibility. Results of immunohistochemistry indicated the rare presence of organisms within the capillaries of lungs in this gorilla. We suggest that the location and rarity of organisms within the lungs were more indicative of hematogenous spread rather than evidence of initial infection via the respiratory tract. The disseminated nature of the infection throughout multiple organs was also consistent with hematogenous spread of the organism.

Achieving an antemortem diagnosis of amoebiasis remains a challenge for physicians and veterinarians, and there are currently no published reports of antemortem diagnosis or successful treatment of B mandrillaris encephalitis in a veterinary patient. Many other medical conditions, including dental disease, severe otitis, bacterial encephalitis, and other diseases affecting the head, neck, or CNS, may produce clinical signs similar to those seen early in the disease course of amoebic meningoencephalitis.[32,33] Early diagnosis in this case was complicated by the presence of the concurrent tooth root abscess combined with the waning and waxing nature of this animal's clinical signs. Advanced cases in humans have been confused with or misdiagnosed before death as other intracranial diseases that are similar in presentation, including viral encephalitis, stroke, brain abscesses, neoplasia, and other protozoal or parasitic infections.[34] There have also been reports in the literature of lesions resembling neoplasia grossly,[11] and the lesions within the pancreas of the current case also resembled a neoplastic process grossly as well as histopathologically. Such lesions could contribute to a postmortem misdiagnosis in some cases.

Some preliminary diagnostic tests may be helpful in raising the clinical suspicion of amoebic encephalitis. Although not pursued in this gorilla, diagnostic evaluation of the brain with CT or magnetic resonance imaging may reveal single or multiple lesions with or without evidence of cerebral edema. Depending on the stage and severity of the infection, the lesions may have a mass-like appearance or include hemorrhage, and have ring enhancement with use of contrast.[35] Whereas lesions identified with imaging can be supportive of a diagnosis, the appearance of lesions is not specific for the disease or a consistent finding in all cases. Collection and analysis of CSF can also be helpful for diagnosis. Analysis of CSF in this gorilla revealed a lymphocytic pleocytosis with a high total protein concentration, which is a common clinicopathologic finding in human cases of B mandrillaris encephalitis.[34] This finding,

however, is not pathognomonic for amoebic encephalitis and may be associated with other diseases. Identification of trophozoites or cysts on cytologic evaluation of CSF is challenging and frequently retrospective in human cases. Other diagnostic tests, including culture of the organism from tissue samples and serologic testing, have limited clinical use because of the length of time required for culture results and challenges related to interpretation of results.[36,37]

A definitive diagnosis of amoebic meningoencephalitis is typically made on the basis of demonstration of the organism in affected tissues or CSF. Biopsy of brain, skin lesions, or other affected organs followed by histopathology, immunohistochemistry, indirect immunofluorescent antibody testing, or PCR assay may lead to a specific diagnosis.[2,38-41] Histopathologic features for lesions affecting the CNS are variable but may include necrotizing vasculitis, meningitis, and encephalitis with suppurative, mixed, or granulomatous inflammation. Amoebic trophozoites or cysts are found intralesionally and are frequently vasculotropic.[5,10,21] Microscopic lesions reported in veterinary cases are similar to those described in humans, and lesions outside the CNS have also been described in both humans and animals. In this gorilla, PCR assay performed retrospectively on a CSF sample that was collected antemortem was positive for *B mandrillaris*. This CSF sample, however, was collected during the later stages of the disease. The initial vague clinical signs, concurrent confounding dental disease, and restricted ability to perform a comprehensive neurologic examination (because of the inherent dangers of conducting physical examinations without anesthesia in this species) delayed the recognition of CNS disease in this case. We do not know whether CSF samples collected at the onset of clinical signs would have yielded a diagnostic sample; however, earlier recognition of this disease as a potential diagnosis may have led us to obtain CSF for testing sooner. Nonetheless, although the sensitivity of CSF analysis and PCR assay early in the course of disease is currently unknown, collection of a CSF sample requires considerably less expertise and is less invasive than a brain biopsy and may lead to a specific diagnosis and facilitate earlier intervention in affected patients.

The presence of *B mandrillaris* organisms within the glomerulus and renal tubules of the kidney as demonstrated in the gorilla of this report suggests the possibility for urinalysis and PCR assay of urine as noninvasive antemortem diagnostic techniques in disseminated cases. Renal involvement is not a consistent finding for this disease, but has been reported previously in a dog, gorillas, and a colobus monkey as well as humans.[11,13,15] However, the use of renal involvement as a means of diagnosis has not yet been reported, and as for results of PCR assay or cytologic analysis of CSF, negative results would need to be interpreted carefully.

Early diagnosis and aggressive treatment would be necessary for a functional recovery adequate for a satisfactory quality of life for a gorilla in a zoological setting. Although there are no established guidelines for treatment of this disease, a small number of human patients have been treated successfully with various combinations of pentamidine, sulfadiazine, fluconazole, clarithromycin, azithromycin, and flucytosine.[42,43] Miltefosine, recently approved for the treatment of leishmaniasis in the United States, has shown promise during in vitro studies and is currently under investigation as a treatment for free-living amoebic infections in human patients.[44] In view of the number of fatal cases of *B mandrillaris* infection reported in gorillas over the past 20 years, clinicians working with these animals should be aware of this disease as an important differential diagnosis for neurologic signs in this species so that appropriate diagnostic testing and targeted treatment may be instituted early in the disease course.

Acknowledgments

Footnotes

a. Zoologix Inc, Chatsworth, Calif.
b. VRL Laboratories, Rockville, Md.
c. Antech Diagnostics, Irvine, Calif.
d. Vector-borne Disease Diagnostic Laboratory, North Carolina State University, Raleigh, NC.
e. Meehan T, Veterinary Advisor, Gorilla Species Survival Plan, Chicago Zoological Society and the Brookfield Zoo, Brookfield, Ill: Personal communication, 2014.

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