

# Primary amebic meningoencephalomyelitis caused by *Naegleria fowleri* in a south-central black rhinoceros (*Diceros bicornis minor*)

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## CASE DESCRIPTION

A 20-year-old female south-central black rhinoceros (*Diceros bicornis minor*) was evaluated because of an acute onset of CNS deficits.

## CLINICAL FINDINGS

The rhinoceros had no history of illness. Clinical signs included acute lethargy, ataxia, and decreased appetite. Hematologic abnormalities included leukocytosis with neutrophilia and a profound left shift. Results of serum biochemical analysis revealed hypophosphatemia but no other abnormalities. Results of a quantitative PCR assay for West Nile virus and an assay for anti-*Neosporium caninum* antibodies in serum were negative; the patient was seropositive for multiple *Leptospira* serovars.

## TREATMENT AND OUTCOME

Antimicrobials and anti-inflammatory agents were administered, but the condition of the rhinoceros worsened overnight; despite treatment with additional anti-inflammatory and antimicrobial agents, IV fluids, and thiamine, it became obtunded and died of respiratory arrest  $\leq 24$  hours later. Necropsy revealed severe, diffuse, suppurative, and histiocytic meningoencephalomyelitis involving the cerebrum, cerebellum, and spinal cord. Amebic trophozoites were observed on histologic examination of affected tissue. Infection with *Naegleria fowleri* was confirmed by results of immunohistochemical analysis and a multiplex real-time PCR assay.

## CLINICAL RELEVANCE

Findings suggested that south-central black rhinoceros are susceptible to the free-living ameba *N fowleri*. Ameba-induced meningoencephalomyelitis should be considered as a differential diagnosis for rhinoceros that have an acute onset of neurologic signs. Diagnosis of *N fowleri* infection in an animal has a profound public health impact because of potential human exposure from the environment and the high fatality rate in people with *N fowleri* infection. (*J Am Vet Med Assoc* 2019;255:219–223)

**A** 20-year-old 1,432-kg (3,150-lb) sexually intact female south-central black rhinoceros (*Diceros bicornis minor*) was evaluated because of an acute onset of profound lethargy, ataxia, and decreased appetite in September 2016. The rhinoceros was born at the Milwaukee County Zoo in Milwaukee, Wis, in January 1996, transferred to an Association of Zoos and Aquariums-accredited zoological institution in Florida in May 1998, and transferred to an exotic wildlife preserve in central Texas in May 2012. Review of the animal's medical record did not reveal any prior health concerns, and husbandry was standard. Vaccinations against tetanus (tetanus toxoid vaccine) as well as rabies, eastern and western equine encephalitis, and West Nile viruses

were current. The rhinoceros had experienced an anaphylactic reaction to a *Leptospira* vaccine in May 2012, and serum anti-*Leptospira* antibody titers had been measured annually since that time without repeating the vaccination.

On initial examination, the rhinoceros appeared severely depressed in demeanor and was minimally responsive to stimulation. The degree of apparent depression was sufficient to allow veterinary staff to approach the animal, perform a limited physical examination, and collect a blood sample from an auricular vein. Rectal temperature was 39.4°C (103.0°F). The rhinoceros had pink mucous membranes, a capillary refill time  $< 2$  seconds, and slight inspiratory dyspnea. Thoracic auscultation did not reveal any

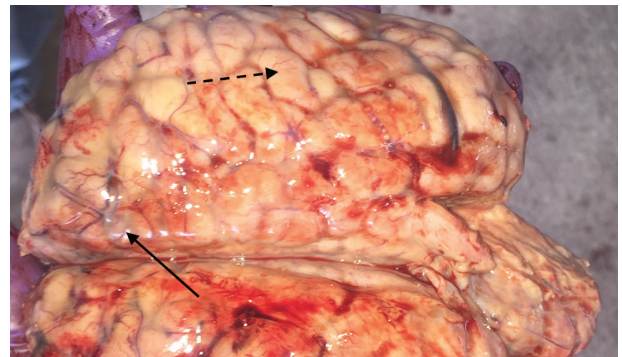
marked cardiopulmonary abnormalities. The menace reflex was absent but palpebral reflexes were intact bilaterally. Positioning of the pupils, eyelids, and globes appeared normal. Pathological nystagmus was not observed. Direct and indirect pupillary light reflexes were appropriate. The rhinoceros appeared to have control of its tongue and was able to swallow. The animal spent several hours in ventral recumbency and would occasionally stand but showed no interest in food or water.

A quantitative PCR assay for West Nile virus was performed along with serum analysis (microscopic agglutination testing) for antibodies against multiple *Leptospira* serovars and *Neospora caninum* at Texas A&M Veterinary Medical Diagnostic Laboratory. Test results for *N. caninum* and West Nile virus were negative, and tests for antibodies against *Leptospira interrogans* serovars *pomona*, *ictero*, *canicola*, and *bratislava* were positive (ie, serum titers  $\geq 800$ ; titers for this patient ranged up to 3,200). A CBC revealed mild leukocytosis ( $13.2 \times 10^3$  WBCs/ $\mu\text{L}$ ; reference range,  $4.4 \times 10^3$  WBCs/ $\mu\text{L}$  to  $11.6 \times 10^3$  WBCs/ $\mu\text{L}$ ) and neutrophilia ( $11.3 \times 10^3$  cells/ $\mu\text{L}$ ; reference range,  $2.4 \times 10^3$  cells/ $\mu\text{L}$  to  $8.3 \times 10^3$  cells/ $\mu\text{L}$ ) with a left shift (band neutrophils,  $0.5 \times 10^3$  cells/ $\mu\text{L}$ ; reference range, 0 to  $0.4 \times 10^3$  cells/ $\mu\text{L}$ ). Serum biochemical analytes were all within reference ranges except for serum phosphorus concentration (0.51 mg/dL; reference range, 2.6 to 6.9 mg/dL). Reference ranges were based on intervals created from the Zoological Information Management System<sup>a</sup> for south-central black rhinoceros. Ceftiofur crystalline-free acid<sup>b</sup> (2.8 mg/kg [1.3 mg/lb]) and flunixin meglumine<sup>c</sup> (0.5 mg/kg [0.23 mg/lb]) were administered IM at the tail head.

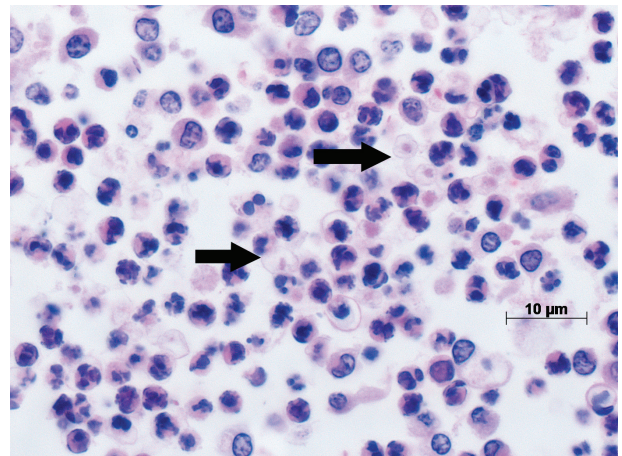
The animal's condition worsened overnight, with more severe ataxia and obtunded mentation observed the next day. The rhinoceros attempted to walk but stumbled into a ditch in the enclosure; following sedation with etorphine hydrochloride<sup>d</sup> (0.34  $\mu\text{g/kg}$  [0.15  $\mu\text{g/lb}$ ], IM), it was moved to an enclosed structure. Naltrexone hydrochloride<sup>d</sup> (7.0  $\mu\text{g/kg}$  [3.2  $\mu\text{g/lb}$ ]) was administered IM for reversal of opiate effects. Five liters of a balanced electrolyte solution<sup>e</sup> (approx 3.5 mL/kg [1.6 mL/lb]) was delivered through an 18-gauge IV catheter in the left auricular vein along with enrofloxacin<sup>f</sup> (1 mg/kg [0.45 mg/lb]) and dexamethasone sodium phosphate<sup>g</sup> (0.03 mg/kg [0.01 mg/lb]). Thiamine hydrochloride<sup>h</sup> (10.0 mg/kg [4.5 mg/lb], IM) was also administered. A few hours later, the rhinoceros had a seizure and bit its tongue and bled into the oral and nasal cavities. Midazolam hydrochloride<sup>d</sup> (0.1 mg/kg [0.05 mg/lb], IM) was used to provide light sedation. Pelvic limb paralysis was observed when the animal attempted to rise. Dexamethasone sodium phosphate<sup>g</sup> (0.07 mg/kg [0.03 mg/lb], IM, q 5 h) was given throughout the rest of the day (5 PM and 10 PM). Two hours after the last dexamethasone treatment, the rhinoceros had respiratory arrest and died.

Necropsy was performed by a private pathology service. Gross findings included swollen gyri in the cerebrum and cerebellum (**Figure 1**). The meninges were cloudy (white to cream-colored and partially opaque). Representative sections of tissue were fixed in 10% neutral-buffered formalin and submitted for histologic examination. All tissues were trimmed and embedded in paraffin blocks. Tissue sections were stained with H&E, periodic acid-Schiff, Gram, and Gomori methenamine-silver stains.

Microscopically, severe, diffuse, suppurative, and histiocytic meningoencephalomyelitis was identified in the cerebrum, cerebellum, and spinal cord. Evidence of inflammation was observed in the choroid plexus. Intralesional amebic trophozoites were seen on H&E-stained slides (**Figure 2**). The amebas were round to ovoid and 5 to 11  $\mu\text{m}$  in diameter with slightly granular and vacuolated cytoplasm. The nuclei were small, weakly basophilic, and often eccentrically located, and each had a prominent, central karyosome. Inflammatory cells (mostly neutrophils with fewer macrophages, lymphocytes, and plasma cells)

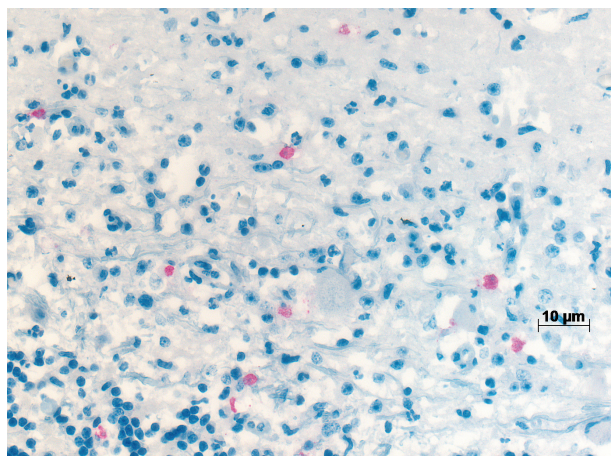


**Figure 1**—Gross photograph of the brain from a south-central black rhinoceros (*Diceros bicornis minor*). Notice swollen gyri (dashed arrow) in the cerebrum and cerebellum. The meninges appear partially opaque (solid arrow).



**Figure 2**—Photomicrograph of a section of the meninges of the rhinoceros in Figure 1. Amebic trophozoites (arrows) are mixed with inflammatory cells. H&E stain; bar = 10  $\mu\text{m}$ .





**Figure 3**—Photomicrograph of a section of choroid plexus from the rhinoceros in Figure 1 revealing multiple trophozoites labeled with anti-*Naegleria fowleri* antibodies (red). Immunohistochemical stain with Mayer hematoxylin counterstain; bar = 10  $\mu$ m.

markedly expanded the meninges and extended multifocally into the cerebral, cerebellar, and spinal cord parenchyma. Perivascular cuffing was observed in associated parenchymal vessels, and there was rarefaction of the neuropil.

Other histologic findings included diffuse lymphoplasmacytic and histiocytic adrenalitis with congestion and hemorrhage. Severe suppurative lymphadenitis with diffuse hemorrhage was present in the tracheobronchial lymph nodes, and the mesenteric lymph nodes had diffuse draining hemorrhage and mild histiocytosis. The heart had evidence of myocardial distress with wavy myofibers and interfiber edema. Centrilobular hepatocellular degeneration and necrosis was also observed with evidence of moderate congestion. Multifocal and moderate lymphoplasmacytic interstitial nephritis with mild congestion was also detected, with bilateral multifocal tubular ectasia and proteinosis. A mild amount of hemosiderosis was observed in the spleen, adrenal glands, lymph nodes, and liver.

Representative samples from the cerebrum, cerebellum, and spinal cord were sent to the CDC Infectious Diseases Pathology Branch and Free-Living and Intestinal Amebas Laboratory for additional testing. Evaluation of H&E-stained slides prepared from the submitted tissues confirmed the presence of amebic trophozoites. Immunohistochemical testing with an immunoalkaline phosphatase technique, with appropriate positive and negative controls, confirmed the presence of *Naegleria fowleri*<sup>1</sup> (**Figure 3**). A multiplex real-time PCR assay was performed as previously described<sup>2</sup> on DNA extracted from formalin-fixed, paraffin-embedded tissue. The assay targeted the 18S small subunit ribosomal rRNA gene, and results were positive for a sequence from *N fowleri* and negative for sequences for 2 other pathogenic free-living amebas, *Balamuthia mandrillaris* and *Acanthamoeba* spp.<sup>2</sup> No evidence of bacterial or fungal pathogens

was identified by use of special stains (Gram and silver stains) on slides of CNS tissues. Immunohistochemical staining for rabies virus<sup>3</sup> and *Leptospira* spp.<sup>4</sup> had negative results. Deoxyribonucleic acid extracted from central nervous tissue was used for a broad-range panbacterial PCR assay targeting conserved 16S rDNA, and the test result was negative.<sup>5</sup>

## Discussion

The thermophilic free-living ameba *N fowleri* is found in freshwater environments throughout the world.<sup>6,7</sup> The ameba is capable of causing primary amebic meningoencephalitis in people when waterborne *N fowleri* enters the nasal sinuses (usually during swimming) and then migrates along the olfactory nerve through the cribriform plate to the brain.<sup>8</sup> Primary amebic meningoencephalitis in people was first described in 1965; > 200 human cases of the disease have been reported, and more than half of these cases were reported in the United States.<sup>7,9</sup> Despite advances in antiparasitic chemotherapy and supportive care, the fatality rate remains > 95% in human patients.<sup>10</sup> The infection is infrequently reported in the veterinary literature, and to the authors' knowledge, there have been no reports of successful treatment and survival in affected veterinary patients. Mice, guinea pigs, sheep, and nonhuman primates have developed meningoencephalomyelitis after experimental infection with *N fowleri*.<sup>11–15</sup> Naturally occurring infections in cattle, sheep, and a South American tapir (which was housed in a zoo in Phoenix, Ariz) have been reported.<sup>16–21</sup>

In people, symptoms of *N fowleri* infection typically start  $\leq$  5 days after exposure and are indistinct from those of other disease processes affecting the CNS, with death usually occurring within 3 to 7 days after the onset of symptoms.<sup>7</sup> Reported clinical signs in animals include anorexia, lethargy, dry cough, mucoid feces, pyrexia, nasal discharge, and acute central neurologic signs (ataxia, facial paralysis, circling, weakness, blindness, and seizures).<sup>17–21</sup> On the basis of a review of current veterinary literature (including reports regarding naturally infected ruminants and a South American tapir), it appears that death in animals occurs  $\leq$  7 days after the onset of clinical signs.<sup>17–21</sup>

*Naegleria fowleri* tolerates temperatures of  $\leq$  45°C (113°F), with a range from 0° to 45°C (32° to 133°F).<sup>6</sup> A history of contact with naturally warm or artificially heated water is the most common risk factor for exposure to the ameba.<sup>10</sup> In outbreaks of *N fowleri* infection in cattle, a common infection source is water in stagnant canals or drinking troughs that reach high temperatures in the summer. Infection may occur when amebas are transferred into the anterior part of the nasal cavity through licking of the nostrils.<sup>18</sup> Although the water supply for the rhinoceros of this report was not tested, exposure was presumed to have occurred at a bog within the enclosure. Ambient air temperatures in the area had

been high (mid-30s celsius [mid-90s Fahrenheit]) at the time of the patient's evaluation. After death of the rhinoceros, the bog was filled in with soil, which prevented other animals from having access to the suspected water source but also prevented water testing.

The presence of adenitis, which is recognized to develop secondary to septicemia, along with changes observed on the CBC suggested that the rhinoceros of this report was likely in the early stages of sepsis. No other disease processes were identified on necropsy, so we considered it possible that septicemia was developing as a secondary process in the presence of primary amebic meningoencephalomyelitis. The observed diffuse supportive lymphadenitis during necropsy was also considered to be secondary to a possible septic process. Observed myocardial and hepatic degeneration and necrosis were interpreted as the result of terminal hypoxia. Valvular changes in the heart were likely subclinical and unrelated to the death of this patient.

It was unknown why the rhinoceros of this report was seropositive for *L. interrogans* serovars *pomona*, *ictero*, *canicola*, and *bratislava*, but it was suspected that this could have resulted from previous vaccinations or environmental exposure. Because the rhinoceros had not been vaccinated against leptospirosis since 2012 (when it had an adverse reaction), it was suspected that this animal had been exposed to the bacteria without signs of clinical disease. On further examination of herd health records, it was noted that a male rhinoceros that had lived in the same enclosure as the female in this report for 1.5 years had died from chronic anemia due to iron storage disease and also had a diagnosis of leptospirosis for which it had been treated in 2015, and this supported the likelihood that our patient had previous exposure to the bacteria. Histologic examination of collected organs did not show any evidence of *Leptospira* infection.

Premortem diagnosis of amebic meningoencephalomyelitis is problematic, and it relies on identification of high polymorphonuclear leukocyte counts and amebas in the CSF.<sup>6,22</sup> Neuroimaging with CT or MRI typically yields unremarkable findings and is generally not helpful in diagnosis.<sup>6</sup> Humoral reactions are usually weak, but circulating antibody titers increase with the duration of infection.<sup>6</sup> Although no successful treatment for veterinary patients has been reported, a few clinical reports in the human medical literature have described successful treatment. In one such report, IV administration of amphotericin B and fluconazole and oral rifampicin treatment resulted in a successful outcome.<sup>23</sup> More recently, miltefosine (a drug used in treatment of breast cancer and leishmaniasis) and voriconazole have shown efficacy against *N. fowleri* during in vitro studies<sup>24</sup> and should be considered for future veterinary therapeutic trials. Prevention of amebic meningoencephalomyelitis should focus on the elimination of warm, stagnant, freshwater sources.

## Acknowledgments

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the CDC.

The authors thank Shantanu Roy, MS, Free-Living and Intestinal Amebas Laboratory, CDC, for assistance performing real-time PCR assays for diagnosis of *Naegleria fowleri* infection; Sherif Zaki, MD, PhD, Wun Ju Shieh, MD, PhD, Rosecelis Martinez, MD, PhD, Jana Ritter, DVM, and Gillian Hale, MD, MS, from the Infectious Diseases Pathology Branch of the CDC for joint review of the case; Brigid Bollweg, Infectious Diseases Pathology Branch Immunohistochemistry Laboratory, for performing immunohistochemical analyses; and Victoria L. Clyde, DVM, for insight and review of the clinical case.

## Footnotes

- Zoological Information Management System, Species360, Bloomington, Minn.
- Excede, Zoetis, Parsippany, NJ.
- Banamine, Merck, Kenilworth, NJ.
- ZooPharm, Windsor, Colo.
- Plasma-lyte, Baxter, Deerfield, Ill.
- Baytril, Bayer Corp, Whippany, NJ.
- Dexaject, Henry Schein Animal Health, Dublin, Ohio.
- Neogen Corp, Lansing, Mich.

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