Multisystemic infection with an *Acanthamoeba* sp in a dog

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Case Description—A 10-month-old Boxer was evaluated for fever and signs of cervical pain.

Clinical Findings—Physical examination revealed lethargy, fever, and mucopurulent ocular and preputial discharge. On neurologic examination, the gait was characterized by a short stride. The dog kept its head flexed and resisted movement of the neck, consistent with cervical pain. Clinicopathologic findings included neutrophilic leukocytosis, a left shift, and monocytosis. Cervical radiographs were unremarkable. Cerebrospinal fluid analysis revealed neutrophilic pleocytosis and high total protein content. On the basis of signalment, history, and clinicopathologic data, a diagnosis of steroid-responsive meningitis-arteritis was made.

Treatment and Outcome—The dog was treated with prednisone (3.2 mg/kg [1.45 mg/lb], PO, q 24 h), for 3 weeks with limited response. Consequently, azathioprine (2 mg/kg [0.9 mg/lb], PO, q 24 h) was administered. Three weeks later, the dog was evaluated for tachypnea and lethargy. Complete blood count revealed leukopenia, neutropenia, and a left shift. Thoracic radiography revealed a diffuse bronchointerstitial pattern. The dog subsequently went into respiratory arrest and died. On histologic evaluation, amoebic organisms were observed in the lungs, kidneys, and meninges of the brain and spinal cord. A unique *Acanthamoeba* sp was identified by use of PCR assay.

Clinical Relevance—This dog developed systemic amoebic infection presumed to be secondary to immunosuppression. The development of secondary infection should be considered in animals undergoing immunosuppression for immune-mediated disease that develop clinical signs unrelated to the primary disease. Although uncommon, amoebic infection may develop in immunosuppressed animals. Use of a PCR assay for identification of *Acanthamoeba* spp may provide an antemortem diagnosis. (*J Am Vet Med Assoc* 2011;238:1476–1481)

A 10-month-old castrated male Boxer (26 kg [57.2 lb]) was evaluated at the University of Georgia Veterinary Teaching Hospital for signs of cervical pain and fever. The dog had been evaluated 2 days previously by the referring veterinarian for an acute onset of lethargy, difficulty rising from a lying position, and a stiff gait in all 4 limbs. On physical examination performed by the referring veterinarian, the dog was clinically normal except for fever (39.3°C [102.74°F]) and signs of cervical pain. Complete blood count revealed neutrophilic leukocytosis (WBC count, 25.08 × 10^3 cells/µL [reference range, 5.50 × 10^3 cells/µL to 16.90 × 10^3 cells/µL]; neutrophil count, 19.92 × 10^3 cells/µL [reference range, 2.0 × 10^3 cells/µL to 12.0 × 10^3 cells/µL]) and monocytosis (2.68 × 10^3 cells/µL; reference range, 0.30 × 10^3 cells/µL to 2.00 × 10^3 cells/µL). Results of serum biochemical analysis were within reference ranges. No remarkable abnormalities were detected on urinalysis except for isosthenuria (urine specific gravity, 1.010). The dog was treated with ampicillin (9.6 mg/kg [4.36 mg/lb], PO, q 12 h), doxycycline (7.7 mg/kg [3.5 mg/lb], PO, q 12 h), and meloxicam (1.8 mg/kg [0.82 mg/lb], PO, q 24 h). The following day, the dog remained febrile. Signs of cervical pain seemed to worsen, and the dog continued to walk with a stiff gait. On the basis of the progression of clinical signs, the dog was referred to the University of Georgia Veterinary Teaching Hospital.

Abbreviations

SRMA	Steroid-responsive meningitis-arteritis
SSU	Small subunit

On admission to the veterinary teaching hospital, the dog was lethargic and febrile (40.3°C [104.54°F]) and had mild mucopurulent ocular and preputial discharge. The remainder of the physical examination findings were unremarkable. Findings on neurologic examination were also normal except for the dog's gait and signs of cervical pain. The dog was ambulatory but had a stiff gait characterized by a short stride in all 4 limbs. On the basis of neuroanatomic localization, the cause was thought to be a diffuse or multifocal disorder affecting the CNS or peripheral nervous system. Dif-

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ferential diagnoses included meningitis (infectious or SRMA), meningomyelitis (infectious or granulomatous meningoencephalomyelitis), diskospondylitis, osteomyelitis, polymyositis, polyneuritis, and, less likely, polyarthritis.

Complete blood count revealed neutrophilic leukocytosis (WBC count, 28.5 × 10^3 cells/µL [reference range, 5.5 × 10^3 cells/µL to 13.9 × 10^3 cells/µL]; neutrophil count, 22.8 × 10^3 cells/µL [reference range, 2.9 × 10^3 cells/µL to 12.0 × 10^3 cells/µL]) with a left shift (0.855 × 10^3 band neutrophils/µL; reference range, 0.0 × 10^3 band neutrophils/µL to 0.45 × 10^3 band neutrophils/µL), monocytosis (2.850 × 10^3 cells/µL; reference range, 0.1 × 10^3 cells/µL to 1.4 × 10^3 cells/µL), and thrombocytopenia (117 × 10^3 platelets/µL; reference range, 235 × 10^3 platelets/µL to 694 × 10^3 platelets/µL). Serum biochemical analysis revealed high alkaline phosphatase activity (263 U/L; reference range, 13 to 122 U/L). Urinalysis revealed isosthenuria (urine specific gravity, 1.008), and sediment examination revealed no abnormalities. There were no abnormalities on lateral radiographs of the cervical vertebral column.

Initial empirical treatment for possible systemic infection consisted of administration of cefazolin sodium (22 mg/kg [10 mg/lb], IV, q 8 h), enrofloxacin (10 mg/kg [4.5 mg/lb], IV, q 24 h), and metronidazole (10 mg/kg, IV, q 12 h). Hydromorphone was administered (0.05 mg/kg [0.023 mg/lb], IV, q 4 h) for analgesia. The dog also received prednisolone sodium succinate (0.5 mg/kg [0.23 mg/lb], IV, once). Fluids (isotonic electrolyte replacement solution[a] supplemented with 16 mEq of KCl/L) were administered (5 mL/kg/h [2.3 mL/lb/h], IV). Approximately 4 hours after the dog received medications and IV fluids, the fever resolved. The following day, the dog was clinically normal. For collection of CSF, the dog was administered butorphanol tartrate (0.15 mg/kg [0.068 mg/lb], IV) and midazolam sodium (0.15 mg/kg, IV) for preanesthetic sedation. General anesthesia was induced with propofol (4 mg/kg [1.8 mg/lb], IV to effect) and was maintained with isoflurane in oxygen after endotracheal intubation. Cerebrospinal fluid was collected from the cerebellomedullary cistern; analysis revealed neutrophilic pleocytosis and a high total protein concentration (nucleated cell count, 4,956 cells/µL [reference range, 0 to 5 cells/µL]; total protein concentration, 259 mg/dL [reference range, < 24 mg/dL]; RBC count, 0 cells/µL). Cytologically, the CSF was composed of 85% nondegenerate neutrophils, 12% macrophages, and 3% lymphocytes. Microbial organisms were not observed. Cerebrospinal fluid was submitted for aerobic bacterial culture and measurement of IgA concentration.

Pending results of aerobic bacterial culture of CSF and measurement of the CSF IgA concentration, the dog was continued on empirical IV antimicrobial treatment (cefazolin, enrofloxacin, and metronidazole) and received a second dose of glucocorticoids (prednisone, 0.76 mg/kg [0.345 mg/lb], PO, once). Four days after admission, the dog was discharged and the owner was instructed to continue administration of the antimicrobials by mouth for 4 weeks.

One week after discharge, the dog was reevaluated at the University of Georgia Veterinary Teaching Hospital for recurrence of lethargy, fever (39.8°C [103.64°F]), and signs of cervical pain. Results of aerobic bacterial culture of the CSF sample obtained during the previous hospitalization were negative. Cerebrospinal fluid IgA concentration for this sample was low (< 33 mg/dL; reference range, 35 to 270 mg/dL). Despite the CSF IgA concentration, the dog was treated with prednisone (3.2 mg/kg, PO, q 24 h) for suspected SRMA, was continued on the previously administered antimicrobials (cefazolin sodium, enrofloxacin, and metronidazole) at the same dosages, and was discharged. On reevaluation 9 days later, the dog was clinically normal except for mild signs of cervical pain. On the basis of the presence of signs of residual cervical pain, azathioprine (2 mg/kg, [0.91 mg/lb], PO, q 24 h) was added to the dog's treatment regimen.

Two weeks later, the dog was reevaluated at the University of Georgia Veterinary Teaching Hospital. No abnormalities were observed on physical and neurologic examination. Complete blood count revealed a neutrophilic leukocytosis (WBC count, 15.1 × 10^3 cells/µL; neutrophil count, 12.986 × 10^3 cells/µL). Serum biochemical analysis revealed high alkaline phosphatase (292 U/L) and alanine aminotransferase (202 U/L; reference range, 12 to 108 U/L) activities. On the basis of the results of the dog's examination, the azathioprine dosage was decreased (2 mg/kg, PO, q 48 h) and prednisone treatment was continued at the same dosage for 2 more weeks, after which the dosage was tapered (1.6 mg/kg [0.73 mg/lb], PO, q 24 h).

After 3 weeks of immunosuppressive treatment, the dog was evaluated at the University of Georgia Veterinary Teaching Hospital for an acute onset of tachypnea and lethargy. On admission, the dog was febrile (39.6°C [103.28°F]). Respiratory rate was 72 breaths/min, and heart rate was within reference limits. Thoracic auscultation revealed harsh lung sounds bilaterally. Complete blood count revealed leukopenia (4.0 × 10^3 cells/µL; reference range, 5.50 × 10^3 cells/µL to 16.90 × 10^3 cells/µL), neutropenia (1.960 × 10^3 cells/µL), and a left shift (1.080 × 10^3 band neutrophils/µL). Serum biochemical analysis revealed high serum alkaline phosphatase (317 U/L) and alanine aminotransferase (110 U/L) activities. No abnormalities were detected on analysis of a urine sample obtained via cystocentesis except for the presence of bacterial rods without the presence of WBCs on examination of the urine sediment. Thoracic radiography revealed a diffuse bronchial and interstitial pattern (**Figure 1**). Tracheobronchial lymphadenopathy was not identified. Over the subsequent several hours, the dog's respiratory rate increased and the dog became dyspneic. Venous blood gas analysis performed to assess ventilation revealed respiratory acidosis with some metabolic compensation (pH, 7.023 [reference range, 7.32 to 7.43]; $Paco_2$, 103 mm Hg [reference range, 29 to 42 mm Hg]; HCO_3 concentration, 27 mEq/L [reference range, 17 to 24 mEq/L]). The dog was placed in an oxygen cage to provide supplemental oxygenation. With the dog in the oxygen cage, hemoglobin oxygen saturation was 100%, as assessed with pulse oximetry. Despite treatment with oxygen and supportive care, the patient's condition continued to decline until ultimately it went into respiratory arrest and died.

At necropsy, the lungs were mottled and contained multiple firm, white-gray, slightly raised foci ranging from 0.5 to 1.2 cm in diameter (**Figure 2**). Similar foci were distributed randomly throughout the kidneys, liver, and heart. The cut surface of both kidneys contained multiple yellow wedge-shaped acute infarcts, extending from the medulla into the cortex. A few areas of the brain leptomeninges were opaque, especially in the sulci of the cerebral cortex. Histopathologic lesions consisted of necrotizing and pyogranulomatous pneumonia, nephritis, and myocarditis. Multiple pyogranulomas contained a central area of necrosis and hemorrhage surrounded by degenerate neutrophils, epithelioid macrophages, multinucleated giant cells, lymphocytes, and plasma cells. Admixed with these inflammatory cells and within the epithelioid macrophages and multinucleated giant cells were moderate numbers of intracellular amoebic organisms in 2 different stages: trophozoites and cysts (**Figure 3**). Trophozoites were spherical and 15 to 30 µm in diameter, with a slightly basophilic granular cytoplasm and round nucleus. Cysts were 15 to 20 µm in diameter and surrounded by an outer thin wall. The nuclei were centrally located with 1 prominent targetoid eosinophilic karyosome. Periodic acid–Schiff and Gomori methenamine silver methods stained only the cyst walls. Histologic examination of the brain revealed scattered clusters of epithelioid macrophages throughout the leptomeninges. Surrounding multiple areas of the dura mater of the spinal cord, especially the cauda equina, and extending into the adjacent adipose tissue were foci of necrosis and granulomatous infiltrate admixed with amoebic organisms. In these areas, medium-sized vessels had fibrinoid change in the wall. Gross and histologic findings were consistent with systemic amoebic infection.

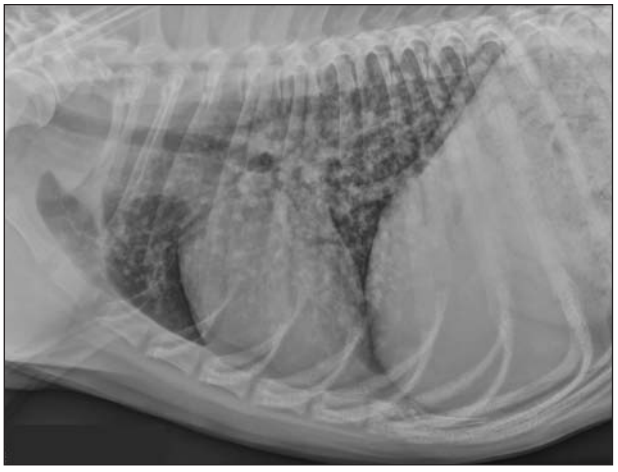

Figure 1—Right lateral thoracic radiographic view of a 10-month-old castrated male Boxer with a subsequent diagnosis of a multisystemic infection with a unique *Acanthamoeba* sp. A severe peribronchial and interstitial pattern is evident diffusely throughout the lungs.

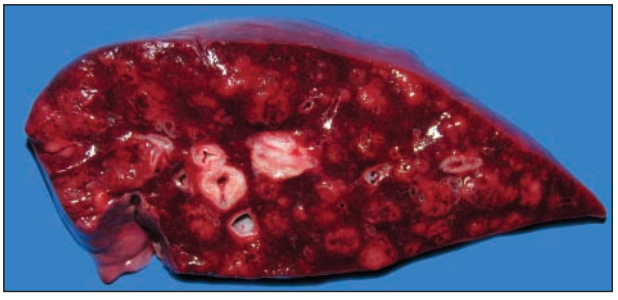

Figure 2—A postmortem specimen of lung from the dog in Figure 1. The cut surface reveals multiple, white-gray, slightly raised foci ranging from 0.5 to 1.2 cm in diameter throughout the parenchyma. The remaining areas are severely congested.

Because an amoebic organism was identified on the necropsy examination, several further means were used to further characterize the infection, including PCR assay and culture. For PCR assay, DNA was extracted from CSF and paraffin-embedded spinal cord, brain, and lung samples as described previously.[1] A second CSF sample had been obtained immediately after death, so that 2 CSF samples were tested (including the sample obtained from the patient during the initial hospitalization). A 238–base pair fragment of the canine *GAPDH* gene was amplified from all CSF and paraffin-embedded tissue specimens to assess the integrity of the nucleic acid in all samples.[2] Previously described PCR primers (AcantF900 and AcantR1100; primers that target portions of the nuclear SSU rRNA gene [rDNA]) were used for amplification of *Acanthamoeba* spp.[3] The 180-bp *Acanthamoeba* PCR assay product was amplified successfully from lung tissue but not from the spinal cord, CSF, or negative controls.

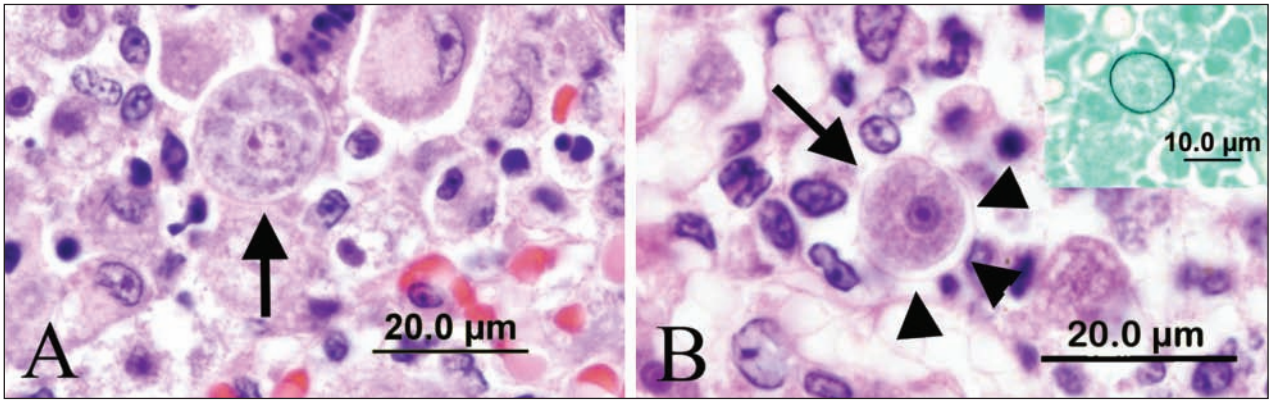

Figure 3—Photomicrographs of sections of the lung from the dog in Figure 1. A—An *Acanthamoeba* trophozoite (arrow) is observed in the lung, which contains pyogranulomatous inflammation. H&E stain; bar = 20 µm. B—The cyst form of the *Acanthamoeba* organism has a characteristic targetoid karyosome (arrow). Notice the thin cyst wall (arrowheads). H&E stain; bar = 20 µm. Inset—Notice the thin cyst wall. Gomori methenamine silver stain; bar = 10 µm.

Kidney and liver tissues were broken up into small pieces and minced; minced tissues were inoculated separately into individual nonnutritive agar plates coated with a layer of *Escherichia coli*, as described previously.[4] Amoebae that grew on the agar plates when examined microscopically had thornlike processes, referred to as pseudopodia, from the surface of the trophozoites, a feature that is characteristic of *Acanthamoeba* organisms.[5] Further, the amoebae differentiated into cysts after 2 weeks of growth, and the cysts had an outer wrinkled ectocyst and a polygonal or star-shaped endocyst. On the basis of these features, the amoebae were identified as *Acanthamoeba* spp group II and designated as CDC:V600.

Samples of kidney and liver tissue were vortexed, placed in medium (amoeba saline solution, peptone, yeast extract, and glucose) in tissue culture flasks, and plated on nonnutritive amoeba saline agar plates seeded with *Enterobacter aerogenes* (CDC strain No. 1998-68) as food. Blocks of agar from plates that yielded growth of *Acanthamoeba* organisms were transferred to liquid culture via media or amoeba saline solution.[6,7] The DNA was extracted from cultures containing *Acanthamoeba* organisms.[b] Following DNA extraction, a PCR assay was used to amplify a portion of the nuclear SSU rDNA gene fragment by use of a previously designed *Acanthamoeba*-specific primer set.[8] Sequencing of the portion of the SSU rDNA gene fragment was completed by use of an automated fluorescent sequencing system[c] and a set of conserved primers and methods that has been used previously.[8]

The partial SSU rDNA gene sequences that were obtained from the kidney and liver samples from the dog in the present report were aligned with a set of > 130 complete sequences (> 2,000 nucleotides in length) of the *Acanthamoeba* nuclear SSU rDNA gene available from GenBank (National Institutes of Health genetic sequence database) with a sequence alignment application.[9,d] This alignment allowed the identification of those complete *Acanthamoeba* spp sequences that most closely matched the sequences obtained from the kidney and liver samples from the patient in this report. Sequences obtained from both tissue sources were identical to each other and were identified on the basis of similarity to other sequences as genotype T1, an as yet unnamed species of the *Acanthamoeba* organism. This is a rare genotype and has thus far only been found in samples derived from animal infections and from humans with predominantly fatal granulomatous amoebic encephalitis. In addition, the T1 sequence in the dog in the present report was rare because it contained some sequence variability in the nuclear SSU rDNA gene fragment, compared with other genotype T1 sequences. These partial nuclear SSU rDNA sequences from the *Acanthamoeba* isolate derived from the tissues of the dog in this report have been deposited in GenBank under the following accession numbers: GQ924681 and GQ924682.

Discussion

The dog in the present report developed systemic infection with a unique *Acanthamoeba* sp. Whether the initial clinical signs were attributable to infection with *Acanthamoeba* or the infection developed secondary to chronic immunosuppression as a result of treatment of SRMA remains undetermined. Steroid-responsive meningitis-arteritis predominantly affects young large-breed dogs.[10–13] There appears to be a breed predilection for Beagles, Bernese Mountain Dogs, Boxers, and Nova Scotia Duck Tolling Retrievers.[11,14–17] Clinically, SRMA is typically characterized by acute onset of signs of cervical pain, stiff gait, and pyrexia.[10,11,13] The diagnosis of SRMA is made through a combination of clinical examination findings and clinicopathologic data. Complete blood count often reveals neutrophilic leukocytosis, occasionally with a left shift, and CSF analysis reveals high protein concentration and nondegenerate neutrophilic pleocytosis.[11,18] Serum or CSF IgA concentrations often are high.[18] Treatment of SRMA typically requires long-term immunosuppression with glucocorticoids.[10,11,13,14,18]

One consequence of long-term immunosuppression is an increased risk of infection. Long-term glucocorticoid treatment has been associated with an increased risk of urinary tract infections.[19,20] Localized brain abscessation has been observed in a dog treated with cyclosporin for perianal fistulas.[21] Development of infection represents the second most common cause of death after immunosuppression in cats undergoing renal transplantation.[22] Although uncommon, systemic amoebic infection has been observed in a dog that had been treated with prednisone.[23,24]

There are several species of free-living amoebae belonging to 4 genera that cause disease in animals and humans.[4,25] The 4 genera of amoebae responsible for CNS disease in animals are *Acanthamoeba* (several species), *Naegleria fowleri*, *Balamuthia mandrillaris*, and the recently described *Sappinia diploidea*.[5] Referred to as amphizoic, these organisms exist as free-living amoebae but also can occasionally invade and parasitize host tissue.[26]

In humans, amoebic infections may be localized to a single organ system (eg, ocular keratitis) or may be disseminated and cause systemic disease.[26,27] Often involving the brain in humans, CNS infections with *Acanthamoeba* spp and *B mandrillaris* typically affect immunocompromised or debilitated individuals resulting in granulomatous amoebic encephalitis.[25] *Naegleria* spp can infect immunocompetent individuals, resulting in rapidly fatal, necrotizing, and hemorrhagic primary amoebic meningoencephalitis.[28] Reported cases of amoebic infections in dogs, resulting in systemic infections and encephalomyelitis, have been attributed to *Acanthamoeba* spp[29,30] and *B mandrillaris*.[23,24] Conversely, documented infections in cattle[31] and a tapir[32] have been localized to the brain with pathologic findings similar to those observed in humans with primary amoebic meningoencephalitis. In the dog in the present report, systemic infection with minimal involvement of the leptomeninges was attributed to infection by an *Acanthamoeba* sp.

Acanthamoeba spp have a global, ubiquitous distribution and are found in a wide range of environments, such as in dust particles in the air, soil, chlorinated pools, sewage, and bottled water, freshwater, and saltwater.[25,26,33] The life cycle of *Acanthamoeba* organisms alternates between a cyst and trophozoite stage. Under

harsh environmental conditions in which there is a lack of nutrients, high temperatures, or lack of water, *Acanthamoeba* spp exist in a resistant cystic form.[25,26,33] *Acanthamoeba* cysts transform into an infective trophozoite under more favorable conditions.[25,26,33]

In previously published reports[23,24,34] of amoebiasis in dogs, the immune system of the affected dogs has not been evaluated. However, in 1 dog infected with *Acanthamoeba castellanii*, T-cell function was determined to be abnormal on the basis of lymphocyte blastogenesis in response to various mitogens, suggesting either immunosuppression or T-cell–mediated immunodeficiency.[34] Although immunocompetence was not typically evaluated in previous reports, some infected dogs had received immunosuppressive treatment prior to the eventual diagnosis of amoebic infection.[23,24] In 1 dog, immunosuppressive treatment with prednisone was used in the treatment of inflammatory bowel disease for 6 months prior to development of disseminated amoebic infection.[24] In another dog, prednisone and lomustine were administered for suspected CNS lymphoma.[23] Whereas the authors of that report[23] later questioned the initial clinical diagnosis of lymphoma, infection was ultimately considered secondary to immunosuppression.

Although speculative, several findings suggest that amoebic infection in the dog in the present report was acquired secondary to immunosuppression. Despite a low CSF IgA concentration, the history, signalment, initial examination findings, and clinicopathologic data were supportive of a diagnosis of SRMA rather than a misdiagnosis of amoebic CNS infection. Although the type of inflammatory cell infiltrate will vary depending on factors related to the host and infecting organism, CSF analysis reported in dogs with confirmed amoebic meningoencephalitis has primarily revealed mononuclear pleocytosis composed of a lymphocytic or a mixed cell population consisting of large mononuclear cells, lymphocytes, and neutrophils.[23,29] Similarly, in humans with *Acanthamoeba* infection, results of CSF analysis are characterized by lymphocytic pleocytosis. In the dog in this report, CSF analysis primarily revealed neutrophilic pleocytosis in which 85% of the inflammatory cells were neutrophils. Moreover, the lack of clinical signs and biochemical abnormalities referable to the lungs or kidneys at the onset of disease makes it unlikely that infection was present initially. Furthermore, the dog experienced a remission of clinical signs for 5 weeks after institution of immunosuppressive treatment. Although transient improvement may have occurred with glucocorticoid treatment, it seems likely that the dog would have deteriorated more quickly following immunosuppression had infection been present at the onset of disease. Finally, the initial clinical signs implicated CNS disease, yet microscopically, there was a paucity of CNS involvement, which tends to counter the possibility of infection at the onset of disease. Consequently, on the basis of CSF analysis, clinical course, and the finding of small numbers of organism within the CNS, amoebic infection in the dog in the present report was likely secondary to immunosuppression.

In humans, infection with *Acanthamoeba* organisms usually results in encephalitis without meningeal involvement, and organisms are rarely identified in CSF.[5] This may explain our inability to identify infection by use of PCR assay of CSF samples in the dog in this report. Only a small number of organisms were identified in the leptomeninges and organisms were not observed in the brain or spinal cord in the present patient. Results of PCR analysis in our patient confirmed the utility of the *Acanthamoeba* PCR assay for lung, kidney, and liver tissue but demonstrated that the organism was not present in the CSF or spinal cord tissues at detectable levels. Therefore, even if there had been a strong index of suspicion for *Acanthamoeba* infection on initial evaluation of this dog, such a diagnosis would not have been made by evaluating CSF via PCR assay.

The amoebic infection in the dog in the present report was concentrated in the lungs and kidneys with only a few organisms observed in the leptomeninges of the spinal cord at necropsy. In humans, the portal of entry is thought to be via inhalation or directly through skin wounds with subsequent hematogenous spread.[5,26] Experimentally, nasal inoculation in mice results in severe pulmonary disease followed by brain infection.[35] In humans, once *Acanthamoeba* organisms gain entrance into the lungs or through skin lesions, the infection subsequently disseminates hematogenously to the CNS.[33] Given the distribution of the organism in the organs in the dog in the present report, inhalation followed by respiratory infection with subsequent hematogenous spread of the organism seems the most likely route of infection. The infection was probably acquired through environmental exposure, given the ubiquitous presence of *Acanthamoeba* spp in soil and water worldwide.[26] Moreover, *Acanthamoeba* spp have been recovered in home environments from sinks, aquaria, and the soil of houseplants.[5]

The prognosis for dogs with systemic or CNS amoebiasis is grave, and to our knowledge, there are no reports of successful treatment. This prognosis may reflect the difficulty in diagnosis given the lack of specific clinicopathologic abnormalities associated with infection coupled with low index of suspicion because of the rarity of infection. In humans, systemic infections and granulomatous amoebic encephalitis also are associated with grave prognoses. However, treatment success in people has been reported for systemic and CNS amoebiasis following combination antimicrobial treatment with trimethoprim-sulfamethoxazole, rifampin, and ketoconazole.[36]

At present, antemortem diagnosis of amoebiasis requires identification of organisms in tissue, typically by use of indirect immunofluorescence staining with rabbit anti-amoeba sera or identification via PCR assay.[37] In humans, a conventional PCR assay is used in the diagnosis of amoebic infections.[38,39] However, because amoebae often are encysted or found as trophozoites within tissues, PCR assay may lack sensitivity when applied to biological samples such as CSF. The present report highlights this limitation, as results of PCR assay performed retrospectively on nucleic acids extracted from both CSF samples obtained from this patient were negative. However, results of PCR assay performed on minced kidney were positive for *Acanthamoeba* spp. Moreover, compared with previous genotypes, the isolate in the present patient showed a

rare sequence variation in the fragment of the nuclear SSU rDNA gene that is used in genotype identification. Interestingly, *Acanthamoeba* organisms have been found in the urine of critically ill patients in Brazil.[40] More recently, real-time PCR assay, in which pathogen load may be quantifiable, has provided for a more rapid diagnosis.[3,41] In the future, such PCR assay techniques may prove to be complementary in the antemortem diagnosis of amoebic infections in animals. Given the pathogenesis of amoebic infections, biological samples such as respiratory secretions and urine specimens may provide a more successful avenue for PCR assay testing. Ultimately, reliable and rapid methods of antemortem diagnosis may enable earlier therapeutic interventions and lead to successful outcomes in affected animals.

a. Normosol R, Abbott Laboratories, Chicago, Ill.
b. DNeasy kit, Qiagen Inc, Valencia, Calif.
c. ABI 3100, Applied Biosystems, Foster City, Calif.
d. CLUSTALX, Mega 3.1 Molecular Evolutionary Genetics Analysis software, The Biodesign Institute, Tempe, Ariz.

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