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Short communication

# Aberrant *Ancylostoma* sp. in the brain of a dog

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ABSTRACT

A 14-month-old, male American Bulldog presented to Texas A&M University Veterinary Medical Teaching Hospital in August of 2012 for anorexia, hydrophobia and gradually worsening neurologic signs. Grossly hemorrhage on the left side of the caudal cerebrum and cerebellum was observed and histologically corresponded with necrohemorrhagic and lymphoplasmacytic encephalitis associated with adult nematodes. Based on morphology and molecular analysis, these were identified as *Ancylostoma* sp.



A 14-month-old, male American Bulldog presented to the Texas A&M University Veterinary Medical Teaching Hospital in August of 2012 for a two-day history of anorexia, one-day history of apparent hydrophobia or dysphagia (staring at the water bowl but refusing to drink) and gradually worsening lethargy and depressed mentation. Approximately ten days prior to presentation right pelvic limb lameness was observed and two days later the owner reported that the left eye was "bloodshot." The patient lived in a suburban environment indoors with one other dog but was allowed outdoors including wooded areas and an adjacent city park for several hours each day. The patient had received no vaccinations and parasite control and treatment history was not provided in the clinical history. On presentation, the patient was approximately 8% dehydrated, quiet, dull, and depressed with generalized ataxia, head pressing, and photophobia. Temperature, heart rate, and respiratory rate were within normal limits. Serum chemistry analysis, complete blood count, and urinalysis were within normal limits.

Differential diagnoses for neurologic signs in a young adult, unvaccinated dog include trauma or congenital disease, as well as infectious agents including rabies virus and canine distemper virus, protozoa such as *Neospora caninum* or *Toxoplasma gondii*, fungi such as Aspergillus spp., or *Cryptococcus neoformans*, and migration of larval helminths. Another possible cause for neurologic signs in a dog is metabolic disease, such as hepatic encephalopathy.

Due to neurologic signs, including apparent hydrophobia, and lack of vaccination, rabies was suspected. No diagnostic imaging was performed. The patient was euthanized and the body submitted for limited post mortem examination, which only requested brain examination and submission for rabies testing.

On postmortem examination, there was extensive hemorrhage of the left side of the brainstem from the caudal colliculi to the caudal cerebellar peduncles (Fig. 1). The appropriate sample was collected and submitted for rabies testing at the Texas Department of State Health Services and the remaining brain was fixed in 10% neutral buffered formalin for histologic evaluation. A negative result was reported for rabies testing. The fixed sections of cerebrum, brainstem and cerebellum were routinely processed, paraffin embedded, sectioned at 5um, and stained with hematoxylin and eosin. Sections of the cerebrum were unremarkable on microscopic examination. In the cerebellum and brainstem there was marked local necrosis and loss of gray and white matter. The sections had extensive hemorrhage mixed with gitter cells, fibrin, edema, lymphocytes, plasma cells, fewer neutrophils, rare eosinophils and multinucleate giant macrophages surrounding cross and tangential sections of adult nematodes (Figs. 2–4 ). Throughout the remaining parenchyma there was significant edema and wide perivascular cuffs of lymphocytes and plasma cells with scattered eosinophils with similar inflammatory infiltrates extending into the meninges. Occasionally vascular walls were brightly eosinophilic and hyaline (fibrinoid necrosis). There were scattered spheroids and neurons with central chromatolysis. Nematodes were approximately 500 μm in diameter with an approximately 15 μm smooth, eosinophilic hyaline cuticle, vacuolated low lateral cords divided into sublaterals, pseudocoelom, bulbous internal cuticular ridges, platymyarian musculature, ovary,

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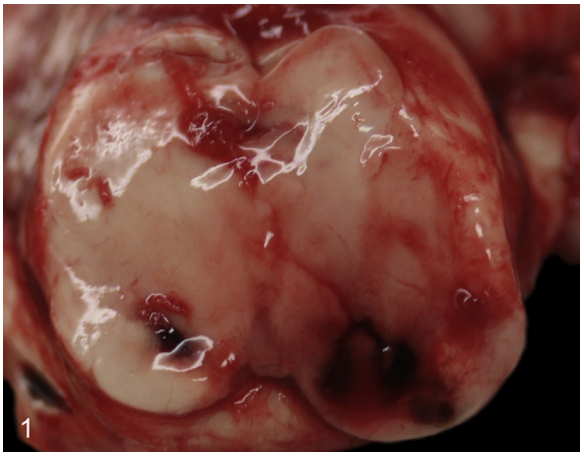


**Fig. 1.** Brainstem at the level of the mesencephalon. Multifocal hemorrhage and necrosis.

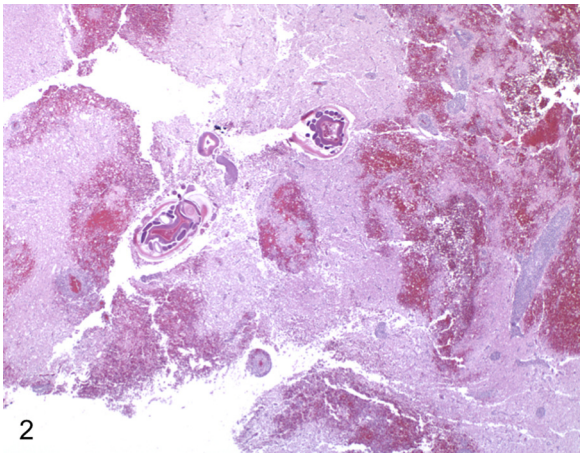


**Fig. 2.** Brainstem. 20× magnification photomicrograph. Hematoxylin and eosin. Necrohemorrhagic encephalitis with intralesional adult *Ancylostoma* spp.

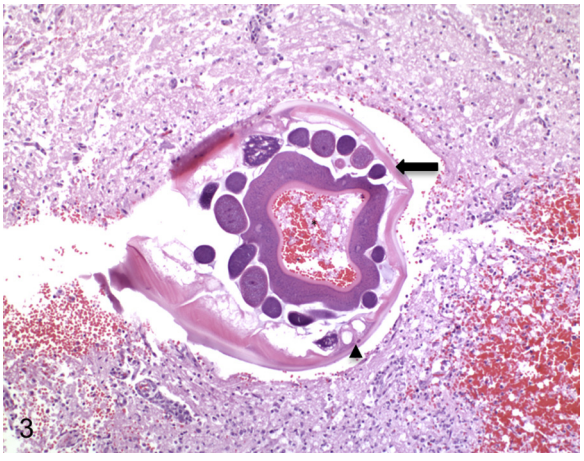


**Fig. 3.** Brainstem. 200× magnification photomicrograph of an adult *Ancylostoma* spp. from the same section as Fig. 2. Cuticle, lateral cord, and intestine containing host erythrocytes indicated by arrow, arrowhead, and star, respectively.

and a large central intestine composed of multinucleate cells with a prominent brush border whose lumen contained a moderate amount of host blood (Fig. 3). Based on these morphologic features, the nematode was identified as an adult female *Ancylostoma* sp.

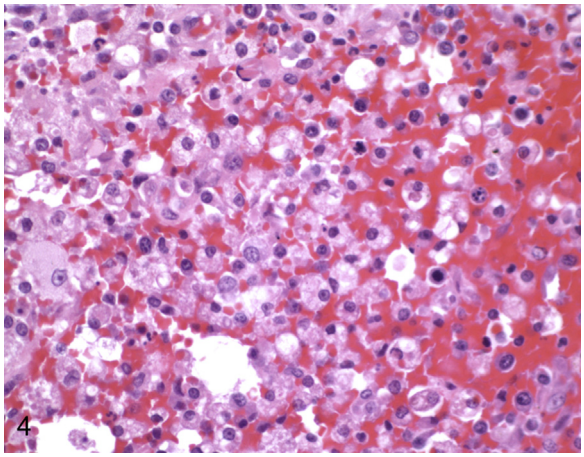


**Fig. 4.** Brainstem. 400× magnification photomicrograph. Hematoxylin and eosin. Lesions in the brainstem were characterized by necrosis and hemorrhage with numerous gitter cells.

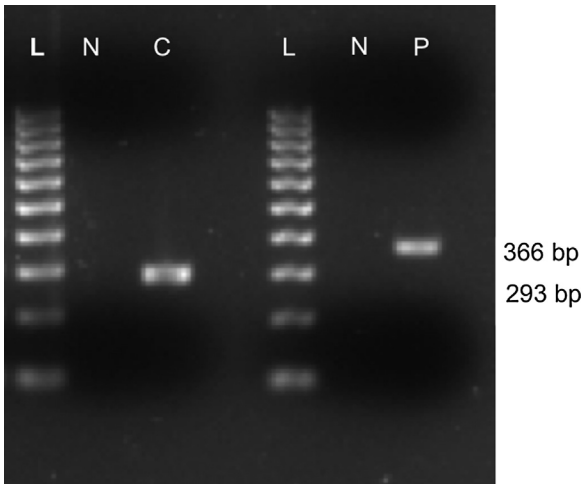


**Fig. 5.** Agarose gel electrophoresis showing amplification of the rRNA gene sequence. Genomic DNA from formalin fixed tissue curls of brain (patient) and intestine (positive control) sections showing hookworm like nematodes was used as template. L: 100 bp molecular ruler, N: negative control, C: patient case, P: positive control.

Generic primers were custom designed to amplify the ribosomal RNA sequence of hookworm species (Table 1). Genomic DNA isolated from formalin-fixed paraffin embedded tissue curls of the brain from the patient, and positive control tissue that showed presence of nematode sections on the intestine was used as template for polymerase chain reaction (PCR). Conventional PCR was performed using Phusion high-fidelity (NEB, Ipswich, MA) polymerase and amplifications were performed on T100 Thermal Cycler (Bio-Rad, Hercules, CA). Amplified PCR products were sequenced, and a blast search against sequences in GenBank was performed to confirm the identity of the nematode in the brain of this patient. Both primer pairs were tested on the patient sample and positive control. Successful amplification of 366 bp product with primer pair-1 was achieved only using positive control tissue as template. The 293 bp product using primer pair-2 was successfully amplified using the patient sample as template (Fig. 5). Since both primers target the ribosomal RNA gene, no further attempts were made to amplify the alternate targets. Blast search of the sequence of both PCR products indicated that the nematode seen in the brain tissue sections was *Ancylostoma caninum*.