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Case Report

Neurocysticercosis in a Rhesus Macaque (*Macaca mulatta*)

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An 8-y-old, intact, male rhesus macaque (*Macaca mulatta*) was sedated to undergo MRI in preparation for the implantation of cranial hardware. During imaging, 9 focal lesions were noted in the brain and musculature of the head. The lesions were hyperechoic with hypoechoic rims. The animal was deemed inappropriate for neuroscience research, and euthanasia was elected. Gross examination revealed multiple round, thick-walled, fluid-filled cysts (diameter, approximately 0.5 cm) in multiple tissues: one each in the left caudal lung lobe, left masseter muscle, and the dura overlying the brain and 8 throughout the gray and white matter of the brain parenchyma. Formalin-fixed sections of cyst-containing brain were stained with hematoxylin and eosin. Microscopic examination and molecular analysis of the *COX1* (*COI*) gene recognized the causative organism as *Taenia solium* at 99.04% identity.

The brain of rhesus macaques (*Macaca mulatta*) is anatomically and functionally similar to that of humans with regard to visual and motor abilities.[10,12,22,24] Rhesus macaques can be readily trained to perform a variety of psychologic tests similar to those used in humans, so that behavioral testing parallels many aspects of neurologic and psychologic testing in humans.[23,30] These characteristics make rhesus macaques a valuable and common model used in behavioral neuroscience research. Although numerous parasitic cestode infections in NHP at research facilities have been reported,[3,5,18,19,21,25,27] few have addressed neurocysticercosis in rhesus macaques. Previous reports in rhesus macaques include a 1936 article which describes *Cysticercus cellulosae* cysts within the subdural and subarachnoid spaces, in "practically every muscle of the body," and in the liver, spleen, and both kidneys as incidental findings.[29] A second report from 1968 describes the incidental finding of neurocysticercosis in the brains of 4 rhesus macaques, but no other tissues were examined.[28] In all reported cases, including the current case, no clinical signs were ever observed.

The parasitic infection of cysticercosis is caused by *Cysticercus cellulosae* cysts, which is the larval form of *Taenia solium*. The larvae can form cysts in a variety of tissues including the muscle and brain. When the cysts occur in the CNS, the condition is called neurocysticercosis. This condition is a major cause of adult-onset seizures in developing countries.[8] Diagnosis is usually made by MRI or CT scans of the brain. In people, the infection can be treated by using antiparasitic and antiinflammatory drugs; occasionally surgery is necessary.[8]

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Rhesus macaques at our institution are housed indoors in an AAALAC-accredited facility. All animals are screened annually for macacine herpesvirus 1, SIV, simian retrovirus types 1 and 2, and simian T-lymphotropic virus. In addition, macaques are screened on a semiannual basis for tuberculosis with intrapalpebral tuberculin testing. Housing and care for these animals is provided in accordance with the standards described in the *Guide for the Care and Use of Laboratory Animals*[20] and the Animal Welfare Act.[1]

An 8-y-old male rhesus macaque was sedated (ketamine, 4 mg/kg IM; dexmedetomidine, 0.05 mg/kg IM) to undergo MRI in preparation for the implantation of cranial hardware in accordance with an IACUC-approved protocol. The animal was captive-bred in China, was received into the facility from Harlan Laboratories (Indianapolis, IN) 4 y prior to presentation, and was experimentally naïve. MRI revealed 9 round (diameter, approximately 0.5 cm), focal hyperechoic lesions with hypoechoic rims (Figure 1). The majority of the lesions were located in the brain parenchyma and occupied both white and gray matter; additional solitary lesions affected the cervical musculature and left masseter muscle. The images were interpreted by a board-certified veterinary radiologist; at the time of imaging, the top differential diagnoses included focal granulomas secondary to parasitic, protozoal, bacterial, or fungal disease and hemorrhagic infarcts.

In light of the poor prognosis for future use in neuroscience research, euthanasia was elected. The animal was first sedated by using intramuscular ketamine and dexmedetomidine and then euthanized by intravenous administration of a barbiturate. Prior to euthanasia, chest radiography and tuberculin skin testing were performed to rule out tuberculosis, and blood was collected for CBC, chemistry, and storage of serum. The results of these tests were within normal limits. No abnormalities were noted on a physical exam performed at the time of euthanasia. A full gross postmortem examination was conducted, and tissues were collected for microscopic examination.

Pathology and diagnosis. Postmortem examination revealed a single cyst each in the left caudal lung lobe and left masseter muscle. Reflection of the calvarium disclosed a third cyst, which was

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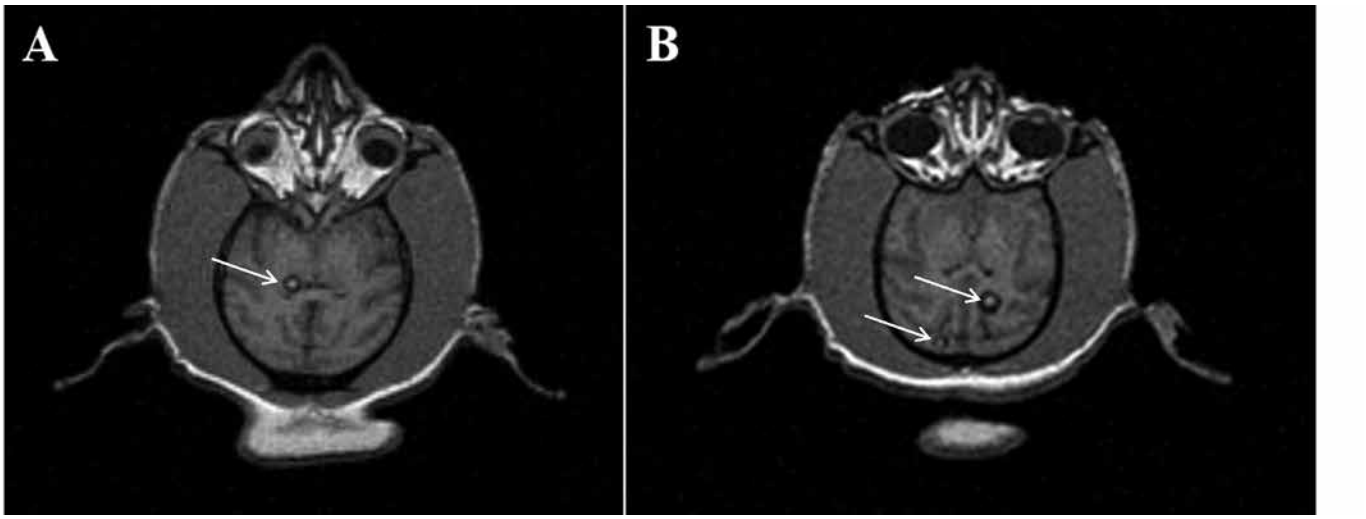

Figure 1. Rhesus macaque brain. T1-weighted MRI images. (A) A large hyperechoic cyst with a hypoechoic rim is seen left of the midline. (B) A similar large cyst is located right of the midline, with a smaller one caudal to the first one and left of the midline (arrows).

located on the dura. Four additional cysts were present on the surface of the brain (Figure 2), and numerous cysts were observed throughout the gray and white matter (Figure 3) on cut section. The cysts were thick-walled, were approximately 0.5 cm in diameter, and contained distinct white to yellow parasitic organisms.

The brain was collected en bloc, placed in formalin, fixed, and sectioned, and cystic lesions were paraffin-embedded, mounted on slides, and stained with hematoxylin and eosin for evaluation by a board-certified veterinary pathologist. Figure 4 illustrates the typical microscopic anatomy of the *Taenia* cysticercus. The cyst comprises an outer capsule and a scolex that is contained within the bladder. The scolex is made up of invaginated basophilic spaces and several inverted papillary projections. Within the papillary projections are calcareous corpuscles (Figure 5) characteristic of cestode tissue. The entire cyst is contained within a fibrous connective tissue capsule, and the surrounding parenchyma is infiltrated by a mixed inflammatory population including macrophages, giant cells and eosinophils.[17]

The cestode from the cyst in the left masseter muscle was sent for molecular diagnosis (Parasitology Laboratory, Wadsworth Center, Albany, NY). DNA was extracted from this tissue by using a QIAamp DNA Mini Kit (Qiagen, Germantown, MD). Briefly, tissue was cut into small pieces and lysed with proteinase K at 56 °C for 1 h. DNA was eluted in a volume of 200 µL. To identify the cestode, a region of the *COI* (*COX1*) gene was amplified by using 2 different forward primers (COI-cestode-F, 5′ GTA AAA CGA CGG CCA GTT TAC KYT RGA YCA TAA GCG TRT DGG-3′; COI-Taenia-F, 5′-GTA AAA CGA CGG CCA GTT TAC TTT RGA TCA TAA GCG DGT WGG 3′) paired with a reverse primer (COI-cestode-R, 5′-AAC AGC TAT GAC CAT GCY TCN GGR TGN CCA AAA AAY CA-3′) designed to detect a broad range of cestodes. The cestode-specific forward primer contained degeneracies to broadly amplify the *COI* genes from many cestodes, where the other forward primer specifically targeted *Taenia*. The amplification reaction volume of 50 µL contained 0.5 µM primers, 200 µM dNTP, Phire Reaction Buffer with 1.5 mM $MgCl_2$ and 1 µL Phire Hot Start II DNA Polymerase (Thermo Fisher Scientific, Waltham, MA). Target DNA was amplified by 45 cycles comprising 98 °C for 5 s, 62.7 °C (COI-cestode-F) or 61.4 °C (COI-Taenia-F) for 5 s, and 72 °C for 10 s, followed by a final extension step at 72 °C for 1 min. Both pairs of primers generated a product of 750 bp (Figure 6). PCR products were purified by using a QIAquick PCR Purification Kit (Qiagen) and were sequenced (Applied Genomic Technologies Core, Wadsworth Center). The amplification product generated by using the *Taenia*-specific primers had a 99.0% match to *T. solium*; the product generated by using the cestode primers also matched *T. solium* (98.9% identity). In both cases, the top 30 matches in the NCBI database all were *T. solium* sequences.

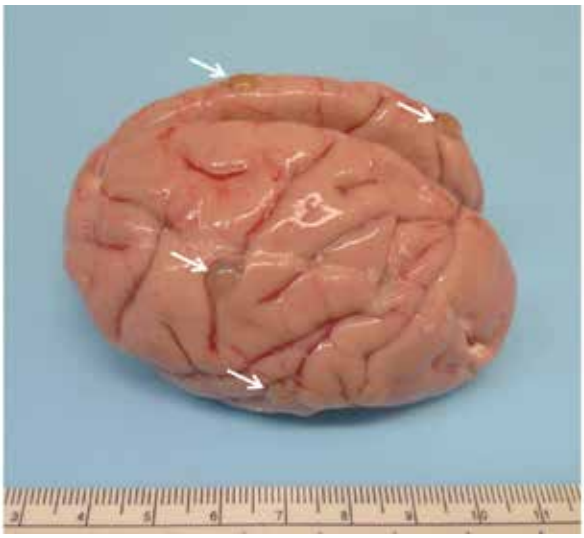

Figure 2. Rhesus macaque brain immediately after removal from the skull. Multiple larval cysts can be seen on the brain's surface (arrows).

Discussion

Cysticercosis is an infection of humans and pigs with the larval stages of *T. solium*. Pigs become infected by ingesting eggs or gravid proglottids, which are shed in the feces of a human carrier. Humans are then infected either by ingesting feces-contaminated food or through reinfection with larvae produced by parasitic cestodes already in the body (autoinfection). After ingestion, the

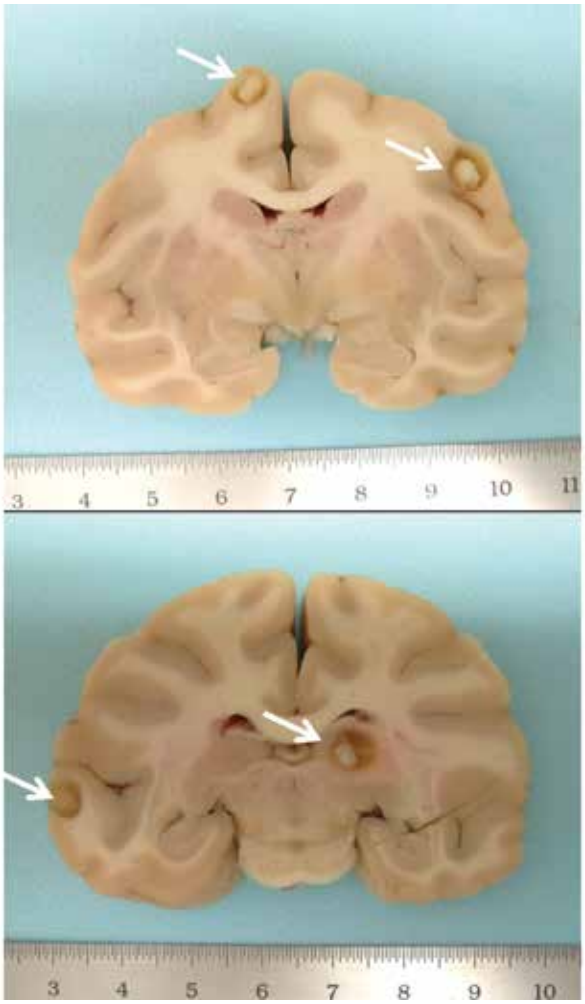

Figure 3. Rhesus macaque brain. Cut formalin-fixed sections with multiple larval cysts present throughout the parenchyma (arrows).

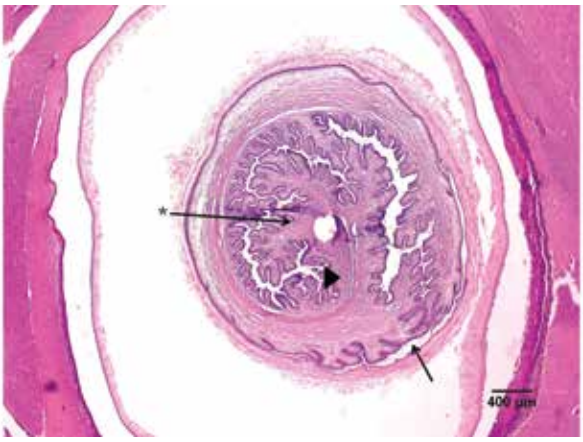

Figure 4. Rhesus macaque brain. Section through the cysticercus of *T. solium*. The cysticercus is made up of a scolex (arrow with asterix), created via invagination of the cyst wall (arrow head). The scolex is surrounded by a bladder (arrow). Hematoxylin and eosin stain, magnification, 2×.

eggs hatch into oncospheres in the intestine, which then invade the intestinal wall and migrate to striated muscles, brain, liver, and other tissues. Once in those tissues, they develop into cysticerci.

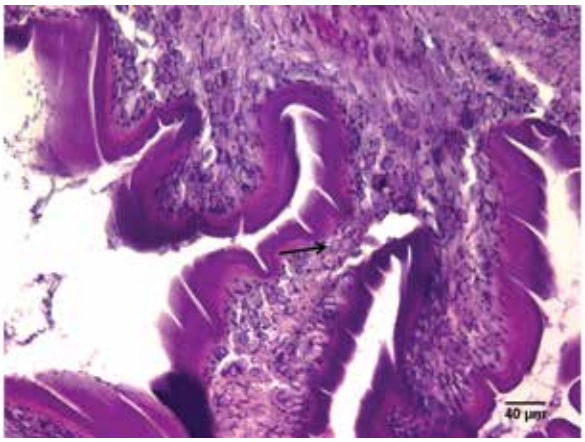

Figure 5. Rhesus macaque brain. Section through the cysticercus of *T. solium*. The papillary projections contained calcareous corpuscles (black arrow) composed of concentric layers of calcium carbonate , which are characteristic of cestode tissue. Hematoxylin and eosin stain; magnification, 20×.

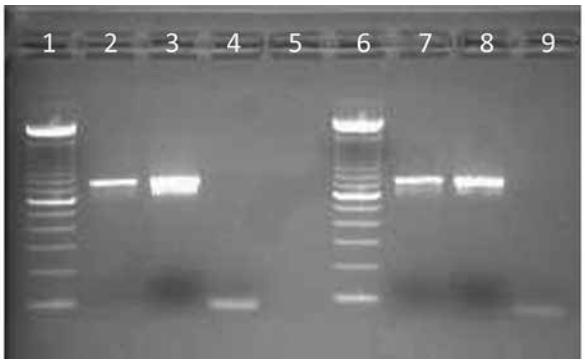

Figure 6. Agarose gel showing *COI* amplification products generated by using DNA extracted from a larval cyst. Lanes 1 and 6, 100-bp ladder; lanes 2 and 7, tissue from cyst; lanes 3 and 8, positive control (cestode); lanes 4 and 9, negative control; lane 5, blank. The products in lanes 2 through 4 were amplified by using the *Taenia* primers; those in lanes 7 through 9 were amplified by using the cestode primers. Both sets of primers generate an amplification product of 750 bp, which is the same size as the amplicon from the positive control.

In humans, cysts that localize in the brain can cause serious sequellae and result in neurocysticercosis. The parasitic life cycle is completed when humans ingest undercooked pork containing cysticerci, which results in human tapeworm infection. In the small intestine, the cysts evaginate, attach by means of their scolex, and develop into adult tapeworms, which can reside in the small intestine for years.[8] Humans are the only definitive host of *T. solium*, in which adult worms develop in the small intestine, and swine serve as the intermediate host, providing the environment for the cysticerci to develop. However, humans and other animals can serve as intermediate hosts and can develop cysts throughout the musculature and organs.[26]

Neurocysticercosis is an infection of the CNS by *Cysticercus cellulosae*, the larval stage of the tapeworm *Taenia solium.* It is the most serious form of cysticercosis and is the most common cause of acquired epilepsy worldwide.[4,6,9,14-16] Neurocysticercosis is a serious public health concern in developing countries in Latin America, Asia, and Africa and is the most common helminthic

infestation of the CNS. Of the estimated 50 million people who are infected annually, 50,000 of them die of neurocysticercosis.[7] The infection can be asymptomatic, but the most commonly reported signs are seizures and headaches. Less common symptoms include confusion, difficulty with balance, brain swelling, and excess fluid around the brain; the most serious symptoms include stroke and death.[8] Our macaque showed no clinical signs.

In humans, the only absolute diagnostic criteria for neurocysticercosis are histologic demonstration of the parasite from biopsy of a brain or spinal cord lesion, cystic lesions showing the scolex on CT or MRI, or direct visualization of subretinal parasites by fundoscopic examination.[13] The presence of clinical signs is not reliable for diagnosis, and serology is often unreliable given that many infected patients are seronegative.[11] In our case, MRI demonstrated the classic cystic lesions, and histologic examination of the brain parenchyma revealed the parasite and its structure. PCR testing of a sample from one of the cystic lesions provided the definitive diagnosis.

In human medicine, treatment options for neurocysticercosis are a cause of debate. The latest review by the American Academy of Neurology recommends albendazole in combination with a steroid to decrease the number of active lesions and to reduce long-term seizure frequency.[2] Evidence supporting or refuting the use of steroid treatment alone is unavailable. Further research is needed to clarify when steroids should be started during the course of antiparasitic treatment and to determine the efficacy of antiepileptic drugs in treating or decreasing occurrence of subsequent seizures.[2] Because this particular macaque was on a neuroscience protocol for which normal neuroanatomy was essential, treatment options were not explored.

Although numerous cestode infections in NHP at research facilities have been reported, only 2 address neurocysticercosis, and neither of these obtained molecular speciation of the parasite. The current case demonstrates the importance of obtaining brain MRI from NHP intended for neuroscience research on their arrival to the facility.

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