Journal of the American Association for Laboratory Animal Science
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Vol 47, No 4
July 2008
Pages 64–67

Visceral and Neural Larva Migrans in Rhesus Macaques

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Large ascarid larvae within granulomas were noted histologically in the mesenteric and pancreatic lymph nodes of 13 of 21 rhesus macaques (*Macaca mulatta*) euthanized as part of an experimental viral pathogenesis study. In addition, 7 of the 13 monkeys had cerebral granulomas, which in 4 animals contained nematode larvae similar to those within the lymph nodes. Despite the lesions, the animals did not show clinical signs associated with the parasitic infections. Characteristics of the larvae included, on cross-section, a midbody diameter of approximately 60 to 80 µm, a centrally located and slightly compressed intestine flanked on either side by large triangular excretory columns, and prominent single lateral cuticular alae. The morphology of the larvae was compatible with *Baylisascaris* spp. Baylisascariasis is a well-described infection of animals and humans that is caused by migrating larvae of the raccoon roundworm, *Baylisascaris procyonis*. A similar species, *B. columnaris*, is found in skunks and can cause cerebrospinal nematodiasis, but most reported cases of baylisascariasis have been due to *B. procyonis*. Our macaques were born free-ranging on an island in the southeastern United States where raccoons, but not skunks, were found to be common inhabitants, indicating that *B. procyonis* was the most likely parasite involved. These cases are similar to the low-level or covert cases of *Baylisascaris* infection described to occur in humans and provide further evidence of the existence of this parasite in the southeastern United States.

Baylisascariasis is a well-described infection of animals and humans that is caused by migrating larvae of the raccoon roundworm, *Baylisascaris procyonis*.[6,10] *B. procyonis* is a common intestinal parasite of the North American raccoon (*Procyon lotor*), which maintains the infection in nature. Female worms produce large numbers of eggs (115,000 to 179,000/worm daily), which are passed in the fecal material. The eggs take 2 to 4 wk to embryonate and become infective, and the thick shell makes them highly resistant to adverse environmental conditions and allows them to remain infective in the soil for years.[10] If infective eggs are ingested by various other mammals, including humans, or by birds, the larvae undergo aggressive somatic migration and enter various tissues, where they become encapsulated in granulomas. The larvae migrate by means of the portal circulation through the liver to the lungs, and are distributed to various tissues including the brain, via the systemic circulation. Larvae migrating in the brain are the most damaging and often result in clinical neurologic disease (neural larva migrans). More than 100 species of birds and mammals in North America, including humans, have developed fatal or severe CNS disease due to *B. procyonis*.[10] Most cases have occurred in the Midwest, Northeast, and on the West Coast, where the parasite has its highest prevalence in raccoons.[10]

Received: 28 Jan 2008. Revisions requested: 25 Feb 2008. Accepted: 3 April 2008.
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In this report, we describe low-level visceral and neural larva migrans due to *B. procyonis* in subclinically infected rhesus macaques in a research setting. Our findings support the occurrence of similar covert infection in humans and other species in endemic areas, including in the southeastern United States, where parasite prevalence and contamination may occur at lower levels.

Materials and Methods

After an uneventful 90-d quarantine, 21 captive-born, clinically healthy, 3- to 5-y-old male rhesus macaques (*Macaca mulatta*) were received from an outdoor breeding facility in the southeastern United States. On arrival, the animals were quarantined for an additional 31 to 45 d before being enrolled in experimental studies. The monkeys were housed and cared for according to the *Guide for the Care and Use of Laboratory Animals*[12] and animal welfare regulations. The animals were housed singly in 6.0-ft^2 biocontainment cages (Primate Products, Woodside, CA) that preclude animal-to-animal contact in order to prevent cross-infection. Monkeys were fed a standard Old World primate biscuit (Harlan-Teklad, Madison, WI) and given a minimum of 1 enrichment food daily. Upon arrival, the macaques were anesthetized with ketamine hydrochloride, and blood and serum samples were collected for serology, complete blood cell count and serum chemistry. The samples were tested for *Cercopithecine herpesvirus 1* (B virus), measles, simian retrovirus D, simian T-cell leukemia virus 1, and simian immunodeficiency virus (SIV). In addition, the animals were tuberculin-tested with mammalian old tuberculin on the upper eyelid and evaluated at 24, 48, and 72 h postinoculation. In addition, fecal samples were collected and Sheather's flotation tests performed for gastrointestinal parasites and ova. Rectal swabs were cultured for *Salmonella* spp., *Shigella* spp., and *Campylobacter* spp. After quarantine, the monkeys were enrolled in an IACUC-approved viral pathogenesis study. The animals were inoculated intracerebrally with live

attenuated tick-borne encephalitis viruses and euthanized on 3, 7, 14, 21, and 30 d postinoculation as required by the study. Necropsies were performed on all animals, and tissue samples from major visceral organs were collected and fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 µm, and processed routinely for light microscopy. Brains and spinal cords were removed, aseptically dissected, and processed for histopathologic examination. We selected 17 coronal-plane brain tissue blocks to include major neuroanatomic compartments: cerebral cortex, hippocampus, basal ganglia, thalamus, midbrain, pons, cerebellum, and medulla oblongata. Spinal cords were divided transversely into 18 tissue samples (6 from each of the cervical, thoracic, and lumbar regions).

Results

Pathogen testing during quarantine was negative in all animals. Complete blood counts and serum chemistry results were within normal ranges. No significant findings were noted during physical examination of the animals. The monkeys were found to be clinically healthy and enrolled in experimental studies. This disposition was not unexpected, because these animals had been tested and treated at the vendor and again at the NIH quarantine facility before arriving to our laboratory. No gross lesions were observed during routine necropsies performed as part of the study. However, on histopathologic examination, large ascarid larvae within eosinophilic granulomas were noted in the mesenteric and pancreatic lymph nodes of 13 out of the 21 (62%) macaques (Figure 1). Of the 13 infected monkeys, 7 (54%) had brain granulomas, and in 4 animals (31%), these granulomas contained nematode larvae similar to those within the lymph nodes (Figure 2). In the 3 animals that had cerebral eosinophilic granulomas without larvae, sectioning most likely missed the organism. The granulomas were characterized by aggregates of foamy, often pigmented, macrophages admixed with degenerate and nondegenerate neutrophils, lymphocytes, plasma cells, eosinophils, and scattered multinucleated giant cells often around a central parasite larva. The larvae were 60 to 80 µm in greatest diameter and had prominent, single lateral alae that were strongly pointed. The larvae also had a large, centrally located, slightly compressed intestine with an open lumen, flanked on either side by prominent lateral cords that supported smaller, roughly triangular excretory columns that were slightly dissimilar in size. Three hypodermal nuclei were visible in the lateral cords, just below the cuticle (Figure 3). In light of these characteristics, the larvae were identified as *Baylisascaris* sp.[3,10] The parasites also were present in the thalamus, and only 1 brain granuloma was observed among the 17 brain sections evaluated per monkey, and none were noted in the 18 spinal cord sections examined per animal. If only 1 to 4 sections per brain had been examined (as is usually done in such cases), the larval lesions might have been overlooked. Despite the lesions, clinical signs, abnormal blood work, and abnormal serum chemistry results were not observed. Although larvae occurred in mesenteric and pancreatic lymph nodes, none were found in the intestinal sections examined.

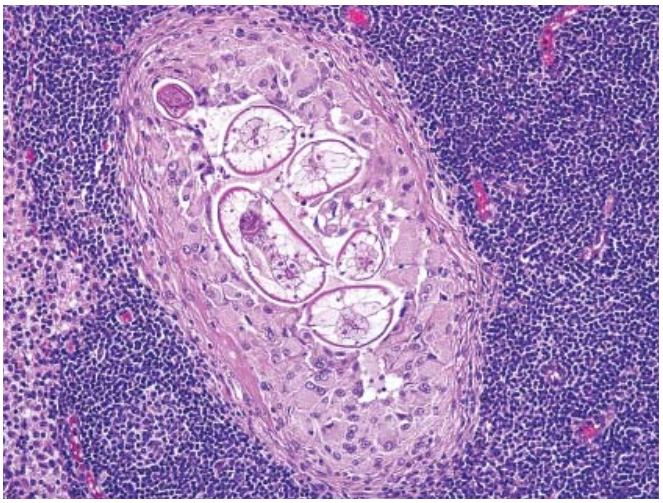

Figure 1. Rhesus macaque mesenteric lymph node section showing cross-sections of a *Baylisascaris* spp. larva within an eosinophilic granuloma. Hematoxylin and eosin stain. Magnification, ×200.

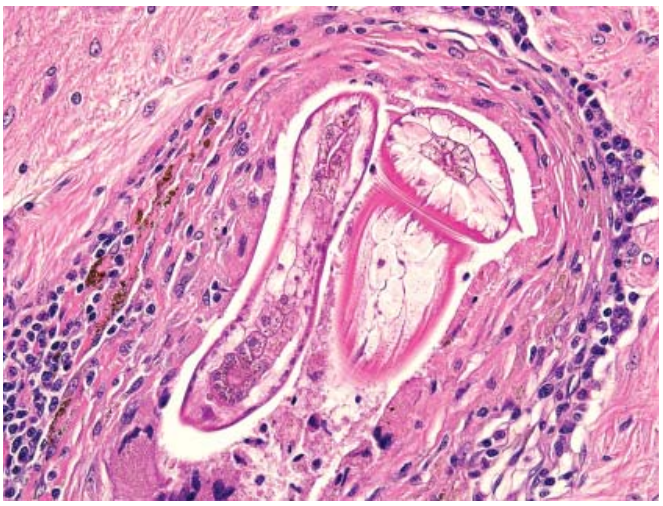

Figure 2. Rhesus macaque brain section showing an eosinophilic granuloma containing cross-sections of a *Baylisascaris* spp. larva similar to those within the lymph nodes. Hematoxylin and eosin stain. Magnification, ×400.

Discussion

Baylisascariasis is a well-described infection in animals and humans that usually is caused by migrating larvae of the raccoon roundworm, *B. procyonis*.[6,10] However, several other species of *Baylisascaris*, including *B. melis* of badgers, *B. columnaris* of skunks, *B. devosi* of fishers and martens, *B. transfuga* of bears, and *B. tasmaniensis* of marsupial carnivores, are potentially capable of causing animal or human disease if sufficient quantities of eggs are ingested.[10,17] Naturally occurring CNS disease caused by *B. columnaris* has been reported to occur in marmosets and emus.[8,11] Recently, fatal disease in Japanese macaques housed with bears and near raccoons was reported in a Japanese zoo.[16] The parasite involved could not be identified conclusively, and the authors suggested that *B. procyonis* was more likely given its greater pathogenicity. Most reported cases have been due to *B. procyonis*, considered the most pathogenic cause of larva migrans in the group.[10]

Clinical signs in cases of *Baylisascaris*-induced neural larva migrans in animals have included head tremors, head tilt, torticollis, opisthotonos, epilepsy, ataxia, partial paresis, posterior paralysis, and lateral recumbency.[1,2,4,7,13,15,16,18] Hematologic and serum chemistry evaluations performed during the acute phase of the disease were within normal ranges in reported cases;[1,15,18] these results may be related to lower overall infection levels in affected animals. In children, in whom infection levels may be much higher and for whom more data are available, parameters may be normal early, but patients usually develop mild to marked leukocytosis, and peripheral and cerebrospinal fluid eosinophilia may be striking.[6,10] Eosinophilic pleocytosis of the cerebrospinal fluid is considered one of the strongest indicators

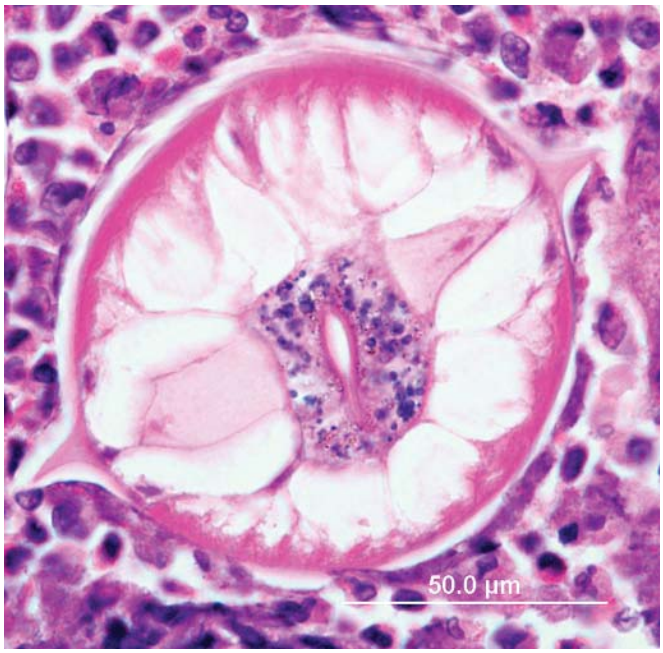

Figure 3. Brain section showing at higher magnification a *Baylisascaris* spp. larva. Larval characteristics on cross-section were a midbody diameter of 60 to 80 µm, prominent single lateral cuticular alae, and a large, centrally located and slightly compressed intestine, flanked on either side by smaller, dissimilar triangular excretory columns. Hematoxylin and eosin stain. Bar, 50.0 µm.

of possible *Baylisascaris* neural larva migrans in humans,[6] and this abnormality has also been seen consistently in experimental animals with clinical disease.[9] In the present cases, no peripheral eosinophilia was observed, probably because of the low infection levels with larvae. Peripheral eosinophilia might not be the best indicator for *Baylisascaris* infection in macaques housed outdoors because of the possibility of infection with other parasites, which are common in macaques housed under these conditions and might also trigger eosinophilia. In vivo, anti-*Baylisascaris* antibodies can be demonstrated in cerebrospinal fluid and serum by ELISA, Western blotting, and indirect immunofluorescent assay.[6] These tests are currently available from the Department of Comparative Pathobiology at Purdue University (West Lafayette, IN) for human patients, but it may be possible to apply them to macaque populations in the future. In addition, molecular methods, such as PCR, are becoming available for identification of *Baylisascaris*, but like serology will only identify the parasite to the genus level.[5] As in our cases, when larvae are recovered or seen in histologic sections, they are easily identified as *Baylisascaris* spp. by using larval morphology,[3,10] with the species involved determined based on the epidemiology of exposure to particular carnivores.[10]

In most cases, treatment of clinical neural larva migrans in humans and animals is unrewarding because infection-induced CNS damage already has advanced by the time clinical signs are observed and baylisascariasis is suspected. Albendazole and diethylcarbamazine are the most promising drugs for the treatment of baylisascariasis, especially when initiated very early (first 3 d) in the course of infection, before the larvae reach the brain.[10] Currently, albendazole is considered the drug of choice in humans and animals, and this agent usually is combined with steroids to control inflammation. Early and aggressive treatment of clinical CNS disease in children by using albendazole has shown encouraging results in several cases, with gradual or marked improvement.[9,14] In addition, gradual improvement was seen in 2 lemurs after extended treatment with albendazole.[10] Improvement after treatment may also be related to the level of infection and extent of CNS damage in affected individuals. Daily administration of pyrantel salts (pyrantel pamoate or tartrate as feed additives or supplements) has great utility as a preventative for *Baylisascaris* larva migrans, especially for animals in known-contaminated environments. These drugs kill the hatched larvae after egg ingestion and even at low daily doses have proven 100% effective in preventing larva migrans and the development of neurologic disease.[10] However, in our cases, giving anthelmintics to the macaques during quarantine would not be expected to lower the number of *Baylisascaris* spp. larvae observed given that (1) the macaques were already infected and larvae were already present in tissues; (2) the larvae are not very susceptible to drugs except during early migratory phase, which occurred before the animals' arrival; and (3) larvae were already walled-off, and at this point they are much less susceptible to any treatment.

In the cases presented, the macaques were born free-ranging on an island in the southeastern United States, where raccoons, but not skunks, were common inhabitants, thereby indicating *B. procyonis* was the most likely parasite involved. Infection levels in the macaques were low, which probably reflects low-level exposure, that is, a low prevalence of infection in the raccoon population on the island and low environmental contamination with eggs.[10] A maximum of 1 granuloma was found per brain, explaining why these animals showed no clinical signs. Neural larva migrans without clinical signs has been seen in rodents infected with either *B. procyonis* or *B. columnaris* and usually involves a single walled-off larva in the brain,[10] similar to what was seen in the macaques described here. Other clinical cases in animals, including primates, have been quite mild or protracted, with subtle CNS disturbances related to low-level infection with few larvae entering the CNS.[9,10] Our cases also support the occurrence of low-level or covert *Baylisascaris* infection in macaques as described for humans, where people can seroconvert to the infection but not develop clinical disease.[10] Cases of clinical neurologic disease certainly would be possible in this colony if any macaques became infected with higher dosages of eggs, for example, from particular areas contaminated by raccoons. Concerning geographic location, these cases provide further evidence of the existence of this parasite in the southeastern United States, where the few available studies indicate that the prevalence of infection in raccoons often has been lower than in other parts of the country. Despite that fact, these cases indicate that infection of exposed animals or people can occur and should be considered in cases of clinical CNS or ocular disease occurring in people from this region.

Acknowledgments

This research was supported by the Intramural Research Program of the National Institutes of Health, National Institute of Allergy and Infectious Diseases (NIAID), Comparative Medicine Branch, the Office of Research Support, and a NIAID contract to SoBran Incorporated. We want to thank Dr Alexander Pletnev and Dr Brian Murphy for providing the tissues and stained sections of brain and spinal cord for this study. We acknowledge the staff of Bioqual Incorporated (Rockville, MD), and the Pathology Associates Division of Charles River Laboratories (Frederick, MD) for histologic support and their excellent assistance in conducting the monkey studies. We also thank Mr Brad Finneyfrock (Bioqual) for compiling the clinical data and Dr David Asher (CBER, FDA) for helpful discussions and comments. This study was presented at the 33rd National Capital Area Branch of the American Association for Laboratory Animal Science meeting in Ellicott City, Maryland, and at the 35th annual workshop of the Association of Primate Veterinarians in Charlotte, North Carolina, 2007.

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