Raccoon Ascarid Larvae (*Baylisascaris procyonis*) as a Cause of Ocular Larva Migrans

Kevin R. Kazacos,* William A. Vestre,† and Evelyn A. Kazacos*

Larvae of the common raccoon roundworm, *Baylisascaris procyonis*, are known causes of visceral larva migrans and CNS disease in animals and human beings. In the present experiments we examined the ability of *B. procyonis* to cause ocular larva migrans (OLM) in subhuman primates, as an indication of its possible ocular zoonotic importance. Squirrel monkeys given 5,000 or 10,000 infective *B. procyonis* eggs *per os* and cynomolgus monkeys given 20,000 eggs had clinical and histologic evidence of OLM, beginning 7 days after inoculation. Clinically, multifocal retinal hemorrhages, white spots, chorioretinitis, inflammatory tracks, vascular sheathing, diffuse retinal degeneration, and motile intraretinal larvae were seen. Histologically, primarily subretinal larvae caused varying degrees of retinal disruption, degeneration and necrosis, retinitis, vasculitis, and perivascular sheathing, primarily with eosinophils. Larvae were also present in choroidal granulomas. It was concluded that *B. procyonis* larvae have marked ability to produce OLM in subhuman primates following oral infection and should be considered as a possible etiology in human ocular disease. Invest Ophthalmol Vis Sci 25:1177–1183, 1984

Baylisascaris procyonis, the common intestinal roundworm of raccoons, is a well-recognized cause of larva migrans in lower animals in North America, in which it usually is associated with central nervous system (CNS) disease.[1–3] Experiments in subhuman primates indicated the marked zoonotic potential of this parasite.[1,3] This was substantiated recently, in the finding of *B. procyonis* as the cause of death, from visceral larva migrans (VLM) and CNS disease, of children in Pennsylvania[4] and Illinois.‡ Epidemiologic studies have indicated the likelihood of contact and infection of human beings with this parasite.[2–5]

Besides causing VLM and CNS disease, an important question concerning *B. procyonis* is its ability to cause ocular larva migrans (OLM) in animals and humans. Several cases of OLM in human beings are suspected of being caused by this parasite.[3,6] Experimentally, OLM was seen in mice, hamsters, gray squirrels, and woodchucks infected with *B. procyonis.*[7] The purpose of the present study was to examine the ability of *B. procyonis* to cause OLM in two species of subhuman primates, as an indication of its possible importance as a cause of ocular disease in human beings.

Materials and Methods

Infective eggs of *B. procyonis* were obtained and prepared as previously described.[1]

Four adult female squirrel monkeys (*Saimiri sciureus*) and four adult male cynomolgus monkeys (*Macaca fascicularis*) were used, in accordance with the ARVO Resolution on the Use of Animals in Research. They were anesthetized lightly by an intramuscular injection of ketamine HCl and inoculated by oral or nasogastric intubation with a suspension of infective *B. procyonis* eggs in saline. Two squirrel monkeys received 5,000 eggs, two received 10,000 eggs, and the cynomolgus monkeys received 20,000 eggs.

The monkeys' eyes were clinically examined, under ketamine anesthesia, by slit-lamp and indirect ophthalmoscopy before and during the studies. The pupils were dilated with tropicamide or phenylephrine HCl-scopolamine HBr. Three of the squirrel monkeys' eyes were examined at 14 days after inoculation

From the Department of Veterinary Microbiology, Pathology, and Public Health* and the Ophthalmology Section, Department of Small Animal Clinics,† School of Veterinary Medicine, Purdue University, West Lafayette, Indiana.

Presented at the Fifth International Congress of Parasitology, Toronto, Canada, August 1982.

Supported in part by grants IND073029 and IND073029V from the United States Department of Agriculture and grant 015455 from the Indiana Department of Natural Resources.

Journal paper number 9672 of the Purdue University Agricultural Experiment Station.

Submitted for publication: October 31, 1983.

Reprint requests: Kevin R. Kazacos, DVM, PhD, Department of Veterinary Microbiology, Pathology and Public Health, Purdue University, West Lafayette, IN 47907.

‡ Fox AS, Kazacos KR, Gould NS, Heydemann PT, and Boyer KM. Fatal meningoencephalitis caused by *Baylisascaris procyonis* in an Illinois child. Unpublished data, 1984.

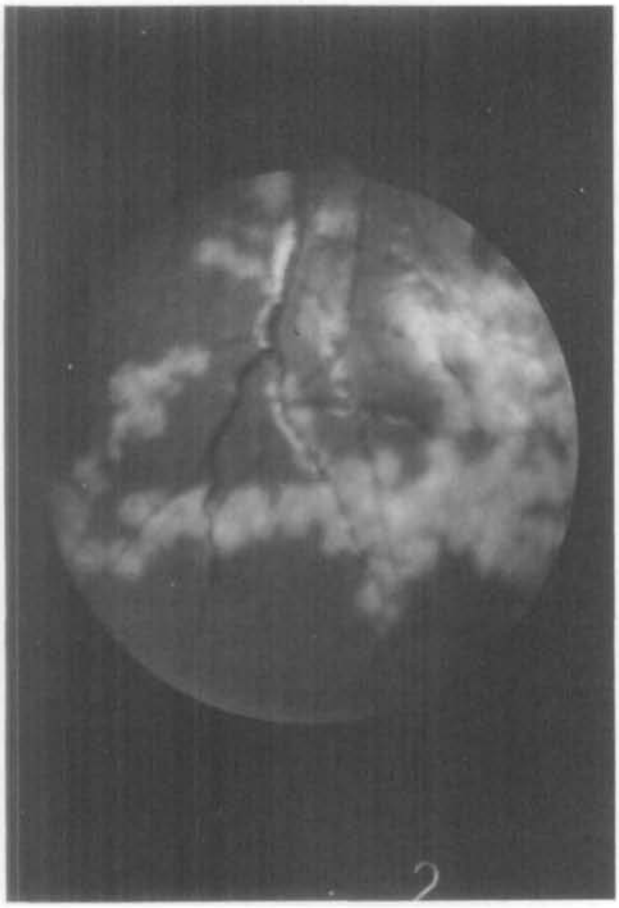

Fig. 1. Fundus of cynomolgus monkey at 8 DAI. Note serpiginous inflammatory tracks, sheathing along retinal vessels, and diffuse retinitis.

(DAI), and the cynomolgus monkeys' eyes were examined every 2-3 DAI.

All of the monkeys were killed or died following the development of CNS disease; the squirrel monkeys were necropsied at 12-19 DAI and the cynomolgus monkeys at 13-22 DAI. The globes were removed immediately and fixed in Zenker's fixative and later washed and processed for histology using paraffin embedding. Histologic sections were cut at 6 μm and stained with hematoxylin and eosin. Five to ten sections were cut and examined from various slices of the globes of three of the squirrel monkeys. The globes of one, high-dose, squirrel monkey and of all cynomolgus monkeys were sectioned serially, with every fifth slide stained and examined.

Results

Ophthalmoscopic Observations

All eyes were ophthalmoscopically normal when the experiments were begun. At 14 DAI, the squirrel monkeys' fundus had focal areas of active chorioretinitis, which were raised slightly with indistinct borders and light gray in color. A round, raised, white focal lesion was seen in the retina, near the optic disc, of the left eye of a high-dose squirrel monkey.

Lesions in the cynomolgus monkeys first were noted at from 7-14 DAI, and consisted of multifocal retinal hemorrhages and white spots, some of which were along retinal vessels, and retinal degeneration and depigmentation. Within 1-3 days, discrete serpiginous inflammatory tracks, multifocal to diffuse areas of retinal inflammation and degeneration, and prominent vascular sheathing were seen (Fig. 1). These lesions became more numerous and confluent, affecting large portions of the fundus, including the maculas, of two monkeys (Fig. 2). One of these monkeys had several visible motile larvae, tracks, and related retinal lesions at 7 DAI, with widespread involvement of the fundus by 11 DAI (Fig. 2); this animal was the first to die from CNS disease, at 13 DAI. Two of the other three cynomolgus monkeys had clinically significant lesions by 14-16 DAI. At this time, some also had multifocal raised white lesions in the fundus.

Histologic Observations

Moderate-to-severe OLM was present in all monkeys. Ocular larvae and lesions were most numerous and extensive in a high-dose squirrel monkey and in two of the cynomolgus monkeys; in the latter, they were most pronounced in the first animal to die from

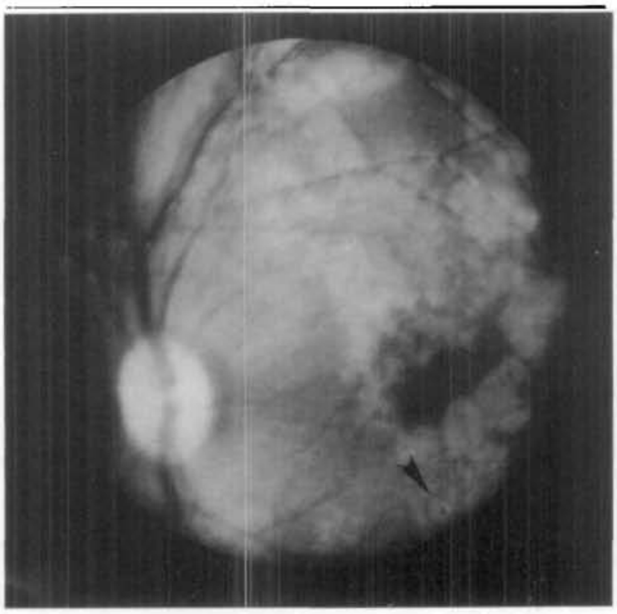

Fig. 2. Fundus of cynomolgus monkey at 11 DAI. Note widespread distribution and confluence of inflammatory tracks, involvement of macular area, diffuse retinitis, and *B. procyonis* larva in the retina (arrow).

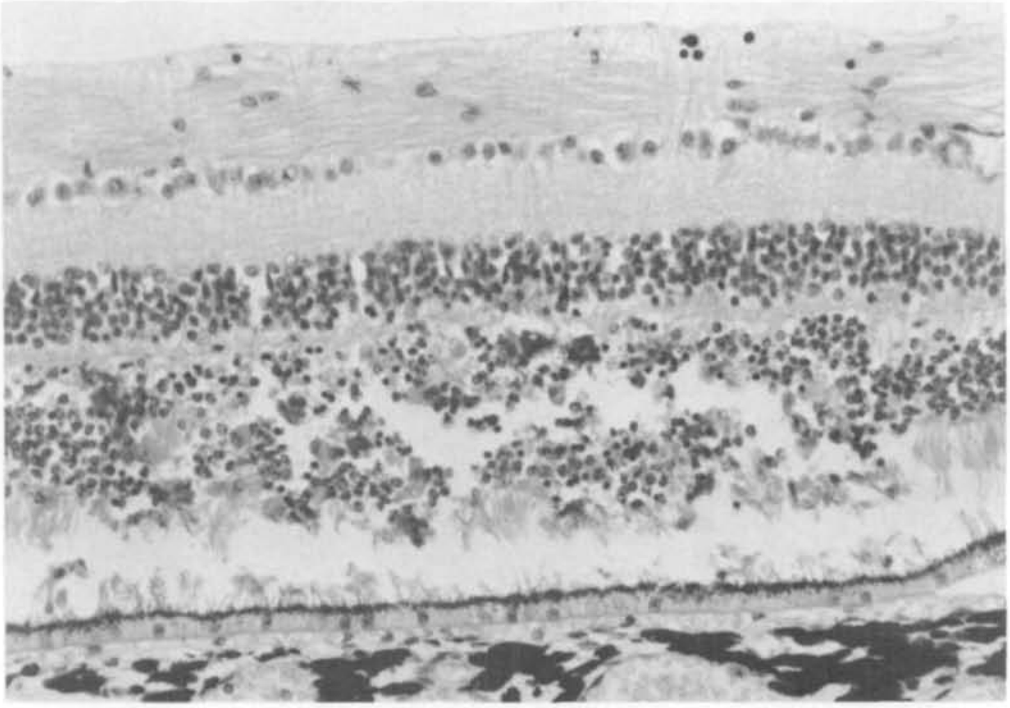

Fig. 3. Histologic sections of cynomolgus monkey globes. Note disruption of outer retinal layers to level of inner nuclear layer, and pyknosis of outer and inner nuclear layer nuclei (*top*). Note subretinal *B. procyonis* larva with associated retinal disruption, pyknosis of nuclei, hyperplasia of the pigment epithelium, and eosinophilic periarteriolar choroiditis (*bottom*) (hematoxylin-eosin, **A**, ×250 and **B**, ×100).

CNS disease. Larvae and lesions were primarily in the retina and choroid, although inflammatory foci and larval granulomas were also occasionally seen in the iris, ciliary body, extraocular musculature, optic nerve, and periocular adipose tissue.

The most common retinal lesion was focal-to-diffuse disruption and degeneration of the outer retinal layers, affecting as much as 2,500–4,000 μm of retina (Fig. 3A). The outer and inner nuclear layer nuclei were pyknotic, and there was hyperplasia and migra-

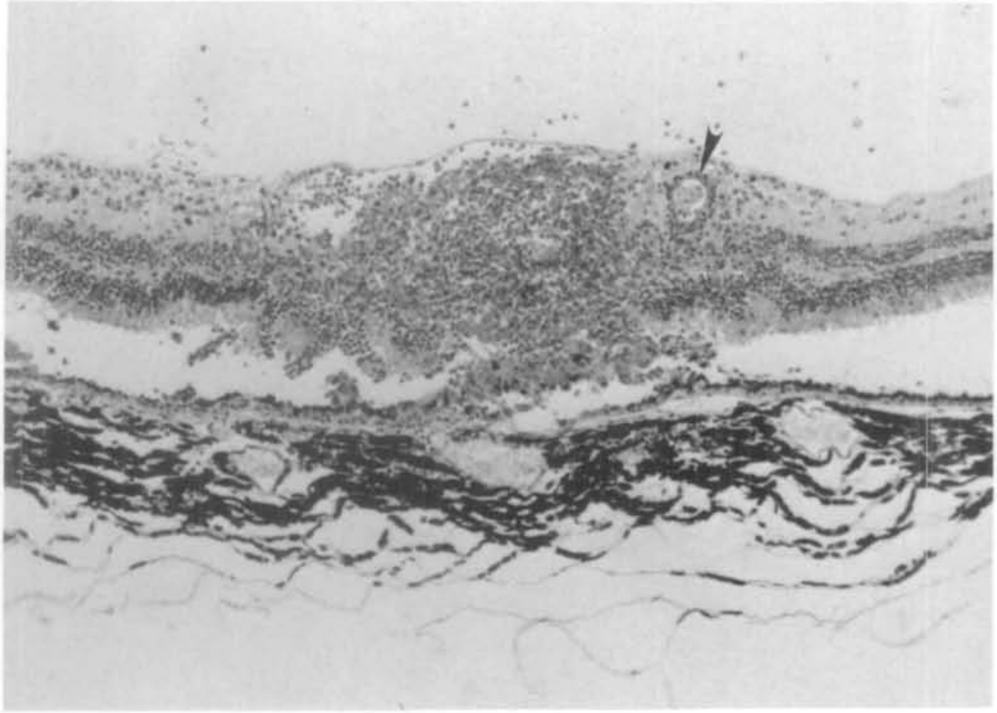

Fig. 4. Histologic section of cynomolgus monkey globe, through a larval migration track with more advanced reaction. Note total retinal disruption, necrosis and inflammation, vasculitis (arrow), hyalitis, hyperplasia and migration of the pigment epithelium, and overlying choroiditis. Diffuse eosinophilic retinitis radiates from both sides of the main lesion (hematoxylin-eosin, ×100).

tion of the pigment epithelium (Fig. 3A, B) and varying degrees of eosinophilic retinitis and vasculitis. Larvae were seen at all depths, including just beneath the inner limiting membrane. However, most were subretinal or embedded in the outer retinal layers, associated with the areas of retinal disruption (Fig. 3B); retinal larvae typically had no inflammatory cells around them. The white focal lesion seen clinically in the squirrel monkey eye was a subretinal coiled larva, causing a focal elevation of the retinal layers, without associated inflammation. Eosinophilic neuritis was sometimes seen in large nerve fibers in the vicinity of these lesions and in the optic nerve.

The cynomolgus monkeys also had intense areas of focal retinal necrosis and inflammation, which were believed to represent a more advanced reaction to larval migration (Fig. 4). These were interpreted as sections of the inflammatory tracks, which were seen clinically, as they could be followed through serial sections. There were total retinal necrosis, disruption, and inflammation measuring about 500 μm wide, with little left of the retina but the internal limiting membrane (Fig. 4). Also present were marked hyperplasia and migration of the pigment epithelium and activation and migration of the Müller cells. The inflammatory infiltrate was primarily of eosinophils but with some neutrophils, macrophages, lymphocytes, and plasma cells; many of the eosinophils were undergoing degranulation. Eosinophilic retinitis flared out up to 2,000 μm on either side of these tracks, and most often involved the nerve fiber layer (Fig. 4). There was an associated necrotizing vasculitis, perivascular sheathing by eosinophils, and a mild eosinophilic hyalitis.

Lesions in the choroid consisted of multifocal choroiditis, associated with adjacent retinitis or with larvae in the choroid undergoing granulomatous encapsulation. Larval granulomas had a centrally located larva in a pool of degranulating and necrotic eosinophils and pigment-laden macrophages (Fig. 5). This was surrounded by macrophages, epithelioid cells, scattered pigment, a rim of fibrous connective tissue, and a peripheral zone of inflammatory cells (primarily eosinophils), which were especially prominent at the poles of the granulomas (Fig. 5). The granulomas averaged 450 μm by 225 μm in size (range 375–500 μm by 188–250 μm). The *Baylisascaris* larvae in the retina and choroid measured an average 72.6 μm by 66.3 μm (range 65.0–81.3 μm by 56.3–75.0 μm) at their greatest diameter, found by serial sectioning. It was estimated from clinical and histologic studies that the eyes contained from one to ten larvae, most often five or less. The distinguishing features of the larvae have been described elsewhere.[1,2]

Discussion

B. procyonis is a common and important cause of larva migrans in mammals and birds in North Amer-

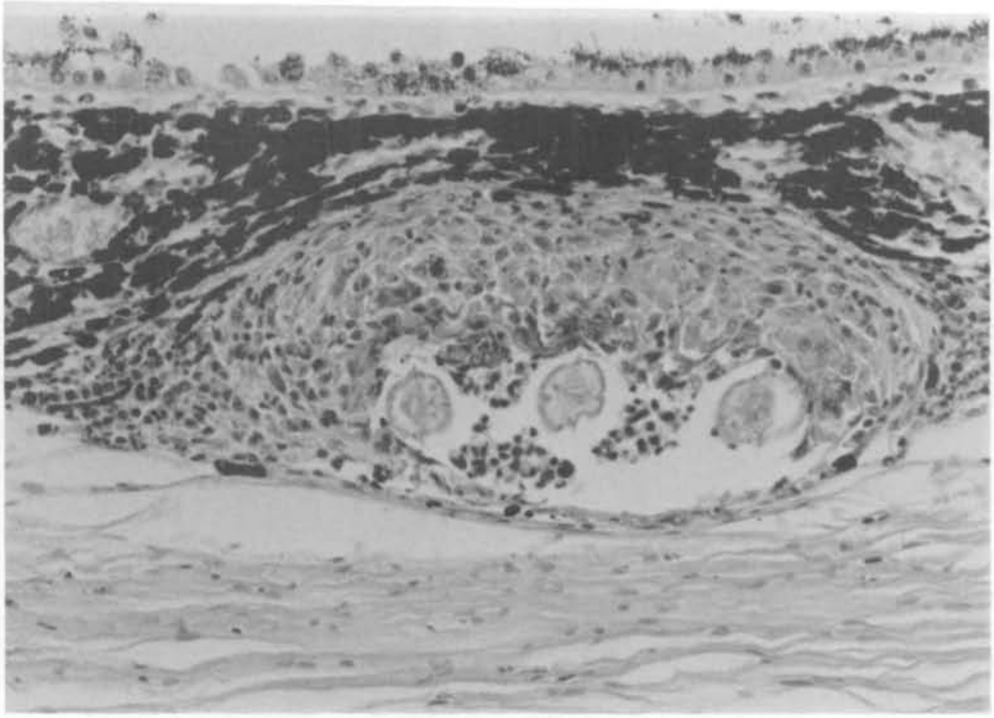

Fig. 5. Histologic section of cynomolgus monkey globe, showing circumscribed *B. procyonis* larval granuloma in the choroid (hematoxylin-eosin, ×250).

ica.[1-3] The present study, other research in subhuman primates[1] and the recent diagnosis of two human fatalities[4,*] indicate that *B. procyonis* should be considered as a cause of larva migrans in human beings. Epidemiologic studies have indicated the ubiquity of this parasite in raccoons, their marked contaminative ability, and the likelihood of contact and infection of human beings with the eggs of *B. procyonis*.[2-5] In our experience, the medical community is generally unaware of this parasite or its marked pathogenic capabilities. The present study indicates that *B. procyonis* has marked ability to produce ocular larva migrans in subhuman primates, and presumably therefore in humans, following oral infection. The larvae of *B. procyonis* are aggressive, reaching the eyes by at least 7 days and causing traumatic, inflammatory, and degenerative lesions therein, primarily affecting the retina and choroid. In related studies, OLM was experimentally produced in mice, hamsters, gray squirrels, and woodchucks following oral infection with *B. procyonis*. Motile larvae were recovered from the eyes of mice beginning at 3 DAI.[7]

There is growing evidence that *Baylisascaris* has produced ocular disease in human beings and gone undiagnosed or misdiagnosed as other conditions or parasites. In 1978, Raymond et al[8] described two cases of clinical OLM in which larvae caused tracks upon the pigment epithelium, and unilateral macular degeneration in one patient; the larvae were destroyed using photocoagulation. Based on their size, the larvae were identified as "filarial-like nematodes." In retrospect, however, these well-reported cases probably represent the first documented cases of human ocular baylisascariasis for the following reasons: (1) the larvae measured 1.6 and 2.0 mm long,[8] which match the usual size of *Baylisascaris* larvae recovered from clinically affected animals and humans[1,3,9,*]; (2) the larva from one patient measured 60 μm in greatest diameter (LA Raymond, personal communication, 1983), which is the average maximum diameter of *Baylisascaris* larvae from clinically affected animals and humans[1-4,9,*]; (3) one of the patients had acquired a *pet raccoon* 6 weeks prior to the onset of ocular symptoms[8]; (4) a likelihood of exposure to *B. procyonis,* especially from pet raccoons, has been established[1-5]; (5) the ocular lesions reported[8] closely resemble those seen in the present study; and (6) except for rare cases such as one from Egypt,[10] filarial nematodes have not been seen or recovered from the retina or choroid but instead are typically found in the anterior chamber and vitreous.[11-13] Lesions similar to those reported[8] have not been associated with ocular filarial worm infections,[10-13] and filarial worms recovered from the eye are much larger, on the order of 10–32 mm in length.[11-13]

In related studies, Gass et al have described an ocular syndrome in humans, which they term "DUSN" or "diffuse unilateral subacute neuroretinitis,"[14-16] characterized early by visual loss, recurrent crops of gray-white retinal lesions that were tracklike in some patients, hyalitis, papillitis, and in several patients, sheathing of retinal vessels.[14–16] Recently, Gass and Braunstein presented evidence that DUSN is caused by at least two different unidentified nematodes, neither of which is *Toxocara canis.*[16] In 12 patients primarily from the southeastern United States, the intraocular nematode measured 400–1,000 μm in length, and in six patients from the upper midwest, which included the two patients of Raymond et al,[8] it measured 1,500–2,000 μm in length. The greatest diameter of the worms was stated as approximately 1/20 their length, or about 20–50 μm and 75–100 μm, respectively.[16]

Both size measurements, but especially those of the larger nematode, match the size range of *Baylisascaris* larvae.[1–4,6,9,*] Many of the lesions of early DUSN[14–16] resemble those seen in the present study. Based on the two size ranges, the authors suggest that DUSN is caused by two different nematodes,[16] which may, indeed, be the case. However, they also simply may represent two ranges on a growth continuum for a single genus or species of parasite, reflecting different ages of larvae, combined with the approximations involved in measuring intraocular nematodes.[6] Again, *Baylisascaris* is a likely candidate. Unlike *Toxocara,* which do not exceed about 400 μm in length, *Baylisascaris* larvae grow considerably following infection, from about 300 μm long at hatching to 1,500–2,000 μm long, the size usually recovered from clinically affected animals and humans.[1,3,9,*] They are 400 μm and larger in about 3–6 days[9] and after that could be represented anywhere on both size ranges given by the authors. As outlined previously, they also could be present in the eye at this time. The case of Raymond et al[8] (case 7[16]) further supports this argument. Instead of measuring about 100 μm in greatest diameter (based on the 1/20 length calculation[16]), the larva actually measured 60 μm in greatest diameter, which places its diameter midway between the authors' two groups.[16] Additionally, 16 of the 18 DUSN patients with larvae[16] are from areas where raccoons are common and where cases of *B. procyonis*-associated disease in animals and humans have been reported.[2–4,6,9,*] In our opinion *Baylisascaris,* especially *B. procyonis* from raccoons, is a probable etiology of many of these cases, and the exposure of these patients to raccoons or skunks should be investigated.[6] As described above, such a relationship has been established for one of these cases (number 7).[8]

The main differences between our results and those of Raymond et al[8] and Gass et al[14–16] are the degree of ocular invasion and resultant damage, and the development of CNS disease in our monkeys. This is explained easily as a dose-related phenomenon. It is well established that a certain percentage of *Baylisascaris* and *Toxocara* larvae enter the brain following oral infection, that fewer larvae enter the eyes, and that both situations are dosage dependent.[7,17–21] In the present study, the monkeys that received the higher dosages, and those that suffered from greater CNS migration, also had more severe OLM. The dosages used in this study are well within the realm of natural infection, based on localized contaminative ability of raccoons,[3] the apparent greater level of infection that occurred in two children,[4,*] and the high levels that are seen in children with VLM due to *Toxocara.*[22] In similar studies on *Toxocara* in primates and guinea pigs, oral dosages of 45,000[23] and 100,000–400,000[21] eggs and intravitreal dosages of 50–100[24] and 500[25] larvae were used.

The usual occurrence under natural conditions, however, is that of low level exposure and infection, with chance migration to the eye, brain, etc., without concomitant clinical VLM or CNS disease.[22] This is supported by recent serologic evidence indicating that 2.8% of apparently healthy persons have been infected with *Toxocara.*[22] The epidemiology of *Baylisascaris* infection seems to parallel that of *Toxocara.*[3] Clinical ocular disease usually results from the migration of a single larva into the eye, causing a progression of lesions from acute to chronic, depending on the duration and extent of migration therein.[7,8,14–16,18,19,22] Due to the development of CNS disease by our monkeys, our results were limited to acute and subacute ocular lesions. However, the clinical lesions were similar to those reported by Raymond et al[8] and in early cases of DUSN.[14–16] Histologically, many of the lesions resembled those seen in acute *Toxocara* OLM[19,24,25] but were more severe probably due to the larger larvae, which caused greater damage. The number of larvae in the eyes was relatively low as compared with other studies,[24,25] more closely approximating what is seen in natural cases[8,14–16] and oral experimental infections.[7,18–22]

We have shown conclusively that *B. procyonis* will produce OLM in primates following oral infection. It is very evident that even a single *Baylisascaris* larva in the eye can produce considerable damage, due to their large size and aggressive behavior. Based on the evidence now at hand, *Baylisascaris,* especially *B. procyonis* from raccoons, should be considered in the differential diagnosis of ocular disease in human beings, and suspected cases should be investigated thoroughly for this possible etiology.

Key words: ocular larva migrans, *Baylisascaris procyonis,* raccoon ascarids, ocular nematodes, parasitic disease

Acknowledgments

The authors wish to thank Drs. Lawrence A. Raymond, Peter M. Schantz, Lawrence T. Glickman, and T. F. Schlaegel, Jr. for their helpful discussion, and Patty Bonney and Cathy Christmas for technical assistance.

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