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Authors
Buyukmihci, NC
Rubin, LF
DePaoli, A

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Protothecosis with Ocular Involvement in a Dog

N. Buyukmihci, VMD; L. F. Rubin, VMD; A. DePaoli, DVM, PhD

SUMMARY

An 8½-year-old Collie dog was referred for evaluation of chronic diarrhea as well as sudden blindness and leukokoria of the right eye. An organism morphologically similar to *Prototheca* sp was recovered from the subretinal fluid and was found at necropsy in the eyes, gastrointestinal tract, lungs, lymph nodes, kidneys, heart, abdominal fat, and omentum.

GRANULOMATOUS chorioretinitis, although relatively uncommon in dogs, is usually caused by fungal,[7,11,13,15] protozoan[1] and bacterial agents,[5] or nematodes.[12] Algal agents also may cause granulomatous inflammation and recently have been recognized with increasing frequency as a cause of both systemic and localized external infections.[2–4,6,8,10,14,16,17] This report documents a case of protothecosis with ocular involvement in a dog.

Case Presentation

On June 1, 1974, a 30.5-kg 8½-year-old spayed female Collie dog in good general condition was referred for evaluation of sudden blindness and leukokoria (Fig 1) that occurred in the right eye about 10 days earlier. For 4 weeks the dog had had intermittent, hemorrhagic diarrhea that had not responded to antibacterial treatment. The dog had been maintained entirely in New Jersey. The right pupil was dilated and did not respond to stimulation with light. On digital palpation the right eye felt softer than the left eye, suggesting lower intraocular pressure. Conjunctival vessels in the right eye were slightly congested, but the anterior ocular segment appeared otherwise normal. The vitreous body of the right eye was cloudy, permitting only faint visualization of the retinal vessels.

In the left eye, pupillary responses and the anterior segment were normal. There was a grayish white, slightly raised lesion about 1 disk diameter wide in the tapetal zone, approximately 2 disk diameters lateral to the disk. Several disk diameters ventral to the disk, in the nontapetal area, were pale white, somewhat fluffy zones. One of these had fresh hemorrhage in the center. General physical examination did not reveal any other obvious abnormalities. The preliminary diagnosis

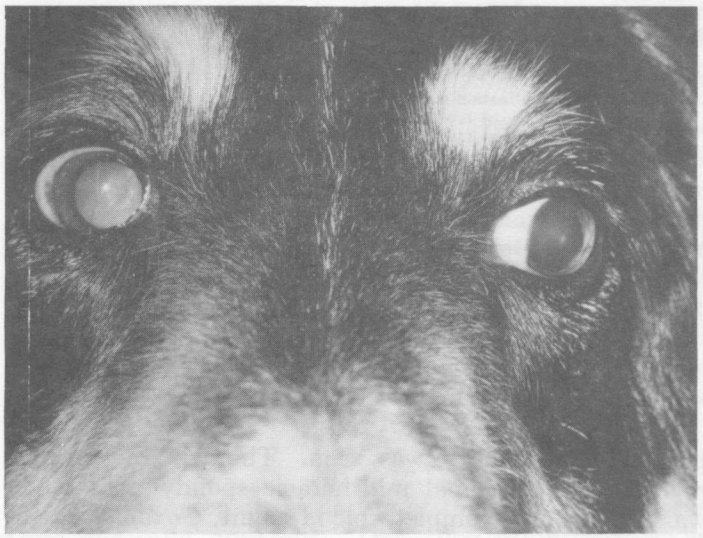

Fig 1—Leukokoria due to vitreous clouding in the right eye of an 8½-year-old Collie.

was bilateral chorioretinitis of unknown cause. The dog was treated orally with 400 mg hetacillin 3 times a day and 1.0 mg dexamethasone twice a day.

The owner reported no improvement in 4 days and the dog was hospitalized. When reexamined, the right eye was unchanged but the lesions in the left eye had enlarged and had increased in number. Also, the left eye had developed areas of bullous retinal separation (Fig 2). Except for tonsillar enlargement, no other

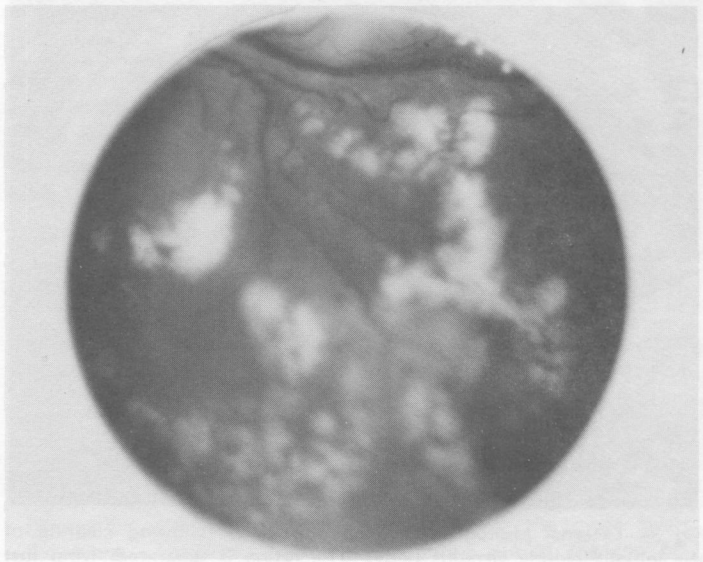

Fig 2—Nontapetal fundus of the left eye, showing many white granulomatous masses within the retina. In the lower left quadrant of the figure, the retina is elevated.

From the Department of Clinical Studies, University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA 19174 (Buyukmihci, Rubin) and the Armed Forces Institute of Pathology, Department of Veterinary Pathology, Washington, DC 20305 (DePaoli). Dr. Buyukmihci is assistant instructor of ophthalmology, Dr. Rubin is professor of ophthalmology, and Dr. DePaoli is chief, division of veterinary pathology. Dr. Buyukmihci's present address is Yale University, School of Medicine, Department of Ophthalmology and Visual Science, 333 Cedar St., New Haven, CT 06510.

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TABLE 1—Clinical Laboratory Results

COMPLETE BLOOD COUNT		
PCV	51%	
Hemoglobin	16.8 g/100 ml	
Red blood cells	6,954,000/cmm	
White blood cells	9,800/cmm	
Differential		
Segmented	80%	
Nonsegmented	1%	
Lymphocytes	11%	
Monocytes	3%	
Eosinophils	5%	
Blood urea nitrogen	26 mg/100 ml	
Lipase	1.5 Sigma-Tietz units	
Amylase	937 Somogyi units	
Cerebrospinal fluid		
Cells	None	
Protein	21.5 mg/100 ml	
TOXOPLASMA TITERS		
Indirect hemagglutination titer		
June 5—negative at 1:64 dilution		
June 19—negative at 1:64 dilution		
Direct hemagglutination titer		
	Untreated	2-Mercaptoethanol treated
June 10	32	4
June 19	32	16
SERUM PROTEIN ELECTROPHORESIS		
Albumin—26.00%		
Globulin		
α_1— 7.00%		
α_2—12.66%		
β_1—13.66%		
β_2—22.33%		
γ —18.33%		

physical abnormality was seen. Thoracic radiographs revealed only minimal midthoracic spondylosis. Blood was drawn for a complete blood count, *Toxoplasma* indirect hemagglutination test, and lipase, amylase, and urea nitrogen determinations. All results were within normal limits (Table 1).

In the afternoon of the 1st day of hospitalization, the vitreous body had cleared in the right eye. A funnel-shaped retinal separation in this eye contained areas of hemorrhage and several small white masses, believed to be granulomas (Fig 3).

The next day (June 6), a complete neurologic examination failed to reveal any abnormalities. Examination of cerebrospinal fluid yielded negative results (Table 1). Fluid from the subneurosensory retinal space was taken and submitted for cytologic examinaton and bacterial and fungal culture. Results of bacterial culture were negative, but a yeastlike organism was recovered after several weeks' incubation. India ink applied to the subneurosensory retinal fluid did not demonstrate any fungal elements; however, Wright's stain revealed numerous ovoid organisms characterized by thin cell walls and large granular nuclei (Fig 4). The cells were approximately 7 to 18 μ long and 5 to 10 μ wide. Some of these organisms were multinucleated and some were phagocytized by macrophages. The rest of the smear consisted of proteinaceous material and numerous inflammatory cells.

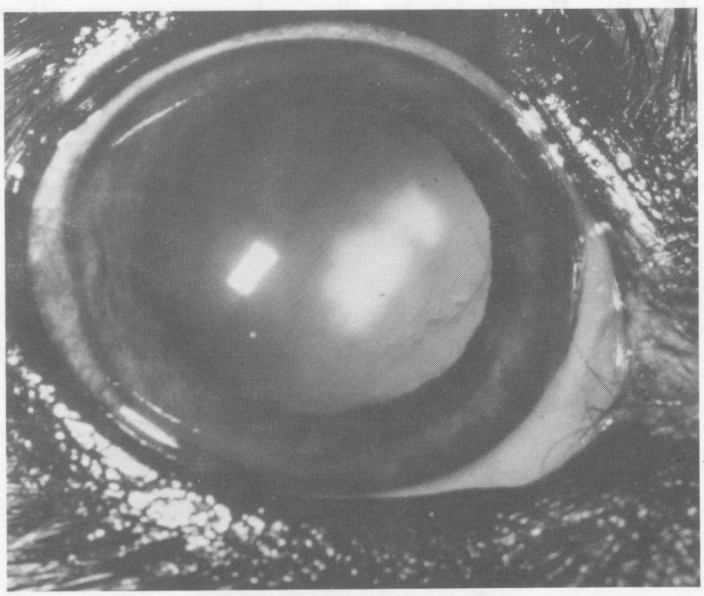

Fig 3—External photograph of the right eye, following clearing of the initially cloudy vitreous body. The retina is separated, lying just posterior to the lens. A large white granuloma lies within the retina.

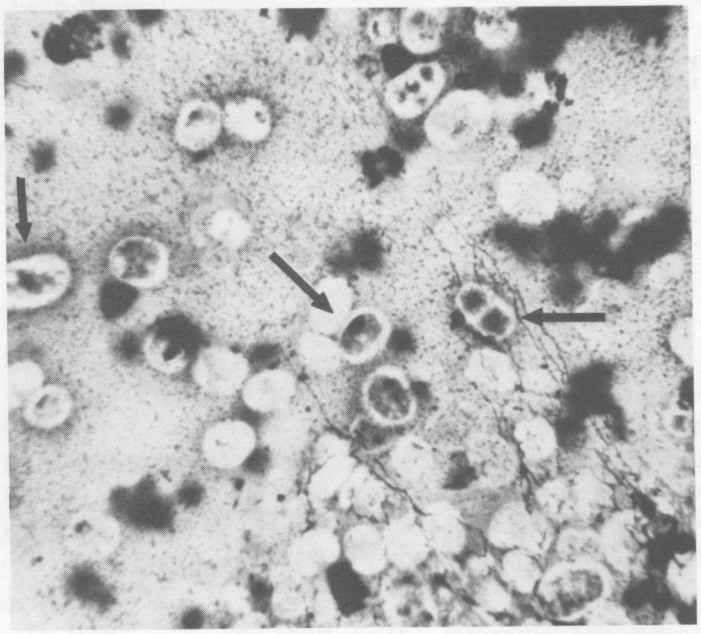

Fig 4—Smear of subretinal fluid obtained from right eye. Several *Prototheca* organisms (arrows) are among the inflammatory cells. Wright's stain; × 360.

Four days later (June 10), a fecal sample was examined but evidence of parasitism (including trophozoites) was not found. Another subneurosensory retinal fluid sample was taken and 2 white mice were each injected intraperitoneally with 0.1 ml of this fluid. Examination of the remaining fluid revealed myriads of the previously described organisms. Serum was submitted for protein electrophoresis and direct *Toxoplasma* agglutination testing (Table 1). The decreased albumin and increased α and β globulins were consistent with a generalized infectious process. The right eye had remained unchanged during this time but the left eye had become progressively worse in that the granulomatous lesions had proliferated and enlarged and the retinal separations had become more extensive. Results of skin tests for histoplasmosis, blastomycosis, coccidioidomycosis, and tuberculosis were negative.

Ten days after admission (June 14), the left eye was enucleated and submitted for histologic examination. Diarrhea, usually hemorrhagic, continued during the 14 days of hospitalization but the general condition of the dog remained good.

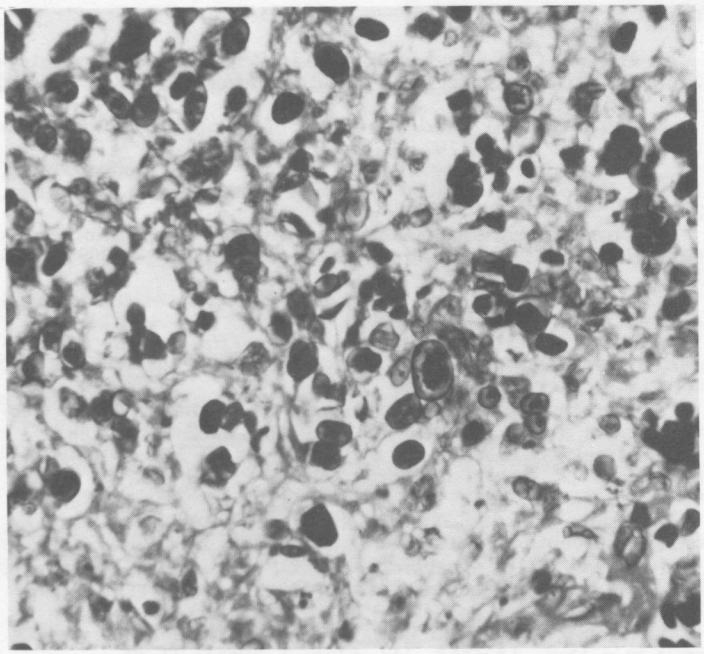

Fig 5—Histologic section from the mesenteric lymph node, demonstrating cell walls of many protothecal organisms. Alcian Blue-PAS-H&E stain; × 260.

On June 19, the dog was euthanatized and necropsied. Serum samples were submitted for a 2nd direct and indirect *Toxoplasma* hemagglutination testing (Table 1).

At necropsy, disseminated small white nodules were found in the serosal surface and wall of the intestines and peritoneal cavity (abdominal fat, omentum) as well as in the myocardium, kidneys, liver, and spleen. Similar small white nodules disrupted the normal architecture of the mesenteric lymph nodes. Several adult *Dirofilaria immitis* were found in the right ventricle and the lumen of the pulmonary artery.

Histologically, the nodules were granulomas containing areas of necrosis and numerous organisms. The organisms were readily observed in hematoxylin and eosin stain and stained deeply with the periodic acid-Schiff reaction. They were round to ovoid, ranging from 5 to 15 μ in diameter, with a prominent nucleus and nucleolus and a hyaline cell wall or theca that was partially birefringent. Many of the larger organisms contained multiple (2–6) daughter cells. These morphologic and reproductive features of the organism are compatible with those reported for *Prototheca*.[14]

The organisms were not limited to sites of gross lesions but were found also in adjoining tissues, where they elicited necrosis and a mild mixed inflammatory cell response (Fig 5). In some areas, anuclear forms of the organism characterized only by cell walls were found. Phagocytosis of organisms by macrophages was a prominent feature, particularly in lymph nodes. In the large intestine, in addition to the grossly visible lesions affecting the serosa and wall, multiple small erosions were in the mucosa. Histologically, these were infiltrated with numerous organisms previously described and with relatively few inflammatory cells. Both eyes had multifocal necrotizing granulomatous chorioretinitis, with large areas of retinal separation and some areas of hemorrhage (Fig 6). Numerous organisms were in the affected portions of the retina as well as in the subneurosensory retinal fluid.

Results of Mouse Inoculation

Four days after intraperitoneal inoculation of mice, 1 of the mice was injected intraperitoneally with 1 ml of sterile saline solution. After several seconds of abdominal massage, 0.2 ml of fluid was removed from the abdomen and submitted for cytologic examination. Several organisms similar to those found in the dog were found in the aspirate, which was otherwise relatively acellular. Most of these organisms were within macrophages.

One of the 2 original mice was killed 31 days after inoculation. Its brain and spleen were ground in sterile saline solution, and 0.3 ml of this suspension was injected intraperitoneally into a 3rd mouse. This 3rd mouse was killed 11 days after inoculation and its kidneys, eyes, brain, spleen, and mesenteric lymph nodes were examined histologically. Neither organisms nor lesions were found.

The 2nd of the 2 original mice was maintained for 7 months' observation, but no abnormality was detected.

Discussion

The granulomatous nature of the intraocular disease was clinically apparent. This type of intraocular inflammatory reaction has been reported with coccidioidomycosis,[13], cryptococcosis,[11] blastomycosis,[15] geotrichosis,[7] toxoplasmosis,[1] tuberculosis,[5] and toxocariasis,[12] as well as protothecosis.[2,10,16] It is apparent that, clinically, our index of suspicion was not attuned to the latter possibility, at least not to the same degree as to the former conditions. The dog failed to respond to skin tests for blastomycosis, tuberculosis, or coccidioidomycosis, although we recognized that the latter disease is not indigenous to the area in which the dog was reared. Results of skin tests for histoplasmosis were also negative. We could find no published report, however, in which

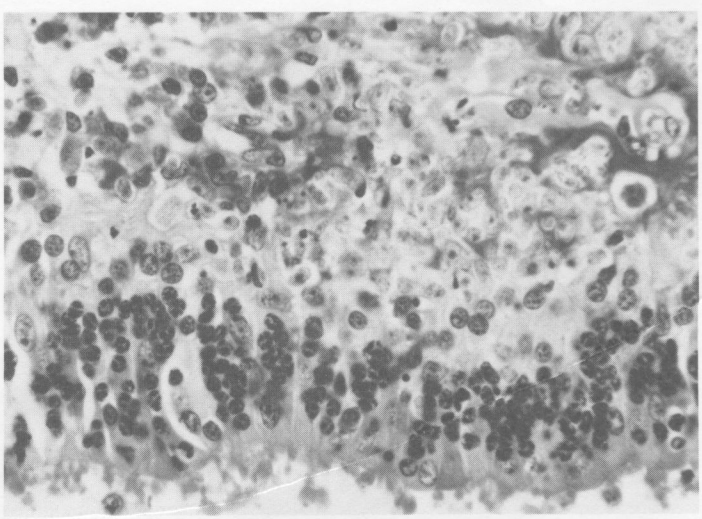

Fig 6—Section from retina of left eye, showing granulomatous inflammation and hemorrhage, principally within the inner layers of the retina. The inner layers of the retina are at the top of the photomicrograph. H&E stain; × 160.

Histoplasma capsulatum has been clearly demonstrated as a cause of uveitis in the dog. After examination of the subretinal fluid, it appeared that cryptococcosis was also an unlikely diagnosis. Although a unicellular organism was identified in smears, it did not grow readily in Sabouraud's agar. This was surprising inasmuch as *Prototheca* are known to grow well in this medium.[14] In retrospect, the yeastlike organism that grew after prolonged incubation may well have been *Prototheca*, because the organism is yeastlike on Sabouraud's agar. However, the diagnosis of protothecosis in this case must rest on the morphologic features of the organism in tissue, features that were compatible with if not identical to those reported for *Prototheca* sp.[14]

Prototheca, a colorless algae of the family Chlorellaceae, has a wide distribution, occurring in Africa, Asia, Europe, and North America.[14] In the United States, animal infections have occurred in California, the East Coast, the West, and in the Midwest.[2,3,8,10,16,17] The epidemiology of the disease is not known, but the organism is generally considered to be an opportunist. Whether the significant rise in direct hemagglutination titer to *Toxoplasma* represented an activation of toxoplasmosis by the protothecal infection or whether the protothecal infection triggered a nonspecific rise in immunoglobulins is not known.[9] *Toxoplasma* organisms were not detected in the tissues examined.

From the cases of protothecosis reported in the dog,[2,10,16,17] as well as from our case, it appears that the combination of chronic diarrhea, often hemorrhagic, and ocular granulomatous inflammation should alert the clinician to suspect protothecal infection.

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