

Heterobilharzia americana infection in a dog

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- ▶ Heterobilharziasis should be suspected in dogs with chronic diarrhea and weight loss that frequent areas where raccoons and lymnaeid snails are prevalent.
- ▶ Heterobilharziasis may be treated successfully with high doses of praziquantel.
- ▶ Use of saline solution sedimentation is essential for the detection of ova and miracidia in feces.
- ▶ Diagnosis of suspected infections in which ova are not evident in feces should be attempted by use of an ELISA for circulating fluke antigens in sera.

A 7-year-old castrated male Golden Retriever cross was evaluated because of chronic diarrhea and weight loss (from 40.9 kg [90 lb] to 27.7 kg [61 lb]). The diarrhea had begun approximately 1 month previously and was characterized as voluminous, mucoid, and bloody and was associated with tenesmus, urgency, and flatulence. A 2-week treatment regimen of enrofloxacin^a and sucralfate dihydrochloride^b did not result in resolution of clinical signs, and the dog was referred.

The diet consisted of a commercially available dog food supplemented with raw meat. Historical dietary indiscretion and pica were reported. The dog had access to a large unfenced area typical of coastal plain topography that contained ponds and drainage ditches bordering a suburban residential development. The dog was found at 6 months of age and had a heavy intestinal worm burden; heartworm test results were positive. The dog received treatment for the intestinal parasites and melarsomine^c for the heartworm infection. Treatment went well, and the dog was maintained on milbemycin^d and fipronil^e year-round. The referring veterinarian had performed fecal flotation and direct smear examinations for parasite ova; results were negative. When the dog was initially evaluated by the referring veterinarian, results of a serum biochemical profile revealed increased total (9.44 g/dl; reference range, 6.0 to 8.0 g/dl) and globulin (6.45 g/dl; reference range, 2.5 to 4.3 g/dl) protein concentra-

tions, and results of a CBC revealed eosinophilia (1.9×10^3 cells/ μ l; reference range, 0.1 to 0.75×10^3 cells/ μ l).

On initial examination at the referral hospital, the dog was quiet and thin with a poor coat and fecal staining of the perineum with a black tarry stool. The popliteal, submandibular, prescapular, and inguinal lymph nodes were large. On abdominal palpation, organomegaly and gas-filled bowels were detected. On transrectal examination, no obvious abnormalities were detected.

A CBC, serum biochemical analysis, tick-borne disease serologic analysis, urinalysis, and coagulation profile were performed. Results of the CBC revealed mild normocytic normochromic nonregenerative anemia, with a PCV of 30% and a mild increase in band neutrophils (900 cells/ μ L; range, 0 to 300 cells/ μ L). Results of the biochemical analysis revealed hyperglobulinemia (7.2 g/dl; range, 2.5 to 4.3 g/dl) and increased alkaline phosphatase (269 U/L; range, 20 to 155 U/L), aspartate aminotransferase (55 U/L; range, 1 to 37 U/L), and alanine aminotransferase (85 U/L; range, 3 to 50 U/L) activities. Serologic results were negative for immunoglobulins against *Ehrlichia canis* and *Borrelia burgdorferi* but were positive for *Rickettsia rickettsii*, with a titer of 1:1,024. The urine was concentrated (specific gravity, 1.030), and 3+ proteinuria was the only abnormality detected. Prothrombin and partial thromboplastin times were within reference ranges.

Thoracic radiography revealed a moderate interstitial pattern and mildly large sternal lymph nodes. Lymph node aspirates of the popliteal and the prescapular lymph nodes were performed and revealed reactive lymphocyte hyperplasia. During abdominal ultrasonography, the splenic capsule appeared moderately irregular, evidence of peritoneal effusion was seen, and the walls of the small intestinal loops were thickened (6 mm) and hypoechoic. Because of the peritoneal effusion, abdominal exploratory was performed.

A standard ventral midline laparotomy inspection of the abdomen and its contents revealed multiple abnormalities including a diffusely thickened irregular firm to sclerotic pancreas, generalized hepatomegaly, generalized mesenteric lymph node enlargement, and multifocal to diffuse miliary transmural nodules in the duodenum, jejunum, ileum, and colon. Biopsy samples of the liver, pancreas, stomach, duodenum, jejunum, lymph node, and colon were obtained. Histologically, granulomas and numerous schistosome ova were scattered throughout the liver, pancreas, small intestine, colon, and a mesenteric lymph node. The stomach was least affected with only a focal microgranuloma in the submucosa and no ova seen. Ova were round to ovoid, approximately 100- μ m thick with a yellowish to clear often collapsed hyalinized wall, and were either empty or contained a developing miracidium. The inflammatory cell infiltrate was similar in all

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involved organs and consisted of single to coalescing granulomas composed of epithelioid macrophages and multinucleate foreign-body giant cells, usually surrounding parasitic ova, admixed with varying amounts of fibrosis, eosinophils, neutrophils, lymphocytes, and plasma cells. Ova and the granulomatous inflammation were distributed transmurally in the small intestine, portally in the liver, and randomly throughout 50% of the pancreatic specimen. Minimal inflammation was evident in the colon and a mesenteric lymph node (Fig 1).

A sample of fluid feces was examined by direct smear, fecal flotation with sodium nitrate, and fecal sedimentation in saline (0.9% NaCl) solution. Results of the fecal flotation were negative; however, the fecal smear and sedimentation revealed large numbers of subspherical ova, each containing a fully developed miracidium (Fig 2). Another sample of feces was sediment-washed in saline solution several times, with the resulting sediment being immersed in deionized water. After immersion (1 to 2 minutes), motile miracidia were seen and subsequently collected from the water column. Some of the miracidia were collected and placed in vials with naïve laboratory-reared Lymnaeid snails, *Pseudosuccinea columella*. A month later, the exposed snails began releasing oculate (with eyespots) cercariae, characteristic of blood flukes. Morphologic characteristics of the ova, miracidia, and cercariae confirmed identification as *Heterobilharzia americana*.^{1,2}

Praziquantel^l (5 mg/kg [2.27 mg/lb] of body weight, PO) was administered. Sera collected from the dog before, during, and following treatment with praziquantel were analyzed by use of an antigen capture ELISA^g for the presence of schistosome circulating anodic antigen (CAA). This ELISA has been used extensively in the diagnosis of schistosomiasis in humans, particularly in seroepidemiologic studies, and is nearly 100% specific for CAA from trematodes of the genus *Schistosoma* that infect humans and cattle.³ Results of the ELISA revealed a moderate concentration (2,003 pg/ml) of circulating antigen in pretreatment serum samples and a lower concentration (973 pg/ml) 1 week after treatment. At this time, the dog still had diarrhea and was shedding ova, and CAA concentrations were only moderately reduced.

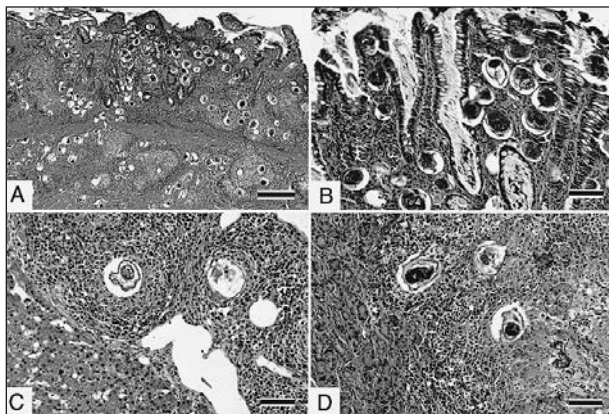


Figure 1—Photomicrographs of specimens of small intestine (A), colon (B), liver (C), and pancreas (D) from a dog infected with *Heterobilharzia americana*. Numerous ova with developing miracidia are evident in each organ. H&E stain; bar = 400 μ m in A; bar = 80 μ m in B–D.

The dosage of praziquantel was deemed too low for clearing blood fluke infections based on reports of schistosomiasis in humans, in which up to 50 mg/kg (22.7 mg/lb) of praziquantel is used.⁴ Therefore, 312 mg of praziquantel was administered subcutaneously, and the oral dosage was increased to 30 mg/kg (13.6 mg/lb). Improvement was not reported until 1 week later when diarrhea stopped and general activity increased; at this time, CAA concentrations were nearly undetectable (51 pg/ml). One month following administration of high doses of praziquantel, no ova were detected on sedimentation, feces were formed, and appetite and activity were normal.

Although serologic results were positive for *Rickettsii*, there were no clinical signs attributable to this disease before or during the time the dog was being evaluated for chronic diarrhea and weight loss or following praziquantel treatment. However, once the dog's appetite returned, he received doxycycline^h (5 mg/kg [2.27 mg/lb], PO) for 21 days because of the high titer.

The morphologic characteristics of the ova and the distribution of lesions in the abdominal viscera were

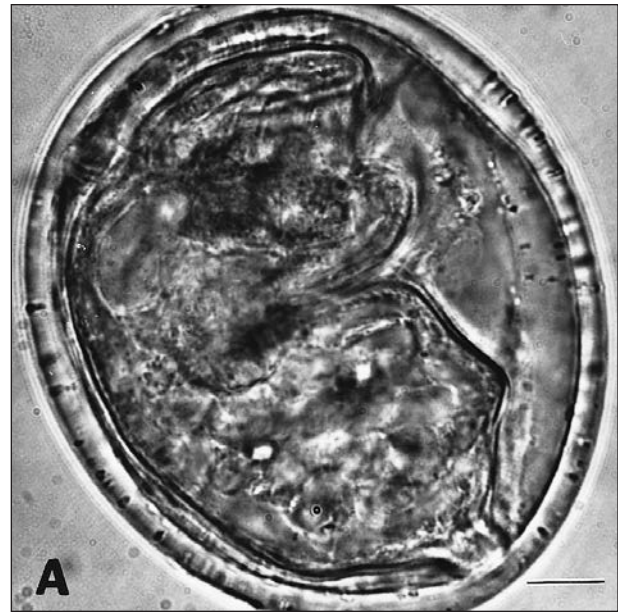


Figure 2—Subspherical ovum of *H americana* (A) containing full-formed miracidium and recently hatched nonoculate miracidium (B). Bar = 10 μ m in A; bar = 20 μ m in B.

typical of those previously reported in dogs and raccoons.⁵⁻⁷ Oviposition usually occurs in the intestinal venules; for those ova that do not make it to the intestinal lumen, many are hematogenously disseminated to distant sites and cause multi-organ failure. The pattern of liver disease seen with heterobilharziasis is likely attributable to the spread of ova from the intestines via portal circulation. Cirrhosis can thereby result in long-standing cases of schistosomiasis.⁵⁻⁷

The habitat where the dog of this report roamed freely was searched for aquatic snails (*P columella* and *Fossaria* spp) known to carry *H americana*. Various invertebrates, mosquito fish, and specimens of *P columella* were found in a drainage ditch near the dog's home. Parasitologic examination of the collected snails revealed that some snails were infected with only the frog lung fluke. This habitat, which provides seclusion and prey, has been observed by the dog's owners to be frequented by raccoons, which is the main reservoir host of *H americana*.

Since Price⁸ first described *H americana*, this digenetic trematode has been reported in 10 naturally infected dogs. All were reported from the southeastern United States: 5 dogs from Texas,⁹⁻¹³ 2 from Florida,^{6,14} and 3 from Louisiana.¹⁵ To the authors' knowledge, the present report is the first of a naturally infected dog from North Carolina.

The details of the life cycle of *H americana* have been described.^{1,16,17} The egg, which is oval without a spine, knob, or hook, contains a fully developed miracidium when passed in the feces of the vertebrate host. Soon after submersion in fresh water, the eggs hatch and release the miracidia, which penetrate the snail hosts of the family Lymnaeidae, specifically *F cubensis* and *P columella*. Mother, then daughter, sporocysts develop in the gastropod host. Fork-tailed cercariae are released from the snail host approximately 25 days after infection. The oculate cercaria infects the vertebrate host by direct penetration of the integument. After approximately 40 days, mature dioecious adult worms are found in the liver, from which they migrate to the mesenteric veins. Although mature after 40 days, ova are not seen in the feces of the host until approximately 68 days after infection.

Raccoons (*Procyon lotor*) are the natural definitive hosts of *H americana* and the most important reservoir host. Raccoons infected with *H americana* have been reported in Texas,^{12,17-19} North Carolina,^{16,19-21} Louisiana,^{15,22,23} Florida,^{7,15,19,21,24} Georgia,²⁵ and Kansas.²⁶ As previously predicted,¹⁹ this parasite has become established in the native raccoon populations of Kansas after southeastern raccoons were translocated to that state by hunting clubs.²⁶ Canids are also highly susceptible hosts. Dogs are the most important domestic definitive hosts; however, other canids such as red wolves, coyotes, and red wolf-coyote hybrids may also be important wildlife reservoirs.²⁷

Two lymnaeid snails, *P columella* and *F cubensis*, are the main snail hosts for *H americana* in North America. It is interesting, however, that natural infections have only been reported in *F cubensis*.¹

The prevalence of *H americana* in raccoons has been reported to range from 1 to 40% in North Carolina,^{19,21}

5 to 71% in Florida,^{19,21,24} 13 to 69% in Louisiana,^{15,22,23} 22% in Texas,¹⁹ and 37% in Kansas.²⁶ All dogs that have been experimentally exposed developed patent infections.^{1,2,28,29} It is reasonable to suggest that in regions of southeastern United States where there are large populations of reservoir hosts (raccoons and feral dogs) and common populations of lymnaeid snail vectors (*P columella* or *F cubensis*), the prevalence of heterobilharziasis in dogs is probably more common than has been reported and has gone undetected because of inappropriate diagnostic testing.

Awareness of appropriate diagnostic testing for *H americana* is especially important, because this parasite may be easily spread to other parts of the United States, as recently reported.²⁶ Because of the low number of reported cases of *H americana* in dogs, more work is needed to determine the feasibility of the CAA assay for use in canine veterinary practices.

As suburban developments encroach on formerly rural areas, heterobilharziasis is likely to become a more important emerging disease in domestic dogs. The accurate diagnosis of *H americana* is facilitated by performing saline sedimentation of feces to observe ova containing a miracidia and by the observation of motile miracidia released from ova exposed to water. Detection of circulating antigen by use of ELISA should be attempted in those instances where history and clinical signs indicate high probability of infection with *H americana*, but ova are not detectable in feces.

^aBaytril, Bayer Corp, Shawnee Mission, Kan.

^bCarafate, Hoechst Marion Roussel, Kansas City, Mo.

^cImmiticide, Rhone Merieux Inc, Athens, Ga.

^dInterceptor, Novartis Animal Health US Inc, Greensboro, NC.

^eFrontline, Merial Ltd, Iselin, NJ.

^fDroncit, Bayer Corp, Shawnee Mission, Kan.

^gThis assay is available on a fee basis by arrangement with Bruce Hammerberg at the College of Veterinary Medicine, North Carolina State University.

^hVibramycin, Pfizer US Pharmaceuticals Group, New York, NY.

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