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Efficacy of a low-dose praziquantel and fenbendazole protocol in the treatment of asymptomatic schistosomiasis in dogs

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Abstract

Background: Established treatment protocols for schistosomiasis (*Heterobilharzia americana*) in dogs are expensive. Anecdotal reports suggest that lower doses of praziquantel, combined with fenbendazole, may eliminate asymptomatic infections.

Objectives: Evaluate the efficacy of a low-dose praziquantel and fenbendazole protocol to manage asymptomatic schistosomiasis in dogs and compare fecal saline sedimentation (FSS) and fecal PCR (FPCR) for therapeutic monitoring.

Animals: Twelve asymptomatic dogs with positive FPCR and FSS results for schistosomiasis.

Methods: Prospective observational study. On day 0, dogs received praziquantel at a median dose of 5 mg/kg PO q8h for 2 days, with fenbendazole at 24 mg/kg PO q24h for 7 days. Fecal PCR and FSS were repeated in all dogs on days 30, 60, and 90.

Results: By day 30, 10 of 12 dogs were negative by FSS, but only 3 of 12 were negative by FPCR. By day 60, all 12 dogs were negative by FSS, and 8 of 12 had become negative by FPCR. By day 90, all 12 dogs remained negative by FSS, but 5 of 12 were positive by FPCR (including 2 that were negative by FPCR on day 60). Three dogs that were positive by FPCR on day 60 were re-treated and subsequently became both FPCR and FSS negative. One FPCR-positive dog developed a mild increase in serum ALP activity, another developed mild hypercalcemia, and a third developed diarrhea.

Conclusions and Clinical Importance: A low-dose praziquantel/fenbendazole protocol may be effective for asymptomatic schistosomiasis in some dogs, but monitoring to ensure treatment success is recommended. Fecal saline sedimentation and FPCR may demonstrate discrepant results, with FPCR being positive more frequently.

KEYWORDS
fecal, monitoring, PCR, saline sedimentation, schistosomiasis, trematode

Abbreviations: ALP, alkaline phosphatase; FDA, Food and Drug Administration; FPCR, fecal PCR for *Heterobilharzia americana*; FSS, fecal saline sedimentation; PCAB, Pharmacy Compounding Accreditation Board.

1 | INTRODUCTION

Heterobilharzia americana is a trematode parasite that causes schistosomiasis in dogs. The parasite is endemic in the Gulf Coast and south Atlantic states, but schistosomiasis also recently has been identified in Kansas, Oklahoma, and Indiana, suggesting an expanding distribution.[1-11] Raccoons are the natural definitive host of *H americana*, but canids are also highly susceptible. The definitive host passes eggs in the feces, which hatch in fresh water, releasing motile miracidia. In turn, miracidia infect the lymnaeid snails that serve as an intermediate host. Cercariae then are released from the intermediate host and penetrate the intact skin of the definitive host, migrating through the lungs and to the liver where they mature to sexually dimorphic adults. Adult schistosomes then migrate to the mesenteric veins, mate, and lay eggs that release proteolytic enzymes that allow them to traverse the intestinal mucosa to the lumen and in doing so induce a pyogranulomatous reaction.[12]

The most commonly reported clinical signs associated with *Heterobilharzia* infection are anorexia, vomiting, diarrhea, weight loss, polyuria, and polydipsia (Graham A, Moshnikova V, Davenport A, et al. *Heterobilharzia americana* infections in dogs: clinical features and outcome in 60 cases (2010-2019). Poster presented at the 2020 ACVIM Forum On Demand (asynchronous)).[10,12] Potential clinicopathologic abnormalities include anemia, eosinophilia, hyperglobulinemia, hypoalbuminemia, hypercalcemia, increased liver enzyme activities, and azotemia (Graham A, Moshnikova V, Davenport A, et al. *Heterobilharzia americana* infections in dogs: clinical features and outcome in 60 cases (2010-2019). Poster presented at the 2020 ACVIM Forum On Demand (asynchronous)).[10,12] Abdominal ultrasound findings consistent with *Heterobilharzia* infection include heterogeneous small intestinal wall layering and pinpoint hyperechoic foci within the small intestine, liver, or mesenteric lymph nodes.[13] Fecal saline sedimentation (FSS) and direct saline smears are utilized for visual detection of *H americana* eggs. An antigen capture ELISA originally designed for detection of *Schistosoma mansoni* in humans has been investigated for the identification of *H americana* antigen in the urine and serum of dogs.[6,12,14] Many PCR assays are available in humans, and a fecal PCR (Schistosomiasis fecal PCR, Gastrointestinal Laboratory at Texas A&M University, College Station, Texas) is commercially available for detection of schistosomiasis in dogs.[14] Diagnosis of schistosomiasis in a dog with clinically evident disease often leads to testing and subsequent identification of infected but asymptomatic animals, such as housemates or other dogs in the local area. Information regarding management of these asymptomatic animals is limited. Standard management recommendations for *H americana* infections typically include variable high doses of praziquantel (7.9-47 mg/kg PO q8-12h for 1-3 days) in conjunction with prolonged fenbendazole treatment (24-50 mg/kg PO q24h for 4-14 days), but these protocols can be cost prohibitive, potentially limiting their use in asymptomatic cases (Graham A, Moshnikova V, Davenport A, et al. *Heterobilharzia americana* infections in dogs: clinical features and outcome in 60 cases (2010-2019). Poster presented at the 2020 ACVIM Forum On Demand (asynchronous)).[10,12] Anecdotal reports suggest that repeated administration of lower doses of praziquantel in conjunction with fenbendazole may eliminate infections early in asymptomatic individuals, before development of overt clinical disease, but to our knowledge no prospective studies have been conducted in this area.

Our study was designed to take advantage of a cluster of asymptomatic *Heterobilharzia*-infected dogs in our local region to address 2 main aims: firstly, to prospectively evaluate the efficacy of a low-dose fenbendazole and compounded praziquantel protocol in the management of asymptomatic schistosomiasis in dogs, and secondly to compare the results of FSS to a fecal PCR (FPCR) available through the Gastrointestinal Laboratory at Texas A&M University for monitoring treatment efficacy. The praziquantel dosage utilized was based on anecdotal use of prophylactic doses of praziquantel (Praziquantel/Praziquantel Combination Products. Plumbs Veterinary Drugs Online) before development of clinical schistosomiasis, and the fenbendazole dosage was based on a previous case report.[15] To our knowledge, no studies have investigated the efficacy of compounded praziquantel suspension in dogs.

2 | MATERIALS AND METHODS

2.1 | Study background

After identification of a symptomatic case of schistosomiasis in a dog at the Mississippi State University College of Veterinary Medicine in early June 2020, it was recommended that dogs living in the same household be evaluated for *H americana* infection. The primary care veterinarian submitted fecal samples from 2 asymptomatic housemates for PCR analysis (Schistosomiasis fecal PCR, Gastrointestinal Laboratory at Texas A&M University, College Station, Texas), both of which were positive for *H americana*. The primary care veterinarian lived near the affected dogs, and subsequently submitted fecal samples for PCR analysis on her own dogs, both of which also were positive for *H americana*. Both dogs were asymptomatic. A statement then was prepared and released by the Mississippi Board of Animal Health alerting veterinarians to the cluster of cases identified in the Mississippi Delta, which led to the identification of more asymptomatic dogs in the region with positive results by *H americana* FPCR. Twenty-five dogs were identified as being *H americana* positive by FPCR analysis in the immediate local area, most of which were also asymptomatic.

2.2 | Study enrollment

From June to July 2020, twelve client-owned dogs managed by their primary care veterinarians were prospectively enrolled in the study. Enrollment criteria were as follows: (a) dog with a positive *H americana* FPCR with or without concurrent positive FSS, (b) no evidence of clinically relevant gastrointestinal, renal, or intestinal disease based on evaluation of history, physical examination findings, and review of a CBC and serum biochemistry profile performed at the time of enrollment, and (c) strict avoidance of suspected infected local water sources in an attempt to minimize re-infection. All owners signed an informed consent form before treatment, which explained that the

proposed therapeutic protocol was not guaranteed efficacious and that, if their asymptomatic dog did not become negative for *H americana* after treatment, or became clinical for schistosomiasis, alternative treatments (ie, higher dosages of praziquantel) might be recommended. The informed consent form also documented that treatment, even at the lower dosages, might be associated with adverse effects on rare occasions. The nature of compounded medications was discussed with pet owners by the primary care veterinarian who prescribed the medication. This study protocol was approved by the IACUC committee of Mississippi State University (protocol # IACUC-20-272).

2.3 | Overview and data collection

At the time of enrollment (day 0), diagnosis was confirmed by evaluation of FPCR results provided by the primary care veterinarian, and an FSS was performed by the investigators. The dogs then were prescribed praziquantel based on recommendations for tapeworm prophylaxis (Praziquantel/Praziquantel Combination Products. Plumbs Veterinary Drugs Online; Table 1) PO q8h for 2 days by their primary care veterinarians, with the praziquantel compounded by the veterinarian's local pharmacy (Clinic Pharmacy, Greenville, Mississippi; see Table 1). The pharmacy was approved by the Pharmacy Compounding Accreditation Board (PCAB) and was registered as a compounding facility with the relevant state board of pharmacy. The praziquantel was formulated utilizing a published recipe (Praziquantel Oral Suspension Formula #4242 Version 4.0, Professional Compounding Centers of America Inc, Houston Texas) that was adjusted from 35 mg/mL to 70 mg/mL because of dog size. United States Pharmacopeia (USP) conventional standards were followed. A Food and Drug Administration (FDA)-approved bulk substance was used and purchased from Professional Compounding Centers of America (PCCA; PCCA, Houston, Texas). Other inert ingredients included silica gel, steviol glycosides, and acesulfame potassium. No flavorings were utilized. An FDA-approved tablet formulation of praziquantel is available for tapeworms in dogs, but was not used in the study. A compounded praziquantel suspension for oral administration was used because of the lack of a commercially available suspension and difficulty in medicating the enrolled dogs using multiple tablets. Concurrently, the dogs were prescribed a low dose of a fenbendazole suspension (Panacur Oral Suspension, Merck Animal Health, USA, De Soto, Kansas; 24 mg/kg PO q24h for 7 days with food), a dosage that was utilized in conjunction with 10 mg/kg PO q8h of praziquantel in a prior case report.[15] A suspension formulation of fenbendazole was utilized to allow for medication of difficult dogs, and to limit variations in the formulations used among subjects. An FDA-approved granule formulation of fenbendazole is available for use in dogs, but was not used in the study. Fecal and serum samples for repeated serum chemistry then were collected on days 30, 60, and 90. If the dog was not negative on FPCR by day 60, evaluation of an additional data point at day 120 was recommended. Fecal saline sedimentation and FPCR techniques are described later.

TABLE 1 Praziquantel oral suspension dosing schedule utilized for asymptomatic schistosomiasis (praziquantel oral suspension formula #4242 version 4.0 adjusted to 70 mg/mL PCCA, Houston, Texas)[a]

Weight of dog	Praziquantel dose (70 mg/mL)	Frequency
<5 lb	0.25 mL	PO q8h for 2 days
6 to 10 lb	0.50 mL	PO q8h for 2 days
11 to 15 lb	0.75 mL	PO q8h for 2 days
16 to 30 lb	1.00 mL	PO q8h for 2 days
31 to 45 lb	1.50 mL	PO q8h for 2 days
46 to 60 lb	2.00 mL	PO q8h for 2 days
>60 lb	2.50 mL	PO q8h for 2 days

2.4 | FSS technique

Feces were collected from a witnessed voided sample. The FSS technique utilized in the study was modified from a previously described technique.[16] Modifications included the use of 1 g of feces instead of 5 g to maintain consistency in tested fecal volume among dogs. A dissection microscope was utilized to evaluate the entire fecal sediment at ×40 magnification.

2.5 | Schistosomiasis FPCR

The FPCR assay utilized was a commercially available conventional PCR assay performed at a single laboratory (Schistosomiasis fecal PCR, Gastrointestinal Laboratory at Texas A&M University, College Station, Texas). The PCR assay utilized specific primers that amplify the variable region of the 18s ribosomal DNA gene of *H americana* (see Supplementary Material 1). Fecal DNA was extracted from 100 to 130 mg of feces using a commercial kit (DNeasy Powersoil Pro Kit, Qiagen, Germantown, Maryland) and the manufacturers recommended protocol. PCR cycling conditions were as follows: 94°C for 5 minutes, 95°C for 14 seconds, 67°C for 30 seconds, 72°C for 45 seconds repeated ×34, and then 72°C for 1 minute. The PCR products then were sequenced in both directions using ABI Terminator Sequencing Mix and were separated using an ABI PRISM 337 DNA Sequencer. During technique development and for the first 2 to 3 years of use, DNA sequences from positive test results were sequenced directly and compared to published PubMed sequences to confirm the identification of *H americana*. During this time, no other schistosoma species were identified (M. Bishop, personal communication). Additionally, during technique development, positive FPCR results were confirmed by FSS and a miracidia hatching technique to confirm assay performance. This approach was used because evaluation of specificity was difficult to perform, because no other schistosome species was available in the United States to prove the PCR was specific only to this schistosome species. Subsequently, fecal samples from animals with *S mansoni* infections that had traveled internationally have been tested and were confirmed to be negative via the PCR assay used in our study (M. Bishop,

personal communication). The laboratory no longer sequences positive results, but cross-reactivity with other agents is considered unlikely based on the analysis of primers against sequences for other organisms that produce similar clinical signs. Every sample was also run, as standard by the laboratory, in conjunction with positive controls (feces spiked with 2 eggs per 100 mg and 20 eggs per 100 mg of feces, with the eggs collected from a dog positive by FSS for *H americana*) and negative controls (healthy dog feces, DNA containing plasmids, and fecal DNA from dogs naturally infected with other parasites common in the United States such as roundworm, hookworm, and tapeworm). A published study has reported the sensitivity of the assay as 1.5 eggs/g of feces, and the assay has been shown to have 100% repeatability (Bishop MA, Suchodolski JS, Steiner JM. Development of a PCR test for the detection of *Heterobilharzia americana* DNA in dog feces. 2008 ACVIM Forum, San Antonio, Texas, 4-7 June 2008). The same study noted positive FPCR results in 2 dogs with naturally occurring schistosomiasis (confirmed by FSS), and a negative control population was studied as part of this study to further assess test specificity (Bishop MA, Suchodolski JS, Steiner JM. Development of a PCR test for the detection of *Heterobilharzia americana* DNA in dog feces. 2008 ACVIM Forum, San Antonio, Texas, 4-7 June 2008).

2.6 | Control group

Ten healthy Walker Hound dogs (5 intact females, 3 spayed females, and 2 intact males) with a median age of 6 years (range, 4-7 years), and considered highly unlikely to have schistosomiasis, were used as a healthy control group to confirm the diagnostic specificity of fecal testing for *H americana*. These dogs were purposely bred research dogs that were raised and maintained in a predominantly indoor research environment with outdoor runs that were not near a water source, and that received monthly deworming treatment (Advantage Multi, Bayer HealthCare LLC, Shawnee, Kansas). All dogs had a freshly voided fecal sample collected from individual kennels, and the feces were submitted for FSS and FPCR at the time of initial enrollment. All 10 healthy control dogs had negative FSS and FPCR at the time of study initiation. No dogs had clinical signs of schistosomiasis.

2.7 | Statistical analysis

Numerical data were evaluated using descriptive statistical analysis. The sensitivity and specificity of each assay for the detection of schistosomiasis was not determined because of lack of histopathology or other readily available reference standard.

3 | RESULTS

3.1 | Asymptomatic schistosomiasis group

The dogs were prescribed a low dose of praziquantel based on body weight ranges as outlined in Table 1. The median dose of praziquantel prescribed was 5.0 mg/kg, the mean dose was also 5.0 mg/kg, with a range from 3.2 to 6.5 mg/kg. At the time of enrollment, all 12 asymptomatic dogs had *H americana* eggs identified in their feces by FSS and, because of the study design, they were also FPCR positive. By day 30 of treatment, 10 of 12 dogs had become negative by FSS, but only 3 of 12 had become negative by FPCR. By day 60, all 12 dogs had become negative by FSS, and 8 of 12 had become FPCR negative. By day 90, all 12 dogs remained negative on FSS, but 5 of 12 were positive on FPCR, 2 of which had been FPCR negative on day 60. By day 120, all 4 of the 4 dogs evaluated were FSS negative and 2 of 4 were FPCR negative (Figure 1 and Supplementary Material 2).

Three of the 4 dogs that were positive by FPCR on day 60 underwent additional treatment prescribed by the primary care veterinarian. One of the 3 dogs was treated using the same-dose protocol used in our study, and the remaining 2 dogs were treated with a higher dosage of praziquantel (Praziquantel Oral Suspension Formula #4242 Version 4.0 adjusted to 70 mg/mL. PCCA, Houston, TX; 10 mg/kg PO q8h for 2 days) in conjunction with the same fenbendazole (Panacur Oral Suspension, Merck Animal Health USA, De Soto, Kansas) dosage and duration used in our study. These 3 dogs subsequently became negative on both FPCR and FSS. Two of the dogs that were positive by FPCR on day 60 also developed clinicopathologic abnormalities on day 60, including mild total hypercalcemia in 1 dog (11.4 mg/dL; reference interval [RI], 8.8-11.2 mg/dL) and a mild increase in alkaline phosphatase (ALP) activity in another dog (176 U/L; RI, 11-140 U/L). The dog with mild hypercalcemia subsequently had a normal total calcium concentration (10.9 mg/dL) on day 90. Unfortunately, ALP activity could not be evaluated on day 90 for the dog with an increased ALP activity on day 60 because of sample condition (4+ lipemia, 1+ hemolysis).

Two dogs became positive by FPCR, after previously having had negative FPCR results, by day 60. One of the dogs initially had failed to be returned for sample collection on day 90 because the dog was clinically well and had prior negative test results on days 30 and 60, but the dog subsequently developed diarrhea, and a sample was submitted on day 110. The dog was positive by FPCR and negative on FSS at that time. Additional treatment was declined, and a day 120 sample was not obtained. The second dog, which became positive by FPCR on day 90 after a previous negative result on day 60, did not undergo re-treatment and remained positive by FPCR at the time of study completion (day 120). This dog had no reported clinical signs of schistosomiasis. Both dogs remained FSS negative.

The remaining dogs had no clinically relevant abnormalities detected on repeated serum biochemistry profiles on days 30, 60, and 90 (all profiles were reviewed by an internal medicine specialist [A. Mackin]). Results were interpreted considering any limitations in sample quality, such as hemolysis or lipemia using guidelines provided by the manufacturer of the biochemistry analyzer (Axcel Liquid Chemistry Analyzer, Alfa Wassermann Diagnostic Technologies LLC, West Caldwell, New Jersey). Results outside of the RI that were considered likely to be a consequence of sample condition alone are noted in Supplementary Material 3.

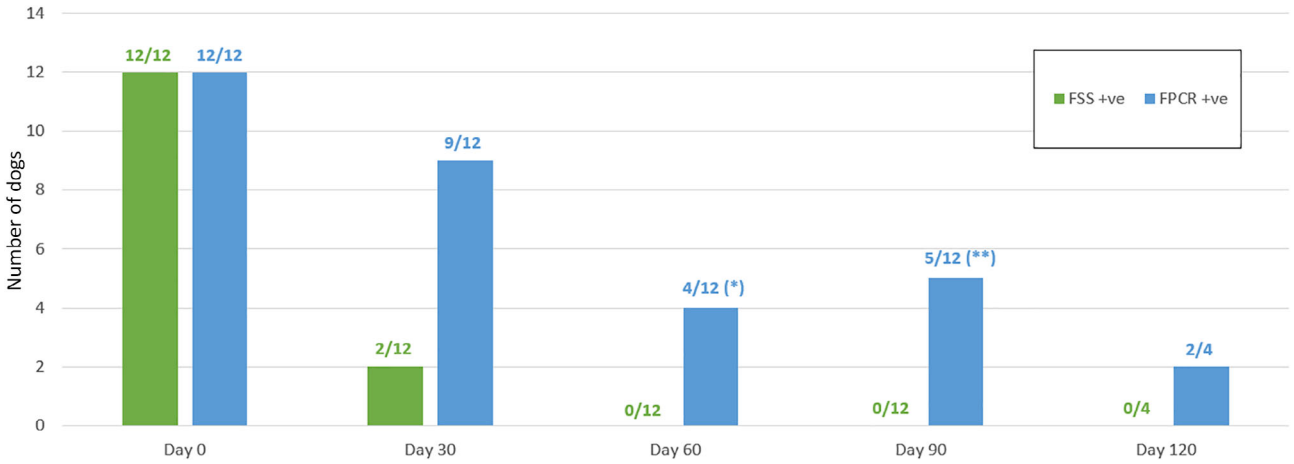

FIGURE 1 Fecal *Heterobilharzia* PCR and saline sedimentation positivity over time in 12 dogs being treated for asymptomatic schistosomiasis. * = 1 previously FPCR negative dog had become FPCR positive, ** = 2 previously FPCR negative dogs had become FPCR positive, +ve = positive test result. FPCR, fecal PCR

4 | DISCUSSION

We evaluated a treatment protocol consisting of repeated low doses of praziquantel (Praziquantel Oral Suspension Formula #4242 Version 4.0, Professional Compounding Centers of America Inc, Houston Texas; median, 5.0 mg/kg PO q8h for 2 days) in conjunction with a low dose of fenbendazole (Panacur Oral Suspension, Merck Animal Health USA, De Soto, Kansas; 24 mg/kg PO q24h for 7 days) for the treatment of asymptomatic *H americana*-infected dogs. The dosages utilized for our study were based on previous anecdotal reports of administration of praziquantel or fenbendazole to eliminate infections before development of clinical disease. The specific praziquantel dosage utilized in our study was based on the prophylactic dosage for tapeworm infection in dogs (Praziquantel/Praziquantel Combination Products. Plumbs Veterinary Drugs Online). Given limited prior evaluation of this treatment protocol, and uncertainty associated with its efficacy, no dogs with symptomatic schistosomiasis or clinicopathologic evidence of systemic effects of schistosomiasis were enrolled in the study.

Currently, no veterinary products are licensed for the treatment of schistosomiasis. Previously recommended treatment protocols therefore are largely based on the extra-label use of praziquantel, in conjunction with fenbendazole. Praziquantel dosages as high as 47 mg/kg have been reported in the veterinary literature. However, this dosage is cost prohibitive for many pet owners. When utilizing the dosage recommended in a recent retrospective study (Graham A, Moshnikova V, Davenport A, et al. *Heterobilharzia americana* infections in dogs: clinical features and outcome in 60 cases (2010-2019). Poster presented at the 2020 ACVIM Forum On Demand (asynchronous); 25 mg/kg PO q8h for 2-3 days)), the cost of praziquantel for a 25 kg dog would be $756 to $1468 for a 2-day course or $1134 to $2202 for a 3-day course (price assessed via online pharmacies on 11.24.20; Chewy Inc, Dania, Florida; 1800 Pet Meds, Delray Beach, Florida). Fenbendazole, in contrast, is comparatively less expensive. The 25 mg/kg dosage of praziquantel that is commonly recommended in clinically affected dogs was based on extrapolation from the veterinary literature on *Paragonimus kellicotti* and, to our knowledge, no comparative studies have been performed evaluating the efficacy of various dosages for schistosomiasis in dogs.[17] In our study, 8 of 12 dogs (66.7%) were both FSS and FPCR negative by day 60 after utilizing a repeated low dose of praziquantel (Table 1) q8h for 2 days at a median dose of 5.0 mg/kg (range, 3.2-6.5 mg/kg) q8h, in conjunction with fenbendazole at 24 mg/kg PO q24h for 7 days. This treatment efficacy compares favorably to the treatment efficacy reported in a recent retrospective study (11/24, 54.2%) of clinically affected dogs utilizing a variety of praziquantel (7.9-47.0 mg/kg PO q24h for 2-3 days) and fenbendazole (24-64 mg/kg PO q24h for 4-14 days) dosages. Direct comparison of these studies is challenging, because differences in disease severity likely impact the results of these studies. This treatment protocol was also well tolerated, with minimal clinically relevant biochemical abnormalities. Some biochemical abnormalities were reported by the clinical pathology laboratory (specifically, increased concentrations of total bilirubin, phosphorus, total protein, and globulin), but were invariably only associated with lipemic or hemolyzed samples, and not in the same dogs when sample condition was normal. Because lipemia, hemoglobinemia, or both are specifically reported by the biochemistry analyzer manufacturer to cause increases in total bilirubin, phosphorus, total protein, and globulin concentrations, this observation strongly suggests that these biochemical abnormalities were caused solely by sample condition (Supplementary Material 3). We cannot however fully exclude the possibility that these changes were associated with infection or treatment.

Despite a reasonable rate of apparent treatment success in our study, 1 dog remained persistently positive on 5 repeated FPCR tests, likely indicating treatment failure, and that other dogs became positive by FPCR after initially becoming negative on FPCR. Potential causes of apparent treatment failure include inadequate drug dose, dog-to-dog variability in drug pharmacokinetics and pharmacodynamics,

failure to clear juvenile parasites, higher initial worm burden, potential re-infection from the environment, or anthelmintic resistance. Praziquantel is effective against trematodes by several proposed mechanisms, including calcium influx, muscle contraction, and surface modifications.[18] Praziquantel is efficiently absorbed after PO administration and is highly protein bound (approximately 80%). In our study, however, dogs were prescribed a compounded formulation of praziquantel, which may have resulted in alterations in composition, bioavailability, and stability among batches, and could have contributed to treatment failure in some dogs. To our knowledge, no studies have been performed comparing the efficacy of compounded praziquantel to the veterinary licensed product. Further study is required to determine the potency, purity, stability, and efficacy of compounded praziquantel suspensions in dogs. Praziquantel is metabolized by cytochrome P450 enzymes and is excreted in the urine.[19] It is therefore also possible that inherited or acquired differences in expression of cytochrome P450 enzymes, or genetic polymorphisms, among individual dogs in our study could have resulted in variable therapeutic efficacy, a hypothesis that to our knowledge has not been tested in dogs.[20] A further potential cause of treatment failure is failure to kill juvenile life stages, thus resulting in the subsequent development of an adult population and failure to clear the infection.[18,21] Additionally, despite attempts by the owners of the dogs in our study to avoid infected water sources, as recommended by the Companion Animal Parasite Council, the potential of environmental re-infection leading to persistently or recurrent positive tests results cannot be excluded. Given widespread utilization of praziquantel in the management of schistosomiasis and other parasites, the development of resistance is a potential concern. However, to date, praziquantel resistance has been reported uncommonly in humans and, to our knowledge, no cases of proven praziquantel resistance have been documented in dogs with schistosomiasis.[22] Praziquantel resistance however has been documented in *Dipylidium caninum* in dogs.[23] Resistance of *D caninum* to praziquantel may be related to high frequency of this parasite and common treatment when compared to schistosomiasis. Fenbendazole is a benzimidazole antiparasitic drug that acts by disruption of intracellular microtubules.[24] Fenbendazole is poorly absorbed after PO administration and has a low incidence of toxicity.[24] Fenbendazole PO suspension was given with food in our study to increase bioavailability.[25] By mechanisms similar to those proposed for praziquantel, fenbendazole may have contributed to treatment failure by anthelmintic resistance, inadequate drug dosage, variability in drug pharmacokinetics and pharmacodynamics, or some combination of these factors. Given that our study had a single treatment arm using a combination of drugs, the treatment effect could have been the result of fenbendazole alone or praziquantel alone. A study with multiple treatment arms, including single-drug treatment groups using fenbendazole and praziquantel, would be needed to determine the contribution of each drug to therapeutic efficacy. A previous study found that a 5 mg/kg PO dose of praziquantel resulted in a maximal concentration of 53.9 ± 9.36 μg/L and an elimination half-life of 7.28 ± 1.22 hours in dogs, but the minimum effective concentration of praziquantel for treatment of schistosomiasis in dogs is unknown and as such the praziquantel dose may have been insufficient.[26] Another study found that, at a 20 mg/kg dosage of fenbendazole PO, the maximal concentration was 0.64 ± 0.11 μg/L and the elimination half-life was 9.33 ± 1.33 hours when fenbendazole was given with food.[27] The same study also found that increasing the dosage of fenbendazole from 20 mg/kg to 100 mg/kg failed to substantially increase the maximal concentration.[27] The minimum effective concentration of fenbendazole for management of schistosomiasis is unknown in dogs.

A further important finding of our study was the diagnostic discrepancy noted between FPCR and FSS in 15 of 52 (28.9%) paired therapeutic monitoring samples. Prior studies have documented that the FPCR assay utilized is highly sensitive for the detection of schistosomiasis and will become positive with, on average, an egg load of 1.5 eggs per gram of feces (Bishop MA, Suchodolski JS, Steiner JM. Development of a PCR test for the detection of *Heterobilharzia americana* DNA in dog feces. 2008 ACVIM Forum, San Antonio, Texas, 4-7 June 2008). The FPCR was negative in all 10 healthy control dogs in our study, suggesting high specificity, as previously reported for experimental real-time PCR assays.[14] Additionally, in all cases of diagnostic discrepancy in our study, the FPCR was positive and FSS was negative. This finding, in combination with the previously reported high diagnostic accuracy of the FPCR assay, suggests that FSS may have lower sensitivity for the detection of *H americana* eggs in feces compared to the detection of DNA by the FPCR assay. Potential supporting evidence for this hypothesis is that no discrepant results were obtained at the time of enrollment, when egg counts were likely to be highest, although unfortunately quantitative fecal egg counts were unavailable to further support this hypothesis. The volume of feces used for each diagnostic test likely impacts sensitivity and should be considered when interpreting our results. Our results likely reflect the nature of these diagnostic tools, and FPCR is dependent on the detection of very small amounts of DNA, which could originate from live or dead organisms, whereas the FSS requires the presence of intact eggs within the fecal sample. However, DNA from disrupted eggs is not anticipated to be present in fecal material for an extended period of time, although a scientific study of this possibility is lacking. All treated dogs in our study became negative on FSS by day 60, and all 12 remained negative by FSS on day 90, as did 4 of 4 dogs tested on day 120. Thus, the high rate of negative FSS results post treatment in our study, despite lingering FPCR positivity in some dogs, most likely indicates a clinically relevant decrease in viable egg-producing parasites without complete clearance of infection. An alternative explanation is death of *Heterobilharzia* parasites with persistence of residual DNA, but persistence of DNA within the feces for >30 days after parasite death is considered unlikely. Although cross-reactivity with other infectious agents has not been directly studied with the FPCR assay utilized here, the DNA primer targets are specific to *H americana* and cross-reactivity is considered unlikely.

Our study also emphasizes the potential for *H americana* to cause asymptomatic infections in dogs. Twenty of 41 (48.8%) dogs evaluated by a single practice in the affected region during the immediate aftermath of a statement release by the Mississippi Board of Animal Health in June 2020 tested positive for schistosomiasis by FPCR, and most of these dogs apparently were in good health. A previous study evaluating the distribution of schistosomiasis in dogs in Texas also

found a substantial number of asymptomatic infections (26/42, 42%) identified incidentally on necropsy or biopsy.[9] It is unknown whether or not some or all of these asymptomatic dogs would go on to develop clinical schistosomiasis. One dog in our study developed diarrhea at the time of a recurrent positive FPCR result, and another 2 dogs developed mild clinicopathologic abnormalities, which subsequently resolved in 1 dog with anthelmintic re-treatment, suggesting that development of clinical signs or clinicopathologic abnormalities is possible in previously asymptomatic dogs. Given the expanding distribution and clinical consequences of this disease, routine screening for *H americana* should be considered in dogs that have been exposed to fresh water in affected areas. Our results also support the use of FPCR over FSS for monitoring treatment in dogs with asymptomatic schistosomiasis, assuming that positive FPCR results post treatment in the absence of clinical signs indicate that live worms still are present.

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CONFLICT OF INTEREST DECLARATION

Dr. Steiner is affiliated with the Gastrointestinal Laboratory at Texas A&M University, which offers a fecal PCR for detection of *Heterobilharzia americana* on a fee-for-service basis. Conflict of interest was managed by ensuring that patient enrollment and shipment of samples was performed at a separate location. The Texas A&M Gastrointestinal Laboratory personnel were blinded to the results of the fecal saline sedimentation test and clinical data on each patient at the time that the PCR was performed.

OFF-LABEL ANTIMICROBIAL DECLARATION

Use of praziquantel and fenbendazole for the treatment of *Heterobilharzia americana* is off-label in the United States.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Approval was granted by the Mississippi State University IACUC. Protocol ID: IACUC-20-272.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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