Pilot Study to Assess the Efficacy of Ivermectin and Fenbendazole for Treating Captive-Born Olive Baboons (*Papio anubis*) Coinfected with *Strongyloides fülleborni* and *Trichuris trichiura*

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In this study, we evaluated the efficacy of combined treatment with ivermectin and fenbendazole (IVM–FBZ) for treating captive olive baboons (*Papio anubis*) infected with *Strongyloides fülleborni* and *Trichuris trichiura*, 2 common nematode parasites of these NHP. Infected baboons were treated for a total of 9 wk with ivermectin (400 µg/kg IM twice weekly) and fenbendazole (50 mg/kg PO once daily for 3 d; 3 rounds of treatment, 21 d apart). Five baboons naturally infected with both *S. fülleborni* and *T. trichiura* (*n* = 4) or *S. fülleborni* alone (*n* = 1) received the combination therapy; an additional baboon infected with both parasites served as a nontreated control. The efficacy of IVM–FBZ was measured as the reduction in fecal egg counts of *S. fülleborni* and *T. trichiura* as determined by quantitative fecal flotation examination after treatment of baboons with IVM–FBZ. All baboons treated with IVM–FBZ stopped shedding *S. fülleborni* and *T. trichiura* eggs by 8 d after treatment and remained negative for at least 161 d. The nontreated control baboon shed *S. fülleborni* and *T. trichiura* eggs throughout the study period. Our results indicate that the IVM–FBZ regimen was efficacious for treating olive baboons infected with *S. fülleborni* and *T. trichiura*.

Abbreviations: EPG, eggs per gram of feces; FEC, fecal egg count; IVM-FBZ, ivermectin-fenbendazole combined therapy

Strongyloides fülleborni and *Trichuris trichiura* are 2 soil-transmitted nematodes of olive baboons (*Papio anubis*). These nematodes are common in both captive^{1,3} and wild^{8,11,13} baboons, and the infections can remain subclinical, cause clinical disease,¹⁰ and confound research projects.⁹ *Strongyloides fülleborni* and *T. trichiura* are zoonotic organisms, and working with infected baboons in contaminated areas poses a risk to human handlers.

S. fülleborni (superfamily Rhabditoidea; threadworms) infects the duodenum and upper jejunum of baboons and other Old World primates.¹² Baboons become infected through skin or oral penetration of third-stage (that is, filariform) larvae. Young baboons can also be infected through the transfer of larvae across the placenta or in colostrum.³ Larvae are carried through the blood to the lungs, where they migrate through alveoli, bronchioles, bronchi, and the trachea; once larvae reach the trachea, they are coughed-up and swallowed. Female S. fülleborni develop and live in the mucosa of the host's small intestines. Eggs are produced from the female worms by parthenogenesis, and diagnosis of infection is most often based on the observation of oval, thin-shelled eggs containing larvae on fecal flotation or of larvae after coproculture of host feces. The prepatent period of S. fülleborni in baboons is unknown but ranges from approximately 1 wk to 1 mo in other species of Strongyloides. Whether S. fülleborni (like S. stercoralis¹⁴) can autoinfect its host is unknown.

Infection of *S. fülleborni* is well-tolerated in immunocompetent hosts but manifests as bronchopneumonia, pulmonary hemorrhage, diarrhea, listlessness, anorexia, emaciation, and reduced growth rate in young or immunocompromised animals.^{3,9}

T. trichiura (superfamily Trichinelloidea; whipworms) infects the mucosa of the large intestines and cecum of primates.^{3,15} Hosts become infected when they ingest an egg containing infective larvae. Eggs of whipworms are long-lived and can last years in contaminated environments. Hosts that are treated for whipworm infection and then reintroduced to contaminated environments often become reinfected, necessitating addition treatments. Larvae are liberated from eggs in the small intestines before they move to the large intestines and cecum. Male and female T. trichiura are found woven intimately in the mucosa, where they reproduce sexually. The prepatent period of T. trichiura in baboons is unknown but is estimated to be 60 to 70 d in humans. Diagnosis is based on the detection of characteristic lemon-shaped eggs with polar plugs in fecal preparations from infected hosts. Clinical signs of trichuriasis in baboons are rare but include diarrhea, lethargy, abdominal pain, weight loss, inappetance, dehydration,^{3,11,15} and intussusception.¹⁰

In general, ivermectin and several other macrocyclic lactones (for example, doramectin, eprinomectin) are used to treat infections of *Strongyloides* spp. in a variety of hosts.² Hosts with infections of *Trichuris* spp. typically are treated with a benzimidazole (for example, fenbendazole, albendazole, mebendazole) or a macrocyclic lactone (for example, ivermectin, moxidectin, milbemycine oxime).² However, treatment and control of dual infection with *Strongyloides* spp. and *Trichuris* spp. is compli-

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cated due to the unique biology of each parasite. Controlling *Trichuris* spp. infections is difficult due to the survivability of eggs in the environment and the organism's long prepatent period.² Treating hosts infected with *Strongyloides* spp. is problematic because the parasite alternates between free-living and parasitic generations, has the capacity for autoinfection, and can exhibit transmammary transmission.¹⁴

In captive baboons in the outdoor convention colony at the University of Oklahoma Health Science Center, the prevalence of S. fülleborni ranges from 39% to 73% and of T. trichiura from 30% to 70%, depending on sampling methodology (for example, amount of feces analyzed, number of samples examined, age of baboons) and diagnostic technique (for example, direct fecal smear, simple fecal flotation, flotation with centrifugation, various flotation media) used. In one study, fenbendazole was more effective than milbemycin oxime against T. trichiura in baboons¹⁶ and that fenbendazole formulated in a primate diet¹⁷ was effective against whipworms. Single-dose ivermectin was ineffective for treating baboons infected with Trichuris spp. at another primate facility.¹ To our knowledge, no published data are available regarding the efficacy of anthelmintics for treating baboons infected with S. fülleborni. Similarly, nothing is known about treating baboons with mixed infections of S. fülleborni and T. trichiura. Here we conducted a pilot study to determine the efficacy of combined treatment with ivermectin and fenbendazole (IVM-FBZ) in captive baboons infected with S. fülleborni and T. trichiura. Our treatment protocol was based on our experience and the difficulty of successfully treating baboons infected with S. fülleborni. All baboons given IVM-FBZ in the current study stopped shedding *S*. *fülleborni* (n = 5) and T. trichiura (n = 4) eggs by 8 d after treatment and remained negative until at least day 161. Our results indicate that the IVM-FBZ regimen was effective for treating baboons infected with S. fülleborni and T. trichiura.

Materials and Methods

Experimental design. Captive-born olive baboons (*Papio anubis*) naturally infected with both *S. fülleborni* and *T. trichiura* (n = 5) or with *S. fülleborni* (n = 1) but without clinical signs of infection were used for the present study. Baboons were individually housed in 3 separate rooms (Table 1). Baboon C2, which was infected with both *S. fülleborni* and *T. trichiura*, served as a nontreated control; the 5 remaining infected baboons were treated with the IVM–FBZ regimen. The efficacy of treatment was determined as the reduction in fecal egg counts of *S. fülleborni* and *T. trichiura* in host feces posttreatment.

Animals. All baboons were housed and cared for according to the standards detailed in the *Guide for the Care and Use of Laboratory Animals.*⁶ Protocols for maintenance and anthelmintic treatment of the baboons were approved by the University of Oklahoma Health Sciences Center IACUC. The animal facilities housing the baboons have maintained full AAALAC accreditation since 1973. Both male and female baboons were used in the present study and ranged from 2 to 21 y in age and 4.7 to 19.7 kg in weight (Table 1). Baboons were housed individually in aluminum cages (floor area, 11 ft²), which were raised above the floor to help prevent reinfection and promote sanitation.

Sample collection and evaluation. Baboons infected with *S. fülleborni* and *T. trichiura* were identified by observation of eggs characteristic of threadworms and whipworms, respectively, during microscopic examination of feces. Quantitative fecal eggs counts (FEC) were accomplished by using double-centrifugation with sugar flotation.¹⁸ Briefly, 5.0 g (weighed to the nearest 0.1 g) of fecal material was thoroughly mixed with 30 mL water,

Table 1. Characteristics of the olive baboons that comprised the study population.

| Baboon | Sex | Age (y) | Weight (kg [lb]) | Parasite(s) |
|--------------|--------|---------|------------------|--------------------------------|
| A1 | Female | 21 | 17.8 (39.2) | S. fuelleborni T. trichiura |
| A2 | Female | 11 | 19.7 (43.3) | S. fuelleborni |
| B1 | Male | 2 | 4.7 (10.3) | S. fuelleborni T. trichiura |
| B2 | Male | 3 | 8.1 (17.82) | S. fuelleborni T. trichiura |
| C1 | Female | 8 | 14.5 (31.9) | S. fuelleborni T. trichiura |
| C2 (control) | Male | 6 | 15.3 (33.7) | S. fuelleborni T. trichiura |

passed through a tea strainer, divided into two 15-mL conical tubes, and centrifuged in a swinging bucket rotor at $176 \times g$ for 10 min. Tubes were decanted without disturbing the sediment and refilled with sugar solution (specific gravity, 1.27). To release the eggs from the plug of debris in the tube bottom, the sediment and sugar solution were mixed thoroughly by using an applicator stick. The tubes again were placed in a swinging bucket rotor, and sugar solution was added dropwise to each tube until a positive meniscus formed. A 22-mm square coverslip was placed over each tube, and the tubes were centrifuged at $176 \times g$ for 10 min. Coverslips were removed and placed on a microscope slide; each 5-g sample yielded 2 coverslips. This quantitative procedure has a sensitivity of approximately 10 eggs per gram of feces (EPG).¹⁸ The EPG of S. fülleborni and T. trichiura were determined by counting the threadworm and whipworm eggs visible under each coverslip at 100× magnification and dividing the sum by the number of grams of feces used. Eggs of S. fülleborni were thin-walled, ellipisoid, measured 40 to 85 µm in length by 20 to 42 µm in width, and contained a larva. Eggs of T. trichiura were lemon-shaped, contained polar plugs on each end, and measured 49 to 65 µm in length by 22 to 30 µm in width. Fecal samples were collected and analyzed on multiple pre- and posttreatment days to calculate the efficacy of the anthelmintic regimen.

Anthelmintic treatment. Infected baboons were treated with fenbendazole (50 mg/kg PO daily for 3 d) for a total of 3 rounds of treatment, with a 3-wk interval between consecutive rounds. Concurrent with fenbendazole therapy, the infected baboons were treated with ivermectin (400 μ g/kg IM) twice weekly for 9 wk. Baboon C2 was the non-treated control and did not receive anthelmintics.

Statistics and determination of anthelmintic efficacy. Mann–Whitney *U* tests were used to compare pretreatment *S. fülleborni* and *T. trichiura* EPG with those of the non-treated control baboon. The efficacy of IVM–FBZ for treating coinfections of *S. fülleborni* and *T. trichiura* in baboons was estimated by calculating the percentage reduction in FEC of *S. fülleborni* and *T. trichiura*:

> [(EPG before treatment – EPG after treatment)/ EPG before treatment] ×100%.⁵

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As recommended by the World Association for the Advancement of Veterinary Parasitology for interpreting FEC reduction tests, descriptive statistics as well as the percentage reduction in FEC of *S. fülleborni* and *T. trichiura* were calculated and reported. In addition, Mann–Whitney *U* tests were used to compare *S. fülleborni* and *T. trichiura* egg-count data before and after treatment. Analyses were performed by using SigmaStat 3.1 statistical software (SyStat Software, Point Richmond, CA).

Results

Before treatment, FEC of infected baboons showed considerable variation in the number of S. fülleborni and T. trichiura eggs shed (Figures 1 and 2). We performed 31 fecal flotations before treatment to establish baseline parasite loads for S. fülleborni. FEC for S. fülleborni ranged from 0.0 to 163.5 eggs per gram of feces; 2 of the 31 samples were negative, corresponding to a false-negative rate of 6.5%. Twelve fecal flotations were performed to establish baseline FEC for T. trichiura from infected baboons. Samples from all T. trichiura infected baboons had eggs detected on each of their pretreatment FEC corresponding to a false negative rate of 0.0%. These tests revealed that whereas baboons A1, B1, B2, C1, and C2 were infected with both S. fülleborni and T. trichiura, baboon A2 was infected with S. fülleborni only (Tables 1 and 2). Comparison of S. fülleborni and T. trichiura egg counts between baboons before treatment and the non-treated control baboon revealed that significantly more (T = 1457.0, P < 0.001) eggs of T. trichiura (median, 26.9) were detected on fecal flotation than were S. fülleborni eggs (median, 3.1).

Treatment of baboons with the IVM–FBZ combination significantly (P < 0.05) reduced FEC for *S. fülleborni* (Table 2) by 8 d and remained negative until at least day 161 d after treatment (Figure 1). The percentage reduction of *S. fülleborni* was 100% for all baboons treated with IVM–FBZ (Table 1). The non-treated control, baboon C2, had a 79% reduction of *S. fülleborni* eggs after the 9-wk period; however, eggs were shed throughout the duration of the study (Figure 1). Similarly, baboons treated with IVM–FBZ demonstrated a significant (P < 0.05) reduction in *T. trichiura* FEC (Table 3), which dropped to 0 by day 8 and remained negative until at least day 161 d after treatment (Figure 2). The percentage reduction of *T. trichiura* eggs in treated baboons was 100% (Table 3). Baboon C2, the non-treated control, had a percentage reduction of –96.9%, indicating an increase in *T. trichiura* FEC after the 9-wk treatment period.

Discussion

FEC reduction tests provide an estimation of the anthelmintic efficacy by comparing parasite loads (that is, no. of eggs per gram of feces) from infected animals before treatment with those after treatment. These tests originally were developed for detecting anthelmintic resistance in domestic animals;^{4,5} however, we here adopted this methodology to determine the efficacy of IVM–FBZ in the treatment of baboons infected with *S. fülleborni* and *T. trichiura*. As demonstrated by the significant reduction in threadworm FEC and the absence of whipworm eggs after treatment, IVM–FBZ were effective for treating infected baboons.

Compared with the information available regarding other species, relatively little is known about treating baboons that are infected with *S. fülleborni* or *T. trichiura*. We are unaware of any published report detailing the treatment of *S. fülleborni* infection in baboons. However, one study compared the efficacy of 400 μ g/kg injectable ivermectin with that of 0.5 mg/kg topical moxidectin for treating rhesus macaques infected with

| Baboon | Sex | Age (y) | Weight (kg [lb]) | Parasite(s) |
|--------------|--------|------------|---------------------|--------------------------------|
| A1 | Female | 21 | 17.8 (39.2) | S. fuelleborni T. trichiura |
| A2 | Female | 11 | 19.7 (43.3) | S. fuelleborni |
| B1 | Male | 2 | 4.7 (10.3) | S. fuelleborni T. trichiura |
| B2 | Male | 3 | 8.1 (17.82) | S. fuelleborni T. trichiura |
| C1 | Female | 8 | 14.5 (31.9) | S. fuelleborni T. trichiura |
| C2 (control) | Male | 6 | 15.3 (33.7) | S. fuelleborni T. trichiura |

Figure 1. Fecal egg-count profile of *Strongyloides fülleborni* in captive baboons before, during, and after anthelmintic treatment.



Figure 2. Fecal egg-count profile of *Trichuris trichiura* in captive baboons before, during, and after anthelmintic treatment.

*S. fülleborni.*⁷ Treatment with either ivermectin or moxidectin decreased the number of *S. fülleborni* eggs detected after treatment, but posttreatment egg counts did not differ significantly from pretreatment counts with either drug, and neither ivermectin nor moxidectin completely eliminated *S. fülleborni* eggs from the treated macaques.⁷ In the current study, intensive, concurrent treatment with 50 mg/kg fenbendazole and 400 μ g/kg ivermectin for 9 wk completely eliminated *S. fülleborni* eggs from treated baboons by 8 d after treatment, and these animals remained egg-negative for at least 161 d.

In a previous study at our institution, the administration of 50 mg/kg fenbendazole for 3 consecutive days was more effective than was 1 mg/kg milbemycin oxime given every 30 d for 3 mo for treating baboons infected with *T. trichiura*.¹⁶ In that study, fenbendazole eliminated shedding of *T. trichiura* eggs in as few as 6 d, and FEC remained 0 through day 65 after treatment. Milbemycin oxime reduced the number of *T. trichiura* eggs shed but never completely eliminated egg shedding.¹⁶ A 5-d course of fenbendazole formulated in a primate diet significantly reduced the number of *T. trichiura* eggs shed from baboons, compared with pretreatment estimates.¹⁷ After the fenbendazole formulated primate diet had been fed for 5 d, FEC for *T. trichiura* were 0 by 7 d after treatment and remained negative for at least 119 d. Conversely, single-dose ivermectin

| | Baboon | | | | | |
|------------------------------|------------------------|------------------------|-----------------------|------------------------|-----------------------|-----------------------|
| | A1 | A2 | B1 | B2 | C1 | C2 (control) |
| Before treatment | | | | | | |
| no. of samples | 4 | 7 | 6 | 7 | 5 | 2 |
| mean ± SE | 45.1 ± 39.6 | 8.6 ± 2.6 | 4.1 ± 0.9 | 15.8 ± 5.7 | 0.08 ± 0.08 | 26.2 ± 5.2 |
| 95% CI | -32.5 to 122.7 | 3.6 to 13.6 | 2.4 to 5.8 | 4.7 to 26.9 | -0.08 to 0.2 | 15.9to 36.5 |
| median | 8.2 | 7.6 | 4.7 | 14.2 | 0 | 26.2 |
| range | 0.4-163.5 | 0.2-17.2 | 0.4-6.0 | 0.2-35.8 | 0.0-0.4 | 21.0-31.5 |
| After treatment | | | | | | |
| no. of samples | 15 | 15 | 11 | 11 | 14 | 16 |
| mean ± SE | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 5.5 ± 2.2 |
| 95% CI | 0.0-0.2 | 0.0-0.2 | 0.0-0.3 | 0.0-0.3 | 0.0-0.2 | 1.2-9.8 |
| median | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.6 |
| range | 0.0-0.0 | 0.0-0.0 | 0.0-0.0 | 0.0-0.0 | 0.0-0.0 | 0.5-28.8 |
| % Reduction in parasite load | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 79% |
| Mann–Whitney U statistics | T = 111.0 P < 0.001 | T = 133.0 P < 0.001 | T = 87.0 P < 0.001 | T = 105.0 P < 0.001 | T = 77.0 P < 0.033 | T = 33.0 P = 0.058 |

Table 2. Number of *Strongyloides fülleborni* eggs per gram of feces in captive olive baboons (*Papio anubis*) before and after combined treatment with ivermectin and fenbendazole

Fecal egg counts after treatment differed significantly ($P \le 0.05$) from that before treatment in all baboons except the control animal.

Table 3. Number of *Trichuris trichiura* eggs per gram of feces in captive olive baboons (*Papio anubis*) before and after combined treatment with ivermectin and fenbendazole

| | A1 | B1 | B2 | C1 | C2 (control) |
|------------------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| Before treatment | | | | | |
| no. of samples | 2 | 2 | 2 | 2 | 2 |
| mean ± SE | 114.4 ± 89.3 | 15.3 ± 2.0 | 23.2 ± 8.4 | 20.4 ± 3.2 | 32.7 ± 26.1 |
| 95% CI | -60.5 to 289.3 | 11.4 to 19.2 | 6.8-39.8 | 14.0-23.6 | -18.4-83.8 |
| median | 114.4 | 15.3 | 23.2 | 20.3 | 32.7 |
| range | 25.1 to 203.6 | 13.3 to 17.2 | 14.9 to 31.7 | 17.2 to 23.6 | 6.6 to 58.8 |
| After treatment | | | | | |
| no. of samples | 16 | 12 | 11 | 15 | 16 |
| mean ± SE | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 64.4 ± 15.4 |
| 95% CI | 0.0-0.2 | 0.0-0.2 | 0.0-0.3 | 0.0-0.2 | 34.3-94.5 |
| median | 0.0 | 0.0 | 0.0 | 0.0 | 48.1 |
| range | 0.0-0.0 | 0.0-0.0 | 0.0-0.0 | 0.0-0.0 | 0.8-191.4 |
| % Reduction in parasite load | 100 | 100 | 100 | 100 | -96.9 |
| Mann–Whitney U statistic | T = 33.0 P < 0.001 | T = 25.0 P < 0.001 | T = 25.0 P < 0.001 | T = 31.0 P < 0.001 | T = 15.0 P = 0.623 |

Baboon A2 was not infected with Trichuris trichiura and therefore is not reported here.

Fecal egg counts after treatment differed significantly ($P \le 0.05$) from that before treatment in all baboons except the control animal.

(concentration not reported) given to 5 baboons infected with *Trichuris* spp. had poor to limited efficacy, because parasite loads increased in 2 of the treated baboons.¹ In our current study, intensive, concurrent treatment using 50 mg/kg fenbendazole and 400 μ g/kg ivermectin for 9 wk completely eliminated *T. trichiura* eggs from treated baboons by day 8 after treatment, and these animals remained egg-negative for at least 161 d.

We here used an intensive deworming regimen involving 2 anthelmintics with different mechanisms of action because 5 of the 6 treated baboons were coinfected with *S. fülleborni* and *T. trichiura*. In addition, in our experience, treating infections of *S. fülleborni* alone or in combination with other parasites can be challenging by using traditional deworming protocols. Given the few animals enrolled in the current pilot study and the lack of ivermectin-only and fenbendazole-only treatment groups, our results should be interpreted with caution. Indeed, adding more animals, treatment groups, and pretreatment samples

likely would minimize the observed variation in the number of parasite eggs shed from infected baboons. For example, control baboon C2 showed a 79% reduction in *S. fülleborni* eggs, whereas the number of *T. trichiura* eggs increased by 96.9% over the same time frame. We attribute these fluctuations in FEC to natural variation, yet we know little about the shedding dynamics of either nematode in baboons.

Intestinal nematode infections typically are treated by using at least 2 anthelmintic doses—the first to eradicate adult worms and the second to eliminate immature stages that survived the initial dose; this second dose is given once enough time has passed for juvenile stages to become susceptible adults. The treatment interval is usually strategically based on the prepatent period of the targeted parasites. In addition, the treatment of hosts infected with threadworms is complicated because *Strongyloides* spp. alternate between free-living and parasitic generations, because *Strongyloides* spp. can undergo Vol 56, No 1 Journal of the American Association for Laboratory Animal Science January 2017

autoinfection, and because *Strongyloides* spp. demonstrate transmammary transmission.¹⁴ The treatment of whipworms is complicated because *Trichuris* spp. eggs are long-lived in the external environment and because *Trichuris* spp. have prolonged prepatent periods.² Regardless of the unique biology of parasitic nematodes targeted and the efficacy of anthelmintic used to treat infected hosts, the success of any anthelmintic program is dependent on preventing and controlling reinfection.

Acknowledgments

We thank the laboratory animal technicians who helped with the collection of the fecal samples during this study. This study was supported by NIH/OD 2 P40 OD010988 (Gary L White) and NIH/OD P40 OD010431 (Roman F Wolf).

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