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Comparison of Various Anthelmintic Therapies for the Treatment of *Trypanoxyuris microon* Infection in Owl Monkeys (*Aotus nancymae*)

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***Trypanoxyuris microon* is a pinworm that infects New World nonhuman primates, including *Aotus nancymae*. Although it typically is clinically insignificant, infection may serve as a significant variable during experimental data analysis. In this study we sought to determine the most effective anthelmintic therapy for eradication of *T. microon* infection in *A. nancymae*. Animals confirmed to be infected with *T. microon* by perianal tape test were treated twice (on days 0 and 14) with pyrantel pamoate, ivermectin, or thiabendazole and evaluated for eggs by daily perianal tape test throughout the entire 28-d period. Successful clearance of eggs was defined as 5 consecutive negative perianal tape tests. Pyrantel pamoate and ivermectin were significantly more effective at egg clearance than were thiabendazole and no treatment. Overall, 100% of the pyrantel pamoate and ivermectin treatment groups were cleared of infection after 2 treatments, whereas only 60% of the thiabendazole group became negative for pinworm eggs. In addition, the time after treatment until clearance was 1 to 2 d for pyrantel pamoate, 2 to 4 d for thiabendazole, and 4 to 6.5 d for ivermectin. These results indicate that pyrantel pamoate was the most effective and rapidly acting anthelmintic for the treatment of adult *T. microon* infection, with ivermectin as a suitable alternative. However because of the potential for continued development of immature stages or reinfection, anthelmintic doses should be repeated after 1 to 2 wk, in combination with effective environmental sanitation.**

Trypanoxyuris microon is the pinworm that most commonly infects *Aotus* monkeys in their natural habitat and captivity.[16] One prevalence study, conducted with 128 *Aotus nancymae*, showed that 54.7% of the monkeys were infected with this pinworm.[8] These nematodes are classified in the family Oxyuridae and, like other genera of pinworms, have a direct life cycle.[4,16] Infection occurs through the ingestion of larval eggs, with the adult worms colonizing the cecum, followed by the migration of gravid female worms to the perianal skin, where eggs are deposited.[2,3,16] The deposition of eggs on the perianal skin is the reason for the effectiveness of the perianal tape test for detecting pinworm infections, although Felt and colleagues describe a lack of circadian-based deposition of *T. microon* eggs, as occurs with human pinworm (*Enterobius vermicularis*) infection.[3]

Infection with the genus *Trypanoxyuris*, which has been shown to cause the least pathology at the intestinal level, is well tolerated by the host and usually asymptomatic.[16] Similar to pinworm infection in other nonhuman primates, irritability associated with anal pruritus and irritation may present clinically in infected owl monkeys.[18] There is even 1 case of an overwhelming infestation, presumably in the cecum or colon, leading to death in a spider monkey.[2]

Despite the usual lack of clinical sequelae, pinworm infection in nonhuman primates has the potential to confound research data through undetermined physiologic, immunologic, or behavioral effects, as have been documented in other laboratory animals.[1,6,7,15,17] Therefore, it is important to eradicate pinworm infections through sound husbandry practices and treatment with an effective anthelmintic. Treatments for nematode infections include benzimidazoles, pyrantel pamoate, and ivermectin.[2,9,16] The purpose of the present study was to determine the most efficacious treatment against *T. microon* in terms of both overall efficiency and rate of clearance after treatment of owl monkeys with oral thiabendazole or pyrantel pamoate or injectable ivermectin.

Materials and Methods

Animals. We selected 19 (9 male, 10 female) *Aotus nancymae* ranging in age from 1 to 6 y from the Naval Medical Research Center Detachment animal colony for assignment to this protocol. The facility is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International; therefore all husbandry and experimental procedures were performed in compliance with the *Guide for the Care and Use of Laboratory Animals*.[10] The experimental protocol was approved by the NMRCD Institutional Animal Care and Use Committee (NMRCD06-1). All animals were confirmed to be positive for *T. microon* by perianal tape test. Animals were single-housed in standard metal cages with nest boxes and perches. A commercially formulated monkey diet (New World Primate Diet 8794N, Harlan Teklad, Madison, WI) was fed daily. The diet was supplemented with a variety of fresh fruits and monkey biscuits purchased from the regional primate center (Instituto Veterinario de Investigacones Tropicales y de Altura, Iquitos, Peru). Distilled water was provided ad libitum. Animals were provided a reverse 12:12-h light:dark cycle that is offset from the normal day so that monkeys can be observed during their active time. Animals were caught in a net while in their cages and manually restrained for perianal tape testing.

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Anthelmintic agents. For this study we used standard oral suspensions of thiabendazole (125 mg/ml, Wedgewood Pharmacy, Swedesboro, NJ) and pyrantel pamoate (250 mg/5 ml, Combantrin, Pfizer, Venezuela) and the standard injectable solution of ivermectin (1 mg/ml; Biomisil 0.1%, Biomont, Lima, Peru) with known potencies.[2,9,16] Thiabendazole and pyrantel pamoate were administered by use of a 5-French sterile feeding tube (Kendall, Mansfield, MA). The feeding tube was flushed with 1.0 ml of sterile water to ensure the full dose was administered. All anthelmintic agents were given once on day 0 and repeated on day 14 at the following doses: thiabendazole, 100 mg/kg orally; pyrantel pamoate, 11 mg/kg orally; and ivermectin, 200 µg/kg subcutaneously.[2,16,18]

Experimental design. A total of 19 *T. microon*-infected animals were distributed into 3 treatment groups of 5 and an infection positive control group of 4 animals. The treatment groups were given anthelmintic therapies of thiabendazole, pyrantel pamoate, or ivermectin. The positive control group was divided in half: 2 animals received 0.3 ml sterile water subcutaneously with a 26-gauge, 1/2-in. needle, and 2 received 2.0 ml sterile water orally, in order to simulate administration routes of the anthelmintic treatments. After the daily perianal tape testing, treatment for all groups was repeated on day 14 with the same anthelmintic.

Starting on day 1 after initial treatment, animals were examined daily for 28 d for the presence of *T. microon* eggs. Unsedated animals were positioned with tail extended and perineum exposed. The perineum was checked for the presence of adult pinworms, urine, or feces. If the perineum was urine-soaked or covered in feces, the area was blotted, not wiped, gently using a 2 × 2 in. gauze pad.[3] When the perineum was dry, an approximately 1-in. piece of 3/4-in., clear cellophane tape was applied directly to the anus. The tape was pressed against the anus 3 times before transferring the tape, sticky side down, to a glass microscope slide. The slide was examined with a light microscope at a magnification of 10× to 40× for the presence of *T. microon* eggs. If 1 or more eggs were observed, the animal was considered to be pinworm positive, and if no eggs were seen, the animal was considered to be negative. After 5 consecutive negative tape tests, which is the standard in human medicine for pinworm detection, the animal was considered to have cleared the infection.[5]

Statistical analysis. The mean time until the first of 5 consecutive days with negative tape tests after 1 or 2 treatments was determined for each drug. The percentage of monkeys that successfully cleared the infection after the first and second treatments was calculated to demonstrate which group had the overall highest success rate for clearance. Effectiveness of treatments was evaluated using the time-to-event approach with the Kaplan-Meier method and log-rank test (Stata 8.0, StataCorp, College Station, TX).

Results

After the first round of treatment, 100% of the animals treated with pyrantel pamoate, 80% treated with ivermectin, and 20% treated with thiabendazole cleared *T. microon*, compared with 0% in the control groups (Table 1). In animals that were successfully cleared of *T. microon* eggs, the average time until clearance, defined as the number of days until the first of 5 consecutively negative perianal tape tests, was 2 d for both the pyrantel pamoate and thiabendazole groups but was 6.5 d for the ivermectin-treated group.

After the first round of treatment, 4 of the 5 animals in the pyrantel treatment group that were previously cleared of adult worms were found to be reinfected with *T. microon* eggs between days 12 and 14, so a second dose of the respective anthelmintic was administered to all treatment groups on day 14 following the daily tape tests (Figure 1). On day 28, 14 days after the second treatment was administered, 100% of the animals that were infected by *T. microon* and given pyrantel pamoate (n = 4) or ivermectin (n = 1) cleared the infection whereas only 50% of those given thiabendazole (n = 2) cleared the infection. No clearance (0%) was detected among the control group. Overall, 100% of the pyrantel pamoate and ivermectin treatment groups were cleared of infection after 2 treatments, whereas only 60% of the thiabendazole group was cleared (Table 1).

Table 1. Successful clearance of *T. microon* eggs (%) between treatment groups after single and repeated dosing

Treatment	1st dose	2nd dose	Total
Pyrantel pamoate orally	5/5 (100)	4/4 (100)	5/5 (100)
Ivermectin subcutaneously	4/5 (80)	1/1 (100)	5/5 (100)
Thiabendazole orally	1/5 (20)	2/4 (50)	3/5 (60)
Sterile H_2O orally	0/2 (0)	0/2 (0)	0/2 (0)
Sterile H_2O subcutaneously	0/2 (0)	0/2 (0)	0/2 (0)

Successful clearance was defined as 5 consecutive negative perianal tape tests.
Data are given as no. of animals negative for *T. microon* eggs/total no. of animals treated.

Figure 1 depicts the resurgence of positive tape tests at day 20 in the pyrantel pamoate group after successful clearance of eggs after the second treatment. In addition, 2 animals from the thiabendazole treatment group were detected to be positive for eggs on days 22 and 27 after clearance after the second treatment (on day 14). The average day of clearance after the second treatment was day 15 of the 28-d study period for pyrantel pamoate and day 18 for both thiabendazole and ivermectin. Table 2 demonstrates that of animals that cleared the infection, the average time after treatment until clearance was 1 to 2 d for pyrantel pamoate, 2 to 4 d for thiabendazole, and 4 to 6.5 d for ivermectin.

Statistical analysis of the data presented in Table 1 indicated clear and significant ($P = 0.001$ for all comparisons) differences between the clearances of eggs in the 4 treatment groups after the first treatment. Pyrantel pamoate was significantly ($P = 0.018$) more effective than ivermectin. Ivermectin was not significantly different from thiabendazole ($P = 0.107$). No significant difference was detected between the clearance of the thiabendazole and control groups ($P = 0.371$), however ivermectin was significantly more effective than no treatment ($P = 0.026$).

There also were statistically significant ($P = 0.002$) differences in clearance of eggs after retreatment at day 14 among the pyrantel pamoate, thiabendazole, and control groups. Because there was only a single animal that remained infected after the initial treatment of ivermectin, which successfully cleared the infection after the second treatment, we were unable to statistically evaluate the drug's effectiveness. Pyrantel pamoate was significantly ($P = 0.008$) more effective than either thiabendazole or no treatment. There was no significant difference between thiabendazole and control groups regarding clearance after retreatment. Comparison of effectiveness between initial treatment and retreatment with pyrantel pamoate and thiabendazole indicated that the rate of clearance did not differ significantly between the first and second dose.

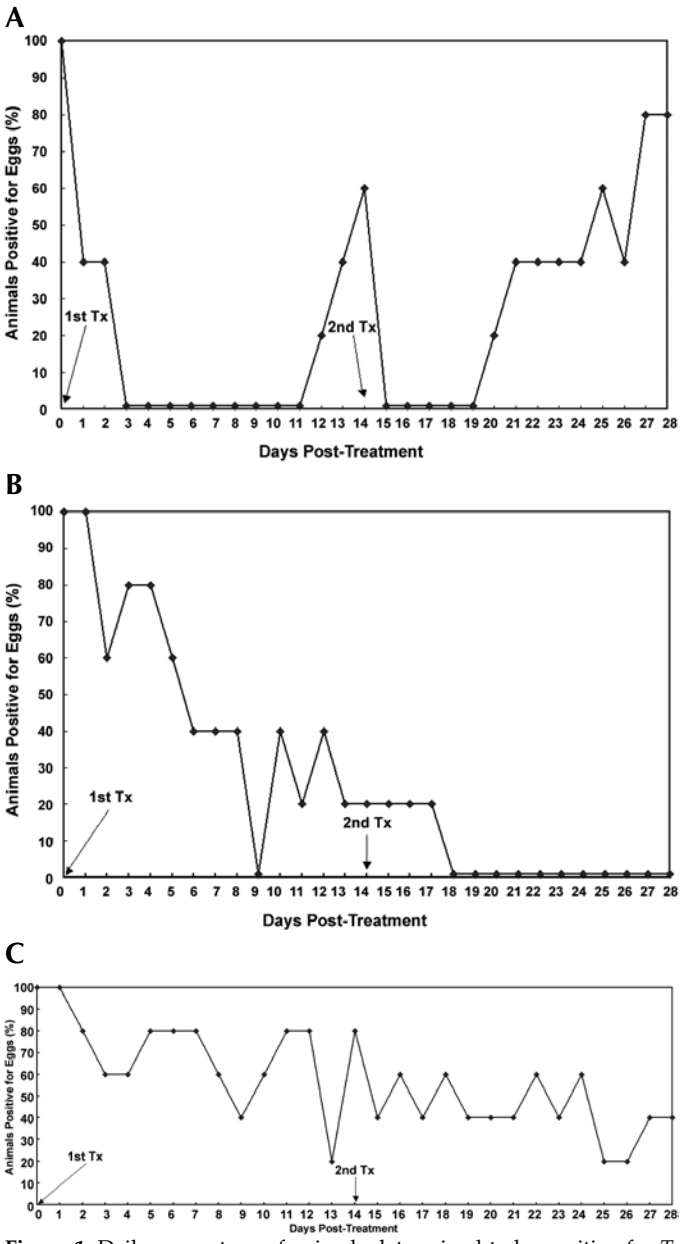

Figure 1. Daily percentage of animals determined to be positive for *T. microon* eggs by perianal tape test after doses of (A) pyrantel pamoate, (B) ivermectin, or (C) thiabendazole on days 0 and 14.

Discussion

We demonstrated that pyrantel pamoate was the most efficacious treatment for killing adult *T. microon* worms after oral dosage (100% of animals successfully cleared) and killed adult worms (1 to 3 d) most rapidly as evidenced by negative tape tests. Pyrantel pamoate was significantly ($P < 0.018$) more effective than ivermectin, thiabendazole, or no treatment at clearing *T. microon* eggs as determined by perianal tape test. Ivermectin was less effective than pyrantel pamoate, having a longer time-to-clearance. However, ivermectin was significantly ($P < 0.026$) better than no treatment and did clear 100% of the group of infection by the end of the study, indicating that this drug may be a useful alternative to pyrantel pamoate.

Pyrantel pamoate, which acts as a depolarizing neuromuscular blocking agent leading to paralysis of the worm,[12] proved to be the most effective treatment in terms of percentage of animals cleared of eggs on perianal tape test and the rate of clearance after treatment. However, our results support previous findings[11] suggesting that pyrantel pamoate may be ineffective against developing eggs and immature larval stages, in view of a resurgence in egg detection after successful clearance of eggs. There was no statistical significance between the first and second doses of pyrantel pamoate in terms of effectiveness. In addition, the pyrantel pamoate dose appeared to lack any pharmacologic additive effect, consistent with data indicating that the drug is rapidly metabolized and excreted into the urine and feces.[12] This finding is consistent with recommendations in human and veterinary medicine to repeat treatment every 1 to 2 wk to eliminate previously unsusceptible stages that have matured.[9,12,13]

Table 2. Average rate (d) of clearance of *T. microon* eggs after single and repeat dosing

Treatment	1st dose	2nd dose
Pyrantel pamoate orally	2 (5)	1 (4)
Thiabendazole orally	2 (1)	4 (2)
Ivermectin subcutaneously	6.5 (4)	4 (1)

Clearance was defined according to the first of 5 consecutive negative perianal tape tests.
The number of animals that cleared the infection is given in parentheses.

The current study also suggests that ivermectin is an effective anthelmintic against *T. microon* infection, because clearance was 80% to 100% after a single dose. Ivermectin's mode of action is to enhance the release of gamma aminobutyric acid at presynaptic neurons. This compound acts as an inhibitory neurotransmitter and blocks the postsynaptic stimulation of the adjacent neuron in nematodes, causing their paralysis and eventual death.[12] A negative aspect of ivermectin therapy was that the average length of time after either treatment until clearance was 4 to 6.5 d. This situation is undesirable, because the prolonged clearance of adults shedding eggs allows more opportunity for the environment to become contaminated with eggs, thereby potentially allowing reinfection of previously treated animals or exposing noninfected animals to infectious material.

Thiabendazole, a benzimidazole, is used to treat many of the helminth infections common in animals and man. The mode of action on the parasite is unknown but may inhibit the helminth-specific enzyme fumarate reductase.[14] Although the drug is recommended in the literature as therapy against *T. microon*, it is our conclusion that single-dose thiabendazole is not an effective therapy.[2] Clearance was between 20% and 50% after 1 or 2 treatments, respectively, and there was no statistical significance between 1 treatment of thiabendazole and no treatment, and only borderline significance between a second dose of thiabendazole and no treatment (Table 1). As with the pyrantel pamoate group, eggs were noted on thiabendazole-treated animals that had been cleared of infection after a second dose of the drug, indicating that treatment should be repeated every 1 to 2 wk until tape test negative. Perhaps thiabendazole would have been more effective if it had been administered for several consecutive days, as is described for various other parasitic infections in animals, although many recommendations for susceptible parasites[12] involve only a single dose which may be repeated in days to weeks, if necessary, as was performed in this study.

As previously proposed in this discussion, the occurrence of

shed eggs most likely is due to continued development of eggs and immature larvae, although reinfection also is possible. Because all treatment groups were housed within the same room, all animals potentially were exposed to contaminated fecal material from untreated animals or animals that did not respond to therapy and therefore were still shedding infectious eggs. Eggs could have been transmitted from positive to cleared animals by direct contact through cage bars or aerosolization of eggs during routine husbandry practices. In addition, monkeys were captured for tape testing in the same net, which may have been contaminated with fecal material. Exposure possibilities underline the importance of an effective anthelmintic therapy regimen combined with routine and thorough disinfection of equipment and room surfaces to prevent reinfection. To enhance elimination of *T. microon* from infected monkeys, further studies should be conducted to elucidate the prepatent period of *T. microon*; having this information would allow repeat dosing of anthelmintic therapy at appropriate intervals to kill all developing stages.

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