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Patterns of Detection of *Strongyloides stercoralis* in Stool Specimens: Implications for Diagnosis and Clinical Trials

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Reported efficacies of drugs used to treat *Strongyloides stercoralis* infection vary widely. Because diagnostic methods are insensitive, therapeutic trials generally require multiple negative posttreatment stool specimens as evidence of drug efficacy. However, only a single positive stool specimen is usually required for study enrollment. To determine the reproducibility of detection of *S. stercoralis* larvae in the stool, 108 asymptomatic infected men submitted 25 g of fresh stool once a week for eight consecutive weeks for examination by the Baermann technique. During the 8-week study, 239 (27.7%) of 864 stool specimens were positive for *S. stercoralis*. Rates of detection of larvae in the stool specimens ranged from eight of eight specimens in 3 (2.8%) men to none of eight specimens in 36 (33.3%) men. Of 43 men for whom *S. stercoralis* was detected in at least two of the first four stool specimens, only 1 (2.3%) man tested negative on all of the next four specimens. In comparison, of 29 men who had detectable larvae in only one of the first four specimens, 22 (75.9%) tested negative on all of the next four samples. Thus, if these 29 men had been enrolled in a therapeutic trial between the first and second sets of four specimens, the efficacy of a drug with no activity against this parasite would have been estimated to be 76%. These data suggest that patterns of *S. stercoralis* detection vary widely among infected persons and that intermittent larval shedding can lead to inflated estimates of drug efficacy. Before a patient is entered in a clinical trial of drug efficacy, four consecutive stool specimens should be examined for *S. stercoralis*; only persons with two or more positive specimens should be enrolled.

Strongyloidiasis is a disease of emerging public health importance. While it is endemic in many tropical areas, *Strongyloides stercoralis* is also indigenous in parts of southeastern United States (14). In immunosuppressed persons, the autoinfective cycle of the organism facilitates disseminated infections that are not infrequently fatal (14).

Diagnosis of strongyloidiasis can be challenging for both the physician and the laboratorian. Microscopic examination of stool specimens for the parasite is insensitive; estimates for a single positive stool examination in cases of uncomplicated infection range from 0 to 66% (6, 7, 10, 11). To overcome this lack of sensitivity, investigators have recommended examination of up to seven stool specimens (19); use of more sensitive and labor-intensive methods of stool examination, such as the Baermann technique (13); use of agar plate cultures (12, 21); and collection of alternative specimens, such as duodenal aspirates (6, 11). Serologic assays have been used in research laboratories for years (4, 18, 20), but they are not generally available for diagnosis. For all of these methods, the reproducibility of positive findings and the patterns of detection of the organism in infected persons have not been well-defined.

Interpreting the results of therapeutic trials of drugs for treatment of strongyloidiasis is difficult because lack of detection of the organism in stool may not be indicative of cure. In treatment studies, from two to eight negative posttreatment specimens have been considered acceptable evidence of treatment efficacy, but only a single positive stool specimen is usually required for enrollment (3, 5, 8, 15–17, 20, 22). Little attention has been given to the reproducibility of a single positive test result, to individual patterns of larval shedding in stool, or to the implications of these patterns for interpretation of clinical drug trials.

To determine the reproducibility and identify patterns of detection of *S. stercoralis* larvae in the stools of infected persons and to assess the adequacy of existing criteria for enrollment in clinical drug trials for this organism, we used the Baermann technique to examine stool specimens from a group of infected men during a period of 8 weeks.

MATERIALS AND METHODS

To screen for intestinal parasites, single stool specimens were collected from 1,304 apparently healthy male soldiers (2) and examined microscopically at the central laboratory of Hospital das Clinicas at Pernambuco Federal University in Recife, Brazil, by the Hoffmann technique (9). Men were eligible to enter and complete the study if they tested positive for *S. stercoralis*, if they had received no anthelmintic drugs during the previous year, and if they remained free of diarrhea during the 8-week study. Infected men who failed to meet these criteria were treated with thiabendazole within 3 weeks of the time that initial stool examination results were available or within 1 week after diarrhea developed. Participants who completed the study received no anthelmintic medications until the end of the study, when they were treated with thiabendazole or ivermectin. Informed consent was obtained from all study participants.

Before treatment, stool specimens were collected from each participant every week for 8 weeks. Twenty-five grams of fresh stool from each specimen was examined by the same technician by the Baermann-Morais technique (13). The total volume of Baermann liquid, 40 ml, was centrifuged, and all the sediment in each sample was examined (up to 100 slides per sample). Larval density was not quantitatively analyzed. Men who developed diarrhea (watery stools) were excluded from the remainder of the study and treated for *S. stercoralis* infection because of the possibility that strongyloidiasis was the cause of the diarrhea. Men who tested negative for *S. stercoralis* in all eight specimens continued to submit stool specimens once a week until larvae were again identified in the feces.

The statistical significance of differences between proportions was determined by using the chi-square or Fisher exact test (two-tailed).

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TABLE 1. Sensitivity of Baermann technique in detecting *S. stercoralis* larvae in eight consecutive weekly stool specimens from 108 men living in Greater Recife, Brazil

Specimen no.	No. (%) of specimens positive for *S. stercoralis*	Cumulative no. (%) of men testing positive for *S. stercoralis*
1	35 (32.4)	35 (32.4)
2	32 (29.6)	53 (49.1)
3	35 (32.4)	64 (59.3)
4	39 (36.1)	72 (66.7)
5	24 (22.2)	72 (66.7)
6	21 (19.4)	72 (66.7)
7	22 (20.4)	72 (66.7)
8	31 (28.7)	72 (66.7)
Total	239 (27.7)	72 (66.7)

RESULTS

All 1,304 soldiers were 18- or 19-year-old men from Greater Recife, Brazil. Of 147 (11.3%) men whose initial stool specimens were positive for *S. stercoralis* by the Hoffmann technique, 34 were excluded because they had received anthelmintic drugs during the previous year and 5 were excluded because they developed diarrhea during the study. Of 108 men who completed the study, 72 (66.7%) had one or more positive stool specimens during the 8-week study period. The 36 men who tested negative for *S. stercoralis* on all eight specimens continued to submit stool specimens once a week; *S. stercoralis* was first detected by the Baermann method in all 36 within 9 to 21 weeks after the beginning of the study. Other intestinal parasites detected in the stool specimens of the 108 study participants included *Ascaris lumbricoides* (41.7%), *Trichuris trichiura* (32.4%), hookworm (46.3%), *Schistosoma mansoni* (13.9%), *Giardia lamblia* (7.4%), and *Entamoeba histolytica* (12.0%).

A total of 864 stool specimens were collected during the 8-week study period. Of the 864, 239 (27.7%) were positive for *S. stercoralis*. Detection rates for individual specimens ranged from 20% for the seventh specimen to 36% for the fourth (Table 1). Among the 108 men who completed the study, detection of larvae in stool specimens was highly variable, ranging from eight of eight specimens in 3 (2.8%) men to none of eight specimens in 36 (33.3%) men (Table 2). The rate of detection during the first 4 weeks of the study was significantly higher than that during the last 4 weeks (32.6 vs. 22.7%; $P = 0.001$). Nonetheless, for individual men, patterns of detection during the first 4 weeks were highly predictive of those during the last 4 weeks of the study (Table 2). For example, all of the men who tested positive for *S. stercoralis* on each of the first four specimens tested positive on at least three of the last four. In comparison, none of the men who tested negative on all four of the first set of specimens tested positive on any of the last four.

The observed variability in detection of *S. stercoralis* was not influenced by the presence of other helminths. However, all 13 men in whom *E. histolytica* was detected on initial stool examination by the Hoffmann technique were found to have *S. stercoralis* larvae in at least one of the eight subsequent stool specimens examined by Baermann's method, compared with 59 (62.1%) of 95 men who initially tested negative for *E. histolytica* ($P = 0.004$).

TABLE 2. Pattern of detection of *S. stercoralis* larvae when the Baermann technique was used to examine eight consecutive weekly stool specimens from 108 men living in Greater Recife, Brazil[a]

No. of first four specimens positive for *S. stercoralis*	No. of men (% of total)[b]	No. of last four specimens positive for *S. stercoralis*				
		4	3	2	1	0
4	6 (5.6)	3	3	0	0	0
3	14 (13.0)	3	2	7	2	0
2	23 (21.3)	1	2	4	15	1
1	29 (26.9)	0	1	1	5	22
0	36 (33.3)[c]	0	0	0	0	36[c]

[a] After being found to be infected on a single specimen examined by the Hoffmann technique, each man had 25 g of stool examined by the Baermann technique once a week for 8 weeks. The results for the first and last four specimens are presented separately.

[b] $n = 108$.

[c] These men continued to submit stool specimens once a week; *S. stercoralis* larvae were detected in the stools of all men 9 to 21 weeks after the beginning of the study.

DISCUSSION

In the absence of treatment, the rate of detection of *S. stercoralis* in this study was low despite examination of multiple stool specimens with one of the most sensitive methods available, and marked variation in patterns of larval excretion was noted. Our findings echo earlier observations by Jones and Abadie, who years before most clinical drug trials for *S. stercoralis* were conducted noted that "initial success in finding larvae in the stool of a patient does not assure that subsequent examinations will yield positive results" (11). Similar findings were recently reported by Sato and colleagues, who showed that 40% of infections were missed when single stool specimens were tested for *S. stercoralis* by the relatively sensitive agar plate culture method (21). Awareness of the low sensitivity of diagnostic methods and of the high degree of individual variation is essential for proper design and interpretation of clinical trials.

The remarkable variation in the reproducibility of larval detection among persons may explain the wide range in efficacy reported for thiabendazole, ivermectin, and other drugs for treatment of *S. stercoralis* infection, since in most studies patients were enrolled after submitting a single positive stool specimen. Our data suggest that patterns of detection of *S. stercoralis* larvae in the feces of infected persons can be characterized on the basis of examination of a minimum of four stool specimens. Before enrollment in a clinical treatment trial, at least two, and preferably three, of these specimens should be positive for *S. stercoralis*. In this study, 29 men tested positive for *S. stercoralis* in only one of the first four stool specimens. Of the 29, 22 (75.9%) men had no detectable larvae in the next set of four stool specimens. Thus, if a therapeutic trial had begun between the first and second set of four specimens, drug efficacy in this group would have been estimated to be 76%. In comparison, of 43 men with at least two positive tests in the first set of four specimens, only 1 (2.3%) man tested negative on all four specimens in the second set.

In addition to their relevance to treatment trials for strongyloidiasis, the findings of our study have several implications for medical practice. Physicians who suspect strongyloidiasis on clinical grounds should consider treatment even if patients test negative for the infection. In addition, persons known to be infected with *S. stercoralis* should be monitored carefully by examinations of stool specimens after treatment, especially if the patients are to receive immunosuppressive therapy. For such persons, examination of several stool specimens before

treatment could help establish whether they are frequent or infrequent excretors of *S. stercoralis* larvae. Symptoms of strongyloidiasis may resolve after treatment, even in the absence of a parasitological cure (4a). Thus, detection of larvae in the stool specimen when symptoms recur may indicate ineffective initial treatment rather than reinfection.

The reasons for the marked variation in detection of *S. stercoralis* among individuals are unknown. Our observations were limited to eight consecutive weeks; we are unable to predict how detection patterns might have changed during a longer period of observation. However, comparison of the first and second sets of four specimens suggests a trend of decreasing presence of larvae in the feces for most study participants. This observation suggests that once candidates for clinical trials are found to have *S. stercoralis* in at least two of four stool specimens treatment should not be delayed. Previous studies have suggested that *S. stercoralis* worm burdens fluctuate in the absence of treatment (5). In this study, 60% of infected men could be characterized as having detectable larvae in the feces infrequently (no more than one specimen positive during the first 4 weeks). The degree to which such persons contribute to transmission of *S. stercoralis* is unknown. It is also unclear whether the different patterns of larval detection in stool specimens are associated with certain symptoms or clinical manifestations of strongyloidiasis; assessment of clinical findings was beyond the scope of this investigation.

Specimens were collected 1 week apart and examined by the Baermann technique. It is unknown whether results would have been similar with more frequent collection of stool specimens or less sensitive techniques for detection of *S. stercoralis*. The agar plate culture method has a sensitivity similar to that of the Baermann technique, but it is less frequently used in Brazil, it appears to be less cost-effective, and it requires more time for test results (12, 13). The Baermann technique is considered one of the most sensitive methods for detection of living *S. stercoralis* larvae in stool specimens, and we examined 25 g of feces, five times more than the usual amount of 5 g. Thus, we consider it unlikely that larvae were present in the stool specimens that tested negative by the Baermann method, despite that fact that all study participants initially tested positive on a single stool specimen that was screened by the Hoffmann technique. We consider it a chance event that larvae were detected on the initial examination, rather than evidence for particularly high sensitivity of the Hoffmann technique (9). The Hoffmann test results were not considered falsely positive because *S. stercoralis* was eventually detected in the stool specimens of all study participants by the Baermann method.

Because the 1,304 soldiers were initially screened by examination of a single stool specimen, a relatively insensitive technique, it is likely that the true prevalence of *S. stercoralis* infection is considerably higher than 11.3% among soldiers in Recife and among the general population. Thus, Greater Recife can be considered a region of hyperendemicity for this parasite (23). The association between *E. histolytica* and increased frequency of detection of *S. stercoralis* larvae remains unexplained. Since diarrhea was a criterion for exclusion from this study, none of these men had amebic dysentery. The predominant strains of *E. histolytica* in Recife appear to be nonpathogenic (1).

In summary, patterns of excretion of *S. stercoralis* larvae vary markedly among infected individuals. Many infected persons appear to rarely have detectable larvae in stool specimens. The data presented here suggest that for clinical trials of drug efficacy, four consecutive stool specimens should be examined for *S. stercoralis* and only persons with two or more positive specimens should be enrolled.

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