

EFFICACY OF STOOL EXAMINATION FOR DETECTION OF *STRONGYLOIDES* INFECTION

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Abstract. To determine the efficacy of stool examination for detection of *Strongyloides* infection, 1,350 stool samples collected in Japan, Brazil, and Thailand were examined by four different methods (direct fecal smear, formalin-ether concentration, Harada-Mori filter paper culture, and agar plate culture). The newly developed agar plate culture method was highly effective; more than 96% of the positive cases were diagnosed by this method. The coprologic examination, however, was not sensitive enough for detecting chronic infections because more than 40% of the positive cases were overlooked even when persons with proven *Strongyloides* infection were re-examined several months later without intervening treatment. Therefore, it is essential to examine stool samples repeatedly to achieve a correct diagnosis, and even so, it is important to note that a negative result does not necessarily indicate the unequivocal absence of the infection.

Strongyloidiasis, which is relatively common in tropical and subtropical areas, is an intestinal parasitic disease resulting from an infection with the nematode *Strongyloides stercoralis*. One of the unique properties of the parasite is its ability to propagate in a host by internal autoinfection. It is probable that autoinfection commonly occurs in human strongyloidiasis and that the phenomenon is responsible for pathogenicity in the parasitic infection. Although the infection often progresses to the fatal hyperinfected state under immunosuppressed conditions, the parasite is usually non-pathogenic in an immunocompetent host.¹

One of the problems related to strongyloidiasis is that it is difficult to detect *S. stercoralis* larvae in stool specimens because the majority of cases involve chronic, low-level infection. In the present study, we attempted to determine the efficacy of stool examination on the diagnosis of strongyloidiasis.

MATERIALS AND METHODS

Stool samples. Stool samples were collected from 1,350 inhabitants in four areas (713 in Okinawa, Japan, 432 in Holambra and Maceio, Brazil, and 205 in Chiang-Mai, Thailand). Additional samples were taken from 123 Okinawan patients with proven strongyloidiasis several months after the initial diagnosis without intervening treatment to reconfirm the presence of fecal larvae.

Stool examination. Four different methods of coprologic examination were compared for efficacy in detecting *Strongyloides* infection. A direct fecal smear and fecal concentration in a formalin-ether solution was considered the conventional method.² Coproculture using a filter paper strip was also performed.³ A new coproculture method using an agar plate developed in Okinawa was also used.⁴ With this technique, approximately 3 g of feces was placed on the surface of a primary agar plate for bacterial culture and incubated for 2–3 days at 28°C. After incubation, the surface of the agar plate was examined under a microscope to find motile larvae that emerged from the fecal mass. With this method, the unique alignment of bacterial colonies on the surface of the agar, which formed along the tracks of wandering larvae, was an indicator of the presence of the larvae. In such cases, a careful search for larvae on the agar surface was performed

and larvae were collected in a small tube by washing the agar surface with phosphate-buffered saline. The larvae recovered were transferred to a glass slide and a drop of iodine solution was added to immobilize them. The larvae were then identified morphologically from those of hookworm and *Rhabditis*. If larval tracks were observed but no larvae were recovered from the agar plate, the stool specimen was examined further (i.e., direct smear, fecal concentration, and/or Harada-Mori culture). The specimen was considered negative if no larvae could be demonstrated by these other methods.

RESULTS

The detection rate of *Strongyloides* larvae by each method is summarized in Table 1. A total of 256 (19.0%) samples were found to be positive. The highest detection rate in all four areas was obtained by the agar plate culture method: more than 96% (246 of 256) of the positive cases were diagnosed using this method. The infection rate estimated by the agar plate culture was 2–3 times higher than those estimated by the other methods.

When the efficacy of the agar plate culture was further determined in relation to the results obtained by the other methods (Table 2), 45–60% of the positive cases were diagnosed only by the agar plate culture. The results indicate that many cases may be overlooked if only the conventional methods are used for the diagnosis. In Table 3, the reconfirmation rates determined by the agar plate culture technique for 123 patients previously proven to have strongyloidiasis are summarized. Patients were examined again before treatment to reconfirm the presence of parasite larvae and to evaluate the diagnostic technique. The presence of larvae could not be detected in as many as 40% of the patients who had a single follow-up examination (direct smear, fecal concentration, filter paper culture, agar plate culture) at five months after the initial diagnosis nor at 18 months in about 12% even when the follow-up examination was repeated on stool specimens collected on three consecutive days. The reconfirmation rate was only 15–24% by a single examination with the conventional methods (direct smear and fecal concentration) and approximately 50% even when the follow-up examination (direct smear and fecal concentration)

TABLE 1
Infection rates of *Strongyloides stercoralis* determined by various examination methods in the four study areas*

Area	No. examined	No. (%) positive	No. (%) positive			
			Direct smear	Concentration	Fecal culture	
					Filter paper	Agar plate
Okinawa (Japan)	713	109 (15.3)	28 (3.9)	44 (6.2)	30 (4.2)	105 (14.7)
Holambra and Maceio (Brazil)	432	49 (11.3)	ND	13 (3.0)	14 (3.2)	46 (10.6)
Chiang-Mai (Thailand)	205	98 (47.8)	ND	54 (26.3)	ND	95 (46.3)
Total	1,350	256 (19.0)		111 (8.2)		246 (18.2)

* ND = not determined.

was repeated three times. The reconfirmation rate increased to approximately 85% with the agar plate culture method when three consecutive stools were examined. The rate, however, was still less than 60% even when the agar plate culture method was applied, if examination was performed only once, indicating that about 40% of the patients may be overlooked at any given examination.

DISCUSSION

The medical significance of strongyloidiasis as a fatal hyperinfection under various immunocompromised conditions has been reported.¹ In Okinawa, the parasitic infection is frequently accompanied by human T cell leukemia virus type-1, causes adult T cell leukemia (ATL).⁵⁻⁷ The parasitic infection often progresses to a fatal infection among patients who develop ATL because the disease is manifested by a severe depression of immunocompetence. Therefore, an exact diagnosis is very important.

The majority of cases involve an asymptomatic chronic infection in an immunocompetent host, and parasitologic diagnosis based on coprologic examination is probably one of the most difficult problems in clinical parasitology. Direct fecal smear, formalin-ether concentration, and filter paper culture (Harada-Mori method) have been conventional methods for detecting larvae in stool specimens. However, these methods are not considered sensitive enough for diagnosis of chronic cases because in such cases, the larvae are present only in very small numbers or are frequently absent from individual stool specimens. In a previous investigation, Jones detected the larvae by direct smear in only 27% of the fecal samples collected from 100 patients who were proven to have strongyloidiasis by examination of duodenal fluid.⁸ As a conclusion, he could not confirm the infection in 20 of the

100 patients despite repeating stool examinations more than five times. Grove has also shown that the positive rate of larval detection was 55% for 44 patients with chronic strongyloidiasis upon initial examination using the filter paper culture method and 68% even after two or more attempts.⁹ In the present study, as shown in Table 3, a single stool examination was only 15-24% effective in reconfirming infection of proven strongyloidiasis cases when the direct smear, formalin-ether concentration, and filter paper culture method were used. Even when the examinations were repeated daily for three days, the reconfirmation rate was approximately 50% by the direct smear and 45% by the concentration method.

Recently, another coproculture method using an agar plate was developed in Okinawa.⁴ Using this method, we were able to detect more than 96% of the positive cases. Because of the large amount of feces used, the method was 2-3 times more effective than conventional methods, showing that approximately 60% of the positive cases were diagnosed by the agar plate culture method than by conventional methods. In a comparative study in which the efficacy of the direct smear, the Baermann method, and an agar plate culture were compared, it was also reported that the diagnostic efficacy of the agar plate culture was as sensitive as that of the modified Baermann method, although the Baermann method has some advantages in terms of cost-effectiveness and time needed to obtain results.¹⁰ The efficacy, however, was still less than 60% if stool examination was performed only once, even when the agar plate culture was applied. It was also our experience that when stool samples from persons who showed a strong positive antibody response to *Strongyloides* antigen were examined, the fecal larvae were actually demonstrated in only 50% of them, indicating that the remaining 50% of the subjects might have occult infections at that particular time.¹¹

The present results indicate that even when using the most sensitive technique, the agar plate culture, several fecal specimens collected on different days should be examined to obtain a correct diagnosis and most importantly, that failure to demonstrate larvae cannot be unequivocally interpreted as the absence of the infection. This is especially true if the patient is to undergo immunosuppressive therapy.

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TABLE 2
Comparison of diagnostic efficacy of agar plate culture and other conventional methods for detection of *Strongyloides* infection*

Results of stool examination		No. (%) of cases			
Agar plate culture	Other methods†	Okinawa (n = 109)	Holambra and Maceio (n = 49)	Chiang-Mai (n = 98)	Total (n = 256)
+	+	52 (47.7)	17 (34.7)	51 (52.0)	120 (46.9)
+	-	53 (48.6)	29 (59.2)	44 (44.9)	126 (49.2)
-	+	4 (3.7)	3 (6.1)	3 (3.1)	10 (3.9)

* n = total number of cases positive for *Strongyloides* infection.

† Examined by direct smear, formalin-ether concentration, and/or filter paper culture.

TABLE 3
Results of re-examination of 123 subjects with proven *Strongyloides* infection*

No. of times examined	No. examined	No. (%) reconfirmed	No. (%) reconfirmed			
			Direct smear	Concentration	Fecal culture	
					Filter paper	Agar plate
Once	90†	54 (60.0)	14 (15.6)	22 (24.4)	15 (16.7)	52 (57.8)
Three times	33‡	29 (87.9)	17 (51.5)	15 (45.5)	ND	28 (84.8)

* ND = not determined.

† Follow-up examination was performed five months after the initial diagnosis.

‡ Follow-up examination was performed 18 months after the initial diagnosis.

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