INTESTINAL PARASITIC INFECTIONS IN LIKUPANG, NORTH SULAWESI, INDONESIA

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Abstract. A parasitological survey was conducted on the inhabitants of 6 villages of Likupang, Minahasa Peninsula, North Sulawesi, Indonesia, in August 1991. A total of 419 fecal samples were examined by using direct smear, flotation, formalin ether concentration, Harada-Mori culture and agar-plate culture techniques. Five nematode and 7 protozoan parasites were detected, while trematode and cestode infection was not observed. Soil-transmitted nematode infections were predominant. Among the younger inhabitants aged less than 15, positive rates of *Ascaris, Trichuris* and hookworm infections were almost same, namely 45.7, 45.3 and 47.7%, respectively. Among the elder people aged 15 or more, positive rate of hookworm infection (89.4%) was much higher than *Ascaris* and *Trichuris* infections (19.3 and 26.1%, respectively). Village to village differences in parasite prevalence, probably due to socio-economic and sanitary-environmental differences were observed. Both *Necator americanus* and *Ancylostoma duodenale* were detected. The agar-plate culture was proved to be an efficient method for detection of hookworm as well as *Strongyloides stercoralis.*

INTRODUCTION

Sulawesi is a large island of Indonesia, located between Kalimantan (Borneo) and Irian Jaya (New Guinea). There have been a few reports on the prevalence of intestinal parasitic infections of humans in North Sulawesi : Stafford *et al* (1976) launched a survey in Gorontalo and Cross *et al* (1977) studied 9 localities of Minahasa Peninsula. In these surveys a high prevalence of soil-transmitted parasite infections was pointed out. However, more than ten years have passed since these surveys, and it is presumed that some changes will have occurred in the parasitological situation in this developing area. The present study was conducted to know the present status of intestinal parasitic infections in Likupang, the northernmost area of Minahasa Peninsula, using five techniques of fecal examination including agar-plate culture, a recently applied method for detection of *Strongyloides stercoralis.* It was also aimed to test the efficacy of the agar plate culture in detection of hookworm infection.

DESCRIPTION OF AREA

Likupang has about 33,000 inhabitants living in 37 villages. Six villages, namely Kokole, Likupang-2, Wineru, Maen, Sarawet and Munte, were chosen for survey. Kokole is located inland, while the other 5 villages are along the coast (Fig 1).

Some social and environmental health conditions of the surveyed area derived by questionnaire are summarized in Table 1. Other important information which may be relevant to parasite prevalence is as follows. People rear cattle, pigs, goats, dogs and chickens for use as meat. Marine fish, squids, prawns and crabs as well as freshwater fish, prawns

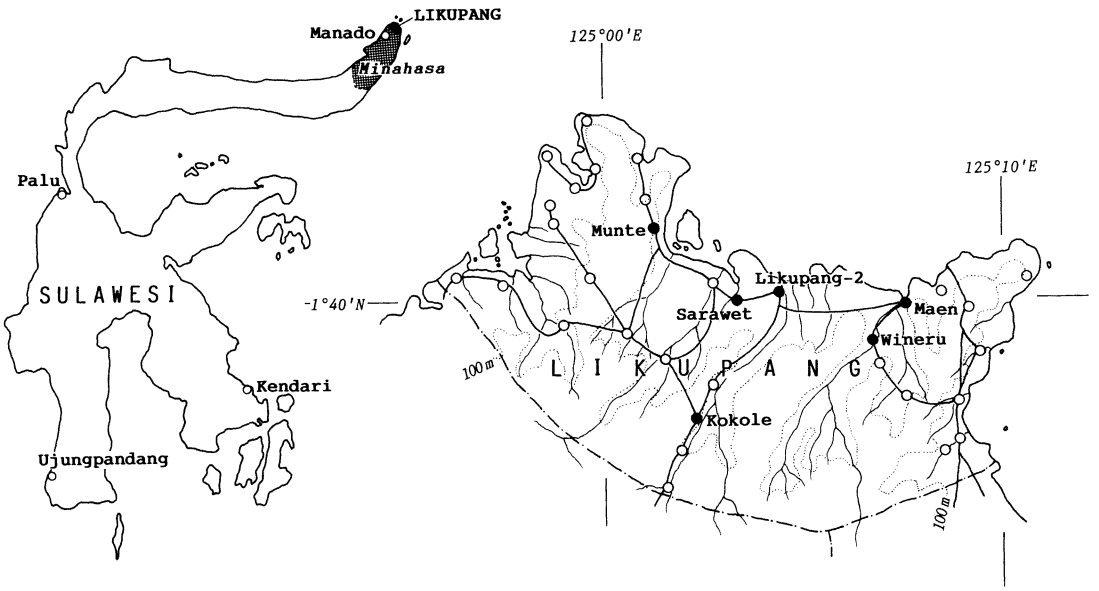

Fig 1—The Likupang area of North Sulawesi.

and crabs are consumed. People also utilize various wild animals such as frogs, snakes, fruit bats and forest rats as food. All kinds of animals are eaten after being well cooked. Vegetables consumed include cabbage, tomato, cucumber, eggplant and onion. Tap water, well water and river water are used for drinking, mostly after boiling.

MATERIALS AND METHODS

Fecal samples were collected from the inhabitants on 1, 3 and 6 August, 1991. Five hundred houses were selected randomly and one person of each house was asked to offer a fecal sample. At the time of fecal collection, a questionnaire on epidemiologic history was completed and blood sampling for malaria survey was done. Results of the epidemiologic and malaria surveys will be published elsewhere in detail. Appropriate anthelmintics were given to the inhabitants who were proven to be infected with parasites.

All fecal samples were examined by formalin-ether concentration and agar-plate culture (Arakaki *et al,* 1988, 1990). If more fecal material was available, the direct smear method, flotation method with saturated sodium chloride solution and Harada-Mori culture were applied. The agar-plate culture and Harada-Mori culture were started on the day of fecal collection, while the other methods were applied within 2 days after collection.

The agar-plate culture was modified from the original method by Arakaki *et al* (1988, 1990) as follows: about 3 g of feces were put in the center of agar-plate in a small plastic dish (diameter 70 mm, height 10 mm), which was then placed in a plastic petri dish (diameter 90 mm, depth 15 mm). About 5 ml of 25% glycerin aqueous solution was added into the space around the small dish in order to trap nematode larva escaping from the agar-plate. Both agar-plate culture and Harada-Mori culture were carried out at room temperature (25-32°C).

The surface of the agar-plate was examined under a dissecting microscope with transmission illumination at 2nd, 3rd and 4th days of culture to find motile nematode larva, winding tracks left by the larva and bacterial colonies developed on the tracks. When a larva was found, it was picked out using a fine curved needle attached at the tip of a small glass rod to be identified under a light microscope. Some culture dishes were examined again after 10 days to collect nematode larvae trapped in the glycerin solution.

Harada-Mori culture was examined at the 10th

Table 1

Social and environmental characteristics of the surveyed area in Likupang, Minahasa, North Sulawesi, according to questionnaire %.

Village	Kokole	Likupang[-2]	Wineru	Maen	Sarawet	Munte	Total
No. people questioned	51	100	48	86	107	100	492
A) Occupation							
Farmer	74.5	18.0	66.7	73.3	59.8	47.0	53.3
Fisherman		47.0		7.0	0.9	12.0	13.4
Construction worker		9.0		1.2		1.0	2.2
Merchant	2.0	2.0			0.9	1.0	1.0
Official worker	7.8	5.0	2.1	2.0	0.9	6.0	3.9
Private company worker	5.9	5.0				1.0	1.8
Others	9.8	14.0	31.3	16.3	37.4	32.0	24.4
B) Religion							
Moslem		64.0	20.8	100.0	76.6	82.0	61.6
Protestant	21.6	36.0	79.2		23.4	25.0	30.3
Catholic	78.4						8.1
C) Staple foods*							
Rice	100.0	93.0	95.8	93.0	98.1	90.0	94.5
Cassava	62.8	17.0	2.3	67.4	5.6	82.0	41.9
Corn	39.2	2.0	22.9	15.1		6.0	10.6
Sweet potato	31.4	2.0	2.3	5.8	0.9	9.0	8.9
Sago	3.9		2.1		0.9		0.1
Others	13.7	3.0	4.2	2.3	4.7	4.0	4.9
D) Footwear outside*							
Shoes	74.5	25.0	25.0	8.1	7.5	13.0	20.9
Sandals	92.2	64.0	81.3	75.6	75.7	78.0	76.0
Others	3.9				0.9	2.0	1.0
Barefooted	2.0	15.0	14.5	17.4	23.4	17.0	16.3
E) Place of bathing*							
Bathroom	70.6	73.0	58.3	5.8	26.2	21.0	38.8
Public bath house	7.8	13.0	16.7	5.8	1.9	8.0	8.1
River	23.5	3.0	16.7	87.2	71.0	4.0	36.2
Others	11.8	16.0	12.5	5.8	1.9	67.0	20.7
F) Frequency of bathing*							
Twice a day	84.3	99.0	72.9	91.9	91.6	95.0	91.3
Once a day	15.7	1.0	27.1	8.1	7.5	5.0	8.5
Every other day					0.9		0.2
G) Place of evacuation*							
Toilet in own house	70.6	31.0	39.6	7.0	65.4	26.0	38.2
Public toilet	11.7	53.0	6.3	1.2	17.8	9.0	18.5
Around house	11.7	4.0	6.3	32.6		13.0	11.0
Bush		5.0	45.8	43.0	13.1	4.0	16.7
River	17.6		6.3	88.4	3.7	3.0	19.3
Seaside		9.0		1.2		44.0	11.0

* With multiple answers.

Table 2

Prevalence of intestinal parasites among inhabitants in six areas of Likupang, North Sulawesi, Indonesia (positive rate by fecal examination, %).

Village/Sex	Kokole	Likupang-2	Wineru	Maen	Sarawet	Munte	Males	Females	Total
Aged less than 15									
No. samples examined	23	82	21	46	40	46	122	136	258
Helminths									
Ascaris lumbricoides	8.7	59.8	19.0	50.0	55.0	39.1	45.1	46.3	45.7
Trichuris trichiura		54.9	23.8	63.0	60.0	30.4	45.9	44.9	45.3
Hookworms	52.2	24.4	71.4	80.4	45.0	45.7	52.5	43.4	47.7
Strongyloides stercoralis		1.2						0.7	0.4
Enterobius vermicularis					5.0		0.8	0.7	0.8
Protozoans									
Entamoeba histolytica				6.5	10.0		2.5	2.9	2.7
E. hartmanni				4.3			1.7		0.8
E. coli	4.3	4.9	9.5	15.2	10.0	6.5	8.2	8.1	8.1
Endolimax nana	4.3		4.8	8.6	7.5		4.1	2.9	3.5
Iodamoeba buetschlii				4.3	2.5		0.8	1.5	1.2
Giardia intestinalis		8.5		4.3	7.5	2.2	4.1	5.9	5.0
Blastocystis hominis	4.3	8.5	9.5	4.3			4.9	4.4	4.7
Aged 15 or more									
No. samples examined	28	15	26	37	34	21	57	104	161
Helminths									
Ascaris lumbricoides	7.1	53.3	11.5	32.4	11.8	9.5	21.1	18.3	19.3
Trichuris trichiura	10.7	53.3	11.5	35.1	35.3	14.3	29.8	24.0	26.1
Hookworms	78.6	73.3	100.0	89.2	94.1	95.2	91.2	88.5	89.4
Strongyloides stercoralis	3.6	6.7		2.7	2.9		5.3	1.0	2.5
Enterobius vermicularis									
Protozoans									
				2.7	5.9		1.8	1.9	1.9
E. hartmanni				2.7	2.9			1.9	1.2
E. coli		6.7	7.7	5.4	5.9	23.8	5.3	8.7	7.5
Endolimax nana	10.7	13.3	19.2	5.4	8.8	4.8	5.3	12.5	9.9
Iodamoeba buetschlii					2.9		1.8		0.6
Giardia intestinalis				2.7	2.9		1.8	1.0	1.2
Blastocystis hominis	7.1	6.7		2.7	2.9		1.8	3.8	3.1

day of culture. The liquid at the bottom of the test tube was examined under a dissecting microscope for the presence of nematode larvae. If a nematode larva was found, 10% formalin solution was added to fix the larva. Then the larva was identified under a light microscope. The identification of nematode larvae was based on Little (1981).

RESULTS

The positive rates of parasitic infections demonstrated are given in Table 2 by locality, sex and age group. Five nematode and 7 protozoan species were detected. In total, 85.0% and 19.8% of the inhabitants were proved to harbor nematodes and protozoa, respectively. No trematode or cestode eggs were observed. In the younger group (<15 years), *Ascaris lumbricoides, Trichuris trichiura* and hookworm infections were found in about a half of the total samples. In the older group (≥ 15 years), the positive rates of *Ascaris* and *Trichuris* infections were lower than in the younger group, while hookworm infection was found in about 90% of the samples (Table 2). The difference in positive rates of these nematode infections was

Table 3

Type of *Ascaris lumbricoides* eggs detected in the feces.

Village	Kokole	Likupang-2	Wineru	Maen	Sarawet	Munte	Total
No. samples examined	4	57	7	35	25	20	148
Fertilized eggs only	1	36	3	24	17	13	94
(%)	(25.0)	(63.2)	(42.9)	(68.6)	(68.0)	(65.0)	(63.5)
Unfertilized eggs only	3	9	4	7	5	5	33
(%)	(75.0)	(15.8)	(57.1)	(20.0)	(20.0)	(25.0)	(22.3)
Mixed		12		4	3	2	21
(%)		(21.1)		(11.4)	(12.0)	(10.0)	(14.2)

Table 4

Comparison of efficacy in detection of hookworm infection by three methods.

	Flotation	Harada-Mori culture	Agar-plate culture	No. of cases	%
	+	+	+	70	43.2
	+	+	−	9	5.6
	+	−	+	4	2.5
	+	−	−	22	13.6
	−	+	+	28	17.3
	−	+	−	15	9.3
	−	−	+	14	8.6
Detected cases	105	122	116	162	
Efficacy*	64.8	75.3	71.6		

\+ Detected; − not detected.

* $\frac{\text{Percentage of detected cases}}{\text{total cases}}$

not remarkable between sexes although they were generally slightly higher in males (Table 2).

There were marked differences in the parasite prevalence among localities. In Kokole, the positive rates of *Ascaris* and *Trichuris* infections were relatively low in both age groups, while that of hookworm infection was moderately high. On the contrary, in Likupang-2, the positive rates of *Ascaris* and *Trichuris* infections were greater than 50% in both younger and elder age groups, but that of hookworm infection in the younger group was about a half of that in Kokole (Table 2). In Maen and Sarawet, the positive rates of *Ascaris* and *Trichuris* infections in the younger group was almost same as in Likupang-2, but those in the older group were lower (Table 2).

The types of *Ascaris* eggs detected were determined in 148 samples as stated in Table 3. Unfertilized eggs were found from more than 50% of the infected inhabitants in Kokole and Wineru where *Ascaris* infection was less than 20% among the younger group (Tables 2, 3). On the contrary, fertilized eggs were passed in feces of more than 75% of the infected persons in the other 4 localities where *Ascaris* infection was found in more than 35% of the younger people (Tables 2, 3).

Hookworm infection was readily detected by

Table 5

Hookworm species identified on the basis of filariform larvae obtained by Harada-Mori culture or agar plate culture of feces.

Village	Kokole	Likupang[-2]	Wineru	Maen	Sarawet	Munte	Total
No. samples examined	12	15	22	49	29	22	149
N. americanus only	12	9	15	27	26	21	110
(%)	(100.0)	(60.0)	(68.2)	(55.1)	(89.7)	(95.5)	(73.8)
A. duodenale only		2		5			7
(%)		(13.3)		(10.2)			(4.7)
Mixed		4	7	17	3	1	32
(%)		(26.7)	(31.8)	(34.7)	(10.3)	(4.5)	(21.5)

agar-plate culture. However, the plates were often invaded by borbolid fly larvae and ants, which made the agar surface dirty preventing detailed observation. Careless handling of the culture dishes often made the glycerin solution flow on the agar-surface inhibiting development of nematode larvae. The efficacy in detection was compared with flotation and Harada-Mori culture on 162 fecal samples. As shown in Table 4, Harada-Mori culture was most effective in detection, followed by agar-plate culture.

On the 2nd to 4th day of agar-plate culture, hookworm larvae were at the rhabditoid stage while *Strongyloides* was at the stage of filariform larva or free-living adult. The trace made by a hookworm rhabditoid larva on the agar surface was large and clear, being easily distinguished from that by filariform larva of *S. stercoralis* when observed under a light microscope at magnification 40x (Figs 2, 3). It was also noticed that the rhabditoid hookworm larvae often invaded into the agar, while *S. stercoralis* larvae were restricted on the agar surface. The filariform larvae of hookworms trapped in the glycerin solution were easily identified because the key characteristics such as buccal "spear" and cuticular striations were well preserved (Figs 4-7).

The species identification of hookworm was made on filariform larvae from a total of 149 fecal samples (Table 5). *Necator americanus* was the predominant species, being observed in more than 85% of samples in every locality. In Kokole, only *N. americanus* was found, while in all other localities *Ancylostoma duodenale* was also detected although often mixed with *N. americanus*. *Strongyloides stercoralis* was detected at a very low positive rate (Table 2). *Strongyloides fuelleborni kellyi* was not observed.

DISCUSSION

The human intestinal parasite fauna in the Likupang area is characterized by a high prevalence of soil-transmitted nematodes as in most areas of Indonesia (Abadi, 1985; Carney *et al,* 1977a,b; Cross and Basaca-Sevilla, 1981; Cross *et al,* 1977; Purnomo *et al,* 1980; Stafford and Joesoef, 1976; Stafford and Dennis, 1980; Stafford *et al,* 1976, 1980). As shown in Table 1, many houses lack latrines, and people defecate around houses, bushes or in rivers, contaminating the environment with parasite eggs and protozoan cysts. Although some houses have water closets, many excreta are discharged directly to the external environment. Moreover, many persons are barefooted or use only simple footwear (Table 1). Thus, they are often exposed to oral and cutaneous infection by parasites. Although Minahasan people consume various kinds of animals, no zoonotic trematode or cestode was detected in the present survey. This may be related to the fact that the people eat these animals well-cooked. In response to the questionnaire, nobody answered that he loved to eat meat raw.

In Sulawesi, the prevalence of parasite species differs from area to area. In general, the level land

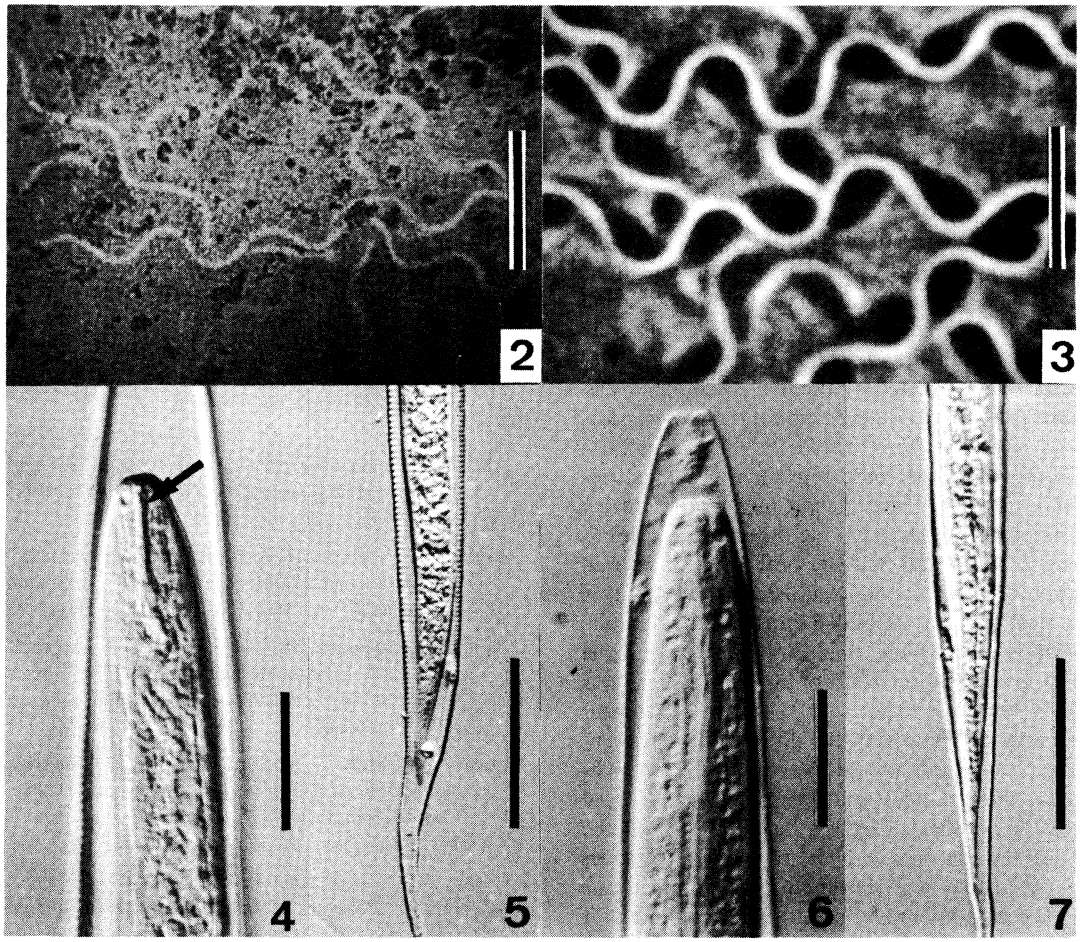

Fig 2—Furrows left by the filariform larvae of *Strongyloides stercoralis* on 4th day of the agar-plate culture (scale bar = 300μm).

Fig 3—Furrows left by the rhabditoid larvae of hookworm on 4th day of the agar-plate culture (scale bar = 300μm).

Fig 4-7—Filariform larvae trapped in glycerin solution around the agar-plate. 4- Cephalic portion of *Necator americanus* showing a distinct buccal "spear" (arrow) (scale bar = 20μm). 5- Caudal portion of *N. americanus* showing distinct cuticular striations on sheath (scale bar = 50μm). 6- Cephalic portion of *Ancylostoma duodenale* (scale bar = 20μm). 7- Caudal portion of *A. duodenale* (scale bar = 50μm).

of South Sulawesi has a high prevalence of *Ascaris* and *Trichuris* infections and low hookworm prevalence (Cross *et al,* 1972; Abadi, 1985). In the highlands of northern South Sulawesi and Central Sulawesi, hookworm infections are highly prevalent but *Ascaris* and *Trichuris* infections are less common (Carney *et al,* 1977a,b). In North Sulawesi, Stafford *et al* (1976) observed in the level land of Gorontalo high positive rates of *Ascaris* and *Trichuris* infections (average 72 and 79%, respectively) and slightly lower rate of hookworm infection (average 54%). Cross *et al* (1977) demonstrated in the level land and low hill areas of Minahasa Peninsula that hookworm infection was the commonest (average 59%), followed by *Ascaris* (45%) and *Trichuris* (22%) infections.

The present parasite rates were close to that recorded by Cross *et al* (1977) in North Sulawesi generally. However, the hookworm prevalence among elder inhabitants in the present survey (average 89.4%) was much higher than in any of previous surveys mentioned above. This may be

due to the difference of detection method. In the previous surveys, only direct smear, Kato's thick smear or formalin-ether concentration were used, and Harada-Mori culture was applied in limited cases. The use of the sensitive methods might have resulted in the high hookworm prevalence found in the present study.

In the present study, village to village differences in positive rates of the helminthic infections were noted (Table 2). In Kokole and Wineru, oral infection with nematodes seems to be relatively rare, although cutaneous infection occurs frequently, as suggested by the low *Ascaris* and *Trichuris* prevalence and the high ratio of *N. americanus,* a skin penetrator (Tables 2, 5). The high ratio of unfertilized *Ascaris* eggs in these localities (Table 3) may also indicate low worm burden (Komiya *et al,* 1962). On the other hand, in Likupang-2, Maen and Sarawet oral infection as well as cutaneous infection occurs commonly because the positive rates of orally-transmitted nematode infections were more than 30% even among the older group and the ratio of unfertilized eggs was 20.0% or lower (Tables 2, 3). These differences probably correlate with the difference in environmental sanitation and socio-economic levels. Kokole is the wealthiest village among the surveyed areas as is reflected by the fact that 70% of the houses have latrines and 75% of people wear shoes outside (Table 1). The economic development due to the recent boom of clove-tree cultivation might have brought an improvement of parasitological condition in some areas of Likupang.

The usefulness of the agar-plate culture in hookworm detection was proven. As shown in Table 4, the efficacy in detection was slightly lower than Harada-Mori culture. However, the efficacy of agar-plate culture was greatly reduced in the present study by the invasion of fly larvae or ants and also by careless handling. If more care is taken, the agar-plate culture can be a more sensitive and more reliable method for hookworm detection. It is an advantage of the agar-plate culture that hookworm and *Strongyloides* are easily distinguished by the shape of furrows left on the agar surface by their larvae on the 2nd to 4th day of culture, and species identification is easily made on the filariform larvae trapped in glycerin solution.

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