INTESTINAL PARASITIC INFECTIONS IN BEKASI DISTRICT, WEST JAVA, INDONESIA AND A COMPARISON OF THE INFECTION RATES DETERMINED BY DIFFERENT TECHNIQUES FOR FECAL EXAMINATION

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Abstract. This study was undertaken to determine the current status of intestinal parasitic infections among schoolchildren in West Java, Indonesia, and to compare the infection rates obtained by three different methods of fecal examination. A total of 285 fecal samples were collected from 131 males and 154 females at a junior high school. Samples were brought to the Department of Parasitology, Faculty of Medicine, University of Indonesia, and were examined for parasites by the Kato-Katz thick smear method (K-K). The residual samples were suspended in more than five volumes of 2% potassium dichromate solution and brought to the Department of Parasitology, Kobe University School of Medicine, Japan, where they were examined for parasites by the Army Medical School method (AMS III) and by the Sucrose Centrifugal Flotation method (SFL). The K-K revealed a total of two helminths with a prevalence of 10% (29/285). In contrast, nine species of parasites, 31% (89/285) positive, were obtained by AMS III, while 10 species, 22% (62/285) were found by SFL. Overall, 12 species of parasites were detected by the three methods: four species of nematoda (*Trichuris trichiura*, *Ascaris lumbricoides*, hookworm, and *Enterobius vermicularis*); five species of protozoa (*Giardia intestinalis*, *Entamoeba histolytica*-like cyst, *E. coli*, *Cyclospora* sp, *Blastocystis hominis*); two unidentified species of nematode eggs; and one unidentified species of mite egg.

INTRODUCTION

It is estimated that 3.5 billion people worldwide are infected with parasites. Soil-transmitted nematodes, such as *Ascaris lumbricoides*, hookworm, and *Trichuris trichiura*, are considered to be the main human parasites: there are now approximately one billion infections with each of these worldwide, resulting in 135,000 deaths per year (WHO, 1997). Chan (1997) focused on an attempt to quantify the disease burden caused by these infections using a recently formulated method: the calculation of Disability Adjusted Life Years (DALYs). According to this calculation, DALYs lost due to infection with intestinal helminths amount to 39, many more are lost to malaria (35.7) and schistosomiasis (4.5).

There have been many epidemiological studies of intestinal parasitic infections in tropical areas using fecal examination as the assay method. Although there are many fecal examination techniques used in field surveys, direct smear methods, primarily the modified Kato-Katz method, are frequently used because of their simplicity. It seems that the real burden of parasitic infection may not be revealed if a survey is carried out by direct smear methods because of their comparative inefficiency. This disadvantage is more evident in areas where

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the infection rate is low. *Cryptosporidium parvum* and *Cyclospora* are causative agents of diarrheal disease and are known to be emerging infectious diseases. Although there have been some reports concerning these protozoan parasites in Indonesia (Fryauff *et al*, 1996; 1999), parasites have been isolated from outcome patients or expatriates, and therefore the precise prevalence of these protozoa among ordinary inhabitants is not known.

The present study was undertaken to determine the current status of intestinal parasitic infections among schoolchildren in West Java, Indonesia, and to compare the infection rates obtained by three different methods of fecal examination. In addition, *Cryptosporidium parvum* and *Cyclospora* were examined by the centrifugal flotation method and viewed by fluorescence microscopy.

MATERIALS AND METHODS

A total of 285 fecal samples were collected from 131 male and 154 female students at a junior high school located in Tambun village, in the Bekasi District, West Java, Indonesia between the 14th and 16th August, 2000. The school is 30 km east of Jakarta. All of the students were approximately 14 years of age; the majority of them were Moslem. Fecal samples were brought to the Department of Parasitology, University of Indonesia, and examined for parasites. The residual samples were suspended in more than five volumes of 2% potassium dichromate solution and brought to the Department of Parasitology, Kobe University School of Medicine, Japan. In Japan, the fecal suspensions were centrifuged at 1,100*g* for 10 minutes, and the sediment thus obtained was considered to be ordinary feces and was used for the fecal examinations. The feces were examined with a direct smear method (Kato-Katz thick smear technique; K-K) and concentration methods (Army Medical School Technique; AMS III and the Sucrose Centrifugal Flotation Technique; SFL). K-K was used only for detecting soil-transmitted helminth eggs, and AMS III and SFL were used for detecting all parasites. *Cryptosporidium parvum* and *Cyclospora* were primarily examined by SFL: briefly, a sucrose solution with a specific gravity of 1.200 was added to 0.5 g of feces (sediment), mixed thoroughly, and centrifuged at 1,100*g* for 10 minutes. Surface solution was recovered using a bacteriological loop and viewed with a phase-contrast microscope (*Cryptosporidium*) or a fluorescence microscope (330-360 μm wavelength; x400 magnification) in the case of *Cyclospora*. Specimens showing no oocysts (cysts) in 40 fields were regarded as negative.

RESULTS

The parasites obtained in this study and their positivity in each test are shown in Table 1. K-K revealed two helminths with a prevalence of 10% (29/285). In contrast, nine species of parasites, 31% (89/285) positivity, were obtained by AMS III, and 10 species, 22% (62/285) positivity, were obtained by SFL. The recovery efficiency of the AMS III was three times higher than that of K-K. Positive rates for male and female students, obtained by AMS III, were 34% and 28%, respectively. Infection rates for each parasite were the highest by AMS III method, except for two cases of *Enterobius vermicularis* and an unidentified mite egg. Overall, 12 species of parasites were detected by the three methods (Table 1): four species of nematoda (*Trichuris trichiura*, *Ascaris lumbricoides*, hookworm, and *Enterobius vermicularis*); five species of protozoa (*Giardia intestinalis*, *Entamoeba histolytica*-like cyst, *E. coli*, *Cyclospora* sp, *Blastocystis hominis*); two unidentified species of nematode eggs; and one unidentified species of mite egg. Of the parasites detected, *T. trichiura* infection was the most common (21% by AMS III), followed by *G. intestinalis* (6%) and *A. lumbricoides* (6%). The infection rate of *T. trichiura* 8% by K-K; this rate increased to 21%, almost threefold, when feces was examined by AMS III.

Of the 16 *A. lumbricoides*-positive samples

Table 1
Parasitic infections among schoolchildren in West Java, Indonesia.

Parasites detected	Prevalence (no. of positive samples) by method		
	K-K	AMS III	SFL
Trichuris trichiura	8 (23)	21 (59)	18 (50)
Ascaris lumbricoides	3 (9)	6 (16)	2 (7)
Hookworm	0 (0)	1 (2)	0 (1)
Enterobius vermicularis	0 (0)	0 (0)	1 (2)
Giardia intestinalis	ND	6 (17)	1 (2)
Entamoeba histolytica[a]	ND	2 (6)	0 (0)
Entamoeba coli	ND	2 (6)	0 (1)
Cyclospora sp	ND	0 (1)	0 (1)
Blastocystis hominis	ND	Positive[b]	positive
Unidentified (2 species)	ND	1 (2)	0 (1)
Unidentified mite egg	ND	0 (0)	1 (3)
Total	10 (29/285)	31 (89/285)	22 (62/285)

[a]*Entamoeba histolytica*-like cyst.
[b]Although the presence of *B. hominis* was confirmed, the infection rate was not obtained.

observed by AMS III, three samples (19%) had fertilized eggs, 11 (69%) had unfertilized eggs, and two samples (13%) had both types of egg. The small, round organisms (11-13 μm in diameter), each having a distinct oocyst wall, were stained with iodine. If the organism contained four nuclei and a central karyosome, we identified it as an *E. hisotlytica*-like cyst. We did not differentiate *E. histolytica* from *E. dispar*. *Cyclospora* was found in two of 285 specimens (1%). We found *B. hominis* in some specimens, but we did not systematically observe and record the infection rate. Three students (all females) excreted unidentified eggs, each of which contained an immature mite (Fig 1).

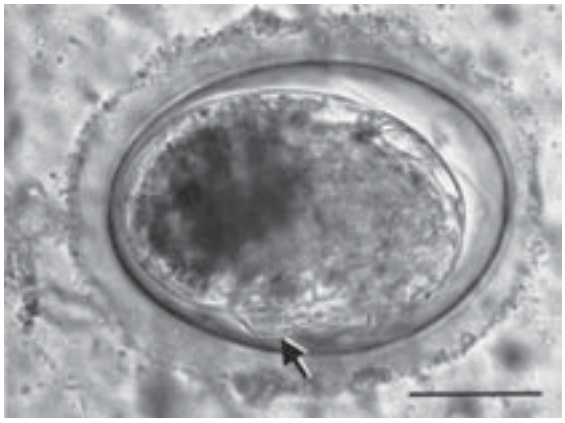

Fig 1–Mite egg recovered from a 14-year-old girl. The egg (120 x 170 μm) has an immature mite, part of the leg of which is shown by the arrow (Bar = 50 μm).

Table 2 shows the number of false negatives produced by AMS III and SFL, respectively. K-K, revealed that 23 samples were positive for *T. trichiura*. This result was used as a baseline and was compared with the results obtained by AMS III and SFL. In AMS III, five (22%) of 23 samples were judged to be negative (false negative); this rate increased to 43% when SFL was used. In the case of *A. lumbricoides*, the rate of false negatives obtained by AMS III was 11% (1/23), which was significantly lower ($p<0.05$; χ^2 test) than that of *T. trichiura* by the same technique. False negative results of *T. trichiura* and *A. lumbricoides* by SFL were two to five times

Table 2
Number of false negatives: AMS III and SFL techniques.

Parasites	No. of positives by K-K	No. of positives and negatives by AMS-III		SFL	
		+	-	+	-
T. trichiura	23 (100)	18 (78)	5 (22)	13 (57)	10 (43)
A. lumbricoides	9 (100)	8 (89)	1 (11)	4 (44)	5 (56)

Figures in parentheses are percentages.

Table3
Number of false negatives in: K-K technique.

Parasites	AMS-III	K-K	
	No. positive	No. positive	No. negative
T. trichiura	59 (100)	18 (31)	41 (69)
A. lumbricoides	16 (100)	8 (50)	8 (50)

Figures in parentheses are percentages.

higher than those obtained by AMS III.

In the same manner, the false negative results produced by K-K were evaluated (Table 3). Of the 285 fecal samples examined, 59 were positive for *T. trichiura* and 16 for *A. lumbricoides* by AMS III (these results were re-examined, and the positivity was confirmed). False negative results obtained through K-K were 50-69%.

Among the 106 positive children detected by any of the three techniques, 82 (77%) were infected with one species of parasite; 19 (18%) were infected with two species; 4 (4%) were infected with three species; and one (1%) was infected with four species of parasites - *T. trichiura*, *A. lumbricoides*, hookworm, and *B. hominis*.

DISCUSSION

Of the 285 fecal samples examined, 89 (31%) were revealed by AMS III to be positive for one or more parasites. This prevalence was much lower than that reported from other places in Indonesia: an 84% positive rate among school-children in West Java (Pegelow *et al*, 1997); a 58% (*A. lumbricoides*) rate in schoolchildren in northern Jakarta (Hadidjaja *et al*, 1998); rates of 85-98% among the inhabitants of other islands (Stafford and Joesoef, 1976). Chan (1997) reported that the proportions of the world's population that are infected with each of these parasites have remained virtually unchanged during the past 50 years. On the other hand, a rapid decline in parasitic infection has been observed among schoolchildren in Taiwan (Chen *et al*, 1991) and Korea (Chai and Lee, 1992). The same phenomenon could have occurred in Jakarta, Indonesia, because the control program on soil-transmitted helminthiases has been carried out since 1987.

The K-K method is known to be simple and semi-quantitative, and is widely used for field surveys in developing countries. The

recovery efficiency of this method has been thoroughly evaluated. Higgins *et al* (1984) compared the recovery efficiency of the McMaster technique with K-K, and found no difference in detection sensitivity. Jones (1976) reported the same recovery rates for both the direct smear and formalin-ether sedimentation methods; similar results were also obtained by Stafford and Joesoef (1976). However, in the present study the detection sensitivity of AMS III was superior to that of K-K. In this study, the K-K method was carried out by Indonesian scientists, and AMS III and SFL were carried out by Japanese scientists. All of the scientists involved in the study had more than 20 years' experience in fecal examinations and it therefore seems likely that there were significant interoperant differences. The differences in the infection rates or the rates of false negatives are likely to be due to the detection sensitivities of the different techniques, indicating that the technique used in a survey might affect its result. The false negative rates of K-K for *T. trichiura* and *A. lumbricoides* were found to be 50% and 69% respectively, indicating that the results obtained by K-K may underestimate the actual prevalence.

Hospital-based cryptosporidiosis study was conducted in Surabaya, East Java, by Katsumata *et al* (1998). They found *C. parvum* oocysts at the rates of 3% in diarrheal and 1% of non-diarrheal patients. We used SFL for *C. parvum*, although no positive was found. Fryauff *et al* (1996) examined small number of expatriates who visited a clinic in Jakarta: *Cyclospora* and *G. intestinalis* were found to be equally common and it was concluded that these protozoa are the dominant pathogenic intestinal parasites in Indonesia; a similar observation was done by Fryauff *et al* in 1999. In our study however, infection rate of *Cyclospora* was only 1%, which was significantly lower than that of *G. intestinalis* (6%). Although there have been many similar fecal examination studies carried out in other areas of Indonesia (Higgins *et al*, 1984; Mangali *et al*, 1994; Pegwlow *et al*, 1997; Toma *et al*, 1999), no report has mentioned mites, probably because the presence of mite eggs is due to eating food that is contaminated with mite eggs. However, if we consider the infection rate (1%) and the number of eggs found per specimens (5-10 eggs per drop of AMS III final concentrate), the infection may not actually be due to the ingestion of contaminated food.

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