SPECIAL REPORT*
INTESTINAL PROTOZOAN INFECTIONS IN MALAYSIA

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Abstract. Intestinal protozoa are found in all communities in Malaysia and among all ethnic groups. Prevalence of intestinal protozoa is not affected by ethnicity but by living conditions. Communties with both basic amenities of safe water supply and proper toilets have lower prevalence than those with one or none of the amenity.

Cryptosporidium is an important intestinal protozoon in Malaysia and should be included in future field and laboratory studies and also in laboratory diagnosis for pathogens. Much interest will be centered on *Blastocystis hominis* in future studies in view that it may be a cause of diarrhea.

INTRODUCTION

An extensive study covering 6 states in Peninsular Malaysia on intestinal parasitic infections done by Bergner and Tantalo (ICNND, 1963) showed that intestinal protozoon infections in humans were commonly found in both urban and rural areas. Other studies also gave the prevalence of intestinal protozoon infections on island populations (Heyneman *et al,* 1967; Nawalinski and Roundy, 1978), among various communities (Sinniah *et al,* 1978; Che Ghani *et al,* 1987; Khairul Anuar and Afifi, 1990), among children (Bisseru and Abdul Aziz, 1970; Sinniah, 1984; Hamimah *et al,* 1982; Kan *et al,* 1987), among different ethnic groups from mainland peninsula (Bisseru and Abdul Aziz, 1970; Che Ghani *et al,* 1987; Khairul Anuar and Afifi, 1990) and in East Malaysia (Christic, 1915; Neo *et al,* 1987; Kan *et al,* 1987; Levy, 1988).

The intestinal protozoa covered by the papers above were mainly *Entamoeba histolytica* and other amebae, and *Giardia intestinalis* and other flagellates. Recently *Cryptosporidium* infection in man has been reported in Malaysia (Mendez *et al,* 1988; Mat Ludin *et al,* 1991; Ng, 1992).

The present paper gives information on human intestinal protozoon infections collected from 1982 to the present (June 1992) by the Division of Parasitology, Institute for Medical Research, Kuala Lumpur. Many of the surveys reported here were part of other studies. The paper also reports on the incidence of *Cryptosporidium* and *Blastocystis* observed in the laboratory over a two-year period.

MATERIALS AND METHODS

In all the stool surveys, labeled containers were distributed to individuals from house-to-house for stool collections and the people were given three days to submit a specimen. Stools were collected daily and the samples were fixed in polyvinyl alcohol immediately in the field. Smears of stool samples were then stained in trichrome and examined for intestinal protozoa in the laboratory. In two studies, stools were collected from children admitted due to gastroenteritis and from children having diarrhea in some villages and these were examined for *Cryptosporidium* only.

Stool samples, usually from chronic diarrheic cases, were submitted by clinicians to the Division of Parasitology. These were examined for protozoa using direct smears, zinc sulphate concentration, trichrome-stained smears and culture in Silva's medium. Semi-diarrheic and watery stools were also stained by the modified Ziehl-Neelsen technique.

RESULTS

General surveys of intestinal protozoan infections

Ten surveys were carried out in various parts of Malaysia, 8 in Peninsular Malaysia and two in Sarawak. Table 1 shows the study areas and the type of communities covered. The types of water supply and the places of defecation are also given.

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Table 1

Surveys for intestinal protozoa in Malaysia.

Year of survey	Place	Ethnic group	Type of community	Main water source	Main places for defecation
Peninsular Malaysia					
1982	Tembeling	mainly Malays	riverine, small-scale farming	river	river bank
1984	Kuala Selangor	mixed	rice growing	treated pipe water	mainly pour-flush
1985	Nenggiri	Orang Asli	riverine, jungle	river	river bank
1986	Pergau	mainly Malays	riverine, rubber small-holdings	river	mainly pour-flush
1987	Lebir	Malays	riverine, rubber small-holdings	river	pour-flush
1987	Ulu Jelai	Orang Asli	riverine, jungle	gravity feed	river indiscriminate
1987	Baling	Malays	rice and rubber	stream	indiscriminate
1991	Bukit Kemandul	Orang Asli	village adjacent to large estates	concrete wells	indiscriminate
Sarawak					
1987	Batang Ai	mainly Ibans	villages adjacent to hydroelectric dam	mainly treated pipe water	pour flush
1989	Bakun	7 local tribes	riverine, small scale farming	gravity feed river	pour flush

Table 2 shows the results of intestinal protozoa which were based on examination of trichrome-stained smears. Protozoan cysts were mainly observed but occasionally trophozoites were also seen. The protozoa without cyst forms, like *Dientamoeba fragilis* and *Trichomonas hominis,* were sometimes seen but because most trophozoites degenerated soon after they were passed out with stool and were often not recognisable, their prevalence rates could not be reported. From 1989, we included *Blastocystis hominis* in our studies.

The total number of people covered was 7,995, and the average response was 50.3% (range 32.7-67.9%). The overall rates for *Entamoeba histolytica* and *Giardia intestinalis* were 8.4% (5.1-21.8%) and 11.1% (5.2-19.1%) respectively. The other *Entamoeba* observed included *E. coli* (13.1%, 4.8-36.1%), *E. hartmanni* (1.1%, 0-5.2%), *E. polecki*

Table 2

Intestinal protozoan infections in various parts of Malaysia.

Place	No. persons	No. examined	Percent with *E. histolytica*	*G. intestinalis*	*E. coli*	*E. nana*	*I. butschlii*	*E. hartmanni*	*C. mesneli*	*E. polecki*	*B. hominis*
Category 1: treated pipe water and pour-flush toilets											
Kuala Selangor	1,410	461	1.5	5.2	4.8	2.6	2.4	0.0	0.0	0.0	-
Category 2: gravity feed water and pour flush toilets											
Batang Ai	421	241	5.8	7.9	6.6	6.2	3.3	2.1	0.0	0.4	-
Bakun	1,821	767	5.1	9.1	13.8	10.2	5.1	0.8	0.5	0.1	5.0
Total	2,242	1,008	5.3	8.8	12.1	9.2	4.7	1.1	0.4	0.2	-
Category 3: gravity feed/concrete wells and no toilets											
Jelai	392	266	12.0	16.9	36.1	12.4	8.6	2.3	4.9	0.8	
Bukit Kemandul	364	196	5.1	13.3	15.3	4.6	5.1	0.5	1.5	0.0	9.2
Total	756	462	9.1	15.4	27.3	9.1	7.1	1.5	3.5	0.4	-
Category 4: river vater and pour-flush toilets											
Pergau	874	558	9.7	9.5	11.6	16.1	8.1	0.9	0.0	0.0	-
Lebir	782	489	11.2	10.6	12.5	11.2	6.5	2.2	1.6	0.0	-
Total	1,656	1,047	10.4	10.0	12.0	13.8	7.4	1.5	0.8	0.0	-
Category 5: river/stream water and no toilets											
Tembeling	876	445	12.6	19.1	-	-	-	-	-	-	-
Nenggeri	718	407	7.4	13.3	20.9	5.7	5.7	0.2	0.0	0.2	-
Beling	337	193	21.8	9.8	24.4	22.8	14.5	5.2	0.5	1.0	-
Total	1,931 (1,055)	1,045 (600)	12.2	15.1	22.0	11.2	8.5	1.8	0.2	0.5	-

(0.2%, 0-4.9%). Other amebae observed were *Iodamoeba butschlii* (5.4%, 2.4-14.5%) and *Endolimax nana* (8.9%, 2.6-22.8%). The other flagellate beside *G. intestinalis* often encountered was *Chilomastix mesneli* (0.7%, 0-4.9%). *B. hominis* (5.8%, 5-9.2%) was sought for only in the last two surveys.

Intestinal protozoa are mainly transmitted through drinking water, food or fingers which had been contaminated by feces. Therefore the type of water supply for normal household use and the places where people defecate would affect the prevalence of these protozoons. In view of these,

Table 3

Scores for intestinal protozoan infections.

Place	Ranks for infection rates (high score = low rate)								Total score
	E. histolytica	*G. intestinalis*	*E. coli*	*E. nana*	*I. butschlii*	*E. hartmanni*	*C. mesneli*	*E. polecki*	
Category 1: treated pipe water and pour-flush toilets									
	5	5	5	5	5	5	5	4.5	39.5
Category 2: gravity feed water and pour flush toilets									
	4	4	3	3	4	4	3	3	28
Category 3: river water and pour-flush toilets									
	2	3	4	1	2	2.5	2	4.5	21
Category 4: gravity feed/concrete wells and no toilets									
	3	1	1	4	3	2.5	1	2	17.5
Category 5: river/stream water and no toilets									
	1	2	2	2	1	1	4	1	14

the communities in the 10 surveys were divided into five categories (Table 2) : 1) with basic amenities of safe water supply (treated pipe water) and proper toilets (pour-flush); 2) with water supply generally uncontaminated by feces (gravity feed) and proper toilets (pour-flush); 3) with proper toilets (pour-flush) but water supply often contaminated by feces (river); 4) with water supply generally uncontaminated by feces (gravity feed or concrete wells) but with no toilet facilities; and 5) with water supply often contaminated by feces (stream/river) and no toilets (indiscriminate defecation or along the river banks).

Ranking scores (1-5) were given to the prevalence rates of 8 intestinal protozoa in the 5 categories with the highest score given to the lowest prevalence (Table 3). Kuala Selangor, belonging to the first category, had the lowest prevalence rates for all the intestinal protozoa and a total score of 38.5. The second category included two communities from Batang Ai and Bakun in Sarawak, whose total score was 28. Pergau and Lebir came under the third category with a total score of 21. The fourth category included two Orang Asli communities, Jelai and Bukit Kemandul, and the total score was 17.5. The last category, consisting of Tembeling, Nenggeri and Baling, had a total score of 14. Statistically it was found that the first category was significantly different from the other 4 categories ($p = 0.001$). There fore the communities with both basic amenities of safe water supply and proper toilets have lower prevalence of intestinal protozoa than those communities with one or none of the amenity.

Table 4 shows the prevalence of amebiasis and giardiasis among the ethnic groups. In Peninsular Malaysia, the Malays (11.1%) had the highest prevalence of amebiasis, followed by the Orang Asli (8.6%). The Sarawakian tribes (6.0%) had lower amebiasis prevalence than the former two ethnic groups. Prevalences of giardiasis among Orang Asli, Malays and the Sarawakian tribes were 13.8%, 11.8% and 9.7%, respectively. There are three ethnic groups in Kuala Selangor in Peninsular Malaysia. The Chinese had the lowest amebiasis rate (0.9%) but the highest giardiasis rate (6.0%). The Indians had the highest amebiasis rate (2.3%) and a giardiasis rate (5.7%) similar to that of the Chinese. The Malays had amebiasis (2.1%) close to that of the Indians and the lowest

Table 4

Protozoan infections among the ethnic groups in Malaysia.

Ethnic group	No. examined	Intestinal amebiasis	Giardiasis
Without both basic amenities of safe water supply and toilets:			
Peninsular Malaysia :			
Malays*	1,689	11.1	11.8
Orang Asli**	998	8.6	13.8
Sarawak :			
Local tribes***	1,008	6.0	9.7
With both basic amenities of safe water supply and toilets:			
Kuala Selangor in Peninsular Malaysia :			
Malays	140	2.1	3.6
Chinese	234	0.9	6.0
Indians	87	2.3	5.7

*Data from Tembeling, Kuala Selangor, Baling, Pergau and Lebir
**Data from Tembeling, Nenggiri, Pergau, Ulu Jelai and Bukit Kemandul
***Data from Bakun and Batang Ai

giardiasis rate (3.6%).

The prevalence rates of both amebiasis and giardiasis according to age and sex are presented in Table 5. The age groups were divided into only four categories because the under seven year olds were preschool children, the 7-12 year old children were attending primary schools, the 13-18 year olds were mostly going to secondary schools; and the rest were all considered as adults.

There was no difference between the males and females with respect to amebiasis and giardiasis. Amebiasis was higher among the adults with women having a higher rate than men. There was a small peak in prevalence among the primary school children followed by the secondary school children having the lowest amebiasis rate. The boys under seven appeared to have a slightly higher amebiasis rate than the girls. Giardiasis was highest among the primary school children, followed by the preschool children, among whom girls had a slightly lower rate than boys. The adults had quite high rates compared to those of the secondary school children. Among the latter, the girls had 3 times the rate of the boys.

Surveys on cryptosporidiosis

Two studies were carried out for cryptosporidiosis. One was carried out in a hospital in Selangor in 1988 and out of 158 cases of children under 5 years old, 4.4% were positive (Mendez *et al,* 1988). In the second study carried out in Kelantan in 1990, 131 stool samples were collected from children under 7 years of age from a hospital and 47 stool samples were obtained from children of the same age groups having diarrhea in a few villages. Cryptosporidiosis rates were 11.4% and 10.6% among diarrheic children in the hospital and in the community, respectively.

Routine examination of chronic diarrheic cases

The Division of Parasitology also receives stool samples of patients with chronic diarrhea. The types of intestinal protozoa diagnosed from the samples obtained from July 1990 to June 1992 are presented. Stool samples from 83 patients

Table 5

Intestinal amoebiasis and giardiasis in Malaysia according to age and sex.

Age (years)	Sex	No. examined	Intestinal amebiasis	Giardiasis
<7	males	314	6.4	19.1
	females	283	3.9	15.5
7-12	males	327	11.1	22.0
	females	296	10.6	21.9
13-18	males	88	2.9	1.3
	females	108	2.8	4.6
>18	males	556	16.9	12.1
	females	645	24.7	13.4
Total	males	1,285	9.1	13.3
	females	1,332	8.9	11.8
	Total	2,617	9.0	12.5

Data from Lebir, Tembeling, Jelai, Bakun and Bukit Kemandul.

were examined for the usual intestinal protozoa while 46 stools, which consistency was diarrheic or semi-diarrheic, were also examined for *Cryptosporidium.* Among the stools examined for *Cryptosporidium,* 23.9% were positive. Among all the stool examined, *Blastocystis hominis* (19.3%) was the most common protozoon seen. *Entamoeba histolytica* was recovered from only one patient (1.2%) and *Giardia intestinals* from 2 other cases (2.4%). *Trichomonas hominis* (3.6%) and *E. coli* (2.4%) were the other protozoa identified.

DISCUSSION

Most intestinal protozoa reported in this paper have been reported in other communities in other parts of Malaysia except *E. polecki* which we observed in 5 communities and *B. hominis* which was sought for in our latest two surveys. Other protozoa that we did not encounter in humans were *Balantidium coli* and *Isospora* species which were recorded on Penang Island by Khairul Anuar and Afifi (1990).

Ow-Yang (1971) reviewed reports of intestinal protozoan infections published from 1900 to 1969. He noted that the earlier studies were mainly on amebic dysentery. Christie (1914) from his observations on hospital cases concluded that 30-40% of the entire population in Sarawak were amebic dysentery carriers. He later studied a community along a river bank and found 59% were positive for amebae. The rates of *E. histolytica* in Asian was 14.5% and giardiasis was 4.2% and infections were higher among the Tamils than Chinese (Fletcher and Jepps, 1927). Davis and Anandan (1969) found 14% of Orang Asli with bloody diarrhea to have *E. histolytica.*

Kan (1988) reviewed publications on amebiasis and giardiasis in Malaysia from 1970 to 1987 and found the prevalence rates from these studies to be from 1.0% to 14.4% for amebiasis and from 2.6% to 25.0% for giardiasis. She also noted that the prevalence was usually under-reported because of many disadvantages in examination of single stool samples. Thus, degeneration of the protozoa, especially the trophozoites, can occur, before the stool samples could be processed; light infections may be missed even by skilled personnel and furthermore, *Giardia* cysts are passed out sporadically.

In previous studies on intestinal protozoa,

many different techniques were used for stool examination. Some techniques have advantages for recovering and/or recognising the protozoa, thereby higher prevalence estimates would be expected. A good method for the study of intestinal protozoa employs preservation of stool in polyvinyl alcohol, followed by trichrome staining. Che Ghani *et al* (1987) even supplied polyvinyl alcohol for direct fixation of stools by individuals in their study. Thus they would probably had detected more cases than other studies. Nevertheless, in spite of technique differences, some comparisons can still be made between studies.

Kan *et al* (1987) reported the prevalence of *E. histolytica* to be as low as 0.8% among the Penans in Sarawak while Neo *et al* (1987) observed a high prevalence of 18.9% among the Ibans also in Sarawak. The present paper reports an overall intestinal amebiasis prevalence of 8.4% with a high of 21.8% in Baling and a low 1.5% in Kuala Selangor, while in Sarawak the prevalence was 5.3% (5.1-5.8%) among the local tribes.

Prevalence of giardiasis was reported to be as low as 1.4% among fishermen in Penang (Khairul Anuar and Afifi, 1990). The overall prevalence of giardiasis in the IMR surveys was 11.1% with the highest in Tembeling (19.1%) and the lowest in Kuala Selangor (5.2%). In Sarawak, the prevalence was 8.8% (7.9-9.1%).

All the surveys reported in this paper were carried out in rural areas and most of the communities studied were of low socio-economic status. Most did not have proper sanitation nor safe water supply except the rice-growing community in Kuala Selangor. Scores were given to the prevalences of protozoon infections grouped into 5 categories depending on the presence or absence of safe water supply and toilets. It was found that prevalence of intestinal protozoa is significantly lower in communities with both basic amenities of safe water supply and proper toilets compared to those with only one or no such amenity. This confirms my previous study (Lai *et al,* 1990) that the introduction of proper-toilets and the availability of piped water, safe for consumption, to an agriculture land scheme village, Ulu Rening in Selangor, resulted in a significant reduction of amebiasis and giardiasis.

In all the previous reports (ICCND, 1963; Bisseru and Abdul Aziz, 1970; Dunn, 1972; Dissanaike *et al,* 1977; IMR, 1977; Nawalinski and Roundy, 1978; Sinniah, 1984; Che Ghani *et al,* 1987; Kan, 1987; Neo *et al,* 1987; Levy, 1988; Khairul Anuar and Afifi, 1990), the prevalences of amebiasis and giardiasis among the different ethic groups were very varied. The Malays had prevalences of 1.2-18.9% for amebiasis and 0.9-17.0% for giardiasis. The Chinese had amebiasis and giardiasis rates ranging from 0% to 16.4%, and 1.5-10.5%, respectively. Prevalences of amebiasis and giardiasis for Indians were 6.1-16.6% and 2.9-9%, respectively. The Orang Asli had prevalence ranges of 1-8.7% for amebiasis and 4.8-25.0% for giardiasis.

The present data show that, generally, there was little difference in the prevalence rates of amebiasis and giardiasis in three ethnic groups, Malays, Orang Asli and the local tribes of Sarawak. This was because most of the people in these groups lived in communities without both basic amenities of safe water supply and toilets. The three ethnic groups, Malays, Chinese and Indians from Kuala Selangor had similar low prevalences for the two infections as they all had both safe water supply and proper toilets. Therefore the factors influencing prevalence of intestinal protozoa were the living conditions like water supply and toilets rather than ethnicity. Che Ghani *et al* (1987) also found similar prevalence rates of amebiasis and giardiasis amongst Malays, Chinese and Indians in urban slums and concluded that this finding could be due to same source of infection, possibly the water supply in the village.

Similarly in previous reports, the prevalences of amebiasis and giardiasis in rural communities were 0.8-18.9% and 1.4-22.8%, respectively while urban communities had prevalences of 2.7-16.2% for amebiasis and 3.1-9.6% for giardiasis. Within the rural or urban communities, living conditions varied from area to area thus the prevalence ranges were large. Che Ghani *et al* (1987) showed people living in rural villages and urban slums to have higher prevalence rates than medical students, who presumably lived in the city.

This report shows no difference in prevalence rates among males and females for both amebiasis and giardiasis. Differences were seen in amebiasis rates of adults, with females having higher rates than males. This was also observed in another IMR study at Ulu Rening (IMR, 1977). ICCND (1963) and Che Ghani *et al* (1987) reported higher

amebiasis rates among females than males while Sinniah (1984) observed school boys having higher rates than girls.

In the studies reported in this paper, the adults had highest amebiasis rates. This was also observed by Nawalinski and Roundy (1978); Sinniah *et al* (1978) and Khairul Anuar and Afifi (1990). The giardiasis prevalence was high among preschoolers and peaked among the primary school children, followed by very low rates among the secondary school children but increased again amongst the adults. As age groupings were very different in various reports, it is not possible to make comparisons except that in three reports, children up to about 10 years had the highest prevalence for giardiasis (ICCND, 1963; IMR, 1977; Sinniah *et al,* 1978).

Cryptosporidium species require special staining techniques for identification so they are usually not sought for in general intestinal protozoae surveys. It was only recently recognized as a cause of diarrhea in humans and has been reported only three times in Malaysia. Cryptosporidiosis incidence of 4% was observed in two studies carried out in pediatric wards in Selangor (Mendez *et al,* 1988) and in Penang (Mat Ludin, 1991). Ng (1992) reported a lower rate of 2% among children from a hospital in Kuala Lumpur. The present paper reports an incidence of 11% among pediatric patients from Kelantan and 10% among diarrheic young children from a community in Kelantan. The prevalence of cryptosporidiosis in normal populations needs to be studied.

The Division of Parasitology began offering diagnosis for *Cryptosporidium* of diarrheic stools only recently. Most of these cases had been screened routinely for other pathogens before a stool sample was sent to our laboratory. A relatively high percentage (23.9%) of positives was obtained over a two-year period. In view of this and the surveys reported above, it is recommended that the diagnosis of *Cryptosporidium* should be routinely checked in clinical laboratories in Malaysia.

The other protozoon of interest is *Blastocystis* which had a high incidence (19.3%), mainly among chronic diarrheic cases received in our laboratory over a two-year period. It is still being debated whether this protozoon is a causative agent of diarrhea.

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