Tropical Biomedicine 24(1): 55–62 (2007)

Prevalence of intestinal protozoa in an aborigine community in Pahang, Malaysia

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Received 22 November 2006; received in revised form 16 February 2007; accepted 21 February 2007

Abstract. The objective was to estimate the prevalence of intestinal protozoa among the aborigines and to determine the problems regarding the infection. The study was carried out in January 2006 in Pos Senderut, Pahang, Malaysia. Samples of faeces were collected from children and adults and these were fixed in PVA and trichrome staining was carried out. From the 130 individuals studied, 94 (72.3%) were positive with at least one intestinal protozoa. Nine intestinal protozoa namely *Blastocystis hominis*, *Giardia lamblia*, *Entamoeba histolytic*a, *Entamoeba coli*, *Endolimax nana*, *Entamoeba hartmani*, *Entamoeba polecki*, *Iodamoeba butschlii* and *Chilomastix mesnili* were detected. The prevalent species were *B. hominis* (52.3%), followed by *G. lamblia* (29.2%), *E. coli* (26.2%) and *E. histolytica* (18.5%). The other species ranged from 1.5 to 10.8%. Among the positive samples, mixed infection with *E. histolytica* and *G. lamblia* was 3.8%, *E. histolytica* and *B. hominis* was 15.4%, *G. lamblia* and *B. hominis* was 17.7%. Triple infection of *E. histolytica*, *G. lamblia* and *B. hominis* was 3.1%. The infection was more prevalent in children below 10 years age group (45.4%) and lowest in the age above 60 years (3.8%). The high prevalence was attributable to poor environmental management, poor personal hygiene and lack of health education.

INTRODUCTION

Intestinal parasitic infections have a worldwide distribution. High prevalence is found in people with low socio-economic status, poor living condition, over crowded areas, poor environmental sanitation, improper garbage disposal, unsafe water supply and unhygienic personal habits. These factors are the cause of major proportion of burden of the disease and death in developing countries (Adamu *et al.*, 2006).

Intestinal parasites are organisms that live in the hosts' intestine and take up the nutrition from the host, and cause abdominal discomfort, dysentery, mechanical irritation of intestinal mucosa, malabsorption syndromes and obstruction. They can be transmitted by fecal-oral route. Children with parasitic infections are usually undernourished and weak. Numerous protozoa inhabit the gastrointestinal tract of humans. This list includes representatives from many diverse protozoan groups. The majoritiy of protozoa are non-pathogenic commensals, or their presence only result in mild disease and also the pathogenic forms (*Giardia lamblia*, *Entamoeba histolytica* and *Blastocystis hominis*). Some of these organisms can cause severe disease under certain circumstances such as severe acute diarrhoea which may lead to chronic diarrhoea, nutritional disorders, dysentery and potentially lethal systemic disease. Factors which increase the likelihood of ingesting material contaminated with fecal

material play a role in the transmission of intestinal protozoa. In general, situations involving close human to human contact and unhygienic conditions promote transmission (Ichhpujani & Bathia, 1998).

Entamoeba histolytica is a protozoan parasite that causes amoebic colitis and liver abscess in developing countries such as Mexico, India and Bangladesh (Gosh *et al.*, 2000). More than 500 million people worldwide are infected and up to 110 000 of those infected die every year (Troll *et al.*, 1997). In a study in Northern Brazil, the prevalence for *E. histolytica* or *E. dispar* was found to be 4.1% (Pinheiro *et al.*, 2004). In Malaysia, Norhayati *et al.* (2003) showed the prevalence of amoebisais varied from 1-14% and Rajeswary *et al.* (1994) showed prevalence of *E. histolytica* among children ranged from 9.0-15.4 with highest in Orang Asli (aborigine) when compared to other ethnic group children. Higher prevalence of *E. histolytica* (21%) was reported by Nor Aza *et al.* (2003).

Giardia lamblia was the most common intestinal protozoan infection and it was found especially in temperate and tropical countries. The prevalence rate of giardiasis was 2 to 5% in developed countries and 20 to 30% in developing countries. In USA and UK, giardiasis was the most commonly reported intestinal protozoan in man. It was estimated that about 200 million people are infected each year in Africa, Asia and Latin America (Norhayati *et al.*, 2003). In the industrialized countries, overall prevalence rate of giardiasis is 2-5%. But in the developing countries, G. lamblia infects infants early in life and prevalence rates of 15-20% in children younger than 10 years are common and children are more frequently infected than adults particularly those who are malnourished (Hesham Al-Mekhlafi *et al.*, 2005). In Malaysian populations Norhayati *et al.* (2003) showed the prevalence varied from 2%-19.4%. Meanwhile Nor Aza *et al.* (2003) reported the prevalence was 8.6% and Rajeswary *et al.* (1994) showed prevalence ranged from 14.8-20% with the highest in an Indonesian.

Blastocystis hominis is the most common human intestinal protozoa worldwide. It was associated with diarrhoea in the tropics and subtropics, since it was found in patients throughout the world. The reported prevalence rates varied from 1.5% to 10% in developed countries, with much higher rates of 30% to 50% in developing countries (Windsor *et al.*, 2001, 2003).

Intestinal parasites are also common in Malaysia, especially among the children and is more prevalent in rural communities. The intestinal protozoan infection rates in Malaysia vary between 18.8% and 91.4%. The prevalence rate of intestinal parasites is high in some rural populations especially among aborigines in Malaysia. They live near rivers or small streams which can be a possible source of infection since ova and cysts of parasites are passed out with faeces into the water (Norhayati *et al.*, 2003). The objective of the present study was to determine the prevalence of intestinal protozoa among the aborigines in Pahang, Malaysia and to determine their problems regarding the infections.

MATERIALS AND METHODS

Study area

The study was carried out in Pos Senderut an aborigine settlement in Pahang. This aborigine settlement comprises of 15 villages and 280 houses with a population of 1772 inhabitants (census record of Kuala Lipis Health Office). Pos Senderut is located 40km from Kuala Lipis town and can only be reached by four-wheel drive and the journey takes one and a half hours. Since all villages were basically similar, 7 villages were randomly selected for this study. The villages were Kg Regang, Kg Muman, Kg Sebeng, Kg Kuala Senderut, Pos Senderut, Kg Poyan and Kg Bukit Long. Most of the houses were built on stills, with bamboo and the roofs were from of 'attaps'. There was no electricity, running water or toilets inside their dwellings.

Collection of stool samples

Verbal consent was obtained from the "Tok Batin" (head of the tribe) of the 7 villages before we started the study. Screw-cap containers, wooden spatulas and zip-lock plastic bags for stool collection were distributed to all household in the selected villages. The study subjects were instructed to fill up stool to half level of the containers. The containers were collected the following and 2 consecutive days thereafter. The specimens were immediately fixed upon arrival at the temporary laboratory set-up in the field. The fixatives were Polyvinyl Alcohol (PVA) solution. The preserved samples were brought to Parasitology Unit, Institute for Medical Research (IMR) for processing and analysis. The Trichrome staining technique was employed for the PVA preserved stools. These PVA preservatives were mainly to detect cyst and trophozoite forms of intestinal protozoa.

In order to get more information about the infection, we asked several simple questions by interviewing the community about the basic amenities available, the use of toilets and problems, water supply, drinking boiled water, personal hygiene practice, education, occupation and knowledge about the parasites, and we also observed the environment of the village such as for cleanliness and presence of animals that can be a source of infection.

Trichrome staining method

PVA faecal emulsion was strained through a filter into a 15 ml centrifuge tube. The tube was centrifuged at 2500 rpm for 5 minutes and supernatant was discarded. The center of the slide was coated with a thin layer of Mayer's albumin by using a fine camel hair brush, a portion of the sediment was lifted and stroked evenly on the albumin coated part of the slide. The smear was stained without letting it to dry.

The slide was placed in tincture iodine for 1 minute. After staining with tincture iodine, the slide was placed in two changes of 70% alcohol for 1 minute each. The slide was placed in Trichrome stain for 8 minutes and then it was placed in acid alcohol for 10 seconds. Then the slide was placed in two changes of xylene for 1 minute each. The slide were mounted using Depex and examines under 100X objective.

Analysis

Detection of intestinal protozoa was based on morphological characteristic of specific protozoa. Analysis was performed using SPSS®, version 11.5. Chi-square analysis was carried out to test for significance between prevalence by gender, age, locality and mixed infections.

RESULTS

A total of 194 stool containers were distributed to seven villages in Pos Sederut, Pahang. However, only 130 stool containers were returned, giving a response rate of 67%. Of the 130 stool samples examined, 94 (72.3%) were found to be infected with at least one of the intestinal protozoa. The highest prevalence rates were due to *B. hominis* (52.3%), *G. lamblia* (29.2%) and the lowest infection rate was due to *E. polecki* (1.5%) (Figure 1). From 94 positive samples, mixed infection with *E. histolytica* and *G. lamblia* was 3.8%, *E. histolytica* and *B. hominis* was 15.4%, *G. lamblia* and *B. hominis* was 17.7% and triple infection (*E. histolytica*, *G. lamblia and B. hominis*) was 3.1% (Figure 2).

From the seven villages studied, two villages (Kg Sebeng and Kg Muman) were excluded from statistical analysis to compare the prevalence of intestinal protozoan infections among these villages since only one sample each was received. The highest prevalence was from Pos Senderut area (80.6%,), followed by Bukit Long (76.9%), Kg Poyan (71.4%), Kg Kuala Senderut (69.4%) and Kg Regang (61.5%). However, there was no statistical difference in protozoan infections among these villages (p value= 0.528).

From 130 stool samples received, 51% were from males and 49% from females.

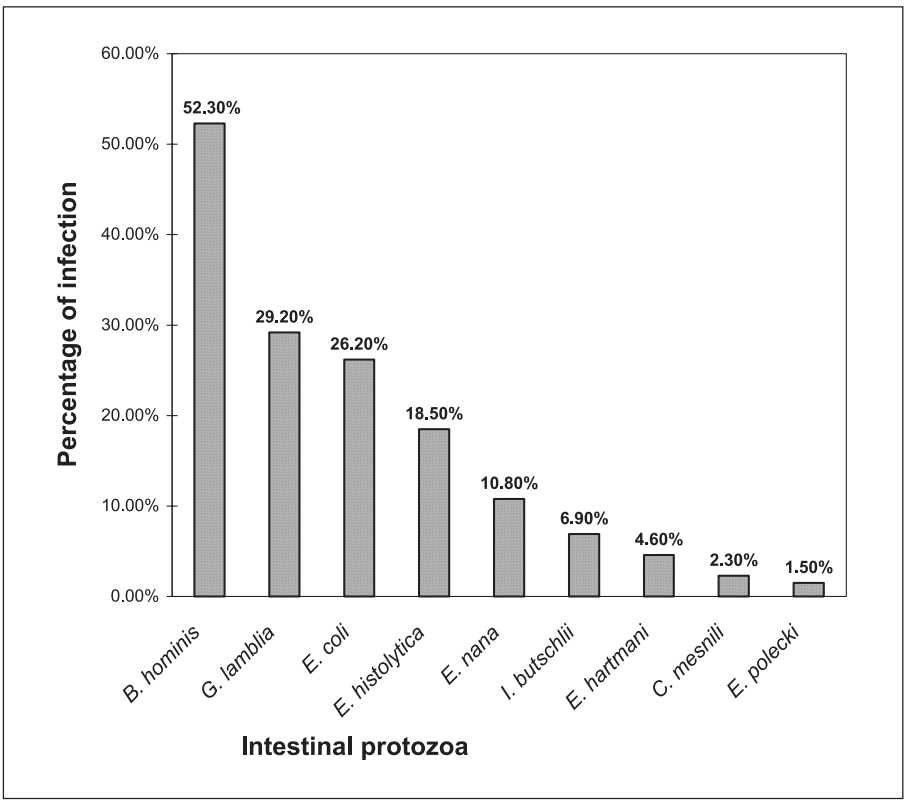

Figure 1. Prevalence of intestinal protozoan infections among the aborigines.

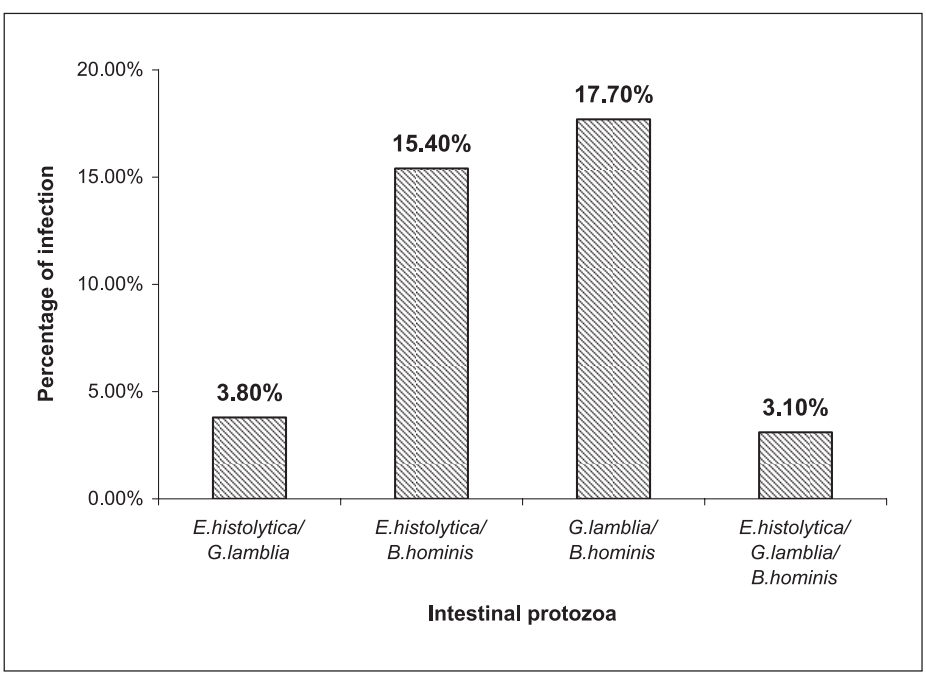

Figure 2. Prevalence of mixed pathogenic intestinal protozoan infection.

Table 1 shows the prevalence of intestinal protozoan infections by gender. Although, the infection was more frequent in females than males it was not statistically significant (P=0.144). The highest infection rate of infection was in the 1-10 years old age group (45.4%) and the lowest infection rate of infection was the 61-70 years age group (3.8%). Detail findings are shown in Table 2.

DISCUSSION

The 72.3% positivity for intestinal protozoa found in this study reflects the high exposure of this community to poor sanitary conditions. Low prevalence of *E. histolytica* (0%) and high prevalence of *G. lamblia* (24.4%) among the children in oil palm estate was detected Sinniah *et al.* (1978). A similar study carried out by Hamimah *et al.* (1982) showed the prevalence for 4.26% intestinal protozoan (2.3% and 2.62% with *E. histolytica* and *G. lamblia* respectively). In Nor Aza *et al.* (2003), the prevalence rates for intestinal protozoa were 21.0% *E. histolytica*, 8.6% *G. lamblia* and only 3.3% for *E. coli*.

The present study revealed a high prevalence of intestinal protozoa with *B. hominis* (52.3%) followed by *G. lamblia*

Table 1. Prevalence of intestinal protozoan infections by gender

Intestinal protozoa	Male 66 (50.8%) (%)	Female*64 (49.2%) (%)	Total 130 (100%) (%)	P value	OR (95% CI)
E. histolytica	15 (22.7)	9 (14.1)	14 (18.5)	0.203	0.556 (0.224-1.382)
G. lamblia	16 (24.2)	22 (34.4)	38 (29.2)	0.204	1.637 (0.763-3.513)
B. hominis	31 (47)	37 (57.8)	68 (52.3)	0.216	1.547 (0.774-3.093)
E. coli	19 (28.8)	15 (23.4)	34 (26.2)	0.488	0.757 (0.345-3.138)
E. nana	7 (10.6)	7 (10.9)	14 (14.8)	0.951	1.035 (0.201-3.138)
E. hartmanii	3 (4.5)	3 (4.7)	6 (4.6)	0.969	1.033 (0.201-5.317)
E. polecki	0	2 (3.1)	2 (1.5)	NC	NC
I. butchlii	4 (6.1)	5 (7.8)	9 (6.7)	0.694	1.314 (0.336-5.129)
C. mesnilii	0	3 (4.7)	3 (2.3)	NC	NC
Total	44 (66.7)	50 (53.2)	94 (72.3)	0.086	1.786 (0.816-3.907)

NC : Not calculated
* Female as reference

Table 2. Prevalence of intestinal protozoan infections by age groups

Age (years)	*E. histolytica* (%)	*G. lamblia* (%)	*B. hominis* (%)	*E. coli* (%)	*E. nana* (%)	*E. hartmanii* (%)	*E. polecki* (%)	*I. butschlii* (%)	*C. mesnili* (%)	Total (%)
<= 10	16.9	44.1	45.8	18.6	8.6	5.1	0	5.1	1.7	45.4
11-20	23.5	29.4	52.9	35.3	23.5	5.9	0	11.8	11.8	13.1
21-30	13.3	13.3	26.7	33.3	0	0	0	0	0	11.5
31-40	22.2	11.1	83.3	33.3	11.1	5.6	2	11.1	0	13.8
41-50	0	0	55.6	44.4	22.2	0	0	22.2	0	6.9
51-60	0	2	57.1	0	0	14.3	0	0	0	5.4
61-70	7	7	80	40	20	0	0	0	0	3.8
Total	18.5	29.2	52.3	26.2	10.8	4.6	1.5	6.9	2.3	

(29.2%) and *E. histolytica* (18.5%). The findings of this study confirmed a trend of high risk infections of intestinal protozoa among the population especially in aborigine settlements as shown by other studies (Rajeswary *et al.*, 1994; Soriano *et al.*, 2001; Norhayati *et al.*, 2003). According to Rajeswary *et al.* (1994), poor socio-economic condition, low standard of sanitation and hygiene, lack of education and failure to use protective materials contributed to the high prevalence of intestinal protozoan infection.

During the survey, the aborigines' behavior, basic amenities available and environmental conditions were observed. This information was used to support the findings of the present study. In this aborigines' settlement, they used water from the river for all purposes such as drinking, cooking, bathing, swimming and washing vegetables.

From this study, nine intestinal protozoa were detected and they were *B. hominis*, *G. lamblia*, *E. histolytica*, *E. coli*, *E.nana*, *E. hartmanii*, *E. polecki*, *I. butschlii* and *C. mesnili*. *Balantidium coli* could not be detected in this study because it requires fresh stool samples and direct stool examination. Intestinal coccidian such as cryptosporidium were not detected as special staining method was not used in this study.

Public health interest in *G. lamblia* is increasing because of the growing recognition of its role as a cause of disease outbreak. *Giardia* cyst may be found in water as a result of the deposition of faecal material from both man and animal Therefore, their stools can easily contaminate water and spread the disease to other communities that totally depend on the river as their main source of water (Rose *et al.*, 1991). *Giardia lamblia* also is a well-known cause of intestinal malabsorption and diarrhoea in human (Safrun *et al.*, 2005). It is the most common infections throughout temperate and tropical countries. Their prevalence varies between 2-5% in developed countries and 20-30% in developing countries. It is estimated that about 200miliion infections occur each year in Africa, Asia and Latin America. In Malaysia infection with giardiasis varies from 2.6-19.4% (Norhayati *et al.*, 2003).

From our observations, we found that although the government provided piped water from the river or stream to houses, the water was not chlorinated. Thus, if the river or stream was contaminated with protozoa, people who drink this untreated water, will become infected. The aborigines do not have the practice of boiling the water before drinking. Thus this will lead to an increase in the transmission of infection.

There was no significant difference in prevalence of intestinal protozoa between males and females. Among the aborigine population of Malaysia, the socio-behavior was the same between males and females. There was no clear cut role in daily routine chores. Therefore, the risk of infection due to intestinal protozoan was the same. Similar results were found in the studies by Nor Aza *et al.* (2003) and Okyay *et al.* (2004) showing that gender is a not a factor contributing to the differences in risk of intestinal protozoan infection.

In this present study, the highest infection rate was found in 10 and less than 10 ($\leq$10) years age group compared to other age groups even though its was not significantly different. Studies by Rajeswary *et al.* (1994), proved the infection rate in children (under-15) increased with age and there is no statistical significance between genders. The prevalence and distribution of intestinal parasites among 456 school children in Gombak, Malaysia showed that 62.9% were positive for one or more parasites. Among them, the prevalence of *E. histolytica* was 9.9% and *G. lamblia* was 14.4%. Infection rate was higher among children from the lower socio-economic groups (Rajeswary *et al.*, 1994). Study by Nor Aza *et al.* (2003) showed that the highest infection rate was found in 11-20 years age group compared to 1-10 years age group, 21-30 years and 31 years and above. Although the younger age group was shown to be more high risk to get

infection of intestinal protozoa than the older age group, the risk of infection was the same for all ages. This is because, by looking at the environment and socio-behavior, all the age groups will get the same infection if the water supply was contaminated. However, the immunity of the younger ages is lower than the older ages. Therefore the prevalence of infection was much higher in younger ages.

Personal hygiene among these aborigines population was very poor. The children tend to have peculiar habits such as not washing hands before and after eating. They also consume raw food especially fruits and sugar cane without washing them. This habit is also common among adults. This is one of the factors for high prevalence of intestinal protozoa. In these villages the government has supplied toilets. However, the use of toilet wasn't practiced in every village. Most of the aborigines never use toilet at all and only 2-3 households used toilet but only for urination. All these villages are located near rivers and streams and they defecate near rivers and streams. The aborigines stated several reasons as to why they did not use toilets. Most of the aborigines have strange beliefs. They believe that if they defecate in small covered places such as toilets especially located near their houses, they will be cursed with some disease. Furthermore, they were not comfortable with the toilet provided by the government. The walls of the toilets were made of "zinc". Therefore, they feel hot and so constricted to use this. It is easier when they defecate near river and streams. They believe the disease will be carried away by the river and streams to other places far from their villages.

The present study showed that high prevalence rate of intestinal protozoa among the aborigines is not only due to non-pathogenic but also pathogenic protozoa. The highest infections were from pathogenic intestinal protozoa, which were *B. hominis*, *G. lamblia* and *E. histolytica*. The aborigines were asymptomatic but the risk of infection is always present.

This present findings have provided the government with baseline data so that it can plan to help the aborigines to be free from the intestinal infections. Although the government has scheduled visits to treat the aborigines on a regular basis, this alone is insufficient to get rid of the infections. They should be educated and provided with better facilities to free them from the problem. Although schools were provided for the children, it will take a long time before the situation can improve. There is a need for adults to be educated as well. The government should play an important role in educating them on how to look after themselves. The aborigine population should be provided with knowledge on life cycle of the protozoa so that they will understand not only the mode of transmission but also the risk of intestinal protozoan infection.

Acknowledgement. The authors wish to thank the Director, Institute for Medical Research, Kuala Lumpur for permission to publish this paper, Dr Rahimi Hassan and Kuala Lipis Health District Office for giving us the permission to conduct field trips and the assistance they gave us during the trip. We also would like to express our special thanks to SEAMEO-TROPMED for funding this project and all staff of Parasitology Unit, IMR who were involved in this research.

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