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Current status of parasitic infections among Pangkor Island community in Peninsular Malaysia

Ahmad, A.F.*, Ngui, R., Muhammad Aidil, R., Lim, Y.A.L. and Rohela, M.
Department of Parasitology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia
*Corresponding author email: arineahmad@um.edu.my

Abstract. Limited data is available on the prevalence of parasitic infections among the island communities in Malaysia with most studies performed between 1960s-1980s. This study was conducted to determine the current prevalence status of parasitic infections among communities living in Pangkor Island Peninsular Malaysia. A total of 131 stool and 298 serum samples were collected and subjected to microscopic examination for intestinal protozoa and helminths and detection of *Toxoplasma gondii* antibodies using commercial ELISA kits respectively. In addition, thin and thick peripheral blood films were microscopically screened for the presence of *Plasmodium* spp. and microfilariae respectively. The overall prevalence of intestinal parasitic infections among Pangkor Island community was 9.9% (13/131) with *T. trichiura* (5.3%) being the most common intestinal parasite detected. Toxoplasmosis was reported in almost 60% of the community with the seroprevalence being significantly high among females (64.7%) compared to males (52.8%) (p=0.038). None of those examined samples were infected with intestinal sarcocystosis, malaria and filariasis. This study revealed that the prevalence of intestinal parasitic infections among Pangkor Island community has been greatly reduced compared to that reported 35 years ago. Massive improvements in the socioeconomic status, personal hygiene, water facilities and sanitation may have contributed to the low prevalence of parasitic infections in this community. Nevertheless, further studies still need to be performed to determine the possible risk factors for the high prevalence of toxoplasmosis in this community.

INTRODUCTION

Parasitic infections are a major cause of great concern particularly in developing countries (WHO, 1987; Haque, 2007; Alyousefi *et al.*, 2011; Ngui *et al.*, 2011a). Among the important parasites are soil-transmitted helminths (*Ascaris lumbricoides*, *Trichuris trichiura* and hookworms), intestinal protozoa (*Entamoeba histolytica* and *Giardia lamblia*), tissue protozoa (*Toxoplasma gondii*), blood parasites (*Plasmodium* spp.) and parasites causing lymphatic filariasis (*Wuchereria bancrofti* and *Brugia malayi*) (WHO, 1987). Worldwide, it has been estimated that > 1000 million, 795 million and 740 million individuals are infected with *A. lumbricoides*, *T. trichiura* and hookworms, respectively (de Silva *et al.*, 2003; Bethony *et al.*, 2006), whereas, more than 50 and 200 million people worldwide are infected with *E. histolytica* and *G. lamblia*, respectively (WHO, 1987; van Hal *et al.*, 2007).

With regards to blood parasites, previous studies have shown that almost 3 billion people are at risk of malaria yearly with 300 - 500 million individuals infected and up to 3 million deaths (Snow *et al.*, 2005; WHO, 2005). Meanwhile, lymphatic filarial nematodes namely *W. bancrofti* and *B. malayi* cause filariasis among humans regardless of age and gender (Michael *et al.*, 1996). Transmission of both diseases to humans is via bites of infected mosquitoes (Vythilingam, 2012). The World Health Organization (WHO) has identified lymphatic filariasis as the second leading cause of permanent and long-term disability

worldwide and studies have estimated that almost 120 million individuals or 2% of the world population are being infected (Zeldenryk *et al.*, 2011). WHO also has included lymphatic filariasis together with soil-transmitted helminths infections as among the top 10 neglected tropical diseases that need to be eradicated by 2020 (WHO, 2010, 2013).

In Malaysia, little is known about the prevalence of parasitic infections among the island communities. Most of the available studies were conducted in 1960s to 1980s which included Tioman (Heyneman *et al.*, 1967), Perhentian Kechil (Balasingam *et al.*, 1969), Penang (Balasingam *et al.*, 1969; Anuar *et al.*, 1978), Pangkor (Nawalinski & Roundy, 1978) and Ketam Island (Sinniah *et al.*, 1988). Among the most commonly detected parasites from these island communities were *T. trichiura*, *A. lumbricoides*, hookworm and *Entamoeba coli* (Heyneman *et al.*, 1967; Balasingam *et al.*, 1969; Anuar *et al.*, 1978; Nawalinski & Roundy, 1978; Sinniah *et al.*, 1988). In addition, there have been two outbreaks of muscular *Sarcocystis* infection reported recently among local and international travellers upon returning from Tioman and Pangkor Islands (CDC, 2012; Abubakar *et al.*, 2013). With this in mind, the present study was conducted to determine the recent prevalence status of parasitic infections among the community in Pangkor Island, Peninsular Malaysia.

MATERIALS AND METHODS

Study population and sample size

Pulau Pangkor (4° 13' 12" N latitude, 100° 33' 18" E longitude) is an island located in the state of Perak, north of Peninsular Malaysia. Briefly, the island has a population of 25 000 with majority of them working as fishermen or are involved in fishing-related industries. To achieve the objective of the study, a minimum sample size required was calculated using a formula by Leedy and Ormrod (2001) based on the latest prevalence of soil-transmitted helminth (STH) infections (60.4%) in Pangkor (Nawalinski & Roundy, 1978). By using the formula, a total of 368 samples were required. About 800 individuals were approached from house to house and at schools but only 345 individuals agreed to participate in this study. Their ages ranged from 1 to 80 years, with a mean ± standard deviation (SD) of 19.32 ± 18.08. The numbers of individual were 38, 214, 10 and 92 out of 354, for the age groups 1-6, 7-12, 13-17 and above 18 years, respectively. Out of these, 214 individuals (62%) provided only blood samples, 47 individuals (13.6%) provided only stool samples and 84 individuals (24.3%) provided both blood and stool samples.

Ethical considerations and samples collection

An ethical clearance was obtained from the ethics committee, University Malaya Medical Centre (UMMC), Malaysia prior to commencement of the study (Reference number: MEC ref. no: 920.16). The island community was also informed that their personal data will be kept confidential, and they could withdraw at any point of the study without giving any reasons. Interested participants were then asked for their consent either in written form (signed) or verbally followed by a thumb print (for those who were illiterate) and from parents or guardians (for children).

Two types of samples (faecal and blood) were requested from each of the interested participant. However, samples were also collected from those individuals who were willing to give only one type of sample. For faecal samples, a wide-mouth crew-capped container was pre-labelled with the participant's name and distributed to all participants. They were then asked to provide a thumb size faecal sample into the container using the scoop attached to the container's lid for collection within the next two days. All faecal samples were then placed in a sealed box and transported to the Department of Parasitology, Faculty of Medicine, University of Malaya immediately, stored at 2-8°C and processed for microscopic examination within 48 h. All the remaining faecal samples were then added with 2.5%

potassium dichromate and kept at 2-8°C for longer storage. For blood samples, a total of 3 ml venous blood was collected in a plain (without anticoagulant) during daytime by a trained medical assistant or nurse. Subsequently, all blood samples were then centrifuged at 1,500 rpm for 10 min to separate the serum from the blood cells. Each of the serum was transferred into a new labelled collection tube and placed in a storage box containing ice packets. The serum samples were then transported to the Department of Parasitology and stored at -20°C until used. For the blood samples in EDTA tubes, a thick and thin blood films were prepared on separate slides immediately, labelled and kept in cardboard slide holders. All thin blood films were fixed with 100% methanol prior to storage.

Microscopic examination of faecal samples and blood films

All faecal samples were concentrated using the formalin-ether concentration technique (Cheesbrough, 1998) and stained with iodine prior to the detection of intestinal parasites (helminths and protozoa) via microscopic examination. For the detection of *Cryptosporidium* oocyst, faecal smears were prepared on glass slides, stained with Ziehl-Neelsen (Cheesbrough, 1998) and examined microscopically. Both thick and thin blood films were stained with Giemsa (Cheesbrough, 1998) for the detection of microfilariae (*B. malayi* sub periodic) and *Plasmodium* spp. respectively.

Serological testing for *Toxoplasma gondii* antibodies

The presence of *T. gondii* antibodies in serum samples was detected using *Toxoplasma gondii* IgM and IgG kits (Trinity Biotech, USA). All procedures for this testing were performed according to the manufacturer's protocols.

Statistical analysis

Statistical analyses were performed where applicable using the statistical package for the social sciences programme for windows version 18 (SPSS, IL, USA). Frequencies were expressed in numbers and percentages. The associations between variables were determined using chi-square (X^2) test. Statistical significance was assumed at $p<0.05$.

RESULTS

Overall results showed that 9.9% (13 out of 131) of the total stool samples were positive for intestinal parasites. Among the 13 positive samples, 8 were positive for a single helminthic infection, 3 were positive for a single protozoan infection, 1 was positive for a double-protozoa infection and 1 was positive for a combination of a single protozoan and an helminthic infection (Table 1). *Trichuris trichiura* (5.3%) was the most common intestinal parasite detected followed by *Blastocystis hominis* (1.5%). Other intestinal parasites detected were hookworm, *Iodamoeba butschlii*, *Entamoeba coli* and *Giardia lamblia* (Table 1). All stool samples were also examined for the presence of *Cryptosporidium* spp. and *Sarcocystis* spp. but none were positive. Higher prevalence of intestinal parasitic infections was seen among the Malay children than that of Chinese children, however the results were statistically not significant (p>0.05).

The seroprevalence of *T. gondii* among the Pangkor Island community by gender, age and ethnic groups is shown in Table 2. Out of 298 blood samples screened, 178 were positive for anti-*T. gondii* antibodies (IgM, IgG or IgM/IgG; data not shown). *T. gondii* seroprevalence was higher in children (134/204; 65.7%) than in adults (44/94; 46.8%). In addition, the seroprevalence of *T. gondii* was statistically higher in females (112/173; 64.7%) compared to males (66/125; 52.8%) (p=0.038). Significantly high *T. gondii* seroprevalence was detected among the Malays (163/247; 66.0%) followed by Indians (2/5; 40.0%) and Chinese (13/33; 28.3%) (p<0.001). Thin and thick blood smears were negative for *Plasmodium* spp. and microfilariae respectively by light microscopy.

Table 1. Intestinal parasites detected in stool samples of Pangkor Island community by gender, age and ethnic groups (N=131)

Variables	N	Intestinal parasites											
		B		T		H		H+E		G		I+B	
		n	%	n	%	n	%	n	%	N	%	N	%
Gender													
Male	71	1	1.4	5	7.0	0	0	0	0	1	1.4	0	0
Female	60	1	1.7	2	3.3	1	1.7	1	1.7	0	0	1	1.7
Age groups (years)													
≤12	107	1	0.93	6	5.6	0	0	1	0.93	1	0.93	1	0.93
≥13	24	1	4.2	1	4.2	1	4.2	0	0	0	0	0	0
Ethnic groups													
Malay	96	1	1.0	6	6.3	1	1.0	1	1.0	1	1.0	1	1.0
Chinese	35	1	2.9	1	2.9	0	0	0	0	0	0	0	0
Total	131	2	1.5	7	5.3	1	0.8	1	0.8	1	0.8	1	0.8

N – total samples examined; n – total positive samples; B – *Blastocystis* spp; T – *Trichuris trichiura*; H – hookworm; H+E – hookworm + *Entamoeba coli*; G – *Giardia lamblia*; I+B – *Iodamoeba butschlii* + *Blastocystis* spp.

Table 2. Seroprevalence of *T. gondii* among the Pangkor Island community by gender, age and ethnic groups (N=298)

Variables	N	*Toxoplasma gondii* seropositive	
		n	%
Gender*			
Male	125	66	52.8
Female	173[a]	112	64.7
Age groups (years)			
1-6	38	14	36.8
7-12	214	117	54.7
13-17	10	3	30.0
18 and above	92	15	16.3
Ethnic groups*			
Malay	247	163	66.0
Chinese	46	13	28.3
Indian	5	2	40.0
Total	**298**	**178**	**59.7**

N – total samples examined; n – total positive samples
*Statistically significant ($p<0.05$)

DISCUSSION

Parasitic infections have always been associated with communities living in poverty, lack of personal hygiene, poor sanitary conditions, low-quality water supply and overcrowding (WHO, 1987; Lim *et al.*, 2009; Alyousefi *et al.*, 2011; Ngui *et al.*, 2011a). Findings of the study indicated that the overall prevalence of intestinal parasitic

infections among the Pangkor community was considerably low with *T. trichiura* as the most common intestinal parasite detected. The results from this study also revealed that almost 1.5% of the island community had hookworm infection while none of the participants was positive for *A. lumbricoides* infection. The absent of the later infection is surprising as it has a similar mode of transmission with *T. trichiura* which is via the faecal-oral route. Moreover, a high prevalence of *A. lumbricoides* infection had been previously reported on this island (Nawalinski & Roundy, 1978).

Besides helminths, intestinal infections caused by protozoa such as *Blastocystis*, *I. butschlii*, and *G. lamblia* were also detected among the Pangkor community either as a single infection or co-infection with other protozoa or helminths. In addition, *E. coli* which is a non-pathogenic amoeba was detected as a co-infection with hookworm in an adult.

In this present study, it was shown that lower prevalence of intestinal parasitic infections was detected among the Chinese (5.7%) compared to the Malays (11.4%). Lower prevalence of parasitic infections had also been reported among the Chinese of Penang Island (Anuar *et al.*, 1978). This could be attributed to the better living conditions of the Chinese as compared to the Malays. It has been well documented in the literature that low socio-economic status is among the major risk factor associated with parasitic infections (Nematian *et al.*, 2004; Ngui *et al.*, 2011a). In Pangkor Island, most of the Chinese adults are involved with huge fishing-related industries that enable them to have better housing (large brick houses, concrete outdoor flooring and adequate sanitation system) and surrounding facilities of wide tarred roads. In addition, the Chinese has the habit of eating using chopsticks and this may help in reducing ingestion of ova/cysts from contaminated or dirty fingers. Previously, a few studies have reported that the higher prevalence of soil-transmitted helminthic infections among the Indians and Malays in Malaysia could be due to their cultural habit of eating with fingers (Bundy *et al.*, 1988; Hanjeet *et al.*, 1991; Ahmed *et al.*, 2011).

In contrast to the Chinese who are in a higher socio-economic status, most of the Malays earn a lesser income. They work as fishermen, employees at the hotels/resorts or own small business. Their houses are poorly constructed (scattered houses and lack of adequate sanitation system) with most houses being built either solely from wood or combination of wood and bricks. During the visits, the kitchens and bathrooms of some houses were observed to be in poor condition with presence of moulds on the walls and floors. Most of the Malay housing areas have narrow untarred roads causing difficulties for vehicles to enter the areas especially during emergency (fire, severe injury or illness) and for garbage or waste collection. The surrounding areas are covered mostly with sandy soil and grasses are hardly seen. Hence, with poor housing environment and sanitation they are at a higher risk of acquiring soil-transmitted helminths and other intestinal parasitic infection.

In Pangkor, although almost all the houses received clean piped water, a few of the Malay households are observed to be still using water from wells or rivers for bathing and washing but not for drinking purposes. These practices may expose them to parasitic infections via accidental ingestion of ova/cysts from contaminated water. In one of the Malay village, Kampung Hujung Kelawai, piped water supply is still not available and the villagers have to depend on natural water that is channelled from a mountain nearby for their daily use. In addition, as the village is situated near the beach, they regularly bathe and wash their clothes in the sea. Therefore, these could be the possible explanations for the detection of parasites in stool samples of two children and an adult from this area (data not shown). The two children aged 12 and 10 years old were infected with *T. trichiura* and *G. lamblia* respectively while the adult was positive for hookworm infection. In fact, *G. lamblia* infection is usually acquired following consumption of water from contaminated water supply. In this study, all but one of the children who were detected positive for intestinal parasites were Malays. This could be possibly due to their active involvement

in outdoor activities. During the visits to the island, most of the Malay children were noted playing outside their houses (playing with soil/sand, riding bicycles and swimming in the river or big puddles) rather than staying indoors.

The findings in this study revealed that the current prevalence of intestinal parasitic infections in Pangkor has dramatically decreased and some parasites are totally absent compared to that obtained in the study performed 3 decades ago. The current findings are most likely due to the improvement in various aspects including health status of the individuals, personal hygiene, sanitation, health care facilities, piped water supply, socio-economic status and health awareness. In addition, sanitation has improved in which most of the houses have their own toilets and proper places to gather rubbish or waste. Furthermore, children have been continuously taught at schools about the importance of personal hygiene. Based on personal communication with Health Inspector of Klinik Kesihatan Pulau Pangkor, in some cases, the children in Pangkor Island were treated with anthelminthic drugs by doctors if they were diagnosed with STH infections. In addition, deworming program among school-age children (1 to 12 years) is still yet to be conducted due to the low incidence of STH infections in the island. Nonetheless, the true prevalence of intestinal parasitic infections in this study might be underestimated since only one stool sample was obtained from each of the participant.

Previously it was reported that the seroprevalence of toxoplasmosis among the general communities (villagers, pregnant mothers and healthy individuals) in Malaysia varied from 10-37% (Nissapatorn & Abdullah, 2004; Ngui *et al.*, 2011b). On the other hand, higher (21-51%) prevalence of toxoplasmosis was reported among immunocompromised patients (HIV) in Malaysia (Nissapatorn & Abdullah, 2004). Further investigations should be done to determine the possible risk factors that may predispose to the high *T. gondii* infection among the Pangkor Island community. During the field trips, many stray cats were seen on the island and had close contact with the Malay community (often fed and let to enter houses). Interestingly, unlike the findings obtained by other researchers which reported a higher prevalence of *T. gondii* IgG than IgM antibodies, the results in this study were the opposite. Based on these findings it could be assumed that most of the island community (children) had a recent exposure to *T. gondii*. On the other hand, studies have reported that in some *T. gondii*-infected individuals, the IgM antibodies may still persist in their blood even after months to years following the first acute infection. Hence, the indication of a recent *T. gondii* infection cannot be relied solely by the presence of IgM antibodies in the infected individuals (Iqbal & Khalid, 2007).

Sarcocystis spp. was not detected in any of the stool samples examined in this study. There was a massive outbreak of muscular *Sarcocystis* that has been reported recently in Pangkor island (Abubakar *et al.*, 2013). It was also revealed in this study that blood parasites namely *Plasmodium* spp. and microfilariae (*B. malayi* sub periodic) were not detected in any of the thin and thick peripheral blood smears respectively in the Pangkor community. In a study by Yap and colleagues, a total of 30/259 people of Penang and 138/286 people of Perhentian Kechil islands were reported to be positive for malaria but none were positive for filariasis (Yap *et al.*, 1968). Despite the absence of these parasites, blood screening among immigrant workers on the island should be performed. Immigrant workers who are from countries (e.g. Nepal, Bangladesh and Indonesia) which are endemic for filariasis and malaria could be the sources of the infection as the infected persons may have the causative agents in their blood and the vectors for transmission are present in Malaysia (Vythilingam, 2012). Furthermore, low-cost international flights to and from these endemic areas for malaria and filariasis may allow easy access of the causative parasites into Pangkor Island as it is one of the favourite holiday destinations for international tourists.

The present study revealed that the prevalence of intestinal parasitic infections among the Pangkor community has

decreased dramatically compared to that obtained from the previous study carried out 35 years ago. Improvement in the socio-economic status, personal hygiene, water facilities and sanitation could be the reasons for the low prevalence of intestinal parasitic infections among the island community. The high prevalence of toxoplasmosis among the Pangkor community should be alerted and preventive and control measures should be made aware of especially to pregnant mothers and of the detrimental effects of the disease on their unborn babies. Finally, although no human malaria and filariasis cases were detected, continuous blood screening should be performed on immigrant workers to avoid silent transmission of the diseases in the community.

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