PARASITIC INFECTION: A RECURRING PHENOMENON IN MALAYSIA

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Abstract. A total of 255 patients including 179 (70.2%) of non-HIV and 76 (29.8%) HIV-infected patients were recruited in this descriptive study. The subjects was significantly found to be male Chinese (157; 61.6% ***vs*** 74; 47.1%) followed by female Malays (98; 38.4% ***vs*** 35; 35.7%) ($p<0.05$). The majority of subjects (124; 48.6%) were in the age group of 21-39 years, however, no statistical difference was found between the various age groups ($p>0.05$). Overall seroprevalence of latent *Toxoplasma* infection was 82/183 (44.8%) being; 3 (3.7%) positive for IgM, 74 (90.2%) for IgG, and 5 (6.1%) for IgG and IgM antibodies. The prevalence was more relatively found in the Chinese (28; 15.3%) and Malays (27; 14.8%) than others ($p<0.05$). While, 23/76 (30.3%) of HIV-positive patients were shown *Toxoplasma* seropositivity. The majority of these subjects (138/181; 76.2%) were significantly asymptomatic ($p = 0.000$), while the others were clinically evident cases of toxoplasmosis. Of this, 37 patients were included in differential diagnosis relating to ocular diseases and only 4 patients were confirmed as having ocular toxoplasmosis. Toxoplasmic encephalitis (TE) was based on presumptive diagnosis, particularly found in 5 patients with AIDS. Seventeen patients were clinically diagnosed as having malaria being; 8 for *P. vivax*, 4 for *P. falciparum*, 3 for *P. malariae*, and 2 for mixed infections. All cases resolved satisfactorily after treatment with antimalarial drugs. Other important emerging parasitic diseases were also detected in these patients including amebiasis (2), blastocystosis (1), cryptosporidiosis (1), filariasis (1), and giardiasis (2) during the time of this study.

INTRODUCTION

Parasitic infections have historically been some of the most common causes of human disease; which pose economic, health, and social problems to the people living in developing countries, including Malaysia. The impact of parasitic infections on human lives has been a topic of great interest in the field of tropical medicine. We therefore conducted this study to determine the prevalence of parasitic infections and the incidence of clinical cases of these diseases from patients admitted to the University of Malaya Medical Center (UMMC), Kuala Lumpur. This data will further enhance the existing baseline information and the implementation of the standard strategies in terms of prevention and proper management.

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MATERIALS AND METHODS

Patients

This retrospective and descriptive study was carried out at the Department of Parasitology, University of Malaya Medical Center (UMMC), Kuala Lumpur, Malaysia. This institution is the oldest university tertiary referral hospital, with 863 beds. It serves as a focus for public services and as a teaching center. Data from a total of 255 patients, including 179 (70.2%) of non-HIV and 76 (29.8%) of HIV-positive patients, were collected and recorded, including laboratory results from blood, serum, and stool samples, in the standardized data collection sheet during May 2004 to February 2006. All positive cases were further investigated, based on the patients' demographic profiles, clinical presentations, relevant laboratory data, and their treatment outcome, from the medical record office, University of Malaya Medical Center. The patients' information was included in the same questionnaire.

Diagnosis of diseases

Various investigations were carefully reviewed according to our study's objectives. The standard

criteria for diagnosing these parasitic infections are as follows:

1. Diagnosis of giardiasis was made by stool examination (concentration technique) for the present of *Giardia* trophozoites or cysts.

2. Diagnosis of cryptosporidiosis was made by the stool examination for the presence of *Cryptosporidium* oocysts and confirmed by modified Ziehl-Neelsen staining techniques.

3. Diagnosis of malaria was made by microscopic identification of peripheral blood smear to detect the presence of *Plasmodium* spp. Also, NOW® MALARIA, USA was serologically used for the antigen detection of *Plasmodium* spp.

4. Diagnosis of seropositivity for *Toxoplasma* infection was via detection of anti-*Toxoplasma* IgG, IgM, or both by either one of the standard ELISA commercial kits (Trinity Biotech, Bray, Ireland; Veda-lab, Alencon Cedex, France) in accordance with the manufacturer's instruction.

5. Diagnosis of seropositive for amobiasis was detected by serologic tests such as IHA or Cellognost® Amoebasis, USA. For filarial infections, peripheral blood smear was used to detect the presence of filarial worms. Few of serological diagnostic kits were available for the antigen detection of Brugia spp and *Wuchereria bancrofti* (NOW® ICT FILARIASIS, USA), also for the antibodies detection of Brugia spp (*BRUGIArapid*™, Malaysia) and *Wuchereria bancrofti* (WB Malaysia). Positive results of these parasitic infections were identified by experienced technicians and confirmed by clinical consultants.

6. Clinical toxoplasmosis was detected in the different two groups of patient, and the diagnoses were determined for a) ocular toxoplasmosis, by clinical presenting signs and symptoms, opthalmoscopic examination, serodiagnosis of *Toxoplasma* infection, and responses to anti-*Toxoplasma* therapy, and b) congenital encephalitis was diagnosed by anti-HIV-positive status, CD4 <200 cells/mm3 (excluded from other of immunocompromised), neurological signs and symptoms, seroevidence of anti-*Toxoplasma* antibodies, and response to anti-*Toxoplasma* therapy.

Statistical analysis

All the findings obtained were entered, edited, and analyzed using statistical software, SPSS version 10 (SPSS Inc, Chicago, USA). The data with quantitative variables were expressed as median and range; whereas, qualitative variables were expressed as frequency and percentage. Statistical analyses were estimated using either the chi-square test or Student's *t* test, where appropriate. A p-value of <0.05 was regarded as statistically significant.

RESULTS

The demographic profiles of the study subjects during May 2004 to February 2006 (21 months) are presented in Table 1. A total of 255 patients, including 179 (70.2%) of HIV-negative and 76 (29.8%) HIV-positive patients were recruited into this retrospective and descriptive study. The age range was from 1 to 81, with a mean of 38.4 ± (SD) 15.31 years. The male:female sex ratio was 1.6:1. The study subjects were significantly found to be male Chinese (157; 61.6% *vs* 74; 47.1%), followed by female Malays (98; 38.4% *vs* 35; 35.7%) ($p < 0.05$). The majority of patients (124; 48.6%) were in the 21-39 year-old age group; however, no statistical difference was found between the various age groups. Interestingly, HIV transmission was found more significantly in males compared with females (56; 35.7% *vs* 20; 20.4%) ($p < 0.05$).

Toxoplasma seropositive and negative status was detected in 181/255 (71.0%) subjects. The overall seroprevalence of *Toxoplasma* infection was 82/181 (45.3%); including 3 (3.7%) positive for IgM, 74 (90.2%) for IgG, and 5 (6.1%) for both IgG and IgM antibodies (Fig 1). The age range was from 1 to 81 years, with a mean of 40 ± (SD) 14.73 years. The sex ratio (M:F) was 1.6:1. The higher prevalent rates were found in males (52; 28.4%) and equally distributed in the subjects between the age group of 20 to 39 and 40 to 59 years (34; 18.8%). However, no statistical difference was found between these associations. Interestingly, the prevalence was significantly related to local racial origins, particularly in Chinese (28; 15.5%) and Malays (27; 14.9%), when compared to others ($p = 0.000$). Twenty-three (30.3%) of 76

Table 1
Demographic profiles of 255 study subjects attended at the University of Malaya Medical Centre (UMMC) during May 2004 to February 2006.

Variable	No. subjects (255)		p-value
	Male n = 157 (%)	Female n = 98 (%)	
Age range = 1-81 years			
Mean = 38.4 (±SD) 15.3 years			
M:F = 1.6:1			
Age group			0.182
≤ 20	11 (7.0)	12 (12.2)	
21-39	75 (47.8)	48 (49.0)	
40-59	50 (32.0)	32 (32.7)	
≥ 60	21 (13.4)	6 (6.1)	
Race			0.023
Malay	35 (22.3)	35 (35.7)	
Chinese	74 (47.1)	29 (29.6)	
Indian	24 (15.3)	20 (20.4)	
Other	24 (15.3)	14 (14.3)	
Country of origin			0.627
Malaysia	131 (83.4)	84 (85.7)	
Outsider	26 (16.6)	14 (14.3)	
HIV status			0.010
Positive	56 (35.7)	20 (20.4)	
Negative or unknown	101 (64.3)	78 (79.6)	
Toxoplasma status			0.879
Positive	52 (33.1)	30 (30.6)	
Negative	61 (38.9)	38 (38.8)	
Unknown	44 (28.0)	30 (30.6)	
Case of toxoplasmosis			0.000
Asymptomatic	153 (97.5)	92 (63.9)	
Ocular toxoplasmosis	2 (1.3)	2 (2.0)	
Toxoplasmic encephalitis	2 (1.3)	4 (4.1)	

HIV-positive patients showed seropositivity for latent *Toxoplasma* infection. The majority of these subjects (138/181; 76.2%) were significantly asymptomatic (p = 0.000), while the others were clinically evident cases of toxoplasmosis. From 37 patients who were included in differential diagnosis related to ocular diseases, 33 of them were suspected as having ocular toxoplasmosis and the other 4 patients were confirmed cases. The life-threatening condition of toxoplasmosis was more commonly found in immunocompromised patients, particularly that involving the brain. From this study, the designation of toxoplasmic encephalitis (TE) was based on presumptive diagnosis, including 1 case in a patient with lymphoma, and 5 cases in AIDS patients.

Concerning malaria, 18 cases were found to be positive for peripheral blood smear (17) or serological (1) detection for malarial infections, including 8 with *P. vivax*, 4 with *P. falciparum*, 3 with *P. malariae*, and 2 with mixed infections. Of these cases, 17 patients were clinically diagnosed as having malaria. Twelve cases were found among locals, including Chinese (6), Malays (5), and an Indian (1). The other five cases were from foreign workers where 4 cases and 1 case came

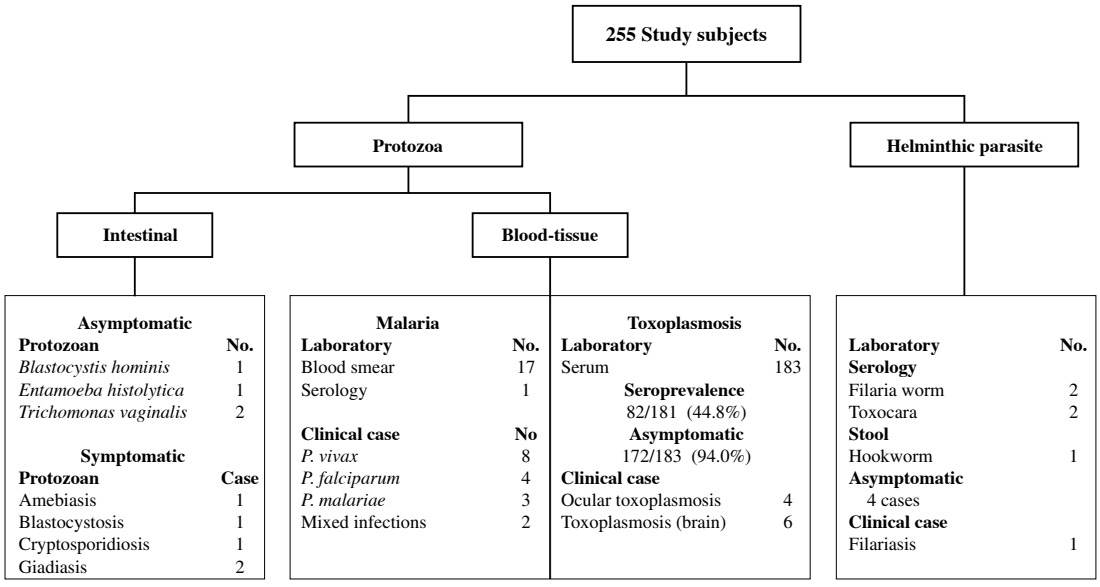

Fig 1- The prevalence and clinically evident cases of parasitic diseases from 255 study subjects during May 2004 to February 2006.

from India and Tanzania, respectively. Malaria occurred more commonly in males (14) and in the younger age group (10) between 20 to 39 years. All cases resolved satisfactorily after treatment with antimalarial drugs.

Regarding helminthic infections, three cases had a seropositive titer for filarial infection, and one case was confirmed clinically as having filariasis. Three cases were asymptomatic with hookworm (1) and *Toxocara* (2) infections. Concerning intestinal protozoan parasites, the majority of positive laboratory investigations were asymptomatic, including 3 for *Blastocystis*, 2 for *Trichomonas*, and 1 for *Entamoeba* infections. Among the symptomatic cases, there was 1 each for amebiasis, blastocystosis, and cryptosporidiosis: in addition, 2 cases were diagnosed for giardiasis. Overall, there was no report of outbreaks, drug resistance, or death relating to any of these parasitic infections during the time of this study.

DISCUSSION

Parasitic diseases have been found in both immunocompetent and compromised hosts in any given geographical distribution. The clinical impacts have been recognized as some of the most common infectious, opportunistic, endemic, and killer diseases in the regional or global arenas.

Overall, the incidence of parasitic diseases was comparatively lower than a previous study conducted in the same setting (Nissapatorn *et al*, 2005). Malaria was the most commonly occurring parasitic disease, and *P. vivax* and *P. falciparum* were the more frequent causes found in this study. Malaria continues to present a significant challenge in tropical countries where over 2 millions people die each year due to this disease. No drug resistance was identified among the affected patients; however, chloroquine resistant falciparum malaria has been reported as early as in 1963, 1995, and 1998 (Jamaiah *et al*, 2005). The continuing expansion of resistance of *P. falciparum* to antimalarial drugs in common use results in the increasing need for different drug combinations (Socheat *et al*, 2003). This is causing great concern, as this species is highly prevalent in tropical Africa, the Amazon, and Southeast Asia (Le Bras *et al*, 2006). Promising results from recent vaccine trials carried out in malaria-naïve and -endemic populations have provided important insights into what will be required of a successful vaccine (Ballou, 2005).

We hope that with this upcoming solution could certainly ease the overwhelming burden relating to malaria in term of control program management in developing countries.

Toxoplasmosis has been listed as a priority among parasitic infections reported in Malaysia. Although this silent parasitic infection has long been afflicting this country, the seroprevalence of latent *Toxoplasma* infection remains high. Toxoplasmosis represents a clinical problem that focuses the attentions of several specialties due to the multiorgan pathology it induces and difficulties in evaluating the activity of the morbid process (Kociecka, 2001). Due to its medical importance, this finding indicated that toxoplasmosis needs a high index of suspicion in suspected cases of ocular disease, particularly in anterior or posterior uveitis. There was no case found in this study of reactivated toxoplasmosis diagnosed after post surgery. Interestingly, one study found that reactivation of ocular toxoplasmosis might develop after LASIK surgery (Barbara *et al*, 2005). Therefore, this infectious disease should be included in differential diagnosis in both medical and surgical involvements with unknown etiological origin and clinical presentations consistent with toxoplasmosis.

Toxoplasmic encephalitis (TE) is based on presumptive diagnosis, and more commonly found in AIDS patents compared with other groups of immunocompromised patients, as indicated in our study as well as others. Neuropathological conditions associated with AIDS are still present in approximately 70-90% of patients and can be the result of HIV itself or of opportunistic infections (Del Valle and Pina-Oviedo, 2006). The majority of patients had secondary reactivation of latent *Toxoplasma* infection that predominantly involved the brain rather than other organs, as also shown in this study. Nevertheless, one study demonstrated that ocular or orbital disease might be the first manifestation of life-threatening systemic toxoplasmosis in HIV-positive patients (Lee *et al*, 2006). However, universal access to HAART has definitely changed both the mortality and morbidity of AIDS (Silva and Araujo, 2005) and has remarkably reduced the incidence of AIDS defining illnesses including TE (Podlasin *et al*, 2006). Due to the benefits of HAART, we support the proposal whereby HIV-infected adult patients who receive effective HAART therapy, primary and secondary prophylaxis against TE can be safely discontinued after the CD4+ T-cell count has increased to more than 200 cells/mm3 for more than 3 months (Miro *et al*, 2006). Nevertheless, it would depend on each case, where there is a need to evaluate the patients' accessibility, availability and affordability of HAART. Then the use of HAART would serve to treat AIDS-related TE fully without the need for chemoprophylaxis. The combination of fansidar and clindamycin is commonly use in treating TE in our hospitals. So far, there has not been any other superior regimen identified for the treatment of TE. The choice of therapy will often be determined by the availability of a therapy such as TMP-SMX, which appears to be an effective alternative therapy for TE in resource poor settings where pyrimethamine and sulfadiazine are not available (Dedicoat and Livesley, 2006). TE is still being seen as one of the most common opportunistic infections in patients with AIDS, particularly in this region.

Gastrointestinal protozoan parasites pose a basic public health concern because of high prevalence rates, particularly in the developing countries. The problem might be more serious because of asymptomatic intestinal parasitic infections (Nuchprayoon *et al*, 2002), as also might be the case in this study. Our data showed 5 cases of symptomatic amebiasis, giardiasis, and cryptosporidiosis in these patients. Of these cases, only cryptosporidiosis was diagnosed in an AIDS patient who presented with chronic diarrhea. Persistent diarrhea and weight loss are the predominant features of cryptosporidiosis (Moolasart *et al*, 1995), and it causes severe and chronic life-threatening gastroenteritis in immunocompromized patients (Zardi *et al*, 2005). Therefore, a high index of suspicion is appropriate for this opportunistic pathogen in HIV-positive patients who have these specific presentations.

Cryptosporidiosis has been one of the most important parasites that is considered by the Centers for Disease Control and Prevention (CDC), Atlanta, as an AIDS-defining illness. In

Malaysia, reports of cryptosporidiosis in patients with AIDS has been very limited (Kamel *et al*, 1994; Nissapatorn *et al*, 2003; Lim *et al*, 2005), and not much attention is given, particularly in clinical practice. The possible explanations are that the routine but specific diagnostic methods have not been established for the laboratory; and, despite the efficacy of passive immunotherapy or some chemotherapeutic agents (*eg*, paromomycin and nitaxozanide), no significant benefits have been demonstrated (Zardi *et al*, 2005). With the introduction of antiretroviral drugs, one study indicated that the increasing immunity due to these drugs might be an important factor in the prevention of opportunistic protozoan infections among HIV-infected patents (Wiwanitkit and Srisupanant, 2006). An increased inhibitory effect was demonstrated when aminoglycoside paromomycin was combined with the protease inhibitors (Hommer *et al*, 2003), and it could be considered as alternative for the development of new drugs that might prove effective against this enigmatic parasite and other related opportunistic parasitic infections (Pozio, 2004). Further studies are required to elucidate the clinical affect of this pathogen, particularly among non-receiving HAART patients, the majority of whom are living in this region.

B. hominis is a cosmopolitan, unicellular, polymorphic protozoan parasite. This organism is one of the most common intestinal parasites found in the large intestine of humans, and the fecal-oral route is the mode of transmission. *B. hominis* has been found in both symptomatic and asymptomatic individuals. This organism however remains controversial and is currently the subject of extensive debate regarding its role as a human pathogen (Nigro *et al*, 2003). In our study, 3 out of 4 positive individuals were asymptomatic. The varying prevalence of *B. hominis* infection from different geographical distributions was comparatively high, up to 46% (Yakoob *et al*, 2004a,b). Further epidemiological surveillance in different groups of the population is required, therefore, to verify the actual prevalence of *B. hominis* infection in this country. The clinical significance of *B. hominis* has been reported worldwide and has certainly gained much attention. In our study, blastocystosis was diagnosed in a patient with symptomatic diarrhea. The role of this organism as a potential intestinal pathogen relating to travelers (Jelinek *et al*, 1997); irritable bowel syndrome (IBS) (Ashford and Atkinson, 1992; Hussain *et al*, 1997); immunosuppressed patients including cancer, hematological malignancy, and HIV/AIDS (Devara *et al*, 1998; Tasova *et al*, 2000; Lebbad *et al*, 2001); hypoalbuminemia and anasarca (Nassir *et al*, 2004); and chronic urticaria (Gupta and Parsi, 2006) has been consistently reported in different settings. In addition, relapse in IBS patients was found to be associated with *B. hominis* (Mylonaki *et al*, 2004). Supporting this clinical aspect, there is a possibility that a subgroup of *B. hominis* could be pathogenic in some patients (Tungtrongchitr *et al*, 2004). With the advance of molecular studies, we should be able to identify this unusual characteristic of *B. hominis* in the near future.

Concerning treatment, metronidazole is considered a standard drug of choice for treating this organism, as was shown in our study. Nevertheless, previous studies have reported that *B. hominis* cysts were resistant, not only to choline (Zaki *et al*, 1996), but also to metronidazole (Zaman and Zaki, 1996; Haresh *et al*, 1999). Interestingly, trimethoprim-sulfamethoxazole was found to be highly effective against this organism (Ok *et al*, 1999) and in some individuals with severe *Blastocystis* infection (Moghaddam *et al*, 2005). In addition, *B. hominis* isolates showed greater *in vitro* susceptibility with furazolidone compared with metronidazole and complete resistance with ciprofloxacin (Yakoob *et al*, 2004a). A recent study suggested that nitazoxanide could be effective in treating *B. hominis* infection in some patients (Rossignol *et al*, 2005). Overall, we recommend further study to elucidate the role of *B. hominis* still; in case of the absence of other causes, *B. hominis* should be considered as a pathogen (Carbajal *et al*, 1997). Also, more clinical drug trial studies, including herbal extracts (Sawangjareon and Sawangjareon, 2005), are required to verify the efficacy of treatments against *B. hominis*.

In this study, only one case was found having clinically evidence of filariasis caused by *Brugia malayi*. Filariasis has been one

of the most important parasitic infections in Malaysia, where only *Wuchereria bancrofti* and *Brugia malayi* are reported to cause human disease. Brugian filariasis accounts for about 13 million cases of the estimated 119 million cases worldwide of active infection and chronic disease (Jamail *et al*, 2005). Almost half of the cases of brugian filariasis are confined to Southeast Asia countries; while India and China account for the other half of the global burden (Michael *et al*, 1996). Improvement in diagnostic techniques for lymphatic filariasis in this country has been remarkably successful and well received. An earlier study showed that monoclonal antibodies have been used to detect circulating antigens and parasite-specific antibodies in filariasis (Abdullah *et al*, 1993). Subsequently, PCR-ELISA has been developed to detect *B. malayi* infection in an area of low endemicity in Malaysia, where more infections were detected, and where it was more reproducible compared to Southern hybridization (Rahmah *et al*, 1998a). Due to its high specificity and sensitivity, serological detection (IgG4 ELISA) would therefore be very useful as a tool in diagnosis if compared to thick blood smear examination (Rahmah *et al*, 1998b, 2001; Lim *et al*, 2001). A further study found that this ELISA format would also be able to demonstrate the decline in IgG4 titer post-treatment (Noordin *et al*, 2003). A rapid dipstick test (Brugia Rapid) has been used to detect specific anti-filarial antibody and has also been tested among suspected cases in our study. The Brugia Rapid is a promising diagnostic tool, not only for detecting *B. malayi*, but also *B. timori* infections; it would be especially useful for the brugian filariasis elimination program in terms of screening and diagnosis, both in Malaysia and related endemic countries worldwide (Rahmah *et al*, 2001, 2003a,b; Noordin *et al*, 2003; Supali *et al*, 2004).

In conclusion, the diversity of parasitic infections is still very much alive in both asymptomatic and symptomatic conditions; which poses a basic public health problem in this tropical rainforest country, Malaysia. Malaria is still the most common parasitic disease; it mostly occurs among locals with a history of traveling to endemic areas. Toxoplasmosis remains one of the most important parasitic diseases, and more efforts in terms of diagnosis and management are needed to curb the primary infection and its consequences of clinical disease. Filariasis is generally well controlled in this country; however, filariasis control programs need to be constantly enforced, particularly in certain endemic areas in this region. Intestinal protozoan parasitic infections share their modes of transmission and pose a significant public health problem. Therefore, more efforts are needed to examine the incidence of symptomatic cases, particularly emerging parasitic infections like cryptosporidiosis or even those with an uncertain pathogenic role, such as that of blastocystosis. Overall, we recommend further studies to be carried out as similar epidemiological and clinical surveillance to monitor closely the trend of parasitic infections periodically.

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