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How common is intestinal parasitism in HIV-infected patients in Malaysia?

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Abstract. Human immunodeficiency virus (HIV)-infected individuals have greater susceptibility to infections by a myriad of microorganisms which can cause significant morbidity and mortality compared to immunocompetent individuals. Of these microbial infections, intestinal parasitic infections (IPIs) however are receiving less attention than bacterial and viral infections, hence, the lack of information of parasitic infections in HIV individuals. Prevalence of IPIs among 346 HIV-infected individuals in Malaysia was determined in this study. The overall prevalence of intestinal parasitic infections (IPIs) was 37.9% (131 of 346) with protozoa infections (18.8%) being more common compared to helminth infections (7.5%). Observed protozoa include *Entamoeba histolytica/dispar* (16.8%), *Cryptosporidium parvum* (12.4%), *Isospora belli* (10.1%), *Cyclospora cayetanensis* (4.9%) and *Giardia duodenalis (intestinalis)* (3.2%) whilst helminthes which were detected comprised of *Ascaris lumbricoides* (13.9%), *Trichuris trichiura* (6.4%) and hookworms (0.6%). Among those 131 infected, 50.4% had multiple infections and 48.9% had single parasitic infection. The CD4 counts were significantly lower (i.e., 200 cells/mm^3) in patients harbouring IPIs. Of those individuals infected with intestinal parasites, 49% were intravenous drug users and 58% were not on any antiretroviral therapy. Most were asymptomatic and had concurrent opportunistic infections (OIs) mainly with *Mycobacterium tuberculosis* infection. These results confirmed that IPIs are ubiquitous among HIV-infected individuals, especially those presenting with low CD4 T cells counts, and provide useful insights into the epidemiology of these infections among HIV-infected patients in Malaysia. It is therefore recommended, that diagnosis of these intestinal parasitic pathogens should be conducted on a routine basis for better management of gastrointestinal illnesses among HIV individuals.

INTRODUCTION

HIV-infected individuals in developing countries such as Malaysia are not only susceptible to opportunistic infections but they are also predisposed to a myriad of enteric pathogens which are endemic in the tropics. Whilst information on bacterial and viral infections is more commonly available to local physicians, unfortunately knowledge of intestinal parasitic infections (IPIs) among HIV patients in Malaysia is still very limited. The lack of knowledge coupled with these intestinal parasites not being included in routine diagnostic testing have unknowingly contributed to these parasites being perceived as "uncommon". However, reports from many regions of the world where HIV/AIDS is endemic have acknowledged that intestinal parasitism is widespread among these populations (Assefa *et al.*, 2009).

Several intestinal parasitic pathogens which have been reported in HIV patients include *Cryptosporidium parvum*, *Isospora belli*, microsporidia (*Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*), *Giardia duodenalis*, *Entamoeba histolytica/dispar*, *Cyclospora cayetanensis*, *Ascaris lumbricoides*, *Trichuris trichuria*, hookworms and *Strongyloidis stercoralis*

(Ramakrishnan *et al.*, 2007; Gupta *et al.*, 2008; Farthing *et al.*, 2009). With impaired immunity especially in patients with low immune level (CD4 counts < 200 cells/mm^3), infections with intestinal parasites may result in diarrheal symptoms (Daryani *et al.*, 2009). Patients with CD4 count of > 180 cells/mm^3 usually have self-limiting infections, whereas most patients with counts < 140 cells/mm^3 develop severe and persistent infections (Current & Garcia, 1991). With the introduction of HAART which partially restores the *immune* function, the incidence of opportunistic parasite infection such as cryptosporidiosis has declined (Hung *et al.*, 2007).

IPIs that are asymptomatic or causes self-limiting diarrhoea in immunocompetent individuals can cause profuse diarrhoea in immunocompromised individuals, generally accompanied by weight loss, anorexia, malabsorption and in some cases fever and abdominal pain (Kurniawan *et al.*, 2009). Patients with some type of immuno-compromised condition and those subjected to immunosuppressive therapy have an increased probability of acquiring IPIs, generally with a high degree of morbidity and mortality in HIV positive individuals worldwide (Chaisson *et al.*, 1998). The parasitic infestations are mainly due to unsanitary conditions (Ramakrishnan *et al.*, 2007).

Several studies have also investigated the existence of interaction between HIV infection and parasitic infections in co-infected individuals (Assefa *et al.*, 2009). The most commonly reported include *C. parvum*, *I. belli*, microsporidia, *G. duodenalis*, *E. histolytica*, *Cyclospora* species (Gupta *et al.*, 2008) and *S. stercoralis*. Some of these parasites may disseminate to other organs such as the bronchia, bile ducts and liver, producing symptoms specific to the organs affected (Botero *et al.*, 2003).

Infection with *G. duodenalis* affects both immunocompetent individuals and immuno-compromised patients, particularly those with common variable or congenital hypogammaglobulinaemia and those in advanced stages of AIDS with prolonged diarrhoea (Mandell *et al.*, 1995). Parasitic infections particularly helminths cause chronic immune activation (Borkow & Bentwich, 2004) in addition to skewing the immune response towards T helper-2 immune responses (MacDonald *et al.*, 2002). In this respect, *A. lumbricoides* can result in an often-fatal obstruction and *T. trichiura* is associated with chronic dysentery and rectal prolapse. Though solid evidences are insufficient, such immune modulation was shown to increase host susceptibility; thereby, promoting HIV infection and disease progression (Brown *et al.*, 2006).

In Malaysia, although IPIs have been widely reported in immunocompetent individuals (Li 1990; Chan *et al.*, 1992; Norhayati *et al.*, 1994; 1997; 1998; DuPont *et al.*, 1995; Kan, 1998), there is still a lack of available data among HIV-infected individuals and whether these parasitic infections are ubiquitous is not known. In order to address this, the present study was undertaken to establish firm data on the prevalence and pattern of parasitic infections among these patients. This information will provide useful insights into the epidemiology of these infections among HIV-infected patients in Malaysia, thus facilitating the understanding of clinical manifestations, diagnosis and treatment management of intestinal parasitic diseases.

MATERIALS AND METHODS

Study design and patients selection

The study was conducted from March 2008 to June 2010 on 346 HIV-infected individuals from three different hospitals in Malaysia, namely: Hospital Sungai Buloh, Selangor; University of Malaya Medical Centre, Kuala Lumpur and Hospital Raja Perempuan Zainab II, Kelantan. Ethical clearance and patient's consent according to the institutional ethical guidelines (IRB Ref. No. 655.17, MOH-NMRR ID: # 09-286-3930) were obtained prior to commencement of study. Single stool sample from 346 patients were collected in sterile screw-capped faecal container with 2.5% potassium dichromate solution as preservative and stored at 4ºC for further analysis.

Stool analysis

Stool samples were subjected to macroscopic examination. Stool consistency was graded by these categories; formed, soft and watery. These samples were concentrated by formal-ether sedimentation technique and the detection of intestinal parasites was examined by wet saline mount and iodine preparation for the presence of ova, larvae and (oo)cysts under 100x and 400x microscopy observation. Modified Ziehl-Neelsen acid fast stain was used for the staining of *Cryptosporidium* and other coccidian parasites and examined under 400x. *Cryptosporidium* oocysts appeared as bright rose-pink sphere (5 ± 1μm) on a pale green background.

Data collection

A standardized form was used to collect information, which includes: (1) socio-demographic characteristics (gender, age and race), (2) clinical and immunological information and (3) treatment regime (antiretroviral drugs). Diarrhoea was defined as three or more watery or loose stools in 24-hour period. Other types of clinical data such as CD4 count and type of opportunistic infections were obtained from the medical record with patient's consent and permission from health authorities.

Statistical analysis

Data entry and analysis was performed using SPSS Version 13 software (Statistical Package for the Social Sciences) programme for Windows (SPSS, Chicago, IL, USA). Descriptive statistics were mainly used to describe the characteristics of the population under study such as age and gender, including the prevalence of intestinal parasitic infections. Statistical significance of differences in proportions was evaluated by Pearson's Chi-square test and student t-test. A significant level of a p-value < 0.05 was used for all tests. Univariate analysis was used to identify the potential risk factors between each variable.

RESULTS

Descriptive analysis

Faecal samples were collected from a total of 346 HIV patients, with 262 samples obtained from Hospital Sungai Buloh (national HIV referral centre for infectious diseases), 63 samples from Hospital Raja Perempuan Zainab II and 21 samples from University Malaya Medical Centre (UMMC). There were 26 (7.5%) children with age ranging from 1 to 13 years with a mean age of 3 years, and 320 (92.5) adults with age ranging from 29 to 42 years with a mean age of 36 years. With regards to gender, there were 302 (87.3%) males and 44 (12.7%) females. There were 185 (53.5%) Malay, 77 (22.3%) Chinese, 42 (12.1%) Indians, 39 (11.3%) foreigners (i.e., Myanmarese) and 2 (0.6%) indigenous East Malaysian. Based on stool macroscopic examination, there were 30 (8.7%) diarrheic stools (symptomatic) whilst 316 (91.3%) stools were formed or soft (asymptomatic).

Since some patients did not give consent for their clinical records to be accessed, clinical information such as mode of HIV transmission, CD4 count, highly active antiretroviral therapy (HAART) and the presence of opportunistic infections were only obtained from 253 patients. Of these 253 patients, 118 (46.6%) were IVDU (intravenous drug users), 39 (15.4%) were heterosexuals, 11 (4.3%) were homosexuals and there were 82 (32.4%) patients who chose not to disclose their mode of transmission. There were 189 (74.7% of 253) patients having CD4 counts < 200 cells/mm^3, 49 (19.4%) having CD4 counts > 200 cells/mm^3 whilst 15 (0.6%) patients did not have any record on CD4 count. At the time of sample collection, there were 119 (47% of 253) patients who were on HAART (highly active anti-retroviral therapy) and 133 (52.6%) who were not on HAART due to reasons such as side effects, non compliance and late presentation.

Prevalence of intestinal parasitic infections (IPIs) among HIV patients

Microscopic examination of IPIs revealed an overall prevalence of 37.9% (131) among 346 HIV patients. There were 65 patients (18.8% of 346) who harboured protozoan parasites and 26 (7.5%) harbouring helminthes whilst 40 (11.6%) patients had both protozoa and helminthes. Protozoan parasites detected included *E. histolytica/dispar* in 58 patients (16.8% of 346), *Cryptosporidium* spp. in 43 (12.4%), *I. belli* in 35 (10.1%), *Cyclospora* spp. in 17 (4.9%) and *G. duodenalis* in 11 (3.2%). Helminthes observed included *A. lumbricoides* in 48 patients (13.9%), *T. trichiura* in 22 (6.4%) and hookworm in 2 (0.6%) (Table 1). Parasitic infections in HIV patients were observed to be mostly mixed infections (66 of 346, 19%) while 64 (18.5%) were single infection. Among the mixed infection cases, combination of *E. histolytica/dispar* and *A. lumbricoides* is the most common while for single infection, *Cryptosporidium* infection was found to be the most prevalent. Most infected cases (118 of 131, 90.1%) were asymptomatic with reference to the consistency of the stool. Based on individual analysis of parasitic infection, there is no significant association between diarrhoea and IPIs. Student t- test analysis showed that there were significant risk of parasitic infection in asymptomatic (non-diarrheic) patients (p=0.000).

Table 1. Prevalence of intestinal parasitic infections (IPIs) according to species among HIV patients (n=346)

Type of species	No.	%
Protozoa		
E. histolytica/dispar	58	16.8
Cryptosporidium spp.	43	12.4
I. belli	35	10.1
Cyclospora spp.	17	4.9
G. duodenalis	11	3.2
Helminthes		
A. lumbricoides	48	13.9
T. trichiura	22	6.4
Hookworms	2	0.6
Protozoa	65	18.8
Helminthes	26	7.5
Protozoa + Helminthes	40	11.6
Total	131	37.9

With regards to IPIs according to age, those aged 13 years and above (118 of 131; 90.1%) had higher prevalence rate compared to those 12 years and below (13, 9.9%) (Table 2). A majority of those 12 years and below had single infections which include protozoan and helminth infections (Table 2). There was only 1 case of mixed infection with both protozoan and helminth in the 12 years and below age group. In contrast, for those aged 13 years and above, 58 (49.1% of 118) had single infection with protozoa, followed by mixed infection of both protozoa and helminthes (39, 33%) while single helminth infection was the lowest (21, 17.8%). Results showed that those above 13 years were harbouring greater diversity of parasites compared to those of 12 years and below (Table 2). Parasites which were found in those above 13 years and not in those of below 12 years include *Cyclospora* species, *G. duodenalis* and hookworms.

Based on gender, there were 114 (87% of 131) male and 17 (13%) female positive with IPIs (Table 2). In male patients, protozoan infections 57 (50% of 114) were more common, followed by mixed infection of both protozoa and helminthes (37, 32.4%) and lastly helminth infections (20, 17.5%) (Table 2). Similarly for female HIV-infected patients, protozoan infections 8 (47% of 17) were also more prevalent, followed by single helminth infection (6, 35.3%) and mixed infection of both protozoa and STH was the lowest (3, 17.6%). Male were infected with more types of parasites whereas female had *E. histolytica/dispar*, *Cryptosporidium* spp., *I. belli*, *A. lumbricoides*, and *T. trichiura* infections. Similar with the findings based on age, parasites which were found in male but not in female were *Cyclospora* spp., *G. duodenalis* and hookworms.

IPIs were also evaluated based on the different ethnic groups in Malaysia (Table 2). Those with IPIs consisted of 72 Malay (55% of 131), 31 Chinese (23.6%), 16 foreigners (i.e., Myanmarese) (12.2%) and 12 Indian (9.2%) (Table 2). For both Malay and Chinese, it was observed that protozoan infections

Table 2. Types of intestinal parasites detected in HIV patients according to age, gender and ethnic group (n = 131)

Parasites	Age group (Years) ≤ 12 (n = 13)	> 13 (n = 118)	Gender Male (n = 114)	Female (n = 17)	Ethnic Groups Malay (n = 72)	Chinese (n = 31)	Indian (n = 12)	Foreigners (n = 16)
Protozoa								
Entamoeba histolytica/ dispar	2 (15.3)	56 (47.5)	54 (47.4)	4 (23.5)	30 (41.7)	15 (48.4)	7 (58.3)	6 (37.5)
Cryptosporidium spp.	4 (30.8)	39 (33.0)	39 (34.2)	4 (23.5)	24 (33.3)	10 (32.2)	4 (33.3)	5 (31.2)
Isospora belli	1 (7.7)	34 (28.8)	30 (26.3)	5 (29.4)	13 (18.0)	13 (41.9)	4 (33.3)	5 (31.2)
Cyclospora spp.	0 (0)	17 (14.4)	17 (14.9)	0	9 (12.5)	3 (9.6)	3 (25.0)	2 (12.5)
Giardia duodenalis	0 (0)	11 (9.3)	11 (9.6)	0	6 (8.3)	3 (9.6)	0	2 (12.5)
Helminthes								
Ascaris lumbricoides	4 (30.8)	44 (37.2)	43 (37.7)	5 (29.4)	24 (33.3)	10 (32.2)	3 (25.0)	11 (68.7)
Trichuris trichiuria	2 (15.3)	20 (17.0)	17 (14.9)	5 (29.4)	10 (13.8)	7 (22.5)	2 (16.6)	3 (18.7)
Hookworm	0	2 (1.7)	2 (1.7)	0	1 (1.4)	1 (3.2)	0	0
Protozoa	7 (53.8)	58 (49.1)	57 (50.0)	8 (47.0)	38 (52.7)	17 (55.0)	7 (58.3)	3 (18.8)
Helminthes	5 (38.5)	21 (17.8)	20 (17.5)	6 (35.3)	16 (22.2)	5 (16.1)	0	5 (31.2)
Protozoa + Helminthes	1 (7.6)	39 (33.0)	37 (33.0)	3 (17.6)	18 (25.0)	9 (29.0)	5 (41.6)	8 (50.0)

were more common (38 of 72, 52.7% and 17 of 31, 55% respectively), followed by mixed infection of both protozoa and helminthes (18 of 72, 25% and 9 of 31, 29% respectively) and lastly helminth infections (16 of 72, 22.2% and 5 of 31, 16.1% respectively) (Table 2). Interestingly, among Indians, there were only protozoan infection (7 of 12, 58.3%) and mixed infections (5 of 12, 41.6%). There was no single helminth infection among Indians. As for foreigners, mixed infection of both protozoa and helminthes were the highest (8 of 16, 50%), followed by helminth infection (5 of 16, 31.2%) and protozoan infection (3 of 16, 18.8%). Further evaluation of types of parasites observed that Malay and Chinese had greater diversity of parasites harboured. It was interesting to note that Indian only had *E. histolytica/dispar*, *Cryptosporidium* spp., *I. belli* and *A. lumbricoides* infections. As for foreigners, all types of parasites found in Malay and Chinese were also found in them except for hookworms (Table 2).

As mentioned above, information such as mode of HIV transmission, CD4 count, highly active antiretroviral therapy (HAART) and the presence of opportunistic infections can only be based on 253 patients. Of these, 98 were infected with IPIs. For the mode of HIV transmission, 48 of 98 (48.9%) were IVDU, 19 (19.4%) were heterosexual and 5 (5.1%) were homosexual. There were 26 patients who were not sure how they acquired the HIV infection (termed as "unknown") (Table 3). Results showed that IVDU, heterosexual and "unknown" harboured all types of parasites. *Cryptosporidium* spp., *G. duodenalis* and hookworm parasites were not found among homosexual. With regards to CD4 count, out of a total 238 patients, 84 had CD4 counts < 200 cells/mm^3 and 14 had CD4 counts > 200 cells/mm^3. It was noted that patients with CD4 counts < 200 cells/mm^3 had a significantly higher risk of parasitic infections (Table 4). Patients with CD4 counts < 200 cells/mm^3 had a significantly high risk of acquiring parasitic infections. The CD4 count was in the range of 2-525 cells/mm^3 with the mean value 92.3% cells/mm^3. Out of 253 patients, 238 had CD4 count information whilst 15 patients did not have any record on CD4 count. In addition to 98 IPIs positive patients, 85.7%, (84) of patients with IPIs were found to have CD4 counts < 200 cells/mm^3 and only 14.3%, (14) of the patients have CD4 counts > 200 cells/mm^3 at the time of diagnosis ($p<0.05$) (Table 4). Based on CD4 classification, it was observed that the number of patients infected with IPIs ranged from 49% (48 of 98) in the category of CD4 counts of ≤ 50 cells/mm^3 group, 18.3% (18 of 98) and 17.3% (17 of 98) in CD4 counts of 51-100 cells/mm^3 and 101-200 cells/mm^3 respectively, whilst 11.2% (11 of 98) in CD4

Table 3. IPIs in HIV patients according to mode of transmission (n=98)

	IVDU n = 48	Heterosexual n = 19	Homosexual n = 5	Unknown n = 26
Protozoa				
E. histolytica/dispar	24 (50.0)	10 (52.6)	2 (40.0)	12 (46.1)
Cryptosporidium spp.	20 (41.6)	7 (36.8)	–	6 (23.0)
I. belli	12 (25.0)	5 (26.3)	3 (60.0)	10 (38.5)
Cyclospora spp.	9 (18.7)	2 (10.5)	1 (20.0)	2 (7.6)
G. duodenalis	7 (14.5)	1 (5.2)	–	2 (7.6)
Helminthes				
A. lumbricoides	18 (37.5)	7 (36.8)	3 (60.0)	8 (30.7)
T. trichiura	6 (12.5)	3 (15.7)	1 (20.0)	6 (23.0)
Hookworm	1 (50.0)	1 (50.0)	–	–

Table 4. IPIs in HIV patients in relation to CD 4 cell counts (n=98)

Parasite	N infected	No. with parasites (%)				
		0-50 cells/mm^3	51-100 cells/mm^3	101-200 cells/mm^3	201-400 cells/mm^3	> 400 cells/mm^3
Protozoa						
E. histolytica/dispar	49	21 (42.9)	11 (22.4)	9 (18.4)	7 (14.3)	1 (2.0)
Cryptosporidium spp.	33	18 (54.5)	3 (9.1)	6 (18.2)	4 (12.1)	2 (6.1)
I. belli	31	14 (45.2)	4 (12.9)	6 (19.4)	7 (22.6)	0
Cyclospora spp.	14	7 (50.0)	3 (21.4)	2 (14.3)	2 (14.3)	0
G. duodenalis	10	6 (60.0)	1 (10.0)	1 (10.0)	2 (20.0)	0
Helminthes						
A. lumbricoides	37	20 (54.1)	8 (21.6)	5 (13.5)	2 (5.4)	2 (5.4)
T. trichiura	16	6 (37.5)	3 (18.8)	5 (31.3)	2 (12.5)	0
Hookworm	2	0	1 (50.0)	1 (50.0)	0	0

* CD4 count < 200 cells/mm^3 84 (85.7%); p=0.044, OR (95% CI) = 2.0 (1.01–3.96)

counts of 201-400 cells/mm^3 and only 3.0% (3 of 98) in CD4 > 400 cells/mm^3. It was observed that *E. histolytica/dispar*, *A. lumbricoides*, *Cryptospordium* spp. and *Isospora* spp. were more common as compare to other parasites. The maximum parasitic isolation was in the group of patients who had CD counts below 200 cells/mm^3 (Table 4).

Interestingly, 63 (64.3% of 98) patients who had IPIs also had concurrent opportunistic infections (OIs) with the most common being *Mycobacterium tuberculosis* infection (37, 58.7% of 63), cerebral toxoplasmosis (11, 17.5%), candidiasis (9, 14.3%) and disseminated or extrapulmonary *M. tuberculosis* infection (8, 12.7%).

DISCUSSION

In Malaysia, IPIs have been widely reported in immunocompetent individuals (Norhayati *et al.*, 1994, 1997, 1998; Kan *et al.*, 1998; Chan *et al.*, 1992; Li, 1990; DuPont *et al.*, 1995), however there is still a lack of available data among HIV-infected individuals as only information on *Cryptosporidium* infection is currently available among Malaysian HIV individuals (Kamel *et al.*, 1994; Lim *et al.*, 2005; Zaidah *et al.*, 2008). In the present study, the overall prevalence of IPIs among HIV-infected patients were found to be considerably higher (37.9% of 346 patients) compared to studies carried out among immunocompetent patients (6.9% to 19.2%) (Nor Aza *et al.*, 2003; Jamaiah & Rohela, 2005).

Globally, information on IPIs in HIV patients are largely concentrated in Asia, Africa and some parts of Middle Europe (Wiwanitkit *et al.*, 2001; Boral *et al.*, 2004; Adesiji *et al.*, 2007; Assefa *et al.*, 2009; Kurniawan *et al.*, 2009; Kulkarni *et al.*, 2009; Berenji *et al.*, 2010). Prevalence rates of IPIs among HIV individuals similar to the present study were also observed in India (35% of 137 patients) (Kulkarni *et al.*, 2009), Turkey (47% of 38 patients) (Boral *et al.*, 2004) and Thailand (50% of 60 patients) (Wiwanitkit *et al.*, 2001). However, studies reported among HIV-infected individuals in Ethiopia (59.8% of 214) (Assefa *et al.*, 2009), Iran (67.7% of 51) (Berenji *et al.*, 2010), Nigeria (79.3% of 150) (Adesiji *et al.*, 2007) and Indonesia (84.3% of 318 patients) have recorded much higher prevalence of IPIs (Kurniawan *et al.*, 2009).

Intestinal parasites noted in the present HIV-infected patients were protozoa such as *E. histolytica/dispar* (16.8%), *Cryptosporidium* spp. (12.4%), *I. belli* (10.1%), *Cyclospora* spp. (4.9%) and *G. duodenalis* (3.2%), and helminthes which include *A. lumbricoides* (13.9%), *T. trichiura* (6.4%) and hookworms (0.6%). These percentages were in accordance with data from Ethiopia (Assefa *et al.*, 2009), whereby the most frequently detected parasites were *Cryptosporidium* spp. (11.4%), *I. belli* (12.2%), *E. histolytica/dispar* (26.5%), *A. lumbricoides* (12.4%), *T. trichiura* (5.6%). Studies from Brazil (Silva *et al.*, 2007), Colombia (Botero *et al.*, 2003), Cuba (Escobedo and Núñez 1999), India (Ramakrishnan *et al.*, 2007; Gupta *et al.*, 2008), Italy (Peruzzi *et al.*, 2006), Iran (Daryani *et al.*, 2009) and Venezuela (Certad *et al.*, 2005) have also noted protozoan infections to be more frequent than helminth infections among HIV patients.

Current study showed that the most common combination of multiple infections is of *E. histolytica/dispar* and *A. lumbricoides* whilst for single infection, *Cryptosporidium* infection was found to be the most prevalent. In Italy, Peruzzi *et al.* (2006) also reported more mixed infections comprising of infections with *Blastocystis hominis*, *E. histolytica* and *G. duodenalis* especially in foreigners as compared to Italian patients. Similarly, in Ethiopia (Assefa *et al.*, 2009), there were higher rate (27.2%) of mixed infection occurring among HIV positive patients compared to HIV negative individuals (OR=2.0; 95% CI 1.2 to 3.6). Berenji *et al.* (2010) also found that in Iran, there were 12.9% of multiple parasitic infections in HIV infected patients.

The high prevalence of *Cryptosporidium* species noted in this study (12.4%) was in agreement with previous study carried out among HIV positive IVDU drug users (Kamel *et al.*, 1994) and in hospitalized patients in Kota Bharu (Zaidah *et al.*, 2008). However, another Malaysian study reported a lower prevalence of cryptosporidiosis among HIV patients (i.e., 3%) (Lim *et al.*, 2005). Globally the prevalence rate of cryptosporidiosis is from 3-50% (Pedersen *et al.*, 1996; Florez *et al.*, 2003). The prevalence of cryptosporidiosis in HIV patients also varies among studies, depending on where the study was conducted, the age of population studied, the stage of disease and the laboratory methods used (Chhin *et al.*, 2006). A number of studies indicated that the prevalence of cryptospori-diosis in HIV-infected patients was in the range of 15% or below (Hunter & Nichols, 2002). However, our results are in accordance with the study carried out in other parts of the world such as Iran (Daryani *et al.*, 2009;), Brazil (Botero *et al.*, 2003), India (Ramakrishnan *et al.*, 2007), Thailand (Nuchjangreed *et al.*, 2008; Srisuphanunt *et al.*, 2008), Cambodia (Chhin *et al.*, 2006), Ethiopia (Assefa *et al.*, 2009) and Nigeria (Okodua *et al.*, 2003).

The overall prevalence of IPIs in the present study was higher in patients above 13 years of age as compared to those 12 years and below. The majority (118 patients) of patients in our study was aged 29-42 years, however; age distribution revealed that patients older than 35 years had increase risk of infection. Such age related increase in IPIs prevalence has been previously reported in adults as well (Certad *et al.*, 2005; Assefa *et al.*, 2009). As there were very limited information of IPIs in HIV patients those of 12 years and below whether within Malaysia or globally, it was interesting to note from this study that infected patients 12 years and below, generally have single infection (protozoa or helminth infection). Co-infection with both protozoa and helminth was only observed in one patient. This study showed that in patients above 13 years were harbouring greater diversity of parasites compared to those 12 years and below. Parasites found in those 12 years and below included *E. histolytica/dispar*, *Cryptosporidium* spp., *I. belli*, *A. lumbricoides*, and *T. Trichiura* whereas; *Cyclospora* spp., *G. duodenalis* and hookworm which were found in patients above 13 years were not seen in those 12 years and below. The prevalence of IPIs in HIV patients also varies among studies, depending on the location of study, the age of population studied, the stage of disease and the laboratory methods used (Chhin *et al.*, 2006).

The present results showed that CD4 count could be used as a potential predicting indicator of protozoa and helminthes infections as those with of CD4 count < 200 cells/mm^3 were significantly at higher risk of acquiring parasitic infection ($p < 0.05$). Further observation based on CD4 classification highlighted that a majority of IPIs were found in HIV-infected individuals having CD4 counts of $\leq$ 50 cells/mm^3. Although IPIs occurred in all CD4 groups, low number of parasitic infections were seen in those with CD4 counts > 400 cells/mm^3. These findings were consistent with other studies that showed parasitic infection usually occurs in patients with CD4 counts of < 100cells/mm^3 (Pedersen *et al.*, 1996; Zali *et al.*, 2004; Chhin *et al.*, 2006; Assefa *et al.*, 2009; Kurniawan *et al.*, 2009). A study in Ethiopia mentioned six-fold higher infection rate of IPIs at CD4 counts of < 200 cells/mm^3 compared with individuals having counts of > 500 cells/mm^3 (OR = 6.3; 95% CI 2.6 to 15.1) (Assefa *et al.*, 2009). In India, researchers determined the pattern of IPIs in different categories of CD4 count on the basis of diarrhoea status (i.e., acute and chronic) and it was reported that IPIs were significantly associated with chronic diarrhoea among those with CD4 count of $\leq$ 50 cells/mm^3 (Daryani *et al.*, 2009). Another study which looked at the types of parasites based on CD4 count in HIV patients with diarrhoea noted that *Cryptosporidium* spp. had the highest prevalence (56.5%, p=0.037) and *E. histolytica* had 28.9% infection (p=0.007) among those in CD4 counts < 200 cells/mm^3 group; both were significant compared with the other CD4 groups (CD4 counts 200–500 cells/mm^3 and CD4 < 500 cells/mm^3) (Sadraei *et al.*, 2005). Similarly, HIV individuals in Indonesia with CD4 cell counts of $\leq$ 50/mm^3 were observed to harbor more types of intestinal parasites such as *Blastocystis*, *Cyclospora*, *Cryptosporidium*, *Giardia*, *Entamoeba* and *Ascaris* compared to those with CD4 count 51-100 cells/mm^3 and 101-200 cells/mm^3 group (Kurniawan *et al.*, 2009).

Unfortunately, individual statistical analysis on the association of opportunistic infection with each intestinal parasite was not significant. Analysis of association between IPIs and OIs were observed, interestingly, 63 (64.3% of 98) patients who had IPIs also had concurrent OIs with the most common being *M. tuberculosis*, cerebral toxoplasmosis, candidiasis and disseminated or extrapulmonary *M. tuberculosis* infection. It is striking that in almost every study from developing regions, *M. tuberculosis* infection is the most frequently identified OIs in HIV patients (Swaminathan & Narendran, 2008; Cain *et al.*, 2009), but none of these studies reported any association of IPIs and OIs in HIV patients.

In conclusion, the high prevalence of IPIs (37.9%) in the present study highlighted the ubiquitousness of these infections among HIV-infected individuals particularly in those with lower CD4 T-cell counts, with or without diarrhoea. The high rates of IPIs, along with profound immunosuppression, may increase risk of developing symptomatic disease such as OIs with mixed infections. With these indications of high prevalence of IPIs in asymptomatic HIV patients, routine screening is utterly crucial and should be instituted in most laboratories at the primary care level, for early treatment and better management of gastrointestinal illnesses among HIV individuals.

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