DETECTION OF OPPORTUNISTIC AND NON-OPPORTUNISTIC INTESTINAL PARASITES AND LIVER FLUKES IN HIV-POSITIVE AND HIV-NEGATIVE SUBJECTS

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Abstract. We assessed the frequency and distribution of infection with opportunistic and non-opportunistic intestinal parasites and the liver fluke, *Opisthorchis viverrini*, in HIV-seropositive and HIV-seronegative subjects. Age- and sex-matched HIV-seropositive (n=78) and HIV-seronegative patients (n=78) from two hospitals in Khon Kaen Province, Thailand, participated in this study from November 1998 to August 2000. These subjects were divided according to the presence of diarrhea and CD4 counts. A single stool sample was obtained and analyzed by using specific techniques. *Opisthorchis viverrini*, was the most common parasite (19.2%) in each group. The prevalence rates of *Cryptosporidium* spp (11.5%) and *Strongyloides stercoralis* (17.9%) in the HIV-seropositive group were significantly ($p<0.05$) higher than those in the HIV-seronegative group (1.0% for *Cryptosporidium* spp and 7.7% for *S. stercoralis* infections). The prevalences of these two parasites were 28% for *Cryptosporidium* spp and 20% for *S. stercoralis* in HIV-seropositives with diarrhea and CD4 counts lower than 100 cells/mm^3, and were higher compared with patients without diarrhea or with high CD4 counts. These results suggest that infection with these parasites increases during HIV infection. The epidemiological distribution of *Cryptosporidium* and *S. stercoralis* may have implications for AIDS-related diseases.

INTRODUCTION

The presence of the opportunistic parasites *Cryptosporidium parvum*, *Cyclospora cayetanensis*, *Isospora belli*, and the microsporidia, are well documented in patients with acquired immunodeficiency syndrome (AIDS) (Goodgame, 1996). These protozoan parasites are not only known as the causative agents of diarrhea in immunocompetent and immunocompromised individuals (Katz and Taylor, 2001), but some of these, such as *Cyclospora*, also cause travelers' diarrhea. These parasites cause significant morbidity and mortality in AIDS patients. Environmental contamination with protozoan or parasitic infective stages occurs through water and food-borne transmission (Marshall *et al*, 1997). Their outbreaks are distributed world-wide (Goodgame, 1996), including in developed countries, such as the USA (Nichols, 2000). They have been recognized as a re-emerging infectious disease (Curry and Smith, 1998).

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Non-opportunistic parasites, such as *Opisthorchis viverrini*, hookworm, *Strongyloides stercoralis, Trichuris trichiura, Taenia* spp and the echinostomes, are frequently encountered in northeastern Thailand (Jongsuksuntigul and Imsomboon, 1998). Similar to opportunistic parasites, some of these infections, such as *S. stercoralis*, may cause diarrhea. Infection with these organisms may contribute to serious morbidity and mortality in both immunocompromized and immunocompetent hosts, especially in HIV-infected individuals and AIDS patients. Little information has been reported about both opportunistic and non-opportunistic parasites in HIV-positive individuals in Thailand. The epidemiology of intestinal parasites in AIDS and/or HIV-infected individuals in northeastern Thailand has not been well documented. Therefore, a study of intestinal parasites and liver fluke infections in HIV-infected individuals and AIDS patients is needed.

We performed a cross-sectional study of HIV-seropositive and HIV-seronegative individuals in Khon Kean Province, northeastern Thailand. We assessed the frequency and distribution of infection with intestinal parasites and liver fluke infections using several specific techniques. To examine both opportunistic and non-opportunistic parasites, we used simple stool smears and formalin-ether concentration techniques. To examine for coccidial parasites, we used a modified Ziehl-Neelsen stain (Casemore *et al*, 1985), and a modified trichrome stain for microsporidia infection (Garcia *et al*, 1994). Positive results for *Cryptosporidium* were further confirmed by using direct fluorescence techniques. To examine *S. stercoralis* infection, we used the agar culture plate technique, which is the most sensitive method for detecting this parasite (Jongwutiwes *et al*, 1999).

MATERIALS AND METHODS

Subjects

The present study was carried out from November 1998 to August 2000 at Srinagarind Hospital and Sirinthorn Hospital, Khon Kaen, Thailand. This study was approved by the ethics committee of Khon Khaen University, Thailand. Individuals who sought medical attention for diarrhea and HIV-positive patients, with or without diarrhea, were asked to participate in the study. All patients understood and signed informed consent forms. Diarrhea was defined as having loose stools at least three times per day. HIV positive status was diagnosed by immunological methods using a passive particle agglutination test kit (SERODIA, HIV-1/2, Fujrebio, Tokyo, Japan) and an enzyme-linked immunosorbent assay. The HIV-positive patients were classified as AIDS based on the presence of typical HIV-related opportunistic infections and CD4 cell counts lower than 200 cells/mm^3. The study population included 78 HIV-seropositive patients (25 diarrheal and 53 non-diarrheal patients) and 78 age- and sex-matched HIV-seronegative patients (28 diarrheal and 50 non-diarrheal patients).

Collection of samples

The blinding of samples was done by the attending physician. About 2-5 grams of a single stool sample was collected from each patient and immediately transferred to the Department of Parasitology, Faculty of Medicine, Khon Kaen University, Thailand. The feces were macroscopically classified into four types according to their consistency: watery, loose, formed and hard. Fifty-five cases in the HIV-positive group were randomly selected out of the 78 HIV-seropositive group for CD4 cell counts.

Sample processing

A fecal sample was immediately examined by direct smear technique. Then, appoximately 2-3 grams of each sample was processed by the formalin-ether concentration technique and a subsequent microscopic examination was performed. The remaining fecal specimen was cultured for *S. stercolaris* larval identification. Each pellet from the concentration technique was smeared on 3 slides. The first was examined by a modified Ziehl-Neelsen stain to identify intestinal coccidian parasites. The second slide was stained using modified trichrome stain to detect microsporidia infection. The last slide was used for samples which had a positive result for the confirmation of *Cryptosporidium* oocysts. The results were verified by direct fluorescence techniques using a kit (Monofluo, Diagnostic Pasteur, France) to detect cryptosporidia. To ensure quality control, positive and negative controls for cryptosporidia, *Cyclospora cayetanensis* and microsporidia were performed in parallel with each set of stainings.

Statistical analysis

Statistical differences between the prevalence of parasites in the HIV-seronegative and HIV-seropositive groups were analyzed using the χ^2-test.

RESULTS

The prevalence rates of intestinal parasites and liver fluke infections are shown in Table 1. The overall prevalence of parasitic infection in the HIV-seropositive subjects was 51.3%, which consisted of *O. viverrini* (19.2%), *S. stercoralis* (17.9%), *Cryptosporidium* spp (11.5%), echinostomes (5.1%), hookworm (3.8%), minute intestinal flukes (2.6%), *Isospora belli*, *Blastocystis hominis*, *Cyclospora cayetanensis*, *Sarcocystis* spp, *Giardia lamblia* and *Taenia* spp (1.3% each). Males had significantly higher parasitic infection rates than females ($p < 0.05$).

There were no statistically significant differences between the HIV-seropositive and HIV-seronegative subjects in the prevalence of *O. viverrini* infection. *Cryptosporidium* spp (11.5%) and *S. stercoralis* (17.9%) infections in the HIV-seropositive groups were significantly higher than in the HIV-seronegative group [*Cryptosporidium* spp (1.3%) and *S. stercoralis* (7.7%), respectively, $p < 0.05$]. The prevalences of the two leading parasites, *Cryptosporidium* spp and *S. stercoralis*, were 28% and 20%, respectively, in the HIV-seropositive patients with diarrhea who had CD4 counts lower than 100 cells/mm^3. The prevalences were higher compared with patients without diarrhea or higher CD4 counts.

Nine out of 78 cases (11.5%) were positive for *Cryptosporidium* spp, all of them males age 25-39 years with CD4 counts lower than 100 cells/mm^3. The consistency of the stool, the size and number of *Cryptosporidium* oocysts are shown in Table 2. Seven out of 9 had diarrhea with formed to watery stools. The number of oocysts observed per high power field ranged from 0-1 to 50-100 with an average size of $4.18 \pm 0.29 \times 3.95 \pm 0.26$ μm. The number of *Cryptosporidium* oocytes was higher in patients with diarrhea than those without diarrhea. In one case (1.3%) of the HIV-seronegative patients, the result was also positive.

DISCUSSION

Our present study constitutes the only known report of a survey focusing on HIV-positive individuals in northeastern Thailand, for not only opportunistic parasites, but other enteric parasites, including liver fluke infection. This study showed that cryptosporidiosis and strongyloidiasis have higher prevalence rates in HIV-positive than in HIV-negative individuals in northeastern Thailand. Both organisms were observed mostly in diarrheal patients with formed to watery stools. Although opisthorchiasis was the most frequently found parasite in this study,

Table 1
Prevalence of intestinal parasitic and liver fluke infections in HIV-seropositive and HIV-seronegative patients with and without diarrhea.

Parasitic infections	HIV-seropositive No. positive (%)			HIV-seronegative No. positive (%)		
	Diarrhea (N = 25)	Non-diarrhea (N = 53)	Total (78) No. pos (%)	Diarrhea (N = 28)	Non-diarrhea (N = 50)	Total (78) No. pos (%)
Cryptosporidium spp	7 (28)	2 (3.8)	9 (11.5)	-	1 (2)	1 (1.3)
Cyclospora cayetanensis	1 (4)	-	1 (1.3)	-	-	-
Isospora belli	1 (4)	-	1 (1.3)	-	-	-
Blastocystis hominis	1 (4)	-	1 (1.3)	-	-	-
Sarcocystis spp	-	1 (1.9)	1 (1.3)	-	8 (16)	8 (10.3)
Giardia lamblia	1 (4)	-	1 (1.3)	-	2 (4)	2 (2.6)
Trichomonas spp	-	-	-	1 (3.6)	-	1 (1.3)
S. stercoralis	5 (20)	9 (17.0)	14 (17.9)	5 (17.9)	1 (2)	6 (7.7)
Hookworm	1 (4)	2 (3.8)	3 (3.8)	3 (10.7)	-	3 (3.8)
C. philippinnensis	-	-	-	1 (3.6)	-	1 (1.3)
O. viverrini	4 (16)	11 (20.7)	15 (19.2)	5 (17.9)	10 (20)	15 (19.2)
Echinostomes	-	4 (7.5)	4 (5.1)	-	2 (4)	2 (2.6)
Minute intestinal flukes	-	2 (3.8)	2 (2.6)	2 (7.1)	2 (4)	4 (5.1)
Taenia spp	-	1 (1.9)	1 (1.3)	1 (3.6)	1 (2)	2 (2.6)
H. diminuta	-	-	-	-	1 (2)	1 (1.3)

Table 2
Stool consistency, CD4 counts, size and number of *Cryptosporidium* oocysts in HIV-seropositive and HIV-seronegative patients with and without diarrhea.

Cases No.	HIV-infection	Diarrhea	Consistency	CD4 count Cell/mm^3	Size (μm)	No. of oocyst/hpf
1	-	-	Formed	ND	(4.03±0.35) x (3.97±0.30)	0-1
2	+	-	Formed	ND	(4.08±0.37) x (3.98±0.34)	5-10
3	+	+	Loose	60	(3.96±0.30) x (3.67±0.41)	0-1
4	+	+	Watery	10	(4.10±0.21) x (3.98±0.20)	20-30
5	+	-	Formed	50	(4.88±0.28) x (4.55±0.39)	5-10
6	+	+	Loose	0	(4.18±0.49) x (3.85±0.46)	50-100
7	+	+	Watery	10	(4.50±0.40) x (4.10±0.31)	30-50
8	+	+	Loose	30	(4.01±0.42) x (3.88±0.33)	5-10
9	+	+	Watery	10	(3.98±0.47) x (3.58±0.49)	30-50
10	+	+	Loose	ND	(4.05±0.41) x (3.97±0.27)	0-1

ND = not determined; hpf = high power field

there was no statistically significant difference in the prevalences between the HIV-positive and HIV-negative groups. In addition, *Isospora belli*, *Blastocystis hominis*, *Cyclospora cayetanensis*, *Sarcocystis* spp, *Giardia lamblia* and *Taenia* spp were observed at low prevalence rates in HIV-positive subjects.

In this study, the prevalent rate of *Cryptosporidium* species was the highest among the various opportunistic intestinal parasites in HIV-positive patients (11.5%), especially in those with diarrhea and with CD4 counts lower than 100 cells/mm^3. Several studies have reported that the prevalence rate of *Cryptosporidium* was 10% in HIV-positive individuals in Songkhla, southern Thailand (Uga *et al*, 1998) and 9.1% in Bangkok (Punpoowong *et al*, 1998). Jirapinyo *et al* (1993) found a rate of 7% in feces obtained from children with chronic diarrhea in Bangkok. The prevalence rate of *Cryptosporidium* infection in this study was lower than a report from the Bamrasnaradura Infectious Diseases Hospital in Nonthaburi, with a reported rate of 20% in AIDS patients (Manatsathit *et al*, 1996), but higher than in other studies done in Bangkok (Waywa *et al*, 2001). The difference in prevalence rates may be due to using different techniques and the different study populations.

Strongyloides stercoralis was also a commonly observed parasitic infection among HIV-infected subjects, which had a significantly higher prevalence rate (17.9%) than HIV-negative persons (7.7%). *S. stercoralis* is a well known cause of diarrheal infection, and has a high prevalence in northeastern Thailand (Jongsuksuntigul *et al*, 2003). It is not clear whether *S. stercoralis* is an opportunistic parasite. Infection with *S. stercoralis* may be more prevalent in AIDS patients due to impaired immunity, which promotes growth of this parasite.

The prevalence rate of opisthorchiasis was the highest among the parasitic infections in the present study. Our data showed that the prevalence of *O. viverrini* infection was the same (19.2%) between HIV-positive and HIV-negative patients, suggesting that opisthorchiasis infection rates are not influenced by HIV-infection status. Similarly, Jongsuksuntigul and Imsomboon (1998) reported that the countrywide prevalence of opisthorchiasis in Thailand was 21.5% with variation between geographical regions. Opisthorchiasis, the most common liver fluke infection in northeastern Thailand, appears to be mainly a rural problem, strongly associated with the habit and frequency of eating raw fish, such as koi-pla (Kurathong *et al*, 1987). Therefore, the results from our study may be used as an indicator to emphasize that HIV-infected patients may live in rural villages as well as in the city.

Other opportunistic parasitic infections were also found in this study in addition to *Cryptosporidium* spp, such as *Cyclospora cayetanensis*, *Sarcocystis* spp, *Blastocystis hominis*, and

Isospora belli. Although our study did not find any microsporidia infections, the prevalence rate for this parasite has been reported to be 27.3% in Bangkok in HIV-infected patients (Punpoowong *et al*, 1998).

Coccidial enteric protozoal infections occur through water and food-borne transmission. HIV-infected individuals with diarrhea may discharge large numbers of oocysts, which may play an important role in the spread of these infections. Our results revealed that HIV-positive patients were more susceptible to infection with *Cryptosporidium* and *S. stercoralis* than HIV-negative patients in northeastern Thailand. Therefore, there is a need for preventive programs for *Cryptosporidium* and *S. stercoralis* infection in rural villages as well as in urban areas in HIV-infected patients. In addition, other enteric parasites, which can cause diarrhea, may need to be periodically investigated as possible re-emerging infectious organisms. Moreover, education programs about AIDS should be provided to the rural communities in northeastern Thailand.

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