OPPORTUNISTIC PROTOZOA IN STOOL SAMPLES FROM HIV-INFECTED PATIENTS

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Abstract. A retrospective study of stool samples of HIV-infected patients from January 1994 to December 1995 submitted to the Department of Tropical Pathology was analyzed. There were twenty-two cases, all of which presented with chronic diarrhea. Result showed that 50% were infected with protozoa. These include *Microsporidium* (27.27%), *Cryptosporidium* (9.09%), *Isospora belli* (4.54%) and *Giardia intestinalis* cysts (9.09%). Other infections were *Candida* sp, *Strongyloides stercoralis* larva and *Opisthorchis viverrini* ova. The data stress the importance of opportunistic protozoa in the HIV-infected patients. Awareness of their existence of the diseases is important areas with increasing number of HIV-infected patients for early detection and proper treatment.

INTRODUCTION

Chronic diarrhea is the most frequent symptom of HIV-infected patients. The prevalence of diarrhea in this group varies from 33% (Pape *et al*, 1994) to 45% (Tarimo *et al*, 1996). Attempts to detect specific pathogens as causes of diarrhea in HIV-infected patients have recently become a focus of interest, since correct treatment can greatly improve the patients' well-being. Conlon *et al*, (1990) studied HIVenteropathy in 63 HIV-infected patients in Zambia and showed that 55% who presented with chronic diarrhea had parasites; the most common were *Cryptosporidium* (32%), *Isospora belli* (16%) and *Strongyloides stercoralis* (6%). In Zaire, *Cryptosporidium* was the most frequently identified intestinal parasite (30%), followed by *Isospora belli* (12%) (Colebunders *et al*, 1988). Similarly in Thailand, among 29 patients with detectable caused of diarrhea, *Cryptosporidium* (20%) has the highest prevalence (Manatsanthit *et al*, 1996).

Opportunistic protozoa play a major role as etiologic agents in chronic diarrhea of HIV-infected patients. Reports from Southeast Asia are very limited. We document the prevalence of different protozoa in HIV-infected patients seen in the Hospital for Tropical Diseases, Bangkok, Thailand.

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MATERIALS AND METHODS

From January 1994 to December 1995, stool samples from HIV-infected patients presented with chronic diarrhea submitted to the Department of Tropical Pathology, Faculty of Tropical Medicine, Bangkok, Thailand were analyzed for possible pathogens. Chronic diarrhea is defined as loose stool of more than three times a day for a period of three weeks. Collected stool samples were immediately fixed in 10% formalin for atleast one hour then filtered with three layers of gauze. The filtered samples were centrifuged at 1,500 cycles per minute for 10 minutes. Excessive supernatant was removed. The precipitate was mixed, smeared on slide as thin film and let dry. Each sample was stained with hematoxylin and eosin stain, Modified Trichrome's stain (Punpoowong *et al*, 1995) -to identify *Microsporidium*, modified acid fast bacilli stain (DMSO method)-to study *Cryptosporidium*, acid fast bacilli stain (Kinyoun's method)-to study acid fast bacilli and periodic acid Schiff's stain-to identify fungal organisms.

RESULTS

During the period of study, twenty-two stool samples from HIV-infected patients presented with chronic diarrhea were collected. The findings are

shown in Table 1. Definite causes were detected in all patients. *Microsporidium* appeared to have the highest prevalence (27.27%), followed by *Cryptosporidium, Giardia intestinalis* cysts (9.09%) and *Isospora belli* (4.54%). Among the non-protozoa detected were *Candida* sp, *Strongyloides stercoralis* larvae and *Opisthorchis viverrini* ova. Four patients (18.18%) had coinfected of *Microsporidium* and *Candida* sp. One patient had both *Strongyloides stercoralis* larvae and *Opisthorchis viverrini* ova. No acid fast bacillus was found in all stool samples.

DISCUSSION

The first documented case of human microsporidiosis was reported in 1959 (Matsubayashi *et al*, 1959). Since the emergence of AIDS, microsporidial infection in HIV-infected patients were documented in 1995 (Modigliani *et al*, 1985). Recently, microsporidia have been reported in up to 39% of AIDS patients with diarrhea (Kotler and Orenstein, 1994) which is comparable to our finding. Diagnosis is difficult because of its small size (1-3 μm) and close resemblance to bacteria. A distinct red belt stripe seen with Modified Trichome's stain is an important feature for recognition of *Microsporidium* un-

Table 1

Opportunistic infection in HIV-infected patients presenting with chronic diarrhea (n=22).

Organisms	No. of cases	%
Microsporidium	6	27.27
Cryptosporidium	2	9.01
Isospora belli	1	4.54
Giardia intestinalis cysts	2	9.09
Candida sp	7	31.82
Strongyloides stercoralis larvae	3	13.64
Opisthorchis viverrini ova	1	4.55

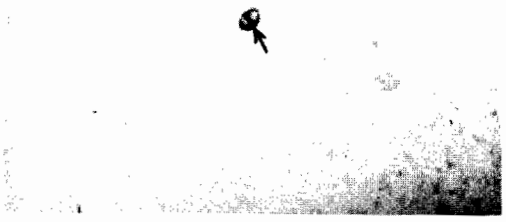

Fig 1–*Microsporidium*, demonstrating microsporidial spore measuring 1-3 μm with red belt stripe ↑ (Modified Trichrome's stain 1,000x).

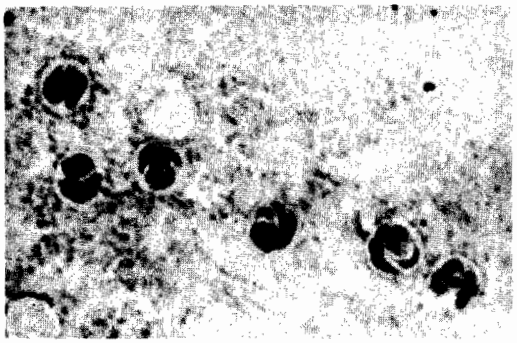

Fig 2–*Cryptosporidium*, oocyst measuring 4-5 μm in diameter and containing four sporozoites (Modified acid fast bacilli stain, DMSO method 1,000x).

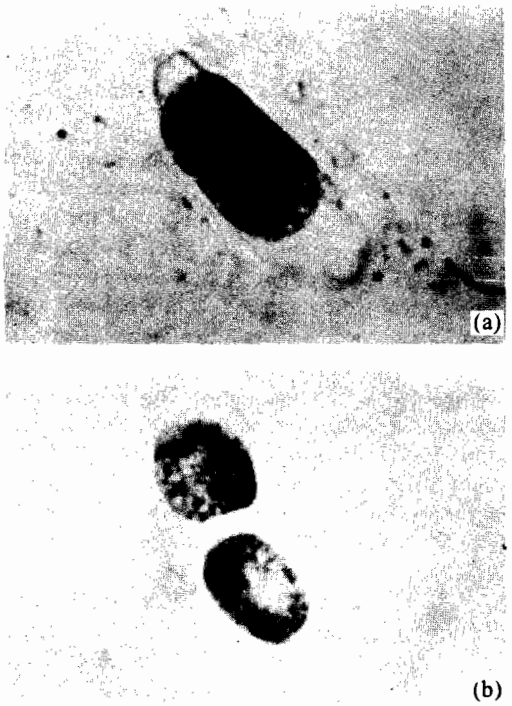

Fig 3–*Isospora belli* showing immature (a) and mature (b) oocysts. They measure 20-30 μm by 10-19 μm (Hematoxylin and eosin stain 1,000x).

der light microscope. Differentiation of species however, can readily be identified by transmission electron microscopy. Newer technics include fluorescence labeled monoclonal antibodies and molecular methods such as polymerase chain reaction with specific primers (da Silva *et al*, 1996). Good results are obtained with albendazole in the treatment of *Microsporidium* (Weber *et al*, 1994).

Cryptosporidium is considered as an emerging infection in HIV-infected patients. It was first reported as a human pathogen in 1976 (Nime *et al*, 1976). A review of nearly 2,000 HIV-infected patients with chronic diarrhea showed *Cryptosporidium* infection in 14% to 24% (Adal *et al*, 1995). Our study showed a lower percentage. This may be due to a small sample size or a reflection of the true percentage in Southeast Asia. Study of larger samples is needed. Diagnosis is established by demonstrating oocyst in stool sample by acid-fast staining, immunofluorescence staining, or PCR. (Laxer *et al*, 1991). Paromomycin can reduce the number of parasite and decrease stool frequency (White *et al*, 1994). *Giardia intestinalis* and *Isospora belli* are another enteric pathogens that can cause diarrhea in immunocompromised hosts. Prevalences of *Giardia intestinalis* and *Isospora belli* in our study are comparable to report from the United States, 6% for *Giardia intestinalis* (Esfandiari *et al*, 1995) and 1-3% for *Isospora belli* (Chui and Owen, 1994).

Fig 4–*Giardia intestinalis* cysts (Hematoxylin and eosin stain 1,000x).

In summary, we document opportunistic protozoa in 50% of stoool samples of HIV-infected patients. Ours are point-prevalence data and may not represent a true reflection of the prevalence of protozoa infection in HIV-infected patients with chronic diarrhea. However, attempt to find the causative pathogens is encouraged, *Microsporidium* and *Cryptosporidium* can correctly be identified only by the use of special stains. Therefore, we recommend a "battery" staining of Modified Trichome's stain, modified acid fast bacilli stain (DMSO method), acid fast bacilli stain (Kinyoun's method) and periodic acid Schiff's stain aside from the routine fresh preparation and hematoxylin and eosin stain for stool samples of HIV-infected patients.

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