CRYPTOSPORIDIUM INFECTION IN HIV-SEROPOSITIVE AND SERONEGATIVE POPULATIONS IN SOUTHERN THAILAND

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Abstract. The prevalence of *Cryptosporidium* infection in 61 HIV-seropositive and 61 HIV-seronegative subjects (aged less than one to 67-year-old) in Songkhla City, southern Thailand was studied by a centrifugal floatation technic using sucrose solution. Most of the HIV-seropositive subjects (72%) were 20 to 39 years old. *Cryptosporidium* oocysts were detected in 10% (6/61) of HIV-seropositive and in 2% (1/61) of HIV-seronegative subjects. Infection rates in these two groups, however, were not statistically significant ($p > 0.05$). The number of *Cryptosporidium* oocysts observed in 20 microscopic fields ranged between one and over 12,000. Among the seven *Cryptosporidium*-positive subjects, six were adults (18 to 42-year-old) and one was three-year-old child. All of the *Cryptosporidium* infected subjects were male, and two of them were passing formed (normal) feces. Biochemical findings revealed dishepatica in five of six *Cryptosporidium* infected HIV-seropositive subjects.

INTRODUCTION

Cryptosporidium parvum is an apicomplexan protozoan parasite,which is known as a causative agent of diarrhea, sometimes profuse and persistent, particularly in small children and immunocompromised individuals who occasionally die of the disease (O'donoghue, 1995; Vakil *et al*, 1996). Infections occur through a fecal-oral route. Though uncommon, extra-intestinal cryptosporidiosis in immunocompromised individuals have also been reported (Ditrich *et al*, 1991; Keusch *et al*, 1995). Infections in immunocompetent persons, however, are self-limiting (symptoms disappear within one or two weeks). More recently, outbreaks of water-borne cryptosporidiosis have occurred in many countries including the USA, England, and Japan. Thus, cryptosporidiosis has recently been recognized as a re-emerging infectious disease (Lisle and Rose, 1995; O'donoghue, 1995; Goldstein *et al*, 1996).

According to WHO (1997), the number of HIV-infected population in the world is 22.6 million and the number is still on the increase. The epidemic of HIV infection hit the Thailand in the late 1980s. Since then, a high prevalence of HIV infection has been observed among drug users, sex workers and their clients in Thailand. It was reported that over 8% of the men attending clinics for sexually transmitted diseases in 1994 were HIV positive (WHO, 1996). HIV infections in Thailand, therefore, have become a serious social problem from the viewpoint of human health as well as economy. Until now, very few studies have been carried out on the actual situations of *Cryptosporidium* infection in HIV-infected individuals or AIDS patients in Thailand. In 1995, Moolasart *et al* made a retrospective study and detected *Cryptosporidium* in 9% (22/250) of HIV-infected individuals with symptomatic diarrhea. Manatsathit *et al* (1996) performed a prospective study and found *Cryptosporidium* in 20% (9/45) of AIDS patients with chronic diarrhea. These studies, however, did not survey *Cryptosporidium* infection among the immunocompetent (HIV-seronegative) persons living in the same area. Furthermore, these two investigations were carried out in the same hospital located in the suburbs of the capital, Bangkok. Hence, the situation in the other areas of Thailand is not yet known.

The purposes of this study were to reveal the prevalence of *Cryptosporidium* infection among HIV-seropositive subjects living in Songkla City, the southern part of Thailand, and to compare the

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result with that obtained from age and sex matched HIV-seronegative individuals.

MATERIALS AND METHODS

The present study was carried out for nine months from October 1995 to June 1996 in Songkhla, a southern city in Thailand. A total of 61 HIV-seropositive (HIV-P) subjects who had been admitted in a teaching hospital of Prince of Songkla University and 61 HIV-seronegative (HIV-N) persons living in same area (control group), thus totaling 122 subjects were included in this study. The diagnosis of HIV infection was made by immunological methods such as passive particle-agglutination test (SERODIA, HIV-1/2, Fujirebio Inc, Tokyo, Japan), rapid immunochromatographic screening assay (HIV 1/2 Sensy-test, Veda Lab, Appert-alencon, France), and enzyme-linked immunosorbent assay. The HIV-N individuals were matched as properly as possible with regard to their age (the maximum error was of four years), sex, and fecal types. The feces were macroscopically classified into five types according to their properties: watery, unform, semiform, form, and hard. Of these, watery and unform were regarded as diarrheal feces.

The detection of *Cryptosporidium* oocysts from feces was performed by the centrifugal floatation technic using sucrose solution of specific gravity of 1.200 as described previously (Uga et al, 1988) and by observing under phase contrast microscope (× 400) in 20 fields. The number of oocysts was not exactly quantitated but was semi-quantitatively measured per gram of feces. Specimens showing no oocysts in 20 fields were regarded as negative.

Statistical significance difference was analyzed by the chi-square test.

RESULTS

Table 1 shows the age and sex distribution of HIV-P and HIV-N populations. The age in either of HIV-P or HIV-N population ranged from one-day-old to 67-year-old with an average of 31-year-old. Among the 61 HIV-P subjects, over 80% were male and 72% of them were in their twenties and thirties (the percentage for the HIV-N was 72%). In this study, 52% of the HIV-P subjects had diarrhea but at the same time, 18% and 3% had a type of form or hard, respectively (Table 2). Table 3 shows age and sex of seven *Cryptosporidium* infected subjects (6 HIV-P and 1 HIV-N) including the types of feces and the number of oocysts detected. *Cryptosporidium* infection rates in HIV-P and HIV-N subjects were 10% (6/61) and 2% (1/61), respectively. However, the difference was not statistically significant ($p > 0.05$). All six *Cryptosporidium* infected HIV-P subjects were adults of over 18-year-old, while the only one person among HIV-N group was a three-year-old child. All of *Cryptosporidium* infected subjects were male. With regard to the types of feces, four were unform, two semiform, and one watery. Among *Cryptosporidium* infected subjects, the highest number of oocysts, more than 12,000 in 20 microscopic fields were detected in a HIV-P 28-year-old male. The numbers of oocysts in other subjects ranged between one to five. Oocysts detected were spherical or elliptical in shape measuring 4 to 6 μm in diameter and contained clear residual body, features identical to that of *Cryptosporidium parvum*. Besides *Cryptosporidium*, detected were *Giardia lamblia* (3%), *Isospora belli* (3%), *Blastocystis hominis* (3%), and *Iodamoe-*

Table 1

Age and sex distribution in HIV-seropositive and-seronegative populations.

	HIV-seropositive			HIV-seronegative		
Age	Male	Female	Total (%)	Male	Female	Total (%)
0-19	5	2	7 (11)	6	2	8 (13)
20-39	39	5	44 (72)	39	5	44 (72)
40-59	5	2	7 (11)	4	2	6 (10)
60-	2	1	3 (5)	2	1	3 (5)
Total	51 (84)	10 (16)	61 (100)	51 (84)	10 (16)	61 (100)

Table 2

Appearance of fecal samples in HIV-seropositive and-seronegative populations.

Type	HIV-seropositive No.	%	HIV-seronegative No.	%
Watery	10	16	9	15
Unform	22	36	21	34
Semiform	16	26	23	38
Form	11	18	7	11
Hard	2	3	1	2
Total	61	100	61	100

ba butshilii (2%) from HIV-P subjects, and *Endolimax nana* (3%), *B. hominis* (3%), and *I. belli* (2%) from the HIV-N persons. However, we have not further investigated these parasites in this study. Table 4 shows the biochemical profile of serum samples from six *Cryptosporidium* infected HIV-P subjects. The A/G ratios tended to be low with a remarkable decrease in the subject Nos. 1, 4, and 6. The levels of GOT and GPT were higher in all the subjects except in No. 3, indicating the disorder of liver function.

Table 3

Cryptosporidium-infected patients in HIV-seropositive and -seronegative populations.

No.	HIV*	Age	Sex	Feces	No. of oocysts /20 field
1	+	18	Male	Unform	2
2	+	28	Male	Unform	> 12,000
3	+	31	Male	Semiform	1
4	+	34	Male	Unform	5
5	+	38	Male	Unform	1
6	+	42	Male	Watery	5
7	-	3	Male	Semiform	1

* Incidences of *Cryptosporidium* in HIV-positive and -negative populations were 10% (6/61), and 2% (1/61), respectively.

DISCUSSION

Opportunistic *Cryptosporidium* infections often occur in HIV-infected individuals, resulting in severe/persistent diarrhea. There are some reports concerning the causative agents for diarrhea in HIV-infected subjects. DuPont and Marshall (1995)

Table 4

Biochemical profiles of HIV-and *Cryptosporidium*- positive sujects.

Items	HIV and *Cryptosporidium* positive subjects 1 *	2	3	4	5	6
Total protain (g/dl)	7.7	7.9	6.9	6.6	7.1	8.1
Albumin (U/l)	3.3	4.1	3.5	1.5	3.9	3.7
Albumin/Globulin	0.75	1.08	1.03	0.29	1.22	0.84
Total bilirubin (mg/dl)	0.52	0.59	0.27	1.97	1.76	0.16
Direct bilirubin (mg/dl)	0.23	ND**	0.15	0.98	1.25	0.06
ALP (IU/l)	170	171	91	1,360	92	123
GOT (IU/l)	133	74	17	105	75	99
GPT (IU/l)	63	38	13	44	82	95
Creatinine (mg/dl)	0.91	1.12	1.45	0.61	ND	1.21
Glucose (mg/dl	94	ND	88	ND	ND	95

*The number indicates the subject number described in Table 3.

**Not done.

reported 18 kinds of possible diarrheagenic pathogens (6 parasites, 9 bacterias, 1 fungus, 2 viruses) in HIV-infected subjects. They found that the infection rate of *Cryptosporidium* was the highest among the various infectious agents. Manatsathit *et al* (1996) also performed a similar study on AIDS patients and detected 11 species of micro-organisms, of which *Cryptosporidium* was the commonest enteric pathogen. Our present study carried out by means of a centrifugal floatation technic using sucrose solution also revealed six types of protozoan parasite with the highest infection rate of *Cryptosporidium*. Jirapinyo *et al* (1993) reported that *Cryptosporidium* was found at the rate of 7% of feces obtained from children with chronic diarrhea, while 131 children without diarrhea were all negative for the parasite. In our study, two of seven *Cryptosporidium* positive subjects had no diarrhea and no relation could be found between *Cryptosporidium* infection and occurrence of diarrhea. Hjlung and Mlbak (1986) have also found 6% of children's feces (without diarrhea) positive for *Cryptosporidium.*

Our present study constitutes the third report of *Cryptosporidium* survey focused on HIV-P individuals in Thailand. *Cryptosporidium* infection rate in HIV-P group was 10% in our study done in southern Thailand. This was lower than that reported by Manatsathit *et al* (1996) in AIDS patients (20%), but was consistent with that reported by Moolasart *et al* (1995) in HIV-P subjects (9%). On the contrary, infection rate of 2% was observed in HIV-N group. This finding was in agreement with that of Jantanavivat *et al* (1991). They also reported 2% of *Cryptosporidium* infection rate in immunocompetent subjects in Thailand. Other studies from Thailand, however, have shown a slightly higher infection rates (ranging from 4 to 8%) (Thamlikitkul *et al*, 1987; Janoff *et al*, 1990; Jongwutiwes *et al*, 1990; Jirapinyo *et al*, 1993) than that of ours. The lower prevalence in our study seems to be due to the inclusion of both children and adults to match their age with those of HIV-P subjects included in this study. *Cryptosporidium* infection rate (10%) in the HIV-P subjects was higher than that (2%) in the HIV-N control group, the difference could not be considered statistically significant. The possible reason for no significant difference is that the HIV-P subjects had been randomly selected without giving consideration to their ages or fecal types. The only one *Cryptosporidium*-infected case in HIV-N group was a three-year-old child discharging semiform feces. It is well known that *Cryptosporidium* infection rate in children is higher than that of adults in spite of their normal immune functions (Jirapinyo *et al*, 1993). We could not obtain the data on CD4 cell numbers in our subjects. However, dyshepatia was observed in five out of six *Cryptosporidium* infected HIV-P subjects. This fact may indicate that the worsening of health conditions of these individuals lead to an opportunistic infection with *Cryptosporidium.*

Cryptosporidium infections have now been recorded in over 170 different host species. Thus zoonotic transmission from animal to human as well as human to human transmission have been exemplified in many reports (O'donoghue, 1995). The environment is contaminated with *Cryptosporidium* oocysts. *Cryptosporidium* transmission through swimming pool, soil, milk, sausage and other sources also occurs (O'donoghue, 1995). It means that we are always at the risk of infection with this protozoan parasite. The immunocompromised individuals, if infected, continue to discharge a large number of oocysts (source of infection), unlike the immunocompetent persons. This indicates that HIV-infected people and/or AIDS patients play an important role of amplifier of *Cryptosporidium* infections, possibly in the epidemic infections or in causing the secondary infection (Goldstein *et al*, 1996). The biggest reported water-borne outbreak of cryptosporidiosis occurred in Milwaukee, USA, in 1993, affecting 400,000 people (Lisle and Rose, 1995; Vakil *et al*, 1996). Among those infected people 400 were reported to have died, most of whom were considered to be HIV-infected or AIDS patients. It is, therefore, concluded that there is a strong need to pay more attention in the health care of HIV infected individuals as well as in considering preventive measures against *Cryptosporidium* infection, particularly in the light of increasing number of immunodeficient individuals in Thailand.

ACKNOWLEDGEMENTS

This study was supported in part by the Japan Society for the Promotion of Science (JSPS).

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