# CASE REPORT

## INTESTINAL MICROSPORIDIOSIS : FIRST REPORTED CASE IN THAILAND

Punnee Pitisuttithum[1], Bhanabhan Phiboonnakit[1], Daungrudee Chindanond[1], Benjanee Punpoonwong[2], Sombat Leelasuphasri[3] and Sirivan Vanijanond[1]

[1]Department of Clinical Tropical Medicine and Hospital for Tropical Diseases; [2]Department of Tropical Pathology, Faculty of Tropical Medicine Mahidol University; [3]Pramongkutklao Hospital, Bangkok, Thailand

Intestinal microsporidiosis is an enteric parasitic infection caused mainly by *Enterocytozoon* species. The organism is unicellular and has characteristic spores containing an extrusible tube which serves as a passage for inoculating host cells. Intestinal microsporidiosis was first reported in AIDS patients in 1985 (Dobbins and Weinstein, 1985; Desportes *et al*, 1985) and is being diagnosed with increasing frequency from various parts of the world (Molina *et al*, 1993; Schattenkerk *et al*, 1991; Orenstein *et al*, 1990). Molina *et al* (1993) had reported rather high prevalence of intestinal microsporidiosis as high as 50% in AIDS patients who had chronic unexplained diarrhea. The diagnosis of this organism has been solely on the ultrastructural identification of this intracellular parasite in biopsy specimens of the small intestines. Later on there were many authors reported various techniques for diagnosing the organism under light microscopy by using stool specimens and duodenal aspirates (Kotler *et al*, 1993; Weber *et al*, 1992; Orenstein *et al*, 1990; Gool *et al*, 1990). Kotler *et al* (1993) were the first to report the sensitivity and specificity of the light microscopic diagnosis of microsporidium by using biopsy specimen. But Orenstein *et al* (1990), and Gool *et al* (1990) reported the practical procedures to diagnose this organism under light microscope.

Here we report a case of intestinal microsporidiosis diagnosed under light microscopy. To the best of our knowledge, it is the first case reported from Thailand.

A 34 years old married male was admitted at Pramongkutklao hospital with the complaint of left hip pain which later was diagnosed as septic hip joint and was found to be HIV positive. His performance status on admission was 70 (Karnofsky score). He also complained of watery diarrhea for indetermine period with a frequency of 3-4 times a day but refused history of weight loss. On examination the patient was thin but not cachectic, oral thrush was present. Other associated opportunistic infection was pulmonary and extrapulmonary tuberculosis. Stool was sent for extensive investigation for ova and parasites as described in the method including culture. His total lymphocyte was 1,105 cells/dl and $CD_4$ count was 28, other biochemistry laboratory findings were still normal at the time of report.

Stool was examined in triplicate by the following methods : simple smear, smear after concentration method using formaline ether, culture for *Strongyloides stercoralis* by Harada and Mori, staining by modified acid fast for cryptosporidium. Smear for microsporidia was prepared separately by preparing from 10 µl of a suspension of unconcentrated liquid stool in 10% formalin (1:3) and allowed to dry at rooms temperature. The slides were fixed in 70% ethanol and stained with modified Gomori's trichrome for 30 minutes, rinsed with acidified alcohol, absolute ethanol and then mounted in permount (modified from Weber *et al*, 1992). The slides were examined under light microscope using oil immersion field. The slides were compared and confirmed by comparison with slides sent by Drs Ralph and Wahlquist, Department of Health and Human Services, Public Health Service, Centers for Disease Control, Atlanta GA, USA.

Stool was negative for cells, mucus and blood. Culture was also negative; *Cryptosporidium, Cyclospora* and *Isospora* were not found. Stool smears prepared by the above method revealed characteristic microsporidian spore of 0.5-1.0 × 1.1 µm, oval in shape stained bright pinkish-red with a pinkish red belt like stripe upto 10 spores were seen (Fig 1).

The patient had been diagnosed as intestinal microsporidiosis after discharge. The patient's diarrhea dissolved without treatment after two weeks of follow up. His performance score at discharge was 90 and is still followed regularly at out patient clinic at Pramongkutklao Hospital.

Fig 1–Microsporidium spore of the patient using modified Gomori trichrome stain (100×).

Intestinal microsporidiosis has been reported recently in higher percentage in HIV positive patients. Upto 7%, 33% and 50% had been reported from Africa, Australia and France respectively (Bretagne *et al*, 1993; Field *et al*, 1993; Molina *et al*, 1993). As a result of improvement of various staining techniques for spores in the stool specimens and alertness of its presence, this new organism now is being recognized as one of the major causes of unexplained chronic diarrhea.

Clinical significance of this infection in HIV positive patients with chronic diarrhea has been reviewed with some controversy. Schattenkerk *et al* (1991) and Bretague *et al* (1993) have reported the relationship between intestinal microsporidiosis and chronic diarrhea, especially in individuals with pronounced cellular immune deficiency (low $CD_4$ cell counts). Asmuth *et al* (1993) reported clinical features of 20 cases of microsporidiosis induced diarrhea as follows : the mean duration of diarrhea was $8.5 \pm 6.9$ month, stool frequency was about $5.7 \pm 2$ per day, the consistancy varied from loose to watery. The other associated gastrointestinal symptoms were bloating and anorexia. But Field *et al* (1993) reported only 33% (36/109) of patients with microsporidia had diarrhea and one of 71 patients who came with other indications had no diarrhea. Cotte *et al* (1993) reported one patients with microsporidia had severe malaborption but no diarrhea. Lastly Rabeneck *et al* (1993) conducted a case-control study to determine whether there was an association between the microsporidian parasite (*Enterocytozoon bieneusi*) and chronic diarrhea in 106 HIV infected men (55 with chronic diarrhea and 51 without). There was no significant difference in the incidence of microsporidiosis between the two groups. In conclusion, patients with intestinal microsporidiosis sometimes present with chronic diarrhea but may not always be the cause of symptoms, like the case reported here. However it warrants further investigation about the role of this microsporidia contributing to disease in HIV positive patients.

Since the number of AIDS cases is increasing in Thailand and nearly half of them presents with diarrhea, the prevalence of this organism is expected to be high in this country. So the first case reported here was just a good start for further evaluating exact prevalence of this infection in this group of population. Usually the invasive procedure is needed to get biopsy speccimens for definitive diagnosis of this organism. But unfortunately the endoscopy is done in this country only when highly indicated especially in AIDS patients so the method used here is a practical method using only stool for the diagnosis.

## ACKNOWLEDGEMENTS

We would like to thank Dr Timothy D Mastro, Chief HIV/AIDS Collaboration, Bangkok, Dr Bryan T Ralph and Dr Suzanne P Wahlquist, Department of Health and Human Services, Centers for Disease Control Atlanta, GA, USA and Dr Dwip Kitiyaporn, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand for his kind assistance in sending us the reference slides.

## REFERENCES

Asmuth DM, De Girolami PC, Federman M, *et al.* Clinical features of microsporidiosis in patients with AIDS. *Clin Infect Dis* 1994; 18 : 819-25.

Bretagne S; Foulet F, Alkassum W, Fleury FJ, Develoux M. Prevalence of *Enterocytozoon bieneusi* spores in the stool of AIDS patients and African children not infected by HIV. *Bull Soc Pathol Exot* 1993; 86 : 351-7.

Cotte L, Rabodonirina M, Pien MA, *et al.* Prevalence of intestinal protozoans in French patients infected with HIV. *J Acquir Immun Defic Synd* 1993; 6 : 1024-9.

Desportes I, Le Charpentier Y, Galain A, *et al.* Occurrence of a new microsporidian: *Enterocytozoon bieneusi* enterocytes of a human patient with AIDS. *J Protozool* 1985, 32 : 250-4.

Dobbins WO, Weinstein WM. Electron microscopy of the intestine and rectum in Acquired Immunodeficiency Syndrome. *Gastroenterology* 1985, 88 : 738-49.

Field AS, Hing MC, Milliken ST, Marriott DJ. Microsporidia in the small intestine of HIV infected patients. A new diagnostic technique and a new species. *Med J Aust* 1993, 158 : 390-3.

Gool TV, Hollister WS, Schattenkerk JE, *et al.* Diagnosis of *Enterocytozoon bieneusi* microsporidiosis in AIDS patients by recovering of spores from feces. *Lancet* 1990; 336 : 697-8.

Kotler DP, Giang TT, Garro ML, *et al.* Light microscopic diagnosis of microsporidiosis in patients with AIDS. *Am J Gastroenterol* 1994; 540-4.

Molina JM, Sarfati C, Beauvais B, *et al.* Intestinal microsporidiosis in Human Immunodeficiency Virus-infected patients with chronic unexplained diarrhea : Prevalence and clinical and biologic features. *J Infect Dis* 1993; 167 : 217-21.

Orenstein JM, Chiang J, Steinberg W, Smith PD, Rotterdam H, Kotler DP. Intestinal microsporidiosis as a cause of diarrhea in human immunodeficiency virus-infected patients a report of 20 cases. *Hum Pathol* 1990; 21 : 475-81.

Orenstein JM. Microsporidiosis in the Acquired Immunodeficiency Syndrome. *J Parasitol* 1991; 77 : 843-64.

Rabeneck L, Gyorkey F, Genta RM, *et al.* The role of microsporidia in the pathogenesis of HIV-related chronic diarrhea. *Ann Intern Med* 1993; 119 : 895-9.

Schattenkerk JKM, Gool TV, Vanketel RJ, *et al.* Clinical significance of small-intestinal microsporidiosis in HIV-1-infected individual. *Lancet* 1991; 337 : 895-8.

Weber R, Bryan RT, Owan RL, *et al.* Improved light microscopical detection of microsporidia spores in stool and duodenal aspirates. *N Engl J Med* 1992; 336 : 1127-8.