

Thesis title	Detection of Microsporidial Spores in Stool of AIDS Patients and non-AIDS Patients with Diarrhea using the Modified Trichrome Blue and the Calcofluor White Stains.
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ABSTRACT

This study reported the infection rates of intestinal parasites between 350 AIDS patients with diarrhea and 350 non-AIDS patients with diarrhea at Bamrasnaradura Infectious Diseases Hospital during May 1996 to January 1997. The results revealed the presence of eight protozoa and six helminths in 173 patients (24.7 %). The rate of patients testing positive for intestinal parasitic infections was statistically higher in AIDS patients with diarrhea than in non-AIDS patients with diarrhea (43.1 % vs 6.3 %, $p < 0.001$). Microsporidia, *Cryptosporidium spp.*, *Isospora belli*, *Blastocystis hominis*, *Strongyloides stercoralis*, *Enterobius vermicularis*, and *Opisthorchis viverrini* were only found in AIDS patients with diarrhea and they were decided to be opportunistic infections in AIDS patients with diarrhea except *E. vermicularis* and *O. viverrini* at $p < 0.05$. Microsporidia were the most common pathogen found in 53 cases (15.1 %) in AIDS patients with diarrhea. Iodine direct wetmount gave identical results to 700 stool examinations with

formalin-ether concentration technique, and the infection rates of intestinal parasites ranged from 0.3 % to 3.4 % but *Cryptosporidium spp.* and microsporidia were not found. *Cryptosporidium spp.* was recognized by the modified Ziehl-Neelsen staining technique in 36 cases (5.1 %) but this technique could not find other parasites. The modified trichrome blue staining technique recognized microsporidial spores in 53 cases (7.6 %) and *Cryptosporidium spp.* in 12 cases (1.7 %). The calcofluor white staining technique detected microsporidial spores in 56 cases (8.0 %) but seven patients were negative by the modified trichrome blue staining technique, were suspected of being falsely positive and four cases were falsely negative by the calcofluor white staining technique. The calcofluor white stain gave 92.5 % of sensitivity, 98.9 % of specificity, 87.5 % of predictive positive value, 99.4 % of predictive negative value, 98.4 % of efficiency, and 939.4 of relative liability when compared with the modified trichrome blue stain. The results were significantly correlated ($p > 0.05$). The calcofluor white stain was rapid, easier, and less expensive than the modified trichrome blue stain, but the fluorescence microscope used was very expensive. Thus, the modified trichrome blue stain could be recommended to set up staining for detection microsporidia in most clinical laboratories.

Clinical feature in AIDS patients with microsporidiosis were lower CD4 counts, CD8 counts, the percentage of lymphocytes, white blood cell counts, T-lymphocyte counts, and CD4/CD8 ratios when compared with AIDS patients without microsporidiosis (mean , 36.6 vs 111.2 cells/ml , 695.9 vs 934.2 cells/ml, 24.2 vs 29.6 % , 5,200 vs 5,659.6 cells/ml, 730.3 vs 1,163 cells/ml, and 0.06 vs 0.16 , respectively). Only T-lymphocyte counts and CD4 counts in AIDS patients with microsporidiosis were statistically difference from AIDS patients without microsporidiosis ($p < 0.05$). The patients with microsporidiosis had longer duration of diarrhea and more body weight loss than the patients without

microsporidiosis (mean 53.1 vs 32.4 days and 0.9 vs 0.8 kg/wk , respectively). Fifty three AIDS patients with microsporidia were 8 more likely to be male than female and the highest infection rate was found in the age groups of 25-34 years old (47.1 % or 25 of 53 cases). The age group of 5-14 years old were not found to have microsporidial spores. In 51 AIDS patients with only microsporidiosis, 33 patients (64.7 %) had diarrhea less than 6 times per day and all of the patients had duration of diarrhea more than 30 days (range , 32 - 150 days). Anorexia, nausea, vomiting, and bloating were commonly found in the patients but they had no fever ($< 37.5^{\circ} \text{C}$).