

CRYPTOSPORIDIUM ACTION IN FECAL AND SPUTUM SAMPLES FROM PATIENTS WITH HIV/AIDS

James S Hutagalung^{1,2}, Erwin Astha Triono³, R.Heru Prasetyo^{1,2}

¹Department of Parasitology, Faculty of Medicine, Universitas Airlangga

²Diploma Program of Medical Analyst, Faculty of Medicine, Universitas Airlangga

³Department of Internal Medicine, Faculty of Medicine, Universitas Airlangga, Dr. Soetomo Hospital, Surabaya

ABSTRAK

Cryptosporidiosis usus pada penderita HIV/AIDS dapat mengalami penyebaran ke paru, namun prevalensi penyebaran paru ini belum diketahui. Tujuan penelitian ini untuk menentukan prevalensi penyebaran cryptosporidiosis usus ke paru pada penderita HIV/AIDS dengan kelainan paru. Sampel dahak dari penderita HIV/AIDS dengan cryptosporidiosis usus dan kelainan paru diperiksa dengan menggunakan teknik pengecatan modifikasi Ziehl Neelsen dan diamati secara mikroskopis pembesaran 1000x dan minyak emersi. Terdapat prevalensi penyebaran ke paru sebesar 72,2% (8/11) cryptosporidiosis usus pada penderita HIV/AIDS dengan kelainan paru. Adanya kelainan paru pada penderita HIV/AIDS selain dicurigai tuberculosis harus juga dicurigai sebagai kemungkinan cryptosporidiosis paru. (FMI 2014;50:245-248)

Kata kunci: Cryptosporidiosis usus, HIV/AIDS, kelainan paru, cryptosporidiosis paru,

ABSTRACT

Intestinal cryptosporidiosis in people with HIV/AIDS may have spread to the lungs, but the prevalence of dissemination to the lung has not been disclosed. The aim of this study was to determine the prevalence of cryptosporidiosis spread of the intestine to the lungs in people with HIV/AIDS with pulmonary disorders. Sputum samples of patients with HIV/AIDS with intestinal cryptosporidiosis and pulmonary disorders were examined using a modified Ziehl Neelsen staining technique and observed microscopically in 1000x magnification and oil immersion. Prevalence of dissemination to the lungs was 72.2% (8/11) in intestinal cryptosporidiosis HIV/AIDS patients with pulmonary disorders. The presence of lung abnormalities in people with HIV/AIDS, in addition to suspicion of tuberculosis, should also lead to the suspicion of pulmonary cryptosporidiosis. (FMI 2014;50:245-248)

Keywords: intestinal cryptosporidiosis, HIV/AIDS, pulmonary disorders, pulmonary cryptosporidiosis

Correspondence: James S Hutagalung, Department of Parasitology, Faculty of Medicine, Universitas Airlangga, Jalan Prof. Dr. Moestopo 47, Surabaya 60131, Indonesia. Email: james_hctv@yahoo.com

INTRODUCTION

The incidence of opportunistic infections in people with HIV/AIDS has been known to occur in HIV/AIDS epidemic. Such conditions causes death of people living with HIV (Lew et al 1997, Kumar et al 2002). In developing countries, opportunistic intestinal parasite *Cryptosporidium* infection is a major cause of diarrhea (Gilson & Buggy 1996, Wiwanitkit 2001, Miao et al 2002). In people with HIV/AIDS who experience chronic diarrhea due to *Cryptosporidium*, the infection may have spread to the lungs and cause pulmonary cryptosporidiosis (Palmieri et al 2005, Markell et al 1999). From Prasetyo's study (2008) it has been known that the prevalence of intestinal cryptosporidiosis in HIV/AIDS patients with chronic diarrhea amounted to 52.46%, but it has not been known how much it spread to the lungs. Lung abnormalities in people with HIV/AIDS has always been associated with tuberculosis, so the presence of lung cryptosporidiosis is

neglected, while the clinical symptoms of lung cryptosporidiosis is difficult to differentiate from clinical symptoms of tuberculosis (Palmieri et al 2005). For HIV/AIDS patients with pulmonary disorders can be dealt with appropriately, it is necessary to improve diagnosis of opportunistic infections in people with HIV/AIDS.

MATERIALS AND METHODS

Faeces and sputum samples were those left from the samples for routine examination of HIV/AIDS patients with chronic diarrhea and lung disorder who were hospitalized in RSU Dr. Soetomo Surabaya. From stool samples obtained, we made preparations on glass objects, and allowed it to dry at room temperature. After drying, fixation was done by adding methanol for 5 minutes, then stained with Modified Ziehl Neelsen staining. Microscopic observation was made by adding

oil immersion and 1000 x magnification. *Cryptosporidium* was found microscopically positive when notching round, smaller than the size of erythrocytes and red colored pink (WHO 2003, Dupont et al 1996). Sputum samples examined were taken from patients who had *Cryptosporidium* positive fecal samples, carried out with a modified Ziehl Neelsen staining technique as the same as those applied in faecal samples. CD4 count of the patient served as secondary data.

RESULTS

Of the remaining stool samples collected from June to August 2013 from HIV/AIDS patients with pulmonary disorders, positive stool samples obtained 11 *Cryptosporidium*, taken from the anaesthetized 6 male patients and 5 female patients with age range between 29 and 55 years, and the number of CD4 was equal to or less than 70 cells/mm³. From 11 patients with *Cryptosporidium* positive feces samples, we checked the rest of the sputum samples, and positive sputum samples was found in 8 *Cryptosporidium*, derived from 4 male and 4 female patients.

Eleven residual sputum samples were collected from people living with HIV and AIDS treated in Dr Soetomo Hospital in the period of June 2013 until August 2013 with age criteria of ≥ 29 years and ≤ 55 years with $CD4 \leq 70$. With a modified Ziehl Neelsen staining method, three samples of stool and sputum showed positive *Cryptosporidium* oocysts. Of 11 sputum and stool samples of people with HIV and AIDS, 8 revealed *Cryptosporidium parvum* in patients with positive sputum HIV/AIDS and containing *cryptosporidium parvum*, with a percentage of 72.70%. While the example of patient stool samples of eleven samples of HIV and AIDS all revealed *Cryptosporidium parvum* (100%).

DISCUSSION

From the results obtained, it is known that the spread of cryptosporidiosis can occur from intestine to the lungs and causes pulmonary cryptosporidiosis, with a prevalence of pulmonary dissemination amounted to 72.2% (8/11). The spread from the intestine to the lung in both male and female patients turned out to have the same opportunities. In terms of the age of the patient is difficult to predict, while the more decisive is the CD4 count, in which the lower the CD4 count, the higher the possibility of its dissemination from intestine to the lungs. A study by Prasetyo (2011) revealed that in patients with severe malnutrition such as the HIV/AIDS barrier formation and release of PGE2 occurred, which

indicates there has been a malfunction of macrophages, as PGE2 is formed and released by macrophages.

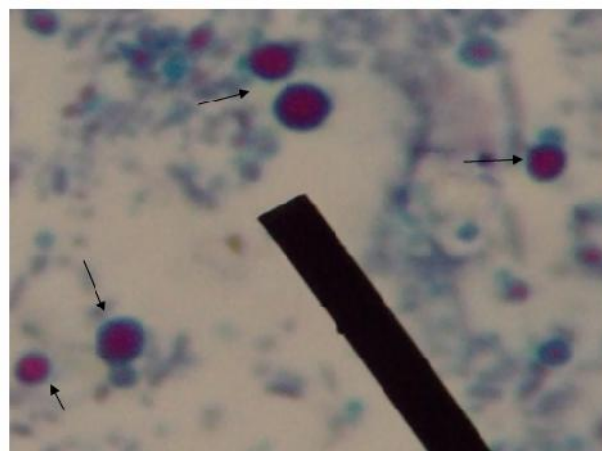


Figure 1. Overview of microscopic positive *Cryptosporidium* oocyst through examination of sputum samples with modified Ziehl Neelsen staining techniques, in magnification 1000 x with oil immersion. *Cryptosporidium* oocysts (arrows) appears in round shape and in reddish-pink color.

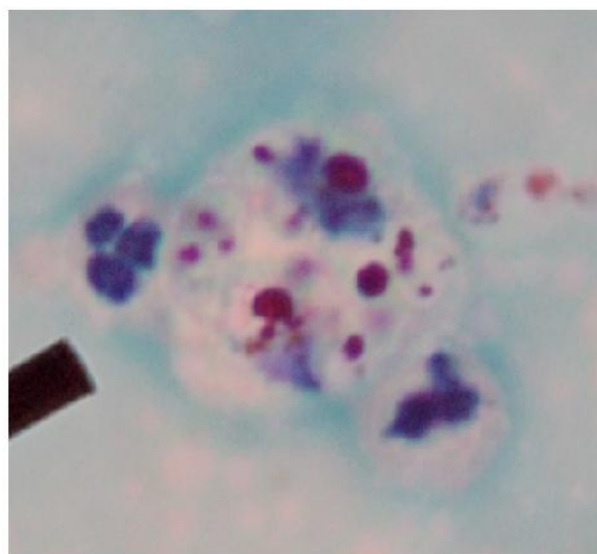


Figure 2. Microscopic profile of positive *Cryptosporidium* oocyst through examination of stool samples with modified Ziehl Neelsen staining technique, 1000x magnification with oil immersion, showing *Cryptosporidium* oocysts in round shape and reddish-pink color.

Table 1. Data on patients' age, gender, CD4 cell count of HIV/AIDS and the results of cryptosporidium from faecal samples and samples of sputum

Number of samples	Age (th)	Sex	CD4 (sel/mm ³)	Examination Result of <i>Cryptosporidium</i> from samples	
1	39	M	50	faeces	sputum
2	47	F	50	Positive	Positive
3	39	M	70	Positive	Positive
4	37	F	50	Positive	Positive
5	55	M	50	Positive	Negative
6	45	F	50	Positive	Negative
7	30	F	50	Positive	Positive
8	29	M	70	Positive	Positive
9	44	M	50	Positive	Negative
10	35	M	70	Positive	Positive
11	54	M	50	Positive	Positive

The spread of cryptosporidium from the intestine to the lung is suspected hematogenously and carried by malfunctioned macrophages. In the macrophages, Cryptosporidium oocysts are not digested, but it can multiply and reach the lungs where Cryptosporidium oocysts are released and lead to the incidence of pulmonary cryptosporidiosis (Mor et al 2010). The incidence of pulmonary cryptosporidiosis can be caused by intestinal cryptosporidiosis in patients who experience vomiting and partially regurgitated material containing oocysts cryptospridium is ingested back and goes into the lung (Mercado et al 2007)

CONCLUSION

This study detected Cryptosporidium oocysts from the stool and sputum samples of patients with HIV/AIDS with chronic diarrhea and pulmonary disorders hospitalized at Dr Soetomo Hospital. From 11 HIV/AIDS patients with intestinal cryptosporidiosis, the prevalence of pulmonary cryptosporidiosis was 72.70%. Either male and female patients with HIV/AIDS suffering from intestinal cryptosporodiosis have equal chance to get lung cryptosporidiosis. CD4 cell counts in HIV/AIDS is getting lower, the greater the chance of suffering from intestinal cryptosporidioasis and the possibility of the spread to the lungs.

REFERENCES

Dupont C, Bougnoux ME, Turner L, Rouveix E, Dorra M (1996). Microbiological findings about pulmonary cryptosporidiosis in two AIDS patients. *Journal of Clinical Microbiology* 34, 227-229

Gilson I, Buggy BP (1996). Cryptosporidiosis in Patients with HIV Diseases. Milwaukee, HIV News-line, Winconsin AIDS Research Consurtium.

Kumar SS, Ananthan S, Lakshmi P (2002). Intestinal parasitic infection in HIV infected patients with diarrhoea in Chennai. *Indian J Med Microbiol* 20, 88-91

Lew EA, Poles MA, Dieterich DT (1997). Diarrheal diseases associated with HIV infection. *Gastroenterol Clin North Am* 26, 259-290

Markell EK, John DT, Krotoski WA (1999). Markell & Voge's medical parasitology 8e 8th ed., Philadelphia, Saunders Elsevier, p 78-82

Mercado R, Buck GA, Manque PA, Ozaki LS (2007). Cryptosporidium hominis infection of the human respiratory tract. *Emerging Infectious Diseases* 13, 462-464

Miao YM, Awad-El-Karim FM, Gazzard BG (2002). Opportunistic protozoan diarrhoea. *Journal of HIV Therapy* 7, 17-20

Mor SM, Tumwine JK, Ndeezi G, Srinivasan MG, Kaddu-Mulindwa DH, Tzipori S, Griffiths JK (2010). Respiratory cryptosporidiosis in HIV-seronegative children in Uganda: potential for respiratory transmission. *Clinical Infectious Disease* 50, 1366-1372

Palmieri F, Cicalini S, Froio N, Rizzi EB, Goletti D, Festa A, Macrí G, Petrosillo N (2005). Pulmonary cryptosporidiosis in an AIDS patients: successful treatment with paromomycin plus azithromycin. *Int J STD AIDS* 16, 515-517

Prasetyo RH (2008). Identifikasi parasit usus pada penderita HIV dengan diare yang dirawat di RSU Dr Soetomo Surabaya. In: Narsonudin, Lusida MI, Widiyanti P, Aksono EB, Rahardjo D (eds.) *Biologi Molecular penyakit infeksi*, 1st ed., Surabaya, Institut of Tropical Diseses Universitas Airlangga, p 59-61

Prasetyo RH (2011). Immunokimiawi PGE2 usus ileum yang kekurangan energi protein. *Indonesian Journal of Clinical Pathology and Medical Laboratory* 17(3).

WHO (2003). Manual of basic techniques for a health laboratory 2nd edition, Geneva, World Health Organization, p 123-125

Wiwanitkit V (2001). Intestinal parasitic infection in Thai HIV-infected patients with different immunity status. BMC Gastroenterol 1, 3