

# Prevalence and Risk Factors of Intestinal Protozoan Infection in HIV/AIDS Patients in Dr. Sardjito General Hospital Yogyakarta

Ershandi Resnhaleksmana<sup>1</sup>, Elizabeth Sutarti<sup>2</sup>, Mahardika Agus Wijayanti<sup>2,\*</sup>

<sup>1</sup>Division of Medical Analyst, POLTEKKES Ministry of Health, Mataram; <sup>2</sup>Department of Parasitologi, Faculty of Medicine Universitas Gadjah Mada

\*)Corresponding author: dikafkugm@yahoo.co.id

## ABSTRACT

**Introduction:** Intestinal protozoa is one of the etiology of gastroenteritis in developing countries. The risk of intestinal protozoan infection increases among HIV/AIDS patients. HIV/AIDS patients with CD4+ T cell < 200 cells/ $\mu$ L are easily infected by intestinal protozoa causing broad clinical symptoms including diarrhea and even death. However, it can be prevented by understanding various risk factors which have role in the pathogenesis of intestinal protozoan infection.

**Objective:** To study the prevalence and risk factors which affect intestinal protozoan infection among HIV/AIDS patients in RSUP Dr. Sardjito Yogyakarta.

**Methods:** Data from 32 HIV/AIDS patients in RSUP Dr Sardjito Yogyakarta in December 2009-March 2010 were obtained by questionnaires, medical records, and macroscopic-microscopic examination of fecal samples with phormol-eter method and acid-fast staining. Data was analysed using Chi square test and multivariate analysis. A p value less than 0.05 is considered as a significantly different.

**Results:** Prevalence of intestinal protozoan infection in HIV/AIDS patients in RSUP Dr Sardjito Yogyakarta was 81.2%. Intestinal protozoa found in fecal examination were *Cryptosporidium* sp. (60.98%), *Microsporidium* sp. (19.51%), *Entamoeba histolytica* (9.76%), *Cyclospora cayetanensis* (4.88%), *Blastocystis hominis* (2.44%), and *Giardia lamblia* (2.44%) (n = 26). Bivariate analysis showed that in female HIV/AIDS patients with clinical stadium 1 and 2, CD4+ T cell  $\geq$  200 cells/ $\mu$ L, had lower risk to be infected by intestinal protozoa (RR = 0.600, 0.065, and 0.026, respectively). Intestinal protozoa were easily found in feces of HIV/AIDS patients with diarrheal symptom. In multivariate analysis, clinical stadium was the most significant factor (Exp<sup>(B)</sup> = 18.85).

**Conclusion:** Prevalence of intestinal protozoan infection in HIV/AIDS patients in RSUP Dr Sardjito Yogyakarta in December 2009-March 2010 was 81.2%. Clinical stadium with moderate and severe symptoms was the most dominant risk factor for intestinal protozoan infection in HIV/AIDS patients.

**Keywords:** risk factor, intestinal protozoa, CD4+ T cell - HIV/AIDS patient

## INTISARI

**Pendahuluan:** Protozoa usus merupakan salah satu etiologi gastroenteritis di negara berkembang. Risiko terjadinya infeksi protozoa usus meningkat pada penderita HIV/AIDS. Penderita HIV/AIDS dengan CD4 + T cell <200 sel /  $\mu$ L akan mudah terinfeksi oleh protozoa usus dan menyebabkan gejala klinis yang luas, termasuk diare dan bahkan kematian. Pemahaman akan berbagai faktor risiko yang memiliki peran dalam patogenesis infeksi protozoa usus diharapkan dapat membantu usaha preventif.

**Tujuan:** Penelitian ini bertujuan untuk mengetahui prevalensi dan factor resiko yang menyebabkan terjadinya infeksi protozoa usus pada pasien HIV/AIDS di RSUP Dr. Sardjito Yogyakarta.

**Metode:** Data didapatkan dari 32 pasien HIV / AIDS di RSUP Dr Sardjito Yogyakarta pada bulan Desember 2009-Maret 2010. Data diperoleh dengan kuesioner, catatan medis, dan pemeriksaan sampel tinja dilakukan secara makroskopik dan mikroskopik dengan metode phormol-eter dan pewarnaan asam-

cepat. Data dianalisis menggunakan uji Chi square dan analisis multivariat. Nilai p kurang dari 0,05 dianggap sebagai perbedaan yang signifikan.

**Hasil:** Prevalensi infeksi protozoa usus pada pasien HIV / AIDS di RSUP Dr Sardjito Yogyakarta adalah 81,2%. Protozoa usus yang ditemukan dalam pemeriksaan tinja adalah *Cryptosporidium* sp. (60,98%), *Microsporidium* sp. (19,51%), *Entamoeba histolytica* (9,76%), *Cyclospora cayetanensis* (4,88%), *Blastocystis hominis* (2,44%), dan *Giardia lamblia* (2,44%) (n = 26). Analisis bivariat menunjukkan bahwa pasien perempuan dengan HIV / AIDS stadium klinis 1 dan 2, sel CD4 + T  $\geq$  200 sel / uL, memiliki risiko lebih rendah untuk terinfeksi oleh protozoa usus (RR = 0,600, 0,065, dan 0,026). Protozoa usus mudah ditemukan dalam tinja pasien HIV / AIDS dengan gejala diare. Dalam analisis multivariat, stadium klinis merupakan faktor yang paling signifikan (Exp ( $\beta$ ) = 18,85

**Simpulan:** Prevalensi infeksi protozoa usus pada pasien HIV / AIDS di RSUP Dr Sardjito Yogyakarta pada bulan Desember 2009-Maret 2010 adalah 81,2%. Stadium klinis dengan gejala sedang dan berat adalah faktor risiko yang paling dominan untuk infeksi protozoa usus pada pasien HIV / AIDS.

**Kata kunci:** faktor resiko, intestinal protozoa, sel CD4+ T, pasien HIV/AIDS

## INTRODUCTION

Prevalence of intestinal protozoan infection in tropical areas is 50-60% of all population in the world. Study on intestinal protozoa in Yogyakarta have been reported, with similar prevalence between one location to another, that is, *Entamoeba histolytica* 3-11%, *Giardia lamblia* 1-8%, *Iodamoeba butschlii* 1-9%, *Endolimax nana* 6-19%, and *Entamoeba coli* 18-19%<sup>1,2,3</sup>.

The increased prevalence of new emerging infectious diseases and reemerging infectious diseases have been associated with the increased in HIV/AIDS patients and other diseases related to the decrease in body immune system. Opportunistic intestinal parasites such as *Cryptosporidium*, *Isospora belli*, *Microsporidia*, *Giardia*, and *Strongyloides stercoralis* cause malabsorption and chronic diarrhea which result in abnormality of intestinal mucosal area. HIV/AIDS patients with severe diarrhea may lose body fluid as many as 3-6 liter daily and they will end with death. Diarrhea is particularly occurred in HIV and AIDS patients who have CD4+ T cell < 200 cells/ $\mu$ L and 26% of them are caused by intestinal protozoa<sup>4,5,6</sup>.

Based on AIDS surveillance data from District Health Office of Yogyakarta, the number of HIV cases in November 2009 were 590 but 294 of

them were diagnosed as AIDS patient. Study on intestinal protozoan diseases in HIV and AIDS patients has not substantially conducted yet, except for several types of particular parasites such as *Toxoplasma gondii* and *Cryptosporidium*. The study of other intestinal protozoa in the feces of HIV/AIDS patients as the etiology of opportunistic infection is need to be clarified.

## MATERIALS AND METHODS

It was an analytical observational study with cross-sectional design, conducted on 32 HIV/AIDS patients in RSUP Dr Sardjito Yogyakarta in December 2009 until March 2010. Inclusion criteria were HIV patients registered in inpatient and outpatient units of RSUP Dr Sardjito. Exclusion criteria were the use of antiparasitic and antibiotic agents within 5-10 days prior parasite examination, age 12- 60 years old. Secondary data obtained from this study was clinical stadium, and CD4+ T cell total number, while other data such as gender, educational level, economic status, employment status, and feces were obtained by interview and direct examination.

HIV and AIDS patient's fecal sample examination was conducted with macroscopic

and microscopic examinations using phormol-ether method and acid-fast staining to find out the types of protozoa in fecal samples. Data was analysed using Chi-square test and multivariate analysis. A p value less than 0.05 is considered as a significantly different.

## RESULTS

Characteristics of HIV/AIDS patients are summarized in Table 1. Total subjects were 32 individuals, consisted of 10 inpatients and 22 outpatients of RSUP Dr Sardjito, Yogyakarta.

From all HIV/AIDS patients, those who suffered from intestinal protozoan infection were 81.2% and those who didn't were 18.8%. Most common intestinal protozoa in HIV/AIDS patient was *Cryptosporium sp.*, found in 25 patients (60.98%). Complete data on various intestinal protozoa found in HIV/AIDS patients were shown in Figure 1.

Based on fecal examination, half of HIV/AIDS patients were not infected by one of the intestinal protozoa, but there were some patients who had multiple infection. Data of HIV/AIDS patients infected with single or multiple intestinal protozoa infection were shown in Table 2.

Characteristics of patients based on CD4+ T cells, feces, and intestinal protozoan infection in HIV/AIDS patients are shown in Table 3.

Characteristics of HIV/AIDS patients based on clinical stadium, feces, and intestinal protozoan infection are shown in Table 4.

On Table 4, there were 2 of 7 HIV/AIDS patients who were asymptomatic, without diarrhea, with positive intestinal protozoa in fecal examination. In this study, there were 8 HIV/AIDS patients with mild symptoms, 6 of them with diarrhea and microscopic examination showed intestinal protozoa in all samples. Other 2 patients showed no diarrhea, but fecal microscopic examination showed 1 sample which positive for intestinal

protozoa. There were 13 HIV/AIDS patients with moderate symptoms, 10 of them with diarrhea and fecal microscopic examination found intestinal protozoa in all samples. Other 3 patients did not have diarrhea, but fecal microscopic examination showed intestinal protozoa in all samples. From 32 HIV/AIDS patients, only 4 who had severe symptoms with diarrhea, and fecal microscopic examination showed intestinal protozoa in all samples.

Frequency distribution of each risk factor and intestinal protozoa infection is shown in Table 5. In HIV/AIDS patients < 30 years old, those who were infected with intestinal protozoa were 84.6% and in patients  $\geq$  30 years old were 78.9% (n = 26), while those who were uninfected in < 30 years old age group were 15.4% and in  $\geq$  30 years old age group were 21.1% (n = 6). In this study, there were 24 HIV/AIDS patients who were males, and 23 of them were infected with intestinal protozoa (95.8%) and 1 was uninfected (4.2%), while from 8 HIV/AIDS patients who were females, 3 were infected (37.5%) and 5 were uninfected (62.5%).

There were 5 HIV/AIDS patients with low educational level, and all were infected by intestinal protozoa; from 27 patients with high educational level, there were 21 who were infected with intestinal protozoa (77.8%), and 6 were uninfected (22.2%). In 12 patients who had low economic status, all were infected with intestinal protozoa and the other 20 had good economic status, 14 of them were infected with intestinal protozoa (70%) and 6 were uninfected (30%). From 32 HIV/AIDS patients, there were 13 patients who were unemployed, 9 of them were infected with intestinal protozoa (75%), and 3 samples were uninfected (25%), while those 20 who had employment, 17 of them were infected with intestinal protozoa (85%) and 3 were uninfected (15%).

In this study, 12 HIV/AIDS patients had no

Table 1. Characteristics of HIV/AIDS patients in RSUP Dr Sardjito Yogyakarta from December 2009-March 2010

| Characteristic                    | Prevalence (%)  |
|-----------------------------------|-----------------|
| Age (years old, average±SD)       | 32.44 ± 7.01    |
| - Minimum age (years old)         | 20              |
| - Maximum age (years old)         | 52              |
| Gender                            |                 |
| - Male                            | 24 (75)         |
| - Female                          | 8 (25)          |
| Educational level                 |                 |
| - Low                             | 5 (15.6)        |
| - High                            | 27 (84.4)       |
| Economic status                   |                 |
| - Low                             | 12 (37.5)       |
| - High                            | 20 (62.5)       |
| Employment status                 |                 |
| - Employed                        | 20 (62.5)       |
| ▪ Private employment              | 7 (21.5)        |
| ▪ Merchant                        | 6 (18.8)        |
| ▪ Laborer                         | 4 (12.5)        |
| ▪ Civil servant                   | 3 (9.4)         |
| - Unemployed                      | 12 (37.5)       |
| Feces                             |                 |
| - Diarrhea                        | 20 (62.5)       |
| - Not diarrhea                    | 12 (37.5)       |
| Clinical stadium                  |                 |
| - Asymptomatic                    | 7 (21.9)        |
| - Mild symptom                    | 8 (25.0)        |
| - Moderate symptom                | 13 (40.6)       |
| - Severe symptom                  | 4 (12.5)        |
| CD4+ T cell (cell/uL, average±SD) | 127.94 ± 173.26 |
| - < 200 cells/μL                  | 25 (78.1)       |
| - 200-499 cells/μL                | 6 (18.8)        |
| - ≥ 500 cells/μL                  | 1 (3.1)         |
| Intestinal protozoan infection    |                 |
| - Positive                        | 26 (81.2)       |
| - Negative                        | 6 (18.8)        |

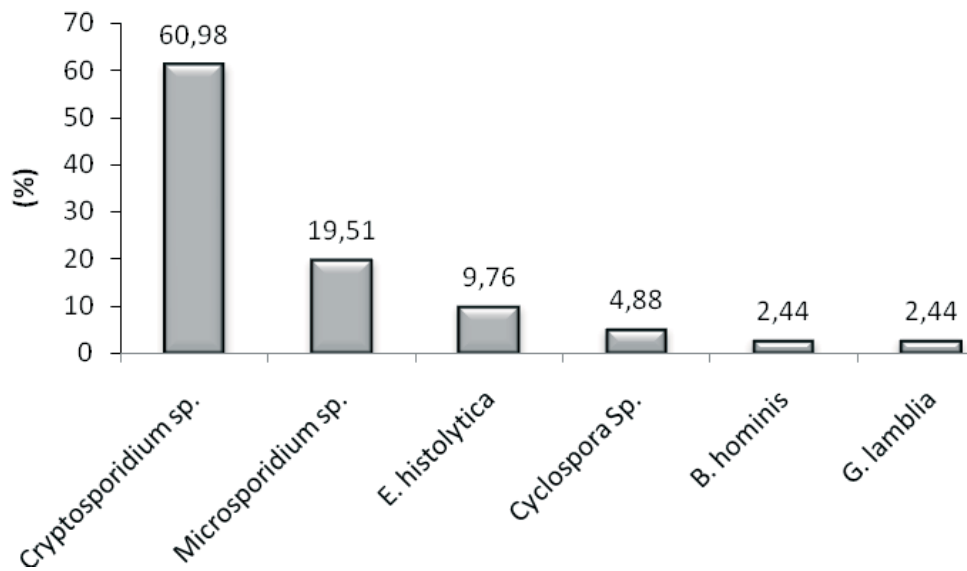


Figure 1. Species and percentage of intestinal protozoa among HIV/AIDS patients in RSUP Dr. Sardjito Yogyakarta in December 2009-March 2010

Table 2. Frequency distribution of single or multiple intestinal protozoan infection in HIV/AIDS patients in RSUP Dr. Sardjito Yogyakarta in December 2009-March 2010

| Single or multiple infection                                      | Total patient |
|---|---------------|
| Single infection  | 12 (46.15%)   |
| Multiple infection  | 14 (53.85%)   |
| Infection >1 intestinal protozoa, CD4+ T cell <200 cells/ $\mu$ L | 13 (92.86%)   |
| Infection >1 intestinal protozoa, CD4+ T cell >199 cells/ $\mu$ L | 1 (7.14%)     |
| Infection >1 intestinal protozoa in clinical stadium 1            | 0 (0%)        |
| Infection >1 intestinal protozoa in clinical stadium 2            | 5 (35.71%)    |
| Infection >1 intestinal protozoa in clinical stadium 3            | 5 (35.71%)    |
| Infection >1 intestinal protozoa in clinical stadium 4            | 4 (28.57%)    |

Table 3. Frequency and species of intestinal protozoa based on number of CD4+ T cell and diarrhea in HIV/AIDS patients in RSUP Dr Sardjito Yogyakarta in December 2009-March 2010

| CD4+ T cell<br>(cells/ $\mu$ L) | Intestinal protozoa        | Diarrhea |          | Non-diarrhea |          | Total |
|---------------------------------|----------------------------|----------|----------|--------------|----------|-------|
|                                 |                            | Positive | Negative | Positive     | Negative |       |
| < 200                           |                            | 18       | 0        | 5            | 2        | 25    |
|                                 | <i>Microsporidium sp.</i>  | 7        | 0        | 1            | 0        |       |
|                                 | <i>Cryptosporidium sp.</i> | 18       | 0        | 4            | 0        |       |
|                                 | <i>Cyclospora sp.</i>      | 1        | 0        | 0            | 0        |       |
|                                 | <i>B. hominis</i>          | 1        | 0        | 0            | 0        |       |
|                                 | <i>E. histolytica</i>      | 4        | 0        | 0            | 0        |       |
|                                 | <i>G. lamblia</i>          | 0        | 0        | 1            | 0        |       |
| 200-499                         |                            | 2        | 0        | 1            | 3        | 6     |
|                                 | <i>Cryptosporidium sp.</i> | 2        | 0        | 1            | 0        |       |
|                                 | <i>Cyclospora sp.</i>      | 1        | 0        | 0            | 0        |       |
| > 500                           |                            | 0        | 0        | 0            | 1        | 1     |
|                                 | Negative                   |          |          |              |          |       |
| Total sample                    |                            | 20       | 0        | 6            | 6        | 32    |

diarrhea, but microscopic examination showed intestinal protozoa in 6 samples (50%), and 6 samples did not show any intestinal protozoa (50%). For other 20 patients with diarrhea, in microscopic examination there were intestinal protozoa in 2 samples (100%). There were 15 HIV/AIDS patients who were asymptomatic and had mild symptoms, 9 of them were infected with intestinal protozoa (60%) and 6 samples were uninfected (40%). There were 17 patients who had moderate and severe symptoms, and all were infected with intestinal protozoa (100%). From 32 HIV/AIDS patients, there were 25 patients with CD4+ T cell < 200 cells/ $\mu$ L, 23 of them were infected with intestinal protozoa (92%) and 2 were uninfected (8%), while in 7 patients with CD4+ T cell  $\geq$  200 cells/ $\mu$ L, 3 were infected with intestinal protozoa (42.9%) and 4 were uninfected (57.1%).

Chi-square analysis on gender, diarrhea, clinical stadium, and CD4+ T cell count of HIV/AIDS patients were significantly different, with p value < 0.05, which meant that gender, diarrhea, clinical stadium, and CD4+ T cell were associated with intestinal protozoan infection in HIV/AIDS patients. OR for gender and CD4+ T cell were 0.026 and 0.065, respectively, which statistically indicated that female were 0.026 times at risk to be infected by intestinal protozoa, while patients with CD4+ T cell  $\geq$  200 cells/ $\mu$ L were 0.065 times at risk to be infected by intestinal protozoa. RR for feces and clinical stadium were 2.000 and 0.600, respectively, which statistically indicated that patients with diarrhea were 2.000 times at risk to be infected by intestinal protozoa, while patients who were asymptomatic and had mild symptoms were 0.600 times at risk to be infected by intestinal protozoa.

Table 4. Frequency and types of intestinal protozoa based on clinical stadium and feces in HIV/AIDS patients in RSUP Dr Sardjito Yogyakarta in December 2009-March 2010

| Clinical stadium | Intestinal protozoa        | Diarrhea |          | Non-diarrhea |          | Total |
|------------------|----------------------------|----------|----------|--------------|----------|-------|
|                  |                            | Positive | Negative | Positive     | Negative |       |
| Asymptomatic     |                            | 0        | 0        | 2            | 5        | 7     |
|                  | <i>Cryptosporidium</i> sp. | 0        | 0        | 2            | 0        |       |
| Mild symptom     |                            | 6        | 0        | 1            | 1        | 8     |
|                  | <i>Microsporidium</i> sp.  | 4        | 0        | 0            | 0        |       |
|                  | <i>Cryptosporidium</i> sp. | 6        | 0        | 1            | 0        |       |
|                  | <i>E. histolytica</i>      | 1        | 0        | 0            | 0        |       |
| Moderate symptom |                            | 10       | 0        | 3            | 0        | 13    |
|                  | <i>Microsporidium</i> sp.  | 2        | 0        | 1            | 0        |       |
|                  | <i>Cryptosporidium</i> sp. | 10       | 0        | 2            | 0        |       |
|                  | <i>Cyclospora</i> sp.      | 1        | 0        | 0            | 0        |       |
|                  | <i>B. hominis</i>          | 1        | 0        | 0            | 0        |       |
|                  | <i>G. lamblia</i>          | 0        | 0        | 1            | 0        |       |
| Severe symptom   |                            | 4        | 0        | 0            | 0        | 4     |
|                  | <i>Microsporidium</i> sp.  | 1        | 0        | 0            | 0        |       |
|                  | <i>Cryptosporidium</i> sp. | 4        | 0        | 0            | 0        |       |
|                  | <i>E. histolytica</i>      | 3        | 0        | 0            | 0        |       |
|                  | Total sample               | 20       | 0        | 6            | 6        | 32    |

Variables of age, educational level, economic status, and employment status were insignificantly different, with p value of 1.00, 0.555, 0.61, and 0.647, respectively, which meant that there were no association between age, educational level, economic status, and employment status of HIV/AIDS patients. Age group < 30 years old, low educational level, low economic status, and employed group had higher possibility to be infected by intestinal protozoa.

From all data obtained, to determine the most dominant risk factor for intestinal protozoan infection, multivariate analysis with logistic regression was conducted. Result of the total analysis of risk factors of HIV/AIDS patients

for intestinal protozoan infection is shown in Table 6.

Table 6 shows that gender, diarrhea, moderate and severe symptom clinical stadium, and CD4+ T cell < 200 cells/ $\mu$ L were associated with intestinal protozoan infection in HIV/AIDS patients with p value < 0.05. Moderate and severe symptom clinical stadium had highest Exp<sup>(B)</sup> of 18.85, compared with other 3 risk factors (0.000, 0.016, 0.909), which statistically indicated that clinical stadium with moderate and severe symptoms was the most dominant risk factor for intestinal protozoan infection in HIV/AIDS patients.



Table 5. Relationship between each risk factor and intestinal protozoan infection in HIV/AIDS patients in RSUP Dr Sardjito Yogyakarta in December 2009-March 2010

| Variables                                  | Intestinal protozoan infection |                | p<br>(CI = 95%) | Odds ratio<br>(OR) |
|--|--------------------------------|----------------|-----------------|--------------------|
|  | Positive (n=26)                | Negative (n=6) |                 |                    |
| Age ≥ 30 years old                         | 15 (78.9%)                     | 4 (21.1%)      | 1.000           | 0.682              |
| Age < 30 years old                         | 11 (84.6%)                     | 2 (15.4%)      |                 |                    |
| Female                                     | 3 (37.5%)                      | 5 (62.5%)      | 0.002*          | 0.026              |
| Male                                       | 23 (95.8%)                     | 1 ( 4.2%)      |                 |                    |
| Low educational level                      | 5 (100%)                       | 0 ( 0.0%)      | 0.555           | 1.286              |
| High educational level                     | 21 (77.8%)                     | 6 (22.2%)      |                 |                    |
| High economic status                       | 14 (70.0%)                     | 6 (30.0%)      | 0.061           | 0.700              |
| Low economic status                        | 12 (100%)                      | 0 (0.0%)       |                 |                    |
| Unemployed                                 | 9 (75.0%)                      | 3 (25.0%)      | 0.647           | 0.529              |
| Employed                                   | 17 (85.0%)                     | 3 (15.0%)      |                 |                    |
| Diarrhea                                   | 20 (100%)                      | 0 ( 0.0%)      | 0.001*          | 2.000              |
| No diarrhea                                | 6 (50.0%)                      | 6 (50.0%)      |                 |                    |
| Stadium I (asymptomatic and mild symptoms) | 9 (60.0%)                      | 6 (40.0%)      | 0.006*          | 0.600              |
| Stadium II (Moderate and severe symptoms)  | 17 (100%)                      | 0 ( 0.0%)      |                 |                    |
| CD4+ T cell ≥ 200 cells/μL                 | 3 (42.9%)                      | 4 (57.1%)      | 0.012*          | 0.065              |
| CD4+ T cell < 200 cells/μL                 | 23 (92.0%)                     | 2 ( 8.0%)      |                 |                    |

Note: \* p value was significantly different if < 0.05

Table 6. Analysis result of the most dominant risk factors of intestinal protozoan infection in HIV/AIDS patients in RSUP Dr Sardjito Yogyakarta in December 2009-March 2010

| Variable                              | p value | Exp (β) |
|---------------------------------------|---------|---------|
| Male                                  | 0.000*  | 0.000   |
| Diarrhea                              | 0.000*  | 0.016   |
| Moderate and severe clinical symptoms | 0.004*  | 18.85   |
| CD4+ T cell < 200 cells/μL            | 0.003*  | 0.909   |

Note : \* p value is significantly different if < 0.05

Exp<sup>(β)</sup> = Odds ratio of risk factor for intestinal protozoan infection

## DISCUSSION

Prevalence of intestinal protozoa in HIV/AIDS patients in RSUP Dr Sardjito Yogyakarta in December 2009-March 2010 was 26 patients (81.2%), and there were 6 who were uninfected (18.8%). The most dominant protozoa that had role in the infection in this study was *Cryptosporidium* sp. (25 patients, 60.98%), followed by *Microsporidium* sp (8 patients, 19.51%), *E. histolytica* (4 patients, 9.76%), *Cyclospora* sp. (2 patients, 4.88%), and *B. hominis* and *G. lamblia* (1 patient each, 2.44%). In this study, prevalence of intestinal protozoa infection was different from that in the previous study, and other pathogenic intestinal protozoa weren't found, such as in India, which found that



the intestinal protozoa in HIV/AIDS patients were *Cryptosporidium* sp. (75%), *G. lamblia* (32%), *Microsporidium* sp (13.3%), and *I. belli* (5.3%), of 75 samples<sup>10</sup>.

Multiple infection by intestinal protozoa commonly found in HIV/AIDS patients was caused by *Cryptosporidium* sp; this intestinal protozoa was possibly the cause of diarrhea, although it might infect patients without any diarrhea or those who had adequate immunity. *Cryptosporidium* sp mostly infects children, elderly, and AIDS patients who have low immunity, and it may cause malabsorption and chronic diarrhea. HIV/AIDS patients with CD4+ T cell < 200 cells/ $\mu$ L had more multiple infection (13 patients, 92.86%) compared with patients with CD4+ T cell  $\geq$  200 cells/ $\mu$ L (1 patient, 7.14%). Multiple infection is occurred because of latent phase of HIV, results in a decrease in CD4+ T cells and destruction of those cells in lymphatic nodes. Similar study found protozoan infection in HIV/AIDS patients with average age of 38 years old in France, such as *Cryptosporidium* sp (37.3%), *B. hominis* (13.7%), *G. lamblia* (5.6%), *I. belli* (2%), *Microsporidium* (2%), and *E. histolytica* (2%) from 81 patients with multiple infection (38.3%)<sup>11</sup>.

Average age of HIV/AIDS patients in this study was 32.44 years old, with minimum age of 20 years old and maximum age of 52 years old. There were 13 patients in < 30 years old the age group (20-30 years old), and there were 84.6% who were infected with intestinal protozoa. There were 19 patients in  $\geq$  30 years old age group (30-52 years old), and there were 78.9% who were infected with intestinal protozoa. Bivariate analysis on age group showed insignificant difference with p value of 1.000 and OR = 0.682, which meant that there was no association between age and intestinal protozoan infection in HIV/AIDS patients in RSUP Dr Sardjito Yogyakarta in December 2009-March 2010, but clinically, the probability of  $\geq$  30 years old age group to have

intestinal protozoan infection was 0.682.

HIV/AIDS patients included in this study were adults. In this age, the body imunity is higher, because it has been developed completely and repeated infections through their life has increased the development of antibody. Meanwhile, in children, immunity has not been developed completely, and in elderly there is poor absorption of nutrition, which decreases the cellular response, such as lymphocyte proliferation, cytokine synthesis, and response of antibody. Similar result in Nigeria showed that the age of HIV/AIDS was insignificantly different, with total intestinal protozoan infection for 20-30 years old age group was 32.9% and for > 30 years old age group was 26.3%. Other study also found the prevalence of intestinal protozoa in patients who did not have HIV/AIDS for 0-9 years old age group was 28%, for 20-40 years old age group was 20%, and for > 50 years old age group was 31%<sup>12,13,14</sup>.

There were 24 male HIV/AIDS patients in RSUP Dr Sardjito Yogyakarta in December 2009-March 2010 and there were 95.8% who were infected with intestinal protozoa, and there were 8 females with 37.5% of them were infected. Bivariate analysis of gender risk factor showed that gender was associated with intestinal protozoan infection, with p value of 0.002 and OR = 0.026. It showed that gender was one of the factors of intestinal protozoan infection in HIV/AIDS patients, but clinically, female had 0.026 times the risk to have intestinal protozoan infection, which meant that males was easier to be infected by intestinal protozoan infection compared with females. It may be caused by androgen hormone in males which are immunosuppressive, while women have estrogen which promote the increase in the immunity to infection. Similar study in Nigeria showed that gender of HIV/AIDS patients was significantly different, with 9 male infected (60%)

and 6 females infected (40%)<sup>12,13</sup>.

Educational level of HIV/AIDS patients in RSUP Dr Sardjito Yogyakarta in December 2009-March 2010 was considered low in 5 patients and all were infected with intestinal protozoa, while 27 patients with high educational level had 77.8% infection by intestinal protozoa. Bivariate analysis of educational level showed that it was not associated with intestinal protozoan infection, with *p* value of 0.0555 and OR = 1.286. It showed that educational level was not a risk factor of intestinal protozoan infection in HIV/AIDS patients, although clinically patients with low educational level had 1.286 times the risk to be infected by intestinal protozoa compared with patients with high educational level. Patients with low educational level was easier to be infected by intestinal protozoa, which probably caused by the lack of knowledge about self- and environmental hygiene related with intestinal protozoa life cycle. Other study in Iran suggested that there was no significant association between education and intestinal protozoan infection<sup>6,14</sup>.

Economic status of HIV/AIDS patients in RSUP Dr Sardjito Yogyakarta in December 2009-March 2010 was grouped by Regional Minimum Wage of Rp 700.000,00, and it was found that there were 12 patients with low economic status and all of them were infected by intestinal protozoa; and there were 20 patients with good economic status and 70% were infected. Bivariate analysis of economic status showed that it was not associated with intestinal protozoan infection, with *p* value of 0.061 and OR = 0.700. The result of the analysis showed that economic status was not a risk factor for intestinal protozoa infection in HIV/AIDS patients, although clinically patients with good economic status had probability of 0.700 to be infected by intestinal protozoa compared with low economic status, which meant that patients with low economic status were easily infected by intestinal protozoa. This

was made possible by the lack of nutrition as the support for the development of body immunity and the lack of support facility for self-, house and environmental hygiene. Previous study in Ethiopia showed that low economic status were easily infected by intestinal protozoa (86%) compared with patients with good economic status (14%)<sup>6,15</sup>.

Employment status of HIV/AIDS patients in RSUP Dr Sardjito Yogyakarta in December 2009-March 2010 consisted of employed (12 patients, 37.5%) and unemployed (12 patients, 37.5%). In employed group, 85% were infected with intestinal protozoa, while in unemployed group, there were 75% who were infected. Bivariate analysis showed that employment status was not associated with intestinal protozoan infection with *p* value of 0.647 and OR = 0.529. It showed that employment status was not a risk factor for intestinal protozoan infection in HIV/AIDS patients, although clinically, unemployed patients had 0.529 times the risk to be infected by intestinal protozoa compared to those who were employed. Infection in employed patients were higher because of the contact of HIV/AIDS patients with infective form of protozoa around the occupational environment and aggravated with the low immunity of HIV/AIDS patients. Previous study in Iraq conducted for a year showed that intestinal protozoan infection was occurred in laborers (19%) and those who worked in the office (10.5%), with *p* > 0.05<sup>14,16</sup>.

There were 20 HIV/AIDS patients with diarrhea in RSUP Dr Sardjito Yogyakarta, and all of them were infected by intestinal protozoa, while 12 non diarrheal patients showed 50% infection. Bivariate analysis on feces of the patients showed that it was not associated with intestinal protozoan infection, with *p* value of 0.001 and OR = 2.000. This analysis showed that intestinal protozoa were easier to be found in diarrheal patients with 2.000 times higher risk

compared with non-diarrheal patients. It was caused by the decrease in CD4+ T cells of HIV/AIDS patients. This is consistent with previous study which found that there was a significant association between diarrhea and intestinal protozoan infection in 99 patients with frequency of 51%, and mostly caused by *Cryptosporidium sp*<sup>17</sup>.

In this study, clinical stadium was classified into stadium I (asymptomatic and mild symptoms), which found in 15 patients (46.9%) and 60% of them were infected by intestinal protozoa; and stadium II (moderate and severe symptoms), which found in 17 patients (53.1%) and all of them were infected. Bivariate analysis on the clinical stadium showed that it was associated with intestinal protozoan infection with p value of 0.006 and OR = 0.600. The result of the analysis showed that clinical stadium was a risk factor of intestinal protozoan infection in HIV/AIDS patients, and clinically, patients who were asymptomatic or had mild symptoms had 0.600 times risk to be infected by intestinal protozoa, which meant that patients with moderate and severe symptoms were easier to be infected by intestinal protozoa. This result was consistent with WHO guideline which suggested that opportunistic infection in HIV/AIDS patients was occurred in clinical stadium 3 and 4.

CD4+ T cells in this study was classified into < 200 cells/ $\mu$ L, which found in 25 patients and 92% of them were infected; and  $\geq$  200 cells/ $\mu$ L, which found in 7 patients, and 42.9% were infected with intestinal protozoa. Bivariate analysis of CD4+ T cells risk factor showed that it was associated with intestinal protozoan infection, with p value of 0.012 and OR = 0.065. Result of the analysis showed that CD4+ T cells was a risk factor of intestinal protozoan infection in HIV/AIDS patients, and clinically patients who had CD4+ T cells  $\geq$  200 cells/ $\mu$ L had 0.065 times risk to be infected by intestinal protozoa, compared

with HIV/AIDS patients who had CD4+ T cells < 200 cells/ $\mu$ L who were easier to be infected.

Result of the study on HIV/AIDS patients in RSUP Dr Sardjito Yogyakarta in December 2009-March 2010 showed that clinical stadium in HIV/AIDS patients had highest value, with  $\text{Exp}(\beta) = 18.85$ . It also showed that clinical stadium was the most dominant risk factor for intestinal protozoan infection in HIV/AIDS patients. This is consistent with WHO statement that opportunistic infection, including intestinal protozoa, was occurred when HIV/AIDS patients were in clinical stadium 3 with moderate symptoms.

## CONCLUSION

Prevalence of intestinal protozoan infection in HIV/AIDS patients in RSUP Dr Sardjito Yogyakarta in December 2009-March 2010 was 81.2%. Clinical stadium with moderate and severe symptoms was the most dominant risk factor for intestinal protozoan infection in HIV/AIDS patients.

## REFERENCES

1. Anonim. Control of tropical diseases. WHO Geneva, 1998.
2. Suyoko, Musfiroh S, Sutarti E, Noerhayati S. Prevalensi parasit usus pada panti asuhan di Yogyakarta. Berkala Ilmu Kedokteran UGM 1980;XII:1-6.
3. Wijayanti MA, Ernaningsih. Parasit usus pada sampel tinja yang dikonsultasikan di Laboratorium Parasitologi Fakultas UGM Yogyakarta selama tahun 1990-1992. Berita Kedokteran Masyarakat 1994;X:(2).
4. Tellez A, Morales W, Rivera T, Meyer E, Leiva B, Linder E. Prevalence of intestinal parasite in the population of Leon, Nicaragua. Acta Tropika 1997;66:119-25.
5. Phiri K, Whitty Cj, Graham SM, Ssembatya-lule G. Urban/rural differences in prevalence

- and risk factors for intestinal helminthic infection in Southern Malawi. *Ann Trop Med Parasitol* 2000;94(4): 381-7.
6. Sandjaja B. *Parasitologi Kedokteran Buku 1 Protozologi Kedokteran*. Jaya Pura : Prestasi Pustaka, 2007.
  7. Wiwanitkit V. Intestinal parasitic infections in Thai HIV-infected patients with different immunity status, Thailand. *BMC Gastroenterology* 2001; 1:3.
  8. Weber J, Pinching A. The clinical management of AIDS and HTLV III infection, in the management of AIDS patient. Ed Miler D. London : Mc Milian, 1986.
  9. Anonim. Statistik Kasus HIV dan AIDS di Indonesia. Laporan. Jakarta : Ditjen PPM & PL, 2009.
  10. Dwivedi KK, Prasad G, Saini S, Mahajan S, Lal S, Baveja UK. Enteric opportunistic parasites among HIV infected individuals: Associated risk factor and imun status. *Jpn J Infect Dis* 2005;60:76-81.
  11. Cotte L, Rabodonirina M, Piens MA, Perreard M, Trepo C. Prevalence of intestinal protozoan in French patients infected with HIV. *J of Acquired Immune Deficiency Syndromes* 1993; 6:1024-9.
  12. Bratawidjaja KG. *Imunologi Dasar*. Jakarta : Balai Penerbit Fakultas Kedokteran UI, 2006.
  13. Okodua M, Adeyeba OA, Tاتفeng YM, Okpala HO. Age and sex distribution of intestinal parasitic infection among HIV infected subjects in Abeokuta, Nigeria. *Online J Health Allied Scs* 2003;4:3.
  14. Kia EB, Hosseini M, Nilforoushan MR, Meamar AR, Rezaeian M. Study of intestinal protozoan parasites in rural inhabitants of Mazandaran province, northern Iran. *Iranian J Parasitol* 2008; 3(1):21-5.
  15. Adamu H, Petros B. Intestinal protozoan infections among HIV positive persons with and without Antiretroviral Treatment (ART) in selected ART centers in Adama, Afar and Dire-Dawa, Ethiopia. *Ethiop J Health Dev* 2009;23(2):133-40.
  16. Raza HH, Sami RA. Epidemiological study on gastrointestinal parasites among. different sexes, occupations, and age groups in Sulaimani district. *J Duhok Univ* 2008; 12(1);317-23.
  17. Awole M, Selassie SG, Kassa T, Kibru G. Prevalence of intestinal parasites in HIV infected adult patients in south western Ethiopia. *Ethiop J Health Dev* 2003; 17(1):71-8.
  18. WHO. WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV related disease in adults and children. *Nlm Classification: 503.1*, 2007.