Intestinal parasitic infections in HIV/AIDS patients presenting with diarrhoea in Jakarta, Indonesia

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Summary We investigated the occurrence of intestinal parasites in Indonesian HIV/AIDS patients with chronic diarrhoea prior to administering antiretroviral therapy. The influence of age, CD4+ cell count and season on parasite occurrence was also studied. In total, 318 unconcentrated stool samples were analysed using Lugol’s iodine and modified acid fast staining to detect intestinal coccidia. Most samples (94.5%) were from males aged 21–40 years with CD4+ counts ≤50 cells/mm³. Parasites were found in 84.3% of samples (single species infections, 71.4%; poly-parasitism, 12.9%), with protozoan pathogens occurring most commonly. Cryptosporidium (4.9%), Cyclospora cayetanensis (4.5%) and Giardia duodenalis (1.9%) were the most frequent single infections, but Blastocystis hominis (72.4%) was the most commonly occurring protist. Cryptosporidium and C. cayetanensis occurred in 11.9% and 7.8% of all (single and mixed) infections. The most common co-infection was with B. hominis and Cryptosporidium (6.3%). Intestinal protozoan pathogens were detected more frequently in cases with CD4+ counts ≤200/mm³. No seasonal influence was determined for Cryptosporidium, C. cayetanensis or B. hominis, but gross seasonal disturbances may have influenced our findings. Intestinal parasites should be looked for routinely in this group of individuals and should be treated to reduce complications and the likelihood of transmission.

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1. Introduction

Infections in HIV-infected individuals can reduce both their quality of life and life span, especially those who are severely immunosuppressed with a CD4+ T-lymphocyte count <200 cells/mm³.1–3 Indonesia currently faces a severe problem with HIV; its incidence has increased rapidly and
Intestinal parasites in HIV/AIDS patients, Indonesia

Abdominal pain and diarrhoea can be the first manifestation of diarrhoea due to intestinal parasites. Diarrhoea is a significant cause of morbidity observed in the majority of studies and is most strongly associated with low CD4+ counts. It is the second leading cause of hospital visits in developing nations. There is a strong negative association between duration of diarrhoea and CD4+ levels.

Intestinal parasitic infections that are asymptomatic or cause self-limited diarrhoea in immunocompetent individuals can cause profuse diarrhoea in immunocompromised individuals, generally accompanied by weight loss, anorexia, malabsorption and, in some cases, fever and abdominal pain. In such patients, the opportunistic parasites Cryptosporidium spp., Strongyloides stercoralis and the microsporidia can disseminate to various organs, including the bronchia, bile and liver ducts, producing symptomatology specific to the affected organ(s).

Between 30% and 60% of HIV-infected patients suffer from infectious diarrhoea, most of which is persistent or chronic, and diarrhoea can be the first manifestation of AIDS that makes the patient seek medical advice/treatment. Parasitic infections are recognised causes of chronic diarrhoea in HIV/AIDS and can result in significant morbidity and mortality.

The season can influence the transmission of protozoan parasites, particularly Cryptosporidium spp. and Cyclospora. Both have been associated with either warmer or wetter seasons, depending on geographical location. Cyclospora cayetanensis is the main protozoal cause of diarrhoea in adult foreign residents during the wet season in Indonesia. To investigate the influence of season on patient presentation to the HIV clinics, the first recorded date of a diarrhoeic patient’s visit was used with respect to the wet (October–March) or dry (April–September) season.

The objective of this study was to determine the range of diarrhoea and whether age, CD4+ cell count or season influenced the occurrence of parasitic infection.

2. Materials and methods

2.1. Study population

A descriptive, prospective, cross-sectional study design was used. All samples analysed in this survey were submitted to the Department of Parasitology, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia, over a 29-month period (November 2004 to March 2007). Standard laboratory investigations of infectious diseases in Indonesian HIV clinics include intestinal parasites, Mycobacterium tuberculosis, and hepatitis B and C viruses. A total of 318 patients aged 5 months to 55 years, with laboratory-confirmed HIV and diarrhoea for >4 weeks, were identified for inclusion in the study. Following stool sample submission, all HIV patients were prescribed co-trimoxazole prophylactically for toxoplasmosis and Pneumocystis pneumonia and those with oropharyngeal candidosis were treated with fluconazole. Samples were not randomised.

2.2. CD4+ cell counts

CD4+ counts were determined by flow cytometry (BD FACSCalibur; BD Biosciences, Franklin Lakes, NJ, USA) in two Jakarta HIV/AIDS centres (Cipto Mangunkusumo Hospital and Dharmas Cancer Hospital), according to WHO recommendations.

2.3. Parasitology

One freshly voided stool was analysed from each person, without concentration, using Lugol’s iodine. Each sample was placed on three separate slides and analysed by trained microscopists in the Department of Parasitology, University of Indonesia. Two unconcentrated smears were air dried, methanol fixed and stained using a modified acid fast technique to detect Cryptosporidium spp., Isospora belli and C. cayetanensis. Intestinal microsporidia were not sought as the stain components were unavailable in Indonesia at the time.

2.4. Statistics

Analyses were performed using SPSS version 12.0 statistical software (SPSS Inc., Chicago, IL, USA). A bivariate analysis was performed to investigate the association between incidence of parasitism and age, seasonal variation and CD4+ counts. A P-value <0.05 was considered significant.

3. Results

Of the 318 HIV patients studied, 80% were from the HIV Clinic, Cipto Mangunkusumo Hospital, and the remainder were from other hospitals in Jakarta. In total, 9 samples were collected in 2004 (from November), 145 in 2005, 148 in 2006 and 16 up until March 2007. Their age stratification was as follows: 3.1% aged <5 years; 2.5% aged 6–20 years; 68.2% aged 21–30 years; 14.5% aged 31–40 years; 3.5% aged 41–50 years; and 1.3% aged >50 years. No information on age was available for 22 individuals. The age range of the majority of the cohort was 21–40 years. Males constituted 94.5% of the study population.

Of the 318 cases, only 160 parasitised cases had CD4+ cell count data. The majority (54%) of cases had CD4+ counts of 50 cells/mm³, 20% and 14% had CD4+ counts of 51–100 cells/mm³ and 101–200 cells/mm³, respectively, whilst only...
12% of cases had CD4+ counts >200 cells/mm³. The frequencies of parasites found in the HIV cases are presented in Tables 1 and 2 and Supplementary Table 1. Of 318 samples analysed, the majority (84.3%; n = 268) contained parasites. With the (possible) exception of *Blastocystis hominis* and *Entamoeba coli*, all were recognised pathogens. Protozoan pathogens were the most prevalent, being found in all but two samples (Table 1). Three cases had intestinal nematode infections, but one was also co-infected with *I. belli*. Single species infections were most common, occurring in 71.4% of cases, whilst 12.9% of cases were infected with two or more species (Tables 1 and 2; Supplementary Table 1). The most common co-infection was *B. hominis* and *Cryptosporidium* sp. (6.3%).

Of the recognised protozoan pathogens, *Cryptosporidium* sp. (4.9%), *C. cayetanensis* (4.5%) and *Giardia duodenalis* (1.9%) were detected most frequently as single infections, yet *B. hominis* (72.4%) was the most commonly occurring protist. Overall, 87.3% (n = 234) of positive samples contained *B. hominis* (73.6% of all samples; Tables 1 and 2). When both single and mixed infections were taken into account, *Cryptosporidium* sp. (11.9%; n = 32) and *C. cayetanensis* (7.8%; n = 21) remained the most frequently detected pathogens (Tables 1 and 2; Supplementary Table 1).

As the majority of the cohort was aged 21—40 years, patient age was divided into three groups (<20 years, 21—40 years and ≥41 years) and the relationship between incidence of parasitism and patient age was analysed. No significant difference was observed (Kruskal—Wallis, P = 0.595), possibly because 82.7% of our cohort fell in the 21—40 years of age category.

A proportion of individuals from each CD4+ count group had samples containing no intestinal parasites (Figure 1). Intestinal polyparasitism occurred in all CD4+ count groups, with the exception of those with CD4+ counts >400 cells/mm³ (Figure 1). Polyparasitism was observed in 12.9% of individuals with intestinal parasites, primarily in those with CD4+ cell counts of ≤50/mm³ (Figure 1; Supplementary Table 1). Based on the CD4+ classification, it was observed that the frequency of polyparasitism ranged from 5% (2/38)

![Figure 1](image-url)  
**Figure 1** Incidence of intestinal parasitoses in relation to CD4+ cell counts. Negative: no parasite found; single: only one type of parasite found; poly: more than one type of parasite found.

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Frequency (%)</th>
<th>n = 318</th>
<th>CD4+ cell count (cells/mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Blastocystis hominis</em></td>
<td>194/268 (72.4)</td>
<td>25/194</td>
<td>0–50</td>
</tr>
<tr>
<td><em>Cyclospora cayetanensis</em></td>
<td>1/12</td>
<td>1/12</td>
<td>51–100</td>
</tr>
<tr>
<td><em>Cryptosporidium</em> sp.</td>
<td>12/268 (4.5)</td>
<td>13/268</td>
<td>101–200</td>
</tr>
<tr>
<td><em>Giardia duodenalis</em></td>
<td></td>
<td>5/268</td>
<td>201–400</td>
</tr>
<tr>
<td><em>Entamoeba histolytica</em></td>
<td></td>
<td>1/268</td>
<td>&gt;400</td>
</tr>
<tr>
<td><em>Ascaris lumbricoides</em></td>
<td></td>
<td>2/268</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>227/318 (71.4)</td>
<td>71/1227</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CD4+ cell count (cells/mm³)</th>
<th>Frequency (%)</th>
<th>n = 318</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–50</td>
<td>80</td>
<td>100</td>
</tr>
<tr>
<td>51–100</td>
<td>40</td>
<td>80</td>
</tr>
<tr>
<td>101–200</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>201–400</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>&gt;400</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

![Table 1](table-url)  
**Table 1** Frequency of occurrence of single parasitic infections among HIV-positive individuals with chronic diarrhoea (n = 318), with specific reference to CD4+ cell count.  

### Notes
- **Table 1**: Frequency of occurrence of single parasitic infections among HIV-positive individuals with chronic diarrhoea (n = 318), with specific reference to CD4+ cell count.
- **Figure 1**: Incidence of intestinal parasitoses in relation to CD4+ cell counts. Negative: no parasite found; single: only one type of parasite found; poly: more than one type of parasite found.
Table 2  Frequency of occurrence of multiple parasitic infections among HIV-positive individuals with chronic diarrhoea (n = 318)

<table>
<thead>
<tr>
<th>Parasites</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blastocystis + Cryptosporidium spp.</td>
<td>17/268 (6.3)</td>
</tr>
<tr>
<td>B. hominis + Giardia duodenalis</td>
<td>9/268 (3.4)</td>
</tr>
<tr>
<td>B. hominis + Cyclospora cayetanensis</td>
<td>9/268 (3.4)</td>
</tr>
<tr>
<td>B. hominis + Isospora belli</td>
<td>1/268 (0.4)</td>
</tr>
<tr>
<td>B. hominis + G. duodenalis + Cryptosporidium spp.</td>
<td>2/268 (0.7)</td>
</tr>
<tr>
<td>B. hominis + Entamoeba coli</td>
<td>2/268 (0.7)</td>
</tr>
<tr>
<td>Strongyloides stercoralis + I. belli</td>
<td>1/268 (0.4)</td>
</tr>
<tr>
<td>Total</td>
<td>41/318 (12.9)</td>
</tr>
</tbody>
</table>

in the CD4+ count 51–100 cells/mm³ group to 22% (6/27) in the CD4+ count 101–200 cells/mm³ group (Figure 1). With the exception of B. hominis, the majority of parasites were detected in individuals with CD4+ cell counts <100/mm³ (Table 1; Supplementary Table 1). Blastocystis hominis infections were detected in all CD4+ groups. Statistical analysis of the occurrence of parasitism (scored as negative, single and multiple species) and CD4+ counts did not show any significant differences between different CD4+ groups (Kruskal–Wallis test, P = 0.694). However, when analysis was restricted to cases with CD4+ cell counts of either <200/mm³ or >200/mm³ and B. hominis-positives were excluded (because B. hominis was distributed in high and low CD4+ count groups), a clear difference was seen, with a higher frequency of single and polyparasitism occurring in individuals with CD4+ cell counts <200/mm³. However, statistical analysis did not show a significant difference (Pearson’s χ² P = 0.185). Further analysis of Cryptosporidium in relation to CD4+ count showed that all Cryptosporidium-positive cases clustered in the CD4+ <200/mm³ group and showed a statistically significant negative correlation with CD4+ count level (Spearman’s correlation coefficient –0.201; P = 0.006).

Analysis of the season when individuals with Cryptosporidium, C. cayetanensis, G. duodenalis and B. hominis first presented revealed no difference in incidence between the wet or dry season for Cryptosporidium, C. cayetanensis or G. duodenalis, either for individuals with single species infections or with polyparasitism (Table 3). However, fewer B. hominis-positive individuals presented in the wet season than in the dry season (Table 3). Further analysis of parasite occurrence by 3-monthly period (Figure 2) failed to reveal

Table 3  Number of individuals presenting with Cryptosporidium spp., Cyclospora cayetanensis and Blastocystis hominis as single or mixed infections with respect to season

<table>
<thead>
<tr>
<th>Parasite(s)</th>
<th>No. (%) with parasites</th>
<th>No. presenting with parasites in the wet season</th>
<th>No. presenting with parasites in the dry season</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptosporidium spp.</td>
<td>13/268 (4.9)</td>
<td>5/13</td>
<td>8/13</td>
</tr>
<tr>
<td>C. cayetanensis</td>
<td>12/268 (4.5)</td>
<td>6/12</td>
<td>6/12</td>
</tr>
<tr>
<td>B. hominis</td>
<td>194/268 (72.4)</td>
<td>77/194</td>
<td>117/194</td>
</tr>
<tr>
<td>B. hominis + Cryptosporidium spp.</td>
<td>17/268 (6.3)</td>
<td>9/17</td>
<td>8/17</td>
</tr>
<tr>
<td>B. hominis + Giardia duodenalis</td>
<td>9/268 (3.4)</td>
<td>4/9</td>
<td>5/9</td>
</tr>
<tr>
<td>B. hominis + C. cayetanensis</td>
<td>9/268 (3.4)</td>
<td>3/9</td>
<td>6/9</td>
</tr>
<tr>
<td>B. hominis + Isospora belli</td>
<td>1/268 (0.4)</td>
<td>1/1</td>
<td>0/1</td>
</tr>
<tr>
<td>B. hominis + G. duodenalis + Cryptosporidium spp.</td>
<td>2/268 (0.7)</td>
<td>0/2</td>
<td>2/2</td>
</tr>
<tr>
<td>B. hominis + Entamoeba coli</td>
<td>2/268 (0.7)</td>
<td>2/2</td>
<td>0/2</td>
</tr>
</tbody>
</table>
an association between Cryptosporidium, C. cayetanensis or B. hominis incidence and seasonal variation.

The seasonal Cryptosporidium pattern differed between 2006 and 2007, as the incidence was higher in July–September 2006 compared with January–March 2007 (Figure 2A). The number of cyclosporiasis cases was higher in 2006 than 2005, being higher in the second half of 2006 (Figure 2B). More B. hominis-positive individuals presented in the dry season than in the wet season, but this was not statistically significant (Pearson’s $\chi^2$ test, $P = 0.876$).

4. Discussion

Here we report on intestinal parasitic infections in HIV/AIDS patients presenting with diarrhoea in Jakarta. The increased association between intestinal parasites, particularly opportunistic parasites, and individuals with reduced immunity due to CD4+ T-lymphocyte depletion in HIV/AIDS is well documented.8–10,17–22 The most frequent intestinal protozoan parasites found were Blastocystis, Cryptosporidium, Cyclospora and Giardia. Other studies identified parasitic protozoa, particularly coccidia, as being dominant parasites in HIV cases with diarrhoea.7–10,22–24 Intestinal parasites recognised as being opportunistic, including Cryptosporidium spp., I. belli and S. stercoralis, were all detected in our cohort. Cryptosporidium sp. (11.9%) and C. cayetanensis (7.8%) were the most frequent pathogens identified, either as single or mixed infections. Hailemariam et al.25 reported that poly-parasitism occurred more commonly among HIV/AIDS patients, and in our HIV/AIDS cohort poly-parasitism occurred most commonly in the ≤50 cells/mm$^3$ CD4+ cell count group (Tables 1 and 2). It was not possible to determine a relationship between case age and incidence of parasitism, probably because the majority of our cohort was aged 21–40 years.

The majority (74%) of HIV-infected individuals presenting with diarrhoea for >4 weeks in our study had CD4+ counts of ≤100 cells/mm$^3$ (normal range 600–1200 cells/mm$^3$) and there were three times as many individuals with CD4+ counts of ≤50 cells/mm$^3$ compared with those with CD4+ counts of 51–100 cells/mm$^3$. Intestinal poly-parasitism occurred in all CD4+ count groups, with the exception of those with CD4+ counts >400 cells/mm$^3$. Decreased immunity in HIV/AIDS predisposes to parasitism7–10,17,18,20,23,24,26 and in our cohort the majority of individuals (71.4%) were infected with one parasite genus. We were unable to demonstrate a statistical association between parasite occurrence (scored as negative, single and multiple species) and decreasing CD4+ count groups. Restricting the analysis to cases with CD4+ cell counts of either ≤200/mm$^3$ or CD4+ >200/mm$^3$ and excluding B. hominis-positives cases (as they were distributed in both high and low CD4+ counts) showed that poly-parasitism clustered in individuals with CD4+ cell counts ≤200/mm$^3$, however this was not statistically significant.

A proportion of individuals from each CD4+ count group had samples containing no intestinal parasites (Figure 1). Whilst we are aware of the importance of other infectious, e.g. intestinal microsporida, viruses, bacteria and fungi, and non-infectious causes of diarrhoea in HIV/AIDS, we did not investigate these due to the unavailability of detection methods in our laboratory. As identified in other studies,7,22 common non-parasitic causes of chronic diarrhoea in our cohort include HIV enteropathy, viral/bacterial infections and unidentified agents.

The dearth of intestinal helminths detected probably reflects the catchment areas of the individuals studied, which was urban rather than rural, rather than the apparent ability of protozoa to out-compete helminths for the intestinal environment. There is a high incidence of helminthiasis in school children in densely populated and slum areas in Jakarta, but not in adults.27 Most of our cases will have visited three to four physicians before presenting to the HIV Clinic at Cipto Mangunkusumo Hospital and have been prescribed antibiotics, but not anthelmintics, which are normally prescribed following a positive stool test at the Department of Parasitology, University of Indonesia, although the possibility that they self-administered anthelmintics cannot be excluded.

As only one freshly voided stool from each person was analysed, without concentration, rather than analysing a formol-ether concentrated sample from three consecutive days, the detection rates for ova, cysts, oocysts and parasites are likely to be underestimates. Furthermore, as an in vitro culture method was used to detect Blastocystis, which is more sensitive that direct microscopy or formol-ether concentration,28,29 this fact coupled with the direct examination of only one stool probably resulted in more efficient detection of Blastocystis infection compared with other intestinal parasites in our cases. This would underestimate the point prevalence data particularly for Cryptosporidium, C. cayetanensis and G. duodenalis. These reasons may account for the Cryptosporidium occurrence rates, which are lower than those quoted for other developing areas.7–22

Of interest is the fact that 7.8% of samples contained C. cayetanensis oocysts, slightly fewer than recorded for Cryptosporidium sp. but more than for G. duodenalis (6.0%; $n = 16$). Co-trimoxazole treatment, which is effective against cyclosporiasis (and isosporiasis), is administered to all patients following stool examination, primarily as prophylactic treatment for toxoplasmosis and Pneumocystis pneumonia.

Neither analysis of wet or dry season nor analysis by 3-monthly period revealed a statistical association between Cryptosporidium or B. hominis incidence and seasonal variation, although an association between cryptosporidiosis and the wet season is well recognised.13,26,30 The seasonal Cryptosporidium occurrence pattern differed between 2006 and 2007. Between 2005 and 2007 there were seasonal anomalies in Jakarta, caused by global warming, resulting in a longer duration dry season and a shorter duration wet season. In January 2007 a massive flood covered 70% of Jakarta, which could have contributed to the higher incidence of cryptosporidiosis during the period January–March 2007. A higher incidence of cyclosporiasis occurred during the period October–December (Figure 2B), which was the start of the wet season. This is in agreement with C. cayetanensis being the main protozoal cause of gastrointestinal illness and diarrhoea in adult foreign residents during the wet season in Jakarta,15 but differs from other regions where an association between Cyclospora occurrence and the dry season has been reported.24,30 More B. hominis-positive individuals presented in the dry sea-
son than in the wet season, but this was not statistically significant. To determine useful trends in these diseases in Jakarta, we recommend analysis for five consecutive years.

*Cyclospora cayetanensis* has been reported in Indonesian residents of Jakarta, rural schoolchildren, expatriates and travellers. Fryauff et al. found that while *C. cayetanensis* was the main protozoal cause of gastrointestinal illness and diarrhoea in adult foreign residents during the wet season, rarely was it a cause of illness in the indigenous population or in children. Infections in rural Indonesian children had characteristically low oocyst density, were asymptomatic and sporadic. The high occurrence of *C. cayetanensis* in adult foreign residents, but not in the indigenous population, during the wet season suggests increased transmission during this season. Fryauff et al. suggested that both repeated exposure due to contaminated food and water during childhood development, resulting in effective clinical and parasitological immunity to *Cyclospora*, and an urban transmission among expatriates resident in Jakarta, based on 'atypical' food preferences, could partly explain this difference. Unlike the data on *C. cayetanensis* in Indonesian expatriates, analysis of our data on whether HIV-positive individuals were more likely to present either in the wet or dry season does not indicate a preponderance in either season for the recognised parasite pathogens (Table 3).

Whilst our data on opportunistic parasites are in agreement with previously published work, the commonness of *B. hominis*, which was detected in 73.6% of samples, was surprising. *Blastocystis hominis* was the most common parasite encountered and, whilst the majority (72.6%) of positives were detected in individuals with CD4+ cell counts of ≤100/mm³, it was also the only parasite detected in individuals with the highest (>400/mm³) CD4+ cell counts. Hailemariam et al. also found that *Blastocystis* was found in significantly higher numbers in HIV/AIDS patients than in controls (P < 0.05).

Controversy remains over the pathogenic potential of *B. hominis*. Ten *Blastocystis* subtypes have been found in humans, but whether the *B. hominis* isolated from our HIV/AIDS cohort fall into a pathogenic subclass remains to be elucidated. Of interest, Stensvold et al. describe a case of refractory blastocystosis in a Danish woman who travelled in Indonesia, caused by subtype 8, whose symptoms and infection resolved following co-trimoxazole treatment.

Parasite endemicity is high (84.3%) among HIV-positive individuals presenting with diarrhoea in Jakarta, therefore we recommend that intestinal parasites should be looked for routinely in this group of individuals. Ideally, one formol-t�tns should be analysed. Furthermore, positive cases should be treated to reduce their complications and the likelihood of transmission to other susceptible humans, including carers and family contacts.

**Authors’ contributions:** AK and TK designed the study; TK, EY and SD carried out the clinical assessment, sought enrolment into the study and checked the validity of all clinical and laboratory (non-parasitological) data; SWD and IPS assisted with sample and data collection, performed the microscopy and entered the data into spreadsheets; AK supervised data collection and wrote the first draft of the paper; AK and HVS analysed and interpreted the data; HVS provided parasite quality assurance and external quality control and co-wrote (with AK) the final version of the manuscript. All authors read and approved the final version. AK is guarantor of the paper.

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**Conflicts of interest:** None declared.

**Ethical approval:** Research and Ethical Committee of the Faculty of Medicine, University of Indonesia, Jakarta, Indonesia.

**Appendix A. Supplementary data**

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.trstmh.2009.02.017.

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