

Prevalence of Intestinal Parasitic Diseases among HIV Positive Patients using Antiretroviral Treatment in Phayao Province, Northern Thailand

Chittakun Suwancharoen* Tanyalak Jaloen-ngam* Wasana Muangwong*

ABSTRACT

Co-infection of intestinal parasites and HIV is widespread, leading to higher morbidity and mortality in tropical countries. Hence, the aims of this study were to determine the prevalence of intestinal parasitic diseases among patients with HIV and their association with immune status. The study was conducted at Phayao and Dok Khamtai Hospitals, Phayao Province, northern Thailand from June 2014 to May 2015. A single stool sample of 458 patients was collected and processed using direct fecal smear, formalin ethyl-acetate concentration and modified Ziehl-Neelsen staining. Basic information was collected on sociodemographics, diarrhea status and current CD4⁺ T-cell counts of each patient. The results showed a high prevalence (33.0%) of intestinal parasites in this population with no significant

difference observed by age ($p=0.296$), sex ($p=0.484$) and diarrhea status ($p=0.947$). The rates of single and multiple infections were 27.4% and 5.2%, respectively. Numbers of *Giardia lamblia* (35.2%), *Cryptosporidium parvum* (28.5%) and *Strongyloides stercoralis* (26.2%) were high among patients whose CD4⁺ T-cell count was lower than 200 cells/ μ L. However, the number of intestinal parasites showed no correlation with CD4⁺ T-cell counts. Therefore, laboratory diagnosis of intestinal parasites is essential for patients with HIV regardless of receiving antiretroviral treatment, CD4⁺ T-cell count or experiencing diarrhea.

Keywords: intestinal parasitic diseases, HIV, CD4⁺ t-cell counts, diarrhea, stool examination



Introduction

Human immunodeficiency virus (HIV) disease and acquired immunodeficiency syndrome (AIDS) are serious issues in Southeast Asia, including Thailand¹. Current epidemiological reports have estimated that 500,000 people are HIV positive in Thailand. One of the major health problems among patients with HIV/AIDS is chronic diarrhea due to pathogen infection^{2, 3}. Importantly, patients with AIDS presenting chronic diarrhea has accounted for 30-60% of American and European patients and the number could reach 90% in developing countries⁴. Similarly, intestinal parasitic diseases spread in most developing countries due to unsanitary conditions, poor hygiene and unclean drinking water. The prevalence of intestinal parasitic diseases among HIV-positive individuals remains a problem in many regions in the world, where only a small number of patients have access to antiretroviral treatment (ART)³.

The progressive decline and collapse of mucosal immunologic functions cause the intermediate or late stages of AIDS. The CD4⁺ T-lymphocyte cells are depleted from the circulating system among patients with HIV/AIDS, which often results in severe diseases and ultimately death. Creating low immune levels, CD4⁺ T-cell counts have decreased below 200 cells/ μ L, and leading to the increased susceptibility to both helminthes and opportunistic intestinal protozoa infections such as

Strongyloides stercoralis, *Trichuris trichiura*, hookworm, *Entamoeba histolytica*, *Giardia lamblia*, *Cryptosporidium parvum*, *Isospora belli* and Microsporidia^{5, 6}. Regarding this phenomenon, the initiation of ART in patients with HIV/AIDS can boost immune responses and protect from acquiring parasitic and other pathogen diseases⁷.

In Thailand, HIV and parasitic diseases are widespread and no routine diagnosis of co-infections is provided in numerous HIV care centers. Few reports and no evidence exist of related studies on the epidemiology of intestinal parasites among HIV positive individuals in northern Thailand. Therefore, this study aimed to determine the prevalence of intestinal parasitic diseases among patients with HIV taking ART with their relation to immune status. Additionally, some opportunistic protozoa including *C. parvum*, *C. cayetanensis* and *I. belli* were included in the study.

Materials and methods

Study sites and population

A cross-sectional study was conducted from June 2014 to May 2015. A total of 458 patients with HIV followed up for their immunological status, were sampled at HIV clinics at Phayao and Dok Khamtai Hospitals, Phayao Province, Thailand. Basic information including sex, age and diarrhea status was recorded by hospital staff or physicians, and

recent CD4⁺ T-cell counts were obtained from their medical records during sample collection. Intestinal parasites were identified by stool examination at the Microbiology and Parasitology Laboratory, School of Medical Sciences, University of Phayao. Patients receiving anti-parasitic treatment were excluded from this study.

Stool specimen analysis

Stool samples were collected in sterile containers according to procedures for collecting feces followed at the HIV clinics. Each fresh stool sample was analyzed within one hour of collecting using direct microscopic examination (wet mount) with normal saline (0.85% NaCl solution) and iodine staining. Then the samples were preserved in 10% formalin and forwarded to the Microbiology and Parasitology Laboratory, University of Phayao. The formalin-stored specimens were analyzed using the formalin-ethyl acetate sedimentation technique and modified Zeihl-Neelsen staining.

Formalin-ethyl acetate sedimentation is the method used for most protozoan and helminth diagnoses. About 7 mL of stool-formalin mixture was filtered through two layers of wet cotton gauze in a conical 15-mL centrifuge tube and centrifuged for 2 minutes at 377 g. The sample was rinsed twice with normal saline. After adding 3 mL of ethyl acetate to the mixture and shaking vigorously for 30 seconds, the mixture was centrifuged

for 3 minutes at 377 g. All of the supernatant fluid was discarded. The sediments were placed on a slide with iodine and examined under a microscope⁸.

To detect oocysts of some opportunistic coccidian parasites (*C. parvum*, *C. cayetanensis*, *I. belli*), modified Zeihl-Neelsen staining was performed. In this technique, the fecal smears were prepared after formalin ethyl-acetate sedimentation and dried on a heating block at 70°C for 5 minutes. The slides were stained with carbol fuchsin, gently heated without boiling over an alcohol lamp for 5 minutes and rinsed with tap water. After decolorizing with 1% HCl acid alcohol, the slides were counterstained with 1% methylene blue for one minute. The sample smears were microscopically examined using an oil immersion objective lens (100X)⁹.

Statistical analysis

Data were analyzed using SPSS, Version 21.0.0, and descriptive statistics were performed. Chi-square and Fisher's exact test were used to determine any associations. A statistical test was considered significant at p-value ≤ 0.05 .

Ethics consideration

This study was approved by the Human Ethics Committee for Clinical and Biomedical Research of the University of Phayao, Thailand (No. 57 02 04 0009). Informed consent was obtained from each participant.



Results

Demographic characteristics of the study population

A total of 458 patients were examined for intestinal parasitic diseases during the study period. In all, 210 (45.9%) and 248 (54.1%) patients were male and female, respectively. The mean age of the study population was

43 ± 8.2 years, varying between 20 to 70 years.

Most of the study participants (54.4%) were in the age range 40 to 49 years, followed by 30 to 39 (21.8%), above 50 (19.0%), and less than 30 (4.8%). Of the total participants, only 40 (8.7%) individuals were reported as having diarrhea, and the remaining participants (91.3%) had no diarrhea (Table 1).

Table 1 Characteristic Distribution of Intestinal Parasitic Infections among the Study Participants.

Factors	Number (%) tested	Number (%) positive for any parasites	X ²	P-value
Sex				
Male	210 (45.9)	64 (30.5)	1.091	0.296
Female	248 (54.1)	87 (35.1)		
Age group (years)				
20-29	22 (4.8)	4 (18.2)	2.454	0.484
30-39	100 (21.8)	35 (35.0)		
40-49	249 (54.4)	84 (33.7)		
≥50	87 (19.0)	28 (32.2)		
Diarrhea status				
Diarrhea	40 (8.7)	13 (32.5)	0.004	0.947
No Diarrhea	418 (91.3)	138 (33.0)		
Total	458 (100)	151 (33.0)		

Prevalence of intestinal parasites among patients with HIV

Patients who had positive results for intestinal parasites accounted for 33.0% of the study subjects (151/458). Intestinal parasitic

diseases were more prevalent among females (87 patients, 35.1%) than among males (64 patients, 30.5%). The age group 30 to 39 years (35 patients, 35.0%) showed the highest prevalence of intestinal parasites while

the least prevalence was observed in the age group 20 to 29 years (4 patients, 18.2%). No significant differences were observed regarding the prevalence of intestinal parasitic diseases in sex and age groups ($\chi^2 = 1.094$, $p = 0.296$ and $\chi^2 = 2.454$, $p = 0.484$, respectively). Concerning the relationship between the diarrhea status and intestinal parasitic diseases, no significant association was observed between the patients experiencing diarrhea (32.5%) and patients without diarrhea (33.0%) ($\chi^2 = 0.004$, $p = 0.947$) (Table 1).

Nine species of intestinal parasites were detected among 179 (39.1%) individuals

including mixed infections that were observed among some participants. A total of 126 (27.5%) patients tested positive for protozoa while 53 (11.6%) patients tested positive for helminthes. The most frequently detected parasites were *G. lamblia* (35.2%), *C. parvum* (28.5%) and *S. stercoralis* (26.2%). The least prevalence (0.6%) was detected equally for *E. vermicularis* and *A. lumbricoides* (Table 2). The prevalence of different intestinal parasites in the study population is illustrated in Figure 1. In all, 126 (27.5%) patients had at least one single parasitic disease, and the rate of multiple infection was 5.2% (Figure 1).

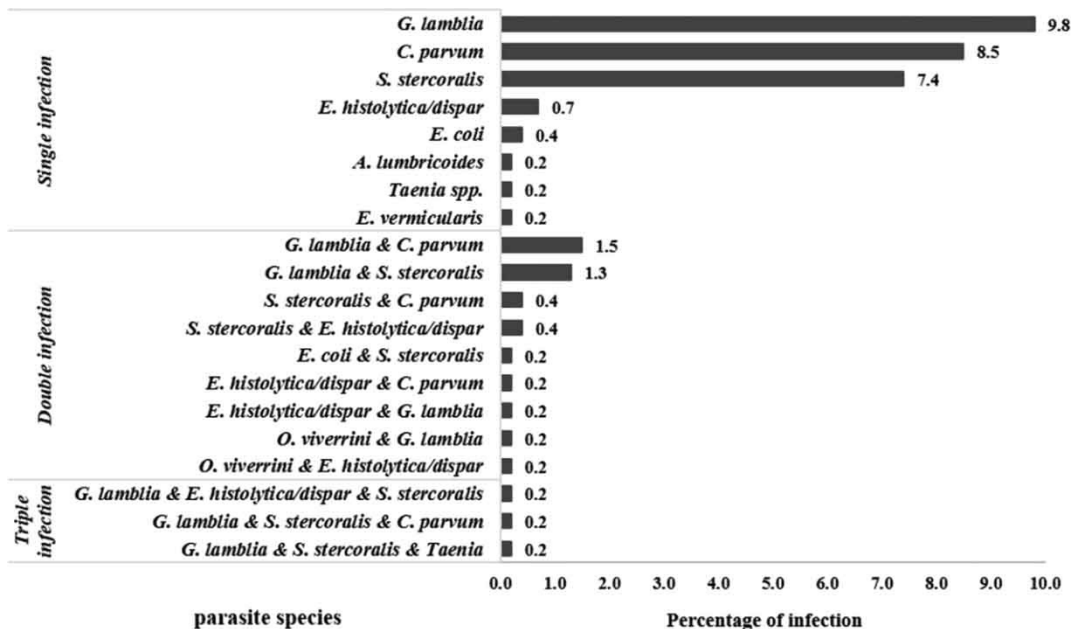


Figure 1 Prevalence of Intestinal Parasitic Infections in the Study Population.



Table 2 Prevalence of Intestinal Parasitic Diseases among Patients with HIV in Relation to their CD4⁺ T-cell counts.

Parasite	Total (%)	CD4 ⁺ T-cell counts			χ^2	p-value
		<200 cells/ μ L	200-499 cells/ μ L	>500 cells/ μ L		
		No (%)	No (%)	No (%)		
Single infection	126 (83.4)	26 (72.2)	59 (93.7)	41 (78.9)	13.000	0.002
Double infection	22 (14.6)	9 (25.0)	4 (6.4)	9 (17.3)	2.273	0.321
Triple infection	3 (2.0)	1 (2.8)	0 (0.0)	2 (3.9)	-	-
Total	151 (100)	36 (23.8)	63 (41.7)	52 (34.5)		
Protozoa					3.184	0.785
<i>Cryptosporidium parvum</i>	51 (28.5)	22 (25.0)	14 (31.1)	15 (32.6)	2.235	0.327
<i>Giardia lamblia</i>	63 (35.2)	32 (36.4)	16 (35.6)	15 (32.6)	8.667	0.013
<i>Entamoeba histolytica/dispar</i>	9 (5.0)	3 (3.4)	2 (4.4)	4 (8.7)	-	-
<i>Entamoeba coli</i>	3 (1.7)	2 (2.3)	0 (0.0)	1 (2.2)	-	-
Helminthes					4.952	0.764
<i>Stroglyoides stercoralis</i>	47 (26.2)	24 (27.3)	13 (28.9)	10 (21.7)	6.936	0.031
<i>Enterobius vermicularis</i>	1 (0.6)	1 (1.1)	0 (0.0)	0 (0.0)	-	-
<i>Taenia spp.</i>	2 (1.1)	1 (1.1)	0 (0.0)	1 (2.2)	-	-
<i>Ascaris lumbricoides</i>	1 (0.6)	1 (1.1)	0 (0.0)	0 (0.0)	-	-
<i>Opisthorchis viverrini</i>	2 (1.1)	2 (2.3)	0 (0.0)	0 (0.0)	-	-
Total	179^a (100)	88 (49.2)	45 (25.1)	46 (25.7)		

a. The number of parasites exceeds the number of infected participants as multiple parasites were examined in some patients

Intestinal parasites in relation to CD4⁺ T-cell counts among patients with HIV

The distribution of intestinal parasitic diseases in relation to CD4⁺ T-cell counts is shown in Table 2. A significant difference was shown among those presenting a single disease ($\chi^2 = 13.000$, $p = 0.002$). The highest prevalence (88 patients, 49.2%) of intestinal parasitic diseases was reported among patients having CD4⁺ T-cell counts below 200 cells/ μ L. *G. lamblia* (36.4%), *S. stercoralis* (27.3%) and

C. parvum (25.0%) were the most common parasites, followed by *E. histolytica/dispar* (3.4%), *E. coli* (2.3%) and *O. viverrini* (2.3%). *E. vermicularis*, *Taenia spp.* and *A. lumbricoides* were detected in one patient each. Among patients with HIV presenting CD4⁺ T-cell counts between 200 to 499 cells/ μ L, 45 had intestinal parasites (25.7%), of which *G. lamblia* was identified at 35.6%, followed by *C. parvum* (31.1%), *S. stercoralis* (28.9%) and *E. histolytica/dispar* (4.4%). Of the CD4⁺

T-cell counts greater than 500 cells/ μ L, intestinal parasites could be identified among 46 patients (25.7%). *G. lamblia* and *C. parvum* were reported equally found in 32.6%, followed by *S. stercoralis* (21.7%) and *E. histolytica/dispar* (8.7%). *E. coli* and *Taenia* spp. were identified in one patient each. Considering the rate of intestinal parasitic diseases by three categories of CD4⁺ T-cell counts, a significant difference was observed regarding *G. lamblia* and *S. stercoralis* ($X^2 = 8.667$, $p = 0.013$ and $X^2 = 6.936$, $p = 0.031$, respectively). However, the prevalence of both protozoa and helminthes showed no correlation with CD4⁺ T-cell counts ($X^2 = 3.184$, $p = 0.785$ and $X^2 = 4.942$, $p = 0.764$, respectively).

Discussion

Intestinal parasitic diseases remain an important problem of morbidity and mortality in tropical countries especially for patients with HIV/AIDS. Our present study reported the only intestinal parasitic diseases focusing on patients with HIV in northern Thailand. The overall prevalence of intestinal parasites reported in this study was 33.0%, which was in agreement with the data from Chokeyhaibulkit *et al.* (33.0%) in Bangkok, Thailand¹⁰. However, the number was lower than that of studies conducted in Lopburi (45.6%)¹¹, King Chulalongkorn Memorial Hospital (50.0%)¹² in central Thailand, and in other countries such as Brazil (44.1%)¹³,

Cameroon (82.6%)¹⁴ and Nigeria (87.8%)¹⁵. The difference could be due to levels of environmental and personal hygiene, economics, occupation, lifestyle and sample size^{2, 4}. Of note, sex, age and diarrhea status did not significantly affect the prevalence of intestinal parasitic diseases in this study population.

The prevalence of intestinal protozoa was about two and one half times higher than that of intestinal helminthes (70.4% vs. 29.6%), which was similarly reported elsewhere^{14,16,17}. About four fifths of the study population was infected by at least one single parasite. Only 5.2% of patients were observed with multiple parasitic infections, which was lower than the number reported by Shimelis *et al.*¹⁷ and Fekada *et al.*¹⁸. *G. lamblia*, *S. stercoralis* and *C. parvum* were the most frequently found parasites in this study. Additionally, *E. histolytica/dispar*, *E. coli*, *Taenia* spp., *O. viverrini*, *E. vermicularis* and *A. lumbricoides* were found at low prevalence rates among patients with HIV.

Our findings showed that the vast majority of intestinal parasites (*G. lamblia*, *S. stercoralis* and *C. parvum*) were found in patients with HIV who had CD4⁺ T-cell counts below 200 cells/ μ L (49.2%), similar to that reported by Wiwannikit *et al.* in Bangkok, Thailand¹². Generally, the cellular immune mechanisms are the main defense against intestinal parasitic diseases. The reduction of



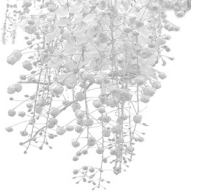
CD4⁺ T-cell counts below 200 cells/ μ L results in the susceptibility to intestinal parasitic diseases, leading to unsuccessful elimination of prior parasitic diseases among patients⁵. In addition, patients with HIV and with CD4⁺ T-cell counts between 200 and 499 cells/ μ L and greater than 500 cells/ μ L in this study presented a similar prevalence at 25.1% and 25.7%, respectively. The observation may be due to the patients taking ART; the use of ART not only greatly reduces the incidence of diarrhea, but restores immunity after ART initiation also. Patients with HIV and with CD4⁺ T-cell counts greater than 200 cells/ μ L may have reduced susceptibility to any pathogen infections. Therefore, our result agreed with the hypothesis that the prevalence of intestinal parasitic diseases increased with lower CD4⁺ T-cell counts among patients with HIV.

In this study, the prevalence rate of *G. lamblia* (35.2%) as non AIDS-defining parasites was the highest among the various intestinal parasites in the studied population. Its prevalence was higher than several reports in Bangkok, Thailand such as Punpoowong *et al.* (9.09%)¹⁹ and Waywa *et al.* (3.8%)²⁰, but a similar prevalence was found in southwestern Ethiopia (32.1%)²¹. The different finding regarding *G. lamblia* could have been due to water and food contaminated by human feces, or owing to poor personal hygiene and sanitation.

The stool examination presented the high prevalence of nematode *S. stercoralis* (26.2%), which was greater than the data observed in many studies in central Thailand such as Bangkok (3.3%)¹² and Nonthaburi (4.4%)²², but still lower than that reported by Cimerman *et al.* in Brazil (30.1%)²³. Importantly, the invasion of *S. stercoralis* in the circulating system, may be due to impaired immunity, and could be associated with hyperinfection syndrome and meningitis with enteric pathogens, significantly increasing the mortality rates among immunodeficiency and immunocompromised patients. As a result, patients with HIV/AIDS have a higher risk of developing strongyloidiasis²⁴.

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C. parvum was a commonly observed opportunistic protozoan infection among patients with HIV. Although the role of CD4⁺ T-cell counts seems to produce no significant difference in the prevalence of *C. parvum* infection, this report showed a high prevalence of *C. parvum* (28.5%). Several studies have reported the prevalence rate of *C. parvum* was 23.3% and 20% in Lopburi¹¹ and Nonthaburi²², central Thailand, respectively and 11.5% among HIV-seropositive patients in Khon Kaen, northeastern Thailand²⁵, and 9.1% in Songkhla, southern Thailand²⁶. In this study, other opportunistic protozoan parasites such as *C. cayatanensis* and *I. belli* were unobserved. The different prevalence rates may have been because of using different methods and



different study subjects. However, our study has some limitation, *Microsporidium* was undetected, which is also reported to be an opportunistic protozoan parasite. Therefore, this result may have underestimated the detection rate of all parasites.

Conclusion

Our findings showed a high prevalence of intestinal parasitic diseases among patients with HIV and with low CD4⁺ T-cell counts despite the availability of ART. These results highlighted the importance of laboratory screening and diagnosis of intestinal parasitic diseases among patients with HIV irrespective of receiving ART, CD4⁺ T-cell counts and diarrhea status.

Acknowledgement

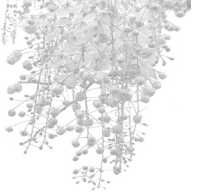
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ความชุกของโรคปรสิตในลำไส้ในผู้ติดเชื้อเอช ไอ วี ที่ได้รับยาต้านไวรัส ในจังหวัดพะเยา ภาคเหนือของประเทศไทย

จิตรกุล สุวรรณเจริญ* ธัญลักษณ์ เจริญงาม* วาสนา เมืองวงศ์*

บทคัดย่อ

การติดเชื้อร่วมระหว่างปรสิตในลำไส้กับเชื้อเอช ไอ วี พบได้อย่างแพร่หลาย ทำให้มีอัตราการป่วยและเสียชีวิตสูงขึ้นในประเทศเขตร้อน การศึกษานี้มีวัตถุประสงค์เพื่อหาความชุกของปรสิตในลำไส้ในผู้ติดเชื้อเอช ไอ วี และประเมินความสัมพันธ์กับสถานะทางภูมิคุ้มกัน โดยเก็บตัวอย่างอุจจาระของผู้ป่วยจากโรงพยาบาลพะเยาและโรงพยาบาลดอกคำใต้ จังหวัดพะเยา ตั้งแต่เดือนมิถุนายน พ.ศ. 2557 ถึงเดือนพฤษภาคม พ.ศ. 2558 จำนวน 458 ตัวอย่างทำการตรวจหาปรสิตในลำไส้โดยวิธีการตรวจอุจจาระอย่างง่าย การปั่นตกตะกอนอุจจาระด้วยฟอร์มาลินและเอทิลอะซิเตท และการย้อมสีแบบ modified Ziehl-Neelsen ซึ่งเก็บข้อมูลพื้นฐานของผู้ป่วยได้แก่ เพศ อายุ ประวัติท้องเสียและปริมาณ $CD4^+$ ล่าสุด ผลการทดลองพบความชุกสูงของปรสิตในลำไส้ร้อยละ 33.0 ซึ่งการติดปรสิตไม่มีความแตกต่างกัน

อย่างมีนัยสำคัญในกลุ่มของอายุ ($p=0.296$) เพศ ($p=0.484$) และประวัติท้องเสีย ($p=0.947$) พบอัตรา การติดปรสิตชนิดเดียวและหลายชนิดร่วมกันร้อยละ 27.4 และร้อยละ 5.2 ตามลำดับ และพบเชื้อ *Giardia lamblia* (ร้อยละ 35.2) *Cryptosporidium parvum* (ร้อยละ 28.5) และ *Strongyloides stercoralis* (ร้อยละ 26.2) ในอัตราที่สูงในผู้ติดเชื้อเอช ไอ วี ที่มีปริมาณ $CD4^+$ น้อยกว่า 200 cells/ μ L อย่างไรก็ตาม จำนวนของปรสิตในลำไส้ที่ตรวจพบไม่มีความสัมพันธ์ กับปริมาณ $CD4^+$ ดังนั้น การตรวจวินิจฉัยปรสิตในลำไส้ จึงมีความจำเป็นในผู้ติดเชื้อเอช ไอ วี โดยไม่ต้อง คำนึงถึงการได้รับยาต้านไวรัส ปริมาณ $CD4^+$ หรือ การมีประวัติท้องเสีย

คำสำคัญ: การติดปรสิตในลำไส้, เอช ไอ วี, ปริมาณ $CD4^+$, ท้องเสีย, การตรวจอุจจาระ