



## Intestinal Parasitosis among Human Immunodeficiency Virus and Tuberculosis Infected Patients from Dharan, Nepal

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### Abstract

Intestinal parasitic infestation reflects a health threat with patients living with HIV & Tuberculosis (TB). Parasitic infections caused by protozoa and helminths are the most common infections worldwide. The present study was done to find out the frequency of intestinal parasitosis among HIV and Tuberculosis (TB) patients of Dharan total of 53 samples (>15 years) from HIV seropositive patients and 28 samples (>20years) from TB patients were collected in a clean, dry and capped fitted container and subjected to macroscopic and microscopic examination for ova, cyst, adult parasites and or segments of parasites. Stool samples were fixed in a 10% formalin-ether solution. Sedimentation technique, with modified acid-fast (Ziehl-Neelsen) staining method, was performed for opportunistic intestinal parasites in both patients. Multi-parasitic infection was noted in the study. The overall prevalence of intestinal parasites was found to be 54 (66.67%). This result had shown that the participation of male patients was comparatively higher than female patients. Out of 81 stool samples, 15 (27.78%) *G. lamblia*, 12(22.22%) *E. histolytica*, 12 (22.22%) *Cryptosporidium parvum*, 6 (11.11%), *Isospora belli* 4 (7.40%) *Microsporidium*, 1(1.85%) *Blastocystis hominis*, 2(3.70%) and *Taenia spp*, 2(3.70%). To prevent this infection appropriate health education should be given to the patients concerning disease transmission, antiparasitic therapy, personal hygiene, and safe drinking water.

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### 1. Introduction

The incidence of parasitic infections was 50% in developed countries while it reached 95% in developing countries like Nepal (Adamu H, 2009) mainly causing severe chronic diarrhea and neurological dysfunction (Konate A, 2003). Coccidia (*Cryptosporidium parvum*, *Isospora belli*, *Cyclospora spp*) and amoebae (*Entamoeba histolytica*) are the etiological agents commonly responsible for the genesis of these intestinal protozoans in HIV-positive persons (Ekejindu IM, 2010). With the evolution before HIV infection, gastrointestinal involvement is frequent and 90% of patients consult for gastrointestinal disorders (Ilboudo D, 1997).

Tuberculosis (TB) is the leading cause of death among infectious intestinal diseases and substantially share similar geographical settings (WHO 2019 & Elias D, 2006). Some studies in Ethiopia reported that

there was a higher prevalence of intestinal parasite infection among TB patients compared to TB-free individuals, while others reported no association between intestinal parasitic infection and TB (Abate E 2012 & Alemayehu M, 2014).

Co-infection of intestinal parasites with tuberculosis (TB) and HIV in humans is one of the important public problems in co-endemic areas, especially in developing countries like Nepal. (Xin-xu Li et al., 2013). Intestinal parasitic infection is one of the major childhood health problems and globally about 3.5 billion people are infected with an intestinal parasite. Among them, 450 million are suffering from its illness (Zemene, 2018 & WHO, 2019). The most important intestinal helminths are soil-transmitted helminths (STHs), such as *Ascaris lumbricoides*, *Trichuris Trichiura*, *Strongyloides stercoralis*, and hookworms where the mode of transmission for *Ascaris* and *Trichuris trichiura* were by faeco-oral route and hookworm by percutaneous or

oral routes (Hotez PJ, 2009). Intracellular intestinal opportunistic infestation (*Isoospora belli*, and *Microsporidium spp*) and non-opportunistic extracellular intestinal protozoan infestation (*Entamoeba histolytica* and *Giardia lamblia*) can result in serious problems in immunocompromised patients (Bayleygn & Yiltok ES, 2021).

The clinical spectrum caused by these parasitic protozoa particularly among HIV-positive patients ranges from asymptomatic infection to severe infection such as chronic diarrhea, dehydration, and mal-absorption (Babatunde SK, 2010). Antiretroviral treatment (ART) increases the expectancy of life and productivity of patients by improving survival and decreasing the incidence of opportunistic infections in people with HIV through reduction of the viral load and increasing the level of CD<sub>4</sub> cells (R. S. Hogg, 1999). A study of co-infection with TB and intestinal helminths suggested that compared with TB patients or healthy controls (HCs), the immune response of coinfecting patients to Mycobacterium tuberculosis (MTB) was decreased (Xin xu- Li, 2013).

We carried out a parasitological survey among infected and non-infected patients consulting in Dharan Healthy Group to understand the epidemiological situation and risk factors of co-infection among HIV and TB patients with intestinal parasites. For this purpose, multiple fecal specimens were examined with a series of diagnostic approaches for intestinal protozoa and helminths and in the meantime, blood specimens were collected for routine examination and detection of antibodies against human immunodeficiency virus (HIV). The ultimate goal of the study was to guide the prevention and control of co-infections including treatment needs of HIV & TB patients, and thus decrease the adverse effects of intestinal parasites on people living with the diseases.

## 2. Materials and Method

The cross-sectional study was conducted at Microbiology Laboratory, Central Campus of Technology, based in Dharan city of Eastern, Nepal. After getting the consent of patients and hospital authority, this study was carried for the duration of six months from August 2019 to January 2020.

### 2.1 Collection of samples

A total of 81 stool samples were collected from HIV-positive patients who were in touch with Dharan

positive group and TB patients who were visiting the public health post center for DOTS. A questionnaire with informed consent was given to each patient accompanying the queries about their clinical history, hygienic practice, and nutritional behavior was filled. A labeled dry, clean, disinfectant-free wide mouth glass container was distributed, and they were asked them to bring about 20 grams (a marble size) stool sample the next morning. After collecting the stool sample, the collected stool samples were brought to the laboratory and were mixed immediately with a 10% equal volume of formal saline. First of all, a drop of saline was taken in a clean, grease-free slide and a small quantity of stool sample was spread over it and then the first examination was done under low power (10x) compound light microscope and the under high power (40x).

### 2.2 Statistical Analysis

The Chi-square test was applied for statistical analysis of results using SPSS version 16. The Association of intestinal infections with different variables was tested. Results were significant if p values were less than 0.05.

## 3. Results and Discussion

In this study, all together 81stool samples were examined. Among 81patients 50(61.72%) males were females and 31(38.27%) were females. This result shows the participation of male patients was comparatively higher than female patients. Out of 81 stool samples, 54 stool samples were found to be positive, among which 15(27.78%) *G. lamblia*, 12 (22.22%) *E. histolytica*, 12(22.22%) *Cryptosporidium parvum*, 6(11.11%) *Isoospora belli* 4(7.40%) *Microsporidium*, 1(1.85%) *Blastocystis hominis*, 2(3.70%) *Taenia spp*, 2(3.70%).

### 3.1 Giardiasis according to gender and type of patients

Prevalence of *G. lamblia* was higher in male (12.34%) patients than in female (7.40%) (Table 1) patients *G. lamblia* was found 12(14.81%) in HIV and 4 (4.93%) (Table 2) in TB patients. The present study has found the prevalence of *G. lamblia* as 1.8% among HIV seropositive individuals and nil among high-risk group populations. The reported prevalence of giardiasis varied from 1.5 to 55% in Nepal and elsewhere in the world (Awole et al, 2003; Cotte et al, 1993; Guk et al, 2005; Hailemariam et al, 2004; Mohandas et al, 2002; Okodua et al, 2003; Sapkota et al, 2004; Zali et

al, 2004). The finding was in agreement with the finding of Awole *et al* (2003) from Southwestern Ethiopia

and Guk *et al* (2005) from South Korea but lower in comparison with studies in different other countries.

**Table 1:** Giardiasis according to the gender of patients

Patient	Parasitosis	Sex		Total	p-Value
		F	M		
HIV	Absent	15	26	41	0.369
	Present	5	7	12	
TB	Absent	10	14	24	
	Present	1	3	4	
Total		31	50	81	

**Table 2:** Giardiasis according to the type of patients

Parasitosis	Patient		Total	p-Value
	HIV	TB		
Absent	41	24	65	0.94348
Present	12	4	16	
Total	53	28	81	

### 3.2 Prevalence of *E. histolytica* according to gender and type of patients

Prevalence of *E. histolytica* was high in male 11(13.58%) patients than in female 1(1.23%) patients (Table 3). *E. histolytica* was found 8(9.87%) in HIV and 4(4.93%) in TB patients (Table 4). However, in Nepal, Rai (2005), reported that *E. histolytica* ranks second among the intestinal protozoan parasites. The reported incidence of intestinal amoebic infections ranged from less than 3 to 28.8%. In a hospital-based

study, the year-to-year incidence ranged from 1.9% to 14.6%. The serological study revealed 24.6% of Nepalese have the anti-amoebic antibody. The prevalence of *Entamoeba histolytica* 35.1% which is higher than other intestinal parasites detected in this study. This is inconsistent with the study carried out in Kenya, *Entamoeba histolytica* (36.7%) being the common parasite, and the Democratic Republic of Saˆo Tome' and Principle, where *Ascaris lumbricoides* (27.6%) were the most prevalent parasite.

**Table 3:** Prevalence of *E. histolytica* of according to gender

Patient	Parasitosis	Sex		Total	p-Value
		F	M		
HIV	Absent	20	25	45	0.021
	Present	0	8	8	
TB	Absent	10	14	24	
	Present	1	3	4	
Total		31	50	81	

**Table 4:** Prevalence of *E. histolytica* of according to patients

Parasitosis	Patient		Total	p-Value
	HIV	TB		
Absent	45	24	69	0.922
Present	8	4	12	
Total	53	28	81	

### 3.3 *Cryptosporidiosis according to gender and type of patients*

Prevalence of *Cryptosporidium parvum* was high in female 8(9.8%) patients than in male 4(4.9%) patients (Table 5). *Cryptosporidium parvum* was found 8(9.8%) in HIV and 4(4.93%) in TB patients (Table 6). The finding of the present study was in agreement with

the finding of Zali *et al* (2004). The prevalence was lower in comparison with the previous study done by Dhakal *et al* (2004), Ghimire *et al* (2004), Sapkota *et al* (2004), and Sherchand *et al* (1996) from Nepal. The diarrheagenic cases in this study were very few and the present study had examined the single stool specimen from the study population. This might be the reason for the low prevalence.

**Table 5:** Cryptosporidiosis according to gender

Patient	Parasitosis	Sex		Total	p-Value
		F	M		
HIV	Absent	15	30	45	0.031
	Present	5	3	8	
TB	Absent	8	16	24	
	Present	3	1	4	
Total		31	50	81	

**Table 6:** Cryptosporidiosis according to types of patients

Parasitosis	Patient		Total	p-Value
	HIV	TB		
Absent	45	24	69	0.922
Present	8	4	12	
Total	53	28	81	

### 3.4 *Prevalence of Isospora belli according to gender and type of patients*

Prevalence of *Isospora belli* was high in male 10(12.34%) patients than in female 2(2.46%) patients (Table 7). *Isospora belli* was found in 6(7.40%) in HIV and was not found in TB patients (Table 8). *Isospora*

*belli* reported in our study shows higher prevalence in comparison to the study done in Yaounde where only 1.9% prevalence was reported and in Congo by Wumba *et al.*, 2010 where 1.7% prevalence was reported. In contrast, this prevalence was as high as 5% in Thailand and 7% in Brazil. In two studies done in China, *I. belli* was not reported (Tian LG, 2012).

**Table 7:** Prevalence of *Isospora belli* according to gender

Patient	Parasitosis	Sex		Total	p-Value
		F	M		
HIV	absent	19	28	47	0.042
	present	1	5	6	
TB	absent	11	17	28	
	present	1	5	6	
Total		31	50	81	

**Table 8:** Prevalence of *Isospora belli* according to types of patients

Parasitosis	Patient		Total	p-Value
	HIV	TB		
Absent	47	28	75	0.064
Present	6	0	6	
Total	53	28	81	

### 3.5 Prevalence of *Microsporidium* according to gender and type of patients

Prevalence of *Microsporidium* was high in male

3(3.70%) patients than in female 1(1.23%) patients (Table 9). *Microsporidium* was found 3(3.70%) in HIV and 1(1.23%) in TB patients (Table 10).

**Table 9:** Prevalence of *Microsporidium* according to gender

Patient	Parasitosis	Sex		Total	p-Value
		F	M		
HIV	Absent	19	31	50	0.76
	Present	1	2	3	
TB	Absent	11	16	27	
	Present	-	1	1	
Total		31	50	81	

**Table 10:** Prevalence of *Microsporidium* according to types of patients.

Parasitosis	Patient		Total	p-Value
	HIV	TB		
Absent	50	27	77	0.680
Present	3	1	4	
Total	53	28	81	

### 3.6 Prevalence of *Hookworm* according to gender and type of patients

The prevalence of Hookworm was 2(2.46%) in male patients and was not found in female patients (Table 11). Hookworm was found 2(2.46%) in HIV and

was not found in TB patients (Table 12). This study revealed the prevalence of 3.6% and 29.7% of *hookworm* infection among HIV seropositive subjects and high-risk group population respectively. The reported prevalence of hookworm infection among HIV/AIDS individuals in Nepal and elsewhere in the world varied from 0.8 to 39.2% (Awole et al, 2003; Hailemariam et

al, 2004; Kaminsky et al, 2004; Modjarrad et al, 2005; Mohandas et al, 2002; Okodua et al, 2003; Sapkota et al, 2004). Hookworm is one of the commonest soil-

transmitted helminthes in Nepal Rai (2005), reported a prevalence of 7% to over 80% in certain communities of Nepal.

**Table 11:** Prevalance of Hookworm according to gender

Patient	Parasitosis	Sex		Total	p-Value
		F	M		
HIV	absent	20	31	51	0.260
	present	0	2	2	
TB	absent	11	17	28	
Total		31	50	81	

**Table 12:** Prevalence of *Hookworm* according to types of patients

Parasitosis	Patient		Total	p-Value
	HIV	TB		
Absent	51	28	79	0.298
Present	2	0	2	
Total	53	28	81	

### 3.7 Prevalence of *Blastocystis hominis* according to gender and type of patients

*Blastocystis hominis* was found 1(1.23%) in female patients and was not found in males (Table 13). *Blastocystis hominis* was found 1(1.23%) in HIV and was not found in TB patients (Table 14). The present study found the prevalence of *B. hominis* as 0.9% among HIV seropositive individuals and nil among the high-risk group population. The reported prevalence elsewhere in the world varies from 2.1 to 14.1% (Awole et

al, 2003; Hailemariam et al, 2004; Mohandas et al, 2002; Prasad et al, 2000; Zali et al, 2004). Few reports from Nepal had reported *B. hominis*. The reported incidence ranged from less than 1.0% to 24.9%. Recently, in a remote hilly region in Far-western Region, it was found to be 1.3%. The prevalence of *B. hominis* in Kathmandu Valley in patients with diarrhea and school attending children were 2.0% and 7.9% respectively. However, one report showed no association between *B. hominis* infection and diarrhea in travelers (Rai, 2005).

**Table 13:** Prevalence of *Blastocystis hominis* according to gender.

Patient	Parasitosis	Sex		Total	p-Value
		F	M		
HIV	absent	19	33	52	0.201
	present	1	0	1	
TB	absent	11	17	28	
Total		31	50	81	

**Table 14:** *Blastocystis hominis* infections according to Patients

Parasitosis	Patient		Total	p-Value
	HIV	TB		
Absent	52	28	80	0.465
Present	1	0	1	
Total	53	28	81	

#### 4. Conclusion

The intestinal parasites were detected from HIV and TB patients by microscopic examination and formalin-ether concentration method. The parasites identified were *G. lamblia*, *E.histolytica*, *Cryptosporidium parvum*, hookworm, *Taenia spp.*, *Isospora belli*, *Microsporidium*, and *Blastocystis hominis*. Most of the parasites are opportunistic among immuno-compromised patients. It may be concluded that in Nepalese HIV and TB infected patients, both intestinal helminthic and protozoal parasitic infections are still highly prevalent. Similarly, the higher prevalence of multiple parasitic infections among such populations indicates severe conditions. The high prevalence of helminthic parasites among such populations indicates the adequate treatment and health education, provision of adequate toilet facilities, and pipe-borne water so that the continual contamination of the environment with ova and larvae of parasite would be greatly reduced. Finally, in the management of HIV and TB infected patients in Nepal with or without diarrheal symptoms, stool examination is still a useful investigation. There was no significant difference between age, sex, religion, ethnicity, syndrome, educational level of parents, family size and family type of patients, and the prevalence of intestinal parasitic infection.

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#### Conflicts of Interest

The authors declare that there is no conflict of interest.

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