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Prevalence of intestinal parasites and its association factors, knowledge, attitude and practice about intestinal parasite among HIV-positive individual in saint peter TB specialized hospital Addis Ababa, Ethiopia

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This is to certify that the thesis prepared by Birhanu Kassaye entitled "Prevalence of Intestinal Parasites and its association factors, Knowledge, Attitude, and Practice about Intestinal Parasites among HIV-Positive Individuals in Saint Peter TB Specialized Hospital, Addis Ababa, Ethiopia." submitted in partial fulfillment of the requirements for the Degree of Masters of Sciences in Clinical Laboratory Sciences (diagnostic and public health microbiology) complies with the regulations of the university and meets the accepted standards concerning originality and quality.

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Abbreviations

AAU = Addis Ababa University

AIDS = Acquired Immune Deficiency Syndrome

ART= Anti-Retroviral Treatment

ARV = Antiretroviral

CBC = Complete Blood Count

CD4 = Cluster Differention 4

CI = Confidence Interval

DC = Data collector

DMLT = Department of Medical Laboratory Technology

EC =Ethiopian Calendar

EDTA= Ethylene diamine tetra acetic acid

EQA= External Quality Assessment

EPHI= Ethiopian Public health Institution

HG = Hemoglobin

HIV = Human Immunodeficiency Virus

HHRT = Highly Active Antiretroviral Therapy

KAP = Knowledge, attitude, and practice

OP = Opportunistic parasites

PI= Principal Investigator

QA = Quality assurance

RPM = Revolution per Minute

SOP = Standard Operational Procedures

SPSH =Saint Peter specialized Hospital

SPSS = Statistical Package for the Social Sciences

WHO = World Health Organization

ZN = Ziehl-Neelsen

Abstract

Background: Intestinal parasitic infections (IPI) and HIV/AIDS have been the leading and persisting public health problems in the world. There vital causes of morbidity and mortality are remarkably high in sub-Saharan Africa, this study aimed to determine the Prevalence of Intestinal parasites and Knowledge, Attitude, and Practice (KAP) among HIV-positive individuals in Saint Peter Hospital, Addis Ababa, Ethiopia

Methods: A cross-sectional study was conducted among patients attending saint peter hospital from December 2019 to May 2020. A total of 328 participants were select by using convenient sampling method. Socio-demographic data and knowledge, attitude and practice were collected using a structured questioner. Stool specimen was collected using clean container and processed and analyzed for parasitological examination using direct wet mount, formal-ether sedimentation, and modified Ziehl-Neelsen staining techniques. Venous blood was collected and the CD4+ T-lymphocyte and hemoglobin analyzed by Presto instrument. The data were analyzed by using SPSS version 23 and P values less than 0.05 were considered statistically significant.

Result: A total of 328 HIV-positive individuals (59.8% female) of age ranging from 13-72 years (mean= 41.8, SD 10.8) participated in this study. The overall prevalence of intestinal parasites among the study participants was 26.2% (86/328), from this 88.4% (76/86) was infected by single parasite, [*Entamoeba histolytica/dispa*r 48.7 % (37/76),*Giardia intestinalis* 30.3% (23/76), Taenia species 10.5% (8/76), Ascaris lumbricoides 3.9% (3/76), Strongyloides stercoralis5.3% (4/76), and *Hymenolepis nana* 1.3% (1/76)]. Co-infection was accounted 11.6% (10/86)[Entamoeba histolytica/dispar and *Giardia intestinalis* 70 % (7/10), *Entamoeba histolytica/dispar* and *Strongyloides stercoralis* 30 % (3/10)] and IP was significantly associated with CD4 count < 200 AOR [4.736 CI (2.338-9.594); P-value <0.001], and also Anemia AOR [3.271 CI (1.069-10.010); P-value 0.038 of all study participants interviewed for their knowledge, attitude, and practices (KAPs), 89.9% had good knowledge, 88.4% had a positive attitude and 77.7% had good practice about intestinal parasitosis.

Conclusion;-Intestinal parasitic infections are still common health problems among HIV/ADIS patients in the study area, so the health professionals need to give attention to parasitological examinations in the routine treatment of HIV/AIDS patients and also give education on these three parts knowledge, attitude and practice, but more focus and follow up on the practice of HIV/AIDS patients on transmission, prevention, and control mechanisms of intestinal parasitosis.

1. Introduction

1.1. Background

Intestinal parasitic infections are widely distributed in the world. The rate of infection is remarkably high in developing countries because of low socio-economic and poor living conditions [1]. Globally, it is estimated that one-third of the population is infected with intestinal parasites most of which are found in tropical and sub-tropical parts of the world. Parasitic infections are an important cause of morbidity and mortality, especially with the emergence of immunosuppressive diseases such as Human Immunodeficiency Virus (HIV/AIDS) [2]. Proper knowledge, good attitude and practice (KAP) against Poor hygiene practice, inadequate sanitary conditions, and lack of awareness of correct mode of transmission are some factors that attribute to high intestinal parasitic infection and significantly mitigate the spreading of intestinal parasitic infection. The majority of intestinal parasites are transmitted by the fecal-oral route while others are transmitted through skin penetration and the Sign and symptoms of intestinal parasitic infections are diarrhea and other intestinal disorders, such as cramping abdominal pains, nausea, vomiting, or low-grade fever. Diarrhea is ordinarily chronic and prolonged in the course of opportunistic diseases with HIV/ AIDS [3].

The causative agents of intestinal parasites are varied from patient to patient and from country to country depending on different reasons some of them are the seasonal variation of pathogens, Endemicity, geographical distribution, and also the immune status of the patient [4]. The most common enteroparasitoses in patients with HIV/AIDS are caused by Strongyloides stercoralis, nematodes, and intestinal coccidian, which usually aggravate the clinical condition of immunocompromised patients and cause refractory infections Because of the chronic and recurrent nature of these infections and The nature of HIV infection is depletion of CD4+ T lymphocytes resulting in overall impairment of the immune response [5]. Therefore, HIV/AIDS patients are more likely to acquire other infections. They are prone to more severe morbidity once the infection is established. They also have disseminated rather than localized infection. Moreover, they are unable to effectively clear infecting microorganisms. All these lead to greater morbidity and mortality, mainly because of opportunistic infections, Opportunistic infections can be of viral, bacterial, fungal, or parasitic origins which usually affect the gastrointestinal [6].

About 80% of deaths of HIV/AIDS patients are associated with opportunistic infections rather than the virus itself, and of these, more than 47% happen due to OIPs (7). Opportunistic protozoan parasites such as *Cryptosporidium* species (spp), *Isospora belli*, and *Microsporidia* spp have been identified in individuals living with HIV/AIDS OIPIs has been a major source of morbidity in tropical countries where HIV/AIDS is endemic [7]. Although there has been an improvement with the introduction of ART for individuals in HIV/AIDS, the presence of OIPIs creates a serious challenge in reducing associated morbidity and mortality. Studies have also shown that the prevalence of OIPs among those patients is high. Nevertheless, with the introduction of combination antiretroviral therapy and also more effective prophylaxis against these 'classic' opportunistic infections, the rate has decreased. However, OIPs still represent a frequent cause of morbidity and mortality in most developing countries [8, 9].

Anemia is the commonest hematological abnormality in patients with HIV. It is associated with increased morbidity and is an indicator of poor prognosis among people with advanced HIV disease, Parasitic infections are commonly associated with anemia due to different reason, The relationship between parasitic infection and anemia is a pathophysiologic type where it is recognized that certain factors play important roles these include metabolic processes of the parasite, the strain, and number, the size, site, age, the parasites, and level of immunity at the time of infection, presence of co-existing diseases or conditions which reduce immune responses and malnutrition [10,11].

Laboratory methods used to diagnose intestinal parasitosis are Formal-ether concentration technique, direct microscopic, modified Ziehl-Neelsen staining methods, and microscopic examination of stool specimen is the most practical method for diagnosis of the intestinal parasite in developing countries [12].

1.2. Statement of the problem

Intestinal parasitic infections are amongst the most common infections in the world. Globally, about 3.5 billion people are infected with an intestinal parasite. Out of whom, 450 million are suffering from its illness [13]. The degree of parasitic infection is remarkably high in sub-Saharan Africa, where the majority of the Human Immunodeficiency Virus HIV/AIDS cases are concentrated. An estimated 80 % of Acquired Immune Deficiency Syndrome (AIDS) patients die of AIDS-related opportunistic infections rather than from HIV itself more than 47% happen due to opportunistic parasitic infections [14].

Diarrhea caused by parasites is one of the major opportunistic illnesses in HIV/AIDS resulting in significant mortality and morbidity despite the advance of antiretroviral therapy (ART) diarrhea is still a common problem of HIV infection and contributes to the reduced life quality and survival of HIV patients, It is estimated that diarrhea occurs in roughly 90% HIV/AIDS patients in developing countries, and 30–60% in developed countries, with Sub- Saharan Africa has the highest burden [8,15,16]. Concomitant infection between HIV and one or more intestinal parasites are common [17].

HIV infection has increased the significance of parasitic infection especially opportunistic intestinal parasites. More importantly, with the emergence of AIDS, epidemiology, as well as the outcome of diseases caused by opportunistic parasites, was significantly modified. But, the effect of HIV on some intestinal parasites is not clearly understood. Overall, either backed by HIV or independently, intestinal parasitic infections have continued to be the major cause of disease in humans [18]

The most intestinal coccidian infection such as *Cryptosporidium*, *Isospora*, *Cyclospora*, and Microsporidia are increasingly becoming prevalent in AIDS patients, Intestinal infection by Cryptosporidiais self-limited but leads to persistent diarrhea in the advanced stage of AIDS and there is no effective treatment available for it. *Isospora* causes chronic diarrhea in AIDS patient but can be treated effectively with available antimicrobials, on the other hand, *S.stercoralisis* important human parasitic infection primarily because of its potential for serious and even lethal disease in immunosuppressed patients,

Undeniably, anemia is the most important clinical problem seen in people living with HIV/AIDS its severity increases as CD4 count declines and it can have serious implications, which may vary from useful and quality of life decrements to an association with disease progression and decreased survival. Parasitic infection is one related to Anemia, and the most prevalent parasites are helminths such as *Ascaris lumbricoides, Hookworm, Trichuris trichiura Strongyloides stercoralis*, and protozoa such as *Giardia lamblia, Entamoeba coli, Entamoeba histolytica, Iodamoeba butschli*i. The helminthic infection causes anemia deficiency by reducing iron uptake from the intestine, directly sucking blood, and interfering directly and indirectly in iron metabolism. Protozoa impact anemia by destructing the intestine mucosal structure that influences micronutrient absorption, such as iron. All these mechanisms also affect the hosts' nutritional status and then alter their immune system [19, 20].

In Ethiopia like most developing countries in Sub-Saharan Africa intestinal parasitic diseases are among the ten top causes of morbidity nationwide due to inadequate knowledge, attitude and improper practice concerning intestinal parasitosis contribute to the high level of prevalence. Moreover, if peoples do not have adequate knowledge, attitude, and practice regarding the disease it is difficult to control [21, 22].

Most of the previous studies conducted in Ethiopia have focused on only prevalence and distribution of intestinal parasitic infections in HIV-positive individuals but the knowledge, attitude and practice of HIV-positive individual about intestinal parasite are not assed. This initiated us to conduct a research on to determine the Prevalence of Intestinal parasites and Knowledge, Attitude, and Practice about Intestinal parasites among HIV-positive individuals in Saint Peter TB Specialized Hospital, Addis Ababa, Ethiopia.

1.3 Significance of the study

Intestinal parasites are highly prevalent in Ethiopia due to socio-economic condition and other unhygienic factors that increase the probability of infection. However, there have been some studies have reported the magnitude of intestinal parasitic infections among HIV patients. The present study is, therefore, aimed to determine the prevalence of intestinal parasites and knowledge, attitude, and practice about intestinal parasites among HIV-positive individuals.

Therefore, this study will provide data for the policy makers, clinicians and other stake holders and also offers information to giving appropriate action to improve the awareness of the patients about intestinal parasite infection and their cause may become an input for clinicians to decide about the need for further care and patient adherence to ART.

2. Literature review

A global systematic review and meta-analysis identified 131 studies that reported microsporidia, Cryptosporidium, and Isospora infection in HIV-infected people. They estimated the pooled prevalence to be 14.0% for Cryptosporidium, 11.8% for microsporidia, and 2.5% for Isospora. A low prevalence of microsporidia and Isospora infection was found in high-income countries, and a high prevalence of Cryptosporidium and Isospora infection was found in sub-Saharan Africa and they also detected a high prevalence of Cryptosporidium, microsporidia, and Isospora infection in patients with diarrhea [23].

Globally, a probable 438.9 million people were infected with H. worm in 2010, 819.0 million with A. lumbricoides, and 464.6 million with T. trichiura. Of the 4.98 million years lived with disability attributable to STH, 65% were attributable to Hook worm, 22% to Ascaris lumbricoides, and the remaining 13% to Trichuris trichiura [24].

A prospective study conducted on Cerebral Toxoplasmosis in HIV-Positive Patients in Clinical Features and Predictors of Treatment in Brazil a total of 55 confirmed or presumptive cases of cerebral toxoplasmosis in HIV positive patients was performed Cerebral toxoplasmosis led to the diagnosis of HIV infection in 19 (35%) patients, whereas it was the AIDS-defining disease in 41 (75%) patients. the overall result is 22 (54%) patients were previously known to be HIV-positive. At diagnosis of cerebral toxoplasmosis, 4 (7%) patients were on highly active ART and 6 (11%) were receiving primary cerebral toxoplasmosis prophylaxis. The mean CD4cell count was 64.2 (69.1) cells /ml 49 patients (78%) showed alterations consistent with toxoplasmosis on brain computed tomography. At six weeks of treatment, 23 (42%) patients had complete clinical response, 25 (46%) partial response, and 7 (13%) died. Change of consciousness, Karnofsky score <70, psychomotor slowing, hemoglobin < 12 mg/dl, mental confusion, Glasgow Coma Scale less than 12 were the main predictors of partial clinical response [25].

A prospective study was conducted in France from 143 consecutive patients were analyzed. The CD4 T cell count median was 32/mm3, and 59% were getting ART diarrhea was existing in 85 patients (59%), 19 of whom (22%) had IPs detected in stools. The overall prevalence IPs was 17%, with microsporidia (n = 6), and Giardia duodenalis (n = 5) cryptosporidia (n = 8), being the most frequent pathogens [26].

In another study conducted in Nigeria blood and Stool samples was collected from each patient the prevalence of anemia was 93.3% while 18% had parasitic infections and there was a significant relationship between CD4 count <200cells/μL and anemia. Cryptosporidium species, A. lumbricoides, hookworm, and Taenia species were associated with anemia [27].

A cross-sectional study was conducted in Kinshasa, Democratic Republic of Congo a total of 242 stool sample were performed using Ziehl–Neelsen staining, real-time polymerase chain reaction (PCR), immunofluorescence indirect monoclonal antibody, nested PCR-restriction fragment length polymorphism, and PCR amplification and sequencing the overall result is Enterocytozoon bieneusi (7.8%),Encephalitozoon intestinalis (0.4%),Cryptosporidium spp. (5.4%),Isospora belli (0.4%), pathogenic intestinal protozoa (2%),nonpathogenic intestinal protozoa (10.6%) and helminths (2.8%) they establish five genotypes of E. bieneusi 2 elder NIA1 and D, and 3 new, KIN1, KIN2, and KIN3. Cryptosporidium parvum (0.4%) and Cryptosporidium hominis (1.6%). on the other hand asthenia (36.4%), diarrhea (34.3%), CD4 count of 100 cells/mm³(31%) and no antiretroviral therapy (ART)(39%).The majority of those with opportunistic intestinal parasites and C. hominis, and all with Cryptosporidium parvum and new E. bieneusi genotypes, had diarrhea, low CD4 counts of 100 cells/mm³, and no ART [28].

A study in Cameroon a total of 52 pre-ART and 248 on-ART HIV patients Stool samples were collected and analyzed for intestinal parasites the overall prevalence of intestinal parasitic infections in pre-ART and on-ART was 84.6 % and 82.3 % respectively with no significant difference observed concerning age and gender all the opportunistic parasites including Cryptosporidium parvum, Cyclospora cayetanensis, Isospora belli, and Microsporidium species were isolated from both groups, with Microsporidium species. significantly associated with CD4+ T cell <200 cells/μl in pre-ART, while Cryptosporidium parvum, Microsporidium spp. and Isospora belli were associated with counts below 200 cells/μl in on-ART Cryptosporidium parvum, was significantly associated with diarrhea in pre-ART meanwhile it was significantly associated with diarrhea in on-ART [29].

A cross-sectional study was conducted in Ilorin, Nigeria a total of 238 Stool samples were examined using direct microscopic and modified Ziehl-Neelsen methods the positivity of intestinal parasites was taken as the presence of worms, oocyst, cyst, ova, or larvae in the stool samples. The overall prevalence of Intestinal parasitic infestation in HIV-positive subjects was 68.5% and was significantly higher than in the HIV-negative controls 49.2%. In HIV-positive

subjects, Cryptosporidium spp. was the commonest (55.0%) parasite isolated. Others were Cyclospora cayetanensis (41.2%), Isospora belli (3.0%), Entamoeba histolytica (8.4%), Giardia lamblia (3.7%), Ascaris lumbricoides (2.5%), Strongyloides stercoralis (1.7%), Trichuris trichiura (0.8%) and Schistosoma mansoni (0.4%). HIV-positive patients with CD4+ T cell count of less than 200cells/ul were more at risk of opportunistic parasites compared to the HIV-negative controls [30].

From a study done by Ahmed, K.S, et al., in Asmara, Eritrean 2017 Knowledge, Attitude and Practice (KAP) Assessment of Intestinal Parasitic Infection A total of 126 stool samples were examined in the study of which 46 (36.50%) were found infected with one or more intestinal parasites. Among the parasites identified, the most common was Hymenolepis nana with a prevalence rate of 35 (76.0%). Here, most of the students 96 (76.1%) were having poor knowledge about the correct mode of transmission of intestinal parasites. The majority of the students 91 (72.2%) practicing defecation in the open air when they are at home, and all 126 (100%) are doing that at school, and a significant number of students were not washing hands after defecation [31].

A study conducted in Bahir dar, Ethiopia on Prevalence of intestinal protozoan infections among individuals living with HIV/AIDS A total sample of 399 HIV-positive individuals were examined for the presence of trophozoites, cysts, and oocysts using direct wet mount, formol–ether sedimentation and modified Ziehl–Neelsen techniques Besides, CD4+ T-cell counts were measured to evaluate the immune status of the study subjects the overall prevalence of 30.6% enteric protozoan infections were recorded. Pre-ART (antiretroviral treatment) individuals were more infected than patients on ART, although this was not statistically significant. The highest prevalence of intestinal protozoan infection was due to Entamoeba histolytica/E. dispar 19.3%, followed by Cryptosporidium spp 5.8%, G. lamblia 4.3%, and Isospora belli 1.3%. A CD4+ T-cell count of <200 cells/ml and the status of being diarrhoeic were significantly associated with the overall prevalence of enteric protozoan infection [32].

A cross-sectional study was conducted in Butajira, Ethiopia a total of 323 study participant was involved in the study Stool sample was collected and identify both common and opportunistic intestinal parasites the overall prevalence of intestinal parasites was 35.9%. Protozoa's (E. histolytica trophozoite, E. histolytica cyst, G. lamblia trophozoite, and G. lamblia cyst), helminths (Tanea spp., A. lumbricoides, S. stercoralis, Hookworm spp, and H. nana), and

opportunistic intestinal parasites (C. parvum, I. belli) were observed in 57 (17.1%), 46 (14.4%), and 28 (8.7%) study participants respectively [33].

Another study conducted in Kombolcha, Ethiopia on Intestinal parasitosis among HIV/AIDS patients who are on anti-retroviral therapy A total of 223 HIV seropositive individuals who are on ART in Kombolcha Health Centre were examined for intestinal parasites the prevalence of intestinal parasites were detected in 13.9% of the 223 study participants and nine different intestinal parasite species were detected and the most prevalent intestinal parasite was *E.* histolytica, which accounts 7.2%. The majority of study participants had the habit of washing their hands before a meal and after the toilet 96.4% and most of the study participants, 56.5% had private toilets [34].

In Aksum, Ethiopia, a study conducted by Tuom .G et al. on Intestinal parasitosis concerning CD4 count and anemia among ART initiated patients on a total of 242 ART-initiated participants the overall prevalence of intestinal parasites was 26.4% and among the six types of parasite recognized Entamoeba histolytica/dispar (18.6%) and Giardia lamblia (2.1%) were the leading, and also lack handwashing before the meal, eating uncooked vegetables, history of taking anti-parasite medication, stool consistency, and anemia were strongly associated with intestinal parasitosis [35].

A cross-sectional study was conducted in Arbaminch, Ethiopia by Getaneh.A et al. A total of 220 patients 10 types of intestinal parasites were identified and cryptosporidium spp were the most frequent parasites detected 8.63% followed by Cyclospora spp 5.9%. Among no opportunistic intestinal parasites, E. histolytica was with the highest prevalence of 3.62% followed by S. stercoralis, G. lamblia, and A. lumbricoides1.81 % each, Taenia spp 1.36%, and Hookworm and H. nana 0.91 % each [36].

A study conducted by Yonatan Kindie and Shiferaw Bekele in South west, Ethiopia on Prevalence and Risk Factors for Intestinal Parasite Infections in HIV/AIDS Patients with Anti-Retroviral Treatment by using Direct wet mount, Formol-Ether Concentration and modified Ziehl-Neelson staining was done from 150 participants only for 120 individuals, and intestinal parasites were detected in 45.0 % of the study participants harboring one or more parasites. Among the detected intestinal parasites, A. lumbricoid accounted for 11.7% followed respectively by E. histolytica 9.2%, S. stercolaris7.5%, and opportunistic parasites 5.0% [37].

A comparative cross-sectional study was conducted among patients attending the clinical laboratory of All African Leprosy and Tuberculosis Eradication and Rehabilitation Training Center hospital, Addis Ababa, Ethiopia. The overall magnitude of intestinal parasite was 35.08%. This proportion was different among study groups with in ART naïve HIV positives patients 39.2%, in HIV negatives 38.83%, and in HIV positive on ART patients 27.14%. Among HIV positive on ART patients Entamoeba histolytica/dispar 15.71%, Giardia lamblia 2.14 %, Ascaris lumbricoides 3.57% Strongyloides stercoralis 5.00%, Trichuris trichiura1.43%, Hookworm 0.00%, Taenia species 0.71% Hymenolepis nana 1.43%, Enterobius vermicularis 0.00, Cryptosporidium parvum 1.43%, Isospora belli 0.71%, Any protozoan parasites 19.29% and Any helminths 12.14%[38]

3. Objectives

3.1 General Objective

To determine the prevalence of intestinal parasites and its association factors, knowledge, attitude, and practice about intestinal parasites among HIV-positive individuals in saint Peter TB specialized hospital Addis Ababa, Ethiopia.

3.2 Specific objectives

- To assess the prevalence of IP among HIV positive individuals in saint peter TB specialized hospital Ababa, Ethiopia
- To assess risk factors of IP among HIV patients in saint peter TB specialized hospital Ababa, Ethiopia
- To assess Knowledge, attitude, and practice about IP among HIV-positive individuals in Saint Peter TB Specialized Hospital Addis Ababa, Ethiopia

4. Materials and methods

4.1. Study area

The study was conducted in St. Peter's Specialized Hospital, which is located in Gullele sub-city, Addis Ababa, Ethiopia. It is found at 9° 4' 5.1312"latitude, and 38° 44' 31.398" longitude, and elevation ranging from 2100 to 3000 meters above sea level. According to the 2011 statistics, the sub-city has a total population of 267,624 with 129,396 males and 138,228 females [39].

St. Peter's Specialized Hospital was established in 1960 GC. The hospital is a TB Specialized Hospital with more than 300 beds and gives different inpatient and outpatient services including HIV counseling and testing, and ART [40].

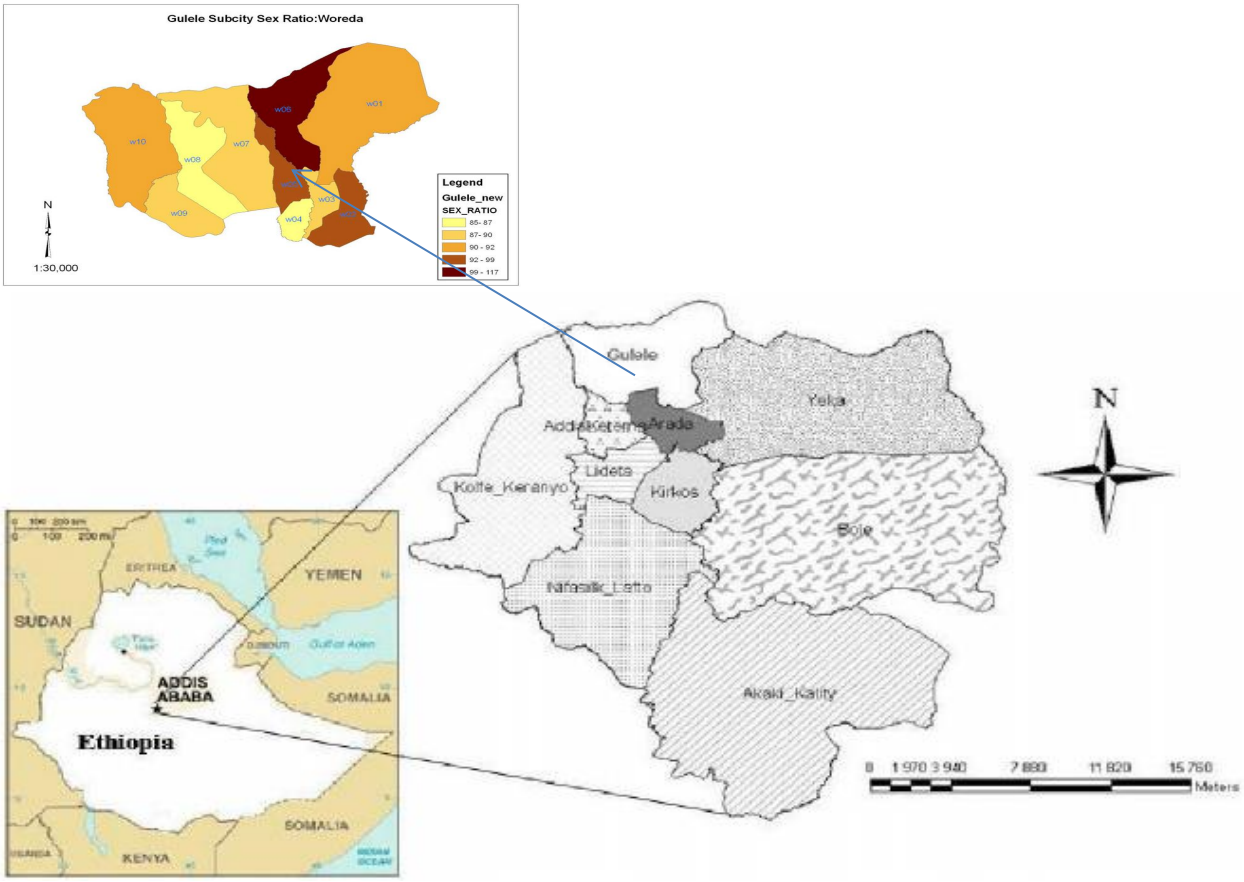


Figure 1: map of Gullele sub-city, Addis Ababa, Ethiopia, 2020

4.2. Study design and period

A cross-sectional study was conducted from December 2019 to May 2020 at St. Peter's Specialized Hospital Addis Ababa, Ethiopia.

4.3. Population

4.3.1. Source population

All HIV-positive individuals who visited St. Peter's Specialized Hospital to get health services during the study period.

4.3.2. Study Population

The study populations were all HIV-positive individuals who meet the inclusion criteria and gave assent/ permission during the study period.

4.4. Inclusion and exclusion criteria

4.4.1. Inclusion criteria

All individuals on ART and volunteered to give consent/ assent to participate were included in the study.

4.4.2. Exclusion criteria

Individuals who were taking anti-parasitic medications within the past two weeks, those with a history or diagnosis of any other acute or chronic disease-causing immunosuppression or anemia, and also individuals who were under the ART regimen containing Zidovudine were excluded from the study because it is known to produce anemia [41].

4.5. Study variables

4.5.1. Dependent variables

Prevalence of intestinal parasite

4.5.2. Independent variables

Socio-demographic factors such as religion, marital status, sex, educational status, age, address, and personal hygiene such as the source of drinking water, a habit of eating uncooked vegetables, habit of handwashing after toilet, habit of handwashing before a meal, the existence of toilet and clinical characteristics such as anemia, CD4 count, stool consistency,

environmental, environmental, diarrhea, World Health Organization (WHO) disease stage, Type of Antiretroviral (ARV) drug and KAPs were the independent variables.

4.6. Sample size calculation and Sampling method

4.6.1. Sample size calculation

Based on a previous study performed in St. Mary Aksum General hospital that reported the prevalence of intestinal parasites in-ART patients 26.4% [35], the sample size was determined at a 95% level of confidence (z = 1.96) and 5% margin of error (d) using the single proportion formula.

$n = Z^2_{\alpha/2} P (1- P)/ d^2$

Where:-

- ✓ "n" is a minimum number of sample size,
- ✓ "Z" is a standard value which is Z=1.96
- ✓ "d" is a marginal error. At 95% confidence interval, marginal error is5%(0.05)

"P" is the prevalence value = 26.4%

$$\mathbf{n = \frac{1.96^2 \times {}^{0.}264\ (1-0.264)}{0.05^2}}$$

$$\mathbf{n = \frac{1.01 \times 0.736}{0.0025}}$$

$$\mathbf{n = 297.344}$$

Thus, the study included at least 297 subjects, but Assuming a 10 % non-response rate, the sample size was: n=297+10 %= 297+30= 328. With these assumptions, the minimum sample size required for the study was 297 and with 10% added for non-respondents, the required sample size was be increased to 327 study participants.

4.6.2. Sampling Method

A convenient sampling technique was employed to include study participants who meet the inclusion criteria until the achievement of the sample size. Samples were collected consecutively until the required sample size was achieved.

4.7. Measurement and Data collection

4.7.1. Data collection procedure

Socio-demographic characteristics, clinical presentation, treatment history, and other variables of the study participants were collected using a structured questionnaire after written informed consent/assent was obtained. Data collectors were identified, trained, and informed to collect the data as per the pre-structured questionnaire.

Stool samples were collected (4 g of formed stool and 10 ml of diarrheic stool) into wide open-necked, carefully labeled, dry, leak-proof, and grease-free transparent stool container by the participants. The participants were instructed on how to collect stool samples at the point of care. These samples were analyzed within one hour of collection for ova, cyst, and parasites. Samples were store in 10 % formol-ether for 24 h and later stained by ZN stain for intestinal Oocysts. After the provision of stool samples, about 4 ml of venous blood was collected into EDTA test tubes. These samples were immediately used for the analysis of the CD4+ T cell counts and Hemoglobin (Hg). All analyses were done at St. Peter's Specialized Hospital Parasitology and immunohematology laboratory.

4.7.2. Laboratory analysis

1. Direct wet mount method

After the collection of stool specimens from each patient was examined by direct wet mount method using normal saline (0.85% NaCl solution) at St. Peter's specialized Hospital Laboratory. Lugol's iodine was used to detect the cyst of intestinal protozoan parasites [38]. The remaining sample was preserved with 10% formalin and examined by formol- ether concentration technique and modified Zeihl– Neelsen method [42].

2. Formolether concentration technique procedures

Formol-ether concentration technique was performed from each stool specimens collected from the study participants. Using a stick, an estimated 1 g (pea-size) of representative feces was emulsified in about 4 ml of 10% formol water contain in a screw-cap tube. Then further 4 ml of 10% v/v formol water was added and mix well by shaking. The emulsified feces were sieved and 4 ml of diethyl ether will add. The tube was mixed for 1 minute and immediately centrifuged at 3000 revolutions per minute (rpm) for 1 min. After centrifugation, the sediment at the bottom of

the tube was transferred to a slide and cover with a cover glass. Then the preparation was examined microscopically using the 10× and 40× objective lenses [42].

3. Modified Ziehl–Neelsen method procedures

Smear from the remaining sediment was stain using Modified Ziehl–Neelsen method. The smear was stained with Carbolfuchsin for 15 min after air-dried and fixed with methanol for 2–3 min. The stain was decolorized with 1% acid alcohol for 15s and Counterstain with methylene blue for 30-60 seconds then the preparation was examined microscopically using the 10× and 40× objective lenses [42].

CD4 and Hemoglobin analysis by BD FACS Presto

When blood is introduced into the BD FACS Presto Cartridge, the specific antibodies bind to the surface antigens on the T lymphocytes and monocytes during the incubation period. When the stained cartridge is inserted into the counter, the dedicated software identifies and counts the CD4+ T lymphocyte absolute and percentage cells, and calculates the hemoglobin concentration. The BD FACS Presto Cartridge also contains immobilized antibodies as a quality control measure which the instrument uses to ensure that the reagents are present and sufficient blood specimen volume has been added [43]

4.8. Quality Assurance

The reliability of the study findings was kept guarantee by implementing quality control measures throughout the whole process of research activity. The questionnaire was pre-tested before the actual study began to make sure that the questions were appropriate and understandable, proper training before the start of data collection, and intensive supervision during data collection by the principal investigator.

All laboratory analyses were carried out using standard operating procedures. Site assessment and pre-test were done before the actual data collection and an adjustment was made accordingly. Adequate stool specimen (4 g of formed stool and 10 ml of diarrheic stool) was collected using carefully labeled, dry, leak-proof, and grease-free transparent stool caps. The specimen was kept free from water, soil, and urine contamination. Specimens contaminated with water, urine, and soil was rejected and the study participants were requested to bring another. External Quality Assessment (EQA) sample which sent from Ethiopian public health institution

(EPHI) used as Positive and negative controls to check the quality of the microscope and the staining solutions. Direct stool examination was performing within 30 min to avoid delay. All microscopic findings and questionnaire-based information were encoded and reported appropriately. CD4+ T cell categorization and anemia definition were making based on the WHO criteria.

4.9. Data analysis and interpretation

For data entry and analysis, SPSS version 23 statistical software was used. Overall socio-demographic, clinical characteristics and specific prevalence were calculated using descriptive statistics of the sample through frequencies and cross-tabulations. The odds ratio was used to determine association at 95% confidence intervals (CI) and $P < 0.05$. hence crude odds ratio of parasitic infections with the independent variables was calculated using bivariate logistic regression analysis. Association was established by multivariable logistic regression analysis of all variables with $p < 0.25$ at bivariate logistic regression analysis at 95% confidence intervals (CI) and $P < 0.05$ was considered for statistical significance. Finally, the results were presented in words, charts, graphs, and tables.

4.10. Ethical considerations

Before starting the study, ethical clearance was be obtained from the Departmental Research and Ethics Review Committee of Addis Ababa University College of Health Sciences, School of Sciences, Department of Medical Laboratory Sciences. Then a letter informing St. Peter's Specialized Hospital and permission was obtained from St. Peter's Specialized Hospital to access data from the study population. All eligible subjects were informed as their participation was voluntary and Information obtained at any course of the study was kept confidential. For children, less than 18 the study aim was explained to all mothers/guardians and then Informed assent was obtained from each child's mother/guardian, after explaining the research work, its confidentiality, protection, and anonymity of data. Positive results were made available to clinicians for decision-making as early as available.

4.11. Dissemination of the result

After conducting the research, the results of the study will be presented to Addis Ababa University, College of Health Sciences and Department of Laboratory Sciences. Besides, the results of the study will be presented to St. Peter's Specialized Hospital community and other concerned bodies. The finding of the study will be also presented to the medical scientific community and the manuscript will be submitted to peer-reviewed journals for publication.

4.12 Operational definition

- ✓ **Opportunistic parasites (OP):** parasites that do not ordinarily produce disease in healthy (immune-competent) individuals but do cause illness in individuals with an impaired defense mechanism [42].
- ✓ **Non Opportunistic parasites:** Most of the parasites which live in/on the body of the host do not cause disease in all groups of individuals [41].
- ✓ **Good knowledge**: individuals who answered ≥50% of the knowledge questions.
- ✓ **Poor knowledge**: individuals who answered <50% of the knowledge questions.
- ✓ **Positive attitude**: individuals who answered ≥50% of the attitude questions.
- ✓ **Negative attitude**: individuals who answered <50% of the attitude questions.
- ✓ **Good practice**: individuals who answered ≥50% of the practice questions
- ✓ **Bad practice**: individuals who answered <50% of the practice questions
- ✓ **Acute diarrhea** is defined as a stool with increased water content, volume, or frequency that lasts less than 14 days [53].
- ✓ **Chronic diarrhea:** diarrhea that lasts for more than four weeks or comes and goes regularly over a long period [53].
- ✓ **Clinical stage 1:** HIV/ADIS patient with Asymptomatic, Persistent generalized lymphadenopathy [52].
- ✓ **Clinical stage 2:** HIV/ADIS patient with Moderate unexplained weight loss (<10% of presumed or measured body weight). Recurrent respiratory tract infections (sinusitis, tonsillitis, otitis media, and pharyngitis) and also Herpes zoster Angular cheilitis recurrent oral ulceration Papular pruritic eruption Fungal nail infections Seborrhoeic dermatitis [52].
- ✓ **Clinical stage 3:** HIV/ADIS patient with Unexplained severe weight loss (>10% of presumed or measured body weight) Unexplained chronic diarrhea for longer than 1-

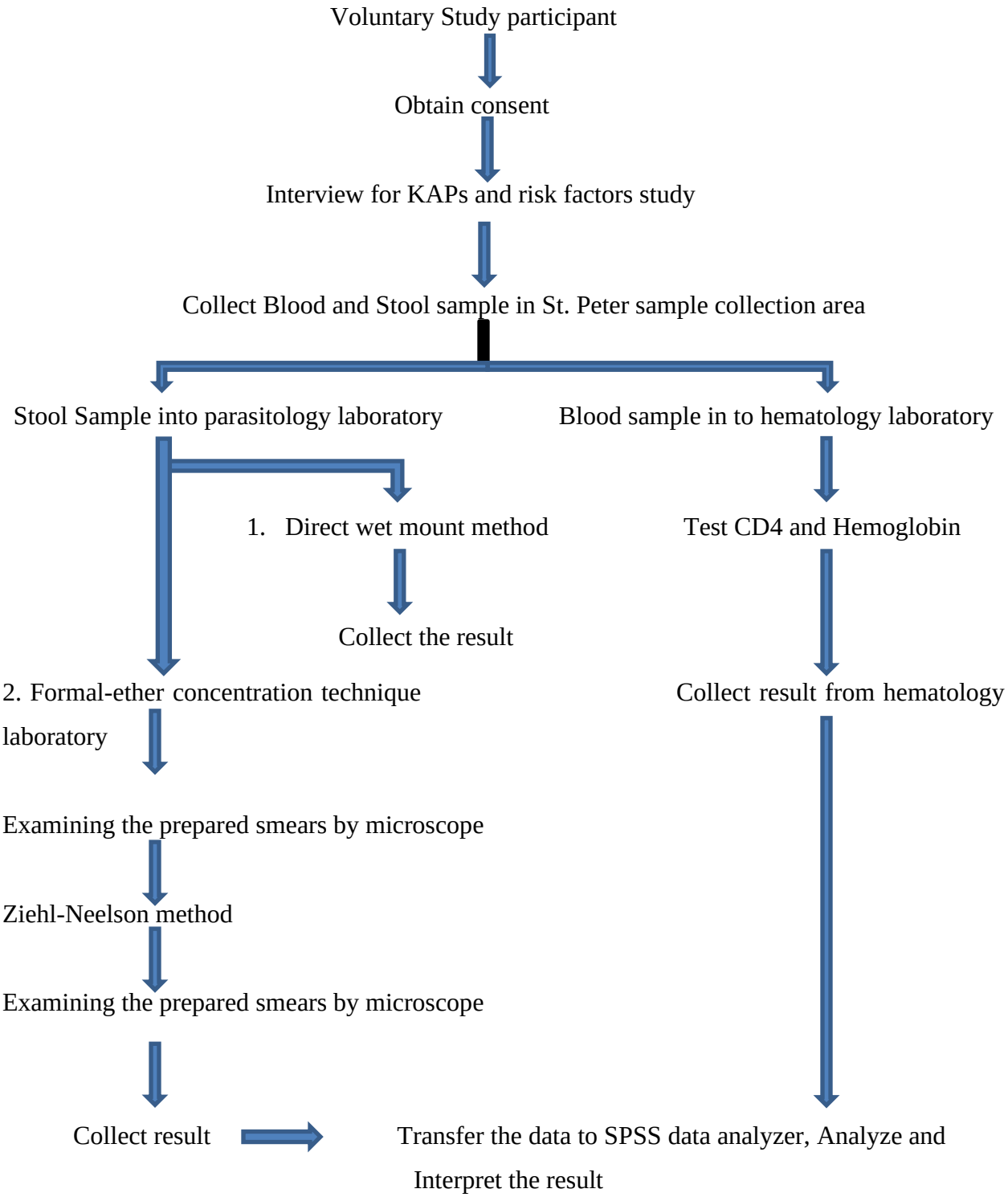
month Unexplained persistent fever (intermittent or constant for longer than 1 month) Persistent oral candidiasis Oral hairy leukoplakia Pulmonary tuberculosis Severe bacterial infections (such as pneumonia, empyema, pyomyositis, bone or joint infection, meningitis, bacteremia) Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis Unexplained anemia (<8 g/dl), neutropenia (<0.5 x 109/l) and/or chronic thrombocytopenia (<50 x 109/l) [52].

- ✓ **Severe anemia** the hemoglobin level less than 7 g/dl
- ✓ **Moderate anemia** hemoglobin level between 7-9.9g/dg
- ✓ **Mild anemia** hemoglobin level between 10-11.9g/dl

5. Workflow

Figure 2 workflow chart St. Peter's TB Specialized hospital Addis Ababa, Ethiopia 2020

The study was conducted as follows:



6. Result

6.1 Socio-demographic characteristics of study subjects

During the study period, 328 were included in the study with a 100 % response rate. Among 328 study participants, 132/328 (40.2 %) were male and 196/328 (59.8 %) were female. The study participants had age ranges between 13 and 72 years with a mean age of 41.80 years (SD=10.8). The majority of the participants were within the age range of 40-49 (114/328; 34.8 %), at primary (1-8) educational level (192/328: 58.8 %), at private employed occupation (164/328; 50.0 %) and married (195/328; 59.7 %). Out of 328 participants, 289 (88.1 %) were urban residents and the rest 34 (11.9 %) were rural dwellers (Table 1).

Variables		**Frequency**	**Percentage (%)**	**Positive for IPs in n (%)**
Age in year	Under 18	2	0.6	
	18-29	28	8.5	12 (14.0)
	30-39	111	33.8	27 (31.4)
	40-49	114	34.8	25 (29.0)
	50 and above	73	22.3	22 (25.6)
Sex	Male	132	40.2	31 (36.0)
	Female	196	59.8	55 (64.0)
Educational status	Illiterate	63	19.2	18 (20.9)
	Primary (1-8)	192	58.5	48 (55.8)
	Secondary (9-10)	43	13.1	15 (17.4)
	Preparatory (11-12)	19	5.8	3 (3.5)
	Higher-level	11	3.4	2 (2.3)
Marital status	Married	195	59.7	46 (53.5)
	Single	94	28.7	31 (36.0)
	Divorced or widowed	39	11.9	9 (10.5)
Current residence	Urban	289	88.1	76 (88.4)
	Rural	39	11.9	10 (11.6)
Occupation	Housewife	102	31.1	25 (29.1)
	Government	44	13.4	9 (10.5)
	Private	164	50.0	46 (53.5)
	Merchant	3	0.9	0
	Student	15	4.5	6 (7.0)

Table 1 Prevalence of intestinal parasites with different socio-demographic characteristics in St. Peter's TB Specialized hospital Addis Ababa, Ethiopia, 2020

6.2 Clinical Characteristics

The overall proportion of subjects with diarrhea was 35 (10.7%) and parasitic infection among patients with acute diarrhea was higher 7/14(50.0%) than patients with chronic diarrhea 2/14 (14.3%), the majority of the participants'CD4 counts 152/328 (46.3%) were between 200- 500 cells/µl. Among CD4 count < 200cells/µl, 200-500 cells/µl, and > 500 cells/µl, respondents, 35(40.7%), 27 (31.7 %) and 24(27.9) % were positive for intestinal parasites respectively. From the total (328) ART initiated HIV positive respondents, stage one, stage two stage three was 95.4%(82/86), 2.3 %(2/86), and 2.3 %(2/86) respectively positive for an intestinal parasite, from a total of 328 participants 17/328 (5.2%) anemic and 13/86(15.1%) had intestinal parasite (IPs). Among subjects with anemia, 3/17(0.9%) had mild anemia, 11/17(3.4%) moderately anemic and 3/17(0.9%) severe anemia and 4/13(30.8%), 7/13 (53.8), and 2/13(15.4%) had intestinal parasites respectively.

Table 2 Laboratory profile of ART patients in relation to the parasite positivity and Clinical Characteristics in St. Peter's TB specialized hospital Addis Ababa, Ethiopia, 2020

Variables		**Participant Frequency& (%)**	**Positive for IP Frequency& %**
Abdominal pain	Present	56 (17.1)	19(22.1)
	Absent	272 (83.2)	67(77.9)
Diarrhea	Present	35 (10.7)	14(16.3)
	Absent	293 (89.3)	72(83.7)
Diarrhea condition	Chronic (4 weeks)	9 (2.7)	2(14.3)
	Acute (1 weeks)	26 (11.0)	7(50.0)
Stool characteristics	Formed	145 (44.2)	28(32.6)
	Soft	136 (41.5)	28(32.6)
	Loose	10 (3.0)	4(11.6)
	Diarrhea	35 (10.7)	24(27.9)
Current WHO HIV stage	Stage 1	323 (97.9)	82(95.4)
	Stage 2	5 (4.5)	2(2.3)
	Stage 3	2 (0.6)	2(2.3)
Cd4 category	Cd4 <200	62 (18.9)	35(40.7)
	Cd4 200-500	152 (46.3)	27(31.4)
	Cd4 >500	114 (34.8)	24(27.9)
Anemia	Yes	29 (8.8)	13(15.1)
	No	299 (91.2)	73(84.9)

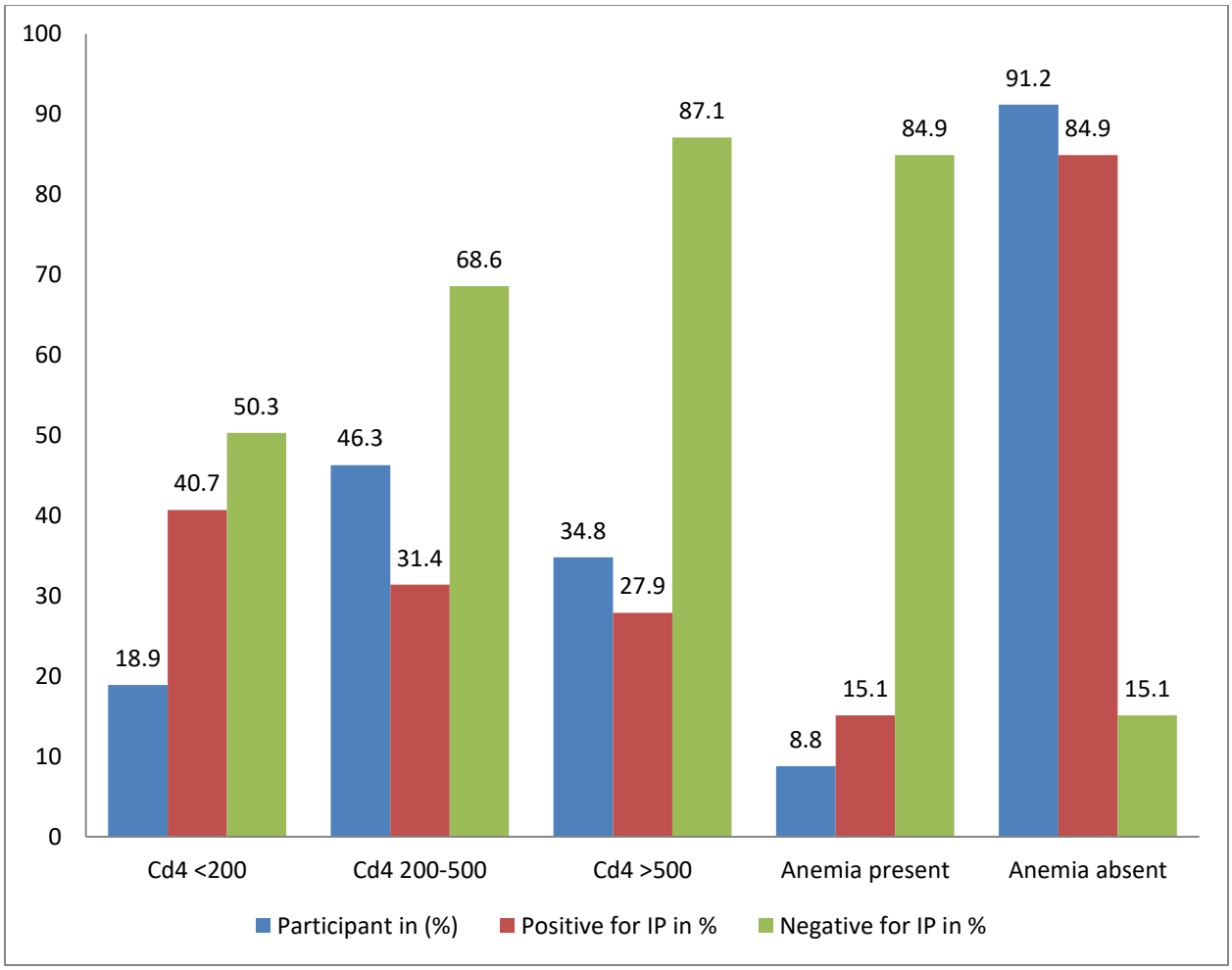


Figure 3: Intestinal parasite in relation to Anemia and CD4 count study participant in St. Peter's TB Specialized hospital Addis Ababa, Ethiopia May 2020.

6.3 The Distribution of Parasite Species

The overall prevalence of intestinal parasites was 26.2% (86/328), specifically *Entamoeba histolytica/dispar* 11.3 % (37/328), *Giardia intestinalis* 7.0% (23/328), and Taenia species 8.0% (8/328) was seized the majority among the study participants. From all, 88.4% (76/86) were infected by single parasites,(*Entamoeba histolytica/dispar* 48.7 % (37/76),*Giardia intestinalis*30.3% (23/76), Taenia species 10.5% (8/76), Ascaris lumbricoides 3.9% (3/76), *Strongyloides stercoralis*5.3% (4/76), and *Hymenolepis nana* 1.3% (1/76)), figure 4

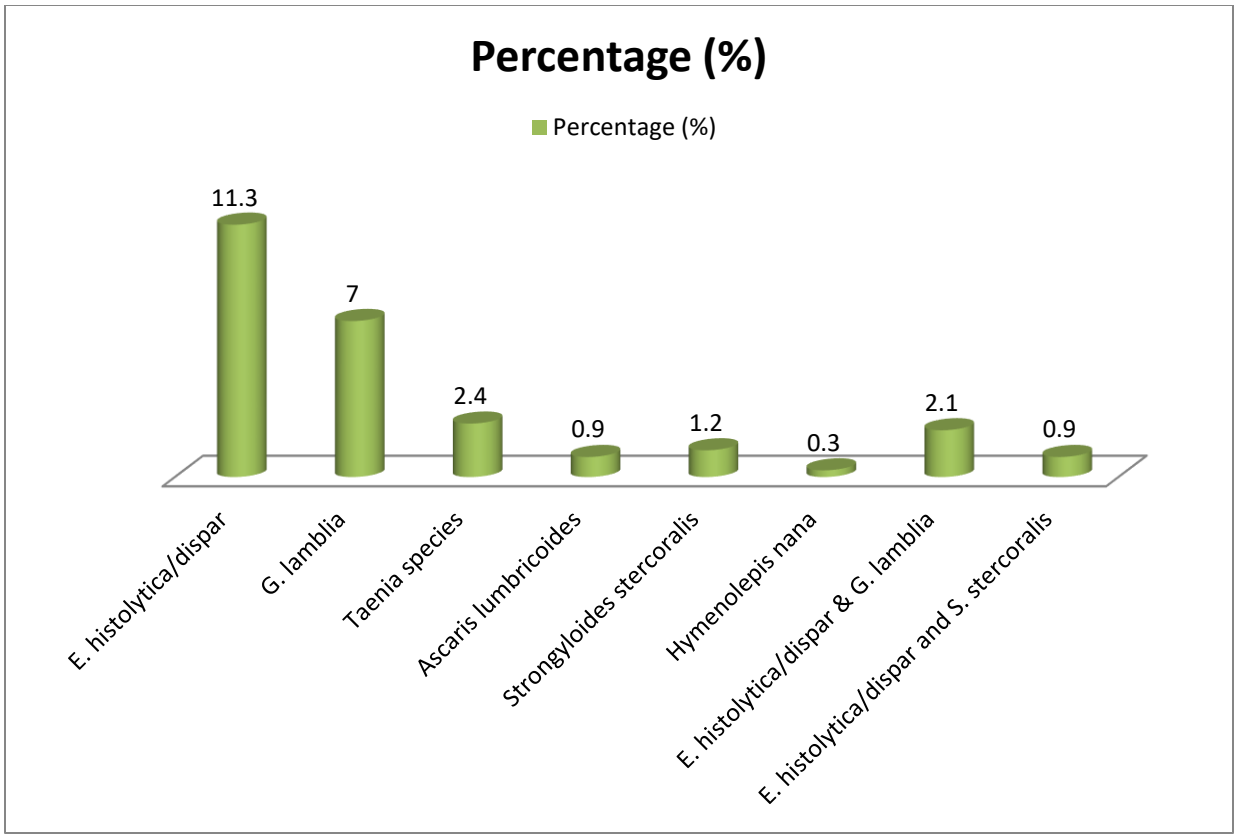


Figure 4: Distribution of intestinal parasites in the study participants in St. Peter's TB Specialized hospital Addis Ababa, EthiopiaMay2020.

6.4 Knowledge, attitude, and practice of study participants towards intestinal parasitosis.

6.4.1. Knowledge about intestinal parasites

All study participants were interviewed for their knowledge, attitude, and practices (KAPs) towards intestinal parasitosis and of the 89.9% (295/328) had good knowledge about the intestinal parasites, way of transmission, prevention of intestinal parasitosis, and treatment of intestinal and remaining 10.1 % (33/328) had poor knowledge. Among the total, 328 (81.1%) of participants had information on what intestinal parasites are. Moreover, 301 (91.8%) of participants know that transmission of the intestinal parasite by eating contaminated food, 296(91.2%) drinking contaminated water, 305(93.0%) eating raw meat, 286(87.2%) Not cut nails regularly, 280 (85.3%) walking barefooted, 315(96.0%) lack of hygiene. Also, the majority of participants had known about sign and symptoms of the intestinal parasite (Abdominal

pain, Diarrhea, Vomiting, Loss of appetite, and Weight loss 92.3%, 97.5%, 89.9%, 88.4%, and 76.2% respectively, Knowledge about the degree of the method of prevention of Treatment with specific medicines 316(96.3%), Use of toilets 295 (89.9%), Provision of safe tap water 321 (97.8%), Avoid defecating in open filed 326 (99.3%) and Personal hygiene 319(97.2%).

Knowledge variables	**Yes % n**	**No % n**	**Total**
Do you know what intestinal parasites are?	81.1(266)	18.9(62)	328
How did you know about the intestinal parasite?			
Friends	10.4(34)		
Health center	54.6(179)		
Radio	14.0(46)		
Other	3.7(12.0)		
How intestinal parasites are transmitted?			
Eating contaminated food	301(91.8)	27 (8.2)	328
Eating raw meat	296(91.2)	32 (8.8)	328
Drinking contaminated water	305(93.0)	23 (7.0)	328
Not cut nails regularly	286(87.2)	43 (12.8)	328
Walking barefooted	280 (85.3)	48 (14.7)	328
Lack of hygiene	315 (96.0)	13 (4.0)	328
What are the sign and symptoms of intestinal parasites?			328
Abdominal pain	303 (92.3)	25 (7.7)	328
Diarrhea	320 (97.5)	8 (2.5)	328
Vomiting	295 (89.9)	33 (10.1)	328
Loss of appetite	290 (88.4)	38 (11.6)	328
Weight loss	250 (76.2)	78 (23.8)	328
Where do you prefer to seek treatment for IP?			
Traditional healer	10 (3.0)	318 (97.0)	328
Health center	320 (97.5)	9 (2.5)	328
Direct to pharmacy	296 (90.2)	32 (9.8)	328
How intestinal parasites are prevented?			
Treatment with specific medicines	316 (96.3)	12 (3.7)	328
Use of toilets	295 (89.9)	33 (10.1)	328
Provision of safe tap water	321 (97.8)	7 (2.2)	328
Avoid defecating in open filed	326 (99.3)	2 (0.7)	328
Personal hygiene	319 (97.2)	9 (2.8)	328
Overall Knowledge			
Good knowledge			**295(89.9%)**
Poor knowledge			**33 (10.1%)**
Total			**328 (100)**

Table 3: Knowledge about intestinal parasitosis, symptoms, transmission, and prevention among the study participants in St. Peter's TB Specialized hospital Addis Ababa, Ethiopia, 2020

6.4.2. Attitude about intestinal parasites

Of a total of 328 participants, the majority 290 (88.4 %) are a positive attitude about transmission, treatment and prevention, and control of intestinal parasite but some participants 38 (11.6%) are a negative attitude, The majority of the participants 289(88.1%) believed that intestinal parasite is a serious disease, from this 60 (69.8%) positive for intestinal parasite and 39 (11.9%) they don't believe intestinal parasite is a serious disease from these participants 26 (30.2%) are positive for an intestinal parasite, and also 187 (57.0%) participants they don't believe taking traditional medication is good to treat intestinal parasites.

Variable	**Yes n (%),**	**frequency & % of IP**	**No n (%),**	**frequency & % of IP**
Do you think intestinal parasitosis is a serious disease?	289 (88.1)	60 (69.8)	39 (11.9)	26 (30.2)
Do you think taking an education against intestinal parasitosis is important?	252 (76.8)	73 (84.9)	76 (23.2)	13 (15.1)
Do you think going to a health facility is important when you feel abdominal discomfort?	141 (43.0)	68 (79.0)	187 (57.0)	18 (21.0)
Do you think taking traditional medication is good to treat intestinal parasitosis?	173 (52.7)	24 (27.9)	155 (47.3)	51 (72.1)
Do you think playing in the soil can cause intestinal parasitosis?	272 (83.2)	48 (55.8)	56 (17.1)	38 (44.2)
Do you think eating raw vegetables can cause intestinal parasitosis?	306 (93.3)	71 (82.5)	22 (6.7)	15 (17.5)
Do you think health education can reduce intestinal parasite prevalence?	326 (99.4)	84 (97.7)	2 (0.6)	2 (2.3)
Do you think hand washes with soap and water can preventive for intestinal parasites?	172 (52.4)	83 (96.5)	156 (47.6)	3 (3.5)
Overall Attitude				
positive attitude				**290 (88.4)**
Negative attitude				**38 (11.6)**
Total				**328 (100)**

Table 4: Attitude of study participants towards intestinal parasitosis in St. Peter's TB Specialized hospital Addis Ababa, Ethiopia, 2020

6.4.3 Practices about intestinal parasites.

The overall practice of participants towards transmition, treatment, and prevention, and control of intestinal parasites are illustrated in Table 5. Of the 10 questions that were asked to measure practice towards intestinal parasite, from this 277/328(77.7 %) is a good practice, about using the toilet (92.7 %), wash your hand after the toilet (86.3 %), cut or clean fingernail (87.2 %), go to

the health center when you feel abdominal discomfort (87.5 %), take medication for intestinal parasite (75.9 %), on the other hand, 73/328 (22.3 %) participant are bad practice to on transmit ion, treatment and prevention and control on the intestinal parasite.

Variable	**Yes n (%)**	**Frequency & % of IP**	**No n (%)**	**Frequency & % of IP**
Do you eat row and/or unwashed fruit?	155 (47.3)	40 (25.8)	172 (52.4)	46 (26.7)
Do you eat raw meat?	111 (35.7)	32 (28.8)	217 (64.3)	54 (24.8)
A water source for drinking?	**Frequency&%**		**Frequency & % of IP**	
Pipe water	198(61.7)		49 (24.7)	
River water	23 (7.0)		5 (2.7)	
Well Unprotect water	71 (21.6)		27 (38.0)	
Bottle water	35 (10.7)		5 (14.8)	
	Yes n (%)	**Frequency & % of IP**	**No n (%)**	**Frequency & % of IP**
Do you use toilet	304 (92.7)	80 (26.3)	24 (7.3)	6 (25.0)
Do defecate on open field	60 (18.3)	18 (30.0)	268 (81.7)	68 (25.3)
Do you wash your hand after toilet/defecation	283 (86.3)	68 (24.0)	44 (13.4)	18 (40.9)
Do you cut or clean finger nail	286 (87.2)	60 (20.9)	42 (12.8)	26 (61.9)
Do you go to the health center when you feel abdominal discomfort	287 (87.5)	74 (25.7)	41 (12.5)	12 (29.2)
Do you take medication for intestinal parasite	249 (75.9)	63 (25.3)	79 (24.1)	23 (29.1)
Do you wear a closed shoe	107 (32.6)		221 (67.4)	
Overall practices				
Good practices				**277 (77.7)**
Bad practices				**73 (22.3)**
Total				**328 (100)**

Table 5: practice of the study participants towards intestinal parasitosis in St. Peter's TB Specialized hospital Addis Ababa, Ethiopia, 2020

6.5 Factors associated with an intestinal parasite

The prevalence of intestinal parasite was higher in females 64.0% (55/86) than males 36.0% and found among the age group of 30-39, followed by 40-49, 50 and above, 18-29 and under 18 are 27(31.4%), 25(29.0%), 22(25.6%), 11(12.8%) 1(1.2%) respectively. Intestinal parasitic infection was significantly associated with CD4 count < 200 AOR [4.736 CI(2.338-9.594); P-value <

0.001], not Cutting or cleaning fingernail AOR[3.438CI(1.381-8.560); P-value 0.008, having Diarrhea AOR[4.810CI (1.510-15.322);P-value 0.010, having moderate Anemia AOR[3.271 CI(1.069-10.010); P-value 0.038, poor Knowledge AOR[9.323 CI(3.902-22.279); P-value < 0.001 and bad practices AOR[5.730 CI(2.767-11.868); P-value < 0.001,The remaining nine (current residence, sex, eat row and/or unwashed fruit, eat raw meat, Defecate on an open field, Abdominal pain, Diarrhea condition, and Anemia status were failed to associate significantly with intestinal parasites in the multivariate model (Table 7 and 8).

Variables		**No. of study participant**	**No. of individuals with IPs**		**COR (95% CI), p-value**	**AOR (95%CI), p-value**
			positive	**Negative**		
Age in year	Under 18	2		2	1	
	18-29	28	11	17	2.31(0.139-38.15)0.558	
	30-39	111	27	84	1.50(0.605-3.720)0.382	
	40-49	114	25	89	0.74(0.384-1.444)0.384	
	50 and above	73	22	51	0.65(0.334-1.271)0.208	
Sex	Male	132	31	101	1	
	Female	196	55	141	0.78(0.473-1.309)0.356	
Marital status	Married	195	46	149	1.54(0.176-13.55)0.695	
	Single	94	37	63	2.46(0.275-21.97)0.420	
	Divorced	33	8	25	1.60(0.162-15.79)0.687	
	Widowed	6	1	5	1	
Current residence	Urban	289	76	213	1	
	Rural	39	10	29	0.966(0.450-2.077)0.930	0.942(0.402-2.211)0.891
Abdominal pain	Present	56	19	38	1.522(0.222-2.819),0.181	
	Absent	271	67	204	1	
Stool consistency	Formed	145	28	117	1	1
	Soft	137	28	109	1.073(0.598-1.927),0.812	
	Loose	11	5	6	3.482(0.991-12.232),**0.052**	0.80(0.030-0.212)**<0.001**
	Diarrhea	35	25	10	10.446(4.504-24.230),**0.000**	4.810(1.510-15.322),**0.010**
Diarrhea condition	No Diarrhea	310	77	233	1	
	Acute (1 weeks)	13	7	6	2.017(0.331-12.298),0.447	

	Chronic (4 weeks)	5	2	3	3.530(1.151-10.825),**0.027**	2.248(0.415-12.184),0.347
Cd4 category	Cd4 <200	62	35	27	4.861(2.447-9.541),**<0.001**	4.736(2.338-9.594),**<0.001**
	Cd4 200-500	152	27	125	0.810(0.439-1.495),0.500	
	Cd4 >500	114	24	90	1	1
Anemia status	Sever (<7)	3	1	2	6.423(0.574-71.880),0.131	
	Mild (10-10.9)	9	3	6	4.586(1.684-12.494),**0.003**	
	Moderate (7-9.9)	17	10	7	1.606(0.392-6.585),0.511	3.271(1.069-10.010),**0.038**
	Normal	299	71	228	1	

(**Note**: COR: crude odds ratio; CI: confidence interval; AOR: adjusted odds ratio; *; Statistical significant association)

Table 6: Prevalence of intestinal parasites and its association with socio-demographic and clinical factors in St. Peter's TB Specialized hospital Addis Ababa, Ethiopia, 2020

Variables		**No. of study participant**	**No. of individuals with IPs**		**COR (95% CI), p-value**	**AOR (95%CI), p-value**
			positive	**Negative**		
Eat row and/or unwashed fruit	Yes	155	40	115	0.960(0.586-1.572),0.872	
	No	172	46	127	1	
Eat raw meat	Yes	117	31	86	1.028(0.618-1.720),0.914	
	No	211	54	156	1	
Use toilet	Yes	304	80	224	1	
	No	24	6	18	1.071(0.411-2.794),0.888	
Defecate on open field	Yes	61	18	43	1	
	No	268	68	199	1.225(0.662-2.266),0.518	
wash hand after toilet	Yes	283	68	216	1	
	No	44	18	26	2.199(1.137-4.254)**0.019**	1.443(0.527-3.953),0.476
Cut or clean finger nail	Yes	286	60	227	1	1
	No	42	26	15	6.558(3.269-13.157),**<0.001**	3.438(1.381-8.560),**0.008**

Knowledge	Good knowledge	295	66	229	1	1
	Poor knowledge	33	20	13	5.338(2.521-11.301),**<0.001**	9.32(3.902-22.27),**<0.001**
Attitude	Positive attitude	290	68	222	1	
	Negative attitude	38	18	20	2.938(1.470-5.872),**0.002**	
practices	Good practices	252	43	213	1	1
	Bad practices	76	43	30	7.470(4.329-13.167),**<0.001**	5.73(2.767-11.86),**<0.001**

(**Note**: COR: crude odds ratio; CI: confidence interval; *; Statistical significant association)

Table 7: Prevalence of intestinal parasites and its association KAP in St. Peter's TB Specialized hospital Addis Ababa, Ethiopia, 2020

7. Discussion

The aim of this study was to assess the prevalence of intestinal parasite and their associated factors and the participant Knowledge, Attitude, and Practice on Intestinal parasites, the overall prevalence rate of intestinal parasites was 26.2%. This prevalence was higher than the study conducted at Benin City Edo State Nigeria (18%), Kombolcha North Central Ethiopia (13.9%), Debre Markos Referral Hospital Northwest Ethiopia (24.2%), Dessie hospital ART clinic Northeast Ethiopia (17.6%), and Hospital Kano Nigeria (11%) [27, 34, 44-46].

This study is lower than the prevalence of intestinal parasites reported from Felegehiwot Referral Hospital Bahir Dar Ethiopia (36%), HiwotFana Specialized University Hospital Eastern Ethiopia (33.7%), Arba Minch Hospital in Southern Ethiopia (28.18%), Butajira Ethiopia (35.9%), and Addis Ababa Ethiopia (35.8%) [3, 32, 33, 36,38], This difference might be due to variations reason such as sociodemographic characteristics, sample size, study period, lifestyles, and study participants might have good knowledge, attitude and practice for the prevention and control of intestinal parasites. On the other hand, this study result is almost similar to a report from St. Mary Aksum general hospital Tigray Ethiopia (26.4%), Debretabor General Hospital Northern Ethiopia (25.3%), and Maputo, Mozambique (26.4) [35, 37, 46].

In the present study, the prevalence of intestinal parasites in Urban 76(88.4%) was higher than in Rural 10 (11.6 %). However, there was no statistically significant difference (P-value >0.05) in the prevalence of intestinal parasites infections and Current residence individual AOR [0.942(0.402-2.211), p-value 00.897], the finding of this study disagreed with findings of previous studies conducted in Axum, St. Mary Hospital where the prevalence of intestinal infection was higher in Rural than Urban [35], This difference might be because socio-demographics and socioeconomic status such as many people share a single toilet and single source water.

In the present study, the prevalence of intestinal parasites in females 55(64%) was higher than males 31 (36%). However, there was no statistically significant difference (P-value > 0.05) in gender and the prevalence of intestinal parasite infections. This finding agreed with the findings of previous studies conducted in Tigray, Ethiopia, and India where the prevalence of intestinal parasitic infection was higher among females than males [35, 4]. On the other hand opposing, the finding of this study differed from the finding of a previous study conducted in Addis Ababa,

Ethiopia where the prevalence of intestinal infection was higher in male than female individuals but there was no statistically significant difference (P-value > 0.05) in the prevalence of intestinal parasites infections between genders [42]. This difference might be due to the lifestyle, occupational behavior, and also the socio-economic status of the participants.

Most of the socio-demographic features in this study were not statistically significant as predictors of the manifestations of intestinal parasite infection among individuals living with HIV/ AIDS. The socio-demographic variables, for example, age, marital status, work status, and level of education were not implicated as determinants of the occurrence of enteric protozoal infection among the study subjects.

The intestinal parasite was more frequent in a patient that had diarrhea than other types of stool samples, and diarrhea was more common in participants with CD4 T cell counts of > 200 cells/μL. This is similar to the studies done in Hawassa, Hiwot Fana Ethiopia, and Axum, St. Mary Hospital [32, 35].

In this study lower prevalence of intestinal helminths, 16(21.3%) than intestinal protozoan parasites 70 (78.7%) was observed. This was in line with studies conducted in Dessie hospital [43]. The laboratory method we used and reduced egg excretion may cause a low detection rate of these parasites in stool sample resulting in a lower number of intestinal helminths.

Among protozoan intestinal parasites Entamoeba histolytica/dispar was the most prevalent 11.3% and this result lower than findings from Eastern Ethiopia (13.5%), Tigray, Ethiopia (18.6%),[3,35] but higher than Butajira Ethiopia (7.2%) Kombolcha Health Centre Ethiopia (7.2%) and Nigeria (5.7%) [33, 34, 46]. The overall prevalence of Gardia lamblia was (7.0%) it was lower than Ilorin, Nigeria (3.7%),[30] and higher than Arba Minch Hospital in Southern Ethiopia (1.8%) [36].

The higher and lower existence of intestinal protozoan parasite may be that the participant's hygiene and sanitation practices such as wash hand after toilet, Cut or clean fingernail and also knowledge, attitude and practice about transmission, prevention and control method of intestinal parasites was clearly related, This study showed that patients who did not cut or clean fingernail were almost 3.4 times more likely [AOR 3.438CI(1.381-8.560),0.008] to have parasitic infection than those who had a habit of hand cut or clean fingernail, at the same time those with the

participant did not good knowledge almost 9.3 times more likely AOR[9.323(3.902-22.279), p-value <0.001] to have the intestinal parasitic infection than the participant have good knowledge Likewise, those with the HIV patient did not good the practice was 5.7 times [AOR5.730CI(2.767-11.868), p-value <0.001 to have the intestinal parasitic infection than those who have a good practice on the way of transmission, prevention, and control of intestinal parasite[31].

A participant who had diarrhea was 4.8 times more likely AOR 4.810CI(1.510-15.322),0.010to have had an intestinal parasitic infection than those who had formed stool and the result is similar to studies done in St. Mary Aksum general hospital, Tigray, Ethiopia [24, 35].

In this study, there is no finding cryptosporidiosis and absence of other opportunistic intestinal parasites among patients with a CD4 cell count of less than 200 cells/µl The absence of opportunistic intestinal parasites in this study is similar to the prevalence reported in other studies among HIV positive patients in Lagos, Enugu in Nigeria the first possible reason for this difference could be that majority of HIV positive patients did not have contact with pets or other animals which are potential sources of transmission of oocyst of coccidian parasites. Another reason could be that the study participants are enrolled in the ART program, managed and monitored by trained ART physicians, and placed on Cotrimoxazole therapy according to the national guidelines [46, 6,] but the finding show CD4 level < 200 were 4.7 times more likely AOR [4.736(2.338-9.594), 0.000] affected by other non-opportunistic intestinal parasites than ART follow up patient CD4 level > 500 which is in line with studies conducted on Northeast Ethiopia, Hawassa Teaching and Referral Hospital Ethiopia and Cameroon [19, 45].

Parasite infection is the cause of known anemia in both HIV and non-HIV patients In this study the rate of anemia was 8.8% it had an association with intestinal parasite especially in Moderate (7-9.9) types of anemia AOR [3.271CI (1.069-10.010),0.038] which is lower than the previous study in HIV patients in Benin City and Edo State Nigeria the prevalence of anemia was 93.3% while 18% had parasitic infections [27] this difference because of different reasons it includes neoplasm, infection, antibody to antiviral agent, dietary deficiencies, medication, bone marrow suppression especially the erythroid lines and blood loss are cause known result anemia [49].

8. Strength and limitation of the study

8.1. Strength

This study has assessed the knowledge, attitude, and practice (KAPs) of participants and we used different techniques (wet mount, formol ether concentration technique, and modified ZN technique) to identify the intestinal parasite.

8.2. Limitation

- We did not include Highly Active Antiretroviral Therapy (HHRT) naive patients.
- A hospital-based cross-sectional study certainly introduces selection bias making generalization impossible.
- We used only single stool samples were collected from each participant.
- In this study we not used other special technique such as Kato-Katz to identify intestinal helminth it may increase the current result of intestinal helminth, molecular techniques, immune-fluorescent techniques which is sensitive for parasites.

9. Conclusion

Intestinal parasitic infections are still common health problems among HIV/ADIS patients in the study area, so the health professionals give to attention parasitological examinations in the routine treatment of HIV/AIDS patients. Most of the participants in the study area had a positive attitude and good knowledge about intestinal parasite but the some of the participants had not good practice so the health educator or health worker gives education on this three parts (knowledge, attitude and practice), but more focus and follow up on the practice of HIV/AIDS patients on transmission, prevention, and control mechanisms of intestinal parasitosis.

10. Recommendations

Based on the finding of the study the following three points were recommended.

1. Health workers give strong health education about transmission, prevention, and control methods of intestinal parasitosis.
2. Stool examination should be routinely performed in the follow-up of patients.
3. Also, further large-scale study by using different diagnostic techniques.

References

1. Asires, A., Wubie, M., and Reta, A., Prevalence and Associated Factors of Intestinal Parasitic Infections among Food Handlers at Prison, East and West Gojjam, Ethiopia. Advances in Medicine, 2019,1-8.
2. Kumma WP, Meskele W, Admasie A. Prevalence of intestinal parasitic infections and associated factors among food handlers in Wolaita Sodo University students caterings, Wolaita Sodo, Southern Ethiopia: A cross-sectional study. Frontiers in public health. 2019 Jun 4;7:140.
3. Teklemariam Z, Abate D, Mitiku H, Dessie Y. Prevalence of intestinal parasitic infection among HIV positive persons who are naive and on antiretroviral treatment in HiwotFana Specialized University hospital. Eastern Ethiopia ISRN AIDS. 2013.
4. Kaniyarakkal V, Mundangalam N, Moorkoth AP, Mathew S. Intestinal parasite profile in the stool of HIV positive patients in relation to immune status and comparison of various diagnostic techniques with special reference to cryptosporidium at a tertiary Care Hospital in South India. Advances in medicine, 2016.
5. Barcelos N, Dias R, Menezes Filho H, Rodrigues R. Opportunistic and non-opportunistic intestinal parasites in HIV/AIDS patients in relation to their clinical and epidemiological status in a specialized medical service in Goiás, Brazil. Revista do Instituto de Medicina Tropical de São Paulo. 2018;60.
6. Nwokediuko S, Bojuwoye B, Onyenekwe B, Apparent rarity of cryptosporidiosis in human immunodeficiency virus (HIV)-related diarrhea in Enugu, South-Eastern, Nigeria. Niger Postgrad Med J.2002;70-73
7. Curry A, Turner AJ, Lucas S. Opportunistic protozoan infections in human immunodeficiency virus disease: review highlighting diagnostic and therapeutic aspects. J Clin Pathol. 1991; 44(3):182.
8. Faye B, Tine RC, NdiayeJL,et al. Impact of intestinal parasites on intensity of HIV infection in senegal. J AntivirAntiretrovir.2010;11-12.
9. Getaneh A, Medhin G, Shimelis T. Cryptosporidium and Strongyloidesstercoralis infections among people with and without HIV infection and efficiency of diagnostic methods for StrongyloidesinYirgalem Hospital, southern Ethiopia. BMC research notes. 2010;3(1):90.

10. Mugisha J, Shafer L, Paal D, Van L, Mayanja B, Eotu H, et al. Anaemia in a rural Ugandan HIV cohort: prevalence at enrolment, incidence, diagnosis, and associated factors. Tropical Med Int Health. 2008; 788–94.
11. McKenna M, McAtee S, Bryan P, Jeun R, Ward T, Kraus J, et al. Human intestinal parasite burden and poor sanitation in rural Alabama. The American journal of tropical medicine and hygiene. 2017 Nov 8;97(5):1623-8.
12. CDC. Laboratory Identification of Parasitic Diseases of Public Health Concern.2013
13. WHO, Prevention and Control of Intestinal Parasitic Infections, WHO Technical Report 1998; 749: 1-86
14. Awole M, Gebre-Selassie S, Kassa T, and Kibru G, Prevalence of intestinal parasites in adult HIV infected patients in South West Ethiopia, Ethiopian Journal of Health Development 2003;17(1):71-78
15. FMOH/FHAPCO. Guidelines for implementation of the antiretroviral therapy program in Ethiopia. Federal HIV/AIDS Prevention and Control Office: Federal Ministry of Health, July 2007
16. Adedayo O and Nasilro R, Intestinal parasitoses, J National Med Association 2004, 96:93-96
17. Hotez PJ, Fenwick A, Savioli L, Molyneux DH. Rescuing the bottom billion through control of neglected tropical diseases. Lancet. 2009;373:1570–5.
18. Ngui R, Ishak S, Chuen CS, Mahmud R, Lim YAL. Prevalence and Risk Factors of Intestinal Parasitism in Rural and Remote West Malaysia. PLoSNegl Trop Dis. 2011;5(3), e974.
19. Nsagha D, Njunda A, Assob N, Ayima C, Tanue E, Kwenti T. Intestinal parasitic infections in relation to CD4+ T cell counts and diarrhea in HIV/AIDS patients with or without antiretroviral therapy in Cameroon. BMC infectious diseases. 2015 Dec;16(1):9.
20. Habtamu B and Kloos H: Intestinal parasitism: In Epidemiology and Ecology of Health and Diseases in Ethiopia, 1st edition, Shama books; 2006:519-538
21. Shah U, Purohit B, Chandralekha D, Mapara M, Co-infection with cryptosporidium, isospora and S. stercoralis in a patient with AIDS- a case report, Ind J Med Micr 2005; 21:137–138

22. Alemu AS, Baraki AG, Alemayehu M, Yenit MK. The prevalence of intestinal parasite infection and associated factors among food handlers in eating and drinking establishments in Chagni Town, Northwest Ethiopia. BMC research notes. 2019 Dec;12(1):302.
23. Wang ZD, Liu Q, Liu HH, Li S, Zhang L, Zhao YK, Zhu XQ. Prevalence of Cryptosporidium, microsporidia, and Isospora infection in HIV-infected people: a global systematic review and meta-analysis. Parasites & vectors. 2018 Dec 1;11(1):28.
24. Pullan RL, Smith JL, Jasrasaria R, Brooker SJ. Global numbers of infection and disease burden of soil-transmitted helminth infections in 2010. *Parasites & vectors*. 2014; 7(1):1.
25. Vidal JE, Hernandez AV, De Oliveira AC, Dauar RF, Barbosa Jr SP, Focaccia R. Cerebral toxoplasmosis in HIV-positive patients in Brazil: clinical features and predictors of treatment response in the HAART era. AIDS Patient Care &Stds. 2005 Oct 1;19(10):626-34.
26. Pavie J, Menotti J, Porcher R, Donay JL, Gallien S, Sarfati C, Derouin F, Molina JM. Prevalence of opportunistic intestinal parasitic infections among HIV-infected patients with low CD4 cell counts in France in the combination antiretroviral therapy era. International Journal of Infectious Diseases. 2012 Sep 1;16(9):e677-9.
27. Akinbo FO, Okaka CE, Omoregie R. Prevalence of intestinal parasites in relation to CD4 counts and anemia among HIV-infected patients in Benin City, Edo State, Nigeria. Tanzania journal of health research. 2011;13(1):8-13.
28. Wumba R, Longo-Mbenza B, Menotti J, Mandina M, Kintoki F, Situakibanza NH, Kakicha MK, Zanga J, Mbanzulu-Makola K, Nseka T, Mukendi JP. Epidemiology, clinical, immune, and molecular profiles of microsporidiosis and cryptosporidiosis among HIV/AIDS patients. International journal of general medicine. 2012;5:603.
29. Mahmud MA, Bezabih AM, Gebru RB. Risk factors for intestinal parasitosis among antiretroviral-treated HIV/AIDS patients in Ethiopia. International Journal of STD & AIDS. 2014 Oct;25(11):778-84.
30. Obateru OA, Bojuwoye BJ, Olokoba AB, Fadeyi A, Fowotade A, Olokoba LB. Prevalence of intestinal parasites in newly diagnosed HIV/AIDS patients in Ilorin, Nigeria. Alexandria journal of medicine. 2017 Jun 1;53(2):111-6.

31. Ahmed, K.S., Siraj, N.M., Fitsumberhan, H., Isaac, S., Yohannes, S., Eman .et al Knowledge Attitude and Practice (KAP) Assessment of Intestinal Parasitic Infection among School Children in Asmara, Eritrea. Health, January 12, 2017, 57-68.
32. Kiros H, Nibret E, Munshea A, Kerisew B, Adal M. Prevalence of intestinal protozoan infections among individuals living with HIV/AIDS at Felegehiwot Referral Hospital, Bahir Dar, Ethiopia. International Journal of infectious diseases. 2015 Jun 1;35:80-6.
33. Gedle D, Kumera G, Eshete T, Ketema K, Adugna H, Feyera F. Intestinal parasitic infections and its association with undernutrition and CD4 T cell levels among HIV/AIDS patients on HAART in Butajira, Ethiopia. Journal of Health, Population and Nutrition. 2017 Dec;36(1):15.
34. Gebretsadik D, Haileslasie H, Feleke DG. Intestinal parasitosis among HIV/AIDS patients who are on antiretroviral therapy in Kombolcha, North Central, Ethiopia: a cross-sectional study. BMC research notes. 2018 Dec;11(1):613.
35. Gebrewahid T, Gebrekirstos G, Teweldemedhin M, Gebreyesus H, Awala A, Tadla K. Intestinal parasitosis in relation to CD4 count and anemia among ART initiated patients in St. Mary Aksum general hospital, Tigray, Ethiopia. BMC infectious diseases. 2019 Dec;19(1):350.
36. Alemu G, Alelign D, Abossie A. Prevalence of opportunistic intestinal parasites and associated factors among HIV patients while receiving ART at Arba Minch Hospital in southern Ethiopia: a cross-sectional study. Ethiopian Journal of health sciences. 2018;28(2):147-56.
37. Kindie Y, Bekele S. Prevalence and Risk Factors for Intestinal Parasite Infections in HIV/AIDS Patients with Anti-Retroviral Treatment in South West Ethiopia. Journal of Tropical Diseases. 2016;4(3).
38. Taye B, Desta K, Ejigu S, Dori G., the magnitude and risk factors of intestinal parasitic infection in relation to human Immunodeficiency virus infection and immune status, at ALERT Hospital, Addis Ababa, Ethiopia. Parasitology international. 2014 Jun 1;63(3):550-6.
39. Sarkar, Dr. Land use and Land Cover Change Detection of GanakbariMauja in SavarUpozila.journal human social scince 2016;16

40. Gullible Sub City –AACA. Addis Ababa.gov.et 2019.Available fromhttp://www.addisababa.gov.et/de/web/guest/gullele-sub-city
41. Koduri PR, Parekh S. Zidovudine-related anemia with reticulocytosis. Annals of hematology. 2003 Mar 1;82(3):184-5.
42. Cheesbrough M, District Laboratory Practices in Tropical Countries, Part 1, United Kingdom, Cambridge University Press 2009
43. Bdbiosiences.com2019 Available from http://www.bdbiosciences.com/ds/europe/tds/23-12808.
44. Missaye A, Dagnew M, Alemu A, Alemu A. Prevalence of intestinal parasites and associated risk factors among HIV/AIDS patients with pre-ART and on-ART attending Dessie hospital ART clinic, Northeast Ethiopia. AIDS research and therapy. 2013 Dec 1;10(1):7.
45. Gietaneh W, Agegne A, Gedif G. Prevalence of Intestinal Parasites and Associated Factors among HIV Patients who had Follow up at Debre Markos Referral Hospital, Northwest Ethiopia, from 2015-2019.
46. Jegede EF, Oyeyi ET, Bichi AH, Mbah HA, Torpey K. Prevalence of intestinal parasites among HIV/AIDS patients attending infectious disease hospital Kano. Nigeria. The Pan African Med. J. 17, 295. Link: https://goo. gl/PcBPRp. 2014.
47. Noormahomed EV. Prevalence of Intestinal Parasites Among HIV Infected and HIV Uninfected Patients Treated at the 1º De Maio Health Centre in Maputo, Mozambique. EC Microbiology. 2017;9:231-40.
48. Oyerinde JP, Odugbemi T, Benson RI, Alonge AA RJ. Investigation of Cryptosporidium in Relation to other Intestinal Parasites at the Lagos University Teaching Hospital, Lagos. West Afr J Med. 1989;8(4):264–9.
49. Akinboet al., Omoregie.,Eghafona, Omoregieet al., Omoregie R, Omokaro EU, et al. Prevalence of anemia among HIV-infected patients in Benin City, Nigeria. Tanzania Journal of Health Research. 2009;11(1).
50. Nyantekyi L, Legesse M, Medhin G, Animut A, Tadesse K, Macias C, *et al*. Community awareness of intestinal parasites and the prevalence of infection among community members of rural Abaye Deneba area, Ethiopia. Asian Pacific journal of tropical biomedicine. 2014; 4(1):152-157.

51. Getachew A. Prevalence and Intensity of Intestinal Parasitic Infections and Associated Risk Factors among Households around Akaki River and Aba Samuel Dam, Addis Ababa, Ethiopia. *SGSHU*.2014; 1(1):1-50.
52. Who.int.Availablefrom:https://www.who.int/hiv/pub/guidelines/arv2013/annexes/WHO_CG_annex_1.pdf
53. Upenn.edu Available from http://www.uphs.upenn.edu/bugdrug/antibiotic_manual/idsadiarrhea.pdf

Annex

Annex I: Participant Information sheet (English and Amharic version

My name is Birhanu. I am a laboratory technologist postgraduate student at Addis Ababa University. Now I am conducting a study entitled the Prevalence of Intestinal parasites and Knowledge, Attitude, and Practice about Intestinal parasites among HIV-positive individuals in Saint Peter TB Specialized Hospital, Addis Ababa, Ethiopia. You are invited to participate in this study. Please read the following statements and ask for any unclear points before you agree to participate. If you agree to be included in this study, I would like to ask you to sign on a document to show your agreement; participate accordingly, and give a clinical specimen. An intestinal parasite is one of the major health problems in our country; the result of the study can be helpful in planning and intervention to solve the problem. Participation in this study is exclusively voluntary. If you are not interested to participate or if you once decide to participate and withdraw yourself at any time, there will be no consequences and you will get all the services provided in the Laboratory. If you decide to participate in this study, you are expected to agree that 4 grams of stool and 4ml of blood will be collected. In addition, you are expected to give answers to some questions about your health and socio-demographic conditions. You need to know that your results might be discussed with another appropriate individual out of this hospital. But your name, address, and phone number will not be disclosed, and rather an identification code will be used in such conditions. The sample collection procedures take about 25 minutes and the interview takes about 15 minutes total of 40 minutes. Specimen collection will not affect and pose no pain on you and the only thing you spend is just your time to fill the questionnaire.

The information in your records is strictly confidential. All the information that you give and the results from your specimen will be used for this study only. Only a limited number of professionals will have access to the information. The information will be encoded in a computer and saved with password protection. Since this study is MSc student research, there will not be payments for participants. But you will also obtain all the results of the analysis for free and communicated to your physician for the appropriate management and also your participation is completely voluntary, and you can refuse to participate or withdraw from the study at any time. Refusal to participate will not result in the loss of medical care provided or any other benefits. You can get your results of the analysis. You may contact me at e-mail

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Participant information sheet (Amharic version)

ስሜ ብርሃኑ እባላለሁ ። እኔ በአዲስ አበባ ዩኒቨርሲቲ የላብራቶሪ ቴክኖሎጂ ባለሙያ የድህረ ምረቃ ተማሪ ነኝ ። አሁን የአንጀት ጥገኛ ተህዋሲያን ስርጭት እና እውቀት ፣ አመለካከት እና ልምምድ ከደም ማነስ ጋር እናCD4 ሕዋስ ቆጠራ በተያያዘ ቅዱስ ጴጥሮስ ቲቢ ስፔሻላይዝድ ሆስፒታል አዲስ አበባ ፣ ኢትዮጵያ ውስጥ በኤች አይ ቪ በተያዙ ታማሚዎች መካከል ነው ። በዚህ ጥናት ውስጥ እንዲሳተፉ ተጋብዘዋል ። ለመሳተፍ ከመስማማትዎ በፊት እባክዎ የሚከተሉትን መግለጫዎች ያንብቡ እና ግልጽ ያልሆኑ ነጥቦችን ይጠይቁ ። በዚህ ጥናት ውስጥ ለመካተት ከተስማሙ ስምምነትዎን ለማሳየት በሰነድ ላይ እንዲፈርሙ መጠየቅ እፈልጋለሁ; በዚህ መሠረት ይሳተፉ እና ክሊኒካዊ ናሙና ይስጡ። በአንጀት ውስጥ ጥገኛ ተህዋሲያን በአገራችን ካሉት ዋነኞቹ የጤና ችግሮች አንዱ ነው ። የጥናቱ ውጤት ችግሩን ለመፍታት በእቅድ እና ጣልቃ ገብነት ሊረዳ ይችላል ። የዚህ ጥናት ተሳትፎ በፈቃደኝነት ብቻ ነው ። ለመሳተፍ ፍላጎት ከሌልዎት ወይም አንድ ጊዜ ለመሳተፍ እና በማንኛውም ጊዜ እራስዎን ለማቆም ከወሰኑ ምንም መዘዞች አይኖሩም እናም በቤተ ሙከራ ውስጥ የሚሰጡትን ሁሉንም አገልግሎቶች ያገኛሉ ። የዚህ ጥናት ተካፋይ ለመሆን ከወሰኑ 4 ግራም(አንድ የሻይ ማንኪያ የሚሆን) የሰገራ ናሙና 4 ሚሊር ደም ይሰበሰባል ብለው ይስማማሉ ተብሎ ይጠበቃል ። በተጨማሪም ፣ ስለ ጤናዎ እና ስለ ማህበራዊ-ስነ-ህዝብ ሁኔታዎ ለአንዳንድ ጥያቄዎች መልስ መስጠት ይጠበቅብዎታል ። የእርስዎ ውጤቶች ከዚህ ሆስፒታል ውጭ ከሌላ አግባብ ካለው ግለሰብ ጋር ሊወያዩ እንደሚችሉ ማወቅ አለብዎት ። ግን የእርስዎ ስም ፣ አድራሻ እና የስልክ ቁጥር አይገለጽም እና ይልቁንም በእንደዚህ ያሉ ሁኔታዎች ውስጥ የመታወቂያ ኮድ ጥቅም ላይ ይውላል ። የናሙና አሰባሰብ አሠራሮች ወደ 25 ደቂቃዎች ያህል የሚወስዱ ሲሆን ቃለመጠይቁ በአጠቃላይ 15 ደቂቃ ያህል ይወስዳል 40 ደቂቃዎች ። የሙከራ ስብስብ ምንም ውጤት አይኖረውም እናም በእናንተ ላይ ምንም ሥቃይ አይፈጥርም እና እርስዎ የሚያጠፋው ብቸኛው ነገር መጠይቁን ለመሙላት ጊዜዎ ብቻ ነው ።

በመዝገቦችዎ ውስጥ ያለው መረጃ በጥብቅ ሚስጥራዊ ነው። የሚሰጡት መረጃ ሁሉ እና ከናሙናዎ የተገኙ ውጤቶች ለዚህ ጥናት ብቻ ያገለግላሉ ። መረጃውን የሚያገኙት ውስን የባለሙያ ባለሙያዎች ብቻ ናቸው ። መረጃው በኮምፒተር ውስጥ ተቀርጾ በይለፍ ቃል ጥበቃ ይቀመጣል ። ይህ ጥናት የ MSc የተማሪ ጥናት ስለሆነ ለተሳታፊዎች ክፍያዎች አይኖሩም ። ነገር ግን ሁሉንም የትንተና ውጤቶችን በነፃ

ያገኛሉ እና ተገቢውን አስተዳደር ለሐኪምዎ ያስተላልፋሉ እንዲሁም ተሳትፎዎ ሙሉ በሙሉ በፈቃደኝነት ነው ፣ እናም በማንኛውም ጊዜ ከጥናቱ ለመሳተፍ ወይም ላለመቀበል ይችላሉ ። ለመሳተፍ ፈቃደኛ አለመሆን የቀረበው የሕክምና እንክብካቤ ወይም ሌሎች ጥቅሞችን አያስገኝም ። የትንተና ውጤቱን ማግኘት ይችላሉ ። ለበለጠ መረጃ በኢ-ሜል Birhanukassaye03@gmail.com ወይም በሞባይል + 251911806783 ሜዲካል ላቦራቶሪ ሳይንስ የምርምር ሥነ ምግባር ቢሮ +251 11 275 5170 ሊያገኙኝ ይችላሉ ።

Annex II: Informed consent form (English and Amharic version)

I undersigned to confirm that, as I give consent to participate after a clear explanation of the objective and purpose of the study to me in the language I understand and a clear understanding of the objectives and purpose of the study & with recognition of my right to withdraw from the study if I change my mind. The questions I raise about the study have been answered satisfactorily.

I_____ do interestingly give consent to

Mrs. _____to give the stool sample and to answer the questions.

Name of Participant: _____

Participant's signature: _____

Name of data collector: _____

Signature of data collector: _____

Date: _____

Informed consent form (Amharic version)

ተሳታፊው በጥናቱ ለመሳተፍሙሉ ፈቃደኛ መሆኑን የሚገልጽበት ቅጽ

የተሳታፊው ስም_____ ስለጥናቱ አስፈላጊ የሆኑትን መረጃዎች አንብቤ ወይም ተነቦልኝ እናም የጥናቱ ዐላማ እና አስፈላጊነት ተረድቻለሁ። በማንኛውም ሰዓት ከጥናቱ የመውጣት መብት እንዳለኝ ተነግሮኛል። ከጥናቱ ጋር የተያያዙ ጥያቄዎችንም ጠይቄ ማብራሪያዎች ተሰጥተዉኛል።

እናም ፍቃደኛ መሆኔ ከታች በመፈረም አረጋግጣለሁ።

እኔ_____ የተባልኩ የተጠየቁትን ጥያቄ መልስ እና የሰገራ ናሙና

ለ_____ ለመስጠት ፈቃደኝነቴን ገልጫለሁ።

የተሳታፊው ፊርማ_____

ቀን_____

የተመራማሪዉ ፊርማ_____ ቀን_____

Annex III: Questioner (English and Amharic version)

ADDIS ABABA UNIVERSITY COLLEGE OF HEALTH SCIENCES, DEPARTMENT OF MEDICAL LABORATORY SCIENCES.

Purpose of the research to determine the Prevalence of Intestinal parasites and knowledge, attitude, and practice about Intestinal parasites among HIV-positive individuals in saint Peter TB Specialized Hospital, Addis Ababa, Ethiopia.

English version Questionnaires

Part one: socio-demographic characteristic

I. Participant code _____

1. Sex A. Male B. Female

2. Age in years: _____

3. Residence A. Urban B. Rural

4. Occupation

A. Housewife B. Government C. Private D. Merchant

E. Student

5. Education Status

A. Illiterate B. Primary (grade1-8) C. Secondary (grade9-10)

D. Preparatory (grade11-12) E. Higher level

6. Marital status

A. Married B. Single C. Divorced or widowed

Part two: Knowledge, Attitude, Practice

A. Questions designed to assess respondents' knowledge about intestinal

1. Do you know what intestinal parasites are? A. Yes B. No

2. How did you know about the intestinal parasite? A. Friends B. Health center C. Health center D. Radio E. Other

3. How intestinal parasites are transmitted?

3.1 Eating contaminated food	A. yes	B. No
3.2 Eating raw meat	A. yes	B. No
3.3 Drinking contaminated water	A. yes	B. No
3.4 Not cut nails regularly	A. yes	B. No
3.5 Walking barefooted	A. yes	B. No
3.6 Lack of hygiene	A. yes	B. No

4. What are the sign and symptoms of intestinal parasites?

4.1 Abdominal pain	A. yes	B. No
4.2 Diarrhea	A. yes	B. No
4.3 Vomiting	A. yes	B. No
4.4 Loss of appetite	A. yes	B. No
4.5 Weight loss	A. yes	B. No

5. Where do you prefer to seek treatment for IP?

5.1 Traditional healer	A. yes	B. No
5.2 Health center	A. yes	B. No
5.3 Direct to pharmacy	A. yes	B. No

6. How intestinal parasites are prevented?

6.1 Treatment with specific medicines	A. yes	B. No
6.2 Use of toilets	A. yes	B. No
6.3 Provision of safe tap water	A. yes	B. No
6.4 Avoid defecating in open filed	A. yes	B. No
6.5 Personal hygiene	A. yes	B. No

B. Questions designed to assess respondents' attitude towards intestinal parasitosis

1. Do you think intestinal parasitosis is a serious disease?

A. yes B. No

2. Do you think take an education against intestinal parasitosis is important?

A. yes B. No

3. Do you think going to a health facility is important when you feel abdominal discomfort?

A. yes B. No

4. Do you think taking traditional medication is good to treat intestinal parasitosis?

A. yes B. No

5. Do you think playing in the soil can cause intestinal parasitosis?

A. yes B. No

6. Do you think eating raw vegetables can cause intestinal parasitosis?

A. yes B. No

7. Do you think health education can reduce intestinal parasite prevalence?

A. yes B. No

8. Do you think hand washes with soap and water can preventive for intestinal parasites?

A. yes B. No

C. Questions designed to assess respondents, practice towards intestinal parasites

1. Do you eat row and/or unwashed fruit?

A. yes B. No

2. Do you eat raw meat?

A. yes B. No

3. Water source for drinking?

3.1 Pipe water	A. yes	B. No
3.2 River water	A. yes	B. No
3.3 Well Unprotect water	A. yes	B. No
3.4 Bottle water	A. yes	B. No

4. Do you use a toilet?

A. yes B. No

5. Do you defecate on the open field?

A. yes B. No

6. Do you wash your hand after a toilet/defecation?

A. yes B. No

7. Do you cut or clean fingernails?

A. yes B. No

8. Do you go to the health center when you feel abdominal discomfort?

A. yes B. No

9. Do you take medication for intestinal parasites?

A. yes B. No

10. Do you wear closed shoes?

A. yes B. No

Part three: Clinical Data

1. Diarrhea

A. Present B. Absent

2. Diarrhea condition

A. Chronic (4 weeks) B. Acute (1 week)

3. Abdominal pain symptoms

A. Present B. Absent

4. Stool characteristics

A. Formed B. Soft C. loose D. Diarrhea

5. Current WHO HIV stage A. I B. II C. III D. IV

6. Current CD4 count (Cells/L or %) ----------

7. CD4 category A. <200 B. 200-500 C. >500

8. Current Hemoglobin level (g/dl) ----------

9. Anemia A. Present B. Absent

10. Anemia status A. Mild (10-10.9) B. Moderate (-9.9) C. Sever <7

የአማርኛ ቅጅ መጠይቆች

የአዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ ፣ የሕክምና ላብራቶሪ ሳይንስ ክፍል።

የምርምር ዓላማ-ከደም ማነስ ጋር በተያያዘ የአንጀት ጥገኛ ተውሳኮችን ብዛት እና እውቀት ፣ አመለካከት እና አሠራር ለመለየት እና በኤች አይ ቪ በተያዙ ታማሚዎች መካከል በሲዲ 4 ቆጠራዎች ውስጥ በአዲስ አበባ ፣ ኢትዮጵያ ውስጥ ።

ክፍል አንድ-ማህበራዊ-ስነ-ህዝብ ባህሪ

I. የተሳትፎ ኮድ _____

1. ፆታ ሀ ወንድ ለ. ሴት

2. ዕድሜ _____

3. የመኖሪያ ቦታ ሀ. የከተማ ለ. ገጠር

4. ሥራ

ሀ የቤት እመቤት ለ.መንግስት ሐ.ግል መ ነጋዴ ሠ ተማሪ

5. የትምህርት ሁኔታ

ሀ ማንበብና መጻፊያ ለ. የመጀመሪያ / ደረጃ 1-8 / ሐ ሁለተኛ ደረጃ (ክፍል 9-10)

መ መሰናዶ (ክፍል 11-12) ሠ ከፍተኛ ደረጃ

6. የጋብቻ ሁኔታ

ሀ ያገባ/ ች ለ. ያላገባ/ ች ሐ.ፍቺ ወይም በሞት የተለየ

ክፍል ሁለት ዕውቀት ፣ አመለካከት ፣ ተግባር

ሀ / ምላሽ ሰጪዎች ስለ አንጀት ያላቸውን እውቀት ለመገምገም የተቀየሱ ጥያቄዎች

1. የአንጀት ተውሳኮች ምን እንደሆኑ ያውቃሉ? ሀ አዎ ለ አይ

2. የአንጀት ተውሳክን እንዴት ያውቁ ነበር? ሀ ከጓደኞች ለ ከጤና ጣቢያ

ሐ. ከጤና ጣቢያ መ ከሬዲዮ ሠ ከሌላ

3. የአንጀት ተውሳኮች እንዴት ይተላለፋሉ?

3.1 የተበከለ ምግብ መመገብ ሀ ይተላለፋል ለ አይተላለፍም

3.2 ጥሬ ሥጋን መመገብ ሀ ይተላለፋል ለ አይተላለፍም

3.3 የተበከለ ውሃ መጠጣት ሀ ይተላለፋል ለ አይተላለፍም

3.4 ጥፍርን በመደበኛነት አለመቁረጥ ሀ ይተላለፋል ለ አይተላለፍም

3.5 በባዶ እግር መጓዝ ሀ ይተላለፋል ለ አይተላለፍም

3.6 የንፅህና አጠባበቅ እጦት ሀ ይተላለፋል ለ አይተላለፍም

4. የአንጀት ጥገኛ ትላትል ምልክቶች እና ምልክቶች ምንድናቸው?

4.1 የሆድ ህመም ሀ ነው ለ አይደለም

4.2 ተቅማጥ ሀ ነው ለ አይደለም

4.3 ማስታወክ ሀ ነው ለ አይደለም

4.4 የምግብ ፍላጎት ማጣት ሀ ነው ለ አይደለም

4.5 ክብደት መቀነስ ሀ ነው ለ አይደለም

5. ለአንጀት ጥገኛ ትላትል ሕክምና የት ይመርጣሉ?

5.1 ባህላዊ መድሃኒት ሀ እመርጣለው ለ አለመርጥም

5.2 ጤና ጣቢያ ሀ እመርጣለው ለ አለመርጥም

5.3 ቀጥታ ከፋርማሲ መግዛት ሀ እመርጣለው ለ አለመርጥም

6. የአንጀት ጥገ ትላትሎች እንዴት መከከላከል ይቻላል?

6.1 ትክክለገኛ መድሀኒት በመውሰድ ሀ ይቻላል ለ አይቻልም

6.2 መፀዳጃ ቤት በመጠቀም ሀ ይቻላል ለ አይቻልም

6.3 ንጹህ የቧንቧ ውሃ በመጠቀም ሀ ይቻላል ለ አይቻልም

6.4 ሚዳ ላይ ባለ መጸዳዳት ሀ ይቻላል ለ አይቻልም

6.5 የግል ንፅህናን በመትበቅ ሀ ይቻላል ለ አይቻልም

ለ / የአንጀት ተህዋሲያን (parasitosis) ምላሽ ሰጪዎችን አመለካከት ለመገምገም የታቀዱ ጥያቄዎች

1. የአንጀት ተህዋሲያን (parasitosis) ከባድ በሽታ ነው ብለው ያስባሉ?

ሀ አዎ ለ አይደለም

2. በአንጀት ተህዋሲያን (parasitosis) በሽታ ላይ ትምህርት መውሰድ አስፈላጊ ነው ብለው ያስባሉ?

ሀ. አዎ ለ አይደለም

3. የሆድ ምቾት ሲሰማዎት ወደ ጤና ተቋም መሄድ አስፈላጊ ነው ብለው ያስባሉ?

ሀ. አዎ ለ አይደለም

4. ባህላዊ መድሃኒት መውሰድ የአንጀት ተህዋሲያን በሽታን ለማከም ጥሩ ነው ብለው ያስባሉ?

ሀ. አዎ ለ አይደለም

5. በአፈር ውስጥ መጫወት የአንጀት ተህዋሲያን ሊያስከትል ይችላል ብለው ያስባሉ?

ሀ. አዎ ለ አይደለም

6. ጥሬ አትክልቶችን መመገብ የአንጀት ተህዋሲያን (parasitosis) ሊያስከትል ይችላል ብለው ያስባሉ?

ሀ. አዎ ለ አይደለም

7. በጢና ጣቢያ የሚሰጡ ትምህርቶች የአንጀት ተህዋሲያን ስርጭትን ሊቀንስ ይችላል ብለው ያስባሉ?

ሀ. አዎ ለ አይደለም

8. እጅን በሳሙና እና በውኃ መታጠብ ለአንጀት ጥገኛ ተህዋሲያን መከላከል ይችላል ብለው ያስባሉ?

ሀ. አዎ ለ አይደለም

ሐ / የጥናቱ ተሳታፊዎች ስለ አንጀትን የሚያጠቁ ትላትሎች በሽታ ያላቸውን ልምድ ለመገምገም የተዘጋጀ መጠይቅ

1. ጥሬ እና / ወይም ያልታጠበ ፍሬ ይመገባሉ?

ሀ. አዎ ለ አይ

2. ጥሬ ሥጋ ትበላለህ?

ሀ. አዎ ለ አይ

3. ለመጠጥ የሚጠቀሙት ውሃ ምንጭ?

3.1 የቧንቧ ውሃ ሀ እጠቀማለው ለ አልጠቀምም

3.2 የወንዝ ውሃ ሀ እጠቀማለው ለ አልጠቀምም

3.3 በደንብ ያልተጠበቀ ውሃ ሀ እጠቀማለው ለ አልጠቀምም

ሐ / መልስ ሰጪዎችን ለመገምገም የታቀዱ ጥያቄዎች ፣ ወደ አንጀት ጥገኛ ተውሳኮች ይለማመዳሉ

1. ጥሬ እና / ወይም ያልታጠበ ፍሬ ይመገባሉ?

ሀ. አዎ ለ አይ

2. ጥሬ ሥጋ ይመገባሉ?

ሀ. አዎ ለ አይ

3. ለመጠጥ የሚጠቀሙትውሃ ምንጭ?

3.1 የቧንቧ ውሃ ሀ እጠቀማለው ለ አልጠቀምም

3.2 የወንዝ ውሃ ሀ እጠቀማለው ለ አልጠቀምም

3.3 በደንብ ያልተጠበቀ ውሃ ሀ እጠቀማለው ለ አልጠቀምም

3.4 የታሸገ ውሃ ሀ እጠቀማለው ለ አልጠቀምም

4. መጸዳጃ ቤት ይጠቀማሉ?

ሀ. አዎ ለ አይ

5. በክፍት ሜዳ ላይ ሰገራ ይጸዳሉ?

ሀ. አዎ ለ አይ

6. ከመፀዳጃ ቤት / ሰገራ በኋላ እጅዎን ይታጠባሉ?

ሀ. አዎ ለ አይ

7. የጣት ጥፍር ይቆርጣሉ ወይም ያጸዳሉ?

ሀ. አዎ ለ አይ

8. የሆድ ህመም ሲሰማዎት ወደ ጤና ጣቢያ ይሄዳሉ?

ሀ. አዎ ለ አይ

9. ለአንጀት ተዋሲያነ መድኃኒት ይወስዳሉ?

ሀ. አዎ ለ አይ

10. ሽፍን ጫማ ይለብሳሉ?

ሀ. አዎ ለ አይ

ክፍል ሶስት ክሊኒካዊ መረጃ

1. ተቅማጥ ሀ አለ ለ የለም

2. የተቅማጥ ሁኔታ ሀ የቆየ (4 ሳምንቶች) ለ የቅብ (1 ሳምንት)

3. የሆድ ህመም ምልክቶ ሀ አለ ለ የለም

Annex IV: Standard Operating Procedures

A. Direct Microscopic Examination

Used to diagnose motile parasites such as the larvae of *S. stercoralis* and trophozoites of *E. histolytica*, *G. lamblia*, helminth eggs, cysts, and oocysts of intestinal protozoa.

Method

a. Place a drop of fresh physiological saline on a slide.
b. To avoid contaminating the fingers and stage of the microscope, do not use too large a drop of saline.
c. Using a wire loop or piece of stick, mix a small amount of specimen, about 2 mg, (matchstick head amount) with the saline. Make smooth thin preparations. Cover each preparation with a Cover glass.
d. Do not use too much specimen otherwise, the preparations will be too thick, making it difficult to detect and identify parasites.
e. Examine systematically the entire saline preparation for larvae, ciliates, helminth eggs, cysts, and oocysts. Use the 10x objective with the condenser iris closed sufficiently to give a good contrast.
f. Use the 40x objective to assist in the detection and identification of eggs, cysts, and oocysts.
g. Always examine several microscope fields with this objective before reporting ‘No parasites found’.
h. Report the larvae, cysts, oocysts, ciliates, and each species of egg found in the entire saline preparation.

B. Formol-ether Concentration technique

Principle

In the Ridley modified method, feces are emulsified in formol water, the suspension is strained to remove large fecal particles, ether or ethyl acetate is added, and the mixed suspension is centrifuged. Cysts, oocysts, eggs, and larvae are fixed and sedimented and the fecal debris is separated in a layer between the ether and the formol water. Fecal fat is dissolved in the ether.

Method

a. Using a rod or stick, emulsify an estimated 1 g (pea-size) of feces in about 4 ml of 10% formol water contained in a screw-cap bottle or tube.
b. Add a further 3–4 ml of 10% v/v formol water, cap the bottle, and mix well by shaking.
c. Sieve the emulsified feces, collecting the sieved suspension in a beaker.
d. Transfer the suspension to a conical (centrifuge) tube made of strong glass, copolymer, or Polypropylene. Add 3–4 ml of diethyl ether or ethyl acetate.
e. Stopper the tube and mix for 1 minute. If using a Vortex mixer, leave the tube unstoppered and mix for about 15 seconds (it is best to use a boiling tube).
f. With a tissue or piece of cloth wrapped around the top of the tube, loosen the stopper (considerable pressure will have built up inside the tube).
g. Centrifuge immediately at 750–1000 g (approx. 3000 rpm) for 1 minute.
h. After centrifuging, the parasites will have sedimented to the bottom of the tube and the faecal debris will have collected in a layer between the ether and formol water.
i. Using a stick or the stem of a plastic bulb pipette, loosen the layer of faecal debris from the side of the tube and invert the tube to discard the ether, faecal debris, and formol water. The sediment will remain.
j. Return the tube to its upright position and allow the fluid from the side of the tube to drain to the bottom. Tap the bottom of the tube to resuspend and mix the sediment. Transfer the sediment to a slide, and cover it with a cover glass.
k. Examine the preparation microscopically using the 10x objective with the condenser iris closed sufficiently to give a good contrast. Use the 40x objective to examine small cysts and eggs.

C. modified Ziehl-Neelsen technique

Principle

The Ziehl-Neelsen staining technique enables the detection of pathogenic coccidian intestinal protozoa in human stool specimens. The three important coccidians that may cause persistent digestive disorders are Cryptosporidium spp., Cyclospora cayetanensis and Cystoisospora belli. The principle behind the ZN technique is that it allows for the detection of so-called “acid-fast organisms” such as coccidian intestinal protozoa and mycobacteria. Once these organisms are stained with a specific dye, they are difficult to decolorize and retain a red color even when treated with a mixture of acid and alcohol (while all other structures present in the sample will

decolorize). Methylene blue is used as a counterstain, hence only the acid-fast organisms will appear as red-colored and can thus easily be detected in a stool sample.

Method

a) Faecal smears are made either directly from the stool sample or the concentration deposit.

b) Allow air to dry.

c) Fix in methanol for 3 minutes.

d) Stain with strong carbon fuchsin for 15-20 minutes.

e) Rinse thoroughly in tap water.

f) Decolourise in acid alcohol (1% HCl in methanol) for 15-20 seconds.

g) Rinse thoroughly in tap water.

h) Counterstain with 0.4% malachite green (or methylene blue) for 30-60 seconds.

i) Rinse thoroughly and air dry.

j) Examine using x40 and x100 objectives

Declaration

I, the undersigned declare that this thesis is my original work in partial fulfilment of the requirement for the master of Diagnostic and Public Health Microbiology. I also declare that it has never been presented in this or any other university and that all resources and materials in the proposal have duly acknowledged.

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