PREVALENCE OF INTESTINAL PARASITE WITH SPECIAL EMPHASIS ON OPPORTUNISTIC INTESTINAL COCCIDIA AND PREDISPOSING FACTORS AMONG HIV PATIENTS ATTENDING ART CLINIC AT WORABE COMPREHENSIVE SPECIALIZED HOSPITAL, WORABE CENTRAL ETHIOPIA

A RESEARCH PROPOSAL TO BE SUBMITTED TO THE SCHOOL OF MEDICAL LABORATORY SCIENCE, HAWASSA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE MASTERS OF SCIENCE DEGREE IN MEDICAL PARASITOLOGY.

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NOVEMBER, 2023

HAWASSA, ETHIOPIA

HAWASSA UNIVERSITY

COLLEGE OF MEDICINE AND HEALTH SCIENCES

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ADVISORS' APPROVAL SHEET

This is to certify that the thesis entitled "PREVALENCE OF INTESTINAL PARASITE WITH SPECIAL EMPHASIS ON OPPORTUNISTIC INTESTINAL COCCIDIA AND PREDISPOSING FACTORS AMONG HIV PATIENTS ATTENDING ART CLINIC AT WORABE COMPREHENSIVE SPECIALIZED HOSPITAL, WORABE CENTRAL ETHIOPIA" to be submitted in partial fulfillment of the requirements for the degree of masters with specialization in Medical Parasitology, the graduate program of the department /school of medical laboratory science and had been carried out by Jemal Hussen under our supervision. Therefore we recommend that the student has fulfilled the requirement and hence here by can submit the thesis to the department.

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We, the undersigned, members of the Board of Examiners of the final open defense by Jemal Husen have read and evaluated his/her thesis entitled "PREVALENCE OF INTESTINAL PARASITE WITH SPECIAL EMPHASIS ON OPPORTUNISTIC INTESTINAL COCCIDIA AND PREDISPOSING FACTORS AMONG HIV PATIENTS ATTENDING ART CLINIC AT WORABE COMPREHENSIVE SPECIALIZED HOSPITAL, WORABE CENTRAL ETHIOPIA" and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirements for the **Master's** degree in **Medical parasitology**.

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SGS Approval	Signature	Date

Final approval and acceptance of the thesis is contingent upon the submission of the final copy of the thesis to the school of graduate studies (SGS) through the department/school graduate committee (DGC/SGC) of the candidate's department.

Stamp of SGS date: ________________

DECLARATION

I hereby declare that this **MSc in Medical parasitology** thesis is my original work and has not been presented for a degree in any other university, and all sources of material used for this thesis have been duly acknowledged.

Name: Jemal Husen Signature: _____________

This MSc in Medical parasitology thesis has been submitted for examination with my approval as thesis advisor.

Tariku Lambiyo (Asst. Prof) Signature: _______________

Place and Date of Submission: _____________________________

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AIDS	Acquired Immune Deficiency Syndrome
AOR	Adjusted Odds Ratio
ART	Anti-Retroviral Therapy
CD4	Cluster of Differentiation 4
CDC	Center of disease control
COR	Crudes Odds Ratio
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
IP	Intestinal parasite
IPI	Intestinal parasitic infection
MZN	Modified Ziehl-Neelsen
OICI	Opportunistic intestinal coccidian infection
OIPs	Opportunistic intestinal parasites
PLWHA	People living with HIV/AIDS
SSA	Sub-Saharan Africa
WCSH	Worabe Comprehsive and Specialized Hospital
WHO	World health organization

ABSTRACT

Introduction: Human Immunodeficiency Virus causes CD4+ T cell depletion and immune system thinning in humans, which increases the vulnerability of HIV patients to opportunistic and other intestinal parasites known to cause diarrhea that can be severe and persistent, leading to dehydration, abnormal electrolyte levels , weight loss, and malabsorption.

Objectives: The present study aimed to determine prevalence of intestinal coccidia and other intestinal parasites and risk factors among Human Immunodeficiency Virus patients monitoring their disease status at ART clinics.

Materials and Methods: Institutional based cross-sectional study was conducted among 400 Human Immunodeficiency Virus patients attending Anti-Retroviral Therapy clinic at Worabe Comprehensive Specialized Hospital from May 2022 to September 2023. Convenient sampling technique was used to recruit study participants. Stool specimens were processed for parasitological examination. A pre-structured questionnaire was used to collect data. Data was entered into Epi-info and analyzed by SPSS version 26 software. A p-value <0.05 in the multivariable logistic regression was considered as statistically significant.

Results: The overall prevalence of intestinal parasites was 34.5%. Eleven different intestinal parasite species were detected. Prevalence of intestinal coccidian in present study was 8.25%. *Cryptosporidium spp*. 7.7% and *Isospora belli* 0.5% were identified intestinal coccidian. In multivariate analysis ,educational status; No formal education , animal contact , ART starting time ; <2 years duration on Anti-Retroviral Therapy ,history of diarrhea and CD4 level; 201-499 cell/mm^3 were significantly associated variables with prevalence of intestinal parasite among study subject.

Conclusions and Recommendations: Intestinal coccidia and other intestinal parasitic infections are still common health problems among HIV patients in the study area. Thus, the health professionals need to give attention to parasitological examinations in the routine treatment of Human Immunodeficiency Virus patients by using specific as well high sensitivity test. Moreover, adherence to Anti-retroviral therapy should strengthen to improve the immune status.

Key words: prevalence, opportunistic intestinal coccidian, ART patients, predisposing factors, intestinal parasite

CHAPTER I: INTRODUCTION

1.1.Background

Infections caused by intestinal parasites are currently the main cause of public health issues worldwide. In low-income nations, particularly in SSA, where the majority of HIV cases are concentrated, infection rates are extraordinarily high (Alemayehu *et al.*,2020).

The primary cause of illness and mortality among HIV patients worldwide is intestinal parasite infection brought on by helminths and protozoa, particularly in resource-poor tropical and subtropical regions (Feleke *et al.*, 2022). Constant immunological activity brought on by intestinal parasites, especially intestinal helminths, is characterized by a dominating Th2 (anti-inflammatory) type cytokine profile, as well as the release of high levels of IgE and eosinophilia. Such immunological profiles negatively affect the ability of Th1 (inflammatory) cytokines to stop HIV virus replication (Tebit *et al.*,2014).

Depletion of CD4+ T cells, which is a side effect of the human immunodeficiency virus, makes people more susceptible to opportunistic infections (Feleke *et al.*,2022). Any time during the course of HIV infection, in particular, opportunistic intestinal coccidian parasite infection can occur; however, infection is established when the CD4+ count is below 200 cells/mm3, which causes severe and life-threatening diarrhea in HIV/AIDS patients (Sah *et al.*,2017). Since the spread of HIV infection and the AIDS epidemic, there has been an increase in the prevalence of protozoan pathogens that cause diarrhea (Aminu *et al.*, 2008). When the cell-mediated immunity is impaired in immunocompromised patients, Strongyloides stercoralis is regarded as an opportunistic intestinal parasite that increases the risk of complex strongyloidiasis (Luvira *et al.*, 2022). Non-opportunistic parasites are also important in diarrheal diseases that occur in individuals living with HIV/AIDS(Shimelis *et al.*, 2016).

Even if the situation for people living with HIV has improved with the introduction of ART, the prevalence of OIPs makes it extremely difficult to lower associated morbidity and death, especially in developing nations (Kiros *et al.*, 2015). Due to a lack of information, a fear of social stigma, and inadequate community access to healthcare, the majority of patients in those countries now seek HIV diagnosis and treatment after the disease has evolved to an advanced stage and the CD4+ T cell count has declined. All of things will cause the patients to have a terrible prognosis, quickly advance their illness, and pass away from opportunistic infections (Bisetegn *et al.*, 2022).

Due to a lack of information, skill, and technique, most routine laboratories at the primary care level in developing country do not test for coccidian parasites, despite the fact that the CDC considers them AIDS-defining opportunistic infections (Gupta *et al.*, 2008) .

The majority of Ethiopian healthcare facilities routinely check HIV/AIDS patients for opportunistic intestinal parasitic infections, although the coccidian parasite is rarely found using the direct saline microscopy diagnostic approach. Information about the frequency of opportunistic intestinal coccidia among this group in Ethiopia is sparse. To our best knowledge, no reliable data on the epidemiology of intestinal coccidia and other intestinal parasites exists among HIV/AIDS patients in the study area. Epidemiological evaluation of intestinal parasitic infection prevalence among HIV sero-positive individual is needed for comprehensive HIV prevention, care and treatment. Thus; this study aimed to investigate the magnitude of intestinal coccidia and other intestinal parasitic infection and its associated risk factors among HIV infected patients attending ART clinics at Worabe Comprehensive Specialized Hospital, Central Ethiopia in order to provide evidence based information, from which medical decisions can be taken.

1.2. Statement of the problems

A serious global public health concern, the human immunodeficiency virus (HIV) has so far claimed the lives of 40.1 million people, of whom 2/3 are in the African region, with the greatest rates of HIV infection in SSA (Sheet *et al*.,2023). Eighty percent of HIV cases worldwide were found in ten Sub-Saharan African nations: South Africa (25%), Nigeria (13%), Mozambique (6%), Uganda (6%), Tanzania (6%), Zambia (4%), Zimbabwe (6%), Kenya (6%), Malawi (4%) and Ethiopia (3%) (Palacios-Lopez *et al.*,2017). Intestinal parasites that formerly only caused sporadic or zoonotic, benign, and asymptomatic infections have evolved into opportunistic parasites that have led to severe diarrhea in HIV patients (Barcelos *et al.*,2018).

The prevalence of gastrointestinal issues in HIV seropositive people brought on by opportunistic parasite infection has dramatically decreased in countries where antiretroviral medications are widely available. But in the majority of African countries, intestinal enteric infections still frequently cause diarrhea, wasting, and weight loss(Kiros *et al.*, 2015).

In underdeveloped nations, it is believed that up to 95% of HIV-infected patients also have parasitic infections(Miressa *et al.,* 2021). About 90% and 30-60% of people with HIV/AIDS in underdeveloped nations and wealthy countries, respectively, experience diarrhea(Shimelis *et al.*,2016). The majority of opportunistic infections (OIPs), particularly in tropical nations where HIV is endemic and which frequently appear when the CD4+ T cell count is below 200 cells/mm3, are thought to be responsible for more than 47% of AIDS patient deaths.(Getaneh et al.,2017.)

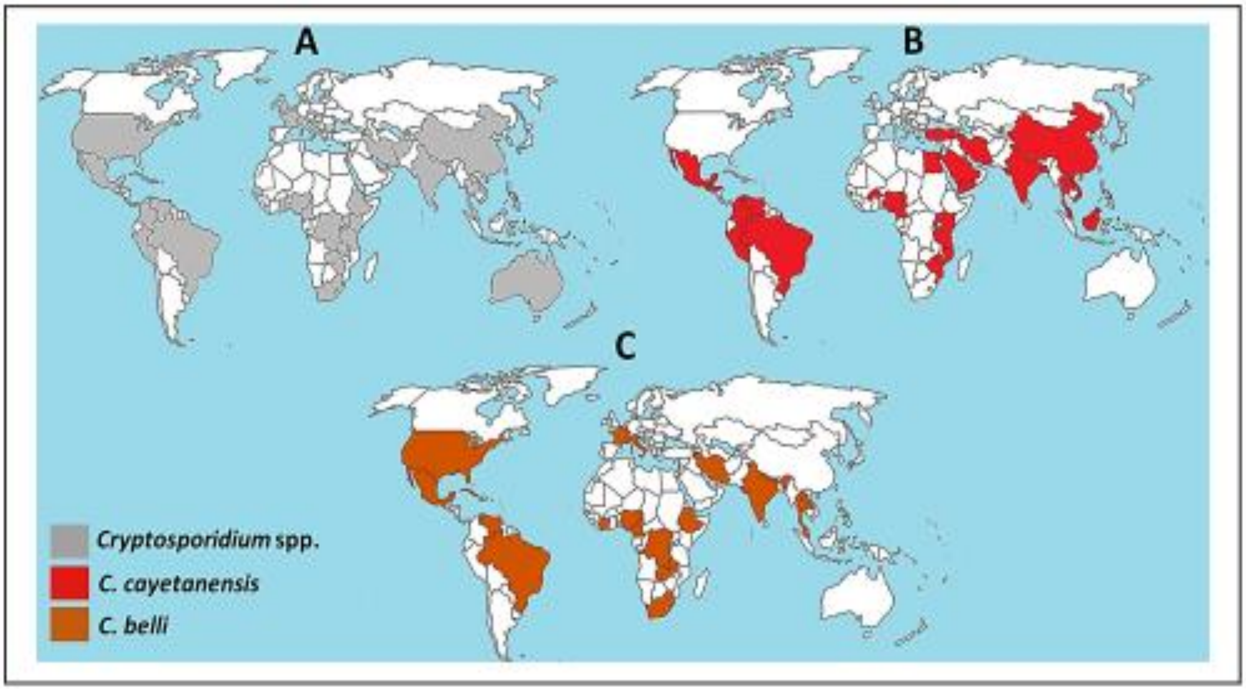

Figure 1:Epidemiological distribution of intestinal coccidian throughout the world, 2023(Contreras-Puentes *et al.*, 2020).

Cryptosporidium is currently one of the most often reported gastrointestinal disorders in immunocompetent and immunocompromised individuals worldwide, with 1-3 and 10% of people excreting oocysts in industrialized and developing countries, respectively(Regina *et al.*, 2021).

Over a million deaths from gastroenteritis linked to Cryptosporidium spp. have been observed in the last quarter of the 20th and the beginning of the 21st centuries, particularly among immunocompromised individuals (Sah *et al.*, 2017). This is primarily Due to its tendency for zoonotic transmission. Additionally, due to its resilience to water purification methods, it has been suggested that the transmission of Cryptosporidium may occur through the ingestion of tainted food and even through water. Infections can also spread from person to person, particularly among homosexually active men. Moreover, the small size (3-5 μm) of oocysts and low infective dose are the major infective potential for *Cryptosporidium spp* transmission(Contreras-Puentes *et al.*, 2020). Also, poverty and malnutrition are some of the factors that contribute to concomitant infections of both HIV and intestinal parasites in Sub-Saharan Africa(Tebit *et al.*,2014).

It was predicted that 39.2% percent of HIV/AIDS patients in Ethiopia had intestinal parasitosis (Abayneh *et al.,* 2022) with ,OIPIs among patients are estimated to be high due to poor level of environmental sanitation and personal hygiene plus contamination of food and drinking water. Ethiopia has an endemic case of cryptosporidiosis, with rates of 7.6-43.6% recorded in HIV/AIDS patients. Water contamination, animal contact, and poor personal hygiene were among possible risk factors for the development of cryptosporidiosis. (Adamu *et al.*, 2014)

CHAPTER II: LITERATURE REVIEWS

2.1. Prevalence of intestinal parasitosis among people living with HIV.

Studies have been conducted on prevalence of intestinal parasites among HIV sero-positive individuals throughout the world at different time. Accordingly, findings in Brazil, Nepal and Cameron revealed that overall prevalence of intestinal parasites among HIV patient were 28.8,38 and 82.6% respectively with intestinal protozoa was more prevalent than intestinal helminthes *E. histolytica*, *I. belli*, *hookworm*, *S.stercoralis C. cayetatensis* and *G. lamblia* were among commonly isolated parasites in studies. Additionally, *B. hominis* was also identified in studies at Brazil and Cameron whereas *A. lumbricoïdes, T.trichiura, H.nana* detected only in investigation conducted at Cameron(Sah *et al.*, 2017),(Barcelos *et al.*, 2018),(Tebit *et al.*, 2014)

Investigation in South Africa suggested that prevalence of intestinal parasites was 30% after screening 600 stool samples among HIV patients following their ART program. The most frequently detected parasites were *Ascaris lumbricoides* followed by *Balantidium coli, Entamoeba coli, Diphyllobothrium latum, Taenia species* and *Schistosoma mansoni* (Ifeoma *et al.*, 2022). Similarly, Study carried out in Burkina Faso displayed that prevalence of intestinal parasites among HIV patients was 73.3 %. The rate of infection was 78.5 and 21.5% for single and mixed respectively and most of detected parasites in the investigation were protozoan (97.9%) with few helminths (2.1%). *G. lamblia* was the most commonly found parasite followed by *Entamoeba coli* (Adama *et al.*, 2017).

Studies on the prevalence and distribution of opportunistic and other intestinal parasites among HIV-positive people in Ethiopia have been done. In Ethiopia, the overall prevalence of intestinal parasite infections among HIV-positive individuals using ART was 40.24%. The Oromia and Amhara regions, respectively, had the lowest (13.9%) and highest (80.3%) prevalence of intestinal parasite infections among the studied papers(Eshetu *et al.*,2016).

According to studies conducted in 2016 at Arbmench south Ethiopia and Kombolcha north Ethiopia prevalence of intestinal parasite among HIV patients was high which were 28.8 and 13.9% respectively. In these findings protozoan was most dominantly detected phylum as compared to helminths. Various types of non-opportunistic and opportunistic intestinal parasites were detected .Specific parasite spp explored commonly in studies were included *E. histolytica, S. stercoralis, A. lumbricoides ,Cryptosporidium spp, H. nana* , S.mansoni, *Cyclospora spp, Isospora belli Trichuris trichuria, Enterobius vermicularis and Taenia spp*(Getaneh *et al*.,2016),(Gebretsadik *et al*.,2018).

Supplementary research carried out at Arsi University eastern Ethiopia in 2017 reveled that intestinal parasitic infection was still common problem among HIV infected group which increases rate of morbidity and mortality. Prevalence of these parasites was identified in study by using different laboratory techniques. Accordingly the overall prevalence observed in finding was 18.4%. Protozoa (*E. histolytica,* and *G. lamblia*) and helminths (*Taenia spp., A. lumbricoides, S. stercoralis, T. trichuria* and *H. nana*) were among detected parasites with protozoan domination seen in investigations(Mesfun *et al*., 2018).

An investigation carried out at Debretabor General Hospital and Dessie Referral Hospital northern part of our country in 2019 told us prevalence of IPIs in HIV positive patients were 25.3 and 21.1% consecutively. Amazingly in both studies detected twelve different types of intestinal parasites using the direct wet mount and formol-ether concentration techniques, including *E.vermicularis, G.lamblia, A.lumbricoides, Cryptosporidium* species *.A. lumbricoides*, *S. stercoralis*, *Taenia Isospora belli, S.mansoni, H.nana and Hookworm.* The dominant intestinal parasite species was *Entameoba histolytica/dispar* in the latter study followed by *E.vermicularis* and *G.lamblia*. The least common parasites detected in this study were *Isospora belli* and *S.mansoni* while *A. lumbricoides*, *S. stercoralis*, and *Taenia species* were the most frequent in the former finding. Additionally the prevalence rates of intestinal parasitic infection with single and multiple were 18% and 23.8% respectively in finding at Debretabor(Alemayehu *et al.*, 2020),(Feleke *et al.*, 2022).

According to a research performed in 2020 in Nekemte south west and Dessie comprehensive hospital north part of Ethiopia the overall prevalence of intestinal parasitic infection among HIV positive individual were 73.3 and 38.1% respectively indicated that infection is still common problem among the group that need special attention. Again in both findings protozoan was the most dominantly detected phylum when compared to helminths. Regarding specific species *G. lamblia, E. histolytica/dispar, A. lumbricoides and S. stercoloaris* were among identified intestinal parasite commonly in both studies. *Taenia, H.nana, Hookworm* and *T.trichiura* were only observed in the former finding with no opportunistic intestinal parasites were detected. *Cryptosporidium spp* was the only opportunistic intestinal coccidia identified in the latter finding. Additionally the most predominantly detected parasite in both findings was *Giardia lamblia* followed *Entamoeba histolytica/dispar*(Miressa *et al.*,2021),(Bisetegn *et al.*, 2022).

Similarly studies in 2021 done at St. Peter's Specialized Hospital in Addis Ababa, Guji south Ethiopia and Mizan-Tepi University south west Ethiopia explored that intestinal parasitic infection was which have been enhancing viral replication among HIV infected individual that negatively affect the morbidity and mortality rate. Consequently, prevalence of this infection was 26.2, 29.1 and 35.1% respectively in these studies. Similar to studies previously discussed higher rate of intestinal protozoan parasites were detected in these findings when compared to intestinal helminths. Furthermore regarding specific spp *G. lamblia, E. histolytica, A. lumbricoides, S. stercoloaris, Taenia spp* and *hookworm* were among commonly detected parasites in the studies. *H.nana* was also observed commonly in findings at St. Peter's Specialized Hospital in Addis Ababa and Mizan-Tepi University while *T.trichiura, S.mansoni, cryptosporidium spps* and *Isospora belli* were seen only in investigation conducted at Mizan-Tepi University. The most frequently detected parasites were *G. lamblia*, followed by *Ascaris lumbricoides* and *E. histolytica/dispar* in Guji, Entamoeba histolytica/dispar followed by *Giardia intestinalis* and *Taenia species* in Addis Ababa and *Entamoeba histolytica/dispar* followed by *Giardia lamblia* in Mizan-Tepi University(Abayneh *et al.*, 2022),(Muleta *et al.*, 2021),(Aliyo *et al.*, 2022)

2.2. Prevalence of opportunistic intestinal coccidian in HIV seropositive patients

Opportunistic intestinal parasites (OIPs) known as coccidian parasites include *Cryptosporidium species*, *Isospora belli* and *Cyclospora cayetanensis* and these parasite diseases are more prevalent in people with HIV/AIDS .Data from a global systematic review and meta-analysis on the opportunistic protozoa infection in HIV patients indicates *Cryptosporidium* spp pooled prevalence found in the range of 0.09- 44% among HIV patients, concurrently recorded in regions such as Africa (2.2-44%), Asia (1.5- 37.9%), Europe (0.09-5.7%), and Latin America and Caribbean (3.3-40.6%). Prevalence of *C. cayetanensis* among the group was ranges from 0.7-36.9% globally. Similarly, high prevalence rates have been observed in populations of Africa (0.7-28.9%), Asia (0.7-24%), Europe (0.7-5.7%), and Latin America and the Caribbean (1.8-36.9%). Moreover, *I. Belli* demonstrated prevalence values ranging from 0.4-31%, which is consistent with data from communities in Asia, Europe, and Latin America and the Caribbean, as well as Africa (0.7-13.3%, 0.4-31.0%, and 3.2-9.9%) respectively(Contreras-Puentes *et al.*, 2020).

Study done in Nepal among HIV patients displayed coccidian parasites comprised of 57.9% of total parasitosis. Out of total coccidian parasites identified *Cryptosporidium spp* was the most prevalent parasite followed by *Cyclospora cayetanensis* and *Isospora belli* respectively(Sah *et al.*, 2017). Similarly research carried out at a tertiary HIV care center in southern part of India indicated that coccidian parasitic infection accounted for about 23.4% of overall parasitic infections, and of these, *Cystoisospora belli* was observed to be the most common cause of diarrhea followed by *Cryptosporidium* spp. and *Cyclospora cayetanensis*. Among the HIV patients with CD4+ T-cell counts <200 cells/µl, *Cryptosporidium* infection was most common followed by infection with *C. belli*. This show that the CD4+ cell play significant role in countering coccidian infection(Swathirajan *et al.*, 2017). Moreover investigation performed in Turkey with 115 HIV positive patients *Cyclospora* and *Cryptosporidium spp*. oocysts were detected in one and two patients, respectively, by staining(Uysal *et al.*, 2017)

Studies at Uganda and Côte d'Ivoire indicated that prevalence of intestinal coccidia parasites in HIV patients were 5.4 and 6.3% respectively from which *Cryptosporidium spp* was the most predominantly detected spp followed by *I. belli and C. cayatenensis* among the group(Assi Fiacr *et al.*, 2021),(Echoru *et al.*, 2015). Similarly finding carried out at Zambia told us overall prevalence of *Cryptosporidium* infection was 9.5% in HIV infected individual(Sinyangwe *et al.*, 2020).

Study carried out on 264 HIV-positive patients at Yirgalem Hospital laid out that prevalence of opportunistic intestinal coccidia was 37.3% with *Cryptosporidium* was the most dominantly detected parasite followed by I.belli and rate of mixed infection in the investigation was 0.75%. Conclude that studies discussed above were indicating that opportunistic intestinal coccidian parasite specifically *Cryptosporidium spp* that account for large portion of morbidity and mortality among the group is still prevalent(Girma *et al.*, 2014).

2.3. Determinant factors

Potential factors determining the transmission of intestinal parasites among HIV infected individuals have been proposed throughout variety of literatures conducted at different part of the world. Correspondingly, studies in Brazil, Côte d'Ivoire, Debretabor General Hospital, Dessie Referral Hospital, St. Peter's Specialized Hospital Addis Ababa, Dessie Comprehensive Specialized Hospital's, Mizan-Tepi university teaching hospital, Aksum, Adigrat, University of Gondar and Guji south Ethiopia identified that CD4 level of HIV positive patients was significantly associated with overall prevalence of intestinal parasite and also its considered as determinant factor for availability OIPs in the study conducted at Arbmench (Barcelos *et al.*,2018),(Assifiacr *et al.*, 2021) ,(Alemayehu *et al* .,2020),(Feleke *et al.*,2022),(Bisetegn *et al.*,2022),(Muleta *et al.*,2021),(Abayneh et *al*.,2022),(Gebrewahid *et al.*, 2019),(Aliyo *et al.*, 2022).

Other clinical factors were also identified in different studies for instance research carried out at Mizan-Tepi university teaching hospital among HIV patients told us that WHO stage (stage II and III) and viral load(>150 copies/ml) were also significantly associated with intestinal parasitic infection. Similarly WHO stage III was defined as predisposing factor for presence of intestinal parasitic infection in study conducted at University of Gondar Northern Ethiopia (Eshetu *et al*.,2016),(Abayneh *et al*., 2022).

Findings at Cameron, Zambia, Uganda, University of Gondar and Guji south Ethiopia were identified source of drinking water as risk factor for prevalence intestinal parasitic infection among HIV patients with those who use lake/spring/river were more vulnerable for intestinal parasite when compared to those who use tap water(Sinyangwe *et al.*, 2020),(Echoru *et al.*, 2015),(Tebit *et al.*,2014),(Eshetu *et al*.,2016),(Aliyo *et al.*,2022).

Latrine was filtered as associated factors for prevalence intestinal parasite among HIV patients in the study conducted at University of Gondar northern Ethiopia in which those groups with lack of toilet at their home were highly infected with intestinal parasite infection(Eshetu *et al*.,2016). Additionally there was a statistically significant association was observed between prevalence of cryptosporidiosis and toilet in the research conducted Felegehiwot Referral Hospital, Bahir Dar. Moreover Unavailability of latrine was associated with *E. histolytica/dispar* and *G. lamblia* prevalence specifically in finding carried out at Adigrat northern Ethiopia among HIV infected groups(Mahmud *et al.*,2014),(Kiros *et al.*, 2015).

Animal contact was identified as risk factor for intestinal parasitic infection among HIV infected patients in studies conducted at different area throughout the world for instance finding carried out in Cameron and Zambia were the witness with those who frequently contact with animal were highly infected by IPIs (Tebit *et al.*,2014),(Sinyangwe *et al.*, 2020). Similarly, it also found that contact with domestic animals was determinant factor for presence of OIPs in the study conducted at Arbmench south Ethiopia(Getaneh *et al.*,2017).

Sex was also another identified predisposing factor in findings carried out at Zambia, Côte d'Ivoire and cape with male participants were highly infected with intestinal parasitic infection when compared with female participants(Assifiacr *et al.*, 2021),(Sinyangwe *et al.*, 2020). Study conducted at Dessie Referral Hospital north Ethiopia alarmed us that hand washing habit after latrine (those who haven't habit of hand washing after defection were more infected with intestinal parasite) , habit of eating raw vegetables and eating raw meat were statically significant factors for prevalence of intestinal parasitic infection among HIV patients(Feleke *et al.*, 2022). Investigation performed at Dessie Comprehensive Specialized Hospital's ART clinic north Ethiopia was also identified habit of eating raw fruit and vegetables as predisposing factors for prevalence intestinal parasitic infection among study participants(Bisetegn *et al.*, 2022).

Studies carried out in 2021 at Mizan-Tepi university teaching hospital and Aksum northern part of Ethiopia among HIV patients were displayed that consuming raw food and eating unwashed raw vegetables were significantly associated variables with intestinal parasitic infection which related with environmental and hygienic practices(Abayneh, 2022),(Gebrewahid *et al.*, 2019).

In study at St. Peter's Specialized Hospital Addis Ababa not trimming or cleaning one's fingernails was explored as predisposing factor for prevalence of intestinal parasite among HIV infected with those who have habit of trimming nail were less vulnerable for the infection(Muleta *et al.*, 2021). Study conducted at Felegehiwot Referral Hospital, Bahir Dar and St. Peter's Specialized Hospital in Addis Ababa displayed status of being diarrheic were significantly associated with the overall prevalence of intestinal parasite and finding conducted in Burkina Faso again observed being diarrheic was determinant factor for prevalence of OIP and OIC respectively(Adama *et al.*, 2017),(Kiros *et al.*, 2015).

Studies among 264 HIV-positive patients in Yirgalem Hospital south Ethiopia and Felegehiwot Referral Hospital, Bahir Dar displayed those with *Cryptosporidium spp*. were also more likely to have chronic diarrhea, weight loss, and chronic vomiting when compared with individuals who didn't infected with parasite(Girma *et al.*, 2014),(Kiros *et al.*, 2015). The finding carried out at Cameron and Debretabor General Hospital north Ethiopia identified educational status as determinant factor for prevalence of IPIs among HIV infected patients with those illiterate and only read and writes study groups were more infected with intestinal parasite. (Tebit *et al.*,2014),(Alemayehu *et al.*,2020). Additionally in multivariate analysis, patients with illiterate had higher rate of opportunistic intestinal parasite in study conducted at Burkina Faso(Adama *et al.*, 2017).

Research performed at Cameron told us occupational status was risk factor for prevalence of intestinal parasitic infection among HIV sero-positive patients with high prevalence was observed among unskilled workers and also there was a statistically significant association between the prevalence of cryptosporidiosis and work status in the study conducted at Felegehiwot Referral Hospital, Bahir Dar(Tebit *et al.*,2014), (Kiros *et al.*, 2015).

Studies in Zambia and Mizan-Tepi university teaching hospital south west Ethiopia among HIV patients verified that marital status was major socio-demographic determinants significantly associated with overall prevalence of intestinal parasite with that high prevalence of intestinal parasitic infection was observed among single status when compared to those of married study participants(Abayneh *et al.*,2022),(Sinyangwe *et al.*, 2020). Exploration in Uganda observed age as determinant factor for presence of intestinal parasite among HIV infected patients whereby the prevalence was tending to be highest in children aged 10-19 years being twice more than occurrence in participants of age group 20-39 years(Echoru *et al.*, 2015).

Investigation conducted at Dessie Referral Hospital north Ethiopia revealed that eating raw meat was statically significant factors for intestinal parasitic infection among HIV infected patients participated in the study(Feleke *et al.*, 2022). Improper hand washing before meal had significant association with prevalence of intestinal parasites among HIV sero-positive patients at study carried out in Aksum(Gebrewahid *et al.*, 2019).

Fact-findings carried out at Mizan-Tepi university teaching hospital south Ethiopia and Kombolcha north Ethiopia among HIV patients told us drinking untreated water was significantly associated variable related with environmental and hygienic practices (Gebretsadik *et al.*,2018),(Abayneh et *al.*,2022). Studies at Mizan-Tepi university teaching hospital south west and Dessie Comprehensive Specialized Hospital north Ethiopia and among HIV patients shows residence of the study participants was identified as the major socio-demographic determinants for intestinal parasitic infection with higher rate of infection was observed among rural residence in the former study whereas urban dwelling was more infected with intestinal parasitic infection when compared to those of rural in the latter study (Bisetegn *et al*., 2022). Residence is again identified as predisposing factor in study conducted at Felegehiwot Referral Hospital, Bahir Dar north Ethiopia With regard to giardiasis that urban dwelling were more infected with infection among HIV infected participants(Kiros *et al.*, 2015).

In generally Intestinal parasitic infections are still common health problems among HIV/ADIS patients. So, combination of public health and clinical strategies is required in the prevention and control of intestinal parasites. Additionally health education on improving personal hygiene and environmental sanitation should be given to tackle the problem.

Conceptual framework

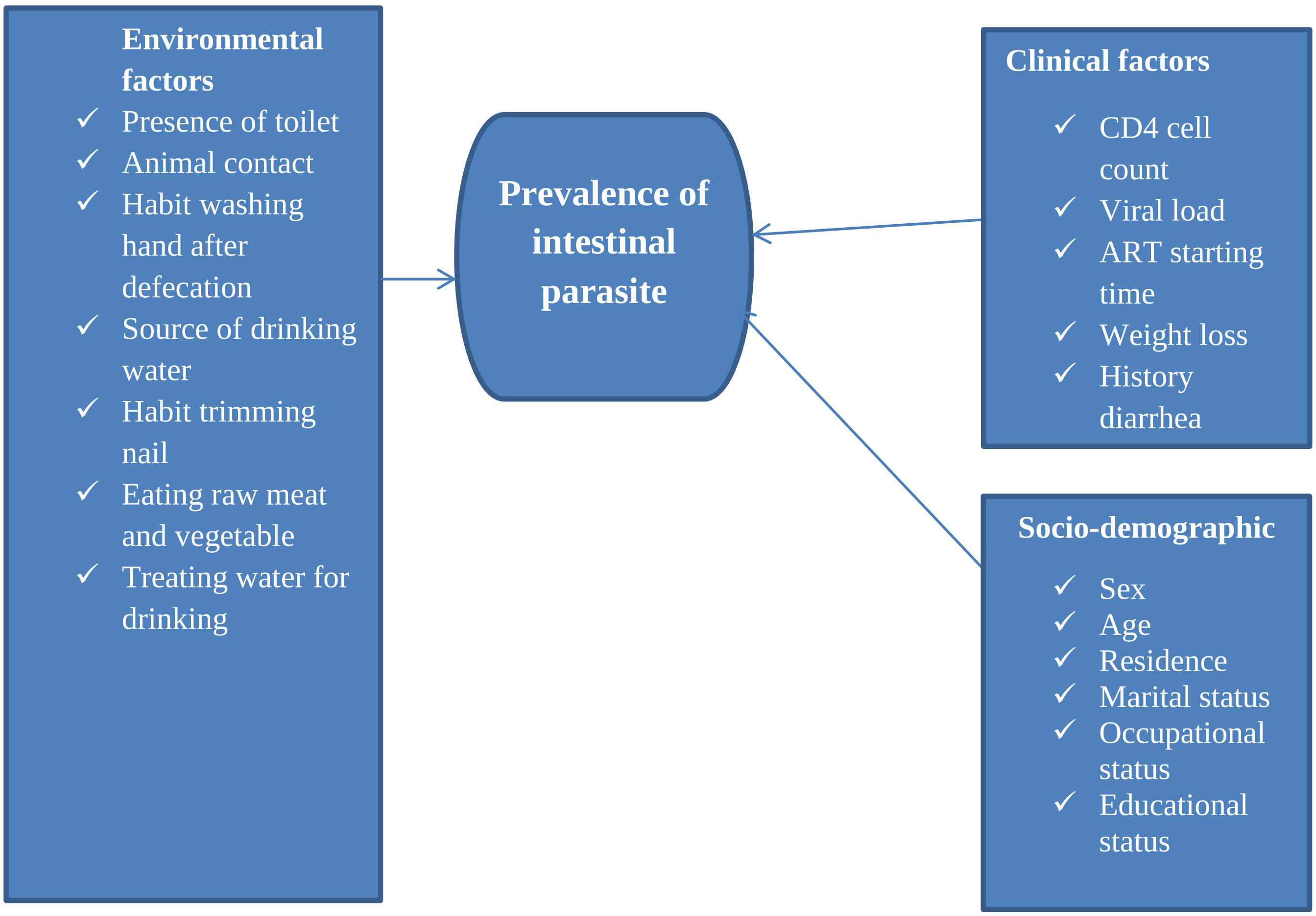

Figure 2: Conceptual framework for determining the prevalence of intestinal parasite with special emphasis on intestinal coccidia and risk factors among HIV patients attending ART clinic at Worabe comprehensive specialized hospital, Worabe, central Ethiopia. Adopted from different literatures (Echoru *et al.*, 2015 ; Eshetu *et al*.,2016; Getaneh *et al.*,2016;Abayneh *et al.*, 2022; Aliyo *et al.*,2022; Assifiacr *et al.*, 2021; Feleke *et al.*, 2022; Bisetegn *et al.*, 2022; Muleta *et al.*, 2021; Alemayehu *et al.*,2020)

CHAPTER III: OBJECTIVE

3.1. General Objective

To assess prevalence of intestinal parasite with special emphasis on opportunistic intestinal coccidia and Predisposing Factors among HIV sero-positive patients attending ART clinic at Worabe Comprehensive Specialized Hospital, Worabe central Ethiopia

3.2. Specific Objective

- To determine prevalence of opportunistic intestinal coccidia and other intestinal parasite infection among HIV patients attending ART clinic
- To identify associated risk factors among HIV patients attending ART clinic.

CHAPTER IV: MATERIALS AND METHODS

4.1. Study area and period

Study was conducted from May 2022 to September 2023 at Worabe Comprehensive Specialized hospital. The hospital is located in Worabe town capital city of Silte zone, located at 172 km to South of Addis Ababa, Ethiopia. The town was found at latitude of 7° 51'N, the longitude of 38° 11'E and altitude of 2113 meters above sea level (*Distancefrom.com*). According to the 2007 national housing and population census, the projected population of Worabe town was about 508,252 and estimated households were 33883. Based on gender 284067 are males and 224185 are females .In the town hotels, floor factors, retail and wholesale, wood and metal works and different governmental and non-governmental organization are there. The town has one Comprehensive Specialized hospital, two health centers, six higher private clinics, and one family guidance association of Ethiopia (*Worabe town administrative health office*).

Worabe Comprehensive Specialized Hospital was established in 2007 G.C and serving more than 1 million population annually. The catchment areas for the hospital include Gurage zone and Alaba zone from central Ethiopia region and east shoa zone from Oromia regional states. Also serves as a referral center for health institutions in Silte Zone. It provides different health services such as outpatients, inpatients, pharmacy and laboratory with full service. The hospital has been providing counseling and testing service for HIV patients since it has been established and currently around 954 HIV patients are following the hospital's ART Clinic. (*WCSH administrative office*)

4.2. Study design

Institutional based cross-sectional study was conducted at Worabe comprehensive specialized hospital of ART Clinic from May 2022 to September 2023 to determine the magnitude and predisposing factors of opportunistic and other intestinal parasite among HIV patients following ART.

4.3. Population

4.3.1. Source Population

All HIV patients attending ART clinics at Worabe Comprehsive Specialized Hospital

4.3.2. Study Population

All HIV-positive individuals attending ART clinics, those who fulfilled inclusion criteria and gave assent/consent during data collection period

4.4. Eligibility Criteria

Inclusion Criteria

All HIV-positive individuals linked to the ART clinic (on ART) were included in the study.

Exclusion Criteria

Individuals, those on specific anthelminthic, anti-protozoan, or who had antacids and treatment for intestinal parasitism in the last two weeks preceding specimen collection, not willing to participate and couldn’t bring stool samples were excluded. Additionally severely ill patients who failed to respond to questions were also excluded. The use of antacids can distort protozoan morphology contributing to the difficulty in identifying the organism.

4.5. Sampling technique and Sample Size Calculation

A convenient sampling technique was employed. Samples were collected consecutively until the required sample size was achieved. Sample size for the 1st objective was calculated using single population proportion formula by assuming confidence interval of 95%, margin error of 5%, the prevalence opportunistic and other intestinal parasites among people with HIV/AIDS was 38.1%(Bisetegn *et al.*, 2022) , which were reported in previous study in Ethiopia.

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

where; n=estimated sample size

Za/2= standard normal value which corresponds 95% of confidence level =1.96

P=prevalance of opportustic and other intestinal parasite

d=tolerable sampling error

$n= (1.96)^2 0.38.1(1-0.38.1)/(0.05)^2$

n=362

Sample size for the 2nd objective was determined using double population proportion formula.

Table 1: Summary of sample size determination for 2nd specific objectives (associated factors) by using Epinfo version 7.2.4 to study prevalence and predisposing factor of opportunistic and other intestinal parasite among HIV patients attending ART clinic,2023.

Variables	Proportion(exposed vs unexposed	Ratio	Power	CI	OR	n	Reference
Consuming raw meat	98.5 and 15%	1	80	95	0.002	10	(Abayneh, 2022)
washing vegetable	29.8 and70.2%	1	80	95	5.5	56	(Abayneh, 2022)
Animal contact	24.49 and 12.3%	1	80	95	0.43	348	(Getaneh *et al.*, 2017)
CD4+ status	24.1 and 6.3%	1	80	95	0.21	148	(Getaneh *et al.*, 2017)

CI: confidence interval, OR: odd ratio, n: sample size

N.B The sample size of second objective was found to be less than the first objective .Therefore; the final sample for the study was 400 after adding 10% non-response rates.

4.6. Data collection

A pre-structured questionnaire through face-to-face interview by counselor nurse was used to collect data regarding socio-demographic characteristics and associated factors for opportustic and other intestinal parasites among patients. The duration on treatment and other information including the most recent CD4 cells counts and viral load were obtained from HIV care clinic registers (esp. ART register). For the purpose of parasitological examination, each study participant was instructed how to collect about 3-5g (equal with container spoon) of stool with plastic stool cup (***Henson***®) and toilet paper. Each sample were labeled with specific code number, and transported to the laboratory within 30 minutes of collection for parasitological analysis. Direct wet mount and concentration techniques were performed to diagnose actively motile protozoan parasites together with other helminthes. Intestinal coccidian parasites were diagnosed using MZN stain.

Laboratory analysis

Fresh stool samples were collected from these patients. In the first time, macroscopic analysis of stool sample was done to determine the consistency, color of the fecal material and element present in the feces such as mucus, blood, and eventually worms (Taenia segment). Secondly collected stool was preserved in 10% formalin in a proportion of 5g of stool in 1.5ml of formalin solution for microscopic examination.

Wet mount technique

A small portion of the fresh specimen was used for direct saline wet mount (0.85% sodium chloride solution) to detect trophozoites of E.histolytica/dispar and other diagnostic stage of helminths and Lugol's iodine solution (for detecting the cystic stage of intestinal protozoan parasites) and the preparation was examined by light microscope at low (10X) and high power (40X) objective lenses as described elsewhere(Taye *et al.*, 2014).

Formol-ether concentration method

Briefly, 1g of stool was placed using an applicator stick in a clear 15ml conical centrifuge tube containing 7ml of formalin saline. The resulting suspension was filtered through a sieve into another conical tube. After adding 3ml of diethyl ether to the formalin solution, the content was centrifuged at 3,200 rpm for 3 min. The supernatant was poured away and sediment was re-suspended. Finally, a smear was prepared from the sediment and observed under a light microscope with 10x and 40x objectives. It's best to detect cyst of protozoa (*E.histolytica/dispar*, *Giardia lamblia)* and eggs of helminths(Alemu *et al.*, 2011), (Bisetegn *et al.*, 2022).

Again concentration method using sodium chloride solution was used to identify hookworm, tapeworm (*Hymenolopis nana*) and *Ascaris lumbricoides*. 1g of stool sample was mixed with 2ml of saturated sodium chloride in beaker and then even suspension was made. Secondly, the solution was sieve in to 15ml of test tube. Thirdly, saturated sodium chloride was added at top of filtered solution almost have to top and final drop of saturated sodium chloride solution was added with help of plastic pipette to make convex solution surface over the brim and coverslip was placed at the top of solution for 30-45 min. Finally coverslip was gentle removed and placed on microscopic slide and examined at 10x and 40x objectives (Zeleke *et al.*, 2021).

Modified Ziehl-Neelsen staining method

A small portion of the fresh stool sample was processed for detection of opportunistic parasites using the MZN method. Concentrated smear from concentration technique of the stool was made on a clean grease-free slide and fixed with methanol for 3 minutes after air dry. Smear was floated with cold carbol fuchsin for 15 minutes and rinsed with water. Preparation was decolorized with 1% acid alcohol (5mL of 37% hydrochloric acid and 495mL of 70% ethanol) for 15 seconds. After rinsing again in tap water, the slide was counter-stained with methylene blue for 30 seconds. Smear examined microscopically for oocysts using a low-power magnification and oil immersion objectives after air drying(Tahvildar-B et al., 2014).

4.7. Operational definitions

ART patients: People who are HIV positive and get started ART follow up.

Opportunistic infections: Infections that frequently occur in individuals with a damaged or weakened immune system (e.g., due to HIV/AIDS)

Diarrhea: The passage of three or more loose or liquid stools per day, or more frequently than the normal bowel habit.

Acute diarrhea: diarrhea that lasted for < 14 days.

Chronic diarrhea: Diarrhea lasting for > 14 days.

Intermittent diarrhea: diarrhea that occur with irregular intervals.

Single infection (monoparasitism): presence of single parasite in a single individual.

Mixed infection (polyparasitism): presence of two or more parasites in a single individual

Intestinal parasitic infection: defined as those who had their stool analysis positive for one or more parasites in many forms: cyst, trophozoite, eggs and/or adult.

4.8. Data Quality management

Structured questionnaire were prepared precisely and translated into the local language Amharic. Additionally pilot study was conducted among 5% of prepared questionnaire at Kibet primary hospital before the actual data collection. Training was given to all data collectors' prior data collection. Every questionnaire was coded for each patient. Daily close supervision was made by principal investigator. All laboratory materials and reagents were checked for expiry and stored appropriately. Performance of the microscope was checked with preserved positive slide. The completeness of data was checked in a daily manner by the main investigator. Liquid stool, sem-solid stools and formed stools were examined within 15-30 min, 1 hr. and up to 24 hours after collection respectively. The quality of stool smear was evaluated by observing the stool smear through newspaper .To minimize missed parasite identification and discrepancy, each slide was re-examined by the senior laboratory technologist. Additionally, coccidian and other intestinal parasite identifications were checked against colored atlas. Moreover positive slides were re-detected by senior laboratory technologist. The results of laboratory examination was recorded on well prepared format carefully and finally attached with the corresponding questioner.

4.8. Study Variable

Dependent Variable

Prevalence of opportunistic intestinal coccidian and other intestinal parasites among ART patients

Independent Variables

Sex
Age
Educational status
Occupation
Trimming nail
Habit of walking on bare foot
Residence
Marital status
Use latrine
Monthly income
Habit of washing fruits/vegetable
Swimming habit
Habit of eating raw meat
Hand washing habit before meal
Hand washing habit after defecation
Water source for drinking
Treatment of water before drinking
Contact with Domestic animals
History of diarrheal in < 3 months
Diarrhea status
ART starting time
CD4 status
Viral load
Reported weigh loss

4.9. Data processing and analysis

Data was entered into Epi-info version 7.2 and analyzed by SPSS version 26 software (IBM Armonk, N.Y.). Descriptive statistics were used to narrate the characteristics of the study population. Data was presented using bar-graph, pie-chart and tables. Binary logistic regression was used to assess the presence and strength of association between the outcome and predictor variables. Multivariate regression analysis was then applied for variables with $p \leq 0.25$ in the bivariate analysis. Again Chi-X^2 was also employed to show the correlation of the dependent variable with the individual independent variable. Association between variables was considered statistically significant only if P-value ≤ 0.05 at 95% confidence level.

4.10. Ethical Clearance

After being thoroughly evaluated and checked for appropriateness, the study got approval by the Ethics Review Board (IRB) of College of Medicine and Health Sciences, Hawassa University, and ethical clearance was attained. Then official collaboration and willingness were obtained from WCSH. After briefly explaining the purpose and objective of the study, written informed consent was taken from each study participant. For participants < 18 years old, written signed assent was obtained from their guardians. Additionally, participant's confidentiality was kept by avoiding personal identifiers and using codes. Positive results of study participants were immediately communicated with the ART clinic physician mentioning the parasitic species and they were treated with appropriate treatment protocol. Moreover, this study was conducted in accordance with the Declaration of Helsinki

CHAPTER V: RESULTS

5.1. Socio-demographic characteristics and associated factors of study subjects

A total of 400 patients (59.3 males and 40.7% females) participated in the study with a response rate of 100%. About 55.5% and 44.5% were urban and rural residence respectively. The majorities 37.8% were aged in between 30-39 with mean of age for study participants was 33.7 years (range, 15–69; SD, 16.8). Of the study participants, (62%), (41.3%) were married and merchant consecutively. Concerning their educational and income status majorities of them were from primary school (40%) and middle income level (51.7%) (Table2).

Table 2: Frequency of Socio demographic characteristics among HIV patients attending ART clinic at Worabe comprehensive and specialized Hospital, 2023 (N=400).

Variables	Category	Frequency	Percent
Sex	Male	237	59.3
	Female	163	40.8
Age group in years	10-19	20	5
	20-29	132	33
	30-39	151	37.8
	40-49	71	17.8
	≥50	26	6.5
Residence	Urban	222	55.5
	Rural	178	44.5
Marital status	Single	65	16.3
	Married	248	62
	Divorced	53	13.3
	Widowed	34	8.5
Occupational status	Gov’t employed	25	6.3
	Self-employed	37	9.3
	Non-employed	27	6.8
	Student	46	11.5
	Merchant	165	41.5
	Farmer	57	14.2
	House wife	28	7
	Other	15	3.8
Income status	High income	104	26
	Middle income	207	51.7
	Low income	89	22.3

Regarding to environmental predisposing factors, almost more than 75% of study participants didn't have exposure with the exception of 83.3% of the study respondents who had no habit of treating water for drinking *(Table3).*

Table 3: Frequency of environmental predisposing factors of HIV patients attending ART clinic at Worabe comprehensive and specialized Hospital, 2023 (N=400).

Variable	**Category**	**Frequency**	**Percent**
Animal contact	Yes	103	25.8
	No	297	74.3
Presence of toilet	Yes	374	93.5
	No	26	6.5
Habit of eating raw meat	Yes	33	8.3
	No	367	91.7
Hand washing habit after defecation	Always	357	89.3
	Sometime	21	5.3
	Never	22	5.5
Hand washing habit before meal	Always	363	90.8
	Sometime	22	5.5
	Never	15	3.8
Treatment of water	Always	28	7
	Sometime	39	9.8
	Never	333	83.3
Habit of washing vegetable	Yes	374	93.5
	No	26	6.5
Swimming habit	Yes	42	10.5
	No	358	358
Habit walking barefoot	Yes	31	7.8
	No	369	92.3
Source of drinking water	Tap	383	95.8
	River/spring/lake	17	4.2

5.2. Clinical characteristics of study participants

The CD4 cell distribution of the study participants indicates majority of them,(56%) were having CD4 count more than 500 cells/mm3 with the mean count of 536 cell/mm^3, median 538 cell/mm^3 and (range 123-1234) .Additionally, more than half (57.8%) of study participants were having viral load <50 copies/ml. Furthermore out of the 400 study participants, (47.5%) started ART follow up 2-5 years ago with the range of 1 week-7yrs ART follow up and majority of the respondents had no history of weight loss (89.5%)(*Table 4*)

Table 4: Frequency of clinical characteristics among HIV patients attending ART clinic at Worabe comprehensive and specialized Hospital, 2023 (N=400).

Variables	Category	Frequency	Percent
CD4-level	<200 cell/mm^3	38	9.5
	201-499 cell/mm^3	138	34.5
	>500 cell/mm^3	224	56
Viral load	>200 copies/ml	38	9.5
	50-200copies/ml	131	32.8
	<50 copies/ml	231	57.8
ART starting time	<2 years ago	120	30
	2-5 years ago	190	47.5
	>5 years ago	90	22.5
Weight Loss	Yes	42	10.5
	No	358	89.5
History of diarrhea <3 month	Yes	49	12.3
	No	351	87.8

5.3. Stool consistence and diarrhea prevalence in the study participants

The present finding identified that most of study respondents stool consistence was soft with 65% (260/400) (*Figure 3).*Among the study participants, diarrhea occurred in 49/400 (12.25%) individuals (Table 4). Of 49 diarrheic individuals, chronic diarrhea was reported in 24/49(48.9%) of the study participants (*Figure 4).*

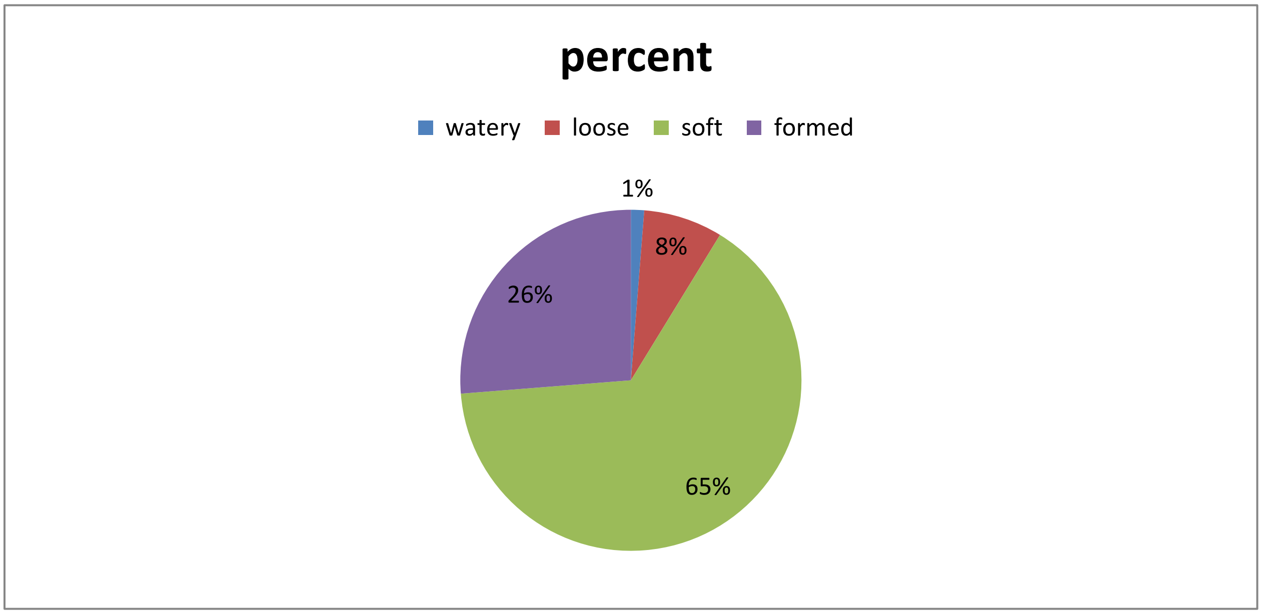

Figure 3: Stool consistency of HIV/AIDS patients attending ART clinic at Worabe comprehensive and specialized Hospital, 2023 (N=400).

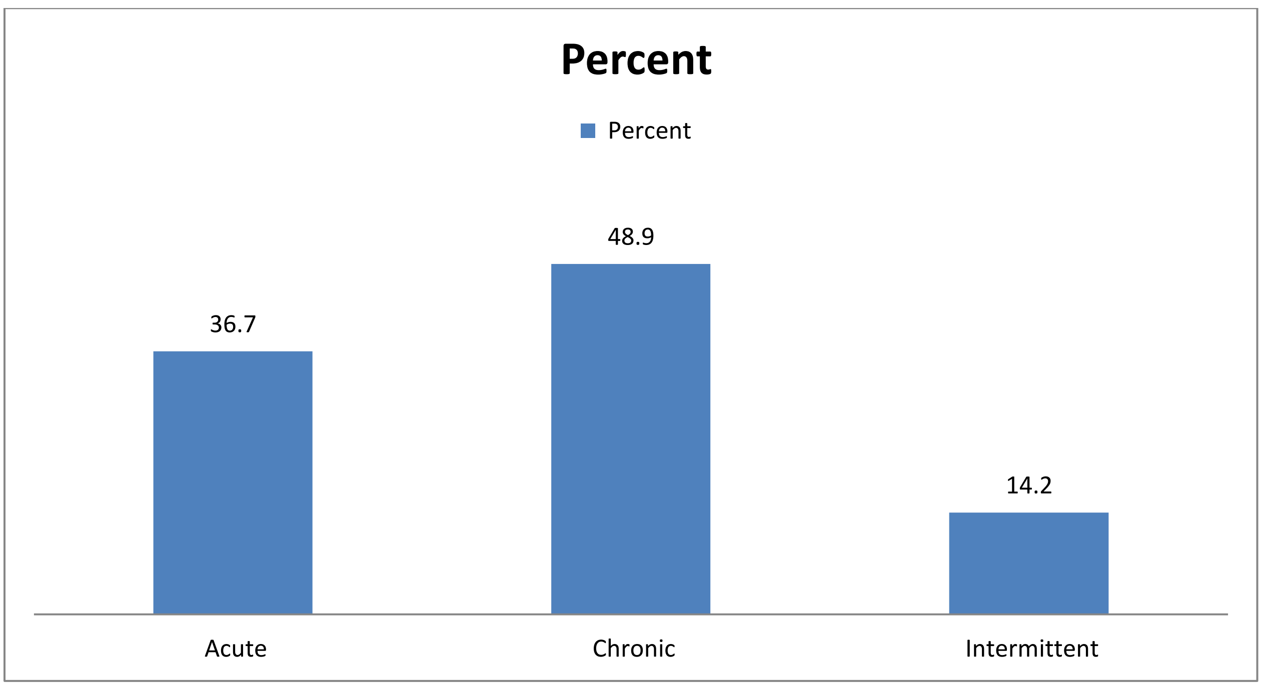

Figure 4: Diarrhea status of HIV/AIDS patients attending ART clinic at Worabe comprehensive and specialized Hospital, 2023 (N=400).

In our finding different laboratory technique such as wet mount, concentration and modified zheil Neelson were employed for detection of both opportunistic and non-opportunistic intestinal parasite among study participants. High detection rate was observed in wet mount technique (normal saline and iodine) followed by concentration technique (formol ether and saturated sodium chloride) with 36 and 23% respectively *(figure 5)*.

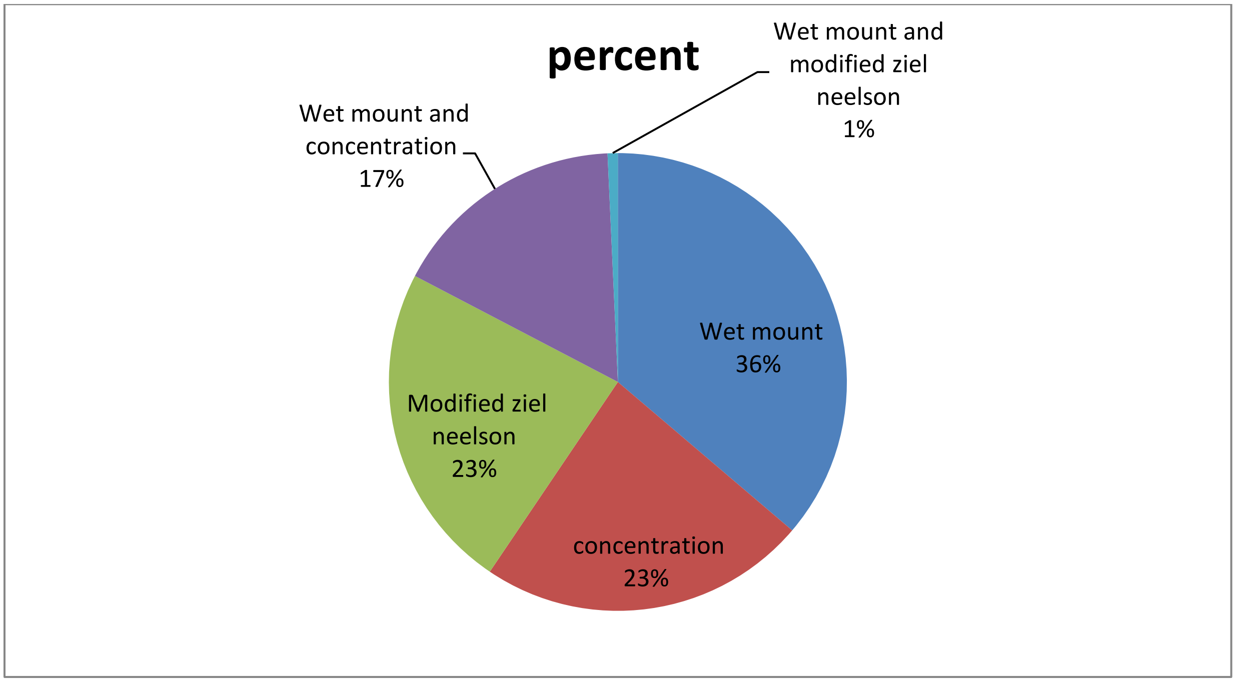

Figure 5: Laboratory techniques employed with their detection rates among HIV patients attending ART clinic at Worabe comprehensive and specialized Hospital, 2023 (N=400).

5.4. Prevalence of intestinal parasites among study participants following ART clinic at WCSH

Various types of non-opportunistic and opportunistic intestinal parasites including intestinal coccidia, intestinal flagellate, amoebae, cestodes and intestinal nematodes were detected. Eleven different intestinal parasites species were detected *(Figure 6).*The overall prevalence of intestinal parasites among the group was 34.5%, from which 76.1 % , 23.9 % were infected with single and mixed parasites respectively. Non-opportunistic parasites were more prevalent in the study 24%.

Prevalence of helminthes and protozoa were observed 40.8% and 59.2% respectively (*Figure 6*) .As far as co-infection is concerned, double and triple were identified in 90.9% and 9.1% respectively. *Ascaris lumbricoides* 24.1% was the most predominantly detected parasite followed by *Entameoba histolytica/dispar* and *Gardia lamblia* 20.6% and 19.5% respectively. On the other hand, *Isospora belli* 1.1% and *H.nana* 0.5% were the least detected parasites *(Figure 6). A.lumbericoides* and *G. lamblia* were the most common parasite combinations detected *(Figure 7).*

Prevalence of opportunistic intestinal parasites (*S. stercoloaris*, *Cryptosporidium spps* and *I. beli*) was 10.5% and from these 7.75% was due to *Cryptosporidium species* followed by S.stercoralis 2.3%. Prevalence of intestinal coccidian in present study was 8.25% .*Cryptosporidium spp.* and *Isospora belli* were among identified coccidian species with 17.8 and 1.1% respectively. *Cyclospora cayentensis* was not identified in present study *(Figure 6).*

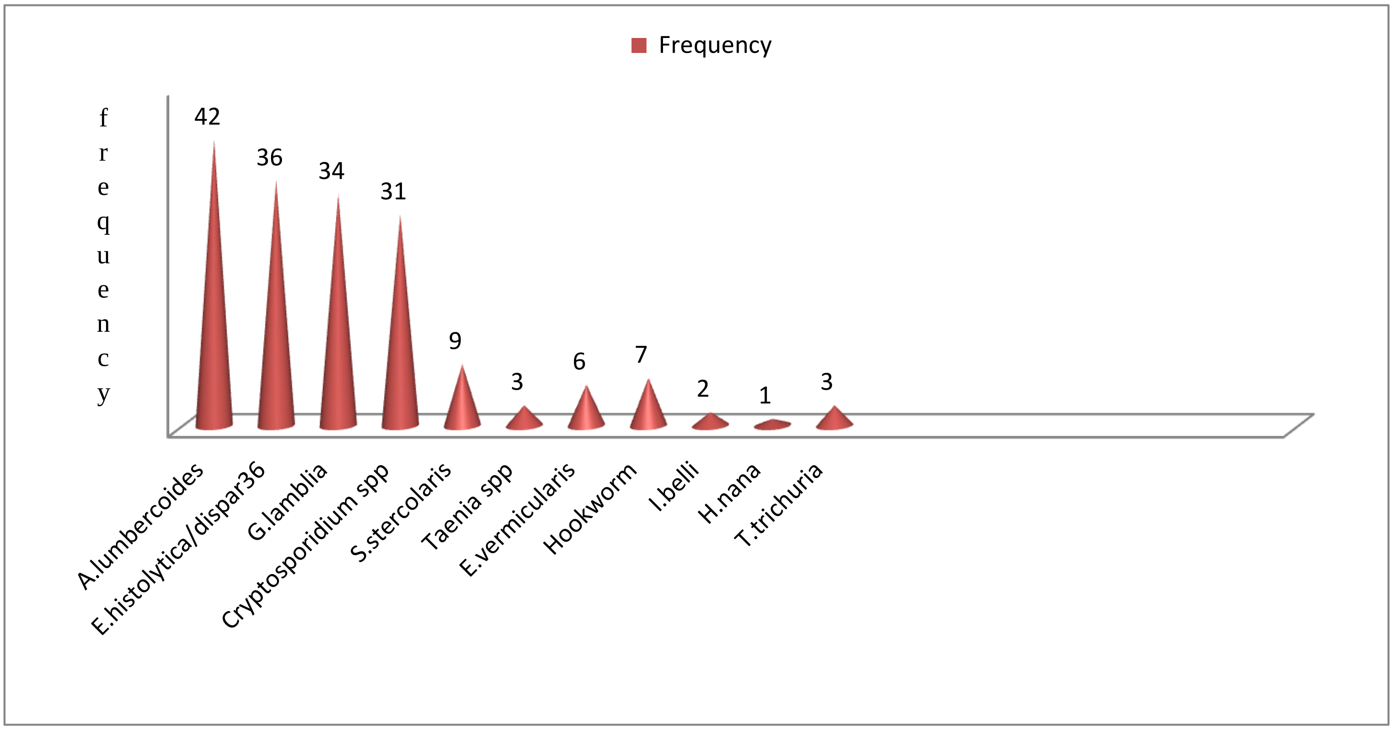

Figure 6: Intestinal parasite species detected among HIV patients attending ART clinic at Worabe comprehensive and specialized Hospital, 2023 (N=400).

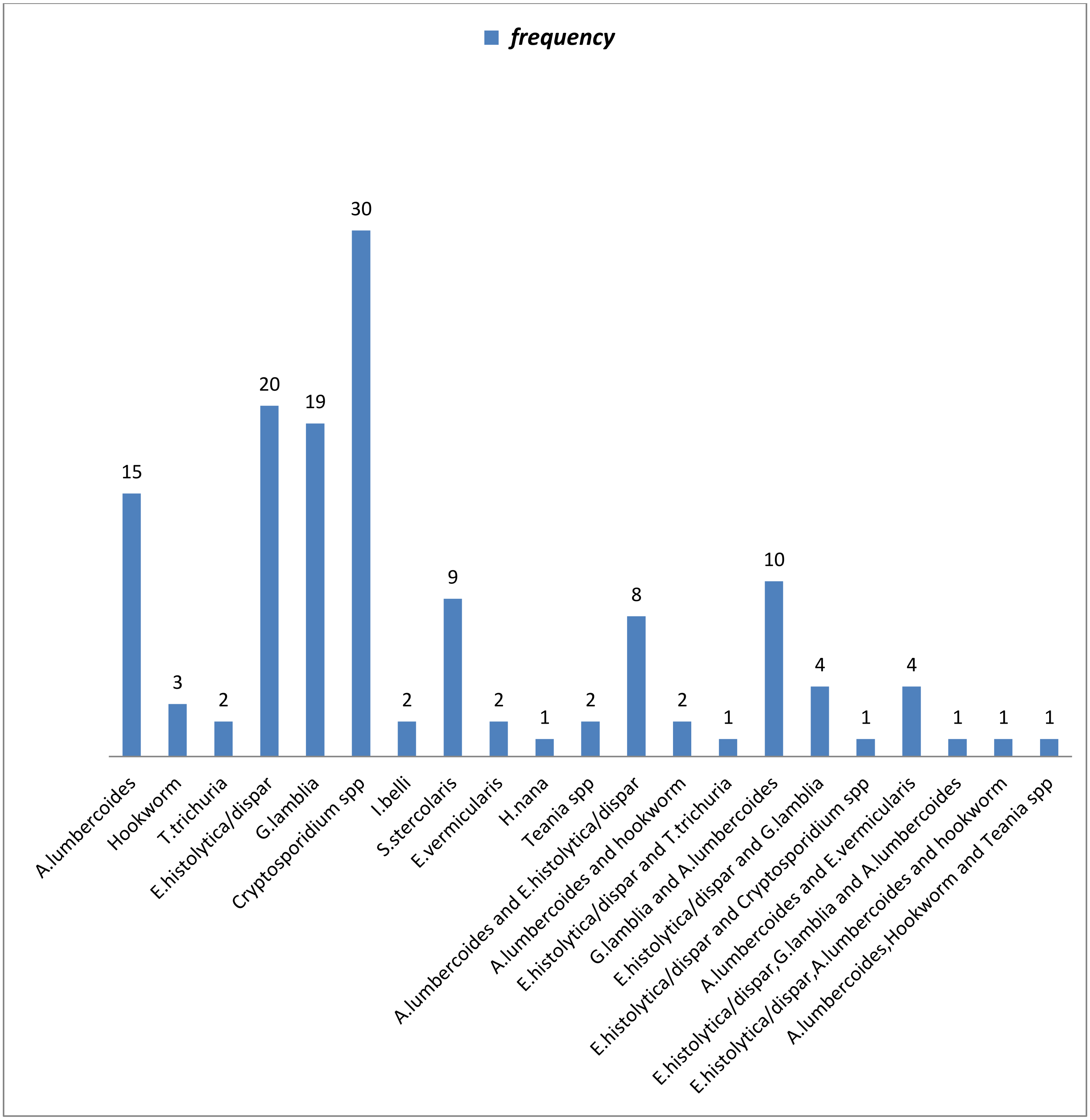

Figure 7: Distribution of intestinal parasites among HIV patients attending ART clinic at Worabe comprehensive and specialized Hospital, 2023 (N=400).

5.5. Over all prevalence of intestinal parasite in relation with socio-demographic characteristics of study participants

The present study revealed that high rate of IPIs was observed in the age group of 20-29yrs (42%); with no significant difference observed [AOR=21.8, 95% CI: 0.85-558, p=0.062] . Additionally males were more affected by IPIs than females. Nevertheless, it's was not statistically significant [AOR=1.37, 95%CI: 0.36-5.2, p=0.64]. Furthermore, merchant (34.7%) was more affected by IPIs than government employee (4.3%). However, the difference was not statistically significant [AOR=1.5, 95%CI: 0.034-66.6, p=0.83] (Table 5). Concerning marital status participants who didn't married and divorced were [AOR=0.25, 95% Cl: 0.012-5.2; P=0.37], [AOR=0.44, 95% Cl: 0.037-5.4; P=0.53] less likely infected respectively with intestinal parasitic infection when compared with widowed group. Individuals who get married were [AOR=1.8, 95% Cl: 0.2-16; P=0.6] times more likely infected with intestinal parasites compared to their counterpart group; however the variable was not considered as predisposing factor in current study *(Table 5).*

Participants living in rural were [AOR = 1.66, 95% CI: 0.5-5.4; P=0.399] more likely infected with intestinal parasite when compared with those who dwelling in urban area but no significantly significant. Regarding to income status those groups with middle and low income level were [AOR=5.1, 95% Cl: 0.69-38; P=0.11], [AOR=4.68, 95% Cl: 0.91-24.2; P=0.06] more likely infected by intestinal parasite respectively when compared to those with high income level, beside no significant association *(Table 5).*

Of socio-demographic characteristics educational status was the only variable that significantly associated with overall prevalence of intestinal parasite among the study participants. The present study suggested that educational status specifically illiterate and primary level attendants were [AOR= 47.9, 95% Cl: 2.8-820.8; P=0.008], [AOR=25.3, 95% Cl: 2.9-219; P=0.003] more likely affected by IPIs respectively when compared with diploma and above level which were statically significant. *(Table 5)*

5.6. Overall prevalence of intestinal parasites in relation with environmental predisposing factors among study participants

HIV patients who didn't have toilets in their homes were 8.7 times [AOR=8.7, 95% CI: 0.76-99.7; p=0.082] more likely infected with intestinal parasites than those who have toilets with no significant association. In multivariate logistic regression analysis, patients who have swimming habit were [AOR=0.21, 95% Cl: 0.029-1.5; P=0.12] less likely infected with intestinal parasite infection when compared with their counterpart but no significant difference seen. Additionally, patients with habit of not washing vegetables were [AOR=0.68, 95% Cl:0.049-9.5;P=0.77] less likely contracted with intestinal parasite infection when standardized against those who have habit of washing vegetables and again it's not considered as risk factor among study participants. Furthermore, HIV patients who have habit of eating raw meat were 0.18 times [AOR =0.18, 95% CI: 0.016-2.1; p=0.17] less likely to infected with intestinal parasite when compared with those who haven't habit of eating raw meat .Yet, it's not considered as determinant for prevalence of intestinal parasite among the group (*Table5).*

In terms of the selected environmental factors; animal contact was significantly associated with overall prevalence of intestinal parasitic infections among HIV infected patients, as shown in (see Table 5) In multivariate logistic regression analysis, patients who had contact with domestic animals in their compound were more likely to acquire IPIs than patients who had no contact with domestic animals (AOR=24.8, 95%CI: 4.7-129.8, P< 0.001) *(Table 5)*

5.7. Overall Prevalence of intestinal parasites in relation with clinical characteristics of study participants

In this study, after multivariate analysis, as shown in *Table-5*, HIV patients whose CD4 counts < 200cell/mm^3 were (AOR = 4.9; 95% CI: 0.03-790; p = 0.54) more likely to be infected with intestinal parasite than those with CD4 counts >500 cell/mm^3 but it's not significantly associated with overall prevalence of intestinal parasitic infection. Regarding viral load ,HIV patients with viral load >200 copies/ml were (AOR = 2.3; 95% CI: 0.014-383; P= 0.75) more likely infected for intestinal parasite infection as equate with those having viral load <50 copies/ml. Paradoxically, those participants who have viral load 50-200 copies/ml were [AOR=0.43; 95% Cl:0.059-3.2;P=0.41] less likely infected with intestinal parasitic infection when compared to those <50 copies/ml virus in their blood but variable was not identified as predisposing factor in present study. As far as weight concerned those participant having history of weight loss were [AOR=0.36, 95% Cl: 0.04-3.2; P=0.36] less likely infected with intestinal parasite when compared to their complement with no significant difference was observed *(Table 5).*

In present study clinical variables as determinant factors for intestinal parasite infection were include ART starting time, diarrhea status and CD4 level. With respect to ART starting time, those who have been started ART <2 years ago were 32 times [AOR = 32.2, 95% CI: 6.9-151.3; p<0.001] more likely to infected with intestinal parasites than those who have started ART >5 years ago (Table 5). Similarly CD4 level; those with CD4 level 201-499 cell/mm^3 were 12.9 times [AOR=12.9, 95% Cl: 1.69-98.3; P=0.014] more likely infected with IPIs when compared with those having CD4 level >500 cell/mm^3. Moreover regarding diarrheal status, those HIV patients who had reported diarrhea were 18.1 times [AOR = 18.1, 95% CI: 2.3-138; p=0.038] more likely infected with intestinal parasites than those who hadn't history of diarrhea *(Table 5).*

Table 5: Bivariate and multivariable logistic regression analysis of intestinal parasite infection with associated factors among ART following patients at Worabe comprehensive and specialized Hospital, 2023 (N=400).

Variables	Category	Total examined N (%)	Parasite detected	COR (95%)	P-value	AOR (95%)	P-value
Age group in years	10-19	20 (5)	5 (3.6)	1.1 (0.28-4.3)	0.88	23.5(0.35-595.8)	0.14
	20-29	132 (33)	58 (42)	2.6 (0.99-6.9)	0.054	21.8(0.85-558)	0.062
	30-39	151 (37.8)	48 (34.7)	1.6 (0.59-4.1)	0.38	5.4(0.28-103.9)	0.26
	40-49	71 (17.8)	21(15.3)	1.4 (0.49-4)	0.53	1.3 (0.052-33)	0.87
	≥50	26 (6.5)	6(4.3)	1	-	1	-
Sex	Male	237(59.3)	64(46.4)	0.45(0.29-0.68)	<0.001	1.4(0.36-5.2)	0.64
	Female	163(40.8)	74(53.6)	1	-	1	-
Marital status	Single	65(16.3)	15(10.8)	0.3(0.12-0.73)	0.008	0.25(0.012-5.2)	0.37
	Married	248(62.3)	86(62.3)	0.53(0.26-1.09)	0.085	1.79(0.2-16)	0.6
	Divorced	53(13.3)	20(14.5)	0.61(0.25-1.5)	0.26	0.44(0.037-5.4)	0.53
	Widowed	34(8.5)	17(12.3)	1	-	1	-
Residence	Urban	222(55.5)	64(46.3)	1	-	1	-
	Rural	178(44.5)	74(53.6)	0.57(0.38-0.86)	0.008	1.66(0.5-5.4)	0.399
Educational status	Illiterate	40(10)	24(17.4)	5.8(2.7-12.6)	<0.001	47.9(2.8-820.8)	0.008*
	Primary	160(40)	83(60.1)	4.2(2.4-7.1)	<0.001	25.3(2.9-219)	0.003*
	Secondary	78(19.5)	6(4.3)	0.32(0.13-0.83)	0.019	7.9(0.6-104.4)	0.11
	Diploma and above	122(30.5)	25(18.1)	1	-	1	-
Occupational status	Gov't employed	25(6.3)	6(4.3)	1	-	1	-
	Self-employed	37(9.3)	9(6.5)	0.63(0.15-2.6)	0.52	6.3(0.16-249.9)	0.33
	Non-	27(6.8)	12(8.7)	0.6(0.17-2.4)	0.51	1.3(0.031-53.1)	0.89

	employed						
	Student	46(11.5)	12(8.7)	1.6(0.43-6)	0.48	5.2(0.19-143.7)	0.33
	Merchant	165(41.3)	48(34.7)	0.71(0.2-2.5)	0.59	1.5(0.034-66.6)	0.83
	Farmer	57(14.2)	37(26.8)	0.82(0.27-2.5)	0.73	0.29(0.014-6)	0.42
	House wife	28(7)	9(6.5)	3.7(1.1-12.3)	0.03	0.45(0.023-9.1)	0.61
	Other	15(3.8)	5(3.6)	0.95(0.25-3.6)	0.94	0.1(0.003-3.98)	0.23
Income status	High	104(26)	41(29.7)	1	-	1	-
	Middle	207(51.7)	76(55.1)	2.1(1.1-3.95)	0.02	5.1(0.69-38)	0.11
	Low	89(22)	21(15.2)	1.88(1.07-3.3)	0.029	4.68(0.91-24.2)	0.06
Presence of toilet	Yes	374(93.5)	132(95.6)	1	-	1	-
	No	26(6.5)	6(4.4)	1.8(0.7-4.6)	0.21	8.7(0.76-99.7)	0.082
Habit washing hand before meal	Always	363(90.8)	124(89.8)	1	-	-	-
	Sometime	22(5.5)	8(5.8)	0.8(0.27-2.2)	0.64	-	-
	Never	15(3.8)	6(4.2)	0.86(0.2-3.3)	0.82	-	-
Treatment of drinking water	Always	28(7)	11(7.9)	1	-	-	-
	Sometime	39(9.8)	15(10.8)	1.3(0.58-2.8)	0.55	-	-
	Never	333(83.3)	112(81.2)	1.2(0.6-2.4)	0.55	-	-
Animal contact	Yes	103(25.8)	75(54.3)	9.9(5.9-16.6)	<0.001	24.8(4.7-129.8)	<0.001**
	No	297(74.3)	63(45.7)	1	-	1	-
Habit of eating raw meat	Yes	33(8.3)	5(3.6)	0.31(0.12-0.83)	0.02	0.18(0.16-2.1)	0.17
	No	367(91.7)	133(96.4)	1	-	1	-
Habit of washing vegetable	Yes	374(93.5)	133(96.4)	1	-	1	-
	No	26(6.5)	5(3.6)	2.3(0.85-6.3)	0.099	0.68(0.049-9.5)	0.77
Swimming habit	Yes	42(10.5)	6(4.3)	0.29(0.1-0.7)	0.006	0.21(0.029-1.5)	0.12
	No	358(89.5)	132(95.7)	1	-	1	-
Habit of walking barefoot	Yes	31(7.8)	8(5.8)	0.64(0.27-1.47)	0.29	-	-
	No	369(92.2)	130(94.2)	1	-	-	-
Source of	Tap	383(95.8)	131(94.9)	1	-	-	-

drinking water	River/spring/lake	17(4.2)	7(5.1)	0.74(0.28-2)	0.55	-	-
Hand washing habit after defecation	Always	357(89.2)	125(90.5)	1	-	-	-
	Sometime	21(5.3)	7(5.1)	1.4(0.55-3.8)	0.46	-	-
	Never	22(5.5)	6(4.3)	1.3(0.36-4.9)	0.67	-	-
Habit of trimming nail	Yes	382(95.5)	131(94.9)	1	-	-	-
	No	18(4.5)	7(5.1)	0.8(0.3-2.2)	0.67	-	-
ART starting time	<2 years	120(30)	109(78.9)	79(32-195.7)	<0.001	32.2(6.9-151.3)	<0.001**
	2-5 years	190(47.5)	19(13.7)	0.89(0.39-1.99)	0.77	0.7(0.16-3.1)	0.64
	>5 years	90(22.5)	10(7.2)	1	-	1	-
CD4 status	<200 cell/mm3	38(9.5)	32(23.1)	46.6(17.6-123.3)	<0.001	4.86(0.03-790)	0.54
	201-499 cell/mm3	138(34.5)	83(60.1)	13.2(7.6-22.9)	<0.001	12.9(1.69-98.3)	0.014*
	>500 cell/mm3	224(56)	23(16.6)	1	-	1	-
Viral load status	>200 copies/ml	38(9.4)	33(23.9)	28.1(10.4-76)	<0.001	2.3(0.014-383)	0.75
	50-200 copies/ml	131(32.8)	61(44.2)	3.7(2.3-6)	<0.001	0.43(0.059-3.2)	0.41
	<50 copies/ml	231(57.8)	44(31.8)	1	-	1	-
History of diarrhea <3 months	Yes	49(12.3)	43(31.1)	9.3(3-6.8)	<0.001	18.1(2.3-138)	0.005*
	No	351(87.8)	95(68.9)	1	-	1	-
History of weight loss	Yes	42(10.5)	9(6.5)	0.5(0.22-1.04)	0.064	0.36(0.4-3.2)	0.36
	No	358(89.5)	129(93.5)	1	-	1	-

COR: Crude Odd Ratio, AOR: Adjusted Odd Ration

5.8. Bivariate analysis for opportunistic intestinal with some predisposing factors of study participants

The present study revealed that participant with CD4 level 201-499 cell/mm^3 was [COR=1.36, 95% Cl: 0.41-4.56; P=0.61] more likely infected with OIP when compared with those of CD4 level >500 cell/mm3; even so no significant association was seen. Similarly those participants that have been started ART 2-5 years ago were [COR=0.46, 95% Cl: 0.13-1.63; P=0.229] less likely infected with OIP when compared with those who have been started ART >5 years ago; though the difference was not significant .Furthermore those study groups with history of animal contact were [COR=0.89, 95% Cl: 0.42-1.88;P=0.76] less likely infected with OIP when compared to their counterpart; however it didn't considered as determinant in current study. Concerning barefoot those attendant who have habit of walking barefoot were [COR=1.3, 95% Cl: 0.43-3.9; P=0.65] more likely infected with infection when compared with those who haven't habit of walking barefoot and again variable was no significantly associated with prevalence of OIP among study participant *(Table 6).*

Bivariate analysis of present study displayed that CD4 level; <200 cell/mm^3 with [COR=160.9, 95% Cl:50.7-509.9;P<0.001], ART starting time;< 2 years ago with [COR=6.8, 95% Cl:2.3-16.6; P<0.001] and Diarrhea status ;acute with [COR=8.75, 95% Cl:1.3-63.4; P=0.032], chronic diarrhea with [COR=7.5, 95% Cl:1.14-49.3;P=0.036] were significantly associated variables with prevalence of OIP and more likely infected when compared with their counterpart *(Table 6).*

Table 6: Bivariate logistic regression analysis of opportunistic intestinal parasitic infection with associated factors among ART following patients at Worabe comprehensive and specialized Hospital, 2023 (N=400**).**

Risk factors	Category	Number examined (%)	Intestinal parasite infection n (%)		COR (95%)	P-value
			No parasite detected	Parasite detected		
walking barefoot	Yes	31(7.7)	27(7.5)	4(9.5)	1.3(0.43-3.9)	0.65
	No	369(92.3)	331(92.5)	38(90.5)	1	-
Animal contact	Yes	103(25.8)	93(25.9)	10(23.8)	0.89(0.42-1.88)	0.76
	No	297(74.2)	265(74.1)	32(76.2)	1	-
ART starting time	<2	120(30)	88(24.5)	32(76.2)	6.8(2.3-16.6)	<0.001
	2-5	190(47.5)	185(51.7)	5(11.9)	0.46(0.13-1.63)	0.229
	>5	90(22.5)	85(23.7)	5(11.9)	1	-
CD4 status	<200	38(9.5)	7(1.9)	31(73.9)	160.9(50.7-509.9)	<0.001
	201-499	138(34.5)	133(37.1)	5(11.9)	1.36(0.41-4.56)	0.61
	>500	224(56)	218(60.8)	6(14.3)	1	-
Diarrhea status	Acute	18(36.7)	4(26.6)	14(41.2)	8.75(1.3-63.4)	0.032
	Chronic	24(48.9)	6(40)	18(53)	7.5(1.14-49.3)	0.036
	Intermittent	7(14.2)	5(33.4)	2(5.9)	1	-

The distribution of opportunistic intestinal parasitic infection in relation to diarrheal status and viral load is presented in (Table 7). The majority (66.6 %) of the study participants with acute diarrhea status were viral load of >200 copies/ml with prevalence rate of (85.7%) for OIP. difference in rate of OIP infection by acute diarrhea was significantly associated in participant with viral load >200 copies/ml (>200 cell/ml; 85.7 %, <50 copies/ml; 14.2%; $\chi2$ =14.1, df = 2, P = 0.001). Additionally in participants categorized under chronic diarrhea status, the rate of opportunistic parasitic infection was higher in patients with viral load >200 copies/ml and again the difference was statically significant ((>200 cell/ml; 88.8%, 50-200 copies/ml; 11.2%, <50 copies/m; l0%; $\chi2$ =15.2, df = 2, P = 0.001). Moreover concerning those of intermittent diarrhea status, rate of infection was equal in both having viral load 50-200 copies/ml and <50 copies/ml.

However, no statistically significant difference was found (50-200 copies/ml 50%, <50 copies/ml 50%; $\chi 2 = 0.18$, df = 2, P = 0.67) *(Table 7).*

Table 7: Distribution of opportunistic intestinal parasite in relation to diarrheal status and viral load in the study population at Worabe Comprehsive specialized hospital, Worabe, central Ethiopia, 2023(N=400)

Diarrhea status	Viral load	N-tested (%)	N-positive (%)	X^2	P-value
Acute	>200 copies/ml	12(66.6)	12(85.7)	14.1	0.001
	50-200 copies/ml	3(16.6)	0		
	<50 copies/ml	3(16.6)	2(14.2)		
	Total	18(100)	14(100)		
Chronic	>200 copies/ml	16(69.5)	16(88.8)	15.2	0.001
	50-200 copies/ml	6(26.1)	2(11.2)		
	<50 copies/ml	1(4.3)	0		
	Total	23(100)	18(100)		
Intermittent	-	-	-	0.18	0.673
	50-200 copies/ml	3(37.5)	1(50)		
	<50 copies/ml	5(62.5)	1(50)		
	Total	8(100)	2(100)		

CHAPTER VI: DISCUSSION

Understanding the epidemiology of intestinal parasitic infection is essential for the effective management of HIV infection. Thus, the present study aimed to assess prevalence of opportunistic intestinal coccidian and other intestinal parasites among HIV patients at central Ethiopia. Accordingly, the overall prevalence of intestinal parasites was 34.5% which is comparable with studies done in Mizan Tepi university (35.1%) and Jimma (37%) south west Ethiopia(Mekonnen, 2012),(Abayneh, 2022).

However, the finding was higher when compared to research performed at different part of the world including; Colombia(29.2%), Brazil(28.8%), South India(23.4%), South Africa(30%), Mozambique(26.5%), Debretabor general hospital(21.1%, Dessie referral hospital north Ethiopia(26.2%), Addis Ababa(21.1%), University of Gondar(26.2%), Arbaminch referral hospital south Ethiopia(29.1%), Arsi university(18.4%) and Kombolcha north Ethiopia (13.9%) with average prevalence of 24.9% (Botero-garcés *et al.*,2021),*(Barcelos et al.*,2018),(Swathirajan *et al.*, 2017),(Ifeoma *et al.*,2022),(Casmo *et al.*,2018),(Alemayehu *et al*.,2020),(Feleke et al.,2022),(Eshetu et al.,2016),(Muleta *et al.*,2021),(Getaneh *et al*.,2020),(Mesfun *et al*.,2017) ,(Gebretsadik et al.,2018). Observed variations may be due to sample size, sensitivity of diagnostic techniques and geographical location.

Again the present finding was lower when standardized with findings conducted in Ethiopia; at University of Gondar 45.3%, Mettu (62.5%), Dessie comprehensive hospital (38.1%) and Adigrat (65%)(Gebrecherkos *et al*.,2016),(Journal *et al.*,2013),(Bisetegn *et al*.,2022),(Mahmud *et al*.,2014). Additionally as compared with other developing countries, this finding was lower when compared with studies carried out at Gabon (53.4%), and Kenya (50.9%) (Lengongo *et al.*, 2020),(Kipyegen *et al*.,2012). Low prevalence in the present study might be due to participants' immunity status and also most of participants haven't history of exposure with most environmental factors.

In generally observed variations may be explained by the difference in geographic location, sample size, sensitivity of diagnostic techniques, methodology, study participants' immunity status, socioeconomic status and access to safe water supply. In addition, the observed variations may be influenced by study period in which nowadays there is a better awareness of the patients about intestinal parasite infection and their cause.

As far as the diversity of parasite species is concerned, several (eleven) types of intestinal parasites were detected, seven of which were helminthes and the remaining four were protozoan parasites which in line with study conducted in University of Gondar northern Ethiopia(Eshetu et al.,2016). The majority of identified parasites in the present study were protozoan which is in agreement with the research performed at Arsi university, Dessie comprehensive hospital, Mettu Karl hospital south west Ethiopia(Mesfun *et al*.,2017),(Bisetegn *et al.*, 2022) and (Solomon *et al*.,2013). Therefore, the prevalence of intestinal protozoa in the current study is a clear indicator of the fecal risk and of the poor level of personal and societal hygiene.

Similar to the previous findings reported from other parts of the world including central Cameron, Jimma south west Ethiopia, Debretabor general hospital north Ethiopia and Dilla south Ethiopia *A. lumbricoides* (42 cases in present study) was the most predominant intestinal parasites found (Ifeoma *et al.*,2022),(Mekonnen *et al.*,2012),(Alemayehu *et al.*,2020) ,(Eriso *et al.*,2015). In contrast to our finding, *E. histolytica* was the predominant parasite detected in research conducted at Kombolcha health center, University of Gondar, Axum northern Ethiopia and Kenya (Gebretsadik *et al.*,2018),(Gebrecherkos et al.,2016),(Gebrewahid *et al.*, 2019) and (Kipyegen *el at.*, 2012). *A. lumbricoides* may be more prevalent in our study due to the parasite's widespread dispersion, eggs' better durability in a variety of external environmental situations, and its unique propensity to produce large numbers of eggs. Furthermore concentration technique specifically using saturated sodium chloride that have better detection capacity for this parasite was incorporated in present study that may contribute for increment of parasite.

E.histolytica was the second (36 case) most abundant parasite among study participants in our finding. This finding is in agreement with investigation conducted at Dessie Referral Hospital, Dessie Comprehensive Specialized, Arsi university, Kombolcha north Ethiopia and Burkina Faso(Feleke *et al.*,2022),(Bisetegn *et al.*,2022),(Mesfun *et al*.,2017),(Gebretsadik *et al.*,2018),(Adama *et al.*,2017). In general, the prevalence of non-opportunistic intestinal parasites like *E.histolytica* and other soil-transmitted helminthes in this study suggested that people with HIV may accelerate the onset of AIDS due to immune system downregulation and also may contribute for increment of mortality and morbidity rates among these groups (Eshetu *et al*.,2016).

Importantly, an opportunistic intestinal parasite (*Cryptosporidium spp, I.belli and S.stercoralis*) was identified among study participants. The overall prevalence of OIP was 10.5 % which in compliance with the findings at Axum north Ethiopia (9.5%) (Gebrewahid *et al.*,2019). However, the finding was lower when compared to those findings previously conducted throughout different part of the world for instance Brazil (28.9%), Burkina Faso (66.7%), Cape south Africa 30.5% and Arbaminch south Ethiopia (17.7%)(Barcelos *et al.*,2018),(Adama *et al.*, 2017),(Ifeoma *et al.*, 2022),(Getaneh *et al.*,2017). This may locate better interventions were being carried out in the study area and also most of our participants had CD4+ T cell counts >200 cell/mm^3 (362/400; 90.5%). Still, prevalence (10.5%) of OIP in this study shall not be neglected and needs special attention for further reduction because of its destructive effect on HIV patients.

In contrast, the prevalence of OIP was higher when compared to investigation performed at Mizan Tepi south west Ethiopia (5.6%) and Dessie comprehensive hospital north Ethiopia (2.2%) (Bisetegn *et al.*, 2022),(Abayneh *et al*.,2022). Variations may be due to sample size.

Similar to the previous findings reported from other parts of the world including Burkina Faso, university of Gondar , Arbaminch hospital south Ethiopia, Axum northern Ethiopia and Mizan Tepi south west part of Ethiopia indicates *Cryptosporidium spp* (with 22.4% in our findings) was the most predominant OIP found(Adama *et al.*, 2017),(Gebrecherkos *et al*.,2016.),(Getaneh *et al*.,2016),(Gebrewahid *et al.*, 2019),(Abayneh *et al.*,2022). Particularly in HIV-positive patients, severe, persistent, and potentially fatal diarrhea is connected to Cryptosporidium spp. (Sannella *et al.*, 2019). Moreover; this study found that the presence of diarrhea was statistically associated with the prevalence of intestinal parasitosis in the study population. Thus, detection of these opportunistic and other intestinal parasites among a significant number of HIV infected individuals in present study revealed that people living with HIV are at high risk of developing life threatening conditions.

The prevalence of the other two opportunistic intestinal parasites identified in our finding (*S.stercoralis* and *I.belli*) are also considerable as they may result in prolonged diarrhea that can reduce nutrient absorption among HIV/AIDS(Eriso *et al.*,2015). Particularly, S. stercoralis, the second-most often found OIP in our findings, is responsible for 60–85% of the death rate in immunocompromised individuals. Such people may get hyper infection syndrome as a result of internal auto infection. By acting as mechanical vectors for various microorganisms, such as intestinal bacteria, the larvae of the parasite can spread to various parts of the body (such as the brain, lung, peritoneum, etc.) and cause severe health consequences or even death. (Evering *et al.*,2006), (Siddiqui *et al.*,2001).

Prevalence of coccidian in present study was 8.25(33/400) which is in agreement with the study conducted at Fiche hospital north west Ethiopia (8.4%), Kumasi-Ghana (8.2%) and Maputo-Mozambique (8.3%) (Adamu *et al*., 2013),(Acquah *et al.*,2012),(Casmo *et al.*, 2018) . Although, lower than investigation carried out at different part of the world i.e. Hawassa south Ethiopia 34.5%,Yaoundé Central Cameron(12.6%),Bobo-Dioulasso-Burkina Faso(26.5%),Alexandria-Egypt (17%), Edo State Nigeria (22.2%) and estado Falcón Venezuela (26.6%)(Girma *et al.*, 2014),(Vouking *et al.*,2014),(Sangaré *et al.*,2015),(Massoud *et al.*,2012),(Akinbo *et al*.,2011),(Cazorla *et al*., 2018). Observed variation may be due to immunity of participants, most of study participants in present study have no exposure with environmental factors and single stool specimen processing.

Furthermore the finding in present study revealed that prevalence of intestinal coccidian among study participant was higher when standardized with research conducted at different side of the global such as Hiwot Fana Specialized University Hospital east Ethiopia (2.2%),Venezuelan community (6.1%), Henan; China (0.7%), Imam Khomeini hospital Iran(0.9%), Germany (1.6%) and Brazil (1.1%)(Zhou *et al.*, 2011),(Teklemariam *et al.*, 2013)(Salehi Sangani *et al.*, 2016),(Chacin-B.*et al*, 2003),(Jelinek *et al.*, 1997) , (Barcelos *et al*.,2018). Observed variation may be due to sample size

Generally variation in prevalence of intestinal coccidian infection in HIV patients could be related to the immune status of HIV patients examined, geographical location, sensitivity of diagnosis technique used and experience of technicians' .Moreover variations may be influenced by study period in which nowadays there is a better awareness of the patients about intestinal parasite infection and their cause.

From coccidian predominantly detected parasite in present study was *cryptosporidium spp* account for 93.7% that in harmony with research conducted at German ,Imam Khomeini hospital Iran ,Edo State Nigeria, Bobo-Dioulasso; Burkina Faso, Fitche Hospital Ethiopia, Hawassa south Ethiopia(Jelinek *et al.*, 1997),(Adamu et *al.*,2013),(Girma *et al.*, 2014),(Salehi S *et al.*, 2016),(Sangaré *et al.*, 2015) and (Akinbo *et al.*, 2011). So, People with HIV should be educated and counseled about the different ways that Cryptosporidium can be transmitted because of its destructive effect.

The highest prevalence (53.6%) among female in present study might be due to women are responsible for all the household duties especially, frequent contact with vegetable, fruit and other raw material for meal preparation. Additionally, in our nation they produce around 29% of the agricultural and livestock that aids in intestinal parasite transmission (Palacios *et al.,*2017). Despite not being statistically significant, in the present study, the majority of intestinal parasites (42%) were detected among HIV/AIDS patients who were in the age range 20-29 years, the most productive and reproductive group in society which is in line with study reports from(Shimelis *et al.*,2016). The potential causes could be attributed to frequent interpersonal interaction, overcrowding in recreational areas and classrooms, and a propensity for sharing things that aids in the transmission of parasites. Regarding the residence, even though no statistical difference was observed, high prevalence (53.6%) among rural might be due farming occupation, drinking untreated water and non-improved water sources *(Table 5).*

Present investigation was tried to describe some proven and hypothesized associated factors for prevalence of intestinal parasitic infection among the study participant and accordingly, educational status, animal contact, individuals who have experience of diarrhea, CD4 level and ART starting time were among identified predisposing factors, as shown in *(see Table 5).*

Multivariate analysis in our finding identified that educational status specifically participants categorized under illiterate and primary level were shown statistical significance with [AOR= 47.9, 95% Cl: 2.8-820.8; P=0.008], [AOR=25.3, 95% Cl: 2.9-219; P=0.003] more likely affected by IPIs respectively when compared with diploma and above level. The finding was supported by studies performed in Burkina Faso, Cameron, Nigeria Benin, Debretabor north Ethiopia, Hawassa south Ethiopia(Adama *et al*.,2017),(Tebit *et al*.,2014),(Akinbo et al., 2017),(Alemayehu *et al*.,2020) and (Shimelis *et al.*,2016). This could result from little information on the importance of sanitation and transmissions way of the infection.

Animal contact was also found as determinant factor for overall prevalence intestinal parasitic infections among study attendant. In view of that participants who had animal contact were about (AOR=24.8, 95%CI: 4.7-129.8, P< 0.001) more likely to be infected by intestinal parasites than those who hadn't animal contact which in line with investigation conducted at Cameron, Zambia, Colombia and Arbmench hospital south Ethiopia(Tebit *et al.*,2014),(Sinyangwe *et al.*, 2020),(Botero-garcés *et al.*, 2021) and (Getaneh *et al.*,2017). Additionally our finding was harmonized with science confirmed theory stated that most of coccidian parasite are zoonotic that transmitted from animal to human including cryptosporidium spp the most detected OIP in present study.

In agreement with other study in Ethiopia south (Arbmench), Patients who stayed <2 years duration on ART had more chance to be infected with IPIs than patients who took ART for >5 years (AOR=29, 95% CI: 5.1-165.3, P<0.001). Again our finding was in line with hypothesis stated that long-term HAART decreased the chances of intestinal parasite infection(Vouking *et al.*, 2014). Furthermore duration of antiretroviral treatment was significantly higher in non-infected patients suggesting an important role of this therapy in the reduction of the risk of intestinal parasitic infection among HIV positive patients(Botero-garcés *et al.*, 2021).

Concerning diarrheal status, those HIV/AIDS patients who had reported diarrhea were 18.1 times [AOR = 18.1, 95% CI: 2.3-138; p=0.038] more likely infected with intestinal parasites than those who hadn't history of diarrhea that in line with studies conducted at different part of the world for instance Felegehiwot Referral Hospital Bahir Dar, University of Gondar northern Ethiopia, Hiwot Fana hospital east Ethiopia, Arsi university, Burkina Faso, Colombia and Indian(Kiros *et al.*, 2015),(Gebrecherkos et al.,2016),(Teklemariam *et al.*, 2013),(Mesfun *et al.*,2017),(Adama *et al.*, 2017),(Botero-garcés *et al.*, 2021) and (Gupta *et al.*, 2008).

According to studies performed at Brazil, Côte d'Ivoire, Burkina Faso, Felegehiwot Referral Hospital Bahir Dar, Debretabor General Hospital, Dessie Referral Hospital, St. Peter's Specialized Hospital in Addis Ababa, Mizan-Tepi university south west Ethiopia, Aksum north Ethiopia ,Arbmench hospital south Ethiopia , University of Gondar, Guji south Ethiopia and Adigrat north Ethiopia was identified that CD4 level <200 cell/mm^3 was determinant or predisposing factor for prevalence intestinal parasite among the group (Barcelos *et al.*,2018),(Assifiacr *et al.*, 2021),(Alemayehu *et al.*,2020),(Muleta *et al.*, 2021),(Abayneh *et al.*,2022).

In contrary our finding hypothesized that those individual with CD4 level 201-499 cell/mm^3 were 12.9 times [AOR=12.9, 95% Cl: 1.69-98.3; P=0.014] more likely infected with IPIs when compared with those of CD4 level >500 cell/mm^3 that confirmed investigation conducted Dessie Comprehensive Specialized Hospital and Benin Nigeria (Bisetegn *et al.*, 2022),(Akinbo *et al.*, 2017). Significant different observed among CD4 level 201-499 cell/mm^3 may be due to most of 60.1% (83/138) our study participants positive for infection were grouped in this category. Our immune system's CD4 T cell is crucial in preventing and controlling intestinal parasite infection in humans. Thus, the HIV virus's destruction of these cells will raise the possibility of intestinal parasite infection, as well as the reactivation and development of opportunistic parasites in the body(Shimelis *et al.*, 2016).

Even while these opportunistic parasites can be acquired at any point during the course of HIV infection, prior research indicates that the majority of OIP infections are developed in individuals with CD4 T-cell counts under 200 cells/ml(Gupta *et al.*, 2008). Accordingly, in bivariate analysis of the present study, significant difference was observed that individuals with CD4 level; <200 cell/mm3 were about 160 times [COR=160.9, 95% Cl: 50.7-509.9; P<0.001] more likely infected with OIP when compared to those of CD4 level >500 cell/mm3. Our finding was in concordance with other studies such as Arbaminch hospital south Ethiopia, Kathmandu-Nepal, Medical College; Jaipur, Hawassa city south Ethiopia, Varanasi India and Burkina Faso which concluded that as CD4 of patients increases opportunistic intestinal parasite decreases inversely(Getaneh *et al.,2017)*,(Sherchan *et al.*,2012),(Vyas *et al.*,2012),(Assefa *et al.*,2009),(Tuli *et al.*, 2008)(Adama *et al.*, 2017) .This raises the question of the role of cell-mediated immunity in host defense against the parasites.

Concerning ART starting time, participant who have started ART < 2 years ago were [COR=6.8, 95% Cl: 2.3-16.6; P<0.001] more likely to get infected with this infection when compared with their counterpart and the difference was significant. This finding is in agreement with the study conducted at Arbaminch hospital south Ethiopia(Getaneh *et al*., 2017). Suggesting an important role of this therapy and it's adherence in the reduction of the risk of opportunistic intestinal parasitic infection among HIV positive patients. Furthermore, regarding diarrhea status ;those with acute and chronic diarrhea were more than 8.7 [COR=8.75,95% Cl:1.3-63.4;P=0.032], and 7.5 [COR=7.5, 95% Cl:1.14-49.3;P=0.036] times more likely to get infected with OIP respectively when compared with those of intermittent diarrhea status which were significantly associated and this is in agreement with the finding conducted at Hawassa University south Ethiopia(Shimelis *et al.*, 2016).

Association of OIP infection with acute diarrheal status was found to be statistically significant in which patients with a viral load > 200 copies/ml were more likely infected. Similarly, statistical difference was observed among participants with chronic diarrhea status in which those groups with viral load >200 copies/ml were more vulnerable to OIP. These results indicate that the chance of detecting OIP is higher among patients' with high viremia and diarrhea status (chronic and acute). This is in line with the guideline for the prevention and treatment of opportunistic infections among HIV-positive patients (Report *et al.*,2009).Additionally our finding was in agreement with study reported that positive correlations between number of excreted eggs and plasma viral load has been documented, with subsequent reduction of viral load following eradication of IPIs(Mukrimaa *et al.*, 2016).

Limitation of the study

There were some limitations in this study .Firstly, Species specific techniques such as Kato-Katz, Bearman's method (Strongyloides stercoralis) and water-ether sedimentation and Weber modified trichrome (for *Microsporidia*) methods were not used due to lack of resources in the laboratory setup and stool samples were collected only once. Hence, the burden of intestinal parasites in this study might probably be underestimated. Secondly, in this study, a non-probability sampling technique was used that limits the relevance of the risk factor analysis to the entire population of PLWHA. Thirdly, cross-sectional nature of the study could only generate a hypothesis about the possible role of certain independent variables on the infection status of these patients but not their causal relationships.

CHAPTER VII: CONCLUSIONS AND RECOMMENDATIONS

7.1. Conclusions

Based on our finding, the results of the present study indicated that the prevalence IPIs is still common among ART patients with both opportunistic and non-opportunistic parasites were detected at different rate. The distribution of intestinal parasites is greatly affected by no formal education, animal contact, reduced CD4+ cell counts, ART starting duration and being diarrheic.

7.2. Recommendations

Health education regarding the source of infection, mode of transmission about intestinal parasitic as well as regular screening, treatment and follow-up is important to limit the effect of intestinal parasitic among HIV positive patients and its disease progression. In addition, this finding calls for establishment of specific diagnostic tests in all health laboratories and train health professionals with special attention to opportunistic intestinal parasites in HIV-positive patients. Moreover, HIV patients with low CD4 + counts should be diagnosed consistently for intestinal parasites and awareness creation should be advocated to be included as an essential component of the ART monitoring strategy for improved patient care. Likely that the analysis of three consecutive stool samples could have increased the number of protozoan parasites to be identified in the study

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Reference

Abayneh, M. Tadesse ,D.(2022) ‘Prevalence of intestinal parasites and associated risk factors among HIV / AIDS patients attending at anti-retroviral treatment (ART) clinic at Mizan-Tepi university teaching hospital , southwest Ethiopia',*Research square*:2(3).PP.6-18

Acquah, F., Tay, S.C.K. and Frimpong, E.H. (2012) ‘Prevalence of Cryptosporidium and Isospora Belli in HIV/AIDS Patients at KomfoAnokye Teaching Hospital, Kumasi-Ghana’, *Int. J. Pure Appl. Sci. Technol*, 8(2), pp. 26–33.

Adama, Z. Abdourahamane, Y. Marcel, P. Sawadogo1, I. Diallo,H. Ibrahim,S. *et al.* (2017) ‘Opportunistic and other intestinal parasites infections among HIV-positive patients in the era of combination antiretroviral therapy and preventive treatment in Ouagadougou, Burkina Faso’, *Journal of HIV for Clinical and Scientific Research*, 4, pp. 8–14.

Adamu, H. Beyene, P. Guoqing, Z. Hailu, K. Said, A.*et al*. (2014) ‘Distribution and Clinical Manifestations of Cryptosporidium Species and Subtypes in HIV / AIDS Patients in Ethiopia’, *PLOS Neglected Tropical Diseases,* 8(4).PP.5-6

Adamu, H., Wegayehu, T. and Petros, B. (2013) ‘High Prevalence of Diarrhoegenic Intestinal Parasite Infections among Non-ART HIV Patients in Fitche Hospital, Ethiopia’, *PLoS ONE*, 8(8), pp. 5–9.

Akinbo, F. Christopher,E. Okaka,H. & Richard, O. (2017) ‘Prevalence of intestinal parasitic infections among HIV patients in Benin City, Nigeria’, *Libyan Journal of Medicine*, 28(2).PP.6-9

Akinbo, F.O., Okaka, C.E. and Omoregie, R. (2011) ‘Prevalence of intestinal parasites in relation to CD4+ counts and anaemia among hivinfected patients in benin city, edo state, Nigeria’, *Tanzania Journal of Health Research*, 13(1), pp. 10–16.

Alemayehu, E. Alemu, G. Aderaw, A. Jemal ,M. Berhanu, K. *et al* .(2020) ‘Intestinal parasitic infections among HIV infected patients on antiretroviral therapy attending Debretabor General Hospital , Northern Ethiopia : a cross-sectional study’, *Research squre* , pp. 1–18.

Abebe, A.Yitayal, S. Gebeyaw, G. Aregaw ,Y. Zelalem ,A.(2011) ‘Opportunistic and other intestinal parasites among HIV / AIDS patients attending Gambi higher clinic in Bahir Dar city , North West Ethiopia', *Asian Pacific Journal of Tropical Medicine*, 4(8), pp. 661–665.

Aliyo, A. and Gemechu, T. (2022) 'Assessment of intestinal parasites and associated factors among HIV/AIDS patients on antiretroviral therapy at Bule Hora General Hospital, West Guji, Ethiopia', *SAGE Open Medicine*, 10. pp.1-7

Aminu, M. and Yakubu, Y.E. (2008) 'Prevalence of asymptomatic intestinal coccidian parasite infections among non-diarrhoeic HIV-positive children in Zaria, Nigeria', *South African Journal of Science*, 104(9–10), pp. 348–350.

Shimelis, A. Berhanu, E. Girmay, M. Zelalem,A. & Techalew, S. (2009) 'Intestinal parasitic infections in relation to HIV/AIDS status, diarrhea and CD4 T-cell count', *BMC Infectious Diseases*, 9, pp. 1–6.

Assi Fiacre-T. Tuo, K. Landry, N. Fulgence, K. Clément K. *et al.* (2021) 'Prevalence of Intestinal Coccidiosis Among Patients Living with the Human Immunodeficiency Virus in Abidjan (Côte d'Ivoire)', *Research Journal of Parasitology*, 16(1), pp. 9–17

B, N. Lorena, d. Regyane ,F. Hélio ,R. de, M. *et al* (2018) 'Opportunistic and non-opportunistic intestinal parasites in HIV/ AIDS patients in relation to their clinical and epidemiological status', *Sao paoul institute of medicine*, 8 ,pp. 1–9.

Bisetegn, H. Melaku, D.Tigist,M.Mihret,K.Mastewal ,B.and Lubaba ,S. *et al.* (2022) 'Prevalence of Opportunistic and other Intestinal Parasites Infections and its Associated Factors among HIV / AIDS Patients attending at Dessie Comprehensive Specialized Hospital , Northeast Ethiopia', *Journal of AIDS & Clinical Research*,12:6 pp.2-5

Botero-garcés, J. Laura ,A. Juan , A. Sofía, G. Ana Luz ,G.*et al.* (2021) 'Prevalence of intestinal parasites in a cohort of HIV- infected patients from Antioquia , Colombia',*Biomédica* 41, pp. 153–164.

Casmo, V. Marianne, L.Salom~ao ,M.Johan, L. (2018) 'Occurrence of Cryptosporidium spp. and Cystoisospora belli among adult patients with diarrhoea in Maputo, Mozambique', *Heliyon*, 4(9), pp. 1–13.

Cazorla Perfetti, D.J., Acosta Quintero, M.E. and Morales Moreno, P. (2018) 'Aspectos epidemiológicos de coccidiosis intestinales en comunidad rural de la Península de Paraguaná, estado Falcón, Venezuela', *Revista de la Universidad Industrial de Santander. Salud*, 50(1), pp. 67–78.

Chacin-Bonilla, L., De Young, M.M. and Estevez, J. (2003) 'Prevalence and pathogenic role of Cyclospora cayetanensis in a Venezuelan community', *American Journal of Tropical Medicine and Hygiene*, 68(3), pp. 304–306.

Contreras-Puentes, N. Diana Duarte-Amador , D. Aparicio-Marenco , A. Bautista-Fuentes,P .(2020) 'Intestinal coccidian: An overview epidemiologic worldwide and Colombia', *Infectio*, 2(2), pp. 112–125.

Defar, A. Birhanu ,K. Asmare, M. Yeabkal, D. Mistre, W. *et al* (2021) 'Knowledge, practice and associated factors towards the prevention of COVID-19 among high-risk groups: A cross-sectional study in Addis Ababa, Ethiopia', *PLoS ONE*, 16(3 March), pp. 1–14.

Echoru, I. Lule ,H. Lisa, M. Mulkah,O. Muhamudu, K.*et al.* (2015) 'Epidemiology of Coccidian Parasites in HIV Patients of Northern Uganda', *British Journal of Medicine & Medical Research* 7(April), pp. 904–913

Eriso, F. (2015) 'Screening for Opportunistic Intestinal Parasites in HIV/AIDS Patients, Attending the Services of Medical Care in Three Different Hospitals, Southern Ethiopia', *Afro-Egyptian Journal of Infectious and Endemic Diseases*, 5(1), pp. 15–23.

Eshetu, T. Getinet, S. Mohammed ,M.Abrham ,A.Habtamu ,W.*et al.* (2016) 'Intestinal Parasitosis and Their Associated Factors among People Living with HIV at University of Gondar Hospital , Northwest- Ethiopia' *Ethiop J Health S*ci, 27(4).PP.412-423.

Evering, T. and Weiss, L.M.(2006) 'The immunology of parasite infections in immunocompromised hosts', *Parasite Immunology*, 28(11), pp. 549–565.

Feleke, D. Abdurahaman, A.Habtye ,B. and Mengaye ,A. (2022) 'Intestinal parasitic infections and associated factors among people living with HIV attending Dessie Referral Hospital , Dessie town , North - east Ethiopia : a cross - sectional study', *AIDS Research and Therapy*, pp. 1–6.

Gebrecherkos, T., Kebede, H. and Gelagay, A. (2016) 'Original article Intestinal parasites among

HIV / AIDS patients attending University of Gondar Hospital , northwest Ethiopia' *Ethiop. J. Health Dev*, 33(2).pp 62-67

Gebretsadik, D., Haileslasie, H. and Feleke, D.G. (2018) 'Intestinal parasitosis among HIV/AIDS patients who are on anti-retroviral therapy in Kombolcha, North Central, Ethiopia: A cross-sectional study', *BMC Research Notes*, 11(1), pp. 1–5.

Gebrewahid, T. Mebrahtu, T. Hailay ,G. Abrham, A.and Kiros ,T.*et al.* (2019) 'Intestinal parasitosis in relation to CD4 count and anemia among ART initiated patients in St. Mary Aksum general hospital, Tigray, Ethiopia', *BMC Infectious Diseases*, 19(1), pp. 1–9.

Getaneh, A. Dagninet, A. Ashenafi, A. (2017) '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', *Ethiop J Health Sci.* 28(2) pp. 2–11.

Girma, M. Wondu ,T. Beyene, P.and Tekola ,E.(2014) 'Cryptosporidiosis and Isosporiasis among HIV-positive individuals in south Ethiopia: A cross sectional study', *BMC Infectious Diseases*, 14(1), pp. 1–6.

Gupta, S. Narang, V.Nunavth ,S.(2008) 'Chronic diarrhoea in HIV patients: Prevalence of coccidian parasites', *Indian Journal of Medical Microbiology*, 26(2), pp. 172–175.

Hotez, P.J. and Kamath, A. (2009) 'Neglected tropical diseases in sub-Saharan Africa: Review of their prevalence, distribution, and disease burden', *PLoS Neglected Tropical Diseases*, 3(8), pp. 2–11.

Ifeoma, A. Teke, A. Boyisi ,A.Olanrewaju ,O.and Dominic ,T. (2022) 'Prevalence of Intestinal Parasites in HIV / AIDS-Infected Patients Attending Clinics in Selected Areas of the Eastern Cape',*Research microbiology*.13 pp. 574–583.

Jelinek, T. Lotze,M . Eichenlaub,S. Löscher,T .Nothdurft,N. (1997) 'Prevalence of infection with Cryptosporidium parvum and Cyclospora cayetanensis among international travellers', *Gut*, 41(6), pp. 801–804.

Solomon,Y. and Teshoma, T. (2013) 'Prevalence of Intestinal Parasites among HIV Seropositive Individuals at Mettu Karl Hospital , Southwest Prevalence of Intestinal Parasites among HIV Seropositive Individuals at Mettu Karl Hospital ', *International Journal of Health Sciences & Research*.7(2).pp.275-289.

Kipyegen, C.K., Shivairo, R.S. and Odhiambo, R.O. (2012) 'Prevalence of intestinal parasites among HIV patients in Baringo, Kenya', *Pan African Medical Journal*, 13, pp. 1–13.

Kiros, H. Endalkachew, N. Abaineh, M.Bizuayehu, K.Melaku ,A. (2015) 'International Journal of Infectious Diseases Prevalence of intestinal protozoan infections among individuals living with HIV / AIDS at Felegehiwot Referral Hospital , Bahir Dar , Ethiopia', *International Journal of Infectious Diseases*, 35, pp. 80–86.

Lengongo, J.V. Princilia ,B.L.Marielle, B.A.Patricia, M.M. *et al.* (2020) 'Prevalence and associated factors of intestinal parasite infection by HIV infection status among asymptomatic adults in rural Gabon', *African Health Sciences*, 20(3), pp. 1024–1034.

Luvira, V. Tanaya, S.Danabhand, P.Kollawat, S. Dorn ,W. *et al*.(2022) 'Strongyloides stercoralis: A Neglected but Fatal Parasite', *Tropical Medicine and Infectious Disease*, 7(10).PP.5--19

Mahmud, M.A., Bezabih, A.M. and Gebru, R.B. (2014) 'Risk factors for intestinal parasitosis among antiretroviral treated HIV / AIDS patients in Ethiopia', *International Journal of STD & AIDS,0(0)* .PP.1-7

Massoud, N.M., Said, D.E. and El-Salamouny, A.R. (2012) ' Prevalence of Cyclospora cayetanensis among symptomatic and asymptomatic immune-competent children less than five years of age in Alexandria, Egypt ', *Alexandria Journal of Medicine*, 48(3), pp. 251–25

Mekonnen, Z. (2012) 'Intestinal Parasitosis among HIV Sero Positive in Jimma , Ethiopia' *Journal of Tropical Disease ,4(1).pp.2-4*

Mesfun, M. (2017) 'Causes for diarrhoea and infection with intestinal parasites among HIV positive patients at a referral hospital in Central Ethiopia', *Research squre* ,pp. 1–15.

Miressa, R.Mebrate ,D.(2021) ‘Prevalence and Predisposing Factors of Intestinal Parasitic Infections Among HIV Positive Patients Visiting Nekemte Specialized Hospital , Western Ethioia’, *HIV/AIDS - Research and Palliative Care*.pp. 505–512.

Mukrimaa, S. Nurdyansyah,G. Eni, F.(2016) ‘Covariance structure analysis of health-related indicators for elderly people living at home, focusing on subjective sense of health’, *Jurnal Penelitian Pendidikan Guru Sekolah Dasar*, 6(11), p. 128.

Muleta, B. Muleta ,K. Asmare ,M. Wube ,T. Yeabkal ,D.*et al.* (2021) ‘Prevalence of intestinal parasites and its association factors , knowledge , attitude and practice about intestinal parasite among HIV positive individuals in Saint Peter TB specialized hospital Addis Ababa , Ethiopia’ *International Journal of Scientific Reports*, 7(9), pp. 448–453.

Palacios-Lopez, A., Christiaensen, L. and Kilic, T. (2017) ‘How much of the labor in African agriculture is provided by women?’, *Food Policy*, 67, pp. 52–63.

Ramezanzadeh, S. Apostolos ,B.Abdol, S.Mohammad ,T. Seyed ,A. *et al.* (2022) ‘Global Burden of Cyclospora cayetanensis Infection and Associated Risk Factors in People Living with HIV and / or AIDS’,*Viruses*, pp. 1–16.

Regina, D. Talita ,C. Bárbara ,B. Carolina, B. Maria, C. *et al.* (2021) ‘Vaccine development for cryptosporidiosis : Systematic review’, *Research, Society and Development*,20(1), pp. 1–8.

Report, M.W. (2009) Guidelines for the Prevention and Treatment of Opportunistic Infections Among HIV-Exposed and HIV-Infected Children ‘*MMWR* ’, 58(11).PP.165-183

Sah, R. Shiva, B.Sarmila, T. Jyoti ,A. Dinesh ,B.*et al.* (2017) ‘Coccidian parasitic infection in HIV positive patients attending Tertiary care Hospital in Nepal’, *BMC Res Notes* 8(07), pp. 432–435.

Salehi, S. Hamed ,M.Shohreh, F.Mostafa ,R. (2016) ‘Prevalence of intestinal coccidial infections among different groups of immunocompromised patients’, *Iranian Journal of Parasitology*, 11(3), pp. 332–338.

Sangaré, I. Sanata ,B.Mamoudou, C.Adama ,Z.Rabila B. *et al.* (2015) ‘Prevalence of intestinal opportunistic parasites infections in the University hospital of Bobo-Dioulasso, Burkina Faso’, *Infectious Diseases of Poverty*, 4(1), pp. 1–6.

Sannella, A. Yupin, S.Ekkarat, W. and Simone ,M. (2019) ‘A retrospective molecular study of Cryptosporidium species and genotypes in HIV-infected patients from Thailand’, *Parasites and Vectors*, 12(1), pp. 1–6.

Sheet, F. (2023) ‘ Global HIV statatics People living with HIV People living with HIV accessing an retroviral therapy New HIV infenctios AIDS- related deaths Popultions’*UNAIDS*,1(1),PP.1-10

Sherchan, J. Ohara ,H.Sakurada, S.Basnet, A.Tandukar,S. *et al.* (2012) ‘Enteric opportunistic parasitic infections among HIV-seropositive patients in Kathmandu, Nepal’, *Kathmandu University Medical Journal*, 10(38), pp. 14–17

Shimelis, T., Tassachew, Y. and Lambiyo, T. (2016) ‘Cryptosporidium and other intestinal parasitic infections among HIV patients in southern Ethiopia : significance of improved’, *Parasites & Vectors*, pp. 1–7.

Siddiqui, A.A. and Berk, S.L. (2001) ‘Diagnosis of Strongyloides stercoralis infection’, *Clinical Infectious Diseases*, 33(7), pp. 1040–1047.

Sinyangwe, N. Joyce, S. John, B. Mumbi, C. and Charles ,M. (2020) ‘Factors Associated With Cryptosporidium Infection Among Adult HIV Positive Population in Contact With Livestock in Namwala’*Frontiers in public health*, 8(2), pp. 1–7.

Swathirajan, C. Ajay ,C.Renu ,B.Neetal ,N.Samir ,J. *et al.* (2017) ‘Occurrence of Enteric Parasitic Infections among HIV - infected Individuals and Its Relation to CD4 T - cell Counts with a Special Emphasis on Coccidian Parasites at a Tertiary Care Centre in’, *Indian Journal of Medical Microbiology*, 35(1), pp. 37–40.

Tahvildar-Biderouni, F. and Salehi, N. (2014) ‘Detection of Cryptosporidium infection by modified ziehl-neelsen and PCR methods in children with diarrheal samples in pediatric hospitals in Tehran’, *Gastroenterology and Hepatology from Bed to Bench*, 7(2), pp. 125–130.

Taye, B. Kassu ,D. Selamawit ,E. Geme, U. (2014) ‘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*, 63(3), pp. 550–556.

Tebit, K. Longdoh ,A.Nguedia ,J. Charlotte, W. Elvis ,A. *et al.* (2014) ‘Prevalence and

Predisposing Factors to Intestinal Parasitic Infections in HIV / AIDS Patients in Fako Division of Cameroon' *American Journal of Epidemiology and Infectious Disease*,5(3),PP. 42-49

Teklemariam, Z. Degu, A.Habtamu ,M. and Yadeta ,D. (2013) 'Prevalence of Intestinal Parasitic Infection among HIV Positive Persons Who Are Naive and on Antiretroviral Treatment in Hiwot Fana Specialized University Hospital, Eastern Ethiopia', *Isrn Aids*, 2013, pp. 1–6.

Tuli, L. Anil, K. Shyam, S. and Tribhuban ,M. (2008) 'Correlation between CD4 counts of HIV patients and enteric protozoan in different seasons - An experience of a tertiary care hospital in Varanasi (India)', *BMC Gastroenterology*, 8, pp. 4–9.

Uysal, H. Gokhan, T.Kevser ,A.Semih, A.Ozer, A. *et al.* (2017) 'The Prevalence of Cyclospora cayetanensis and Cryptosporidium spp in Turkish patients infected with HIV-1'*Acta Parasitologica*, 62(3), pp. 557–564.

Vouking, M. Patrice, E. Claire V. Carine N. (2014) 'Prevalence of intestinal parasites among HIV patients at the Yaoundé Central Hospital, Cameroon', *Pan African Medical Journal*, 18, p. 136

Vyas, N., Pathan, N. and Aziz, A. (2012) 'Enteric pathogens in HIV-positive patients with diarrhoea and their correlation with CD4+ T-lymphocyte counts', *Tropical Parasitology*, 2(1), p. 29.

Zeleke, A. Ayenew, A.Adane, D.Yalewayker ,T.Meseret, B.*et al.* (2021) 'Evaluation of Hookworm Diagnosis Techniques from Patients in Debre Elias and Sanja Districts of the Amhara Region, Ethiopia', *Journal of Parasitology Research*, PP.3-7

Zhou, Y. Biao, L.Qiang ,W.Rongjun ,W.Fuchun ,J. *et al.* (2011) 'Prevalence and molecular Characterization of Cyclospora cayetanensis Henan, China ' *Emerging Infectious Diseases*, 17(10), pp. 1887–1890

Appendices

Appendix I: Questionnaire

Questionnaire to "determine prevalence and predisposing factors of opportustic intestinal coccidian and other intestinal parasite among HIV patients attending ART clinic at Worabe comprehensive and specialized hospital from May 2022 to September 2023.

Part I**:** Socio-demographic information of Study participants**.**

Code **__________**

S. No	Questions	Possible response
1	Age (in year)	___________
2	Gender	1.Male 2.Female
3	Marital status	1.Single 2.Married 3.Divorced 4.Widowed
4	Education status	1.Illiteracy 2.Primary 3.Secondary 4.Diploma and above
5	Residence	1.Urban 2.Rural
6	Occupational status	1. Gov't employed 2.Self-employed 3.Not employed 4.Merchant 5.Farmer 6.Student 7.House wife 8.Other
7	Monthly income (EB) (Getaneh *et al.*,2017)	1.High income level (>2000) 2.Middle income level (1000-2000) 3.low income level (<1000)

Part II**:** Environmental predisposing factors for opportunistic and other intestinal parasites among study participants

S.No	**Questions**	**Possible response**
1	Presence of toilet	1.Yes 2.No
2	Hand washing habit before meal	1.Always 2.Sometime 3.Never
3	Treatment of water before drinking	1.Always 2.Sometime 3.Never
4	Contact with domestic animal	1.Yes 2.No
5	Habit of eating raw meat	1.Yes 2.No
6	Habit of washing vegetables/fruit	1.Yes 2.No
7	Swimming habit	1.Yes 2.No
8	Habit of walking on bare foot	1.Yes 2.No
9	Source of water for drinking	1.Tape water 2.River/spring water/lake
10	Hand washing habit after defecation	1.Always 2.Sometime 3.Never
11	Habit of trimming nail	1.Yes 2.No
12	History of diarrhea in <3 months	1.Yes 2.No
13	If **yes** diarrhea status	1.Acute 2.Chronic 3.Intermittent

Part III**:** Information on patient's clinical characteristics

S.No	**Questions**	**Possible response**
1	CD4 level (Abayneh *et al.*, 2022)	1.<200cell/mm3 2.201-499 3. >500 cell/mm3
2	Viral load (Botero-garcés *et al.*, 2021)	1.>200 copies/ml 2.50-200 copies/ml 3.< 50 copies/ml
3	ART starting time(Getaneh *et al.*, 2017)	1.<2 years ago 2. 2-5 years ago 3.>5 years ago
4	Reported weight loss	1.Yes 2.No

Name of data collector__________________________________sign____________date________

Name of investigator___________________________________sign____________date________

አባሪ I፡ መጠይቅ

በወራቤ አጠቃላይ እና ስፔሻላይዝድ ሆስፒታ በአርት ክሊኒክ ውስጥ በኤች አይ ቪ ታማሚዎች መካከል የአጋጣሚ የአንጀት ኮሲዲያን እና ሌሎች የአንጀት ጥገኛ ተውሳኮችን ስርጭት እና ተጋላጭነት ለመወሰን መጠይቅ።

ክፍል አንድ፡ የጥናት ተሳታፊዎች ማህበረ-ሕዝብ መረጃ። (ክበብ)

S. No	ጥያቄዎች	ሊሆን የሚችል ምላሽ
1	ዕድሜ (በዓመት)	__________
2	ጾታ	a. ወንድ b. ሴት
3	የጋብቻ ሁኔታ	a. ነጠላ b. ያገባ c.የተፋታ d. መበለት
4	የትምህርት ደረጃ	a.መሃይምነት b.ዋና c.ሁለተኛ ደረጃ d.ዲፕሎማ እና ከዚያ በላይ
5	የመኖሪያ ቦታ	a.ከተማ b. ገጠር
6	የሙያ ደረጃ	a.መንግስት ተቀጠረ b.በራስ ተቀጣሪ c. አልተቀጠረም d.ነጋዴ e.ገበሬ f. ተማሪ g. የቤት ሚስት h.ሌላ
7	ወርሃዊ ገቢ (በብር)(ደፋር እና ሌሎች፣ 2021)	a. ከፍተኛ የገቢ ደረጃ (> 2000) b. መካከለኛ ገቢ ደረጃ (1000-2000) c. ዝቅተኛ የገቢ ደረጃ (<1000)

ክፍል II፡ በጥናቱ ተሳታፊዎች መካከል ለአጋጣሚ እና ለሌሎች አንጀት ጥገኛ ተህዋሲያን አካባቢያዊ ቅድመ ሁኔታዎች።

S.No	ጥያቄዎች	ሊሆን የሚችል ምላሽ
1	የመጸዳጃ ቤት መኖር	a. አዎ b.አይ
2	ከምግብ በፊት የእጅ መታጠብ ልማድ	a. ሁልጊዜ b. አንዳንድ ጊዜ c. በጭራሽ
3	ከመጠጣትዎ በፊት የውሃ አያያዝ	a.ሁልጊዜ b. አንዳንድ ጊዜ c. በጭራሽ
4	ከቤት እንስሳት ጋር መገናኘት	a. አዎ b.አይ
5	ጥሬ ሥጋ የመብላት ልማድ	a. አዎ b.አይ
6	አትክልቶችን / ፍራፍሬዎችን የማጠብ ልማድ	a. አዎ b.አይ
7	የመዋኛ ልማድ	a. አዎ b.አይ
8	በባዶ እግር የመራመድ ልማድ	a. አዎ b.አይ
9	ለመጠጥ የሚሆን የውሃ ምንጭ	a. የቴፕ ውሃ b. ወንዝ / ምንጭ ውሃ / ሐይቅ
10	ከመጸዳዳት በኋላ የእጅ መታጠብ ልማድ	a. ሁልጊዜ b. አንዳንድ ጊዜ c. በጭራሽ
11	ጥፍር የመቁረጥ ልማድ	a. አዎ b.አይ
12	በ 3 ወር ውስጥ የተቅማጥ ታሪክ	a. አዎ b.አይ
13	አዎ ከሆነ የተቅማጥ ሁኔታ	a.አጣዳፊ b. ሥር የሰደደ c. የሚቆራረጥ

ክፍል III፡ የታካሚ ክሊኒካዊ ባህሪያት መረጃ (ክበብ)

S.No	ጥያቄዎች	ሊሆን የሚችል ምላሽ
1	ሲዲ4 ደረጃ (አባይነህ እና ሌሎች፣ 2022)	a. <200cell/mm3 b. 201-499 cell/mm3 c. > 500 cell/mm3
2	የቫይረስ ጭነት (Botero-garces et al., 2021)	a.>200 ቅጂዎች / ml b.50-200 ቅጂዎች / ml c.< 50 ቅጂዎች/ሚሊ
3	ART መነሻ ጊዜ (ጌታነህ እና ሌሎች፣ 2017)	a.<2 ዓመታት በፊት b. 2-5 ዓመታት በፊት c.> 5 ዓመታት በፊት
4	ሪፖርት የተደረገ ክብደት መቀነስ	a.አዎ b.አይ

Appendix II: Patient information Sheet

You have been selected to be part of this study because you have been ART patients. The purpose of this study is to determine prevalence and predisposing Factors of opportunistic intestinal Coccidian and other intestinal parasites among HIV/AIDS patients attending ART clinics at Worabe Comprehensive Specialized Hospital like you. Your participation in this study is totally voluntary and will involve answering questions and providing a stool sample for the study. There is no risk or discomfort during the collection of a stool sample. If you are uncomfortable answering a question, you can leave. Your answers to the questions will be kept confidential, and the data will be coded to prevent the identification of individual participants. You will benefit from this study by knowing your status of intestinal parasitic infection. The assessed information about the prevalence of intestinal parasites and associated factors among ART patients will be used as baseline data for government policy.

Contact address

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አባሪ II፡ የታካሚ መረጃ ወረቀት

እርስዎ የአርት ሕመምተኞች ስለሆናችሁ የዚህ ጥናት አካል እንድትሆኑ ተመርጠዋል። የዚህ ጥናት አላማ እንደ እርስዎ በወራቤ ኮምፕሬሄንሲቭ ስፔሻላይዝድ ሆስፒታል የአርት ክሊኒኮችን በሚከታተሉ የኤችአይቪ/ኤድስ ህሙማን መካከል የቫይረሱ ተጋላጭነት እና አጋላጭ ሁኔታዎችን ኦፖርቹኒስቲክ ኢንስቲትናል ኮሲዲያን እና ሌሎች የአንጀት ጥገኛ ተውሳኮችን ለመለየት ነው። በዚህ ጥናት ውስጥ ያለዎት ተሳትፎ ሙሉ በሙሉ በፈቃደኝነት ነው እና ጥያቄዎችን መመለስ እና ለጥናቱ የሰገራ ናሙና ማቅረብን ይጨምራል። የሰገራ ናሙና በሚሰበሰብበት ጊዜ ምንም አይነት ስጋት ወይም ምቾት አይኖርም. ጥያቄን መመለስ ካልተመቸህ መተው ትችላለህ። ለጥያቄዎቹ የሚሰጡት መልስ በሚስጥር ይያዛል፣ እና ውሂቡ የተናጠል ተሳታፊዎችን መለየት ለመከላከል ኮድ ይሆናል። የአንጀት ጥገኛ ኢንፌክሽን ያለበትን ደረጃ በማወቅ ከዚህ ጥናት ተጠቃሚ ይሆናሉ። ስለ አንጀት ጥገኛ ተህዋሲያን ስርጭት እና በ ART ታካሚዎች መካከል ተያያዥ ምክንያቶች የተገመገመው መረጃ ለመንግስት ፖሊሲ እንደ መነሻ መረጃ ሆኖ ያገለግላል።

የእውቂያ አድራሻ

• መርማሪ - Jemal Husen

• ኢሜል – mohammedjemalhusen@gmail.com

• ሞባይል- +251 942450256

Appendix III: Informed consent form

I, the undersigned, have been informed and understand that the purpose of this particular research project is to find out prevalence and predisposing factors of opportunistic intestinal Coccidian and other intestinal parasites among HIV/AIDS patients attending ART clinics at Worabe Comprehensive Specialized Hospital. I have been told that it is my full right not to participate, to discontinue, or to participate in the study. If I do not want to participate, I am not obliged to answer the questionnaire. I have been told that there will be no negative impact on whatever decision I make. Hence, with this understanding, my honest participation will contribute to generating information that can be used in the fight against intestinal parasites. Signature of the participants:

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Date:

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አባሪ III፡ በመረጃ የተደገፈ የፈቃድ ቅጽ

በወራቤ ኮምፕሬሄንሲቭ ስፔሻላይዝድ ሆስፒታል የኤችአይቪ/ኤድስ ህሙማን የኤችአይቪ/ኤድስ ህሙማንን ስርጭትና አጋላጭ ሁኔታዎችን ኦፓፖርቹኒስቲክ ኢንስቴስቲንታል ኮሲዲያን እና ሌሎች የአንጀት ጥገኛ ተውሳኮችን ለማወቅ እንደሆነ ከዚህ በታች የተፈረመኝ ተረድቻለሁ እና ተረድቻለሁ። አለመሳተፍ፣ አለማቋረጥ ወይም በጥናቱ አለመሳተፍ ሙሉ መብቴ እንደሆነ ተነግሮኛል። መሳተፍ የማልፈልግ ከሆነ መጠይቁን የመስጠት ግዴታ የለብኝም። በማንኛውም ውሳኔ ላይ ምንም አይነት አሉታዊ ተጽእኖ እንደማይኖር ተነግሮኛል. ስለዚህ በዚህ ግንዛቤ ውስጥ የእኔ ቅን ተሳትፎ የአንጀት ጥገኛ ተውሳኮችን ለመዋጋት የሚረዱ መረጃዎችን ለማመንጨት አስተዋፅኦ ይኖረዋል።

የተሳታፊዎች ፊርማ;

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Appendix IV: Informed assent form

I, undersigned parents /guardians have been informed that the objective of the study to determine Prevalence and Predisposing Factors of opportunistic intestinal Coccidian and other intestinal Parasites among HIV/AIDS patients attending ART clinics at Worabe Comprehensive Specialized Hospital. I requested for the participation of my child in the study to give his/her stool sample for diagnosis of intestinal parasitic infection and related information accordingly. Therefore, with full understanding of the importance of the study, I agreed voluntarily to provide the requested sample from my child for the research purposes.

Signature of the guardians/family:

____

Date:________________

አባሪ IV፡ በመረጃ የተደገፈ የፍቃድ ቅጽ

በወራቤ ኮምፕሬሄንሲብ ስፔሻላይዝድ ሆስፒታል የኤችአይቪ/ኤድስ ህሙማን የኤችአይቪ/ኤድስ ህሙማንን የመስፋፋት እና ቅድመ ሁኔታ ሁኔታዎችን ለማወቅ የጥናቱ አላማ በስም የተፈረመ ወላጆች/አሳዳጊዎች ተነግሮናል። ልጄ በጥናቱ ውስጥ እንዲሳተፍ ጠየቅኩት የአንጀት ተውሳክ ኢንፌክሽን እና ተያያዥ መረጃዎችን ለመመርመር የሰገራ ናሙናውን እንዲሰጥ ጠየቅሁት። ስለዚህ የጥናቱ አስፈላጊነት ሙሉ በሙሉ በመረዳት ከልጄ የተጠየቀውን ናሙና ለምርምር ዓላማ ለማቅረብ በፈቃደኝነት ተስማማሁ።

የአሳዳጊዎች/ቤተሰብ ፊርማ፡-

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ቀን፡-____________________