



Status of Sanitary Practices and Amebiasis and Giardiasis Among Patients Visiting Haik Health Center, South Wollo, Northeast Ethiopia

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## **List of abbreviations/acronyms**

AIDS	Acquired Immuno-Deficiency Syndrome
AOR	Adjusted odd ratio
CDC	Center for Disease Control and Prevention
CI	Confidence interval
COR	Crude Odd Ratio
DNA	Deoxyribonucleic Acid
Exp (B)	Expected Beta
GI	Gastro Intestinal
HHC	Haik Health Center
HIV	Human Immuno-deficiency virus
IPIs	Intestinal parasite infections
IPPIs	Intestinal protozoan parasite infections
KAP	Knowledge Attitude Practice
NaCl	Sodium chloride
NGO	Non-governmental organizations
SPSS	Statistical Package for Social Science
USA	United States of America
UV	Ultra violet
WHO	World Health Organizations

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## Abstract

Amoebiasis and giardiasis are among major causes of diarrhea in least-developed tropical and subtropical regions including Ethiopia. Recent information on these diseases is essential to design suitable control interventions in a specific setting. This study was conducted to detect amoeba and giardia infections and explore the status of some universal risk factors in Haik town and its surroundings, northeast Ethiopia. Patients visiting Haik health center (HHC) between November 2015 and April 2016 and suspected of amoebiasis and giardiasis were invited to participate in the study. Socio-demographic and environmental variables linked to amoebiasis and giardiasis were extracted using a semi-structured questionnaire. Stool samples were collected and screened using the direct-smear microscopy. Data were analyzed using univariate and multivariate logistic regression analysis with 95% confidence interval (CI) and p-value <0.05 considered statistically significant. The overall prevalence of intestinal protozoan infection was 10.6% (n=500). Of these, 2.2% were *Entamoeba histolytica/dispar/moshkovskii* and 8.4% *Giardia intestinalis*. About half of the participants were farmers and thus had lower education level with poor hygienic habits. Latrine coverage was good (60.8%), but still considerable number (39.2%) of the participants defecate in the field. Substantial number (56.2%) of participants had no practice of post-toilet hand wash. About 46% of the participants never attended health education. Substantial number (252) of people used tape water (which is relatively better protected). However, still a large number of people (248) were using unprotected water (pond and pump, spring and river). These all conditions could create ideal ground for amoeba and giardia transmission although the current amoebiasis or giardiasis cases were low. Patients who defecate in the field, do not practice post-toilet hand wash and those had unprotected drinking water were at significantly higher risk of being amoeba or giardia-positive. Adjusted odds ratio (AOR) and 95% CI were respectively 11.56, 4.33-33.38; 5.18, 1.71-15.71; 8.47, 2.4-29.45. The p-value was <0.0001 at all instances. Amoeba and Giardia are important health problems of the study participants. There for, prompt intervention strategies should be designed and implemented to upgrade status of sanitarian practices including provision of adequate safe water supply, health education on personal as well as personal hygien.

**Keywords:** HHC, *E. histolytica/dispar/moshkovskii*, *G. intestinalis*, prevalence, risk factors

## 1. Introduction

Parasitic protozoa are eukaryotic, single-celled microorganisms. They possess one, rarely two nuclei (nuclear reproductive forms). Reproduction is asexually by binary or multiple fission or sexually (Kayser *et al.* 2005). Intestinal parasitic protozoa are known to affect all vertebrate and many invertebrate species and are able to adapt to life in virtually all body sites of their hosts.

Prevalence rates of intestinal parasite in low-income countries ranges from 30-60%, as compared to less than 2% in the high-income countries (Shubeir *et al.* 2000). Intestinal parasitic infections (IPIs) prevalence is linked to lack of sanitation, access to safe water and improper hygiene, thus occurring wherever there is poverty. People of all ages are affected by the cycle of prevalent parasitic infection. Low-income countries located at tropical climates and at low altitudes, such infections remain a serious medical and public problems among the poor, who are the direct victims of the low socioeconomic conditions poor personal and environmental hygiene overcrowding and limited access to clean water.

Coccidia are small intestinal protozoan parasite that live in the intestine tract of dogs and cats. They grouped under class sporozoa an exclusively parasitic group that typically requires alternation of sexual and asexual reproduction in its life cycle. Coccidians cause parasitic disease called coccidiosis (Ettinger and Feld-Man 1995).

Giardiasis, amoebiasis and coccidiosis may induce life-threatening diarrhea specially in children and acquired immuno-deficiency syndrome (AIDS) cases (Benedict 2013). Nevertheless, there is general lack of interest and these infections are among neglected tropical diseases. Similarly, in Ethiopia the infections are little addressed at community level apart from treating passively presented cases at health centers. Most of the people who get treatment at health centers come from rural areas where no clean water source, toilet coverage and awareness about the infections severely lack. Health facility records show high number of amoebiasis or giardiasis cases across the country. But integrated approaches of controlling amoebiasis and giardiasis are not in place. Current information is vital to understand the level of the problem at a health facility for future community-level interventions. This study was, thus, designed to investigate the status of the problem and associated sanitary practices among patients examined at Haik health center (HHC), northeast Ethiopia, to contribute towards that end.

## **2. Objectives**

### **2.1 General objective**

The objective of this study was to assess the status of sanitarian practices and amebiasis and giardiasis among patients visiting HHC.

### **2.2 Specific objectives**

To determine the status of sanitarian practices.

To compare the prevalence of these two parasites.

To identify the KAP of study participants towards IPIs.

To asses sanitarian practices in relation to amoeba and giardia positivity.

### 3. Literature review

Numerous protozoa inhabit the gastro-intestinal tract of humans. This list includes representatives from many diverse protozoan groups. The majority of these protozoa is non-pathogenic commensals, or only result in mild disease. Some of these organisms can cause severe disease under certain circumstances. Intestinal protozoa cause significant morbidity in children and as opportunistic infections in HIV/AIDS and immunosuppressed patients in low-income countries who are already malnourished or have limited access to medical services (Nissapatorn 2008). Consequently, these patients will suffer from repeated severe diarrhea episodes that can be fatal (Kurniawan *et al.* 2009; Gupta *et al.* 2008; Eza *et al.* 2006).

#### 3.1 Amebiasis

Amebiasis is a widespread parasitic disease caused by the protozoa *E. histolytica*. *E. histolytica* is morphologically identical with the apathogenic *Entamoeba dispar*. They can be identified by means of zymodem and DNA analysis with monoclonal antibodies (Kayser *et al.* 2005) *E. histolytica* is estimated to inflict severe disease in 48 million people and 100 000 deaths per year (Petri and Singh 1999). *E. histolytica* is the causative agents of amebic dysentery and amebic liver abscess. It is one of the leading causes of death from parasitic diseases (Davis *et al.* 2009). Amoebic dysentery from *E. histolytica* is the second most common cause of death from parasitic disease worldwide after malaria (Stanley 2001 and 2003). The most common manifestation of amebic infection is dysentery and liver abscess, but infections of the lung, heart and brain also occur (Haque *et al.* 2003). In low-income countries amebic infection depends largely on cultural habits, ages, levels of sanitation, crowding and socio-economic status (Petri *et al.* 2000). However in confirmatory diagnostic analysis using PCR solution hybridization enzyme linked immunoassay *E. dispar* was identified in only 21 of 232 cases while no *E. histolytica* DNA was detected (Kebede *et al.* 2003).

##### 3.1.1 Life cycle of *E. histolytica*

*Entamoeba histolytica* exists in two life stages as cyst and trophozoite. Cysts measure 10-15mm in diameter and typically contain four nuclei. *Entamoeba histolytica* requires a single host to complete its life cycle. When mature tetra-nucleated cyst from contaminated food or drink or from hands contaminated with feces is ingested it excysts in the small intestine to produce metacystic trophozoite by a process of binary fission. The immature trophozoites

migrate to the colon and grow to become mature trophozoite stage, multiply by binary fission to invade the mucus membrane of the large intestine. Sometimes it can perforate the intestinal wall causing extra-intestinal amoebiasis. The trophozoite stage may pass with diarrhea or dysentery. Occasionally or usually trophozoites are ingested by man with contaminated food or water but they will be killed by stomach acids, since they have no protective covering. After a period of growth and multiplication, encystment occurs in the large intestine (Mekete and Adem 2003).

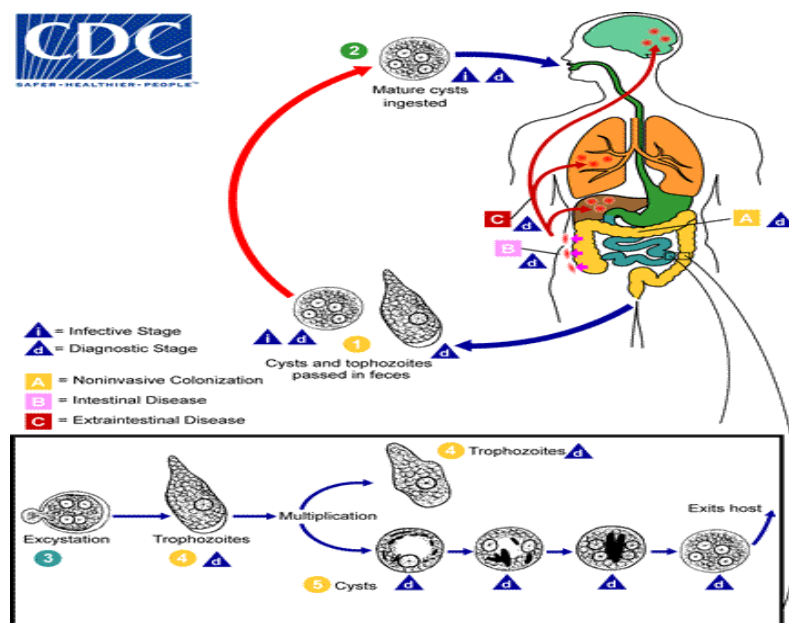


Figure 1 Life cycle of *E. histolytica* (Source <http://www.cdc.gov/dpdx/> Accessed August 20/2016)

### 3.1.2 Epidemiology of *E. histolytica*

The epidemiology of *E. histolytica* has been complicated by the mid-1990's re-description of *E. histolytica* into two species: *E. histolytica*, which is pathogenic, and *E. dispar*, which is not (WHO 1997). However from recent studies *E. dispar* was identified as infection agent using PCR solution hybridization enzyme linked immunoassay among patients with diarrhea in Wonji and Akaki, Ethiopia (Kebede *et al.*2003). The bulk of previous studies on the prevalence of *E. histolytica* were based on stool microscopy, which is of low sensitivity and specificity and cannot distinguish *E. histolytica* cysts or trophozoites from *E. dispar* or *E. moshkovskii*. Thus the conclusions that 10% of the world's population is infected with *E. histolytica* are probably an overestimate (Stanley 2003). Furthermore *E. histolytica* affects

about 10% of the world's population or 500 million people however this infection can be as high as 25% in low income tropical countries. *E. histolytica/dispar* species are found throughout the world but like many other intestinal protozoa, they are more common in tropical countries or other with poor sanitary conditions (Mehraje et al. 2008).

In high-income countries, populations at risk for *E. histolytica* infection include returned travelers or expatriates and immigrants from endemic countries and most cases of invasive disease are imported. In a retrospective review of medical records of more than 34,000 HIV-infected patients in the US (Lowther *et al.* 2000) 111 (0.3%) patients were diagnosed as having *E. histolytica* or *E. dispar* infection, and only 2 had extra-intestinal amoebiasis.

Over the past several years, many investigators in Japan, Taiwan, Korea, and Australia have found that amoebiasis is increasingly diagnosed among HIV-infected MSM (Hung *et al.* 2005 and 2008; Park *et al.* 2007; Stark *et al.* 2008; Stark *et al.* 2006) an estimated 90% of *E. histolytica* infections are asymptomatic, prospective studies of asymptomatic carriers indicate a risk of invasive disease of 5/56 (9%) over the course of 6-12 months (Irusen *et al.* 1992). Invasive disease has a high mortality, estimated at >10% for either dysentery severe enough to require hospitalization or liver abscess. Accordingly, the risk of low-income invasive disease, as well as decreased transmission, are rationales for treating asymptomatic carriers of *E. histolytica*, however WHO recommends against treatment of asymptomatic patients when only a morphologic diagnosis by stool examination is available (i.e., *E. histolytica/dispar*).

Similarly amoebiasis and giardiasis are common cause of intestinal protozoan parasitic infections thought Ethiopia. The prevalence of amoeba ranges from 0-3 % (Haile *et al.* 1994). In other previous study conducted at Tikur Anbesa hospital showed that *E. histolytica* trophozoite was the most common reported parasite with 13.6% prevalence rate (Petri and Sing 1999). But the prevalence of *E. histolytica* in Ethiopia may differ because of misleading microscopic techniques of stool examination (Kebede *et al.* 2004).

### **3.1.3 Diagnosis**

Laboratory diagnosis of intestinal amoebiasis is based on examination of a fresh diarrheic or dysenteric fecal specimen or rectal scraping for motile amoebae using saline. Examination of formed or semi-formed feces for cyst stages is also done. Such stool can be examined by

direct saline and/or iodine smear, and zinc sulphate floatation or centrifugal floatation method. Specimens must be examined without delay otherwise identification of the trophozoites becomes impossible because the amoeba lose their motility, extrude vacuoles containing red cells, round up with the recognition that *E. histolytica* is morphologically identical but genetically distinct from *E. dispar*, cysts, formerly reported as *E. histolytica* should be now reported as *E. histolytica /dispar/moshkovskii*. Since the above method relies on microscopic examination by morphology of protozoa, it is difficult to identify among protozoan with similar morphology. The only way to differentiate these organisms is using zymodem and DNA analysis with monoclonal antibodies.

### **3.1.4 Mode of transmission**

The infection is due to transmission of mature cysts with contaminated foods (fruits,vegetables) drinking water or fecally contaminated hands. The greatest risk is associated with the carrier of the disease,especially, when they are engaged in the preparation and handling of food. Cysts have been found to remain viable for several days in faeces and for at least 8 days in soil at 28-34°C. They also remain infective in water, and wet soil depending on the temperature. They are easily killed by desiccation on the surface of hands and by boiling. Health education, improvement of sanitation and personal hygiene are suggested as realistic measures to reduce the transmission of the parasite.

## **3.2 Giardiasis**

Giardiasis is caused by infection with the enteric pathogenic intestinal flagellate, *Giardia intestinalis* (*G.intestinalis*) (syn. *G.lambli*a, *G. duodenale*). The incidence is estimated at 200 million clinical cases per year (Partovi *et al* 2007). The prevalence of giardiasis is very high in low income countries where the living standard of the society is very low (Endeshaw *et al.* 2004). *G.intestinalis* is flagellate protozoan parasite that grouped within the order diplomonadida and family hexamitidae. There are three known species of the genus giardia but *G. intestinalis* being the only species to infect human. The parasite inhabits the duodenum and upper jejunum where the alkaline pH is favorable for growth.

### **3.2.1 Life cycle *G. intestinalis***

*G. intestinalis* exists into two forms as cyst and trophozoite. Viable cysts are ingested with contaminated food or drinking or by person to person contacts. The cysts pass unharmed by gastric acid through the stomach to the small intestine. Encystation, normally occurs in the

duodenum producing in mature trophozoite. The trophozoite invades the intestinal mucosa causing fatty diarrhea and resists the ordinary peristaltic movements by adhering to the microvillus border the epithelial cells using a ventral disk-shaped sucker Encystation of the trophozoite occur in the colon and rectal region. The infective cysts are voided in stools for another infection.

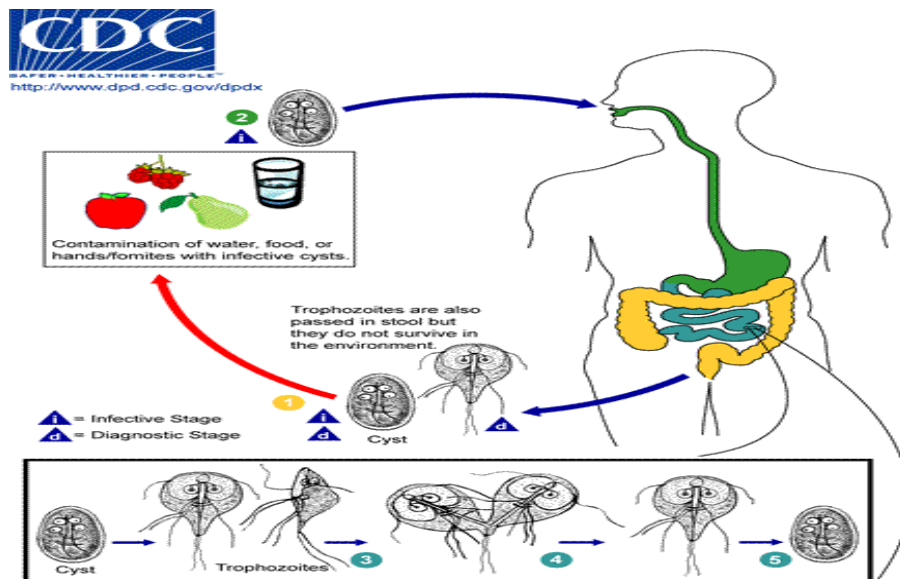


Figure 2 Life cycle of *G. intestinalis* (Source <https://www.dpd.cdc.gov/> Accessed August 20/2016)

### 3.2.2 Epidemiology of *G. intestinalis*

*G. intestinalis* has a cosmopolitan distribution (Feng *et al.* 2011). High-risk groups include infants, young children, international adoptees, travelers, immuno-compromised individuals, and patients with cystic fibrosis. In resource-limited settings, the prevalence of giardiasis has been reported to be as high as 20-40% (Feng *et al.* 2011). The highest rates of infection in resource-limited areas occur among children < 5 years. Many individuals with *G. intestinalis* identified in stool samples are asymptomatic, a point highlighted by studies that identified giardia more commonly in the stool of asymptomatic individuals than among individuals with acute diarrhea (Muhsen *et al.* 2012).

Outbreaks, which often provide the best epidemiological evidence for cryptosporidiosis and giardiasis, tend to be associated with municipal and recreational water systems (Dib *et al.*

2008; Lobo *et al.* 2009; Castro *et al.* 2008-2009; Ggaczyk *et al.* 2009) and crowded human ecologies, such as daycare centers (Carvalho *et al.* 2009; Tashima *et al.* 2009).

Giardiasis is wide spread in Ethiopia. A countrywide survey of giardiasis, using formol-ether concentration method, among schoolchildren and residents showed overall prevalence rates of 8.9% and 3.1% respectively. The corresponding rate for non-school children (5-19 years of age), however, was 4.4% showing that the School children are more significantly infected than their school children counter parts (Hailu *et al.* 1995). *G. intestinalis* infection was generally found to be more prevalent in children than in adult. Among children of school population those in their first decade of life were more affected (Mekete and Adem 2003).

*G. intestinalis* cysts are highly resistant to environmental conditions, being able to survive in cold mountain streams, stomach acid, and chlorine and even in UV-treated wastewater (Li *et al.* 2009; Caccio *et al.* 2003). *G. intestinalis* is consequently the cause of many of the outbreaks occurring in recreational water facilities (Yoder *et al.* 2008). Outbreaks (along with an increase in bacterial infections such as cholera) occur in periods of heavy rain when water management systems are overwhelmed, such as the annual floods in some areas.

### **3.2.3 Laboratory diagnosis**

Most, but not all, cases of giardiasis can be confirmed by stool examination. Finding the trophozoite and cyst stages in stool specimen. The stool is usually offensive, bulky, pale, mucoid (fatty), diarrheic (watery) but there is no blood in the stool. Several specimens collected at different time need to be examined because trophozoites and cysts are excreted irregularly. *G. intestinalis* examination needs differentiation from other intestinal flagellates like *Pentatrichomonas homin* (Mekete and Adem 2003).

### **3.2.4 Transmission of *G. intestinalis***

The parasite transmitted indirectly by contaminated water. *G. intestinalis* is frequently identified as the etiologic agent in waterborne diarrhea outbreaks from contaminated surface water that has been ineffectively filtered or pretreated. Thus contaminated water source include surface waters, shallow wells and household water from either of these sources.

## **4. Materials and Methods**

### **4.1 Study area**

The study was conducted at Haik town, northeast Ethiopia. Haik town is 430km from Addis Ababa. Administratively Haik town belongs to Tehuledere *woreda*. The *woreda* has border with Ambasse *woreda* in the north and west, Worebabo *woreda* in the East and Dessie town in the south (fig. 3). The *woreda* is one of densely populated part of the South Wollo zone and as well from the region. The *woreda* is characterized by agro-ecologically moist midland, the annual rainfall 1145.65 and the mean annual temperature is 18.45°C (Tehuledere Woreda communication office).

In HHC there are eight health officer sixteen nurses, three lab Technologists and nineteen administrative workers. The lab room is well organized with light microscope, fluorescent microscope and necessary chemicals are available. It is estimated that 52800 patients get treatment from HHC(HHC administrative).

### **4.2 Study design and population**

The study was a cross-sectional survey based at Haik health center (HHC). A semi-structured questionnaire was prepared and general information was collected from the community to increase the quality and compatibility of information about their source of infection. The study was conducted from November 2015- April 2016. The target population for the study was all patients suspected of amoebiasis and giardiasis and examined at HHC. From these 500 patients were included in the study using convenient sampling technique.

### **4.3 Stool collection and processing**

A single fresh stool was collected from consented study subjects and was processed by direct wet mount method. Normal saline was added in the stool emulsified on slides. Observed for motile intestinal parasites and trophozoites was under light microscope at 10X and 40x magnification. Lugol's iodine staining was also done to observe cysts of the intestinal parasites as described in Cheesbrough (Cheesbrough 2007).

### **4.4 Data analysis**

Stool samples results and socio-demographic data were analyzed using univariate and multivariate logistic regressions analysis at 95% confidence intervals. Statistical significance

was at p-value less than 0.05. All statistical analyses were performed using the SPSS IBM version 16.0 software.

#### **4.5. Ethical clearance/considerations**

The ethical clearance was obtained from college of natural science Zoological science department. Written informed consent was obtained from each participants or guardians for individuals below 17 years old. Further, no loss or harm came to the study participants of this study. In addition confidentiality of data and information from this study was maintained.

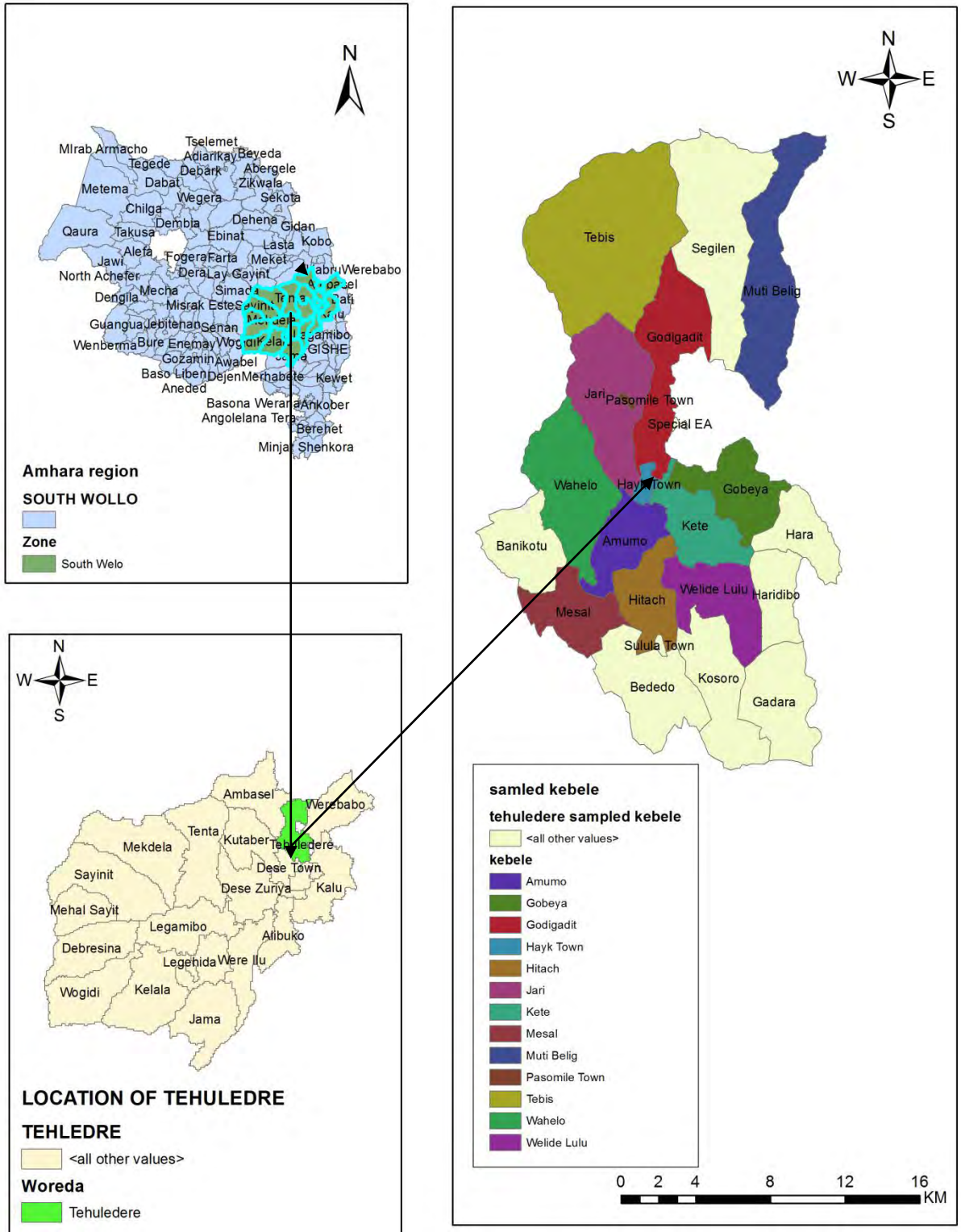


Figure 3 Map of study area

## **5. Results**

### **5.1 Socio-demography**

Out of 500 participants, 278(55.6%) were males and 222(44.4%) females. Majority 341(68.2) were above 15 years old followed by 116(23.2%) and 43(8.6%) in the age groups 6-15 and 1-5 respectively. Their educational status was illiterate 239(47.8%), read/write 156(31.2%), primary school and above 105(21%). Concerning their residence, 245(49%) were from Haik town and 255(51%) from surrounding rural areas. Most of the participants (248(49.6%) were farmers. There were 82(16.4%), 42(8.4%), 67(13.4%), 61(12.2%) merchants, government employees, daily laborer and housemaids respectively.

### **5.2 Amoeba and giardia prevalence and associated factors**

The overall prevalence of amoeba and giardia was 53(10.6%), giardia (8.0%) and amoeba 2.6%. The prevalence of intestinal protozoa in the age groups (years) 1-5, 6-15 and >15 was 6.9, 7.1 and 12.0% respectively. But there was no statistically significant difference in amoebiasis or giardiasis prevalence with respect to age ( $p=0.151$ ). Out of 222 females and 278 males examined, 22(4.4%) and 31(6.2%) were positive, respectively. Although the proportion of males positive for either amoeba or giardia was slightly higher for males than females the difference was not statistically significant ( $p=0.650$ ). The stool examinations revealed that the prevalence of amoeba and giardia in Haik town was 4(1.6%) and in the rural community were 49(19.2%) without statistically significant difference. Univariate and multivariate analysis results are indicated in tables 1 and 2.

The prevalence of giardia or amoeba was significantly higher among participants who eliminated stool in field compared to those in toilet (adjusted odd ratio (AOR) 11.56, 95% CI 4.0-33.38,  $p<0.0001$ ). Participants who had no experience of hand washing after toilet had significantly higher risk of getting amoebiasis or giardiasis compared to those who had the experience (AOR 7.13, 95% CI 3.04-28.4,  $p<0.0001$ ). Similarly patients who attended health education regularly were at significantly lower risk than those that attended sometimes (AOR 4.71, 95% CI 0.86-25.57,  $p<0.0001$ ). Regarding source of household use of water, there was statistically significant difference in amoebiasis and giardiasis positivity among patients whose water source was spring and river water than those who had tap water (AOR 4.88, 95% CI 1.36-17.40,  $p=0.014$ ). In case of the knowledge of transmission of intestinal protozoan parasites, Participants who responded 'no' had significantly higher risk of being

exposed to the infection than their counterparts (AOR 3.13, 95% CI 1.04-4.6, p 0.028). Among patients who responded ‘no’ about their knowledge of preparation of vegetables using disinfecting chemicals were statistically significant (AOR 5.18, 95% CI 1.71-15.71, p 0.004).

Table 1 Binary logistic regression analysis of socio-demographic factors in relation to amoeba/giardia-positivity at HHC, northeast Ethiopia, November 2015-April 2016 (N=500)

Variables	Alternatives	n(%)	pos, n(%)	COR	95%CI	p-value
Age(year)	1-5	43(8.6)	3(6.90)	0.54	0.16-1.85	0.33
	6-15	116(23.2)	9(7.70)	0.61	0.28-1.30	0.207
	>15	341(68.2)	41(12.0)	1.00		
Sex	Male	278(55.6)	31(11.2)	1.14	0.64-2.03	0.65
	Female	222(44.4)	22(9.90)	1.00		
Educational status	Illiterate	239(47.8)	40(16.7)	2.18	1.20-6.51	0.016*
	Read/write	156(31.2)	6(3.80)	0.56	0.18-1.71	0.310
	Primary school/ above	105(21)	7(6.70)	1.00		
Occupation	Farmer	248(49.6)	30(12.0)	2.66	0.78-9.02	
	Merchant	82(16.4)	7(8.50)	1.80	0.78-9.00	0.407
	Gov't employee	42(8.4)	4(9.50)	2.00	0.44-7.22	0.360
	Daily work	67(13.4)	9(13.4)	3.01	0.43-9.60	0.112
	Servant	61(12.2)	3(4.90)	1.00	-	-
Residence	Urban	245(49)	4(1.60)	1.00	-	-
	Rural	255(51)	49(19.2)	14.3	1.35-4.8	0.004*
stool elimination	In toilet	304(60.8)	10(3.20)	1.00	-	-
	In field	161(32.2)	36(22.3)	8.40	4.00-17.5	0.000*
	Both	35(7)	7(20.0)	7.30	2.50-2.00	0.000*
Hand wash after toilet	Yes	219(43.8)	6(2.70)	1.00	-	-
	No	281(56.2)	47(16.7)	7.13	2.98-17.0	0.000*
health education	Usually	106(21.2)	15(14.1)	0.47	0.25-0.90	0.024*
	Some times	74(14.8)	4(5.40)	0.32	0.11-0.96	0.001*
	Never	230(46)	34(14.7)	1.00	-	-
Water source	Tape water	252(50.4)	6(2.30)	1.00	-	-
	Pond and pump	42(8.4)	3(7.50)	3.15	0.07-13.1	0.000*
	Spring	105(21)	14(14.3)	6.30	2.35-16.9	0.000*
	River	109(21.8)	30(27.5)	17.3	6.70-42.7	0.000*
Disinfecting vegetables	Yes	259(51.8)	8(3.08)	1.00	-	-
	No	241(48.2)	45(18.6)	7.20	3.31-15.6	0.000*
parasite transmission	Yes	211(42.2)	5(2.30)	1.00	-	-
	No	289(57.8)	48(16.6)	8.20	3.20-20.9	0.000*
Clean water provider	Gove's only	203(40.6)	35(17.2)	2.70	1.10-6.70	0.290
	Ngo's	19(3.8)	2(10.5)	1.50	0.28-8.30	0.610
	Society it self	193(38.6)	10(5.10)	0.72	.025-2.01	0.537
	All	85(17)	6(7.00)	1.00	-	-
Disposal of garbage	Burning	173(34.6)	12(6.90)	0.89	0.18-4.20	0.888
	River	94(18.8)	17(18.00)	2.60	0.57-12.2	0.214
	Open ground	207(41.4)	22(10.60)	1.42	0.31-6.40	0.644
	Burning	43(8.6)	2(7.60)	1.00	-	-

CI: confidence interval, COR: crude odds ratio, n: number, %: percent, pos: positive, \*statistically significant (p<0.05), Gove's: government, NGO: nongovernment organization

Table 2 Multivariate logistic regression analysis of socio-demographic factors in relation to amoeba/giardia-positivity at HHC northeast Ethiopia, November 2015-April 2016 (N=500)

Variable	Alternatives	n	pos in %	COR	AOR	95% CI of AOR	p-value
Residence	urban	245	4(1.60)	1.00	1.00	-	-
	Rural	255	49(19.2)	14.33	8.47	1.16-12.8	0.028
Way of stool elimination	In toilet	304	10(3.10)	1.00	1.00	-	-
	In field	161	36(22.3)	8.60	11.56	4.00-33.3	0.000
Attending health education	Both	35	7(20.0)	7.35	8.43	1.68-41.8	0.009
	Usually	106	15(14.1)	0.47	2.14	0.68-6.76	0.192
	Sometimes	74	4(5.40)	0.32	0.47	0.01-0.17	0.000
Source of water	Never	230	34(14.7)	1.00	1.00	-	-
	Tape water	252	6(2.30)	1.00	1.00	-	-
	Pond and pump	42	7(16.6)	3.15	3.04	0.50-18.20	0.223
	Spring	105	12(11.4)	6.30	4.88	1.36-17.4	0.014
Disinfecting vegetables	River	101	29(28.7)	17.3	14.35	4.29-47.9	0.000
	Yes	259	8(3.08)	1.00	1.00	-	-
parasite transmission	No	241	45(18.6)	7.203	5.18	1.71-15.7	0.004
	Yes	211	7(3.30)	1.00	1.00	-	-
Hand wash after using toilet	No	289	48(16.6)	8.26	3.84	1.15-12.8	0.028
	Yes	219	6(2.73)	1.00	1.00	-	-
	No	281	47(16.7)	7.13	9.30	3.04-28.4	0.000

AOR: Adjusted odds ratio CI: confidence interval, COR: crude odds ratio, n: number, %: percent, pos: positive, \*statistically significant (p<0.05)

## 6. Discussion

The current prevalence (10.6%) is higher compared with 4% among food vectors in Accra, Ghana reported by Ayeh-kumi *et al.* (2009). The most possible reasons are presence of dirty source and unprotected water, limited health education, open field elimination of stool practices, improper preparation of raw vegetables, and poor knowledge of parasite transmission and awareness of the disease. However, this prevalence is smaller than 26% prevalence rate reported by Mengste (2014) in Debre markos town among HIV positives.

The most prevalent parasite in the study area was *G. intestinalis* (8.4%), which is higher than 4.3% in Awuramba reported by Yihineu (2011), 4.4% in Anambra state reported by Emmy-Egbi *et al.* (2012), 6.4% in Nigeria reported by Auta *et al.* (2013), 6.7% in Babile town Eastern Ethiopia reported by Tadesse (2005), 6.6% in Teda Health center North Gondar, 4.9% in Gorgora and chuahit health center North Gondar reported by Abate *et al.* (2013). The possible reasons might be the presence of poor hand wash habit, open field stool elimination practices, contamination of food and water. Nevertheless, the current giardia prevalence is lower than 69.4% prevalence among Delgi schoolchildren North Gondar reported by Ayalew *et al.* (2011), 16.9 in selected primary school children in Wukro town Tigray Region North Ethiopia reported by Eleni *et al.* (2014) 11.8% among primary school children in Motta town reported by Mulusew (2014) respectively.

The rate of *E. histolytica/dispar/moshkovskii* prevalence in this study (2.2%) was relatively lower compared to finding among small farming village, near Lake Tana, Ehiopia (5.7%) (Dagneu *et al.* 2005) and findings of semi-pastoralist tribes in lower Omo Valley, Southwestern Ethiopia by Teklehaymanot (2009) (16 %). On the other hand, the rate was relatively higher compared to the finding of Mojo health center, eastern Ethiopia 2% in the year 2009 (Bayssa 2013). The rate was also higher compared to the other previous studies by Lowther *et al.* (2000) 0.3% and 1.7% prevalence rate reported by Benedict (2011) of the Hospital hill primary schoolchildren.

The prevalence of intestinal protozoan infection in relation to sex according to this study indicated that males and females were exposed equally like the findings of Yihenew (2011) in Awuramba and Neighboring community. This might be due to the fact there is relatively similar understanding between males and females, but it is against the findings of Abebe and

Alenu (2015) that showed higher prevalence in females than males among Wollega university students.

Majority of the study participants (68.2%) were in the age group >15 years and has no a significant difference in the infection prevalence of amoeba and giardia within this study. This implies that infection by intestinal protozoan parasite does not depend up on age with respect to this study but giardiasis or amoebiasis affects mostly children.

The prevalence of amoeba and giardia infection was higher among patients those that came from rural area to the study clinic than that of urban area. This enables that there is knowledge, attitude and practice gap between the two communities. This was similar with a study at Wollega university students' in 2015; the students from rural areas have higher prevalence than their counterparts. That is why communities from rural areas are engaged in dirty environments and they usually use river or spring which are not treated well (Abebe and Alemu 2015).

Latrine coverage or use was good (60.8%), but still considerable number (39.2%) of study participants eliminate their stool in the field. Furthermore, statistically significant association was observed between infection prevalence and stool elimination in field among the study participants. This further increases the risk of significantly high parasite prevalence of the infection by the parasites. This has a direct relation with possession of toilet. Similar findings were also reported by Yihinew (2011) indiscriminate stool elimination increases the risk of significantly high prevalence among communities. Absence or lack of proper hand wash after toilet and before meal elevate prevalence of intestinal infections. From this study 56.2% participants did not wash their hand after toilet. Even though this result was relatively lower than the findings of 98.8% reported by Vivas (2010) among school children in Angola, hand wash after toilet was one of transmission of giardia or amoeba for patients examined at HHC.

Most of the study participants (79%) were uneducated (illiterate, read and write) this status of education contributes for infection prevalence of amoebiasis or giardiasis in these study participants. Still about half of the participants were farmers and thus had lesser education, poor hygienic habits. This in turn could result in higher risk of infection.

Knowledge and awareness of a given society is enhanced by health education. In this study 46% of study participants never attend health education and there was statistically significant association between health education and the infection prevalence of giardia and amoeba. This implies that significant number of people have no sufficient information about intestinal protozoan diseases. Health education measures create awareness of the dangers associate with exposure of persons to IPIs. These measures include encouraging infected persons to seek early treatment, boiling water before drinking and adequate cooking and sanitary handling of food items before eating. Health education also includes measures reading and writing (Benedict 2011). Lack of knowledge was one of risk factors that contributed for this higher rate of infections among the study participants.

The other factor that exposed the study participants for amoeba and giardia in this study was eating of unwashed and improperly prepared vegetables. The reason may be due to the contamination of vegetables with fecal materials in the farm. Growing of vegetables in fecal-polluted gardens is conducive for transmission of intestinal parasites.

Substantial number of people used tape water (which is relatively better protected). However, still a large number of people two hundred forty eighty (248) were using unprotected water (pond and pump, spring and river). The result of the study also revealed significant association between giardia or amoeba prevalence and with these water sources. This condition could create ideal ground for amoeba and giardia transmission although the current fecal sample result was low. This may arise from contamination of water with animals and human waste that flood into the water bodies the communities were using. Over 70 species of protozoan and helminthic parasites can infect humans through food and water contaminations (Pozio 2003).

## **7. Conclusion and recommendation**

Status of sanitary practices of patients examined at Haik health center is low and most of risk factors for amoeba and giardia infections prevail in the area much remains to be done. Amebiasis and giardiasis are among the health problems for the study participants therefore, household and community sanitary status must be upgraded. Moreover the diagnostic method used could not detect cysts efficiently. Thus, more robust methods using increased sample size, and multiple samples per individual need to be employed to understand the level of infection. Scaling-up of access to safe water, latrine, education and health facility is required. At the root of all these is poverty and lack of interest. It must be somehow addressed as poverty and chronic diseases such as amoebiasis and giardiasis are reciprocal. The finding of the study calls for further investigations in the study site.

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#### **Web address**

<http://www.dpd.cdc.gov> / Life cycle of *G.intestinalis* Accessed August 20/ 2016

<http://www.cdc.gov>/ Life cycle of *E. histolytica* Accessed August 20/2016

## 9. Annexes

### Annex 1. Written consent form

Signature\_\_\_\_\_ Code No \_\_\_\_\_

Name of the study participant \_\_\_\_\_ Age \_\_\_\_\_ Sex \_\_\_\_\_

Name of Physician\_\_\_\_\_ Health center\_\_\_\_\_

I have been informed about a study that plans to investigate the "Status of sanitary practices and Amebiasis and Giardiasis among Patients Visiting Haik Health Center, North Ethiopia" which helps in understanding the prevalence of parasitic infection in relation with knowledge, attitude and practice of different communities towards parasitic disease. At the same time, it enables concerned body in designing better control and preventive measures of parasitic diseases in the study.

For this study, I was requested to give stool sample for intestinal parasites identification. He also informed me that all laboratory results would be kept in secret. Moreover, I was clearly informed that my participation in this study is completely voluntary and I have right to withdraw from participating in this study and in so doing there will be no impact on the overall management of my conditions. Refusal to participate will not result in loss of medical care provided or any other benefit. I was given enough time to think over before I signed this informed consent. It is therefore; with full understanding of the situation that I gave informed consent and cooperate at my will in the course of the study.

Name

(participant)\_\_\_\_\_ Signature \_\_\_\_\_ Date \_\_\_\_\_

Name (Investigator)\_\_\_\_\_ Date \_\_\_\_\_

Name (Wittiness)\_\_\_\_\_ Signature \_\_\_\_\_ Date \_\_\_\_\_

**Annex 2. Written consent form (Amharic version)**

**የውል ስምምነት መጠየቂያ ቅጽ**

የተሳታፊው ስም-----እድሜ-----ጾታ----- የሚሰጥር ቁጥር-----

የበተሙከራ ባለሙያ ስም-----የጤና ጣቢያው ስም-----

የዚህ ጥናት አላማ ወደ ጤና ጣቢያው ከሚመጡ ታካሚዎች መካከል የጽዳት ልማድ እና በአንጀት ጥገኛ ትዋህሲያን አማካኝነት የሚመጣ በሽታ ትስስርና ስርጭት በመለየት፤ የታካሚዎችን ስለበሽታው ያላቸውን እወቀት፤ አመለካከትና ስለሚወሰዱ ርምጃዎች ተገንዝቦ ተገቢ የሆነ እርምጃ በሚመለከተው አካለ እንድወሰድ መሆኑ ተገልጾልኛል።

በመሆኑም ለዚህ ጥናት የሚሆን የሰገራ ናሙና እንድሰጥ ተጠይቄያለሁ። የምርመራ ወጭቱ ሚስጥራዊነቱ የተጠቀ እና ተሳትፎዎ ሙሉ በሙሉ በፈቃደኝነት ላይ የተመሰረተ ሲሆን ከጥናቱ እራሴን ባገል ከጤና ጣቢያው ሌሎች አገልግሎቶች ላይ ምንም አይነት አድልኦ እንደማይደርስብኝ ጭምር ተገልጾልኛል። ስምምነቱን ስሞላ በቂ ጊዜ ተሰጥቶኝ አስቤባት በመሆኑ በጥናቱ ሙሉ በሙሉ ፈቃደኛ ሆኜ ተሳትፈዋል።

### ANNEX 3. Questionnaire

Date -----

Code number -----

This questionnaire is about socio-demographic health status of the study community. It will help the investigator to find out the study participants trends, knowledge, attitude and practices towards the parasites. You are requested to give your answer honestly the information you gave in the questionnaire will be used and handled confidentially.

Please, circle the letter of your choice, tick the boxes that are correct for you or write the figures

#### Part I

##### A. Study participant's identification

Woreda: \_\_\_\_\_

Kebele: \_\_\_\_\_

Household number: \_\_\_\_\_

Age: \_\_\_\_\_

Sex: Male  Female

Education: Illiterate:  Read and write only  Years of formal education \_\_\_\_\_ Others Specify: \_\_\_\_\_.

##### B. Information about occupation

Farmer  Merchant  Government employee  Day worker   
Servant  Others specify \_\_\_\_\_

#### Part II

##### A. Information about knowledge, attitude, practices towards the parasitic diseases.

1. Where do you eliminate your stool?

A. Toilet B. Field C. Both D. no idea

2. Do you wash your hands after toilet?

A. yes B. no

3. Do you wash your hands before eating food?

A. always B. usually C. sometimes D. never

4. Do you attend health education from health centers and from extension workers?

A. usually B. some times C. never

5. What type of water source do you use?  
A. tap water B. pump and pond water C. spring D. river
6. If your water source is grouped under pump or spring, is there a nearby toilet?  
A. yes B. no
7. Do you eat vegetables without proper washing and cooking?  
A. yes B. no
8. Do you know how can vegetables that need no cooking be prepared?  
A. yes B. no
9. Do you know ways of intestinal parasites transmission?  
A. yes B. no
10. According to you, provision of clean water for the society is carried out by  
A. Government only B. NGO's C. the society itself D. all
11. As to you, intestinal protozoan parasitic disease is a/an  
A. ordinary disease B. serious if not treated C. no idea
12. How do you dispose your home garbage?  
A. by burning B. into the river C. open ground D. by burying

**ANNEX 4. Questionnaire (Amharic version)**

**የጽሁፍ መጠይቅ**

ይህ መጠይቅ የተዘጋጀው በአንጀት ጥገኛ ተዋህስኖን ምክንያት ስለሚመጣ በሽታ ህብረተሰቡ በቂ ልማድ፣ እውቀት እና ስለበሽታው ዝንባሌ መኖሩን እና አለመኖሩን ለማረጋገጥ ነው።

ስለሆነም እርስዎ ጥያቄዎችን በደንብ ካነበቡ በኋላ በታማኝነት መልስ እንድሰጡ በትህትና እየጠየኩ የሚሰጡት ማንኛውም መረጃ በሚስጥር ይያዛል

እርስዎ መልስ ብለው የመረጡትን ፊደል በማክበብ እና በሣተኑ ላይ ✓ ምልክት በማስቀመጥ ይሙሉልኝ

**ክፍል አንድ**

ሀ. የጥናቱ ተሳታፊ መለያዎች

ወረዳ \_\_\_\_\_ ቀበሌ \_\_\_\_\_ የቤት ቁጥር \_\_\_\_\_  
ዕድሜ \_\_\_\_\_  
ጾታ \_\_\_\_\_ ወንድ \_\_\_\_\_ ሴት \_\_\_\_\_  
የትምህርት ደረጃ \_\_\_\_\_ የመደበኛ ትምህርት የክፍል ደረጃ \_\_\_\_\_  
ሌላ ካለ ይገለጹ \_\_\_\_\_

ለ. የሥራ ሁኔታ

አርሶ አደር \_\_\_\_\_ ነጋዴ \_\_\_\_\_ የመንግስት ሠራተኛ \_\_\_\_\_ የቀን ሰራተኛ \_\_\_\_\_ የቤት ሰራተኛ \_\_\_\_\_  
ሌላ ካለ ይገለጽ \_\_\_\_\_

**ክፍል ሁለት**

በዚህ ክፍል በጥገኛ ተዋህስኖን ዙሪያ ላይ የእርስዎ እውቀት ዝንባሌና የሚወስዱትን እርምጃ በተመለከተ አጥኝው መረጃ የሚሰበሰቡበት ክፍል ነው።

1. የት ትፀዳዳለህ /ሽ?  
ሀ. በሽንት ቤት ለ. ሜዳ ላይ ሐ. በሁለቱም መ. ሀሳብ የለኝም አላውቅም
2. ከሽንት ቤት ከተጠቀሙ በኋላ እጅዎን ይታጠባሉ?  
ሀ. አዎ ለ. አልታጠብም
3. እጅዎን ከምግብ በፊት ይታጠባሉ?  
ሀ. ሁልጊዜ ለ. አልፎ አልፎ አልፎ ሐ. አልታጠብም
4. በጤና ጣቢያዎችና በጤና ኤክስቴንሽን ባለሙያ የሚሰጡ የጤና ትምህርት ይከታተላሉ?  
ሀ. ሁልጊዜ ለ. አልፎ አልፎ ሐ. አልከታተልም
5. ውሃ የምትቀዱት ከየት ነው?  
ሀ. ቧንቧ ለ. ከኩሬና ከጥልቅ ጉድጓድ የሚቀዳውን ሐ. ምንጭ መ. ወንዝ

6. የውሃ ምንጭቱ የቧንቧ ውሃ ከሆነ በየጊዜው የውሃ ማከሚያ ይደረግበታል?  
 ሀ. ሁልጊዜ ለ. አልፎ አልፎ ሐ. ተደርጎ አያውቅም መ. መረጃ የለኝም
7. አትክልትና ፍራፍሬዎችን በአግባቡ አጥበውና አብስለው ይመገባሉ?  
 ሀ. አዎ ለ. አይደለም
8. ሳይቀቀሉ የሚበሉ የጓሮ አትክልቶች እንዴት እንደሚዘጋጅ ያውቃሉ?  
 ሀ. አዎ ለ. አላውቅም
9. የአጀት ተዋህሶያኑ መተላለፊያ መንገድ ያውቃሉ?  
 ሀ. አዎ ለ. አላውቅም
- 10 እንደእርስዎ አስተሳሰብ ንፁህ ውሃ ለማህበረሰቡ የማቅረብ ግዴታ ያለበት ማን ነው?  
 ሀ. መንግስት ብቻ ለ. የእርዳታ ድርጅቶች ሐ. ማህበረሰቡ መ. ሁሉም ተቀናጅተው
- 11 ህመም ሲገጥመዎ የሚያማክሩት ማንነው?  
 ሀ. የጤና ባለሙያዎችን ለ. የባህል ሀኪሞችን ሐ. ምንም አላደርግም
- 12 የቤት ቆሻሻዎችን በምን መንገድ ያስወግዳሉ?  
 ሀ. በሚቃጠል ለ. ወንዝ በመጣል ሐ. ሜዳ ላይ በመጣል መ. በመቅበር

**Annex 5:** Giardia and *E. histolytica/dispar/moshkovshkii* distribution among study participants by sex and residence.

Patient ID	No	SEX	Residence	Type of parasite
S8	1	F	Haik town	Giardia
S21	2	F	Neighbor	Giardia
S29	3	M	Neighbor	Giardia
S31	4	F	Neighbor	Giardia
S35	5	M	Neighbor	Giardia
S37	6	F	Neighbor	Giardia
S39	7	M	Haik town	<i>E. histolytica/dispar/moshkovshkii</i>
S40	8	M	Neighbor	<i>E. histolytica/dispar/moshkovshkii</i>
S48	9	F	Haik town	<i>E. histolytica/dispar/moshkovshkii</i>
S49	10	M	Haik town	Giardia
S61	11	F	Haik town	Giardia
S65	12	M	Neighbor	Giardia
S69	13	M	Neighbor	<i>E. histolytica/dispar/moshkovshkii</i>
S93	14	M	Haik town	Giardia
S110	15	M	Neighbor	<i>E. histolytica/dispar/moshkovshkii</i>
S114	16	M	Neighbor	<i>E. histolytica/dispar/moshkovshkii</i>
S149	17	F	Haik town	Giardia
S159	18	M	Neighbor	Giardia
S177	19	F	Haik town	Giardia
S183	20	F	Neighbor	<i>E. histolytica/dispar/moshkovshkii</i>
S186	21	F	Haik town	Giardia
S193	22	M	Neighbor	<i>E. histolytica/dispar/moshkovshkii</i>
S203	23	F	Neighbor	Giardia
S216	24	M	Neighbor	Giardia
S219	25	F	Haik town	Giardia
S233	26	M	Neighbor	Giardia
S241	27	M	Neighbor	Giardia
S250	28	M	Neighbor	Giardia
S251	29	M	Neighbor	Giardia
S257	30	M	Neighbor	Giardia
S259	31	F	Neighbor	Giardia
S267	32	M	Neighbor	Giardia
S294	33	M	Neighbor	Giardia
S295	34	M	Haik town	Giardia
S296	35	F	Neighbor	Giardia
S300	36	M	Haik town	Giardia
S301	37	M	Neighbor	Giardia
S302	38	F	Haik town	Giardia
S304	39	M	Neighbor	Giardia
S308	40	M	Haik town	Giardia
S312	41	M	Neighbor	Giardia
S322	42	F	Neighbor	Giardia
S343	43	M	Neighbor	Giardia
S357	44	M	Neighbor	<i>E. histolytica/dispar/moshkovshkii</i>
S390	45	F	Neighbor	Giardia
S401	46	F	Neighbor	Giardia
S408	47	F	Neighbor	<i>E. histolytica/dispar/moshkovshkii</i>
S464	48	F	Neighbor	Giardia
S477	49	M	Neighbor	<i>E. histolytica/dispar/moshkovshkii</i>
S483	50	M	Neighbor	Giardia
S487	51	M	Neighbor	Giardia
S491	52	F	Neighbor	Giardia
S494	53	F	Neighbor	Giardia

## **Declarations**

I, the undersigned, declare that this Thesis is my original work and all source materials used are duly acknowledged.

Name	Haji Mamo
Signature	_____
Date	_____

**Statement of the supervisor(s)**

This Thesis has been approved for submission to the Department Zoological Sciences for public defense.

Name            Hassen Mamo(PhD)

Signature       \_\_\_\_\_

Date             \_\_\_\_\_