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COMMUNITY BASED PREVALENCE OF INTESTINAL PARASITIC INFECTION AND ASSOCIATED RISK FACTORS STUDY IN YEKA AND ARADA SUB-CITY OF ADDIS ABEBA, ETHIOPIA.

BY:-BEHAILU TSEGAYE (BSc)
ADVISER: MISTIRE WOLDE (MSc, PhD)
: DESSIE ABERA (MSc)

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GRADUATE STUDIES

This is to testify that the thesis prepared by Behailu Tsegaye: entitled "COMMUNITY BASED PREVALENCE OF INTESTINAL PARASITIC INFECTION AND ASSOCIATED RISK FACTORS STUDY IN YEKA AND ARADA SUB-CITY OF ADDIS ABABA,ETHIOPIA" and submitted in partial fulfillment of the requirements for the degree of Master in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology Specialty) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

External Examiner________________________Signature____________Date____________________

Internal Examiner________________________Signature____________Date_________________

Advisor:Dr Mister Wolde (PhD) Signature_____________Date________________________

Advisor: Mr. Dessa Abera (MSC) Signature _________Date______________________

____

Chairman of the Department or Graduate Program Coordinator

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Abbreviations

AAU	Addis Ababa University
AU	Africa Union
CDC	Centre for Disease Control
CI	Confidence Interval
EPG	Eggs Per Gram
EPG	Eggs Per Pram
Exp (B)	Expected Beta
GRDP	Gross Regional Domestic Product
IPI	Intestinal Parasitic Infection
KK	Kato-Katz technique.
RPM	Rotations Per Minute
SOP	Standard Operating Procedure
SPSS	Statistical Package for Social Science
SSA	Sub-Sahara Africa
STH	Soil Transmitted Helminthes
UN	United Nations
UNECA	United Nations Economic Commission for Africa
WHO	World Health Organization

DEFINITION OF OPERATIONAL TERMS

Definitive hosts: the hosts in which sexual development of helminthes occur.

Hygienic behavior: Washing hands with soap and water during the critical times.

Hygiene Practices: Ways of preventing intestinal parasitic infections including how
To wash hands properly using soap and water, and critical times for hand washing.

Intestinal parasitic infections: Intestinal protozoan and helminthic infestations confirm by stool examinations.

Public Health Interventions: This is a process of curtailing infections among the school going children by installing tippy taps, providing soap and water for hand washing.

Sanitation practices: Proper methods of using latrines and keeping them clean.

Abstract

Background: Intestinal parasitic infections an important public health problem in developing countries. Low socioeconomic conditions, lack of access to potable drinking water, poor personal hygiene and environmental sanitation are the factors associated with intestinal parasitic infection.

Objective: to determine the prevalence of intestinal parasitic infections and associated risk factors in selected sub cities of Addis Ababa, Ethiopia.

Methods: Community-based cross-sectional study was performed in Yeka and Arada sub cites of Addis Ababa from January to June 2019. A total of 382 study participants were selected by using multistage sampling technique. From each participant, socio-demographic data was collected by using standardized questionnaire and risk factors analyzed by using SPSS version 20 for analysis value less than 0.05 considered as statistical significant. In addition, stool samples were collected and analyzed by direct wet mount, formal ether concentration techniques and kato techniques. Moreover, parasite load was estimated by using WHO criteria for degree of parasitemia..

Results: Out of the total 382 participants prevalence of intestinal parasites was 10.5%. Among these, *Entamoeba histolytica/dispare*(6.01%) was the most common, followed by *Giardia lamblia* (1.8%), *Ascaris lubricoide* (1%) ,*Hookworm* (0.8%) and *Strongloid stercolaris* (0.3 %). In addition, 0.5% of positive cases were double infection, *Entamoeba histolytica/dispare* and *Giardia lamblia*. Intestinal parasitosis was seen more in female (5.75 %) than male (4.18).According to WHO criteria the intensity of helminthic infection was light. Intestinal parasitic associated risk factors such failure to wash hands with soup before meal, a habit of eating undercooked vegetables had Statistical significant p value less than 0. 05.

.**Conclusion***:* The intensity of helminthic infection was light; this might be related to low prevalence of intestinal parasitic infection. In addition, intestinal parasitic infection was associated with proposed risk factors. This indicates the need for additional preventive and control mechanism. Furthermore, large scale studies including assessment of environmental sanitation, and healthy education need to undertake the problem.

Keywords: Intestinal Parasitic Infection, Risk Factors, Addis Ababa.

1. Introduction

1.1 Background

Intestinal parasites (IPs) are organisms living in the body of other organism having some metabolic dependence (1). Generally, these parasites are more common in tropics and subtropics than elsewhere in the world. Intestinal parasites can be categorized into two groups; protozoa and helminthes these parasites cause various intestinal symptoms including abdominal bloating, cramps, constipation, diarrhea, lack of appetite and vomiting (2). Intestinal protozoan's are single cell organisms, whereas helminthes are multicellular organisms. Intestinal protozoans, such as *Entameba histoltica, Gardia lamblia* whereas most common soil-transmitted helminthes (STH) includes *Ascaris lumbricoides*, *Hook worms and Trichuris trichiura* are associated with depletion of nutrient of the host for their own nutrition, damage host tissue, and interfere on immune balance (3).

Regarding transmission of parasites, intestinal parasites acquired without any intermediate host directly through contact with parasite eggs includes (*A. lumbricoides and T. trichiura*) or larvae (in the case of hookworm) that thrive in the warm and moist soil (3). *A. lumbricoides and T. trichiura are* ingested from contaminated foods, raw vegetables, and water and also larvae develop and migrate to their final habitat in the intestine (4). As adult worms, STH can live for years in the human gastrointestinal tract and their eggs are excreted with faeces of infected persons. While *A. lumbricoides and T. trichiura* feed on their host's intestinal food content, hookworms suck blood and fluids from grasping and cutting gut tissue (3; 4).The eggs *of Ascaris lumbricoides* develop best in sandy, damp soils; resistant to cold and disinfectants (5).
The infective stages of *Ascaris lumbricoides* are embriyonated eggs having enormous capacity of withstanding the environmental extremes of urban environments. Viable *Ascaris lumbricoides* have been recovered in soil samples more than 10 years after being first deposited because of a lipid layer that makes them resistant (6).On the life cycle of helminthes, there are three main developmental stages: eggs, larvae and adults. Adult worms infect definitive hosts (those in which sexual development occurs) whereas larval stages may be free-living or parasitize invertebrate vectors, which are their intermediate hosts (7).

On the other hand, protozoa are unicellular parasites that inhabit the human intestinal tract. Most of these protozoa are non-pathogenic commensal, or causing only mild disease (8). In terms of disease burden and prevalence, here focused on two common intestinal protozoa; i.e. *Giardia lambla* also often referred to as *G. lambliaor; G .duodenalis,* causing *giardiasis and Entamoeba histolytica,* causing amoebiasis. The life cycle of both intestinal protozoa species is simple consisting of a cyst stage (long-lived infective stages) and a motile trophozoite stage. Once ingested, cysts transform to the trophozoite stages, during which they take up nutrients and undergo asexual replication, while some develop into cysts again. Cysts are characterized by a resistant wall and once excreted in stool, they maintainthe life cycle by further faecal-oral spread (9; 10). Both, *E. histolytica and G. intestinalis* are transmitted through contaminated water and food; however the latter is relatively uncommon for *G. intestinalis* (11). *E. histolytica, E.dispar, and E. moshkovskii*, are morphologically identical in both their cyst and tropozhoite stages (12; 13).Majority (about 90%) of individuals infected with *Entamoeba species* are colonized by the nonpathogenic strain *E. dispar* (14;15).

Intestinal parasites expected to be prevalent is almost negligible amount in big cities and relative hygienic environments. In this regard, the prevalence of IP in Addis Ababa should be relatively lower than all other big cites IP prevalence rates, in Ethiopia. Thus this research addresses the prevalence of IP in selected sub cities of Addis Ababa, Ethiopia.

1.2 Statement of the problem

Intestinal parasitic infestation represents a large and serious medical and public health problem in developing countries (16). It is estimated that 3.5 billion people are affected, and 450 million are ill as a result of these infections (17). In addition to this 270 million pre-school children and over 600 million of school children are living in areas where the parasites are intensively transmitted and are in need of treatment and preventive interventions (18). Annually, 16 million deaths occur due to the intestinal parasitic disease worldwide (19). according to (WHO; 2012) report, in Ethiopia the two-third proportion of children aged 1–14 years requiring preventive chemotherapy which mainly involve deworming of infected children (20) .

Approximately 4 to 10% of carriers infected with *E. histolytica* develop clinical disease within a year, and amoebic dysentery is considered the third leading cause of death from parasitic disease worldwide (21) .According to WHO report, approximately 500 million people are infected worldwide annually with *E. histolytica* resulting in symptomatic illnesses and death in about 50 million and 100,000 persons, respectively (22).However, it is believed that since 90% (450 million) of infections are due to *E. dispar*, while 10% (or 50 million) are infections with *E. histolytica* (23).

Apart from causing morbidity and mortality, infection with intestinal parasites has known to cause iron deficiency leading to poor school performance and absenteeism, growth retardation and other physical and mental health problems in children (24).Among intestinal parasites, the WHO considers soil-transmitted helminthes (STH) as the most common infections in vulnerable populations (25) .On a larger scale, people living below the poverty line in low-income countries, especially young women and young pregnant women, their infants, and children, are at a high risk of IPI (26).

Intestinal parasitic infections (IPIs) are a major concern, mostly in developing countries, particularly in sub-Saharan Africa (SSA),Including Ethiopia due to the low level of environmental and personal hygiene, contamination of food and drinking water that result from improper disposal of human excreta (23, 27).

In addition, lack of awareness of simple health promotion practices is also a contributing factor (28).and also In Ethiopia; intestinal parasitic infection is sixth of the top ten causes of morbidity

amongst children (29). According to the Ethiopian Ministry of Health more than half a million annual visits of the outpatient services of the health institutions are due to intestinal parasitic infections (30).Addis Ababa's water is a major public health concern that could lead to the spread of cholera and other water-borne diseases .With almost 40% of the vegetables consumed in Addis derived from fields irrigated with waste waters, high concentration of metals in food threaten people's health and wellbeing (31).

1.3 Significance of the study

- ❖It can be used by those who are responsible for planning health development activity.
- ❖Providing epidemiological information on the current situation of IPIs prevalence& local risk factors for the community.

2. Literatures Review

The WHO (2012) estimates 25% of humans are infected with *Ascaris lumbricoides*, while *Necatora mericanus* and *Ancylostoma duodenale* (hookworms) infects over a billion people (20).They are widely distributed in tropical and subtropical areas, with the greatest numbers occurring in sub-Saharan Africa, the Americas, China and East Asia. In South America, a systematic review and geostatistical meta-analysis by Chammartins *et al*. found that a population-adjusted prevalence of infection with *Ascaris lumbricoides* was 15.6%, with *Trichiuris trichiura* was 12.5%, and with hookworms was 11.9% from 2005 onwards (32).And also similar cross-sectional study was done in Saudi Arabia found that *Entamoeba histolytica/dispa*r the most predominant intestinal parasite (8.2%), followed by *Giardia lamblia (6.5%) (32).*

The WHO (2012) report indicates that in Africa, the number of children at risk of infection by soil transmitted helminthes is 280 million in 42 countries (33).In 2001, the Fifty Fourth World Health Assembly resolved to attain by 2010 a minimum target of regular administration of chemotherapy to at least 75% and up to 100% of all school-age children at risk of morbidity from the disease (34). The school preventive chemotherapy has covered 25% of the school age children in the period between 2006 and 2009 (WHO, 2012)(35).A study by Abate *et al,* in Ethiopia found *Ascaris lumbricoides* was the most predominant parasite (23.2%) followed by hookworms(6.6%),*Hymenolepis nana* (1.5%), *Enterobius vermicularis*(0.4%), and *Strongyloides stercoralis* (0.2%) (35).

And also Community-based, cross-sectional study was done in Jimma town among a young adult population with a mean age of 23 (+16) from October 2004- January 2005.From their research they found that the most dominant intestinal parasite was *T. trichiura* 124 (16.4%),followed by *A. lumbricoides 44 (5.8%).* they conclude that Intestinal parasitosis is highly prevalent in the study area (36).In another similar study conducted in Butajira, Ethiopia, that included mothers (mean age 26.8 (+6.3) showed an overall prevalence of any such infection being 43.5% (95% CI 40.2-46.8%)(38).Likewise Community-based, cross-sectional study conducted among highland and lowland dwellers in Gamo area, South Ethiopia with, a mean age of the participants was 25 ± 19. From this they Found that the prevalence of *Entamoeba histolytica/dispar* was the highest 98(11.4%), followed by *Giardia lamblia* 91(10.6%), *Ascaris lumbricoides* 67(7.8%),

Strongyloides stercoralis 51(5.9%), hookworm 42(4.9%), from this study they conclude that the high prevalence of intestinal parasitic infections among the lowland and highland dwellers in Gamo area (38).

In another cross-sectional study conducted among street dwellers in Addis Ababa with the mean age of the study participants were 28.4+12.4 years (age ranged from 4 to 75 years). From this study they Found that the most prevalent parasites were *Ascaris lumbricoides*(34.9%), *Trichuris trichiura* (22.8%), *Giardia lamblia*(9.6%) and *Entamoeba histolytica/dispar*(8.2%). according to this survey they conclude that high prevalence of intestinal parasitic infections in street dwellers in Addis Ababa.(39). In another similar cross-sectional study was conducted at Gandhi Memorial Hospital with pregnant women who came for regular ANC follow-up with the mean age of the study participant was 27.6 (+4.3) year .(age ranged from14 to 39 years). Found that the most prevalent parasites were *Entamoeba histolytica 39 (12.4%), followed by Ascaris lumbricoid 10 (3.2%),Giardia lamblia 7 (2.2%),Trichuris trichuria 5 (1.6%), Hookworm 4 (1.3%),strongloyid strcolaris 3(*1.0%) were found*.* AOR of *5.95 (95% CI 1.68, 21.14)* however*,* statistical significance was not achieved. (40).

Intestinal protozoan infections are distributed worldwide (41). While most infections and death from these parasitic diseases affect people in developing countries, they also cause significant illness in developed countries (42).It is estimated that 12% of the population of the world are infected with *Entamoeba histolytica*, 10% of whom are likely to have symptoms (43).These infections occur worldwide with the prevalence disproportionately increased in developing countries. This has been associated with poor socioeconomic conditions and sanitation levels which facilitates contamination of food or water by *E. histolytica* cysts. Infection with *E. dispar* occurs approximately 10 times more frequently than infection with *E. histolytica* (44).

Several studies was indicated a high prevalence of intestinal protozoan infections in Ethiopia, Egypt, and Cote D'ivoire(34).In Ethiopia, the prevalence of *Entamoeba histolytica* was found to be 13.8% (47). In Egypt there was a prevalence of >21% (46). In Cote D'Ivoire the prevalence of *Entamoeba histolytica* and *Giardia lamblia* was 18.8% and 13.9% respectively.
In other previous study conducted at TikurAnbesa hospital showed that *E. histolytica* trophozoite was the most common reported parasite with 13.6% prevalence rate (45). Community-based,

cross-sectional study was done in Jimma town from October 2004- January 2005. From this study they found that the prevalence of amoebiasis and giardiasis was 3.1% and 3.6%, respectively. These are within the range of the nation-wide prevalence rate for amoebiasis and giardiasis (36).

According to the World Health Organization (WHO) reports, *Giardia lamblia* is one of the most common parasites which affect nearly two thirds of people worldwide (33). Giardiasis has a worldwide distribution, but it is more common in areas with poor sanitary conditions and insufficient water treatment facilities. In developed countries, nearly 2% of adults and 6-8% of children get infected (46). The prevalence is significantly higher in developing regions of the world where nearly 33% are infected (47). Giardiasis is a common cause of diarrheal disease in all age groups, especially in children (42).

3. Objective

3.1. General objective

- ❖To determine the prevalence of intestinal parasitic infection and associated risk factor among the community of Yeka and Arada sub city in Addis Ababa; Ethiopia

3.2 Specific objectives

- ❖To determine the magnitude of intestinal parasites among people live in Yeka and Arada sub city in Addis Ababa; Ethiopia

- ❖To assess associated risk factors of intestinal parasitic infection among people live in Yeka and Arada sub city in Addis Ababa; Ethiopia.

- ❖To determine the intensity of intestinal helminthes among people live in Yeka and Arada sub city in Addis Ababa; Ethiopia.

4. Materials and Methods

4.1 Description of the Study Area

The study was conducted in Addis Ababa, capital city of Ethiopia. The city was established in 1886, and located at 9°1′48″N 38°44′24″E coordinates. Addis Ababa has an area of 527 km2 and an estimated population of 3,040,740 as stated by CSA (48).Addis Ababa is the also political center being the head quarter for the African Union and United Nations Economic Commission for Africa. Addis Ababa, nearly 80% of the inner city areas are defined as slum (48).Nearly 70% of the houses are dilapidated and lacking basic facilities and urban infrastructure services (31).According to World Bank the city has only capacity for picking up 45-80% of solid waste (48).

According to the national census, 99.9%of the housing units of Addis Ababa had access to safe drinking water, while 14.9% had flush toilets, 72.27% pit toilets and 14.3% had no toilet facilities (31). Only 14% of the households are linked to the sewage system.(31). In the city, around 70 public toilets are available to those who do not have domestic toilet facilities. However, the public toilets are not evenly distributed in the City and most of them are not accessible to the inhabitants. Thus, all available vacant spaces within the City (green areas, road sides and riverbanks) are common defecation sites (31).

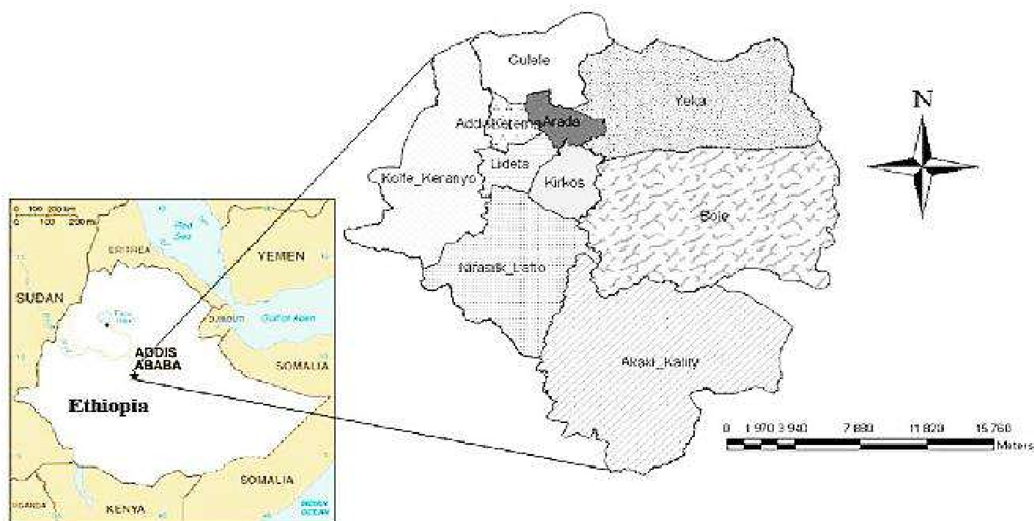


Figure 1: Map of Addis Ababa and its ten Sub Cities (Source: https://www.researchgate.net/figure/Map-of-Addis-Ababa-City_fig1_281460707 retrieved at 6:15pm Jan 15, 2018)

As it is shown in Figure 2 Arada sub city is located in the northern area of the city, nearby the center. It borders with the districts of Gullele, Yeka, Kirkos, Lideta and Addis Ketema subcities. It has area of 9.9 sq.km and population size of 225,999 (31). It is the second most densely populated subcity with 22,805 per sq.m. It has ten Woredas administered under its jurisdiction. Out of the ten Woredas, two Woredas (6, 10) were drawn to be the study site from Arada sub city.

On the other hand, Yeka sub city is located in the northern east area of the Addis Ababa, bordered with the districts of Arada, Bola, Gullele and Kirekos sub cities. It has area of 85.98 km square and population size of 368,418 people (33). And 4,284.9 people live in one kilometer square. It has Woredas administered under its jurisdiction. Out of the thirteen Woredas two Woreda*s* (3, and 6) were drawn to be the study site from Yeka subcity and also the number of household of Yeka and Arada sub-city 90,195, 49,564 respectively.

4.2 Study Design and Period

A Community-based cross-sectional study was conducted from January to June 2019 to determine the prevalence of IPIs and associated risk factors.

4.2.1. Source population: the population from Yeka and Arada sub -city.

4.2.2 .Study Population: the household from Woreda 6 and, 3 of Yeka and Woreda 6 and 10 of Arada sub city of Addis Ababa who was apparently healthy; eligible and came to healthy centers of Woreda.

4.3 . Eligibility criteria

4.3.1 Inclusion criteria: -Volunteer population; did not take any anti-intestinal parasitic drug within 2 weeks.

4.3.2 Exclusion Criteria: -Non-volunteers population; who took any anti- intestinal parasitic drug within two week.

4.4. Study Variables

4.4.1. Dependent Variables:-The prevalence of intestinal helminthes and protozoa parasites Status.

4.4.2. Independent Variables:- Sex; Age; Parent education status, Parent occupation; Protected shoe ;Hand washing with soap after deification

4.5.1. Sample Size Calculation

The sample size was determined using the single proportion population formula (49). Cross-sectional survey: n = Z2 p (1-p)/d2 Where, P =50 %since there was no studies conducted concerning the present topic in the study area; to achieve the maximum sample size. d = absolute precision and is taken as 0.05 and Z = 1.96 at 95% confidence interval .n=1.962x0.50x0.50/.052, this gives a sample size of 384. To minimize errors arising from the likelihood of non-compliance, five percent of the sample size was added, giving a final sample size of 404.

N = (Zα/2)2 * (1-p) * (p)/(d)2

Where n=sample size estimated

α = level of significance

z = at 95% confidence interval Z value (α = 0.05) =>Zα/2=1.96d= Expected margin of error =0.05

P =50 % since there was no studies conducted concerning the present topic in the study area

n =1.962x0.50x0.50/.052

n=384+5% Contingency for the unknown circumstance.

n=404

4.5.2. Sampling method

The Addis Ababa city has a population of 3,040,740 and is divided into 10 sub-cities. Among the ten sub city of Addis Abeba, Yeka and Arada were selected purposely. Because of resources and budget constraint from each sub city by a lottery system woreda 3 and 6 from Yeka and 6 and 10 from Arada were selected. A proportionate sampling was conducted to define the number of households from each woreda for the survey. The total households in each woreda is multiplied by calculated sample size (404) and divided by the number of total households in selected woredas (woreda6&10 of Arada and woreda3&6 of Yeka sub- cities) as shown by Table1.

The sample size in each woreda == total household in each woreda*calculated sample size (404)

Number of total households in the selected woreda

The number of sample from single proportion population formula proportionally allocated to the number of households in each woreda. The house numbers of the households within the woredas were used as a sampling frame.

Table, 1 Allocation of the study participants from each of the selected woredas

Sr number	Sub city	woreda	Total number of household in the woreda	Sample obtained
1	Arada	6	6935	116
		10	4465	74
2	Yeka	3	4707	79
		6	8084	135

Based on their population size four woredas were randomly selected from two sub-cities. Two woreda (4, 10) from Arada sub- city and two woreda (3, 6) from Yeka sub city were included in the study. Similarly, 190 (116 from Woreda, 6) and 74 from woreda, 10) samples were obtained from Arada sub city and214 (79 from woreda, 3, 135 from woreda, 6) samples were obtained from Yeka sub- city settings. And also offices, shops and other public houses were excluded. Convenience sampling was utilized to select household from each *woredas* in order to undertake the survey.

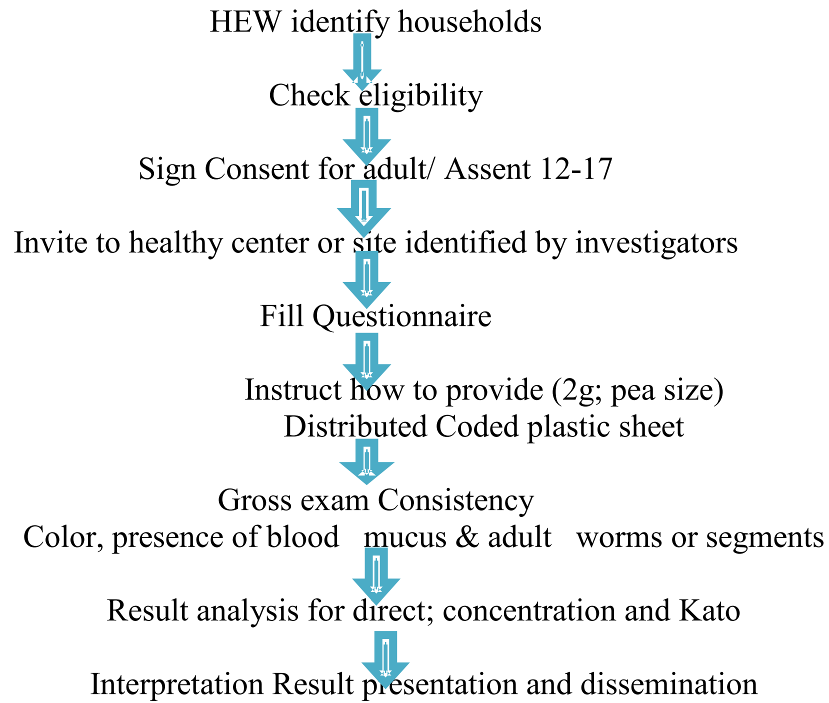
4.5.3 .Methods of Data Collection

4.5.3.1 .Interview with Structured Questionnaires

A structured questionnaire was prepared originally in English according to the research objectives. According to local situation the questionnaire was translated in to the local language i.e., Amharic. Healthy professionals were recruited for data collection. Pre-survey visit was made to see the place, the number of population in selected areas and the convenient time for data collection.

After the aim of the survey was explained to each participant/guardian, the participants were interview about intestinal parasites, types of toilets utilization, source of drinking water, source of food and feeding habit using structure questionnaire. In case of children younger than 17 years, their parents/guardians were interviewed. Information on socio-demographic characteristics of the individuals was also including in the questioner.

Figure 2, Work flow



4.5.4. Laboratory analysis

Direct wet Preparation of Fresh Stool

❖PRINCIPLE

- The value of wet preparations lies in the fact that certain protozoa trophozoites retain their motility which may aid in their identification. Definitive identification however may not be possible, especially for amoeba, since the nuclei of trophozoites and cysts are often not clearly visible.
- Wet preparations on fresh unpreserved liquid stool should be performed and examined as soon as possible (within 30 minutes of passage) and on soft/formed stool within 60 minutes of passage provided that prior arrangements have been made with the lab. Wet preps can also be used to determine schistosome egg viability ("flame" cells). (Wet preps can also be performed on SAF fixed specimens but motility is lost since the fixative kills the parasites.)(50).
- **Formalin-ethyl acetate sedimentation concentration**
- This procedure leads to recovery of all protozoan cysts and oocysts, helminthes eggs and larvae present in the stool specimen; it is recommended as being the easiest to perform and the least subject to technical error, allowing recovery of the broadest range of parasitic elements. The specimen can be fresh or fixed stool. The preparation will often contain more debris than that obtained with the flotation and other procedures.
- Note: This technique is not recommended for eggs of Fasciola spp. and larvae of Strongyloides stercoralis

4.5.4.3 Kato–Katz technique

❖ PRINCIPLE

Most organisms release eggs sporadically and therefore there is no correlation between burden and the number or organisms seen in a stool sample. Schistosome eggs can be released at a steady rate therefore it is desirable to get an accurate measure of the number of eggs in a given amount of sample so that worm burden can be inferred. The Kato thick smear method accomplishes this by depositing a reproducible amount of material on the slide (50).

Procedure

4.6. Data Quality Control

A standardized questionnaire was used to collect data of socio-demographic characteristics for determining the prevalence of intestinal parasitic infection. To ensure the quality of data, first the questionnaire were pretested. Training was given for the data collectors and supervisors before the actual data collection. Every day after data collection, questionnaires was review and check for completeness, accuracy and clarity by principal investigator.

4.7. Quality control

4.7.1. Pre-analytical

The processes of selecting appropriate tests, ordering, collecting, identifying and labeling(three label system i.e PID, CODE, LSN), handling, and transporting biological samples were performed as per the standard guideline. The Process of accepting samples by the laboratory, centrifuging, diluting, and sorting the biological samples all the process of pre-analytical steps were performed according to standard operating procedure. Aseptic techniques were implemented in all the steps of specimen collection.

4.7.2. Analytical

All materials equipment and procedures were adequately controlled. Detect each stage of intestinal parasites in less than 20 min of stool sample collection. To increase the quality of result, each stool sample was examined by triple test such as direct wet mount; concentration and kato–techniques.

4.7.3 .Post-analytical

All of the extracted information (filled questionnaire and laboratory findings) were checked for legibility, completeness, consistency and placed in secure location. Cross-checking and data cleaning were done. During data cleaning and cross-checking missing information was obtained by going back to the questionnaire and laboratory records. The data were stored in a CD as a backup. All laboratory isolates were stored as per the SOP of the study site

4.8. Data Management

All data obtained from patients/guardians was kept on a secured, password protected computer. Hard copies of the data collection sheets were kept securely locked and archived to protect Client's confidentiality.

4.9. Data analysis and interpretation

The collected data were coded and entered in to SPSS software version 20 to perform the statistical analysis. The base line characteristics of the study population were summarized using medians and ranges for continuous variables, simultaneously proportions and frequencies for categorical variables. Internal comparison was made using logistic regression to determine the independent effect of the variables by calculating the strength of the association between infection and risk factors using odds ratio (OR) and 95% confidence interval (CI). Crude and adjusted OR was computed using bivariate and multivariate logistic regression analysis respectively. P value less than 0.05 (5%) was considered statistically significant.

5. Ethical considerations

The study was conducted after ethically reviewed and approved by the Department of Research & Ethical Review Committee DRERC of Department of Medical Laboratory Science College of Health Sciences, Addis Ababa University. Formal latter was written from the DMLT and permission was obtained from Addis Ababa health bearu.

6. Result

Socio demographic characteristics

A total of 404 residents were selected for investigation. Of these because of inability to provide specimen and due to previous drug intake, 4.9 %(n=19/404) and 0.7% (n=3/404) were excluded respectively. Therefore, finally, a total of 382 respondents were included in the study. Among the study subjects, 136(35.6%) were males and 246 (64.4%) were females. female to male ratio of 1:1.8. The mean age of study subjects was 29.6(±15) years with a minimum and maximum age of 5 and 80 years respectively (Table, 2).

The risk factors associated with intestinal parasite in relation to socio-demographic characteristics were examined by regression analysis. In this study 246(64.1%) household Heads were mothers/females and the remaining 136(35.6%) were fathers/males. The highest percentages of household heads were government workers 143(37.4%), followed by housewives and private employee with 66(17.3%) and (30(7.9%), respectively.

From the total household, 36 (9.4%) were unable to read and write, 27(7.1%) were able to only read and write, 158 (41.4. %) were in primary (1-8), 64(16.8%) secondary level and 97(25.4 %) had higher institution certificate/diploma. Regarding hand washing habit before eating and after toilet 322(84.3%) household had a habit of washing and 60(15.7%) household had no habit of washing. Tap water supply was the source of water 382 (100%) for domestic purposes, (Table, 2).

Table 2, Socio demography and associated risk factors.

Background characteristics		Frequency	Percentage
Sex	Female	246	64.4%
	Male	136	35.6%
	Total	382	100%
Age group	<15	86	22.5%
	15-24	53	13.9%
	25-85	242	63.4%
	85+	1	0.3%
	Total	382	100%
Education	Illiterate	36	9.4%
	Read &write	27	7.1%
	Primary(1-8)_	158	41.4%
	Secondary(9-12)	64	16.8%
	Collage	97	25.4%
	Total	382	100%
Occupation status	Student	117	30.6%
	House wife	66	17,3%
	Government employee	143	37.4%
	Private employee	30	7.9%
	Other (specify)	26	6.8%
	TOTAL	382	100%
monthly income	<500	7	1.8%
	500-2000	105	27.1
	2001-4000	63	16.5%
	4001-6000	56	14.7
	6001-8000	3	0.8%
	>8000	7	1.8%)
	Missing	141	38.9%
	Total	382	100%
Eating raw meat	Yes	185	48.4%
	No	197	51.5%
Hand washing habit	Yes	322	84..3%
	No	60	15.7%
eating undercooked vegetables	Yes	260	68%
	No	122	31.9%
food prepared on the road	Yes	183	47.9%
	No	199	52.1%
Sources of water	Pipe water	382	100%
	Other(river ,stream)	0	0%

Association of intestinal parasitic infection with risk factor

The overall prevalence of IPIs was 40(10. 5%). Among these positive cases, 23 (6.02 %) were females and 17 (4.50%) were males. Regarding age group classification of cases, those with age range of 25–84 years were more infected than those in 15-24 years of age and <15 age groups of study subjects [20(5.2%) vs. 12(3.14%)and8(2.09 %.)], respectively. But, this was not statistically significant difference (P>. 0.05). (Table,3)

In this study significant relationships were observed between risk factors for acquiring intestinal parasitic infection and in some of socio-demographic, and environmental or behavioral factors. Among the total study participants 322 (84%) had hand washing habit before eating &after toilet. Household heads who were unable to read and write and also only those who read and write had the risk of their children to acquire the intestinal parasitic infection than household heads who had higher educational level with statistically significant difference ($p < 0.05$). In addition, among the study participants 260(68%) had eating under cooked vegetables before than households who had not 124 (32.4.%). This showed that households with undercooked vegetables condition had a more likelihood to be infected than those who had cooked vegetables condition (Odds ratio12.36, 95% CI 4.344to 35.170, $p < 0.0001$).

Households who had no habit of hand washing were the risk to be infected by intestinal parasite infection than those who had hand washing habit (Odds ratio 2.10 95% CI (1.183-3.747)) with statistically significant difference ($p < 0.001$) (Table 3 and 5).

Table3 Bivariate logistic regression analysis of potential risk factors associated with parasitic infection in Yeka and Arada sub city of Addis Ababa; Ethiopia Jan-Jun2019.

Independent variable		Intestinal parasites			
		No examined	No infected	Crude OR (95%CI)	p-value
Sex	Female	246(64.4%)	23(6.02%)	0.826(0.385-1.770)	0.623
	Male	136(35.5%)	17(4.45%)	1	
Age group	<15	86(22.5%)	12(3.14%)	130135(0.0001-000)	1.000.
	15—24	53(13.9%)	8(2.09%)	1(0.0001-000)	1.000
	25-85	242(63.4%)	20(5.2%)	714812(0.0001)	1.000
	85+	1(0.3%)	(0%)		
Education	Illiterate	36(9.4%	14(3.66%)	2.694(0.26-17.036)	0.292
	Read &write	27(7.1%)	8(2.09%)	1.796(0.177-18.249)	0.620
	Primary(1 -8)_	158(41.4%)	10(2.61%)	4.557(1.323-15.692)	0.016
	Secondary (9-12)	64(16.8%)	3(0.78%)	1.561(0.297-7.7443)	0.617
	C0llage	97(25.4%)	5(1.3%)	1	
Occupation status	Student	117(30.6%)	20(5.23%)	4478541(.001-000)	0.998
	House wife	66(17,3%)	4(1.047%)	512848(.001-000)	0.998
	Government employee	143(37.4%)	13(3.4%)	1.000	1.000
	Private employee	30(7.9%)	3(0.78%)	1.000	1.000
	Other (specify)	26(6.8%)	(0%)	1	
	TOTAL	382(100%)	40(10.5%)		
Eating raw meat	Yes	185(48.4%)	32(8.37%)	0.056(0.013-0.235)	0.0001
	No	197(51.5%)	8(2.09%)		
Hand washing habit	Yes	322(84.2%)	10(2.61%)	3.221(1.897-5.470)	0.0001
	No	60(15.7%)	30(7.8%)		
eating under cooked vegetables	Yes	260(68%)	12(3.14%)	2.268(1.303-3.947)	0.0001
	No	122(31.9%)	28(7.32%)		

Note, Other, Priest, monks

($P < 0.05$) indicates statistically significant differences

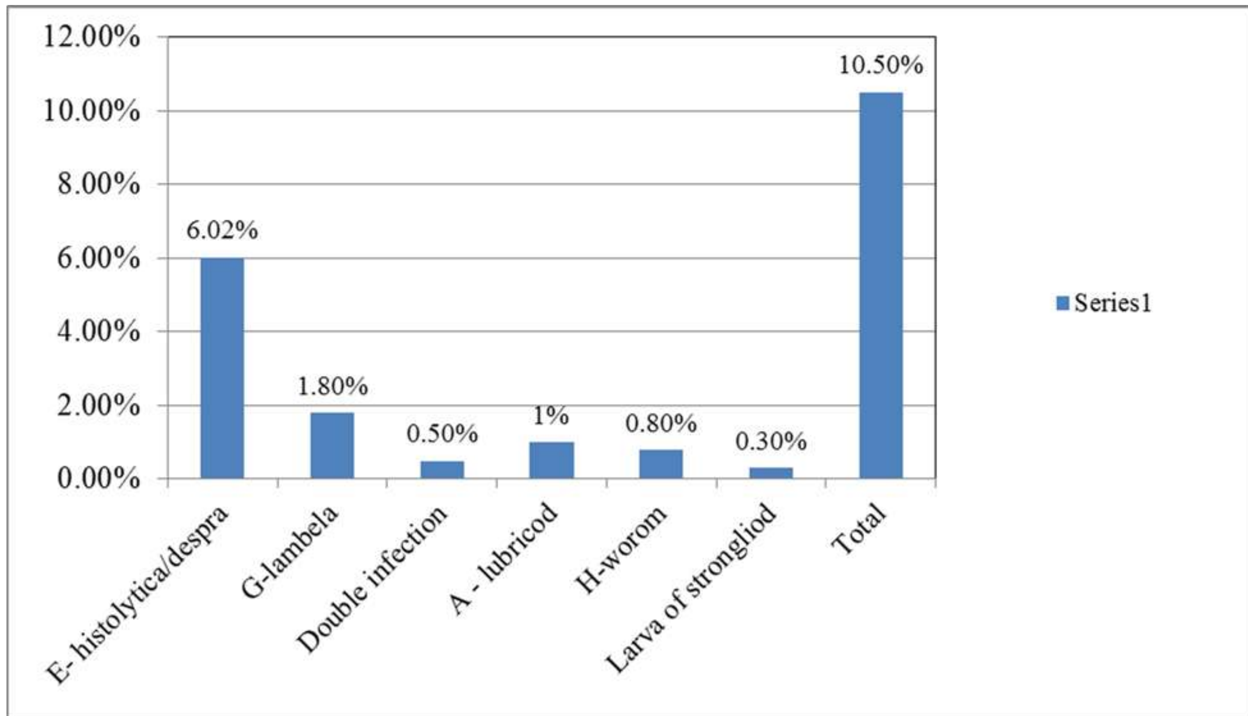
Five intestinal parasite *A. lumbricoides*, *Hookworm species*, *Srongliod strcolaris* species, *E. histolytica/dispar* and *Giardia lamblia* were identified among the Population of woreada (3,6) As well in Yeka sub-city and woreada (4,10) Arada sub-city of Addis Ababa. The overall Prevalence of helminthic infections was 8(2.09%). Among the helminthic infections, *A. lumbricoides 4* (1%) being the most predominant, followed by *Hookworm species* 3(0.8%), while only 1(0.3%) had *strongliod strcolaris* in the study area. Intestinal parasitosis was seen more in female 22 (5.75 %) than male 16(4.18%).

Regarding to protozoal infection, the overall prevalence was 32(8.4 %), multiple infection were 2(0.5%) (*E. histolytica/dispar, Giardia lamblia)*. The highest prevalence rate was due to *E. histolytica/dispar* 24(6.1%) and followed by 8(2.09%) *Giardia lamblia*. The prevalence rate in *E.histolytica/dispar* and *Giardia lamblia* parasite was higher in females. However, it was not with statistically significant difference ($p > 0.05$) bellow the (fig, 4)

The prevalence of intestinal protozoa in the age groups (years) <15, 15-24 and 25-84 was 12 (3.14%.), 8(2.09%) and 20(5.2%) respectively. In addition to this, there was not statistically significant difference in amoebiasis or giardiasis prevalence with respect to age ($p>0.05$).with regarding educational status the prevalence of intestinal parasite. Illiterate, Read &write, Primary (1-8), Secondary (9-12) and Collage &above, 14(3.66%), 8(2.09%), 10(2.61 %), 3(0.78%) and 5(1.3%) (Table, 4) Of the 382 study subjects, 126 (32.9%), 115 (30.1 %), 71 (18.5. %) and 70 (18.3%) were taken Yeka woreda six, Arada woreda six, Yeka woreda three and Arada woreda ten respectively. In this study the total prevalence of Yeka sub-city 27(7.03%) was higher than Arada sub-city 13(3.33%) (Table 3 and 4).

Table,4.Prevalence of IPI in Yeka and Arada sub-city according sex and Woreda distribution from January to June 2019 Addis Ababa Ethiopia.

Name of Woreda		Sex	No of examined	No of infected
Yeka	Woreda six	Male	44(11.5%)	7(1.83%)
		Female	82(21.4%)	13(3.40%)
		Sub –total	126(32.9%)	20(5.2%)
	Woreda three	Male	43(11.2%)	4(1.047%)
		Female	28(7.3%)	3(0.78%)
		Sub –total	71(18.5%)	7(1.83%)
Arada	Woreda six	Male	40(10.4%)	3(0.78%)
		Female	75(19.6%)	4(1.047%)
		Sub –total	115(30.1%)	7(1.83%)
	Woreda ten	Male	25(6.5%)	3(0.78%)
		Female	45(11.7%)	3(0.78%)
		Sub –total	70(18.3%)	6(1.57%)



Distribution of intestinal parasite

Fig,3**.** Prevalence of each intestinal parasite based on wet mount, formol-ether concentration technique and kato technics among Yeka and Arada sub city of Addis Ababa, Ethiopia from January to June, 2019.

Table 5. Multivariate logistic regression analysis of potential risk factors associated with parasitic infection in Yeka and Arada sub city of Addis Ababa; Ethiopia Jan-Jun 2019.

Independent variable		Intestinal parasites			
Risk factors		No examined	No infected	Adjusted OR (CI 95%)	p-value
Education	Illiterate	36(9.4%)	14(3.66%)	0.371(0.59- 2.347.)	0.292
	Read &write	27(7.1%)	8(2.09%)	0.557(0..55- 5.656)	0.620
	Primary(1-8)_	158(41.4%)	10(2.61%)	0.219(0.064-0.756)	0.016
	Secondary(9-12)	64(16.8%)	3(0.78%)	0.660(0.129-3.371)	0.617
	Collage	97(25.4%)	5(1.3%)	1	
Eating raw meat	Yes	185(48.4%)	32(8.37%)	0.202(0.041-0.987)	0.048
	No	197(51.5%)	8(2.09%)		
Hand washing habit	Yes	322(84.2%)	10(2.61%)	2.105(1.183-3.747)	0.011
	No	60(15.7%)	30(7.8%)		
eating under cooked vegetables	Yes	260(68%)	12(3.14%)	12.361(4.344-35.170)	0.0001
	No	122(31.9%)	28(7.32%)		

($P < 0.05$) indicates statistically significant differences

Helminthic infection intensities

Helminthic infection intensities were estimated based on duplicate Kato-Katz thick smears. The overall geometric mean faecal egg count was 600EPG from this A. lumbricoides was 384EPG and the respective estimate for H. worm was 216EPG.According to WHO standard all infections were light intensity. The intensities of helminthes infection can be described in the table below.

Table.6 WHO standard of Helminthic infection intensities

Sr Nu	Helminthes	Light-intensity	Moderate intensity	Heavy-intensity	EPG	Intensity
1	A lumbricoid	1-4999epg	5000-49999epg	>50 000 epg	384	Light
2	H worm	1-1999epg	2000-3999epg	>4 000 epg	216	Light

WHO cut-offs. The intensities of helminthes infection can be described in terms of eggs per gram

7. Discussions

It is known that the transmission of intestinal parasites depends on the presence of infected individuals, poor sanitation and principally, the socio-economic and behavioral factors in the population (50). The overall prevalence intestinal parasites infection in the current study was 10.5%.This was similar with a study conducted in Haik Health center Wollo, North east Ethiopia with 10.6% prevalence (51).And also nearly similar with study conducted in Saudi Arabia in the prevalence rate of 12% (52). .

However, the current prevalence (10.5 %) was higher compared with 4% among food vendors in Accra, Ghana. This variations could be attributable to the differences in geographic area and cultural practices (54).But the prevalence of the current study was lower than similar studies conducted in Ethiopia with the prevalence rate range 27% - 83% (36, 37, 38, 49,40).The possible explanations for the discrepancy between the present and previous study finding might be the difference in the quality of drinking water source, better awareness of study participant, variation in the environmental condition.

On the present study the prevalence of *A. lumbricoid*, Hook worm and *Srongloid stricolaris* was 1%, 0.8% and 0.3% respectively. This finding was lower than the study conducted in other studies in Ethiopia, such as by Wegayehu, *et al* and Mengistu *et al* (38, 39). The explanation for this prevalence of helminthes difference was due to quality of drinking water source, variation in the environmental condition, the awareness of study subject and socio-economic conditions, educational status of the study subjects. In addition our study result was lower than the study conducted in Jimma with the prevalence rate of *Ascaris lumbricoides, Hookworm and Trichuris trichiura* was (14.7%), (20.4 %) and (3.3%) respectively. According to WHO standard all helminthic infections were light intensities. This difference might be due to variation socio-economic conditions, variation in the environmental condition, the awareness of study subject and the difference in the quality of drinking water source. The cut off point for classification of intensity of parasitic infection was set according to the thresholds proposed for use by a WHO Expert Committee (54)..

The finding of the current study was lower than the study conducted in Côte d'Ivoire with prevalence rate of *hookworm, T. trichiura* and *A. lumbricoides* were 20.8%,15.4% and 13.1%, respectively(55).Regarding intestinal helminthic infection the intensities all helminthic infections were light intensities. This variation might be due to the differences in geographic area and cultural practices.

The prevalence of intestinal protozoa infection in our study was 8.4%. This result was higher than the study conducted Saudi Arabia with the prevalence rate, 5%(52).The possible explanations for this study due to the differences in geographic area and cultural practices. However, the prevalence of intestinal protozoa infection in our study was lower than similar other studies conducted in Ethiopia with the prevalence rate of 22% and 26% in gamo and Benishangul-Gumuz respectively. The possible explanation was the difference in the quality of drinking water source,socio-economic conditions, variation in the environmental condition and improved awareness of the population and hence contributing to lower prevalence of parasitic infections.

Similarly, the prevalence rates of *E. histolytica/dispar* 6.2% lower than the conducted in Addis Ababa 8.4% (40). However, our study was higher than the study conducted in Saudi Arabia with the prevalence rate of *E. histolytica/dispar* 2%(52).This variation might be due to the differences in geographic area and cultural practices. The final result of this research indicates that the use of drinking water from improved sources (standpipe) is a protective factor for infections with *E. histolytica/E. dispar, G. lambila*. Similar findings were observed on Kyrgyzstan and Côte d'Ivoire respectively (43, 56, 57).

Variation of results due to gender differences was not observed in the present study. This result was similar to a study conducted by Wegayehu *et al* (37).And also significant difference among the age groups was not observed in the current study. The association was not statistically significant in the above two cases ($p > 0.05$). Though IPIs were not significantly associated with sex, the prevalence is higher in female participant (5.75 %) as compared to male participant (4.18%).The possible reason was not clear. One of the factors strongly associated with intestinal parasite infection in this study was low educational level of children mothers'. This finding was similar with the results of other study Pinar *et al* (58) and Wördemann *et al*(59). This is more likely that parents of children at high level of education provide better sanitation condition for

their children than low educational level parents. The association was statistically significant ($p < 0.05$).

Intestinal parasitic infections were significantly associated with poor hand washing practice($p < 0.05$).The likelihood of acquiring infections among study participant who do not practice hand washing before eating and after defecation was 2.1times higher than among those who had good hand washing practice. Similar association of intestinal parasites and hand washing habit of study participants were reported from Ethiopia (60), 61). This is probably due to low knowledge of the study subject about the feco-oral transmission of intestinal parasite through their unwashed hands.

Moreover, intestinal parasitic infections were significantly associated habit of eating unwashed/undercooked vegetables ($p < 0.05$) .The likelihood of acquiring infections among study participant who had the habit of eating undercooked vegetables was 12 times higher. Similar report in Bahir Dar in growing of vegetables in faecally-polluted gardens was all found to be conducive for transmission of geohelminthes and intestinal protozoa (62). The reason might be due to the contamination of vegetables with fecal materials in the farm and contamination of the vegetation with wastes in home produce conducive environment for transmission intestinal protozoa.

8. Limitation and strength

8.1. Limitation

- ❖Only one stool sample was collected from each participant. Previous research has shown that multiple stool sampling enhances the sensitivity of helminthes and intestinal protozoa diagnosis.

- ❖In the process of conducting this research, the researcher had encountered challenges such as budget constraints.

8.2 .Strength

- ❖Exploiting available parasitological method.
- ❖The study was conducted at the community level.

9. Conclusion and recommendation

9.1 Conclusion

Intestinal parasitosis in Yeka and Arada sub-cty were relatively lower than expected national, urban and rural settings of Ethiopia. The Prevalence intestinal parasite significantly related to lack of wash hands with soup before meal and a habit of eating undercooked vegetables. This study alerts the need for implementing additional preventive and control mechanisms to reduce the risk of parasitic infection on the study areas. Furthermore, large scale studies are needed to assess more potential risk factors for intestinal parasitosis.

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11. Appendixes

I. Standard operating procedure

- Direct Wet Preparation of Fresh Stool

❖ PRINCIPLE

- The value of wet preparations lies in the fact that certain protozoa trophozoites retain their motility which may aid in their identification. Definitive identification however may not be possible, especially for amoeba, since the nuclei of trophozoites and cysts are often not clearly visible. Wet preparations on fresh unpreserved liquid stool should be performed and examined as soon as possible (within 30 minutes of passage) and on soft/formed stool within 60 minutes of passage provided that prior arrangements have been made with the lab. Wet preps can also be used to determine schistosome egg viability ("flame" cells). (Wet preps can also be performed on SAF fixed specimens but motility is lost since the fixative kills the parasites.)

❖ SPECIMEN

- Fresh liquid stool (within 30 minutes of passage - by prior arrangement with lab)
- Duodenal or small bowel aspirate in SAF or if fresh - within 30 minutes of collection by prior arrangement with lab.
- Abscess sample
- Respiratory sample
- CSF sample
- Urine
- Stool in SAF (no motility possible)
- External QC samples
-

❖ **MATERIALS**

Reagents

- Normal saline (0.85%)
- Lugols iodine (commercial product—Snap N' Stain).
- Preparation:
- Potassium iodide 10 gms
- Powdered iodine crystals 5 gms
- Distilled water 100 mls

Preparation

1. Dissolve potassium iodide and iodine crystals in distilled water in a flask or bottle using a magnetic stirrer.

2. The potassium iodide solution should be saturated with iodine with some excess Crystals left on the bottom.
3. Store in a tightly stoppered brown bottle protected from the light.
4. Label the bottle with the expiration date of one year.
5. Dilute a portion 1:5 with distilled water for routine use (working solution). Place this Working solution in a dropper bottle and discard when the color lightens (within 14Days).

Equipment:

- Pasteur pipettes
- Glass microscope slides
- Applicator sticks
- Glass cover slips, (22 x 22 mm)
- Sharps disposal container
- Light microscope with ocular micrometer and set for Kohler illumination

QUALITY CONTROL

For direct smear:

1. Check the working iodine solution each time it is used
2. Iodine should be the color of strong Orange Peko tea, discard if it is too light.
3. Protozoan stained with iodine should contain yellow gold cytoplasm, brown glycogen Material and paler refractile nuclei. The chromatoidal bodies may not be as clearly visible as in a saline mount.
4. The microscope should be calibrated (within the last 12 months)
5. All QC results should be appropriately recorded and any "out- of-control" results Referred to the laboratory director for action.
6. Ensure that reagents and chemicals used are not expired.Safety note: Universal precautions should be observed

PROCEDURE

1. Place one drop of 0.85% NaCl on the left side of the slide and one drop of iodine(Working solution) on the right side of the slide.
2. Take a small amount of fecal specimen and thoroughly emulsify the stool in salin and iodine using an applicator stick. The sample should be spread thinly enough that news print can barely be read when the slide is placed on top of text.
3. Slide a 22mm cover slip at an angle into the edge of the emulsified fecal drop. Pus the cover slip across the drop before allowing it to fall into place.
4. Systematically scan the entire 22mm cover slip with overlapping fields with the 10xObjective.
5. Switch to high dry (40X objective) for more detailed study of any suspect eggs or Protozoa

PROCEDURE NOTES

1. If a fresh or unpreserved sample is received Only process if the duration from passage is known. This is usually only done by prior arrangement with the lab.Perform a wet direct mount on liquid samples if received within 30 minutes of passage and on soft/formed stools within 60 minutes of passage and examine for motile trophozoites.
2. Describe the consistency of the specimen, e.g. bloody, watery, loose, soft or formed. Request a repeat specimen in SAF if only a fresh specimen was sent.
3. Examine specimens macroscopically for the presence of adult worms, proglottids,scoleces and other abnormal conditions. Use applicator sticks to break up the stools as necessary.
4. The sample on the slide should be spread thinly enough that newsprint can barely be read when the slide is place on top of text.
5. The microscope light should be reduced for low power observation since most organisms will be overlooked with a bright light illumination should be regulated so that some of the cellular elements and feces should be refractile.
6. Iodine solution will help make the nuclei more visible. However it is not useful for confirming motility since it kills trophozoites.In preserved specimens the SAF replaces the saline and can be used in the direct smear; however no motility will be visible since the organisms are killed in SAF
7. To prevent contamination of iodine solution, the drops of iodine working solution should be placed on the slide before the specimen is added.
8. Duodenal aspirates can be examined directly with or without added saline.
9. If the slide is to be kept for any period of time, the edges of the cover slip may be sealed With Vaseline in order to prevent evaporation.

REPORTING

- Protozoan stained with iodine should contain yellow gold cytoplasm, brown glycogen material and paler refractile nuclei. The chromatoidal bodies may not be as clearly visible as in a saline mount. Protozoal trophozoites, cysts and helmithes eggs and larva can be seen and identified. However results from the direct smear should be considered presumptive and should be definitively confirmed with concentrates and direct smears.

LIMITATIONS

1. Once iodine is added the organisms will be killed and motility will be lost.
2. Specimens that arrive in the lab already preserved do not require a direct smear examination. Concentration and permanent stain smears should be performed instead.
3. Direct smears are normally examined at low (x100) and high dry (x400) power. Oil immersion examination (x1000) is not recommended since organism morphology is often not clear.

➢ Formalin-ether Concentration Method

❖ PRINCIPLE

Fecal concentration is a routine part of the ova and parasite examination and allows the detection of small numbers of organisms that may be missed by using a direct wet smear. Sedimentation methods use centrifugation to concentrate the protozoa, helminthes ova and larva in the bottom of the tube. Ether is used as an extractor of debris and fat from the feces.

❖ SPECIMEN

- Stool preserved in SAF
- Preserved duodenal aspirates
- External QC aspirates
- Aspirates from abscesses

❖ MATERIALS

Reagents

- Normal saline (0.85%)(Commercial product, PML)
- Diethyl ether (Commercial product, PML)
- 10% neutral buffered formalin (pH 7.0) (Commercial product, PML)

IF MADE IN HOUSE:

- Formaldehyde (Commercial product) 1200mls
- Na_2HPO_4 10.7gr
- NaH_2PO_4 0.23gr
- Distilled water 10.8 L
- Triton X-100 (commercial product) 12mls

1. Mix thoroughly before dispensing. Smaller quantities can be prepared.
2. Titrate pH to 7 using concentrated HCl or NaOH.

Normal Saline Wash Solution (commercial reagent, PML):

IF MADE IN HOUSE:

NaCl-- 34gm

distilled water --4000mL

Triton X-100 (commercial product, BDH) 4mL

.

❖ Equipment:

- Fume hood
- safety centrifuge
- Microscope with ocular micrometer and set for Kohler illumination
- Funnel filter – disposable (PML)
- Applicator sticks
- Centrifuge tubes and caps (PML)
- Pasteur pipette
- Sharps discard
- Cotton tip applicator
- Glass microscope slides
- Cover slips (22 x 40 mm)

❖ QUALITY CONTROL

1. Check all reagents each time they are used and ensure that formalin and saline appear clear without any visible contamination.
2. Ensure that all reagents and chemicals have not expired.
3. The microscope should be calibrated (within the last 12 months)
4. All QC results should be appropriately recorded and "out-of-control" results should be referred to laboratory director for action.
5. Whenever possible, one technologist will read the concentrate and a different one will read the smear. Discordant results will be recorded and resolved with the lab director before reporting.
6. Whenever possible multiple samples from the same individual should be read by a different technologist

❖ Special Safety Notes

➢ Ether is highly flammable and should only be used in a fume hood and should be disposed of in the appropriate hazardous waste container Formalin is hazardous and all processing

should be performed in a fume hood. Stool samples should be treated as biohazards and universal precautions are always indicated.

- Formalin-ethyl acetate sedimentation concentration
- This procedure leads to recovery of all protozoan cysts and oocysts, helminthes eggs and larvae present in the stool specimen; it is recommended as being the easiest to perform and the least subject to technical error, allowing recovery of the broadest range of parasitic elements. The specimen can be fresh or fixed stool. The preparation will often contain more debris than that obtained with the flotation and other procedures.

Note: This technique is not recommended for eggs of Fasciola spp. and larvae of Strongyloides stercoralis

Procedure

1. Mix about 1 g of faeces (size of a hazelnut) with 10 mL of fixative (SAF or formalin 5–10%), and leave for at least 30 minutes.
2. Strain the suspension into a 15 ml conical tube through a sieve or double layer of gauze allocated into a small funnel and centrifuge at 500 *g* for 10 minutes.
3. Remove the supernatant and break the sediment with a wooden toothpick.
4. Add 7 mL of saline to the sediment; seal the tube with a stopper and mix.
5. Add 3 mL of ethyl acetate (or gasoline or ether. Caution: these reagents should be handled with special care as they are very volatile and may cause explode), seal the tube with a rubber stopper (check that it is tightly closed) and shake vigorously for 30 seconds.
6. Wait 15–30 seconds and carefully remove the stopper.
7. Centrifuge at 500 *g* for 3 minutes.
8. The contents in the tube will separate in four layers, starting from the bottom:sediment (containing the parasitic elements), saline, plug of faecal debris, and top layer of ethyl acetate (or ether or gasoline).
9. Detach the plug of debris from the tube wall with the help of an applicator stick.Pour off the top three layers by inverting the tube with a brisk movement.
10. Mix the sediment with the remaining liquid (if needed, add a few drops of saline).

11. Place a drop of the sediment on a slide and cover with a coverslip. A Lugol stained preparation can be placed on the same slide.
12. Examine using a microscope.

➢ Kato–Katz technique

❖ PRINCIPLE

Most organisms release eggs sporadically and therefore there is no correlation between burden and the number or organisms seen in a stool sample. Schistosome eggs can be released at a steady rate therefore it is desirable to get an accurate measure of the number of eggs in a given amount of sample so that worm burden can be inferred. The Kato thick smear method accomplishes this by depositing a reproducible amount of material on the slide.

❖ SPECIMEN: any fresh stool sample that has not been refrigerated

❖ Materials and reagents

- applicator sticks
- screen, stainless-steel, nylon or plastic: 60–105 mesh size
- Template, stainless-steel, plastic or cardboard. Templates of different sizes have been produced in different countries.
- A hole of 9 mm on a 1-mmthick template will contain 50 mg of faeces; a hole of 6 mm on a 1.5-mmthick template, 41.7 mg; and a hole of 6.5 mm on a 0.5-mm thick template,20 mg.
- The templates should be standardized, and the same size template should always be used, to ensure the repeatability and comparability of prevalence and intensity data.
- spatula, plastic
- microscope slides (75 x 25 mm)
- hydrophilic cellophane, 40–50 g, strips 25 x 30 or 25 x 35 mm
- flat-bottom jar with lid
- forceps
- toilet paper or absorbent tissue

- newspaper
- glycerol–malachite green or glycerol–methylene blue solution (1 ml of 3%aqueous malachite green or 3% methylene blue added to 100 ml of glycerol and 100 ml of distilled water and mixed well). This solution is poured onto the cellophane strips in a jar and left for at least 24 h be fore use.

QUALITY CONTROL

- Egg counts are subject to errors therefore multiple egg counts on the same specimen should be performed
- Ensure that the microscope has been calibrated in the last year and that the results of the calibration are displayed on the microscope base.
- As it is not possible to have a positive control specimen to use with this procedure,the technologist should review the appearance and size of the organisms present to ensure that they match reference material (i.e. Bench Aids for the Diagnosis of Intestinal Parasites (WHO)).

REPORT

- If no eggs found report “No ova found per gram of stool”
- If eggs are found report for example “20 schistosoma ova found per gram of stool

LIMITATIONS OF PROCEDURE

- Adult schistosomes settle in the host’s blood system and therefore it can take weeks to months for the eggs to be passed.
- A negative result may indicate that adults are present but that eggs are not being passed

Procedure

- Place a small mound of faecal material on newspaper or scrap paper, and press the small screen on top so that some of the faeces are sieved through the screen and accumulate on top.
- Scrape the flat-sided spatula across the upper surface of the screen to collect the sieved faeces.
- Place the template with a hole on the centre of a microscope slide, and add faeces from the spatula so that the hole is completely filled. Pass the side of the spatula over the template to remove excess faeces from the edge of the hole.
- Remove the template carefully so that the cylinder of faeces is left on the slide.

- Cover the faecal material with the pre-soaked cellophane strip. The strip must be very wet if the faeces are dry and less so if the faeces are soft. If excess glycerol solution is present on the upper surface of cellophane, wipe with toilet paper.
- Invert the microscope slide and firmly press the faecal sample against the hydrophilic cellophane strip on another microscope slide or on a smooth hard surface. The faecal material will be spread evenly between the microscope slide and the cellophane strip. It should be possible to read newspaper print through the smear after clarification.
- Carefully remove the slide by gently sliding it sideways to avoid separating the cellophane strip or lifting it off. Place the slide on the counter with the cellophane upwards. Water evaporates while glycerol clears the faeces.
- Read the slide after 30–60 min at ambient temperature. (The exact time should be determined in each setting to avoid over-clearing of hookworm eggs, which become transparent with time and are thus impossible to see.)
- Examine the smear systematically, and record the number of eggs of each species.
- The multiplication factors used to obtain the number of eggs per gram from the number of eggs per slide are: 20 if using a 50-mg template, 50 if using a 20-mg template and 24 if using a 41.7-mg template.

II. Assent form for children aged 12-17 years

I have read the information above, or it has been read to me. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction. I voluntarily assent that I would participate in this study provided my parents/guardians give their consent.

To give my stool ☐
To give my urine ☐
To collect my blood ☐ and be a participant in this study and understand that I have the right to withdraw from the study at any time ☐.

Print name of participant, date and signature or thumb impression of participant

____ ____ /____ /____ (dd/mm/yy) ___________

If illiterate;

Print name of independent literate witness, date and signature of witness (if possible, this person should be selected by the participant and should have no connection to the research team)

____ ____/____/_____ (dd/mm/yy) ___________

Phone number (parents/guardians)__________

Print name of researcher, date and signature of researcher

____ ______/____/_____ (dd/mm/yy) _______

III. Consent form for adults (≥18 years)

I have read the information above, or it has been read to me. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction. I voluntarily consent that I would participate in this study.

To give my stool ☐
To give my urine ☐
To collect my blood ☐ and be a participant in this study and understand that I have the right to withdraw from the study at any time ☐.

Print name of participant, date and signature or thumb impression of participant

____ ____ /____ /____ (dd/mm/yy) ___________

If illiterate;

Print name of independent literate witness, date and signature of witness (if possible, this person should be selected by the participant and should have no connection to the research team)

____ ____/____/_____ (dd/mm/yy) ___________

Phone number________________________

Print name of researcher, date and signature of researcher

____ ______/____/_____ (dd/mm/yy) _________

IV. Consent form for parents/guardians

I have read the information above, or it has been read to me. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction. I voluntarily consent that my child participates in this study (provided he/she gives assent for children 12-17 years).

To give his/her stool ☐

To give his/her urine ☐

To collect her/his blood ☐ and be a participant in this study and understand that I have the right to withdraw my child from the study at any time ☐.

Print name of participant, date and signature or thumb impression of participant

____ ____ /____ /____ (dd/mm/yy) ________

If illiterate;

Print name of independent literate witness, date and signature of witness (if possible, this person should be selected by the participant and should have no connection to the research team)

____ ____/____/_____ (dd/mm/yy) ___________

Print name of researcher, date and signature of researcher

____ ______/____/_____ (dd/mm/yy) _______

V. Consent form for children 12-17 years 12—17 ዓመት ለሆኑ ህፃናት የስምምነትቅፅ)

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የዓይነምድርናሙናለመስጠት☐

የሽንትናሙናለመስጠት☐

ደምለመቀዳት ☐

እናበዚህጥናትተሳታፊለመሆን፣በማንኛውምሰዓትከጥናቱለመውጣትመብትእንዳለኝምተረድቻለሁ☐.

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_______________________________________ _____ /_____ /_____ (ቀን/ወር/ዓመተምህረት)

ያልተማሩከሆኑ;

የተማሩገለልተኛእማኝሰውስም፣ቀንናፊርማ (ከተቻለይህሰውበተሳታፊውቢመረጥናከተመራማሪአባላትግኑኝነትየሌለውቢሆን)

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ስልክቁጥር (የወላጅወይምአሳዳጊ)_______________

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_______________________________________ _______/_____/______ (dd/mm/yy) __________

VI. Consent form for adults (≥18 years)(18 ዓመትእናከዚያበላይለሆኑአዋቂዎችየስምምነትቅፅ)

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የዓይነምድርናሙናለመስጠት☐

የሽንትናሙናለመስጠት☐

ደምለመቀዳት ☐እናበዚህጥናትተሳታፊለመሆን፣በማንኛውምሰዓትከጥናቱለመውጣትመብትእንዳለኝምተረድቻለሁ☐.

የተሳታፊስም፣ቀንእናፊርማ (ወይምአሻራ) ከዚህበታችይፃፉ

____ ____ /____ /____ (ቀን/ወር/ዓመተምህረት)

ያልተማሩከሆኑ;

የተማሩገለልተኛእማኝሰውስም፣ቀንናፊርማ (ከተቻለይህሰውበተሳታፊውቢመረጥናከተመራማሪአባላትግኑኝነትየሌለውቢሆን)

____ ____/____/_____ (dd/mm/yy) ___________

ስልክቁጥር_____________

የተመራማሪውስም፣ቀንናፊርማ

____ ______/____/_____ (dd/mm/yy) _________

VII. Questionnaires to be filled by health professionals

Part I. General information

Code Number___________________ Region __________________ Zone ___________________

Woreda____________ / city /_sub city ______________________ Kebele __________________

Part II. Personal information

Age (in years)______________________

Sex _________

No.	Questions	Responses
	Part III. SOCIO-DEMOGRAPHIC INFORMATION	
	Educational status	Illiterate Read and write Primary (1-8) Secondary (9-12) College diploma/degree and above
	Occupation	Student House wife Government employee Private employee Farmer Others (specify) ________________________
	Marital status	Single Married Divorced Widowed Not applicable (children)
	Religion	Orthodox Christian Muslim Protestant Catholic Others (Specify) _____________________
	Residence	Rural 2. Urban
	Questions 7-12 are additional questions to Students	
	Father's Age	____________
	Mother's Age	____________
	Father's Educational Level	Illiterate Read and write Primary (1-8) Secondary (9-12) College diploma/degree and above
	Mother's Educational Level	______________
	Father's Occupation	

	Mother's Occupation	
	Monthly income (in birr collected from salary, rent, and other income)	_______________Birr
	Family Size (Number of People)	________
	Source of water	Pipe Spring water Well water River Other sources (specify)

	Source of water	Pipe Spring water Well water River Other sources (specify)
	Did you eat undercooked/raw meat?	Yes 2. No
1	When did you last take anthelmintic and /or anti-protozoa medication	This week 2.the last two week 3.not at all
2	Where does your family get water for domestic use?	1.Tap water 2.Rever water 3.stream water
3	What type of toilets do you use while at home?	1.Pit latrine 2.flush toilet
4	Do you drink water that is not boiled	1.yes 3 other 2.No
5	Do you boil drinking water while at home?	1.yes 2.No 3 ,other
6	Hand washing habit before eating and after defecation	1.yes 2.No
7	Do you wash fruits before eating	yes no
8	Do you walk barefoot?	1.yes 2.No
9	Do you bath or go swimming in the rivers, swamps?	1.yes 2.No
	Check list for environmental sanitation in schools	

10	Name of school	1 private 2 .government
11	What is the source of water in your school?	1.pipe 2.Rever water 3.stream
12	What type of sanitary facilities does the school have for the students?	Pit-latrine 2.flush toilet 3. Pit-latrine& flush toilet

Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate: BehailuTsegaye(BSc.)

Signature: ____________________

Date of submission: ____________________

This thesis has been submitted with our approval as advisors.

Advisor: Mister Wolda (MSc, PhD)

Signature: ______________________

Date: ______________________

Place: Addis Ababa, Ethiopia.

Advisor: DessaAbera(MSc**)**

Signature: _____________________

Date: _____________________

Place: Addis Ababa, Ethiopia.