ADDIS ABABA UNIVERSITY
COLLEGE OF HEALTH SCIENCE
SCHOOL OF ALLIED HEALTH SCIENCE
DEPARTMENT OF MEDICAL LABORATORY SCIENCE

Prevalence of *Helicobacter pylori* and intestinal parasite and their associated risk factors among school children at Selam Fire Elementary School in Akaki Kality, Addis Ababa, Ethiopia

By: Abebe Worku (BSc)

Advisors: Kassu Desta (MSc, PhD fellow)

Mistire Wolde (MSc, PhD)

A thesis submitted to Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Science, in partial fulfillment of the requirements for the degree of master in Clinical Laboratory Science (Diagnostic and public health microbiology).

June /2017/

Addis Ababa, Ethiopia

ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF ALLIED HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

This is to certify that the thesis prepared by Abebe Worku, entitled: Prevalence of *Helicobacter pylori* and intestinal parasite and their associated risk factors among school children at Selam Fire Elementary School in Akaki Kality, Addis Ababa, Ethiopia and submitted in partial fulfillment of the requirements for the degree of Master of Science in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

BY: ABEBE WORKU (BSc)

Approved by the Examining Board

Chairman, Dep. Graduate Committee	**Signature**
_____	_____
Advisors	**Signatures**
_____	_____
_____	_____

Internal Examiner	Signature	Date
_____	_____	_____
External Examiner	Signature	Date
_____	_____	_____

Acknowledgment

Primarily my heartfelt thanks go to the Almighty God, And my all Families

Then my thanks go to Addis Ababa University, Department of Medical Laboratory Sciences for arranging a program to conduct my MSc Thesis work.

I would like to express my sincere gratitude and deep appreciation to my advisors, Kassu Desta (MSc, PhD fellow) and Dr Mistre Wolde (MSc, PhD) whose advice and support made this work fruitful. Their guidance was very clear since the beginning of the process and their input always valuable in term of giving direction and helps to solve problems in this thesis.

Finally, my special thanks also go staffs working in Selam Fire Health Center Laboratory; and Akaki kality Sub City education Bureau and to Selam Fire Elementary School director, and all staffs, in which the research has been conducted, also the school children who participated in this study.

Table of Contents

List of tables **Page**

List of figures **Page**

List of abbreviations

Ag: Antigen

ENAO: Ethiopian National Accreditation Office

ELISA: Enzyme linked immune sorbent assay

FECT: Formal ether concentration technique

HP: Helicobacter pylori

HpSA: Helicobacter pylori stool antigen

ICT: Immune chromatographic test

IgG: immunoglobulin G

IPI: Intestinal parasitic infection

KG: Kindergarten

MALT: Mucosa-associated lymphoid tissue

MUAC: Mean Upper Arm Circumference

NaCl: Sodium Chloride

PTA: Parents Teachers Association

SOP: Standard operating producers

SPSS: Statistical package for social study

STH: Soil transmitted helminthes

UK: United Kingdom

WGO: World Gastroenterology Organization

WHO: World health organization

Operational definitions

Helicobacter pylori stool Antigen test (HPSA): is a lateral flow chromatographic immunoassay for the qualitative detection of *H. pylori* antigen in human faecal specimen.

Prevalence: is a measurement of all individuals affected by the disease at a particular time.

Co-infection: the simultaneous presence of two or more infections, which may increase the severity and duration of one or both

Children: A person between birth and puberty.

School children: a child attending school.

A Primary school: is a school for children between the ages of 5 and 18.

Abstract

Background: The prevalence of *H. pylori* infection mainly acquired during childhood and may be persisting throughout life and it has been found high in developing countries; this prevalence is related to low socioeconomic status, and Intestinal parasitic infections are among the major public health problems in Sub-Saharan Africa. Their distribution is mainly associated with poor personal hygiene, environmental sanitation and limited access to clean water. There is limited information on the burden of the *H. pylori* and intestinal parasites in Ethiopia and this research will address such gap.

Objectives: To assess the prevalence of *Helicobacter pylori* and intestinal parasite and their associated risk among Elementary School Children.

Methods: A cross sectional study was conducted to determine the burden and risk factors associated of *H. pylori* and intestinal parasite among in 422 school children. The study was conducted between March to June 2017. Multiple sampling methods were used, to collect the data. The stool samples were tested for intestinal parasite using direct wet mount and concentration techniques and stool antigen test for *H. pylori*. Information from the laboratory analysis and questionnaires were entered into SPSS version.20 for analysis.

Results: A total of (n=422) students have been participated in this study 55.2 %(n=233/422) 44.8% (n=189/418) were female and male respectively. Age of range (4-18) years, with mean age of 11.16±SD years [95% CI 10.82-11.5], the mean weight of 30.99 ± SD Kg [95 % CI 29.9 -32.08], the mean height 1.36 ± SD m [95% CI 1.34-1.38], *Helicobacter pylori* antigens were detected in 14.6% (n=61/422) ,and 6%(n=25/189) 8.6%(n=36/233) male and female respectively. Intestinal parasite were detected in 23.7 %(n=100/422), 10.4 %(n=44/189) 13.3 %(n=56/422) male and female respectively. The co-infection for *HP* and IPI was present in 4.5 %(n=19/422). The age of study subject, educational status/ monthly income status of their family/guardians, overcrowding and some sanitary practice were a risk factor for the development of intestinal parasite and *H. pylori* infection.

Conclusions: The prevalence of *H. pylori* infection is 14.6 %, and IPI is 23.7 %, this burden of IPI among school children call mass de-worming which is going on in some schools. Moreover further studies are required to understand the role of *HP* and IPI on the overall growth of children and school performance.

Key words: *H. pylori*, intestinal parasite, School Children, Selam Fire Elementary School

1. INTRODUCTION

1.1 Background

In the early 1980s, gastroenterologist Barry Marshal and his pathologist colleague, Rober Warren, found spiral-shaped bacteria in about half of the routine biopsies, obtained from patients attending the gastroenterology consultation, and their presence was closely associated with mucosal inflammation[1].

H. pylori cause acute and chronic gastritis, and can cause duodenal and gastric ulcers. There is strong epidemiological evidence to implicate *H. pylori* gastritis in marginal B cell mucosal lymphomas. Although these significant diseases are typically found among adults, there are clear parallels with gastro duodenal disease in children. In particular, Peptic ulceration, abdominal pain in the absence of peptic ulceration and Gastro-esophageal reflux diseases are pediatric disorders have been associated with *H. pylori*[2].

The natural history of *H. pylori* infection in children has not yet been extensively studied, but there are several reports that affected children develop a chronic gastritis, localized especially in gastric antrum, similar to adult. The majority of infected children remain asymptomatic, but the inflammatory response may result in an ulcerogenic process, also the prevalence of *H .pylori* associated peptic ulcer in children is not clearly known. It is thought to be low, based on the studies of large pediatric endoscopy unit which report an incidence of 5-9 new peptic ulcer, cases per year. *H. pylori* are crucial factor in the pathogenesis of peptic ulcer, especially duodenal ulcer, since almost all children with the disease were positive for the bacterium [3].

Intestinal Parasitic Infections (IPIs) constitute the greatest single worldwide cause of illness and disease. 3.5 billion Individuals have been infected with intestinal parasites, of these 450 million individuals developed diseases Africa, more specifically Sub-Saharan Africa, parasitic infections are the major public health problem and most of the victims are children [**4**]. Parasites are one of the important casual agents of diarrhea, loss of weight, abdominal pain, nausea, vomiting, lack of appetite, abdominal distention and Iron deficiency anemia [**5**]. Currently, the protozoan parasite (*Entameoba histolitica and Giardia intestinalis*) and the soil transmitted helminthes (*Ascaris lumbricoides, Trichuris trichiura*, and *Hookworm*) are the leading intestinal parasites which cause significant morbidity and mortality in the world [**6**].

For instance, recent estimates indicated that approximately 1472, 1298 and 1049 million people have round worm, hookworm and whip worm infection, respectively [**7**]. However, the incidence and prevalence of intestinal parasitic infections varies within and across the countries due to environmental, social and geographical factors [**8**].

In children, soil-transmitted helminthes is the cause of common health problems, in most instances, associated with stunting of linear growth, physical weakness and low educational achievement. These is due to their immune systems are not yet fully developed and they also habitually play in faecally contaminated soil. Those problems are predominant in tropical areas [9].

In Ethiopia, intestinal parasitic infection is sixth of the top ten causes of morbidity amongst children. Different studies conducted in different regions depicted that the prevalence and possible associated factors are different [10].

So far, guidelines for the management of Hp infection in children recommend endoscopy to exclude other pathological causes for the child's symptoms [11]. The reasoning behind this recommendation is that no specific complex of clinical symptoms and signs has been established for children. Cultures of gastric tissues have a specificity of 100%, but a relatively low sensitivity of 38-80%. PCR testing in gastric tissue can detect genes associated with virulence factors and antibiotic resistance. The 13C-urea breath test (UBT) and the monoclonal stool test have been validated well in children older than 6 years for the detection as well as the eradication control of Hp. Unfortunately these non-invasive tests have not sufficiently been validated in younger children, below the age of 6 years[12].

The diagnosis of intestinal parasite is initially based on clinical signs and symptoms and Confirmed by the presence of cysts, trophozoites, ova, and larva stage etc, in stool samples [13]. The direct wet preparation is more useful for detection of characteristic motility of trophozoite [14]. Diagnosis of intestinal parasite by conventional microscopic methods following the application of fecal concentration techniques, especially Zinc sulphate flotation and centrifugation remains a relatively reliable indicator of infection [15]. Enzyme immunoassay (ELISA) is highly sensitive and specific. For these reasons, in the last years, Molecular techniques particularly polymerase chain reaction (PCR) based procedures have greater sensitivity and specificity than the conventional diagnostic methods for diagnosis of intestinal parasite [15].

1.2 Statements of the problems

In various regions of sub-Saharan Africa, for example, 61–100% of the population may harbor *H. pylori* infection; young children have the highest prevalence [16].

A lack of proper sanitation, safe drinking water, and basic hygiene, as well as poor diets and overcrowding, play a role in determining the overall prevalence of *H. pylori* infection [17].

The greatest burden of soil-transmitted helminthes (STH) occurs among children in developing countries, where there is poor hygiene and sanitation [18].

In severe cases the number of parasites may grow so large that the intestines become blocked. Some infections cause specific complications: *Amebiasis* can affect the liver, lungs and brain; parasites migrating through the lungs may cause difficulty in breathing; and hookworm infection can cause anemia and malnutrition, which can affect growth and development in children [19].

Multiple infections with several different parasites are common and their harmful aspects are often aggravated by coexistent with malnutrition or micro environment [20].

In the absence of Clean, functioning and adequate toilets will result in children to defecate in and around the school compound. In such situations the school and its surroundings are likely to become infested with parasitic helminthes. In the absence of the availability of convenient hand washing facilities Children dipping their unwashed hands into a shared drinking-water supply are a typical route of contamination infectious diseases which can be spread via the faecal-oral route [21].

To the best of our knowledge, there is no study conducted in this area in particular and in Ethiopia in general, about *H. pylori*, and intestinal parasite infection among school children, hence conducting this study and address this issue will be fill the existing gap.

1.3 Significance of the study

This study would helpful to see which type of parasite is more prevalent and the prevalence of *H. pylori* infection in school children and which type of predisposing risk factors contribute more to existence of both infection or for each of infection.

This study would help us to design strategies that involve schools about school health services, which provides invaluable support for schools in order to achieve the collective goals of promoting healthier environments.

The findings of this study would help in strengthening the information available so far and would be helped policy makers to design effective strategies to combat intestinal parasitic infections and *H. pylori* in the study area.

This study provided the current prevalence of *H. pylori* infection and its associated risk factors among the study subjects and used to plan intervention activities in the future. Lastly the study served as base line data for the upcoming researchers in this area.

2. LITERATURE REVIEW

2.1. Prevalence of *H. pylori* infection

The prevalence of *H. pylori* and associated diseases has been highly inconsistent worldwide. In industrialized countries there is generally a low prevalence of *H. pylori* infection and yet a relatively high prevalence of gastric cancer. On the other hand, some countries with high *H. pylori* prevalence have low gastric cancer prevalence, particularly among the Asian countries. Prevalence of *H. pylori* infection is high in less developed Asian countries like India, Bangladesh, Pakistan, and Thailand, and is acquired at an early age than in the more developed Asian countries like Japan and China. The frequency of gastric cancer, however, is very low in India, Bangladesh, Pakistan and Thailand compared to that in Japan and China. Similar enigma has been reported from Africa as compared to the West [22].

The search identified population-based studies reporting frequency of *Helicobacter pylori* infection primarily from Asia and the Middle East. Several studies used stool antigen testing; others used serologic testing, carbon-13 urea breath testing, or urine antigen testing [23]. Prevalence of infection with *H. pylori* varied between 7% in a study conducted among asymptomatic children in the Czech Republic, 24 to 92% in Pakistani population [24].

A study was conducted in China on children and adults in two regions of China with both a low and a high incidence of gastric cancer, reported that the prevalence of *H. pylori* was significantly lower in 2006 when compared to the early 1990s, with a decrease in the prevalence between 5 and 28%, depending on the population under study. Only one study compared prevalence of *H. pylori* infection within the same population using different diagnostic tests and reported no statistically significant difference in the prevalence of infection when the stool antigen test was used, compared with serologic testing [25].

In a rural village of Linqu Country, Shandong Province, China, a study of 98 children found that nearly 70% of those aged 5-6 years were infected with the organism, a rate similar to that reported for adults in that area, suggesting that most infection takes place early in childhood [26].

In developing countries, *H. pylori* infection is markedly more prevalent at younger ages than in developed countries. According to World Gastroenterology Organization (WGO) 2010 the Prevalence of *H. P*ylori in Ethiopia was 48% in age between 2-4, 80% at the age of 6 and 95% in adult's population [17].

In developing countries, where majority of children are infected before the age of 10, the prevalence in adults peaks to more than 80% before 50 years of age. In developed nations, serologic evidence of *H. pylori* is rarely found before 10 years of age, but increases to 10% in those between 18 and 30 years of age and to 50% in those older than 60 [27].

The increased prevalence of infection with age was initially thought to represent a continuing rate of bacterial acquirement throughout one's lifetime. However, epidemiologic evidence now indicates most infections are acquired during childhood even in developed countries. Thus, the frequency of *H. pylori* infection for any age group in any locality reflects that particular cohort's rate of bacterial acquisition during childhood years [28].

Infection with *H. P*ylori is relatively common in Africa, and the organism is the main cause of at least 90% of duodenal ulcers and 70% of gastric ulcers. Studies conducted in various parts of Africa have revealed high Sero-prevalence of infection (61-100%) which differs from country to country and between different racial groups within each country [29].

2.2. Risk factors for *H. pylori*

Several epidemiological studies have examined risk factors for *H. pylori* infection, with lower socio-economic conditions being the most consistently identified. However, social classifications by occupation, level of education or earning are merely markers for groups of people sharing certain characteristics or practices and not a specific cause of infection. Studies of adults have revealed a stronger association between *H. pylori* infection and childhood living conditions than for current living conditions, thus supporting acquisition early in life. The risk of introduced recall bias when adults and elderly were asked about living conditions before the age of 5 years should not be ignored. However, studies performed among children have confirmed the finding of an inverse association between socio-economic conditions and *H. pylori* infection [30].

Ayse et al. reported from eastern Turkey a very high prevalence of *H. pylori* (64.4%) among300 children. The risk factors for acquiring the infection were the low economic status and larger sibling size of the family. However, no significant difference between children whose parents were from different educational levels was found suggesting that the very high prevalence of *H. pylori* in eastern Turkey depends on environmental factors [31].

Person-to-person transmission of *H. pylori* has been suggested in a number of studies pointing at domestic overcrowding early in life as an important risk factor for infection [33].

A common exposure to infection could, however, not be excluded. Two studies from the UK (Whitaker et al. 1993, Webb et al. 1994) identified childhood crowding, increasing number of siblings and bed sharing as possible risk factors for transmission of the organism. Although statistical analyses could not separate the relative importance of the three, the findings indicated transmission via close personal contact early in life [32].

Several studies investigated putative risk factors for *H. Pylori* infection. Gender and age do not seem to be associated with an increased risk of infection. Indeed, most studies reported no significant difference of *H. pylori* infection between men and women, both in adults and in children .No-significant association was found between infection and age in the adult population. Moreover, several factors related to residence have been found to be associated with the infection. Indeed, living in a rural area, in crowded homes, and having contaminated sources of drinking water were risk factors for *H. pylori* infection [33].

Several socioeconomic factors have been associated with *H. pylori* infection. In particular, subjects with a low socioeconomic status, measured also as a low family income, had a higher likelihood of carrying *H. pylori* infection. Furthermore, an inverse association between educational level and *H. pylori* infection was found in the majority of the studies; indeed, except for two cases, individuals with lower educational levels had a higher risk than those with a higher education. The same association concerning the parents' education was also found in studies on children [34].

High prevalence of human infection seen in Africa and the world at large are an indication that effective public-health interventions need to be developed; while the variations seen in the prevalence of infection between and among populations may point to the fact that parameters such as age, cultural back-ground, genetic predisposition, socio-economic status and environmental factors all play a role in the acquisition and transmission of *H. pylori* [35].

Within countries, there may be similarly wide variation in prevalence between the more affluent urban populations and the resource-poor rural populations. A lack of proper sensitization, good drinking water and poor diet seem to play a role in the high prevalence of infection [36].

2.3. Prevalence of intestinal parasite

Intestinal parasitic infections which are caused either by protozoa or helminthes or both are among the most widespread of human infections worldwide. It is estimated that as much as 60% of the World's population is infected with intestinal parasites which may play a significant role in morbidity due to intestinal infections [37].

The most common intestinal parasitic infections in the world are *Ascaris lumbricoide*,*Trichuris trichuria* and *Hook worms*. [38] Also the study found, in India shows that approximately two-third (63.94%) of the school children was infected with intestinal parasitic infection. In another study performed in India showed a low prevalence (29.2%) of intestinal parasitic infection when compared to the present study [39].

The prevalence of intestinal parasites was investigated in a primary school located in kubia Junior, Saopaulo state Brazil, of 219 school children of which 123 (56.1%) were found to be infected with one or more parasite species [40].

Study conducted at western city, turkey showed that about 456 stool specimen were collected and 145(31.8%) were infected with one or more intestinal parasites, 29(6.4%) of the students were infected with more than one parasites, 26(5.7%) with two parasites and about 3(0.7%) infected with three parasites. The three most common parasites were *E.vermicularis. G.lamblia and E.coli* intestinal parasites prevalence were higher in rural than urban area [41].

A cross sectional survey conducted in Ethiopian on intestinal infection in asymptomatic children in south western Ethiopia in July 2005 showed that the overall prevalence rate of intestinal helminthes was 57.4% with *T.trichiura* (31%) *A.lumbricides* (30.5%), *H.nana* (14.3%) and *hook worm* (4%) [42].

Another study conducted in Jiren School, Jimma town, showed that the overall intestinal parasite prevalence rate of 68.4% and *A.lumbricoides* was the most prevalent parasite which accounted 52.2% and *T.trichiura* was the second parasite with 18.6% and *S.mansoni* was the least intestinal parasite which was (0.3%) [43].

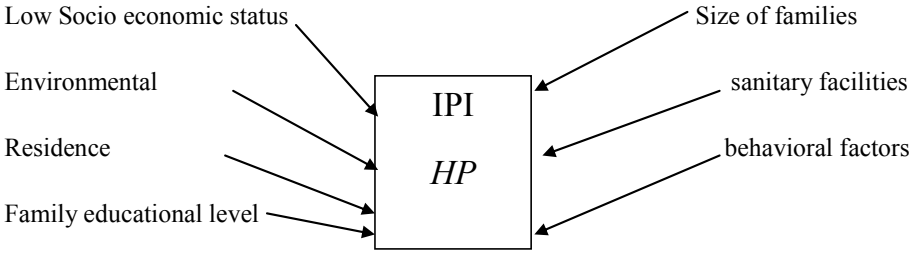
2.4. Risk factors for intestinal parasite

Most studies show that potential risk factors with the prevalence of intestinal parasites among school children. Socio-demographic, Environmental, behavioral factors and different sanitation facilities had a significant contribution for the presence of IPIs. Among the potential risk factors, the unavailability of washing facilities constructed at home had also a contributing effect for the presence of intestinal parasites. Home cleanness condition also had contribution for the existence of IPIs [44].

They are closely associated with low household income, poor personal and environmental sanitation, overcrowding conditions, and limited access to clean water, tropical climate and low latitude [45].

Study conducted in, Delgi, School children in south Gondar of Ethiopia, the finding showed school children who had no toilet with washing facilities in their home were more likely to acquire the IPIs than those who had the facilities. However, the difference was not statistically significant ($p > 0.05$). An open defecation system of latrine in the living environment could have a significant contribution for the occurrences of IPIs ($P < 0.05$). The highest prevalence of IPIs was also found in children who had no toilet at their vicinity compared to those who had toilet at/around their home. This might have contribution due to the absence washing facility and exposure of children to parasites in open defecation system [46]. Based on the above literature review no data is available in Ethiopia context comprising both *H. pylori* and IPI. Hence this study is required to fill the gap.

Figure 1: Conceptual framework:



I have adopted this framework from different literature this risk factor could be for either of infection [reference, 32, 34, 35, and 44].

3. OBJECTIVES

3.1 General objectives:

To assess the prevalence and risk factors associated with *Helicobacter pylori*, and intestinal parasites among Selam Fire Elementary School Children form a duration of March to June 2017.

3.2 Specific objective:

- To determine the prevalence *Helicobacter pylori* among school children.
- To determine the prevalence *intestinal parasite* among school children.
- To assess the risk factors associated with *H. pylori* and intestinal parasite among school children.

4. MATERIALS AND METHODS

4.1. Study design

A cross-sectional, institutional-based study was conducted.

4.2. Study area

The study was conducted in Selam Fire Elementary School (SFES)which is located in Akaki Kality Sub City in wareda 3, Addis Ababa, Ethiopia, according to the Sub City educational system (educational statistic annual abstracts, 2008 E.C) report the number of Elementary school are around 68which are 20 governmental and 48 privates and others.

According to the SFES annual reports (statically data) the School was built in 2002E.c, by the communities with the governments support; it is also governed under Addis Ababa city education Bureau, the school located having an area of 15,274 square meters, organized with 58 teachers with different level of study and departments and 35 administrative staff, The total number students enrolled in this school are 1,174.it has water facility, but has no hand washing facility especially at toilet, it has seven toilets or dry toilet, it has eighteen (18) room or teaching class each of them have 56 square meter Area , the school also has enough area for playing and studying. The school has two set up, pre-school which has KG students with total number of 184 from this there are 92 female and 92 male, And the primary school has two cycle structure the first cycle is from grade 1-4 has 421students from this, there are 184 male, and 237 female, and the second cycle from grade (5-8) has 557 students from this, there are 238 male, and 319 female, in all of these area the lower limit of age was 4 years and the above limit it was reach up to 18 years.

4.3. Study duration

The study was conducted in the period from March to June, 2017.

4.4. Population

4.4.1. Source population

All of Selam Fire Elementary school students who were attending the classes during the study period.

4.4.2. Study population

Selam Fire Elementary School students who have been involved in the study based on the inclusion and exclusion criteria were the study population.

4.5Inclusion and exclusion criteria

4.5.1. Inclusion criteria

In the study documented all the school students who have been enrolled were included in the study after consent with parents/guardians.

4.5.2. Exclusion criteria

Those students were taken. *H. Pylori* treatments for the last two week.

4.6 Variables of the study

4.6.1. Independent variables:

Age, sex, weight, pre-sample antibiotic history, Socio demographic factors, parents educational status, parents income, hygiene practice like hand washing, environmental conditions (latrine, water source etc.)

4.6.2. Dependent variables:

Burden of *H. pylori* infection, and intestinal parasites

4.7 Sample size determination and sampling

4.7.1. Sample size determination

Different studies in different parts of the country also reported different prevalence rates of intestinal parasites in school children, also resa5rch has not been conducted the prevalence of *H. pylori* in school children. So that to determine appropriates for the population sample maximum value 50 % had been used.

$$N = \frac{Z^2 P (1-P)}{D2}$$

Where Z= 95% confidence interval (1.96)

P = Estimated prevalence rate (50%), = (0.90)

D = Marginal of sampling error

N = minimum sample size

$$= \frac{(1.96)^2.0.50(1-0.50)}{0.05^2} = \frac{3.8x.0.50\ (0.50)}{0.0025} = \mathbf{384}$$

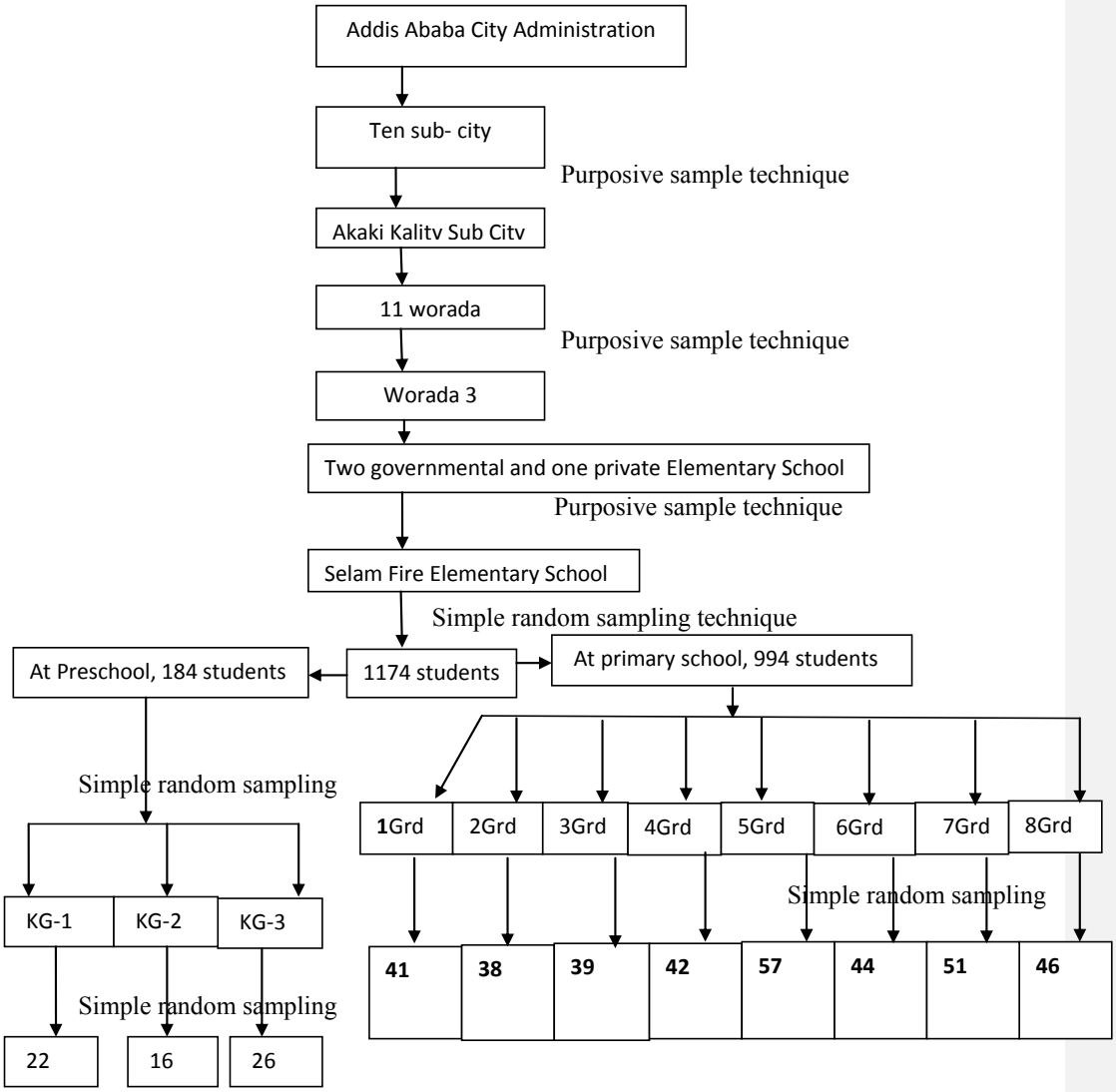
Therefore by adding 10% non-response rates, a total of **422** study subjects were participated in the study.

4.7.2. Sampling procedures

Multiple sampling methods were used , such as a purposive sampling technique was used to select these sub city, Akaki Kality, which is located at western parts from the center of Addis Ababa, and comprises of 11 woarda, and 27 local kebele, the total population is around 2,739,551 (1,305,387 male, and 1,437,164 female).

Simple random sampling technique was used to include study participants who meet the inclusion criteria until the achievement of the sample size, And to represent a proportional distribution from the various arms /grade of classes. Each arm/grade of class was given an equal chance of being selected. Selection of the various arms /grade were made by Simple random sampling technique this is assured by about (422/1174) or 35 % chances were given at each of classes, Consenting students in the class (es) selected was interviewed while stool samples collected immediately after the interview from the respondents.

Figure: 2 A diagrammatic representations of sampling procedures.



Note: Stu: students, Grd: grade, no = students number

4.8 Data collection tools and procedures

4.8.1 Demographic characteristics and exposure to risk factors

Structured questionnaire was prepared with English version and translated to Amharic and retranslated to English for data analysis and interpretation of results. Before the actual data collection time, the questionnaire was pre-tested on 20 of the study subjects (5% of the sample), in Aste Tewdrose Elementary school, which is located at worda 3 , in Akaki Kality sub city to check for any missing options, ambiguity and clarity.

4.8.2 Specimen collection and transportation

Students were advised to pass the stool samples directly into a plastic cup with a tight fitting lid. About 20-40 grams of formed stools or 5-6 spoonfuls for watery stools was collected. All specimens were labeled with patient's name, age, sex, and date of collection. The safety was assured by using universal safety guide line, and National health and safety guideline, by Wearing personal protective equipments and other personnel protective equipments. The participant's students were informed and well instructed by data collectors how handling the sample and their safety to reduce the contamination, after toilet, and giving a sample the participants were also informed to clean their hands with soap and clean water after the collection of the sample.

4.8.3 Laboratory techniques

4.8.3.1. Direct wet mount:

A small sample of Faeces was placed on a glass slide and mixed with a drop of 0.9% solutions of NaCl and the slide was covered with a glass cover slip and examined for the presence of intestinal parasites at 10× and 40 × magnifications [47].

4.8.3.2 Formal ether concentration technique (FECT):

About 1 g of Faeces (pea-size) will be emulsified in 4 ml of 10% formal saline. 3-4 ml of 10% formal saline were added and mixed well by shaking. Then sieved in a beaker and transferred to centrifuge tube. A 3- 4 ml of diethyl ether were added, stoppered and mixed for 1 minute. Then centrifuged at 750-1000 rpm for 1 minute, layers of faecal debris, ether and formal saline were discarded by using plastic bulb pipette. The sediment were re-suspended, mixed and transferred to slide, covered with cover glass, and then it was examined microscopically using (10 xs, 40 xs) [48].

4.8.3.3 *H. pylori* antigen rapid test (*H. pylori* Ag rapid test):

The source of *Hp*, Wondfo was one of the earliest high tech. biological companies focusing on rapid diagnostic in china: as described by manufacturer the use of rapid immune chromatographic test (ICT): for the qualitative detection of *H. pylori* antigen in fresh fecal samples. Instructions given by the manufacturer were followed. Stool collection device were opened and using collection stick to pierce the stool sample, then the collection stick was replaced to stool device and was shake vigorously. On the test device, 2 drops of the solution was dispended into the sample well. Results may be read after 15 minutes of adding the specimen, the performance of these test kit has been compared with *H. pylori* ELISA detection kit , accordingly the fact given from manufactures it has (99.1% ,99.6%) , and (99.2%, 96.6%) sensitivity and specificity respectively [49].

4.9. Data management and quality control

Data was collected by pre tested questioner and clean sample collection material with leak proof and a lid. Data collectors were identified, trained and informed to collect the data as per the pre-structured questionnaire, and Interviews were conducted by two trained research assistant's that has more than a three years' work experience. Also they were trained in how to use the instrument and how they should introduce themselves and the research objectives modestly to the students/parents/guardians during the interview. The daily analysis was supervised by Personal investigator. For laboratory analysis Pre-analytical, analytical and post-analytical stages of quality assurance that is incorporated in Standard operating procedures (SOPs) was strictly followed. The purpose of the study as well as any related harm and benefits were explained to the study participants accordingly. Demographic data and potential risk factor of *H. pylori*, and intestinal parasitic infection, MUAC, weight, pre-sample antibiotic history, parental /guardian per-individual monthly income was recorded.

4.9.1 Pre-analytical phase

First of all we were asked the parents/guardians verbally and by written consent/assent for their willingness and then we were filled all the information on the preformed questioner, we were also took, weigh, height, and MUAC measure, finally by labeling the stool cup/container with participants identification number and information was informed them to bring the sample. The specimen quality assured by stool specimen rejection criteria of the health center laboratory which is indicated in SOP, following collection, specimens has been transported with ice bag at about 20c ^{o} to the Selam Fire health center laboratory within 20-30 minutes. Which is situated about 100 meter away from the study area.

4.9.2 Analytical phase

The sample was analyzed at Selam Fire Health Center which is located at nearby the study area, this Health center is still participated, and it has two Star levels ENAO assessment program. The test was performed by the well experienced laboratory technicians/technologist and continuously was supervised by principal investigator. A collected sample was tested once for Stool Ag for *H. pylori*, and for intestine parasite. All materials, equipment and Procedures have been adequately controlled. Stool Ag test reagents were evaluated using a control band indicator on test kit. Standard operating procedures (SOPs) of the health center laboratory for both tests (stool Ag test and stool examination) were strictly followed and the results were checked by the supervisors.

4.9.3 Post-analytical phase

The results were recorded with identification number. In order to avoid the errors in the results of the test, the reporting has been repeatedly checked and evaluated by the head of the department and principal investigator. The laboratory result was given a free of charge for parents/guardians at tested day or whenever they came with their identification number. For students with positive for either of *H. pylori* and intestinal parasite or for co infection we were linked to the health center, for medical treatments. Every laboratory test results were interpreted based on the SOPs of SFHCL, AARL.

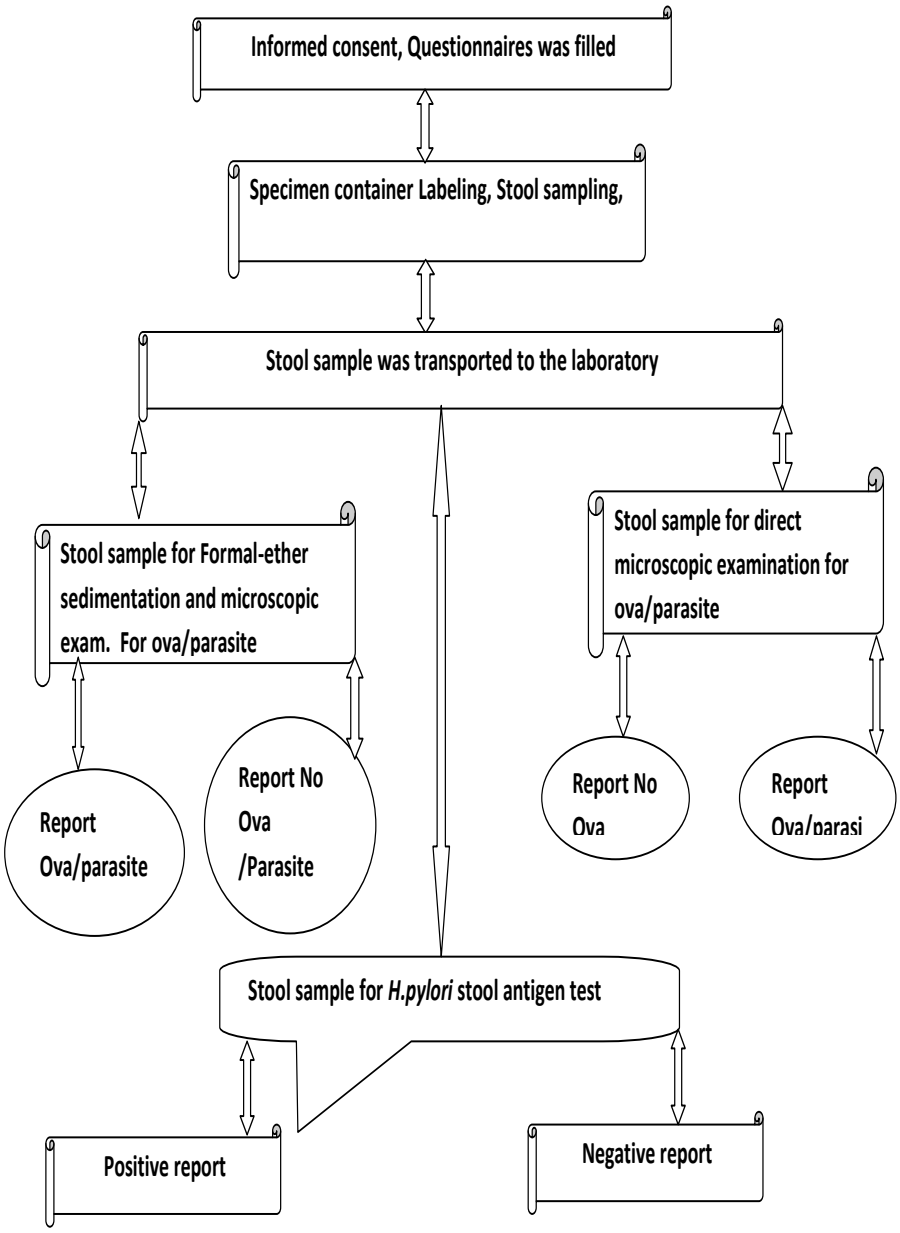


Figure 3: Work flow of stool sample for *H. pylori* stool antigen test, direct microscopic stool examination and formol-ether sedimentation and microscopic examination for ova/parasite

4.10 Data processing and analysis

Data was coded and entry analysis were done using SPSS statistical software version 20. The descriptive statistics were calculated & binary and multiple logistic regression analysis was used to see the relation between dependent variable and independent variables. The association was assessed by using chi-square test. Variables that was showed a significant association were selected for further analysis. In all cases P-value less than 0.05 was considered as statistically significant. The strength of the association was interpreted using an odds ratio in a 95% confidence interval. Finally, the results were presented on words, charts, graphs and tables.

4.11 Ethical considerations

This research project has been approved by department of ethical and review research committee (DRCRC) of the Department of Medical Laboratory Sciences, CHS, and School of Allied Health Science of AAU, and Addis Ababa public Health Research and emergency management core processes, During the planning of the study, the researchers approached the authority's in-charge of the selected schools particularly the principals and the Local Education District Officer in-charge of public schools with formal letters to obtain permission to carry out the work in the schools and also explained the study objectives.

There was very minimal risk associated with the process of sampling and data collection. For all confirmed *H. pylori* infection or intestinal parasite infection, we were linked to health center by informing their parents/guardians to get treatment. We were provided a laboratory results with free of charge. All the information contained within the study was kept confidential.

5. RESULTS

5.1 Socio demographic characteristics of the study subjects

From 1174 students of Selam Fire Elementary School, only 422 students were selected. About 55.2% (n=233/422) of student were female resulting male to female ratio of 1:1.3. Children with age groups 10-15 years were the highest population 59.6% (n=249/422), Age of range (4-18) years, with mean age of 11.16±SD years [95% CI 10.82-11.5] none of the school children had severe malnutrition based on medium upper arm circumference (MUAC). (Table **5.1)**

Table5.1. Socio demographic characteristic of Selam Fire Elementary School children.

Variable	Frequency	Percent
Age		
4-9	124	29.4
10-15	253	60.0
16-18	45	10.7
Sex		
Male	189	44.8
Female	233	55.2
MUAC		
Normal	268	63.5
Moderate	154	36.5
Residence		
Urban	402	95.3
Rural	20	4.7
Family/guardians income		
Low	396	93.8
Medium	26	6.2
Family/guardians level of education		
Illiterate	175	41.5
Read and write	61	14.5
Primary school	72	17.1
Secondary school	69	16.4
Above secondary	45	10.7

In this study,97.9%(n=413/422) children live in house with bed room of 3 or low and majority of the child live in a family size of 6 and above 54.5%(n=230/422), and 87.2 %(n= 368/422) did not had history of de-wormed. **(Table 5.2)**

Table5.2 Shows the distribution of the study population by household population characteristics, and student's behavioral characteristic in School children.

Variable	Frequency	Percent
No of bed room		
< 3	413	97.9
> 3	9	2.1
No person do live in house		
< 6	192	45.5
> 6	230	54.5
Abdominal pain		
Yes	134	31.8
No	288	68.2
De-worm		
Yes	54	12.2
No	368	87.8
Source of water		
Tap/bono water	402	95.3
Bottled water	1	0.2
Mineral/ground water	19	4.5
Type of toilet		
Pit latrine	389	92.2
Flush toilet	1	0.2
Open field	32	7.6
Hand washing after using toilet		
Always	296	70.1
Some times	71	16.8
Not at all	55	13.0

5.2. Prevalence of *H .pylori* and associated of risk factors

Helicobacter pylori antigens were detected in children giving an overall prevalence of 14.6 % (n=61/418), (95% CI 1.82-1.88), 8.6% (n=36/233) in female and 5.9 %(n=25/189) in male children (x^2 = 0.310, 95% CI=1.82-1.88). The prevalence of *H .pylori* among age group of 4-9, 10-15, and 16-19 is 10(2.4%), 42(10.0%), and 9(2.2%) respectively. According to the study subject the peak age was 10-15 year and prevalence was 10.0 % (X^2 = 6.16, p value= 0.046). (**Table5.3**). In this study the high prevalence of *H. pylori* infection were in age group of 10-15, in accounting 10%(n=42/249), and majority of these infection were occurred in children whose their family/guardians low level of education, also majority of infection were occurred in children with less than 3 bed room in the house. (**Table 5.3**)

Comment [u1]: Not clear

Table 5.3 Association between risk factors and *H. Pylori*

Variables and categories	N	*HP,* Positive (%)	X^2, 95%CI, OR	P value
Age			6.16	0.046
4-9	124	10(2.4%)		
10-15	253	42(10%)		
16-18	45	9(2.2%)		
Sex			0.42(0.48-1.45)	0.52
Male	189	25(5.9%)		
Female	233	36(8.6%)		
Family/guardians educational			10.53	0.03
Illiterate	171	29(6.9%)		
Read and write	61	13(3.1%)		
Primary school	72	12(2.8%)		
Secondary school	69	6(1.4%)		
Above secondary	45	1(0.4%)		
Family/guardians income			4.7(0.8-0.88)0.85	0.03
low	396	61(14.6%)		
Middle	26	0(0.0%)		
No of bed room			0.08(0.17-11)1.4	0.77
< 3	413	60(14.2%)		
> 3	9	1(0.4%)		
No person do live in house			0.04(0.6-1.6)0.94	0.83
< 6	192	27(6.4%)		
> 6	230	34(8.2%)		
Total	**2532**	**366(14.5%)**		

Table5.4. Association between risk factors and *Intestinal parasites* in school children

Variables and categories	N	*IPI,* Positive (%)	X^2, 95%CI, OR	P value
Age			11.33	0.003
4-9	124	16(3.8%)		
10-15	253	71(16.8%)		
16-18	45	13(3.1%)		
Sex			0.033(0.6-1.5)0.96	0.86
Male	189	44(10.5%)		
Female	233	56(13.3%)		
Family/guardians education			9.6	0.04
Illiterate	175	48(11.4%)		
Read and write	61	12(2.8%)		
Primary school	72	23(5.5%)		
Secondary school	69	11(2.6%)		
Above secondary	45	6(1.4%)		
Family/guardians income			3.93(0.9-17)3.95	0.04
low	396	98(23.2%)		
Middle	26	2(0.5%)		
No of bed room			2.19(0.1-1.4)0.38	0.14
< 3	413	96(22.7%)		
> 3	9	4(1.0%)		
No person do live in house			5.3(0.37-0.93)0.58	0.02
< 6	192	36(8.6%)		
> 6	226	64(15.3%)		
De-worm				
Yes	54	11(2.6%)	0.38(0.39-1.61) 0.8	0.54
No	368	89(21.1%)		
Source of water			12.6	0.002
Tap/bono water	402	89(21.1%)		
Bottled water	1	1(0.2%)		
Mineral water	19	10(2.4%)		
Type of toilet			27.8	0.00
Pit latrine	389	80(19.0%)		
Flush toilet	1	1(0.2%)		
Open field	32	9(4.5%)		
Hand washing after using a toilet			245.5	0.00
Always	296	14(3.3%)		
Some times	71	32(7.6%)		
Not at all	54	54(12.8%)		
Total	**4220**	**900(21.3%)**		

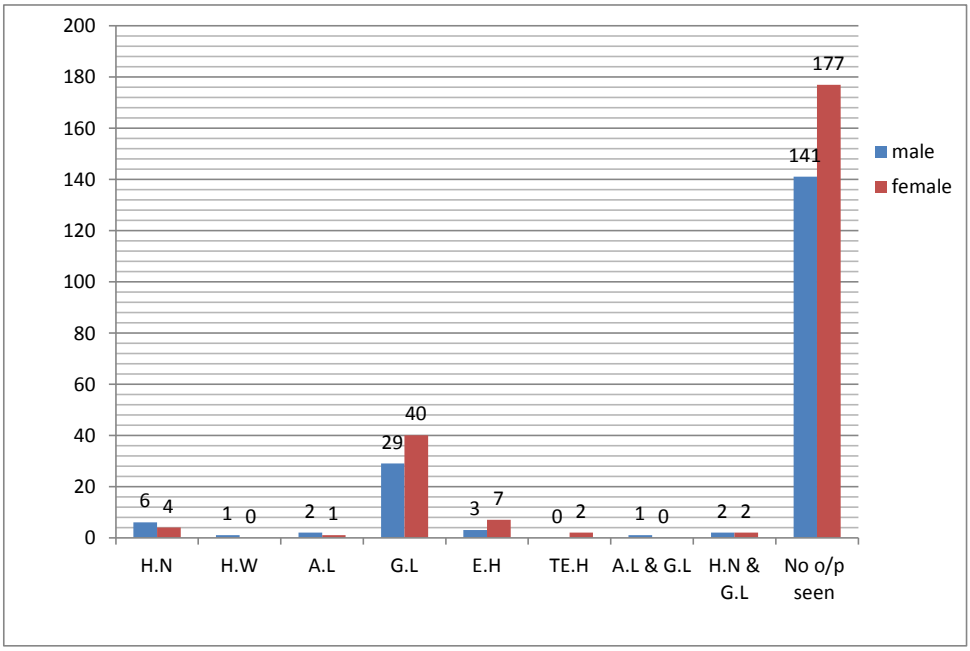
5.3. Prevalence of intestine parasite and associated risk factors

Intestinal parasite were detected in children giving an overall prevalence of 23.7% (n=100/422) (95% CI 1.72-1.8), 13.3% % (n=44/233) in female and 10.5 %(56/189) in male children. (**Table 5.3**) The distribution of IPI in female children was, 1(0.2%) ova of *Ascaris Lumbricode*, lowest prevent, and 40(9.6%) cyst of *Giardia Lamblia*, which is the most prevalent. And in male the distribution the highest prevalence was 29(6.9%) cyst of *Giardia lamblia, and* the lowest prevalence was 1 (0.2%) ova of *Hookworm*. In both gender the occurrence of multiple infection in one individual was very minimum. (Fig **4**)

The frequency of *IPI* colonization was not significantly different between females and males p = 0.952, and the highest prevalence of *IPI was seen* among age group of 10-15 years 16.8 %(n=71/253) and in age group of 16-19 years 3.1 %(n= 13/45) had the lowest prevalence of IPI (P value of 0.003), according to these study there were a significant association the between the prevalence IPI with age. (**Table 5.4**)

Comment [u2]: DO NOT WRITE EVERYFINDINGS IN TEXT (WORDS) THE DETAIL SHOULD BE SHOWN IN TABLES PLESAE FOLLOW FOR ALL YOUR RESULTS !!!

Figure 4: the distribution of intestinal parasite infection among the gender of children



Key:

H.N – Ova of *H. Nana*

H.W- *Ova of Hookworm*

A.L- *Ova Ascaris Lumbricoid*

G.L- *Cyst of Giardia Lamblia*

E.H- *Cyst of Entambea Histolytical*

TE.H- *Trophozoite of Entambea Histolytical*

Out of 422 study subjects co-infection was observed in 4.5 %(n=19/422), of children had *HP a*nd IPI. (X^2= 2.19 p=0.14), OR= 1.56, 95%CI= 0.86-2.84),in this study we had observed a common risk factors which is significantly associated with both infection, like Family/guardians educational level and their monthly income. (**Table 5.5**)

Table 5.5 The co infection of IPI with *H. pylori* among the study groups

		H. pylori		Total
		Positive	Negative	
IPI	Positive	19	81	100
	Negative	42	280	322
	Total	61	361	422

P= 0.14

6. DISCUSSION

6.1 The prevalence of *Helicobacter pylori* and risk factors

The overall prevalence rate of 14.6% obtained in this prospective cross sectional study is low and suggests that *H. pylori* infection was not significant in the asymptomatic school children. As compared to study conducted in Pakistani children was between 24 and 92%, and in China on children in between 5 and 28%, however these prevalence was affected by test methods, and variation from country to country [**24**, **25**].

The prevalence of *H. pylori* 2.4 % in the age group 4-9, and increased to 10% in the age group 10-15., and 2.2 % among age group of 16-18 and also majority of students number was under 10-15 age categories. From study subjects the increased prevalence of infection with age was initially thought to represent a continuing rate of bacterial acquirement throughout one's lifetime, as previous study conducted in developing countries where majority of children are infected before the age of 10 and the prevalence in adults peak up to 80% (**28**)

Comment [u3]: Controvercial statement please be consistant if the prevalence is low how it is significant.

The prevalence of *H. pylori* infection in age group of 4-9 years was very small; as compared to study conducted WGO 2010 which was reported 80%, however. The reduced prevalence of *H. pylori* in study were related to the study subjects were asymptomatic. The difference in socio-economic factors, educational status of their family/ guardians and life style could also contribute for their difference [**17**].

This study was strongly agreed with in studies showed that risk factors such as gender do not seems to be associated risk factor and no significant association were founded among gender from the general study population [**33**].

Ayse.et.al reported from eastern turkey, also shown, the high prevalence of *H. pylori* infection not a significant different where parents were different education level. In contrast to this study found a significant relationship between *H. pylori* prevalence, and family/guardians educational status (p= 0.030.) [**31**].

Study conducted in eastern turkey has shown as large family size and overcrowding was risk factors. This study also strongly agreed and a significant. (p= 0.03) in 3 bed room or less 14.2% (n=60/423) and also *HP* was higher in study subjects living more than six people in the household 8.2 %(n=34/230) **[31, 33**]

In previous study, several socioeconomic factors have been associated with *H. pylori* infection [**33, 34**]. In particular, subjects as a low family income had a higher likelihood of carrying *H. pylori* infection. This study agreed and founded a significant Prevalence rates were higher in children of low socioeconomic class compared with those of increased socioeconomic class.

Study conducted in UK showed that, bed sharing, family size was possible risk factors. However, According to this study the prevalence of *H. pylori* were almost not different, also statistically insignificant in families with large number peoples do lives than less, although, majority infection to this organism were occurred among who shared bed, but insignificant as risk**[32].**

6.2 The prevalence of intestinal parasite and risk factors

Different studies which were conducted in different parts of the world showed that a different prevalence rate, in India showed two third of 63-94% **[38].** , in kubia Junior, Saopaulo state Brazil, of 219 school children of which 123 (56.1%) **[39]**. in asymptomatic children in south western Ethiopia in July 2005 showed that the overall prevalence rate of intestinal helminthes was 57.4%, Another study conducted in Jiren School, Jimma town, showed that the overall intestinal parasite prevalence rate of 68.4%.[**42**]. As compared to the above studies showed that prevalence of IPI among study group was low, the overall prevalence of intestinal parasite was 23.7 %(n= 100/422), and 20.4 %(n=86/422) for Protozoan, and 3.32 %(n=14/422) for Protozoan Helminthes, and multiple infection 0.96 %(n=4/422).

In this the most prevalent intestinal parasite were cyst of *Giardia Lamblia* 17.1% (n=72/100), and the least was ova of Hookworm 0.2 %(n= 1/100). As compared this study agreed with in Study conducted at western city, turkey showed that the three most common parasites were *E.vermicularis. G.lamblia and E.coli* intestinal parasites prevalence were higher in rural than urban area [**19**]. And a cross sectional survey conducted in Ethiopian in July 2005 showed in asymptomatic children with *T.trichiura* (31%) *A.lumbricides* (30.5%), *H.nana* (14.3%) *and hook worm* (4%) [**37, 38**]

In our study showed that the prevalence of IPI in different age group significantly increased, its prevalence with age was initially thought to represent a continuing rate of intestinal parasite acquirement throughout one's lifetime. Also it's the prevalence among the gender of the children not significantly associated, however. Majority of these were significantly detected in children with parents/guardians with low monthly income, and level of

educational status. As compared this study is agreed with studies conducted in that potential risk factors with the prevalence of intestinal parasites among school children. Socio-demographic, Environmental, behavioral factors and different sanitation facilities had a significant contribution for the presence of IPIs [**38**, **43**].

Overcrowding index such as family size and number of bed room in the house is risk factors. [**43**]. in our study the prevalence of IPI among children were significantly increased in families with large number people than less. However, majority of the IPI infection among children were insignificantly occurred in families with sharing a bed room.

Different sanitary activity and facilities had a significant contribution for the prevalence of IPI among school children [**43, 44].** In our study not practicing hand washing after using toilet was significant factors for observing a high prevalence of IPI among children, and, majority of the prevalence IPI observed in study participants which used tap/bono water than other; this could be due to contaminated and poor hygiene. Even though, majority of study participants used a pit latrine and they had a significantly high prevalence of IPI among children. according to this perspective study showed that the prevalence rate was in consisted with the poor health practices were responsible ,especially the poor habits of hand washing after using toilet, families/guardians awareness, educational status, and economic factors.

7. Limitation of the study

1. The limitation of this study was lack of quantitative confirmatory test. The test should be confirmed by enzyme-linked immunosorbant assay (ELISA) stool Antigen test, because the Linear *Helicobacter Pylori Ag* cassette is limited to the qualitative detection of *H. Pylori* antigen in human fecal specimen.
2. This study done in one governmental elementary school and the data was collected from those students who learned to this school during the data collection period. It doesn't include other elementary school in the city of Addis, as well as in this study area in Akaki Kality sub City that may underestimate findings.

8. CONCLUSIONS AND RECOMMENDATIONS

8.1 Conclusion

The study concluded that *IPI* infection was high prevalent compared with *H. pylori* infection in the study area, prevalence rates of with *H. pylori* and *IPI* were (14.6%), (26.7%) respectively in subjects under study by using stool Ag test, and direct wet mount and FECT respectively. Females were found to be more affected than males in infection by both pathogens. The prevalence of *IPI* and *H. pylori* was higher in the age group 10-15 years old than other age groups this is due to large number of study sample under this group. The study concluded that of *IPI* and *H. pylori* is possibly a burden in asymptomatic study area, due to social, economical, and environmental risk factors.

8.2 Recommendations

1. In this study educational status, monthly income status of their family/guardians was positively associated with *H. pylori* prevalence. Overcrowding, educational, and monthly income level of families/guardians, poor sanitary practice of the students, water source positively associated with *IPI*. Thus, minimizing overcrowded condition, and improving the living standard of the society, health education in the area is mandatory.
2. Improvement of health measures such as personal hygiene, and water purification are useful ways for elimination of the infection, and these should be done in the school.
3. Further studies with the aid of more advanced techniques such as PCR, ELISA are recommended to assess the presence of *IPI* and *H. Pylori*.
4. The school de-worming program should be strengthened, to reduce and eliminated intestinal parasitic infection.

9. REFERENCE

1. Warren JR. Unidentified curved bacilli on gastric epitilium in active chronic gastritis, 1983; 321:1273-1275.

2. Dr David I Campbell Gastric cancer and Helicobacter pylori: a combined analysis of 12 case control studies nested within prospective cohorts. Gut 2001; 49:347 53.

3. Gold BD, colletti RB, Abbott M, Czinn SJ, Elitsur Y, Hassall E, et al. Helicobacter Pylori infection in children: recommendation for diagnosis and treatment. Journal and paediatric Gastroenterology and nutrition 2000:31; 490-97.

4. Hall A, Hewitt G, Tuffrey V, de Silva N , A review and meta-analysis of the impact of intestinal worms on child growth and nutrition. Matern Child Nutr Suppl 2008: 118-236.

5. Evans AC, Stephenson LS not by drugs alone: the fight against parasitic helminthes. World Health Forum 2001, 16: 258-261.

6. Albonico M, Crompton DW, Savioli L Control strategies for human intestinal nematode infections, Adv Parasitol (2000) 42: 277-341.

7. Bentwich Z, Kalinkovich A, Weisman Z Immune activation is a dominant factor in the pathogenesis of African AIDS. Immunol Today 1999, 16: 187-191.

8. Astizarian Garcia H, Espenosa Cantenallo M, Castanon G, Chavez-Munguia B, Martinez Palomo A Giardia lamblia: Effect of Infection with Symptomatic and Asymptomatic Isolates on the Growth of Gerbils (Meriones unguiculatus). Experimental parasitology 2000, 95:128-135.

9. Rao VG, Sugunan AP, Murhekar MV, Sehgal SC Malnutrition and high childhood mortality among the Onge tribe of the Andaman and Nicobar Islands.Public Health Nutr 2006,9: 19-25.

10. Asrat AY, Tewodros DE, Alemayehu WO prevalence and risk factors of intestinal parasites among delgi school children, North Gonder. Ethiop J Health and Biomed Sci 2011 3:75-81.

11. Koletzko S, Jones NL, Goodman KJ et al. Evidence-based Guidelines from ESPGHAN and NASPGHAN for Helicobacter pylori Infection in Children. J Pediatr Gastroenterol Nutr 2011; 53(2):230-243.

12. Guarner J, Kalach N, Elitsur Y, Koletzko S. Helicobacter pylori diagnostic tests in children: review of the literature from 1999 to 2009. European Journal of Pediatrics2010; 169(1):15-25.

13- Julio, C., Vilares, A., Oleastro, M., Ferreira, I ., Gomes, S., Monteiro, L., Nunes, B., Tenreiro, R. and Angelo, H. Prevalence and risk factors for Giardia duodenal is infection among children: A case study in Portugal Parasites & Vectors, 2012. 5(22):1-8.

14- John, D.T. and Petri, W. A. J.Medical Parasitology. 9th edition,USA. 2006.48 -55, 393-395.

15- Salman, Y. J. Efficacy of some laboratory methods in detecting Giardia lamblia and Cryptosporidium parvum in stool samples. Kirkuk University Journal, 2014 9(1):7-17.

16. Roland N. Ndip, Alertia E. Malange Takang, Juliet E. A. Ojongokpoko1, Henry N. Luma Helicobacter pylori isolates recovered from gastric biopsies of patients with gastro-duodenal pathologies in Cameroon: current status of anti bio-gram. Tropical Medicine and International Health 2008; 13 (6) 45-56.

17. R.H. Hunt, S.D. Xiao ,F. Megraud ,R. Leon-Barua ,F. Bazzoli S. van der Merwe et; al World Gastroenterology Organization Global Guidelines, Helicobacter pylori in developing countries; Journal of Digestive Diseases 2011; 12; 319-326.

18. WHO Expert Committee on Comprehensive School Health Education and Promotion Geneva World Health Organization, 2011

19. Center for disease control and prevention, Human Diseases and Conditions. http://www.cdc.gov 2011

20. Berna G.pinnar O. intestine parasite prevalence and related risk factors in school children, western city, 2005,10: 4-64

21. Leslie J & Jamison DT Health and nutrition consideration in education planning. 1. Education consequences of health problems among school-age children. 2. The cost and effectiveness of school-based interventions. Food and nutrition bulletin, 2000J, 12, 3:191-214.

22. Graham DY, Lu H, Yamaoka Y. African, Asian or Indian enigma, the East Asian Helicobacter pylori: facts or medical myths. J Digest Dis 2009; 10: 77-84.

23. Naito Y, Shimizu T, Haruna H, Fujii T, Kudo T, Shoji H, et al. Changes in the presence of urine Helicobacter pylori antibody in Japanese children in three different age groups. Pediatr Int 2008; 50: 291-4.

24. Javed M, Amin K, Muhammad D, Husain A, Mahmood N. Prevalence of H. Pylori. Professional Med J 2010; 17: 431-9.

25. Shimoyama T, Oyama T, Matsuzuka M, Danjo K, Nakaji S, Fukuda S. Comparison of a stool antigen test and serology for the diagnosis of Helicobacter pylori infection in mass survey. Helicobacter 2009; 14: 87-90.

26. Thomas JE, Dale A, Bunn JE, Harding M, Coward WA, Cole TJ, Weaver LT. Early Helicobacter pylori colonization: the association with growth faltering in the Gambia. Arch Dis. Child. 2004, 89(12): 1149-1154.

27. Jackson L, Britton J, Lewis SA, McKeever TM, Atherton J, Fullerton D, et al.A population-based epidemiologic study of Helicobacter pylori infection and its association with systemic inflammation. Helicobacter 2009; 14: 460-5.

28. Rowland M, Daly L, Vaughan M, Higgins A, Bourke B, Drumm B, et al. Age-specific incidence of Helicobacter pylori. Gastroenterology 2006; 130: 65-72.

29. Ndip RN, MacKay WG, and Farthing MJG, Weaver LT. Culturing Helicobacter pylori from clinical specimens: Review of microbiologic methods. J. Pediatr. Gastroenterol.Nutr.2003, 36: 616-622.

30. Mendall MA, Goggin PM, Molineaux.Childhood living conditions and Helicobacter pyloriseropositivity in adult life. Lancet 2000; 339:896-7.

31. Ayse Selimoglu M, Ertekin V, Inandi T. Seroepidemiology of Helicobacter pylori infection in children living in eastern Turkey. Pediatr Int 2000; 446:6669.

32. Whitaker CJ, Dubiel AJ, Galpin OP. Social and geographical risk factors in Helicobacter pylori infection. Epidemiol Infect 1999; 111:63-70.

33. Hanafi MI, Mohamed AM, Helicobacter pylori infection: seroprevalence and predictors among healthy individuals in Al Madinah, Saudi Arabia. J Egypt Public Health Assoc 2013; 88:40–5.

34. Zhu Y, Zhou X, Wu J, Su J, Zhang G. Risk factors and prevalence of Helicobacter pylori infection in persistent high incidence area of gastric carcinoma in Yangzhong city. Gastroenterol Res Pract 2014; 481-365

35. Segal I, Ally R, Mitchell H. Helicobacter pylori: an African perspective. Q. J. Med. 2001. 94: 561-565.

36. Dale A, Thomas JE, Darboe MK, Coward WA, Harding M, Weaver LT. Helicobacter pylori infection, gastric acid secretion, and infant growth. J. Pediatr. Gastroenterol.Nutr. 2000, 26(4): 393-397.

37. [No authors listed] Prevention and control of intestinal parasitic infections, Report of a WHO Expert Committee. World Health Organ Tech Rep Ser 2001, 749: 1-86.

38. Curtale F, Pezzotti P, Sharbini AL, al Maadat H, Ingrosso P, Saad YS, et al. Knowledge, perceptions and behavior of mothers toward intestinal helminthes in Upper Egypt: implications for control, Health Policy Plan. 2001; 13(4):423–32.

39. Virk KJ, Prasad RN, Prasad H. Prevalence of intestinal parasites in rural areas of district Shahjahanpur, Uttar Pradesh. J Commun Dis. 1999; 26(2):103–8.

40. Teklehaymanaot t. Intestinal parasitosis among kara& kwego semi pastoralist tribes in lower OMO valley. South Western Ethiopia EJHD 2009, 23(1): 57-62.

41. Piva O.Ozlem., Prevalence of intestinal parasitic infection in western city Turkey.2004: 4-64

42. Haila Amlak A. intestinal parasitic in Asymptomatic children in south west Ethiopia EJHS July 2005.vol 15(2).

43. Girmay H. chali Taddese M. intestinal parasitism among Jiren elementary and junior secondary students in south western Ethiopia.2001: 8(1); 37-41

44. Erko B, Medhin G, Birrie H: Intestinal parasitic infection in Bahir Dar and risk factors for transmission. Trop Med 1995, 37:73–78.

45. Amare Mengistu, Solomon Gebere-Selassie, and Tesfaye Kassa: Prevalence of intestinal parasites among urban dwellers in South West Ethiopia.Ethoip.J. Hlth.Dev.2007, 21(1):12-17

46. Ayalew A, Tewodros D, Alemayehu W: Prevalence and risk factors ofintestinal parasites among Delgi school children, North Gondar Ethiopia J Paras and Vec. Bio 2011, 3(5):75–81.

47. Chakarova, B. Comparative evaluation of the diagnostic methodsfor detection of Giardia intestinalis in human fecal samples. Trakia Journal of Sciences, 2010.8(2):174-179.

48. Cheesbrough, M. District laboratory practice in Tropical Countries.Part1.Egyptian editions, Cambridge.2000, 193-198.

49. Wondfo, one step *H.pylori* feces Test; catalog No. W028-S

10. LIST OF ANNEXES

Annex I: English version of participant information sheet

I. English version of Participant information sheet

Department of Medical Laboratory Science, Collage of Allied Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia, 2017

Title of the Research Project: Prevalence of *Helicobacter pylori* and intestinal parasite and their associated risk factors among school children at Selam Fire Elementary School in Akaki Kality Sub City, March to June 2017, Addis Ababa, Ethiopia

Introduction: First of all I would like to thank you in advance for your cooperation and consent in participation in this study. Please read or listen when it is read for you about the general information of the study. If you have any question regarding the study please ask freely.

Background information

Background: The prevalence of *H. pylori* infection mainly acquired during childhood and may be persisting throughout life, it has been found high in developing countries; this high prevalence is related to low socioeconomic status, Intestinal parasitic infections are among the major public health problems in Sub-Saharan Africa. Their distribution is mainly associated with poor personal hygiene, environmental sanitation and limited access to clean water. There is limited information on the burden of the *H. pylori* and *intestinal parasites* in Ethiopia and this research will be addressed such gap.

Aim of the study

The purpose of this study was determined the Prevalence of *Helicobacter pylori* with *intestinal parasite* and their associated risk factors among school children; since most school children are asymptomatic, besides these the study were focused on to evaluating their burden and socio-demographic, lifestyle, and environmental hygiene conditions as associated possible risk factor.

Study Duration

The study was conducted in the period from March to June 2017.

Benefits for participants

Study participants were not having any financial incentives or other inducements from participating on this study. However, based on the diagnosis result, we were linked to health center by consenting to parent/guardians to get treatment. We were provided a laboratory results with free of charge. Most importantly, the result of the study was beneficial to design effective prevention and control measure for Elementary School children. Hence, you are indirectly benefiting other children and the society in this respect.

Risks and complication

There are no anticipated risks to you and your child participation. Stool sample were taken from the students once by clean sample container. During sample giving it was expect form students, after toilet they should be washed their hands with plane water and soap.

Confidentiality

There is no sensitive issue that you were asked related with your social desirability but any information that is obtained in connection with this study and that can be identified with you and your child will remain confidential. Participants were not being prohibited to stop or withdraw at any time from the study. Only interested participants can retrieve their own lab result using their code number. The information collected about you and your child were coded using numbers. No personal information was disclosed to third party or will not appear in any report from this study.

Assurance of Principal Investigator

I put my signature below to confirm you that I take over the responsibility for the scientific ethical and technical conduct of the research project and for provision of progress reports for all stakeholders of the research project.

Abebe Worku (PI)

Signature: _____ Date: _____

Note: If you have any questions about this study, you should feel free to ask now or anytime throughout the study by contacting

PI Address: Abebe Worku: Department of Medical Laboratory Sciences, Collage of Allied Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia,2017

E-mail:-workuabebe249@gmail.com,

Mobile/phone no: +251-912685504

Fax no:-

Po Box: - 36, Addis Ababa, Akaki, Ethiopia, 2017

Department Address: Department of Medical Laboratory Science, Collage of Allied Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia

Phone no: +251-112-75-51-70

Fax no: +251-112-75-46-69

E-mail:- SMLT@ethionet.et

Po-Box: - 1176, Addis Ababa, Ethiopia, 2017

Annex II. Amharic version of the information sheet

ቅፅ 1 ፡ለተሳታፊው በቂ መረጃ ለመስጠት የተዘጋጀ ቅጽ (ትርጉም በአማርኛ) አዲስ አበባ ዩኒቨርሲቲ የድህረ ምረቃ ትምህርት፡፡

የተመራማሪውስም፡አበበወርቁ

የድርጅቱ ስም፡ አዲስ አበባ ዩኒቨርሲቲ፡የጤና ሳይንስ ኮሌጅ ፡የአላይድ ጤና ሳይንስ ት/ት፡

ህክምና ላቦራቶሪ ሳይንስ ክፍል

ርዕስ፡ የኤች ፓይሎሪ ባክቴሪያ እና የሆድ ውስጥ ትላትሎች በአንደኛ ደርጃ ት/ቤት በልጆች ላይ ስላለው ስርጭት ምክንያት እና መንሴዎችን ለይቶ ለማወቅ የሚል ነው፡፡

መግቢያ ፡ በመጀመሪያ ለዚህ ጥናት ለመሣተፍ እና ለመተባበር ፍቃደኛ በመሆኖ እጅግ አመስግናለሁ ፡፡ በመቀጠል ስለጥናቱ አላማ እና ሁኔታ ለማወቅ እባኮ መጠየቁን ያንብቡት ወይም እንዲነበብሎት ይጠይቁ ፤ ማንኛውንም ጥያቄ ስለ ጥናቱ ካሎት እባኮ በነጻንት ይጠይቁ፡፡

የጥናቱ ዋና አላማ ፡ የጥናቱ አላማ የተለያዩ የጨጋራ ህመሞች ምክንያት የሆነውን የኤች ፓይሎሪ ባክቴሪያ እና የሆድ ውስጥ ትላትሎች በአንደኛ ደርጃ ት/ቤት በልጆች ላይ ስላለው ስርጭትና ፡እና ምክንያትና መንሴዎችን ለይቶ ለማወቅ ነው፡፡ ይህም የሚረዳው እርሶዎ በሚሠጡት መልስ እና ከልጆሆ በሚገኘው መረጃና የላብራቶረ ውጤት ላይ በመንተራስ ለኤች ፓይሎሪ ባክቴሪያ እና ለሆድ ውስጥ ትላትሎች ስርጭት ምክንያት የሆኑትን መተላለፊያ መንገዶችን ለመግታት እንዲየስችል ነው፡፡

የጥናቱ ጊዜ፡ ጥናቱ በሚካሄድበት ት/ቤት የተማሪዎች ብዛት የሚወስን ሲሆን 5ወር እና ከዛም በላይ ሊወስድ ይችላል፡፡

የጥናቱ ሂደት፡ ለዚህ ጥናት እውን መሆን የእርሶዎን እና የተማሪውን ተሳትፎ እንፈልጋለን በዚህ ጥናት ለመሳተፍ ፈቃደኛ ከሆኑ የስምምነት ቅጹን መረዳትና መፈረምይጠበቅቦዎታል ከዛም ህብረተሰብ ነክ እና የህክምና መረጃዎች መጠይቁ ላይ ይሞላሉ ወይም ስለ ልጁ የሎትን መረጃ በመጠየቁ ላይ ይሞላሉ፡፡ናሙና የሚስበሰበው ከልጆት ሠገራ ላይ ነው ምርመራውም ወዲየሁኑ ወይም በነጋታው በትምህርት ቤቱ አቅራቢያው ካለው በስላም ፍሬ ጤና ጣቢያ ላብራቶሪ ይከናወናል፡፡

ሊከሰት ስለሚችሉ ስጋቶችና ምቾት መንገዶች ፡ለጥናቱ በሚወስደው ችግር ስጋት አይኖረውም ምክንያቱም የጥናቱ ናሙና አወሳሰድ የተለየ አይደለም ወይም ልጆትን ምንም አይነት ችግር አይፈጥረም ምክንያቱም ለምርመራው የምንጠቀመው ስገራ በመሆኑ እና ለመስጠት አስቸጋሪ ስላልሆነ፡፡

የተሳታፊዎች ጥቅሞች፡ በዚህ ጥናት በመሣተፎ ምንም አይነት ገቢ አያስገኝም ነገር ግን ባክቴሪያው እና የተለያዩ የሆድ ትላትሎች ያለባቸውን ተማሪዎችን የላብራቶሪ ውጤታቸውን ለቤተሰባቸው ወይም ለአሳዳጊ እናሳውቃለን፡፡ ከዚያም ከጤና ጣቢያ ገር ግንኙነት በመፍጠር እንዲታከሙ ማመድርግ ነው፡፡

ሚስጥራዊነት፡ለጥናቱ ተብለው የተስባስቡ የግልዎ እንዲውም የልጆዎ መረጃ ሚስጢራዊነቱ የተጠበቀ ነው ፡፡

ክፍያ፡ በዚህ ጥናት በመሳተፍዎ እርሶም ሆነ ልጆትም የሚያገኙት ምንም ልዩ ክፍያ የለም፡

የተሳታፊውመብት፡ የእርሶም ሆነ የልጆት ተሳትፎዎ ሙሉ በሙሉ በፈቃደኝንት ላይ የተመስረት ነው፡፡ፈቃደኛ ካልሆኑ በዚህ ጥናት ያለመሳተፍ መብትዎ የተጠበቀ ነው ፡፡

የጥናቱ ፈቃድ/ህጋዊነት፡- የዚህ ጥናት ህጋዊነት በዲፓርትመንታል ምርምር እና ስነምግባር ቅኝት ኮሚቴ ፤ አዲስ አበባ ዩኒቨርሲቲ፤ ኮሌጅ ኦፍ ሄልዝ ሳይንስ ስኩል ኦፍ አላይድ ጤና ሳይንስ እና በአዲስ አበባ ጤና ቢሮ ፡ ፈቃድ ያገኘ ነው፡፡

መረጃ ስለማግኘት ፡- ይህን ጥናት አስመልክቶ ምንም አይነት ጥያቄ ወይም ማብራሪያ ቢያስፈልግዎት ፡

1. አዲስ አበባ ዩኒቨርሲቲ፡ የጤና ሳይንስ ኮሌጅ፡የአላይድ ጤና ሳይንስ ት/ት ፡ ህክምና ላቦራቶሪ ሳይንስ ዲፓርትመንት

ሰ.ቁ ፡ +251 -112-75-51-70 ፋክስ ፡ +251-112-75-46-69

ኢ-ሜል ፡ SMLT@ethionet.etፒ.ኦ.ቦክስ ፡ 1176 ፡ አዲስ አበባ ፡ ኢትዮጵያ፣2017

2. **የጥናቱ ተመራማሪ አበበወርቁ (በአዲስ አበባ ዩኒቨርሲቲ የማስተር ተማሪ)**

ሰ.ቁ፡ +251 912685504

ኢ-ሜል ፡ workuabebe249@gmail.com

ፒ.ኦ.ቦክስ ፡ 36 ፡ አዲስ አበባ ፡ ኢትዮጵያ፣2017

Annex III. **English version of Consent form**

We are doing this research to find out more about the Prevalence of *H. pylori*, intestinal parasite and associated risk factors among Elementary school children; the choice that you have to take part in it is up to you. We would still take good care of you no matter what you decide. We want you to ask us any questions that you have any time. If you decide to be in the research, you may need a clean stool container for sample so we could test some of your child stool sample. We would ask you to read questions on a piece of paper or listen when read for you. Then you would mark your answers on the paper or tell your answer. A person on the research team would ask you questions. Then you would say your answers out loud. Some of the questions might be hard to answer. You can say 'no' to what we ask you to do for the research at any time and we will stop. This research will not help you and you would not be paid. But we hope it will help your child and other children. It is also OK to say yes and change your mind later. You can stop being in the research at any time. If you want to stop, please tell the researcher. Take the time you need to make your choice. If you want to be in the research after we talk, please sign below.

We will write our name too. This shows we talked about the research and that you want to take part. I _____ hereby give my consent for giving of the requested information and allowing my child participation for this study.

Participant code_____Signature_____date_____

Annex IV. Amharic version of the consent form

ቅፅ1 **የፍቃደኝነት መጠየቂያ**

ይህ ጥናት በኤች ፓይሎሪ ባክቴሪያ እና በሆድ ውስጥ ትላትሎች ህመምን በአንደኛ ደርጃ ት/ቤት በሉ ልጆች ላይ ያለው ስርጭት ለማየትና የዳሰሳ ጥናት ለማካሄድ የሚገለጽ ሲሆን መሳተፍ ከፈለግህ/ሽ ምርጫው ባንተ/ች የሚወሰን በማንኛውም ሰአት ማንኛውንም ጥያቄ መጠየቅ ትችላለህ/ሽ።በጥናቱ ለመሳተፍ ከወሰነክ/ሽ ለምርመራ ጥቂት የስገራ ናሙና ከልጆ ይወሰዳል ወይም መስጠት ይጠይቃል።ወረቀቱ ላይ ለሰፈሩት ጥያቄዎች አንብበህ/ሽ ተገቢውን ምላሽ ወረቀቱ ላይ ሙላ/ይ ወይም እንዲነበብ በመጠየቅ ተገቢውን ምላሽ መስጠት ይጠበቅቦታል ከጥናት ቡድኑ አባል ለሚጠይቅህ/ሽ ድምፅህን/ሽን ከፍ አድርገህ/ሽ መልስ ስጥ በማንኛውም ሰአት በጥናቱ አልሳተፍም የማለት መብት አለህ/ሽ።በዚህ ጥናት ምንም አይነት የገንዘብ ክፍያና የተለየ ጥቅም አታገኝም/ኚም ሆኖም ግን በዚህ የጥናት ዉጤት ልጆትን እና ሌሎች በዚህ እድሜ ክልል ላሉ ልጆች ይጠቅማል።

በማንኛውም ሰአት ጥናቱን ማቆም ትችላለህ/ያለሽ።ምርጫህን/ሽን ለማሳወቅ ጊዜ ወስደህ/ሽ አስብበት።በመጨረሻም ለመሳተፍ ከወሰንክ/ሺ ስምና ፊርማህን/ሽን ከታች ባለው ክፍት ቦታ ላይ አስፍር/ሪ።

እኔ ---------- የተባልኩ ግለሰብ ይህን ሁሉ በመገንዘብ በምርምሩ ላይ መረጃና የልጄ የስገራ ናሙና እንዲወሰድ ተስማምቻለሁ።

ፊርማ---------- ቀን----------

Annex V. English version of Assent form

I have been informed about the research which will be conducted in Addis Ababa, in Akaki Kality Sub City in Selam Fire Elementary School which plans to determine the Prevalence of *H. pylori*, intestinal parasite, and Associated Risk factor of among Elementary school children. The objective and the application of the study were briefly explained to me. Moreover, I have been well informed of my right to refuse information, decline to cooperate and drop out of the study if I want. It is therefore with full understanding of the situation that I agreed to give the informed assent voluntarily to the researcher for my child to give stool sample for the mentioned study. I agreed that the specimen will be tested for *H. pylori* stool antigen test and intestinal parasite. I have had the opportunity to ask questions about the project and received clarification to my satisfaction in a language I understand. I have been also informed that results for the analysis of stool will be given with free of charge whenever I came with unique code number, to those with a positive finding they will be liked to health center for treatment.

I _____ hereby give my assent for giving of the requested information and my child's stool for this study.

Participant code: _____ Signature: _____ Date: _____

Annex VI. Amharic version of the Assent form

ቅጽ 2 የፈቃደኝነት ማረጋገጫ (ለቤተሰብ/አሳዳጊ)

በአዲስ አበባ ከተማ በአቃቂ ቃሊቲ ክፍለ ከተማ በስላም ፍሬ አንደኛ ደረጃ ት/ቤት ላይ የኤች ፓይሎሪ ባክቴሪያና የሆድ ውስጥ ትላትሎች ህመምን ስርጭትና የበሽታው አጋላጭ ሁኔታዎችን ለማጥናት በሚል ርእስ ላይ በሚደረገው ጥናት ላይ ለመሳተፍ ሲሆን፤ የጥናቱ አላማና ጥቅም በሚገባ ተገልፆልኛል፡፡በመጠይቁ ላይ የሚሞላው የኔ ሙሉ መረጃም በሚስጥር እንደሚያዝ ተነግሮኛል፡፡

በተጨማሪም በጥናቱ ውስጥ ልጄን አለማሳተፍ መብቴ እንደሆነና በማንኛውም ጊዜ ከጥናቱ በራሴ ውሳኔ መውጣት እንደምችል በሚገባ ተረድቻለሁ፡፡

ስለሆነም ሁኔታውን በሚገባ በማጤን በፍቃደኝነት ልጄን በምርምሩ ላይ ለማሳተፍ ለተመራማሪው ፍቃደኝነቴን ሰጥቻለሁ፡፡በተጨማሪም ልጄ የሚሰጠው የስገራ ናሙና ለተጠቀሰው ጥናት ብቻ እንደሚውል ተነግሮኝ ተስማምቻለው፡፡ማንኛውም ያልገባኝን ነገር የመጠየቅ እድል ተሰጥቶኝ በሚገባኝ ቋንቋ መልስ አግኝቻለሁ፡፡

በተጨማሪም የላብራቶሪ ምርመራ ውጤት ምንም አይነት ክፍያ ሣልጠየቅ በነጻ እንደሚስጠኝና ነገር ግን ከእኔ የሚጠበቀው በወቅቱ መለያ ቁጥር ይዞ መቅረብ ብቻ እንደሆነ ተነግሮኛል፡፡ከዚያም የጨጋራ ባክቴሪያ እና የሆድ ውስጥ ትላትሎች ላለባቸው ልጆችን ከጤና ጣቢያ ገር ግንኙነት በመፍጠር እንዲታከሙ እንደሚያደርጉ ነው፡፡

እኔ ---------- የተባልኩ ግለሰብ ይህን ሁሉ በመገንዘብ በምርምሩ ላይ ስለልጄ መረጃና የስገራ ናሙና እንዲወሰድ/እንዲሰጥ ተስማምቻለሁ፡፡

Annex VII . English version of Questionnaire

Addis Ababa University Collage of Health Sciences, School of Allied Health Science
Department of Medical Laboratory Science.

Questionnaires: for the demographic characteristics, assessment of risk factors for *H. pylori*, intestinal parasitic for students, who were learning at Elementary School:

Participant Identification

School name _____ Year _____ Participant code no: _____

Participants address (Sub city) _____ Telephone _____ signature_____

Grade _____Block_____

Data collector name _____date _____signature_____

Demography of the child

Part one: - For Children from KG level to grade eight

1. Age in yr. _____
2. Weight in kg._____
3. Height. _____
4. MUAC_____
5. Sex? 1. Male 2. Female

6. Residence of the children?

   1. Rural 2. Urban

7. Shoe wearing habit?

   1. Sometimes 2. Always 3. Not at all

8. Does the child have a habit of washing hands after using toilet?

   1. Sometimes 2. Always 3. Not at all

Part two: - For Family/Guardian

1. Age in yr. _____
2. Sex?

   1. Male 2. Female

3. Residence of family /guardians?

   1. Rural 2. Urban

4. Does the child report abdominal pain more than 3 times per week?

   1. Yes 2. No

5. Does the students have taken / given a de-worming before the last six weeks?

   1. Yes 2. No

6. How much family /guardian monthly income?

1. <1000
2. 1000-1500
3. 2000-2500
4. 3000-3500
5. > 4000

7. How many bedrooms do you have in the house?

   1. 1-2

   2. 3-5

   3. ≥ 5 above

8. How many persons do live in the house?

   1. < 4
   2. 4-6
   3. >6

9. What is family or guardian level of education?
    1. Illiterate
    2. Read and write
    3. Primary school
    4. Secondary school
    5. above secondary school
10. What is your family/home drinking water Source?
    1. Tap/ bono water
    2. Bottled water
    3. Boiled tap water
    4. Mineral water
11. Do you have a Toilet in your house?

    1. Yes 2. No
12. What type of toilet used in your house?
    1. Pit latrine
    2. Flush toilet
    3. Open field

Annex VIII: Amharic version of Questionnaire

የጨንራ ህመምች ምክንያት የሆነውን የኤች ፓይሎሪ ባክቴሪያ እና የሆድ ውስጥ ትላትሎች በአንደኛ ደርጃ ት/ቤት በልጆች ላይ ስላለው ስርጭትና፡ አጋላጭ ምክንየትና መንሴዎችን ለይቶ ለማወቅ ለማጥናት የተዘጋጀ ቃለ መጠየቅ ።

የተሳትፎመለያ

የት/ቤቱ ስም_____ዓ/ም_____መላያ ቁጥር : _____

አድራሻ (ክፊለ ከተማ) _____ ስልክ ቁጥር _____ ፊርማ_____

የት/ ደርጃ _____ህንፃ ቁጥር _____

የመርጃ ስብሳቢ ስም _____ቀን _____ ፊርማ_____

ክፍል አንድ ፡- ከ KG — 8 ኛ ክፊል ላሉ ተማሪዎች

መሠረታዊመረጃ

1. እድሜ ----------
2. ክብደት ----------
3. ቁመት ----------
4. የክንድ መጠን ልኬት ----------
5. *ፆታ* 1. ወንድ 2. ሴት
6. *መኖሪያቦታ*?
    1. ገጠር
    2. ከተማ
7. ጫማየማድረግልምድአለው/ አላት?
    1. አንዳንዴ 2. ሁልጊዜ 3. አይ የላትም/ውም
8. መጸዳጃ ቤት ከተጠቀመ/ች በዋላእጅየመታጠብልምድአለው/አላት?
    1. አንዳንዴ 2. ሁልጊዜ 3. አይ የላትም/ውም

ክፍል ሁለት፡- ለቤተሠብ/ ለአሳዳጊዎች ;

1. እድሜ ----------
2. ፆታ 1. ወንድ 2. ሴት
3. መኖሪያቦታ?
    1. ገጠር
    2. ከተማ
4. ልጆት በሳምንት ከ ሶስት ጊዜ በላይ የሆድ ህመም ስሜት ይስማዋል?
    1. አዎ ይስማዋል 2. አይ አይስማውም
5. በት/ቤቱ የፀር ሆድ ትላትልመድሀኒትተስጥቶየውቃል?
    1. አዎ ያውቃል 2. አይ አያውቅም
6. የቤተሠብ/ የአሳዳጊ ወርሀዊ ገቢ?
    1. <1000
    2. 1000-1500
    3. 2000-2500
    4. 3000-3500
    5. > 4000
7. ስንት መኝታ ክፍል ቤት አለዎት ?
    1. 1-2
    2. 3-5
    3. ከ 5 በላይ
8. ምን ያህል ስው በቤት ውስጥ ይኖራል?
    1. < 4
    2. 4-6
    3. > 6

9. የአሳዳጊው /የወላጅ የትምህርት ደረጃ?

   1. ያልተማረ
   2. ማንበብና መጻፍ
   3. አንደኛ ደርጃ ትምህርት
   4. ሁለተኛ ደርጃ ትምህርት
   5. ከሁለተኛ ደርጃ ትምህረት በላይ

10. ቤተሠቡ ለመጠጥ የሚጠቀመው ውሃ አይነት?

   1. የቧንቧውሃ
   2. የታሸገ የፕላስቲ
   3. የፈላ የቧንቧ ውሃ
   4. የጉድጋድ /የከርስ ምድር ውሃ

11. በቤት ውስጥ የመጸዳጃ ቤት አሎት?

   1. አሥ አለኝ 2. አይ የለኝ

12. ቤተሠብ የሚጠቀመው የመጸዳጃ ቤት አይነት?

   1. ባለ ጉድጎድ ሽንት ቤት
   2. ውሃ መልቀቂያ ያለው ሽንት ቤት
   3. ሜዳ ላይ መጸዳዳት

Annex IX: Standard operational Procedure for Laboratory investigation

Well-trained laboratory technologist/technician was collected stool in order to ensure that appropriate stool specimen is obtained and quality control for *H. pylori* stool antigen test and intestinal parasite.

1. Standard operational procedures (SOP) for *Helicobacter pylori* Stool Antigen test

1.1 Purpose

The *H. pylori* Ag Rapid test is a lateral flow chromatographic immunoassay for the qualitative detection of *H. pylori* antigen in human faecal specimen. It is intended to be used by professionals as a screening test and as an aid in the diagnosis of infection with *H. pylori*. Any reactive specimen with the *H. pylori* Ag Rapid test must be confirmed with alternative testing method(s).

The *H. pylori* Ag Rapid test uses a colloid gold conjugated monoclonal anti-*H.Pylori* antibody and another monoclonal anti-*H. pylori* antibody to specifically detect *H. pylori* antigen present in the faecal specimen of an infected patient. The test is user friendly, accurate, and the result is available within 15 minutes.

1.2 Test Principle

The *H .pylori* Ag Rapid test is a sandwich lateral flow chromatographic immunoassay. The test strip consists of: a burgundy colored conjugate pad containing monoclonal anti- *H. pylori* antibody conjugated with colloid gold (anti-H.P conjugates) and a nitrocellulose membrane strip containing a test band (T band) and a control band (C band). The T band is pre-coated with another monoclonal *anti-H.P* antibody, and the C band is pre-coated with goat anti-mouse IgG antibody. When an adequate volume of extracted faecal specimen is dispensed into the sample well of the test cassette, the specimen migrates by capillary action across the cassette. *H. pylori* antigens if present in the specimen was bind to the anti-*H. Pylori* conjugate. The immune complex is then captured on the membrane by the pre-coated antibody, forming a burgundy colored T band, indicating an *H. pylori* positive test result. Absence of the T band suggests that the concentration of *H. pylori* antigens in the specimen is below the detectable level, indicating an *H. pylori* negative test result.

Reagents and Materials Provided

1. Individually sealed foil pouches containing:

- ✓ One cassette test device.
- ✓ One desiccant.

2. Sample extraction tubes, each containing 2ml of extraction buffer.

3. Plastic droppers for transferring watery stool.

4. One package inserts (instruction for use).

1.3 Test Procedure

1. Bring the specimen and test components to room temperature if refrigerated or frozen. Mix the specimen well prior to assay once thawed

2. When ready to test, open the pouch at the notch and remove the test strip. Place the strip on a clean, flat surface.

3. Fill the plastic dropper with the specimen. Holding the dropper vertically, dispense 1 drop (about 30-45 µL) of specimen into the sample pad making sure that there are no air bubbles. Then add 1 drop (about 35 – 50 µL) of Sample Diluents immediately and wait for 15 minutes

4. Set up timer

5. Results read within 15 minutes.

1.4 Test Quality Control

The test contains an internal control (C band) which should exhibit a burgundy colored band of the immune complex of goat anti-mouse IgG/mouse IgG-gold conjugate regardless of the color development on the T band. If the C band does not develop, the test result is invalid and the specimen must be retested with another device.

Interpretation of Assay Result of *H. pylori* test

1. Negative Result: If only the C band is developed, the test indicates that no detectable *H. pylori* antigen is present in the specimen. The result is negative.

2. Positive Result: If both C and T bands are developed, the test indicates the presence of *H. pylori* antigen in the specimen. The result is positive.

3. Invalid: If no C band is developed, the assay is invalid regardless of any color development on the T band as indicated below. Repeat the assay with a new test device. Excess faecal specimen can lead to invalid test results; if this is the cause, re-sample and re-test (see instructions for collection of specimen).

Safety precaution

Consider any materials of human origin as infectious and handle them using standard bio-safety procedures.

2. SOP for Direct stool examination

2.1 Purpose of the test

For detection and identification of parasites in wet mount preparation of stool.

2.2 Principle of the test

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.

2.3 Test Procedure

1. Place a drop of fresh physiological saline on one a slide, to avoid contaminating the fingers and stage of the microscope, do not use too large a drop of saline

2. Using a wire loop or piece of stick, mix a small amount of specimen, about 2 mg, (matchstick head amount) with the saline .Make smooth thin preparations. Cover preparation with a cover glass. Sample from different areas in and on the specimen or preferably mix the faeces before sampling to distribute evenly any parasites in the specimen. Do not use too much specimen otherwise the preparations will be too thick, making it difficult to detect and identify parasites.

3. Examine systematically the entire saline preparation for larvae, ciliates, helminthes eggs, cysts, and oocysts. Use the 10x objective with the condenser iris closed sufficiently to give good contrast. Use the 40x objective to assist in the detection and identification of eggs, cysts, and oocysts. Always examine several microscope fields with this objective before reporting 'No parasites found'.

4. Report the number of larvae and each species of egg found in the entire saline preparation.

3. SOP for Stool Sedimentation Concentration technique

3.1 Purpose of the test

Sedimentation methods (using centrifugation) lead to the recovery of all protozoa, oocysts, spores, eggs, and larvae present; however, the preparation contains more debris. If one technique is selected for routine use, the sedimentation procedure is recommended as being the easiest to perform and least subject to technical error.

3.2 Principle

By centrifugation, this concentration procedure leads to the recovery of all protozoa, eggs, and larvae present; however, the preparation contains more debris than is found with the flotation procedure. Ethyl acetate is used as an extractor of debris and fat from the feces and leaves the parasites at the bottom of the suspension.

The formol ether sedimentation concentration is recommended as being the easiest to perform, allows recovery of the broadest range of organisms, and is least subject to technical error.

3.3 Test Procedures

1. Using a rod or stick, emulsify an estimated 1g (pea size) of faeces in about 4 ml of 10% formol water contained in a screw cap bottle or tube from the surface and several places in the specimen.

2. Add a further 3–4 ml of 10% v/v formol water, cap the bottle, and mix well by shaking.

3. Sieve the emulsified faeces, collecting the sieved suspension in a beaker.

4. Transfer the suspension to a conical (centrifuge) tube made of strong glass, copolymer, or polypropylene. Add 3–4 ml of diethyl ether or ethyl acetate.

5. Stopper the tube and mix for 1 minute. If using a Vortex mixer leave the tube unstoppered and mix for about 15 seconds (it is best to use a boiling tube). * Do not use a rubber bung or a cap with a rubber liner because ether attacks rubber.

6. With a tissue or piece of cloth wrapped around the top of the tube, loosen the stopper (considerable pressure will have built up inside the tube).

7. Centrifuge immediately at 750–1 000 g (approx. 3000 rpm) for 1 minute. After centrifuging, the parasites will have sedimented to the bottom of the tube and the faecal debris will have collected in a layer between the ether and formol water

8. Using a stick or the stem of a plastic bulb pipette, loosen the layer of faecal debris from the side of the tube and invert the tube to discard the ether, faecal debris, and formol water.

9. Return the tube to its upright position and allow the fluid from the side of the tube to drain to the bottom. Tap the bottom of the tube to re-suspend and mix the sediment. Transfer the sediment to a slide, and cover with a cover glass.

10. Examine the preparation microscopically using the 10objective with the condenser iris closed sufficiently to give good contrast. Use the 40 objective to examine small cysts and eggs. To assist in the identification of cysts, run a small drop of iodine under the cover glass

3.4 Microscopic result interpretation

1. No ova of parasite seen, if there is no finding.

2. Examine systematically the entire saline preparation for larvae, ciliates, helminthes eggs, cysts, and oocysts.

Annex X: Data entry work sheet for participants' laboratory test results

Ro: no	Grade	Age	Sex	Weight	Height	MUAC	Helicobacter stool Ag test		Intestinal parasites	
							Positive	Negative	Formol-ether concentration methods	Direct microscopy (wet method)
001										
002										
003										
004										
005										
006										
007										
008										
009										
010										
011										
012										
013										
014										

Annex XI: Selection participants from various arms /grade would made by balloting/lottery method as described in the above. As summarized as follow:

Level of School	Total number of students enrolled		Chance of students to be selected is 35%		Total number of participant /students from each grade
Pre-school	Male	Female	Male	Female	
KG -1	30	36	10	12	22
KG -2	24	20	8	8	16
KG -3	38	36	13.	13	26
Total	92	92	31	32	**64**
Grade 1A	23	26	9	10	19
Grade 1B	15	36	8	13	21
Grade 2A	20	32	8	11	19
Grade 2B	21	28	8	10	18
Grade 3A	24	34	8	12	20
Grade 3B	24	31	8	12	20
Grade 4A	24	35	9	12	21
Grade 4B	33	25	12	9	21
Grade 5A	35	45	12	16	28
Grade 5B	37	43	13	15	28
Grade 6A	26	37	9	13	22
Grade 6B	30	34	10	12	22
Grade 7A	30	42	10	16	26
Grade 7B	30	45	10	16	26
Grade 8A	17	25	6	11	17
Grade 8B	17	25	6	10	16
Grade 8C	18	23	6	8	14
Sum Total	516	658	**186**	**289**	**422**

Annex XII: Declaration

As thesis advisor, I hereby certify that I have read and evaluated this thesis prepared under my guidance, by Abebe Worku ; entitled: 'Prevalence of *Helicobacter pylori* and intestinal parasite and their associated risk factors among school children at Selam Fire Elementary School in Akaki Kality, March to June 2017, Addis Ababa, Ethiopia

Abebe Worku (BSc) _____ _____

Principal investigator Signature Date

I recommend it to be submitted as fulfilling the thesis requirement.

Advisor	Signature	Date
Kassu Desta (MSc, PhD fellow	_____	_____
Mistire Wolde (MSc PhD)	_____	_____