

**PREVALENCE OF INTESTINAL PARASITIC INFECTIONS AND
THEIR ASSOCIATIONS WITH ANTHROPOMETRIC
MEASUREMENTS OF SCHOOL CHILDREN IN YESHIMEBET
PRIMARY SCHOOL, HARAR, HARARI REGION, ETHIOPIA**

MSc THESIS

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**Prevalence of Intestinal Parasitic Infections and their Associations with
Anthropometric Measurements of School children in Yeshimebet
Primary School, Harar, Harari Region, Ethiopia**

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Dedication

I dedicate this thesis to my family for their endless love, support, and encouragement during this study.

STATEMENT OF THE AUTHOR

By my signature below, I declare and affirm that this Thesis is my original work. I have followed all ethical and technical principles of scholarship in the preparation. Data collection, data analysis and compilation of this thesis. Any scholarly matter that is included in the thesis has been given recognition through citation.

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BIOGRAPHICAL SKETCH

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LIST OF ACRONMYS AND ABBREVIATIONS

BMI	Body Mass Index
GIT	Gastro Intestinal Tract
HAZ	Height-for-Age Z-score
HU	Haramaya University
IPI	Intestinal Parasitic Infection
NCCLS	National Committee on Clinical Laboratory Standard
NCHS	National Center for Health Statistics
SD	Standard Deviation
SSA	Sub-Saharan Africa
SPSS	Statistical Package for Social Science
STH	Soil Transmitted Helimenths
TDS	Trichuris Dysentery Syndrome
WAZ	Weight-for-Age Z-score
WHO	World Health Organization

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Prevalence of Intestinal Parasitic Infections and their Association with Anthropometric Measurements of School children in Yeshimebet Primary School, Harar, Harari Region, Ethiopia

ABSTRACT

Intestinal protozoan and helminthes parasite infections are the major public health problems in many developing countries including Ethiopia. The objective of the present study was to determine the prevalence of intestinal parasitic infections and their association with anthropometric measurements among school children in Yeshimebet

Primary School of Harari Region, Ethiopia. The study was a school based cross sectional survey, involving 422 participants (241 males and 181 females, age ranging from 10-18 years). Structured and pre-tested questionnaires were administered to gather relevant information on demographic data of the school children. The study was conducted based on written consent. Stool samples were processed for microscopic examinations using direct wet mount and formal-ether concentration methods. Weight and height were taken to assess body mass index (BMI) of each study participant. The data were analyzed using SPSS version 16 and anthropometry calculating software (Anthroplus). The overall prevalence of intestinal parasitic infections was 28.7 % (26.6% for males and 31.5% for females). The prevalence of intestinal protozoan parasite, Entamoeba histolytica and Giardia lamblia infections was 7.1% and 5.9%, respectively. Similarly the prevalence of helminth infections with, Hymenolepis nana, Hookworm and Enterobium vermicularis was 10.2%, 3.3% and 2.1%, respectively. The prevalence of intestinal parasitic infections was significantly associated with risk factors such as, washing hands before meal, education level and availability of latrine ($p= 0.000, p=0.000$ and $p=0.000$) . Most of the socio-demographic factors, family size, parents' occupation, source of water, washing hands after toilet and residence were not significantly associated with intestinal parasitic infections. The percentage of anthropometric indices of study participants was 59.5%, 1.9%, and 17.3% for underweight, stunting, and wasting, respectively. Underweight school-children (59.5%) had a high prevalence of parasitic infection as compared with other anthropometric indices (Wasting and Stunting). In general intestinal parasitic helminth infections represent a public health problem in the school children of Harar town.

Key words: Anthropometric measurement, Body mass index, Ethiopia, Harar, Intestinal parasite.

1. INTRODUCTION

Intestinal parasitic Infections (IPIs) constitute the greatest worldwide cause of disease. 3.5 billion individuals have been infected with intestinal parasites, of these 450 million individuals developed diseases (WHO, 2008 and Hall *et al.*, 2008). Intestinal 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 (Evans and Stephenson, 1995). In Africa, more specifically in the Sub-Saharan Africa (SSA), intestinal parasitic infections are the major public health problems and most of the victims are children (Hall *et al.*, 2008).

Intestinal parasites are found in the gastrointestinal tract of humans. Over 70 species of protozoan and helminths parasites can infect humans through food and water contamination. Intestinal parasites are more prevalent among the poor, who are negatively affected by low socio-economic conditions, poor personal and environmental hygiene, overcrowding and limited access to clean water (Amare *et al.*, 2007).

The common intestinal protozoan parasites of human are *Entamoeba histolytica/dispar*, *Giardia lamblia/intestinalis*, *Cryptosporidium parvum* and *Cyclospora cayetanensis*. Common parasitic helminths (worms) that infect humans are belong to two phyla, Platyhelminthes and Nematodes. These are subdivided into nematodes (roundworms), cestodes (tape worms) and trematodes or flukes (WHO, 2000).

The distribution of intestinal parasitic infection depends on many factors. These include geographic and socio-economic factors, such as climate, poverty, malnutrition, personal and community hygiene, population density, unavailability of drinking water and poor sanitary facilities (Nematian *et al.*, 2008).

The worldwide prevalence of intestinal infections caused by pathogenic protozoan species is also reported to be high. Amoebiasis is estimated to inflict severe disease in 48 million individuals around the globe (Petri and Singh, 1999). The global prevalence of giardiasis is suspected in millions of people. The most common intestinal parasite among

adults and child groups surveyed were *E. histolytica/dispar*, *G. intestinalis*, *A. lumbricoides* and *T. trichiura* (Eckmann and Gillin, 2001).

The most prevalent and important helminths in developing countries are the soil-transmitted helminths such as: roundworm, whipworm and hookworm. About 400 million school-age children around the world are infected while 500 to 600 million people are at risk of becoming infected (Lang, 2005).

Studies in children from rural as well as urban areas of the Kashmir valley, India, have reported that at least one intestinal helminth parasitic species was found in 71.2 % of the sampled population. The prevalence of *Ascaris lumbricoides* was highest (68.3%), followed by *Trichuris trichiura* (27.9%), *Enterobius vermicularis* (12.7%) and *Taenia saginata* (4.6%) (Wani *et al.*, 2008).

Many infections caused by intestinal parasites are cosmopolitan in distribution (Crompton, 2002). According to Mehraj *et al.* (2008), *A. lumbricoides* infects 1.221 billion people, *T. trichiura* 795 million, hookworms 740 million, *E. histolytica* 500 million and *G. lamblia* about 200 million people worldwide. The greatest number of intestinal parasitic infections occur in tropical and subtropical regions of Asia, especially China, India and Southeast Asia, as well as Sub-Saharan Africa (de Silva *et al.*, 2013).

According to Teklehaimanot *et al.* (1998), younger children are more susceptible to *A. lumbricoides* infection than older children. It could be transmitted through the contamination of the environment, especially the soil where the children usually play in the open fields and eat food without washing hands. Thus, as age increases exposure to intestinal nematode infection decreases possibly due to improved personal hygiene. Intestinal parasitic infections also cause serious public health problems in Ethiopia. Moreover, a number of surveys have shown that intestinal parasites are prevalent in varying magnitudes (Berhanu and Girmay, 2003). The prevalence of hookworm, *A.*

lumbricoides, *T. trichiura*, *H. nana*, and *Taenia spp* were estimated to be 16%, 37%, 30%, 28.3% and 22.3%, respectively (Tadesse *et al.*, 2008). The distribution of IPI varies by place and with age (Belayhun *et al.*, 2010). WHO also indicated that the prevalence of helminth infections in Ethiopia ranges from 31-57.8% (WHO, 2003 as cited in Borkow and Bentwich, 2004). The prevalence of Hookworm, *G. lamblia* and *E. histolytica* in South Gonder were 23.6%, 22.8% and 21.6%, respectively (Mulat *et al.*, 2013).

School children in many developing countries including Ethiopia harbor the most intense infections with those intestinal protozoan parasites and helminths (Warren *et al.*, 1993). Therefore, treatment of this age group which is easily accessible through the school system achieves optimal improvements in health status and educational performance of school age children. However, the role of intestinal parasitic protozoan and helminths in causing morbidity and mortality and their pathogenesis differ from species to species. Similarly, the distribution and prevalence of various species of intestinal parasites also differ from region to region because of several environmental, socio economic and geographical factors. Hence, study on the prevalence of various intestinal parasitic infections is a prerequisite not only for formulation of appropriate control strategies but also to predict risk for communities under consideration (Nasiri *et al.*, 2009).

In Ethiopia, intestinal parasitic infections are major public health problems. Therefore, it was very crucial to know the distribution of intestinal parasitic infections and its association with anthropometric measurements of children. Since there was no previous report on the prevalence of intestinal parasitic infections in the present study area, this research was undertaken to determine the prevalence of intestinal parasitic infections in relation to anthropometric measurements among school children of selected primary schools in Harar town, Harari Regional State, Eastern Ethiopia.

General Objective

- The main objective of this study was to determine the prevalence of intestinal parasitic infections and their associations with anthropometric measurements of school children in Yeshimet Primary School of Harar town, Harari Regional State, Eastern Ethiopia.

Specific Objectives:

1. To determine the prevalence of intestinal parasitic infections among school children in the study population
2. To identify the major protozoan and helminth intestinal parasitic species of school children in the study population.
3. To analyze the associate of intestinal parasitic infection with anthropometric measurements of school children in the study population.

2. LITERATURE REVIEW

2.1. Human Intestinal Parasitic Species

Intestinal parasitic infections (IPI) are global health problems causing clinical illness in 450 million inhabitants in developing countries (Quihui *et al.*, 2006). Parasites found in the intestine can be categorized into two groups; as protozoan and helminths. The major intestinal parasites of global public health concern are the protozoan species such as *E. histolytica* and *G. intestinalis*, soil transmitted helminthes *A. lumbricoides*, *T. trichiura*, hookworm and schistosomiasis (WHO, 2000). Helminthic infections are enhanced by poor socio-economic conditions, lack of sanitary facilities, improper disposal of human feces, insufficient supplies of potable water, poor personal hygiene, poor housing conditions and lack of education (WHO, 1996).

The level of harm caused by intestinal parasite infection to the health of individual and communities depend on: the parasite species, the nature of the interaction between the parasite and the concurrent infections, the intensity and course of infection and nutritional and immunological status of the population (WHO, 2007; Hamdan *et al.*, 2010). IPI and helminths in particular, are associated with increased risks for nutritional anemia, protein energy malnutrition and growth retardation in children, poor increase in body weight in pregnancy, intrauterine growth retardation, and low birth weight (Rodriguez-Morales *et al.*, 2006). Children infected with soil-transmitted helminths (STHs) have poor educational

level and performance at school and a high level of truancy, thus impacting on their future earnings and productivity (Miguel, 2004; Hotez, 2004).

2.1.1. Human Intestinal Protozoan Parasitic Species

The common protozoan parasites include *Entamoeba histolytica/dispar*, *Giardia lamblia/intestinalis*, *Cryptosporidium* and *Cyclospora species*. Even though the majority of protozoa occur as free living organisms in the soil, moist, marine or fresh water environments, a substantial number also exists as mutual, commensal or parasite. Protozoan parasites are known to affect all species of vertebrates and many invertebrates. They are able to adapt to life in virtually all body sites of their hosts (Neva and Brown, 2012). *Entamoeba histolytica/dispar* is an intestinal parasite that characterized by possessing clear protoplasm which form pseudopodia. These pseudopodia are the means by which the organisms move and use for feeding purposes. The two species *E. histolytica* and *E. dispar* are morphologically identical but pathologically distinct (WHO, 2013). *E. histolytica* is the pathogenic genus that causes colitis and liver abscess in the tropics. Amebiasis is spread widely from pole to pole Infection usually occurs by ingestion of water or food contaminated by faecal matter, but most infections occur due to the non-invasive species (Stanley, 2003).

Giardia lamblia/intestinalis (also known as *Giardia duodenalis*) is a unicellular flagellated intestinal protozoan parasite of humans isolated worldwide and ranked among the top 10 parasites of man, causing giardiasis (Fayer *et al*, 2000). The parasite attaches to the epithelium by a ventral adhesive disc, and reproduces via binary fission. Giardiasis does not spread via the bloodstream, nor does it spread to other parts of the gastrointestinal tract, but remains confined to the lumen of the small intestine (Harrison, 2008). *Giardia* trophozoites absorb their nutrients from the lumen of the small intestine, and are anaerobes. If the organism is split and stained, its characteristic pattern resembles

the familiar "smiley face" symbol. Chief pathways of human infection include ingestion of untreated sewage, a phenomenon particularly common in many developing countries; contamination of natural waters also occurs in watersheds where intensive grazing occurs (Hogan *et al.*, 2010).

Cryptosporidium species are very small intestinal protozoa. They dwell in the stomach or in the small intestine of mammals, birds and reptiles. This apicomplexa parasite infects humans and animals globally. Up to now eight valid *Cryptosporidium* species have been reported to be capable of infecting humans (Rimhanen-Finne, 2006). It can cause gastrointestinal illness in a wide variety of mammals, including humans, cattle, sheep, goats, pigs and horses Worldwide (Fayer, 1997).

Cyclospora cayetanensis is the only species found in humans, and it is apparently restricted to this host. *Cyclospora cayetanensis* originally referred to as "cyanobacterium-like bodies" has been recognized as a waterborne pathogen and reclassified (Bendall, 1993; Ortega, 1993). It has been associated with several waterborne outbreaks worldwide.

2.1.1.1. The Life Cycle of Human Intestinal Protozoan Parasitic Species

There are two diagnostic life cycle stages commonly seen in parasites, the cysts and the adult trophozoite stage. The trophozoite stage can be detected directly on a slide without concentration. Cysts require concentration (Petri and Singh, 1999). The life cycle of *E. histolytica* includes the motile and invasive trophozoite and the infective cyst. Infection is acquired primarily through the ingestion of infective cyst forms present in fecally contaminated water and food (Petri and Singh, 1999). The trophozoite measures 10-50 micro meter and contains a single nucleus; whereas, the cyst is 10-15 micrometer in diameter and contains four nuclei. *E. histolytica* cysts are resistant to gastric juices present

in the human stomach, chlorination desiccation and capable of surviving in a moist environment for several weeks (Neva and Brown, 1994).

After ingestion of contaminated water or food, the cyst wall is dissolved in the upper gastrointestinal tract and the organism excysts with in the lumen of the small intestine. During excystation, nuclear division is followed by cytoplasm division, giving rise to eight uninucleated trophozoites. Trophozoite *E. histolytica* epithelial cells line the gastrointestinal tract. Once penetration of the intestinal mucosa is achieved, dissemination to other organs, extra intestinal infections, usually the liver, can occur. Trophozoites which dwell in the colon multiply encyst and are passed in the stool from where further spread is possible (Clark *et al.*,2000).

Giardia lamblia reproduces by binary fission. This is a type of reproduction in which one cell divides into two new cells by mitosis during the growth cycle. The components of the cell multiply so that each daughter cell is a complete copy of the parent cell. This parasite has a simple direct life cycle consisting of an infective cyst and a vegetative trophozoite. The cyst of *G. lamblia* is elliptically shaped and contains two to four nuclei (Heresi and Cleary, 2006).

The round or oval shaped cysts, which are the infective form of the protozoa, are approximately 11-14µm long and 7-10µm wide (Garcia, 1999). After ingestion, the cysts pass unharmed by gastric acid through the stomach to the small intestine. Excystation normally occurs in the duodenum. Infection with *G. lamblia* is usually confined to the upper small intestine but also has been observed in the bile duct and gall bladder of ill patients. The structure of the cyst makes the organism very resistant to environmental factors and disinfection and it is the transmittable form that causes the infection. Identification of the parasite is usually made by microscopic examination of direct fecal smear for either trophozoites or cysts in the feces (Neva and Brown, 2004).

The life cycle of *Cryptosporidium* is monoxeous. Its life cycle is short and the infection may be short lived or may be persistent for months completed within the gastrointestinal tract of a single host (Fayeret *et al.*, 2000). Subsequent to oocyst ingestion and activation in the upper GI tract, the organism's excyst to release sporozoites. The oocyst is spherical in shape measuring 3-6µm in diameter and it may be either thick or thin walled (Ramirez *et al.*, 2004;Abhay *et al.*, 2009). The resistant stage that is found usually in the environment is the thick walled oocyst excreted together with feces (Fayeret *et al.*, 2000).

The life cycle of *Cyclospora* is unknown; however environmental data suggest that *Cyclospora*, like *Cryptosporidium* species, is a water-borne parasite. The oocysts of *C.cayetanensis* are spherical, measuring 8-10µm in diameter and the mature oocyst contains 2 sporocysts. Oocysts of *Cyclospora cayetanensis*, are twice as large in comparison with *C.parvum* and are not sporulated (do not contain sporocysts - upon excretion).When freshly passed in stools, the oocyst is not infective thus, direct fecal-oral transmission cannot occur; this differentiates *Cyclospora* from another important coccidian parasite, *Cryptosporidium* (Markell *et al.*, 2000).

In the environment, sporulation occurs after days or weeks at temperatures between 22°C to 32°C, resulting in division of the sporont into two sporocysts, each containing two elongate sporozoites. The oocysts excyst in the gastrointestinal tract, freeing the sporozoites which invade the epithelial cells of the small intestine. Inside the cells they undergo asexual multiplication and sexual development to mature into oocysts, which will be shed in stools. The potential mechanisms of contamination of food and water are still under investigation (Markell *et al.*, 2000).

2.1.2. Human Intestinal Helminthic Parasite Species

Parasitic helminthes (worms) that infect humans belong to two phyla, Platyhelminths and Nematodes. The common intestinal helminthes are trematodes (flukes) includes *Schistosomia mansoni*), nematodes (round worms) includes *Ascaris lumbricoide*, *Trichuris trichiura* and hook worms (*Necator americanus* and *Ancylostoma duodenale*) and cestodes (tape worms) includes *Hymenolepis nana*, *Taenia saginata* and *Taenia solium*. The major soil-transmitted helminthes considered to be of global public health concern are *A. lumbricoides*, *T. trichiura*, and Hook worm among others (Awasti *et al.*, 2003). Helminthic infections are enhanced by poor socio-economic conditions, lack of sanitary facilities, improper disposal of human feces, insufficient supplies of potable water, poor personal hygiene, poor housing conditions and lack of education (WHO, 2007).

Helminthes infection has been linked with an increased risk for several nutritional, protein-energy malnutrition and reduced physical growth and development in infants and children (Stephenson *et al.*, 2000). Over one billion of the world's population is estimated to be infected with these parasites: two billion are at risk (Montresor *et al.*, 1998). Children are reported to be at especially increased risk for severe infections and the morbidity and mortality associated with these (Bethony *et al.*, 2006).

Trematodes: trematodes (flukes) are leaf shaped with an outer cover called the tegument which may be smooth or spiny. Most trematodes are hermaphroditic and most of the body consists of reproductive organs and their associated structures. Schistosomiasis is chronic water related parasitic disease caused by blood flukes of the genus *Schistosoma*. It is the most important disease in terms of its public health and socio economic impact next to malaria, and is still a major helminthes infection at the beginning of the 21st century in

many developing countries of the tropics. The disease is endemic in 74 tropical developing countries (George and Mohb, 2000). People become infected when coming in contact with water containing schistosome infected snails.

Nematodes: nematodes (round worms) are non-segmented helminthes, relatively simple structured organisms. They possess bilateral symmetry and a complete digestive tract with 11 oral and anal openings; they taper to a relative point at both ends. They are also found to have separate sexes, with the male being smaller than the female, ranging in size from a few millimeters to over a meter in length. Nematodes infections have a wide spread distribution being found in both temperate and tropical climates. They can be found in fresh water, in the sea and in soil. About 85% of Nematodes infections are asymptomatic (Arfaa, 1994).

Ascaris lumbricoides (roundworms) are dioecious, with male and female organs in separate individuals, and have a direct life cycle (no intermediate hosts). It is characterized by great size. Males are 2–4 mm in diameter and 15–31 cm long. The males' posterior end is curved ventrally and has a bluntly pointed tail. Females are 3–6 mm wide and 20–49 cm long. The vulva is located in the anterior end and accounts for about a third of its body length. Uteri may contain up to 27 million eggs at a time with 200,000 being laid per day. Fertilized eggs are oval to round in shape and are 45-75 μm long and 35-50 μm wide with a thick outer shell. Unfertilized eggs measure 88-94 μm long and 44 μm wide (Roberts *et al.*, 2009).

A. lumbricoides is a strong parasite. This quality is due, in part, to the resilient nature of its eggs, which are capable of surviving a wide range of hot and cold temperatures,

chemicals, and other extreme conditions (O’Lorcain and Holland, 2000). The eggs of *Ascaris* are one of the most resilient of the helminthes eggs and can remain infective for years embedded in the soil (Gilgen and Mascie-Taylor, 2000).

A. lumbricoides infection is acquired through the ingestion of infective eggs from fecally contaminated food or water. Since the eggs are very sticky, they readily adhere to raw finites and vegetables, which are washed with contaminated water endemic areas. *Ascaris* eggs may be found on eating or cooking utensils, or under the finger nails. They also may circulate in household dust and air where they are inhaled or swallowed (O’Lorcain and Holland, 2000).

Trichuris trichiura is commonly known as whip worm, due to the whip-like form of the body. *T. trichiura* infection is estimated to affect around 1049 million persons worldwide. Of these, 144 million are children of pre-school age and 233 million are of school age. These nematodes are most commonly seen in tropical climates and in areas where sanitation is poor (Bethony *et al.*, 2006).

Necator americanus and *Ancylostoma duodenale* are the two major species of hookworm known to infect humans. Hookworms are estimated to infect 151 million people worldwide and cause mortality in another 65.000. Mature hookworms typically inhabit the jejunum where they attach to the intestinal mucosa with their ventral teeth (Hotez *et al.*, 2004). The worms derive their nourishment by feeding on the villous tissue and sucking blood at the point of attachment (Gilgen and Mascie-Taylor, 2000).

Hookworm infection is acquired by invasion of the infective larval stages through the skin. *A. duodenale* larvae are also orally infective. Following host entry, the larvae undergo a journey through the vasculature, then the lungs and other tissues, before they enter the

gastrointestinal tract and molt twice to become one-centimeter-long adult male and female worms (Hotez *et al.*, 2004).

Cestodes: cestodes are tapeworm, specialized flatworms, looking very much like a narrow piece of adhesive tape. Tapeworms are the largest, and among the oldest, of the intestinal parasites that have plagued humans and other animals since time began. The most important cestodes affecting humans and animals in Ethiopia are *Taenia saginata*, and *Hymenolepis nana*, the former due to the custom of eating raw meat and the later due to unhygienic foodconsumption with contaminated hands and fingers that allow the ingestion of eggs from the faeces of an infected person (Belete and Kloos, 2006).

The adults of *Taenia saginata* and *Taenia solium* live in the intestine and are very large worms, i.e. several meters in length. Proglottids as well as eggs appear in faeces. The eggs of the two species are identical; they are round to oval in shape, measuring 35-43 μm in diameter and have a thick, radially-striated shell. The egg contains a 6-hooked embryo called an oncosphere or hexacanth. These eggs must be handled with extreme care because the egg of *Taenia solium* is infective to humans and produces cysticercosis (WHO, 2004).

The adult worm of *Hymenolepis nana* found in the intestine. It is very small, only a few centimeters long. The egg is unique in its appearance. It is small, measuring 30-47 μm in diameter with a thin, colorless shell. The membrane surrounding the hexacanth embryo has 4-8 filaments arising from each pole that fill much of the space between the embryo and the shell (WHO, 2004).

2.1.2.1. Life Cycle of Human Intestinal Parasitic Helminthes

The life cycles of most helminthes follow the same pattern (Stephenson *et al.*, 1989). Adult worms of genera *Necator americanus* and *Ancylostoma duodenale* parasitise the upper part of the human small intestine, while roundworms parasitise the entire small intestine. Adult *trichuris* (whipworms) live in the large intestine, especially the caecum (Despommier *et al.*, 2005). The parasites can live for several years in the human gastrointestinal tract. Human beings are regarded as the only major definitive host for these parasites, although in some cases *ascaris* infections can also be acquired from pigs (Crompton, 2001).

The soil-transmitted helminthes vary greatly in size, and female worms are larger than males. After mating, each adult female produces thousands of eggs per day, which leave the body in the feces. People become infected with *T. trichiura* and *A. lumbricoides* by ingesting the fully developed eggs (Despommier *et al.*, 2005).

Ascaris lumbricoides infections in humans occur when an ingested infective egg releases a larval worm that penetrates the wall of the duodenum and enters the blood stream. From here, it is carried to the liver and heart, and enters pulmonary circulation to break free in the alveoli, where it grows and molts. In 3 weeks, the larvae pass from the respiratory system to be coughed up, swallowed, and thus returned to the small intestine, where they mature to adult male and female worms. Fertilization can now occur and the female produces as many as 200,000 eggs per day for a year. These fertilized eggs become infectious after two weeks in soil; they can persist in soil for 10 years or more (Murray *et al.*, 2005).

N. americanus and *A. duodenale* hookworm eggs hatch in soil. The larvae moult twice to become infective third-stage larvae, which are non-feeding but motile organisms that seek out higher ground to improve the chance of contact with human skin. After skin penetration, they enter subcutaneous venules and lymphatic vessels to access the host's afferent circulation. Ultimately, the larvae become trapped in pulmonary capillaries, enter the lungs, pass over the epiglottis, and migrate into the gastrointestinal tract (Hotez. *et al.*, 2004). About 5–9 weeks are needed from skin penetration until development of egg-laying adults. *A. duodenale* larvae are also orally infective, and lactogenic transmission during breast feeding has been postulated. Soil-transmitted helminths do not reproduce within the host. This feature is crucial for understanding of the epidemiology and clinical features of soil-transmitted helminthes infections, as well as the approaches to their control (Hotez. *et al.*, 2004).

Infection with *Trichuris* occurs via the oral-fecal route by the ingestion of infective eggs from contaminated food, hand or water. These then pass through the stomach to the small intestine where they hatch. The larvae penetrate the cell of the small intestine coming to lie above the lumina to undergo four molts. The immature adults emerge and are passively transported to the large intestine where they mature and embed their thin whip-like anterior into columnar cell. The adult whipworms develop within 60-90 days after initial infection (Stephenson *et al.*, 2000).

2.2. Major Clinical Symptoms of Human Intestinal Parasitic Infections

Clinical signs and symptoms of intestinal protozoan infection include diarrhea, epigastric pain, wasting and impaired absorption of vitamin B12. When these symptoms occur, there is an incubation period of 3-25 days, followed by acute onset of watery, sometimes

explosive diarrhea without blood, bloating, and abdominal pain, which last for 3-4 days in acute infection. In most individuals the infection with *Giardia* is self-limiting, but chronic infection can occur, resulting in similar but intermittent signs for months (Glaas, 2010). Since Symptoms of soil-transmitted helminthes infections are different for each but, in general they are characterizing by hemorrhages, deficient in blood coagulation and undernourishment. Soil transmitted helminthes infections can degenerate into cancer tumors. The location and burden of the worm mostly determine the type and degree of morbidity observed in the host (Awolaju and Morenikeji, 2009).

During the migratory phase of *Ascaris*, large number of larvae may induce allergic reactions and host sensitization resulting in asthma, coughing, and shortness of breath, fever, skin rash and eosinophilia. Mature worms in the small intestine cause clinical signs and symptoms such as abdominal pain and distention, nausea, vomiting and diarrhea (O'Lorcain and Holland, 2000).

The clinical spectrum of *trichuriasis* varies from asymptomatic infection to *Trichuris* dysentery Syndrome (TDS), which is characterized by chronic mucous-bloody *trichuriasis*. Blood loss occurs from both the feeding activities of the parasites and extensive damage to the colonic mucosa (Crompton, 2000; Gillespie, 2001). Rectal bleeding and prolapse are also associated with moderate infection include epigastric and lower abdominal pain, vomiting, diarrhea, flatulence and weight loss. In severe infections, the worm may be observed embedded in the edematous rectal mucosa accompanied by moderate eosinophilia. Furthermore, growth stunting associated with decreased collagen synthesis has been reported in children (Stephenson *et al.*, 2000). The most severe manifestation of heavy infection among children is the TDS, which is associated with chronic dysentery, rectal prolapse, anemia, and growth stunting (Stephenson *et al.*, 2000). Clinical sign and symptoms of hookworm include pneumonia in heavily infected children

during larval migration and epigastric pain and iron deficiency anemia during the intestinal phase of the infection (Awolaju and Morenikeji, 2009; Crompton, 2000).

2.3. Transmission of Human Intestinal Parasitic Infection

The main transmission route for most intestinal parasites is fecal–oral. Protozoan parasites, such as *Giardia lamblia*, *Entamoeba histolytica* and helminthic parasites, including *A.lumbricoides*, hookworms and whip worm (*Trichuris trichiura*), are transmitted via contaminated water and food (Chan *et al.*, 1994).

Over 70 species of protozoan and helminths parasites can infect humans through food and water contamination. Intestinal parasites are more prevalent among the poor, who are negatively affected by low socio–economic conditions, poor personal and environmental hygiene, overcrowding and limited access to clean water (Amare *et al.*, 2007).

2.4. Epidemiological Distribution of Human Intestinal Parasitic Infetion

2.4.1. Global Distribution of Human Intestinal Parasitic Infections

Intestinal protozoan and helminth parasite infections are widely distributed throughout the tropics and subtropics. Climate is an important determinant of transmission of these infections, with adequate moisture and warm temperature essential for larval development in the soil (Brooker and Michael, 2009 and Brooker *et al.*, 2006). Equally important determinants are poverty and inadequate water supplies and sanitation (de Silva *et al.*, 2003). In such conditions, protozoa and helminth species are commonly co – endemic.

There is evidence that individuals with many intestinal parasitic infections have even heavier infections with protozoa and helminth parasitic infections (Raso *et al.*, 2013). A study estimated that *Ascaris lumbricoides* infects 1,221 million people, *Trichuris trichiura* 795 million, hookworms infect 740 million and about 200 million peoples were infected by *G. lamblia* (Brooker *et al.*, 2006). Approximately, 85% of the Neglected Tropical

Diseases (NTDs) burden results from protozoa and helminth parasite infections (Hotez and Kamath, 2009). Moreover, it has been estimated that intestinal helminth parasite infections account for 12% of the total disease burden and about 20% of disability adjusted life years (DALYs) lost due to communicable diseases in children, globally (Awasthi *et al.*, 2010).

2.4.2. Major Human Intestinal Parasitic Infections in Ethiopia

As a result of low level standards of living, poor environmental sanitations and ignorance of simple health promoting factors, intestinal parasitism is very high. Even though the prevalence of individual parasite varies in different parts of the country, *Ascaris lumbricoides* is the most prevalent intestinal parasite. Many reports in Ethiopia indicated that the most prevalent soil transmitted helminthes are *A. lumbricoides* followed by *Trichuris trichiura* (Shibru and Teklemariam, 1986).

Reports from different parts of Ethiopia showed different prevalence rate of the intestinal protozoan parasites. The prevalence of *Cryptosporidium* infection in children with diarrhea ranged from 3.3% in Jimma, 5.6% in Addis Ababa to 9% in Northwestern Ethiopia (Mengistu *et al.*, 2007). In a study conducted in South Western Ethiopia, the prevalence of Giardiasis was 13.7% though the rate is much lower than *A. lumbricoides* (Mulat *et al.*, 2013). Intestinal protozoan parasites including Giardia, Cryptosporidium and amoeba are widely distributed in the country (Karanis *et al.*, 2007).

Another study was conducted in Babile town, eastern Ethiopia on 415 school children and *Hymenolepis nana* was the most prevalent followed by hook worms (Girum, 2005). In Ethiopia, infection of intestinal parasite remains among the most ubiquitous and serious health problem with strikingly high prevalence rates of the major protozoan and nematode

infections. According to Ministry of Health (2000), intestinal parasitism accounts for 8.5% of all male and 10.4% of all female outpatients' visit in the country.

2.5. Factors Associated with Intestinal Parasitic Infections

2.5.1. Climate, Water and Season

Climate and topography are crucial determinants of the distribution of nematode and protozoa infections (Brooker, 2007). Nematode infections are highly affected by surface temperature (Brooker *et al.*, 2003), altitude, soil type, and rainfall (Kariuki *et al.*, 2004). Wetter areas exhibit increased transmission, and in some endemic areas, intestinal nematode infections exhibit marked seasonality. Recent use of geographical information systems and remote sensing has identified the distributional limits of intestinal nematode on the basis of temperature and rainfall patterns (Brooker and Michael, 2009).

2.5.2. Poverty, Sanitation and Urbanization

Parasitic infections depend for transmission on environments contaminated with egg-carrying feces. Consequently, intestinal parasites are intimately associated with poverty, poor sanitation, and lack of clean water. The provision of safe water and improved sanitation are essential for the control of parasitic infections. The populations in developing countries live in conditions that are highly conducive to the acquisition of parasitic infestation. Poor hygiene, crowded household conditions, dietary habits, education level of the community and deficient sanitation mark their day-to-day life (Culha *et al.*, 2007).

2.5.3. Behavior, Household Clustering and Occupation

Specific occupations, household clustering, and behaviors influence the prevalence infections particularly for hookworm, in which the highest intensities occur among adults (Brooker *et al.*, 2004). Engagement in agricultural pursuits, for example, remains a common denominator for hookworm infection.

2.5.4. Age Dependency

The high prevalence rate of intestinal infection in children is attributed to many factors particularly environmental and personal hygiene. For reasons not well understood, compared with any other age group, school-aged children (including adolescents) and preschool children tend to harbor the greatest numbers of intestinal worms and as a result experience growth stunting and diminished physical fitness as well as impaired memory and cognition (Crompton and Nesheim, 2005)

2.6. Diagnosis of Human Intestinal Parasitic Infections

There are different types of diagnostic techniques that are used for diagnosis of intestinal parasitic infection. For research purposes, surveys must be designed with methods that provide a high combination of sensitivity and specificity. However, for surveillance, the use of a diagnostics is based on cost-effectiveness, i.e. time and resources required per test, simplicity, robustness and community given priority. It should also be remembered that, while Kato-Katz thick smears should be read quickly for yielding the highest hookworm egg counts (Raso *et al.*, 2004).

Diagnostic techniques play a vital role in providing the scaffolding that medical personnel and disease control managers rely on when deciding which infections are the most threatening for an individual patient or for the entire community. Due to lack of progress in the detection of low intensity infection, the spatial distribution and burden of many STH infections are not well understood. This issue is an important reason why STH infections are often neglected (Hotez *et al.*, 2008).

Definitive diagnosis of intestinal parasitic protozoan is made by identification of cysts and trophozoites in the feces. Identification is usually made by microscopic examination of direct fecal smears for either trophozoites or cysts in the feces but the diagnosis of

soil-transmitted helminthes usually relies on finding the eggs of the parasite in the stool of infected individuals (Raso *et al.*, 2004). Since infections with multiple intestinal parasitic protozoan and helminthes species are the norm rather than the exception in the developing world, there is a need for well-trained laboratory technicians and quality control measures to ascertain accurate, species-specific diagnosis (Raso *et al.*, 2004)

2.7. Prevention and Control of Intestinal Parasitic Infections

2.7.1. Treatment

Based on different age group, endemicity of the parasite and use of antimicrobial therapy vary. Currently, there are different groups of drugs available to giardiasis infection and other intestinal parasites. The most common anti-giardia drug is metronidazole (Gardener and Hill, 2001). Unlike other drugs, metronidazole is quickly and completely absorbed and penetrates body tissues and sections such as saliva, breast milk, Semen and Vaginal secretions (Gardner and Hill, 2001)

2.7.2. Health Education

Health education and promotion of healthy behaviors can play a key role in reducing the incidence of human intestinal parasitic infections. However, the effectiveness of those activities in reducing transmission of infection varies according to different reports. In some cases, health education can decrease costs, increase levels of knowledge, and decrease re-infection rates. Health education efforts can build trust and engage communities in aspects that are crucial to the success of public health initiatives (Lansdown *et al.*, 2002).

2.7.3. Improved Sanitation

Control programs based on sanitation aim to reduce or interrupt transmission, prevent re-infection and gradually reduce worm loads. However, to be effective in a short period of time they need to be combined at their first stage with chemotherapy. Long term

sanitary control programs need to add elements to improve the economic conditions of a region, to ensure a reliable and permanent sanitation system and have permanent health education programs (Gillespie, 2009).

School age children harbor the most intestinal parasitic infections with roundworm, hookworm and whip worm. Therefore, treatment of this age group which is easily accessible through the school system achieves optimal improvements in health status and educational performance. In many developed countries, the prevalence rate of the parasitic infection has been significantly reduced as a result of improved standard of living, without the adoption of specific control measures. The principal measures that should be included in a control program consist of massive and periodic treatment of the human population to prevent environmental contamination, sanitary excreta disposal, and provision of potable water and health education for the purpose of encouraging personal hygiene habit in the population (Sackey *et al.*, 2012).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

Harar is the capital of Harari Region, Ethiopia. The city is located on a hill top in the eastern extension of the Ethiopian high lands, about 500 kilometers from the national capital Addis Ababa at an elevation of 1885 meters. Based on figures from the central statistical agency in 2017, Harar had an estimated total population of 245,000, of whom 124,000 were males and 121,000 were females. (CSA 2013)

3.2. Design of the Study

The study involved a descriptive cross-sectional survey of major intestinal protozoan and helminth parasitic infections from school children and the associated risk factors among school children at YeshimetElementary and Junior Secondary School, HararTown; Eastern Ethiopia. Association of intestinal parasitic infections with anthropometric measurements of school children was also investigated.

3.3. Study Population, Sample Size Determination and Sampling

Technique

All students from grade 5 to grade 8 in the selected school, who were willing to participate and signed the consent form included in the present study. Since, there was no previous investigation conducted on the same title in the study area, P value of 0.5 was taken to ensure a sample size large enough to satisfy the precision and confidence constraints. By taking this in to consideration, the sample size for single population was calculated based on the 95% confidence limits and 5% sampling error by using the statistical formula described below. In estimating the sample size (n), 50% prevalence, for Z value for 95% confidence interval is 1.96 and 5% precision was used to determine n using the statistical formula: (Kish and Leslie 1965).

$$n = \frac{Z^2 P (1-P)}{d^2}$$

Where: n = sample size

Z = Z (statistic) for a level of confidence

d = precision (confidence interval)

p = expected prevalence or proportion

Based on the above formula, the sample size (n) was calculated as follows:

$$n = \frac{(1.96)^2 (0.5) (0.5)}{(0.05)^2}$$
$$= 384$$

Based on the above formula, the study participants were selected using a stratified random sampling technique. To minimize errors arising from the likelihood of non compliance, ten percent of the sample size was added to the normal sample. Four hundred twenty two (422) school children were chosen to participate in the study. The students were first stratified according to their grade level (grade 5 to grade 8 and sex). A quota was allocated for each grade and sex. Finally, random sampling techniques were used to select students from each grade and sex by using class rosters as the sample frame.

Table 1 Total population of the school and sample size of the study participants

Total Number of students	Selected students
--------------------------	-------------------

Grade	male		female		total	male		female		Total
5	188	195	383	69	56	125				
6	161	152	313	64	49	113				
7	132	123	255	57	41	98				
8	110	99	209	51	35	86				
<hr/>										
Total	591	569	1160	241	181	422				

Exclusion criteria

Those students who have been treated for any intestinal parasitic infection in the past three months at the time of the present study were excluded.

3.4. Method of Data Collection

3.4.1. Questionnaire Survey

Data related to socio-demographic characteristics (variables) of the study subjects, risk factors (drinking water source, personal hygiene and life skill practice, residence of children, basic knowledge on parasitic infections, availability of latrine in the close vicinity of their home) that predispose school children to parasitic infections, and common signs and symptoms of intestinal protozoan and soil-transmitted helminth parasitic infections were gathered using pre-tested and structured questionnaire prepared in English and translated in Amharic language for each of the selected students.

3.4.2. Stool Sample Collection Procedures

Stool collection was made by disposable plastic cups and applicators that were distributed to each study subject along with brief instructions on how to collect the stool. Then they were advised to bring stool sample of about three grams of their own. Stool samples were protected from contamination with water, soil and urine. Information about name, sex and age and stool examination result of each student was recorded by the researcher. Stool samples were examined within three hours after collection for the cysts and ova of intestinal parasites by direct microscopic examination. The unique code of each student was labeled on the container. The stool samples were carried to Hiwot Fana Hospital on the same day of collection for parasitological examination and enumeration.

3.4.3. Anthropometric measurements

The anthropometric measurements of the school children included weight and height by using the standardized procedures mentioned in Gibson (2005) and body mass index (BMI) was calculated using the formula, $BMI = \text{weight in kg} / (\text{height in m})^2$, Weight was taken without shoes and minimum clothing using weighing scale and was recorded to the nearest 0.1 Kg. Height was measured to the nearest 0.1 cm using a measuring tape. The age of each child was recorded during sample collection. All the data were transformed and expressed in Z-scores and calculated using anthropometry calculating software program, AnthroPlus (WHO, 2007). Under-nutrition was defined for a child, who had less than -2 z-scores (-2SD) from the National Center for Health Statistics (NCHS) median reference population values (WHO, 2007).

3.5. Laboratory Parasitological Examination Procedures

3.5.1. Direct wet mount technique

Direct wet mount technique was used to assess the overall prevalence of intestinal parasitic infections in study participants. The direct wet mount was processed by conventional iodine to identify the presence of motile intestinal parasites, cysts, eggs and trophozoites under the light microscope at 10x and 40x magnification. Saline solution was used to observe cysts of intestinal parasites (Singh *et al.*, 2004). About one gram of stool sample was emulsified with 3-4 ml normal saline, and then a drop of emulsified sample was placed on a clean microscopic glass slide, then a few drops of iodine solution was added and covered with a cover slip. The presence of intestinal parasites ova and cyst were observed under the microscope (Lindo *et al.*, 1998).

3.5.2. Formol-ether Concentration Method

Using an applicator stick, about two g of preserved stool sample was placed in a clean 15 ml conical centrifuge tube containing 10 ml formalin. The sample was suspended and

mixed thoroughly with applicator stick. The resulting suspension was filtered through a sieve (cotton gauze) into a beaker and the filtrate was poured back into the same tube. The debris trapped on the sieve was discarded. After adding three ml of ether to the mixture and hand shaken, the content was centrifuged at 2000 rpm for three minutes. The supernatant was poured away and the tube was replaced in its rack. Iodine stain was added into the sediments. Finally, the preparation was examined under the microscope with $\times 10$ and $\times 40$ objectives for the presence of ova, cysts and trophozoite of parasite (WHO, 2000).

3.6. Data Analysis

Prevalence of intestinal protozoan parasites as well as prevalence of STH was analyzed using SPSS, Windows Version 20. Anthropometric indices were computed using the calculator mode of anthropometry calculating software program AnthroPlus (WHO, 2007). Wasting, stunting and underweight were defined as z- score values of less than -2 SD (standard deviation), which is below what is expected on the basis of international growth reference scale (WHO, 2007). Descriptive statistics were applied to indicate the prevalence of intestinal parasitic infections and nutritional status as percentages and proportions. The significance of the differences in frequency distribution was tested by using chi-square analyses. P-values less than 0.05 were considered statistically significant.

3.7. Data Quality Control

During data collection, all the activities of the work were carefully monitored and supervised. First, a meeting was conducted with the laboratory technicians and nurses to discuss the need for quality data. To ensure quality control, all the laboratory procedures including collection and handling of specimens were carried out in accordance with standard protocols. All the reagents were checked for contamination each time they were used. To ensure general safety, disposable gloves were worn and universal bio-safety

precautions were also followed at all times. The microscope used for this research was adjusted, and the objectives and oculars used for the calibration procedure were used for all measurement done with the microscope. The calibration factors for the 10x and 40x objectives were posted on the microscope for easy access. The weight scales were checked at the beginning of each working day. To ensure accurate identification of parasite species, bench aids for the diagnosis of intestinal parasites and diagrams of various parasite ova and larvae from the parasitological were used as reference.

3.8. Ethical Consideration

Permission was obtained from Hareri Health Bureau, Educational Office and School Principals. The objective of the study was explained to the teachers, school principals and the study participants. The samples were collected from consented school children. Individuals diagnosed positive for any intestinal parasite infections were treated free of charge with appropriate drug which was provided by the health professional. The prescription on how to use the drug was given by the health workers of the University Hospital.

4. RESULTS AND DISCUSSION

4.1. Socio-Demographic Characteristics of Study Participants

A total of 422 students were sampled to participate in the study. Of these, 241 were males and 181 were females who participated in the present study. About 241 (57.1%) of the children were males and 181(42.9%) were females. The age distribution ranged from 10-18 years; 161 (38.2%) students were in the age group 10-12 years, 238 (56.4%) were 13-15 years and 23 (5.4%) were 16-18 years old. More than 50% of the sample study participants were in the age group of 13-15years old (Table 2).

As shown in Table 2, the majority of the households 312 (73.9%) had <5 family size while 110 (26.1%) households had >5 family size. About 338(80.1%) of them also indicated that they have pipe water supply for domestic uses. However, 84 (19.9) % were using ground water. With regard to parents' education, 189 (44.8%) and 233 (55.2%) said that they were illiterate and literate, respectively. About 364 (86.3%) of the students' households with latrines in close vicinity of their homes. The remaining 58(13.7%) did not have latrines at their homes. Children who washed hands before meals were 390 (92.4%) while those who did not wash hands before meals were 32 (7.6%). About 167 (39.6%) participants were washing their hands after toilet and the remaining 255 (60.4%) were not washing. About 350 (82.9%) of the households did not treat water before drinking and 72 (17.1%) households treated their water before drinking. From a total of 422 study participants, about 407 (96.4%) of the children were living in urban areas while 15 (3.6%) of the children having in rural areas (Table 2).

Table 2. Socio-demographic characteristics of study participants in Yeshimebet primary school from March-May, 2017

Character	Frequency	Percents (%)
Sex: Male	241	57.1
Female	181	42.9
Age group (years) 10-12	161	38.2
13-15	238	56.4
16-18	23	5.4
Family size: <5	312	73.9
>5	110	26.1
SW Pipe	338	80.1
Ground	84	19.9
WHBM yes	390	92.4
No	32	7.6
WHAT Yes	167	39.6
No	255	60.4
AVL Yes	364	86.3
No	58	13.7
EDL Illiterate	189	44.8

WT	Literate	233	55.2
	Yes	72	17.1
RES	No	350	82.9
	Urban	407	96.4
	Rural	15	3.6

Key:- AVL= availability of latrine, EDL=education level, RES=residences, SW =source of water, WHAT =washing hands after toilet, WHBM=washing hands before meal ,WT=waater treatment

4.2. Prevalence of Intestinal Parasitic Infections in School Children

Prevalence of intestinal parasitic infections among the school-children at Yeshimebet Primary School by age and sex of the respondents is presented. Of the total 422 children examined, 28.7 % (121/422) of them were positive for intestinal parasitic infections (Table 3). Of these, the prevalence of intestinal parasitic infection among the infected children was 26.6 % (64/422) and 31.5 % (57/422) for males and females, respectively.

In this study, the prevalence of intestinal parasitic infections for the age group 10-12 years was 22.1% in males and 50% in females, for the age group 13-15, it was 26.6% for males and 18.2% for females, however for the age group 16-18 years it was 44.4% and 80% for males and females respectively (Table 3). The higher prevalence of intestinal parasitic infection was seen in the age groups of 10-12 and 16-18 years which indicated that

younger children are more exposed since they usually play in the open fields and eat food without washing hands.

In the present study, the result revealed that there is similar prevalence to the result of the study conducted in school- aged children in Babile, Eastern Ethiopia (27.2%) by Girum (2005) and from Hossaena, 27.9%, by Baruda (2013).

The prevalence of protozoan parasites in male and female school children was 28 (11.6%) and 27(14.9%), respectively (Table 3). Similarly, the prevalence of helminthic parasites in male and female students was 36 (14.9%) and 30 (16.6%), respectively (Table 3). Several studies have reported a higher prevalence of infection in boys than girls (Jejaw *et al.*2015; Yentur Donie *et al.* 2015), but some have reported different findings (Mukhiya *et al.* 2012; Sah *et al.*2013).

The prevalence of the two intestinal protozoan parasites among the three age groups of the participants of the study was 16.1%, 10.5% and 17.4% for 10-12, 13-15 and 16-18 years, respectively (Table 3).

As the result in Table 3, shows the overall prevalence of helminth parasitic infection in the study participants was 15.6%. Of these, the prevalence of the three major Helminth parasites among the age groups of 10-12 years, 13-15 years and 16-18 years was 17.4%, 12.2% and 39.1%, respectively (Table 3). The possible explanation for the highest prevalence of helminth infection observed in higher aged children may be due to higher chance of harboring more than one helminthes infections as individuals stay longer in endemic areas.

Generally, the prevalence of intestinal parasitic infections was higher in the age group of 10-12 years old (36.05%) and in the age group of 16-18 years (65%) school children. This was because younger people have lower resistance to parasitic infections and their defense systems are not fully developed in children. In addition to this, children were more

exposed to overcrowded conditions (schools, nurseries, playgrounds etc). Higher prevalence of parasitic infections among school-children may occur due to the poor sanitary conditions in schools (Oguntibeju, 2006). Children usually do not take care of their personal hygiene. For instance, they play in contaminated outdoor environments, in and around disposal sites (which can certainly cause serious health problems), problems of absence of latrine and lack of basic life skills, such as washing hands before and after meals (Abu Mourad, 2004).

Table 3 . Prevalence of intestinal protozoan and helminth parasites by age and sex of examined children in Yeshimebet Primary School from Mar-May, 2017.

Age group And sex	No exam	Protozoan No post(%)	Helminths No post(%)	Both No post(%)	X ² -value	p-value
10-12						
Male	95(59)	9(9.5)	12(12.6)	21(22.1)	12.272	0.001
Female	66(41)	17(25.8)	16(24.2)	33(50)		
13-15						
Male	128(53.8)	16(12.5)	18(14.1)	34(26.6)	2.369	0.124
Female	110(46.2)	9(8.2)	11(10)	20(18.2)		
16-18						
Male	18(78.3)	3(16.7)	6(33.3)	9(50)	1.433	0.231
Female	5(21.7)	1(20)	3(60)	4(80)		

All age						
Male	241(57.1)	28(11.6)	36(14.9)	64(26.6)	0.976	0.323
Female	181(42.9)	27(14.9)	30(16.6)	57(31.5)		
Total	422(100)	55(13)	66(15.6)	121(28.7)		

The reason for the higher prevalence of helminth parasites than protozoan infection in this study could be due to modes of transmission of the parasites. Protozoan parasites are transmitted through contaminated hands, food etc whereas helminths parasites are transmitted by fecal-oral means and skin penetration (eg hookworm). The difference in

parasitic infections between sexes that was higher in females than males could be due to modes of transmission of the parasites, sample size determination, study population and the methods used could attribute to this observed difference in detections of various parasites.

The study result showed that from 28.7% of positive cases of school children, the prevalence of intestinal protozoan and helminth parasitic infections were found to be 13% and 15.6%, respectively (Table 3). Thus, it was interesting to find that the intestinal helminth infection seemed to be more of a problem than the protozoan parasites. There are differences with variations in geography, socio-economic conditions, and cultural practices of the population under consideration. Studies conducted in school students in Turkey (Okyay *et al.*, 2004) and Afghanistan (Korzeniewski *et al.*, 2015), showed that the prevalence of intestinal parasites was 31.8% and 39.1%, respectively, which were higher than this study.

4.3 Major Intestinal Protozoan and Helminth Parasites Identified In Examined Children

This study indicated that from 422 school-children, 121 (28.7%) were positive for intestinal parasites. Of these, 26.6% and 31.5% were males and females, respectively. *Entamoeba histolytica* and *Giardia lamblia* were the major protozoan parasites identified from the school- children with the prevalence of 7.1% and 5.9%, respectively (Table 4).

Similarly, the major helminth parasites identified were *H.nana*, *Hookworm* and *E.vermicularis* with the prevalence of 10.2%, 3.3% and 2.1% respectively. These three helminth parasites were found with an overall prevalence of 15.6% (Table 3). *H.nana* was the most prevalent helminth species in these school children. In present study, there is high prevalence of helminth parasites. The high prevalence of helminths, such as *A. lumbricoides*, in other studies (Ayalew *et al.* 2011; Oliveira *et al.* 2015), are probably due

to the low living standards and environmental health, as well as people's lack of awareness of the sanitary basics (Ayalew *et al.*2011).

The prevalence of hookworm infection was lower than *H.nana* but greater than *E.vermicularis* among the present study participants i.e., 3.3%. This was lower than the prevalence reports of 46.9% by Tilahun (2010), 33.3% by Mengistu (2014) and 9.5% by Tadesse (2013). On the other hand, the findings of the prevalence of hookworm infection recorded in the present study were almost above the former report (1.0%) in North Ethiopia, Adwa (Lemlem *et al.*, 2008). Variability in endemicity or prevalence of these infections might be due to low sensitivity of the diagnostic method, variation in the degree of environmental contamination and inability of the helminth eggs to withstand diverse temperature could partly explain the observed difference as reported by Mazingo *et al.* (2010). This variation could also indicate that infection rates depend on such factors as local personal hygienic and sanitary conditions.

Table 4.Major intestinal protozoan and helminth parasites identified in examined children in yeshmabet primary school from March-May, 2017.

Age group	No exam	Protozoan		Helminths			X ² -value	p-value
		Eh No post(%)	Gl No pos(%)	Hn No post(%)	Hw No post(%)	Ev No post(%)		
10-12								
Male	95(59)	7(7.4)	2(2.1)	11(11.6)	0(0)	1(1.1)	18.952	0.002
Female	66(41)	9(13.6)	8(12.1)	11(16.7)	4(6.1)	1(1.5)		
13-15								
Male	128(53.8)	8(6.2)	8(6.2)	12(9.4)	1(0.8)	5(3.9)	6.758	0.239
Female	110(46.2)	4(3.6)	5(4.2)	5(4.5)	4(3.6)	2(1.8)		
16-18								
Male	18(78.3)	1(5.6)	2(11.1)	2(11.1)	4(22.2)	0(0)	4.191	0.381
Female	5(21.7)	1(20)	0(0)	2(40)	1(20)	0(0)		
All age								
Male	241(57.1)	16(6.6)	12(5)	25(10.4)	5(2.1)	6(2.5)	4.345	0.501
Female	181(42.9)	14(7.2)	13(7.2)	18(9.9)	9(5)	3(1.7)		
Total	422(100)	30(7.1)	25(5.9)	43(10.2)	14(3.3)	9(2.1)		

Eh=*Entamoeba histolytica*, Gl=*giardia-lamblia*, Hn=*Hymenolopisis nana*, Hw=*Hookworm*, Ev=*Enterobium vermacularis* .

Regarding the number of intestinal parasitic infections per individual, there was no individual with more than one parasite found in the study subjects. Similar study done by Solomon (2006) in Welayta Zone, Southern Ethiopia has reported 35.9% prevalence of helminth infections among school-children. The major helminth parasites identified during this study were *Hookworm*, *A.lumbricoides* and *T.trichiuria* with the prevalence of 25.6%, 12.3% and 10.5%, respectively (Solomon, 2006). According to the report of Solomon (2006), the prevalence of Hookworm infections was significantly higher ($P < 0.001$) in the age group of 14 years and above than younger age groups. Similarly, in this study the prevalence of Hookworm infections was higher in the age group of 16-18 years than other age groups. Similarly, hookworm frequently exhibits a steady rise in intensity of infection with age, peaking in adulthood (Bethony *et al.*, 2011).

As indicated from the result the prevalence of *E.histolytica* (7.1%) and *G.lamblia* were higher in the age group of 10-12 and 16-18 than others. Higher prevalence of parasitic infections in the age group of ≥ 16 years may occur due to the poor sanitary conditions in the schools (Oguntibeju, 2006). In different to this, the results reported by other studies in Iran (Taheri *et al.*, 2011; Daryani *et al.*, 2012) and other countries (Korze-niewski *et*

*al.*2015). There was also no significant difference between the prevalence of intestinal parasites and age.

4.4. Association of Intestinal Parasitic Infections with Socio Demographic Characteristics of the School Children

This study has also identified the relationships between socio-demographic factors of the school-children and the prevalence of intestinal parasitic infections. The overall prevalence of each intestinal parasite species diagnosed in the study of school children and the proportion of different socio-demographic factors were presented in Table 5.

Out of the 121 positive participants of the study, 92 (29.5%) and 29 (26.4%) were with less than five and greater than five family size, respectively. From 338(80.1%) pipe water users, 47(13.9%) were found to be positive for protozoan parasites and 56(16.6%) for helminthic infection. Whereas from the 84(19.9%) ground water users, 8(9.5%) were found to be positive for protozoan and 10(11.9%) for helminthic infection. The result shows that among 33(17.5%) protozoan and 45(23.8%) helminth parasitic infections of 78(41.3%) participants of the study were with illiterate parents. About 22(9.4%) protozoan and 21(9%) helminth parasitic infections, 43(18.5%) participants of the study were with literate parents. In this study, education was the major risk factor for the prevalence of intestinal parasites ($p=0.000$ (Table 5). The present study showed significant association between parents' literacy level and the intensity of the parasitic infection, which was consistent with other studies in Iran (Masoumeh *et al.*,2012).

The students' households with latrines in close vicinity in their homes 364(86.3%), 43(11.8%) were found to be positive for protozoan parasite and 48(13.2%) for helminthes infection. The remaining 58(13.7%) of the students' households did not have latrines in their homes were found to be positive for protozoa and helminthes, that is 12(20.7%) and

18(31%), respectively. The same thing from 167 (39.6%) of the students' who wash their hands after toilet, 23(13.8%) were found to be positive for protozoan parasite and 29(17.4%) for helminthes infection. The remaining 255(60.4%) of the students' who did not wash hands after toilet were found to be positive for protozoa and helminthes, that is 32(12.5%) and 37(14.5%), respectively. 390 (92.4%) of the students' who wash their hands before meal, 43(11%) were found to be positive for protozoan parasite and 56(14.4%) for helminthes infection. The remaining 32(7.6%) of the students' who did not wash hands before meal were found to be positive for protozoa and helminthes, that is 12(37.5%) and 10(31.3%), respectively.

There was statistically significant association between prevalence of parasitic infection and latrine availabilities and washing hands before meal ($p=0.000$, $p=0.000$) (Table 5).

The prevalence rate of intestinal helminths in terms of family size was found to be 29.5%, and 26.4% for family size of ≤ 5 and >5 , respectively. The majority of the school children (82.9%) of the pupils drink water without any form of treatment and the prevalence of helminths and protozoan among them were 14.9% and 13.7% respectively.

			(%)	(%)			
Family size							
<5	312(73.9)	44(14.1)	48(15.4)	92(29.5)	0.817	0.650	0.420
>5	110(26.1)	11(10)	18(16.4)	29(26.4)			
SW							
Pipe	338(80.1)	47(13.9)	56(16.6)	103(30.5)	0.576	3.464	0.063
Ground	84(19.9)	8(9.5)	10(11.9)	18(21.4)			
WHBM							
Yes	390(92.4)	43(11)	56(14.4)	99(25.4)	0.178	23.532	0.000*
No	32(7.6)	12(37.5)	10(31.3)	22(68.8)			
WHAT							
Yes	167(39.6)	23(13.8)	29(17.4)	52(31.1)	1.243	0.991	0.319
No	255(60.4)	32(12.5)	37(14.5)	69(27.1)			
AVL							
Yes	364(86.3)	43(11.8)	48(13.2)	91(25)	0.362	13.006	0.000*
No	58(13.7)	12(20.7)	18(31)	30(51.7)			
EDL							
Illiterate	189(44.8)	33(17.5)	45(23.8)	78(41.3)	0.313	27.706	0.000*
Literate	233(55.2)	22(9.4)	21(9)	43(18.5)			
WT							
Yes	72(17.1)	7(9.7)	14(19.4)	21(29.2)	1.044	0.023	0.880
No	350(82.9)	48(13.7)	52(14.9)	100(28.6)			
RES							
Urban	407(96.4)	53(13)	62(15.2)	115(28.3)	1.270	0.183	0.669
Rural	15(3.6)	2(13.3)	4(26.7)	6(40)			

Key:- AVL= availability of latrine, EDL=education level, RES=residences, SW=source of water, WHAT =washing hands after toilet, WHBM=washing hands before meal ,WT=water treatment, OD=odds ratio, *=significant

The existence of intestinal parasitic infections depends on environments contaminated with egg-carrying feces. Consequently, intestinal parasites are intimately associated with poverty, poor sanitation, and lack of clean water. The provision of safe water and improved sanitation are essential for the control of parasitic infections. People in developing countries live in conditions that are highly conducive to the acquisition of parasitic infestation. Poor hygiene, crowded household conditions, dietary habits, education level of the community and deficient sanitation mark their day-to-day life (Culha *et al.*, 2007)

Regarding residences; out of 407 (96.4%) sampled school children, 53(13%) and 62(15.2%) were found to be positive for protozoan and helminthes parasitic infections, respectively. From the remaining 15(3.6%) rural participants of the study, 2(13.3%) and 4(26.7%) were found to be positive for protozoan and helminthes parasitic infections respectively.

In this study, intestinal parasitic infections are highly prevalent. This is due to children have a higher risk of infection than adults because they spend more time at home and would be engaged in risky behaviors such as playing on and with soil. Thus, sanitation and hygiene are priorities for the action of reducing the prevalence of intestinal parasitic infections (WHO, 1997). Safe disposal of fecal material and proper life skills, such as hand washing are also an effective barrier for the transmission of intestinal parasitic infections (Curtis *et al.*, 2000).

Children are more receptive to learning and are very likely to adopt healthy behaviors at younger age. They can also be agents of change by spreading what they have learned in the school to their family and community members. Enhanced, comprehensive knowledge

about these issues should be used to improve low-cost but highly effective programs that will meaningfully attenuate the burden of transmissible diseases among students in rural settings (Lopez-Quintero *et al.*, 2009).

Generally, in this study the prevalence of intestinal parasitic infections and some risk factors such as , education level, washing hand before meal and availability of latrine are statistically significant ($p=0.000$, $p=0.000$ and $p=0.000$) respectively.

Improper hygiene in children is closely associated with parents' level of education. Environmental factors known to cause STH parasites are related to water supply and availability of toilets and behavioral habits. According to Ziegelbauer *et al.* (2012), systematic review and Meta-Analysis study on effect of Sanitation on Soil-Transmitted Helminth Infection found that the availability and use of sanitation facilities were associated with a reduction in the prevalence of infection with soil-transmitted helminthes.

4.5 Anthropometric Measurements of School-Children

As recommended by WHO (2007),the anthropometric measurements of children in the present study were compared with an international reference population defined by the U.S. National Centre for Health Statistics (NCHS) and accepted by the U.S. Centers for Disease Control and Prevention (CDC).Each of the three nutritional status indicators described below was expressed in standard deviation units (Z-scores) from the median of the reference population (Table 6).

Each of these indicators, BMI-for-age, weight-for-height, and height-for-age provides different information about growth and body composition, which is used to assess nutritional status. The height-for-age index is indicator of linear growth retardation and cumulative growth deficits. Children whose height-for-age Z-score is below minus two

standard deviations (-2 SD) from the median of the reference population are considered short for their age (stunted) and are chronically malnourished. Children who are below minus three standard deviations (-3 SD) from the median of the reference population are considered severely stunted. Stunting reflects failure to receive adequate nutrition over a long period of time and is also affected by recurrent and chronic illness. Height-for-age, therefore, represents the long-term effects of malnutrition in a population and does not vary according to recent dietary intake (CSO, 2005).

In the present study, the prevalence of BMI-for-age underweight (<5th percentile) which were an indicator for being underweight for 10-18 years of age was 59.5%. BMI - for - age under 5th percentiles was 61.4% for males and 56.9% for females (Table 6). Moreover, BMI - for - age percentiles of 5th - 85th, and \geq 85th were calculated for analyzing the status of normal growth and to assess risks for overweight and /or obesity, respectively.

In the present study the prevalence of underweight was 59.5% which is higher than the prevalence of underweight (25.7%) reported for Malaysian school children (Zulkifli *et al.*, 2000), and with Tilahun (2010) who reported 30.7% in Debub Achefer district. Aleminesh (2013) reported 33.9% in Mojo from eastern Ethiopia. The variations might be due to nutritional status and environmental sanitation (Vikram *et al.*, 2008).

As revealed in Table 5, 251(59.5%), 73(17.3%) and 8(1.9%) of the study children aged 10-18 showed underweight, wasting and stunting, respectively. Of these 61.4% were males and 56.9% were females for underweight, 17% males and 17.7% females for wasting and 1.7% males and 2.2% females for stunting. Prevalence of wasting and stunting was almost the same between boys and girls in this age group. Even though there

was no statistically significant difference in underweight, there was difference between males and females, i.e.61.4% males and 56.9% females (Table 6).

Other study done on school-children of Babile town by Girum Tadesse (2005) showed that wasting was the predominant manifestation of malnutrition (11.6%) followed by stunting (5.4%) and underweight (5.2%). As compared with this study, there was a higher proportion of underweight and wasting with 59.5% and 17.3%, respectively. Stunted students show a higher prevalence of parasitic infection than the other anthropometric measurements (Girum, 2005).

Table 6 Prevalence of BMI-for-age, height-for-age, and weight-for-height status among males and females of study school children aged 10-18 years in Yeshimebet Primary School From March-May, 2017

Age group	Sex	Examined children		Nutritional Indictors					
				Under weight		Wasting		Stunning	
		F	%	F	%	F	%	F	%
10 – 18	Male	241	57.1	148	61.4	41	17	4	1.7
	Female	181	42.9	103	56.9	32	17.7	4	2.2
	Total	422	100	251	59.5	73	17.3	8	1.9
X ²				2.314		0.320		0.168	
P-value				0.314		0.858		0.682	

4.6. Prevalence of Underweight and/or Thinness in the School Children

BMI-for-age is the recommended indicator for assessing thinness, overweight and obesity in children 10-19 years (WHO, 2009). Therefore, in the current study, the prevalence of BMI-for-age under 5th percentiles which were an indicator for being underweight for 10-18 years of age was 59.5%. Of which, 61.4% was for males and 56.9% was for females. In addition, BMI-for-age of normal weight was calculated for analyzing the status of normal growth for 10-18 years of age was 167(39.6 %). Of which, 92 (38.2%) was males and 75(41.4) was females .In addition to these there was low risk for overweight among the study school children 4(0.9%) of which, 0.4% and 1.7% was for males and females respectively (Table 7).

10 – 18	Male	241	57.1	148	61.4	92	38.2	1	0.4
	Female	181	42.9	103	56.9	75	41.4	3	1.7
	Total	422	100	251	59.5	167	39.6	4	0.9

Key: BMI= Body Mass Index

The prevalence of underweight among age group 10-18 years in the present study (59.5%) was higher than the prevalence of underweight (36%) reported from Southern Ethiopia

(Birmeka, 2007). On the other hand, the prevalence reported for Abchikeli and Ayalew Mekonnen Elementary school children was 30.7% by Tilahun (2010).

Generally, in the present study, the prevalence of underweight (59.5%) among age group 10-18 years were found to be higher than those of both the regional and national rates, where wasting was 6.5% both nationally and regionally, and underweight was 29.7% regionally, but 20.8% nationally (FMOH, 2005).

The higher rates of acute malnutrition in the present study may be due to inadequate dietary intake, cultural, religious, or other factors associated with low socio-economic development such as differences by place of residence and mother's education level as reported by EDHS (2005).

4.7. Association of Intestinal Parasitic Infection and Physical Growth of School Children

This study has also analyzed correlation between anthropometric indices of the school-children and the prevalence of intestinal parasitic infections. The overall prevalence of each intestinal parasitic species diagnosed via school children employed in the study and the proportion of different anthropometric measurements is presented in Table 8.

The prevalence of intestinal parasitic infections was 69(27.5%) in underweighted students. The association between intestinal parasitic infections and nutritional status was statistically insignificant ($p=0.579$).

The prevalence of underweight among age group 10-18 years in the present study was greater than with the prevalence of underweight 9.9% reported by Alemineh (2013) among school children in Mojo and the prevalence of underweight (5.2%) reported by Girum (2005) among school children in Babile, Eastern Ethiopia. The variations in prevalence of underweight might be due to differences in nutrition and types of staple food the communities live upon (Gezahegn, 2008)

Table 8. Association of Intestinal Parasitic Infection with anthropometric measurements children in yeshmabet primary school from March-May, 2017.

Nutritional indicator	Numbers	Intestinal parasite infections		OR	X ² -value	p-value
10-18	Frequency %	Negat(%)	posti(%)			
BMI for age <5th perc	251(59.5)	182(72.5)	69(27.5)	0.913	1.092	0.579
5th to 85th perc	167(39.6)	118(70.7)	49(29.3)			
>85th perc	4(0.9)	2(0.5)	2(0.5)			

OR: odds ratio, Perc=percentile

5. SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1 Summary

The objective of the present study was to determine the prevalence of intestinal parasitic infections and their associations with anthropometric measurements among school-children of Harar, Harari Rregion, Ethiopia. The design of the study was a cross-sectional epidemiological investigation involving a sample population of 422 school-children who were selected by stratified random sampling method from Yeshimebet Primary School during 2017 Academic year.

A total of 422 stool samples were collected and examined using direct wet-mount Formol-ether Concentration and Modified Ziehl-Neelsen Method. After screening of 422 stool samples, the overall prevalence of intestinal parasitic infections was 28.7% (26.6% of males and 31.5% of females) with the prevalence of 13% and 15.6% of intestinal protozoan and helminth parasites respectively. The prevalence of intestinal protozoan parasites, *E. histolytica* and *G.lamblia* was 7.1% and 5.9%, respectively. Similarly, the prevalence of helminth parasitic infections *H.nana*, *Hookworm* and *E.vermicularis* was 10.2%, 3.3% and 2.1%, respectively.

The prevalence of intestinal parasitic infections was significantly associated with some of the factors, such as source of water, education level, washing hand before meal and availability of latrines ($p=0.063$, $p=0.000$, $p=0.000$ and $p=0.000$), respectively. Even though, there were high parasitic infections, they were not statistically associated with some socio-demographic factors, such as family size, washing hand after meal, treatment of water and residence.

Anthropometric measurements of school children measured and the relationships with the prevalence of intestinal parasitic infections were analyzed. The prevalence of intestinal parasitic infections was higher in underweight (59.5 %) students than wasted and stunted pupils. Underweight, wasted and stunted school-children had a prevalence of 59.5%, 17.3 and 1.9%, respectively.

5.2 Conclusion

This study confirms the presence of different intestinal parasites in the school children of Yeshmabet primary school. The major intestinal parasite species diagnosed in the school children of Yeshmabet primary school were *E.histolytica*, *G.lamblia*, *H.nana*, *Hookworm* and *E.vermicularis*. The findings in the present study showed that intestinal parasitic helminth infections were the major public health problems in the school-children of Harar town. *H.nana*, *Hookworm* and *E.vermicularis* infections were common for the school children. *E. histolytica* and *G.lamblia* infections were found as a dominant species of intestinal protozoan parasites diagnosed in the stool samples of the school-children. The result of this study also showed high prevalence of malnutrition among the school children.

5.3 Recommendations

Based on empirical results of this study and to reduce the prevalence of intestinal parasitic infections to a low level and to increase the knowledge and awareness about the causes of intestinal parasitic infections, the following recommendations are forwarded.

- Local health sector and any concerned bodies should collaborate with school health program for delivering health education to increase the knowledge, attitude, and practice of school children as to how transmissions of intestinal parasite infection is prevented such as improving of personal hygiene, use of safe water and environmental sanitation.
- Health education programs should be intensified in the area and beyond to bring awareness of intestinal parasite transmission and prevention such as high standard of hygiene practices to both the children and the parents.
- School health programs for the assessment of malnutrition and health education particularly for school children and parents and in general on how to prevent malnutrition should also be carried out.
- For better evaluation of the epidemiology of intestinal parasite in the area, in-depth studies should be made on socio economic factors, such as, source of water, latrine usage, and hand washing practices.

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APPENDIX

Appendix 1 Questionnaire

Dear respondents, the main purpose of this study is to find out the prevalence of intestinal parasitic infections and their association with anthropometric measurements among students. It is also very important to create awareness on its prevalence and controlling measure among all concerned bodies. Therefore, I kindly request you to give your genuine response for each question. Thank you!

1. Age _____

2. Sex: M _____ F _____

3. What is your family size?

A. <5

B. > 5

4. Residence

A. Urban

B. Rural

6. Caregiver education level A. Illiterate B. Literate

7. Did your child wash his/her hands after toilet?

A. Yes B. No

8. If your answer is yes for question no 7 what is the mode of his/her hand washing?

A. only water B. water and soap

9. Did your child wash his/her hands before meal?

A. yes B. no

10. Where do you get drinking water for your household?

C. Pipe water D. Ground water

11. Do you treat water before drinking?

A. Yes B. No

12. Do you have a toilet facility?

A. Yes B. No facility/ Open defecation

Appedix 2. Consent form

For participation as volunteer in the research undertaking

Code number-----

Name of the study subject -----

Explanation on procedures and condition of the agreement

I am from Haramaya University, School of Biological Science and Biotechnology. I am here to study The Prevalence of intestinal parasitic infections and their association with anthropometric measurements of school children in Yeshimeet primary school. The objective of this study is to determine prevalence of intestinal parasitic infections and their associated with anthropometric measurements of school children in Yeshimebet primary school. The information obtained from this study will provide the current status intestinal parasites in the study area.

I am asking you to participate in the study for intestinal parasitic infections investigation. The investigation will involve collection of faeces for parasitological examination and interview through pre-structured questionnaire for demographic and associated risk factors of the disease.

If the investigation is confirmed intestinal parasite, you will be treated with appropriate drug. The information that you provide me in the questionnaire and the results of the laboratory investigation would be kept confidential.

If you have any questions regarding the purpose of the study, you have the right to ask question and get clarification. It is your right to refuse this study if you are not interested to participate in the study.

Finally, if you have understood the explanation well enough, I am asking you kindly to participate in this study, and put your signature as illustrated below.

With full understanding of the situation that I agreed to give the informed consent voluntarily to the researcher. I agree that I am contributing to design and implement prevention and control strategies of the disease in the study area, by participating in this project.

Signature of participants (parent) _____ Date _____

Signature of investigator (researcher) _____ Date _____

Appedix 3. Laboratory Form

Name _____

Code _____

Laboratory Parasitological Examination Procedures.

Microscopic Examination of Stool:

1. Direct wet mount, formol ether and modified zeil neelson methods

Status of the infections:

A. Single infection with _____

B. Double infection with _____ and _____

C. Multiple infections with _____, _____ and _____

2. Anthropometric measurements:

A. Height _____

B. Weight _____