Prevalence and Intensity of Soil-Transmitted Helminth Parasitic Infections and their Associations with Anthropometric Measurements of School Children in Tulu Nageso Primary School, Oromia Regional State, Ethiopia

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Prevalence and Intensity of Soil-Transmitted Helminth Parasitic Infections and their Associations with Anthropometric Measurements of School Children in Tulu Nageso Primary School, Oromia Regional State, Ethiopia

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Merga Gonfa

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APPROVAL SHEET

HARAMAYA UNIVERSITY
POST GRADUATE PROGRAM DIRECTORATE

As thesis research advisors we herby certify that we have read and evaluated this thesis prepared, under our guidance, by **Merga Gonfa**, entitled **"Prevalence and Intensity of Soil-Transmitted Helminth Parasitic Infections and their Associations with Anthropometric Measurements of School Children in Tulu Nageso Primary School, Bokoji Town, Arsi Zone, Oromia Region, Ethiopia".** We recommend that it can be submitted as fulfilling all the thesis requirements.

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Name of Co. Advisor	Signature	Date

As member of the board of the examiners of the open defenses of the examination, we certify that we have read and evaluated this thesis properly which was prepared by **Merga Gonfa**. We recommended that the thesis can be accepted as fulfilling the *Thesis* requirement for the degree of *Master of Science* **in Biology**.

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Name of Internal Examiner	Signature	Date
______________________	_________________	_______________
Name of External Examiner	Signature	Date

DEDICATION

I dedicated this thesis to my beloved wife **Wagane Tadesa** who scarified a lot to bring me up to this level and my father **Gonfa Kefeni** and my mother **Angatu Gurmu** for their support extended to me the success in my life.

STATEMENT OF THE AUTHOR

First, I declare that this thesis is my original work and that all source of material used for this thesis have been duly acknowledge. This thesis has submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science at the Haramaya University and is deposited at the University Library to be made available for borrower under the rule of the library. I truly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree.

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Place: - Haramaya University, Haramaya

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BIOGRAPHICAL SKETCH

The author was born in Arsi zone on November 19, 1982. He attended his primary and secondary classes at Albaso and Kersa Schools, respectively. He completed secondary school classes in 2002 at Kersa High School. Then he was enrolled at Jimma Teachers' Training College Diploma program and studied from 2003 to 2004. After his graduation, he taught Biology in Shino high school from 2005 to 2007. In 2007, he joined summer program at Mekelle University and graduated with Bachelor of Education in Biology in 2010. From 2014 until 2016, the author has been teaching at Bokoji Preparatory School. He then joined the postgraduate program of the Department of Biology, Haramaya University, to study M.Sc. in Biology in 2013.

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LIST OF ABBREVIATIONS/ACRONYMMS

BMI	Body Mass Index
DALYs	Disability Adjusted Life Years
EPGs	Eggs Per Gram of Faeces
HAZ	Height-for-Age Z-score
IRERC	Institutional Research Ethics Review Committee
MOH	Ministry of Health
NCCLS	National Committee on Clinical Laboratory Standard
NTDs	Neglected Tropical Diseases
SEM	Standard Error Mean
SPSS	Statistical Package for Social Sciences
STH	Soil-Transmitted Helminthes
WHA	World Health Assembly
WHO	World Health Organization
WAZ	Weight-for-age Z-score
WHZ	Weight-for-Height Z-score

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ABSTRACT

Soil-transmitted helminth parasitic infections constitute major public health challenges among school children in developing tropical and sub-tropical countries. Therefore, the objective of the present study was to determine the prevalence and intensity of soil transmitted helminth parasitic infections and their associations with anthropometric measurements at Tulu Nageso Primary School of Oromia Region, Ethiopia. The design was cross sectional survey, involving 348 participants (185 males and 199 females, ages ranging from 6-16 years) who were selected by stratified random sampling method. Stool samples were processed for microscopic examinations using direct wet mount technique and Kato-Katz method. Weight and height of children were taken to assess body mass index (BMI) of each study participant. The Data were analyzed using statistical package for social sciences (SPSS version 16) and anthropometry calculating software (Anthroplus). The National centre for Health Statistics (NCHS) growth chart reference was used to estimate the prevalence of underweight/thinness and stuntedness among study participants. The overall prevalence of soil- transmitted helminth parasitic infection was 19.7 % (22.7% for males and 17.0% for females). Double infection with two soil-transmitted helminthe p arasites was found in 0.8% (3/384) of the study participants. The prevalence of Ascaris lumbricoides, Trichiuris trichiura and hookworm infections were 15.1%, 2.8%, and 1.0%, respectively. The mean ± SEM egg counts of A. lumbricoides, T. trichiura and hookworm were 111.9±15.9 (range 24-480), 63.9±10.9 (range 24-480), and 36± 6.9 (range 24-48) eggs per gram of faeces, respectively. The anthropometric indices of study participants in 6-9 years age group were 3.1%, 6.7%, and 6.1% for underweight, stunting, and wasting, respectively while the prevalence of underweight/thinness in the 10-16 years age group was 4.1%. The present study did not find any statistically significant (p=0.98) association between soil-transmitted helminth parasitic infections and anthropometric indices (p>0.05). Coordinated work with health officers and school community is required on the prevention of STH infection. Chemotherapy has to be complemented with other measures such as health education, provision and use of latrines to control transmission of soil-transmitted helminthiasis in the study area.

Keywords*: Anthropometric measurement, Body mass index, Bekoji, Intensity, School-children, Soil-Transmitted Helminth*

1. INTRODUCTION

Parasitic helminths (worms) that infect humans belong to two phyla, Phylum Platyhelminths (flatworms) and Phylum Nematode (roundworms) (Maizels and Yazdanbakhsh, 2003; Hotez *et al*., 2008). Soil-transmitted helminths (STHs) refer to a group of round worms that are transmitted through contact with fecally contaminated soil (Hotez *et al*., 2008). These are among the most major health problems in many developing countries, especially among children (Parajuli *et al*., 2009; Goodman *et al*., 2007; Mukhopadhyay *et al*., 2008). The main soil transmitted nematode species are *Ascaris lumbricoides, Trichuris trichiura* and the hookworms (*Necator americanus* and *Ancylostoma duodenale*) (WHO 2002; Utzinger and Keiser, 2004; Bethony *et al*., 2006).

Intestinal helminth parasitic infections affect over one quarter of the world's population (Bethony *et al.,* 2006; Brooker *et al*., 2006), of which 300 million suffer from associated severe morbidity and about 400 millions of which are school-age children (Rodriguez-Morales *et al*., 2005). More than 150 million school age children are severely affected by intestinal parasitic worms (Jukes *et al.,* 2008). Helminth parasites, particularly intestinal nematodes are major public health problems in tropical and subtropical countries of the world.

Helminth parasitic infections cause decreased body resistance, retardation of physical and mental development of children, indigestion, diarrhea, anorexia and lack of memory, increased morbidity rate, greater incidence of abortion, sterility, stillbirth and impaired lactation, anemia, pneumonia, bronchitis, appendicitis, increased susceptibility to various non-helminthic diseases (Parajuli *et al*., 2009) . The high prevalence rate of the parasite was correlated with poverty, poor sanitation and impoverished health services (WHO, 2002).

In recent years, academic institutions, medical agencies, and public health officers have shown an increasing interest in helminth parasites infections, recognized as a priority health problem for large parts of the population in many developing countries (Osazuwa *et al*., 2011). The global prevalence and number of cases of intestinal helminth parasite infection in school age children have been estimated to be Ascaris 35% (320 million), Whipworm 25% (233 million), Hookworm 26% (239 million) and others 14% (128 million) (Partnership for Child development, 2011). The world development report (World Bank, 1993) ranked intestinal helminths as one of the main causes of disease in children. The estimates of the total number of people infected with soil-transmitted helminth parasites stand about 807-1,221 millions for *A. lumbricoides,* 604-795 millions for *T. trichiura* and 576-740 millions for the hookworms (de Silva *et al*., 2003: Bethony *et al*., 2006).

In children, intestinal parasitic infections, particularly soil-transmitted helminthiasis is the cause of common health problems in tropical countries. Younger children are predisposed to heavy infections with intestinal parasites since their immune systems are not yet fully developed (Rao *et al*., 2006), and they also habitually play in faecally contaminated soil. In addition to considerable mortality and morbidity, infection with intestinal helminth parasites has been found to profoundly affect a child's mental development, growth and physical fitness while also predisposing children to other infectious agents (Parajuli *et al*., 2009).

In Ethiopia, as in other developing countries, high prevalence of STH parasites infection is attributable to factors associated with low socio-economic status. Such factors include poor personal hygiene and environmental sanitation, with low household income, overcrowding and lack of clean water supplies for most parts of the country. For instance, Ethiopia is one of the poorest countries in supplying quality drinking water and latrine coverage in the world (Mengistu *et al.,* 2007).Report from 2000 study shows that Ethiopia had only 12% latrine coverage while Kenya had 87% (Kumie and Ali, 2005 as cited in Mengistu *et al.,* 2007).

The availability of safe and relatively inexpensive drugs for both schistosomiasis (praziquantel) and STHs parasites (albendazole and mebendazole) has made control through chemotherapy a potentially affordable option, even in resource-poor countries (Handzel *et al.,* 2003). Consequently, in May 2001 and January 2009 the World Health Assembly (WHA) recognized the place where control measures including chemotherapeutic interventions have been implemented in a sustainable way, as demonstrated in several countries, mortality, morbidity and transmission showed dramatically decrement (WHA, 2001). However, understanding the epidemiology of major intestinal helminth parasite infection in a particular place is crucial to design appropriate preventive and control measures.

In different places of Ethiopia, the prevalence of STHs parasites has been reported by different researchers (Alemayehu *et al*., 1998; Erko and Medhin, 2003; Nyantekyi *et al*., 2010; Reji *et al*., 2011). Intestinal parasitic infections cause serious public health problem in Ethiopia. Moreover, a number of surveys have shown that STHs are prevalent in varying magnitudes (Reji *et al*., 2011). Similarly, the prevalence of hookworm, *Ascaris lumbricoides,* and *Trichuris trichiura was* estimated to be 16%, 37% and 30%, respectively (Tadesse *et al.,* 2008). And their distribution varies by place and with age (Belyhun *et al.,* 2010).

As Leykun (2000) reported, the overall prevalence of intestinal parasites was 49% in south Gonder, 27.2%, and 43.7% in Babile town and around Lake Zway, respectively (Girum, 2005; and Gezehang, 2008). *A. lumbricodes* and *T. trichiura* are co-existing and highly prevalent with increase in altitude. The highest prevalence of infection was recorded at an altitude more than 2400 m above sea level and it was also known by commonly affecting school age children.

Of 301 school children who were studied in south western Ethiopia, 68.4% harbored one or more parasites (Alemeshet *et al*., 2010). In that study from the total ten species identified, hookworm was the leading (62.5%) followed by *Ascaris lumbricoides* (52.2%), *T. trichiura* (18.6%), *E. histolytica* (13.5%), *G. lamblia* (6.7%), while *C. parvum* was the least (0.3%) (Alemeshet *et al*., 2010).

Another study was conducted in Babile town, eastern Ethiopia on 415 school children and *Hymenolepis nana* was the most prevalent followed by hookworms (Girum, 2005). In Ethiopia, infection of intestinal parasite remains among the most ubiquitous and serious health problems 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.

World Health Organization also indicated that the prevalence of helminth parasite infections in Ethiopia ranges from 31-57.8% (WHO, 2003 as cited in Borkow and Bentwich, 2004). But there was lack of empirical sources on intestinal helminthiasis among school children in Bokoji, Eastern Ethiopia. There were no previous studies which show the associations between anthropometric indices (child growth) and intestinal parasite infections among school children. Therefore, the present study was undertaken to investigate the association of intestinal parasite infections with anthropometric status (child growth) in Bokoji Primary school children.

For this reason, the present study was aimed to provide the current epidemiological information on STH with respect to prevalence, intensity and association with anthropogenic measurements of school children in Tulu Nageso, around Bokoji in Eastern Ethiopia. Therefore, this study was believed to provide recent and valuable information to be used as a base line data for further study on the area.

Objective of the study

General Objective

To determine the prevalence and intensity of soil transmitted helminth parasitic infection and their association with anthropogenic measurements of school children in Bokoji town, Arsi Zone, Oromia Region

Specific objectives

1. To identify the major soil-transmitted helminth parasites among school children in the study area.
2. To determine the prevalence of soil transmitted helminth parasitic infections among school children in the study area.
3. To determine the intensity of soil-transmitted helminth parasitic among school children in the study area;
4. To determine the association between the prevalence and intensity of soil-transmitted helminth infections with anthropometric measurement of primary school children in the study area.

2. LITERATURE REVIEW

2. 1. Soil-Transmitted Helminthes Parasites Infections

The term 'soil-transmitted helminthes' include various nematodes together that infect humans and share a common source of infection - soil contaminated by faecal matters. The main species are *Ascaris lumbricoides, Trichuris trichiura* and the hookworms (*Necator americanus* and *Ancylostoma duodenale*) Hotez *et al*., 2008). They are considered together because it is common for a single individual, especially a child living in a less developed country, to be chronically infected with all three worms. Such children have malnutrition, growth stunting, intellectual retardation, and cognitive and educational deficits (Parajuli *et al*., 2009).

The adult worms share a common location the intestinal tract and their numerous eggs reach the environment via the faeces but they differ in their modes of transmission and infection. *A. lumbricoides* and *T. trichiura* are transmitted orally by ingestion of mature eggs. After hatching, *T. trichiura* larvae directly reach the colon and mature while *A. lumbricoides* larvae leave the intestinal tract, migrate through various organs including the lung, ascend the trachea, are swallowed and again reach the gastro-intestinal system where they develop into adult worms (Bethony *et al*., 2006).

N. americanus and *A. duodenale* eggs can be found in warm, moist soil where they will eventually hatch into first stage larvae, or L1. L1, the feeding non-infective rhabditoform stage, will feed on soil microbes and eventually molt into second stage larvae, L2. L2, which is also in the rhabditoform stage, will feed for approximately 7 days and then molt into the third stage larvae, or L3. L3 is the filariform stage of the parasite, that is, the non-feeding infective form of the larvae. The L3 larvae are extremely motile and will seek higher ground to increase their chances of penetrating the skin of a human host. The L3 larvae can survive up to 2 weeks without finding a host (Reji *et al*., 2011).

A. duodenale worms are grayish white or pinkish with the head slightly bent in relation to the rest of the body. This bend forms a definitive hook shape at the anterior end for which hookworms are named. They possess well developed mouths with two pairs of teeth. While males measure approximately one centimeter by 0.5 millimeter, the females are often longer and stouter. Additionally, males can be distinguished from females based on the presence of a prominent posterior copulatory bursa (Markell *et al.,* 2006).

N. americanus is very similar in morphology to *A. duodenale*. *N. americanes* is generally smaller than *A. duodenale* with males usually 5 to 9 mm long and females about 1 cm long. Whereas *A. duodenale* possess two pairs of teeth, *N. americanes* possesses a pair of cutting plates in the buccal capsule. Additionally, the hook shape is much more defined in *Necator* than in *Ancylostoma* (Markell *et al.,* 2006).

Ascaris lumbricoides is characterized by its 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 micrometers long and 35-50 micrometers wide with a thick outer shell. Unfertilized eggs measure 88-94 micrometers long and 44 micrometers wide (Roberts *et al*., 2009).Adult *Trichuris trichiura* are approximately 40-50 mm in length, the posterior end is thick and the anterior 2/3 of the body is slender, giving a “whip like” shape to the worm; hence the name whipworm (Reji *et al*., 2011).

2.2. The Life Cycle of Soil-Transmitted Helminth Parasites Infections

Soil-transmitted helminths are group of parasitic nematode worms causing human infection through contact with parasite eggs or larvae. The life cycles of ascaris, trichuris, and hookworm follow a general pattern. The adult parasite stages inhabit the gastrointestinal tract (ascaris and hookworm in the small intestine; trichuris in the colon), reproduce sexually, and produce eggs which are execrated with human faeces and deposited in the external environment (Stephan and Richard, 2001). Adult worms survive for several years and produce large number of eggs after 4-6 weeks. Eggs can remain viable in the soil for several months (A. *lumbricoides* and T.*trichiura*) and larvae several weeks (hookworm), dependent on prevailing environmental conditions. They share a common location, the intestinal tract and their numerous eggs reach the environment via the faeces but they differ in their modes of transmission and infection.

A. lumbricoides and T. *trichiura* are transmitted orally by ingestion of mature eggs. After hatching, *T. trichiura* larvae directly reach the colon and get matured while *A. lumbricoides* larvae leave the intestinal tract, migrate through various organs including the lung, ascend the trachea, are swallowed and again reach the gastro-intestinal system where they develop into adult worms. Hookworm eggs already hatch in the soil and third-stage larvae (L3) penetrate the human skin and reach the blood circulation. After migration that resembles the way taken by *A. lumbricoides* larvae, they also settle and mature in the intestinal tract (Bethony *et al*., 2006).

2.3. Morbidity and Public Health Effects due to Soil-Transmitted Helminth Parasite Infections

Light and moderate soil-transmitted helminth infections are often associated with little or no acute disease whereas heavy infections can lead to life-threatening conditions like intestinal obstruction (*A. lumbricoides*), acute dysentery (*T. trichiura*) and severe blood loss and anemia (hookworm). Of particular public health significance are the chronic impairments resulting from untreated infections of any intensity. However, these chronic and unspecific health effects are often difficult to measure and their association with a particular parasite is even more challenging (Worku *et. al.,* 2009). Since *A. lumbricoides* and *T. trichiura* prevalence and infection intensities peak among children and young adults and uncontrolled hookworm prevalence remain high throughout adulthood, most effects resulting from infections with the former two parasites are seen among children while hookworm related morbidity is also found in adults, particularly women of child-bearing age (Hotez *et al.,* 2006).

Intestinal parasitic infections are considered a public health problem of worldwide importance for reasons of their high prevalence, widespread distribution, and effects on health (Worku *et. al.,* 2009). Intestinal parasite infection risk is reported to be elevated in infants and children compared to other age groups. In addition, they are reported to disproportionately suffer from the nutritional health, and developmental consequences of intestinal parasitic infections (Worku *et. al.,* 2009). Morbidity and mortality caused by intestinal parasitic infection is usually more pronounced in children compared to adults due to their higher nutritional requirements and less mature immune systems (Guyatt, 2000). Furthermore, the risk for poor clinical outcomes is reported to be increased in those children who were already malnourished prior to becoming infected (Stephenson *et al*., 2000a). The chronic malnutrition-intestinal parasitic infection cycle which commonly begins during childhood in many developing populations also has been linked with decreased work capacity and productivity in adolescents and adults **(**Guyatt, 2000).

2.3.1. Infection Intensity

The infection intensity is not uniformly or randomly distributed among individuals. It is highly over-dispersed. In other words, most individuals have a few or no worms, while a few hosts harbor a disproportionate number of worms. This has been described for all of the major intestinal nematode species: *A.lumbricoides*, hookworm, and *T.trichiura* (Reji *et al*., 2011).

The heavily infected individuals are often described as 'wormy individuals' and these people are both at risk of the more severe complications of infection and act as an important source of transmission. The reasons for over-dispersion are both genetic and environmental, although it is often difficult to tease these different components apart, as families not only share their genes but their environment. It is clear that the risk of re-infection is related to the pre-treatment infection density, and that is also true within families'. The risk of infection depends on behavioral factors that are notoriously difficult to study (Brooker *et al.,* 2006).

There is no simple relationship between prevalence and intensity. For most helminth species, the initial rises in intensity with age closely mirror that of prevalence. Maximum intensity occurs at a host age which is parasite and species-specific and depends on the basic biology of the parasite longevity and is independent of local transmission rates. For Ascaris and Trichuris, maximum worm burdens occure at 5-10 years of ages, where as for Hookworms it occurs at 20-25 years of age (Saathoff *et al*., 2004).

2.3.2. *Ascaris lumbricoides*

Ascaris lumbricoides, a soil transmitted round worm, is reported to infect at least one fourth of world's population (Crompton, 1994). In most countries where geo-helminths are endemic, school age children experience the highest prevalence and intensity of infection, particularly with *A. lumbricoides* and *T. trichiura* (Jukes *et al.,* 2008). Annual morbidity associated with this parasite has been estimated by WHO at 60,000 with another 250 million people said to be at risk for acquiring the infection age (Saathoff *et al*., 2004).

Both domestic and wild animals are common reservoirs for *A. lumbricoides. A. lumbricoides* is a robust parasite due to the resilient nature of its eggs, which are capable of surviving a wide range of hot and cold temperatures, chemicals, chemical disinfectants and other extreme conditions (Neva and Brow, 1994). The eggs of ascaris are one of the resilient of the helminth eggs and can remain infective for many years embedded in the soil (Gilgen and Mascie-Taylor, 2000).

Ascaris is the largest of the human intestinal parasitic nematodes, in which both mature male and females usually inhabit the jejunum, where they feed on the-semi- digested food present in the host (Neva and Brow, 1994). They also produce or secrete antitrypsin and thus are capable of adequately competing with the host system for ingested proteins and resist the normal peristaltic movement of the gut by assuming an S-shaped configuration, pressing their outer cuticle against the columnar epithelium of the host (Katz *et al*., 1989).

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 fruits and vegetables, which are washed with contaminated water or fertilized with contaminated night soil. In highly endemic areas, ascaris eggs may be found on eating utensils, or under the fingernails. They may also be found in household dust and air where they are inhaled or swallowed (O'Lorcain and Holland, 2000).

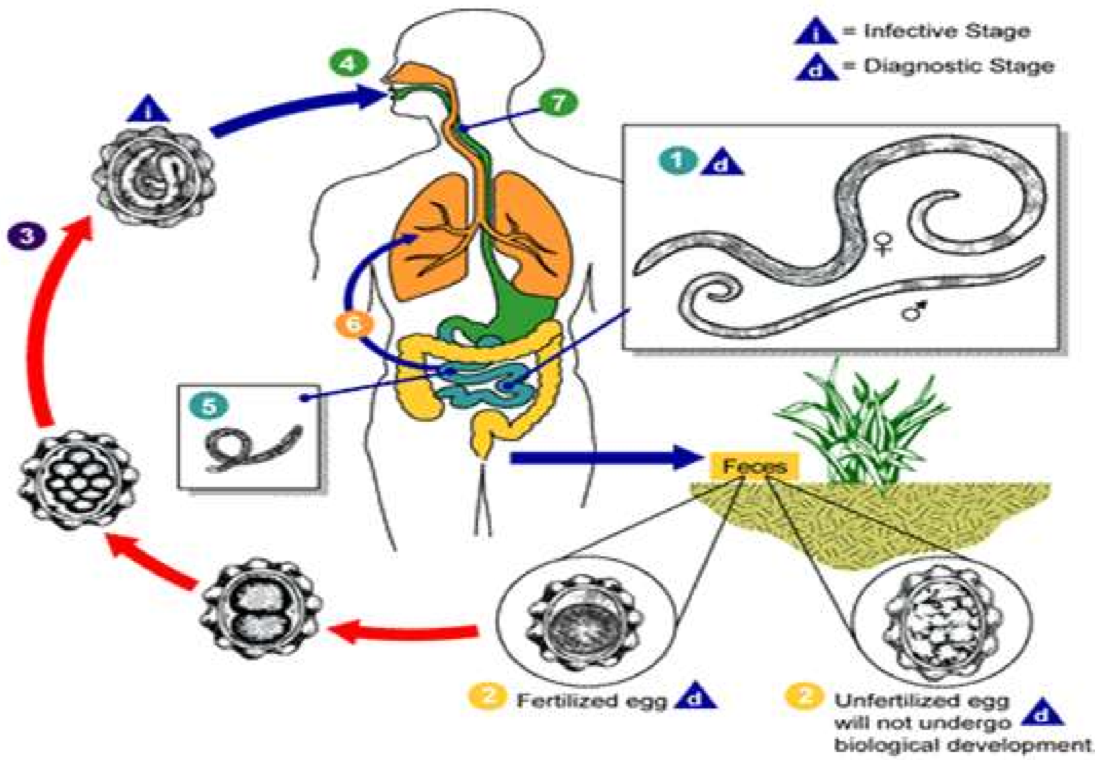

Figure 1: Life cycle of *Ascaris lumbricoides* (Source: CDC, 2012)

The location and burden of worms mostly determine the type and degree of morbidity observed in the host. During the migratory phase, large numbers of larvae may include host sensitization, resulting in asthma, coughing, and shortness of breath, fever, skin rash, and eosinophilia(Parajuli *et al*., 2009). After the worm gets matured in the intestine, clinical signs and symptoms may include abdominal pain and distension, nausea, vomiting and anorexia.

In young children, adult worms can aggregate in the ileum and cause partial obstruction, because the lumen is small. *A. lumbricoides* has been observed to decrease micronutrients and vitamin A absorption, probably by causing a structural abnormality of the mucosa in the small intestine (Curtale *et al*., 1995 and Stacky, 2001).

2.3.3. Whipworm (*Trichuris trichiura*)

Trichuriasis or whipworm infection is caused by *Trichuris trichiura*. The infection is estimated to affect around 347 million persons worldwide. Of these, 114 million are children of pre-school age and 233 million are of school age (Chan, 1997). Humans are the primary host for infections caused by *Trichuris trichiura* but the species has been detected in some non-human primates (Horii and Usuii, 1985).

The infection of specific host with *Trichuris trichiura* occurs through the oral-fecal route caused by ingestion of infective eggs from contaminated foods, hands or water. Eggs then pass through the stomach to the small intestine where they hatch. The larvae penetrate the cells of the small intestine and coming to lie above the lamina propria where they undergo number of molts (Kataz *et al*., 1989; Neva and Brow, 1994). The cecum is the preferential site for invasion although heavy infections will extend throughout the colon and even distally to the rectum to obtain nutrients, particularly for the mature parasites (Gilgen and Mascie-Taylor, 2000; Hotez, 2000). It feed on the enterocyte synctium and may also ingest enterocytes, leucocytes and mucosal fluids (Pawloski, 1984.)

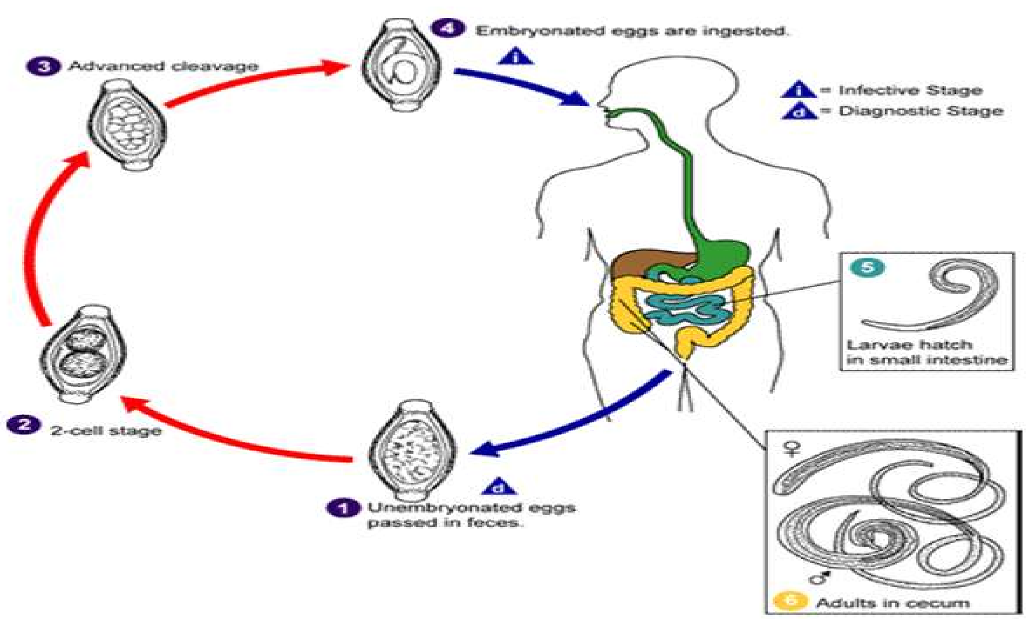

Figure2: Life cycle of *Trichuris trichiura* (Source: CDC, 2011)

The adult parasite leads both an intracellular and an extracellular existence, with the anterior end embedded in epithelial tunnels within the intestinal mucosa and the posterior end located in the lumen. Inflammation at the site of attachment from large numbers of whipworms results in colitis. Longstanding colitis produces a clinical disorder that resembles inflammatory bowel disease, including chronic abdominal pain and diarrhea, as well as the sequel of impaired growth, anemia of chronic disease, and finger clubbing Trichuris dysentery (Parajuli *et al*., 2009). Syndrome is an even more serious manifestation of heavy whipworm infection, resulting in chronic dysentery and rectal prolapsed.

2.3.4. Hookworm

Hookworm infection in humans is caused by two species, namely *Necator americanus* and *Ancylostoma duodenale* are transmitted through contact with contaminated soil with third-stage infective larvae, which either penetrate the skin (*N. americanus* and *A. duodenale*) or when they are ingested (*A. duodenale*) (Hawdon and Hotez, 1996).

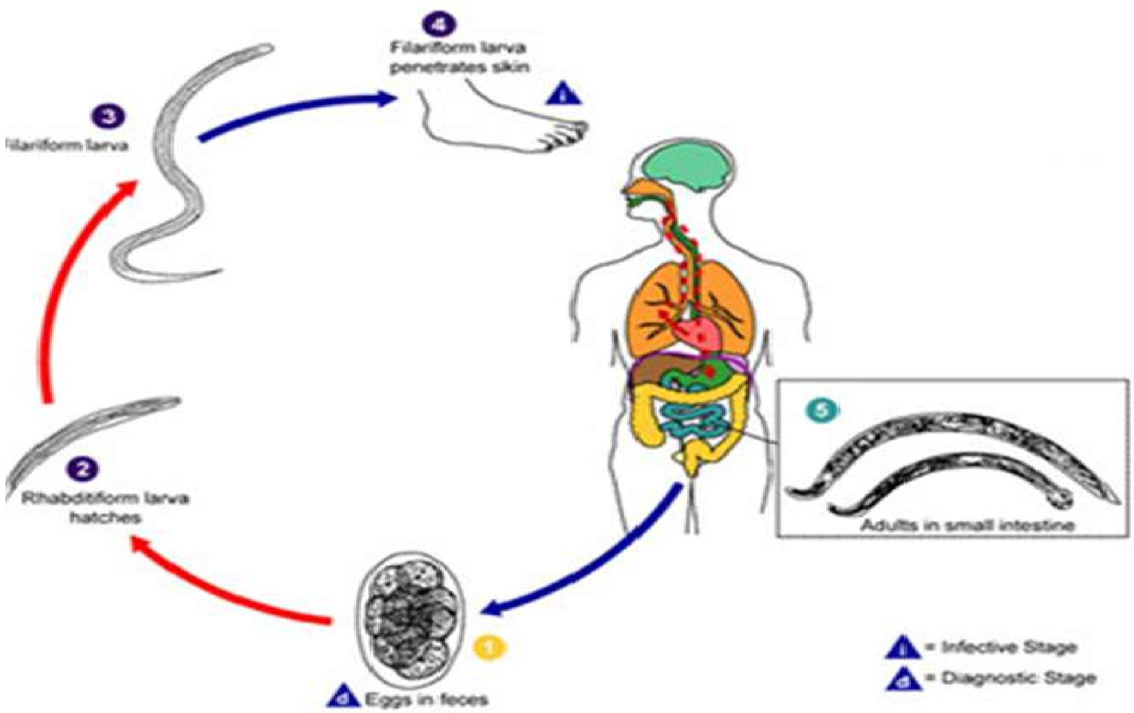

Figure3: Life cycle of Hookworm (Source: USAID, 2010)

Hookworm infection is one of the most common chronic infections with an estimated 1.3 billion cases globally and accountable for 65,000 deaths annually (Silva *et al*., 2003). The presence of more than 40 adult worms in the small intestine is estimated to be sufficient to reduce host hemoglobin concentrations below 11 g/dl, (Lwambo *et al*., 1992); although the exact number depends on several factors including the species of hookworm. *A. duodenale* causes more blood loss and reduce the host iron reserves than *N. americanus* (Hotez *et al*., 2004).The clinical manifestations of hookworm disease resemble those of iron-deficiency anemia from other causes.

Soil-transmitted helminth infections are widely distributed throughout the tropics and subtropics. Recent estimates suggest that *A. lumbricoides* infects 1.221 billion people, *T. trichiura* 795 million, and hookworms 740 million world widely. The greatest numbers of soil-transmitted helminth 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*., 2003). Based on previous estimates derived in 2002 (de Silva *et al*., 2003), it is estimated that 198 million people in Sub-Saharan Africa (SSA) are infected with hookworm (29% of the region's population), including 40–50 million school-aged children (Brooker *et al.,* 2006; Brooker *et al*., 2006). Approximately one-third of the world's hookworm today occurs in SSA, with the greatest number of cases occurring in Nigeria (38 million) and the Democratic Republic of Congo (DRC, 31 million), followed by Angola, Ethiopia, and Cote d'Ivoire (10–11 million). Hookworm is the most widely distributed (Neglected Tropical Disease) NTD in SSA and it is pervasive throughout the region (including both rural and urban areas) except in some parts of extreme southern Africa (Brooker *et al.,* 2006).

Compared to hookworm, both ascariasis and trichuriasis exhibit a more patchy distribution in SSA, with the highest prevalence occurring in equatorial Central and West Africa, eastern Madagascar, and southeast Africa (Brooker *et al.,* 2006). In contrast to the high rates of ascariasis and trichuriasis in South Africa (Saathoff *et al*., 2004), hookworm is less common except in KwaZulu-Natal (Mabaso *et al*., 2004). 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, 2000).

Equally important determinants are poverty and inadequate water supplies and sanitation (de Silva *et al.,* 2003)

2.4. Factors that Influence Transmission of Soil-Transmitted Helminth Parasites

Among the conditions influencing the development of these infections are poor sanitary conditions, lowering resistance of the host, population explosion, inadequate control of vectors and infection of reservoirs, increased migration, and military operation and travelling around the world are quite common (Jongsuksuntigul *et al.,* 1993; Olsen *et al*., 2001 and Stephenson *et al*., 2002). This disease can undermine child development, educational achievement, reproductive health and social and economic development (Allen *et al*., 2002 and Bennet *et al*., 2000,) and some of these parasitic infections can cause morbidity and mortality (Worku *et. al.,* 2009).

Infection occurs through accidental ingestion of eggs or penetration of the skin by infective stages of STHs. The transmission of ascariasis and trichuriasis are by the fecal - oral route of infected food or water ingestion. Similarly, enterobiasis and hymenolepidiasis are also transmitted through ingestion of fecally contaminated food or water with infective stages of egg of the worm. The transmission of hookworm infections and strongyloidiasis involves filariform larval penetration of any parts of the skin exposed to contacts with contaminated soil or water (Asaolu and Ofoezie, 2003). But, the larvae of *A. duodenale* are acquired by both skin penetration and ingestion of contaminated water/food, whereas the larvae of *N. americanus* are acquired exclusively by skin penetration (WHO, 1990).

Soil type favorable for the development of larvae of *N. americana* is sandy clay soil, while sandy loam soil is conducive for the larvae of *A. duodenale.* Ascaris' eggs development requires hard clay soil (Kloos and Tesfayohanis, 1993). In general, the environmental phase for most of the parasites has specific requirements but they have also high possibility to adapt themselves to different environmental conditions (WHO, 1980).

Poor handling of personal hygiene and environmental sanitation, use of human excreta as fertilizers, lack of shoes, low household income, overcrowding living style and limited access to clean water are the major causes that favor intestinal worm infections (Bhargava, 2001; MOH, 2004; Wordermann *et al.,* 2006; Mengistu *et al.,* 2007; Ostan *et al.*, 2007; Gamboa *et al.*, 2009; Niyyati *et al.*, 2009).

2.4.1. Environment

Climate and topography are crucial determinants for the distribution of helminth infections (Brooker, 2007). Helminths transmitted by vectors are limited to landscapes in which host and vector come together in the same habitat, resulting highly focal distribution. In the case of onchocerciasis, the distribution and incidence of the disease are limited by bio-geographic variations favorable to exposure to the black fly vectors (Bockarie and Davies, 2000; Bockarie *et al.,* 2000). Soil-transmitted helminths are highly affected by surface temperature, altitude, soil type, and rainfall (Brooker, 2003). 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 (Brooker and Michael, 2003).

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). Equally important determinants are poverty and inadequate water supplies and sanitation (de Silva *et al.,* 2003). In such conditions, soil transmitted helminth species are commonly co-endemic. There is evidence that individuals with many helminth infections have even heavier infections with soil transmitted helminthes (Raso *et al*., 2004).

2.4.2. Heterogeneity

Heterogeneity in the worm burden among different individuals infected with the same helminth is a hallmark feature of helminth epidemiology. A consequence of such heterogeneity is the aggregated distribution of helminth infection in endemic communities, such that a small proportion of hosts are rapidly, frequently, and/or heavily infected (Brooker *et al.,* 2006).

For example, 70% of the worm burden occurs in 15% of the infected individuals at a given time point (Albonico *et al*., 1999). The aggregated distribution of helminth infection has led some to hypothesize that certain "wormy" people are "predisposed" to heavy infection from as yet undefined genetic, immunogenetic, ecological, behavioral, and social factors (Parajuli *et al*., 2009). Predisposition refers to studies in which intensity of infection prior to anthelminthic treatment positively correlates with intensity of re-infection 12–24 months after treatment (Schad and Anderson, 1985). The bases of both heterogeneity and predisposition to helminth infection have yet to be fully elucidated. However, among the major factors under consideration are age, household clustering, and genetics.

2.4.3. Age Dependency

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, 2002).

Much epidemiologic research has focused on heterogeneity in the intensity of helminth infection by age (Chan *et al*., 1996). Changes with age in the average intensity of infection tend to be convex, rising in childhood and declining in adulthood. For *Ascaris lumbricoides* and *Trichuris trichiura,* the heaviest and most frequent infections are in children aged 5–15 years, with a decline in intensity and frequency in adulthood (Gilles, 2003). In contrast, hookworm frequently exhibits a steady rise in intensity of infection with age, peaking in adulthood (Bethony *et al*., 2002).

2.5. Diagnosis of Soil Transmitted Helminth Parasite Infections

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

For the control of the neglected tropical diseases such as the food-borne trematodiases and schistosomiasis and the common soil-transmitted helminthiasis, there is tendency to emphasize research development of new drugs and vaccines, while the critical importance of quality-assured diagnostic tests is receiving far less attention. Moreover, assessment of efficacy of intervention and verification of disease elimination and early warning systems strongly depends on reliable diagnostic assays (Peeling *et al.,* 2006). The diagnosis of soil-transmitted helminthes parasite infections usually relies on finding the eggs of the parasite in the stool of infected individuals (Raso *et al.,* 2004).

In the diagnosis of intestinal parasites, a wide variety of laboratory methods can be employed (Ahmadi *et al*., 2007). The choice of a particular technique for routine use is influenced by its affordability, simplicity, sensitivity and level of professionalism or technical skill involved (NCCLS, 1997; WHO, 2000). Stool microscopy using direct wet mounts, formal-ether concentration and the Kato-Katz technique offers many relative advantages over other diagnostic methods for detecting intestinal parasites (Parija and Srinivasa, 1999; Bogoch *et al*., 2006). Since infections with multiple helminth species are norm rather than exception in the developing world, there is a need for well-trained laboratory technicians and quality control measures to ascertain accurate, specific diagnosis (Raso *et al.,* 2004).

2.6. Treatment of Soil Transmitted Helminth Parasite Infections

Recommended drugs used in the treatment of soil-transmitted helminthes parasites are albendazole, mebendazole; older drugs including pyrantel, tiabendazole and niclosamide are also recommendable (Heelan, 2004). Infections can be treated with drugs called ascaricides. The treatment of choice is Mebendazole. The drug functions by binding to tubulin in the worms' intestinal cells and body-wall muscles. Nitazoxanide and ivermectin can also be used (Roberts *etal.* 2009). Piperazine citrate is also used to paralyzes the worms (Maguire, 2005) rendering them unable to resist the peristalsis action of the host's intestine and so are expelled in the feces. This drug is highly effective and was once the drug of choice for treating intestinal obstruction; however, it can be neurotoxic and hepatotoxic and is no longer widely available.

Growth stunting is sometimes reversible with specific anti-helmintic treatment and supplemental oral iron. Intellectual and cognitive impairments and delays are also associated with chronic heavy infections (Ramzan *et al.,* 2009).These deficits sometimes reverse following anti-helminthic therapy, particularly when treatments are linked to psychological support (Stephenson *et al.,* 2000).

2.7. Prevention and Control of Soil Transmitted Helminth Parasite Infections

The regulation of helminth populations is a complex process, influenced by host immunological and nutritional status, age and breed of the animal. The interaction between helminth parasite infections and nutrition can be considered from two interrelated points of view: the influence of the helminth parasite infections on the host's physiology and nutrition and the effect of host nutrition on the helminth populations, *i.e.* their establishment, persistence and reproductive capacity (Kaunas, 2007).

A well structured control strategy needs to be based on local and accurate data concerning the epidemiology, definition of appropriate chemotherapy and health education campaign sanitation monitoring and evaluation program (Keiser and Utzinger, 2008). All these component need to be integrated into the prevailing system of primary health care and must be based on multisectoral collaboration (WHO, 2008),a goal often difficult to carry out in practice, which is why it is common to find control programs based on some of these elements and with limited results. The basic control programs, oriented for treating patients' chemotherapy, sanitation, oriented to stop or disconnect the exposure to the oral faecal transmission, and education, the key used as an instrument to implement the two ways of control programs.

Control programs based on sanitation aim to reduce or interrupt transmission, prevent re-infection and gradually reduce worm loads (Bundy, 1994). 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 programmers 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.

2.7.1. 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 (Ramzan *et al.,* 2002).

The development of effective helminth control is possible because of the availability of proven, cost-effective and logistically feasible intervention strategies. A key issue for the optimal use of an anti-helmintic drug is to decide when and how frequently to treat the population of concern. Common drugs used are albendazole, pyrantel, mebendazole, tiabendazole and niclosamide (O'Lorcain and Holand, 2000). Others drugs used are benzimidazoles, levomisole and praziquantel (Brooker *et al.,* 2004)

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was conducted in Bokoji Town, Arsi Zone, which is located at 231km east of Addis Ababa. Bokoji town is bordered by Lemmu and Bilbilo kebeles or neighbouring kebeles in the East by Chiba Michael in the West by Taminya Aware in the South by Dewa Bursa and in the North by Bokoji Nageso (Fig.1). The study area was chosen because the school is located at the center of the cluster schools and is the most accessible site for the study. The schools have contained two shifts, from grade one to four and grade five to eight other shifts. The toilet availability, water availability, total facility and sanitary status are well good at the study areas.

The altitude of the study area is 2740 meters above sea level. The mean annual rainfall is between 1000 mm to 2000 mm; and reaching its peak from end of July to the middle of August. The type of soil in the study area is clay soil and loam soil. The weather is *Woinadega* with the temperature ranging from 12^{o}C to 26^{o}C. Bokoji town covers a total area of 3409 hectare (Bokoji Government Communication Affairs, 2014). As presented in the 2014 annual reports of the Bokoji Governmental Communication Affairs, the total population was 53,250 people, of whom 26,806 were females and 26,444 were males, respectively. The main sources of income in the area are trade and agriculture, where the farming system is characterized by mixed small-scale production of crops and livestock.

There is one secondary and preparatory school, and three primary schools in Bokoji town. The study was carried out in one primary school namely, Tulu Nageso Primary School. There were different organizations to provide social services where people get health services: one governmental health center, one new higher hospital which has started functioning since 2007 E.C. Five medium private clinics also provide health services for the community.

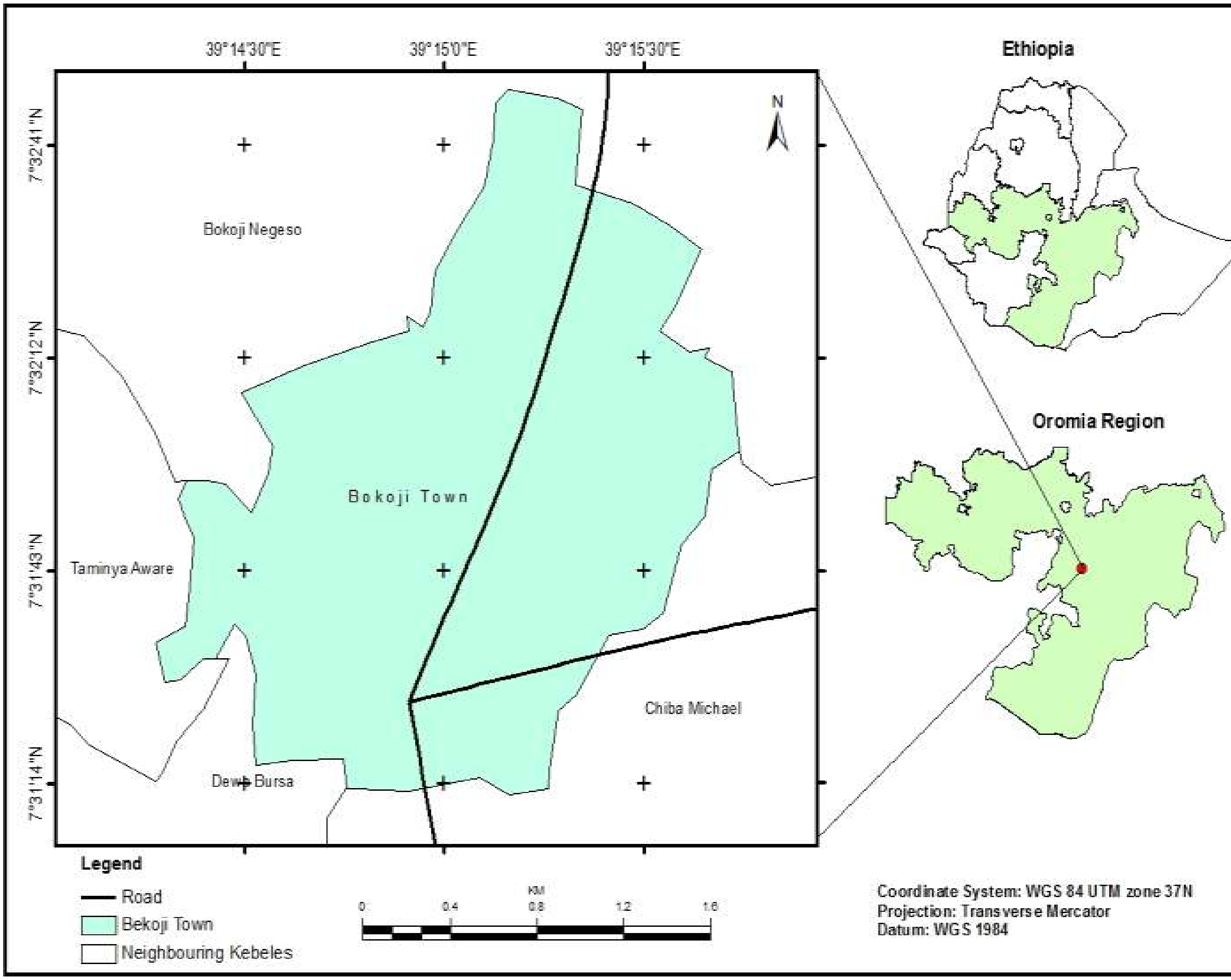

Fig.1.Map of the study area (Source; Bokoji Government Communication Affairs, 2014)

3.2. Study Design

The study design was a cross-sectional parasitological survey (which involved laboratory investigation) of soil-transmitted helminth parasitic infections of school children from Tulu Nageso Primary School. The study was conducted from February-July, 2016. Prevalence and intensity of soil-transmitted helminth parasitic infections of school children in the study area as well as the association of prevalence and intensity with anthropometric measurements of school children were determined.

3.3. Study Population and Sample Size Determination

In order to determine the sample size, widely used statistical formula was employed. Accordingly, the sample size (n) was determined using the following formula $(Z\alpha/2)^2$ p (1-p)/d^2 (Kish and Leslie, 1965). Since the overall prevalence rate (p) of soil-transmitted helminth parasite infections was not known for the study area, p was taken to be 50%. For the calculation, a 95% confidence interval (z) and a 5% margin of error (d) was used.

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

$$n = (1.96)^2 0.5 (1-0.5)/0.05^2 = 384$$

Where, n = sample size, P = prevalence of STHs (50%)

d = marginal error b/n the samples and population (0.05)

Z α/2 = critical value at 95% certainty (1.96),

Therefore, three hundred eighty four (384) study participants were included in the present study, (Table 1)

Stratified random sampling method was utilized to select the sample population. The students were first stratified according to their grade level (grade 1 to 8). A quota was allocated for each grade level and each classroom. A random sampling technique was employed to select students from each grade and classroom by using class rosters as the sampling frame.

Exclusion criteria: Students who were allowed to participate voluntarily in the study were those who never had treatment for any intestinal parasitic helminth infections during the last 3 months and those who were attending classes at the time of sample collection.

3.4. Sampling Techniques

The total population of grade 1-8 students aged 6-16 year enrolled during the 2015/2016 academic year in Tulu Nageso Primary School was **1057**. Of these, **551** were females and **506** were males (Table 1).

To select the study participants, the students were first stratified according to their grade level (1 to 8). Proportional sample size was allocated for each grade and each class room. Finally, the sample children were selected using stratified random sampling method.

Table: 1. Total population of the school and sample size of the study from the Selected school

School Name	Grades	Student population			Sample population		
		Male	Female	Total	Male	Female	Total
Tulu Primary School	1-4	330	383	713	124	125	249
		176	168	344	61	74	135
Total	1-8	506	551	1057	185	199	384

3.5. Methods of Data Collection

3.5.1. Stool Sample Collection

Before collecting the stool sample the aim of the study was explained to the school principal, other concerned authorities and students. During the stool collection, young children were provided with a small plastic sheet and applicator (very young children were provided with stool cup), and instructed to bring proper stool sample, the size of the tip of the thumb/the size of soya bean, about 3g of fresh stool. A portion of the specimen was processed using Kato- Katz technique (Peters, *et al.,* 1980; Birrie and Medhin, 1996). Microscopic examination of stool sample and counting of helminth ova was done on the same day of collection. Duration between sampling stool and processing was morning and afternoon, respectively.

3.5.2. Anthropometric measurements of school children

Anthropometric measurements (height and weight) of the selected participants (school children) consisting of both sexes, was recorded by employing standard procedures mentioned in Gibson (2005). Weights and heights of the pupils were recorded using a scale to the nearest 0.1 kilograms (kg) and 0.1 centimeters (cm) which used balance and meter, respectively, and the pupils were made to wear minimum clothing and no shoes. Anthropometric data of body mass index (BMI) was calculated by the formula: BMI=weight (kg)/height ^{2}(m^2). The anthropometric measurements were categorized into wasting, stunting, underweight, and BMI as per the guidelines of the WHO and wasting, stunting and underweight was defined as Z-score values of less than -2 SD (Standard Deviation), which was below the expected level on the basis of the international growth reference scale (Rolland-Cachera, 1993). Height- for-age, Weight-for-age and Weight-for- height denote stunting, wasting and underweight, respectively (WHO, 2006). Two different sets of nutritional indicators were used as recommended by World Health Organization for 6-9 years and above (adolescent) 10-18 years. Nutritional values below -2Z-scores (Standard deviations) were taken as cut-off points for being malnutrition as recommended by WHO, (1995).

$$\text{BMI}=\frac{Weight(kg)}{Height(metersquer)}$$

Applying the classification of the World Health Organization (2000), the BMI scores can be categorized into four main groups:

- Underweight group (< 20.0 kg/m2);
- Normal (≥ 20.0 and < 25 kg/m2);
- Overweight (≥ 25 and < 30 kg/m2);
- Obese (≥ 30 kg/ m2).

3.6. Parasitological Examination Procedure

3.6.1. Macroscopic examination of the stool

The collected stool sample were physically characterized based on consistency, color, presence and absence of parasites and other macroscopic features by the health personnel. The consistency of the stool was categorized as formed, soft, loose or watery.

3.6.2. Direct Wet Mount Technique

Direct smears were prepared with normal saline for microscopic examination. About 1g of stool sample was emulsified with 3-4 ml normal saline and a drop of emulsified sample was added on a glass slide, then a few drop of iodine solution were added and was covered with a cover slip. The preparation was first examined by 10X objective lens, then 40X for detailed identification of parasites under low light intensity by using mono ocular microscope. After STH eggs were identified, those positive individuals were treated with appropriate drugs by health professionals in the study area.

3.6.3. Kato-Katz Method

Briefly, the Kato template (with hole) was placed in the centre of a microscope slide. With a wooden applicator stick, a small amount of faecal material was placed on the "Pergamon" absorbent sheet and a small sized nylon mesh screen was pressed on top of the faecal material so that, some of the faeces was sieved through the screen and accumulated on top of the screen. A spatula was used to scrape across the upper surface of the screen so that sieved/filtered faeces were accumulated on the spatula.

The collected sieved faecal specimen was added in the hole of the template so that it was completely filled. The side of the spatula was used to pass over the template to remove excess faeces from the edge of the hole. The template was carefully removed from the slide so that a cylinder of faeces was left on the slide. The faecal material was covered with pre-soaked hydrophilic cellophane strip (Bogoch *et al*, 2006).

The microscope slide was then inverted and the faecal sample was firmly pressed against the hydrophilic cellophane strip on a smooth hard surface (a glazed tile), to spread faecal material evenly. The slide was carefully removed by gently sliding it sideways to avoid separating the cellophane strip or lifting it off. The slide was placed on the bench with the cellophane upwards. After 30 minutes incubation at room temperature (allowing water to evaporate and glycerol to clear the faecal material), the Kato thick smear was microscopically examined, with the X10 and X40 objectives, in a systematic manner so that the entire cover slip area was observed, and the number of eggs of each species was recorded. Having used what WHO recommended, 41.7 mg template (WHO, 1991), the number of eggs per gram (epg) of faeces was obtained by multiplying the number of eggs found by a factor of 24.

In the subjects studied, the intensity of helminth parasitic infection was measured by using the Kato-Katz technique (Galvani, 2005). Intensity of infection was estimated indirectly by counting the mean number of eggs per gram of faeces (epg) (Brooker *et al*. 2007), and categorized using thresholds recommended by the World Health Organization. Accordingly, it was indicated for hookworm infections to be light (1 – 1999 epg), moderate (2000 – 3999 epg), and heavy infection (≥ 4000 epg), for *A.lumbricoides* light, (1 - 4999 epg), moderate (5000 - 49,999 epg and heavy infection (≥ 50,000 epg), for *T. trichiura* light, (1 - 999 epg), moderate, (1000 – 9999 epg) and heavy (≥ 10,000 epg). Soil-transmitted helminth infections of moderate and high intensity in the gastrointestinal tract produce clinical manifestations, with the highest intensity of parasites being show in children (Chan *et al.*, 1994).

3.7. Methods of Data Analysis

SPSS, Windows version 16, was used for data analysis. Anthropometric indices were computed using the calculator mode of anthropometry calculating software, AnthroPlus (WHO, 2007). Descriptive statistics such as percentage, frequency, standard error of mean, mean and range were determined for each soil-transmitted infection. Descriptive and inferential (chi-square) statistical test was used to examine differences for proportions. Wasting, Stunting and underweight was defined as Z score values of less than-2SD (Standard Deviation), which was below what was expected on the basis of the international growth reference scale (Dean *et al*., 1994). The significance of the differences in the frequency distribution was tested by using chi-square analysis. P-values less than 0.05 were considered, statistically significant.

3.8. Data Quality Control (QC)

To ensure quality, all the laboratory procedures including collection and handling of specimens were carried out in accordance with standard protocols (WHO, 1991; NCCLS, 1997). To ensure general safety, disposable gloves were worn and universal bio-safety precautions (NCCLS, 2002) were also followed at all times.

The calibration factors for the 10x and 40x objectives was posted on the microscope for easy access; and 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 WHO (1994), and diagrams of various parasite ova and larvae from the parasitological was reviewed.

3.9. Ethical Consideration

Ethical approval for Monitoring & Evaluation (M & E) surveys was obtained from Arsi Health Bureau. Before the data collection period, a letter was written by Arsi Health Bureau about the objective of the study to the Bokoji Health Office, Educational office and Tulu Nageso primary school.

At the beginning of the study, the objectives and the purpose of the study was explained to the school principal, other concerned authorities and students. Only a laboratory technician was taken the stool sample and all activities in clinical examination as well as diagnosis were supervised by health personnel. Individuals diagnosed positive for STH infections was treated free of charge with anti-helminths drug (Mebendazole) prescribed by the physician which was donated by the sites of health centre. Laboratory samples were taken from the pupils of the selected area and signed on consent form.

4. RESULTS AND DISCUSSION

4.1 Age and Sex Distribution of the Study Participants

The age distribution ranged from 6 to 16 years; 163 (42.44%) students were in the age group 6-9 years, 177(46.1%) were in the age group of 10-12 years and 44(11.5%) were in the age group of 13-16 years old (Table 2). A total of 185 (48.2%) of the respondents were males whereas 199 (51.8%) were females (Table 2).

Table2. The age and sex distribution of the study participants in Tulu Nageso Primary School, Eastern Arsi, Ethiopia,

Age group(in years)	Male	Female	Both Sexes
	Number (%)	Number (%)	Number (%)
6-9	87(53.3)	76(46.6)	163(42.4)
10-12	78(44.0)	99(55.9)	177(46.1)
13-16	20(45.4)	24(54.5)	44(11.5)
All age groups	185(48.2)	199(51.8)	384(100)

4.2. Prevalence of Soil-Transmitted Helminth Parasitic Infections among School Children

The prevalence of soil-transmitted helminth parasitic infections among primary school children at Bokoji town by age and sex of the study participants is presented in Table 3. Of the total 384 children examined, 19.7 % (76/384) were positive for one or more of the STH parasites (Table3). Of these, the prevalence of STH parasitic infections was 17.0 % (34/199) and 22.7 % (42/185) for females and males, respectively. In the present study, male pupils showed a bit higher prevalence of STH parasitic infection than females. Although gender were not a significant risk factor for the prevalence of STH parasitic infections (Wani *et al*., 2010). .But there was no statistically significance difference in prevalence of STH parasitic infections between males and females in the present study

(Table 3). The slight difference may be due to the fact that; males were more engaged in playing outdoors particularly in faecally contaminated soil. So, environmental contamination is one of the most common ways of transmission which would contribute for sex associated infection (WHO, 1987). The prevalence of STH parasitic infections among the age groups 6-9 years, 10-12 years and 13-16 years was 20.8%, 21.4% and 9.0%, respectively (Table 3).

Table3. Prevalence of soil- transmitted helminth parasitic infections by age and sex of school children in Tulu Nageso Primary School

Age group (In years).	Male		Female		Both sexes		X^2	p-value
	No. Ex	No. Pos. (%)	No. Ex. (%)	No. Pos. (%).	No. Ex. (%)	No. Pos. (%)		
6-9	87	22 (25.2)	76	12 (15.7)	163	34 (20.8)	0.015	0.913
10-12	78	18 (23.0)	99	20 (20.2)	177	38 (21.4)	0.902	0.442
13-16	20	2 (10.0)	24	2 (8.3)	44	4 (9.0)	0.019	0.909
All age Groups	185	42 (22.7)	199	34 (17.0)	384	76 (19.7)	0.075	0.930

Pos. = positive, Ex. = Examined, No. =Number

Generally, in the present study, the prevalence of STH parasite infections among the age group of 10-12 years (21.4%) was higher than the other age groups, but it was statistically insignificant (p=0.980) (Table3). In all age groups the prevalence of STHs was relatively low. But comparatively very low in the age groups 13-16 years. The higher prevalence rate was seen among the 10-12 years age group due to highly risk for environmental contamination, especially, the soil where the children always play and eat food without washing their hands. In the present study, the result was revealed that there exists lower prevalence of STH infection as compared to the result of the study conducted in school-aged children in south Gonder and around Lake Zway, Eastern Ethiopia (Girum, 2005).

The differences in findings among different studies can be explained by variations in geography, socio-economic conditions, and cultural practices of the population under consideration. The category of the study population, the methods employed for stool examination, and the time of study may also have contributed to the differences (Mazingo *et al*. 2010) .

4.3. Major Intestinal Soil-Transmitted Helminths Identified in Examined Children

The major soil-transmitted helminthes parasite species identified in the stool samples of the school children were *Ascaris lumbricoides*, *Trichuris trichiura,* and hookworm species with the prevalence of 15.1%, 2.8%, and 1.0%, respectively (Table 4). These three soil-transmitted helminth parasites were found with an overall prevalence of 19.7% (76 of 384 children). The predominant parasite was *A. lumbricoides* which was observed in 58 (15.1%) students followed by *T. trichiura* in 11 (2.8%) of the examined students. The high prevalence of *A.lumbricodes* infection in the present study was due to environmental sanitation, common practice of eating street foods and poor personal hygiene. The least prevalent parasite in the present study was hookworm, which were 4(1.0%) (Table4).

A. lumbricoides, *T.trichiura*, and hookworm are the most prevalent helminth parasite worldwide (Crompton *et al*., 1999). In the present study, *A. lumbricoides* and *T. trichiura* were found to be the major prevalent STHs parasite infections among the study participants in the present study area. Similar study done on the prevalence of *A. lumbricoides*, hookworm and *T. trichiura* in Wolayta, southern Ethiopia, showed that the prevalence of *A. lumbricoides*, hookworm, and *T. trichiura* was 40%, 25% and 14.7%, respectively (Lopiso *et* al., 2002). Another study done in Southern Nigeria showed that the overall prevalence by species was 76.89%, 54.60%, and 29.24% for *A. lumbricoides,* hookworm and *T. trichiura*, respectively (Nmor *et al*., 2009). From what Girum (2005) reported, the prevalence in Eastern Ethiopia was 6.7% for hookworm, 4.3% for *A. lumbricoides* and 3.9% for *T. trichiura* infection. As compared to others the prevalence of *A. lumbricoides* infection in the present study was lower than that of the prevalence of the same parasite (20.9%) reported in south Ethiopia, Abosa, around Lake Ziway by Gezahegn (2008).

On the other hand, the prevalence of *A. lumbricoides* (15.1%) (Table 4) found in the present study was higher than what Tesfamichael and Teklemariam (1983) found in Ziway Island (4.1%), the result that Girum (2005) reported in Babile (4.3%) and the prevalence that was reported in Adwa, 6.4% (Lemlem, 2008).

Table4. Major intestinal soil-transmitted parasites identified among children in Tulu Nageso Primary School,

Age group(years) and sex	No. Examined (%)	Soil-transmitted helminth parasites			Multiple infection
		Al	Tt	Hw	
		No. pos. (%)	No.pos. (%)	No.pos. (%)	No. pos. (%)
6-9					
Male	87	20(22.9)	1(1.1)	1(1.1)	0
Female	76	8(10.5)	3(3.94)	1(1.3)	0
10-12					
Male	78	12(15.4)	2(2.6)	2(2.6)	1(1.3)
Female	99	16 (16.2)	3(3.0)	0	2(2.0)
13-16					
Male	20	2(10)	0	0	0
Female	24	0	2(8.3)	0	0
All age Groups					
Male	185	34(18.4)	3(1.6)	3(1.2)	1(0.5)
Female	199	24(12.1)	8(4.0)	1(0.5)	2(1.0)
Total	384	58(15.1)	11(2.8)	4(1.0)	3(0.8)

Al=Ascaris lumbricoides, Tt=*Trichuris trichiura and* HW=Hookworm

The prevalence of *T. trichiura* in the school children of Tulu Nagesso Primary School was 2.8%. This was similar with the former reported (3.6%), in Babile (Grum, 2005), 2.3% in Debub Achefer district, northwest Ethiopia (Tilahun, 2010) and 2.5% in Zarima town, northwest Ethiopia (Abebe *et al*., 2011). However Abraham (2003) conducted a study in south west Ethiopia and found 57.4% prevalence of *T. trichiura* infection. The prevalence of the same parasite infection in south Gonder was 21.08% (Mengistu, 2010) as in south Ethiopia, Abosa, around Lake Ziway (Gezahegn, 2008) which was 19.3%, much higher than the prevalence of *T.trichiura* infection in the present study (2.8%).

The low prevalence of geo-helminths attribute to the fact that STHs require hot and humid weather and wet soil. Because the present study was undertaken in highland area which has cold weather and soil, environmental factors can affect the survival of the ova of these parasites in the external environment so that rate of transmission can be hindered (Zulkifli *et al*., 2000).

The prevalence of hookworm infection was lower among the present study participants i.e., 1.0%. This was lower than the prevalence of 46.9% reported by Tilahun (2010), 33.3% by Mengistu (2010) and 14.3% by Gezahegn (2008). On the other hand, the finding of the prevalence of hookworm infection recorded in the present study was almost similar with the former report 1.0% in North Ethiopia, Adwa by Lemlem, (2008). Variability in prevalence of this infection might be due to the 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 rate depends on such factors as local personal hygienic and sanitary conditions.

Regarding the number of STHs parasite infections per individuals, more than one parasite was found in few study participants. Multiple infections were seen in 3 cases out of the total 384 examined stool samples of the school-children with the prevalence of 0.78 %. The prevalence of multiple infection (0.78%) was lower as compared to the studies carried at different places of Ethiopia, 8.9% in south Ethiopia, Abosa, around Lake Ziway (Gezahegn, 2008) and 8.6% in Debub Achefer district, northwest Ethiopia (Tilahun, 2010).

Even though there was no significance difference in the prevalence of multiple STH parasite infections, there was a difference in the prevalence of multiple STH parasite infections between male and female students in the present study population. Thus, in the present study, male students showed a lower prevalence of multiple STH infections (0.5%) than that of the female (1.0%) (Table4).

4.4. Intensity of Soil-Transmitted Helminth Infections among School Children

Most helminth parasite infections were light with no moderate and heavy infection based on thresholds proposed by WHO (Montresor *et al*., 2002). Total egg count for *Ascaris lumbricoides,* hookworm and *Trichuris trichiura* ranges from 24-480, 24-48 and 24-480, respectively (Table 5). The total mean egg count for *Ascaris lumbricoides,* hookworm and Trichuris *trichiura* infections were 111.9±15.9, 36±6.9 and 63.3±10.9, respectively (Table 5).

Epidemiologic research has been focused in the intensity of helminth parasite infections by age (Chan *et al*., 1996). With changes in age, the average intensity of infection tends to be complex, rising in childhood and declining in adulthood. For *Ascaris lumbricoides* and *Trichuris trichiura,* the heaviest and most frequent infections occur in children aged 5–15 years, with a decline in intensity and frequency in adulthood (Gilles, 1996). This could be due to high level of soil contact activity and low personal hygiene in the youngest age groups. Although heavy hookworm infections also occur in childhood, frequency and intensity commonly remain high in adulthood, even among the elder people (Bethony *et al.*, 2002). This trend was also observed in other studies (Brooker et *al*., 2004).

Similarly, in the present study, the highest mean egg count (108 and 66) was recorded in the age group of 6-9 years for *Ascaris lumbricoides* and *Trichuris trichiura,* respectively (Table 5). In contrast to this, medium intensity of hookworm infection was observed in age group 6-9 years but it was similar in 10-12 age groups and not seen in 13-16 years age group with mean egg count 36, 36 and 0, respectively (Table 5). Brooker *et al*. (2004) had also reported that the age-intensity profile for hookworm considerably increased with age until adulthood and then formed plateaus. The total mean egg count for *Trichuris trichiura* was significantly higher in female children than in male. The observed higher intensity of *Trichuris trichiura* infection in female children compared to male children could be attributed to the different pattern of soil contact activities between them. There was significant difference in the total mean egg count for hookworm which was significantly higher in male than in female children (Table 5).

Table5. Mean ± SEM egg counts of soil- transmitted helminth parasites by age and sex of examined children in Tulu Nageso Primary School, Eastern Ethiopia

Age group (in year) and Sex	No. Examined	*Ascaris lumbricoides*		Hookworm		*Trichuris trichiura*	
		Mean ±SEM	Range	Mean± SEM	Range	Mean ±SEM	Range
6-9							
Male	87	93.6±23.9	24-480	48±6.9	24-48	24±10.9	24-120
Female	76	144±56.9	24-480	24±0	24-24	80±26.7	48-120
Total	163	108±23.3	24-480	36±12.1	24-48	66±20.5	24-120
10-12							
Male	78	74±18.1	24-240	36±12.1	24-48	36±12	24-120
Female	99	153±37.1	24-480	0	0	72±28.2	24-120
Total	177	112.3±23.6	24-480	36±12.1	24-48	57.6±18.3	24-120
13-16							
Male	20	60±12.1	48-72	0	0	0	0
Female	24	0	0	0	0	72±24.2	48-96
Total	44	60±12.1	48-72	0	0	0	0
All age groups							
Male	185	84.7±16.3	24-480	40±8.1	24-48	32±8.1	24-480
Female	199	150±30.5	24-480	24±0	24-24	75±12.5	24-480
Total	384	111.9±15.9	24-480	36±6.9	24-48	63.3±10.9	24-480

KEY: SEM=Standard Error of Mean,

4.5. Anthropometric Measurements of School Children

As shown in, the overall prevalence of stunting among age group of 6-9 years was 6.7 % of which 8.0% were males and 5.3% were females (Table 6). There was no severe stunting as compared to previous report, but it was moderate. On the other hand, 3.1% of the school children aged 6-9 were underweight, of which 2.6% were females and 3.4% were males. The prevalence of wasting among the same age group was 6.1 % of which males and females were 4.5% and 7.8%, respectively. There was no a significant difference in the prevalence of stunting, wasting and underweight between males and females (P>0.05).

Table6. Frequency and percent prevalence of underweight, stunting and wasting status among study children aged 6-9 years in Tulu Nageso Primary School, Eastern Ethiopia

Study subjects	No. Examined	Underweight (WAZ) Frequency (%)	Stunting (HAZ) Frequency (%)	Wasting (WHZ) Frequency (%)
Male	87	3(3.4)	7(8.0)	4(4.5)
Female	76	2(2.6)	4(5.3)	6(7.8)
Both	163	5(3.1)	11(6.7)	10(6.1)

As recommended by WHO (2007), the anthropometric measurements of children in the survey 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 were expressed in standard deviation units (Z-scores) from the median of the reference population.

Each of these indicators, height-for-age, weight-for-height, and weight-for-age provides different information about growth and body composition, which is used to assess nutritional status. The height-for-age index is an 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).

As shown in Table 6, 11 (6.7%), 5 (3.1%) and 10(6.1%) of the study children aged 6-9 showed, stunting, underweight and wasting, respectively. Of these, 2.6% were females and 3.4% were males for Underweight, 8.0% males and 5.3% females for Stunting and 4.5 % males and 7.8 % females for wasting.

Malnutrition is a considerable health problem with prevalence ranges of 4 - 46% in developing countries (Thomas *et al.,* 2001). In the present study, the prevalence of stunting observed among school children 6.7%, was relatively lower as compared to previous findings in Attat area (48.3%) in Ethiopia (Birmeka, 2007). The prevalence of stunting observed among school children in Tanzania was 42.5% (Lwambo *et al.,* 2000) while a prevalence of 40.2% was observed among school children in Malaysia (Zulkifli *et al.,* 2000). Since stunting is a type of chronic malnutrition which begins in childhood, this variation might be due to difference in breast feeding of an infant with breast milk.

Generally, in the present study, the prevalence of wasting (6.1%) and underweight (3.1%) among age group 6-9 years were found to be much lower than those of both the regional and the national rates, where wasting was 6.5% both nationally and regionally, and underweight was 29.7% regionally, but 20.8% nationally (FMOH, 2005). However, stunting (6.7%) was not a serious problem in comparison to the Ethiopian Demographic and Health Survey report which was 51.3% (CSO, 2005). The low rates of malnutrition in the present study in comparison with previous study may be due to improved living condition of the community (suitable food intake).

The overall prevalence of stunted (6.7%) was lower than the prevalence (33%) reported by WHO (2000) in developing countries and Shankya *et al*. (2004) reported the prevalence of stunting to be 21.5% in Nepal. The variations in the prevalence of stunting in age group 6-9 years might be due to differences in nutrition and types of staple food the communities live upon or they were infected by some other diseases (Gezahegn, 2008). In the present study prevalence of underweight (3.1%) and wasting (6.1%) was lower than that of the study conducted by Tilahun (2010) in North west Ethiopia which was 20.8% and 17.9% for prevalence of underweight and wasting, respectively.

4.6. Prevalence of underweight or thinness in age group 10-16 years

In older children, i.e. above 10 years, weight-for-age is not a good indicator as it cannot distinguish between height and body mass in an age group where many children are experiencing the pubertal growth spurt and may appear as having excess weight (by weight-for-age) when in fact they are just tall. BMI-for-age is the recommended indicator for assessing thinness, overweight and obesity in children 10-19 years (WHO, 2009). Therefore, in the present study, the prevalence of BMI-for-age under 5^{th} percentiles which was an indication for being underweight for 10-16 years of age was 9(4.1%) (Table7). Of which, 5(5.1%) was for males and 4(3.3%) was for females. In addition, BMI-for-age percentiles of 5^{th} - 85^{th}, and > 85^{th} were calculated for analyzing the status of normal growth and to assess risks for overweight and/or obesity, respectively. However, there was no any risk for overweight among the study school children (Table 7).

The prevalence of underweight among age group 10-16 years in the present study was (4.1%) was much lower than the prevalence of underweight (36%) reported from Southern Ethiopia (Birmeka, 2007) and the prevalence reported for Abchikeli and Ayalew Mekonnen Elementary school children (30.7%) (Tilahun, 2010). The high variations may be due to differences in family living standard that is due to difference in adequate nutrition or staple food types.

Table7. Prevalence of underweight/or thinness in the age group 10-16 years in Tulu Nageso Primary School, Eastern Ethiopia

Gender	No. Examined	Underweight Frequency (%)	Healthy Frequency (%)	Overweight Frequency (%)
Male	98	5(5.1)	93(94.9)	0
Female	123	4(3.3)	119(96.7)	0
Total	221	9(4.1)	212(95.9)	0

4.7. Association of Intestinal Parasite Infections with Anthropometric Measurements

The prevalence rate of under nutrition, based on Height-for-age (Stunting), Weight-for-age (underweight) and Weight-for-height (wasting) among age group 6-9 years in the school children was 54.5%, 40% and 20%, respectively (Table 8). The prevalence of underweight among age group 10-16 years in the current study (33.3%) was lower than that of the prevalence reported (36%) from Southern Ethiopia (Birmeka, 2007) and higher than the prevalence reported from Debub Achefer District, Northwest Ethiopia, (30.7%) by Tilahun (2010) and the prevalence reported for Malaysian school children (25.7%) (Zulkifli *et al.*, 2000) (Table 8). The variations may be probably due to differences in nutrition and types of staple food the communities live upon.

Even though the prevalence of malnutrition was very high among the study participants, in this cross-sectional study; a significant association was not found between intestinal parasite infections and nutritional status (Table 8).

In the present study, the prevalence of malnutrition was very low among the study participants compared to the previous report; a statistical significant association was not found between intestinal parasite infections and nutritional status (Table 8). This finding consistent with previous study, which revealed an independence of anthropometric scores on the overall rate of intestinal helminthic infections (Girum, 2005). Similar results have also been reported from the study conducted in Lowland Bolivia (Tanner *et al.*, 2009), in rural Kelantan and Northern Rwanda (Zulkifli *et al.*, 2000) .This could suggest that other factors such as poverty, poor health and sanitary conditions, limited knowledge of nutritional matters among certain households, and fluctuations in incomes which affect the nutritional status may be predominant among the study subjects as reported by Andrade *et al.* (2001). In addition to this, lack of adequate nutrients caused by high intensity infections in a critical period can prevent the normal growth spurt in pre pubertal and pubertal children (Andrade *et al.*, 2001).

Table.8. Association of intestinal parasite infections with anthropometric measurements of children in Tulu Nageso Primary School, Eastern Ethiopia

Age group (in year)	No. examined	Intestinal parasites				
		No. Pos (%)	No. Ne. (%)	OR (95%CI)	χ^2	P-value
6-9	163	34(20.9)	129(79.1)			
Stunting				1.679	0.250	0.909
Yes	11	6(54.5)	5(45.5)	(0.952-2.325)		
No	152	8(5.3)	144(31.6)			
Underweight						
Yes	5	2(40)	3(60)	0	0.250	0.807
No	158	10(6.4)	148(93.6)			
Wasting						
Yes	10	2(20)	8(80)	0.235	9.000	0.543
No	153	6(3.9)	147(96.1)	(0.474-1.270)		
10-16	221	42(19)	179(81.0)			
Underweight						
<5thpercentile	9	3(33.3)	6(66.7)	0.597	2.250	0.698
>5th percentile	212	39(18.4)	173(81.6)			

Key= OR= Oddis ratio; 5th …….5th percentile

5. SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1. Summary

The objective of the present study was to determine the prevalence and intensity of STH parasite infection and their associations with anthropometric measurements of school children in Bekoji town Eastern Arsi. The design of the study was a cross-sectional parasitological survey involving school-children from grade 1-8 in Tulu Nageso Primary School Bekoji town, Oromia Region. The study was conducted during April-June 2016.

A total of 384 stool samples were collected and examined using direct wet mount and Kato-Katz techniques. The overall prevalence of soil-transmitted parasite infections was 19.7%. The prevalence of *A.lumbricoides, T.trichiura* and hookworm was found to be 15.1%, 2.8% and 1.0%, respectively. Multiple STH parasites infection was found in 0.8% of the study participants. The mean egg count and ranges of *Ascaris lumbricoides*, hookworm and *Trichuris trichiura* were 111.9, 36 and 63.3, respectively.

The prevalence of Stunting (HAZ), Wasting (WHZ) and Underweight (WAZ) amongst the study participants was 6.7%, 6.1% and 3.1%, respectively (Table 6). The chi-square test analysis showed that there was a higher prevalence of wasting, underweight and stunting. The overall prevalence of poor nutritional status was higher in males than in females with respect to each of the three nutritional indices. Statistical significant association was not found between intestinal parasite infections and anthropometric indices. The possible reasons might be due to other multifactorial problems such as shortage of a balanced diet.

5.2. Conclusion

The soil-transmitted helminth parasitic species detected in the present study area included *A. lumbricoides*, *T. trichiura,* and hookworms. *A. lumbricoides* and *T. trichiura* were the dominant parasites in the school-children. The result of this study also showed low prevalence of malnutrition among the school children. The results showed that the burden of STHs parasite infections was within the range of light infections, and there were no cases with moderate and heavy infection of STH.

5.3. Recommendations

Based on findings of this study, the following recommendations are forwarded:

- Local health sector should collaborate with school health program for delivering health education to increase the knowledge, attitude and practice of school children as to how intestinal STH parasite infections are transmitted and prevented.
- Regular deworming program for school children should also be put in place to keep STH parasite infections intensities low.
- School health program for the assessment of malnutrition and health education particularly for school children and parents and/ or guardians in general on how to prevent malnutrition should also be carried out.
- Establishment and maintenances of a network for treatment of intestinal parasitic infections and provision of health education program in primary schools.
- Provision of mass-treatment with appropriate anti parasitic drugs and giving health education with every six months in the primary schools.

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7. APPENDIX

7.1. Appendix I

LABORA TORY FORM

For laboratory use only

Name_____________________ **Age**____________**Sex**_________ **code**____________

Laboratory Parasitological Examination Procedures

Macroscopic Examination of Stool:

1. Direct wet mount methods

A. Positive **B. Negative**

Status of the infections:

Single infection with____________________

Double infection with ___________________and ________________

Triple infection with ___________________, ___________________and _____________.

2. Kato- Katz Technique:

A. Positive **B. Negative**

Ascaris lumbricoides eggs/slide ____________________ epg _____________

Hook worm eggs/slide ______________ epg _____________

Trichuris trichiura eggs/slide _______________ epg______________

Status of the infections:

Single infection with___________________

Double infection with___________________ and ________________

Triple infection with ____________, _________________and _____________.

3. Anthropometric measurements:

a. Height __________
b. Weight __________
c. BMI_____________

7.2. Appendix II

Table 9.Intensity of infection with *Ascaris lumbricoides*, hookworm and *Trichuris trichiura* by age group and sex among children

Age (in year) and Sex	No.exa Mined	*A.lumbricoides*			*Hookworm*			*T.trichiura*		
		Light (%) 1-4,999	Moderate (%) 5000-49,999	Heavy (%) >50, 0000	Light (%) 1-1,999	Moderate (%) 2000-3,999	Heavy (%) ≥4000	Light (%) 1-999	Moderate (%) 000-9,999	Heavy (%) 10,000
6-9										
Male	87	20(22.9)	-	-	1(1.1)	-	-	1(1.1)	2(4.2)	-
Female	76	8(10.5)	-	-	1(1.3)	-	-	3(3.9)	2(4.7)	-
Total	163	28(17.2)	-	-	2(1.2)	-	-	4(2.5)	4(4.4)	-
10-12										
Male	78	12(15.4)	-	-	2(2.6)	-	-	2(2.6)	4(2.9)	-
Female	99	16(16.2)	-	-	-	-	-	3(3.0)	2(2.5)	-
Total	177	28(15.8)	-	-	2(1.1)	-	-	5(2.8)	6(2.2)	-
13-16										
Male	20	2(10)	-	-	-	-	-	-	-	-
Female	24	-	-	-	-	-	-	2(8.3)	-	-
Total	44	2(4.5)	-	-	-	-	-	2(4.5)	-	-

7.3. Appendixes III

Written consent form

Code No ___________

Name of the study participant________________________ Age _______Sex__________

Name of Physician______________________ Study site/Health center___________

I have been informed about a study that plans to investigate the **"Prevalence and Intensity of Soil-Transmitted Helminth Infection and their association with Anthropometric Measurements among School Children in Tulu Nageso Primary School, Bokoji Town, Arsi Zone, Oromia Region, Ethiopia".** I am requesting your children to participate in this study which would require his /her response to obtain stool sample. There is no any health related risk in participating. When your children are found positive for intestinal parasites they will receive standard drugs free of charge. The information in your records is strictly confidential.

Your participation in this study is completely voluntary and you can refuse to participate or free to withdraw yourself from the study at any time. Refusal to participate will not result in loss of medical care provided or any other benefits. Do you understand what has been said to you? If not, you have the right to get proper explanation.

I, the undersigned have been informed about the study objectives. I have also been informed that all the information is to be kept confidential and that I have the right to decline from or to cooperate in the study. Therefore, with full understanding of the study objective I agree to give the informed consent voluntarily to the researcher to identify the intestinal parasites of my children.

This consent form has been readout to me in my own language, and I understand the content and I am voluntarily consent to participate in the study.

Name (participant) ___________________________ Signature _________ Date________

Name (Witness) ______________________________Signature _________ Date________

Name (Investigator) __________________________ Signature _________ Date ________

Xalayaa Walii Galtee

Lakk .Koodii__________

Maqaanama hirmaatuu____________________umrii___________ saala______________

Maqaa nama laaboraatoorii hojjatuu__________ buufata fayyaa______________

Ani hojjataan qorannoo kanaa kaayyoo qorannoo kanaa hirmaattotaaf karaa ifa ta'een "**babal'ina fi baay'ina raammoo maxxantuu biyyeedhaan daddarban (nematode) fi walitti dhufeenya inni antirooppoomeetirik meezyarmantii waliin qabu mana barumsa Tulluu Nageessoo, Magaala Boqojjii Godina Arsii, naannoo oromiyaa, itiyoophiyaa** ibseera. Maatiin barattoota keessan udaan kennuun akka deebii isaan nuuf kennan isin gaaffanna. Rakkoo fayyaatiin kan walitti qabatu tokkoollee hin jiru. Ijoolleen raammoo garaa qaban bilisaan qorsi ni kennamaaf. Ragaaleen argaman hundinuu iccitiidhaan ni eegamu.

Hirmaannaan isin qorannoo kana keessatti gootan fedha keessaniin kan ta'ee fi yeroo barbaaddaniitti dhiisuu akka dandeechani fi dhiisuun keessaniif tajaajila fi bu'aa argachuu qabdan akka hin dhabne isnii ibsina. Wanta jedhamuu barbaadame hubattanii? Hin hubanne taanaan odeeffannoo gahaa argachuuf mirga guutuu qabdan.

Ani maqaan koo eerame, galma qorannoo kanaa hubadheera. Akkasumas odeeffannoon akka iccitiidhaan eegamu hubadhee jira. Yeroon barbaadettis qorannoo keessaa akka bahuu danda'u hubadhee jira. Kanaafuu, hubannoo guutuu argachuun xalaayaa walii galtee kana fedhii kootiin raammoon garaa daa'imman kiyya keessa jiru akka ilaallamu qorataa waliin walii galee jira.

Xalayaa walii galtee kun afaan dhalootaa kootiin naaf dubbifamee jira, anillee qabiyyee isaa waanan hubadheef fedhii kootiin qorannoo kana keessatti nan hirmaa dha.

Maqaa Hirmaattotaa ______________________ mallattoo _______ guyyaa_________

Maqaa maatii ____________________________ mallattoo _______ guyyaa_________

Maqaa qorataa____________________________ mallattoo _______ guyyaa_________

7.4 Appendix IV

Procedure of Kato- Katz Technique

A. Kato- Katz Technique

1. Place a small amount of faecal material on scrap paper and press the small on top.
2. Scrap with flat-sided spatula across the upper surface of the screen to collect the sieved faeces.
3. Place template with 41.7mg whole size on the center of a microscope slide and add faeces from the spatula so that the hole is completely filled. Pass the side of the Spatula over the template to remove the excess faeces from the edge of the hole
4. Remove the template carefully so that the cylinder of faeces is left over the slide.
5. Cover the faecal material with the pre-soaked cellophane strip.
6. Invert the microscope slide and firmly press the faecal sample against the cellophane strip on another microscope slide or smooth hard surface.
7. Carefully remove the slide by gently sliding it side ways to avoid separating the cellophane strip or lifting it off.
8. For all except hookworm eggs, keep the slide for one or more hours at ambient temperature to clear the faecal material prior to examination under the microscope.
9. Examine the smear systematically and count the number of eggs of each species of the parasite. Multiply the number of eggs a factor depending on size of the template to report egg per gram of stool.

7.5 Appendices V Experiment done at Aklilu Lemma pathiobiology Institution

7.6. Appendices VI During slide prepared in Aklilu Lemma's Pathiobiology Institution

7.7. Appendices VII Prepared slide in Aklilu Lemma Pathiobiology Institution