Intestinal parasitic Infections among School-age Children In Mekaneselam Health Center, Borena, Northeast Ethiopia

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Acronyms

WHO	World Health Organization
IPIs	Intestinal Parasitic Infections
CSA	Central Statistics Agency
ORDA	Originations for Rehabilitation and Development of Amhara
MFM	Menschen für Menschen
SPSS	Statistical Package for Social Sciences
Eh/d/m	*Entamoebahistolytica/dispar/moshkovskii*
Gl	Guardia lamblia,
Hw	Hookworm
Al	*Ascaris lumbricoides*
MHC	Mekaneselam Health Center

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Abstract

Intestinal parasitic infections (IPIs) are still quite common in low-income countries including Ethiopia, particularly in children due to low-quality drinking water, poor personal and environmental sanitation. Periodic evaluation of the current status of IPIs in a locality is a prerequisite to better control these health threats. Thus, the objective of this study was to assess the prevalence and determinant factors of IPIs among school-age children in Mekaneselam, northwest Ethiopia. The study was conducted at Mekaneselam health center and its design was cross-sectional and retrospective investigation. Stool samples collected in November 2015 - May 2016 were analyzed using the direct-wet method. Retrospective data recorded between November 2014 and May 2015 was also extracted from the health record system. Structured questionnaire was used to gather data on environmental, socio-demographic and behavioral variables. Data analysis was done using the chi-squared test. In the retrospective investigation, out of 314 patients examined 249(79.3%) had microscopy-confirmed IPIs. Ninety (28.7%) *Entamoeba histolytica/dispar/moshkovskii,* 84(26.8%) *Giardia lamblia,* 14(4.5%) hookworm and 61(19.4%) *Ascaris lumbricoides* were detected. Totally 227 fresh stool samples were examined in the cross-sectional survey and 133(58.6%) were positive for IPIs. There were 51(22.5%) *E. histolytica/dispar/moshkovskii*, 57(25.1%) *G. lamblia*, 15(6.6%) hookworm and 10(4.4%) *A. lumbricoides* cases. The results indicated that IPIs were common health problem among the school-age children. Source of drinking water and hand washing practice before meal were significantly associated with positivity for IPIs (X^2=0.166, p=0.809). Thus, there is a need for intensive and habitual health education for behavioral changes related to personal hygiene and mass treatment for effective control of IPIs in the study area.

Keywords: Prevalence, *Entamoeba histolytica/dispar/moshkovskii*, *Giardia lamblia, Ascaris lumbricoides,* hookworm

1. Introduction

Parasites are defined as organisms that obtain food and shelter from other organisms, the host. For a parasitic to be defined as intestinal, it must have an intestinal life cycle stage. Moreover, it may have life cycle stage in the heart, circulation, lung, tissue, and other animals on the environment. Helminths and protozoa are natural parasites found in the intestine of man which are eukaryotes, unicellular or multicellular, microscopic or macroscopic.

Globally, about 3.5 billion people are affected by intestinal parasitic infections and cause clinical morbidity in approximately 450 million individuals (WHO 1997). Developing countries are reported to be the most affected and within these, the majority of the cases occur among school aged children (WHO 1996; Montresor et al. 1998).

The distribution of intestinal parasitic infection depends on many factors. These include sociodemographic variables associated with poverty, reduced access to adequate sanitation, potable water, and health care as well as the prevailing climatic and environmental conditions (Mata 1982; WHO 1996; Montresor et al. 1998). Young children are reported to be disproportionately affected by IPI's compared to adults due to their increased nutritional requirements and less developed immune systems (Scrimshaw 1994).

Entamoeba histolytica is estimated to inflict severe disease in 48 million individuals around the globe (Petri and Singh 1997). Severe *E. histolytica* infections in children often cause dysentery and bloody diarrhea; amebic liver abscess may also occur although they are less common (Petri and Singh 1999).

Giardia lamblia is the most common protozoal infection of the human small intestine (Eckmann and Gillin 2001). Giardia infection is associated with the malabsorbtion of fats, carbohydrates, and vitamins, especially vitamins A (Solomons 1982). Recent authors also have linked it with reduced serum hemoglobin levels (Curtale et al. 1998). Improvement of sanitation, clean water supplies and health education reduces parasites transmission in the long term (Bukenya 1987; Stephenson et al. 1993).

Ethiopia like any other low income country in the tropics is heavily affected by IPIs due to very poor personal and environment hygiene, poor water quality and toilet coverage. Studies on the prevalence of intestinal parasites in different parts of the country and identifying high risk groups in the community are important to design appropriate intervention strategies. The aim of this research is to detect the prevalence of IPIs and the status of associated risk factors among school children in MHC.

2. Objectives

2.1 General objective

The objective of the study is to evaluate the prevalence of IPIs and associated risk factors among school age children treated at MHC.

2.2 Specific objectives

The specific objectives of the study were;

1. To assess the prevalence of IPIs among school children treated in MHC.
2. To identify the dominant intestinal parasite among school children in MHC.
3. Determine the risk factors associated with occurrence of IPIS.

3. Literature review

3.1 Intestinal Parasitic Infections

Intestinal parasitic infections (IPI's) enjoy a wide global distribution. They are estimated to affect an estimated 3.5 billion people, most of whom are children residing in developing countries (WHO 2000). The major IPI's of global public health concern are the protozoan species *E. histolytica* and *G. lamblia* and the soil transmitted helminthes *Ascaris lumbricoides*. The incidence and prevalence of these parasitic pathogens varies both between and within countries. The majority of infections are associated with poverty conditions such as reduced access to safe drinking water, adequate sanitation and hygiene, housing, and inadequate access to health care (Mata 1982; Montresor et al. 1998).

They are also affected by poor family and community hygiene and sanitation practices and prevailing climatic and environmental conditions (Jemaneh 1998). These conditions lay the stage for the continuous transmission of the IPI's (Mata 1982; Montresor et al. 1998; Crompton 1999). IPI's caused by pathogenic helminthic and protozoal species are endemic throughout the world. They affect an estimated 3.5 billion persons and cause clinical morbidity in approximately 450 million (WHO 2000).Developing countries are reported to be the most affected and within these, the majority of the cases occur among school aged children (WHO 1996; Montresor et al. 1998).The distribution of IPI depends on many factors. These include socio-demographic variables associated with poverty such as reduced access to adequate sanitation, potable water, and health care as well as the prevailing climatic and environmental conditions (Mata 1982; WHO 1996; Montresor et al. 1998).

The economic burden caused by hookworm, roundworm and whipworm infections is high Intestinal parasites are those types of entero-parasites which infect the lumen and lining tissue of the lumen of the small and large intestine. These include: nematodes, cestodes, protozoan parasites. Positive cases of entero-parasitoses (intestinal) were defined as the "presence of parasites' egg, larvae, cyst or the parasite in fecal specimen" (Morales-Espinoza et al. 2003). Intestinal parasitic infections are among the most common health problems generally in global level, in Ethiopia in particular (Mengistu et al. 2007). Similar to other developing countries, wide distribution of intestinal parasites in Ethiopia is due to low level of environmental sanitation, personal hygiene, food and water contamination with human excreta (WHO 1981) and unaware of simple health promotion practices such as personal hygiene, food hygiene, etc.(Zein 1988; Kloos and Tesfayohannes 1993).

These parasites are commonly transmitted through ingestion of contaminated food and/or water as a result of poor sanitation and hygiene. In some instances, transmission occurs through close contact between infected and non-infected individuals as in infected food handlers and consumers, respectively (Neghab et al. 2005).

Young children are reported to be disproportionately affected by IPI's compared to adults due to their increased nutritional requirements and less developed immune systems (Scrimshaw 1994). Intestinal parasitic infections in this age group have been linked with significantly reduced growth (Stephenson et al. 1993) and an increased risk for protein-energy malnutrition (Stephenson et al.2000) including growth stunting (Oberhelman et al. 1998; Tsuyuoka et al. 1999), iron-deficiency anemia (Stoltzfusetal.1997), and reduced cognitive/psychomotor development (Nokes et al. 1992; Callendar et al. 1994; Sakti et al. 1999).

3.2 Intestinal Protozoan Infections

The protozoa are an extremely diverse group of unicellular organisms occurring in almost all of the ecological niches known to humans, including the bottom of hot springs and the edges of ice flows. Even though the majority of protozoa occur as free-living organisms in the soil, moist, marine or freshwater environments, a substantial number also exist as mutualisms, commensals or parasites (Melhorn 1988; Katz et al. 1989). Protozoan parasites are known to affect all species of vertebrates and many invertebrates. They are able to adapt to life in virtually all body sites of their hosts. Their characteristic high infectivity enhances their pathogenicity within the host (Katz et al 1989; Neva and Brown 1994).

Among the many species of intestinal protozoa, *E. histolytica* and *G. lamblia* are potentially pathogenic and in many parts of the world either or both organisms constitute a public health problem (WHO 1987). The prevalence of *E. histolytica* infections differs from geographic area to another, and severity varies from one case to another (Walsh et al., 1988). Rapid urbanization, especially in tropics is often associated with increased poverty, poor housing and unsanitary conditions (Wilson et al. 1998). *G. lamblia*, *E. histolytica* and *E. coli* can be transmitted orally by drinking infected water and both are environmental contaminants of the water supply. The water supply is really an important risk factor for giardiasis and several large outbreaks of giardiasis have resulted from the contamination of municipal water supplies with human wastes (Wilson et al. 1998).

3.3 *E. histolytica* infection

E. histolytica infection is common in most developing countries. It also becoming more frequent in the United States and other developed countries as the result of increasing tourism abroad and a rising number of refugees and other immigrants and non- immigrants originating in endemic countries (Petri and Singh 1999). The two species *E. histolytica* and *E. dispar* are morphologically identical but pathologically distinct (WHO 1997; Petri and Singh 1999). Together they infect around 10% of the world's population (Walsh 1986). However, only *E. histolytica* is capable of causing disease (WHO 1997). Colonization with E. dispar is said to be three times more common than *E. histolytica* in developing countries while it is ten times more common in developed nations (Petri and Singh 1999). *E. histolytica* is reported to be responsible for approximately 50 million cases of invasive amoebiasis (Petri and Singh 1999) and upwards of 100,000 deaths/year (WHO 1997). Thus, it is ranked second only to malaria as the cause of mortality due to a protozoal infection (WHO 1997). The parasite normally inhabits the large intestine but is also capable of invading other organs such as the liver, brain and spleen (Petri and Singh 1999).

The majority of amoebic infections are reported to occur in Central America, South America, Africa and Asia. These are often associated with poor water and food hygiene and sanitation practices (Petri and Singh 1999). Humans are the most significant reservoir of infection even though morphologically identical amoebae have been isolated from certain domestic and wild animals (Katz et al. 1989).The lifecycle of *E. histolytica* includes the infective cyst and the invasive trophozoite forms. Infection is acquired primarily through the ingestion of infective cyst forms present in focally contaminated water and food. It also can be transmitted by person-to-person contact (Petri and Singh 1999). The quadrinucleate cysts measure approximately 10 – 20µm in diameter and are resistant to gastric juices present in the human stomach.

The amoeba excysts in the small intestine by releasing four metacystic amoebae that divide into eight trophozoites which then move down to the large intestine (Neva and Brown 1994; Katz et al.1989; Garcia 1999; Petri and Singh1999).Intestinal stasis allows the amoeba to establish a foothold in the cecal are of large intestine even though a portion may be swept along to the sigmoidorectal region or even out of the body (Neva and Brown 1994). The chances of establishing a foothold are improved when the volume of food intake is low and the number of parasites is high (Neva and Brownb 1994).

The trophozoites measure approximately 10-60µm in size (Garcia 1999). These adhere to the colonic mucin glycoproteins via a galactose and N-acetyl-D- galactosamine specific lectin (Stanley and Reed 2000). Host colonic cells are killed via the induction of an apoptotic cascade (Petri and Singh 1999). Colonic lesions formed by the trophozoites vary from mucosal thickenings to characteristic flask shaped ulcerations to necrosis of the intestinal wall (Neva and Brown 1994; Petri and Singh 1999). The trophozoites are typically found in the periphery of the necrotized tissues. Reproduction is by binary fission or the formation of cysts. These pass out with the stool and are immediately infective (Neva and Brown 1994). In the extraintestinal invasion no cysts are formed and trophozoites proliferate solely by binary fission (Katz et al. 1989).

Asymptomatic infection with *E. histolytica* is defined as the presence of cysts in stools in the absence of colitis or extraintestinal infection. These healthy carriers may pass millions of cysts in the stool per day as the trophozoites multiply in the intestinal lumen (Petri and Singh 1999 WHO 1997, Neva and Brown 1994). Approximately, 90% of all intestinal *E. histolytica* infection is asymptomatic. However, even asymptomatic infection is associated with a small but significantly increased risk for developing invasive amoebiasis (Petri and Singh 1999; Mohamed et al. 2000). Clinical symptoms of acute intestinal amoebiasis include diarrhea, bloody stool that may contain necrotic mucous, abdominal pain, tenderness and fever (Petri and Singh 1999). Symptoms of amoebic liver abscess usually involve fever, right upper abdominal tenderness/ pain, weight loss and colitis (Katz et al.1989; Neva and Brown 1994; Petri and Singh 1999). Amebic liver abscesses 7-10 times more common in men than women but this sex difference in risk has not been reported for children (Petri and Singh 1999). Amoebiasis infection can be controlled through proper treatment and/or disposal of raw sewage and maintaining clean water supply including the protection of open wells, springs and rivers from contamination with sewage and feces. The risk for infection can also be reduced via the adequate boiling of drinking water or treatment of water with chlorine or iodine. The exterior of raw vegetables and fruits should also be washed with soap and soaked in vinegar for 15 minutes prior to consumption (Petri and Singh 1999).

3.4 Giardia infection

Giardiasis is caused by infection with the enteric pathogenic intestinal flagellate, *G. lamblia*. It is considered the most common human intestinal protozoan infection. Humans are the preferred host but morphologically identical organisms have been isolated in dogs, beavers, ungulates and other mammals (Katz et al. 1989). The frequent availability of hosts and reservoirs for the protozoan has contributed to the high prevalence of this infection in many industrialized countries (Juckett 1996). The transmission of the parasite is mainly by the fecal-oral route but also occurs by human-to-human transmission (Mata 1982; Garcia1999). *G. lamblia* (also known as *G. duodenalis* or *G. intestinalis*) is a unicellular, flagellated intestinal protozoan parasite of humans isolated worldwide and is ranked among the top 10 parasites of man (Wolfe 1992; Farthing 1997). *G. lamblia* is the most common Protozoan intestinal parasite isolated worldwide as causative agents of diarrhoea.

Epidemiological studies suggest that the parasite is responsible for about 5% of acute diarrhoea and 20% of chronic diarrhoeal illness in the world (Thompson et al. 1993). Giardia exists in two forms: the infective cyst and the not invasive trophozoite which typically inhabits the duodenum and upper jejunum of humans (Neva and Brown 1994). The round or oval shaped cysts, which are the infective form of the protozoan, are approximately 11-14µm long and 7-10µm wide (Garcia 1999). After ingestion the cysts pass unharmed by gastric acid through the stomach to the small intestine. Infection with Giardia is usually confined to the upper small intestine but also has been observed in the bile duct and gall bladder of ill patients (Ibid). Identification of the parasite is usually made by microscopic examination of direct fecal smears for either trophozoites or cysts in the feces.

Clinical symptoms of giardiasis include diarrhea steatorrhea, epigastric pain, wasting, hypoalbuminemia and impaired absorption of folate and vitamin B12 (Neva and Brown 1994;

Solomons 1982). The organism has been found in more than 40 animal species (Meyer, 1994). Nowadays there are five species of Giardia known to infect different animal species: *G. lamblia* in mammals including man, rodents, reptiles and possibly birds; Giardia muris in rodents, birds and reptiles, G.agilis in amphibians (Filice, 1952); G. ardae in the great blue heron (Erlandsen et al., 1990); and G. psittaci in the budgerigar (Erlandsen and Bemrick, 1987).

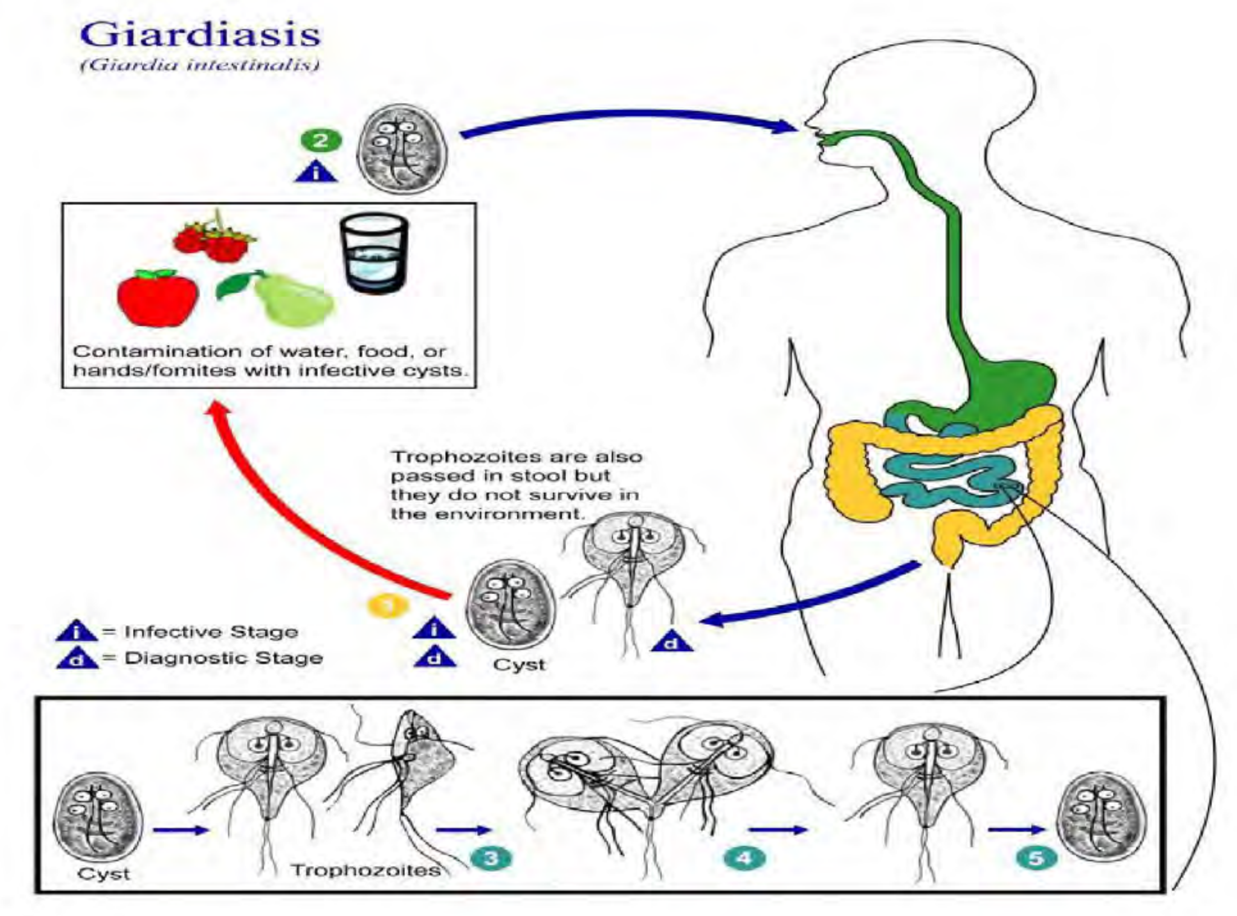

Figure 1 the life cycle of Giardia   Source http://www.dpd.cdc. Gov/dpdx

3.5 Intestinal Helminthes Infections

The three major soil transmitted helminthes considered to be of global public health concern are *A. lumbricoides* (roundworm), *Trichuris trichiura* (whipworm), and *Ancylostoma duodenale* or *Necator americanus* (hookworm). Over one billion of the world's population is estimated to be infected with these parasites; two billion are more said to be at risk (Montresor et al. 1998). Children are reported to be at especially increased risk for severe infections and the morbidity and mortality associated with these (Chan et al 1994). Helminth, infection has been linked with an increased risk for several nutritional anemias, protein-energy malnutrition, and reduced physical growth and development in infants and children (Connolly and Kvalsvig 1993; Nokes and Bundy 1994; Stephenson et al. 2000).

3.6 *A. lumbricoides* and hookworm

A. lumbricoides, a soil transmitted helminths, is reported to infect at least one-fourth of the world's population (Crompton 1994). Annual morbidity associated with the parasite has been estimated by the WHO at 60,000 with another 250 million people said to be at risk for acquiring the infection (Montresor et al. 1998). Both domestic and wild animals are common reservoirs for A. lumbricoides. It should be noted that some controversy existed regarding whether Ascaris suum, the pig round worm, is genetically identical to *A. lumbricoides* (Anderson 1995; Peng et al. 1998).

Ascaris lumbricodes is a robust parasite. This quality is due, in part, to the resilient nature of its eggs, which are capable of surviving a wide range of hot and cold temperatures, chemicals, chemical disinfectants and other extreme conditions (Neva and Brown 1994). The eggs of Ascaris are one of the most resilient of the helminth eggs and can remain infective for years embedded in the soil (Gilgen and Mascie–Taylor 2000; Crompton 1994). Ascaris is the largest of the human intestinal parasitic nematodes. Mature male and females can grow from 15–30 cm and 20- 35cm in length, respectively. Both usually inhabit the jejunum where they feed on the semi-digested food present in the host (Neva and Brown 1994). They also secrete antitrypsins. And thus, are capable of adequately competing with the host system for ingested proteins. They 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. This allows them to move against peristalsis (Katz et al. 1989). The mature female lays approximately 200,000 eggs per day, which then pass out in the feces in an unembryonated form (Thein Hlaing 1993; Crompton 1994).

Under favorable condition such as adequate moisture and shade the eggs embryonate to form the second stage larva in which they remain until they are subsequently ingested (Thein Hlaing

1993; Crompton 1994). 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 or cooking utensils, or under the fingernails. They also may circulate in household dust and air where they are inhaled or swallowed (O'Lorcain and Holland 2000). Ingested eggs hatch in the duodenum to release the larvae, which penetrate the intestinal wall to reach the venules. From here, the larvae are carried passively to the liver via portal circulation. The larvae subsequently migrate to the heart through the inferior vena cava and are carried to the lungs by the pulmonary circulation were they lodge in the alveolar spaces where and undergo their second and third molt. Next, third stage larvae break out of the alveolar space and migrate up the trachea into the pharynx were they are swallowed. After arriving at the small intestine, they undergo one more molt before they are transformed into the mature worm. These mature forms then mate and lay more eggs (Neva and Brown 1994). This process, which starts with the ingestion of infective eggs and ends with the production of eggs, takes approximately 2-3 months (Thein Hlaing 1993). Adult worms have a life span of 1-2 years. Adult females can generate eggs for up to 1 year (Thein Hlaing 1993; O'Lorcain and Holland 2000).

The location and burden of the worms mostly determines the type and degree of morbidity observed in the host. During the migratory phase, large numbers of larvae may induce host sensitization resulting in asthma, coughing, shortnes of breath, fever, skin rash and eosinophilia (O'Lorcain and Holland 2000; Neva and Brown 1994). After the worms mature in the small intestine clinical sign and symptoms may include abdominal pain and distension, nausea, vomiting and anorexia. Vitamin A, fat and protein malabsorption also may be present (ibid). Less frequent clinical complications include intestinal obstruction, intestinal perforation, and blockage of the bile duct, acute pancreatitis or appendicitis due to the migration of worms. The infection is estimated to affect around 1049 million persons worldwide. Of these, 114 million are children of preschool 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 mature male and female whipworm inhabit the transverse and descending colons where they embed their narrow anterior portion in the host's columnar epithelium cells to obtain nutrients (Gilgen and Mascie-Taylor 2000). The worms feed on the enterocyte synctium but may also ingest enterocytes, leucocytes and mucosal fluids if they happen to penetrate below the basal membrane of the same (Pawloski 1984). Their posterior wider portion hangs in the gut lumen allowing the effective emission of eggs. Both sexes live for a span of approximately two years. The adult female can deposit from 3000 – 5000 eggs per day (Katz et al 1989). Fertilized eggs deposited with the feces are undeveloped and must embryonate before they can become infective. The duration of egg maturation is approximately 18–25 days if the ambient temperature is optimal, i.e., 20 – 30ºC (Katz et al 1989; Neva & Brown 1994). Trichuris eggs are more sensitive to the environment compared to those of *A. lumbricoides* eggs. Temperatures that fall below –9ºC or rise above 52ºC kill the eggs. Direct sunlight within 15 hours also kills the eggs (Stephenson et al. 2000).

Infection with Trichuris occurs via the oral-fecal route caused by the ingestion of infective eggs from contaminated food, hands or water. These then pass through the stomach to the small intestine where they hatch. The larvae penetrate the cells of the small intestine coming to lie above the lamina propria where they undergo four molts. The immature adults emerge and are passively transported to the large intestine where they mature and embed their thin, whip-like anterior into the columnar cells. The adult whipworm develops within 60–90 days after initial infection (Mehlhorn 1988; Katz et al 1989; Neva and Brown 1994; Stephenson et al 2000).

3.7 Global Situation of intestinal parasites

Intestinal parasites are top global health problem with prevalence of about 67% (Morales-Espinoza et al. 2003) and are distributed virtually throughout the world, with high prevalence rates in many regions (WHO 1987); whereas amoebiasis, ascariasis, Hookworm and trichuriasis are among the ten most common infections (WHO 1987). According to WHO (2000), an estimate of 3.5 million people have been infected and around 450 million children are ill due to intestinal parasitic infection. They affect child health and development and slow down growth while reducing adults' productivity and work capacity (Ngrenngarmlert et al. 2007); affect psychomotor development of the children, caused iron deficiency anemia, mental problem in children (Mengistu et al. 2007). Moreover, they cause economic burden of medical expenses and compounding the personal burden of disease and disability (WHO 2006). Intestinal parasitic infections are caused by both micro-parasites (protozoans) and macro-parasites (helminths) (WHO 1980). The global prevalence and intensity of intestinal protozoan and helmithic infections in man have shown considerable variation in distribution and in seasonal occurrence due to geographical and climatic factors and to human activities changing the environment and improving sanitation (WHO 1980). Intestinal parasites have clear social and environmental determinants, with high prevalence in region with deficiency of sanitation, potable water supplies, education and adequate dwelling condition (Ostan et al. 2007). High prevalence of intestinal parasitic infection is reported by different researchers over the entire world, particularly in developing countries, such as Africa. For example, Geltman et al. (2003) reported that high prevalence of intestinal parasite infection (56% of 1,254 refugees had intestinal parasites) among African refugees was detected. Among these refugees, 40% were infected with helminthes and 52% was with protozoan parasites.

At least 50% of the population in the world is affected with one or more species of helminthic parasites (Guerrant et al. 2001). According to Feachen et al. (1978), in tropical countries, particularly, in those with wide spread of poverty and low standards of hygiene, the rate of infection with intestinal parasites is considerably greater than this. An estimation of 850 million people (WHO, 1996) and 1.5 billion people (Crompton 1999) (quarter of the population in the world) are infected with *A. lumbricoides*. This high infection of ascariasis is concentrated in developing world with poor sanitation and personal hygiene (de` Silva et al. 1997).

In addition to helminths, there is a wide range of worldwide intestinal protozoa infection on humans; but the range of species and their prevalence has been high in developing countries with low standards of environmental sanitation and hygiene, whether in temperate or the tropical locations. The protozoan species that infect the human intestinal tract include: *G. lamblia*, Cyclospora caytanensis, Entameoba histolytica/dispar, the Cryptosporidium and Microsporidia species and Isospora belli. Most of these parasites are either strict or opportunistic intestinal parasites and their presence in stool specimen indicates that they are obtained through contamination of food or water (Mills and Goldsmid 1995).

The mortality and morbidity due to intestinal parasitic infections are relatively low when expressed as percentage of infected people, but because of their high prevalence, the total number of deaths and cases of diseases are rather high in relation to bacterial, viral or other infections. Hence the effect of intestinal parasitic infection on communities cannot be neglected. The transmission of most of the intestinal parasites reflects the level of sanitation and the availability and quality of water. For instance, the high prevalence of Ascariasis is good indicator of improper fecal disposal and high prevalence of Giardiasis frequently reflects the lack of water or its quality. Others such as Hookworm interfere with nutrition while others such as taeniasis etc., spread in contaminated food (WHO 1987).

3.8 Intestinal parasites in Ethiopia

Different researchers have reported that intestinal parasitic infection is widely distributed in Ethiopia (Haile et al. 1994; Erko et al. 1995; Erko and Medhin 2003; Andargie et al. 2006; Mengistu et al. 2007 etc.). Both geohelminthiasis and schistosomiasis are common in the country (Erko and Medhin, 2003). Studies by Haile et al. (1994) and Jemaneh 1998) showed that the high prevalence and distribution of intestinal parasites in Ethiopia may be due to the effect of altitude, urbanization, irrigation, and resettlement within the country. Mengistu et al. (2007) reported that parasitic helminthic infections are the second most predominant cause of outpatient morbidity in the country. Several factors such as climate, humidity, socioeconomic status, customary of nutrition, wars, immigration and water reservoir, etc., play an important role in the distribution and frequency of intestinal parasites (Yilmaz et al. 2007).

The most important intestinal parasites predominantly distributed in the county include: A. lumbricoides, *G. lamblia*, hookworm, *Hymenolepis nana*, *T. trichiura*, *E. vermicularis*, *E. histolytica*/*dispar*; with varying prevalence in different areas of the country. For example, Mengistu et al. (2007) showed that *T. trichiura*, *A. lumbricoides*, *E. histolytica*/*dispar*, *G. lamblia*, *Strongyloides stercoralis*, *H. nana*, intestinal schistosome, *Taenia saginata*, *E. vermicularis* and hookworm with prevalence of 60.9%, 40.9, 17.1% 13.9%, 17.5%, 2.1% 5.0%, 2.3%, 14.8% and 1.1% respectively were diagnosed from study groups in Jimma, south-western Ethiopia. In addition, Legesse and Erko (2004) reported that stool diagnosis from 259 surveyed students for intestinal parasites, 83.8% had one or more intestinal parasites which include hookworm (60.2%), *S. mansoni* (21.2%), *T. trichuria* (14.7%), Taenia species (13.9%), *E. histolytica*/dispar (12.7%), *A. lumbricoides* (6.2%), *G. lamblia* (6.2%) and *S. stercoralis* (5.8%), in descending order of prevalence.

Moreover, Jemaneh (1998) reported that the distribution of infection prevalence of the three common helminths; *A. lumbricoides*, Trichuris trichiura and the hookworms in school children in several communities of three altitudinal regions in Ethiopia have been shown to be different. That is, the prevalence of *A. lumbricoides* infection was 29% in the highlands, 35% in the temperate areas and 38% in the lowlands. The prevalence of hookworm infection was highest in the lowlands (24%) followed by the temperate (15%) and highland (7%) areas and the difference was significant ($p < 0.0001$), while T. trichiura infection exhibited similar prevalences in all altitudinal regions (13% on the average).

Stool samples examination based on morphology are commonly employed for the detection of the parasite ova, larvae, cyst or the parasite (Ngrenngarmlert et al. 2007). But, there has been the development of new approaches to the diagnosis, treatment, and prevention of intestinal protozoan parasites which can resolve the problem of morphological similarity between some intestinal protozoan parasites such as the pathogenic *E. histolytica* and the non-pathogenic E. dispar which have similar morphology and could pledge difficulty in differentiating them with microscopic diagnosis. Antigen-detection tests are now commercially available for the diagnosis of all three major intestinal protozoan parasites.

However, the diagnosis and treatment of intestinal helminthic infections have not been changed much, and the traditional microscopic method can be used for their diagnosis (Rashidul 2007). Despite substantial investment and research, no vaccines are yet available for prevention of human intestinal parasitic infections. Prevention is based on avoidance strategies which are based on sanitary disposal of feces, keeping personal hygiene and provision of purified water. Generally, diagnosis, treatment, prevention and control of the common intestinal parasites require the good functioning of the health centers in a horizontal approach (WHO 1987). In connection with the hygiene and sanitary based control of intestinal parasites, deworming of helmintheiasis on targeted groups of the community (children of age above 5 years old) has been implemented in Ethiopia.

4. Materials and methods

4.1 The study site

The study was conducted in Borena Wereda of Amhara national regional state and part of south Wollo northeast Ethiopia (fig. 2). Borena Woreda is located at a distance of 578km from Addis Ababa. It is located in Amhara National Regional State and is part of South Wollo Zone which is bordered on the north by Amhara Saint, on the south by Wogdi Woreda, on the west by the Abay River which separates it from East Gojjam and on the east by Legambo Woreda. The absolute location of Woreda lies between 10°44'00'' to 11°05'00" latitude and 38°45´00'' to 39°11'00'' longitude (Borena woreda communication office 2014).

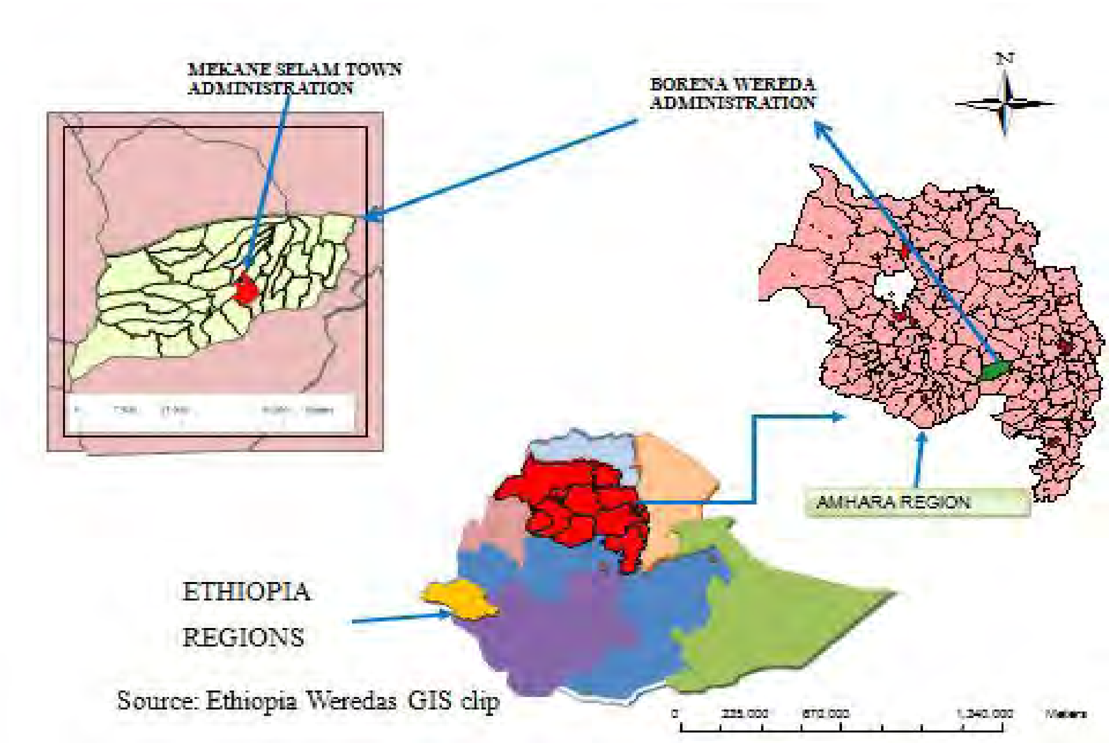

Figure 2

Figure 2 Location of the study area

The altitude of this Woreda ranges from 500 meters above sea level at the bottom of the canyon of the Abay River to 3200 meters in the north east corner. This Woreda has four climatic zones: 1% Wurch, 20% Dega, 47% Woynadega and 32% Kola. The mean annual rainfall is1500mm and the mean annual temperature is 23˚c (Borena Woreda Communication Office 2014). According to Central Statistical Agency of Ethiopia (CSA 2007), Borena Woreda has an estimated total population of 163,588 of which 82,936 males and 80,652 females. Over the past few years, the Organization for Rehabilitation and Development in Amhara (ORDA) and the NGO named Menschen fiir Menschen (MfM) have been constructing a number of rural water supply sources in this area through a community- based approach with a goal of improving the health status and the livelihood of rural communities. Even do so, potable water supply coverage remains very low in the rural areas (BWWSO 2015).

4.2 Study design and population

Retrospective (November 2014-May-2016) and cross-sectional surveys (November 2015-may 2016) were carried out for assessment of the prevalence of IPIs among school-age children at MHC. The study population included school-age children (5-14 years old).Thus the sampling technique was convenience sampling method. Data on socio-demography, document analysis, and personal households as well as knowledge, attitude and practices (KAP) of participant about IPIS were captured using a structure questionnaire. Direct observations were made to verify verifiable information. . During data collection parents of children participated to respond to questionnaire.

4.3 Stool sample collection and analysis

During stool collection, disposable plastic cups and spoon were distributed to each study subject along with brief instructions on how to collect the stool. They were also advised to fill up the disposable plastic cup about the size of the tip of the thumb (approximately 5g of stool) of fresh stool using disposable spoon that was given with the container. Each sample was labeled properly. The stool samples were transported to MHC on the same day of collection for analysis. Stool samples were diagnosed for the presence of intestinal parasites using direct wet-mount method. The processed stool samples were checked for the presence of intestinal parasite ova or cysts under light microscopy using objectives 10 xs and 40 xs. Identification of the parasite species was done on the basis of morphology and size by the principal investigator assisted by experienced laboratory technicians and referring the parasitological laboratory manual (Cheesbrough 1990). Direct wet mount technique was used to assess the overall prevalence of IPI in the study area. The direct wet mount was processed by conventional iodine to identify the presence of motile intestinal parasites, cysts, egg and trophozoite under light microscope at 10X and 40x magnification. Saline was used to observe cysts of intestinal parasites (Singh et al.

2004). About 2g of stool samples was emulsified with 3-4 ml normal saline, and then a drop of emulsified sample placed on a clean microscopic glass slide, then a few drops of iodine solution was added and it was covered with a cover slip. The presence of intestinal parasites ova and cyst was observed under the microscope (Lindo et al. 1998).

4.4 Data analysis

SPSS (statistical package for social sciences) version 16.0 soft ware was used for statistical data analysis. Descriptive statistics was used to give a clear picture of population characteristics such as age, sex, and the prevalence of intestinal parasites. Association of risk factors and proportion of intestinal parasites was made using chi-square test. Statistical significance was defined at P-values less than 0.05.

4.5 Ethical considerations

The study was reviewed and approved by the higher management of MHC. The identity of the participants was confidential. Finally the study participants were treated with the standard regime for freely by local health professionals.

5. Results

5.1 Study population

Retrospective data

In the past eight months (September 2014-May 2015) 314 IPI suspected patients were registered on MHC laboratory logbook. Out of these, 149(47.5%) were males and 165(52.5%) females. In the age 5-9 were 160 and the rest were 10-14 years old (table 1). The overall prevalence of IPIs among all age groups of school-age children was 79.29%.

Table 1 Distribution of IPIs identified among children at MHC northeast Ethiopia, November 2014-May 2015 (n=314)

Description		Eh/d/m	Gl	Hw	Al
Age(year)	N	no.(%)	no.(%)	no.(%)	no.(%)
5-9					
Male	77(48.1)	21(27.3)	13(16.9)	2(2.6)	12(15.6)
Female	83(51.9)	18(21.7)	15(18.1)	6(7.2)	8(9.6)
10-14					
Male	72(46.8)	27(37.5)	22(30.5)	2(2.8)	17(23.6)
Female	82(53.2)	24(29.2)	31(37.8)	4(4.8)	19(23.2)
Total	314(100)	90(28.7)	84(26.8)	14(4.5)	56(17.8)

Eh: *Entamoeba histoly/dispar/moshkovskii* Gl: *G. lamblia*, Hw: Hookworm, Al: *A. lumbricoides*

5.2 Results of the current survey

The overall prevalence of IPIs was 58.8%. There were 4 different species or types. These were *E. histolytica/dispar/moshkovskii* 51(22.5%), *G. lamblia* 57(25.1%), hokworm 15(6.6%) and *A. lumbricoides* 10(4.4%). Significantly higher prevalence of IPIs occurred among 5-9 years old children (61.9%) compared to those aged 10-14 (49.9%) (table 2). Age group 5-9 has lower infection with hookworm 11.8% male and 5.6% female as compare to *G. lamblia* 35.3% male and 22.2% female. *E. histolytica/dispar/ moshkovskii, A. lumbricoides* and *G. lamblia* infections were shown to be the most frequent infection in the age group of 5-9 years old. A relatively frequent *E. histolytica/dispar/moshkovskii* and *A. lumbricoides* infection was seen in the age group 10-14 years old. *E. histolytica/dispar/moshkovskii* infection was most frequent in the age group 10-14, while hookworm was the lower infection

Table 2 Distribution of IPIs identified among school-age children at MHC northeast Ethiopia, in the current cross-sectional, November 2015-May 2016 (n=227)

Age(year)	N	Eh/d/m no.(%)	Gl no.(%)	Hw no.(%)	Al no.(%)
5-9					
Male	51(48.6)	14(27.5)	18(35.3)	6(11.8)	2(3.9)
Female	54(51.4)	9(16.7)	12(22.2)	3(5.6)	1(1.9)
10-14					
Male	66(54.1)	15(22.7)	16(24.2)	4(6.1)	1(1.5)
Female	56(45.9)	13(23.2)	11(19.6)	2(3.6)	6(1,1)
Total	227(100)	51(22.5)	57(25.1)	15(6.6)	10(4.4)

Eh: *Entamoeba histolytica/dispar/moshkovskii*, Gl: *G. lamblia*, Hw: hookworm, Al: *A. lumbricoides*

5.3 KAP data

Analysis showed that there was no significant difference between male and female children parasitic infection in the present study (p=0.175). Some of the parents and children were complaining of having signs and symptoms related to intestinal parasites such as abdominal pain and abdominal cramp 23.8%, bloating 11.4% and nausea 13.3%, bloody diarrhea 3.8%, diarrhea without blood 4.8%, loose stool 0.95% and fatty stool 4.8% were diagnosed parasitic.

Among the questionnaire that was requested whether study participants have used to washing their hands before meal and after toilet use 90.5% reported that they have washed their hands before meal and after toilet. Among the participants who owned toilet 20.0% and from those without toilet 80.0% were determined to possess intestinal parasites. Other risk factors in relation to intestinal parasite infection were drinking water sources; pond 0%, river 24.0%, spring 64.0% and Tap water 24.0% found in the study participants were infected with intestinal parasites.

Comparison of parasite prevalence between those who were drinking raw and boiled water showed that those drinking raw water were at more than eight times at risk of infection. In addition, participants who reported of disposing household wastes through incineration/ buried under ground 48.0% and on the open field 52.0% were found infected with intestinal parasites. Moreover, those participants who consume raw meat 48.0% and uncooked vegetables, 52.0% were also found infected by the same. From the information obtained, it was obvious that IPI were associated with easily avoidable risk factors such as absence of toilet, custom of eating raw meat and uncooked vegetables and drinking raw water (table 3)

Table 3 Socio-demography and other variables of children treated at MHC for IPIs, northeast Ethiopia 2015 2016 (N=227)

Variable	**N**	**IPI-positive n(%)**	**X^2**	**p-value**
Sex				
male	141	32(60.0)		
female	86	21(40.0)	3.8345	0.683
Toilet				
Yes	74	15(20.0)		
No	153	43(80.0)	5.5512	0.004
Post-toilet/pre-meal hand wash				
Yes	160	22(40.0)		
No	67	32(60.0)	3.1666	0.001
Drinking water				
Pond	17	0		
Spring	142	39(64.0)		
River	15	6(24.0)		
Tap water	53	13(24.0)	2.1335	0.004
Utilization of drinking water				
Raw	153	47(88.0)		
Boiled	74	7(12.0)	4.8190	0.001
Beef consumption				
Yes	87	25(48.0)		
No	140	28(52.0)	7.9121	0.056
Waste disposal				
Incineration	112	26(48.0)		
Open field	115	28(52.0)	1.2051	0.002
Uncooked vegetables				
Yes	104	35(64.0)		
No	123	20(36.0)	8.9567	0.098

IPIs: intestinal parasitic infections, p: p-value: X^2: chi-squared MHC: Mekaneselam health center

6. Discussion

Among the widely prevalent intestinal parasites in low-income countries (Rao et al. 2003), four were detected in the present study. The 24.6% total prevalence detected in the present study was lower than the average prevalence (46.3%) of the retrospective clinical record obtained from MHC and other studies conducted in different parts of Ethiopia. For example, Roma and Worku (1997) had reported an infection prevalence of 89.4% among schoolchildren in Wondo-Genet Zuria, southern Ethiopia and Mengistu et al. (2007) with 83% prevalence among urban dwellers in southwest Ethiopia, had reported higher prevalence than the present study. But it was comparable to that reported by Tadesse (2005) which was 27.2%. The higher prevalence in this study might be due to favorable climatic and environmental conditions coupled with poor water supply and other sanitary practices or facilities.

E. histolytica/dispar/moshkovskii with prevalence of 10.83% was the most prevalent parasitic infection in the present study which is relatively lower than the study reported by Ali et al. (1999) with prevalence of 21.9%, Mengistu et al. (2007) with prevalence of 17.1% among urban dwellers in southwest Ethiopia; but much higher than that reported by Woldemichael et al. (1999) with prevalence of 0.5%, from a study conducted in Western Abaya, Ethiopia. However, it is nearly similar with that reported by Legesse and Erko (2004) with prevalence of 12.7% in a study conducted among schoolchildren in a rural area close to the southeast of Lake Langano.

A. lumbricoides was the second most prevalent parasite in this study with prevalence of 7.6% which is lower than that reported by several other investigators in Ethiopia. For example, Jemaneh (1998) assessed schoolchildren in several communities of three altitudinal regions and found 29% in the highlands, 35% in the temperate areas and 38% in the lowlands. Merid et al. (2001) recorded 76.9% prevalence among children at Lake Awassa area, south Ethiopia. On the other hand, the current study is similar with the finding of Woldemichael et al. (1999) from Western Abaya (10%).

However, lower prevalence of *A. lumbricoides* (3.9%) than the present study was reported by Tadesse (2005) among school children in Babile town, eastern Ethiopia. Hookworm with prevalence of 3.6% in the present study was lower in prevalence than that reported by Tadesse

(2005) which is 10.1% among school children in Babile town, eastern Ethiopia, but with nearly similar prevalence (5%) by Mengistu et al. (2007) among urban dwellers in southwest Ethiopia. Hookworm was found to be 1.99% which is very low compared other reports as high as 40% (Erko et al. 1995) from Bahir Dar; Jemaneh (1998) with prevalence of 7-24%; Tadesse (2005) 6.7%; Mengistu et al. (2007) with 17.5%. In other sub-Saharan African countries such as Nigeria the prevalence hookworm infection among school children reaches 54.6% (Nmor et al. 2009).

G. lamblia with prevalence of 1.81% in the present study was relatively lower than the 3.1% by Mengistu et al. (2007), but higher than Woldemichael et al. (1999) with prevalence of 0.3%. *G. lamblia* infection was significantly different between males and females. This may be due to the fact that females are more often involved in food processing and handling activities than males, hence water and food contamination could be the possible infectious cause (WHO 1987).

Multiple parasitic infections in the present study (3.1%) much more lower than other reports elsewhere in Ethiopia as well as Nigeria. For instance, Mengistu et al. (2007) documented 56.7%, Nmor et al. (2009) 39.40%.

Toilet possession was not significantly associated with protection from IPIs suggesting that the toilets were not being properly utilized. On the other hand, direct utilization of spring water for drinking was significantly associated with IPIs particularly *E. histolytica/dispar/moshkovskii*. This finding together with the overall highest prevalence of this parasite implies waterborne transmission of the protozoa among the children. This calls for provision of safe drinking water.

The data showed that pond, river and tap waters were less likely causes of amoeba infection compared to springs. Because of chlorination tap water is relatively best protected and might have contained very low intestinal parasites whose probability of infection would be very low. As river water is continually flowing down stream, intestinal parasites may be less concentrated to cause infection. The pond water might not have been contaminated with surface runoff or flood water or sewage. On the other hand, spring water could be easily contaminated with flood water and fecal matter contained in dust carried by wind.

According to the food habit interviewed, the utilization of uncooked vegetables was significantly associated with *E. histolytica/dispar/moshkovskii* infection and this is similar with other studies (Erko et al. 1995, Myarango et al. 2008). This could be due to the fact that uncooked vegetables served as food may be contaminated with intestinal parasites through fecal contaminated water as well as by food handlers. But, regarding waste disposal, there was no significant association between IPIs and the method of waste disposal indicated in the interview response. Seasonal floods wash away parasites in the wastes and seasonal fluctuations may account for this lack of association.

7. Conclusion and recommendations

IPIs were common health problem among the school-age children. This study has also shown that children mothers' educational level, nail hygienic status, and hand washing practice before meal were closely associated with the prevalence of IPIs. Thus, there is a need for intensive and habitual health education for behavioral changes related to personal hygiene and mass treatment for effective control of IPIs in the area. Further research to understand the epidemiology and clear picture of IPIs based on large sample size including all age groups is necessary. There should be mass-scale deworming

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9. Appendices

Annex I 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 intestinal parasitic infections among school age children treated at Mekaneselam health center north west Ethiopia which helps in understanding the prevalence of parasitic infection in relation with knowledge, attitude and practice of different communities towards parasitic disease. At the same time, it enables concerned body in designing better control and preventive measures of intestinal parasitic diseases in the study area.

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

Name (participant)____________________________Signature____________Date__________

Name (Wittiness)______________________________Signature ________ Date_________

Name (Investigator)_____________________________Signature _________ Date ________

Appendix II. Questionnaire

The purpose of this questionnaire is to gather data on intestinal parasites in school age children in Mekaneselam health center. It is conducted with the aim of obtaining information to the research as the partial fulfillment for Master of Science in biology. To attain its objective you are kindly requested to give reliable information. The researcher assures you that your response will be confidential and only to be used for the purpose of the study; moreover, when filling this questionnaire you don't need to write your name.

Thank you in advance

Part 1: demographic information

Sex; - male____ Female___

Age: 20-25 years____ 26-30 years______ 31 years and above _____

Educational status: Illiterate ------- Read and write only--------

Years of formal education others (Specify) -------------------

Part 2: questionnaire

Please, circle the letter of your choice that are correct for you .

1. Have you ever encountered abdominal pain? A. yes B. no

If your answer is yes which kinds of symptom that you observed during intestinal discomfort?

A. cramps B. bloating C. nausea and vomiting during intestinal discomfort?

2. Which kinds of sign and symptoms did you observed when you defecate?

A. blood diarrhea B. Non bloody diarrhea C. Loose fatty stool

3. Did you take treatment for this discomfort? A. yes B. No

4. Do you wash your hands before meal and after latrine use?

A. yes B. No

5. Do you have a toilet? A. yes B. No

6. Where do you get drinking water from? A. spring B. pond C. river and pipe

7. How do you use drinking water? A. boiling B. chlorine treatment C. Direct

8. How do you dispose household wastes? A. bury underground (incinerate) B. Open field

9. Have you ever eaten raw meat? A. yes B. No

10. What about uncooked vegetable? A. yes B. No

11. Where do get treatment serves for intestinal parasites? A. Government health center B. private clinic and pharmacies

Appendix III Questionnaire (Amharic version)

የዚህ መጠይቅ ዋና አላማ "በመካነሰላም ጤና ጣቢያ እይተከሰተ ያለዉን የአንጀት ጥገኛ ተህዋስያን ያላቸዉን ጉዳት እንድሁም በምን ሁኔታ ላይ እንዳለ መጠቆም ሲሆን በተለይም የጥናቱ ዋና አላማ እድሜአቸዉ ለትምህርት የደረሱ ህፃናት ላይ ያለዉን የኢኮኖሚ የማህበራዊ እና የስነ-ልቦና ጉዳት በአሁኑ ሰአት እና ባለፉት ወራት ያደረሰዉን ተፅእኖ እና የወደፊት የመፍትሄ አቅጣጫ ለማስቀመጥ ታስቦ የተዘጋጀ ነዉ:: ስለዚህ የጥናቱን አላማ ለማሳካት እና ከግብ ለማድረስ ትክክለኛዉን መረጃ እንድሰጡኝ በትህትና እየጠየኩ መጠይቁን ሲሞሉ ስምዎትን መፃፍ አይጠበቅበዎትም::

ስለ ትብብርዎ እናመሰግናለን

ክፍል 1 ስነ-ህዝብ መረጃ መጠይቅ

ፆታ ወ------ ሴ--------

እድሜ 20-25 አመት 26-30 አመት 31 አመት እና በላይ

ክፍል 2 መጠይቅ

ትክክለኛዉን መልስ ምረጥ/ጭ

1. ሆድዎ አካባቢ የህመም ስሜት ተሰምቶዎት ያውቃል? ሀ. አዎ ለ. አያዉቅም

አዎ ብለው ከመለሱ ምልክቱ ምን አይነት ይሆን? ሀ. የአንጀት ጡንቻ መሸማቀቅ ለ. የእብጠት ስሜት መ. ማቅለሽለሽ እና ማስታወክ

2. ሲፀዳዱ ምን አይነት ምልክት ያስተዉላሉ? ሀ. ደም የቀላቀለ ተቅማጥ ለ. ደም የሌለዉ ተቅማጥ መ. ልልና ስብ ያለው ተቅማጥ

3. ለበሽታዉ ህክምና አድርገው ያዉቃሉ? ሀ. አዎ ለ. ኣያዉቁም

4. እጅዎትን ምግብ ከመመገብዎ በፊት እና ከሽንት ቤት መልስ ይታጠባሉ? ሀ. አዎ ለ. አልታጠብም

5. መፀዳጃ ቤት አለዎ? ሀ. አዎ ለ. የለኝም

6. የሚጠጣ ዉሃ ከየት ይቀዳሉ? ሀ. ከምንጭ ለ. ከኩሬ መ. ወንዝ እና ቧንቧ ዉሃ

7. የመጠጥ ዉሃን ዕንደት ያከማሉ? ሀ. በማፍላት ለ. ክሎሪን በመጨመር መ. በቀጥታ(ጥሬ ዉሀ)

8. የቤት ቆሻሻን እንደት ያስወግዳሉ? ሀ. በመቅበር እና በማቃጠል ለ. ሜዳ ላይ በመድፋት

9. ጥሬ ስጋ በልተው ያውቃሉ? ሀ. አዎ ለ. በልቶ አላዉቅም

10. ያልተቀቀለ አትክልትስ? ሀ. አዎ ለ. በልቶ አላዉቅም

11. የአንጀት ጥገኛ ተህዋስያንን የት ይታከማሉ? ሀ. መንግስት ጤና ጣቢያ ለ. ግል ክሊኒክ እና መድሃኒት ቤት ስለ ትብብርዎ እናመሰግናለን

10. Declaration

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

Name Yimam Ali

Signature_______________________________________

Date ____

11. Statement of supervisor(s)

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

Name Hassen Mamo (PhD)

Signature____________________________Date__________________________________