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BIOMEDICAL SCIENCE**



**MALARIA AND INTESTINAL PARASITE INFECTIONS AND CO-
INFECTIONS IN TACH GAYINT DISTRICT, SOUTH GONDAR ZONE**

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LIST OF ABBREVIATIONS

CDC = Center for Disease Control

CQ = Chloroquine

DALY = Disability Adjusted Life Year

MOH = Ministry of Health

HIV/AIDS = Human Immuno Deficiency Virus/ Acquired Immuno Deficiency Syndrome

Ig = Immunoglobulin

IL = Interleukin

MoE = Ministry of Health

Msal = Meter above sea level

RBC = Red Blood Cell

RPM = Rotation per Minute

SP = Sulfadoxine Pyrimethamine

STH = Soil Transmitted Helminthes

TDC = Trichuris Dysentery Syndrome

TH = T- helper

TNF = Tumor Necrosis Factor

WHO = World Health Organization

ABSTRACT

To assess the level of intestinal parasite and malaria infection, a total of 806 consenting individuals were randomly selected from Tach Gayint District. The prevalence of malaria, intestinal protozoa and helminth infections and co-infections in the area were determined. The study was conducted in two seasons, November/December, 2008 (after the big rains) and April/May, 2009 (after the small rains). Blood film determination, direct wet mount and concentration techniques were respectively used for diagnosis of malaria and intestinal parasites. The overall prevalence of intestinal parasites was the same in the two seasons, 67.28% (November/December) and 67.20% (April/May), indicating lack of variation in intestinal parasite burden during the two dry periods of the year. However, the prevalence of hookworm, *S. mansoni* and *A. lumbricoides* were significantly higher in the age groups below 14 years ($P < 0.05$). The prevalence of *T. trichiura* in school children was much higher (21.08%) than in the adult population (3.33%) ($P < 0.001$) and the cumulative prevalence of *H. nana* in school children (7.98%) was also significantly ($P = 0.003$) higher than in the adult population (3.30%). These findings indicated that the hygienic condition of the children is much worse than that of the adults and requires extra attention for improvement. On the other hand, the prevalence of malaria showed the typical seasonal pattern, with high peak transmission (11.17%) in November/December and low transmission (5.46%) in April/May. Malaria prevalence in the two seasons was at levels that are of public health concern ($> 5\%$). This indicates that malaria control program in the study area was inadequate or was not being properly implemented. Furthermore, the double, triple and quadruple co-infections involving malaria and intestinal parasites were indications of high burden of parasite infection in the study area. On the whole, the findings of the present study have provided an empirical evidence for the need to implement effective malaria and intestinal parasite control measures in Tach Gayint District.

Key word: Intestinal, Helminth, Protozoa, Malaria, Co-infections, Tach Gayint.

1. INTRODUCTION

Intestinal helminth and protozoan infections are among the most common infections worldwide. Every year, nearly one million deaths result from malaria infection (Barnes *et al.*, 2009). The prevalence of intestinal parasites in children varies in different regions of the world. It is particularly high in poor and developing countries due to use of contaminated drinking water, inadequate sanitary conditions, and poor personal hygiene (Wadood *et al.*, 2005). Ethiopia has one of the lowest quality drinking water supply and latrine coverage in the world. As a report from 2000 study shows Ethiopia had only 12% latrine coverage while Kenya had 87% (Kumie *et al.*, 2005).

The World Health Organization (WHO) reported that *Ascaris lumbricoides* and invasive amoebiasis are the most common public health problems in Africa (WHO, 1987 cited in Mengistu *et al.*, 2007). Several studies indicated that the prevalence of parasitic infections were high in the lower altitudes including southwestern Ethiopia. Many reports illustrated that *A. lumbricoides* is the most prevalent intestinal parasite in different communities usually occurring together with *Trichuris* infections (Wodemichael *et al.*, 1999). The prevalence of taeniasis alone ranges from 1- 48%, (Wodemichael *et al.*, 1999). Schistosomiasis is common in northern region as compared to south and south west regions of Ethiopia. Amoebiasis and giardiasis are common causes of intestinal protozoal infections throughout the nation. The prevalence of amoebiasis ranges from 0-4% and that of giardiasis is 3-23% (Haile *et al.*, 1994). Over one billion of the world's population is chronically infested with soil-transmitted helminthes and 200 million are

infested with schistosomiasis. Schistosomiasis is more common in the northern regions as compared to the south and south west regions of Ethiopia (Lo *et al.*, 1988). In spite of the number of measures taken to control parasites, parasitic infections are still prevalent and cosmopolitan especially in the tropical regions. Among tropical diseases, malaria has been identified as one of the most important public health problems and is the most important human parasitic disease, affecting over 200 million people and causing more than one million deaths each year (Suyaphun *et al.*, 2002).

South Gondar, like many malarious regions of Ethiopia, is prone to unstable and epidemic malaria. In the region, malaria is seasonal and follows the rain with the highest incidence in November and December (Emerson *et al.*, 2008). Accurate information is needed not only to improve our knowledge of malaria epidemics, but also to assess progress of malaria control initiatives that aim to decrease deaths from malaria worldwide by 50% by 2010 (Nabarro and Tayler, 1998).

Associated with chronic diarrhea and malnutrition, the intestinal parasites often compromise the physical and intellectual development, especially among the youngest age groups in a population (Mengistu *et al.*, 2007). Severe parasites can lead to blood loss, tissue damage, spontaneous abortion, and death. The distribution of protozoan and helminth infection depends on conditions such as suitable climate and human activities such as population movement and poor sanitation (Tadesse, 2005).

1.1. Malaria

Malaria is a major human health threat in tropical and subtropical regions of the world (Minakawa *et al.*, 2006). Malaria kills about 1 million people each year (Pierce and Miller, 2009). About 90% of all malaria deaths in the world occur in Africa, south of the Sahara (Guerra *et al.*, 2008). The majority of infections in Africa is caused by *P. falciparum* (Van Schaijk *et al.*, 2008; Nago *et al.*, 2008), which is the most dangerous of the four human malaria parasites (Guerra *et al.*, 2008), transmit by the vector *Anopheles gambiae*.

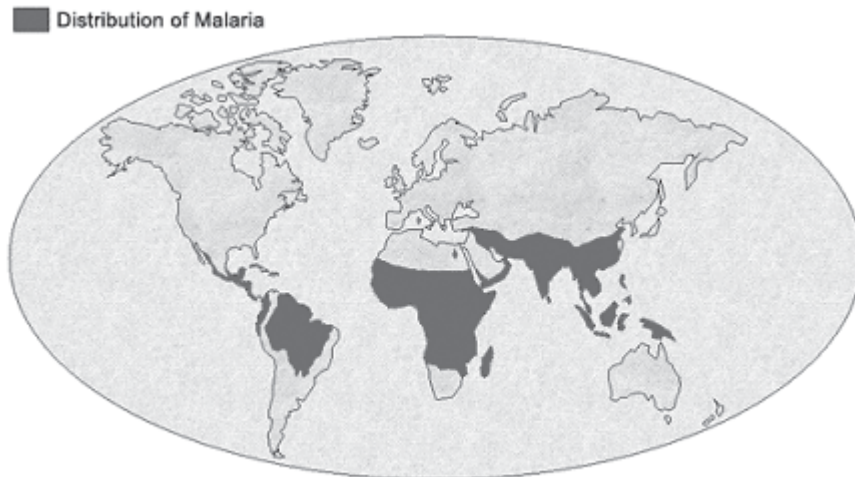


Figure1. Global distribution of malaria (Reproduced from CDC website http://www.cdc.gov/malaria/distribution_epi/distribution.htm. 04/06/2009)

Malaria constitutes a major public health problem and impediment to socioeconomic development in Ethiopia. It is estimated that about 75% of the total area of the country and 65% of the population is estimated to be at risk of infection (MOH, 2007). *P. falciparum* accounts for about 60% of the cases of malaria; *P. vivax* accounts for about 40% (Ramos *et al.*, 2005). The switch from the previously predominant *P. vivax* to *P. falciparum* has been reported in some

areas of Ethiopia (Ramos *et al.*, 2005). Drug resistance of *P. falciparum* to sulfadoxine-pyrimethamine (SP) and chloroquine (CQ) and insecticide resistant *Anopheleous* mosquito is frequent and intense in some areas of the country and can be accounted for the switch to *P. falciparum* (Schunk *et al.*, 2006). However this situation is reversed due to utilization of effective drugs such as Artemisin based combination therapy. An important feature in the epidemiology of malaria in Ethiopia is the brevity of transmission season that precludes the development of immunity that favours periodic epidemics attended by high mortality. The peak transmission of malaria occurs during the months of September to December (MOH, 2007).

Migration into malarious areas, changing agricultural practices such as irrigation projects, increased demands on public health systems due to the spread of HIV/AIDS in developing countries, and long term climatic changes such as global warming, contribute to the growing incidence of malaria (Sachs and Malaney, 2002; Lindblade *et al.*, 2000; Hume *et al.*, 2003). In Ethiopia malaria control has an integrated approach, that is vector control with long lasting Insecticide Treated Nets, Insecticide Residual Spray, early diagnosis and treatment, environmental modification and education that help to reduce malaria (MoH, 2007).

The major clinical features of malaria include, severe anemia due to reduced production and increased destruction of RBCs, cerebral complications due to micro-vascular obstruction in the brain, which causes impaired consciousness, convulsions and long term neurological deficits metabolic acidosis, reduced tissue perfusion, hypoglycaemia, hypoxia due to respiratory distress and pulmonary pathology and placental infection during pregnancy (Mackintosh *et al.*, 2004). Whereas severe headache, fever, vomiting, sequestration of RBCs, anemia and loss of appetite

are the clinical feature of uncomplicated malaria (Mackintosh *et al.*, 2004). In areas of stable malaria transmission, very young children and pregnant women are at highest risk for malaria morbidity and mortality. Severe anemia and cerebral malaria constitute the major cause of death, mostly in children under the age of 5 years (Malaguarnera *et al.*, 2002).

In areas with stable parasite transmission, *P. falciparum* malaria is concentrated among young children because protective immunity to the disease is acquired over a period of years of repeated infections. Pregnant women constitute a notable exception to this rule, since they are highly vulnerable to infection, particularly during a first pregnancy (Chaisavaneeyakorn *et al.*, 2005). Adult women in areas of stable transmission have a high level of immunity, but this is impaired especially in the first pregnancy. Al-Serouri *et al.*, (2000), showed that asymptomatic malaria parasitemia affects impaired cognitive function and school achievement in Yemen school children.

In Ethiopia 75% of the land is potentially endemic to malaria (MOH, 2005). This leads to mortality and morbidity of most children (Eggena *et al.*, 2006). There are three principal ways in which malaria can contribute to death in young children. First, an overwhelming acute infection, which frequently presents as seizures or coma (cerebral malaria), may kill a child directly and quickly. Second, repeated malaria infections contribute to the development of severe anemia, which substantially increases the risk of death. Third, low birth weight frequently the consequence of malaria infection in pregnant women is the major risk factor for death in the first month of life.

Of all *Plasmodium spp* *P. falciparum* is the most life threatens species in Africa. Attachment of

erythrocytes infected by *Plasmodium falciparum* to receptors of the microvasculature is a major contributor to the pathology and morbidity associated with malaria. Adhesion is mediated by the *P. falciparum* erythrocyte membrane protein 1 (PfEMP-1), which is expressed at the surface of infected erythrocytes and is linked to both antigenic variation and cytoadherence. Adhesion of infected erythrocytes in various tissues a process called sequestration is thought to be key to the pathogenesis of *Plasmodium falciparum*. Sequestration is thought to be the result of interaction between parasite –encoded variant surface antigens on the outer membrane of IEs and a range of host receptors (Hviid L, 2007).

1.2. Intestinal Protozoa

Numerous protozoa inhabit the gastro-intestinal tract of humans. The majority of intestinal protozoa is non-pathogenic commensals, or only result in mild disease. Some of these organisms can cause severe disease under certain circumstances. Apicomplexa and microsporidia species, which normally do not evoke severe disease, can cause severe and life-threatening diarrhea in AIDS patients and other immunocompromised individuals (Chen *et al.*, 2007). Intestinal protozoa are transmitted by the fecal-oral route and tend to exhibit similar life cycles consisting of a cyst stage and a trophozoite stage. Fecal-oral transmission involves the ingestion of food or water contaminated with cysts. Some of the trophozoites will develop into cysts instead of undergoing replication. Factors which increase the chance of ingesting materials contaminated with fecal material play a role in the transmission of these intestinal protozoa. In general, situations involving close human-human contact and unhygienic conditions promote transmission.

Several members of the genus *Entamoeba* infect humans. High prevalence of intestinal amoebiasis is commonly reported by microscopy in Ethiopia (Kebede *et al.*, 2004). Among these only *E. histolytica* is considered pathogenic and the disease it causes is called amoebiasis or amebic dysentery. *E. dispar* is morphologically identical to *E. histolytica*, but is not pathogenic. Genetic and biochemical data indicate that the non-pathogenic *E. dispar* is a distinct species (Kebede *et al.*, 2004 and Stanley, 2003). The two species are found throughout the world, but like many other intestinal protozoa, they are more common in tropical countries or other areas with poor sanitary conditions. It is estimated that up to 10% of the world's population may be infected with either *E. histolytica* or *E. dispar* and in many tropical countries the prevalence may approach 50%. It is also estimated that about 100,000 deaths and 50 million cases of amoebiasis occur per year in the world and humans are the only host of *E. histolytica* and there are no animal reservoirs (Haque *et al.*, 2003).

Upon ingestion the cysts pass through the stomach and excyst in the lower portion of the small intestine, and undergo repeated rounds of binary fission (Stanley, 2003). The amebas can also metastasize to other organs and produce an extra intestinal amoebiasis (Haque *et al.*, 2003). The non-invasive disease is often asymptomatic, but can cause diarrhea or other gastro-intestinal symptoms such as abdominal pain or cramps. This non-invasive infection can persist or progress to an invasive disease in which trophozoites penetrate the intestinal mucosa and kill the epithelial cells (Stanley, 2003).

Giardia lamblia has a worldwide distribution and is the most common protozoan isolated from human stools. The incidence is estimated at 200 million clinical cases per year (Partovi *et al.*,

2007). The prevalence of giardiasis is very high in developing countries where the living standard of the society is very low. Endeshaw *et al.* (2004) reported a prevalence of 20.8% among diarrheal patients referred to EHNRI. However, Ayalew (2006) reported a prevalence of 38% among children from eastern Ethiopia (Dire-Dawa). Trophozoites of *G. lamblia* might damage the brush borders of enterocytes and impair activity of mucosal enzymes, particularly the disaccharidases causing carbohydrate and fat malabsorption (Partovi *et al.*, 2007). *Giardia* exhibits a typical fecal-oral transmission cycle. The infection is acquired through the ingestion of cysts. Factors leading to contamination of food or water with fecal material are correlated with transmission.

Person-to-person transmission is the most prevalent mode of transmission and the risk factors are close human contact combined with unhygienic conditions. The ingested cyst passes through the stomach and excystation takes place in the duodenum, the trophozoites are predominantly found attached to epithelial cells of the small intestine (especially the duodenum and jejunum) and are rarely found in stools, except in the cases of severe diarrhea.

1.3. Intestinal Helminths

Intestinal helminths are widely distributed mostly in tropical and sub tropical regions of Asia, especially China, India and South East Asia as well as Sub-Saharan Africa. It was estimated that almost 2 billion people are infected by one or more of intestinal helminthes (Hotez *et al.*, 2004). Of the 1-2 billion soil-transmitted helminth infection worldwide, approximately 300 million infections result in severe morbidity, which are associated with the heaviest worm burdens (Hotez *et al.*, 2004). In Ethiopia the prevalence and distribution of intestinal helminthes varies

from place to place (Erosie *et al.*, 2002; Legesse and Erko, 2004; Jemaneh, 2000; Merid *et al.*, 2001; Tadesse, 2005).

Epidemiological studies of most human intestinal helminthes indicated that adolescents and adults are more resistant to infection and re-infection than children. Due to the greatest risk for acquiring heavy infections with hookworms, *Ascaris* and *Trichuris* infections, the children will suffer anemia, acute ascariasis, intestinal obstruction, hepatobiliary ascariasis, *Trichuris* dysentery syndrome (TDS), or rectal prolapse, and physical growth retardation associated with cognitive and educational impairments (Abebe *et al.*, 2003; Montresor *et al.*, 2002; Kirwan *et al.*, 2009).

Hookworm infection in humans is caused by two species of nematodes, *Necator americanus* and *Ancylostoma duodenale* (Jemaneh and Tedla, 1985). Both of the human hookworm species are known to exist in Ethiopia (Armstrong and Tadesse, 1975). The infection is acquired by skin penetration of filariform larva during contact with contaminated soil or water or by ingestion of contaminated water. The infection occurs in areas where the standard of living of the population is low. Sanitary and environmental conditions favor the development of filariform larvae and infection of hosts (Jemaneh and Tedla, 1985). In spite of considerable advances in chemotherapy and control, hookworms rank amongst the wide spread of soil-transmitted intestinal helminth parasites and affect a significant proportion of the world population (Bundy *et al.*, 1995). The infection is very intense in the tropics and sub-tropics with an estimated 740 million cases (De Sylva *et al.*, 2003), and the global DALYs for hookworm is estimated to be 22.1 million life years lost (Chan, 1997). A study done by Hawdon and Hotez (1996) showed that hookworms cause severe anemia and malnutrition in developing countries of the tropics. Hookworms

contribute to iron deficiency by actively feeding on blood from the capillaries in the intestinal mucosa, resulting in significant gastro-intestinal hemorrhage, loss of serum proteins, and intestinal inflammation (Olsen *et al.*, 1998). Children under five years are particularly vulnerable to disease caused by soil-transmitted helminths (STH). Periodic deworming has been shown to improve growth, micronutrient status (iron and vitamin A), and motor and language development in preschool children (Kirwan *et al.*, 2009).

Blood loss occur when the worms use their cutting apparatus to attach themselves to the intestinal mucosa, sub mucosa and contact their muscular esophageal to create negative pressure, which sucks a plug of tissue into their buccal capsules (Hotez *et al.*, 2004). Capillaries and arterioles are ruptured both mechanically and chemically through the action of digestive enzymes of the hookworms (Hotez and Pritchard, 1995). Moreover, the hookworms release anti-clotting agents to the blood stream, to flow blood the blood continuously (Stanssens *et al.*, 1996). Hookworm infections also contribute to poor appetite and decreased food intake (WHO, 1996).

Trichuris trichiura, the whipworm, is the most common in the warm moist tropical and sub tropical countries. Heavy infection of *T. trichiura* has long been known to be associated with anemia, protein-energy malnutrition and chronic diarrhea and dysentery (Wong and Tan, 1961 as cited by Stephenson *et al.*, 2000). Furthermore, the effect of helminthes and *T. trichiura* infections on cognitive function has been reported (Partovi *et al*, 2007). Some studies also have showed anemia was prevalent in children with heavy infection of *T. trichiura* (Stephenson *et al.*, 2000).

A. lumbricoides is a well-known cause of malnutrition, intestinal obstruction, biliary colic and pancreatitis (Basavaraju and Schantz, 2006). *A. lumbricoides* is estimated to infect a quarter of the world's population. Because sexually mature female worms produce characteristic eggs, infections can be diagnosed by direct microscopy of faeces. However, this diagnosis does not indicate whether the infected person is diseased, because morbidity is related to the number of worms living in the intestine (Hall, 2000). Many reports illustrated that *A. lumbricoides* is the most prevalent intestinal parasite in different communities usually occurring together with *Trichuris* infections (Mengistu *et al.*, 2007). Hookworm infection, strongyloidiasis and enterobiasis are also public health problem though the magnitude is lesser compared to ascariasis. Although *A. lumbricoides* infection is generally more prevalent in Africa, the occurrence of *Plasmodium* co-infections is also high and, has been associated with iron deficiency anemia and abdominal pain (Egwyenyenga *et al.*, 2001). *A. lumbricoides* infection in primary school children also affected cognitive function iron deficiency anemia, growth retardation in children and other physical and mental health problems (Pinaraih *et al.*, 1998 as cited by Partovi *et al.*, 2007). *P. falciparum* infection with *A. lumbricoides* is also found to protect cerebral malaria (Nacher *et al.*, 2002).

Schistosomiasis is one of the tropical diseases caused by digenetic trematodes belonging to the genus *Schistosoma*. The disease affects both human and animal health. It is also the second parasitic disease next to malaria by affecting large number of people in the world (Steinmann *et al.*, 2006). Schistosomiasis in humans is mainly caused by *Schistosoma haematobium*, *S. mansoni* and *S. japonicum*. Other two species, *S. mekongi* and *S. intercalatum* also infect humans

(Wu and Halim, 2000; WHO, 2007). *S. haematobium* alone is believed to infect more than 193 million people in the tropics with more than 85% infections occurring in Africa (Richens, 2004).

In Ethiopia, both *S. mansoni* and *S. haematobium* pose considerable public health and socioeconomic problem. In the country, distribution of schistosomiasis is highly focal and discontinuous (Erko *et al*, 1997; Kloss *et al*, 1988). *S. mansoni* has been reported in places with an altitude range between 650 and 2400 above sea level (Erko *et al*, 1997); most transmission sites of *S. mansoni* infections are in agricultural communities along streams between 1300 and 2000 m above sea level which are infested with *Biomphalaria pfeifferi*, the major snail intermediate host in the country (Kloss *et al*, 1988).

The life cycle of schistosome parasite requires snail intermediate hosts and definitive hosts. In the life cycle, fresh water snails are essential for the survival of the parasite because they are sites for enormous replication of the larval stages (Wu and Halim, 2000). Human beings get infected by schistosome parasite when contacting infested surface waters by the infective larval stage called cercariae which bore through skin. Adult parasites form pairs with an opposite sex and reside in mesenteric venules or plexus venules of bladder and release eggs through faeces or urine depending on the parasite species (Wu and Halim, 2000).

There are different important pathogenic species of *Taenia*, these are: *Diphyllobothrium latum* (fish tape worm), *H. nana* (dwarf tapeworm), *H. diminuta* (rate tapeworm), *T. saginata* (Beef tape worm), *T. solium* (pork tape worm), *Echinococcus granulosus* (Hydatid tapeworm), *E. multilocularis* (Hydatid tape worm) and *Diphylidium caninum* (dog tape worm). Tape-worms

inhabit the intestinal tracts of vertebrates, and the larvae inhabit the tissues of vertebrates and invertebrates. The low economic standard, poor sanitation and ignorance of simple health promotion practices favor the wide distribution of intestinal helminths in Ethiopia. Several studies in the country have also revealed that intestinal parasite infections are widely distributed with high prevalence rates (Merid *et al.*, 2001).

H. nana infection has cosmopolitan distribution and most commonly infects humans, especially school-aged children, particularly most common in children aged 4-10 years, in dry, warm regions of the developing world (Robert and Tolan, 2009). Humans become infected with *H. nana* when they ingest infective eggs, most commonly by direct fecal-oral exposure. *H. nana* affects millions of people, worldwide. Estimated rates of infection in various regions range from 0.1-58% (Robert and Tolan, 2009). Human infection with *H. diminuta* results from accidental ingestion of insects (immature fleas, flour beetles, meal worms, cockroaches) that carry the parasite in their body cavities (Robert and Tolan, 2009).

1.4. Malaria Intestinal Protozoa and Helminth Co-infections

Even though most common co-infections of malaria are with intestinal helminths (Mwangi *et al.*, 2006; Helmby, 2007), intestinal parasites and protozoan infections are amongst the most common infections worldwide. It is estimated that some 3.5 billion people are affected, and that 450 million are ill as a result of these infections, the majority being children in the poor community (Mengistu *et al.*, 2007). Recent studies show that malaria and hookworm infections are widespread throughout tropical and subtropical countries. Both hookworm and malaria are

significant causes of anemia in pregnant women. Intestinal protozoa, helminth and malaria co-infections occur in the tropical and subtropical countries. The prevalence of hookworm infection among reproductive age females in sub-Saharan Africa is estimated to be as high as 32% (Basavaraju and Schantz, 2006). In Africa, over a quarter of school aged children are at high risk of coincidence infection and consequently at enhanced risk of clinical diseases (Brooker *et al.*, 2006).

P. falciparum and helminth co-infections result in lower birth weight than *P. falciparum* infections alone (Egwyonyenga *et al.*, 2001). Malaria and soil-transmitted helminthes (STH) are widely co-endemic and are largely a burden of the poor. Preliminary data suggest a relationship between helminth infection and malaria incidence in addition to helminth-associated reduction in the severity of *Plasmodium* spp. infections (Basavaraju and Schantz, 2006). The biology of the parasite and the host, climate, socio-economic status of the population and the like in the area are the major factors that influence the epidemiological and geographical patterns of infections and co-infections. Climate determines the survival of the mosquito vector of the malaria and the free living and infective stage of the helminth (Brooker and Michael, 2000; Hay *et al.*, 2000). Low level of education and poverty associated with poor malaria prevention and poor access to effective anti-malarial drugs enhance the chance to be infective (Asaulo and Ofoezie, 2003). Such household related risk factors may partially explain the empirical observation that malaria as well as helminth infections tend to cluster within certain households (Shapiro *et al.*, 2005).

Infection with helminth appears to polarize the immune response towards T-helper-2 type, characterized by high level of cytokines such as interleukin-4 (IL-4), IL-5, IL-13 and high serum

level of immunoglobulin-E (IgE) (Hartgers and Yazdanbakhsh, 2006). The helminth induced Th-2 shift may have complex consequences on malaria, decreasing anti-sporozoite immunity, but protecting against severe malaria (Nacher, 2002). Helminths induced Th-2 is thought to drive the antibody responses of individuals co-infected with malarial parasites towards the production of the non-cytophilic subclasses, but protection against malaria is highly associated with the presence of the cytophilic subclasses (Druilhe *et al.*, 2005). In rodents a significant decrease in tumor necrosis factor (TNF) has been observed associated with an increased *P. chabaudi* parasitemia, after their co-infection with *S. mansoni* (Helmbj *et al.*, 1998).

Co-infection of malaria parasite with intestinal helminth has shown to affect drug efficacy in animal model (Kalenga *et al.*, 2004). Malaria parasite had shown to modulate the immune response to helminth infections. The proliferative Th-2 responses to schistosome antigens were found to be suppressed up to 1 month after malaria infection (Helmbj *et al.*, 1998). Infections with intestinal helminthes may alter susceptibility to clinical malaria and malaria may influence the clinical consequences of helminth infection by exacerbating their morbidity. Splenomegally associated with *S. mansoni* infection is found to be exacerbated by chronic malaria in children (Booth *et al.*, 2004). However recent studies have shown that *S. mansoni* and *S. haematobium* infections are found to increase incidence of malaria fever (Booth *et al.*, 2004). *S. mansoni* appears to increase malaria attack (Sokhna *et al.*, 2004), and incidence of severe malaria by repeated vomiting and convulsions.

This study was conducted in the area since no such previous study has been reported. Knowledge about the prevalence of malaria and intestinal parasites in Tach Gayint District will be essential for the initiation and implementation of parasite control programmes in the region.

Statement of hypothesis

Malaria, intestinal protozoa and helminths are significantly prevalent in Tach Gayint District, South Gondar, Amhara Region.

2. OBJECTIVE OF THE STUDY

2.1 General objective

The general objective of this study is:

- To assess and document the prevalence of malaria, intestinal protozoa and helminths and co-infections with them in Tach Gayint District, South Gondar, Amhara Region.

2.2 Specific objectives

The specific objectives of this study are:

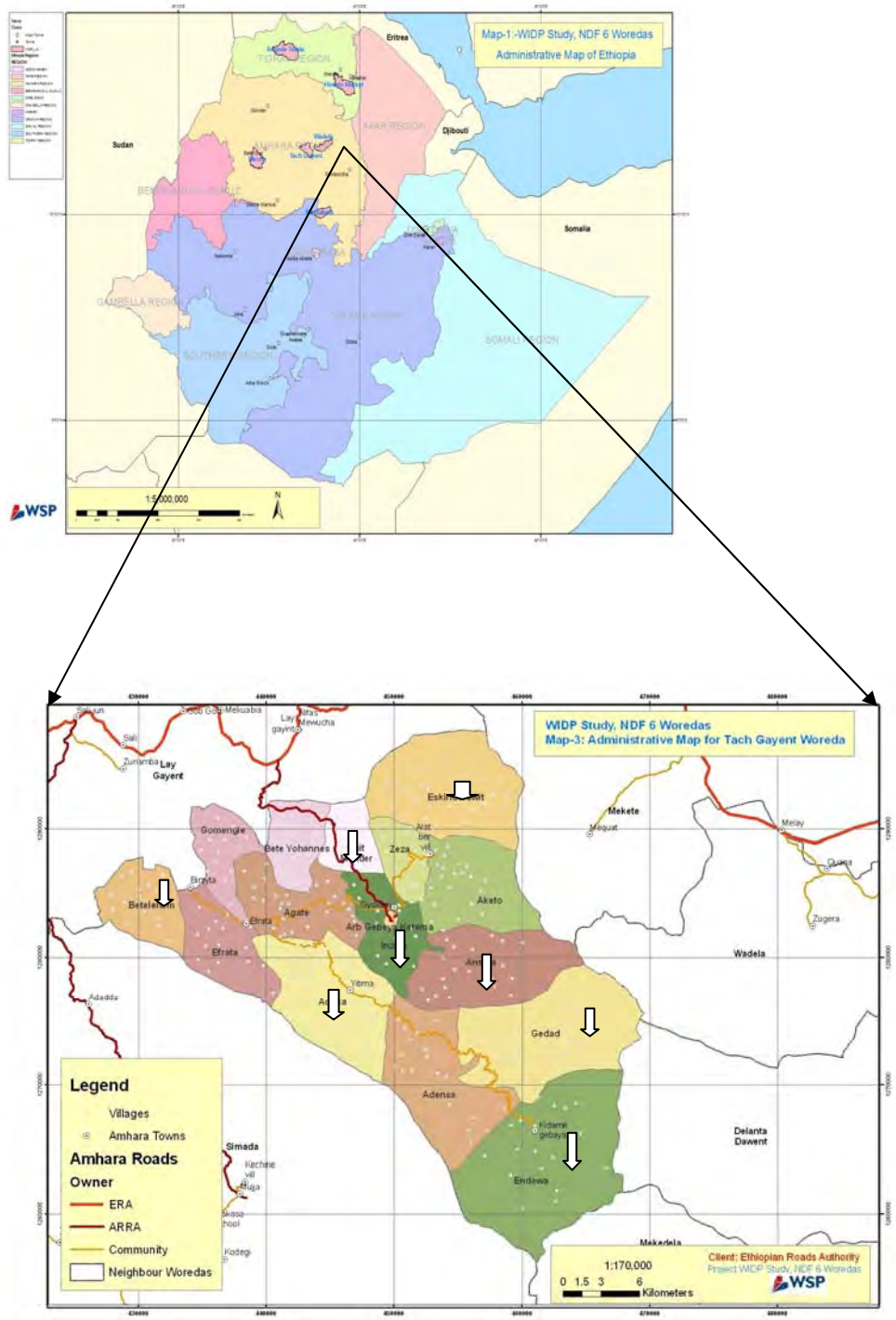
- To determine the prevalence of *P. vivax*, *P. falciparum*, *G. lamblia*, *E. histolytica*, and some helminthes infection and co-infection in the study area.
- To compare the association of malaria and intestinal protozoa and helminths co-infections in the study area.
- To identify factors that maintains endemicity of the parasite agents.

3. MATERIALS AND METHODS

3.1. Study area

Tach Gayint is one of the 105 districts in Amhara National Regional State of Ethiopia. It is bordered on the south by the Bashilo River, which separates the district from the South Wollo Zone, on the west by Simada, on the north by Lay Gayint, and on the east by the North Wollo Zone. The major town of the District is Arb Gebeya.

Tach Gayint is located at 780km to the North of Addis Ababa. The district is also located 24 km far from the main roads of Ethiopia, located at $13^{\circ} 45'$ N latitude, and $35^{\circ} 46'$ to $40^{\circ} 25'$ E longitude and the altitude of the district range from 1800 masl-3000masl. The district contains: Dega, Woina-dega and Kolla zones. The study is carried out in randomly selected kebeles of the district, in November/December, 2008 and April/May, 2009 G.C. According to the district's natural hazardous management and control office report the majority of the district's population is engaged in mixed agricultural activities. The major crops in the district are: Teff, maize, sorghum, wheat, barley and some oily crops. The major animals reared in the district are cattle, sheep, goat, donkey, horse, dog poultry etc.



∩ = Study sites (Kebeles in the district)

Figure 2. Map of Tach Gayint District, South Gondar, Amhara National Regional State (The Map shows the different Kebeles where samples were collected for the study)

3.2. Study population

The study population was from selected kebeles of the district. Based on figures published by the Central Statistical Agency in 2007, this district has an estimated total population of 114,326, of whom 57,132 were males and 57,194 were females; 4,610 (4.03%) of its population are urban dwellers, which is less than the Zone average (8.3%). Tach Gayint has an estimated population density of 137 persons per square kilometer, which is less than the Zonal average of 169.21 persons per Km².

Totally 806 consent study participants were examined for malaria, intestinal protozoa and Helminth parasite infections in two seasons. The sample size to this study was estimated by taking the prevalence 50% as there was no study reported in the area. Then the sample size was determined following the formula $n = \frac{Z^2 P (1-P)}{d^2}$. Where, Z= 1.96, P= prevalence of the diseases (50%) and d= precision (0.05) (Naing *et al* 2007). From the total of 16 kebeles in the district, the study includes a randomly selected people in eight kebeles; all ages and sex of the people were included in the study area. The mean age of the total study population was 20.88 ± 14.3 (ranging from 1 to 72).

3.3. Laboratory methods

The choice of diagnostic techniques depends on available equipment and reagents, experience, and considerations of time and cost. The techniques used in this study are: Direct Wet Mount Microscopic Examinations and Formalin-Ether concentration techniques. Throughout the study the techniques used to diagnose malaria, intestinal protozoa and helminthes were the same.

3.3. 1. Diagnosis for malaria parasites

Blood Film determination for malaria parasites

Laboratory technicians collected the samples and malarial infections were determined from thick and thin films of finger-prick blood fixed and stained with Giemsa stain. Thick and thin blood smear wear prepared for each subject from capillary blood by finger prick using sterile lancet. The thick smear was stained with Giemsa solution and the thin smear was fixed with methanol before stained with Giemsa solution. Each blood smear was observed under the oil immersion objective of the microscope. The thick smear was used to determine whether the malaria parasite was present or not after observing 100 fields of vision. The thin smear was used to identify the type of *Plasmodium* species.

3.3.2. Diagnosis for intestinal parasites

Direct Wet Mount Microscopic Examinations for Detection of Intestinal parasites

A drop of saline was placed on slides and a small amount of faeces were placed on the microscope slide using tooth pick, or applicator stick, and mixed in drop and covered by cover slip. Finally the samples were examined microscopically at high power magnification.

Formalin-Ether Concentration technique for stool Examination

This concentration procedure is efficient in recovering protozoan cyst, helminth egg and larva including operculated and schistosome eggs. The formalinized specimen was thoroughly stirred and a sufficient quantity was strained through gauze in to a 15ml. pointed centrifuge tube to get the desired amount of sediment. Then saline was mixed and thoroughly mixed and centrifuged at

2000-2500 rpm for 1 minute. The supernatant was decanted and washed again with tap water if desired. 10ml. of 10 percent formalin was added to the sediment and mixed thoroughly. Then 3ml. of ether was added and shaken vigorously in an inverted position for a full 30 seconds, and then the stopper was removed carefully. The resulting solution was centrifuged at 1500 rpm for about 1 minute, and four layers were produced. The three top layers were decanted carefully, and adhering debris were removed from the top with a cotton swab. The remaining sediment was mixed with the small amount of fluid that drains back from the slides of the tube or a small drop of formalin or saline was added. Finally iodine and unstained mounts were prepared for microscopic examination (Peters *et al.*, 1980).

3.3.3. Data analysis

Statistical analysis was carried out using SPSS Version 15 Software. A significance level of 0.05 was used for all tests.

3.3.4. Ethical considerations

Diagnosis was done using sterile and disposable materials. Only a laboratory technician took the blood sample and all activities in clinical examination as well as diagnosis were supervised by health personnel. Individuals diagnosed positive for malaria intestinal protozoa and/or intestinal helminth infections were treated free of charge. Those individuals who were positive for malaria (chloroquine and coartem) were provided. (Metronidazole; 750 gm t.i.d for 5-7 days Tinidazole; 2 gm p.o. for 3 consecutive days) were provided for those diagnosed positive for intestinal protozoa. Those positive for helminth infections were provided with anti-helminthic drug

(Albendazole; 400mg single dose for Ascaris, Trichuris, and Hookworm. As alternative Mebendazole; 500mg single dose /Niclosamide/Praziquantel 600mg single dose for *Taenia spp.*), were provided as per Ethiopian Ministry of Health guideline. Only volunteer individuals were included in the study. Ethical clearance was obtained from the ethical clearance committee of Department of Biology, Addis Ababa University.

4. RESULTS

In November/December, 2008 the percentage of males and females infected by at least one parasite was 152 (70.05%) and 123 (66.13%), respectively. In the study conducted on April/May, 2009 the percentage of males and females infected at least by one parasite was 146 (67.28%) and 125 (67.20%), respectively.

The overall prevalence of the parasites in November/December, 2008 and in April/May, 2009 was 275 (68.24%) and 271 (67.25%), respectively. Except malaria, the other parasitic diseases did not show significant difference in between the two study seasons. The prevalence variations in most parasitic diseases were detected in different age groups of the study population; among these *T. trichiura* *H. nana*, *Plasmodium spp.* hookworm and *A. lumbricoides* were the parasites that showed significant differences in age groups in the study population. Some of these parasites were highly prevalent in children than young and adult population. Generally from all the detected parasitic diseases in Tach Gayint district (in November/December, 2008 and April/May, 2009) only *Plasmodium spp.* had showed significant difference in the study seasons.

4.1. Malaria

In November/December, 2008, from a total of 403 study participants, 45 (11.17%) were found to harbor one species of *Plasmodium* parasites in their blood, in the second season (April/May, 2009), the prevalence of malaria had decreased to 22 (5.46%). In both study seasons, *P. falciparum* 41 (61.19%) and *P. vivax* 26 (38.80%) were the only malaria parasites detected in the study area. The difference in the prevalence of malaria after the big rains (11.17%) and after the small rains

(5.46%) was statistically significance ($P = 0.003$). In the study conducted from April/May, 2009 the prevalence of malaria had been decreased to 5.46%.

4.2. Intestinal protozoa

Intestinal protozoa are more prevalent in the district; among these giardiasis and amoebiasis are the two major intestinal protozoa that are more prevalent in the study area. The prevalence of *G. lamblia* in the study conducted on November/December, 2008 had showed statistically significant difference in different age groups of the study population. The difference were observed between the age groups 15-19 and > 19 ($P = 0.04$).

4.3. Intestinal Helminth

Many of study subjects were infected either with hookworm, *Ascaris lumbricoides*, *Taenia spp.* *T. trichiura* or *H. nana*. The most prevalent infection was *A .lumbricoides* followed by hookworm and *Taenia spp.* infections. *T. trichiura* was the least prevalent in the study population. The prevalence of malaria, intestinal protozoa and helminths in November/December, 2008 and April/May, 2009 is depicted in Table.1.

Table.1. The prevalence of malaria, intestinal protozoa and helminthes in selected kebeles of Tach Gayint District, South Gondar Zone, Amhara National Regional State, in November/December, 2008.

		A/Gebeya	Agat	Aduka	Anseta	Bettelheim	Eskenderawit	Gedoda	Quit-Mender
No. Examined		50	51	51	50	51	50	50	50
<i>P. falciparum</i>	Nov/Dec	0(0.00%)	2(3.92%)	7(13.73%)	4(8.00%)	8(15.68%)	2(4.00%)	4(8.00%)	0(0.00%)
<i>P. vivax.</i>	Nov/Dec	0(0.00%)	1(1.96%)	6(11.76%)	2(4.00%)	6(11.76%)	1(2.00%)	2(4.00%)	0(0.00%)
<i>G. lamblia</i>	Nov/Dec	3(6.00%)	4(7.84%)	11(21.57%)	4(8.00%)	16(31.73%)	8(16.00%)	9(18.00%)	3(6.00%)
<i>E.histolytica/dispar</i>	Nov/Dec	11(22.00%)	9(17.64%)	12(23.53%)	9(18.00%)	11(21.57%)	8(16.00%)	7(14.00%)	9(18.00%)
<i>S. mansoni</i>	Nov/Dec	2(4.00%)	3(5.88%)	8(15.68%)	3(6.00%)	9(17.64%)	2(4.00%)	3(6.00%)	0(0.00%)
<i>Taenia spp.</i>	Nov/Dec	13(26.00%)	6(11.76%)	3(5.88%)	8(16.00%)	2(3.92%)	5(10.00%)	9(18.00%)	3(6.00%)
<i>H/worm</i>	Nov/Dec	1(2.00%)	3(5.88%)	14(27.45%)	11(22.00%)	16(31.73%)	8(16.00%)	10(20.00%)	1(2.00%)
<i>T. trichiura</i>	Nov/Dec	3(6.00%)	6(11.76%)	8(15.68%)	13(26.00%)	2(3.92%)	5(10.00%)	9(18.00%)	0(0.00%)
<i>A. lumbricoides</i>	Nov/Dec	7(14.00%)	12(23.53%)	14(27.45%)	11(22.00%)	10(19.60%)	17(34.00%)	19(38.00%)	4(8.00%)
<i>H. nana</i>	Nov/Dec	1(2.00%)	3(5.88%)	4(7.84%)	4(8.00%)	2(3.92%)	4(8.00%)	4(8.00%)	0(0.00%)

Table.2. The prevalence of malaria, intestinal protozoa and helminthes in selected kebeles of Tach Gayint District, South Gondar Zone, Amhara National Regional State, in April/May, 2009.

		A/Gebeya	Agat	Aduka	Anseta	Bettelheim	Eskenderawit	Gedoda	Quit-Mender
No. Examined		50	51	51	50	51	50	50	50
<i>P. falciparum</i>	April/May	0(0.00%)	2(3.92%)	4(7.84%)	2(4.00%)	3(5.88%)	1(2.00%)	2(4.00%)	0(0.00%)
<i>P. vivax</i>	April/May	0(0.00%)	1(1.96%)	2(3.92%)	1(2.00%)	2(3.92%)	1(2.00%)	1(2.00%)	0(0.00%)
<i>G. lamblia</i>	April/May	4(8.00%)	3(5.88%)	10(19.60%)	8(16.00%)	14(27.45%)	4(8.00%)	11(22.00%)	2(4.00%)
<i>E.histolytica/dispar</i>	April/May	12(24.00%)	9(17.64%)	11(21.57%)	7(14.00%)	11(21.57%)	9(18.00%)	7(14.00%)	8(16.00%)
<i>S. mansoni</i>	April/May	1(2.00%)	3(5.88%)	8(15.68%)	2(4.00%)	7(13.73%)	3(6.00%)	4(8.00%)	0(0.00%)
<i>Taenia spp.</i>	April/May	14(28.00%)	4(7.84%)	5(9.80%)	4(8.00%)	3(5.88%)	6(12.00%)	9(18.00%)	4(8.00%)
<i>H. worm</i>	April/May	1(2.00%)	2(3.92%)	17(33.33%)	10(20.00%)	19(37.25%)	16(32.00%)	14(28.00%)	0(0.00%)
<i>T. trichiura</i>	April/May	1(2.00%)	6(11.76%)	7(13.73%)	13(26.00%)	2(3.92%)	5(10.00%)	8(16.00%)	1(2.00%)
<i>A. lumbricoides</i>	April/May	8(16.00%)	13(25.49%)	16(31.73%)	13(26.00%)	11(21.57%)	15(30.00%)	18(36.00%)	5(10.00%)
<i>H. nana</i>	April/May	1(2.00%)	4(7.84%)	4(7.84%)	3(6.00%)	4(7.84%)	2(4.00%)	3(6.00%)	0(0.00%)

Table.3. The prevalence of malaria, intestinal protozoa and helminthes in Tach Gayint District, South Gondar Zone, Amhara National Regional State, in November/December, 2008 and April/May, 2009.

Cases	Prevalence of parasites		
	November/December, 2008 (n=403)	April/May, 2009 (n=403)	P. value ^a
<i>Plasmodium spp.</i>	45 (11.17%)	22 (5.46%)	P = 0.003
<i>Giardia lamblia</i>	58 (14.39%)	56 (13.90%)	P = 0.800
<i>E. histolytica/dispar</i>	76 (18.86%)	74 (18.36%)	P = 0.800
<i>S. mansoni</i>	30 (7.44%)	27 (6.70%)	P = 0.680
<i>Taenia spp.</i>	49 (12.16%)	49 (12.16%)	P = 1.000
Hookworm	64 (15.88%)	79 (19.60%)	P = 0.160
<i>T. trichiura</i>	46 (11.41%)	43 (10.67%)	P = 0.700
<i>A. lumbricoides</i>	94 (23.33%)	99 (24.57%)	P = 0.680
<i>H. nana</i>	22 (5.46%)	21 (5.21%)	P = 0.870
Total	275 (68.24%)	271 (67.25%)	P > 0.05

^a = Any P-value less than or equal to 0.05 is statistically significant

In the study conducted on November/December, 2008, the prevalence of malaria in different age groups had showed statistically significant difference (P < 0.001). The prevalence of malaria showed statistical difference in the age groups between 6-14 and > 19 (P < 0.001). In the study conducted in November/December, 2008 *Plasmodium spp.* hookworm *T. trichiura* and *A. lumbricoides* had showed statistically significance differences in different age groups (P <

0.001). The prevalence difference in *T. trichiura* was mainly observed in between the age groups 1-5 and > 19 (P = 0.02) and in between the age groups 6-14 and > 19 (P < 0.001). In *E. histolytica/dispar* the statistical difference appeared in between the age groups 6-14 and 15-19 (P = 0.02), and in between the age groups 6-14 and > 19 (P < 0.001).

Table.4. The age related prevalence of malaria and some intestinal helminths in Tach Gayint District, South Gondar Zone, Amhara National Regional State, November/December, 2008.

Parasites	Age			P. value ^a
	1-5	6-14	≥15	
No. Examined	31	144	228	
<i>Plasmodium spp.</i>	2 (6.45%)	6 (4.17%)	37 (16.23%)	P < 0.001
<i>S. mansoni</i>	2 (6.45%)	16 (11.11%)	12 (5.26%)	P < 0.041
Hookworm	2 (6.45%)	38 (26.39%)	24 (10.53%)	P < 0.001
<i>T. trichiura</i>	8 (25.81%)	24 (16.67%)	14 (6.14%)	P < 0.001
<i>A. lumbricoides</i>	12 (38.71%)	52 (36.11%)	30 (13.16%)	P < 0.001
<i>H. nana</i>	5 (16.13%)	10 (6.94%)	7 (3.07%)	P < 0.032

^a = Any P-value less than or equal to 0.05 is statistically significant.

In the study conducted on April/May, 2009 Malaria, Hookworm, *T. trichiura*, *A. lumbricoides* and *H. nana* showed statistically significant differences in different age groups (P < 0.001). In April/May, 2009 where there is no much rain the prevalence of the malaria had decreased in all

age groups of the study population. *T. trichiura*, *A. lumbricoides* and *H. nana* were the other parasites that showed statistically significant differences in different age groups of the study population. The difference in prevalence of *T. trichiura* was observed in between the age groups 1-5 and 6-14 ($P < 0.001$), 1-5 and 15-19 ($P < 0.001$) and in between the age groups 1-5 and > 19 ($P < 0.001$). In between the age group 6-14 and 15-19 ($P < 0.001$), and in between the age group 6-14 and > 19 ($P < 0.001$).

The prevalence of *A. lumbricoides* had a significant difference between different age groups of the study population. The differences were appeared in between age group of 1-5 and 6-14 ($P < 0.001$), in between 1-5 and 15-19 ($P < 0.001$) and in between 1-5 and > 19 ($P < 0.001$). In *H. nana* the prevalence difference appeared in between age groups of 1-5 and 6-14 ($P < 0.001$), 1-5 and 15-19 ($P < 0.001$) and in between age groups of 1-5 and > 19 ($P < 0.001$). Hookworm showed statistically significant difference in different age groups of the study population. The statistically significance difference was mainly observed in the age groups between 6-14 and 1-5 ($P = 0.03$), and between 6-14 and > 19 ($P < 0.001$). *T. trichiura* and ascariasis had showed statistical difference prevalence in different age groups of the study population.

Table.5. The age related prevalence of *Plasmodium* spp. and some intestinal helminths in Tach Gayint District, South Gondar Zone, Amhara National Regional State, April/May, 2009.

Parasites	Age			P. value ^a
	1-5	6-14	≥15	
No. Examined	37	138	288	
<i>Plasmodium spp.</i>	0 (0.00%)	2 (1.45%)	20 (8.77%)	P < 0.001
<i>S. mansoni</i>	1 (2.70%)	15 (10.87%)	11 (4.82%)	P < 0.05
Hookworm	5 (13.51%)	46 (33.33%)	28 (12.28%)	P < 0.001
<i>T. trichiura</i>	20 (54.05%)	22 (15.94%)	1 (0.44%)	P < 0.001
<i>A. lumbricoides</i>	22 (59.46%)	41 (29.71%)	36 (15.79%)	P < 0.001
<i>H. nana</i>	6 (16.22%)	9 (6.52%)	6 (2.63%)	P < 0.001

^a = Any P-value less than or equal to 0.05 is statistically significant.

The prevalence of *Plasmodium spp.* and *H. nana* was statistically significantly different in different sexes. In the first study season, the prevalence of *Giardia lamblia* (P = 0.96) and *E. histolytica/dispar* (P= 0.80) did not show statistically significant difference in different sexes. The prevalence of malaria in females was highly decreased than males. The prevalence of malaria in males and females was statistically different (P < 0.001). None of the intestinal helminth showed statistically significant difference in different sexes except *H. nana*. The prevalence of *H. nana* among females (8.06%) was significantly higher than males (2.76%) (P = 0.021).

4.4. Malaria Intestinal Protozoa and Helminths Co-infections

During the study, there were co-infections of malaria with intestinal helminths and protozoa. Amoebiasis, giardiasis and hookworm are the major parasitic diseases that have co-infections with malaria. *H. nana* was the least parasite that had co-infection with malaria in the two study seasons. The following tables show co-infection of malaria with other intestinal protozoa and helminthes, in the study area.

Table.6. Prevalence of malaria co-infection with different intestinal protozoa and helminths in Tach Gayint District, South Gondar Zone, Amhara National Regional State, in November /December, 2008 and April/May, 2009.

Co-infections	Prevalence in November	Prevalence in April /May	P. value ^a
	/December, 2008 (n = 403)	2009 (n = 403)	
Malaria/ <i>A. lumbricoides</i>	6(1.49%)	5(1.24%)	P = 0.76
Malaria/Hookworm	6(1.49%)	6(1.49%)	P = 1.00
Malaria/ <i>S. mansoni</i>	4 (0.99%)	1(0.25%)	P = 0.18
Malaria/ <i>T. trichiura</i>	4 (0.99%)	1(0.25%)	P = 0.18
Malaria/ <i>G. lamblia</i>	10(2.48%)	2(0.50%)	P = 0.02
Malaria/ <i>E. histolytica/dispar</i>	11(2.73%)	5(1.24%)	P = 0.13
Malaria/ <i>Taenia spp.</i>	8(1.99%)	4(0.99%)	P = 0.25
Malaria/ <i>H. nana</i>	2 (0.50%)	1(0.25%)	P = 0.56

^a = Any P-value less than or equal to 0.05 is statistically significant.

In the study conducted on April/May, 2009 Malaria hookworm, *A. lumbricoides* and *E. histolytica/dispar* were the major parasites that had co-infections with malaria. From the overall co-infections, there were single, double, triple and quadruple infections. The amounts of infections were greater in single and double infections than triple and above infections.

Table.7. Single and co-infections of malaria, intestinal protozoa and helminths in Tach Gayint District, South Gondar Zone, Amhara National Regional State, November/December, 2008.and April/May, 2009.

Infections/Co-infections	Nov/Dec, 2008	April/May, 2009	P. value^a
Single	150 (37.22%)	134 (33.25%)	P = 0.24
Double	70 (17.37%)	86 (21.34%)	P = 0.18
≥Triple	55 (13.65%)	51(12.66%)	P > 0.05

^a = Any P-value less than or equal to 0.05 is statistically significant.

5. DISCUSSION

This study may be considered the first study conducted on the prevalence and association of malaria, intestinal protozoa and helminth infections and co-infections in Tach Gayint District, as no published records exist. As knowledge of the distribution extent of malaria, intestinal protozoa and helminth infections in a given community is a prerequisite for planning and evaluating health intervention programs (Tadesse, 2005), the information obtained through the present study is of vital relevance.

Tach Gayint has suitable climate and topography conducive for transmission of malaria, intestinal protozoal and helminth infections. Climate and soil type which determine the distribution of intestinal worm infections (Mabaso *et al.*, 2003) are also characteristic of Tach Gayint. Although it has been shown that the infection rate of malaria, intestinal protozoa and helminths could vary from place to place due to differences in the socio-economic and educational status of the population (Zhou *et al.*, 2004), such variations would not be expected in the study area as there is no apparent variations in these conditions.

As shown by Investigators elsewhere (Asaulo and Ofoezie, 2003), the areas which have better socio-economic status have lower prevalence of helminth, malaria and intestinal protozoal infections than the areas with low socio-economic status. Thus, the high prevalence of helminth and protozoal intestinal parasites is a reflection of the poor socio-economic condition of Tach Gayint District (personal communication: Tach Gayint Natural Disaster Management and Control Office).

The transmission of malaria in the area is seasonal and unstable and hence acquired immunity will be expected to be low (Lindsay and Martens, 1998). This is unlike the case of year round stable transmission, where the community usually develops protective immunity.

Brooker and Michael (2000) and Hay *et al.*, (2000), have shown that peak transmission of malaria occurs following the main rainy season and a minor transmission peak occurs following light rainy season in the tropics. Therefore the relatively low peak transmission in April/May, 2009, following the light rains, was to be expected in the study area. However, it was interesting to find that the prevalence of malaria in males was significantly higher than in females ($P = 0.001$) in April/May, 2009. This finding might be explained by the fact that, in Tach Gayint District males spend the early part of the night working in their farms where they might be easily infected by exophagous mosquito bites, whereas most females do not have such risk as they normally are engaged in indoor household chores. At the time where there is no much rain, the water near the residences dries up and only the adult individuals that may move far from their residences might be exposed to outdoor infection due to the presence of irrigation and other water bodies. Thus, this might explain why those under 14 years old had lower cases of malaria, particularly, in the seasons after the light rains.

In Tach Gayint most of the people use unsanitary drinking water and live under inadequate hygienic conditions, and experience poor personal hygiene that favors high prevalence of intestinal protozoal and helminth infections.

The study finding of no difference in the prevalence of *G. lamblia* and *E. histolytica/dispar* in the different seasons suggests the year round transmission of the pathogens to be contaminated drinking water. The prevalence of *G. lamblia* and *E. histolytica/dispar* in the two study seasons was greater than 18% which is consistent with what was reported by (Haque *et al.*, 2003; Wadood *et al.*, 2005), in many tropical countries the prevalence of these may approach 50%.

The high prevalence of *G. lamblia* in preschool children was similar to the findings of Mengistu *et al.*, (2007), who reported *Giardia* trophozoites and *H. nana* to be significantly higher in preschool children. On the other hand, since adults are known to eat more raw and undercooked vegetables than children, in the study area, more cases of *E. histolytica/dispar* in adults than in children might be due to this feeding habit of adults.

Although the District Health Office had reported the existence of deworming programme, hookworm infection ranked second among the widespread intestinal helminth parasites and affected a significant proportion of the population especially in some kebeles of the District. As the age group 6 to 14 years old are known to actively make soil contact at the play around and it is to be expected that the prevalence of hookworm was significantly higher in this age group ($P < 0.001$), whereas the age group under 5 are too young to play around and make soil contact activities, therefore exposure to the infective filariform larvae would be relatively low (Bundy *et al.*, 1995; De Sylva *et al.*, 2003). As Tadesse (2005) showed, wearing shoes regularly has a significant contribution to the low prevalence rate of hookworm infections. Therefore, since most

adults in the study area regularly wore shoes, it was to be expected that the prevalence of hookworm would be relatively low in the age group above 19 years.

Among STH *S. stercoralis* and *E. vermicularies* were not common in the study area, whereas *T. trichiura*, *A. lumbricoides* and *H. nana* were the other parasites that showed statistically higher prevalence among the age groups below 14 years. This is related to the activities of the children that more frequently play with soil and other contaminated materials exposing them to the ingestion of ova as food contaminants. Furthermore, even though infection of *H. nana* can occur in persons of any age because of exposure to infected human feces, school-age children have more risk of hymenolepiasis. According to Robert and Tolan (2009), infection of *H. nana* is most common in children aged 4-10 years, in dry, warm regions of the developing world with an estimated rate of infection in various regions ranging from 0.1-58%. Therefore, the higher prevalence in the age group below 5year old, in this study can be due to direct transmission of the parasite through fecal-oral exposure as shown elsewhere by Robert and Tolan (2009). On the other hand, as a result of protective immunity following cure from infections during early age in life infection in adults tends to clear spontaneously, and hymenolepiasis is uncommon among this age group (Robert and Tolan, 2009).

In this study, the prevalence of *S. mansoni* infection was found to be similar in males and females even though gender-associated difference has been reported by other workers (Assefa *et al.*, 1998; Abebe *et al.*, 2001). This finding might be associated that, in the study area both females and males have the same activities up to they became adults. Although it was not detected in the present study, higher prevalence of schistosomiasis was also reported in males than in females in Northern Ethiopia (Abebe *et al.*, 2001). The existence of more outdoor

activities among the active age group (6-14) than the other age groups could be one of the reasons for the high prevalence of *S. mansoni* in this age group. This is supported by the observation in the area, that of the adults and those under 5 years old did not play or swim in local water bodies, reduces the chances for their exposure to cercariae-infested waters. In addition, the significantly reduced *S. mansoni* prevalence following the age of puberty is consistent with what was reported for the protective effects of puberty-related hormones and acquired immunity in adults (Abebe *et al.*, 2003; Montresor *et al.*, 2002).

In the study conducted in November/December, 2008 those children under 5 were least infected by *S. mansoni* as they were too young and may not have contacts with water bodies. The prevalence of *S. mansoni* in the 6-14 year olds was higher than in other ages in the study population, due to the greater contact of these age group individuals with water bodies. It has been shown by many that the amount of exposure, such as swimming in the water bodies is a determining factor for the spread of schistosomiasis (Erko *et al.*, 1997; Kloos and Lemma, 1974; Birrie *et al.*, 1998; Kloos, 1993).

In Tach Gayint District some kebeles such as Bethlehem 16 (16.00%) and Aduka 16 (15.68%) had relatively high number of *S. mansoni* cases as the kebeles are irrigated. According to Jemaneh (2000) the prevalence of schistosomiasis among Tach Gayint school children (Tatek Lesira primary school) was about 1.7%, which is much lower than the present finding. This discrepancy can be explained by the fact that Jemaneh had considered only one primary school, which is found in the main town of the district and did not sample other potential schistosomiasis endemic areas, which are included in the present study. The difference in the methods employed

for stool examination-Formalin ether concentration and direct wet mount used in this study versus the kato-katz used in the schistosomiasis study may have contributed to the difference observed between the two findings. In this study due to time and budget constraints the kato-katz technique was not used for schistosomiasis diagnosis as the result of which its prevalence may have been under estimated.

Although it may be argued that in highly contaminated environments, focal transmission in defecation grounds may not be the single most important site for geo-helminth infection, it is a significant univariate risk factor for infection of many geo-helminths (*Ascaris*, hookworms and *Trichuris*) (Traub *et al.*, 2004). In the community of the present study, children were particularly at high risk as they were defecating in sites that are already polluted by faeces and therefore can be exposed to more frequent and heavy infection than adults.

Eventhough the prevalence and distribution of intestinal helminths varies from place to place because of several environmental, social and geographical factors (Erosie *et al.*, 2002; Legesse and Erko, 2004; Jemaneh, 2000; Merid *et al.*, 2001; Tadesse, 2005), the prevalence of *Taenia spp.* (12.16%) was within the same range reported from other parts of Ethiopia (Wodemichael *et al.*, 1999). This can be accounted for by the ubiquitous tradition of raw beef consumption in Ethiopia.

In the study area the intestinal protozoa *Giardia* and *E. histolytica/dispar* had considerable co-infections with malaria in Nov/Dec, 2008, which is characteristic of a wet season where both malaria and the intestinal protozoa are highly prevalent (Traub *et al.*, 2004). In

November/December, 2008 study, co-infection of malaria and *G. lamblia* was significantly higher than that in April/May, 2009 ($P = 0.02$). According to Ayalew *et al* (2008), high number of giardiasis cases result during high rain fall months. This finding may be associated with the presence of flood storm that may wash fecal materials into drinking water bodies. *Giardia lamblia* have been implicated in many water-born diarrheal out-breaks both in adults and children that are exposed to contaminated drinking water and swimming in lakes and rivers (Serpil *et al.*, 2005).

Overlapping distributions of intestinal helminths and malaria result in high rate of malaria and helminth co-infections (Adrienne *et al.*, 2005). In many regions of Sub-Saharan Africa, intestinal helminth infections particularly hookworm disease overlaps geographically with *falciparum* malaria (Guyatt and Snow, 2001). The co-infection of malaria and hookworm were relatively common in Aduka and Bethlehem in Tach Gayint District. Aduka and Bethlehem, two of the malarious kebeles of the district had the major co-infection of malaria and intestinal helminths resulting from favorable physical environment and ecology. In most tropical regions hookworm has high co-infection with malaria (Mwangi *et al.*, 2006; Helmby, 2007). The basis for this was explained by Hotez *et al.*, (2004) who hold that hookworms have a wider thermal tolerance than *A. lumbricoides* and *T. trichiuria*, adapting them to tropical temperature that increases the chances of co-infection with malaria.

Poor environmental sanitation and climatic conditions (hot, wet and humid) favor the persistence of mosquito vectors of malaria and the free living infective stage of hookworm larva (Brooker and Michael, 2000; Hay *et al.*, 2000). In Africa over a quarter of school aged children are at high

risk of coincidence infection and consequently at enhanced risk of clinical diseases. On the whole, the high co-incident infections detected in Tach Gayint District is consistent with what was reported for much of Sub-Saharan Africa (Brooker *et al.*, 2006).

6. CONCLUSIONS

1. The findings of the present study showed that malaria, intestinal protozoa and helminthes were more prevalent in children than in adults in Tach Gayint District.
2. Malaria was seasonal and relatively more prevalent in November/December, 2008 than in April/May, 2009.
3. The prevalence of *S. mansoni* was higher in the age group 6-14 years than in other age groups of the study population.
4. The prevalence of *Taenia spp.* (most probably *Taenia saginata*) was very low in children under 5, whereas the prevalence of *H. nana* was very high in children under 5years of age.
5. The prevalence of hookworm was very high in the age group 6 to19 years, but very low in children under 5 and in those above 19 years of age.
6. *T. trichiura* and *A. lumbricoides* were more prevalent in children under 5 than in other age groups of the study population.
7. Multiple infections with malaria, intestinal protozoa and helminths were common.

7. RECOMMENDATIONS

- 1.** Public education program on personal hygiene, proper use of latrines, and improved sanitation should be provided to prevent and reduce the rate of worm infections.
- 2.** The existing deworming programme must be redesigned for more coverage and efficiency to address the health problems in Tach Gayint District, particularly in the kebeles where intestinal helminthes are more prevalent.
- 3.** Improvement in the malaria control measures such as chemical, biological, and environmental management techniques that are being implemented must be instituted in the district.
- 4.** Further detailed investigation by using the Kato-katz method should be conducted to determine the intensity of schistosomiasis in the area.

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APPENDIX.I

Written consent form

I am conducting a study to malaria intestinal protozoa and helminth infections and co-infections. You are being to participate in this study if you agree. I would like to obtain finger prick blood sample in filter paper and stool samples in a plastic sheet from you or your children, which would be used only to detect the presence of malaria and the mentioned intestinal parasites. There is no serious risk in participating, but you may experience a small pain during finger pricking. When you or your children are found positive for malaria and the intestinal protozoa and helminths, you will receive 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 or any other benefits.

Do you understand what has been said to you? If you have any question you have the right to get proper explanation. I am informed to my satisfaction the purpose of this study and the nature of laboratory investigations. I am also aware of my right to opt out of the study at any time during the course of the study without having to give reasons for doing so.

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

Study code no-----

Name-----

Signature-----Date-----

Witness Name-----Signature-----Date-----

Investigator Name-----Signature-----Date-----

APPENDIX.II

የስምምነት ቅፅ

በወባ እና በአንጀት በሽባ ወጪ ላይ ጥናት ስና ምርምር በማድረግ ላይ ነኝ። ስርዕሪ ፈቃደኛ ከሆኑ በጥናቱ ላይ አንዲሳተፉ ተጋብዘዋል። በጥናቱ ላይ ተሳተፎ ከሆኑ ከስርዕሪ ወይንም ከቤተሰብዎ ላይ ለምርምር የሚያገለግል የደም እና የሰገራ ናሙና በመስጠት ስንዲተባበሩኝ በትህትና ስጦታቸዎቼ ላይ። ይህም በሚሆንበት ጊዜ ከመጠነ ፍ የሰጣት ህመም ወይንም ምንም አይነት የጤና ችግር አይኖርም።

በምርመራው ወቅት በስርዕሪ ወይንም በቤተሰብዎ ላይ የወባ እና የአንጀት በሽባ ቢገኝ ለስርዕሪ ወይንም በቤተሰብዎ ከወጪ ነፃ የሆነ ደረጃውን የጠበቀ መድሀኒት ተጠቃሚ ይሆናሉ። የስርዕሪ/ቤተሰብዎ በዚህ ጥናት ላይ መካተት የስርዕሪ ሙሉ ፈቃድ ሲሆን በጥናቱ ላለመካተትም ሆነ ከተካተቱም በኋላ ያለምንም ቅድመ ሁኔ ፈቃደኝነትዎን የማንሳት መብትዎ ሙሉ በሙሉ የተጠበቀ ነው።

ስርዕሪ በጥናቱ ላለመካተት መወሰንም በስርዕሪም ሆነ በቤተሰብዎ ላይ ያገኙት የነበረን የህክምና ተጠቃሚነትንም ሆነ ሌላ ችግር ፈፅሞ ሊያስከትልብዎ አይችልም። ለስርዕሪ የተባለውን ነገር በትክክል ተረድተውተል? ጥያቄ ካለዎት መጠየቅ እና ማብራሪያ የማግኘት መብት አለዎት።

ስለ ጥናቱ ዓላማ ምርመራ እና ሒደት ተገልጿልኛል። ምክንያት ሳያስፈልገኝ ከጥናቱ ለማቋረጥ አንደምችል ተረድቻለው። ይህ የስምምነት ቅፅ በአፍ መፍቻ ቋንቋዬ አንብቤ/ተነባልኝ በትክክል ተረድቼ በራሴ ፈቃደኝነት በጥናቱ ለመካፈል ተስማምቻለው። ለዚህም በፊርማዬ አረጋግጣለው።

መለያ ቁጥር _____ የጥናቱ ስፍራ _____

ስም _____ ፊርማ _____ ቀን _____

የምስክር ስም _____ ፊርማ _____ ቀን _____

የጥናቱ ባለሙያ ስም _____ ፊርማ _____ ቀን _____

