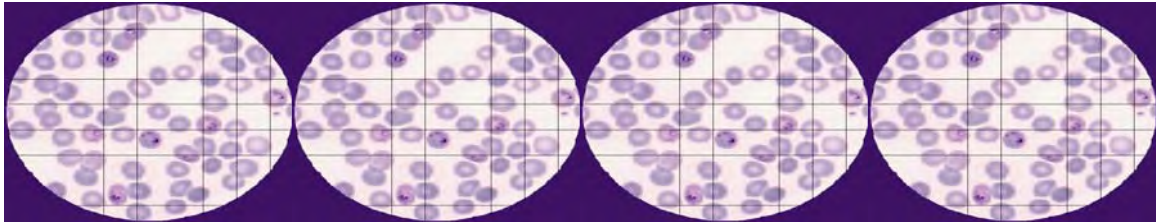




**MALARIA/INTESTINAL HELMINTH CO-  
INFECTIONS AND ANEMIA IN ANTSOKIA-GEMZA  
DISTRICT, AMHARA NATIONAL REGIONAL  
STATE**



**BY**

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## LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
CI	Confidence Interval
CSA	Central Statistics Agency
°C	Degree Celsius
BMI	Body mass index
g/dl	Gram per deciliter
epg	Egg per Gram
FMOH	Federal Ministry of Health
EPO	Erythropoietin
IDA	Iron deficiency anemia
IFN	Interferon
IL	Interleukin
masl	meter above sea level
MUAC	Mid-upper arm circumference
OR	Odd Ratio
PCV	Packed cell volume
PRBC	Parasitized or infected red blood cell
RBC	Red blood cell.
SPSS	statistical package for social sciences
Spp	Species
TDS	Trichuris Dysentery Syndrome
TNF	Tumor necrosis factor
UNICEF	United Nations Children's Fund
WHO	World Health Organization

## **ABSTRACT**

*The study was conducted on 595 individuals aged 5–65 years old residing in Antsokia-Gemza District, North Shoa Zone, Amhara National Regional State, Northern Ethiopia. The aim of the study was to investigate the extent of association of malaria, intestinal helminth infections and malaria/intestinal helminth co-infections with anemia in the community. Blood was collected by finger pricking to determine the malaria parasite species involved, the parasitaemia and haemoglobin concentration. Haemoglobin concentration was measured by using a portable spectrophotometer (Hemocue HB 201). The Kato-Katz technique of stool examination was used to determine prevalence and intensity of intestinal helminth infections. The overall prevalence of malaria, intestinal helminth infections, and co-infections were 43.1%, 31.5%, and 14.0%, respectively. The overall prevalence of anemia and severe anemia was 32.9% and 1.8%, respectively. Prevalence of anemia in individuals with malaria, intestinal helminth infection, and malaria/intestinal helminth co-infections was 31.5%, 25.0% and 45.3%, respectively. Prevalence of anemia was significantly higher and mean haemoglobin concentration was significantly lower among individuals with malaria/intestinal helminth co-infections as compared to those with only malaria or intestinal helminth infections. Univariate analysis identified malaria (OR = 2.325,  $p < 0.001$ ) and malaria/hookworm co-infection (OR = 6.133,  $p < 0.001$ ) as significant risk factors of anemia in the community. Malaria/hookworm co-infections were found to have contributing effect to low haemoglobin level. However, the overall prevalence of anemia was lower than what has been reported from a similar study in Wolayita, Southern Ethiopia. Consumption of teff as a staple food, in the present study area, was speculated as a possible factor contributing to the relatively lower prevalence of anemia as compared to the non-teff staple food region in Wolayita. Implementations of malaria control measures and institution of deworming programs to prevent parasitic infections are recommended to reduce anemia prevalence to the level below what is of public health concern in the study area.*

**Key words:** *malaria, anemia, intestinal helminths, co-infections, Antsokia-Gemza District, Ethiopia*

# 1. INTRODUCTION

## 1.1 Anemia

Anemia is a common disease condition, which is often caused by multiple factors and is defined as the reduction in haemoglobin concentration below cutoff level established by the World Health Organization (WHO, 2001) by taking age, sex and altitude into account and by considering the genetic background of the individuals under investigation. The specific haemoglobin cutoffs recommended by the World Health Organization are: haemoglobin concentration less than 11 g/dl for children aged below 6 years and pregnant women; less than 12 g/dl for children aged 14 years or below and non-pregnant females aged 15 years or more; and less than 13 g/dl for males aged 15 years and above. Severe anemia is defined as haemoglobin concentration of less than 7g/dl.

Anemia is one of the world's most widespread public health problems. Around half the population of developing countries is iron deficient and 47% of non-pregnant women and 60% of pregnant women have anemia world-wide (Crompton, 2000). The World Health Organization has reported that more than 2 billion people are anemic worldwide (WHO, 2001).

In Ethiopia, however, the magnitude and importance of iron deficiency anemia (IDA) as a public health problem is disputed (Adish *et al.*, 1999). Some studies reported iron deficiency anemia rates of around less than 18% (Gebremedhin *et al.*, 1976; Gies *et al.*, 2003) while others have reported rates of around 25% (Zein and Assefa, 1987). It has been held that the low level of anemia in some parts of the country is due to the high daily intake of iron, which is estimated to be between 180 and 500 mg, which is 10–20 times the suggested daily requirement (Hofvander, 1968). This high intake is attributed to consumption of the staple cereal, teff (*Eragrostis teff*), which contains 90 mg of iron per 100 g (Hofvander, 1968). It is also stated that teff contains two to three times more iron than other cereals and the fermentation process during its preparation enhances the bioavailability of iron (Gies *et al.*, 2003). The prevalence of iron deficiency anemia was

reported as 8% in areas where teff (*Eragrostis teff*) was the main staple and 30.5% in areas where sorghum was the main staple (Haidar *et al.*, 1999). In spite of this high intake of iron, some studies have reported a high prevalence of anemia, even in teff-consuming communities (Zein and Assefa, 1987). Therefore, anemia in Ethiopia may not be only due to the low dietary intake of iron (Adish *et al.*, 1999).

Anemia, especially iron deficiency anemia, leads to weakness, poor physical growth, reduced appetite and a compromised immune system – decreasing the ability to fight infections and increasing morbidity – and is also thought to impair cognitive performance and delay psychomotor development (Crompton, 2000). Severe anemia is known to be associated with a reduction in the oxygen carrying capacity of circulating blood resulting in tissue hypoxia.

Recent macroeconomic estimates suggest that the average impact of iron deficiency anemia could be as large as 4 percent of GDP in less developed countries (Horton and Ross, 2003). An indication of the public health importance of iron deficiency anemia may be discerned from the plan of action implemented at the World Summit for Children in New York in 1990, which included the following goals: a reduction in the incidence of low birth weight (2.5 kg or less) to no more than 10%; a one third reduction from 1990 levels in iron deficiency anemia among women (World Bank, 1993).

## **1.2 Causes of Anemia**

Causes of anemia are multi-factorial and include physical injuries, nutritional deficiencies—of iron, folate and vitamin B-12 and parasitic diseases such as malaria and hookworm. The relative contribution of each of these factors to anemia varies greatly by geographical location, season, and dietary practice (Pawlowski *et al.*, 1991).

Iron deficiency anemia is the common type of anemia. Several studies have identified the main causes of iron deficiency anemia as increased physiological demands, inadequate iron intake and abnormal loss of blood as a consequence of parasitic infection and physical

injuries. Individuals during pregnancy, menstrual periods and childhood need high amount of iron due to the nature of their physiology. It has been pointed out that for some women and adolescent girls it is hardly possible to satisfy their daily iron requirements even with good quality, iron-fortified diets (Viteri, 1994).

Vitamin B-12 or folate deficiency causes megaloblastic anemia. Both Vitamin B-12 and folate are required for DNA replication and inhibition of transcription of DNA to RNA. Lack of B-12 or folate means that RNA builds up and this makes the cells too large and structurally non-functional. In sub-Saharan Africa, iron and folate deficiencies are the most common causes of anemia (Baker and DeMaeyer, 1979). Vitamin B-12 deficiency may be an unrecognized contributor to anemia in this region of the world due to reliance of the population on grains as dietary staples and low consumption of foods of animal origin, which are the primary source of dietary vitamin B-12.

Some viral infections, some drugs, exposure to radiation and toxins can induce aplastic anemia, which is associated with the failure of marrow stem cells production (Heimpel, 2000).

Intrinsic defects, like hereditary spherocytosis, glucose-6-phosphate dehydrogenase deficiency, pyruvate kinase deficiency, thalassaemia and sickle-celled anemia cause hemolytic anemia (Mentzer and Wagner, 1989). These defects occur as a result of disorders in the structure or synthesis of haemoglobin; deficiencies of enzymes that provide the red cell with energy or protect it from chemical damage; and abnormalities of the proteins of the red cell's membrane. There are also extrinsic defects, which cause hemolytic anemia due to autoimmune and mechanical destruction of red blood cells (Mentzer and Wagner, 1989).

### 1.3 Malaria and Anemia

Malaria is caused by four *Plasmodium* species parasites – *P. vivax*, *P. falciparum*, *P. malariae* and *P. ovale*. The parasites are transmitted by different species of female *Anopheles* mosquitoes. Although the genus *Plasmodium* was identified as a causative agent of malaria before a century, it is still responsible to cause 300–500 million cases and 1–3 million deaths annually (WHO, 2005). Africa remains to carry the greatest burden of malaria cases and deaths in the world (Worrall, 2004). Malaria is a major public health problem in Ethiopia. 75% of the land of the country is potentially endemic to malaria, which puts 45 million people at high risk, and 4-5million people estimated to get infected annually (FMOH, 2005). It has been consistently reported as one of the three leading causes of morbidity and mortality in the past years (FMOH, 2004).

The population at risk is increasing rapidly due to the spread of drug-resistant parasites, especially *P. falciparum*. The problem gets worse by the emergence of insecticide-resistant *Anopheles* mosquito vectors. In addition, migration into malarious areas, changing agricultural practices such as irrigation projects, increased demands on public health systems due to the spread of HIV in underdeveloped 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). It has been estimated that if no effective intervention strategies are undertaken, the number of malaria cases will double every 20 years (Sachs and Malaney, 2002; Snow *et al.*, 1999; Breman, 2001).

The major clinical features of malaria include, severe anemia due to reduced production and increased destruction of RBC, cerebral complications due to microvascular 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).

Anemia is one of the severe clinical outcomes of malaria and remains one of the most important public health problems in malaria-endemic areas (Crawley, 2004). Malaria causes anemia through haemolysis and increased splenic clearance of infected and uninfected red blood cells and cytokine-induced dyserythropoiesis (Menendez *et al.*, 2000). A single overwhelming episode of malaria or repeated episodes due to re-infection may result in life-threatening anemia, metabolic acidosis and, if untreated, death (Ekvall *et al.*, 2003). Severe anemia is estimated to account for more than half of all childhood deaths from malaria in Africa (Murphy and Breman, 2001). A study in Uganda demonstrates that strong association exists between severe anemia and profound coma in children with cerebral malaria (Idro, 2003).

In the pathogenesis of acute malaria, RBCs are directly destroyed by infecting parasites. Parasite multiplication causes rupture of pRBCs, which results in decreased haemoglobin concentration (Mackintosh *et al.*, 2004). Recent studies have demonstrated that *P. falciparum* infected RBC are recognized and phagocytosed by monocytes and macrophages in an opsonin-independent manner (Serghides *et al.*, 2003). Acute malaria infection is generally associated with reduced total erythropoietic activity. Chang and Stevenson (2004) pointed out that during *P. falciparum* infections, reticulocyte levels are inappropriately low, reflecting suppression of the normal response of erythropoietin (EPO) (Fig. 1). Ferrokinetic studies in acute and chronic malaria show that RBC iron utilisation, which is a measure of effective erythropoiesis, is reduced (Srichaikul *et al.*, 1969; Knuttgen, 1987). Weatherall *et al.* (1983) and Abdalla (1990) did also observe severe dyserythropoiesis in patients with chronic *P. falciparum* malaria.

Patients with severe malarial anemia appear to have an increased ratio between the pro-inflammatory cytokine (tumor necrosis factor, TNF) and the anti-inflammatory cytokine (interleukin, IL-10) (Kurtzhals *et al.*, 1998), and it has been proposed that inflammatory cytokines may be a causative factor for malarial anemia (Biemba *et al.*, 1998). Persistence of such dysregulated inflammatory response is believed to be the cause of extended bone marrow suppression during malarial attack (Helleberg *et al.*, 2005).

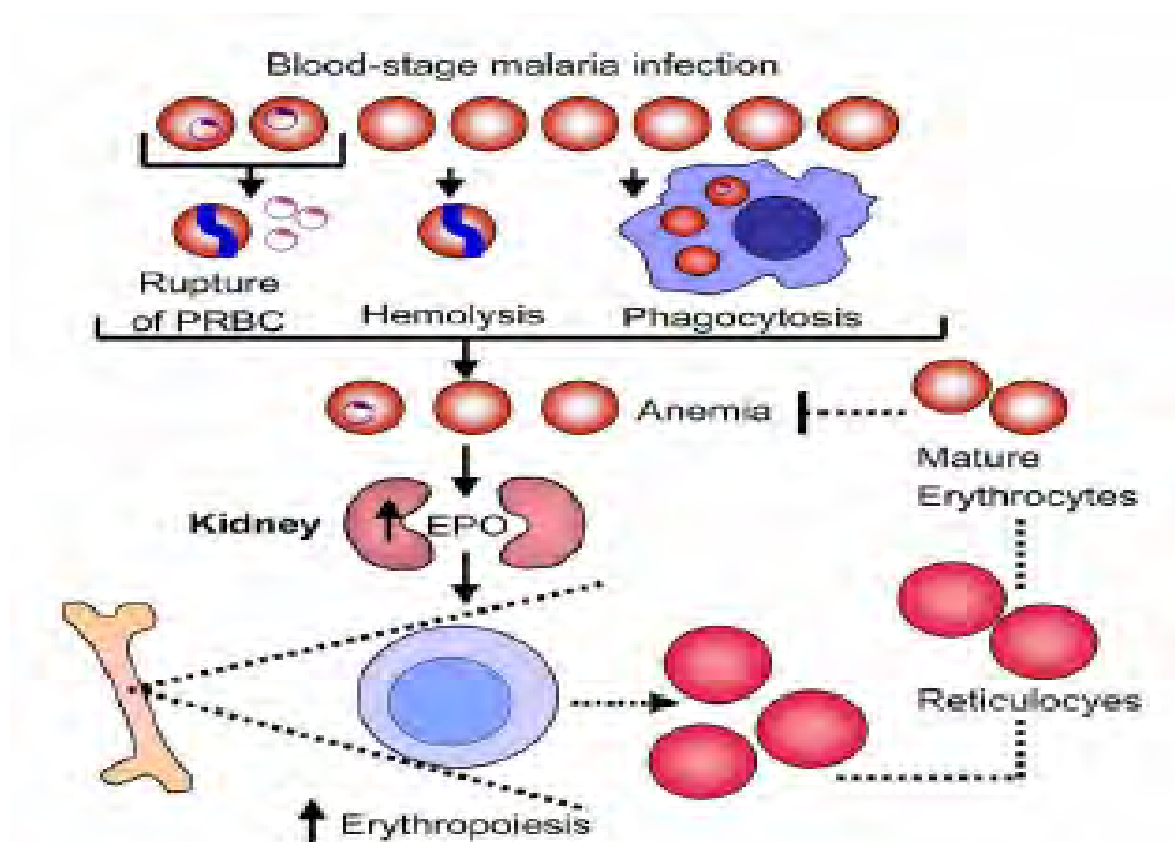


Figure 1. Pathogenesis of malarial anemia (Source: Chang and Stevenson, 2004). Malarial anemia is due to a number of factors including rupture of pRBC, haemolysis and phagocytosis of infected and uninfected RBC. In response to anemia not related to infection or chronic disorders, EPO production is up-regulated in the kidney and travels via the blood to the bone marrow where it promotes erythropoiesis leading to an influx of immature erythrocytes or reticulocytes into the blood to alleviate anemia. However, if erythropoiesis is not optimal as often occurs during malaria, the anemia becomes severe and death of the host ensues. Dash lines indicate that the response may not be optimal in malaria.

### 1.4 Intestinal Helminths and Anemia

The WHO estimates that almost 2 billion people are infected with one or more of intestinal helminths, accounting for up to 40% of the global morbidity from infectious diseases, exclusive of malaria (Hotez *et al.*, 2003). The greatest numbers of soil-transmitted helminth infections occur in tropical and subtropical regions of Asia, especially China, India and Southeast Asia, as well as Sub-Saharan Africa. Of the 1-2 billion soil-transmitted helminth infections worldwide, approximately 300 million infections result in severe morbidity,

which are associated with the heaviest worm burdens (Hotez *et al.*, 2003). In Ethiopia, the prevalence and distribution of intestinal helminths varies from place to place (Ali *et al.*, 1999; Erosie *et al.*, 2002; Legesse and Erko, 2004; Jemaneh, 2000; Merid *et al.*, 2001; Tadesse, 2005).

Epidemiologic studies conducted throughout the developing world point out school-aged children as the population at greatest risk for acquiring heavy infections with hookworms, *Ascaris* and *Trichuris* infections (Montresor *et al.*, 2002). These children suffer the consequences of iron deficiency anemia, acute *Ascaris* intestinal obstruction, hepatobiliary ascariasis, *Trichuris* dysentery syndrome (TDS), or rectal prolapse. Physical growth retardation, cognitive and educational impairments caused by heavy chronic infection are even more significant. Furthermore, hookworms may be directly immunosuppressive and could promote increased susceptibility to concurrent viral and other infections, including HIV/AIDS and malaria. These infections also contribute to poor appetite and decreased food intake. Each can contribute to malnutrition, anemia or other states of poor health, which in turn, can lead to impairment of learning and poor school performance. It is important to note that the stunting of children's growth is not readily recognized, because it occurs almost imperceptibly over time (WHO, 1996). Thus, the full impact of helminth infections is greatly under-reported.

Some of the major intestinal helminths that may cause anemia include hookworms, *Ascaris lumbricoides*, *Trichuris trichiura* and *Schistosoma* species. The proposed mechanisms of these infections causing anemia include direct blood loss (Crompton, 2000), nutritional theft and impairment of the appetite due to immunological factors (Stephenson *et al.*, 2000). The disability-adjusted life-years (DALYs) for intestinal nematodes, like hookworm, *Ascaris lumbricoides* and *Trichuris trichiura*, combined is 39.0 million life-years lost, while that for malaria, which is inherently more overtly disabling, is similar, at 35.7 million life-years lost (Chan, 1997).

### 1.4.1 Hookworms

Hookworm infection in human is caused by two species of nematodes, *Necator americanus* and *Ancylostoma duodenale*. The infection is acquired by skin penetration of filariform larvae during contact with contaminated soil or water or by ingestion of contaminated water, in the case of *A. duodenale*. Despite considerable advances in chemotherapy and control, hookworms rank amongst the most widespread of soil-transmitted intestinal helminth parasites and affect a significant proportion of the world population (Bundy *et al.*, 1991). The infection is very intense in the tropics and subtropics with an estimated 740 million cases (de Sylva *et al.*, 2003). The global DALYs for hookworm is estimated to be 22.1 million life-years lost (Chan, 1997). 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 (Tedla and Jemaneh, 1985). Both human hookworm species are known to exist in Ethiopia (Armstrong and Tadesse, 1975).

Hookworm infection has long been known to be associated with iron deficiency anemia, and data from human studies generally confirm an inverse correlation between intensity of infection, as estimated by fecal egg counts, and blood haemoglobin levels (Crompton, 2000). A study done by Hawdon and Hotez (1996) showed that hookworms caused severe anemia and malnutrition in developing countries of the tropics. Hookworms contribute to iron deficiency by actively feeding on blood from capillaries in the intestinal mucosa, resulting in significant gastrointestinal hemorrhage, loss of serum proteins, and intestinal inflammation (Kalkofen, 1974; Olsen *et al.*, 1998). Blood loss occurs when the worms use their cutting apparatus to attach themselves to the intestinal mucosa and sub mucosa and contract their muscular esophagi 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 anticlotting agents to the blood stream, which makes the blood flow continue (Stanssens *et al.*, 1996).

The degree of iron-deficiency anemia (IDA) induced by hookworms is determined by the intensity and type of the species (Pawlowski *et al.*, 1991). Depending on the status of host iron, a hookworm burden of 40 to 160 worms may be associated with low haemoglobin level (Bundy *et al.*, 1995). The relationship between hookworm infection and IDA was obtained by measuring blood loss from children and correlated the losses with the intensity of the hookworm infection as measured indirectly by means of egg counts (Stoltzfus *et al.*, 1996). As a result, average loss of blood in the stools was found to increase by 0.825 ml/g faeces for each 1000 epg (Crompton, 2000). On the other hand, infection with *A. duodenale* causes greater blood loss than infection with *N. americanus* does. For instance, in Zanzibar, among children who were infected only with *N. americanus* hookworms, the prevalence of hypoferritinemia (ferritin level, <12 µg/liter) was 33.1%, whereas in children who were also infected with *A. duodenale* hookworms, the prevalence was 58.9% (Stoltzfus *et al.*, 1997).

#### **1.4.2 *Trichuris trichiura***

*Trichuris trichiura*, the whipworm, is most common in the warm, moist, tropical and subtropical countries. It is also found in temperate climates. An estimated 1049 million persons harbor *Trichuris* (Crompton, 1999) including 114 million preschool age children and 233 million school-age children, 5-14 years (Chan, 1997). Trichuriasis has been estimated to cause 6.4 million disability-adjusted life-years (DALYs) to be lost (Chan, 1997).

Heavy infections of *T. trichiura* have long been known to be associated with anemia, protein-energy malnutrition and chronic diarrhea and dysentery (Wong and Tan, 1961 cited in Stephenson *et al.*, 2000). *Trichuris* infection causes a significant blood loss and heavy infections can cause iron deficiency anemia in children. Some studies showed anemia was prevalent in children with heavy infection of *T. trichiura* and these children have been found to show significantly lower haemoglobin (Stephenson *et al.*, 2000).

Synergism appears to occur between pre-existing anemia and *T. trichiura*, due to blood loss and probably due to appetite suppression *via* tumor necrosis factor (TNF)- $\alpha$  (Stephenson *et al.*, 2000). *T. trichiura* may also involve significant blood loss with little opportunity for the re-absorption of iron given the location of the worms in the large intestine (Bundy and Cooper, 1989). Therefore, trichuriasis can cause considerable blood loss and heavily infected children often are anemic.

However, in relatively well-nourished children with lighter infections, anemia is unlikely to develop solely from the infection. It is likely that there are both direct and indirect mechanisms responsible for the anemia. The direct mechanism is via bleeding in the large bowel, especially bleeding ulcers in the rectum of children with *Trichuris* Dysentery Syndrome (TDS). Sometimes erythrocytes may exude into the gut lumen from the gut mucosa after damage by the presence of worms. In humans, from 1 ml to 10 ml of blood per infected person may be lost daily and experiments on animal model system showed that a heavy infection caused iron deficiency anemia in pigs (Bundy and Cooper, 1989). The indirect mechanism is likely to be elevated TNF- $\alpha$  levels which may produce or aggravate pre-existing anemia by decreasing appetite and thus food intake and iron intake; this is a systemic mechanism that could occur in any child with sufficiently elevated TNF- $\alpha$  levels independent of direct blood loss from the lower gastrointestinal tract (Stephenson *et al.*, 2000). Some studies show that anti-helminth treatment improves the haemoglobin level significantly in school age children, who were heavily infected with *Trichuris trichiura* (Robertson *et al.*, 1992).

### **1.4.3 *Ascaris lumbricoides***

*Ascaris lumbricoides* infects quarter of the world population (Crompton, 1994). It is highly prevalent intestinal helminth species in areas where there is poor sanitation due to low socio-economic status and educational level. The DALY for *A. lumbricoides* is 10.5 million life-years lost. Most *Ascaris* infections are of a chronic form and are widely considered to significantly impair childhood nutrition, especially in areas where poor growth and ascariasis are common; the infection is most likely to affect bodily growth, fat

absorption, vitamin A absorption, iodine absorption, lactose digestion, and protein absorption (Stephenson and Holland, 1987; Crompton, 1994). Some studies pointed out that *Ascaris lumbricoides* infection didn't affect iron absorption and directly cause iron deficiency anemia; but it might aggravate anemia resulting from malnutrition (Islek *et al.*, 1993).

#### **1.4.4 *Schistosoma* Species**

Schistosomiasis remains a global public health problem: an estimated 600 million individuals are at high risk of infection, and over 200 million are infected at any given time (Stephenson, 1993). Anemia is an important manifestation of chronic schistosomiasis, even in low intensity infection (Leenstra *et al.*, 2006; Friedman *et al.*, 2005). Eggs translocation across the intestinal or bladder walls may result in faecal or urine blood loss with subsequent iron deficiency anemia (Stephenson and Holland, 1987). Schistosomiasis also may produce anemia by inducing a proinflammatory cytokine-mediated dyserythropoiesis, as seen in anemia of inflammation (Konijn, 1994). Anemia in acute or chronic inflammation is mediated by decreased erythropoietin production or responsiveness of erythrocyte precursors in the bone marrow, decreased erythrocyte life span, shunting the bioavailability of iron to storage forms and reduced uptake of dietary iron in the gut (Mahmoud and Woodruff, 1972). Schistosomiasis may also produce anemia secondary to increased sequestration of erythrocytes or increased hemolysis in the spleen of persons with schistosomiasis-associated splenomegaly (Woodruff *et al.*, 1966 cited in Leenstra *et al.*, 2006).

### **1.5 Malaria and Intestinal Helminths Co-Infections**

Malaria is widely co-endemic with the major intestinal helminth infections, hookworm infection, ascariasis, trichuriasis and schistosomiasis; although the geographical congruence is highest with that of hook worm due to its wider thermal tolerance (Mwangi *et al.*, 2006; Helmby, 2007). Therefore, co-infections by these two groups of parasites are common. Recent analysis shows that malaria and hookworm infection are widespread

throughout Sub-Saharan Africa and over a quarter of school-aged children in this region appear to be at high risk of coincident infection and consequently at enhanced risk of clinical disease (Brooker *et al.*, 2006).

Several environmental and host-specific factors influence the epidemiological and geographical patterns of infection by these two groups of parasites. Climate, biology of the parasites and socio-economic status of the population in endemic areas are among the major factors. 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). The result of many investigations reveal that low level of education and poverty are not only associated with poor malaria prevention and poor access to effective anti-malarial drugs but also determine hygienic and water contact behaviour, thereby influencing exposure to infective stages of helminths in the external environment (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 helminths has a profound effect on the immune system. It appears to polarize the immune response towards T-helper-2 (Th2) type, characterized by high levels of cytokines such as interleukin-4 (IL-4), IL-5, IL-13 and high serum levels of immunoglobulin-E (IgE) (Hartgers and Yazdanbakhsh, 2006). It has been suggested that the helminth induced Th2 shift may have complex consequences on malaria, decreasing anti-sporozoite immunity but protecting against severe malaria (Nacher, 2002). The acquisition of resistance to *P. falciparum* reflected an isotype imbalance, i.e. the result of the host's ability to develop antibodies of the 'proper' isotypes (i.e. the cytophilic IgG3 and IgG1) and a reduction of the proportion of non-cytophilic isotypes (i.e. IgG2, IgG4 and IgM) of the same specificity. Th2 cytokine milieu induced by helminth infection is thought to drive the antibody response of individuals co-infected with malarial parasites towards the production of the non-cytophilic subclasses, whereas protection against malaria is associated with the presence of the cytophilic subclasses (Druilhe *et al.*, 2005). Evidence

has been obtained in rodents about the influence of helminths on the immune response to malaria. A significant decrease in tumor necrosis factor (TNF), associated with an increased *P. chabaudi* parasitaemia, and has been observed in mice after their co-infection with *S. mansoni* (Helmby *et al.*, 1998). Recent studies on animal models have demonstrated that concurrent nematode infection impaired the development of protective immunity against malaria infection. Co-infection with *P. chaubadi* and *Heligmosoides polygyrus* (nematode) was found to have a pronounced detrimental effect on the development of immunity against malaria (Su *et al.*, 2005). It has also been stated that the level of protective immunity induced by malaria vaccine has been reduced in mice in the presence of a concurrent nematode infection (Su *et al.*, 2006). Co-infection with malaria parasite and intestinal helminth was also shown to affect drug efficacy in animal model (Legesse *et al.*, 2004). Legesse and colleagues (2004) pointed out that *S. mansoni* caused delayed parasite clearance of *P. burghei* in *S. mansoni* infected mice. Malarial parasites had also been shown to modulate the immune responses to helminth infections. The proliferative Th2 responses to schistosome antigens were found to be suppressed up to 1 month after malaria infection (Helmby *et al.*, 1998).

Interaction between malaria parasites and helminths could work in either direction. Infections with intestinal helminths may alter susceptibility to clinical malaria and malaria may influence the clinical consequences of helminth infections by exacerbating their morbidity. Splenomegaly associated with *S. mansoni* infection is found to be exacerbated by chronic malaria in children (Booth *et al.*, 2004). Recent studies show that intestinal helminth infections appear to influence some clinical outcomes of malaria. *Schistosoma mansoni* and *S. haematobium* infections are found to increase incidence of malaria fever (Booth *et al.*, 2004). Moreover, *Schistosoma mansoni* appears to increase malaria attack (Sokhna *et al.*, 2004) and incidence of severe malaria by repeated vomiting and/ or coma and convulsions (Le Hesran *et al.*, 2004). Moreover, studies on animal model showed that *S. mansoni* enhanced *P. burghei* parasitaemia and increased mortality of the host (Legesse *et al.*, 2004).

However, in several studies, intestinal helminths infections appear to attenuate some severe clinical outcomes of malaria. A recent study concludes that underlying schistosomiasis is associated with protection against clinical falciparum malaria in an age-dependent manner (Lyke *et al.*, 2005). Infection with *Ascaris lumbricoides* is found to protect cerebral malaria (Nacher *et al.*, 2000); helminth infections was found to be associated with protection of cerebral malaria and increased nitrogen derivatives concentrations in Thailand (Nacher *et al.*, 2002); helminth infections are found to reduce jaundice and renal failure (Nacher *et al.*, 2001a); they also reduce organomegaly during acute malaria. *S. haematobium* infection appears to reduce the malaria parasite density during co-infections (Lyke *et al.*, 2005). It has also been reported that hookworm was associated with relatively lower oral temperature (Nacher *et al.*, 2001c). Nacher (2002) explained how *P. falciparum* might have benefited from helminth infected host and how hosts might have benefited from helminths. As helminths protect their host from *P. falciparum*, a major killer, they protect their habitat and secure their survival and reproduction (Nacher, 2002).

Anemia is an important consequence of both malaria and intestinal helminth infections and severe public health problem of the tropics. Malaria is a significant contributor to anemia, operating through a number of mechanisms, including hemolysis and phagocytosis (Mackintosh *et al.*, 2004); whereas intestinal helminths like hookworm causes anemia as result of significant loss of blood from intestinal mucosa (Hotez *et al.*, 2004). Since the mechanisms by which malaria and intestinal helminth infections such as hookworm infections cause anemia differ, it is possible that their impact on haemoglobin level is additive (Mwangi *et al.*, 2006; Brooker *et al.*, 2006). Patients with clinical falciparum malaria and co-infected with intestinal helminths have been reported to show increased anemia and decreased reticulocyte counts (Nacher *et al.*, 2001b; Basavaraju and Schantz, 2006).

Since malaria and intestinal helminth infections have distinct mechanisms of causing anemia, co-infections with these parasites would be expected to have an exacerbating effect on the severity of this clinical outcome.

## **2. OBJECTIVES OF THE STUDY**

### **2.1 General Objectives**

- To investigate the contributions of malaria/intestinal helminth co-infections in the development of anemia in malaria endemic areas

### **2.2 Specific Objectives**

- To assess the prevalence of anemia in cases with malaria and intestinal helminth infections and co-infections in the study area
- To compare the association and severity of anemia with malaria, intestinal helminth infections and malaria/intestinal helminth co-infections

### **3. MATERIALS AND METHODS**

#### **3.1 Study Areas**

The study was carried out at Mekoi health centre, in Antsokia – Gemza District, North Shoa Zone, Amhara National Regional State, Ethiopia. Antsokia – Gemza is located some 350km away from Addis Ababa to the Northeast of the country. Sixteen Kebele's are found in the District. The District is estimated to cover some 386.1 square kilometers areas of land (CSA, 2005). It shares the same border with Oromia zone to the north-east, Efrata-Gidim District to the south, and Gishe-Rabel and Gera-Keya Districts of the Amhara National Regional State to the west (Fig. 2). The altitude of the District ranges from 1400masl to 3050masl (World Vision Ethiopia, 1999). According to the information gained from the Agricultural Office of the District, the mean annual temperature ranges from 10°C to 21.5°C and the mean annual rain fall of the District ranges from 1200mm to 1800mm. The District has three distinct agro-ecological zones, dega (11.3%), woinadega (44.1%) and kola (44.6%). The dominant soil type of the District is loam soil.

Based on figures published by the CSA (2005), this District has an estimated total population of 102,767, of whom 51,599 were males and 51,168 were females in the year 2005. The percentage of individuals of age between 5 and 14 was 24.7% and that of individuals between 15 and 65years was 53.6%. The dominant ethnic group is Amhara, which is followed by Oromo ethnic group.

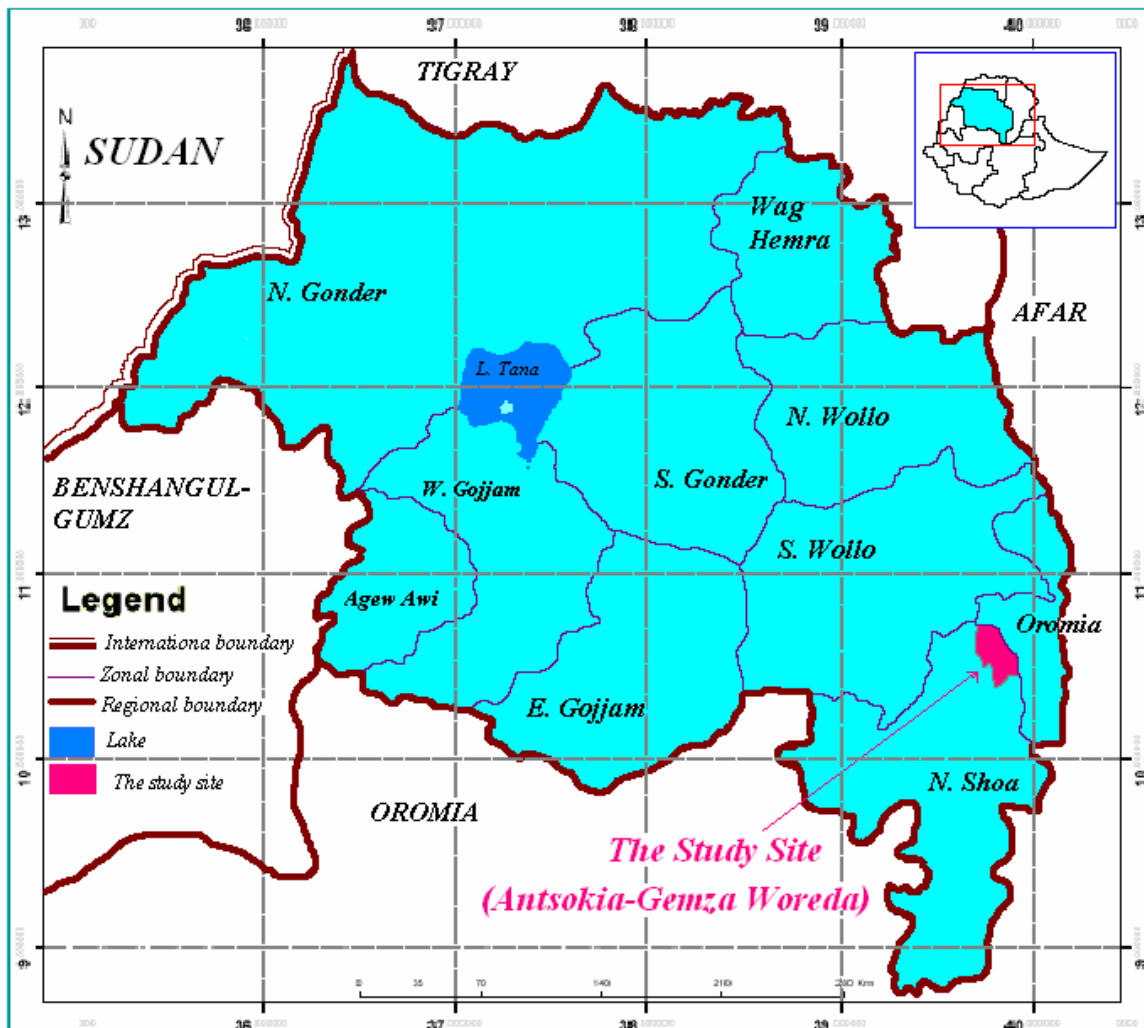


Figure 2. Map of Amhara National Regional State

The majority (90%) of the population of the District is engaged in mixed agricultural activities. The major crops grown in the District are teff (*Eragrostis teff*), sorghum, maize, wheat, barley and pulses (World Vision Ethiopia, 1999). Various vegetables and fruits are also grown in the area. According to the information obtained from the District's Agricultural Office, the major staple food is 'injera' (local bread) made of teff, sorghum, wheat and barley or their mixture. Pulses, fruits and vegetables are largely used as cash crops. Cattle, goats, camels, sheep and poultry are the major animals reared by most farmers.

There are several infectious diseases in the District. Malaria and intestinal parasites are the most prevalent infectious agents and important public health problems in the area. 14 'Kebeles' of the District are malarious and the District's Health Office has consistently reported malaria as one of the top ten diseases and the leading causes of morbidity and mortality. There are three health centers in the District: Mekoi, Majette and Ambo Wuha, with professional staff that include health officers, nurses, laboratory technicians, pharmacy technicians and other supportive staff. There are several health posts in the District, each of which is staffed with health extension workers.

The general nutritional status surveys, on the most vulnerable group of the society (children under 5 years, pregnant women and lactating mothers), were carried out in the District by the year 2005/06. The survey was done in three rounds by the medical staff of the District's Health Office and health centers. During the surveys, subjects were checked for bilateral edema, mid-upper arm circumferences (MUAC) and body mass indices based on the guideline developed by Federal Ministry of Health and UNICEF (FMOH-UNICEF, 2005). The last two surveys (2<sup>nd</sup> and 3<sup>rd</sup> round) were carried out during the present study. Results of the two rounds of the survey have been obtained from the District's Health Office. Around 15637 children under 5years and 2449 pregnant women and lactating mothers were screened for Weigh-height body-mass-index (BMI) and mid-upper arm circumference (MUAC). Only 1.0% of children were found to show those indicators of malnutrition – BMI less than 80%, MUAC less than 12cm and with bilateral edema. 2.4% of pregnant women and lactating mothers were found to have MUAC less than 21cm (Table 1). Based on this result, the prevalence of acute malnutrition was very low and is of little public health concern.

Table 1. Summary of the nutritional status survey on the most vulnerable groups of the community in Antsokia-Gemza District, Amhara National Regional State, Northern Ethiopia, 2005/2006 (Source: Antsokia – Gemza District Health Office)

Indicators of malnutrition	Children under 5 years	Pregnant women and Lactating mothers
Number screened for BMI* and MUAC**	15637	2449
BMI<80%	114 (0.73%)	---
MUAC<12cm	171 (1.1%)	---
MUAC<21cm	---	60 (2.4%)
Bilateral edema	10 (0.07%)	---
Total cases with acute malnutrition	153 (1.0%)	60 (2.4%)

\*BMI=body mass index

\*\*MUAC=Mid-upper arm circumference

### 3.2 Study Subjects

The study participants included 457 consenting local residents, between the ages of 5 and 65 years old who visited the Mekoi health center. In addition, 138 healthy volunteers, who didn't seek any medical treatment, were also registered for this study after informed consent. Individuals that were suspected for HIV infection and pregnant and lactating women were excluded from the study because of some low level of anemia that has been detected through a nutritional survey by the District's Health Centres.

Sample collection was done in three seasons, after the heavy rains (November-December, 2005), after small rains (May-June, 2006) and again after the heavy rains (October-November, 2006). Each sample was diagnosed for malaria and intestinal helminth infections in the field and the positive cases reported to the health center physician. Then, every sample was packed in appropriate container and transported to the Biomedical Sciences Research Laboratory at the Department of Biology, Addis Ababa University, for further investigations.

### **3.3 Laboratory Methods**

#### **3.3.1 Diagnosis for Malaria Parasites**

The Giemsa solution was prepared in the Biomedical Sciences Research Laboratory, Department of Biology, AAU. Preparation of the Stock Giemsa solution was done by adding 3g Giemsa powder in 66ml of glycerin and heated for 2 hours in 60°C water bath. Then 66ml methanol was added to the resulting solution. The content was shaken well and put in dark place for a week. Then the solution was filtered, poured into darker container and again put at dark place for several weeks. Then the matured stock solution was transported to the study site. Working solution was prepared by taking 1ml of the stock solution and diluting it 1:10 in distilled water. Excess Giemsa working solution was discarded after a day and a fresh solution was prepared before use.

Thick and thin blood smears were 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. And the thin smear was used to identify the type of *Plasmodium* species and determine parasitaemia. Parasitaemia was determined by counting the number of infected RBCs per 1000 RBCs in the thin smear and expressed in terms of percentage (Warhurst and Williams, 1996).

#### **3.3.2 Diagnosis for Intestinal Helminths**

Clean stool cap, soft paper and stick applicator were provided for each subject and asked to bring stool sample. Thick smear was prepared according to the modified Kato-Katz method (Peters *et al.*, 1980). Malachite green-glycerine solution was prepared by mixing 100ml of distilled water and 100ml glycerin and adding 1ml of 3% malachite green solution (Peters *et al.*, 1980).

A portion of the stool sample was taken by clean stick applicator and put on a piece of news paper. By pressing the linen mesh on the sample, the filtered soft portion of the stool was taken by using wooden spatula. Then it was placed on the hole of a plate, which was on the slide. This hole contains 41.2g of stool sample. After removing the plate from the slide, the sample was covered with a piece of cellophane tape, which was soaked in malachite green-glycerine solution, and made to spread by pressing with another slide (Peters *et al.*, 1980). The prepared slide was examined under middle and high power objectives of the microscope. Helminth's eggs were counted and multiplied by 24 to determine the number of eggs per gram of stool (epg). Priority was given to the hookworm egg count since the egg of this nematode gets over clearance after a couple of hours from the smear.

### **3.3.3 Determination of Haemoglobin Concentration**

The haemoglobin level in the capillary whole blood was quantitatively determined by using a portable haemoglobin spectrophotometer, Hemocue Hb 201 analyzer (*HemoCue*, Ängelholm, Sweden), and specially designed microcuvette, the Hemocue Hb 201 Microcuvette (*Hemocue*, Ängelholm, Sweden), which were purchased from SETEMA limited Ethiopia. This Hemocue technique is based on the optical measuring microcuvette of a small volume and a short light path. The capillary whole blood from fingertip of each subject was taken by using sterile lancet. After rubbing the finger tip three times using sterile cotton, a drop of blood was allowed to enter the optical window of the microcuvette, which has a volume of 10 $\mu$ l according to the manufacturer, by capillary action and spontaneously mixed with the reagent in the optical window. Then the microcuvette was placed into the cuvette holder of the analyzer for photometric determination of haemoglobin. The concentration of the haemoglobin was read from the analyzer in g/dl.

### **3.4 Data Analysis**

Statistical analysis was carried out using SPSS Version 13.0 software. Chi-square tests and ANOVA were used to test for differences in proportions and means. Pearson's correlation test was used to compare parasite intensities and haemoglobin level. Univariate analysis was used to identify predictors of anemia. A significant level of 0.05 was used for all tests.

### **3.5 Ethical Consideration**

The ethical aspect of this study was approved and clearance was obtained from the Ethical Committee of Biology Department, Addis Ababa University. The objective of the study was explained to the study participants – patients and healthy volunteers, at the time of specimen collection. Every sample was taken when the participant agreed to give the sample following informed consent. The blood samples were taken from each study participants by qualified laboratory technicians by using sterile and disposable blood lancet. All used blood lancets and cotton were properly disposed in to safety box and burnt to avoid risk of contaminations. Participants, who were positive for malaria parasites, intestinal helminths as well as anemic individuals were treated free of charge in the health center.

## 4. RESULTS

A total of 595 individuals were randomly included in the study from those individuals who visited Mekoi Health Centre (a total of 457) and healthy volunteers from Mekoi Secondary School and some public places (a total of 138) after obtaining informed consent (Table 2). The number of male participants was higher (63.4%) than that of female participants (36.6%). The median age of the study participants was 22 years (ranging from 5 to 65 years) and that of the control group was 17 years (ranging from 6 to 24).

Table 2. The sex and age distribution of study participants in the malaria/intestinal helminth co-infection and anemia study in Antsokia-Gemza District, Amhara National Regional State, Northern Ethiopia, 2005/2006

Study Participants	Female		Male		Total
	Age (5-14)	Age (15-65)	Age (5-14)	Age (15-65)	
Control*	14	41	16	67	138
Study subjects**	16	147	38	256	457
Total	30	188	54	323	595

\* Control = include individuals who didn't seek any medical treatment during sample collection.

\*\* Study Subjects = includes individuals who came to Mekoi health centre for medical treatment.

### 4.1 Malaria

From a total of 457 patients, 197 (43.1%) were found to harbor at least one species of *Plasmodium* parasites in their blood. *P. falciparum* accounted for the majority of malaria cases whereby 143 (31.3%) of the total cases were detected as *P. falciparum* positive and 51 (11.2%) were *P. vivax* positive. 3 (0.7%) were double infections by both species of *Plasmodium* parasite (Table 3). Malaria was significantly ( $p < 0.05$ ) more prevalent in males (47.2%) than in females (35.5%) (Table 3).

Table 3. Cumulative annual *Plasmodium* species prevalence among different sex of the study participants in Antsokia-Gemza District, Amhara National Regional State, Northern Ethiopia, 2005/2006

<i>Plasmodium</i> species	Female (F)	Male (M)	Total	Significant differences ( $p < 0.05$ )
	163	294	457	
<i>P. falciparum</i> Prevalence (%):	24.5% (40/163)	35.0% (103/294)	31.3% (143/457)	M > F
<i>P. vivax</i> Prevalence (%):	11.0% (18/163)	11.2% (33/294)	11.2% (51/457)	NS
Mixed Infections Prevalence (%):	0	1.0% (3/294)	0.7% (3/457)	NS
Total Prevalence** (%)	35.6% (58/163)	47.3% (139/294)	43.2% (197/457)	M > F

\*\* Significant at  $p < 0.01$

NS = not significant

Among the malaria positives the proportion of children (age: 5-14years) (66.7%) was more than adults (age: > 14years) (40.0%). Furthermore, the prevalence of *P. falciparum* was significantly lower ( $p < 0.05$ ) in females (24.5%) than in males (35.0%) and was significantly higher ( $p < 0.01$ ) in children (50.0%) than in adults (28.8%); whereas the prevalence of *P. vivax* was uniformly distributed among the sexes and age groups (Table 4).

Table 4. Cumulative annual *Plasmodium* species prevalence among different age groups of the study participants in Antsokia-Gemza District, Amhara National Regional State, Northern Ethiopia, 2005/2006

<i>Plasmodium</i> species	Children (a)	Adults (b)	Total	Significant differences ( $p < 0.05$ )
	54	403	457	
<i>P. falciparum</i> ** Prevalence (%):	50.0% (27/54)	28.8% (116/403)	31.3% (143/457)	a > b
<i>P. vivax</i> Prevalence (%):	14.8% (8/54)	10.7% (43/403)	11.2% (51/457)	NS
Mixed Infections Prevalence (%):	1.8% (1/54)	0.4% (2/403)	0.7% (3/457)	NS
Total Prevalence**	66.7% (36/54)	40.0% (161/403)	43.2% (197/457)	a > b

\*\* Significant at  $p < 0.01$

NS = not significant

The prevalence of malaria significantly ( $p < 0.001$ ) varied in different seasons – 43.9% in November-December, 2005; 31.6% in May-June, 2006; and 55.1% in October-November, 2006 with *P. falciparum* being the commonest species in all three seasons. No mixed *Plasmodium* species infection was detected following the small rains, May-June, 2006 (Fig. 3).

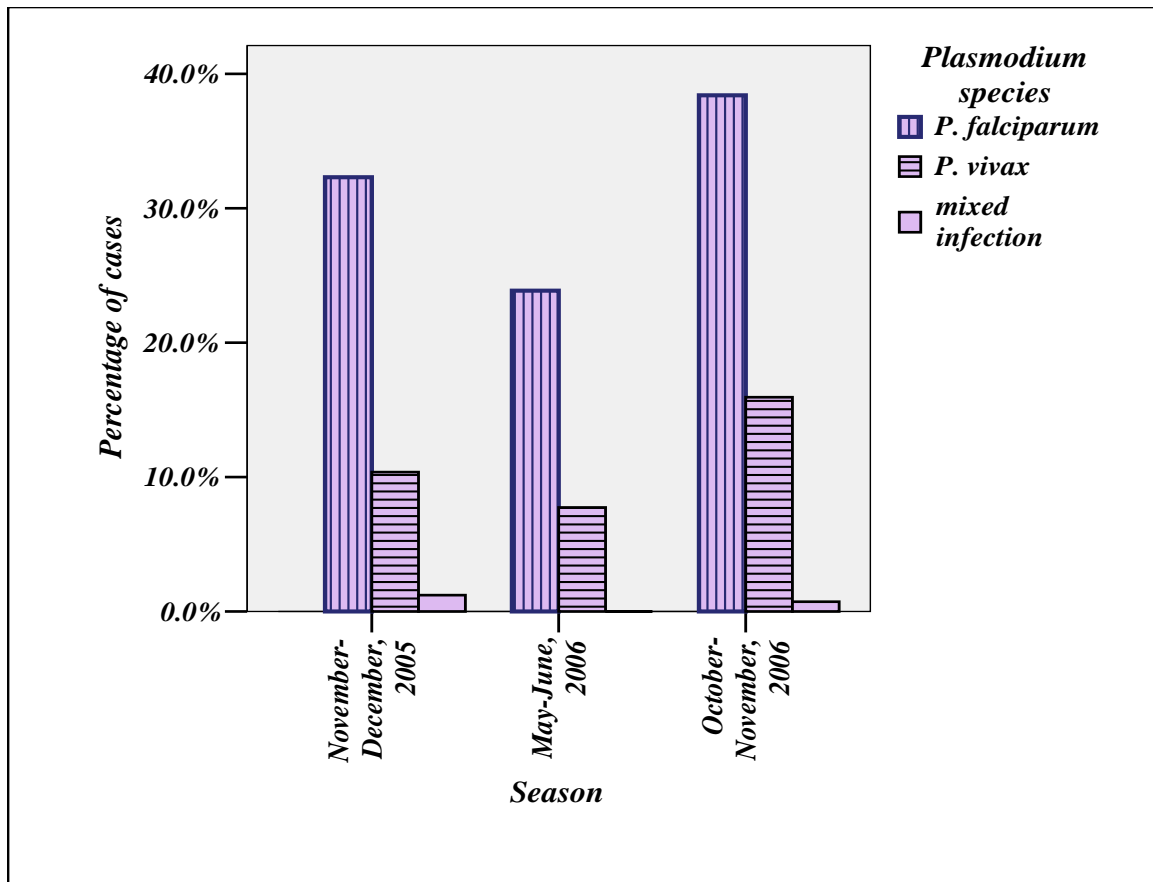


Figure 3. Seasonal prevalence of *Plasmodium* species infections in Antsokia-Gemza District, Amhara National Regional State, Northern Ethiopia, 2005/2006

The mean parasitaemia of *P. falciparum* was 4.7% (range: 0.6% - 16.4%) while that of *P. vivax* was 4.2% (range: 0.1% - 11.2%). There was also no significant association either with age or with sex and parasitaemia.

## 4.2 Intestinal Helminth Infections

Seven species of intestinal helminths were detected from the stool samples examined (Table 5). 31.5% (144 out of 457) of the study participants were found infected with at least one species of intestinal helminth parasite. Out of these, *Ascaris lumbricoides* was found in 78 (17.1%) individuals; Hookworm in 49 (10.7%) individuals; *S. mansoni* in 34 (7.4%) individuals; *T. trichiura* in 13 (2.8%) individuals and other helminths (*Enetrobius vermicularis*, *Taenea* species and *Hymenolepis nana*) in 22 (4.8%) individuals. Overall, *A. lumbricoides* consisted of 54.2% of the total intestinal helminth infections. The second and third most prevalent intestinal helminth species were hookworm and *Schistosoma mansoni* species, which constituted 29.9% and 27.8% of the cases, respectively. Multiple infections constituted 27.1% of the helminth infections (Table 5). In general, intestinal helminth infections were found to be more common in males than in females although the association was not significant. The observed differences in helminth infections between the age groups was statistically significant ( $p < 0.05$ ) with 57.4% of children and 28.0% of adults infected with at least one intestinal helminth species.

Table 5. Prevalence of intestinal helminth infections among different age groups of the study participants in Antsokia-Gemza District, Amhara National Regional State, Northern Ethiopia, 2005/2006

Intestinal Helminth Species	Children (a)	Adults (b)	Total	Significant differences ( $p < 0.05$ )
	52	403	457	
<i>A. lumbricoides</i> Prevalence (%):	25% (13/52)	16.1% (65/403)	17.1% (78/457)	NS
Hookworm Prevalence (%):	17.3% (9/52)	9.9% (40/403)	10.7% (9/457)	NS
<i>S. mansoni</i> Prevalence (%):	17.3% (9/52)	6.2% (25/403)	7.4% (34/457)	a > b
<i>T. trichiura</i> Prevalence (%):	3.8% (2/52)	2.7% (11/403)	2.8% (13/457)	NS
Other Intestinal Helminths* Prevalence (%):	9.6% (5/52)	4.2% (17/403)	4.8% (22/457)	NS
Double infections Prevalence (%):	9.6% (7/52)	5.4% (22/403)	6.3% (29/457)	NS
Triple infections Prevalence (%):	0	1.7% (7/403)	1.5% (7/457)	NS
Quadruple infections Prevalence (%):	0	0.7% (3/403)	0.7% (3/457)	NS
Total prevalence (%)**	63.5% (33/52)	28.0% (113/403)	31.5% (144/457)	a > b

\*Other intestinal helminths = *Enterobius vermicularis*, *Hymenolepis nana* and *Taenia* spp.

\*\* Significant at  $p < 0.01$

NS = not significant

Prevalence of each intestinal helminth species and multiple intestinal helminth infections were found to be higher in children than in adults (Fig. 4). *A. lumbricoides*, hookworm and multiple intestinal helminth infections were found to be relatively more prevalent in males than in females. *S. mansoni*, *T. trichiura* and other intestinal helminth infections appeared more common in females than in males. But these differences were not statistically significant.

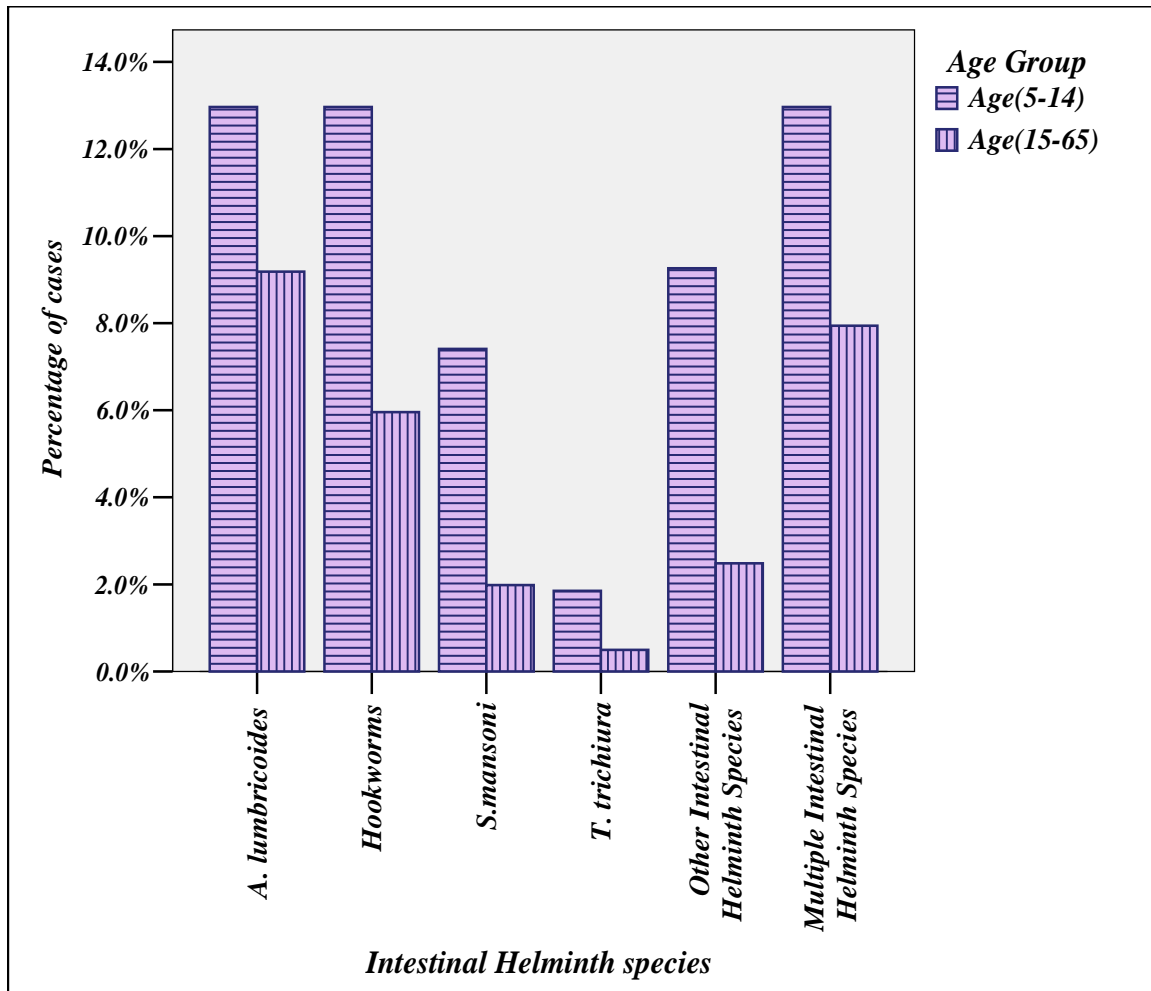


Figure 4. Percentage distribution of intestinal helminth species infections among different age groups in Antsokia-Gemza District, Amhara National Regional State, Northern Ethiopia, 2005/2006

\*Other intestinal helminths = *Enterobius vermicularis*, *Hymenolepis nana* and *Taenia* species

The mean epg of *A. lumbricoides* was 6,390.77 (range: 24 – 120,000); the mean epg of hookworm 385.47 (range: 24 – 2,472); the mean epg of *S. mansoni* 456 (range: 24 – 5,016); and that of *T. trichiura* 125.54 (range: 24 – 720). The mean intensity of *A. lumbricoides* seemed to decrease as the age increase although this was not statistically significant. Hookworm, *S. mansoni* and *T. trichiura* intensities of infections did not show any observable trend with respect to age. However, there was no significant relationship between mean epg and different sex and age groups for each intestinal helminth species.

### 4.3 Malaria and Intestinal Helminth Co-Infections

A total of 64 (20.3%) malaria infected cases were found to be infected with at least one species of intestinal helminth. Malaria/*A. lumbricoides* co-infection was the highest with a prevalence of 17.8%. The second more frequent co-infection was malaria/hookworm with 13.7% (Table 6). The over all prevalence of malaria/intestinal helminth co-infections was 14.0% (64 out of 457).

Table 6. Prevalence of co-infection of malaria with different species of intestinal helminths in Antsokia-Gemza District, Amhara National Regional State, Northern Ethiopia, 2005/2006 (n=197)

Co-infections	Number of cases	Prevalence
Malaria/ <i>A. lumbricoides</i>	35	17.8%
Malaria/Hookworm	27	13.7%
Malaria/ <i>S. mansoni</i>	9	4.6%
Malaria/ <i>T. trichiura</i>	4	2.0%
Malaria/other intestinal helminthes*	6	3.0%

\*Other intestinal helminths = *Enterobius vermicularis*, *Hymenolepis nana* and *Taenia* Spp

#### 4.4 Anemia

The mean Haemoglobin concentration of the study participants was 13.231g/dl, ranging from 5.4g/dl to 18g/dl; and that of the controls was 14.846g/dl, ranging from 8.5g/dl to 17.7g/dl. The overall prevalence of anemia (haemoglobin concentration <12g/dl for children and adult women or haemoglobin concentration <13g/dl for adult men) was 32.9% in infected subjects and 3.6% in the control group; and prevalence of severe anemia (Haemoglobin concentration <7g/dl) was 1.8% in the study subjects and none in the control (Table 7). *Plasmodium* or helminth infected females were found to have significantly lower ( $p<0.05$ ) mean haemoglobin concentration (12.803g/dl) than that of infected males (13.423g/dl). Infected children were found to have significantly ( $p<0.05$ ) lower mean haemoglobin concentration (12.323g/dl) than infected adults (13.446g/dl).

Table 7. Comparison of prevalence of anemic levels between control and study subjects infected with either malaria or intestinal helminths in Antsokia-Gemza District, Amhara National Regional State, Northern Ethiopia, 2005/2006

Anemic level	Control	Infected subjects**
Normal*	133 (96.4%)	181 (65.3%)
Anemic	5 (3.6%)	91 (32.9%)
Severely anemic	0	5 (1.8%)

\* Normal = haemoglobin concentration above 12g/dl for adult women and children and haemoglobin concentration above 13g/dl for adult men

\*\* Infected subjects = Study subjects infected with either malaria or intestinal helminths

The prevalence of anemia was higher in males (35.1%) than in females (27.9%) and higher in children (35.8%) than in adults (32.1%); but these differences were not statistically significant. Infections with *Plasmodium* and intestinal helminth species, in general, were associated with lower mean haemoglobin concentrations as compared to the control (Fig. 5).

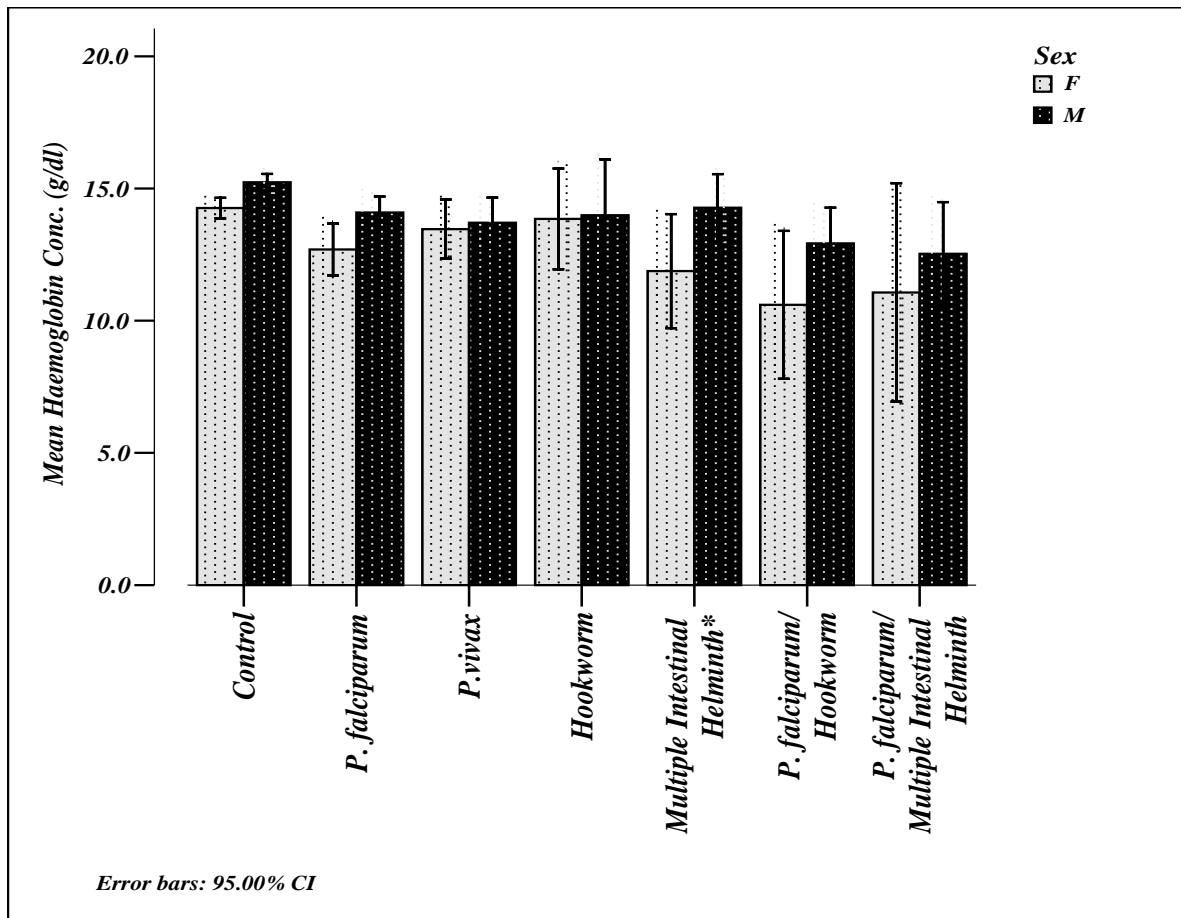


Figure 5. Mean haemoglobin concentration in various infected and co-infected study participants and the control group in Antsokia-Gemza District, Amhara National Regional State, Northern Ethiopia, 2005/2006

\*Two or more intestinal helminths in a single study subject

#### 4.4.1 Anemia and Malaria

Anemia was significantly associated with malaria whereby 31.6% (42/133) and 1.5% (2/133) of the *Plasmodium* infected subjects were found to be anemic and severely anemic, respectively. It was noted that 27.5% and 2.2% of *P. falciparum* infected subjects were anemic and severely anemic, respectively while 35.9% of *P. vivax* infected subjects were anemic, with no severely anemic cases (Table 8). However, infections with both species of *Plasmodium* were found to be significantly ( $p < 0.001$ ) associated with lower mean haemoglobin concentration when compared with the control group (Fig. 5). Double

infection with both malaria parasites was associated with lower mean haemoglobin concentration and anemia when compared to single infections. Parasitaemia of both species of *Plasmodium* was found to be negatively and significantly ( $p<0.01$ ) associated with the level of haemoglobin.

Table 8. Prevalence of anemia in different *Plasmodium* species in Antsokia-Gemza District, Amhara National Regional State, 2005/2006

Anemic level	<i>P. falciparum</i>	<i>P. vivax</i>	Mixed <i>Plasmodium</i>
Anemic	27.5% (25/91)	35.9% (14/39)	100.0% (3/3)
Severely anemic	2.2% (2/91)	0	0

#### 4.4.2 Anemia and Intestinal Helminth Infections

Intestinal helminth infections were collectively associated with low level of haemoglobin. The overall prevalence of anemia and severe anemia in intestinal helminth infected subjects was 25.9% (20/80) and 2.5% (2/80), respectively.

Infection with *A. lumbricoides* was associated with relatively lower haemoglobin level as compared to the controls although it was not significant enough to cause anemia. Hookworm infection was associated with relatively lower haemoglobin concentration as compared with the control group although it was not significant enough to cause anemia. On the other hand, infection with *S. mansoni* was associated with much lower mean haemoglobin concentration and anemia as compared with all other intestinal helminth

infections and the control. Multiple intestinal helminth infections were associated with lower mean haemoglobin concentration as compared to the controls.

#### **4.4.3 Anemia and Malaria/Intestinal Helminth Co-Infections**

Malaria/intestinal helminth co-infections on the whole were associated with lower haemoglobin concentration as compared to the controls and those infected by malaria and intestinal helminths alone. *P. falciparum*/intestinal helminth co-infections were more significantly associated with lower mean haemoglobin concentration ( $p < 0.001$ ) (Fig. 5). Thus, the prevalence of anemia and severe anemia in malaria/intestinal helminth co-infections was 45.3% (29/64) and 1.6% (1/64), respectively.

When single intestinal helminth and malaria co-infections considered, it was shown that malaria/*A. lumbricoides* co-infection to be associated with a relatively lower mean haemoglobin concentration when compared with single infections and the controls. In particular, *P. falciparum*/*A. lumbricoides* co-infection was associated with lower haemoglobin level and higher prevalence of anemia as compared with the respective parasites alone. But there was no severe anemia in these co-infection cases (Table 9).

Malaria/hookworm co-infection was significantly ( $p < 0.001$ ) associated with lower haemoglobin levels as compared to infections with *Plasmodium* species and hookworm alone as well as the control group. Anemia was highly prevalent in malaria/hookworm co-infections. The prevalence of anemia in the malaria/hookworm and *P. falciparum* and hookworm co-infections was 60.0% and 50%, respectively (Table 9). However, there no severe anemia was detected in the co-infections.

Furthermore, *P. falciparum*/multiple intestinal helminth co-infections were significantly associated with lower mean haemoglobin concentration as compared with infections with *P. falciparum*, multiple intestinal helminths alone and the control ( $p < 0.05$ ) (Fig. 5).

Table 9. Relationship of various parasitic infections and co-infections to anemia among the study participants in Antsokia-Gemza District, Amhara National Regional State, Northern Ethiopia, 2005/2006

Infections	Prevalence of anemia	Significant differences ( $p < 0.05$ )
Control (C)	3.6% (5/138)	NA
<i>P. falciparum</i> (Pf)	27.5% (25/91)**	Pf > C
<i>P. vivax</i> (Pv)	35.9% (14/39)**	Pv > C
Hookworm (Hw)	18.2% (2/11)*	Hw > C
<i>A. lumbricoides</i> (Al)	21.7% (5/21)**	Al > C
<i>S. mansoni</i> (Sm)	62.5% (5/8)**	Sm > C
Multiple Intestinal Helminths <sup>a</sup> (mih)	24.0% (6/25)*	mih > C
<i>P. falciparum</i> / <i>A. lumbricoides</i> (Pfal)	50.0% (8/16)*	pfal > C
<i>P. falciparum</i> /Hookworm (PfHw)	58.8% (10/17)*	Pfhw > C, Pf, mih, Hw, Al
<i>P. falciparum</i> /Multiple Intestinal Helminths (Pfmih)	38.5% (6/13)*	Pfmih > C, mih

<sup>a</sup> Two or more intestinal helminth species in a single study subject

\* The observed difference is significant at  $p < 0.05$

\*\* The observed difference is significant at  $p < 0.01$

NA = not applicable

Univariate analysis of anemic status (normal vs anemia/severe anemia) as dependent variable and infections and co-infections as risk factors showed that malaria, malaria/hookworm co-infection and malaria/multiple intestinal helminth co-infections were identified as risk factors for anemia (Table 10). *A. lumbricoides* and hookworm and multiple intestinal helminth infections alone co-infection with malaria and *A. lumbricoides* were not found to be significant risk factors for anemia. However, co-infection with malaria and *A. lumbricoides* was highly but not significantly associated to increased risk for anemia.

Table 10. Univariate analysis of anemic status as dependent variable and malaria, intestinal helminth infection and malaria/ intestinal helminth co-infections as risk factors for anemia in Antsokia Gemza District, Amhara National Regional State, Northern Ethiopia, 2005/2006

Risk Factors	OR	95.0% CI for OR		<i>p value</i>
		Lower	Upper	
Malaria **	2.325	1.507	3.588	0.000
Hookworm infection	0.833	0.178	3.905	0.817
Malaria/Hookworm co-infection**	6.133	2.449	15.357	0.000
<i>A. lumbricoides</i> infection	1.046	0.381	2.876	0.930
Malaria/ <i>A. lumbricoides</i> co-infection	2.404	0.974	5.935	0.057
Multiple intestinal helminth infection <sup>a</sup>	1.098	0.447	2.698	0.839
Malaria/Multiple intestinal helminth co-infection *	3.924	1.350	11.404	0.012

\*Significant at  $p < 0.05$

OR = Odds Ratio

\*\*Significant at  $p < 0.01$

CI=confidence interval

<sup>a</sup> Two or more intestinal helminth species in a single study subject

## 5. DISCUSSIONS

In general, anemia is a major public health problem and is highly prevalent throughout the world (Crompton, 2000); its magnitude being very severe in almost all countries of sub-Saharan Africa (WHO, 2001). However, the overall prevalence of anemia, 32.9%, and severe anemia, 1.8%, in the present study was not very high. This finding is significantly different from the finding of Aragie (2006), who conducted a similar study at Areka District (Wolayita, Southern Ethiopia) and reported an overall prevalence of anemia and severe anemia to be 45.1% and 7.8%, respectively. It is also slightly lower than the findings of Mekonnen (2006), who also conducted a similar study on children less than 5 years of age in Dembia (North Gonder, Amhara Region) and reported an overall prevalence of 34.8%. The most plausible explanation for these differences is that variations in the major staple food of the three areas, teff (*Eragrostis tef*) in the present study area and other crops (corn, root crops, enset) in Areka, may contribute to the lower prevalence. It is well known that teff is believed to be a good source of iron and its fermentation enhances the bioavailability of iron (Hofvander, 1968; Haidar *et al.*, 1999; Gies *et al.*, 2003). According to Hofvander (1968), teff is believed to contain around 90mg iron in 100g of the grain; and in some teff producing areas of Ethiopia, the daily iron intake is 10 to 20 times higher than the suggested daily requirements (Hofvander, 1968). Moreover, the results of the nutritional status surveys carried out by the District Health Office on the most vulnerable groups in the area seem to support this notion. According to the results of this nutritional survey, malnutrition did not appear as a serious public health problem in the study area. The other explanation can be the low number of consenting women study participants and absence of children under the age of 5 years in the present study. Thus, the present study findings may not be directly comparable with the reports of Aragie (2006) and Mekonnen (2006) as it does not adequately include women and the under 5 years old children, which are known to be highly vulnerable to anemia due to their physiology (Viteri, 1994).

The higher prevalence of anemia in children than in adults might be due to the fact that infections with *Plasmodium* parasites and different species of intestinal helminths were more prevalent in children than in adults.

The high prevalence of malaria in the present study, 43.2%, was comparable with the situations elsewhere (Aragie, 2006). The higher prevalence of malaria observed in males than in females may have been due to exophagous transmission because the activities of most males in the study area, such as farming and keeping cattle, as observed at the time of sample collection, oblige the males more than the females to stay outside of their houses in the evening. This would expose them to the bite of exophilic mosquitoes increasing the probability of getting infected with the malaria parasites. Furthermore, the higher prevalence of malaria observed in children of age group 5-14 years old, in the present study, shows that children are at higher risk to malaria than adults; and it may be explained by the recent observation that immunity against malaria parasites in children might take relatively longer time to be established than in adults (Druilhe *et al.*, 2005). It has been shown long ago that immunity against blood stage malaria builds up progressively in children in endemic areas and becomes fully effective by the age of 15-25 years (McGregor, 1972 cited in Druilhe *et al.*, 2005).

The significantly higher seasonal prevalence of malaria in October-November, 2006, is in agreement with the general fact in malaria endemic areas in Ethiopia. Malaria epidemic gets its peak just after heavy rain, September-November, in those malaria endemic areas. But the difference between the first season, November-December, 2005, and the third season, October-November, 2006, was due to the delay in starting sample collection in the first season.

Malarial anemia is a major cause of morbidity and mortality in malaria endemic areas (Chang and Stevenson, 2004). In the present study, anemia is observed to be associated with malaria cases. 31.6% and 1.5% of the malaria cases are associated with anemia and severe anemia, respectively. This investigation is significantly different from the finding of Aragie (2006), who reported the prevalence of 58% anemia and 10.4% severe anemia. This

difference would be expected because of the low background prevalence of anemia in the study area largely due to the iron rich staple food.

The mean haemoglobin concentration is significantly lower in malaria infected cases as compared to the uninfected ones. These finding is in line with a number of previous investigations. Several studies (Chang and Stevenson, 2004; Helleberg *et al.*, 2005) had demonstrated that malaria is the major cause of reduced haemoglobin concentration and anemia in endemic areas. The proposed mechanisms of malarial anemia include erythrocyte lysis and phagocytosis, increased sequestration of parasitized erythrocytes and their destruction by the immune system as well as reversible suppression of the bone marrow response to erythropoiesis (Abdalla *et al.*, 1990). Anemia develops when this accelerated removal of erythrocytes is not compensated by the bone marrow (Helleberg *et al.*, 2005).

Double infections with *P. falciparum* and *P. vivax* were found to be associated with more severe anemia when compared with single infections. This may be due to the synergistic effect of the two malaria parasites on aggravating anemia (Mayxay *et al.*, 2001).

Negative correlation was observed between intensity of *P. falciparum* infection and haemoglobin level in the present study. The observation that low haemoglobin levels are associated with higher intensity of malaria parasites is in agreement with other reports (Helleberg *et al.*, 2005; Chang and Stevenson, 2004) that parasite multiplication results in decreased haemoglobin level due to rapture of parasitized red blood cells as well as destruction of non-parasitized red blood cells because of reduced red cell deformability (Dondorp *et al.*, 1999) and autoimmune haemolysis (Ritter *et al.*, 1993; Woodruff *et al.*, 1979).

The 33.1% overall prevalence of intestinal helminth infections in the present study is comparable with the findings of Tadesse (2005) and Aragie (2006), from other localities in Ethiopia indicating a more or less similar unhygienic situation that predisposes to geohelminth infections in the country. The fact that children are found to be more infected

than adults is consistent with records from elsewhere those helminth infections are generally most prevalent and intense in school-age children (Bundy and Medly, 1992).

Comparison of the prevalence of specific helminth parasites with what was reported from other localities in the country showed significant differences. Tadesse (2005) from Eastern; Ali *et al* (1999) from Western and Erosie *et al* (2002) from Southern Ethiopia have reported different prevalence values for hookworm infection, ascariasis, trichuriasis, schistosomiasis mansoni, etc. These differences may be explained by variations in geographical and socio-economic conditions of the population in the different study areas in addition to differences in the category of the study population.

The association of intestinal helminth infections with low haemoglobin level in the present study is in agreement with what was reported previously by Larocque *et al* (2005). The proposed mechanism of anemia in intestinal helminth infections includes direct blood loss, for example due to hookworm infections, malnutrition, and impairment of appetite due to some immunological factors (Hotez *et al.*, 2004; Stephenson *et al.*, 2000).

Single infection with *A. lumbricoides* appeared to contribute non-significantly to low haemoglobin level. This observation supports the previous findings of Islek *et al* (1993), who showed that *A. lumbricoides* infection does not lead to iron malabsorption and iron deficiency anemia; but it may aggravate through malnutrition. Thus, the non-significance of lowered haemoglobin level was because the intensity of *A. lumbricoides*, in the present study, was low and moderate. It has been suggested that high worm load would be required to cause low haemoglobin concentration (Crompton and Whitehead, 1993) as a result of malnutrition. Single infections with hookworm seem to be less significant in affecting haemoglobin level in this study. This finding was contrary to several previous reports (Aragie, 2006; Bulto *et al.*, 1992; Bundy *et al.*, 1995; Tsegaye *et al.*, 1999; Hawdon and Hotez, 1996; Hotez *et al.*, 2004). The discrepancy may be due to the involvement of the less virulent hookworm species, i.e. *N. americanus*, and the low intensity of infection in the study subjects. It has been shown that the appearance and persistence of anemia, occurs only when the loss of blood is induced by a great number of hookworms (Crompton and

whitehead, 1993). The nutritional background, particularly consumption of teff, may be another contributor to this variation. The type of hookworm species available in the area may also be the cause of this discrepancy (Stoltzfus *et al.*, 1997). *N. americanus* is a hookworm species to be found in the study area as previous reports (Tedla and Jemaneh, 1985) would show. Tedla and Jemaneh (1985) have identified *N. americanus* in the nearby towns Ataye, Karakore and Dessie. These places, especially Ataye and Karakore, are neighboring towns and have almost similar climate and soil type as Antsokia-Gemza District, the present study area.

The significant association of *S. mansoni* infection with low haemoglobin level and anemia is supported by the reports from other investigations (Leenstra *et al.*, 2006; Stephenson and Holand, 1987; Konijn, 1994). According to Leenstra *et al* (2006), anemia is associated with chronic schistosomiasis, even in low intensity. Translocations of eggs across the intestinal and bladder walls and some immunological factors have been suggested to contribute to the blood loss and anemia in schistosomiasis (Stephenson and Holand, 1987; Konijn, 1994; Friedman *et al.*, 2005).

In prevalence of malaria/intestinal helminth co-infection, *P. falciparum*/*A. lumbricoides* and *P. falciparum*/hookworm take the largest share. This finding supports the observations of Mwangi *et al* (2006), which hold that geographical congruence of *P. falciparum* and helminth infections to be greater for hookworm. The results of recent analysis based on geographical information systems indicate that, in Africa, hookworm is the most geographically wide-spread of the three main types of intestinal helminths while *A. lumbricoides* and *T. trichiura* are restricted to Equatorial regions and human schistosomiasis has focal distribution throughout the tropics (Brooker *et al.*, 2006; Mwangi *et al.*, 2006).

The observed association of malaria/intestinal helminth co-infection with lower haemoglobin levels as compared to infections with only malaria and intestinal helminths alone is in agreement with the finding of Nacher *et al* (2001b), who concluded that during malaria, preexisting helminth infections are associated with decreased haemoglobin

concentration and reticulocytosis, which may aggravate malarial anemia. Studies in Thailand, has revealed that concurrent helminth infections among malaria patients were associated with increased malaria parasite gametocyte carriage, which was again negatively correlated with haemoglobin concentration (Nacher *et al.*, 2001d). The prevalence of anemia in malaria/hookworm co-infection in the present study, 60%, was in agreement with the finding of Aragie (2006), who reported a prevalence of 59%. However, the prevalence of anemia in malaria/*A. lumbricoides* co-infection, 38.1%, was lower than the finding of Aragie (2006), who reported a prevalence of 48.4%. This difference is most probably due to the difference in the background prevalence of anemia in the two study areas.

One of the consequences of co-infection with malaria and hookworm is the increased risk of anemia (Brooker *et al.*, 2006). The high prevalence of anemia in malaria and hookworm co-infection in the present study suggests the synergistic effect of these two parasites on aggravating anemia. Malaria contributes to reduce haemoglobin concentrations through a number of mechanisms, mainly by increasing rates of destruction and removal of parasitized and non-parasitized red cells and decreasing the rate of erythrocyte production in the bone marrow (Chang and Stevenson, 2004). Therefore, some of the mechanisms that cause anemia during malaria are associated more with the acute clinical states (e.g. hemolysis or cytokine disturbances), whereas chronic or repeated infections are more likely to involve dyserythropoiesis (Menendez *et al.*, 2000). By contrast, hookworm causes anemia through the process of intestinal blood loss (Hotez *et al.*, 2004) and the degree of pathology is actually related to the intensity of worm infection (Stoltzfus *et al.*, 1997). Given the distinct mechanisms by which malaria parasites and hookworm reduce haemoglobin concentrations, it is probable that malaria and hookworm would be additive in their ability to cause anemia (Brooker *et al.*, 2006).

Malaria and *A. lumbricoides* co-infection has also been observed to be associated with low haemoglobin level and anemia as compared to single infections and the control. Moreover, though not significant, it has been identified as one of those risk factors for anemia. This

finding is in agreement with previous observation by Nacher *et al* (2001b), whereby *A. lumbricoides* co-infection has been found to protect from cerebral malaria (Nacher *et al.*, 2000) although it would aggravate some clinical courses of malaria such as anemia.

Identification of malaria, malaria/hookworm co-infection and malaria/multiple intestinal helminth co-infections as risk factors for anemia in the present study, on the whole, is in agreement with several earlier findings (Chang and Stevenson, 2004; Brooker *et al.*, 2006; Mwangi *et al.*, 2006; Nacher *et al.*, 2001b; Aragie, 2006). Patients with malaria and co-infected with intestinal helminths have been shown to have increased risk of anemia (Nacher *et al.*, 2001b; Basavaraju and Schantz, 2006).

## 6. CONCLUSIONS AND RECOMMENDATIONS

### Conclusions:

- Infections with malaria parasites and different species of intestinal helminth were significantly associated with low haemoglobin level and high prevalence of anemia.
- The low level of malnutrition together with consumption of teff as a staple food in the study area may have contributed to the lower prevalence of anemia and severe anemia.
- Simultaneous infections with malaria parasites and multiple intestinal helminths in a single host may have exasperating effect on the severity of anemia.
- Co-infection with malaria parasites and hookworm was identified as the most prominent risk factor for anemia.

### Recommendations:

- Because co-infection is associated with higher prevalence of anemia, integrated control of malaria parasites and intestinal helminths is very crucial to reduce the prevalence of anemia to the level below what is of public health concern.
  - Better sanitation, proper disposal of wastes, avoiding unsafe water-contact behaviour, using latrines and control of snails are helpful measures.
  - Mass deworming programs, which can be implemented through the existing school system, must be considered.
- Further study must be conducted to obtain better information on the contribution of different helminth species to anemia during co-infection with malaria. These studies must:
  - Include children under the age of 5years.
  - Determine the hookworm species involved.

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## **Declaration**

I, the undersigned, hereby declare that this M. Sc. thesis is my original work. It has not been presented for a degree in any other university and that all sources of materials used for the thesis have been duly acknowledged.

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