ASSESSMENT OF PUBLIC HEALTH IMPLICATION OF MALARIA-HOOKWORM CO-INFECTION IN ASENDABO, SOUTH WEST ETHIOPIA

Fekadu Demissie

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By

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Prof. Beyene Petros (Advisor)

Dr. Amha Kebede (Advisor)

Aug 3, 2007

DEDICATION

To my family and friends (F) & (Lala) and the troubles I have faced all through the study….thank you….to the influences you made on me in shaping my present personality….

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My honored thanks goes to my respected advisors professor Beyene Petros and Dr Amha Kebede for their fine mind in sharpening the thesis from the beginning to the end.

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ABBREVIATIONS AND ACRONYM

AOR	Adjusted Odds Ratio
a s l	above sea level
CDC	Center for Disease Control and Prevention
CI	Confidence Interval
CM	Cerebral Malaria
COR	Crude Odds Ratio
CSA	Central Statistics Agency
DPD	Division of Parasitic Diseases
epg	egg per gram of stool
GIS	Global Information System
Hb	Hemoglobin
HRP-2	Histdin rich Protein-2
ICT	Immuno-Chromatographic Test
MOH	Ministry of Health
NPV	Negative Predictive Values
PCR	Polymerase Chain Reaction
PPV	Positive Predictive Values
PfHRP-2	Plasmodium falciparum Histdin Rich Protein-2
pLDH	Plasmodium Lactate Dehydrognase
RBM	Roll Back Malaria
RDT	Rapid malaria Diagnostic Test
SLS	Sodium Lauryl Sulfate
SNNPR	South Nation and Nationalities People Region
STH	Soil Transmitted Helminth
UNICIF	United Nation Children's Fund
WHO	World Health Organization

ABSTRACT

Malaria and geo-helminth infections are causes of severe illness and poor economic growth. The present study aimed at investigating the public health importance of Malaria-hookworm co-infection with emphasis on anemia the leading co-morbid disease condition *in Asendabo, south west Ethiopia during the months of October and November 2006. A total of 370 suspected malaria cases and additional 100 school children participated in the study. Modified Kato-Katz and formal-ether concentration techniques were used for stool examination. Harada Mori hookworm culture was established for identification of hookworm species. Thick and thin blood films and Paramax-3™ rapid antigen capture assay were used for detection of malaria parasites. A total of 61.6% individuals were positive for at least one intestinal helminth and/or protozoan infection. Hookworm was the most prevalent (38%) followed by Ascaris (19.2%) and Trichuris trichiura (10.3%). The majority (92.0%) of hookworm infections were N. americanus and the rest (8.0%) were A. duodenale. Furthermore, 32.4% of the study participants were positive for either of the two Plasmodium species of which 64.3% were P. falciparum and the rest 35.7% were P. vivax. Compared with Geimsa stained microscopy, Paramax-3™ was sensitive (90.1%) and specific (93.3%) for identification of total malaria with positive and negative predictive values of 86.4% and 95.0%, respectively. Hemoglobin measurement detected 27.6% anemic cases and both hookworm and malaria infections were significantly associated with anemia (P<0.05). 20.8% of study participants were co-infected with malaria and any helminth. Hookworm and malaria positive individuals had low mean hemoglobin concentration than their respective negative counter parts and the difference was significant (P<0.05). Furthermore, mean hemoglobin concentration was significantly lowered in malaria-hookworm co-infected individuals than in individuals infected with either hookworm or malaria infection alone (F=69.39, P=0.000). The study has revealed that malaria co-infections with hookworm as well as other intestinal helminths worsens hemoglobin loss and that individuals co-infected must receive prompt health care to control anemia morbidity / mortality. Furthermore, de-worming of the population must be considered concurrently with malaria control.*

Keywords:**-Hookworm, Malaria, Co-infection, Paramax-3 ™, Anemia, Helminth, Asendabo, Ethiopia.**

1. INTRODUCTION

Throughout evolutionary history humans have been infected with parasites. Today, it is estimated that over a third of the world's population, mainly those individuals living in the tropics and sub-tropics, are infected with parasitic helminths or one or more species of Plasmodium (Mwangi *et al.,* 2006). The ubiquity of these parasites results in high rate of co-infection (Peney & Andrews, 1998). An interaction between helminths and malaria could work in either direction. Helminth infection may alter susceptibility to clinical malaria or malaria may influence the clinical consequences of helminth infection. And there is a growing interest in investigating the consequences of co-infection.

The large scale geographical distribution of malaria and helminths are determined largely by climate, which determines the survival of the mosquito vectors of the malaria parasite and of the free living stages of the helminths (Mwangi *et al.,* 2006).

The results of recent analysis based on geographical congruence of helminth infection indicate that, in Africa, hookworm is the most geographically wide-spread of the three main types of soil-transmitted helminth (STH). Consequently, the co-infection of *P. falciparum* and helminth infection is the highest for hookworm. Further more, hookworm appears to have a wider thermal tolerance than *A. lumbricoides* and *T. trichuria* making the worm to successfully adapt to tropical temperature and widen the chance of co-infection with malaria (Hotez *et al.,* 2004; Peney & Andrews, 1998).

An important consequence of co-infection (hookworm-malaria, in particular) is anemia, a severe public health problem in the tropics. Malaria is a significant contributor to anemia among young children and pregnant mothers, operating through a number of mechanisms, including haemolysis and phagocytosis, whereas hookworm infection is an acknowledged significant cause of anemia as a result of intestinal blood loss (Hotez *et al.,* 2004). Since the mechanisms by which malaria and hookworm infections cause anemia differ, it is possible that their impact on hemoglobin level is additive.

1.1. Malaria

Malaria is an infectious disease caused by a group of protozoan parasite belonging to the genus plasmodium (CDC, 2006). One hundred and seven countries and territories are reported as areas under malaria transmission (Figure 1), making 3.2 billion people at risk. Present estimates are around 350–500 million clinical disease episodes annually. In addition, malaria is the leading cause of under-five mortality (20%) and constitutes 10% of the continent over all disease burdens. It also accounts for 30-50% of the in-patient admission and up to 50% of out patient visit in areas of high malaria transmission (RBM/WHO/UNICEF, 2005).

Around 60% of the cases of clinical malaria and over 80% of the deaths occur in Africa south of the Sahara. More than one million Africans die from malaria each year (most are children under 5 years of age). And one child dies of malaria somewhere in Africa every 20 seconds and there is one malarial death every 12 seconds somewhere in the world (CDC, 2004). In addition to acute disease episodes and deaths in Africa, malaria also contributes significantly to anemia in children and pregnant women with adverse birth outcomes such as spontaneous abortion, stillbirth, premature delivery and low birth weight, and overall child mortality (World Malaria Report, 2005).

In Africa today, malaria is understood to be both a disease and a cause of poverty. Annual economic growth in countries with high malaria transmission has historically been lower than in countries without malaria. Economists believe that malaria is responsible for a growth penalty of up to 1.3% per year in some African countries (Malaria in Africa. http://www.rbm.who.int/).

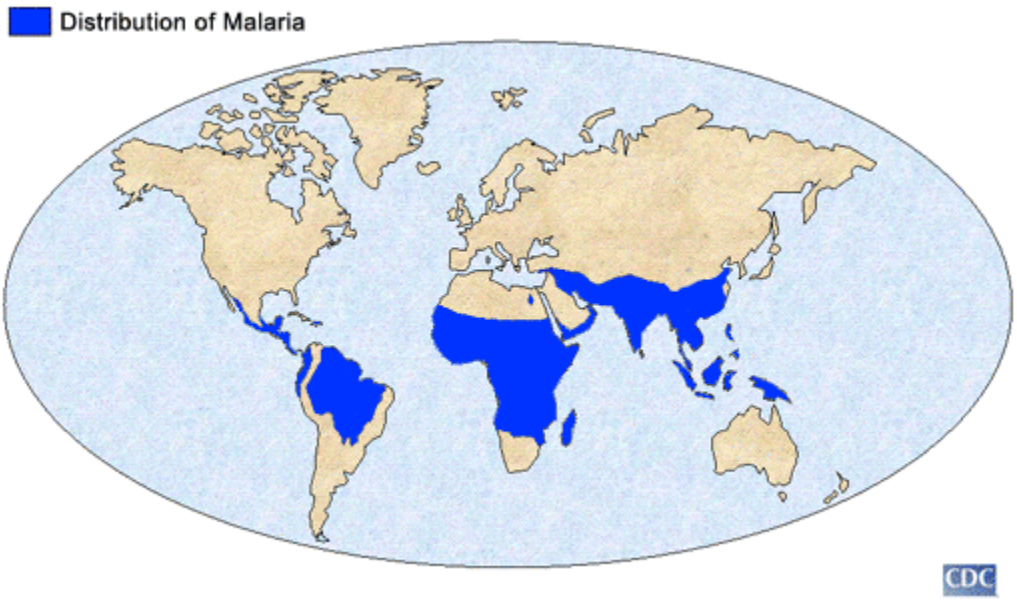


Figure 1: Geographic distribution of malaria globally (2002) http://www.cdc.gov/malaria/images/graphs/geodistribution.gif/

Malaria is a major public health problem in Ethiopia. It is found in about 75% of the total area of the country, and 40 million (>65%) of the total population is at risk of infection (Tulu, 1993; MOH[a], 2004). Every year more than 4 million clinical cases are reported from health facilities and communities. This accounts for 10-40% of all outpatient consultation and 13-26% of all inpatients admission in various health facilities with corresponding proportional mortality rate of 13-35% (MOH[b], 2004).

Malaria transmission in Ethiopia is seasonal and depends on altitude and rainfall. Transmission usually occurs at altitudes <2000 meter above sea level (m a s l). The two main seasons for transmission of malaria in Ethiopia are September–December, after the heavy summer rains, and March–May, after the light rains. *Plasmodium falciparum* and *P. vivax* are the predominant human malaria species and account for about 60% and 40% of cases, respectively (Tulu, 1993).

Malaria epidemics are frequent and widespread in Ethiopia. Most of the areas affected by epidemics are highland or highland fringe areas (mainly areas 1000–2000 m a s l), in

which the population lacks immunity to malaria. Occasionally, transmission of malaria occurs in areas previously free of malaria, including areas >2000 m a s l, in which the microclimate and weather conditions are favorable for malaria (Tulu, 1993).

Similar pattern of seasonality is observed in Asendabo town south west of Ethiopia, Oromiya regional state (the study site). Two years monthly average malaria prevalence indicates September- December are the highest period of time when malaria transmission reach the highest hit (Figure-2) followed by Apr-May.

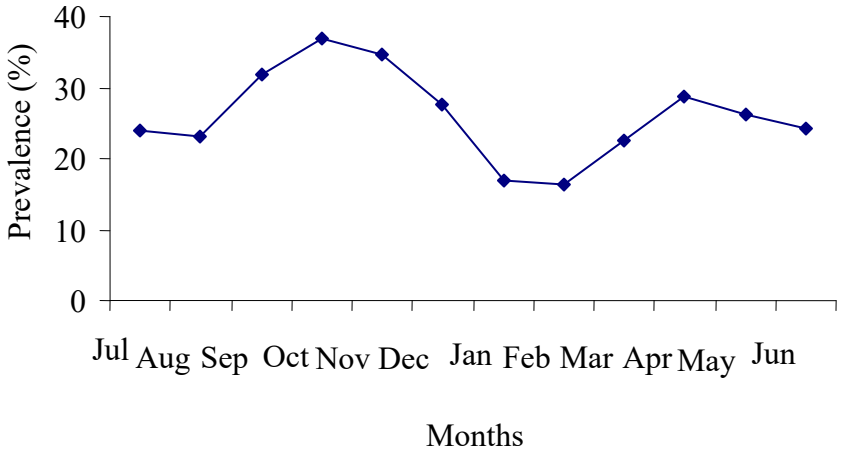


Figure 2: Two years (2005-2006) monthly average malaria prevalence in Jimma zone, Asendabo health center (Source: health center documentation, 2006).

Four species of the genus plasmodium cause nearly all malaria infection in human (CDC, 2006). These are *Plasmodium falciparum*, *P. vivax, P. malarae* and *P. ovalae.*

Human infection begins when a female anopheline mosquito inoculates sporozoites from its salivary glands during a blood meal (Figure 3). These microscopic motile forms of the malaria parasite are carried via the blood stream to the liver, where they invade hepatic parenchymal cells and begin a period of asexual reproduction. Inside the hepatocyte, each sporozoite develops into tens of thousands of merozoites which can invade many RBCs upon release from the liver (Louis, *et al*., 2002). Depending on the species the development in the liver cell requires upto 16 days. These merozoites invade RBCs and undergo a second multiplication, which lasts 48-72 hours and produce 16 – 20 merozoites

(Louis *et al*., 2002; Katharine & Donald, 2004). After a series of asexual cycle in *P. falciparum* or immediately after release from liver in *P. vivax*, *P. ovalae*, and *P. malarae* some of the parasites develop into morphologically distinct, long lived sexual forms (gametes) that can be ingested with the blood meal of a biting female anopheline mosquito (Niloofar *et al*., 2004).

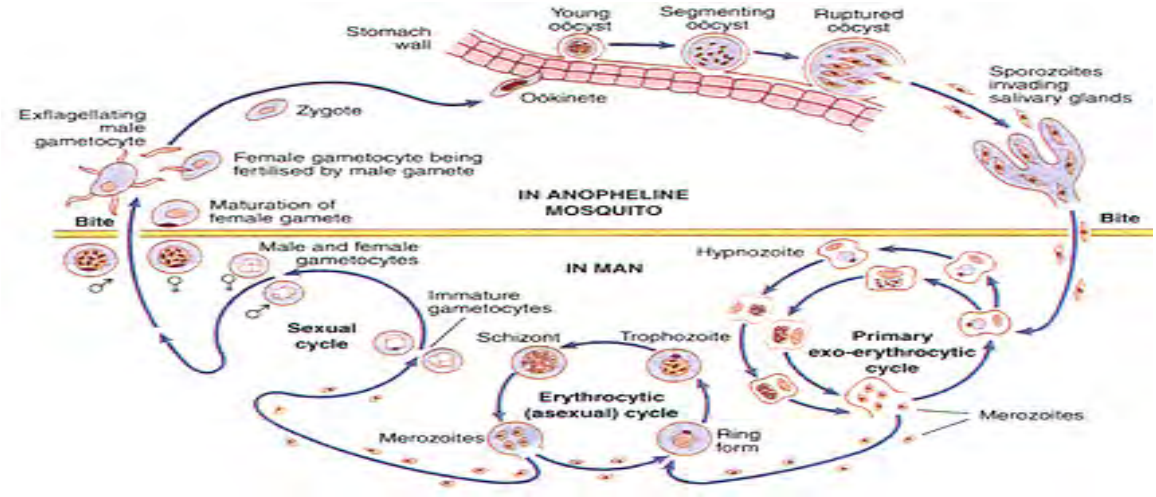


Figure 3: Malaria life cycle (http://www.malariasite.com/.)

Malaria symptoms may appear on average 12 days after inoculation of sporozoites into the blood stream. The first symptoms and signs of malaria are associated with the rupture of erythrocytes. Released parasite toxin presumably triggers a host immune response to produce macrophages to secrete TNF-α and Il-1 (Lucia & Salvitor, 2002)

Fever is the cardinal feature of malaria. Other common symptoms include chills, headache, myalgias, nausea, and vomiting, diarrhea, abdominal pain, and cough is occasionally seen (Louise *et al*., 1994). As the disease progress example, some patients may develop the classical malaria paroxysm with short period of illness alternating with symptom free periods. Malaria paroxysm comprises three successive stages. The first is a 15 to 60 minute cold stage characterized by shivering and a feeling of cold. Next comes the 2 to 6 hours hot stage, in which there is fever, some times reaching 41^{o} C, flushed , dry skin, and often headache, nausea, and vomiting. Finally, there is the 2 to 4 hour sweating stage during which the fever drops rapidly and the patient sweats. In all types of

malaria the periodic febrile response is caused by rupture of matured schizonts. In *P.vivax* and *P.ovale*, brood of schizonts matures every 48 hours; the periodicity of fever is tertian and hence tertian malaria, whereas in *P. malariae* disease, fever occurs every 72 hours (quartan malaria). The fever in *P. falciparum* malaria may occur every 48 hour, but is usually irregular, showing no distinct periodicity. These classic fever patterns are usually not seen early in the course of malaria, and therefore the absence of periodic, synchronized fevers does not rule out a diagnosis of malaria (James & Stephen, 2002).

Severe malaria is a complicated syndrome almost exclusively caused by *P. falciparum* (Qijun *et al*., 2000). Approximately 5-10 million *P. falciparum* infected individuals per year develop complication during acute infection. The over all mortality from severe malaria varies from 15 to 30% with the highest mortality resulting from cerebral malaria (CM), metabolic acidosis, and pulmonary edema (May & Nicholas, 1999). Other complications include severe malaria anemia, placental malaria, hypoglycemia, renal failure, pulmonary edema and high fever (Menendez *et al*., 2000; Warrell & Molyneux, 1990).

In malaria patients, prompt and accurate diagnosis is a key to effective disease management. The two diagnostic approaches currently used most often, are clinical diagnosis and microscopic diagnosis. However, both do not allow a satisfactory diagnosis of malaria. Clinical diagnosis is the most widely used approach; however, the symptoms of malaria are rather nonspecific and overlap those of other febrile illnesses (WHO, 1999). A diagnosis of malaria based on clinical grounds alone is, therefore, unreliable and, when possible, should be confirmed by laboratory tests. Microscopic examination of thick blood film is currently the standard method for malaria diagnosis (Nicholas *et al.,* 1999). This method is relatively simple and has low direct costs, but it is time consuming, labor intensive, affected by quality of staining, the expertise, and alertness of the microscopist and its reliability is questionable particularly at low levels of parasitemia and in the interpretation of mixed infection (Nicholas *et al.,* 1999; Jamshaid *et al.,* 2002; Craig *et al.,* 2002).

Recently, rapid antigen detection methods have been developed for areas in which reliable microscopy may not be available. Studies have indicated that rapid antigen captured methods are very sensitive to detect the antigen even with low parasitemia. In addition it requires almost no experience and can be carried out under field situation with out the need for slides and microscopes, centralized laboratories and trained manpower (Nicholas *et al.,* 1999).

These tests are based on the detection of antigen(s) released from parasitized red blood cells (Moody, 2002). In the case of *Plasmodium falciparum*, these new methods are based on detection of *P. falciparum* Histidine-Rich Protein 2 (HRP-2) or *Plasmodium*-specific lactate dehydrogenase (pLDH). Species-specific pLDH isoforms have been used to develop a test for *Plasmodium vivax* (Moody, 2002; Jamshaid *et al.,* 2002).

There are wide ranges of commercial rapid assay types in the market today. The sensitivity and specificity of each of these tests have been assessed in a range of clinical situations (Taddese & Mekete, 2001; Craig *et al.,* 2002; Jamshaid *et al.,* 2002; Moody, 2002). In Ethiopia, there were limited numbers of studies done to assess the applicability of these kits in the county's set up. Taddese and Mekete (2001) have shown the ICT prototype can be interchangeably used with microscopy with sensitivity and specificity 85.7% and 98.3%, respectively. And it is generally agreed that there should be an evaluation phase before any rapid malaria antigen capture assay is put in use in a country's health system. Therefore, as one component of this study we have tried to evaluate performance characteristics of an Indian company product Paramax-3^{TM} (Zephyr Biomedicals) along other main objectives of this thesis work.

Basically Paramax-3^{TM} incorporates similar antigen detection approaches with other rapid antigen capture assays. It utilizes the principle of immunochromatography in which test sample flows through the membrane assembly of the device after addition of the clearing buffer, the colored colloidal gold conjugates of anti-HRP-2 antibody, anti *P.vivax* specific pLDH antibody and anti pan specific pLDH antibody complexes the HRP-2 corresponding pLDH in the lysed sample. This complex moves further on the

membrane to the test region where it is immobilized by the monoclonal anti *P. falciparum* HRP-2 antibody and / or monoclonal anti *P.vivax* specific pLDH antibody and / or monoclonal pan specific pLDH antibody coated on the membrane leading to formation of a pink / purple colored band in the respective regions which confirms a positive test result. Absence of a colored band in the test region indicates a negative test result for the corresponding antigen. The un-reacted conjugate along with the rabbit anti-sera colloidal gold conjugate and unbound complex if any, move further on the membrane and are subsequently immobilised by anti-rabbit antibodies coated on the membrane at the control region, forming a pink / purple band. This control band serves to validate the test performance (Zephyr Biomedical, 2007).

1.2. Hookworm infection

Hookworm infection in humans is caused by helminth nematode parasites *Necator americanus* and *Ancylostoma duodenale* and is transmitted through contact with contaminated soil. It is one of the most common chronic infections with an estimated 1.3 billion cases globally and directly accountable for 65 000 deaths annually (Silva *et al.,* 2003). Disability adjusted life years as a quantitative measure of a disease burden reveals that hookworm infection outranks African trypanosomiasis, Dengue, Chagas' disease, Schistosomiasis, and Leprosy (Hotez *et al.,* 2003).

Hookworms are dioecious and have a direct life history (Figure-4) (Crompton, 2000). After the adult worms have mated in the small intestine, mature female hookworm produces between 5000 and 25,000 eggs each day. In warm, moist condition out side the body, further development occurs with the formation of a first stage (L1) rhabditiform larva. After the second moult the larvae develop into infective (filariform) larvae in 7 days. Filariform larvae move freely in sandy soil and where temperature and moisture are optimal for the survival (Nicholas *et al.,* 1999). Larvae penetrate skin of host (e.g. bare feet), circulate to lungs where they penetrate alveoli, move up bronchi and are swallowed. Then, attach by mouth to small intestinal mucosa and suck blood. Pre-patent period (time from skin penetration to egg production) is 4-5 weeks. Adults can live 5-15 years (Crompton, 2000; Hotez *et al.,* 2004; Pawlowski, 1991)

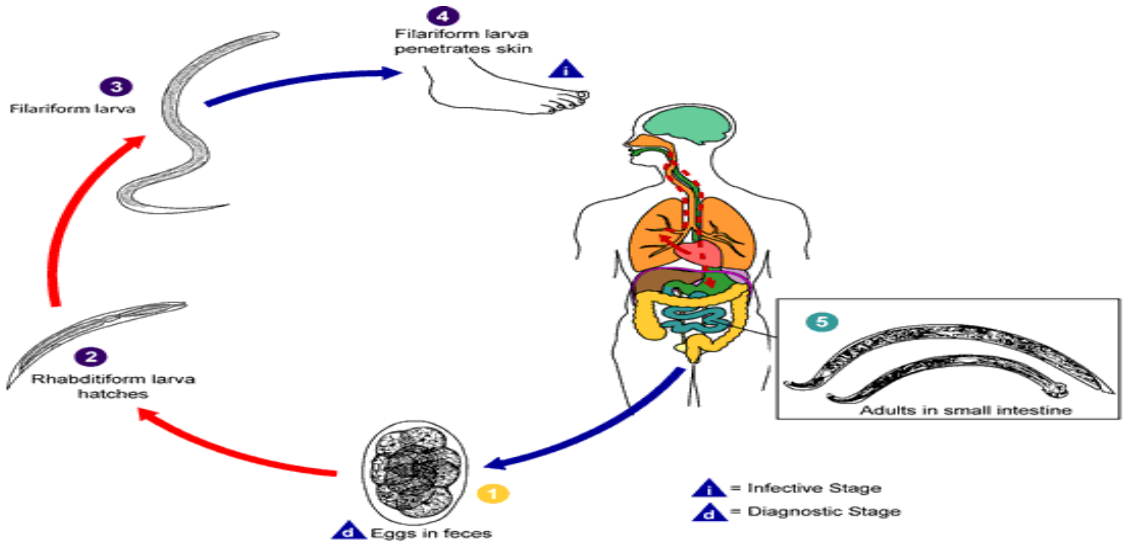


Figure 4: Life cycle of hookworm (CDC, 2006)

The two species (*Ancylostoma duodenale* and *Necator americanus*) of hookworm that infect human exhibit differences in their pathogenicity, mode of transmission, geographic distribution, and biology and these differences may influence the morbidity of hookworm disease (Crompton, 2000). *A. duodenale* is geographically more restricted in distribution and is found at higher elevation, and in more extreme climates, whereas *N. americanus* is the most common hookworm worldwide. It is responsible for 90% of human hookworm infection that occur in tropical and subtropical regions of the world (DPD, 2006). In some localities, the distribution of the species overlaps. Furthermore, it has been suggested that *A. duodenale* can be vertically transmitted from mother to infant (Gerhard, 1991; Navitsky *et al.,* 1998), however, the larvae have not yet been demonstrated in human milk or colostrums. *A. duodenale* undergoes developmental arrest in human host which lasts for about eight months. This survival adaptation enables *A. duodenale,* the species with shorter adult life span, to survive the dry season in the host avoiding release of eggs into hostile environment (Gerhard, 1991).

Earlier studies (Jemaneh & Tedla, 1984; Armstrong & Chane, 1975) conducted in various parts of Ethiopia showed the existence of both species with *N. americanus* accounting for

larger share. Jemaneh and Tedla (1984) reported 92.5% and 7.5% prevalence rate for *N .americanus* and *A. duodenale*, respectively in Gojam whereas the same group demonstrated *N. americanus* as a sole agent of hookworm infection in Gonder. Armstrong and Chane (1975) on the other hand, showed a species distribution of 78% for *N .americanus* and 22% for *A. duodenale* at three different localities in the country.

In an attempt to highlight regional distribution of hookworm species in the country, Tedla and Jemaneh (1985) showed out of 95 communities surveyed in 10 administrative regions 82 of them had hookworm infection and in 20 of those communities the two species were found sympatrically. In 6 of the 20 communities, people with mixed infections were recorded. But, when mixed infections were found, the occurrence was rare, and only one person in each of those 6 communities harbored both species (Tedla & Jemaneh, 1985).

In contrast to these major anthropophilic species, three species of zoonotic hookworm are minor causes of disease in humans. *A. ceylanicum* infects dogs and cats and can also infect humans occasionally but it is not considered as an important pathogen. The dog hookworm *A. caninum*, causes human eosinophilic enteritis in northeastern Australia and *A. braziliense* causes cutaneous larva migrans (Prociv & Croese, 1996).

Climate and soil types determine the distribution of the worm. Areas with sandy soil with clay content of less than 15%, warm temperatures and relatively high rainfall favor the highest prevalence (Mabaso *et al.,* 2003).

The overall prevalence and intensity of hookworm infection are higher in males than in females, in part because males have greater exposure to infection. However, women and young children have the lowest iron stores and are therefore most vulnerable to chronic blood loss as the result of hookworm infection (Stoltzfus *et al.,* 1997).

For many common helminthic infections, including ascariasis, trichiuriasis, and schistosomiasis, the intensity of infection usually peaks during childhood and adolescence. In contrast, there appears to be considerable variation in the age–intensity profile of hookworm infection. Although the hookworm burden may be heavy in

children, especially those in sub-Saharan Africa, the most commonly recognized pattern is a steady rise in the intensity of infection during childhood, with either a peak or a plateau in adulthood (Figure 5). The observation that the intensity of hookworm infection increases with age has led to the suggestion that hookworms can either evade or suppress host immune responses (Hotez *et al.,* 2004).

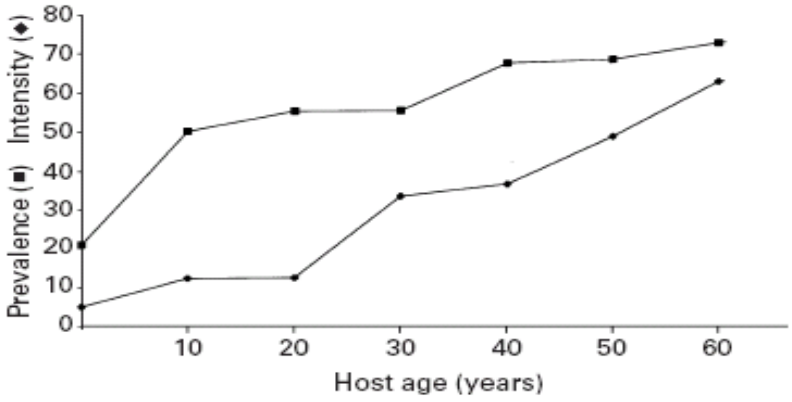


Figure 5: Age prevalence and intensity curve for hookworm infections (Chandiwana, 1990).

The major hookworm-related injury in humans occurs when the adult parasites cause intestinal blood loss (Hotez *et al.,* 2004).The term "Hookworm disease" refers primarily to the iron-deficiency anemia that result from moderate or heavy infection. Blood loss occurs when the worms use their cutting apparatus to attach themselves to the intestinal mucosa and submucosa and contract their muscular esophagi to create negative pressure, which sucks a plug of tissue into their buccal capsules. Capillaries and arterioles are ruptured not only mechanically but also chemically, through the action of hydrolytic enzymes. To ensure blood flow the adult hookworms release anti-clotting agents (Stanssens *et al.,* 1996).

Clinical manifestations of hookworm disease are the consequences of chronic intestinal blood loss. Iron-deficiency anemia occurs and hypoalbuminemia develops when blood loss exceeds the intake and reserves of host iron and protein (Crompton, 2000; Stoltzfus *et al.,* 1997) depending on the status of host iron, a hookworm burden (i.e., the intensity

of infection, or number of worms per person) and species of hookworm involved. Because infection with *A. duodenale* causes greater blood loss (Table-1) than does infection with *N. americanus,* (Necator 0.03 ml/day, *Ancylostoma* 0.15 ml/day), the degree of iron-deficiency anemia induced by hookworms depends on the species (Crompton, 2000).

Table 1: Hookworm species and their association with blood loss (Crompton, 2000)

	N. americanus	***A. duodenale***
Intestinal blood loss in ml per day/ worm	0.03	0.15
Number of worms causing a blood loss of 1ml/day	25 (14-50)	5 (4-7)
Blood loss (ml/day) per 1000 epg	1.3 (0.82-2.24)	2.2 (1.54-2.86)
Iron loss(mg/day) per 1000 epg stool	0.45	0.76
Worm burden responsible for 1000 epg	32	11

Hookworm infection is considered a major health threat to adolescent girls and women of reproductive age, with adverse effects on the outcome of pregnancy (Bundy *et al.,* 1995).The World Health Organization estimates more than half of the pregnant women in developing countries have problems related to iron-deficiency anemia (WHO, 2002). Severe iron-deficiency anemia during pregnancy has been linked to increased maternal mortality, impaired lactation, and pre-maturity and low birth weight. An estimated 44 million pregnant women are infected with hookworm worldwide, with 7.5 million in sub-Saharan Africa alone. Estimates in Kenya and Nepal suggest that hookworm infection causes 30 percent and 41 percent, respectively, of moderate or severe cases of anemia among pregnant women (Stoltzfus *et al.,* 1997).

In children, chronic hookworm disease retards physical growth (Stephenson *et al.,* 1989) which is sometimes most apparent at puberty. More recent evidence suggests that hookworm infection also has subtle yet profound adverse effects on memory, reasoning ability, and reading comprehension in childhood (Stephenson *et al.,* 1989). Most of these effects are probably attributable to the presence of iron-deficiency anemia. Infants and preschool children are particularly vulnerable to the developmental and behavioral deficits caused by iron-deficiency anemia and two analyses indicate that hookworm infection remains an important contributor to anemia in this age group (Brooker & Michael, 1999)

Egg counts, expressed as epg (eggs per gram of faeces), give an indirect measure of the intensity of infection. The higher the egg count, the more female worms are assumed to be present. Knowing intensity or load is important for relating healthy variables such as blood hemoglobin concentration, for assessing anti-helminthic drug efficacy and for monitoring the progress of control programs (Montresor *et al.,* 1998).

Thresholds proposed by WHO expert committee for the classes of intensity for helminths in stools is grouped as light intensity infection, moderate intensity infection, and heavy intensity infection. For hookworm the epg is 1-1,999 for the light intensity infection and 2,000-3,999 for moderate intensity infection and greater than 4000 for heavy intensity infection (Montresor *et al.,* 1998)

Identification of the species of hookworm is achieved either by checking the morphology of the buccal apparatus of adult worms obtained by expulsion chemotherapy or by studying the morphology of larval stage cultured from eggs. In all cases correct identification is important to ensure control measures are applied most effectively (Pawlowski, 1991).

Three culture techniques are recommended for diagnosis of hookworm infection in routine laboratories, namely Formalin-ethyl acetate concentration, Harada-Mori and agar plate culture (Pawlowski, 1991; Sirima & Vichit, 1999). Studies showed Harada-Mori cultivation is the best method to detect hookworm infection. Not only the high detection

rate but also other advantages such as low cost, reusable equipment and the safe discarding of used filter paper (Sirima & Vichit, 1999).

In Harada-Mori culture technique, fresh is feces smeared on a strip of filter paper and the paper is placed in a tube containing small amount of water. Larvae move (against capillary movement of water) down ward to the bottom of the tube within seven to ten days and survive for several days. Then larvae are killed while it is in the tube and a drop of the sediment taken for observation under microscope. Identification is based on key morphological differences in esophagus and tail regions as explained by Pawlowski (1991).

1.3. Malaria and intestinal helminth co-infection and disease outcomes

Overlapping distributions of intestinal helminth and malaria result in a high rate of co-infections (Adrienne *et al.,* 2005). In laboratory studies there is evidence suggestive of both synergism and antagonism in nematode and protozoa co- infections (Mathieu, 2002).

Infection with helminths has a profound effect on immune system resulting in polarization towards Th_2 , characterized by high level of cytokines such as interleukin-4 (IL-4), Il-5, Il-13 and high levels of immunoglobulin E (IgE) (Adrienne *et al.,* 2005; Hartgers & Yazdanbakhsh, 2006). The profound effect of helminth infection on the immune system might be expected to influence the immune response against malaria parasite and affect the course of an infection (Hartgers & Yazdanbakhsh, 2006)

Animal model studies have been intensively used to analyze the result of co-infections with helminths on the course of protozoan particularly malaria infection. Rats co-infected with *Strongyloides ratti* and *Trypansoma brucei* have increased survival over those with *T. brucei* infection alone (Onah *et al.,* 2004). Similarly, protection from cerebral malaria is conferred upon mice with pre-existing *Brugia pahangi* infection (Kirsten *et al.,* 2005). However, mice with ova-producing *S. mansoni* infections have increased parasitemia with

P. chabaudi infection (Helmby *et al.,* 1998) and delayed parasite clearance after chloroquine treatment of *P. bergehi* compared with those with *Plasmodium* species infection alone.

Limited studies of clinical interaction in human co-infection have also indicated contradicting reports. Pre-existing helminth infection is associated with protection from cerebral malaria in Thai adults and reduced malaria-related renal and liver abnormalities (Mathieu, 2002). Other recent studies have reported a protective effect of infection with *Schistosoma haematobium* on *P. falciparum* infection. These studies have shown *S. haematobium* had a protective effect on infection by decreasing *P. falciparum* densities as compared to helminth-free children (Briand *et al.,* 2005). Furthermore, early study performed in 1978 also described that anti-helminth treatment in a high-transmission area was followed by an increase in symptomatic malaria infection (Murray, 1978).

Conversely, a negative interaction has been noted with increased incidence of uncomplicated *P. falciparum* malaria in individuals with intestinal helminth infection. Study in northern Senegal showed that the incidence of malaria attacks to be higher in children positive for infection with *Schistosoma mansoni* (Hartgers & Yazdanbakhsh, 2006). In another study in Senegal risk of clinical malaria was reduced in helminth-free children compared to children positive for Ascaris, hookworm or Trichuris (Spiegel *et al.,* 2003; Hesran *et al*., 2004). From the stand points of the various studies conducted so far, the possible conclusion one can draw is that helminth infection seems to increase the susceptibility to malaria infection and light infection such as fever, whereas they might protect against some complications of severe malaria.

The largest burden of clinical disease resulting from infection with *P. falciparum* and helminths is carried by populations living in sub-Saharan Africa. Among the three STH the congruence of *P .falciparum* and hookworm is the greatest in Africa (Mwangi *et al.,* 2006).

Anemia has been revealed as perhaps the most important of the leading co-morbid disease conditions. In the case of hookworm and malaria, Brooker *et al.* (2000) have shown that in Kenya the two types of anemia—anemia from hookworm and the anemia from malaria—can build on each other to produce profound reductions in hemoglobin. Among the neglected tropical diseases, hookworm, accounts for up to 35% and 73% of the iron-deficiency anemia and severe iron-deficiency anemia in Africa, respectively (Stoltzufus *et al.,* 1997).

Hookworm causes iron-deficiency anemia through intestinal blood loss, whereas malaria causes anemia by several different mechanisms, including increased destruction of both parasitized and non-parasitized red blood cells and dyserythropoiesis resulting from host production of inflammatory cytokines, especially tumor necrosis factor and macrophage migration inhibitory factor (Mathieu, 2002; Hotez *et al.,* 2004).

Severe anemia resulting from helminth poly-parasitism and malaria produces several adverse health consequences among three particularly important African subpopulations: pregnant women, children, and individuals with HIV. In coastal Kenya, malaria was identified as the most important cause of anemia in primigravidae, whereas hookworm attained increased importance among multigravidae (Mathieu, 2002).

Therefore, clinical studies on impact of concomitant infection between malaria and hookworm on anemia and other related health indicators are research gaps suggested to be studied further. This work is an attempt made to assess public health implication of malaria and hookworm co-infection among study subjects in communities south west of Ethiopia.

2. OBJECTIVES OF THE STUDY

General objective

To determine the prevalence of Malaria-hookworm co-infection and its possible association with severe anemia

Specific objectives

1. To determine the effect of Malaria-hookworm co-infection on hemoglobin concentration in the study population
2. To identify the dominant species of hookworm in the study area
3. To determine prevalence of other geo-helminths and protozoan parasites in the study subjects
4. To determine sensitivity, specificity, positive and negative predictive values of the rapid test, Paramax-3 ^{TM} , in malaria diagnosis

3. METHODS AND MATERIALS

3.1. The study site

The study was conducted in Asendabo health center in Omo Nada Woreda, Jimma Zone, Oromiya regional state. The health Center is found in Asendabo town, one of the two towns in Omo Nada woreda. The boundaries of the woreda are Tiro- Afeta Woreda in the North, Sekoru woreda in the North East, SNNPR in the South East, Dedo town in the South West, Kersa Woreda in the North West (Figure-6). The town is situated at an altitude of 1725m a s l.

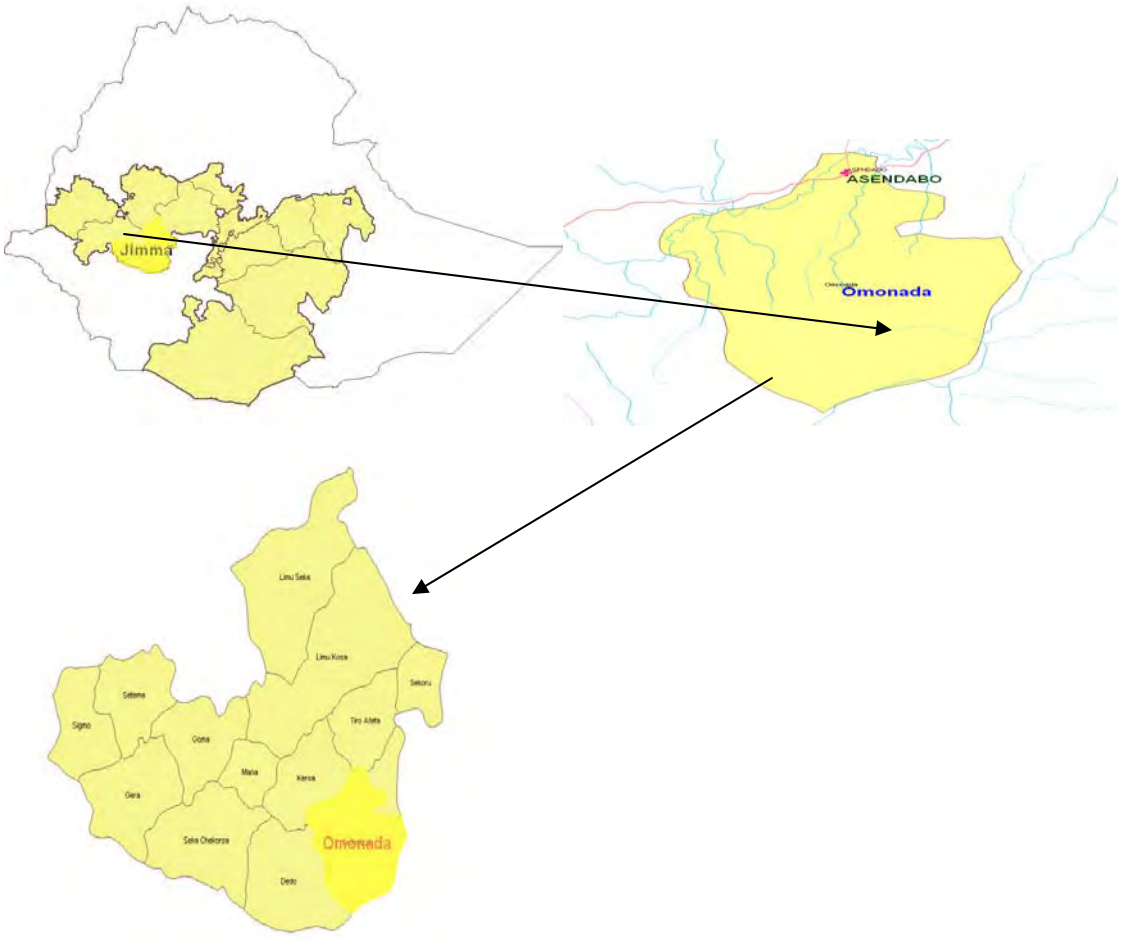


Figure 6: Geographical location (sketch map) of the study site in the country together with Woredas bordering it (Source act GIS)

Five years average annual rain fall was 1131.08 mm with bimodal rainy seasons. The highest malaria prevalence (36.9%) was recorded in October following high rain fall in the preceding the months (Figure-7). The monthly average maximum and minimum temperatures were 27.6 ^{o}C and 13 ^{o}C for five consecutive years (2000-2004) (Figure-8). Although relative humidity data for the town is not available, it is expected to share similar humidity situation with that of Jimma town (45 Km away) for which three times a day monthly average humidity for five consecutive years (2000-2004) was 71% (Source National Metrological Service Agency Addis Ababa Ethiopia and Health center's documentation).

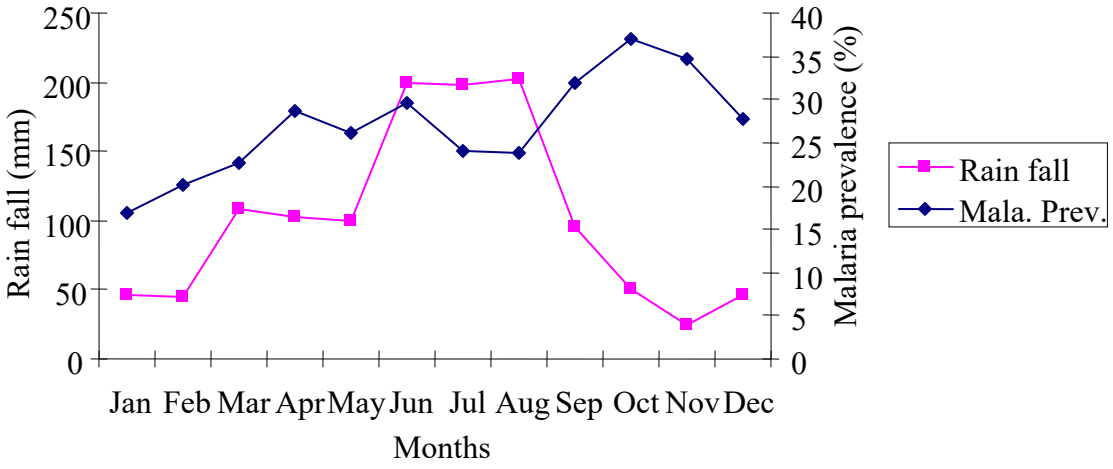


Figure 7: Mean monthly average rain fall in mm for Asendabo town in conjunction with mean monthly malaria prevalence, South West Ethiopia 2000-2004.

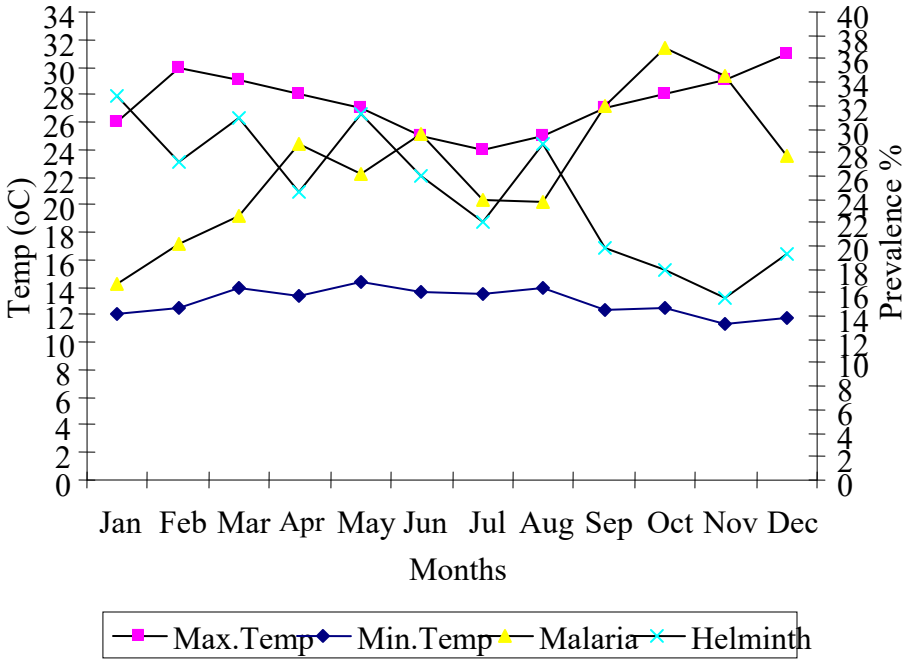


Figure 8: Five years (200-2004) monthly average maximum and minimum temperatures in conjunction with mean monthly malaria and helminth prevalence, Asendabo town, South West Ethiopia

The health center is one of the training health centers found in Jimma zone, established in 1975 E.C, with estimated catchment population of about 49,817, among which rural and urban comprising of 81% and 19% respectively for the year 1999 E. C (CSA, 2005). The Health center provides mostly preventive and curative services to the 9 kebeles including Asendabo Kebele out of the 41 kebeles found in the Woreda.

3.2. Study design

A cross-sectional study design was initiated during the study period (October to December 2006). All febrile cases (suspected malaria cases) with malaria laboratory request, excluding pregnant women and children below the age of five years, were included in the study. All consenting study subjects were requested to provide venous blood and fresh stool samples at the time of visit.

3.3. Sample size estimation

A total of 384 study subjects were expected to be included in the study considering 95% confidence interval , 50% expected proportion (there is no previous epidemiological studies on malaria-worm co-infection), and 5% margin of error. And another 100 school children were taken for hookworm species identification.

Total study subjects (n) = $\frac{z^2 p q}{d^2}$ = 384 (Daniel, 1998).

Where P = 50%, q= 50%, Z= 1.96 (95%), d= .05

3.4. Socio-demographic variables

Most indicative variables of socio-demographic status of the study subjects were collected using pre-tested structured questionnaire (See annex-I). Information on income found to be highly biased as individuals were not able to tell their income in clear terms and hence omitted from the study questionnaire.

3.5. Blood collection and processing

About 5ml venous blood sample was collected from each study subject following standard safety pre-cautions. Part of the sample was used for thick and thin blood film preparation and the other part taken to Jimma University specialized hospital for hemoglobin measurement (annex-II).

3.5.1. Thick and thin blood film preparation

Thick and thin blood films were made as indicated elsewhere (Cheesbrough, 1998). The slides were stained using Giemsa stain prior to fixation in case of thick film and after fixation with methanol in case of the thin film.

3.5.2. Counting parasite density in thick blood film

Parasite density was determined by counting number of parasites against 100 WBC. Then parasites per µl of blood were calculated as follows (Cheesbrough, 1998).

$$\text{Parasites/µl} = \frac{\text{WBC/ µl X parasite counted against 100 WBC}}{100}$$

Where WBC is total white cell count and assumed to be 8000 cell/µl for each individual.

3.5.3. Paramax-3^{TM} chromatographic malaria rapid test

Paramax-3^{TM} is a rapid self-performing, qualitative, two sites sandwich immunoassay utilizing whole blood for the detection of *P. falciparum* specific histidine rich protein-2 (Pf HRP-2), *P. vivax* specific pLDH and pan malaria specific pLDH. The test can be used for the specific detection of *P. falciparum* and *P. vivax* malaria, differentiation of other malarial species and for the follow up of antimalarial therapy. The procedure is summarized as follows: Gently a drop (about 5µl) of anticoagulated or fresh whole blood was allowed to touch the test area of the strip. Then five drops of cleaning buffer were added so that the blood diffuses slowly along the pad. At the end of 15 minutes the result is read as indicated in Figure 9.

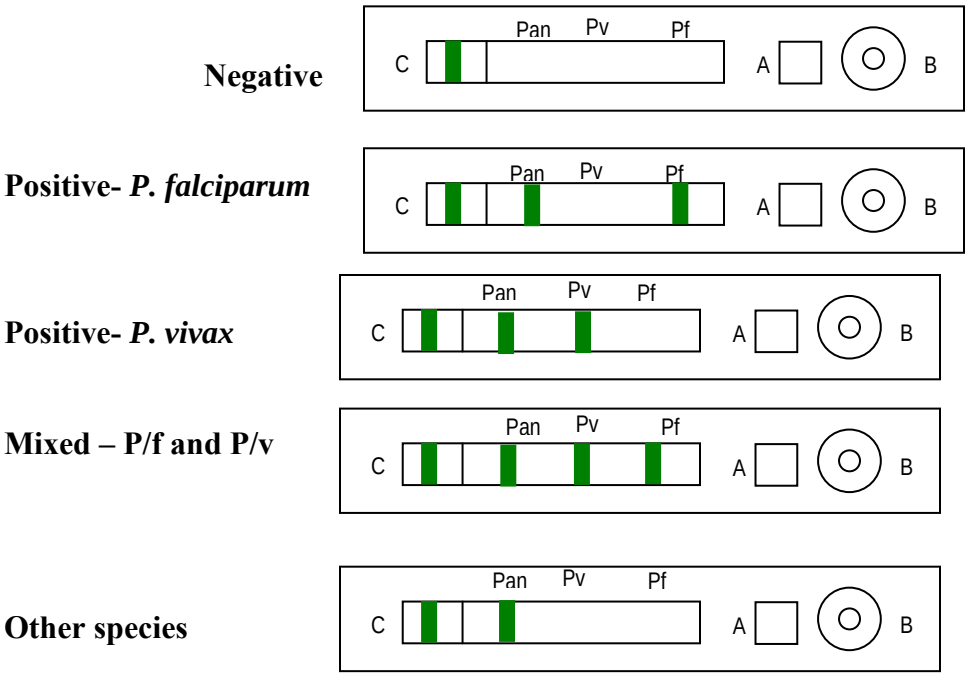


Figure 9: Pictorial depiction of Paramax-3 ^{TM} in identification and speciation of malaria parasites (The mark A indicates the area where blood is applied and B indicates where buffer is added)

3.5.4. Sysmex Hematology analyzer Hemoglobin determination

Sysmex hematology analyzer utilizes cyanide free solution sodium lauryl sulfate (SLS). In this procedure erythrocytes are hemolyzed by the action of SLS and Triton X-100 and the eluted hemoglobin is converted into SLS hemoglobin which has maximum

Spectorophotometric absorbance at 539nm. The reading is converted into hemoglobin concentration by in-built reading system (http://WWW. Patientstorm.us/patients)

3.6. Stool collection and processing

A single fresh stool was collected with labeled and clean cup. A portion of the sample was preserved for later preparation of formal-ether concentration technique using 10% formalin while the rest was utilized for preparation of on the spot modified Kato Katz smears (annex-III).

3.6.1. Modified Kato-Katz method

In order to avoid effect of malachite green on hookworm ova, modification was made on Kato-Katz procedure. Briefly after weighing about 41.7 mg stool using template the sample was mixed with normal saline on slide and covered with big sized cover slip instead of malachite soaked cellophane. EPG (egg per gram of stool) was calculated by multiplying count by conversion factor i.e. 24 (Cheesbrough, 1998).

3.6.2. Formalin-ether concentration

A portion of preserved stool samples were processed by formalin-ether concentration method at Jimma University School of medical laboratory as described by Cheesbrough (1998). Briefly the preserved stool sample was sieved with cotton gauze and transferred to 15ml centrifuge tube. Then 7ml of 10% formalin and 3ml of diethyl ether was added and centrifuged for 2 minutes at 2000rpm. The supernatant was decanted and the residues were transferred to microscope slides and observed under light microscope at10X and 40 X magnifications for the presence of cysts and ova of the parasites.

3.6.3. Hookworm culture

Test tube culture (Harada-Mori technique) was set up. Briefly thin film of feces was spread on one side of the middle third of a 13 by 120 mm strip of filter paper and placed in a 15 ml conical tip centrifuge tube containing about 3 ml distilled water. The culture was kept for 7 to 10 days at 28^{o}C, adding water daily as needed to keep the water level well above the bottom end of the filter paper. For identification, the larvae were transferred to slide and mounted under a cover glass. Morphological key differences such as esophagointestinal junction, esophageal bulb, buccal spears and striations on sheath in tail region were used to identify the species (Pawlowski, 1991).

3.7. Ethical clearance

Ethical clearance was obtained from the ethical committee of Biology Department, Addis Ababa University. Positive individuals were treated according to standard guideline for malaria and helminth infection.

3.8. Data analysis

Statistical analyses were performed using SPSS software version 13. Data were analyzed by use of Chi square test (χ 2) for association and one way ANOVA for comparing means and binary logistic analysis for risk analysis and Kappa test for degree of agreement. Values were considered to be statistically significant when P-values are less than 0.05.

4. RESULTS

4.1 Description of the study subjects

From 384 individuals recruited for co-infection study, 14 were excluded from the study because of failure to submit stool and/or blood specimen. Over response rate was 96.4%. Therefore, during the whole study period a total of 370 suspected malaria patients and a separate sample of 100 school children for malaria-hookworm co-infection study and for hookworm species identification were included in the study, respectively. Mean age for co-infection study was 23 years with male to female ratio 0.97:1. Age by sex distribution of the study subjects is summarized in Table 2.

Table 2: Distribution of the study participants by age and sex, Asendabo, south West Ethiopia, Oct-Nov 2006

Age Category (in Years)	Males (N)	Females (N)	Total N (%)
5-14	50	54	104 (28.1)
15-24	55	68	123(33.2)
25-34	38	34	72(19.5)
35-44	22	13	35(9.4)
45-54	12	8	20(5.4)
55+	6	10	16(4.3)
Total N (%)	183 (49.5)	187 (50.5)	370(100)

The majority (88.4%) of the study participants were Oromo and Muslims (84.1%). Of these, farmers made the majority (39.7%). Most (43 %) use streams or rivers as their water source. And 87% of them had latrines and 51.6% of the participants wore shoes always (Table3).

Table 3: Socio-demographic characteristics of the study participants, Asendabo, South West Ethiopia, Oct-Nov, 2006

Socio-demographic variables	Male	Female	Total (%)
Ethnicity			
Oromo	156	171	327(88.4)
Dawuro	11	4	15(4.0)
Amhara	6	6	12(3.2)
Yem	4	3	7(1.8)
Keffa	3	2	5(1.3)
Others	3	1	4(1.0)
Religion			
Muslim	155	156	313(84.6)
Christian	27	29	57(15.4)
Occupation			
Farmer	90	57	147(39.7)
Student	59	71	130(35.2)
House wife	0	37	30(8.2)
Gov't emp.	10	8	18(4.8)
Others	17	13	45(12.1)
Educational status			
Litrate	84	74	158(42.7)
Iliterate	99	113	212(57.3)
Water source			
Pipe	31	38	69(18.0)
Well	66	74	140(38.6)
Stream	86	75	161(43.4)
Latrin availablity			
Yes	159	163	322(87)
No	24	24	48(13)
Shoe- wearing			
Always	86	105	191(51.6)
Some times	68	70	138(37.3)
Not at all	29	12	41(11.1)

4.2. Helminth infection

All the 370 stool samples, per study participant, were properly submitted. Of these 228 (61.6%) were positive for one or more intestinal helminth parasites. The most prevalent geo-helminths found were hookworm, *Ascaris lumbricoides* and *Trichuris trichiura* with respective frequencies of 141 (38.1%), 71(19.2%) and 38(10.3%) for each species (Table 4). None of the samples submitted were positive for schistosome ova while very few samples were positive for protozoan cysts.

Table 4: Helminth species prevalence in the study participants by age group, Asendabo, south west Ethiopia, Oct-Nov, 2006.

Age category	**Total examined n**	**Parasites species**						
		Hw n(%)	**Al n(%)**	**Tt n(%)**	**Hn n(%)**	**Ev n(%)**	**Tae. n(%)**	**Ss n(%)**
5-14	104	37(36)	24(23)	16(15)	1 (0.9)	2(3)	0(0)	0(0)
15-24	123	55(45)	25(20)	10(8)	4(2.0)	3(2.4)	1(0.7)	0(0)
25-34	72	28(39)	9(13)	4 (6)	2(3.0)	1(1.3)	0(0)	0(0)
35-44	35	11(31)	8(23)	5(14)	1(2.8)	1(2.5)	0(0)	1(2.8)
45-54	20	7(35)	2(10)	1(5)	0(0)	0(0)	0(0)	0(0)
55+	16	3(19)	3(19)	2(13)	0(0)	1(6.1)	0(0)	0(0)
Total (%)	370 (100)	141 (38)	71 (19)	38 (10)	8 (2)	8 (2)	1 (0.3)	1 (0.3)

Where Al= *Ascaris lumbricoides*, Hw= Hookworm, Tt= *Trichuris trichiura H*, Ev= *Enterobius vermicularis*, Tae= Taenia species, Ss= *Strongyloides stercolaris* , Hn= *Hymenolepis nana*

.

Single infection was noted in 82.5% (N=118) of the positive cases while the rest 17.5% (40/228) harbored multiple infections with two or three different species at the time of examination. The most frequently observed multiple infection was between hookworm and *Ascaris lumbricoides* (N=17) followed by Ascaris and *Trichuris trichiura* (N=10) (Figure-10).

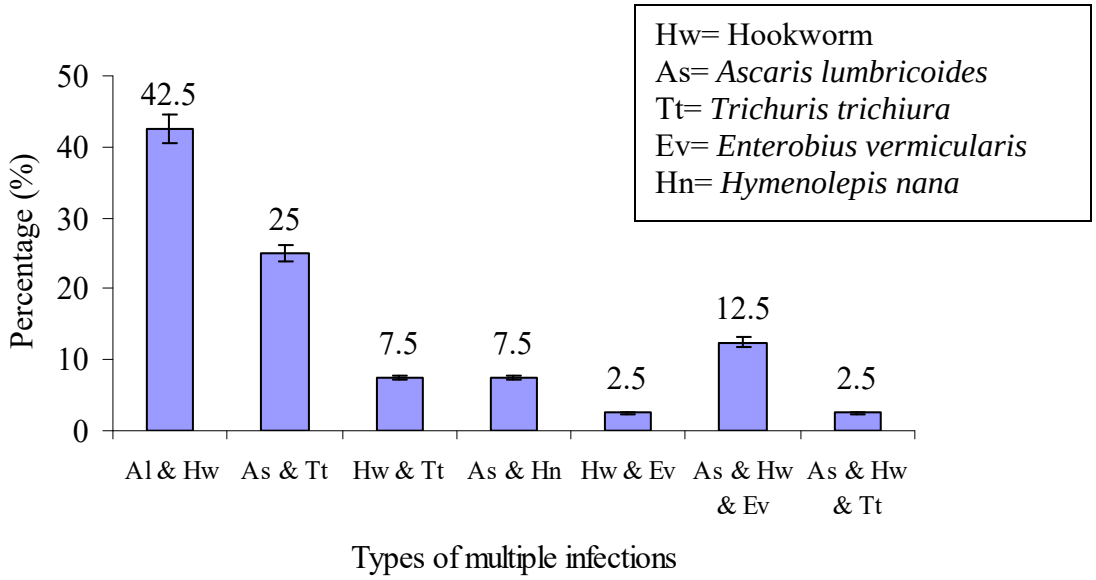


Figure 10: Percentage distribution of helminth mixed infections among study participants, Asendabo, south west Ethiopia, Oct – Nov, 2006

Although there was observable variation in proportion of hookworm infected individuals, for example, between sexes (male=39.3% and female 36.9%), among shoe wearing habits (Always =34.6%, Some times=38.4 and Not at all=53.7%) and others, it was found that the difference in the prevalence rate was not significant at $P<0.05$ significances level for each condition (Table-5).

Table 5: Some selected socio-demographic variables by hookworm infection among study participants, Asendabo, south west Ethiopia, Oct-Nov, 2006

Variable	Hookworm		Statistics
	Total examined	Positive (%)	
Age			
5-14	104	37(40)	
15-24	123	55(48)	
25-34	72	28(34)	χ^2= 5.287
35-44	35	11(29)	P= 0.382
45-54	20	7(20)	
55+	16	3(30)	
Total	370	141(38)	
Sex			
Male	183	72(39.3)	χ^2= 0.235
Female	187	69(36.9)	P= 0.628
Total	370	141(38)	
Occupation			
Farmer	155	68(43.8)	
Student	130	48(36.8)	
House - wife	37	11(29.7)	χ^2= 5.702 P= 0.336
Gov't emp.	18	5(28.0)	
Others	30	9(30.0)	
Total	370	141(38)	
Water source			
Pipe	69	22(31.9)	χ^2= 2.028
Well	140	52(37.1)	P= 0.363
Stream	161	67(41.6)	
Total	370	141(38)	
Latrine availability			
Yes	322	122(37.9)	χ^2= 0.051
No	48	19(39.6)	P= 0.822
Total	370	141(38)	
Shoe- wearing			
Always	191	66(34.6)	χ^2= 5.231
Some times	138	53(38.4)	P= 0.730
Not at all	41	22(53.7)	
Total	370	141(38)	

The highest age specific hookworm prevalence was observed in age group between 15-24 years of age followed by slow drop at later ages (Figure 11). Figure 11 also shows a familiar line graph for other major STH and overall age specific helminth prevalence with peak prevalence at age group between 5-14 years.

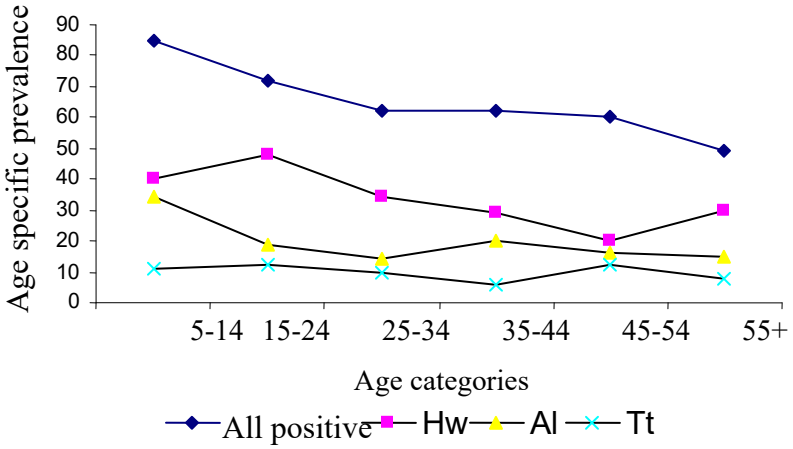


Figure 11: Age specific prevalence curve for the big three STH among study participants, Asendabo, south west Ethiopia, Oct-Nov, 2006

Geometric mean for hookworm egg count was 268.4 eggs per gram of stool with minimum and maximum counts ranging between 48 eggs/gm and 5023 eggs/gm, respectively. The heaviest egg count (5023 eggs/gm) was recorded in 16 years old boy. In addition, highest age specific geometric mean egg count for hookworm infection was observed in the age range 15-24 with gradual fall as age increases. Both age (F=0.712; P=0.156) categories and sex (F=0.516; P=0.474) did not show statistically significant difference in geometric mean egg count or infection intensity.

According to World Health Organization (Montresor *et al.,* 1998), hookworm infection intensity classifications, our result showed, 60.9% of hookworm positive subjects suffered from light hookworm intensity infection, 31.9 % from moderate intensity infection and only 6.3% of individuals harbored heavy intensity infection (Figure 12). Intensity of hookworm infection was not associated with age groups (P=0.493; χ^2 =4.939) and sex (P=0.731; χ^2 = 1.290).

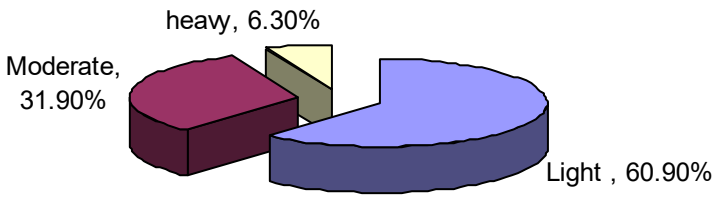


Figure 12: Proportion of hookworm positive study participants by infection intensities, Asendabo, south west Ethiopia, Oct-Nov, 2006

A separate sample (N=100) of school children with mean age 12.9 range 10-18 was taken in order to identify the dominant hookworm species in the area. Among the 100 students, 93 properly submitted fresh stool samples (93% response rate) and the rest failed to produce samples and hence excluded from the study. Direct microscopy was done before Harada Mori culture. It was found that 38(40.8%) specimens were positive for hookworm, 13(14%) were positive *Ascaris lumbricoides* and 5(5.4%) were positive for *Trichuris trichiura*. After 10 days of well-established Harada Mori culture almost all 97% (37 out of 38), hookworm positive stool samples were hatched into third stage filariform larva. Detail morphological examination revealed that 34(92%) of the larvae

were *N. americanus* and 3(8%) were *A. duodenale*. None of the culture showed mixed infection (infection by the two species at a time) (Figure 13).

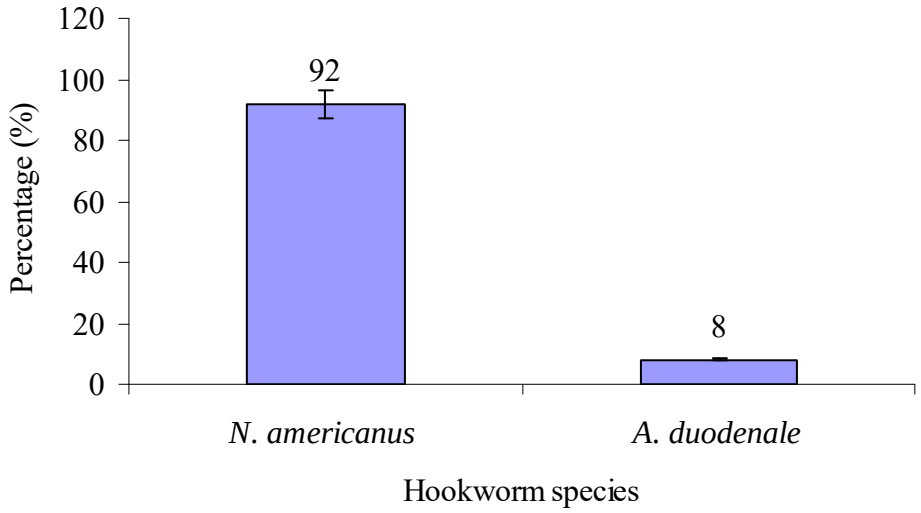


Figure 13: Hookworm species distribution among positive school children in Asendabo elementary school, south west Ethiopia, Jan, 2007

4.3. Malaria case detection

During the study period a total of 370 Giemsa stained thick and thin blood films were examined. At least 100 oil immersion fields were checked before reporting a negative result. Accordingly, 120 (32.4%) of the study participants were positive for either of the two Plasmodium species. Mixed infection and heamoparasites other than malaria were not detected in all slides. *P. falciparum* was identified in 77(64.2%) of the positive cases while *P. vivax* in 43(35.8%) of the positive cases. Although sex and age did not show significant association with risk of malaria infection, the most attacked age group was the age group 5-14 years old individuals (Table 6).

Table 6: Malaria distribution by age and sex among study participants, Asendabo, south west Ethiopia, Oct-Nov, 2006

Variable	Total examined N	Malaria positive N (%)	Statistics
Age			
5-14	104	39(37.5)	
15-24	123	39(31.7)	
25-34	72	23(31.9)	χ^2=17.457
35-44	35	10(28.5)	P=0.065
45-54	20	6(30.0)	
55+	16	3(19.0)	
Total (%)	370	120(32.4)	
Sex			
Male	183	65(35.5)	χ^2=4.245
Female	187	55(29.7)	P=0.120
Total	379	120(32.4)	

Along slide blood film examination (Giemsa stained microscopy), each blood sample was simultaneously tested for malaria parasite with a rapid malaria antigen capture assay (Paramax-3^{TM}). Out of 120 malaria positive cases (combined *P. falciparum* and *P. vivax*), Paramax-3^{TM} identified 90% (108/120) of them correctly and 92.8% (234/252) negative cases with an agreement (Kappa = 0 .823) (Table 7). And Paramax-3 ^{TM} read discordant results in 29 tested individuals. Some of the discordant cases (N=17) were labeled as false positives (11 *P falciparum* and 6 *P. vivax*). The remaining 12 were labeled as false negatives (8 *P. falciparum* and 4 *P. vivax*) (Table 8).

Table 7: Total malaria parasite detection: microscopy versus Paramax-3™ among study participants, Asendabo, south west Ethiopia, Oct –Nov, 2006

Paramax-3™	Microscopy		Total (%)	Statistics
	Positive	Negative		
Positive	108	17	125	Kappa=0.823
Negative	12	233	245	
Total	120	250	370	

Similarly, Paramax-3™ equally identified 88.3% (68/77) of *P. falciparum* and 90.7% (39/43) of *P. vivax* as determined by microscopy. On the contrary, Paramax-3™ wrongly labeled one microscopically positive *P. falciparum* subject as *P. vivax* and produced eight negative results that were positive for *P. falciparum* under microscopy (Table 8). In the case of *P. vivax*, Paramax-3™ labeled only four *P. vivax* cases as negatives.

Table 8: Species specific parasite detection: microscopy verses Paramax-3™, among study participants, Asendabo, south west Ethiopia, Oct-Nov, 2006

Paramax-3™	Microscopy			Total
	P/f	P/v	Negative	
P/f	68	0	11	79
P/v	1	39	6	46
Negative	8	4	233	245
Total	77	43	250	370

Other test indices such as Positive Predictive value (PPV), Negative Predictive value (NPV) and test accuracy for combined malaria diagnosis were 89.7%, 95%, and 92.1%, respectively. Paramax-3™ was sensitive (96.7%) and specific (99.1%) in the diagnosis of *P. falciparum* malaria infection, with PPV, NPV and accuracy of 97.0%, 96.7%, and 81.4%, respectively. The corresponding sensitivity and specificity for the diagnosis of *P.*

vivax malaria were 90.7% and 98.7%, respectively with an NPV of 98.3% and a PPV of 92.0%.

Paramax-3^{TM} showed 82.7%, 91.4% and 100% sensitivity at parasite density levels of 100-100/ µl, 1001- 10,000/ µl and > 10,000/ µl, respectively. That means sensitivity increased as parasite density increased and reached 100% as parasite density approached a parasite count of more than 10,000/ µl.

Mean malaria parasite density was 3873/µl with maximum and minimum values ranging between 160/ µl and 16231/ µl. Many of the positive cases (67.5%) had parasite count between 1001- 10,000/ µl (Figure 14). Furthermore, 40% of males and 28% of females had parasite density between 1001 and 10,000/ µl. Mean parasite count between males and females was not significantly different (F=0.018; P=0.895). Similarly the mean parasite count difference was not significant among age groups (F=0.459; P=0.806)

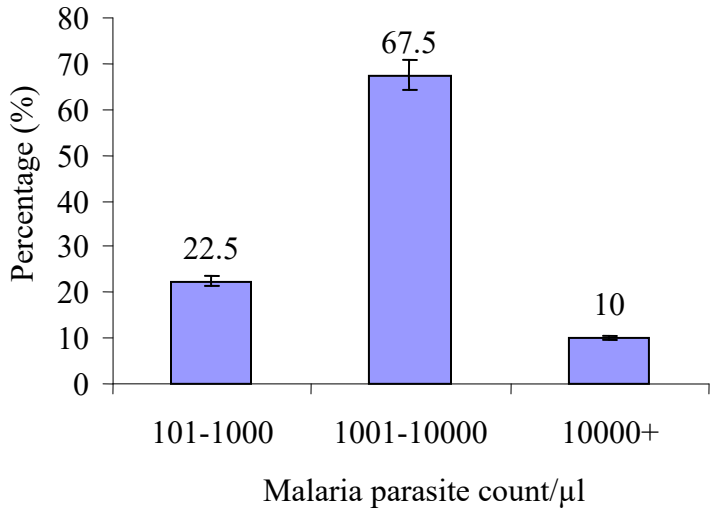


Figure 14: Percentage distribution of malaria positive cases by classes of parasite density among study participants, Asendabo, south west Ethiopia, Oct-Nov, 2006

4.4. Hemoglobin measurement

Hemoglobin determination was carried out at Jimma university specialized hospital using automated Sysmex hematology analyzer. Accordingly, mean hemoglobin concentration was 12.4 gm/dl ranging between 6 gm/dl to 20.10 gm/dl with standard deviation of 2.374 gm/dl. Thus 27.6% (102/370) of the subjects were anemic and among the anemic 53.9% (55/102) were female and 46.0 %(47/102) were males.

From 102 anemic subjects, 26.5% (27/102) had hookworm infection without concurrent malaria infection and 19.6% (20/102) were positive for malaria parasites without co-infection with hookworm whereas, 18.6% (19/102) anemic individuals were free of both malaria and helminth infections.

Anemia was significantly higher in hookworm positive subjects (χ^2=7.019; P=0.008). Similarly, anemia was significantly associated with malaria infection (χ^2=10.309; P=0.001). Adjusted odds ratios for both hookworm infection (AOD=1.40, P=0.006; CI=0.322-0.826) and malaria infection (AOD=2.224; P=0.001; CI=0.278-0.727) revealed that both infections were predictors of anemia in the study participants. In case of malaria infection, positive individuals were two times more prone to anemia than their negative counter parts.

On the other hand, although, age groups, and sex specific anemia prevalence revealed age group 5-14 and females as the most affected, the difference was not statistically significant. In addition, stool result (helminth positive versus negatives individuals) did not differ significantly in anemia prevalence (χ2=2.162; P= 0.141) (Table 9)

Table 9: Prevalence of anemia by selected variables among study participants, Asendabo, south west Ethiopia, Oct-Nov, 2006

Variable	Anemia		% anemic	Statistics
	Anemic N	Non-anemic N		
Age				
5-14	40	64	38.5	
15-24	26	97	21.1	$\chi^2 = 13.524$
25-34	18	54	25.3	P=0.079
35-44	9	26	25.7	
45-54	5	15	25.0	
55+	4	12	25.0	
Sex				
Male	47	136	25.6	χ^2=0.644
Female	55	132	29.4	P=0.422
Stool				
Positive (any helminth)	69	159	30.3	χ^2=2.162
Negative	33	109	23.2	P=0.141
Hookworm				
Positive	50	91	35.5	χ^2=7.019
Negative	52	177	22.7	P=0.008
Malaria				
Positive	46	74	38.3	χ^2=10.309
Negative	56	194	22.7	P=0.001

Based on WHO recommended cut off values for different age groups, sex and physiological conditions anemic individuals were categorized into severe (hemoglobin < 7.0 gm/dl), moderate (7.0-10.0 gm/dl) and mild (10.0-11.0 gm/dl) categories. Accordingly, 14.7% (15/102), 35.5% (36/102) and 50.0% (51/102) of anemic cases, respectively suffered from severe, moderate and mild anemia.

4.5. Malaria and hookworm co-infection

Examination of stools and blood films for co-infection with malaria and intestinal parasites revealed an overall co-infection in 77 (20.8%) individuals. When hookworm and malaria co-infection alone were analyzed, 44 (36.7 %) of malaria positive individuals were found to harbor hookworm (Figure 15). Statistically significant association was not observed between intestinal parasite positive and negative subjects in acquiring malaria infection (P=0.486) and similarly significant difference was not observed between hookworm positive and negative subjects in acquiring malaria infection (P=0.692).

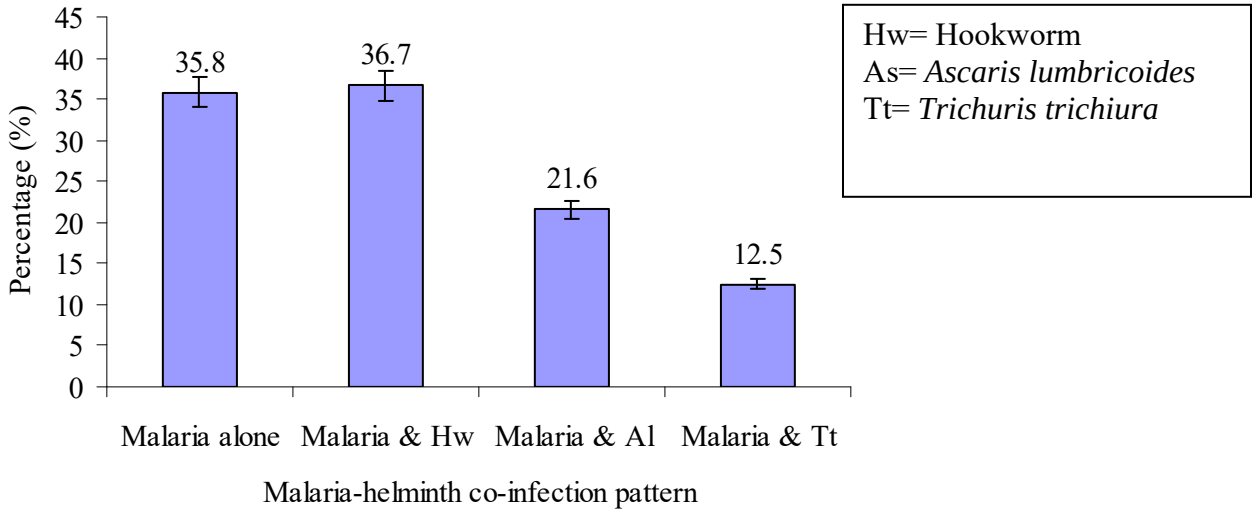


Figure 15: Malaria-helminth co-infection pattern for major STH among study participants, Asendabo, south west Ethiopia, Oct-Nov, 2006

Among the 44 subjects who were co-infected with malaria and hookworm, 18% (8/44) were severely anemic, 27.3% (12/44) moderately anemic, 6.3% (3/44) mildly anemic and the remaining 47.7% (21/44) were non-anemic. Furthermore, 72 % of the co-infected individuals had malaria parasite count between 1001 and 10,000/µl and 50% of them had light intensity hookworm infections. Co-infection was not significantly different between age groups (χ^2 =2.918; P=0.713) and the sexes (χ^2 =2.831; P=0.092).

Looking at anemia versus malaria-hookworm co-infection, a total of 23 (22.5%) subjects were found to be anemic. And anemia was found to be significantly associated with co-infection (χ^2=12.264; P=0.000).

The mean hemoglobin concentration in hookworm or malaria infected individuals was lower than their respective negative counter parts. And the mean difference was statistically significant (Table 10). Similarly, strong significant difference in mean hemoglobin concentration was observed in subjects co-infected with malaria and hookworm (F=27.9; P=000) when compared with those subjects infected with either malaria or hookworm alone or free from both infections.

Mean malaria parasite count was also higher in subjects co-infected with Malaria-hookworm than individuals free of hookworm infection alone and the mean difference was statistically significant (F= 0.882; P=0.022) (Figure 16).

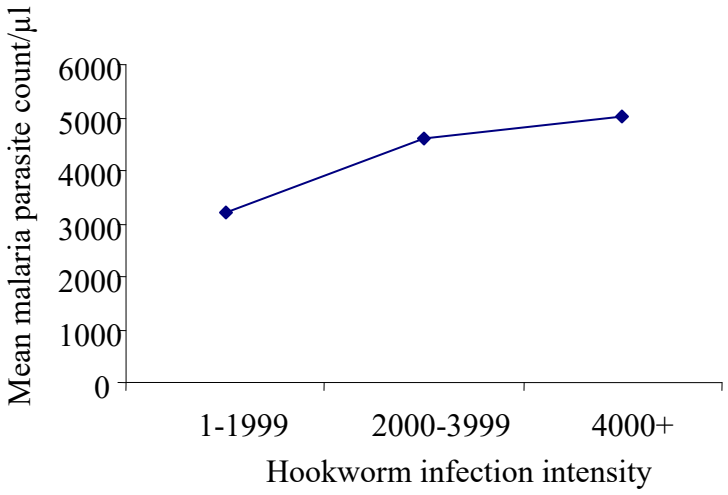


Figure 16: Pattern of mean malaria parasite count increment among malaria-hookworm co-infected study participants, Asendabo, South west Ethiopia, Oct-Nov, 2006

Table 10: Mean hemoglobin concentration differences by status of infection among study participants, Asendabo, south west Ethiopia, Oct-Nov, 2006

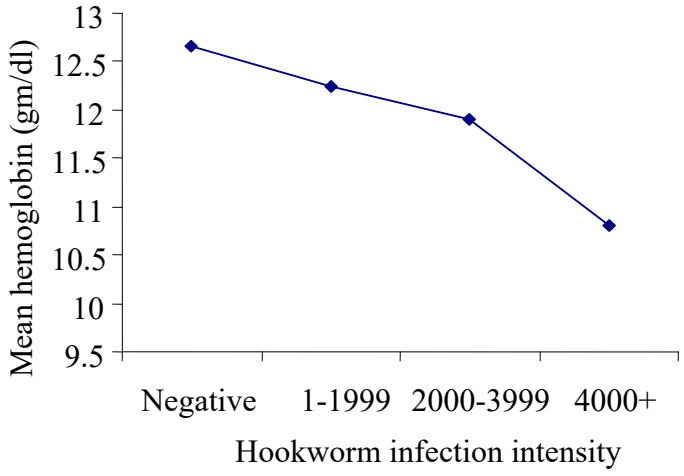
Infection	Mean Hb. (gm/dl)	ANOVA One way	95% CI
Hookworm *			
-Positive	12.03	F=6.281	11.6-12.5
- Negative	12.66	P=0.013	12.4-12.9
Malaria **			
-Positive	11.70	F=17.245	11.2-11.3
-Negative	12.76	P=0.000	12.5-13.0
Co-infection[+]			
-co-infected	10.73	F=27.90	9.8-11.6
-Non-co-infected	12.65	P= 0.000	12.2-12.7

* Hookworm positive without concurrent malaria infection

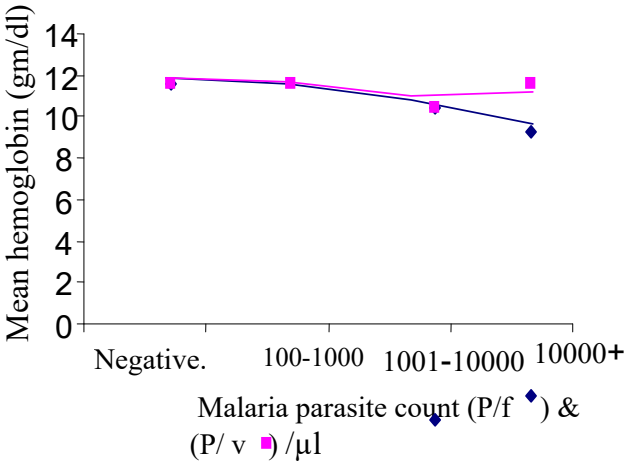
** Malaria infection without concurrent hookworm infection

[+] Co-infection (malaria plus hookworm)

Mean hemoglobin concentration for subjects with malaria intensity level between 100-1000/µl was 12.48 gm/dl whereas the mean hemoglobin was 10.93 gm/dl for those subjects with malaria intensity level greater than 10,000/µl. Similarly, hemoglobin concentration decrement was observed as hookworm infection intensity increased (Figure 17 A and B) and the mean hemoglobin difference among hookworm infection intensities and malaria parasite counts categories were statistically significant with F= 7.412; P=0.000 and F=3.163; P=0.025, respectively for both conditions.



(A)



(B)

Figure 17: Hookworm infection intensity (A) and malaria parasite density level (B) by mean hemoglobin concentration among study participants, Asendabo, south west Ethiopia, Oct-Nov, 2006

A mean hemoglobin reduction of 2.04 gm/dl was observed between Co-infected individuals (10.73 gm/dl) and individuals free of both malaria and helminth (12.77 gm/dl) co-infection (both negatives) as depicted in Figure 18.

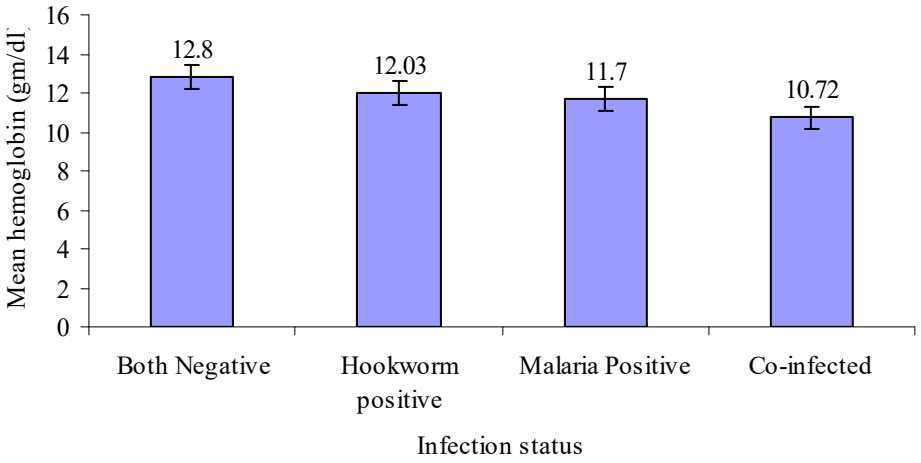


Figure 18: Mean hemoglobin reduction pattern among study participants by their infection status, Asendabo, south west Ethiopia, Oct-Nov, 2006

5. DISCUSSION

The overall prevalence of intestinal helminth infections (61.6%) in this study, was relatively lower than what was reported for schoolchildren by Ibrahim *et al.* (1999) (86.2%) from the same study area, and that of Roma and Worku (1997) (89.4%) from Wondo-Genet area, SNNP region. This difference is to be expected because the present study included all age groups, which would include adult population that is known to have relatively lower parasite prevalence.

Helminth species prevalence pattern in the study population was similar to that reported from Uganda (Adrienne *et al.,* 2005) whereby hookworm was the most prevalent (32.1%) followed by *Ascaris lumbricoides* (17.4%) and *Trichuris trichiura* (8.1%). On the other hand, a number of other studies (Haileamlak, 2005; Roma & Worku, 1997; Erko & Medhin, 2003) have shown a different species prevalence pattern whereby A. *lumbricoides* was the predominant species in Wondo-Genet (Erko & Medhin, 2003) and *Trichuris trichiura* from south west Ethiopia (Haileamlak, 2005). This difference in helminth parasite species prevalence pattern might be explained by altitude and soil type differences, which are known to influence species distribution of geo-helminth parasites (Tesfamichael & Kloos, 1983).

Age specific prevalence for major STH parasites in this study showed highest peak in the age group between 5-14 years. This is similar to the finding from south west Ethiopia (Haileamlak, 2005) and others from elsewhere (Mwangi *et al.,* 2006). Contrary to what was reported on age specific prevalence for hookworm infection by Crompton (2000) and Hotez *et al.* (2004) that prevalence of hookworm infection rises steadily from early age, levels off in later childhood and then remains stable during adulthood, the present study showed an almost similar age specific prevalence with that of other STH, which is characterized by a steady rise at early age, peak at around 15 years, and a decline then after. Similar results to the present findings were reported from Paraguay (Nora *et al.,* 1999) and from Nigeria (Udonski, 1980). This difference in the age-specific prevalence pattern of hookworm infection between the reports of Crompton (2000) and Hotez *et al*. (2004) and that of the present one may be due to age factor (previous exposure) and

concomitant immunity which might protect older people from repeated hookworm infection.

The fact that both hookworm species are present in the study area is in agreement with previous studies conducted elsewhere in the country (Jemaneh & Tedla, 1884; Armstron & Chane, 1985; Tedla & Jemaneh, 1985). With regard to the prevalence of both hookworm species, our finding was similar with that of Jemaneh and Tedla (1984), who reported 7.5% for *A. duodenale* and 92.5% for *N. americanus* from Gonder area. Unlike some studies (Armstron & Chane, 1975; Tedla & Jemaneh, 1985) who reported mixed infection by both hookworm species in a single individual, no concurrent infection by the two hookworm species was detected. This could possibly be explained by the fact that mixed infection is usually a rare incidence as indicated by Tedla & Jemaneh (1985) for whom only one person in each of the six community assessed harbored both species.

Compared with golden standard microscopy, the overall sensitivity, specificity, PPV, NPV and test accuracy achieved by Paramax-3 ™ in this study were comparable with many other Rapid malaria Diagnostic Test (RDT) evaluation studies (Singh & Sasena, 2002; Mboera *et al.,* 2006; Taddesse & Mekete, 2001; Kodisinghe *et al.,* 1997). Paramax-3 ™ overall agreement (Kappa= 0.823) with the reference method is well above the cutoff kappa value (0.7) set for any analytical agreement test (Smith, 2003). The observed lack of perfect agreement could be due to the inherent problem associated with the reference method employed in the study. In some situations antigen capture assays are more superior to microscopy (Kodisinghe *et al.,* 1997) in detecting much lower parasite density.

The false positive discordant cases observed in this study were due to the superior sensitivity of RDTs test that provide a more precise diagnosis of patients infected with *P. falciparum* by detecting parasite that could be missed by traditional blood film investigation (Taddese & Mekete, 2001; Singh & Saena, 2002; Biruk *et al.,* 1999). This is because of the sequestration of *P. falciparum* infected red blood cells in microcirculation (Warrell & Molyneux, 1990), which allows HRP-2 antigen to be detected by the antigen

capture method while the blood film would reveal no parasitemia in the peripheral blood circulation.

The other logical explanation for the false positive result for both *P. falciparum* and *P. vivax* cases could be the preceding anti-malarial treatment which might have cleared out circulating parasitemia while leaving circulating antigenaemia for subsequent 3 to 5 days after treatment. In this study treatment status of those discordant individuals before testing were not ruled out. Furthermore, false positive *P. falciparum* could also be recorded in subjects positive for rheumatoid factors particularly with RDTs such as ICT prototype because of cross-reactivity (Alessandro *et al.,* 1998).

The false negative readings by Paramax-3 ^{TM} were identified as *P. falciparum* under microscopy. This could be due to insufficient antigenaemia during early malaria infection as shown by Juntra *et al.* (1996). The antigen HRP-2 is derived from trophozoite, and it is possible that during early infection the trophozoite may not have expressed enough HRP-2 to be detected by the test. This may suggest that there is no correlation between the positivity of the test and the parasitaemia, which is mainly due to small ring forms, not trophozoites. The level of circulating HRP-2 should correlate better with the trophozoite load than with the number of circulating small ring forms (Juntra *et al.,* 1996). The other plausible explanation might be Pf HRP-2 and pLDH (in cases of *P. vivax*) deleted mutant genes might have caused variation in the expression of HRP-2 (Neeru *et al.,* 2005) and pLDH hence resulting in absence of immunological reaction.

Paramax-3 ^{TM} misidentified one *P. falciparum* case as *P. vivax*. It was not clear why this happened. But some reports showed there would be cross-reactivity in the common antigen band between *P. falciparum* and *P. vivax* (Postigo *et al.,* 1998). At any rate, the finding of this study showed the Paramax-3 ^{TM} fulfill the by WHO (1988). Therefore, it is reasonable to recommend the use of Paramax-3 ^{TM} as an alternative to microscopy for the diagnosis of malaria with all test precautions and standards are followed properly. In particular in regions that lack electric power for efficient use of microscopy.

On the basis of Geimsa stained microscopy, peak season malaria species distribution *P. falciparum* (64.3%) and *P vivax* (35.7%) reported in this study very closely resembles what is known in the country for years (Tulu, 1993). Lack of previous malaria-hookworm co-infection studies in the country poses difficulty on making rigorous discussion on the problem. However, Aragie (2006) showed much higher malaria-hookworm co-infection prevalence from Areka (SNNP) than this study. This could be because of the difference between study participants with respect to age distribution, the educational level and geographical location.

The present study did not reveal individuals positive for helminth infections to be more predisposed to malaria infection and vise-versa. Although, Adrienne *et al*. (2005) reported similar finding from southwest Uganda where they reported no significant association of even the most heavily helminth infected individual with risk of malaria, a study conducted in a Thai mountain village by Nacher *et al*. (2001) had showed that helminth infected individuals were more likely to develop malaria than helminth free subjects. This discrepancy in the study finding may only be rectified through a longitudinal study design.

According to Mathieu *et al.* (2001), Th_2 bias in helminth infected patients might be beneficial in controlling blood stage parasites whereas it might be detrimental in IFN-γ mediated immunity against liver stage parasites. Thus, sporozoites could be more successful at infecting hepatocyts in helminth infected patients. A shift towards Th_1 responses might be more successful at inducing anti-sporozoite responses but it might also up-regulate adhesion molecules thus favoring sequestration of blood stage parasites. Alternatively, a helminth-mediated shift towards Th_2 responses could render the host more susceptible to liver stage parasite and favor Plasmodium reproduction but protect the host from severe complication of blood stage malaria.

In the present study, malaria helminth co-infected individuals, on the average were detected to have a higher mean parasite density than malaria positive subjects without helminth infection. This is in agreement with finding from animal model (Helmby *et al.,*

1998) and human studies (Hesran *et al.,* 2004) that have shown malaria parasitemia to increase in helminth-malaria co-infected subjects. However, others (Briand *et al.,* 2005; Hartgers & Yazdanbakhsh, 2006) had reported *S. haematobium* to have host protective effect by decreasing *P. falciparum* densities as compared to helminth-free children.

This seems to suggest that, association between malaria susceptibility and helminth infection may be influenced by the type of helminth involved, the intensity of infection and the age of the population studied (Hotez *et al.,* 2006). Therefore, it appears inappropriate to make a firm statement on the role of helminth co-infection in malaria pathogenesis and cross protection on the basis of current understanding of co-infection immunology. As a result, further well defined studies will be required to elucidate the underlying mechanisms and unravel the possible interactions in malaria-helminth co-infection.

Some studies in the country such as Zein and Assefa (1987), Adish *et al.* (1999); Aragie (2006), reported relatively a higher anemia prevalence rates of 47.2 %, 42 % and 45.1% in different localities in Ethiopia, respectively than the present study (27.6%). Although it is not easy to compare our finding with the above studies it is possible to suggest that the observed low anemia rate might be because of the majority of hookworm positive cases harbored light infection intensity (60.9%) and the lesser pathogenic *N. americanus* species. Furthermore, basic socio-economic, culture, helminth, and malaria transmission dynamics in the present study area different from the above study areas.

Mean hemoglobin concentration for subjects with heavy hookworm infection was lower when compared with moderate and light intensity infection. This is in agreement with similar studies (Hotez *et al.,* 2004; Crompton, 2000; Pawlowski, 1991). The basis for hookworm mediated anemia has been elucidated by Stoltzufus *et al.* (1996) who measured blood loss from children and correlated the loss with intensity of hookworm infection as measured indirectly by means of egg counts. They showed that, on average the loss of blood in stools was found to increase by 0.825 mg/g of feces for each 1000

epg. Crompton (2000) also had shown hemoglobin concentration falls as intensity of hookworm infection rises.

Michele *et al.* (2000), from Nepal, further showed malaria and hookworm co-infection to be the stronger predictors of moderate to severe anemia. Study from Nigeria (Egwunyenga *et al.,* 2001) also showed mean hemoglobin values of malarial mothers with intestinal helminth infections to be lower than those without intestinal helminth infections. This is due to the additive effect of both pathogens causing depletion in host hemoglobin (Brooker & Michael., 1999). Malaria causes anemia by several different mechanisms, including increased destruction of both parasitized and non-parasitized red blood cells and dsyerythropoiesis resulting from host production of inflammatory cytokines (Hotez *et al*., 2006). On the other hand, hookworm causes anemia as a result of chronic intestinal blood loss (Crompton, 2000).

6. CONCLUSIONS AND RECOMMENDATIONS

Conclusions

1. The present study has pointed out Malaria-helminth (in particular hookworm) co-infection is a common encounter in the communities living around the study area. It was further noted, co-infection with malaria and hookworm aggravate hemoglobin loss leading to severe anemia.
2. The study also identified the existence both hookworm species in the area with *N. americanus* being the dominant one.
3. Relatively higher burden of helminth infection was seen in the study area.
4. Paramax-3^{TM} was proven efficient in the diagnosis of malaria if all necessary precautions are considered particularly in areas without electricity.

Recommendations

Based on the present findings we recommend the following points:

1. Public health planners should incorporate the concept of Malaria-helminth co-infection in communicable diseases control and prevention strategies as co-infection will seriously affect the outcomes of intervention measures such as malaria vaccine trials, anemia prevention or control.
2. Because blood loss due to hookworm infection differs on the type of hookworm species involved due attention should be given with regard to treating hookworm positive individuals.
3. Environmental and personal hygiene measures should be enforced to curb the high burden of helminth infection seen in the study participants.
4. As it was observed in this study and in the others too, malaria-helminth co-infection studies were a topic of debate among researchers. Therefore, we forward further more well defined co-infection study with case control prospective study design should be proposed so that possibly all immunological and epidemiological questions raised would be addressed.
5. Finally, a study which synchronizes hookworm species identification process with hemoglobin determination for each subject involved in co-infection study should be initiated so that species specific hemoglobin loss could be appreciated.

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8. ANNEX-I: Socio-demographic data Collection format

Code	Age	Sex	Ethnicity	Religion	Education	Occupation	Water source	Latrine availability	Shoe wearing

Ethincity
1. Oromo
2. Dawuro
3. Amhara
4. Yem
5. Kefa
6. Others

Religion
1. Muslim
2. Christian

Education
1. Literate
2. Illiterate

Occupation
1. Farmer
2. Student
3. Hose wife
4. Gov't employee

Water source
1. Pipe
2. Well
3. Stream/river

Latrine
1. Yes
2. No

Shoe
1. Always
2. Some times
3. Not at all

Annex-II Stool collection format

Patient ID_____

Sample Id_____

Modified Keto Ktaz Examination Result
Ova/ larvae/ Seen:

1. Hookworm___ Count/gm
2. Ascaris_____
3. *Trichuris trichuiria*_____
4. *H. nana*____
5. Strongloyid stercolaris___
6. *Entrobius vermicularis*____
7. Taenia Spp _____

Formol Ether Concentration technique
Ova/Cyst seen

1. Hookworm___
2. Ascaris_____
3. *Trichuris trichuiria*_____
4. *H. nana*____
5. *Entrobius vermicularis*____
6. Taenia Spp _____
7. Protozoan cysts

Annex-III Blood sample collection format

Patient ID_____
Sample Id_____

Malaria examination result/Microscopy 1. Positive 1.1-P/f
1.2-P/v
2. Negative

Paramax-3 ™ examination result 1. Positive 1.1-P/f
1.2-P/v
2. Negative

Annex- IV Sysmex Hemoglobin measurement format

Patient ID_____
Sample Code____

Hemoglobin result____ mg/dl

DECLARATION

This thesis is my original work, has not been presented for a degree in any universities and that all sources of materials used for the thesis has been gratefully acknowledge.

Name Fekadu Deimissie

Signature _____

Date July23, 2007

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