# INTESTINAL HELMINTHS AND ANEMIA IN A MALARIA ENDEMIC AREA, AREKA, WOLYTA ZONE

**By**

**SOLOMON ARAGIE**

*A Thesis Submitted the Graduate Studies Program, Addis Ababa University in partial fulfillment of the Requirements for the Degree of Master of Science in Biology*

**ADDIS ABABA UNIVERSITY**
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# ADDIS ABABA UNIVERSITY
# SCHOOL OF GRADUATE STUDIES

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Approved by Examining Board

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# LIST OF ABBREVIATIONS

ANOVA = Analysis of variance

DALY = Disability Adjusted Life Year

EPG = Egg Per Gram

g/dl = gram per deciliter

Hgb = Hemoglobin

ID = Iron Deficiency

IDA = Iron Deficiency Anemia

FAO = Food and Agriculture Organization

M s l = Meter above sea level

MCHC = Mean Corpuscular Hemoglobin Concentration

MCV = Mean Corpuscular Volume

NO = Nitric Oxide

RNI = Reactive Nitrogen Intermediates

RBC = Red blood cell.

SNNPR = Southern Nations Nationalities and Peoples' Region

SPSS = Statistical package for social sciences.

TDS = Trichuris Dysentery Syndrome

TNF = Tumor Necrosis Factor

UNICEF = United Nations Children's Fund

UNCHA = United Nations Office For Coordination Of Humanitarian Affairs

WHO = World Health Organization.

# ABSTRACT

The overall aim of the study was to determine the contribution of intestinal helminth infections to the prevalence of anemia caused by malaria in a population living in a poor small town, Areka. Malaria and helminth infections were highly prevalent in the study population. The severity of anemia due to malaria and helminth infections alone and in malaria–helminth co-infections was assessed. Patients (n = 767) who visited Areka Health Center, students from Areka Senior Secondary School and persons who accompanied patients to the Health Center were randomly screened for anemia, malaria and helminth infections. Uninfected individuals (n = 291) were taken as controls. Anemia was defined as Hemoglobin(Hgb) less than 11 g/dl in children < 6 years, < 12 g/dl in children 6-14 years and females >14 years and < 13 g/dl in males above the age of 14 years. The prevalence of anemia in cases (individuals who were positive for either malaria or helminth infections) was 45.1 % and in the control group 14.8 %. Severe anemia (Hgb < 7 g/dl) was 7.8 % in the malaria and/or helminth infected and was none in the controls. Infected children less than 6 years of age were more anemic, with an overall prevalence of 52.8 %. Anemia was more prevalent in infected adult females as compared to infected adult males (37.6 % and 22.6 %, respectively). Children less than 6 years of age were more vulnerable to malaria, whereas children 6-14 years old had a higher prevalence of *A. lumbricoides* (18.2%) & *T. trichiura* (14.2%). Hookworm infection prevalence was the highest (19.4%) in the age group14 years and above. The main risk factors for anemia were identified as malaria in all age groups, hookworm infection in adults and malaria-hookworm co-infection in children below the age of 14 years. Data indicated that no association with anemia could be drawn for *A. lumbricoides* and *T. trichiura* infections. Anemia control should include prevention and treatment of malaria and hookworm infections and by a deworming program of the general population. Nutritional iron deficiency prevalent in the study community may have exacerbated anemia. Thus dietary iron supplementation in the nutrition of the general population must also be considered to control anemia in the population studied.

# 1. INTRODUCTION

Anemia is defined as a reduction in the oxygen-carrying capacity of the blood as a result of fewer circulating erythrocytes than is normal or a decrease in the concentration of hemoglobin (Hgb) (WHO, 1972). Hemoglobin concentration levels below which anemia is likely to be present are usually categorized as follows: children 6 months – 6 years: 11 g/dl; children 6-14 years: 12 g/dl; adult males: 13 g/dl; non-pregnant females: 12 g/dl; and pregnant females 11 g/dl. Severe anemia has been defined as < 7g/dl (WHO, 1989).

Anemia may result from defects at any stage of red cell and hemoglobin production or when an increased rate of red cell destruction (hemolysis) exceeds the capacity of the bone marrow to mount a compensatory increase in production. Changes in the relationship between red blood cell count and plasma volume may also result in a reduced hemoglobin concentration. Such changes occur physiologically in pregnancy where red blood cell volume is increased less markedly than plasma volume (Brabin, 1985).

Anemia is a major health problem worldwide affecting approximately 30 % of the world's population (De Maeyer and Adiels -Tegman, 1985). The problem is predominantly found in developing regions (especially South Asia and Sub-Saharan Africa) where 36 % of the total population was estimated to be anemic compared to only 8 % in developed nations (WHO, 1992). Infants, preschool children, adolescents and women of child bearing age particularly pregnant women, are at a great risk of developing anemia. According to WHO (1992), 56 % of pregnant women in the developing countries and 18 % in the developed countries are anemic, and in Africa the estimated prevalence in pregnant women is

50 % - 60 % and an estimated 37 % of school age children are also affected. However, adult males may also be at risk, especially where there is inadequate food intake and / or a frequent parasitic infection, a situation which is common in developing countries. The estimated prevalence of anemia for this group is 18 % and 3 % in developing and developed countries, respectively (De Maeyer and Adiels-Tegman, 1985). Several community and hospital-based studies have documented the magnitude of anemia in malaria endemic areas of Africa. In community studies, the prevalence of anemia in children ranged from 49-89 %, and the prevalence of severe anemia from 1.3-6.4 % (WHO, 1990).

Anemia has not been considered as a serious public health problem in Ethiopia. This is because in adults rates of iron deficiency are presumed to be relatively low due to the staple food, Teff ( *Eragrostis teff*), whose iron content is largely enriched from the soil on which it is threshed (Hofvander, 1968). Despite this, higher rate of 57 % have been reported in high risk groups such as pregnant women in rural Ethiopia (Desalegn 1993). Shamebo (1987) reported anemia, excluding anemia due to chronic diseases as one of the common hematological disorders in hospitalized patients accounting for 21.1% of the disorders. Iron deficiency anemia, megaloblastic and hemolytic anemia due to *P .falciparum* were the common types of anemia reported by (Shamebo, 1987). A relatively high prevalence of anemia in the Northern part of Ethiopia was reported with a prevalence rate of 40.5 % (Zein and Assefa, 1987) and 47.2 % in children was reported from this same localities by Zein (1991). Among the young children (6month- 6 years old) an overall anemia prevalence of 47 % was determined while a survey in the rural town of Ijaji found a similar rate, 48 % among preschool children (Iannotti, *et al*., 1998). The etiologies of anemia were intestinal helminth infections and poor iron bioavailability of the diet. No national prevalence data for anemia are reported in Ethiopia.

Anemia has detrimental physical, social and economical effects. Even mild to moderate anemia affects the sense of well being, resulting in fatigue, stress and decrease in work capacity. Severe anemia that occurs in developing countries are a major cause of maternal mortality and morbidity (WHO, 1992) and attributed as a direct or indirect cause of about 26 % of maternal death in Africa. Severe anemia may cause cardiac failure and death, whereas chronic anemia is considered to be only contributory, especially in cases of hemorrhage and infections (Brabin, 1985). Anemia during pregnancy is also associated with an increased risk of intrauterine growth retardation, premature delivery and low birth weight, resulting in an increase in prenatal mortality (Brabin *et al*., 1990; Fleming, 1989).

The most severe consequences of Iron Deficiency Anemia (IDA) are the impaired mental development and physical coordination in infants and toddlers which are presumably due to cerebral changes (Grantham-McGregor & Ani, 2001). Longitudinal studies have shown that these effects are not fully reversible by treatment with iron, since children with IDA during infancy continued to have poorer cognition and school achievements, and had more behavior problems into middle school, although IDA had successfully been treated during infancy (Lozoff *et al*., 2000). IDA is also associated with reversible abnormalities of immune function; however, there is no conclusive evidence that IDA increases the rate of infections. Further, reduced work performance and productivity have also been associated with IDA and are probably related to reduced oxygen transport (Gilgen *et al*., 2001).

The physical manifestations of anemia include the nonspecific symptoms of anemia, such as tiredness, lethargy, and a general feeling of lack of energy (Beard, 2001). Further, the clinical symptoms of anemia include glossitis, angular stomatitis and esophageal webbing (abnormalities to the mucosa of mouth and esophagus), blue sclera (blue tinge to whites of eyes), and koilonychias (spoon nails).

IDA is also associated with the perversions of taste which can lead to the consumption of non-food items (pica) or a craving for ice (pagophaga) (Beard, 2001; Lynch & Green, 2001).

Anemia can be classified by cytometric schemes (i.e., those that depend on cell size and hemoglobin-content parameters, such as Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin Concentration (MCHC)), erythrokinetic schemes (those that take into account the rates of RBC production and destruction), and biochemical/molecular schemes (those that consider the etiology of the anemia at the molecular level) (Harris and Kellermeyer, 1970).

Cytometric classification of anemia makes use of parameters that depend on cell size and Hb content. Accordingly anemia can be categorized into three groups (Harris and Kellermeyer, 1970).

1. Normochromic, normocytic anemia (normal MCHC, normal MCV). These include: anemia of chronic disease, hemolytic anemia (those characterized by accelerated destruction of RBC's), anemia of acute hemorrhage and aplastic anemia (those characterized by disappearance of RBC's precursors from the marrow)
2. Hypochromic, microcytic anemia (low MCHC, low MCV). These include: iron deficiency anemia, thalassemias and anemia of chronic disease.
3. Normochromic, macrocytic anemia (normal MCHC, high MCV). These include: vitamin B12 deficiency and folate deficiency

Causes of anemia in malaria-endemic regions of Africa, is frequently multifactorial in origin. The Common causes include:

***1.1. Nutritional deficiency*:** that is, iron deficiency (ID), Folate deficiency, Vitamin B12 and Vitamin A deficiency.

### 1.1.1. Iron Deficiency (ID)

ID develops when the amount of iron absorbed from the diet is lower than the amount of iron needed to cover the physiological iron requirements (Cook & Finch, 1979). Consequently, the population groups most at risk to develop ID are those with the highest iron requirements. These are infants, children, adolescents, and pregnant women who have additional iron needs due to growth as well as women of childbearing age who have higher iron losses due to menstrual blood loss (Cook & Finch, 1979).

ID is one of the most common and widespread nutritional disorders in the world. De Maeyer and Adiels-Tegman (1985) estimated, based on the evaluation of 523 studies, that 30 % of the world's population was anemic due to ID. Estimates of the prevalence of iron deficiency anemia (IDA) from 1990-1995 were similar to an earlier report with approximately 2 billion people being anemic (WHO/UNICEF, 2001).

ID can be caused by multiple factors; low dietary iron intake is one of the reasons and can, for example, result from energy restriction or a diet low in iron (Baynes, 1994). Low iron absorption can further be a result of poor dietary quality rather than low iron intake (Bothwell *et al*. 1989). For example, in Venezuela comparisons of the diets consumed by the different socio-economical classes showed that while iron content was higher in the diet of the lower social classes, the intake of meat and ascorbic acid was substantially lower and that of phytate (which inhibits iron absorption) was substantially higher than in higher social classes (Taylor *et al*., 1995). This was explained by the high cost of meat and fresh fruits which limits their consumption in underprivileged population groups, in developing as well as industrialized countries (Marx, 1997). It is thus not surprising that ID is most common among groups of low socio-economic status (WHO/UNICEF, 2001). Further, it has been shown that in industrialized countries members of minority groups frequently suffer from IDA (Wandel, 1993), which has been

associated with the lack of dietary adjustment to the new environment (Marx, 1997).

ID can further be caused by increased blood loss or decreased iron absorption due to pathological causes. For example, hookworm and schistosomiasis infections cause blood loss from the intestinal mucosa. Infections with schistosomiasis can further cause hematuria (Stephenson, 1993). Pathological causes of gastrointestinal blood loss that also affect individuals include oesophagitis, gastritis, peptic ulcers, neoplasm, inflammatory bowel disease, and hemorrhoids (Beveridge *et al*., 1965).

Although the pathogenetic mechanism has so far not been fully understood, gastric *Helicobacter pylori* infections have been shown to cause IDA, as reviewed by Barabino (2002).

Other population groups that have an increased risk for ID due to high losses are blood donors and athletes. The increased iron need of athletes, especially of runners, is predominately due to additional gastrointestinal losses resulting from intravascular hemolysis. However, increased iron losses with sweat and increased demand for myoglobin and iron containing respiratory enzymes are further possible reasons for the so-called exercise-induced anemia (Weaver & Rajaram, 1992). Blood donations result in a substantial loss of iron (250 mg per donation) and have been associated with a decline in serum ferritin levels (Finch *et al*., 1977).

### 1.1.2. Folate Deficiency

Folate deficiency occurs when absorbed folate does not meet requirements over time. Folic acid is present in all foods of plant and animal origin, particularly liver, leafy vegetables, fruit, pulses, and yeast. In a mixed diet, absorption may be around 70%. An individual may become folate depleted as a consequence of a folate-deficient diet, often seasonal, or from malabsorption

following systemic infections or tropical sprue (Fleming, 1989). Pregnancy and lactation exert a particularly high demand for folate, and demand may have pathological consequences as a result of malarial hemolysis or hemoglobinopathies. Tissue folate stores may be depleted in up to one-third of pregnant women worldwide (FAO/WHO, 1970) and its prevalence may be particularly high in Africa and Asia (Fleming, 1989). Folate deficiency may lead to anemia (Fleming and Werblinska 1982), increased susceptibility to infections, low birth weight, neural tube defects, delayed growth in early childhood and adolescence (Brabin, 1982) and an increased risk of coronary heart disease (Morrison *et al*., 1996). It has been described as a frequent complication of protein-energy malnutrition in both West and southern Africa (Margo *et al*., 1978) and as contributing to anemia (Fleming and Werblinska, 1982).

### 1.1.3. Vitamin B12 deficiency

Vitamin B12 is present in animal foods, but not plants (unless contaminated with Bacteria), and is stored in the liver for long periods. Depletion of body vitamin B12 stores takes years, and vitamin B12 deficiency resulting from nutritional inadequacy is rare. Its deficiency produces megaloblastic anemia due to its role in folate metabolism (Fleming, 1989).

Common symptoms of megaloblastic anemias are weakness in the legs, difficulty in walking and balancing, tingling and numbness in the hands and feet, and nerves becoming highly responsive to any mild stimulation. Vitamin B12 deficiency can lead to mental confusion, mild depression, apathy or irritability and can progress to dementia (Fleming and Werblinska, 1982). Victims may also suffer from loss of appetite and weight, indigestion and periodic diarrhea. In its early stages, these symptoms can be reversed but if the deficiency is not caught within a few months, the damage can be permanent. Vitamin B12 is required for proper nerve function, including the nerve cells or neurons in the brain needed for memory. Vitamin B12 also

works with two other B vitamins, folate or folic acid and vitamin B6, to maintain proper levels of homocysteine, which is naturally produced in the body as a result of the breakdown of proteins. If homocysteine levels become elevated, such as when B vitamins are low, the risk of heart disease, stroke and Alzheimer's disease and certain forms of dementia increases (Fleming and Werblinska, 1982).

### 1.1.4. Vitamin A deficiency

Vitamin A is involved in mobilizing stored iron, and poor vitamin A status has been reported to be associated with altered iron metabolism and iron-deficiency anemia (Mejia and Arroyave 1982). Sommer and West (1996) have summarized studies in which vitamin A supplementation was found to produce a hemoglobin response, mostly on the order of about 1 g/dl. In a randomized controlled trial in anemic preschool children in Ethiopia, iron or vitamin A supplemented children showed an increase in hemoglobin (1.9 and 1.2 g/dl, for iron and vitamin A supplementation, respectively). However, the largest increase in hemoglobin (2.2 g/dl) occurred in children receiving both vitamin A and iron (Abdulaziz, 1997). Also in a randomized placebo-controlled trial in Nepal, weekly supplementation with 23 000 IU vitamin A as retinol or beta-carotene resulted in a 45 % reduction in anemia (defined as low maternal hemoglobin <10 g/dl) among women who did not have hookworm infection (Stoltzfus *et al.*, 1997).

## *1.2. Malaria*

Malaria is thought to be the primary cause of severe anemia (Hgb < 7 g/dl) in at least 50 % of subjects living in malaria-endemic areas (Anstey *et al.*, 1999). A study in Tanzania has confirmed the role of malaria as the largest contributor to the etiology of severe anemia in infants in highly endemic areas, accounting for about 60 % of all cases, compared with iron deficiency,

which accounted for about 30 % of severe anemia episodes (Menendez *et al*. 1997).

Severe hypoglycemia and anemia affect African children and pregnant women in holoendemic areas (Marsh, 1992). Guyatt and Snow (2001) suggested that approximately 400,000 pregnant women develop moderate or severe anemia each year in Sub-Saharan Africa as a result of malaria infection.

In holo- or hyper-endemic areas, there is an increased incidence of maternal anemia associated with *falciparum* malaria (Greenwood *et al*., 1989), but most women are asymptomatic. Severe anemia is often a complication of acute febrile malaria in children, but may also occur in people with asymptomatic *P. falciparum* infection (Anstey *et al*., 1999; Kurtzhals *et al*., 1999).

Anemia is an inevitable consequence of malaria resulting from a combination of mechanisms, principally increasing rates of destruction and removal of parasitized and non-parasitized red cells and decreasing the rate of erythrocyte production in the bone marrow (Philips and Pasvol, 1992). Some of the mechanisms of 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). The various pathological mechanisms involved in anemia are complex, although evidence suggests that TNF is involved in the bone marrow depression and erythrophagocytosis seen during the terminal *P. vinckei* infections in mice (Rickman *et al*., 1991).

## *1.3. Intestinal Helminth infections*

Intestinal helminths affect over one quarter of the world's population at any one time.

chronic blood loss due to hookworm, Schistosomes and infection with *Trichuris trichiura*, particularly, Trichuris dysentery syndrome (TDS) are a

significant contributor to anemia (Cooper and Bundy 1989). *Ascaris lumbricoides* may also contribute (Stoltzfus *et al*., 1997), but in isolation it is unlikely to result in anemia.

### 1.3.1 Hookworm

Hookworm infection in humans is caused by an infection with the helminth nematode parasites *Necator americanus* and *Ancylostoma duodenale* and is transmitted through contact with contaminated soil with third-stage infective larvae, which either penetrate the skin (as do both *N. americanus* and *A. duodenale*) or when they are ingested (*A. duodenale* only) (Hawdon and Hotez, 1996). *N. americanus* is the most common hookworm worldwide, whereas *A. duodenale* is more geographically restricted. It is one of the most common chronic infections, with an estimated 740 million cases in areas of rural poverty in the tropics and subtropics (De Silva *et al*., 2003). The greatest number of hookworm cases occur in Asia, followed by sub-Saharan Africa (De Silva *et al*., 2003).

The major hookworm-related injury in humans occurs when the adult parasites cause intestinal blood loss (Hotez and Pritchard, 1995). The term "hookworm disease" refers primarily to the iron-deficiency anemia that results from moderate or heavy infection. 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. Capillaries and arterioles are ruptured not only mechanically but also chemically, through the action of hydrolytic enzymes (Hotez and Pritchard, 1995). To ensure blood flow, the adult hookworms release anticlotting agents (Stanssens *et al*., 1996). The hookworm ingests a portion of the extravasated blood. Some red cells undergo lyses, thereby releasing hemoglobin, which is digested by a cascade of hemoglobinases that line the gut of the parasite.

In chronic intestinal blood loss, iron-deficiency anemia and hypoalbuminemia develops when blood loss exceeds the intake and reserves of host iron and protein (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) of 40 to 160 worms is associated with hemoglobin levels below 11 g/dl (Bundy *et al*., 1995). However, other studies have shown that anemia may occur with a lighter hookworm burden (Oisen *et al*., 1998). Because infection with *A. duodenale* causes greater blood loss (0.2 ml blood /day) than does infection with *N. americanus*,( 0.03 ml blood /day) the degree of iron-deficiency anemia induced by hookworms depends on the species (Oisen *et al*., 1998). When iron stores in the host become depleted, there is a direct correlation between the intensity of hookworm infection (typically measured by quantitative egg counts) and the reduction in hemoglobin, serum ferritin, and protoporphyrin levels (Stoltzfus *et al*., 1997).

Hookworm infection is considered to be a major health threat to adolescent girls and women of reproductive age, with adverse effects on the outcome of pregnancy (Bundy *et al.*, 1995). An estimated 44 million pregnant women are infected with hookworm worldwide, with 7.5 million in Sub-Saharan Africa alone (Crompton, 2000). And, the World Health Organization estimates that because of increased physiological demands for iron during pregnancy combined with malnutrition, 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 prematurity and low birth weight (WHO, 2002).

### 1.3.2. *Trichuris trichiura*

*Trichuris trichiura* causes host injury both through direct effects by invading the colonic mucosa and through the systemic effects of infection. The cecum is the preferential site for invasion although heavy infections will extend throughout the colon and even distally to the rectum (Hotez, 2000). The adult parasite leads both an intracellular and an extracellular existence in which the attenuated anterior end becomes embedded in epithelial tunnels it creates by secreting novel pore forming proteins (Drake *et al*, 1994), while the larger posterior end extrudes into the lumen. Inflammation at the site of attachment results both from disruption of the normal colonic architecture as well as host inflammation comprised of macrophages and pro- inflammatory cytokines in the lamina propria (MacDonald *et al*, 1994). Several investigators have pointed out the clinical similarities between the pediatric colitis caused by *Trichuris* infection and more established causes of inflammatory bowel disease such as Crohn's disease and ulcerative colitis. These clinical features include impaired growth, anemia of chronic disease, and even finger clubbing (Hotez, 2000) The most severe manifestation of heavy infection among children is the *Trichuris* Dysentery Syndrome (TDS), which is associated with chronic dysentery, rectal prolapse, anemia, and growth stunting (Stephenson *et al*, 2000). Growth stunting is sometimes reversible with specific anthelminthic treatment and supplemental oral iron. Intellectual and cognitive impairments and delays are also associated with chronic heavy infections (Drake *et al*, 2000). These deficits sometimes reverse following anthelminthic therapy, particularly when treatments are linked to psychological support (Stephenson *et al*, 2000).

### 1.3.3 *Schistosomes*

*Schistosomes*: *S. mansoni* and *S. japonicum*, which are intestinal parasites, and *S. haematobium*, which infects the bladder and urinary tract can cause

anemia (WHO 1991). Although all three forms can cause severe anemia, the role of *S. haematobium* (urinary schistosomiasis) as a cause of anemia in populations is more firmly established than that of intestinal schistosomiasis (WHO, 2002). The geographic distribution of endemic *S. haematobium* is limited to Africa and the Middle East (WHO, 2002). In severe infection, blood in urine can be observed visually (red, brown, or foggy urine). Schistosomiasis is transmitted by swimming or wading in bodies of water that are habitats for infected snails. Infection is usually most prevalent and severe in children, older boys, and men, who are more likely to swim. Where urinary schistosomiasis is endemic, it should be considered in the treatment of severe anemia and in school-based anthelminthic chemotherapy programs (WHO 1991).

## *1.4. HIV/AIDS and other Chronic Diseases*

Up to 70 % of people with AIDS are anemic (Bain, 1997). The mechanisms include anemias of chronic disorders e.g. tuberculosis; HIV actions on stem cells and other precursors; an imbalance of growth factors due to HIV actions on macrophages, fibroblasts and T-cells; uncontrolled parvovirus B19 infection; nutritional deficiencies including vitamin B12, folate, pyridoxine; over dosage with trimethoprim, pyrimethamine, antiretroviral drugs; and anti-red cell antibodies (Bain, 1997). Severe anemia in nonpregnant adults requiring admission to African hospitals is virtually all due to the anemias of chronic disorders secondary to HIV and tuberculosis (Fleming, 1997). In a Kenyan study, the attributable mortality fractions for severe anemia and HIV infection among women of reproductive age were 31 % and 75 % (Zucker *et al*. 1994). Anemia was found in 55 % of these women and severe anemia (Hgb less than 7g/dL) in 6 %. Severely anemic women were significantly more

likely to be HIV positive (38 % Vs 17 %) and more likely to die (10.7 % Vs 1.4 %) than other women, respectively.

Anemia is also seen in individuals suffering from chronic infections and noninfectious inflammatory diseases (such as rheumatoid arthritis), and neoplasms (Harris and Kellermeyer, 1970). The molecular basis for anemia of chronic disease is not well known.

### *1.5. Hemoglobinopathies*

Among several types of anemia caused by inherited abnormalities of RBCs, the most common are sickle cell anemia and thalassemia. These disorders are other known causes of anemia reported in Africa (Fleming, 1997). Where health services are inadequate, mortality is high among those with the severe type of Hemoglobinopathy (Fleming, 1997).

Sickle cell anemia is an inherited disease predominantly of the black race (Fleming, 1997). This anemia is characterized by abnormal hemoglobin structure and sickle-shaped RBCs. The abnormal sickle-shaped RBCs are damaged or destroyed as they pass through the circulatory system.

Thalassemias are a group of inherited anemias caused by abnormal hemoglobin(Harris and Kellermeyer, 1970). The abnormal hemoglobin may cause abnormal red blood cells as well as low hemoglobin levels. Thalassemias most commonly affect people of Mediterranean descent, but some types also affect peoples of Africa, Asia, India, and the South Pacific (Harris and Kellermeyer, 1970).

### *1.6. Malaria and Helminthic concomitant infections*

Anemia undoubtedly is a major health problem in malarious areas of tropical Africa and its cause is frequently multifactorial. In many regions of Sub-Saharan Africa, intestinal helminth infections particularly hookworm disease overlaps geographically with *falciparum* malaria where much of the

morbidity associated with both disease results from anemia (Guyatt and Snow, 2001). Because of other factors that frequently contribute to anemia in many malarious areas including malnutrition and genetic factors; it is difficult to estimate the percentage of anemia in a particular population that can be attributed to intestinal helminth infections. However, there are attempts to determine the contribution of malaria and hookworm in the development of anemia in malaria endemic areas of Africa. For instance, Disability Adjusted Life Year (DALY) reported the percentage of malaria related anemia to be 18 % of all anemias in endemic areas and a higher prevalence of malaria related anemia among pediatric communities in three countries of Africa (Murray and Lopez 1996). The study also showed that in areas with a low prevalence of *P. falciparum* infection (less than 25 %) the median prevalence of mild anemia was 31.7 %, whereas in areas where the prevalence of *P. falciparum* infection was in excess of 25 %, the median prevalence of anemia was 75 %. Mendenze *et al.* (1997) reported the role of malaria as the largest contributor to the etiology of severe anemia in infants in highly endemic areas, accounting for about 60 % of all cases compared to other causes of anemia. Some studies showed the association of high prevalence of anemia to malaria by estimating the Hgb concentration improvements with treatment of *P. falciparum* infection. For example, Verhoeff *et al.* (1997) reported an average improvement of at least 1g/dl in the moderately anemic, and for more anemic individuals improvement is greater, reaching estimates of 3 g/dl with treatment of *P. falciparum.*

It has been observed that hookworm infection is also strongly correlated with anemia among children and pregnant women in malaria-endemic areas. For instance, estimates in Kenya and Nepal suggest, respectively hookworm infection accounts for 30 % and 41 % of moderate and severe cases of anemia among pregnant women (Hgb less than 9 g/dl) (Stoltzfus,1997). However, the extent of association of intestinal helminth infections with anemia among all age groups, in malaria endemic areas is largely unreported.

The occurrence of intestinal helminth infections is high and a number of surveys on intestinal helminth parasites in Ethiopia have been carried out (McConnell and Armstrong, 1976; Tedla and Jemaneh, 1985). Hence the species, prevalence and distribution of the parasites are well known for most parts of Ethiopia.

Some authors also reported the association of hookworm infection and anemia in both malaria endemic and non endemic areas of the country (Shamebo, 1987; Zein and Assefa, 1987; Desalegn, 1993; Tsegaye, *et al*.1999). However, the contribution of intestinal helminth infections as well as malaria in the development of anemia, the concomitant occurrence of *malaria* and intestinal helminth infections and their clinical manifestations in malarious areas of the country have not been dealt in any detail in Ethiopia.

# 2. OBJECTIVES

## *2.1 General objective*

- To determine the contribution of intestinal helminth infections (Hookworm, *Trichuris trichiura* and *Ascaris lumbricoides*) in the development of anemia in a malaria endemic area.

## *2.2 Specific objectives*

- To assess the prevalence of anemia in a malaria endemic area.
- To determine the risk of developing anemia in malaria as well as intestinal helminth infections in endemic areas
- To compare the severity of anemia due to malaria, intestinal helminth infections and malaria/intestinal helminth co- infections.

# 3. MATERIALS AND METHODS

## *3.1 The Study area*

The study was conducted in Areka, Bolosso Sore district of Wolyita Zone found in the South Nations Nationalities and Peoples' Region (SNNPR). Bolosso Sore district is located 415 kms south of Addis Ababa and 30 kms away from the zonal capital, Sodo (Fig.1). Areka, the district main town was chosen for this study for reasons of accessibility. The area is located at an altitude range of 1730 and 1830 m.s.l. There is a high population density in the area which is estimated to be about 450 persons per $km^2$. The area like most parts of the country has two periods of rainfall; the "big" and "small" rains of June to September and March to April, respectively, receiving an average annual rainfall of about 1528.1 mm. The average annual temperature is 19.5 $^0$C and the yearly maximum and minimum temperature is about 25.6 $^0$C and 13.7 $^0$C, respectively.

The population is predominantly Wolyta and agriculture is the main source of income in the area, where the farming system is characterized by small-scale production of mixed crops and livestock. In this area, several root crops (yam, cassava, taro and sweet potato) as well as enset are planted soon after a "small" rain from April to June. Cereals, such as teff, wheat, maize and beans are also planted during the "big" rains, which last from June to September.

From the dietary information, the major staple diets are maize and enset followed by sweet potato and a combination of maize and enset and maize and sweet potato. Food security is fragile in this woreda due to a high population density, large family size, small land holdings per household and heavy reliance on rain (Amcross, 2000). Nutrition survey carried out in Bolosso Sore woreda by Oxfam in 2000 found that an overall prevalence of 45.1 % global malnutrition of which 20.4 % was severe (Oxfam, 2000). This

indicated that malnutrition rates were alarmingly high in Bolosso Sore Woreda and it has recorded one of the highest levels of malnutrition when compared with some of other woreda nutrition surveys (Oxfam, 2000). This high prevalent malnutrition and non- haem iron diets consisting of maize, enset and root crops which predominates in the area may dispose the population to iron deficiency anemia.

There are no published reports on the relative prevalence of malaria and anemia in the town of Areka. But, according to the records of health facilities in the town, there is a high prevalence of malaria, *P. falciparum* being the dominant causative agent. For instance, among patients who visited Areka health Center in 2002 / 2003 and 2003/ 2004 about 41.7 % and 42.4 % respectively were identified as malaria patients. Data from these medical records indicate malaria as the principal cause of morbidity amongst the top ten causes. The occurrence of intestinal helminth infection is also high (Lopiso, *et al.*, 2002).

Medical care for patients is primarily provided by Areka Health Center, which is staffed with one health officer, three medical laboratory technicians, five nurses and other few auxiliary staff members.

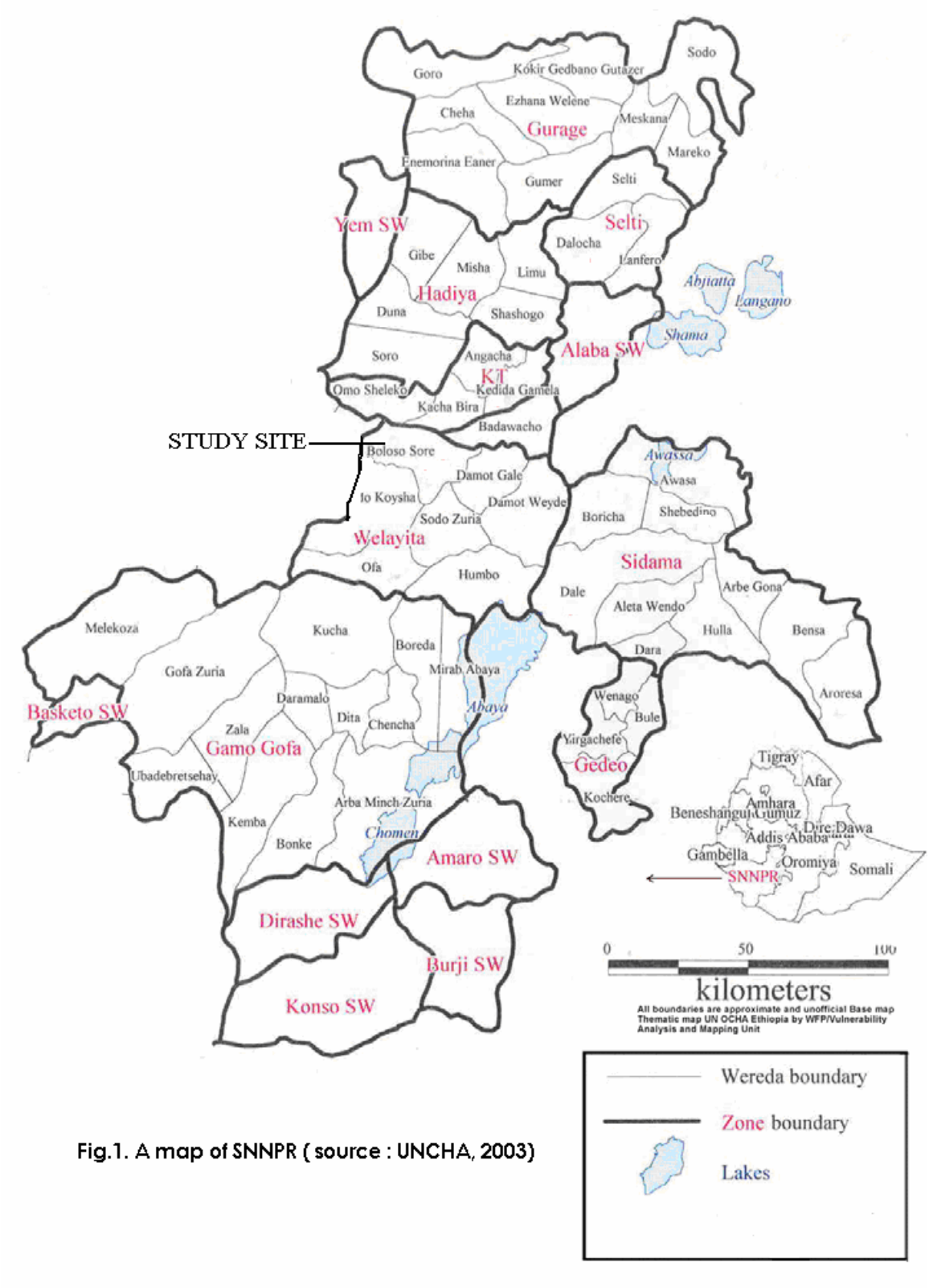


Fig.1. A map of SNNPR ( source : UNCHA, 2003)

## *3.2 The Study population*

The study population consisted of all age groups (range: 6 month to 65 years old), who visited Areka Health Center for medication, students from Areka Senior Secondary School and from people who accompanied patients to the Health Center during the periods of October – November 2004( after a "big" rain) and from January - April 2005 ( after a "small" rain) . Subjects were randomly selected and screened for anemia, malaria and intestinal helminth infections. A total of 767 subjects were enrolled and according to their parasitological manifestations patients were grouped into three Sub-groups (1) only malaria positive (2) only intestinal helminth infections positive (Hookworm, *Ascaris lumbricoid*es, and *Trichuris trichiura*) and (3) positive for both malaria and intestinal helminth infections. Around 291 uninfected subjects were taken as control.

## *3.3. Parasitological examination*

### *Kato-Katz Method for determination of helminth infections*

Stool samples were processed using 41.7 mg templates according to the modified Kato–Katz technique (Peters *et al.,* 1980). A small amount of faecal material was placed on scrap paper and a piece of nylon screen was pressed on top so that some of the faeces sieved through the screen and accumulated on top. A flat-sided spatula was scraped across the upper surface of the screen to collect the sieved faeces. A template was placed on the slide and the sieved faeces was added with the spatula so that the hole in the template was completely filled. The spatula was passed over the filled template to remove excess faeces from the edge of the hole. The template was removed carefully so that a cylinder of faeces was left on the slide. The faecal material was covered with a pre-soaked cellophane strip. The slide was inverted and the

faecal sample was pressed firmly against the hydrophilic cellophane strip to spread evenly. The slide was then placed on the bench with cellophane upwards to enable the evaporation of water while glycerol cleared the faeces. For all helminths, except hookworm eggs the slide was kept for one or more hours at room temperature to clear the faecal material, prior to microscopic examination.

***Blood Film determination for malaria parasites***

Malarial infections were determined from thick and thin films of finger-prick blood fixed and stained with Giemsa stain. Thick films were considered negative if no parasite was found in 100 high power fields (100 x objectives) (WHO, 1980). With positive slides, the percentage of parasitaemia was determined by counting the number of infected red cells in 1000 RBCs and converted to percentage. Parasite species were identified from thin films.

## *3.4. Hemoglobin Determination*

Peripheral blood was collected from a finger prick using a safety lancet. One drop was taken for hemoglobin measurement and further sample from the same finger prick was taken for parasitological analysis. Hemoglobin level was determined in finger prick blood using a portable, battery-operated hemoglobinometer (HemoCue TM, Angelholm, Sweden) (Cohen and Seidi-Friiedman,1988). According to the manufactures instruction, site for blood collection was cleaned with alcohol-soaked cotton and pricked with a blood lancet, and wipe away with dry cotton. The next drop of blood was used to fill the cuvette by touching the cuvette tip into the middle of the drop of blood until completely filled. The filled cuvette was then put on the holder and pushed into the HemoCue instrument. The Hgb value was displayed in g /dl after approximately 45 seconds.

Anemia was defined as a Hgb value less than 11 g/dl (in children < 6 years), less than 12 g/dl (in children 6-14 years and in female adults above the age of 14 years) and less than 13 g/dl (in male adults above the age of 14 years). Severe anemia is defined as Hgb value of less than 7 g/dl (Akinkugbe, 1980; WHO, 1989).

## 3.5. Ethical considerations

Diagnosis was done using sterile and disposable materials. Only a laboratory technician took the blood sample and all activities in clinical examination as well as diagnosis were supervised by health personnel. Individuals diagnosed positive for malaria and/or intestinal helminth infections were treated free of charge. Those individuals who were positive for malaria were provided with appropriate anti-malaria drugs (Quinine and Fansidor) as per Ethiopian Ministry Of Health recommendation. Those positive for helminth infections were provided with anti-helmenthic drug (Mebendazole). Only volunteer patients were included in the study. Ethical clearance was obtained from the ethical clearance committee of Department of Biology, Addis Ababa University.

## 3.6. Statistical analysis

The estimated sample size to establish the prevalence of intestinal helminths and malaria was 412 for all age groups, assuming that the prevalence of intestinal helminths and malaria was 25.5% and 42%, respectively with a precision of ± 10%.

Statistical analysis was carried out using SPSS Version 13 Software. Chi-square tests were used to test for differences in proportions, while the ANOVA test was used to assess differences in intensities of infection. A significance level of 0.05 was used for all tests. Univariate analysis was used to identify predictors of reduced Hgb value.

# 4. RESULTS

A total of 767 subjects were randomly selected from patients who visited Areka Health Center, Areka Senior Secondary School and from people who accompanied patients to the health center. Of which 336 (204 cases and 132 control) and 431 (274 cases and 157 control) were included in the big and small rain seasons, respectively. The mean age of the total population was 17.6 (range: 6 month – 65 years) and the male to female proportion was 55.5 % and 45.5 %, respectively. The age and sex distribution of the study population is depicted in Table 1.

Table1. The age and sex distribution of the study participants, Areka, 2004 /2005.

| STUDY PARTICIPANTS | < 6 YEARS | | 6 – 14 YEARS | | > 14 YEARS | | TOTAL |
|---|---|---|---|---|---|---|---|
| | M | F | M | F | M | F | |
| Cases [a] | 45 | 39 | 62 | 46 | 159 | 125 | 476 |
| Controls | 30 | 35 | 21 | 16 | 109 | 80 | 291 |
| Total | 75 | 74 | 83 | 62 | 268 | 205 | 767 |

[a] Cases are individuals who were positive for either malaria or helminth infection.

**Malaria infection**

From a total of 767 blood smears collected from individuals of all age groups, 40.9 % of participants were found to be infected with malaria parasites. There was no significant difference in prevalence of malaria in between the two seasons (37.4% and 40.6% in small and big rains season, respectively). The parasite species detected were *Plasmodium falciparum* and *Plasmodium vivax*. The prevalence of *P. falciparum* and *P. vivax* was 30.2 % and 9 %, respectively. There were 11 cases (1.5 %) of mixed infections of *P. falciparum* and *P. vivax*. The mean parasitemia was 2.8 % (range: 0.5 % – 13.8 %). And there was no significant difference in mean parasitemia. The prevalence and parasitemia of malaria infection by age and sex is shown in (Table 2).

The prevalence of *P. falciparum* was significantly higher in children less than 6 years of age (47.6 %) than in children 6-14 years (46%) and adults greater than 14 years of age (32.4 %) ($P< 0.05$). But no sex difference was associated with the prevalence of malaria in all age groups, female (39%) and male (37.5%). Mean parasitaemia was significantly negatively correlated with age ($P < 0.05$) (Fig.2).

**Table 2.**Prevalence and intensity of helminth and malaria infections by age and sex in the participants, Areka, 2004/200.

| INFECTIONS | < 6 YEARS | | 6 – 14 YEARS | | > 14 YEARS | |
|---|---|---|---|---|---|---|
| | M | F | M | F | M | F |
| | 75 | 74 | 83 | 62 | 268 | 205 |
| Any helminth Prevalence (%) | 1.3 | 6.8 | 27.7 | 25.8 | 29.1 | 25.4 |
| Hookworm Prevalence (%) | 4 | 4.8 | 17.8 | 13.6 | 23.4 | 18.0 |
| Intensity : mean (epg) | 2248 | 254.4 | 709 | 396 | 410 | 800 |
| *A. lumbricoides* prevalence (%) | 2.8 | 4.0 | 16.4 | 20.0 | 10.8 | 16.6 |
| Intensity: mean (epg) | 2100 | 4300 | 2566 | 1356 | 2374 | 2584 |
| *T .trichiura* Prevalence (%) | 4.0 | 5.2 | 7.2 | 21.2 | 12.3 | 10.3 |
| Intensity mean(epg) | 595 | 120 | 268 | 539 | 248 | 445 |
| Double helminth infection Prevalence (%) | 1.3 | 2.5 | 9.2 | 16.2 | 8.6 | 8.8 |
| Triple helminth infection Prevalence (%) | 0 | 1.4 | 1.0 | 3.0 | 2.4 | 2.9 |
| *P. falciparum* Prevalence (%) | 47.9 | 46.2 | 42.6 | 48.9 | 35.0 | 29.1 |
| Mean percent Parasitemia | 4.8 | 4.4 | 3.7 | 3.5 | 2.9 | 2.8 |
| *P. vivax* Prevalence (%) | 20 | 17.6 | 9.6 | 8.1 | 3.7 | 10.2 |
| Mean percent Parasitemia | 3.7 | 4.5 | 5.8 | 4.3 | 3.2 | 4.2 |
| Malaria-any helminth co- infection prevalence (%) | 5.3 | 8.1 | 19.3 | 16.1 | 14.6 | 17.1 |
| Malaria-hookworm co-infection prevalence (%) | 4.0 | 5.4 | 14.5 | 8.5 | 12.9 | 11.2 |
| Malaria – *A. lumbricoides* co-infection prevalence (%) | 0 | 4.1 | 6.0 | 9.7 | 3.0 | 6.3 |
| Malaria- *T. trichiura* co- infection prevalence (%) | 2.7 | 1.4 | 4.4 | 9.7 | 5.2 | 5.9 |

Abbreviations:
Epg: eggs per gram

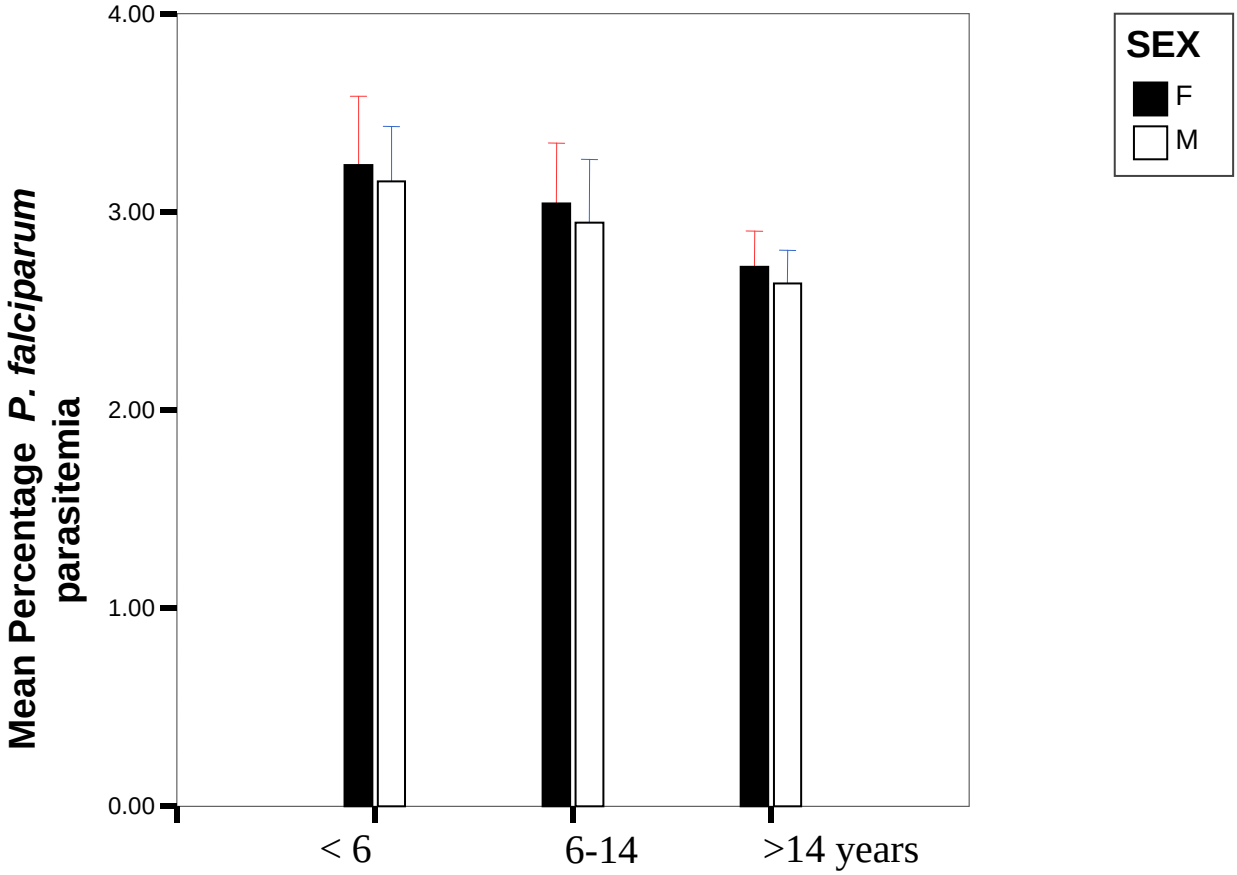


**Fig.2.** Mean Percentage *P. falciparum* parasitaemia by sex and age group, Areka,2004/2005.

**Intestinal helminth infections**

Overall, 35.9 % of study subjects were infected either with hookworm, *Ascaris lumbricoides* or *Trichuris trichiura*. The most prevalent infection was hookworm infection (25.6 %) followed by *A. lumbricoides* (12.3 %) and *T. trichiura* (10.5 %). Mixed infections were relatively less common (8 % double and 2.2 % triple infection) (Table.2). The prevalence of hookworm infection was significantly higher (P< 0.001) in the age group 14 years and above than

younger age groups (Table. 3). The prevalence of *A. lumbricoides* and *T. trichiura* infections were significantly higher ($P < 0.001$) in 6 - 14 years than other age groups (Table 3). On the other hand, the prevalence of hookworm, *A. lumbricoides* and *T. trichiura* infections were not significantly different in both sexes.

Estimation of intensity of infection with intestinal helminths was based on the amount of egg load in the stool. It was determined that most helminth infections were light with only few moderate and rare heavy infections. Egg count for hookworm, *A. lumbricoides* and *T. trichiura* ranged from 24 to 9672 (mean 619.55), 48 to 14592 (mean 2443.14) and 24 to 3240 (mean 356.65) per gram of stool, respectively.

Chi-square test showed the prevalence of moderate and heavy infection with hookworm (>1000 epg) and with *A. lumbricoides* (> 5000 epg) to be significantly higher ($P < 0.05$) in the 6-14 years of age than in older and less than 6 years of age (Table 3). On the other hand, the prevalence of moderate and heavy infection of *T. trichiura* (>1000 epg) was significantly higher ($P< 0.05$) in the age group < 6 years than in other age groups (Table 3). No significant variation was seen with regard to sex.

The chance of harboring more than one infectious agent concomitantly by an individual was significantly greater ($P < 0.001$) in the age group 6 - 14 years (double infection) and in the age group above 14 years of age (triple infection) (Table. 3).

**Table 3**.Determination of percent prevalence and intensity of helminth infections in different age groups, Areka, 2004/2005.

| Age group | Hookworm | | | *A. lumbricoides* | | | *T. trichiura* | | | Double Helmin. Inf | Triple Helmin. Inf |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Overall prevalence | Intensity (epg) | | Overall prevalence | Intensity (epg) | | Overall prevalence | Intensity (epg) | | Overall prevalence | Overall prevalence |
| | | 1-1000 | > 1000 | | 1-5000 | > 5000 | | 1-1000 | > 1000 | | |
| <6 years | 4.0 | 87.5 | 12.5 | 3 | 84.6 | 15.4 | 4.9 | 87.5 | 12.5** | 1.8 | 0.6 |
| 6-14 years | 15.8 | 85.4 | 14.6** | 18.2*** | 60.0 | 40.0** | 14.2*** | 90.5 | 9.5 | 12.8*** | 2.0 |
| >14 years | 19.4*** | 90.2 | 9.8 | 13.7 | 86.2 | 13.8 | 11.3 | 92.6 | 7.4 | 8.6 | 2.7** |

Key:

*** The mean difference is significant at P<0.001(Chi- square test)

** The mean difference is significant at P< 0.05 (Chi- square test)

NB. All tests were compared between age groups.

**Anemia Prevalence**

The overall mean Hgb concentration of cases was 11.7g/dl (range: 3.2 to 18.8 g/dl) and that of control group was 13.7 g/dl (range: 9.2 to 18.5 g/dl). And the Hgb concentration of cases in between the two seasons was not significantly different (11.8 g/dl Vs 11.7 g/dl in the big and small rains season,

respectively). Anemia was prevalent in 45.1 % of cases and in 14.8 % of the control group. Severe anemia (Hgb less than 7 g/dl) was prevalent in 7.8 % of cases and non in the control group.

The Chi square test showed anemia to be significantly higher ($P < 0.001$) among children of age less than 6 years with a steady decrease of prevalence with increasing age (Table 4). In conformation of findings on anemia, the mean Hgb concentration was significantly lower ($P < 0.001$) in the age group less than 6 years compared to other age groups in infected individuals (Fig.3). Also, prevalence of severe anemia (Hgb < 7g/dl) was significantly higher ($P < 0.001$) in the age group less than 6 years than other age groups of cases (Table 4). The prevalence of anemia and severe anemia was higher in adult (age greater than 14 years) females than males ($P < 0.001$) (Table 4). In concurrence with anemia determinations the mean Hgb concentration was significantly lower ($P < 0.005$) in female than male adult cases and controls (Fig. 3).

**Table 4**. The percent prevalence of anemia and severe anemia in different age groups and sexes (Comparison among age groups and between sexes in all age groups), Areka, 2004/2005

| | < 6 years | | | 6 – 14 years | | | > 14 years | | |
|---|---|---|---|---|---|---|---|---|---|
| | cases | | | cases | | | cases | | |
| | M | F | total | M | F | total | M | F | total |
| Anemia | 23/45 (51.1%) | 32/59 (54.2%) | 55/104 (52.8%) *** | 29/62 (47%) | 21/46 (45.6% | 50/108 (46.2 %) | 36/159 (22.6%) | 47/125 (37.6%) *** | 83/284 (29.2%) |
| Severe anemia | 10/45 (22.2%) | 13/59 (22%) | 23/104 (22.1%) *** | 3/62 (4.8%) | 1/46 (2.2%) | 4/108 (3.7%) | 3/159 (1.9%) | 11/125 (8.8%) *** | 14/28 (4.9%) |

Key:

*** The mean difference is significant at $P<0.001$(Chi-square test)

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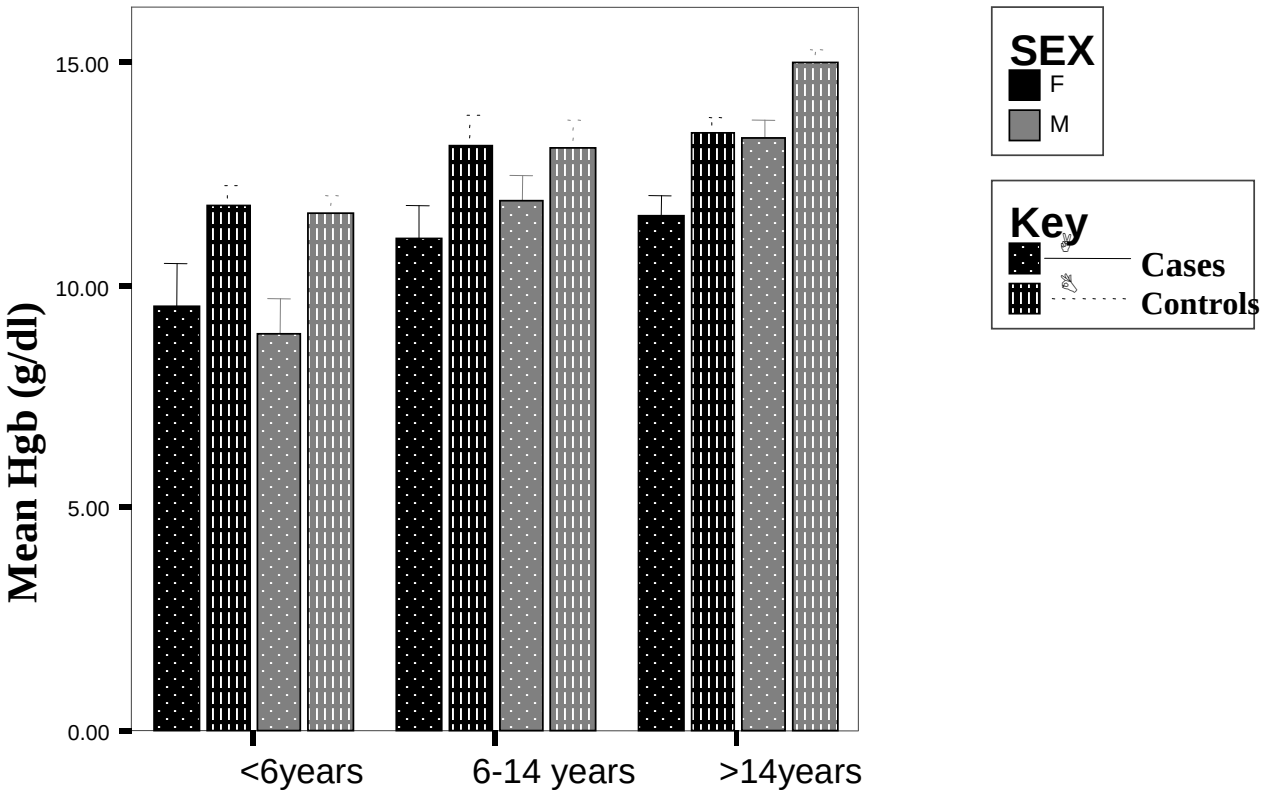


**Fig.3**. Mean Hgb concentration by age and sex of cases and control group, Areka,2004/ 2005

**Anemia and Infections**

The prevalence of anemia was significantly higher ($P < 0.001$) among malaria positive cases (58%) than any helminth (23.4 %), hookworm (27.9%), *A. lumbricoides* (13.8 %), and *T. trichiura* (15.6 %) positive cases and in the control group (14.8 %). And severe anemia was also significantly higher among malaria positive cases (10.4 %) (Table 5).This was expressed as a significantly lower ($P < 0.001$) mean Hgb concentration in malaria positive cases compared to the control group (Fig.4). Anemia was significantly associated with malaria parasitemia in that, the geometric mean malaria parasitemia was significantly higher among anemic (2.5%) than non-anemic (1.5%) malaria positive cases ($P< 0.05$). Significantly reduced ($P < 0.01$) mean Hgb concentration was detected in the malaria positive cases of less than 6 years (Fig.6). Female malaria positive cases above the age of 14 years were more anemic than the males of the same age category ($P < 0.05$) (Fig .6).

**Table 5**. Percent prevalence of anemia and severe anemia among cases with malaria, any helminth and malaria / helminth co-infections, Areka, 2004/ 2005.

| | Prevalence | Significant differences ($p< 0.05$) |
|---|---|---|
| Malaria [a] | | |
| Anemia (%) | 58*** | **a > b, c, d, e, j** |
| Severe anemia (%) | 10.4*** | **a > b, c, d, e, j** |
| Any Helminth [b] | | |
| Anemia (%) | 23.4** | **b > d, e, j** |
| Severe anemia (%) | 0.6 | NS |
| Hookworm [c] | | |
| Anemia (%) | 27.9** | **c > d, e, j** |
| Severe anemia (%) | 2.8*** | NS |
| Ascaris [d] | | |
| Anemia (%) | 13.8 | NS |
| Severe anemia (%) | 1.1 | NS |
| Trichuris [e] | | |
| Anemia (%) | 15.6 | NS |
| Severe anemia (%) | 0.0 | NS |
| Malaria & any helminth [f] | | |
| Anemia (%) | 58.3*** | **f > b, c, d, e, j** |
| Severe anemia(%) | 14.8*** | **f > b, c, d, e, j** |
| Malaria &hookworm [g] | | |
| Anemia(%) | 59*** | **g > b,c, d, e, j** |
| Severe anemia(%) | 24.1*** | **g < b, c, d, e, j** |
| Malaria &Ascaris [h] | | |
| Anemia(%) | 48.4*** | **h > b, c, d, e, j** |
| Severe anemia(%) | 14.1*** | **h > b, c, d, e, j** |
| Malaria & Trichuris [i] | | |
| Anemia(%) | 54.1*** | **i > b,c, d, e, j** |
| Severe anemia(%) | 21.6*** | **i > b, c,d, e, j** |
| Control [j] | | |
| Anemia (%) | 14.8 | NS |
| Severe anemia (%) | 0.0 | NS |

Chi-square test

*** The mean difference is significant at P<0.001.
** The mean difference is significant at P< 0.05
NS. Non - significant difference.
N.B. comparisons were among all groups

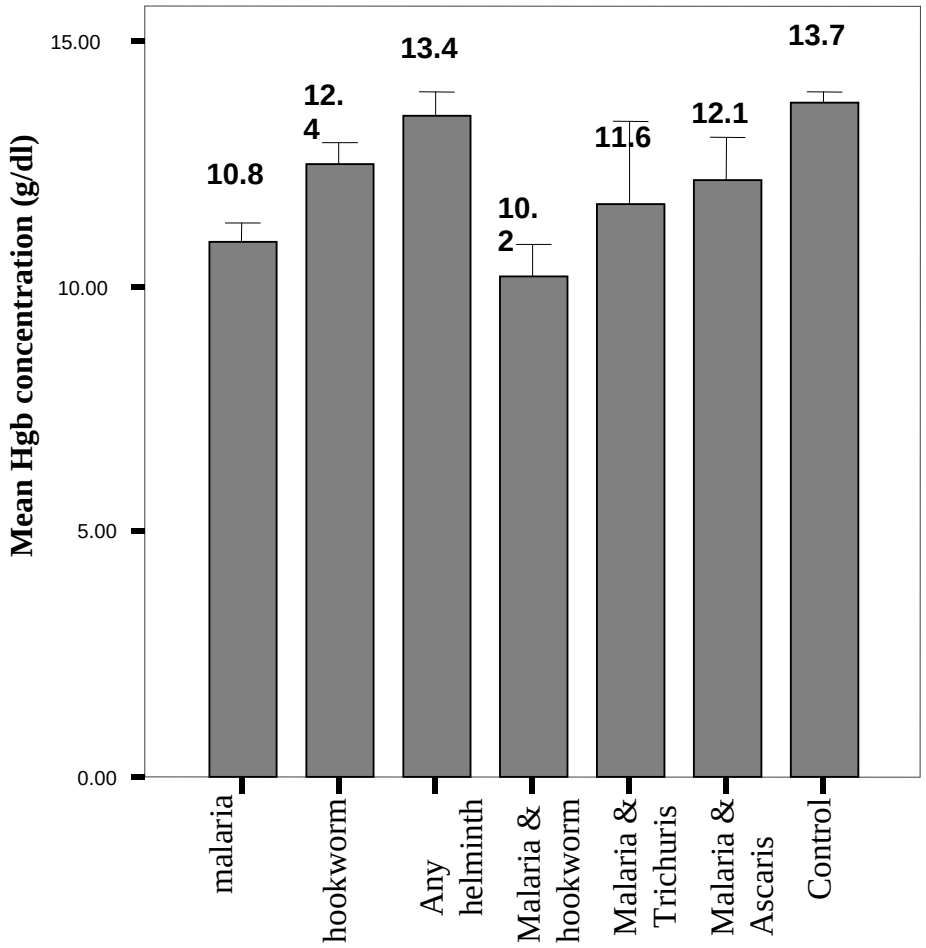


**Fig.4**.Mean Hgb concentration in malaria, hookworm, any helminth, malaria & hookworm, malaria & *Trichuris*, malaria & *Ascaris* infections and control group, Areka, 2004/2005.

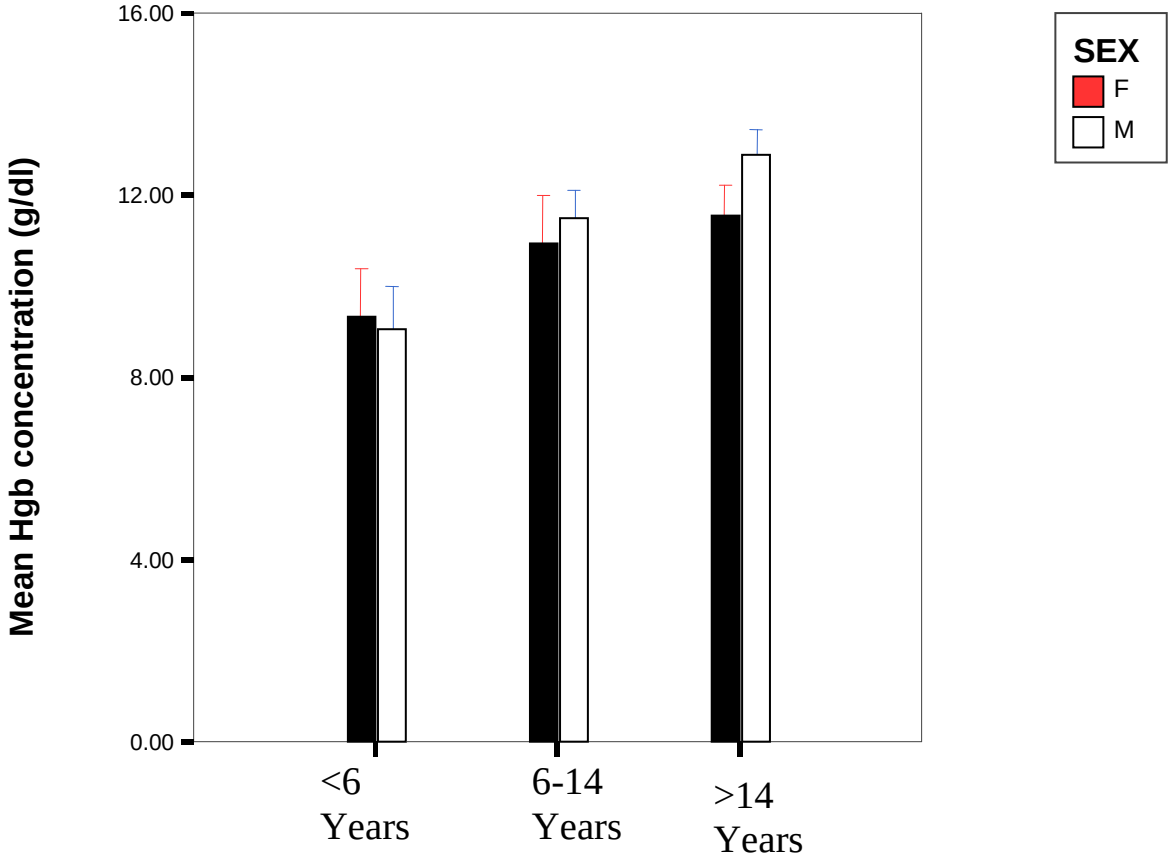


**Fig.5.** Mean Hgb concentration by age and sex of *P. falciparum* positive cases, Areka, 2004/ 2005.

The prevalence of anemia among participants infected with any helminth (23.4%) and hookworm (27.9 %) infections showed a significantly higher ($P < 0.05$) prevalence of anemia compared to *A. lumbricoides*, *T. trichiura* infections and the control group (Table 5). Also, a significantly higher proportion ($P < 0.05$) of cases who had an egg count of greater than 1000 epg

of hookworm were anemic compared to light infections ( less than 1000 epg). There was also a significant difference (P< 0.05) in the mean hemoglobin concentration among subjects with heavy and light hookworm infection (Fig. 7).

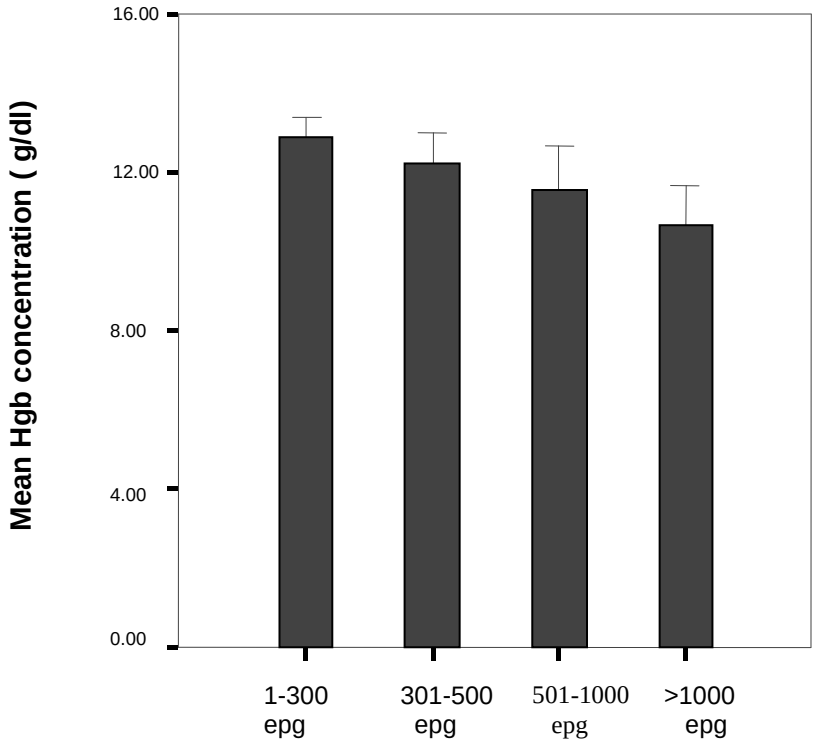


**Fig 6**. Mean Hgb concentration among cases infected with heavy and light hookworm infection, Areka, 2004/ 2005.

From the total study population about 14.1 % were infected with malaria and any helminth, 10.2 % with malaria and hookworm, 4.8 % with malaria and *A. lumbricoides* and 4.8 % with malaria and *T. trichiura*.

The prevalence of anemia among cases who were co-infected with malaria and any helminth , malaria and hookworm, malaria and *A. lumbricoides* and malaria and *T. trichiura* was significantly higher than among cases who were infected with any helminth, hookworm, *A. lumbricoides, T. trichiura* and among the control group ( $P < 0.001$) (Table 5). However, there were no statistically significant differences observed between the prevalence of anemia among cases who were infected with only malaria, malaria and any helminth, malaria and hookworm, malaria and *A. lumbricoides* and malaria and *T. trichiura* co-infections. The prevalence of severe anemia was also significantly higher among cases who were infected with malaria and any helminth, malaria and hookworm, malaria and *A. lumbricoides* and malaria and *T. trichiura* co-infections than among cases who were infected with any helminth, only hookworm, only *A. lumbricoides*, only *T. trichiura* and the control group ($P < 0.001$) (Table 5).

The mean hemoglobin concentration of cases harboring both malaria and hookworm, malaria and *A. lumbricoides* and malaria and *T. trichiura* was significantly reduced compared to cases who were infected with any helminth, hookworm, *A. lumbricoides*, *T. trichiura* and in the control group ($P < 0.001$ ) (Fig. 5 ).

In Univariate analysis of cases *P. falciparum*, hookworm and *P. falciparum* - hookworm co-infection were identified as significant predictors of anemia. Since significant interaction between age and Hgb level was found, the effects of *P. falciparum*, hookworm and *P. falciparum*-hookworm co-infections were therefore assessed separately for different age groups. This showed *P. falciparum* to be significant predictor of anemia in all age groups, while hookworm infection significantly predicted anemia in age group greater than14 years and *P. falciparum*–hookworm co-infection was a significant predictor in the age groups less than 6 and 6- 14 years (Table 6).

**Table 6**.Univariate analysis with Hgb as dependent variable; *P. falciparum*, helminth and malaria-helminth co-infections as risk factors for anemia in cases, Areka, 2004/200

| Age group | Risk factors | Dependent variable Hgb ≤ 11g/dl Vs Hgb > 11 g/dl<br>OR ( 95% CI) | Value |
|---|---|---|---|
| Less than 6 years | *P. falciparum* | 0.226 | 0.001* |
| | Hookworm | 0.086 | 0.073 |
| | *A. lumbricoides* | 0.3 | 0.25 |
| | *T. trichiura* | 0.05 | 0.39 |
| | *P. falciparum* / hookworm co-infection | 0.051 | 0.0015* |
| | *P. falciparum / A. lumbricoides* | 0.014 | 0.32 |
| | *P. falciparum / T. trichiura* | 0.077 | 0.062 |
| 6 to 14 years | *P. falciparum* | 0.094 | 0.05* |
| | Hookworm | 0.27 | 0.26 |
| | *A. lumbricoides* | 0.04 | 0.41 |
| | *T. trichiura* | 0.17 | 0.44 |
| | *P. falciparum* / hookworm co-infection | 0.03 | 0.049* |
| | *P. falciparum / A. lumbricoides* | 0.013 | 0.25 |
| | *P. falciparum / T. trichiura* | 0.010 | 0.202 |
| Greater than 14 years | *P. falciparum* | 0.04 | 0.001* |
| | Hookworm | 0.018 | 0.05* |
| | *A. lumbricoides* | 0.014 | 0.15 |
| | *T. trichiura* | 0.078 | 0.219 |
| | *P. falciparum* / hookworm co-infection | 0.02 | 0.25 |
| | *P. falciparum / A. lumbricoides* | 0.04 | 0.37 |
| | *P. falciparum / T. trichiura* | 0.01 | 0.41 |

**Keys**

* The mean difference is significant at P<0.05

OR: Odds Ratio

# 5. DISCUSSION

The estimated overall prevalence of anemia in the population of Sub-Saharan Africa is 36 % and an estimated 37 % of school age children are affected (WHO, 1992). In malaria endemic areas of Africa the overall prevalence of anemia ranges from 49 to 89 % in children (WHO, 1990). In the present study, a 52.8 % anemia detected in children falls within the range reported by WHO. This finding is higher than what was reported from different parts of Ethiopia. For instance, Zein (1991) had reported 47.2 % from Northern Ethiopia, Iannotti, *et al*. (1998) from Ijaji 47 % and Adish *et al*. (1999) from Tigray 42%. This difference is to be expected because on the average, children from malaria endemic areas have lower Hgb levels than children in areas without malaria transmission (Bruce-Tagoe *et al*., 1977).

The observed decrease in prevalence of anemia with increasing age and significantly higher prevalence in adult females than males can be explained by the fact that children during growth spurts and menstruating women have higher physiological demands for iron (Viteri, 1997).

The causes of anemia are known to be complex and multifactorial (Newton, *et al*., 1997). Several studies have identified malaria as the primary cause of anemia, while other studies cite an iron deficient diet as an important cause of anemia (Tatala *et al*., 1998). It is known that inadequate intake of iron is one of the most important factors in iron deficiency anemia (Baynes, 1994). In several developing countries the intake of iron from diet is more than adequate. For instance, in some parts of Ethiopia, the daily intake of iron is estimated to be between 180 and 500 mg/day which is 10 to 20 times higher than the suggested daily requirement (Hofvander, 1968). This high intake is attributed to consumption of the staple cereal, teff (*Eragrostis teff*) which contains 90mg of iron per 100g is mainly due to its contamination with iron rich clay soil (Hofvander, 1968). 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). According to the information obtained from a nutrition survey in Ofa district of Wolyta zone, the major staple diet is maize (60%) followed by enset (17%) and sweet potato (6.3%). Combination of enset and maize make up 11.4% and maize and sweet potato combined constitute 4.7%, while teff constitutes only 0.4 % (Amcross, 2000). Since Areka is a typical Wolyta community, both agro-ecologically and in terms of economic activities, the data would hold true to the study population. It can also be noted that the nutrient sources listed above, as in many developing countries, lack variety and contain substances, such as tannins, phenols and fiber that have been shown to inhibit the absorption of iron (Haghshenass, 1972). It has also been shown that the intake of meat and ascorbic acid, which improve the absorption of non-haem iron, is substantially lower and that of phytates, which inhibits iron absorption is substantially higher in such communities that obtain iron from non-haem sources (Taylor *et al*., 1995). Therefore, the low dietary iron intake and low iron absorption from non-haem sources may have contributed to the higher background prevalence of anemia in the study population.

Hookworm, *A. lumbricoides* and *T. trichiura* are the most prevalent helminths worldwide (Warren *et al*., 1993). The intensity of Hookworm and *T. trichiura* with 1-1000 epg is considered as light and with >1000 is considered as moderate and heavy infection but *A. lumbricoides* infection with 1-4999 epg is mild and greater than 5000 epg is considered as moderate and heavy infection (Atukorala and Lanerolle, 1999). The highest rate and intensity of helminth infections observed in Children aged 6-14 years, except for hookworm and triple helminth infections, for which the age group of above 14 years had highest rate of infection, is in agreement with Sadun (1955), who observed the prevalence of intestinal helminths to show an increase up to the ages of 10-14 years and a remarkable decrease in adults. Hinz (1967) also had concluded that age 6-12 show the highest prevalence rate. Similarly

Kightlinger *et al*. (1995) noted that children usually become infected with hookworm, *A. lumbricoides* and *T. trichiura* from 6-12 months to 3 years of age and hookworm, and *A. lumbricoides* infections increase with age, reaching highest prevalence in late adolescence and 4 to 10 years of age, respectively. The high proportion of hookworm infection in the age group > 14 years could be explained by the fact that adults in this age group have high outdoor activities such as farming and walking barefoot in moist soil, resulting in high exposure to infective hookworm larvae in the soil. The possible explanation for the highest prevalence of triple helminth infections observed in adults would be the chance of harboring more than one helminth infections concomitantly by an individual may become higher as individuals stay longer in endemic areas.

Although, these three helminths were highly prevalent in the population studied, only hookworm was associated with low Hgb concentration. Hookworm is an important cause of anemia worldwide (Roche and Layrisse, 1966) and this is expressed as a negative correlation between hookworm load and Hgb level (Pritchard *et al*., 1991). In addition, whether or not a person with hookworm infection develops anemia depends on the worm species and load, duration of infection, body iron store, dietary intake and absorption and physiological iron requirements. On the other hand, a low hookworm load can cause anemia in people whose intake of iron is low and whose iron stores are already depleted (Pawlowski *et al*., 1991). In contrast, when high iron intake is available hookworm will not cause anemia even with high worm load. This was demonstrated in children in Botswana by Michaelsen (1985). The present study demonstrated a high proportion (27.9 %) of hookworm infected cases to be anemic compared to uninfected individuals (14.8 %). The high proportion of anemia in the hookworm infected cases indicates that hookworm infection possibly accounts for some of the anemia in this population. By taking the differences between the prevalence of anemia in infected and uninfected Venezuelans, Lyrisse and Roche (1964) reported the percentage of individuals

with anemia due to hookworm to be 16%. Similarly, Aster (1995) reported a 15 % anemia due to hookworm by taking the differences between the prevalence of anemia in infected and non-infected subjects in Wolisso, South Western Ethiopia. The present study has revealed a comparable level (13.1%) of prevalence of anemia due to hookworm infection in Areka, Southern Ethiopia.

The analysis on the impact of hookworm load on Hgb showed the intensity of infection to be significantly associated with low Hgb level in the study population. Although, most of our study subjects had light hookworm infections, heavy infection with hookworm load greater than 1000 epg was significantly correlated with low Hgb level. This significant association between intensity of infection and low Hgb level is in agreement with studies from other populations. For in stance, in the study of Stephenson *et al*. (1985), which was conducted in Kenyan school children, a significant negative correlation between Hgb level and hookworm load was observed where about 36% of the subjects had what is generally regarded as heavy infection. Seboxa and colleagues (1986) also reported a significant negative correlation between Hgb level and hookworm load in their heavily infected subjects with mean ova load of 11,392 epg from Gondar, North Ethiopia. The association of only high hookworm burden (greater than 1000 epg) on low Hgb level, determined in the present study, is in contrast with some studies in which even light hookworm infections significantly correlate with low Hgb level. For example, in a study in preschool children on the Kenyan coast (Brooker *et al*., 1999), hookworm egg counts starting with 200 epg were found to be associated with lower Hgb concentration. Infection with the more virulent hookworm species, *A. duodenale* is implicated in the Kenyan report whereas; involvement of the less virulent hookworm species, *N. americanus* may be the cause in the present study (Tedla and Jemaneh, 1985). Similarly, Latham and coworkers (1983) and Oisen *et al*., (1998), who enrolled children and adults in a study in

western Kenya, had shown that 300 or more hookworm epg were significantly correlated with low Hgb. In contrast, in Nigeria, Akinkugbe (1980) and in Tanzania, Tatala *et al*. (1998) reported Hgb concentration not to be influenced by hookworm infection among preschool children. This finding may be due to the relatively low prevalence in their study population (less than 10%) or these may have been light hookworm infections and given the young age, the infection may have been recently acquired and did not progress to chronicity to be associated with anemia (Roche and Layrisse, 1966). Further more, the hookworm species involved may have been the less virulent, *N. americanus*.

Stoltsfuz *et al*. (1998) have shown that infection with *A. duodenale* in Zanzibar school children has a great impact on anemia than infection with *N. americanus*. In the present study although, the two species of hookworm were not differentiated *N. americanus* would be expected to be the predominant species in Wolyta as can be deduced from the reports of Tedla and Jemaneh, (1985).

Although, no relationship between *A. lumbricoides* intensity and Hgb status in both children and adult population was detected in the present study, *A. lumbricoides* infection was associated with lower Hgb values in Zanzibar school children (Stoltzfus *et al*., 1997). In contrast to this, and in agreement with our findings, other studies (Taren *et al*., 1987; Islek *et al*., 1993) failed to find any relationship between high *A. lumbricoides* intensity of infection and anemia.

Similar to what was reported by some studies (Stoltzfus *et al.*, 1997), no significant association of *T. trichiura* infection with low Hgb concentration was observed in the present study. However, although not statistically significant, intensity of *T. trichiura* infections greater than 1000 epg showed a negative trend of association with Hgb. In contrast to our observation Cooper *et al*. (1997) showed the relationship between low Hgb level and heavy infection with *T. trichiura* dysentery syndrome. However, since *T. trichiura*

dysentery syndrome was not observed in the present study population, the two findings may not be comparable.

Although, the prevalence of malaria was not statistically significant in between the two seasons, it was higher in the big rain than in the small rains season. It is well known that, in most malaria areas of Ethiopia, the main malaria transmission season is from September to November but the minor rainy season (January to March), also facilitates mosquito breeding and transmission, so a short peak usually occurs in malarious areas during this time (MOH, 1999). Evidences from the cumulative number of positive cases in the study area in the past two years, suggested that the peak transmission occurs in September of the big rains season. In our study, the timing of the survey may have had a significant impact on the relatively low prevalence rates detected in the big rains season, which was conducted towards the end of the peak transmission season.

The significantly higher prevalence of anemia (58 %) in malaria positive cases confirms earlier observation of Murray and Lopez (1996) in studies from three African countries, who noted that the prevalence of anemia could reach up to 75% in the areas where the prevalence of *P. falciparum* infection was in excess of 25 %.

Children less than 6 years old suffered from significantly reduced Hgb and the mean *P. falciparum* parasitemia was significantly higher in them. This observation that malaria parasitemia is associated with lower Hgb concentration confirms earlier observation of MacGregor *et al*. (1966) in the Gambia. These findings were also consistent with the condition in other endemic areas notably, Kenya and Malawi (Shulman *et al*.,1996). It is possible that higher parasite density increases the rate of hemolysis, resulting in anemia in patients who cannot produce new red blood cells at an equally accelerated rate. Erythropoeisis might be limited by folate deficiency, iron deficiency, insufficient erythropoietin production or cytokine mediated

suppression of the bone marrow response to erythropoietin (Brabin, 1982). A greater proportion of circulating parasites might also cause more iron to be incorporated into hemozoin complexes (Brabin, 1982).

In contrast to the present finding, there was no direct association of malaria density and Hgb level in spite of significantly higher prevalence of anemia in children and adults of malaria patients in Zanzibar and in Western Kenya (Stoltsfuz *et al*., 1997; Oisen *et al*., 1998). This lack of association in Zanzibar and Kenya can be explained by the fact that children have several malaria attacks yearly which will decrease their Hgb concentration to constantly low levels. The slowness of the Hgb response means that insufficient time is available to restore Hgb values between attacks, resulting in a lack of fluctuation in response to parasitemia (Oisen *et al*., 1998).

The steady decrease in the prevalence of anemia and *P. falciparum* infection with age is consistent with the fact that recurrent exposure leads to acquisition of immunity to malaria in the older age groups (Marsh, 1992). The significantly reduced Hgb concentration observed in female than in male malaria positive cases of age greater than 14 years may be due to increased nutritional demands during menustral period in the females, which can contribute to anemia (Viteri, 1997).

This study has demonstrated that cases with malaria-helminth co-infections had significantly higher prevalence of anemia. This finding is comparable to what was reported by Egwunyenga *et al*. (2001), who reported the occurrence of low Hgb concentration in pregnant women co-infected with *Plasmodium* parasites and helminths in Kenya. The increased prevalence of anemia in co-infected cases may be attributed to chronic blood and iron loss due to worm infections in addition to the loss due to malaria.

The determination that malaria-*A. lumbricoides* and malaria-*T. trichiura* co-infections were associated with anemia is in agreement with the report of

Nacher *et al*. (2001), who observed a significant difference in Hgb concentration in malaria-helminth co-infected study patients and patients with malaria infection alone. Although, *A. lumbricoides* and *T. trichiura* infections were not significantly associated with anemia independently, they have been shown to aggravate anemia when they concomitantly infect with malaria (Stoltzfus *et al*., 1997).

Iron is absorbed through the intestinal wall in the duodenum and jejunum and it is known that its absorption could be impaired by the presence of *A. lumbricoides* in this part of the intestine (Islek *et al*., 1993). Also, as *T. trichiura* lives in the luminal epithelium, mainly of the larger intestine, it has been shown to be associated with the anemia that is mediated through erythrocyte loss from the gut (Cooper and Bundy, 1989). Another possible explanation for these findings could be that helminth infections lead to decreased erythropoeisis through Nitric oxide (NO) release (Korbut and Gryglewski, 1993). Since NO can reduce erythrocyte deformability, it could lead to increased red blood cell destruction (Dondrop *et al*., 1999). Nacher *et al*. (2001) observed a significantly higher concentration of Reactive Nitrogen Intermediates (RNI) in helminth-infected than uninfected malaria patients.

No single risk factor was identified as a predominant predictor of anemia. Odd ratios of 2 or less suggest the multifactorial nature of anemia (Adish *et al*., 1999).

The findings on the associated risk factors for anemia that were identified in the present study, such as *P. falciparum* in all age groups, hookworm infection in the age group greater than 14 years and *P. falciparum*-hookworm co-infection in the age groups less than 14 years of age are in agreement with other reports from western Kenya (Oisen, *et al*., 1998). Brooker *et al*. (1999) had identified malaria and hookworm as significant predictors for anemia in pre-school children in Kenyan coast. However, in contrast with the present finding, they did not observe the predictive value of malaria-helminth co-infections for low Hgb level.

# 6. CONCLUSIONS AND RECOMMENDATIONS

The findings reported in the present study show that anemia is a major health problem in the study area and it is more prevalent and severe in children less than 6 years old. The most important parasitic infections associated with anemia were malaria, heavy hookworm infection and malaria-helminth co-infections. Malaria-helminth co-infections, especially that of malaria–hookworm were most frequently associated with anemia. Furthermore, malaria in all age groups especially in children below 6 years old, hookworm infections in the age group greater than 14 years old and malaria–hookworm co-infection in the age groups below 14 years of age were the most important factors associated with anemia in the study area.

There is need to ensure adequate prevention and treatment of anemia in the study area. Since, it is difficult to assess the relative contributions of different etiologic agents, the most appropriate approach to prevent anemia would be launching interventions that are linked to the primary causes of anemia in a population. Therefore, the integrated malaria control programs such as, control of the mosquito vector, use of chemotherapeutic agents and control using insecticides, must be strengthened to reduce the high level of anemia in the study area. Anti-helminthic treatments must be seriously considered to protect the population from exacerbation of anemia by intestinal helminth infections. Control of these infections can be achieved through regular treatment with inexpensive, single dose and highly effective drugs.

Apart from damages caused to the gastrointestinal system, helminth infections cause anemia, poor physical growth and intellectual development. Studies from the industrialized countries illustrate the role of deworming in improving the intellectual development and cognition that have a substantial impact on professional competence later in life. And, since, it has been shown

that successful deworming programmes are considered one reason for these countries' subsequent economic explosions, similar programs must be designed to address the health and development problems in Areka area, in particular and the whole country in general. Furthermore, food supplementation to increase iron and folate intake and health education on anemia prevention must be provided in the community. Environmental control of infectious agents causing anemia must also be promoted. This needs to be carried out on a continuous basis and integrated with other ongoing community health programmes.

In the present study no attempt was made to rule out the contribution of other etiologic agents such as, chronic infections (e.g. HIV), nutritional deficiencies and hemoglobinopathies and biochemical analysis for the determination of stored iron in the study population were not done. Therefore, there is need for more population–based studies that carefully monitor the iron status of the population, identification of the types of anemia and investigation of the possible contribution of other infectious agents and nutritional deficiencies among the high risk groups of the population.

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## Declaration

I, the undersigned, declared that this thesis is my own original work, has not been presented for a degree to any other university and that all sources of materials used for the thesis have been duly acknowledged.

Name : SOLOMON ARAGIE

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