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MOLECULAR BIOLOGY PROGRAM UNIT

EFFECT OF INTESTINAL HELMINTH INFECTIONS AND DEWORMING ON ANAEMIA AMONG SCHOOL CHILDREN IN TIKUR WUHA ELEMENTARY SCHOOL, JIGA, NORTHWESTERN ETHIOPIA

A Thesis

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By

Yonas Yimam

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LIST OF ABBREVIATIONS AND ACRONYMS

AAU	Addis Ababa University
ACC/SCN	Administrative Committee on Coordination/ Sub-Committee on Nutrition
B12	Vitamin B12
CI	Confidence intervals
DALYs	Disability Adjusted Life Year
DCCP	Diseases Control Priorities Project
DNA	Deoxy- ribonucleic acids
EPG	Eggs per gram
FAO	Food and Agriculture Organization
Hb	Hemoglobin
IDA	Iron deficiency anaemia
masl	Meter above sea level
OR	Odds ratio
RBC	Red blood cell
SPSS	Statistical package for social sciences
STHs	Soil-transmitted helminthes
USAID	United States Agency for International Development

ABSTRACT

Soil-transmitted helminths (STHs) and schistosomes are the major public health problems in the vast majority of developing countries including Ethiopia. Both helminthic groups are known to significantly contribute to anaemia. This study was aimed to assess the effect of intestinal helminth infections and deworming to anaemia among school children. A cross-sectional study was carried out and 403 school children were selected using systematic random sampling technique from Tikur Wuha Elementary School, Jiga, Northwestern Ethiopia from February - March, 2011. Stool samples were processed for microscopic examinations using double Kato-Katz and average fecal egg counts were used. Hemoglobin was determined using Hemocue HB 201 analyzer. Data was analyzed using the statistical package for social science (SPSS). The overall prevalence of STHs and schistosome infections among the school children was 58. 31%. Single, double, triple and quadruple infections were 41.19%, 15.38%, 1.49 and 0.25%, respectively. The prevalence of hookworm, *Schistosoma mansoni, Ascaris lumbricoides*, *Trichuris trichiura*, *Enterobius vermicularis*, and *Hymenolepis nana* infections were 46.90%, 24.57%, 4.22%, 1.74%, 0.5% and 1.24%, respectively. The current study showed that intestinal helminth infections particularly hookworm and *S. mansoni* were positively associated with anaemia ($P < 0.05$). The overall pre-treatment prevalence of anaemia was 14.64%, while anaemia associated with intestinal helminth infections was found to be 11.91%. After deworming, there was a rise in the mean hemoglobin of school children from 12.73±1.18 pretreatment level to 13.96±1.21 g/dl post-treatment level ($P= 0.000$). The result revealed that following deworming, prevalence of both intestinal helminth infections and anaemia associated with intestinal helminth infections were reduced from 58.31% to 12.41% and 11.91% to 8.44%, respectively. The present study showed that deworming as part of helminth control decreases intestinal helminth infections and improves hemoglobin concentration among school children. Deworming program should be included as a strategy for the control of anaemia in school children where there is high prevalence of intestinal helminth infections.

Key words/ phrases: Anaemia, deworming, school children, STHs, schistosomiasis

1. INTRODUCTION

In the vast majority of developing tropical and subtropical regions of the world, helminth infections, particularly those caused by soil-transmitted helminths (STHs) and schistosomes, constitute major public health and developmental challenges (Uneken, 2010). They are associated with poverty and underdevelopment and are most prevalent in the poorest communities of the developing world (Montresor *et al*., 1998; WHO, 2002).

Current estimates indicate that 4.5 billion individuals are at risk of STHs infections and the global estimate of number of cases of *Ascaris lumbricoides* is 807 million, *Trichuris trichiura* 604 million, hookworms (*Necator americanus, Ancylostoma duodenale*) 576 million, and schistosomes 207 million (Bethony *et al.,* 2006; Hotez *et al.,* 2008). Estimates of disability-adjusted life years (DALYs) lost due to these helminth infections differ greatly from one source to another (WHO, 2002; DCPP, 2008). However, total DALYs lost annually may range from 4.7 million to 39 million (DCPP, 2008).

Because STHs are transmitted through poor sanitation and hygiene, and schistosomiasis by contact with infected freshwater streams and lakes, school-age children are typically at increased risk resulting in high prevalence and intensity of infection due to high level of exposure (Montresor *et al.,* 1998; WHO, 2002). Helminthic infections often lead to iron deficiency anaemia (IDA), protein energy malnutrition, stunting (a measure of chronic under nutrition), wasting (a measure of acute under nutrition), listlessness and abdominal pain (Van der Werf *et al.,* 2003; Bethony *et al.,* 2006), and may negatively affect class-attentiveness of schoolchildren (Berhe *et al.*, 2009).

The etiology of anemia varies from country to country and includes deficiencies of essential nutrients such as micronutrients (e.g., iron, vitamin A, vitamin C, folate, riboflavin, and vitaminB12), physical injuries (e.g., hemorrhages, abnormal menstrual bleeding), genetic abnormalities (e.g., thalassemia, sickle-celled anemia), parasitic infections (e.g., hookworms, malaria, etc), systemic infections (e.g., malaria, HIV, tuberculosis), and cancer (USAID, 2004;

Haidar and Pobcik, 2009). The link between hookworm infection and anemia is well known (Stolzfus *et al.,* 1997). Recent large scale studies have suggested links between heavy intensities of *S. mansoni* infections with anemia and lowered haemoglobin level (Koukounari *et al.,* 2008). Moderate or high intensities of *T. trichiura* are also associated with higher risks of anaemia in the presence of other STHs (Robertson *et al.,* 1992). On the other hand, the impact of *A. lumbricoides* on anaemia is less clear (Ezeamama *et al.,* 2008).

The four basic approaches to the prevention of iron deficiency anemia are supplementation with medicinal iron, education and associated measures to increase dietary intake, the control of infection and the fortification of the staple food with iron (Al-Mekhlafi *et al.,* 2008; Ramzan *et al.,* 2009). A central principle of the most approach to develop anemia reduction strategies is that the interventions should be linked to the primary causes of anemia in a population or population group (USAID, 2004). Administration of intestinal anthelminthic agents has been proposed as an additional intervention to reduce anemia in populations with a relatively high prevalence of intestinal helminthiasis (Gulani *et al.,* 2007). Periodic mass deworming of communities at high risk of helminth infections reduces anemia by storing iron and improving growth in children (de Silva, 2003).

Therefore, intestinal helminth infections and anaemia among schoolchildren are not only health problems, but also pose considerable problem to socio-economic development in developing countries like Ethiopia. Deworming is one of the strategies to prevent and control iron deficiency anemia in area where intestinal helminth infections are endemic. Hence, it is necessary to determine the impact of intestinal helminth infections and deworming on anemia among schoolchildren to generate data that would guide control programs.

2. ANAEMIA

Anaemia is defined as a condition where there is less than the normal hemoglobin (Hb) level in the body (Tolentino and Friedman, 2007). Hemoglobin levels indicative of anaemia have been established by World Health Organization (WHO) for specific population groups: for children 6 months to 5 years of age anaemia is defined as Hb level < 11g/dl, children 5–11 years of age Hb

< 11.5 g/dL, adult males Hb < 13 g/dL, non-pregnant females Hb < 12g/dL, and pregnant females Hb < 11g/dL. Severe anemia is defined as Hb < 7.0 g/dL (WHO, 2001). There are, however, mild-to-moderate forms of iron deficiency in which the host is yet anemic, but the tissue are functionally iron deficient (Bruner *et al.,* 1996). In physiological terms, anemia occurs when there are an inadequate number of red blood cells or an inadequate amount of hemoglobin for the body to function properly (USAID, 2004).

Worldwide, more than 2 billion people are anemic (WHO, 2001; Casey *et al*., 2009).The highest prevalence of anemia exists in the developing world where its causes are multi-factorial (WHO, 1996). In the developing world, over half of all pregnant women are anemic (Crompton, 2000), 42% of children less than five years of age and 53% of children 5–14 years of age are anemic (ACC/SCN, 2000).

In Ethiopia, the magnitude and importance of iron deficiency anemia as a public health problem is still disputed. Some studies in the past have documented the problem as being rare in Ethiopia attributed to consumption of "*teff*" a cereal, which has high iron content mainly due to contamination with the soil (WoldeGabriel*,* 1993). On the other hand, others have concluded the issue to be a mild to moderate public health problem (Adish *et al.,* 1999). FAO (2010) reported that more than half of under fives and more than a quarter of women are anemic in Ethiopia due to low consumption of foods of animal as the main cause, compounded by high incidence of malaria and other parasitic diseases.

IDA impairs cognitive performance, causes poor physical growth, delays psychomotor development and productivity, reduces appetite, leads to low birth weight, hypoxia, compromises immune system and increases morbidity (Bobonis *et al*., 2004). Moreover, iron deficiencies early in life are thought to potentially inhibit the function of neurotransmitters, compromising brain function and may also lead to severe anaemia (Stoltzfus *et al.,* 1997). These overall impacts lead to a decrease in work capacity and fitness of individuals (Bobonis *et al*., 2004).

Anaemia is particularly common in individuals infected with soil-transmitted helminths or schistosomes (Bates *et al.,* 2007). Hookworm infection is causally linked to anaemia in human (Stolzfus *et al.,* 1997). The link between human schistosomiasis and anemia has also been documented (Friedman *et al.,* 2005). Moderate or high intensities of *T. trichiura* are also associated with anaemia (Brooker *et al.,* 2006), while the impact of *A. lumbricoides* on anaemia is less clear (Ezeamama *et al.,* 2008).

2.1. Intestinal Helminth-Induced Anaemia

Hookworm infection is causally linked to anaemia in humans (Stoltzfus *et al.,* 1997; Olsen *et al*., 1998; Hotez *et al.,* 2004). Schistosome (Friedman *et al.,* 2005; Kraemer and Zimmermann, 2007) and moderate or high intensity *Trichuris* (Stephenson *et al.,* 2000; Ramdath *et al*., 1995) infections are also associated with anaemia. Furthermore, it is speculated that *Trichuris* infection may exacerbate anemia in the presence of other helminth infections, particularly hookworm (Robertson *et al.,* 1992; WHO, 2002). *Ascaris* infection is known to influence nutritional status but its impact on anemia is less clear (Ezeamama *et al.,* 2008). Nevertheless, two epidemiologic studies have found associations between this infection and anaemia (Stoltzfus *et al.,* 1997; Curtale *et al.,* 1993). The relative contribution of individual helminth species to anaemia is governed by different mechanisms for initiating and maintaining anaemia (Ezeamama *et al.,* 2008). In spite of the real biologic and physiologic differences between species, there is relative consensus that these helminth species contribute in varying degrees to anaemia (Ramdath *et al.,* 1995; Stephenson *et al.,* 2000).

2.1.1. Hookworm Infection and Anaemia

Hookworm infection in humans is caused by two species, namely *N. americanus* and *A. duodenale* and is transmitted through contact with contaminated soil with third-stage infective larvae, which either penetrate the skin (*N. americanus* and *A. duodenale*) or when they are ingested (*A. duodenale*) (Hawdon and Hotez, 1996). Both of human hookworm species are known to exist in Ethiopia (Armstrong and Tedla, 1985). Hookworm infections occur in almost half of sub-Saharan Africa's poorest people, including 40–50 million school-age children and million pregnant women in whom it is a leading cause of anaemia, and under nutrition (Hotez and Kamath, 2009). Basically, IDA and poor iron status are the hallmarks of hookworm disease, and is well known worldwide (Crompton and Nesheim, 2002).

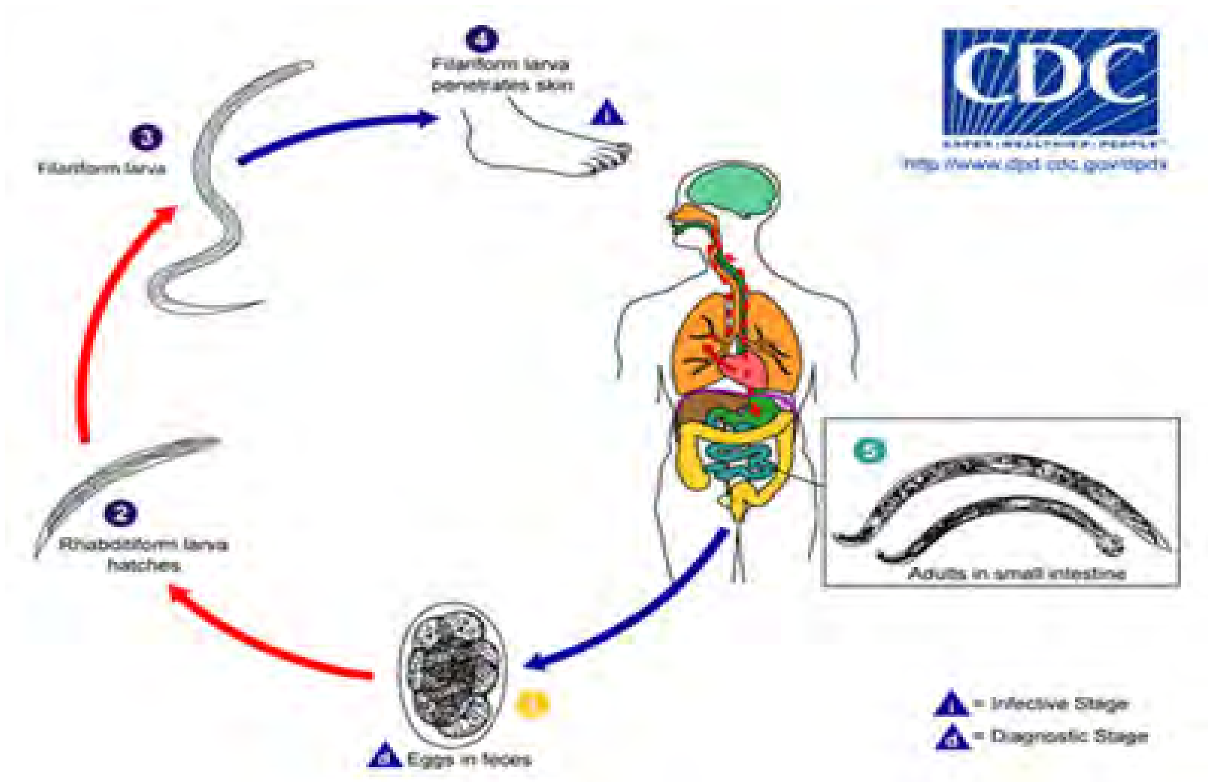

Figure 1: Life cycle of Hookworm(Source: USAID, 2010)

Hookworms contribute to IDA by actively feeding on blood from the capillaries and arterioles in the intestinal mucosa, causing further gastrointestinal hemorrhage, loss of serum proteins, and intestinal inflammation (Olsen *et al*., 1998). Blood loss occurs when the worms use the cutting apparatus to attach themselves to the wall of intestinal mucosa, and contract their muscular esophagi to create negative pressure, which sucks a plug of tissue into their buccal capsules (Hotez *et al.,* 2004).The digestive enzymes of the hookworms help to rupture blood capillaries both mechanically and chemically (Hotez and Pritchard, 1995). Moreover, they release anticoagulant compounds into the blood stream, which makes the blood leaking out continues (Stanssens *et al*., 1996). Therefore, the amount of blood loss in hookworm infections is strongly and linearly correlated with worm load /fecal egg counts (Muhangi *et al.,* 2007). Depending on the status of the host iron content, 40 to 160 hookworm burdens may be associated with low Hb level (Bundy *et al.,* 1995).

2.1.2. Schistosomiasis and Anaemia

Schistosomiasis is a chronic and debilitating disease which is draining the economic development in much of the tropics, where it is concentrated. It is the second parasitic disease next to malaria by affecting large number of people in the world (Steinmann *et al*., 2006). The three major schistosome species (*S. haematobium*, *S. mansoni* and *S. japonicum)* that cause human disease exist in 74 developing countries. Approximately 600 million individuals reside in endemic regions and 200 million are thought to be infected (Savioli *et al.,* 2004). In Ethiopia, both *S. mansoni* and *S. haematobium* pose public health and socioeconomic problems (Erko *et al.,* 1997; Kloss *et al.*, 1988).

Although adult *Schistosoma* flukes feed on blood in the vessels around the bladder and intestine, the anemia caused by schistosomes is mainly due to blood loss caused by the parasite eggs. Female schistosomes release their eggs into the smallest blood vessels they can reach, where many eggs become wedged. The surface of each egg is covered with sharp spines, and these help to cut into the lumen of the gut or bladder as the eggs are forced through the vessel walls. In *S. haematobium* infection, the site of penetration bleeds into the bladder, making the urine pink or even red (hematuria). Typically, however, the color is usually seen in only the last few drops, but the prevalence of urinary hematuria can be used to estimate the prevalence of *S. haematobium* (Kraemer and Zimmermann, 2007).

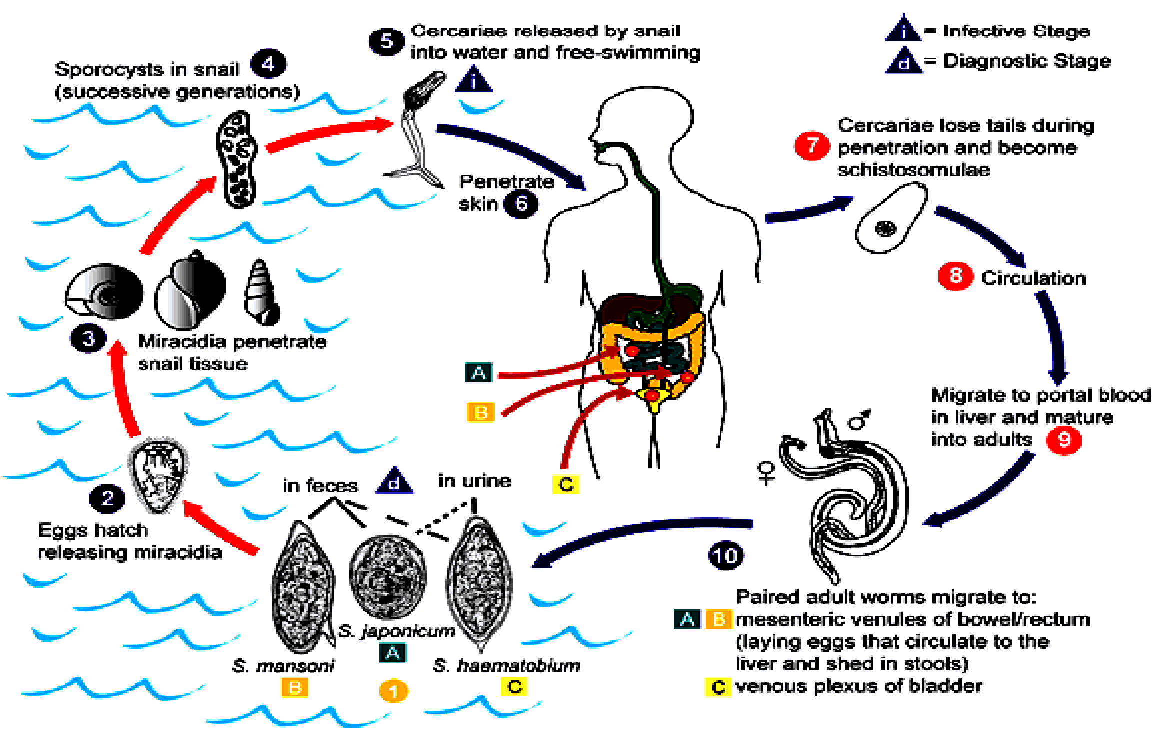

Figure 2: The life cycle of human schistosomes (Source: CDC, 2003)

The mechanisms by which schistosome infections lead to anemia have been debated for decades. Four proposed mechanisms are: (i) iron deficiency due to extra-corporeal loss; (ii) splenic sequestration; (iii) autoimmune hemolysis; and (iv) anaemia of inflammation (Friedman *et al.,* 2005).

Infection with *S. mansoni* and *S. japonicum* can lead to hepatosplenomegaly. The eggs produce inflammation and gross amputation of the intrahepatic veins, portal and periportal granulomas and eventually a coarse perilobular fibrosis (pipe-stem) (Andrade *et al.,* 1992) which leads to hepatosplenomegaly, portal hypertension and esophageal varices then gastrointestinal tract bleeding, the most common cause of death from this disease(Andrade *et al.,* 1992). In support of autoimmune hemolysis as a potential mechanism of anemia in schistosomiasis, the survival of RBCs decreases in animals infected with irradiated cercariae (no egg production, less liver disease and splenomegaly), which was thought to be the result of an autoimmune hemolytic process **(**Mahmoud and Woodruff, 1973)**.** Schistosomiasis may cause anemia by inducing proinflammatory cytokine-mediated dyserythropoiesis, as seen in anemia associated with inflammation (Konijn, 1994; Means, 2000). Schistosomiasis also cause iron deficiency due to extra-corporeal loss as the eggs are forced through the vessel walls (Friedman *et al.,* 2005).

2.1.2. Trichuriasis and Anaemia

Trichuriasis or whipworm infection is caused by *T. trichiura*. Humans are the primary host for infections caused by *T. trichiura* but the species has been detected in some non- human primates (Horii and Usuii, 1985). Infection with *T. trichiura* occurs through the oral-fecal route caused by ingestion of infective eggs from contaminated foods, hands or water. Eggs then pass through the stomach to the small intestine where they hatch. The larvae penetrate the cells of the small intestine and coming to lie above the lamina propria where they undergo number of molts (Katz *et al*., 1989; Neva and Brown, 1994). The cecum is the preferential site for invasion although heavy infections will extend throughout the colon and even distally to the rectum to obtain nutrients, particularly for the mature parasites. It feeds on the enterocyte synctium and may also ingest enterocytes, leucocytes and mucosal fluids if they happen to penetrate below the basal membrane of the same (Stephenson *et al.,* 2000).

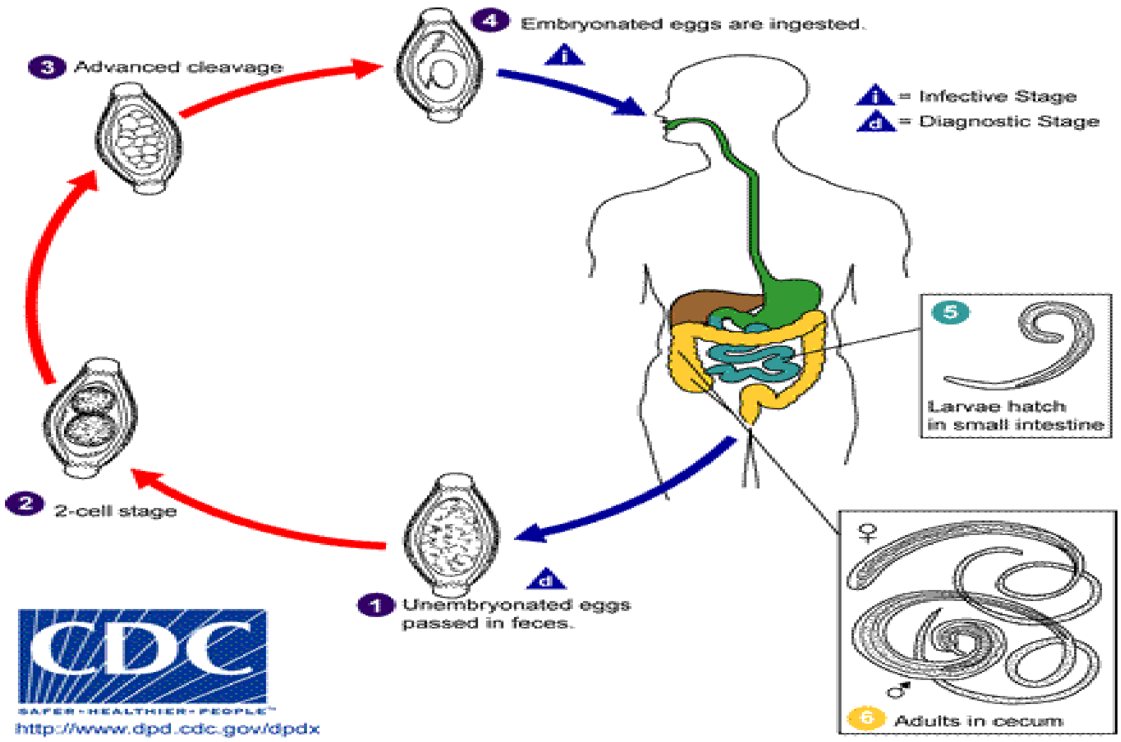

Figure 3: Life cycle of *Trichuris trichiura*(Source: CDC, 2011)

Heavy infections with *T. trichiura* have been reported to cause IDA (Ramdath *et al*., 1995). Trichuriasis often occurs concurrently with hookworm infections and so may accelerate the onset of IDA (WHO, 2002). Although blood loss can be a feature of *T. trichiura* infection, it is less prominent than in hookworm infections. However, there are both direct and indirect mechanisms in trichuriasis which is responsible for anemia. The direct mechanism is via bleeding in the large bowel, with Trichuriasis Dysentery Syndrome (TDS) or rectal prolapse. The indirect mechanism is systemic. This occurs likely with elevated levels of tumor necrosis factor alpha (TNF-α), which reduces appetite, and thus food (iron) intake (Stephenson *et al.,* 2000). This could occur in any child and the effects may produce, or aggravate pre-existing anemia.

2.1.4. Ascariasis and Anaemia

A. lumbricoides, a soil-transmitted round worm, is reported to infect at least one fourth of world's population (Neva and Brown, 1994). In most countries where geo-helminths are endemic, school age children experience the highest prevalence and intensity of infection; particularly with *A.*

lumbricoides and *T. trichiura (*Hall *et al.,* 1997) both domestic and wild animals are common reservoirs for *A. lumbricoides.* Approximately 1,500 million people are infected with *Ascaris*, or about 25 % of the world's population. Ascariasis is concentrated in developing countries with poor sanitation and in tropical areas, where the eggs survive the longest in the environment. An estimated 59 million people are at risk of some morbidity, with 1.5 million children suffering from Ascaris infections that will result in permanent growth retardation, even if the infection is cured (Crompton, 1999).

Infection is acquired through the ingestion of infective eggs from focally contaminated food or water. Since the eggs are very sticky, they readily adhere to raw fruits and vegetables, which are washed with contaminated water or fertilized with contaminated night soil. In highly endemic areas, *Ascaris* eggs may be found on eating utensils, or under the fingernails. They may also be found in household dust and air where they are inhaled or swallowed (O'Lorcain and Holland, 2000).

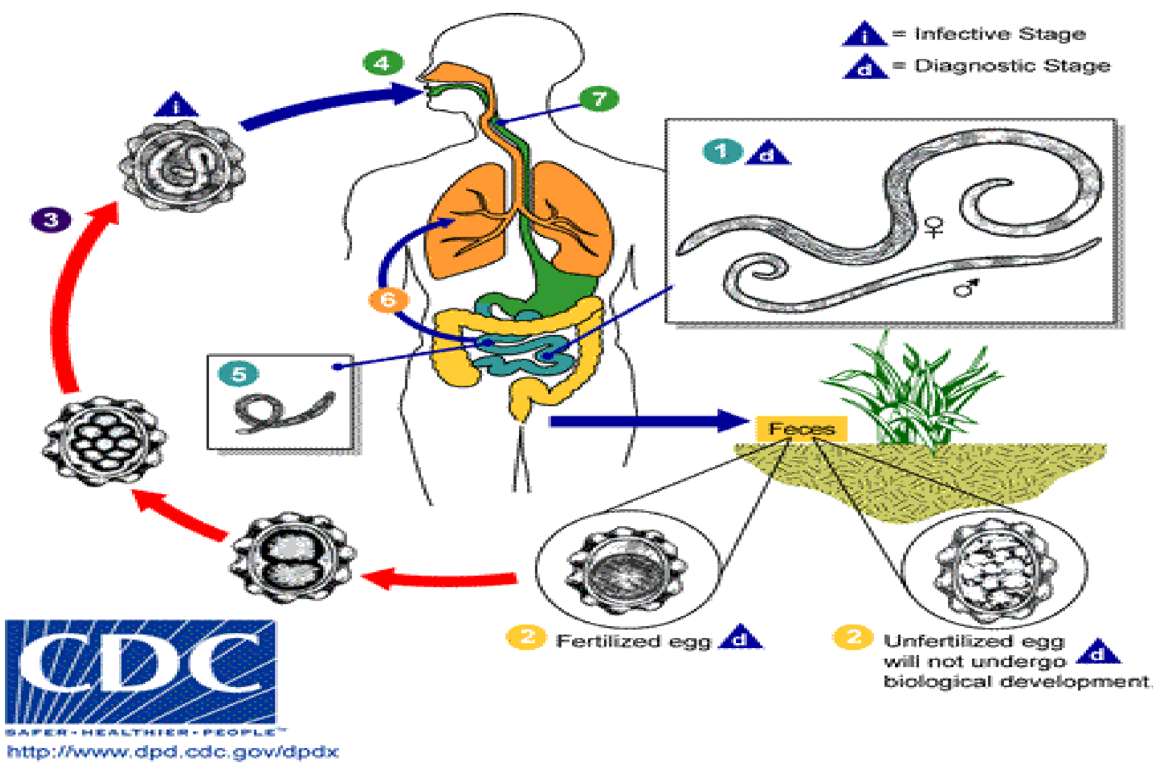

Figure 4: Life cycle of *Ascaris lumbricoides*(Source: CDC, 2011)

Infection with *A. lumbricoides* has been linked to low hemoglobin concentrations in Zanzibari (Stoltzfus *et al.,* 1997) and Nepalese (Curtale *et al.,* 1993) children. How this worm causes anemia is uncertain, as the worm feeds on gut contents rather than on blood. Infection is associated with poverty and poor diet and it has been reported to cause anorexia (Crompton and Nesheim, 2002). Undoubtedly, worms compete with the host for food, and infestation is linked to poor growth. Heavy infection may cause malabsorption of iron, as the worm lives in the duodenum or jejunum, where iron absorption occurs, and low-grade inflammation may contribute to anemia of chronic inflammation (Crompton and Nesheim, 2002).

2.1.5. Polyparasitism and Anaemia

Individuals living primarily in rural areas of low-income countries commonly harbor multiple parasitic infections, including infection with multiple helminth species (Ezeamama *et al.,* 2008). In particular, intestinal schistosomiasis and polyparasitic soil-transmitted helminth infections constitute major public health problems in sub-Saharan Africa (Stoltzfus *et al.,* 1997). There are few human studies of the morbidity implications of polyparasitism, which may elicit a range of biologic interactions between the host's immune system and the invading parasites. One possible form of interaction is synergism, which implies that the adverse health effect associated with multiple species infection is greater than the sum of adverse effects for individual species (Ezeamama *et al.,* 2005). There is an inverse relationship between the number of parasites in an infected person and their Hb level. For example, low-intensity polyparasite infections in children in the Philippines are associated with 5-fold increases in prevalence of anaemia (Hb <11 g/dl) (Ezeamama *et al.,* 2005). Moderate/high intensity infections with three or four soil-transmitted helminths and *S. japonicum* are associated with 8-fold increases in anaemia (Bates *et al.,* 2007).

3. DEWORMING

Anthelmintic drug treatment ("deworming") is aimed at reducing morbidity by decreasing the worm burden. Drugs recommended by WHO for reducing morbidity due to soil- transmitted helminthiasis are albendazole, levamisole, mebendazole, and pyrantel (WHO, 2002). The benzimidazole anthelmintics, mebendazole and albendazole, are commonly used to remove these infections (Benothey *et al.,* 2006). Albendazole is recommended as a single dose of 400 mg and mebendazole either as a single dose of 500 mg or as 100 mg twice daily for three days. Some are now discouraging the latter because of concern about compliance in completing the full 3-day treatment (USAID, 2004). For reducing morbidity due to schistosomiasis, WHO recommends praziquentel which is effective against all schistosome species. It is mostly marketed as 600 mg tablets, with a recommended standard regimen of 40 mg/kg of body weight in a single dose (WHO, 2002).

Albendazole and mebendazole are the two most widely used benzimedazoles for the treatment at individual patient level and community-based control of STHs. The benzimidazoles act directly within the gastrointestinal tract binding to the nematodes β-tubulin, which impairs vital cellular processes and lead to parasite death (Martin *et al.,* 1997). Praziquantel, an acylated quinoline-pyrazine that is active against all schistosome species, is now the most widely used. . The drug acts by paralysing the worms and damaging the tegument (Gryseels *et al.,* 2006). Orally administered praziquantel results in rapid and almost complete absorption; time to maximum concentration in serum is 1-2 hour (Leopold *et al.,* 1978). Praziquantel undergoes first-pass liver clearance with an observed plasma half-life of 1-2 hour. Metabolites are inactive, the most abundant of which is the 4-hydroxycy-clohexyl-carbonyl analogue. Praziquantel is bound to plasma proteins (~ 80%) and is primarily eliminated via urine. Praziquantel has a very rapid onset of action and disrupts the Ca^{+} homeostasis in schistosomes (Gryseels *et al.,* 2006).

Several controlled trials have demonstrated a positive impact of anthelminthic treatment on the iron status of school children (Guyatt *et al.,* 2001; Gulani *et al.,* 2007; Smith and Broker, 2010). Routine administration of intestinal anthelminthic drugs results in a marginal increase in haemoglobin (1.71 g/l), which could translate on a public health scale into a small (5% to 10%) reduction in the prevalence of anaemia in populations with a relatively high prevalence of intestinal helminthiasis (Gulani *et al.,* 2007).

4. STATEMENT OF THE PROBLEM

The anaemia task force guides a situational analysis that compiles what is known of anaemia prevalence and causes, related policies and programs, the economic context of anaemia, and the political commitment to address it (USAID, 2004). In Ethiopia, even though studies have shown the association of intestinal helminth infection with anaemia (Worku *et al*., 2009), there are no detail studies on the impact of deworming on anaemia. Therefore, there is a need to carry out further studies on the impact of intestinal helminth infections and deworming on anaemia on high risk groups of the society in this country. Such information is required to guide policy makers in deciding on the type of preventive and control strategies in controlling intestinal helminth induced anaemia.

In Jiga, Northwestern Ethiopia, there are no previous studies on the impact of intestinal helminth infections and deworming on anaemia among school children. However, according to clinical reports of Jiga Health Center, worm infections are currently listed as among the top reason why people visit health facilities. Therefore, the present study was undertaken to investigate the impact of intestinal helminth infections and deworming on anaemia among Tikur Wuha Elementary School children.

5. SIGNIFICANCE OF THE STUDY

Knowledge about the effect of intestinal helminth infections and deworming to anaemia is important to institute preventive and control measures as well as for evaluation of intervention programs.

6. RESEARCH HYPOTHESIS

Anaemia is particularly common in individuals infected with soil-transmitted helminths or schistosomes. Deworming is one of the strategies to control anaemia in areas where there is a high risk of intestinal helminthiasis. Based on this consideration, intestinal helminth infections will be positively associated with anaemia while deworming reduces worm burden and anaemia in Tikur Wuha Elementary School, Jiga, Northwestern Ethiopia.

7. OBJECTIVES OF THE STUDY

7.1. General Objective

The main objective of this study was to assess the association of deworming to anaemia among school children in Tikur Wuha Elementary School, Jiga, Northwestern Ethiopia.

7.2. Specific Objectives

- To determine the prevalence of soil-transmitted helminthiasis and schistosomiasis among school children in Tikur Wuha Elementary School, Jiga, Northwestern Ethiopia.
- To determine the prevalence of anaemia among school children in Tikur Wuha Elementary School, Jiga, Northwestern Ethiopia
- To determine the effect of deworming on worm burden
- To assess the association of intestinal helminth infections and anaemia.

8. MATERIALS AND METHODS

8.1. Study Design

A cross-sectional study was conducted from February to March 2011 to assess the impact of intestinal helminth infections and deworming on anaemia among school children in Tikur Wuha Elementary School, Jiga, Northwestern Ethiopia.

8.2. The Study Area

The study was conducted in Jiga, Jabi Tehnan District, Amhara National Regional State, Ethiopia. Jiga is located about 375 Km from Addis Ababa on the road side to Bahir Dar. It is situated in Western Gojjam Zone between Dembecha and Finote Selam towns.

Jiga has an altitude of 1850 masl above sea level. The town has 14, 245 inhabitants. It has 430 private and 14 public pipe water facilities and 1057 latrine owned by private, public and institution (offices, hotels). It has also one health center, two primary and one high school. The town was one of the sites for pilot schistosomiasis control project study instituted by Aklilu Lemma Institute of Pathobiology in collaboration with various local, regional and national institution which was implemented from 1985 to 1987 (Yeneneh *et al.,* 1996; Kloss *et al.,* 1993). Tikur Wuha Elementary School, selected for the present study, is on the main road in Jiga town.

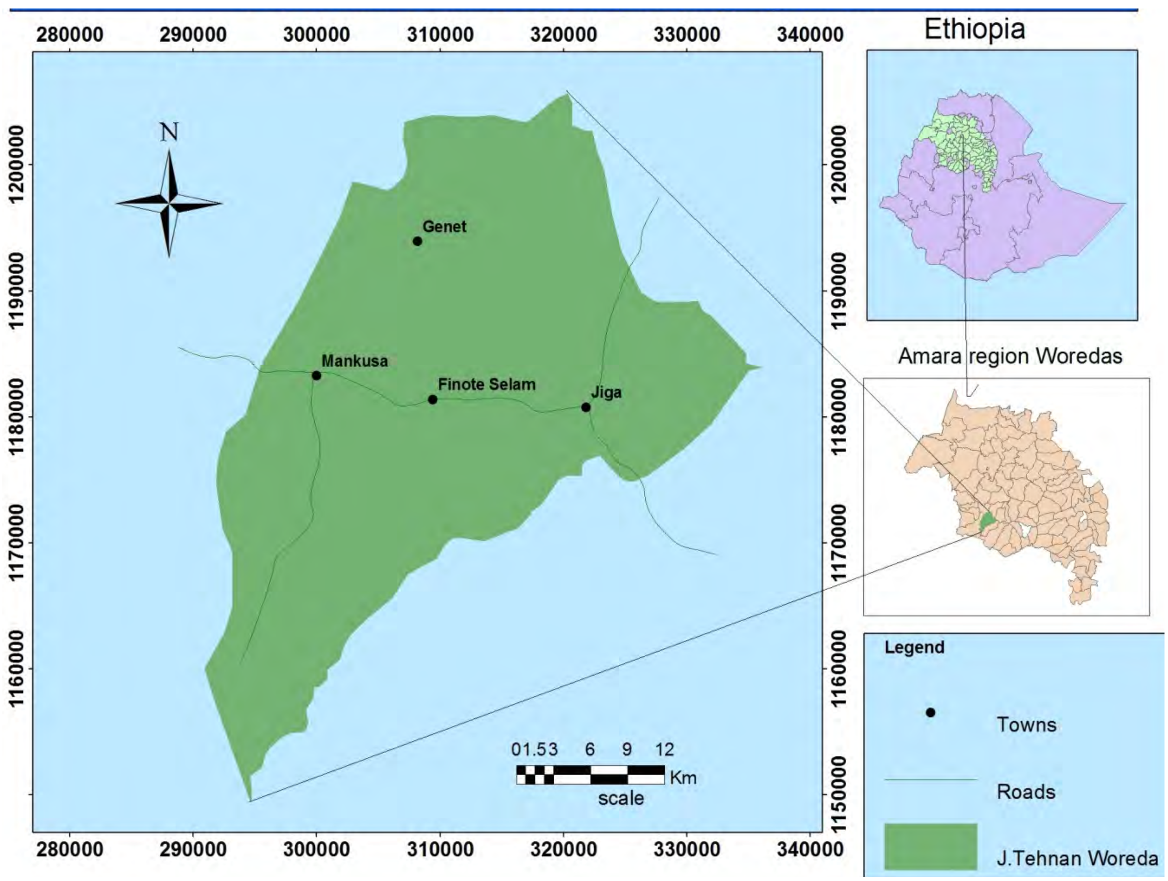

Figure 5: Map of Jabi Tehnan Woreda showing the study site (Source: Modified from Ethio-GIS)

8.3. Study Population

The study population consisted of school children (6-15years) from Tikur Wuha Elementary School. Tikur Wuha Elementary School is located on the main road in Jiga town. The school age children were selected as the study population with the aim to represent the community they live in for prevalence of infection. That is, primary school children can be used as an index for assessing community prevalence (Guyatt *et al*., 1999). A total of 403 children were randomly selected from enrolled at the school.

8.4. Sample Size Determination and Sampling Technique

8.4.1. Sample Size

The sample size (n) was estimated using the statistical formula (Daniel, 1995),

$$n = \frac{z^2 p (1-p)}{d^2},$$

Where n = sample size; z = z statistic for a level of confidence; d= precision; p= expected prevalence or proportion

Since the overall prevalence of helminth infections is not known for the study area, p was taken to be 50%. For the calculation, a 95% confidence interval (z) and a 5% margin of error (d) was used. Hence, the estimated sample size was 384.

To compensate for the likelihood of non-compliance, 5% of the samples were added to the initially estimated sample size (384). Hence, this addition of contingency (5% of 384), gave 19. As a result, 403 school children were chosen to participate in the study.

8.4.2. Sampling Technique

To select the study subject, the students were first stratified according to their educational level (Grade 1 to Grade 8). Allocation of student was done proportional to the number of students in each grade. Finally, the study subjects were selected using systematic random sampling by using class roster as the sampling frame. Every fourth study subject was selected after the first study subject.

8.5. Data Collection Procedure

8.5.1. Kato-Katz Technique

The school children were provided with small clean plastic sheets and clean wooden applicator stick. They were then informed to bring sizable stool sample of their own. Then the stool samples were processed for microscopic examination using double Kato–Katz slides employing a template delivering 41.7 mg of stool (WHO, 1991), and average fecal egg count were used.

Briefly, portion of the fecal specimen was taken by clean wooden spatula and was forced through the nylon screen to separate fecal materials from the large debris. The screened fecal material was transferred to the template which was laid flat centrally on a microscope slide. The template hole was completely filled with screened fecal material and was leveled to the surface of the template. The cellophane square which was soaked in malachite green-glycerin solution was placed over the fecal specimen. The specimen was made to spread evenly under the cellophane tape by pressing it with another glass slide (prepared for this purpose).

The prepared Kato-Katz slides were examined systematically under the middle (100X) objectives of the microscope for STHs and schistosomes ova. Hookworm eggs were examined within 30 minutes after preparation. Negative samples were re-examined on the same day at the same time by another laboratory technician and the researcher. Eggs counted for each species of STHs and schistosomes were recorded and later were converted into eggs per gram of stool (EPG), multiplying by a factor of 24. Infection intensity (light, moderate, and high) was classified as described by World Health Organizations criteria (WHO, 2002).

8.5.2. Hemoglobin Determination

Blood was collected by finger puncture using disposable lancet and Hb was determined by a portable hemoglobinometer (Hemocue, Hemocue HB 201 analyzer) to determine anaemia (WHO, 2001).

The tip of the middle finger was cleaned with alcohol-soaked cotton and was pricked with a disposable blood lancet, and two drops of blood was wiped away with dry cotton. The next drop of blood was used to fill the microcuvette by touching the microcuvette tip into the middle of the drop of blood until completely filled by avoiding air babble. The filled microcuvette was then put on the microcuvette holder and pushed into the Hemocue instrument. The Hb value was displayed in g /dl after approximately 30 seconds was then recorded. School children with hemoglobin levels lower than 11.5g/dl and 12g/dl were considered as anemic for age ranges of 6-11 and 12-14 years (WHO, 2001).

8.6. Ethical Consideration

Prior to the commencement of the study, the project obtained ethical clearance from the Ethical Clearance Committee of the Department of Biology. Permission was also obtained from Jiga, local education authority of the school and health center to conduct the study. The sample was collected from assented children. Finger puncture was done with safe disposable lancet. Children who were found positive for *S. mansoni* were treated with praziquantel at the single oral dose of 40 mg/kg of body weight while STHs were treated with albendazole (400mg) in a single dose (WHO, 2002). Albendazole and praziquantel were co-administered by health professionals for schistosomiasis and soil-transmitted helminthiasis (WHO, 2006). Finally, the school children and staff were given health education on personal hygiene and environmental sanitation by the principal investigator.

8.7. Quality Control

Standard operating procedures were used for specimen collection and processing to obtain a good quality data. The data collection was supervised by the researcher. Ten percent of samples were randomly selected and rechecked blindly. The use of two different techniques, Kato - Katz and Formol-Ether concentration for diagnosis of the parasite was also used as a quality control. Each Hemocue photometer was checked for calibration every morning before use.

8.8. Statistical Analysis

The data was computerized using Excel 2007, cleaned and checked against original document before analysis. All statistical analyses were performed using SPSS for windows version 15 statistical package. Descriptive statistical tests were applied to calculate the prevalence of STHs and schistosomiasis and anemia as percentages and proportions. To test the null hypothesis, inferential statistical analyses of comparisons between two categorical variables was carried out using Pearson chi-square (x^2) test to verify the relationship between independent factors and the outcome variables. Logistic regression analysis (Odds ratios, OR) was used to determine association of intestinal helminth infections with anemia. The 95% CI was used to show the accuracy of data analysis. Probabilities less than 5% ($P < 0.05$) for null hypothesis testing were considered statistically significant.

9. RESULTS

9.1. Profile of study participants

A total of 403 study participants from grade 1 to grade 8 were enrolled in this study. Out of 403 study participants, 186(46.15%) were male and 217(53.85%) female. 147(36.48%) students were 6-10 years old and 256(63.52%) were 10 to 15 years old. Out of 403 study participants, 235(58.31%) were found to be positive for soil-transmitted helminthes and schistosome and 59(14.64%) were anaemic (Table 1).

Table 1: Profile of study participants in Tikur Wuha Elementary School, Jiga, Northwestern Ethiopia, February - March, 2011

Characteristics		%(No of study subjects)
Age groups in years	6-10 years	36.48(147)
	11-15 years	46.15(256)
Sex	Male	46.15(186)
	Female	53.85(217)
Anaemic	Mild	13.15(53)
	Moderate	1.49(6)

9.2. Prevalence of Intestinal Helminth Infections

The overall prevalence of STHs and schistosome infections among school children in Tikur Wuha Elementary School, Jiga, Northwestern Ethiopia was 58. 31%. Single, double, triple and quadruple infections were 41.19%, 15.38%, 1.49% and 0.25%, respectively. The prevalence of hookworm, *Schistosoma mansoni, Ascaris lumbricoides*, *Trichuris trichiura*, *Enterobius vermicularis*, and *Hymenolepis nana* infections were 46.90%, 24.57%, 4.22%, 1.74%, 0.5% and 1.24%, respectively. There was no single infection for *Enterobius vermicularis* and *Hymenolepis nana*, but found in mixed infections.

Table 2: Prevalence of intestinal helminth infections among school children in Tikur Wuha Elementary School, Jiga, Northwestern Ethiopia, February - March, 2011

Helminth infections	% (Positive)
Hookworm species	46.90(189)
Ascaris lumbricoides	4.22(17)
Trichuris trichiura	0.25(1)
Schistosoma mansoni	24.57(99)

No heavy infection was observed for hookworm, *A. lumbricoides* and *T. trichiura*. For *S. mansoni,* only 1 child was found to be heavily infected (Table 2). In this study, the majority of infections were light except few children who had moderate intensity of infections.

Table 3: Intensity of infection for STHs and schistosomiasis among school children in Tikur Wuha Elementary School, Jiga, Northwestern Ethiopia, February - March, 2011

Helminths and classes of intensity(epg)	% (Number)
Ascaris lumbricoides	
Light (1-4,999)	3.97(16)
Moderate (5,000-49,999)	0.25(1)
Hookworm species	
Light (1-1,999)	46.90(189)
Trichuris trichiura	
Light (1-999)	1.49(6)
Moderate (1,000-9,999)	0.25(1)
Schistosoma mansoni	
Light(1-99)	19.60(79)
Moderate (100-399)	4.71(19)
Heavy(≥400)	0.25(1)

The prevalence of infection did not differ significantly by sex for hookworm, *A. lumbricoides* and *T. trichiura*, but there was significance difference by sex for *S. mansoni* infection. The prevalence of *S. mansoni* infection among males was found to be higher than that of females and the association was statistically significant ($P<0.05$). There were no statistically significant differences in the prevalence of STHs and schistosomiasis between age groups.

Table 4: Prevalence of STHs and schistosomiasis by sex and age groups among school children in Tikur Wuha Elementary School, Jiga, Northwestern Ethiopia, February - March, 2011

Helminths	**%(Number) infected**					
	Male	**Female**	**P value**	**6-10 years**	**11-15 years**	**P value**
Al	1.49(6)	2.73(11)	0.359	1.74(7)	2.48(10)	0.665
Hw	21.59(87)	25.31(102)	0.963	18.11(73)	28.78(116)	0.347
Tt	0.74(3)	0.99(4)	0.860	0.743(3)	0.99(4)	0.713
Sm	13.65(55)	10.91(44)	0.031	7.69(31)	16.87(68)	0.241

Al =*Ascaris lumbricoides*, Tt =*Trichuris trichiura,* Hw = Hookworm and Sm = *Schistosoma mansoni* (Table 3).

9.3. Association of Intestinal Parasitic Helminth Infection and Anaemia

The overall prevalence of anaemia associated with STHs and schistosomiasis among school children was 11.91% (48/403). Only 2.27% of anaemia was attributable to non-intestinal helminth infections. The prevalence of anaemia was significantly higher among hookworm and *Schistosoma mansoni* positive children ($P<0.05$). No association was observed between anaemia and *Ascaris lumbricoides* and *Trichuris trichiura* in the present study ($P< 0.05$).

Table 5: Association of intestinal helminth infection and anaemia (hemoglobin) among school Children, Jiga, Northwestern Ethiopia, February - March, 2011

Helminths	%(infected number)	%(number) anaemic	OR(95% CI)	P value
Hookworm	46 .90(189)	20.63(39)	2.28(1.26- 4.12)	0.001*
A. lumbricoides	4.22(17)	17.64(3)	0.86(0.22-3.32)	0.720
T. trichiura	1.74(7)	14.29(1)	0.72(0.08-6.47)	0.979
S. mansoni	24.57(99)	26.26(26)	2.73(1.51-4.95)	0.000*

9.4. Effect of Deworming on Intestinal Helminth Infections and Anaemia Burden

9.4.1. Effect of Deworming on Intestinal Helminth Infections

The prevalence of hookworm, *Schistosoma mansoni*, *Ascaris lumbricoides* and *Trichuris trichiura* infections was reduced from 46.9% to 4.22%, 24.57% to 4.47%, 4.22% to 0.25% and 1.74% to 0.5%, respectively, due to deworming (Figure 2).

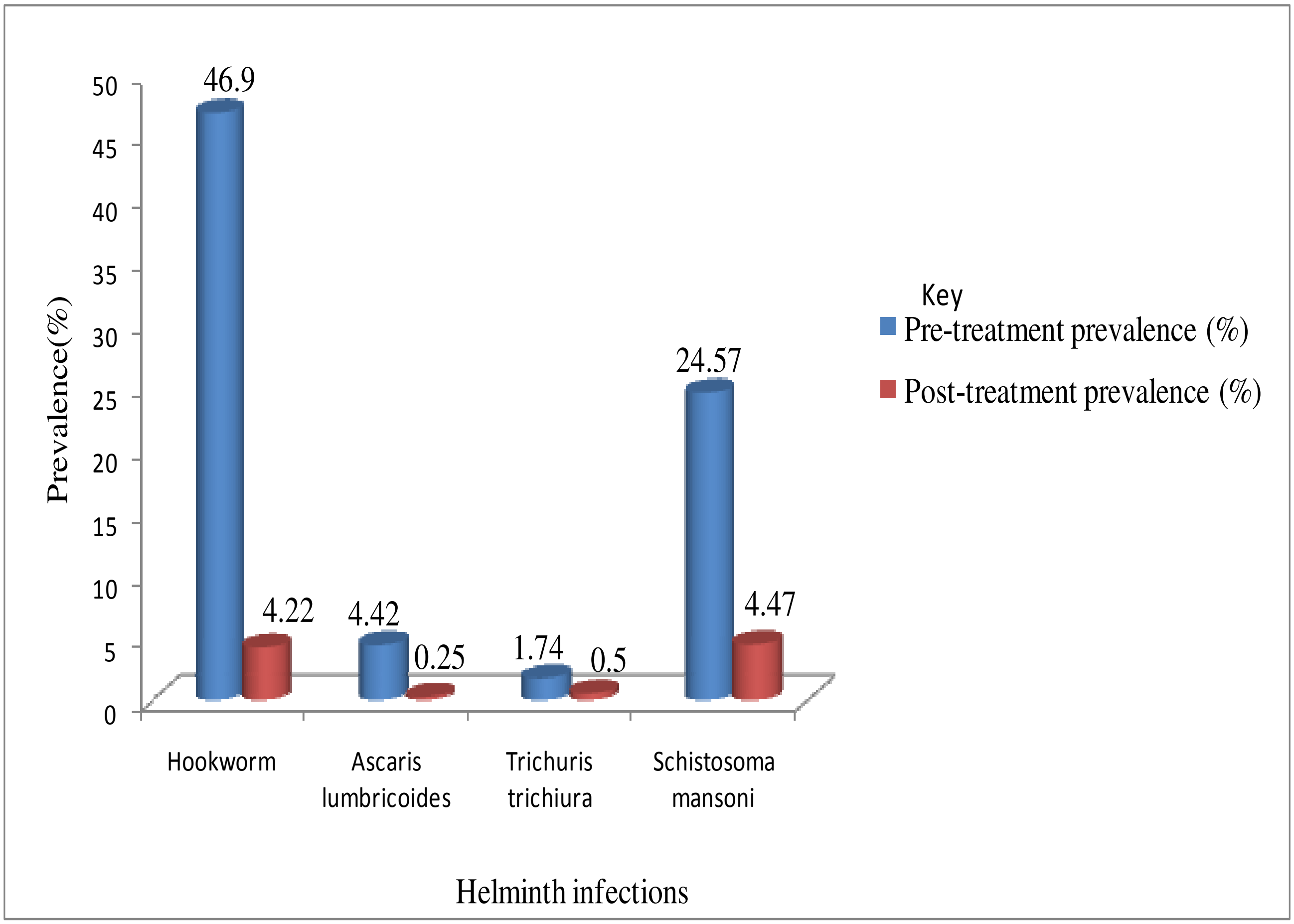

Figure 6: Pre- and post-treatment prevalence of intestinal helminth infections among school children in Tikur Wuha Elementary School, Jiga, Northwestern Ethiopia, February - March, 2011

9.4.2. Effect of Deworming on anaemia

After anthelminthic treatment, there was a rise in the mean hemoglobin of school children who were dewormed from an initial pre-treatment mean hemoglobin of 12.73±1.18 g/dl to a post-treatment of 13.96±1.21 g/dl (paired t-test = 6.8502; *P* = 0.000). Following deworming, the prevalence of anaemia was reduced from 11.91% to 8.44 % (Table 6).

Table 6: Effect of deworming on anaemia among school children, Jiga, Northwestern Ethiopia, February - March, 2011

Anaemia	**Pre-treatment (235)**	**Post-treatment (235)**	**t-test**	**P-value**
Mean (SD) hemoglobin level(g/dl)	12.73(1.18)	13.09(1.21)	6.850	0.000
Prevalence of anaemia	11.91%	8.44%		
Mild anaemia	10.92%	7.94%		
Moderate anaemia	0.99%	0.50%		

10. DISCUSSION

Knowledge about the causes of anaemia in a given community is very crucial for planning and evaluation of intervention programs. The present study assessed the effect of intestinal helminth infections and deworming to anaemia among Tikur Wuha Elementary School children, Northwestern Ethiopia. The overall pre-deworming anaemia in this study was 14.64%, which was mild. A similar prevalence rate was reported in children in Northern Ethiopia (Addish *et al.,* 1998). On the other hand, the prevalence of anaemia observed among school children in the present study was much lower than the prevalence of anaemia (48%) among Northern Kenya school children (WHO, 1996). The lower prevalence of anaemia in the present study could be attributed to consumption of "*teff*", a cereal which has high iron content mainly due to contamination with the soil (WoldeGabriel, 1993).

The overall prevalence of intestinal helminth infections in the present study was 58.31%, and this was similar to prevalence of infection (55.6%) reported among school children in Gondar, Northwestern Ethiopia (Worku *et al*., 2009). Nevertheless, the prevalence of intestinal helminth infections observed in the current study was relatively lower than previous reports from other parts of the country (Erko and Medhin, 2003; Legesse and Erko, 2004; Tadesse, 2005). These findings indicate that helminth infections very considerably in occurrence between districts and regions. The differences in prevalence among the different communities appear to be associated with environmental sanitation, water supply and socioeconomic status of households, although this needs to be verified in more extensive follow up studies. Other factors related to macro-and micro-environment, time of study, method of examination, etc., do also contribute to the differences in the prevalence and distribution of these intestinal helminths.

In the present study there was no significant difference in the prevalence of STHs and schistosomes between age groups. This might be due to similar exposure risk to STHs and schistosomes between these age groups. There was significant difference between genders in prevalence of *S. mansoni*. The prevalence of *S. mansoni* infection among males was found to be higher than that of females and the association was statistically significant ($P<0.05$) and this was similar with other study (Assefa *et al*., 1998). This could be explained by the fact that males stay most of the time outdoors, playing, swimming in rivers compared to females who seldom participate in such activities (Erko *et al*., 1997).

Analysis of the class of intensities of infection for hookworm, *S. mansoni, A. lumbricoides* and *T. trichiura* showed the infection to be light for hookworm, light, moderate and heavy for *S. mansoni*, light and moderate for both *A. lumbricoides* and *T. trichiura.* This agrees with the finding of Anderson and May (1991) that for all the major human STHs and schistosome infections, worm burdens exhibit a highly aggregated (overdispersed) distribution so that most individuals harbor just a few worms in their intestines, although a few hosts harbor disproportionately large worm burdens.

The current study showed significant association of anaemia with hookworm infections. This observation agrees with the earlier studies done in other parts of Ethiopia (Tsegaye *et al.,* 1999). A light hookworm load can cause anaemia in people whose intake of iron is low and whose iron stores are already depleted (Stoltzfus *et al.,* 1997). Similarly, the present study showed that light intensity of hookworm infections had significant associations with anemia. Bates *et al.* (2007) also reported that heavy and light intensity of hookworm infections showed very strong relationships with anaemia. On the other hand, (Legesse and Erko, 2004) reported that an association was not found between hookworm infection and low haematocrit value of school children in southeast of Lake Langano. The possible explanations for the observed discrepancy between these findings could be variations in hookworm species and load, duration of infection, body iron stores and dietary intake and absorption.

In this study, *Schistosoma mansoni* was positively associated with anaemia. This agrees with studies from Kenya, Niger and Tanzania that have shown hemoglobin levels of children and pregnant women to correlate negatively with egg count in both *S. mansoni* and *S. haematobium* infections (Stoltzfus *et al.,* 1997; Gilgen *et al.,* 2001).

On the other hand, association was not observed between ascariasis, trichuriasis and anaemia in the present study. This is consistent with the report of Bates *et al.* (2007), but inconsistent with the study from Zanzibari (Stoltzfus *et al.,* 1997) and Nepalese (Curtale *et al.,* 1993) in which *A.*

lumbricoides has been linked to low hemoglobin concentrations. Nevertheless, the mechanism by which this worm causes anemia is uncertain, as the worm feeds on gut contents rather than on blood (Kraemer and Zimmermann, 2007). The current study showed that there was no significant association between trichuriasis and anaemia. This agrees with the previous report of Osazuwa *et al.* (2011).

Anthelmintic drug treatments keeps the infection below the level of public health importance and improve iron stores (de Silva, 2003). The current study showed that deworming was associated with reduced prevalence of intestinal helminth infections and anaemia. Similarly, other studies also indicated that anthelmintic treatment delivered as part of a helminth control programme can decrease infection and morbidity among schoolchildren and improve hemoglobin concentration (Gilegen and Mascie-Taylor, 2001; Khan *et al.,* 2005; Gulani *et al.,* 2007).

After deworming, there was a rise in the mean hemoglobin of school children, from 12.73±1.18 pre-treatment level to 13.96±1.21 g/dl post-treatment level. In agreement with the present study, studies from Indonesia, Panama, Philippines, Sierra Leone, South Africa, Sri Lanka, Tanzania and Uganda reported that administration of intestinal anthelmintic agents improve hemoglobin concentration (Robertson *et al.,* 1992; Stoltzfus *et al.,* 1997; Bhargava *et al*, 2003; Guyatt *et al.,* 2001; de Silva, 2003; Koukounari *et al .,* 2008). However, contrary to the present study, de Silva (2003) reported that albendazole and mebendazole treatment of children targeting *Trichuris trichiura and* hookworm did not have such benefit. Such variations may probably be due to variations in the effectiveness of deworming which includes anthelmintic treatment efficacy, post-treatment reinfection and presence of other related causes of anaemia.

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11. CONCLUSION AND RECOMMENDATIONS

The current study showed high prevalence result of intestinal helminth infections among school children in the study area. Anaemia was found to be mild (14.64%). Intestinal helminth infections, particularly hookworm and *Schistosoma mansoni* were associated with anaemia in the study area. The present study also showed that deworming as part of helminth control can decrease intestinal helminth infections and improve hemoglobin concentration among school children.

Therefore, based on the finding of the study, the following points are recommended.

- ❖ Deworming of school children twice a year should be considered as one of the main strategies for the control of anaemia in the study area.

- ❖ Further studies should be conducted on the other causes of anemia and long term effect of deworming on anaemia among school children in the study area.

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13. APPENDICES

Annex 1: For STHs and schistosomiasis school survey

- Participant identification

1.1 Name of the school ____

1.2. Student's code __________

1.3 Student's name ____

1.4 Grade and section___________________

1.5 Age__________________

1.6 Sex 1. Male ☐ 2. Female ☐

1.8 Duration of stay_______________________

- Laboratory Data

Results of stool examination

Schistosoma mansoni eggs/ slide ___ eggs/gram __________

Ascaris lumbricoides eggs/slide____________ eggs/gram___________

Trichuris trichiura eggs/slide____________ eggs/gram___________

Hookworm eggs/slide____________ eggs/gram___________

Others_________________, _____________________, ____________________

Annex 2: Laboratory procedure

- Kato - thick technique Procedure

Materials**:**

Spatula	Previously soaked pre-soaked cellophane
Applicator sticks	Plastic sheet
Template delivering 41.7mg of stool	Microscope slides
Screen with specific mesh size	Forceps

Procedure

1. Put a screen over the sizeable stool specimen on the plastic sheet
2. Scrap the stool under the screen with spatula so that stool will be sieved out of the screen
3. Collected the sieved stool with spatula and fill it in the holes of templates which each holes form approximately 41.7mg stool sample
4. Remove the template carefully so that the cylinder of feces is left on the slide.
5. Cover the slide with the stool with pre-soaked cellophane
6. Gently slide over the sample with another slide so that the stool spread evenly
7. Leave the prepared slide for at least 24 hrs to clear the smear
8. Examine the slide with microscope for the presence of STHs and schistosome egg.
9. Count the number of eggs encountered per each slide

Annex 3: Consent form

For participation as volunteer in research undertaking

I am a post graduate student from the Faculty of life Science, Department of Microbiology, Cellular and Molecular Biology Program Unit, at Addis Ababa University. I am here to study the impact of intestinal helminth infections and deworming on anaemia in your school. I am requesting your children to participate in this study which would require his /her response to obtain stool sample, a drop of blood sample from finger. There is no any health related risk in participating. When you or your children are found positive for intestinal helminthiasis and anemia, you will receive standard drugs free of charge. The information in your records is strictly confidential.

Your participation in this study is completely voluntary and you can refuse to participate or free to withdraw yourself from the study at any time. Refusal to participate will not result in loss of medical care provided or any other benefits. Do you understand what has been said to you? If not, you have the right to get proper explanation.

I, the, undersigned have been informed about the study objectives. I have also been informed that all the information is to be kept confidential and that I have the right to decline from or to cooperate in the study. Therefore, with full understanding of the study objective I agree to give the informed consent voluntarily to the researcher to identify the parasites and measure hemoglobin concentration of my children.

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

Study Code No _________Study area_____________________________________

Name_____________________________Signature____________Date__________

Witness

Name____________________________ Signature ________ Date_________

Investigator

Name ________________________________ Signature _________ Date ___