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SCHOOL OF GRADUATE STUDIES

DETERMINANTS OF ANAEMIA IN PREGNANT WOMEN WITH EMPHASIS ON INTESTINAL HELMINTHIC INFECTION AT BUSHULO HEALTH CENTER SOUTHERN ETHIOPIA

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A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES OF ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN MEDICAL PARASITOLOGY

BAMLAKU TADEGE TSEHAYU

ABSTRACT

Anaemia is a common and serious problem in pregnancy. Parasitic infections contribute to iron deficiency anemia in pregnant women. The objective of the study was to assess determinants of anaemia with emphasis on intestinal helminthic infection in pregnant women. A cross-sectional study was carried out among pregnant women who came for their antenatal care follow up at Bushulo health center, Tula woreda, Southern Ethiopia from July – August 2008. A total of 374 pregnant women were screened for intestinal parasitic infection using direct microscopy and formol ether concentration techniques and level of haemoglobin was determined using haematocrite screening technique. Other determinants of anaemia during pregnancy were also assessed using a structured questionnaire. Data were analyzed using SPSS for windows version 13.0. Statistical tests were performed at the level of significance of 5%. Prevalence of intestinal parasite infection in pregnant women was 58.2%. Out of 218 intestinal parasite infected women 88(40.4%) had more than one intestinal parasitic infection. Prevalence of anaemia in this study subjects was 51.9% and the mean haematocrite level was 34%. Anaemic women were 14 times likely to have hookworm plus other intestinal helminthic infection (p value = .000), 2 times likely to have birth interval less than two years (p value = .018), 2 times likely not to have shoe wearing habit (p value = .045) and 2 times likely not having taken iron during pregnancy (p value = .025). It is suggested that all ANC attendants should be screened for anaemia and intestinal helminthic infection at their first visit and those anaemic and helminthic infected women should be treated properly. Health education should be given to pregnant women about family planning, importance of wearing shoe, importance taking iron and the community as a whole to use latrine.

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Acronyms

ANC- Antenatal care

HB- Haemoglobin

IUGR- Intrauterine growth retardation

LBW- Low birth weight

PCV- Packed cell volume

UNICEF- United nation international children emergency fund

WHO- World health organization

10X- Ten times magnification

40X- Forty times magnification

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1. INTRODUCTION

Anaemia is one of the most widespread public health problems, especially in developing countries. It impaired cognitive development, reduced physical work capacity and in severe cases increased risk of mortality particularly during prenatal period (WHO, 2001). Anemia in pregnant women is defined hemoglobin levels less than 11g/dL (WHO, 1996). It is usually caused by iron deficiency, which is the most common nutrient deficiency in the world. It has been estimated that, at any one time in developing countries, half of the population (mainly children and women of reproductive age) is affected by anemia (Hercberg and Galan, 1992). During pregnancy, approximately 75% all anaemia diagnosed are due to iron deficiency (Sifakis and Pharmackides, 2000).

Iron deficiency is highest in population sub groups that are peak rates of growth; namely infants, young children and pregnant women. Pregnancy is the time during which the risk for developing iron deficiency anaemia is greatest as iron requirements are substantially higher than average (Zavaleta *et al.*, 1993, Allen, 1997). Furthermore, WHO considers that women in developing countries may be pregnant for as much as one half of their reproductive lives and therefore are at increased risk of anaemia during this time (WHO, 1996).

In the developing world, young women, pregnant women and their infants and children frequently experience a cycle, where under nutrition (macro nutrient and micronutrient) and repeated infection; including parasitic infections, lead to adverse consequences that can continue from one generation to the next. Among parasitic infections, malaria and intestinal helminthes co-exist widely with micro nutrient deficiencies and contribute importantly to anaemia and this cycle retard growth and development (Steketee, 2003). .

An estimated 44 million pregnant women are infected with hookworm worldwide, with 7.5 million in sub–Saharan African alone. Hookworm infection is considered a major health threat to adolescent girls and women of reproductive age, with adverse effects on the outcome of pregnancy (Peter *et al.*, 2004). Hookworm infections induce deficiencies of iron, total energy, protein and possible folate and zinc (Nurdia *et al.*,

2001). Severe iron deficiency anaemia during pregnancy has been linked to increased maternal mortality, impaired lactation prematurity and low birth weight (Peter *et al.*, 2004). Estimates in Kenya and Nepal suggest that hookworm infection causes 30 percent and 41 percent, respectively, of moderate or severe cases of anaemia among pregnant women (haemoglobin level, < 9 g per deciliter. The association between hookworm infection and anaemia is greatest in multigravidas (Peter *et al.*, 2004). Studies in Africa and Asia reported a higher prevalence of anaemia and its association with women of age < 20 years, third trimester of pregnancy, rural residents and multi–parous women (Singh and Fong, 1998).

Anaemia in pregnancy is also related to different socio-demographic, dietary and economic factors (15, 16). Mother's age < 20 years, educational status, economic position, and antenatal care were significantly associated with anaemia during pregnancy in a study conducted in India (Bechuram *et al.*, 2006).

In Ethiopia, anaemia is the most frequent morbidity among pregnant women with the prevalence raging from 23–66.5% (Tadios, 1996, Gebremedin, 2004). There is an urban rural difference in the prevalence of anaemia. As indicated by studies in Asendabo and Mettue, anaemia among pregnant women was consistently higher in the rural women compared to the urban counterparts (Tadios, 1996, Gebremedin, 2004).

The purpose of this study is therefore to assess determinants of anaemia in pregnant women attending antenatal care at Bushulo health center.

2. LITERATURE REVIEW

Anaemia in pregnancy is related to different socio-demographic factors (15, 16). Age, educational status, economic position, antenatal care and different parasitic infections have been found to be significantly associated with anaemia during pregnancy (Bechuram *et al.*, 2006; Stephenson *et al.*, 2000, WHO, 2002; Guyatt and Snow, 2001).

2.1. Anaemia

The health conscious world community has come to realize that anaemia, the majority of which is due to iron deficiency, has serious health and functional consequences (Alien *et al* 1994), is wide spread especially among tropical low income populations and that most of its nutritional component is controllable with a very high benefit/cost ratio. Women of fertile age and pregnant–lactating as well as their infants and young children are particularly affected (WHO, 1991).

It is estimated that more than 2 billion people are iron deficient globally. Among these people, 1.2 billion become severely anaemic. About 90% of all anaemia has an iron deficiency component. In the developing world nearly half of the population has iron deficient. However the industrial world is not free from it; 11% of its population has iron deficiency (WHO, 1991).

According to WHO, 1993 estimate, anaemia ranked the 8th leading cause of disease in girls and women in the developing world. Data collected from all over the world indicate that more than 2 billion people (men, women and children) are anaemic by WHO criteria. The most affected groups in approximately descending order are pregnant women, the elderly, school children and adult men. In developing countries, prevalence rates in pregnant women are commonly estimated to be in the range of 40%- 60%. Among non pregnant women this is 20%-40% and in school aged children and adult men the estimate is around 20% (WHO, 1993).

It was estimated that up to 56% of all non pregnant women living in developing countries were anaemic by WHO standard (Hb < 12g/dl), compared with 18% in industrialized countries (WHO, 1991). The greatest burden of anaemia is born by Asia

and Africa where it is estimated that 60% and 52% of women, respectively, are anaemic and between 1% and 5% are severely anaemic (Hb<7 g/dl) (WHO, 1992).

Anaemia increased maternal morbidity and mortality. It has been reported that close to 500,000 maternal deaths occur every year, vast majority taking place in developing world. Anaemia is thought to be the major contributory cause of death in 20 – 40% of these maternal deaths (WHO, 2001, McDermot, 1996). Furthermore it has been estimated that 16–20% of all maternal deaths are associated with iron deficiency anaemia (Crompton, 2000).

The unacceptable high prevalence of anaemia in developing countries could be an underestimate; data from rural areas is still lacking, the actual prevalence rates for many individual countries are not known, and there are very few community-based surveys (WHO, 1991).

A study conducted between July 1997 and June 1998, the prevalence of all anaemia (Hb < 11 g/dl) in a population of urban women (n = 4708) attending antenatal clinic at queen Elizabeth Hospital in Blantyre was 57.1% and the prevalence of severe anaemia (Hb < 7g/dl) was 3.6%. In rural area (Namitambo health center in chiradzulu district) prevalence of anaemia and sever anaemia in pregnant women (n = 2293) was 72% and 4% respectively (Van, 2000). In Peru, 35% of women of reproductive age and 50% of pregnant women were found to be anaemic (Zavaleta, 1993).

In Ethiopia anaemia is one of a serious health problem in pregnant women. Prevalence rates as high as 40.5% in the general population and 47.2% in children (WHO, 1993) were reported from North–West Ethiopia. Higher rates about 57% have also been reported in pregnant women in Jimma, Ethiopia (Desalegn, 1993).

2.1.1 Malaria Parasites

Four species of malaria parasites can infect humans under natural conditions: *Plasmodium falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. Malaria infection is endemic across the tropics and subtropics; affects people in more than 90 countries; causes 300–500 million infections each year; and is estimated to lead to approximately 1 million deaths each year, mostly in young children (Murphy and Breman, 2001). Most infections and the most severe morbidity and mortality are caused by *Plasmodium falciparum*. The other three human malaria parasites (*P.*

vivax, *P. malariae* and *P. ovale*) contribute to fewer infections and to more moderate disease and relatively few deaths (Mendis, 2001). *Plasmodium vivax* and *P. ovale* have dormant liver stage parasites ("hypnozoites"), which can reactivate ("relapse") and cause malaria several months or years after the infecting mosquito bite. *Plasmodium malariae* produces long-lasting infections and if left untreated can persist asymptomatically in the human host for years, even a lifetime. Most *P. falciparum* infections and consequences are in sub-Saharan Africa, but Asia, Southeast Asia and the Americas are also sites of transmission for this parasite.

It is estimated that each year over 30 million women become pregnant in malarious areas of Africa, with most living in areas of stable malaria transmission (WHO, 2003). Although the vast majority of women with malaria infections during pregnancy remain asymptomatic, infection increases the risk of maternal anemia and delivering a low-birth-weight (LBW) baby. LBW (<2,500 g) is an important risk factor for infant. Although a few reports of adverse consequences of *P. vivax* in pregnancy exist (Nosten *et al*., 1999), *P. falciparum* is the only human malaria parasite that is more common in pregnant than in non-pregnant women and is the only human parasite with a clear and substantial adverse effect on pregnancy, nutrition during pregnancy and pregnancy outcome (Steketee *et al.,* 2001).

2.1.1.1 Transmission

Malaria is transmitted among humans by female mosquitoes of the genus Anopheles. Female mosquitoes take blood meals to carry out egg production, and such blood meals are the link between the human and the mosquito hosts in the parasite life cycle. Of the approximately 430 known species of *A*nopheles, only 30-50 transmits malaria in nature. The successful development of the malaria parasite in the mosquito (from the "gametocyte" stage to the "sporozoite" stage) depends on several factors. The most important is ambient temperature and humidity (higher temperatures accelerate the parasite growth in the mosquito) and whether the Anopheles survives long enough to allow the parasite to complete its cycle in the mosquito host ("sporogonic" or "extrinsic" cycle, duration 10 to 18 days). Differently from the human host, the mosquito host does not suffer noticeably from the presence of the parasites (Mendis, 2001).

2.1.1.2 Pathophysiolpgical process

Erythrocytes infected with *P. falciparum* congregate in the maternal placental vascular space where the sinusoidal and low pressure blood flow, and possibly parasite adherence to endothelial

cells, allows parasites to sequester and replicate. An active immune response involving antibody production, cytokine release and a cellular response (principally a basophilic monocytic macrophage response) is frequently observed in malaria-infected placentas (Beeson *et al*., 2001, Ismail *et al*., 2000). The infection and, possibly, aspects of the immune response contribute to poor pregnancy outcomes of prematurity and fetal intrauterine growth retardation (IUGR). These adverse consequences appear to be mediated through several different pathways. The effect on prematurity is not entirely clear, but women with an active parasite infection and a fetus exposed to parasitized maternal erythrocytes may develop an immunologic response that contributes to stimulus of early onset of labor (Steketee *et al*., 2001).

The effect of malaria on IUGR appears to be basic to the system of nutrient transport to the fetus. High-density or prolonged parasite infection in placental blood and the consequent cellular immune response may require substantial nutrients and thus leave fewer nutrients (glucose and oxygen) available for passage to the fetus. In addition, histopathologic studies of malaria-infected placentas demonstrate thickening of cytotrophoblastic membranes, which may alter nutrient transport to the fetus (Ismail *et al*., 2000). Although the details of these biological processes are difficult to study except when the placenta is delivered, the overwhelming evidence of many studies suggests a clear adverse effect of malaria on LBW and prematurity (Steketee *et al*., 2001).

Malaria also clearly contributes to anemia throughout life and specifically during pregnancy. In a recent review of studies of *P. falciparum*–related anemia in pregnant women, (Guyatt and Snow, 2001) suggest that approximately 400,000 pregnant women develop moderate or severe anemia (hemoglobin <80 g/L or hematocrit <0.25) each year in sub-Saharan Africa as a result of malaria infection.

Malaria due to *P.falciparum* may cause severe anaemia in pregnancy. It is estimated that in sub Saharan Africa 23 million pregnant women are exposed to malaria infection annually. Women in their first and second pregnancies living in an endemic area are at higher risk of acquiring malaria than non-pregnant or multigravidae, due to reduction of an appropriate immune response to the malaria parasite (WHO, 1993).

Studies have shown that maternal anemia contributes independently to LBW through IUGR and to infant mortality. Although the specific biological processes are not clearly delineated, the

contribution of moderate and severe anemia to poor oxygen transport to the developing fetus is a likely mode of action for anemia's adverse effect on fetal growth. In addition, malaria-associated anemia in the mother likely has important consequences on her outcome whereby already anemic women are at increased risk of severe consequences (e.g., hypotension, shock, death) even with a moderate ante- or postpartum hemorrhage (Brabin, and Piper, 1997, Ismail *et al*., 2000).

Malaria in early childhood has long been thought to contribute to lasting under nutrition. Recently, in intervention studies with very malaria-specific interventions (e.g., insecticide-treated bed nets), malaria prevention was shown to substantially reduce the frequency of early stunting in children, suggesting that malaria contributes directly to stunting and that this is reversible in early childhood. This malaria effect on stunting then contributes further to the cycle noted above where poor nutrition in childhood (e.g., early and recurrent infection with *P. falciparum*) leads to short and small reproductive-age women who risk a poor pregnancy outcome (Kuile *et al*., 2003).

2.1.1.3 Routine Laboratory Diagnostic Method for malaria

A. Stained Blood Films

The accepted laboratory practice for the diagnosis of malaria is the preparation and microscopic examination of blood films stained with Giemsa, Wright's, or Field's stain (Warhurst, 1996). Blood obtained by pricking a finger or earlobe is the ideal sample because the density of developed trophozoites or schizonts is greater in blood from this capillary-rich area (Gille, 1993). Blood obtained by venipuncture collected in heparin or Sequestrine (EDTA) anticoagulant-coated tubes is acceptable if used shortly after being drawn to prevent alteration in the morphology of white blood cells (WBC) and malaria parasites. Both thick and thin blood films should be prepared.

Thick blood film

The thick blood film concentrates the layers of red blood cells (RBC) on a small surface by a factor of 20 to 30 and is stained as an unfixed preparation using Field's stain or diluted Wright's or Giemsa stain. The thick blood film provides enhanced sensitivity of the blood film technique and is much better than the thin film for detection of low levels of parasitemia and reappearance of circulating parasites during infection

recrudescence or relapse. The lysis of the RBC during the staining process can make the process of scanning for parasites more difficult until experience is gained in finding the parasites among the WBC and platelets (Warhurst 1996).

Thin blood film

The thin blood film is methanol fixed and stained with diluted Giemsa or Wright's stain using buffered water at pH 7.2 to emphasize the parasite inclusions in the RBC. Because of the fixed monolayer of RBC available in this procedure, the morphological identification of the parasite to the species level is much easier and provides greater specificity than the thick-film examination. The thin blood film is often preferred for routine estimation of the parasitemia because the organisms are easier to see and count. The ability to count parasites in sequential blood films enables the response to therapy to be monitored, particularly for *P. falciparum* infections (Warhurst, 1996).

Treatment of malaria during pregnancy is highly effective in clearing or reducing placental infection, anemia and LBW consequences. Because of the high frequency of *P. falciparum* infection in many African settings, a preemptive approach using intermittent preventive treatment at regularly scheduled antenatal clinic visits and providing insecticide-treated bed nets for each pregnant woman has been shown to be highly effective; this approach was adopted by the Roll Back Malaria partnership and established as policy in a number of countries (Steketee *et al.*, 2001, Kuile *et al.*, 2003, Nahlen, 2000). Studies have shown that additional supplementation of iron and other micronutrients; possibly including vitamin A and folate should be coupled with antimalarial use for anemia and LBW prevention in pregnancy (Richard, 2003a).

2.1.2 Intestinal Helminthic infection

Helminths (the word is derived from the Greek meaning "worms" (Faust *et al.*, 1970). There are two major phyla of helminths. The nematodes (also known as roundworms) include the major intestinal worms (also known as soil-transmitted helminths) and the filarial worms that cause lymphatic filariasis (LF) and onchocerciasis, whereas the platyhelminths (also known as flatworms) include the flukes (also known as trematodes), such as the schistosomes, and the tapeworms (also known as the cestodes), such as the

pork tapeworm that causes cysticercosis. The most common helminthiases are those caused by infection with intestinal helminths, ascariasis, trichuriasis, and hookworm, followed by schistosomiasis and LF. Practically speaking, this means that the inhabitants of thousands of rural, impoverished villages throughout the tropics and subtropics are often chronically infected with several different species of parasitic worm; that is, they are polyparasitized (Hotez, 2007).

The soil-transmitted helminthiases are ancient diseases that continue to cause misery and disability in poor populations. About 2 billion harbor these infections worldwide, of which 300 million suffer associated severe morbidity. Of the total number infected, an estimated 400 millions are school-age children. In 1999, WHO estimated that schistosomiasis and soil-transmitted helminthiasis represented more than 40% of the disease burden due to all tropical diseases, excluding malaria (WHO, 2003).

According to World Health Organization estimate, globally there are 800-1000 million cases of the round worms *(Ascaris lumbricoides)*, 700-900 million cases of the hook worm *(Necator americanus* and *Ancylostoma duodenale)* and 500 million cases of the whip worm *(Trichuris trichura*) (WHO, 1986).

Although acute symptoms of infection are uncommon, numerous studies have shown a consistent association between intestinal nematode infection and diminished food intake and weight loss (Gyorkos *et al.*, 2004, Richard., 2003b). The total amount of work a women can do in a day definitely decrease when she is anaemic, what ever the cause is, and pregnancy plus helminth infections produce a double burden for a women in some rural farming community(Stephenson *et al.*, 2002). Women may even acquire helminth infections in the process of growing the family's food and thus increase their degree of anaemia in pregnancy, as for example, in Vietnam, where insufficiently composed human faeces may be used as fertilizer on vegetable crops (Humphries *et al.*, 1997). Intestinal parasitic infection especially helminthes, increase anaemia in pregnant women (Otieno, 1999).

Geohelminth infections in pregnancy have been associated with iron deficiency, maternal anemia, and impaired nutritional status, as well as decreased infant birth weight, intra-uterine growth retardation, and adverse birth outcomes (WHO, 2002).

Hookworm infections can cause or exacerbate iron deficiency and anemia. Blood loss can be a feature of *Trichuris trichiura* infection, but it is less prominent than in hookworm infection; however, it often occurs along with hookworm infections and so may accelerate the onset of iron-deficiency anemia. *Ascaris lumbricoides* infections are commonly asymptomatic, although clinical complications of extra-intestinal or high numbers of ascaris have been well described. *A. lumbricoides* infection has been associated with impaired fat digestion, reduced vitamin absorption, and temporary lactose intolerance, and treatment has shown to improve nutritional status (Stephenson *et al*., 2000, WHO, 2002)

A study conducted in Venezuela, where more than a thousand young asymptomatic pregnant women were evaluated a high prevalence of intestinal parasitosis (more than 70%), higher than those previously reported for pregnant women in Congo (9%), Nigeria (12.5%), Mexico (38.2%), Brazil (45.1%) and 69.2% at different sites in Indonesia. From infected pregnant women in Venezuela half of them had mixed infections, due to different parasite species, which represented a significant risk to have anaemia, almost twice than those women who did not. Parasitic infected pregnant mother not only presented a higher frequency of anaemia but also significant lower level of haemoglobin and haematocrit and obviously higher levels of eosinophilia (Alfonso *et al.*, 2006).

In the study conducted in Iquitos, Peru, the over all prevalence were 47.22% for hookworm, 82.25% for trichuris, and 63.92% for ascaris. Only 9.31% of the pregnant women were free of any parasite infection; 20.25% of the women had a single infection, 38.96% had two infections and 31.48% were infected with all three worm infections. The prevalence of trichuris and hookworm co-infection was 44.05% (Renee *et al*., 2005).

In Ethiopia, soil transmitted helminthic infections are frequently reported and highly prevalent. *Ascaris lumbrcoides (A. lumricoides)* and *Trichuris trichuria (T. trichuria)* are wide spread in Ethiopia but prevalence rate vary considerably: rates are lowest in the low land and dry areas of the country than in more humid high lands (Jemaneh, 1998).

In studies done in 13 administrative regions between 1978- 1981 in Ethiopia *A. lumbricoid* (43.5%) was the most prevalent parasite, followed by *T. trichuria* (25.5%) and hookworm (10.3%). The highest rate of multiple infections was observed in the age of 10-19 years, followed by the 20-29 years and those below 10 and above 60 years had the lowest rates (Tedla, 1986). Prevalence of intestinal helminthic infection in a study conducted among fisher children at Lake Awassa area was, *A. lumbricoides* (76%), hookworm (62.5%) and *Schistosoma mansoni* (*S. mansoni)* (33%) (Merid *et al.*, 2001). Wide distribution of intestinal helminthes in Ethiopia like other developing countries is attributable to low socio-economic status and poor sanitation, absence of safe drinking water supplies and inadequate medical care.

2.1.2.1 Hookworm

The hookworm is a parasitic nematode worm that lives in the small intestine of human. Two species of hookworms commonly infect humans, *Ancylostoma duodenale* and *Necator americanus*.

A. Biological Life Cycle

N. americanus and *A. duodenale* eggs can be found in warm, moist soil where they will eventually hatch into first stage larvae, or L1. L1, the feeding non-infective rhabditoform stage, will feed on soil microbes and eventually molt into second stage larvae, L2. L2, which is also in the rhabditoform stage, will feed for approximately 7 days and then molt into the third stage larvae, or L3. L3 is the filariform stage of the parasite, that is, the non-feeding infective form of the larvae. The L3 larvae are extremely motile and will seek higher ground to increase their chances of penetrating the skin of a human host. The L3 larvae can survive up to 2 weeks without finding a host. It is important to note that while *N. americanus* larvae only infect through penetration of skin (Hawdon, 1996).

A. duodenale can infect both through penetration as well as orally. After the L3 larvae have successfully entered the host, the larvae then travel through the subcutaneous venules and lymphatic vessels of the human host. Eventually, the L3 larvae enter the lungs through the pulmonary capillaries and break out into the alveoli. They will then travel up the trachea to be coughed and swallowed by the host. After being swallowed, the L3 larvae are then found in the small intestine where they molt into the L4, or adult worm stage. The entire process from skin penetration to adult development takes about 5–9 weeks. The female adult worms will release eggs (*N. americanus* about 9,000-10,000 eggs/day and *A. duodenale* 25,000-30,000 eggs/day) which are passed in the feces of the human host. These eggs will hatch in the environment within several days and the cycle with start a new (Hawdon, 1996).

B. Pathology

Hookworm infection is generally considered to be asymptomatic, but it is an extremely dangerous infection because its damage is “silent and insidious.There are general symptoms that an individual may experience soon after infection. Ground-itch, which is an allergic reaction at

the site of parasitic penetration and entry, is common in patients infected with *N. americanus*. Additionally, cough and pneumonitis may result as the larvae begin to break into the alveoli and travel up the trachea. Then once the larvae reach the small intestine of the host and begin to mature, the infected individual will suffer from diarrhea and other gastrointestinal discomfort. However, the "silent and insidious" symptoms are really mainly related to chronic, heavy-intensity hookworm infections. Major morbidity associated with hookworm is caused by intestinal blood loss, iron deficiency anemia, and protein malnutrition (Hotez and Pritchard, 1995). They result mainly from adult hookworms in the small intestine ingesting blood, rupturing erythrocytes, and degrading hemoglobin in the host (Hotez *et al*., 2005).

Hookworm infection causes mechanical laceration and enzymatic damage to the mucosa of the small intestine leading to approximately 0.05 ml/dl of blood loss per adult *Necatur americnus* and approximately 0.25 ml/dl per adult *Ancylostoma duodenale* (Huddle *et al*., 1999). This long-term blood loss can manifest itself physically through facial and peripheral edema; eosinophilia and pica caused by iron deficiency anemia are also experienced by some hookworm-infected patients. Recently, more attention has been given to other important outcomes of hookworm infection that play a large role in public health. It is now widely accepted that children who suffer from chronic Hookworm infection can suffer from growth retardation as well as intellectual and cognitive impairments (Hotez *et al*., 2005). Additionally, recent research has focused on the potential of adverse maternal-fetal outcomes when the mother is infected with hookworm during pregnancy.

In contrast to most intestinal helminithiasis, where the heaviest parasitic loads tend to occur in children, hookworm prevalence and intensity can be higher among adult males. The explanation for this is that hookworm infection tends to be occupational, so that plantation workers, coalminers and other groups maintain a high prevalence of infection among themselves by contaminating their work environment. However, in most endemic areas, adult women are the most severely affected by anemia, mainly because they have much higher physiological needs for iron (menstruation, repeated pregnancy), but also because customarily they have access to much poorer food than the men (Bethony *et al*., 2006).

C. Epidemiology

Necator americanus predominates in the Americas, sub-Saharan Africa, Southeast Asia, China and Indonesia, while *A. duodenale* predominates in the Middle East, North Africa, India and (formerly) in southern Europe. It is estimated that between 576-740 million individuals are infected with Hookworm today. Of these infected individuals, about 80 million are severely affected (Bethony *et al.*, 2006). The major etiology of hookworm infection is *N. americanus* which is found the Americas, sub-Saharan Africa, and Asia. (Hotez *et al.*, 2005). *A. duodenale* is found in more scattered focal environments, namely Europe and the Mediterranean. Most infected individuals are concentrated in sub-Saharan Africa and East Asia/the Pacific Islands with each region having estimates of 198 million and 149 million infected individuals, respectively. Other affected regions include: South Asia (50 million), Latin America and the Caribbean (50 million), South Asia (59 million), Middle East/North Africa (10 million) (Bethony *et al.*, 2006). A majority of these infected individuals live in poverty-stricken areas with poor sanitation.

It is well known that both hookworm species exist in Ethiopia at varying levels of prevalence in different geographical locations (Tedla and Jemaneh, 1980). The extensive work of study also indicates that the two species coexist in different part of Ethiopia, including the Melka Sedi area.

D. Hookworm-related Anemia in Pregnancy

Data from the early 1990s suggest that 44 million of the developing world's 124 million pregnant women harbored hookworm infection (Huddle and Gibson, 1999). Numbers like this have led to an increased interest in the topic of hookworm-related anemia during pregnancy (Gyorkos *et al.*, 2006).

The gastrointestinal blood loss, mal-absorption and appetite inhibition may further aggravate the iron, zinc and protein energy deficiencies and the anaemia of pregnancy (Huddle and Gibson, 1999). An association was found between the presence of hookworm eggs in stool in the last trimester of pregnancy and low haemoglobin concentration. Studies in Kenya and Zanzibar also found an association between the presence of hookworm and anaemia in both children and adults and this was particularly significance in pregnant women (Mary *et al.*, 2005). In a previous study

moderate, and heavy hookworm infection and co-infection of moderate and heavy hookworm and Trichuris infections were found to have a statistical significant association with anaemai (Renee *et al.*, 2005). A study conducted among pregnant women at Jimma university hospital showed a statistical significance difference between anaemic and non-anaemic cases with hookworm infection (Belachew, and Legese, 2006)

With the understanding that chronic hookworm infection can often lead to anemia, many people are now questioning if the treatment of hookworm could effect change in severe anemia rates and thus also on maternal and child health as well. Most evidence suggests that the contribution of hookworm to maternal anemia merits that all women of child-bearing age living in endemic areas be subject to periodic anthelmintic treatment. The World Health Organization even recommends that infected pregnant women be treated after their first trimester (Bethony *et al.*, 2006). Regardless of these suggestions, only Madagascar, Nepal and Sri Lanka have added deworming to their antenatal care programs (Brooker *et al.*, 2008). This lack of deworming of pregnant women is explained by the fact that most individuals still fear that anthelmintic treatment will result in adverse birth outcomes. But a 2006 study by Gyorkos *et al.* should assuage these fears. They found that when comparing a group of pregnant women treated with mebendazole with a control placebo group, both illustrated rather similar rates in adverse birth outcomes. The treated group demonstrated 5.6% adverse birth outcomes, while the control group had 6.25% adverse birth outcomes (Gyorkos *et al.*, 2006).

Furthermore, another study illustrated that treatment for hookworm infection actually led to positive health results in the infant. The study concluded that treatment with mebendazole plus iron supplements during antenatal care significantly reduced the proportion of very low birth weight infants when compared to a placebo control group (Larocque *et al.*, 2006). So far studies have validated recommendations to treat infected pregnant women for hookworm infection during pregnancy.

2.1.2.2 Schistosomiasis

Schistosomiasis, principally caused by *S. haematobium*, *S. japonicum* and *S. mansoni*, is endemic in 74 countries and infects more than 200 million people worldwide (WHO,

2000). These parasites have a complex, indirect life cycle involving an intermediate snail caused primarily by schistosome eggs, which are deposited by adult worms in the blood vessels surrounding the bladder or intestines. Urinary schistosomiasis, in which the bladder is affected, is caused by infection with *S. haematobium*, which occurs mainly in Africa. Intestinal schistosomiasis results from infection with *S. mansoni*, which occurs in the Middle East, South America, and Africa, and from infection with *S. japonicum*, which occurs in parts of China and the Philippines (Ross *et al.*, 2002). Two other schistosome species are known to cause intestinal schistosomiasis in restricted geographical areas: *S. intercalatum*, found in Central Africa, and *S. mekongi*, found in Cambodia and the Lao People's Democratic Republic. Ethiopia is one of the endemic countries for both *Schistosoma mansoni* and *Schistosoma haematobium*. Infection caused by *Schistosoma mansoni* has a wide geographical distribution in Ethiopia (McConnell and Armsrong, 1976).

A serious acute illness accompanied by fever and lymphadenopathy, known as Katayama Syndrome, can result from heavy schistosome infections. Chronic disease is mostly due to perforation of blood vessels and entrapment of eggs by host tissues. The host's reaction to entrapped eggs results in granuloma formation. *S. haematobium* causes bladder wall pathology, leading to ulcer formation, hematuria, and dysuria. Granulomatous changes and ulcers of the bladder wall and urethra can lead to bladder obstruction, dilatation, secondary urinary tract infections and subsequent bladder calcification, renal failure, lesions of the female and male genital tracts, and hydronephrosis. *S. haematobium* is also associated with increased risk of bladder cancer. The morbidity commonly associated with *S. mansoni* infection includes lesions of the liver, portal vein, and spleen, leading to periportal fibrosis, portal hypertension, hepatosplenomegaly, splenomegaly, and ascites. Schistosomiasis also causes chronic growth faltering and can contribute to anemia (Ross *et al.*, 2002).

Women of reproductive age may experience genital tract infection with disease in the pelvis affecting the renal system and the genital tract including salpingitis and tubal obstruction with possible ectopic pregnancy. As a systemic disease that causes anemia, schistosomiasis may have consequences similar to those described for hookworm infection. Investigators have demonstrated this blood loss and anemia from *S. mansoni*, *S. japonicum* and *S. haematobium*

(Farid *et al*., 1967**).** Some case reports of congenital infection exist and *S. haematobium* eggs have been recognized in placental blood (Renaud *et al*., 1971), but other than the anemia noted above, there is little documentation of other widespread pregnancy-associated consequences of schistosomiasis. Because the treatment for schistosomiasis with praziquantel is relatively simple and considered safe at least in the second and third trimester of pregnancy, case management during pregnancy can be considered and would likely have important benefits in endemic settings (WHO, 1999).

2.1.2.3. Epidemiology of helminth infections

With the exception of *Strongyloides stercoralis*, helminths do not replicate within the human host. This fundamental aspect of helminth biology establishes a set of transmission dynamics quite different than those for viruses, bacteria, fungi, and protozoa. For example, prevalence, which is the proportion of persons in a defined population at a given time point infected with the helminth (Fletcher and Fletcher, 2005), is seldom used as the only measure to assess the epidemiological situation for that helminth infection, because morbidity is associated with the number of worms infecting the host (i.e., the worm burden) rather than the absence or presence of infection.

Prevalence is commonly combined with worm burden (also referred to as the "intensity of infection"), which is commonly measured by the number of eggs per gram (EPGs) of feces for intestinal helminths and schistosomes (Anderson, 1982). Based on EPGs and their association with morbidity, individuals are classified into categories of light, moderate, and heavy infection (Montresor *et al*., 1998). Furthermore, in the case of soil-transmitted helminths, both prevalence and intensity of infection to classify communities into transmission categories category I (high), category II (medium), and category III (low). These transmission categories are assigned according to both the number of heavily infected people in the community (greater or less than 10%) and the prevalence of infection (greater or less than 50%). For example, a community with greater than 50% prevalence but less than 10% heavy infection would be considered a category II transmission community (Montresor *et al*., 1998).

Climate and topography are crucial determinants of the distribution of helminth infections (Brooker, 2007). Helminths transmitted by vectors are limited to landscapes in which host and vector come together in the same habitat, resulting highly focal distribution. For example, the

distribution of schistosomiasis reflects the biotic and abiotic features (i.e., climatic, physical, and chemical factors) that affect the survival and development of the snail vector (Sturrock, 1993). Soil-transmitted helminths are highly affected by surface temperature (Brooker, 2003), altitude, soil type, and rainfall (Appleton and Gouws, 1996).

Much epidemiologic research has focused on heterogeneity in the intensity of helminth infection by age. Changes with age in the average intensity of infection tend to be convex, rising in childhood and declining in adulthood. For *Ascaris lumbricoides* and *Trichuris trichiura,* the heaviest and most frequent infections are in children aged 5–15 years, with a decline in intensity and frequency in adulthood (Chan *et al*, 1996; Gilles, 1996). In contrast, hookworm frequently exhibits a steady rise in intensity of infection with age, peaking in adulthood (Bethony, 2002).

An increasing number of studies of helminth epidemiology have shown that it is common for individuals to be infected with more than one species of helminth (Brooker, 2000), in concurrent intestinal nematode and schistosome infections. A number of epidemiological studies have indicated that individuals infected with multiple species of helminth often harbor heavier infections than individuals infected with a single helminth species (Booth, 1998, Needham, 1998). An important consequence of simultaneous infection with the parasites that cause hookworm, schistosomiasis, and malaria is severe anemia (Brooker, 2006, Mwangi, 2006).

2.1.2.4. Laboratory Diagnostic Methods for Intestinal Parasites

In order to diagnose intestinal parasites, many methods can be considered. The choice of a particular technique will depend on its affordability, ease to carry out, its effectiveness and level of professionalism involved. Examples of these methods are DNA probes, PCR and direct fluorescent antibody methods, which are highly sensitive but are too costly to be used in the developing countries. It has been proved that direct stool smear, formol-ether and salt flotation techniques in stool microscopy offers many advantages over other diagnostic methods of detecting intestinal parasites. If performed correctly, these methods are sensitive, simple and economical (Parija and Srinivasa, 1999).

Direct stool smear, formol-ether concentration and flotation methods are used in diagnosing intestinal parasites by hospital and researchers in developing countries

because of their affordability, simplicity and sensitivity (Bearer, 1950, Allen, 1970). For the fact that the density of the parasite in the faeces is low, direct smear method is useful for the observation of motile protozoan trophozites and examination of exudates, but is not recommended solely for the routine examination of suspected parasitic infections (Arcari *et al.*, 2000). Therefore there is need for increase probability of finding the parasite in the faecal samples to allow for accurate diagnosis, hence there is need to concentrate. Though direct stool smear technique is quick to prepare and inexpensive when compared with modified formol-ether concentration methods, it can miss parasites (ova, cysts and larvae) if concentration is too low or if too much debris or fat is present. Sand, seeds or other faecal debris can make fixing cover slips onto slide difficulty. Most diagnostic centers show high performance to this method, owing to the fact that it is inexpensive and non-time –consuming thereby disregarding the consequences of misdiagnosis, which has led to prevalence of parasitic diseases and morbid conditions (Barnabas and Aboi, 2005).

Formol-ether concerntration techniques employ the use of ether or ethyl acetate as a lipid solvent and 10% formol saline as a fixative. This method recovers most ova, cysts and larvae and maintains their structure. In a recent study by Oguoma and E. Kwunife ether concentration technique is a very effective means of stool examination followed by the direct stool smear method. However formol-ether concentration technique is not used by many laboratories due to the expensive nature of ether or ethyl-acetate (Oguama and Ekwunife, 2007).

2.1.2.5. Prevention and control of soil transmitted helminthiasis

To achieve sustainable control of worm infections, while maintaining high coverage of anthelminthic treatment among children and pregnant mothers, it is essential to ensure access to safe water, adequate sanitation facilities and good hygiene and sanitation facilities and good hygiene and sanitation practices at the community level (WHO, 2004)

A comprehensive control strategy according to WHO, 2004 for helminth infection should include: ensuring wide availability of anthehelminthics for schistosomiasis and soil-transmitted helminth infections in all health services in endemic areas; ensuring good case management of symptomatic cases; regular treatment of all children at risk - including adolescent girls, through

school and community base initiatives; treating pregnant women at risk, through antenatal care and other women's health programme; ensuring a safe water supply and adequate sanitation facilities in all schools, ensuring provision of adequate water and sanitation facilities at household/ community level; promoting good hygiene and sanitation practices among schoolchildren; caregivers and to the communities (hand-washing, use of latrines; use of footwear) through community capacity development activities.

2.1.3 Other related factors for anaemia

Anaemia in pregnancy is also related to different socio-demographic, dietary and economic factors (Singh and Fong, 1998, Peter RG, 1995). Mother's age < 20 years, educational status, economic position, and antenatal care were significantly associated with anaemia during pregnancy in a study conducted in India (Bechuram *et al*., 2006). Iron deficiency is often nutritional in origin. One of the major contributory factors in developing countries is consumption of plant based food containing insufficient iron, especially insufficient available haem iron from meat. Iron is obtained in the form of non-haem iron from vegetables and as a haem iron from meat. Haem iron obtained about two to three times better than non-haem iron (Van den Brock, 2003).

Pregnant women from subsistent farming households in rural Africa usually consume predominately plant- based diets; intakes of animal source foods are low. Consequently micronutrient intakes are often inadequate, a problem often exacerbated in these settings by malabsorption induced by infection and bacterial overgrowth. Hence coexisting deficiencies of iron, zinc vitamin A, vitamin B-12, and folate have often been reported and associated with increased risk of maternal complications and poor fetal outcomes (Gibson *et al*., 2008).

In Sidama Zone of Southern Ethiopia maize and fermented enset products are the major staple foods contributing up to 90% of energy. Nevertheless dietary iron deficiency is not the major cause of anaemia during pregnancy. Such low intakes of animal products concomitant with infection and bacterial overgrowth may place pregnant women in Sidama at high risk of vitamin B-12 deficiency and possibly folate deficiency, especially in this malaria area, because some antimalarial drugs interfere with folate metabolism (Abebe *et al*., 2008).

Consumption of enset fermented by vitamin B-12 producing microorganisms may have increased vitamin B-12 levels in the diets and in plasma while at the same time enhancing non

haem iron absorption. Two micronutrients (zinc and iron status) and two non dietary factors, infection and gravida were the major predictors of haemoglobin (Gibson *et al*., 2008).

The commonest cause of anaemia during pregnancy includes iron and foliated deficiency aggravated by short birth intervals, and parasitic infections (WHO; 1991, http://www.infoforhealth.org/pr/l13edsum.shtmle). A study conducted at Jimma university hospital in pregnant women showed a statistical significant difference between anaemia and birth interval (Tefera and Yosef, 2006). Iron deficiency occurs if the amount of iron absorbed is too little to meet the body's demand. This may be due to insufficient iron in take, reduce bioavailability of dietary iron, increase iron requirements during pregnancy or a period of growth (WHO, 1998). Iron absorption is enhanced when consumed with foods high in vitamin C such as orange juice but substances in coffee and tea inhibit iron absorption (Cook and Monsen, 1997). WHO recommends that all pregnant women be supplemented with 60mg iron daily, in a pill that usually contains 400µg folic acid (WHO, 1998). A study conducted in rural Vietnam showed that taking iron tabelates significantly and positively associated with haemoglobin concentration in pregnant women (Aikawa, 2006).

2.1.4 Hemoglobin determination methods

The prevalence of anaemia in a population is best determined by using a reliable method of measuring haemoglobin concentration *(*Villanova, 1994*)*. Compared with the cost and difficulty of biochemically assessing the prevalence of iodine deficiency and vitamin A deficiency, the determination of the prevalence of anaemia in a population is relatively simple and inexpensive. The only methods generally recommended for use in surveys to determine the population prevalence of anaemia by haemoglobinometry are the cyanmethemoglobin method in the laboratory and the HemoCue system.

The cyanmethemoglobin method for determining haemoglobin concentration is the best laboratory method for the quantitative determination of haemoglobin. It serves as a reference for comparison and standardization of other methods *(*Villanova, 1994*)*. A fixed quantity of blood is diluted with a reagent (Drabkins solution) and haemoglobin concentration is determined after a fixed time interval in an accurate, well-calibrated photometer.

The HemoCue system is a reliable quantitative method for determining haemoglobin concentrations in field surveys *(*Van Schenck *et al.*, 1986*)*, based on the cyanmethemoglobin method. The HemoCue system consists of a portable, battery-operated photometer and a supply of treated disposable cuvettes in which blood is collected. The system is uniquely suited to rapid field surveys because the one-step blood collection and haemoglobin determination do not require the addition of liquid reagents. Survey field staff without specialized laboratory training has been successfully trained to use this device. The HemoCue system gives satisfactory accuracy and precision when evaluated against standard laboratory methods *(*Johns and Lewis, 1989*)*. The packed cell volume (PCV) is used as a simple screening test for anaemia, as a reference method for calibrating automated blood count systems and as a rough guide to the accuracy of haemoglobin measurements (WHO, 1998). The microheamatocrit method has an adequate level of accuracy and precision for clinical utility (WHO, 2000).

3. SIGNIFICANCE OF THE STUDY

Parasitic infection especially hookworm infection contribute most to anemia in pregnant women. Prevalence of parasitic infection is huge in most parts of our country where there is poor personal hygiene and environmental sanitation (Tedla, 1986). In addition to parasitic infection other factors like nutrition, poor antenatal care service and socio-demographic contribute to anaemia during pregnancy. There is no previous data about the prevalence of parasitic infection and prevalence of anaemia in the study area. This study attempted to assess prevalence of parasitic infection and to assess determinants of anaemia in pregnant women at the study area. Information on this regard is necessary to determine the existing significant variables related anaemia so as to guide the antenatal care service to work towards alleviating the problem with an appropriate care.

4. OBJECTIVE

4.1. General objective:

To assess determinants of anaemia with emphasis on intestinal helminthic infection in pregnant women at Bushulo health center in Southern Ethiopia

4.2. Specific objective:

1. To determine the prevalence of intestinal helminthic infection in pregnant women.
2. To evaluate the level of association between intestinal helminthic infection and anaemia in pregnant women.
3. To identify other determinants of anaemia in pregnant women.

5. MATERIALS AND METHODS

5.1 Study design

A cross–sectional study was conducted to assess determinants of anaemia with emphasis on helminthic infection in pregnant women attending antenatal care at Bushulo health center.

5.2 Study area

The study was conducted from July 1, 2008 to August 30, 2008 at Bushulo health center. Bushulo health center is located at a distance of 7km from the city of Awassa in rural setting close to the Lakeshore and 282km from Addis Ababa. The Catholic church of Awassa founded Bushulo health center in 1979. The health center runs by the Franciscan missionaries of Mary on non-profit making basis. The altitude of the area is 1697 m above sea level. The climate is warm with mean annual temperature 20.9 ^{0}C and mean rainfall 997.6mm. The catchment area of Bushulo health center includes the following kebeles: Finichawa, Tulo, Alamura and Tula with total population of 26, 909. From these populations, 15,535 are females. Finichawa and Tulo spread near the Awassa Lakeshore. The common agricultural products in the study area include enset, sorghum, and maize. Enset and sorghum are the common traditional dietary habit in the area (SNNPRG, 2007).

5.3 The study population

Pregnant women attending antenatal follow up at Bushulo health center were a study population for this particular study.

5.3.1 Study subjects

Pregnant women attending health center for their antenatal care during the study period who met the inclusion criteria were the study subjects.

5.4 Sample size determination and Sampling techniques

5.4 .1 Sample size

The required sample size for this study was calculated based on the prevalence rate of 67% of hookworm infection reported from the previous study done on intestinal helminthic infection and schistomiasis in the community in Delo Awraja Bale administrative Region Southern Ethiopia (Wodimanehu, *et al.*, 1997), and the 95% confidence interval and 5% marginal error, sample size (n) was determined using the following statistical formula (Danile, 1995).

$$n = \frac{Z^2 P (1 - P)}{D^2}$$

D = margin of error between the sample and the population.

n = sample size

Z = 95% confident interval

P = prevalence rate of 67% based on the previous study

$$n = \frac{1.96^2 \times 0.67 (1 - 0.67)}{0.05^2} = 340$$

By adding 10% for none response the final sample size was 374.

5.4.2 Sampling techniques

Women who came for antenatal care follow up were assessed and those pregnant women who met the inclusion criteria during the study period included in this study.

5.5 Inclusion criteria

- Resident in the study area
- Pregnant women
- Not having received anthehelminthic treatment for the last 6 months
- Given consent to participate

5.6 Exclusion criteria

- Pregnant women who are severely sick because of medical conditions (diabetes, renal or cardio-respiratory diseases, and chronic hypertension) for which follow up are required. Such type of pregnant women was screened by a medical doctor at a health center.
- Non pregnant women

5.7 Data collection procedure

5.7.1 Questionnaires

A structure questionnaire was used to obtain socio-demographic information, present and past history in pregnant women, environmental related factors and dietary habit. The questionnaire was developed in English and then translated into Amharic language. Public health nurses who can speak the local language (sidamigna) obtained training on data collection procedure for this particular study to attain standardization and maximize interviewer reliability. The data collectors were regularly supervised by the principal investigator for proper data collection.

5.7.2 Parasitological examination

5.7.2.1 Collection of stool specimen

Stool specimen containers were given to each pregnant woman with toilet tissue paper and clean applicator stick after questionnaire administration to bring fresh stool specimen.

Orientation was given to the women on how to collect sufficient amount and contamination free stool specimen. Women were requested to bring the stool sample immediately to process and examine within two hours. The laboratory technician checked whether sufficient amount of stool specimen was collected or not during receiving of sample.

5.7.2.2 Stool specimen examination methods

5.7.2.2.1 Direct microscopy method

Stool smear was prepared using saline for direct microscopic identification of intestinal helminthes and protozoa infection. Two slides were prepared for each pregnant woman. Direct smear was examined by 10X and 40 X microscopic magnifications.

5.7.2.2.2 Concentration method

Formol ether sedimentation technique was used following standard operational procedure to concentrate wide range parasites from stool specimen with minimum damage to their morphology (Monica, 1998). Smear was prepared from fecal deposit and examined by 10X and 40X microscopic magnifications.

5.7.3 Blood specimen examination

5.7.3.1 Blood film examination

Pregnant women were screened for the presence of malaria infection by collecting blood from finger prick. Giemsa stained of thick and thin smears were prepared to determine the presence or absence of malaria parasite.

5.7.3.2 Measurement of anaemia

The packed cell volume (PCV) was used as a simple screening test for anaemia, as a reference method for calibrating automated blood count systems and as a rough guide to the accuracy of haemoglobin measurements. According to WHO guidelines pregnant women are normal with haemoglobin concentration 110g/L or 33% haematocrit value and above (WHO, 1998).

The microhaematocrit method is carried out on blood contained in capillary tubes 75mm in length and having an internal diameter of about 1mm. The tubes may be plain for use with anticoagulated blood samples. The centrifuge used for capillary tubes provides a centrifugal force of 12,000g, and 5minutes centrifugation result in a constant PCV. The microheamatocrit method has an adequate level of accuracy and precision for clinical utility (WHO, 2000).

5.8. Study variables

5.8.1 Dependent variable

- Anaemia of pregnant women

5.8.2 Independent variable

- Parasitic infection
- Age
- Availability of latrine
- Shoe wearing habit
- Water supply
- Iron supplement
- Gestational period
- Marital status
- Residence
- Educational status
- Parity
- Economic status
- Occupation
- Blood loss
- Place of birth
- Antenatal care
- Nutritional status
- Contraceptive
- Bed net
- Abortion

5.9. Quality assurance

To ensure reliable data collection

- Training was given on data collection procedures for interviewers.
- Training was given for senior laboratory technicians to apply standard operational diagnostic procedures.
- The data collection, application of standard procedure, accuracy of test results was supervised by principal investigator.
- Some specimens were taken for cross checking of the accuracy of laboratory results.
- Close follow up by the investigator during data collection process was done.

- Filled questionnaires were collected after checking for consistency and completeness

5.10. Ethical consideration

The research proposal was first approved by the Department of Microbiology, Immunology and Parasitology (DMIP), ethically cleared by Institutional Reviewer Board Faculty of Medicine, and endorsed by the Faculty Academic Commission. Before starting data collection, a supportive letter was written by Medical Faculty of Addis Ababa University about the objective of the study to Awassa regional health office and to Bushulo health center.

To ensure confidentiality it was anonymous type whereby names of the study subjects was not written on the questionnaire. Those pregnant women who were anaemic and positive for parasitic infection got an appropriate treatment accordingly. All pregnant women who had intestinal helminthic infection were treated with mebendazol after their first trimester. Pregnant women of the first trimester were informed to get treatment with antehelmenthic in their second trimester. Pregnant women infected with S. mansoni were informed to get treatment after delivery. Anaemic pregnant women were treated with ferrous sulfate (WHO, 1998).

5.11. Data Analysis

Data were entered, cleaned and edited using SPSS for windows version 13.0. Dependent variable frequencies, percentage, mean, range and proportion were calculated. The association between anaemia (haematocrit < 33% or haematocrit ≥ 33%) and its independent variable was examined by chi-square test analysis. Multivariate logistic regression analysis was used with variables significant at the p value < 0.05 being kept in the final model. Variables which are biologically important and showed significant association in the previous studies included in multiple logistic regression analysis if in the chi-square test analysis; they had a P value of < 10%.

6. RESULTS

6.1. Socio-demographic of study subjects

Total of 374 informed and consented pregnant women who came for their antenatal follow up were enrolled in this study. The mean age of the attendants was 25.96 years old (range from 18-40). Seven (1.9%) were 18 years old and five (1.3%) were greater than 36 years old. Majority of the study group were 19- 36 years old. Two hundred nightly (77.5%) of the women came from rural and eighty four (22.5%) came from urban. Out of 374 attendants 250 (66.8%) illiterate, 38(10.8%) read and write, 59 (15.8%), grade 1-6, and 27(7.2%) were ≥ grade 7. One hundred nightly seven (52.7%) pregnant women were house wife and 140 (37.4%) were farmers. Three hundred forty eight women (93%) were married. The mean monthly income of the women was 338.28 birr (range from 70 – 1000).

The presence of anaemia was assessed based on socio-demographic characteristics of the study subjects. Age, residence, occupation, income family, religion, marital status and educational status were taken as study variables to see the out come of dependent variable. There was a statistical significant difference between all socio demographic variables and anaemia except educational status with chi-square test analysis (table 1).

Access to drinking water was primarily from community installation, such as pipe water functioning only at certain times of the day. Two hundred seventy five (73.5%) have a locally constructed latrine at a house hold level. The presence of anaemia was assessed based on environmental related factors. Water and latrine were taken as a study variable to see the out come of dependent variable. There was statistical significance difference between anaemia and latrine (Table 1).

Table1:- Anaemia with socio-demographic characteristics and environmental related factors in pregnant women attending antenatal follow up at Bushulo health center, 2008

Characteristics		**Anaemic** (N=194) Number (%)	**Non-anaemic** (N=180) Number (%)	Total number	Chi-square test value	P value
Age group	≤ 18	0(0)	7(3.9)	7	10.474	0.033*
	19-24	72(37.1)	62(34.4)	134		
	25-30	100(51.5)	81(45)	181		
	31-36	20(10.3)	27(15)	47		
	37-42	2(1)	3(1.7)	5		
residence	Urban	35 (18)	49(27.2)	84	4.52	0.034*
	Rural	159(82)	131(72.8)	290		
occupation	Farmer	88(45.6)	52(28)	140		
	Housewife	89(45.8)	108(60)	197		
	Merchant	17(8.7)	20(11.1)	37		
Income family	< 350	114(58.8)	77(42.8)	191	12.196	.002*
	350-500	61(31.4)	67(37.2)	128		
	> 500	19(9.8)	36(20)	55		
Educational status	illiterate	139(72.6)	111(61.7)	250	7.54	.056
	Read - write	17(8.8)	21(11.7)	38		
	1-6	30(15.5)	29(16.1)	59		
	≥ 7	8(4.1)	19(10.5)	27		

religion	Christian	181(93.3)	156(86.6)	337	4.607	.032*
	Muslim	13(6.7)	24(13.3)	37		
Marital status	married	180(92.7)	168(93.3)	348	0.978	.044*
	Single	7(3.6)	6(3.3)	13		
	Others	7(3.6)	6(3.6)	13		
latrine	yes	131(67.5)	144(80)	275	7.465	.006*
	No	63(32.5)	36(20)	99		
water	Tap water	192(99)	180	372	1.866	.172
	river water	2(1)	0(0)	2		

* Significant (p value > .001), ** Highly significant (p value ≤ .001)

6.2. Parasitic infection

Intestinal parasites: parasitic infection among pregnant women was assessed and found that 218 out of 374 pregnant women were infected with intestinal parasites. Malaria was screened for all study subjects but no malaria cases were identified from all study subjects.

Out of 218 helminthic infected women 40.4 % (88 women) showed more than one intestinal helminthic infection. *A. lumbricoid* was the leading parasite among pregnant women.

The over all prevalence of *A. lumbricoid* in pregnant women was 48.4%.The prevalence of anaemia among intestinal helminthic infected and non-intestinal helminthic infected pregnant women was 64.7% and 34% respectively.

Table 2- Distribution of intestinal parasites in pregnant women attending antenatal care at Bushulo health center, 2008

Intestinal parasite	Number	Percent
No ova parasite	156	41.7

A.lumbricoid	106	28.3
T.trichuria	10	2.7
Hookworm	9	2.4
S.mansoni	5	1.3
Hookworm with others	55	14.7
S.mansoni with other*	16	4.3
A.lumbricoid and *T.trichuria*	16	4.3
A.lumbricoid with Taenia species	1	0.3
Total	**374**	**100.0**

Other* represents *S.mansoni* with *A.lumbricoid* and *T.tricuria*

Other** represents hookworm with *T.trichuria*, *A.lumbricoid*, taenia and hymenolepis

Prevalenc of intestinal parasites was assessed at different kebeles as shown in (table 3). *S.mansoni* was investigated from Finichawa and Tulo kebeles. Finichawa and Tulo are located surrounding Lake Awassa. High prevalence of intestinal parasites (70.5%) was investigated among Finichawa study subjects followed by Tulo (67%).

Table 3:- Prevalence of intestinal parasitic infection from different kebeles in pregnant women attending antenatal care at Bushulo health center, 2008

Investigation	**Kebele**					**Total**
	Alamura	**Tulo**	**Awassa**	**Finichawa**	**Tulla**	
	n (%)	n (%)	n (%)	n (%)	n (%)	
No ova parasite	63(60.6)	36(33.3)	2(50)	23(29.5)	32(40)	156
A.lumbricoid	29(27.9)	24(22.2)	1(25)	32(41)	20(25)	106
T.trichuria	5(4.8)	4(3.7)	0	0	1(1.25)	10
Hookworm	0	1(.92)	0	1(1.3)	7(8.75)	9
S.mansoni	0	4(3.7)	0	1(1.3)	0	5
Hookworm with others	4(3.8)	25(23.1)	1(25)	9(11.5)	16(20)	55
S.mansoni with other	0	8(7.4)	0	8(10.2)	0	16
A.lumbricoid and *T.trichuria*	3(2.9)	6(5.5)	0	3(3.8)	4(5)	16
A.lumbricoid with taenia species	0	0	0	1(1.3)	0	1
Total	**104(100)**	**108(100)**	**4 (100)**	**78(100)**	**80(100)**	**374**

The prevalence of anaemia among preganent women infected with hookworm plus other helminthes was 62/67(92.5%) whereas the prevalence of anaemia among non-intestinal helminthic infected women was 54/156(34.6%), (table 4). This indicates that not being infected with intestinal parasite may reduce prevalence of anaemia.

The presence of anaemia was assessed with intestinal parasite infection to see the out come of the study variable. There was a statistical significant difference between intestinal parasitic infection and anaemia (Table 4).

Table 4:- Prevalence of anaemia in relation to Helminthic infection in pregnant women attending antenatal care at Bushulo health center, 2008

Characterstics		**Anaemic** (N=194) Number (%)	**Non-anaemic** (N=180) Number (%)	Total number	Chi-square test value	P value
Helminthic infection	No ova parasite	54(27.8)	102(56.7)	156		
	Hookworm plus other helminthes	62(32)	5(2.6)	67	74.308	0.000**
	S. mansoni plus other helminthes	15(7.7)	2(1.1)	17		
	All other helminthic infection	64(33)	70(38.9)	134		

** Highly significant (p value ≤ .001)

6.3. Anaemia

The over all prevalence of anaemia in this study population using a cut off level 11g/dl (33% haematocrite) was 51.9%. The mean haematocrite level was 0.34 (34%) range from 22% – 45%. Prevalence of anaemia among rural women was higher than prevalence of anaemia among urban women i.e., 159/290(54.8%) and 35/84(41.6%) respectively.

Anaemia was assessed based on different localities and there was a statistical significant difference between anaemia and localities when Tula kebele was taken as reference. High prevalence of anaemia (62.8%) was registered from Finchiwa antenatal care attendants followed by Alamura and Tulo respectively.

Table 5:- Prevalence of anaemia in pregnant women attending antenatal care at Bushulo health center from different kebeles, 2008

Kebele	Anaemic	Non-anaemic	Total	Chi-square test(p value)
	Number(%)	Number(%)		
Alamura	58(55.8)	46(42.2)	**104**	
Tulo	57(52.8)	51(47.2)	**108**	
Finichawa	49(62.8)	29(37.2)	**78**	12.23(.006)
Tulla	29(36.2)	51(63.8)	**80**	
Total	**193**	**177**	**370**	

The prevalence of anaemia was 39.2%, 54.5% and 57.5% for premigravida, multigravida and grandgravida respectively. The result showed that the occurrence of anaemia increase with gravidity in that anaemia is more prevalent in mothers with large number of pregnancies than mothers with few or one pregnancy.

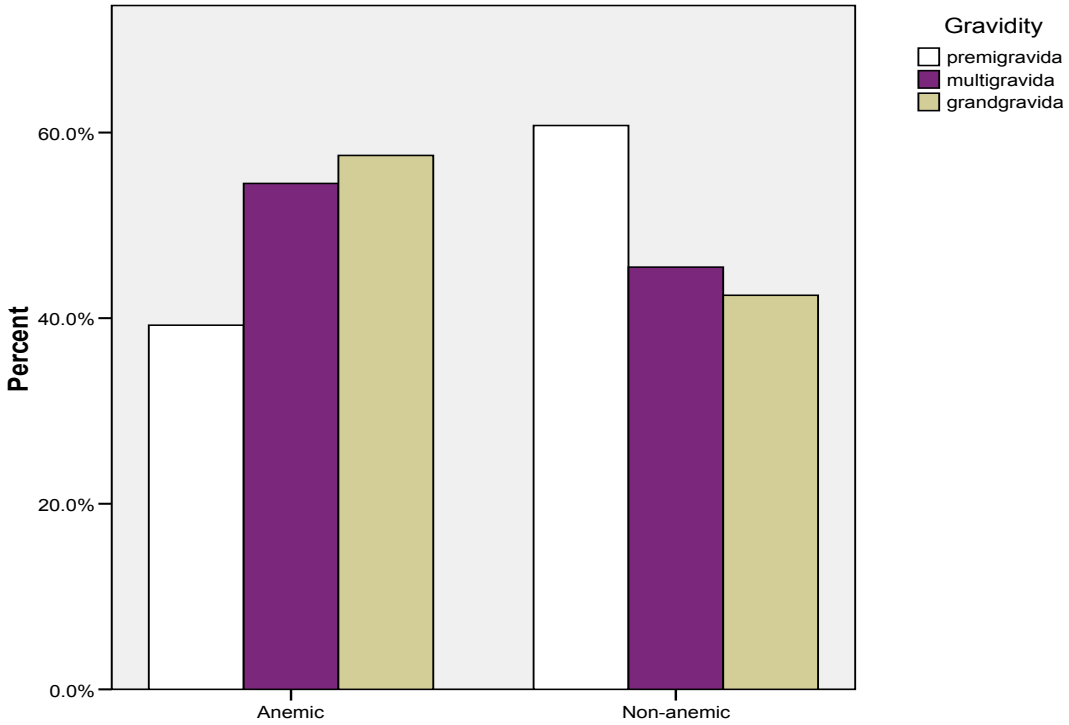

Figure 1. Prevalence of anaemia in pregnant women by their gravidity during the study period at Bushulo health center, 2008

Prevalence of anaemia among pregnant women with birth interval greater than or equal to two years was (90/137(65.7%) which is higher than prevalence of anaemia among pregnant women with birth interval greater than two years (69/153(45%) (Table 6). Prevalence of anaemia in first trimester, second trimester, and third trimester was 50.5%, 52.2% and 56.4% respectively. The trend of anaemia showed that as gestational age increase, the occurrence of anaemia also increases.

Pregnant women attending the previous pregnancy antenatal care showed less prevalence of anaemia comparing to those pregnant women who did not attend the previous antenatal care51.3% and 61.1% respectively. The prevalence of anaemia among pregnant women who did not attend the current ANC regularly was much higher (74%) than women who did attend current ANC regularly (48.4%) (Table 6).

Pregnant women who had previous blood loss during previous delivery showed high prevalence as compare to pregnant women who did not have blood loss during previous delivery 62% and 53% respectively. Place of delivery also showed difference in prevalence of anaemia (56% in pregnant women who delivered at home and 50% in women who delivered at health institute).

The prevalence of anaemia in pregnant women who took iron tablets and who did not take iron tablets during pregnancy was 39% and 55.8% respectively. Contraception users' pregnant women showed lower prevalence of anaemia (47.6%) comparing to none users (54%). Wearing shoe also showed lower prevalence of anaemia comparing to non shoe wearing pregnant women (35% and 66%), respectively (Table 6).

The presence of anaemia was assessed based on different pregnancy variables of the study subjects. Trimester, gravida, birth interval, abortion, twins, place of delivery, ANC follow up, blood loss, use of contraceptive, taking iron tablets, use of bed net, history of malaria and shoe wearing were taken as study variables to see the out come of dependent variable. Birth interval, gravida, taking iron tablets, shoe wearing, abortion and current regular ANC attendant showed a statistical significant difference with anaemia (Table 6).

Table 6:- Distribution of anaemia in pregnant women attending antenatal care with different variables at Bushulo health center, 2008.

Characteristics		**Anaemic** (N=194) Number (%)	**Non-anaemic** (N=180) Number (%)	Total number	Chi-square test value	P value
Trimester	< 16 weeks	89(45.9)	87(84.4)	176		
	16-28 weeks	83(42.8)	76(42.2)	159	.448	.799
	> 28 weeks	22(11.5)	17(9.4)	39		
Gravida	premigravida	31(16)	48(26.7)	79		
	multigravida	121(62.4)	101(56.1)	222	6.603	.037*
	grandgravida	42(21.6)	31(17.2)	73		
Birth interval	≤ 2 years	90(56.6)	47(35.9)	137	12.37	.000**
	> 2 years	69(43.4)	84(64.1)	153		
Abortion	yes	35(18)	15(8.3)	50	7.598	.006*
	no	159(81.9)	169(91.6)	328		
Twins birth	yes	6(3.1)	9(5)	15	.882	.348
	no	188(96.9)	171(95)	359		
Place of delivery	home	129(18.1)	101(77.1)	230	.712	.399
	Health center	30(18.9)	30(22.9)	60		
Previous pregnancy followed up	yes	97(60.6)	92(69.7)	189	2.607	.106
	no	63(39.4)	40(30.3)	103		

Regular follow up of current pregnancy	yes	158(81.4	168(93.3)	326	11.799	.001**
	no	36(18.6)	12(6.7)	48		
Bleeding in previous pregnancy	yes	36(22.5)	22(16.7)	58	1.546	.214
	no	124(77.5)	110(83.3)	234		
Bleeding in current pregnancy	yes	9(4.6	13(7.2)	22	1.125	.289
	no	185(95.4)	167(92.8)	352		
Taking iron tablets	yes	37(19	56(31.1)	93	7.243	.007**
	no	157(81	124(68.9)	281		
Use of contraceptive	yes	60(30.9)	66(36.7)	126	1.376	.241
	no	134(69.1)	114(63.3)	248		
Use of bed net	yes	136(70.1)	127(70.6)	263	.009	.924
	no	58(29.9)	53(29.4)	111		
History of malaria attack	yes	37(19.1)	31(17.2)	68	.215	.643
	no	157(80.9)	149(82.8)	306		
Shoe wearing	yes	61(31.4)	112(62.2)	173	35.58	.000**
	no	133(68.6)	68(37.8)	201		

Only 21(5.6%) of the respondents indicted that they used to eat animal food (milk and meat) daily and 178(47.6%) of them used to eat animal food once a month. One hundred sixty (42.8%) and forty three (11.5%) of the respondents indicted that they used to eat green leafy vegetables once a week and once a month respectively. Three hundred fifty nine (96%) of the study subjects had the habit of drinking coffee immediately after meal and ninety six (25.7%) of them had fruit after meal (table 3).

The presence of anaemia was assessed based on different dietary habits to assess the out come of dependent variable. Eating animal food, eating green leafy vegetables

and taking fruit after meal showed a statistical significance difference with anaemia (Table 7).

Table 7:- Distribution of anaemia in relation to dietary in take habit in pregnant women attending antenatal care at Bushulo health center, 2008.

Eating habit		**Anaemic** (N=194) Number (%)	**Non-anaemic** (N=180) Number (%)	Total number	Chi-square test value	P value
Frequency of eating animal food	Every day	6(3)	15(8.3)	21		
	Every two day	12(6.2)	32(17.7)	44	20.107	.000**
	Once a week	69(35.5)	62(34.4)	131		
	Once a month	107(55.1	71(38.4)	178		
Frequency of eating green leafy vegetables	Every day	32(16.5)	25(13.9)	57		
	Every two day	45(23.2)	69(38.3)	114	10.142	.017*
	Once a week	92(47.4)	68(37.8)	160		
	Once a month	25(12.9)	18(10)	43		
Taking tea or coffee immediately after meal	yes	183(94.3)	176(97.8)	359	2.883	.090
	no	11(5.7)	4(2.2)	15		
Taking fruit	yes	39(20.1)	57(31.7)	96	6.544	.011*

after meal	no	155(79.9)	123(68.3)	279		

Educational status, birth interval, taking iron tablets, shoe wearing and hookworm co-infection with other intestinal parasites showed statistical significant association with anaemia using multivariate logistic regression analyses (table 8A and table 8B)

Table 8A:- Multivariate logistic regression analysis of anaemia by determinants of anaemia in pregnant women attending antenatal follow up at Bushulo health center, 2008.

Characteristics		**Anaemic** (N=194) Number (%)	**Non-anaemic** (N=180) Number (%)	**Total number**	**OR(95% CI)**	**P value**
residence	Urban	35 (18)	49(27.2)	84	1	
	Rural	159(82)	131(72.8)	290	.42(.15, 1.19)	.106
occupation	Farmer	88(45.6)	52(28)	140	.86(.23,3.13)	.774
	Housewife	89(45.8)	108(60)	197	.72(.23, 2.27)	.825
	Merchant	17(8.7)	20(11.1)	37	1	
Educational Status	illiterate	139(71.6)	111(61.7)	250	5.9(1.07, 32.5)	.041*
	Read - write	17(8.8)	21(11.7)	38	4.5(.74, 26.8)	.101
	1-6	30(15.5)	29(16.1)	59	7.25(1.19, 43.9)	.031*
	≥ 7	8(4.1)	19(10.5)	27	1	
Income family	< 350	114(58.8)	77(42.8)	191	1.48(.53, 4.11)	.448
	350-500	61(31.4)	67(37.2)	128	1.43(.52, 3.97)	.482
	> 500	19(9.8)	36(20)	55	1	
religion	Christian	181(93.3)	156(86.6)	337	1.22(.45, 3.23)	.693
	Muslim	13(6.7)	24(13.3)	37	1	
latrine	yes	131(67.5)	144(80)	275	1	
	No	63(32.5)	36(20)	99	1.25(.59, 2.63)	.565
Gravida	premigravida	31(16)	48(26.7)	79		
	multigravida	121(62.4)	101(56.1)	222	.67(.24, 1.84)	.439

	grandgravida	42(21.6)	31(17.2)	73		
Birth interval	≤ 2 years	90(56.6)	47(35.9)	137	2.18(1.14, 4.17)	.018*
	> 2 years	69(43.4)	84(64.1)	153	1	
Abortion	Yes	35(18)	15(8.3)	50	1.36(.56, 3.32)	.500
	No	159(81.9)	169(91.6)	328	1	
Regular follow up of current pregnancy	yes	158(81.4)	168(93.3)	326	1	
	No	36(18.6)	12(6.7)	48	2.72(1.11, 4.17)	.071
Taking iron tablets	Yes	37(19	56(31.1)	93	1	
	No	157(81	124(68.9)	281	2.27(1.11, 4.54)	.025*
Shoe wearing	yes	61(31.4)	112(62.2)	173	1	
	No	133(68.6)	68(37.8)	201	2.08(1.01, 4.16)	.045*
Frequency of eating animal food	Every day	6(3)	15(8.3)	21	1	
	Every two day	12(6.2)	32(17.7)	44	.99(.20,4.91)	.994
	Once a week	69(35.5)	62(34.4)	131	1.58(.37,6.82)	.538
	Once a month	107(55.1	71(38.4)	178	2.15(.49,9.34)	.306
Frequency of eating green leafy vegetables	Every day	32(16.5)	25(13.9)	57	1	
	Every two day	45(23.2)	69(38.3)	114	.51(.19,1.33)	.169
	Once a week	92(47.4)	68(37.8)	160	.57(.22, 1.46)	.240
	Once a month	25(12.9)	18(10)	43	.98(.31, 3.08)	.970

Table 8B:- Multivariate logistic regression analysis of anaemia by determinants of anaemia in pregnant women attending antenatal follow up at Bushulo health center, 2008.

Characteristics		**Anaemic** (N=194) Number (%)	**Non-anaemic** (N=180) Number (%)	**Total number**	**OR(95% CI)**	**P value**
Taking fruit after meal	yes	39(20.1)	57(31.7)	96	1	
	no	155(79.9)	123(68.3)	279	1.88(.81,4.34)	.134
Taking tea or coffee immediately after meal	yes	183(94.3)	176(97.8)	359	1.95(.40,9.41)	.404
	no	11(5.7)	4(2.2)	15	1	
Helminthic infection	No ova parasite	54(27.8)	102(56.7)	156	1	
	Hookworm plus other helminthes	62(32)	5(2.8)	67	14.2(4.25, 47.25)	.000**
	S. mansoni plus other helminthes	15(7.7)	2(1.1)	17	4.43(.47, 41.89)	.193
	All other helminthic infection	64(33)	70(38.9)	134	1.39(.74, 2.62)	.303

* Significant (p value > .001), ** Highly significant (p value ≤ .001)

7. DISCUSSION

Anaemia is a common and serious problem in pregnancy. It needs to be addressed at community level as well as during antenatal care. Iron deficiency anaemia has multifactorial causes in developing countries. The study has shown that anaemia is prevalent in pregnant women attending antenatal follow up at Bushulo health center. The prevalence of anaemia in the rural women was higher than prevalence of anaemia in urban women. Similar results obtained in a study conducted at Asendabo and Mettue, anaemia among pregnant women was consistently higher in rural women compare to the urban counter parts (Gebremedin, 2004, Tadios, 1996). The prevalence of anaemia obtained in this study(51.9%) was almost consistent with the report of the previous studies 50% in Peru (Zavaleta *et al.*, 1993), 40-60% in developing countries pregnant women(WHO, 1993), but some how lower prevalence of anaemia compare to prevalence of anaemia in pregnant women in Jimma(57%)(Desalegn, 1993).

Anaemia was also assessed at different kebles and high prevalence of anaemia was found from Finchiwa antenatal care attendants. Finchiwa is located near Awassa lake shore and the area is endemic to *S. msnsoni*. The presence of *S. mansoni* in addition to other intestinal helminthes and less antenatal care service in the kebele may increase the prevalence of anaemia at finchiwa. There was low Prevalence of anaemia in pregnant women who came from Tulla. Comparing to other kebeles there is a better

antenatal care service and iron supplements at Tula kebele that may reduce the prevalence of anaemia.

On chi-square test analysis there was statistical significant differences between anaemia and socio-demographic variables including residence, religion, occupation, marital status, monthly income, and latrine although only educational status showed a statistical significant difference with anaemia on multivariate logistic regression analysis which indicates the lower chance of confounding effect of these variables on anaemia. A study conducted on risk factors of anaemia during pregnancy among pregnant women in India showed a statistical significant association between education and anaemia which is consistent with the current study (Bechuram *et al.*, 2006). This finding indicates the need for strengthening of interventions related to education to women to create awareness of antenatal care, balanced diet during pregnancy and family planning.

Parasitic infections contribute to iron deficiency in developing countries. The burden of disease imposed on helminth-infected in child bearing age, especially during pregnancy may be one of single most important contribution of intestinal parasitic infections to the calculation of their global disease burden. The total amount of work a women can do in a day definitely decreases when she is anaemic, whatever the cause is, and pregnancy plus helminh infections produce double burden for women in some rural farming communities(Stephenson *et al.*, 2002). Women may even acquire helminth infection in the process of growing the family's food thus increase their degree of anaemia in pregnancy.

The prevalence of intestinal parasites identified in this study (58.2%) is higher than the prevalence reported for pregnant women in Congo (9%), Nigeria (12.5%), Mexico (38.2%), Brazil (Sao Paulo State, 45.1%) and lower than Indonesia (69.7%) and Brazil (Rio dejaneiro State, 69%) (Alfonso *et al.*, 2006). This might be due to the difference in geographical location and economic status.

The prevalence of individual intestinal parasite in pregnant women at bushulo health center was recorded as, *A.lumbricoid* 28.3%, hookworm 2.4%, *S.mansoni* 1.3% (table2).

A.lumbricoid was the leading parasite (48.8%) followed by hookworm (17.1%). The occurrence of helminthic infection at high rates among pregnant women is an indicative of faecal pollution of soil, improper utilization of latrine and poor personal hygiene in the study area.

The prevalence of intestinal helminthic infection among pregnant women was 64.7%. About 41% helminthic infected women had mixed infections, due to two or more than two different parasite species which might increase the burden and intensity of helminthic infection which could increase prevalence anaemia, almost twice as compare to those who did not infected with intestinal helminthes.

In this study although all intestinal parasitic infections showed a statistical significance difference with anaemia on chi-square test analysis, only hookworm plus other intestinal parasitic infection showed a statistical significant difference with anaemia on multivariate regression analyses (P = .000), OR (95% CI): 14.173(4.251, 47.25). Those pregnant women infected with hookworm plus other intestinal parasitic infections were fourteen times likely to be anaemic. Previous study report showed that an association between moderate and heavy hookworm infection and anaemia was strengthened when there was co-infection with moderate and heavy *T.trichuria* infection (Mary *et al.*, 2005).

Another study also showed a significant association between hookworm infection and anaemia (Renee *et al.*, 2005; Belachew and Legese, 2006). Infections causing chronic blood loss such as parasitic infection with hookworm, increase iron requirement. Hookworm infection is described to be one of the principal causes of iron deficiency anaemia. This finding indicates the need for strengthening of interventions related to screening intestinal parasitic infection for pregnant women during their first antenatal care service.

No malaria parasite was found in all study subjects. The reason may be the study period was not malaria season, indoor residual spraying of insecticides, the accessibility of bed net use for all kebele and no study subjects came with malaria clinical manifestation

On the assessment of anaemia with pregnancy variables, there was a statistical significant difference between anaemia and birth interval, gravida, taking iron tablets,

shoe wearing, abortion and current regular ANC attendant on chi-square test analysis, but only birth interval, taking iron tablets, shoe wearing remain statistically significant difference with anaemia on multivariate logistic regression analysis which indicates the lower chance of confounding effect of other variables on anaemia.

Pregnancy with a short birth interval leads to iron deficiency anaemia as iron requirements are substantially higher than the average (WHO, 1991). Short birth interval increases risk for uterine rupture. There was a significant association between birth interval and anaemia (p = .018), OR (95% CI): 2.180(1.141, 4.166. This result was consistent with a study conducted in Jimma hospital (Belachew and Legese, 2006). Pregnant women with short birth interval less than or equal to two years were two times likely to be anaemic. Appropriate time after each pregnancy for recuperation and replenishment of nutrient stores and circulating levels is a minimum of three to five years (http://www.infoforhealth.org/pr/l13edsum.shtmle). This finding indicates the need for strengthening of interventions related to child spacing and awareness especially in rural women.

Iron deficiency occurs if the amount of iron absorbed is too little to meet the body's demand. This may be due to insufficient iron intake, reduce bioavailability of dietary iron intake and increase iron requirements during pregnancy. Malabsorption and loss of appetites due to intestinal parasitic infection may also cause iron deficiency. In this study there was a significance association between iron intake and anaemia (p=.007), OR (95%CI): 2.60(1.29, 5.23). This finding was consistent with the report of the previous study taking iron tablets significantly and positively associated with haemoglobin concentration in pregnant women (Aikawa $et\ a.,l$ 2006). Pregnant women who did not take iron during pregnancy were greater than two times likely to be anaemic than those who took iron supplement. WHO recommends that all pregnant women be supplemented with 60mg iron daily, in a pill that also usually contains 400µg folic acid (Stoltzfus and Drefuss, 1998). An additional benefit of iron supplementation during pregnancy is the opportunity to improve the maternal iron stores postpartum. This could reduce the risk of anaemia during lactation and in subsequent pregnancy.

There was a statistical significance difference between anaemia and shoe wearing habit (p= .025), OR (95% CI): 2.19(1.10, 4.33). Similar result was obtained in a previous

study (Belachew and Legese, 2006). Pregnant women walking barefoot were two times likely to be anaemic. Most rural pregnant women attending antenatal care walk barefoot; even those women who have shoe do not wear regularly. They wear shoe when they come to town for antenatal care and for marketing. Walking barefoot may predispose to hookworm infection and the consequence will result iron anaemia especially in pregnant women.

Prevalence of anaemia was high in the third trimester and grand gravida which indicates the need for proper follow up of pregnant women starting from the first trimester to the third trimester and the need of attention to grandgravida mothers. Prevalence of anaemia among aborted mothers was much higher (75%) as compare to non-anaemic mothers which indicates that previous history of pregnant women should be taken during their first antenatal care visit to properly manage aborted mothers. Regular ANC attendant pregnant women showed low prevalence of anaemia but, there was high prevalence of anaemia (74%) in non-regular ANC attendant pregnant women. Antenatal care initiated in the first trimester facilitates early diagnosis of anaemia and allows treatment at the periphery so that the condition can be corrected before delivery.

One of the major contributory factors in developing countries is consumption of plant based food containing insufficient iron, especially insufficient available haem iron from meat (Van den Brock, 2003). Iron absorption is enhanced when consumed with foods high in vitamin C such as orange juice but substances in coffee and tea inhibit iron absorption (Cook and Monsen, 1997). The current study tried to assess different dietary risk factors for anaemia. Eating animal food, eating green leafy vegetables and taking fruit after meal showed a statistical significance difference with anaemia on chi-square test analysis but none of them showed a statistical significant difference with anaemia on multivariate analysis which may be due to no difference in eating habit between anaemic and non-anaemic group.

8. CONCLUSION

- The current study showed high prevalence of anaemia and intestinal helminthes among pregnant women in the study area.
- There was high prevalence of mixed intestinal parasitic infection among pregnant women.
- The prevalence of anaemia was high in third trimeste, grandgravida, aborted mothers and pregnant women who did not attend current ANC regularly.
- Anaemia in pregnant women found to have association with:
    - ✓ Hookworm plus other helminthes
    - ✓ Absence of taking iron during pregnancy and walking barefoot
    - ✓ Birth interval less than or equal to two years
    - ✓ Less educational status

9. RECOMMENDATIONS

- All pregnant women attending antenatal care should be screened for soil transmitted parasites at their first visit.
- To prevent recurrence parasitic infection pregnant women should be advised to use footwear, improve sanitation and personal hygiene.
- Pregnant women should be encouraged to have regular ANC follow up.
- Routine supplementation of iron to pregnant women starting at their first prenatal visit should be encouraged during ANC follow up.
- Health education on family planning information should be given to pregnant women.
- The community should be encouraged to use latrine to reduce the burden of high prevalence of parasitic infection in the area.
- Further investigation on each determinant of anaemia at different areas should be done.

10. References

Abebe Y., Bogale A., Hambidge M., Stoecker J., Arbide I, Teshome A, Krebs F., Westcott E., Bailey B. (2008). Inadequate intakes of dietary zinc among pregnant women from subsistent households in Sidama, Southern Ethiopia. *Publ Hth Nutr,* in press.

Aikawa R., Ngyen K., Sasaki S., Binns W. (2006). Risk factor for iron deficiency anaemia among pregnant women living in rural Vietnam. *Pub Health Nutr*. **9**(4): 443-8.

Alfonso J., Rodríguez M., Rosa A., Cynthia C., Melissa A., Marisela R., Henry P., Oscar U., Gloria G., Nestor R, Andrea M.(2006). Intestinal Parasitic Infections among Pregnant Women in Venezuela. Infect Dis Obstet Gynecol. **10**(1155): 23125.

Alien K., Lonneradal B. (1994). The consequences of iron deficiency and anaemia in pregnancy. In Nutrient Regulation during Pregnancy, Lactation and Infant growth. Plenum Press, New York, 121 – 133.

Allen V., Ridley S. (1970). Further observation on the formol ether concentration for faecal parasites. *J Clin Pathol*, **23**: 343-352.

Allen H. (1997). pregnancy and iron deficiency: unresolved issues. *Nutr Rev*. **55**: 113– 232.

Anderson M. (1982). Population dynamics of infectious diseases: theory and applications. Chapman and Hall. London, United Kingdom, pp. 377.

Appleton C., Gouws E. (1996). The distribution of common intestinal nematodes along an altitudinal transect in KwaZulu-Natal, South Africa. *Ann. Trop. Med. Parasitol.* **90:**181-188

Arcari M., Boxendine A., Benette E. (2009). Diagnosing medical parasites through coprological techniques. (2000). Available online at (www.soton.ac.uk/ceb/diagnosis/v) date of access 16 July 2009.

Awashi B., Bundy A., Savioli L. (2003). Helminthes infection. *BJM.* **227**: 431 – 433.

Barnabas M., Aboi K. (2005). Missed diagnosis of schistosoliasis leading to unnecessary surgical procedures in Jos University Teaching Hospital, *Trop Doctors,* **35**:96-97.

Bearer C. (1950). The standardisation of faecal smear for estimating egg production and worm burden. J. *Parasitol*, **36**:451-6.

Bechuram M., Vikal T., Ranjan G. (2006). Risk factors of anaemia during pregnancy among the Garo of Meghalaya, India. *Human Ecology Special Issue*, **14**(1):27-32.

Beeson G., Reeder C., Rogerson J., Brown V. (2001). Parasite adhesion and immune evasion in placental malaria. *Trends Parasitol*. **17**: 331–337.

Belachew T., Yosef L. (2006). Risk factors for anaemia among pregnant women attending antenatal clinic at Jimma University Hospital, *Ethiop Med J*, **44**, No.3.

Bethony J. (2002). Emerging patterns of hookworm infection: influence of aging on the intensity of Necator infection in Hainan Province, People's Republic of China. *Clin. Infect. Dis.* **35:**1366-1344.

Bethony P., Brooker S., Hotez P. (2006). Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm, *Lancet* **367**:1521–1532.

Booth M., (1998). Associations among multiple geohelminth species infections in schoolchildren from Pemba Island. *Parasitology*. **116:**85-93.

Brabin B., Piper C. (1997). Anaemia-and malaria-attributable low birth weight in two populations in Papua New Guinea. *Ann. Hum. Biol*. **24**: 547–555.

Brooker S., Hotez J., Bundy P. (2008). Hookworm- Related anaemia among women: A Systemic Review. *PLoS Negl Trop Dis* **2**(9): 291.

Brooker S. (2000). Epidemiology of single and multiple species of helminth infections among school children in Busia District, Kenya. *East Afr. Med. J.* **77:**157-161.

Brooker S. (2003). Mapping soil-transmitted helminths in Southeast Asia and implications for parasite control. *Southeast Asian J. Trop. Med. Public Health*. **34:**24-36.

Brooker S. (2006). The co-distribution of *Plasmodium falciparum* and hookworm among African schoolchildren. *Malar. J.* **5:**99.

Brooker S. (2007). Spatial epidemiology of human schistosomiasis in Africa: risk models, transmission dynamics and control. *Trans. R. Soc. Trop. Med. Hyg*. **101:**1-8.

Chan M., Guyatt L., Bundy A., Medley F. (1996). Dynamic models of schistosomiasis morbidity. *Am. J. Trop. Med. Hyg.* **55:**52-62.

Cook J., Monsen R. (1997). Vitamin C, the common colds and iron absorption. *Am J Clin Nutr*, **30**:235-241.

Crompton W. (2000). The public health importance of hook worm disease. P*arasitology* **121**:39 – 50.

Demaeyer M., Dallman P.,Gurney M., Hallberg L., Sood K., Srinkantia G. Prevention and controlling iron deficiency anaemia through primary health care. Geneva: WHO, 1998.

Desalegn S. (1993). Prevalence of anaemia in pregnancy in Jimma town, Southwestern Ethiopia. *Ethiop Med J*, **31**:251-58.

Farid Z., Bassily S., Schulert R., Raasch F., Zeind S., Rooby S., Sherif M. (1967). Blood loss in chronic *Schistosoma mansoni* infection in Egyptian farmers. *Trans. R. Soc. Trop. Med. Hyg*. **61**: 621–625.

Faust C., Russell F., Jung C. (1970). Craig and Faust's clinical parasitology . 8th edition. Philadelphia, Lea & Febiger. p. 251.

Fletcher H., Fletcher W. (2005). Clinical epidemiology: the essentials. 4th edition. pp. 288

Gebremedin S. (2004). Prevalence of anaemia and its predisposing factors in pregnant mothers attending antenatal care in Mettu Carl hospital.

Gibson S., Abebe Y., Stabler S., Allen H., Westcott E., Stocecker J., Krebs F., Hambidge M. (2008). Zinc, Graivda, infectiona and but not Vitamin B12 or folate status predict hemoglobin during pregnancy in Southern Ethiopia. *J Nutr* **138** (3): 581 – 6.

Gilles H**.** (1993)**.** Diagnostic methods in malaria. *In:* Essential malariology, Gilles HM, Warrell DA and Arnold P E (ed.), 3rd ed., London, United Kingdom. p.78

Gilles H. (1996). Soil–transmitted helminths (geohelminths). *In: Manson's tropical diseases.* G. Cook, editor. W.B. Saunders. London, United Kingdom. pp. 1369–1412.

Guyatt L., Snow W. (2001). The epidemiology and burden of Plasmodium falciparum-related anemia among pregnant women in sub-Saharan Africa. *Am. J. Trop. Med. Hyg*. **64** (*S1–2*), 36–44.

Gyorkos L., Csapia G. (2006). Lack of risk adverse birth outcomes after deworming in pregnant women. *Pediatric infect Dis,* **25**(9): 791-794.

Gyorkos W., Casapia M., Gotuzzo E., Dendukuri N., Soto C., Maclean D., Larocque R. (2004). Improving maternal and new born health in hook worm – endemic areas by adding a single-dose anthelminthic to prenatal care. Global Forum for Health Research. Forum 8, Mexico City.

Hawdon H. (1996). Hookworm: developmental biology of the infectious process Current Opinion in Genetics and Development **6**(5):618-623

Hercberg S., Galan P. (1992). Nutritional anaemia Baillieres *Clin Haematol* **5**:143 – 168.

Hotez P., Bethony J., Bottazzi E., Brooker S., Buss P. (2005). Hookworm: "The Great Infection of Mankind". *PLoS Med,* **2**(3): 67

Hotez J., Pritchard I. (1995). "Hookworm infection". *Sci. Am.* **272** (6): 68–74.

Hotez J. (2007). Control of neglected tropical diseases. *N. Engl. J. Med*. **357:**1018-1027.

Huddle M., Gibson S., Cullinan R. (1999). The impact of malaria infection and diet on the anaemia status of rural pregnant Malawian women. *European J Clin Nutr* **53** (10): 792 – 801.

Hughes A. (1991). Anaemia pregnancy. Maternal Health and Safe motherhood. Division of family health, WHO.

Humphries L., Stephenson E., Pearce J., Dan T., Khan T. (1997). The use of human faeces for fertilizer is associated with increased intensity of hookworm infection in Vietnamese women. *Trans Roy Soc Trop Med Hyg*. **9**1(5): 518-520.

International Council for standardization in Haematology. (1998). Recommendations for reference method for determination by centrifugation of packed cell volume of blood. *J Clin Path,* 331- 2.

Ismail R., Ordi J., Menendez C., Ventura J., Aponte J., Kahigwa E., Hirt R., Cardesa A., Alonso L. (2000). Placental pathology in malaria: a histological, immunohistochemical, and quantitative study. Hum. *Pathol*. **31**: 85–93.

Jemaneh L. (1998). Comparative prevalence of some common intestinal Helminthic infections in different altitudinal regions in Ethiopia. *Ethiop Med J,* **36**: 1-8.

Johns L., Lewis M. (1989). Primary health screening haemoglobinometry in a tropical community. *Bull WHO*, **67**:627-633.

Kuile O., Terlouw J., Kariuki K., Howard A., Mirel B., Hawley A., Friedman F, Shi P., Kolczak S., Lal A., Vulule M., Nahlen L. (2003). Impact of permethrin-treated bednets on malaria, anemia and growth in infants in an area of intense perennial malaria transmission in western Kenya. *Am. J. Trop. Med. Hyg*., in press.

Larocque C., Gotuzzo M., Soto R., Gyorkos L. (2006). A double-blind randomized controlled trial of antenatal mebendazole to reduce low birth weight in hookworm endemic area of Peru. *Trop Med Int Health*, **11**(10): 1485-1489.

Makandala C. (2003). The effect of malaria in pregnancy on maternal anaemia and birth weight in rural Malwi A maser's paper submitted to the faculty of the University of North Carolina at Chapel Hill.

Mary A., Akanmori D. (2005). Determinants of anaemia in pregnancy in Sekyere West District Ghana. *Ghana Med J*, **39**(3): 102-107.

McConnell E., Armsrong C. (1976). Intestinal parasitism in fifty communities on the central plateau. *Ethio Me d J,* **14**: 159-169.

McDermot M., Slutsker L., Steketee W., Wirima J., Breman G., Heymann L. (1996). Prospective assessment of mortality among a cohort of pregnant women in rural Malawi. *AMJ Trop Med HYg*. **55**:66 – 70.

Mendis K., Sina J., Marchesini P., Carter R. (2001). The neglected burden of *Plasmodium vivax* malaria. *Am J Trop Med Hyg*, **64** (*S 1–2*), 97–106.

Monica C. (1998). District Laboratory Practice in Tropical Countries, Part one. PP 99-100.

Montresor A., Crompton W., Bundy A., Hall A., Savioli L. (1998). Guidelines for the evaluation of soil-transmitted helminthiasis and schistosiasis at community level. http://whqlibdoc.who.int/hq/1998/WHO_CTD_SIP_

Murphy C., Breman G. (2001). Gaps in the childhood malaria burden in Africa: cerebral malaria, neurological sequelae, anemia, respiratory distress, hypoglycemia, and complications of pregnancy. *Am J Trop Med Hyg*, **64** (*S 1–2*), 57–67.

Mwangi W., Bethony M., Brooker S. (2006). Malaria and helminth interactions in humans: an epidemiological viewpoint. *Ann. Trop. Med. Parasitol.* **100:**551-570.

Nahlen L. (2000). Rolling back malaria in pregnancy. *N Engl J Med,* **343**: 651–652.

Needham C. (1998). Epidemiology of soil-transmitted nematode infections in Ha Nam Province, Vietnam. *Trop Med Int Health,* **3:**904-912.

Nosten F., McGready R., Simpson A., Thwa L., Balkan S., Cho T., Hkirijaroen L., Looareesuwan S., White, J. (1999) .Effects of *Plasmodium vivax* malaria in pregnancy. Lancet **354**: 546–549.

Nurdia S., Sumarni S., Suyoko., Hakim M., Winkvist A. (2001). Impact of intestinal helminthes infection on anaemia and iron status during pregnancy: a community based study in Indonesia. *SouthEast Asian J Trop Med Pub Health,* **32** (1):14 – 22.

Oguama M., and Ekwunife A. (2007). The need for a better method: comparism of direct smear and formol-ether concentration techniques in diagnosis of intestinal parasites. *Int J Trop Med*. **3**(2).

Otieno B. (1999). Tropical diseases can harm pregnancy. Network **19** (2): 5 -7.

PAHO. (1998). Washington, DC: Pan American Health Organization; 1998. Informe de una reunion de consulta sorbe el uso de quimioterapia para el control de la morbilidad debida a nematodos transmitidos por el suelo en humanos.

Parija C., Srinivasa J. (1999). Viewpoint: The neglect of stool microscopy for intestinal parasites and possible solutions. *Trop Med Int Health*. **4** (7):522-4.

Peter J., Simon B., Phil J., Bethony M., Maria E., Alex L., Shuva X. (2004). Hookworm infection, *N Eng J Med,* **351**(8): 799 – 807.

Peter G. (1995). Iron bioavailability from diets by different socio-economic strata of Venezulan population. *J Nutr*, **125**(7): 1860-8.

Renaud R., Brettes P., Carrie J., Castanier C., Renaud L., Chesnet Y., Doucet J., Loubiere R. (1971). Bilharziasis of the internal female genital organs. *Rev. Fr. Gynecol. Obstet.* **66**: 1–18.

Renee L., Martin C., Edduardo G., Theresa G. (2005). Relation ship between intensity of soil – transmitted helminthes infection and anaemia. During pregnancy. *AM J Trop Med Hyg*, **73**(4): 783 – 789.

Reproductive Health. New findings on birth spacing: three to five years is the optimal interval, http://www.infoforhealth.org/pr/l13edsum.shtmle server programs birth spacing optimal interval. Date of accessed October 5, 2008.

Richard S. (2003a). Supplement: Nutrition as a Preventive Strategy against Adverse Pregnancy Outcomes Pregnancy, Nutrition and Parasitic Diseases. *J. Nutr.* **133**:1661S-1667S.

Richard S. (2003b). Pregnancy, nutrition and parasitic diseases. Division of parasitic diseases, national center for infectious diseases, centers for disease control and prevention, Atlanta, GA, *J. Nutr*. **133**: 16615 -16675.

Ross G., Bartley P., Sleigh C. (2002). Shcistosomiasis. *N Engl J Med*. **346**:1212.

Shibiru T. (1986). Intestinal helminthiasis in Ethiopia. Helminthologica **23**:43-48.

Sifakis S., Pharmackides G. (2000). Anaemia in pregnancy Ann NY *Acad Sci,* **90**:125 – 136.

Singh B., Fong F, Arikumeron S. (1998). Anaemia in pregnancy, a cross sectional study in Singapore, *European J Clin Nutr*, **52**(1) 65- 70.

SNNPRG. (2007). Tula woreda Administration Finance and Economic Development Department.

Steketee W., Nahlen L., Parise E., Menendez C. (2001). The burden of malaria in pregnancy in malaria-endemic areas. *Am J Trop Med Hyg*, **64** (*S. 1–2*), 28–35.

Steketee W., Wirima J., Hightower W., Slutsker L., Heymann L., Breman J G. (1996). The effect of malaria and malaria prevention in pregnancy on offspring birth weight, prematurity, and intrauterine growth retardation in rural Malawi. *Am J Trop Med Hyg,* **55** (*S 1*), 33–41.

Steketee W. (2003). Pregnancy nutrition and parasitic diseases. *J Nutr,* **133** (Sl2): 16615 – 16675.

Stephenson S., Latham C., Ottesen A. (2000). Malnutrition and parasitic helminth infections. *Parasitology* **121**: S23–S38.

Stephenson S., Latham C., Ottesen A. (2002). Malnutrion and parasite helminth infections. *Parasitology*, **12**(7): 23-38.

Stoll R. (1962). On endemic hookworm, where do we stand today? *Exp Parasitol* **12**:241-252.

Stoltzfus J., Drefuss L. (1998). Guidelines for the use of iron supplement to prevent and treat iron deficiency anaemia. Geneva: International nutrition anaemia consultative group /UNICEF/ WHO.

Sturrock F. (1993). The intermediate hosts and host-parasite relationships. In: Human Schistosomiasis. Jordan P, Webbe G and Sturrock RF, ed. CAB International. Wallingford, United Kingdom. 33–85.

Tadios Y. (1996). Prevalence of anaemia and its risk factors among pregnant mothers following antenatal in Asendabo health center.

Tedla S., Jemaneh L., (1980). Distribution of Ancylostoma duodenale and Necator americanus in Ethiopia. *Ethiop Med J*, **23**:149-58.

Van den Brock R. (2003). Anaemia and micronutrient deficiencies. *British Med Bull*, **67**: 149-160.

Van den Brock R., Rogerson J., Mhango G., Kambala B., White A., Molyneux E. (2000). Etiology of anaemia in pregnancy in southern Malawi. *Am J Clin Nutr*, **72**(2):247S – 53S.

Van Schenck H., Falkensson M., Lundberg B. (1986). Evaluation of "HemoCue", a new device for determining haemoglobin. *Clin Chem*, **32**:526-529.

Villanova A. (1994). Reference and selected procedures for the quantitative determination of haemoglobin in blood: approved standards. 2nd ed., National Committee for Clinical Laboratory Standards.

Warhurst C., Williams E. (1996). Laboratory diagnosis of malaria. *J Clin Pathol*, **49:**533–538.

Merid Y., Mamdouh H., Mekit G., Teklemariam S. (2001). Intestinal Helminthic infection among children at Lake Awassa area, Southern Ethiopia. *Ethiop. J. Health Dev*. **15**(1):31-37.

Wodimanehu T., Birrie H., Yemaneh H. (1997). Schistosomiasis and intestinal helminthic infections in Delo Awraja, Bale administrative region South Ethiopia. *Ethiop J Health Dev*. **11**(3): 183 -188.

World Health Organization. (1991). Controlling iron deficiency. ACC/SCN state of the art series Nutrition Policy Discussion Paper No.9.

World Health Organization. (2004). Prevention and control of schisstosomiasis and soil transmitted helmithiasis.

World Health Organization /UNICEF. (2001). Iron deficiency anaemia, assessment, prevention and control: a guide for programme managers.

World Health Organization. (1986). Statistic quarterly reported disease prevention and control.

World Health Organization. (1991). National strategies for overcoming micronutrient malnutrition. Document EB 8.9/27. Executive board, 89th session.

World Health Organization. (1992). The prevalence of anaemia in women a tabulation of available information Geneva, WHO, WHO/MCH/ MSM/92.2; 119 – 124.

World Health Organization. (1993). Prevention and management of severe anaemia in pregnancy. WHO, WHO/FHE/MSM/ 93 – 5.

World Health Organization. (1996). Report of the WHO informal consultation on hookworm infection and anaemia in girls and women. Geneva: WHO, CTD/SIP/ 96.1.

World Health Organization. (2000). WHO recommended method for determination of packed cell volume by centrifugation prepared by Expert Panel on Cytometry of International Council for Standardization in haematology Document WHO, DIL 00 2 1 9.

World Health Organization. (2002). Prevention and control of schistosomiasis and soil-transmitted helminthiasis: report of a WHO expert committee.

World Health Organization. (2003). Schistosomiasis and soil-transmitted helminthiasis: an unprecedented opportunity for control.

World Health Organization. (2004). Prevention and control of Schistosomiasis and Soil-transmitted helminthiasis.

World Health Organization. http://www.who.int/ctd/schisto/burdens.htm

Schistosomiasis. Accessed Dec. 1, 2000.

World Health Organization. (1999). Monitoring Helminth Control Programs (WHO/CDS/CPC/SIP/99.3).

World Health Organization /UNICEF. (2003). Africa malaria report. WHO/CDS/MAL/2003.1093.

Zavaleta N., Berlanga R., Lonnerdal B., Brown H. (1993). Prevalence and determinants of iron deficiency anaemia in a representative sample of pregnant women in Lima, Peru,. Pan American Health Organization.

ANNEX

ANNEX I: LABORATORY REQUESTING AND RECORDING FORMAT

ADDIS ABABA UNIVERSITY MEDICAL FACULTY DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY AND PARASITOLOGY

Parasitological investigation and haemoglobin determination at Bushulo health center in pregnant women, Tula woreda.

1. Personal data

1.1 Code no. ……

1.2 Age …….

1.3 Address …….

1.4 Occupation …….

1.5 Date of sample collection ----------

2. Laboratory data

2.1 Physical examinations

2.1.1 Consistency of the stool

Formed ☐ Semi-formed ☐

Soft ☐ watery diarrhea ☐

Bloody diarrhea ☐

2.1.2 Appearance of stool

Blood stained ☐ mucus ☐ Normal ☐

2.1.3 If macroscopic worm is present, write the type of worm ----------

2.2 Microscopic examination

2.2.1 Direct microscopic examination

A. No ova parasite

B. Types of ova parasite seen----------

C. Other intestinal protozoa seen

2.2.2 Concentration technique

D. No ova parasite seen

E. Types of ova parasite seen----------

F. Other intestinal protozoa seen----------

2.2.3 Blood film examination

G. No haemo parasite seen

H. Types of haemoparasite seen----------

Haemoglobin concentration----------

Name of investigator _____

Signature _____ Date _____

ANNEX IIa: Questionnaire

Assessment of Anaemia Determinants			Remark
1.	How old are you?		
2.	Where do you live?	A. Urban B. Rural	
3.	What is your occupation?	A. Farmer B. Housewife C. Merchant	
4.	What is your monthly income?		
5.	What is your educational status?	A. Illiterate B. Read and write C. 1 – 6 D. 7 – 12	
6	What is your marital status?	A. Married B. Single C. Others	
7.	What is your religion?	A. Christian B. Muslim	
8.	Do you have latrine?	A. Yes B. No	
9.	If you have latrine do you wash your hand after latrine?	A. Yes B. No	
10.	Do you wash your hand before meal?	A. Yes B. No	
11.	From where do you get water?	A. Protected spring B. River	
12.	Do you wear shoe?	A. Yes B. No	
13.	Do you eat meat and animal products?	A. Yes B. No	
14.	If you eat meat and animal products how many times?	A. Daily B. Every 2 day C. Every week D. Once a month	
15.	Do you eat green leafy vegetables?	A. Yes B. No	
16.	If you eat green leafy vegetables how many times?	A. Daily B .Every 2 day C. Every week C. Once a month	
17.	Do you take tea or coffee immediately after meal?	A. Yes B. No	
18.	Do you eat fruit after meal?	A. Yes B. No	

19.	What is your gestational age?		
20.	How many children do you have?		
21.	What is your gravidity?	A. Premigravida B. Multigravida C. Grandgravida	

ANNEX IIb: Questionnaire

Assessment of anaemia determinants			Remark
2.	Where did you deliver your babies?	A. Health institute B. home	
3.	Was there any blood loss in your previous delivery?	A. Yes B. No	
4.	Did you follow antenatal care in your previous pregnancy?	A. Yes B. No	
5.	Do you follow antenatal care for the current pregnancy?	A. Yes B. No	
6.	Was there any blood loss in your current pregnancy?	A. Yes B. No	
7.	At what interval did you deliver your babies?	A. ≤ 2 years B. > 2 years	
8.	Was there any abortion in your pregnancy?	A. Yes B. No	
9.	If you say yes for question 29 how many times?		
10.	Do you use contraceptive?	A. Yes B. No	
11.	Did you become infected with malaria for the last one year?	A. Yes B. No	
12.	Have you taken iron supplement the current pregnancy?	A. Yes B. No	

13.	Do you have anti- malaria bed net?	A. Yes B. No	
14.	If you say yes for question 34 do you use frequently?	A. Yes B. No	

ANNEX III: Amharic version of questionnaire

በአዲስ አበባ ዩኒቨርሲቲ የህክምና ፋኩሊቲ የማይክሮ ባዮሎጂ፣ ኢሚዩኖሎጂና ፓራሳይቶሎጂ የትምህርት ክፍል

ሙሉ ፈቃደኛ በሆኑ ነፍሰጡር እናቶች ላይ የአንጀት ጥገኛ ተዋህሲያንና ሌሎች መንስኤች ለደም ማነስ ያላቸውን እገዛ ለማጥናት የተዘጋጀ መጠይቅ

በዚህ ጥናት ለመካፈል የደምና የሰገራ ናሙና በመስጠትና ለሚቀርብልዎት ቃለመጠይቆች ምላሽ ለመስጠት ሙሉ ፈቃደኛ ነዎት?

አዎን-------- አይደለም-------

የጤና ተቋሙ ስም _____

የጥናቱ ተካፋይ መለያ ቁጥር _____

	ስነ ማህበራዊ እና ህዝባዊ መጠይቅ		ምርመራ
1	ዕድሜዎት ስንት ነው?		
2	የት ነው የሚኖሩት?	ሀ. ገጠር ለ. ከተማ	
3	ሥራዎት ምንድ ነው?	ሀ. ገበሬ ለ. የቀን ሰራተኛ ሐ. ነጋዴ መ. የቤት እመቤት ሰ. ሌላ (ይጠቀስ)	
4	የወር ገቢዎት ስንት ነወ?		
5	የጋብቻ ሁኔታ	ሀ. ያገባች ለ. ያላገባች ሐ. አግብታ የፈታች መ. ባል የሞተባት	
6	እምነትዎት ምንድ ነው?	ሀ. ኦርቶዶክስ ለ. ኘሮቴስታንት ሐ. ካቶሊክ መ. ሙስሊም ሠ. ሌላ	

7	የትምህርት ደረጃዎት ስንት ነወ?·	ሀ. ማንበብና መፃፍ እችላለሁ ለ. ማንበብና መፃፍ አልችልም ሐ. ከ1-6 ክፍል መ. ከ7-12 ክፍል ሠ. ከ12 በላይ	
8	ሽንት ቤት አልዎት?	ሀ. አዎ ለ. የለም	
9	ሽንት ቤት ካልዎት ከሽንት ቤት መልስ እጅዎትን ይታጠባሉ?	ሀ. አዎ ለ. አልታጠብም	
10	ምግብ ከመመገብዎ በፊት እጅዎትን ይታጠባሉ?	ሀ. አዎ ለ. አልታጠብም	
11	የመጠጥ ውሃ ከየት ነው የሚጠቀሙት?	ሀ. ንጽሕናው ከተጠበቀ ምንጭ ለ. ንጽሕናው ካልተጠበቀ ምንጭ ሐ. የቧንቧ ውሃ መ. ወንዝ	
12	ጫማ ያደርጋሉን?	ሀ. አዎ ለ. አላደርግም	
13	ስጋና የእንስሳት ውጤቶችን ይመገባሉን?	ሀ. አዎ ለ. አልመገብም	
14	ስጋና የእንስሳት ውጤቶችን የሚመገቡ ከሆነ ለምን ያህል ጊዜ?	ሀ. በየቀኑ ለ. ከሁለት ቀን አንዴ ሐ. በሣምንት አንዴ መ. በወር አንዴ	
15	አረንጓዴ ቅጠላ ቅጠሎችን ይመገባለን?	ሀ. አዎ ለ. አልመገብም	
16	አረንጓዴ ቅጠላ ቅጠሎችን የሚመገቡ ከሆነ ለምን ያህል ጊዜ?	ሀ. በየቀኑ ለ. ከሁለት ቀን አንዴ ሐ. በሣምንት አንዴ	

		መ. በወር አንደ	
17	ካረገዙ ስንተኛ ወርዎት ነው?		
18	ስንት ልጆች አልዎት?		
19	ለስንት ጊዜ አርግዘዋል?		
20	መንታ ልጆች ወልደው ያውቃሉ?	ሀ. አዎ ለ. አልወለድኩም	
21	ከዚህ በፊት የወለድዋቸው ህፃናት የት ነው የተወለዱት?	ሀ. ጤና ተቋም ለ. ቤት	
22	በወሊድ ጊዜ የደም መፍሰስ ችግር አጋጥምዎት ያውቃል?	ሀ. አዎ ለ. አላጋጠመኝም	
23	ልጆችን በምን ያህል ጊዜ አራርቀዉ ይወልዳሉ?	ሀ. ከሁለት አመት በታች ለ.ሁለት አመት እና ከዚያ በላይ	
24	ዉርጃ አጋጥምዎት ያዉቃልን?	ሀ. አዎ ለ. አላጋጠመኝም	
25	ለጥያቄ 24 መልስ አዎ ከሆነ ስንት ጊዜ?		
26	የወሊድ መከላከያ እንክብል ይጠቀማሉን?	ሀ. አዎ ለ. አልጠቀምም	
27	ቡና ወይም ሻይ ከምግብ ጋር ይጠቀማሉ?	ሀ. አዎ ለ. አልጠቀምም	
28	ከምግብ በኃላ ፍራፍሬ ይመጋባሉን?	ሀ. አዎ ለ. አልጠቀምም	
29	በፀረ-ወባ ኬሚከል የተነከረ የአልጋ አጎበር አልዎት?	ሀ. አዎ ለ. የለም	
30	ለ ለጥያቄ 29 መልስዎ አዎ ከሆነ አዘዉትረዉ ይጠቀማሉን?	ሀ. አዎ ለ. አልጠቀምም	
31	ከዚህ በፊት በነበርዎት የእርግዝና የቅድመ ወሊድ ክትትል ያደርጉ ነበር?	ሀ. አዎ ለ. አልአደረኩም	

32	**በአሁኑ የእርግዝና ጊዜ ውስጥ ደም መፍሰስ አጋጥምዎት ያውቃልን?**	**ሀ. አዎ** **ለ. አላጋጠመኝም**	
33	**በአሁኑ የእርግዝና ጊዜ የቅድመ ወሊድ ክትትል አድርገዋልን?**	**ሀ. አዎ** **ለ. አላደረኩም**	
34	**በአሁኑ የእርግዝና ጊዜ ለደም ማነስ የሚሰጥ መድኃኒት ወስደዋልን?**	**ሀ. አዎ** **ለ. አልወሰድኩም**	
35	**ባለፈው አንድ አመት ጊዜ ውስጥ በወባ ታመው ያውቃሉን?**	**ሀ. አዎ** **ለ. አልታመምኩም**	

ANNEX IV: Consent form

Code number_____

Consent form

A.A.U., Department of Microbiology, Immunology and Parasitology

This is an agreement request to assess determinants of anaemia with emphasis on helminthic infection in pregnant women. With this request those pregnant women who are attending antenatal follow up at Bushulo health center and willing to participate in the study will be invited to assess determinants of anaemia.Based on the laboratory investigation anaemic and positive parasite infected pregnant women will be treated free of any cost.

Objective of the study

Questionnaires will be requested on determinants of anemia to assess anaemia. On the request paper your name or your identities will not be mentioned. Samples and information given by the participants will serve only for this research not for any other purpose. You have every right to accept or refuse participation of this study.

You will be requested to give small amount of blood and stool. Blood will be collected from the tip of your finger using sterile lancet. There will be some pain during pricking of your finger but not harmful to your health. If you are agree to give samples you will be requested to answer for questionnaire.

Are you willing to participate with this study by giving blood and stool sample and answer to the requests? A. Yes B. No

Thank you

Name of health institute_____

Patient name_____ Signature_____ Date _____

Name of data collector_____ Signature_____ Date _____

ANNEX V: Amharic version of the Consent form

የስምምነት መጠየቂያ ቅጽ

የጥናቱ ተካፋይ መለያ ቁጥር_____

በአዲስ አበባ ዩኒቨርሲቲ የሕክምና ፋካልቲ የማይክሮ ባዮሎጂ ኢሚዮኖሎጅና ፓራሳይቶሎጅ የትምህርት ክፍል

በነፍሰ ጡር እናቶች ላይ የአንጀት ጥገኛ ተዋሲያንና ሌሎች መንስኤዎች ለደም ማነስ ያላቸውን እገዛ በተመለከተ ለማጥናት የተዘጋጀ የስምምነት መጠየቂያ ቅጽ::

በመጠይቁም በቡሾሎ ጤና ጣቢያ አገልግሎት ከሚያገኙ ነፋሰጡር እናቶች ውስጥ በጥናቱ ለመካፈል ፈቃደኛ የሆኑ አንዲሁም መመዘኛውን በሚያሟሉት ላይ የአንጀት ጥገኛ ተዋሐሲያን እንዲሁም ሌሎች መንስኤዎች ለደም ማነስ ስርጭት ያላቸው እገዛ ምን እንደሚመስል ለማጥናት የተዘጋጀ ነው::

የደምና የሰገራ ናሙና ምርመራ በማድረግ በሚገኘው ውጤት መሠረት የአንጀት የጥገኛ ተዋሲያንና የደም ማነስ ችግር ካለብዎት ከጤና ጣቢያው ባሙያዎች ጋር በመተባበር ተገቢውን የሕክምና አገልግሎት ያገኛሉ::

<u>የጥናቱ አላማ</u>

ለደም ማነስ መንስኤ በሆኑት ዙሪያ ቃለ መጠይቅ ይቀርብሎታል:: በመጠይቁም ላይ ስሞዎን ወይም የእርስዎን ማንነት የሚገልፅ ማንኛውንም ነገር አይጠቀስም ወይም አይያያዝም:: የሚሰጡትም መረጃም ሆነ ናሙና ከዚህ ጥናት ውጭ ሆነ ከእርስዎ ጋር ለተገናኘ ለሌላ ጥቅም በፍፁም አይውልም::

በዚህ ጥናት የሰገራና የደም ናሙና በመስጠት እንዲሁም ለሚቀርብልዎት ቃለ መጠይቆች ምላሽ በመስጠት ሙሉ ተሳታፊ ለመሆን ፈቃደኛ ነዎት?

አዎን ከሆነ መልሱ

የጤና ተቋሙ ስም_____

የጥናቱ ተካፋይ ስም_____ ፊርማ

ቀን_____

የመረጃ ሰብሳቢው ስም_____ ፊርማ_____
ቀን_____

ANNEX VI: DECLARATION

I, the undersigned, declare that this MSc thesis is my original work, has not been presented for a degree in Addis Ababa University or any other universities. I also declare that all sources of materials used for the thesis have been duly acknowledged.

Name of the candidate _____

Signature _____

Place _____

Date of submission _____/_____/_____

This thesis has been submitted for examination with my approval as university advisor.

Name of advisor _____

Signature _____

Place _____

Date of submission _____/_____/_____

Name of examiner _____

Signature _____

Place _____

Date of submission _____/_____/_____