

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



**THE HEALTH IMPACT OF INTESTINAL HELMINTH  
INFECTIONS AMONG PODOCONIOSIS PATIENTS IN  
WOLAITA ZONE, SOUTHERN ETHIOPIA**

*A Thesis Submitted to the School of Graduate Studies of Addis Ababa University  
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## TABLE OF CONTENTS

ACKNOWLEDGEMENTS .....	i
TABLE OF CONTENTS .....	ii
LIST OF TABLES .....	iv
LIST OF FIGURES .....	vi
LIST OF APPENDIXES.....	vi
LIST OF ABBREVIATIONS AND ACRONYMS .....	vii
Abstract:.....	viii
1. INTRODUCTION .....	1
1.1. Intestinal helminth infections .....	1
1.1.1. Anemia and intestinal helminth infections .....	2
1.1.2. Hookworm infection.....	3
1.1.3. Pathophysiology and clinical disease of hookworm.....	4
1.1.4. Hookworm prevention and control strategies.....	7
1.2. Podoconiosis.....	8
1.2.1. Etiology, pathology and genetic predisposition .....	10
1.2.2. Prevention and management of podoconiosis .....	13
1.3. Podoconiosis and hookworm .....	14
1.3.1. Geographical overlap.....	14
1.3.2. Occupational overlap.....	15

1.4. Anemia .....	15
2. OBJECTIVES .....	19
3. MATERIALS AND METHODS.....	20
3.1. Study area.....	20
3.2. Study design .....	22
3.3. Parasitological examination of stool .....	22
3.4. Blood film screening for malaria parasites .....	23
3.5. Anemia determination .....	23
3.6. Sample size calculation .....	25
4. ETHICAL CONSIDERATION .....	25
5. STATISTICAL ANALYSIS .....	26
6. RESULTS .....	27
6.1. Overall prevalence of intestinal helminth infection .....	27
6.2. Anemia prevalence and its relation to helminth infection.....	31
6.3. Clinical stages of podoconiosis .....	37
7. DISCUSSIONS.....	40
8. CONCLUSIONS.....	46
9. RECOMMENDATIONS .....	47
10. REFERENCES .....	48
11. APPENDIXES .....	57

## LIST OF TABLES

Table 1. The age and sex distribution of the study participants in Wolaita Zone, Southern Ethiopia (April and May, 2010).....	28
Table 2. Prevalence of intestinal parasites among podoconiosis patients (cases) and non- .....	29
Table 3. Prevalence of helminth polyparasitism among podoconiosis patients (cases) and non-podoconiosis individuals (controls) in Wolaita Zone, Southern Ethiopia (April and May, 2010).....	30
Table 4. Prevalence of intestinal helminth infection among podoconiosis patients by highland and lowland <i>woredas</i> in Wolaita Zone, Southern Ethiopia (April and May, 2010).....	30
Table 5. Comparison of mean Hgb level between podoconiosis patients (cases) and non-podoconiosis individuals (controls) without considering helminth infections in WolaitaZone, Southern Ethiopia (April and May, 2010). .....	32
Table 6. Comparison of anemia prevalence between podoconiosis patients (cases) and non-Podoconiosis individuals (controls) in relation to helminth infections in Wolaita Zone, Southern Ethiopia (April and May, 2010). .....	33
Table 7. Comparison of helminth infections and anemia severity among podoconiosis patients in Wolaita Zone, Southern Ethiopia (April and May, 2010). .....	34

Table 8. Shoe wearing and hookworm infection between podoconiosis patients (cases) and non-podoconiosis individuals (controls) in Wolaita Zone, Southern Ethiopia (April and May, 2010). .....	35
Table 9. Logistic regression analysis of helminth infection on hemoglobin values among podoconiosis patients in Wolaita Zone, Southern Ethiopia (April and May, 2010). .....	36
Table 10. Clinical staging of podoconiosis patients and prevalence of major intestinal helminth infections in Wolaita Zone, Southern Ethiopia (April and May, 2010). .....	37
Table 11. Clinical stages and duration of disease among podoconiosis patients in relation to hemoglobin level in Wolaita Zone, Southern Ethiopia (April and May, 2010). .....	38

## LIST OF FIGURES

Figure 1. The Life Cycles of <i>Necator americanus</i> and <i>Ancylostoma duodenale</i> (Hotez <i>et al.</i> , 2004). .....	6
Figure 2. Soft ‘water-bag’ swelling in a 20-year-old man suffering from podoconiosis (Photo: Ewenet GebreHanna, with permission) (Davey <i>et al.</i> , 2007). .....	11
Figure 3. Hard and fibrotic type of swelling of the foot of a podoconiosis patient (Davey <i>et al.</i> , 2007). .....	12
Figure 4. A map of Wolaita Zone, SNNPR (Finance and economic development bureau, 2009)	21
Figure 5. Photographs showing: A/ sample collection in the field and B/ microscopic examination of preserved stool samples at biomedical research laboratory, Department of Biology, Addis Ababa University. ....	24
Figure 6. Clinical manifestations of podoconiosis in Wolaita: Stage 1 (A), Stage 2 (B) and Stage 3 (C) (photograph taken in the field, April and May, 2010). ....	39

## LIST OF APPENDIXES

Appendix I. Consent form .....	57
Appendix II. Checklist of information on the study participants .....	58

## LIST OF ABBREVIATIONS AND ACRONYMS

Hgb/Hb	Hemoglobin
Hct	Hematocrit
NTD	Neglected tropical disease
WHO	World health organization
CDC	Center for disease control and prevention
STH	Soil transmitted helminths
DALY	Disability-adjusted life years
MFTPA	Mossy Foot Treatment and Prevention Association
SAF	Sodium acetate- acetic acid-formaldehyde
P	Expected prevalence or proportion
OR	Odds ratio
SD	Standard deviation
X <sup>2</sup>	Chi-square statistical analyses
Fe	Iron

**Abstract:**

Intestinal helminth infections are most common diseases of the poor and a potential cause of anemia in developing countries. Podoconiosis is a non-filarial “elephantiasis” of the lower legs in farmers that work on loamy clay soil and is a chronic complication that does not have effective treatment. Soil contact being the mode of transmission of geo-helminths and the cause of podoconiosis, the aim of this study was to investigate the health impact of helminth infections on podoconiosis patients in Wolaita Zone, southern Ethiopia. A total of 480 (384 podoconiosis patients and 96 controls) study participants were enrolled in the study. The study showed an overall prevalence of 57.8% helminth parasites among podoconiosis patients and 37.5% among the controls. Out of the helminth positive podoconiosis patients, 3.9% had triple and more infections, 12.8% double and 41.1% had single infections. Among the control study participants, triple and more infections were rare (1%), whereas 7.3% double and 29.2% single infections were detected. Shoe wearing in relation to hookworm infection among the podoconiosis patients was assessed and most had no practice of appropriate shoe wearing. Hemoglobin (Hgb) and Hematocrit determination techniques used to determine anemia showed mean Hgb value between podoconiosis patients ( $12.85 \pm 1.69$ ) and controls ( $14.71 \pm 1.69$ ) to be significantly different ( $P < 0.05$ ) showing that more podoconiosis patients were anemic. On the whole, anemia was twice as likely among helminth-infected podoconiosis patients than their helminth-free counterparts. Furthermore, hookworm-infected podoconiosis patients were 3.4 times more likely to be anemic than hookworm infected non-podoconiosis individuals ( $P < 0.05$ ). Double helminth infections, with hookworm included, further increased the risk of being anemic among podoconiosis patients (OR=3.9,  $P=0.032$ , 95% CI=1.124, 13.523). On the other hand, the overall prevalence of intestinal geo-helminth infection decreased with increasing clinical stages of podoconiosis, implying that with severe and complicated podoconiosis, the individual would be seriously incapacitated to work on the farm and hence will have a limited contact with soil to be exposed to helminth infections. The study has provided good evidence to justify selective initiation of deworming and iron rich nutrient supplementation to improve the wellbeing of podoconiosis patients under resource poor conditions in Wolaita.

**Key words:** Podoconiosis, Helminths, Anemia, Hookworm, Wolaita

## **1. INTRODUCTION**

### **1.1. Intestinal helminth infections**

Intestinal helminths are common parasitic infectious agents of humans in the world mainly in developing countries (Hotez et al., 2008). More than 2,000 million people are infected by soil-transmitted helminths (STH) worldwide, of which more than 300 million suffer from associated severe morbidity. STH infections are widely distributed in tropical and subtropical areas, especially in poor populations (Montresor *et al.*, 2002 as cited in Abahussain, 2005). Climate is an important determinant factor for transmission of these infections in the tropical and subtropical areas, with adequate moisture and warm temperature essential for larval development in the soil. Equally important determinants are poverty and inadequate clean water supplies and sanitation. In such conditions, soil transmitted helminth species are commonly coendemic. Morbidity and rate of transmission of STH infections are directly related to the number of worms harbored in the host. Intensity of infection is measured by the number of eggs per gram of faeces, generally by the Kato-Katz fecal thick-smear technique (Bethony *et al.*, 2006).

Intestinal parasitism has been widespread in Ethiopia. Parasitic helminthic infections are the most predominant causes of outpatient morbidity in the country (Mengistu *et al.*, 2007). Several studies have indicated that the prevalence of parasitic infections is higher at lower altitudes in Ethiopia (Jemaneh, 1998). In Ethiopia, the coverage of safe drinking water and latrine is very low (Kumie and Ali, 2005; WHO/UNICEF, 2006). Poor environmental sanitation and climatic conditions (hot, wet and humid) were reported to favor the persistence of parasite ova in Ethiopia (Tesfa-Yohannes and Kloos, 1988; Mengistu *et al.*, 2007).

### **1.1.1. Anemia and intestinal helminth infections**

Although soil transmitted helminths (STH) do not cause significant mortality when compared with many other parasitic diseases, they do cause substantial morbidity. Infections with STH have a pronounced impact on nutrition, growth, physical fitness, cognitive function and anemia in infants, school-age children and adults. These infections have long term effects and are measured in terms of disease impact statistics, such as disability-adjusted life years (DALYs) (Horton, 2003; Bethony *et al.*, 2006).

One of the oldest established nutrition and infection relationships is the one that describes how STH nematodes lead to iron deficiency anemia. It has long been established that high intestinal worm burdens with *Ascaris lumbricoides*, *Trichuris trichiura*, *Ancylostoma duodenale* and/or *Necator americanus* lead to anorexia, decreased food intake and weight loss, decreased nutrient absorption and utilization, and gut pathophysiology. When these conditions occur with extensive parasitosis, the accompanying intestinal blood loss can result in moderate to severe anemia requiring treatment. In most developing countries, STH or gastrointestinal nematode infections are important predictors of anemia and the severity of anemia is proportional to worm burdens (Koski1 and Scott, 2003).

Some intestinal helminths in particular stand out to cause anemia because of their widespread prevalence and distribution that result in hundreds of millions of human infections. These include hookworm, schistosomes and trichuris. Chronic blood loss due to infection with these helminths is a significant contributor to anemia (Cooper and Bundy 1989).

### **1.1.2. Hookworm infection**

Human hookworm infection is caused by helminth parasites of species *Necator americanus* and *Ancylostoma duodenale*. *N. americanus* is the predominant etiology of human hookworm infection worldwide, whereas *A. duodenale* occurs in more scattered focal environments. This infection is one of the most common chronic infections, and has a prevalence of 740 million cases in areas of rural poverty in the tropics and subtropics (De Silva *et al.*, 2003). The greatest number of hookworm cases occurs in Asia, followed by sub-Saharan Africa (De Silva *et al.*, 2003). High hookworm transmission also occurs in southern China, the Indian subcontinent, and the Americas. In all regions, there is a striking relationship between hookworm prevalence and low socioeconomic status. Hookworm's neglected status partly reflects its concentration among the world's poorest 2.7 billion people who live on less than \$2 a day (De Silva *et al.*, 2003; Hotez *et al.*, 2005).

For many common helminthic infections, including ascariasis, trichuriasis, and schistosomiasis, the intensity of infection usually peaks during childhood and adolescence (Bundy, 1995). In contrast, there appears to be considerable variation in the age intensity profile of hookworm infection. Although the hookworm burden may be heavy in children, especially those in sub-Saharan Africa, the most commonly recognized pattern is a steady rise in the intensity of infection during childhood, with either a peak or a plateau in adulthood (Hotez *et al.*, 2004). The observation that the intensity of hookworm infection increases with age is due to its occupational nature and has led to the suggestion that hookworms can either evade or suppress host immune responses (Loukas and Prociv, 2001).

In Ethiopia, both species of hookworm are found (Leykun and Tedla, 1984) with *N. americanus* the predominant species (Tedla and Jemaneh 1985). In general, hookworm infections are widely distributed in the humid western and southwestern lowlands where moisture is abundant through most of the years and where the average temperature of the coldest month is above 18 ° c. Microclimate, man-made soil environment as in irrigated areas and the use of footwear also influence hookworm transmission in the country (Kloos *et al.*, 1980).

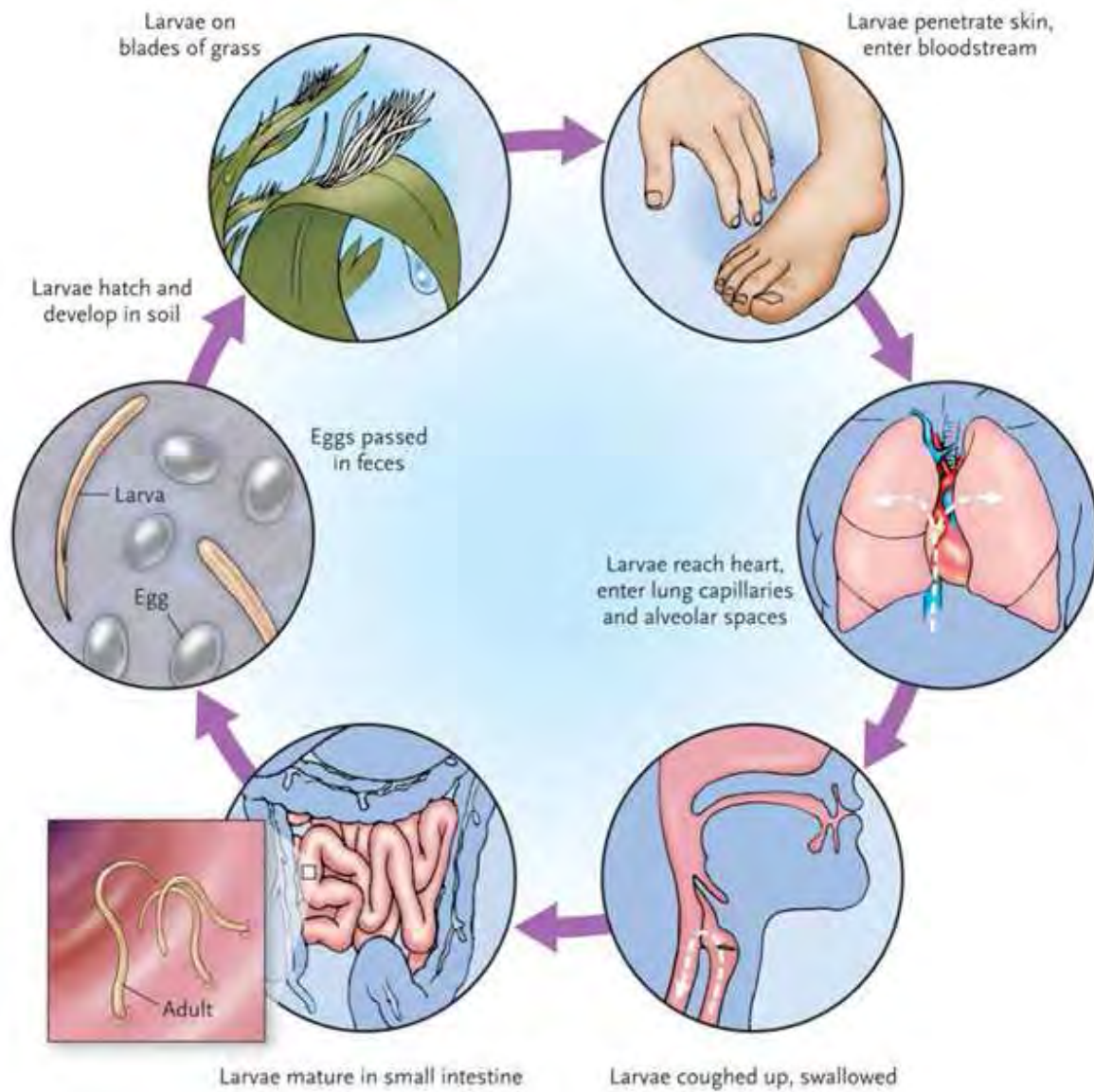
### **1.1.3. Pathophysiology and clinical disease of hookworm**

Hookworm infection is acquired by invasion of the infective larval stages through the skin (*A. duodenale* larvae can also enter through oral route). Following host entry, the larvae undergo a journey through the vasculature, then the lungs and other tissues, before they enter the gastrointestinal tract and molt twice to become one-centimeter-long adult male and female worms. The worms mate and the female hookworms produce up to 30,000 eggs per day, which exit the host's body in the feces (Figure 4). Because hookworms do not replicate in humans, the morbidity of hookworm is highest among patients that harbor large numbers of adult parasites. Estimates of the intensity of hookworm infection are typically obtained by using quantitative fecal egg counts as a surrogate marker for worm burden. The World Health Organization defines moderate intensity infections as those with 2,000–3,999 eggs per gram of feces, and heavy-intensity infections as those with 4,000 or more eggs per gram (Hotez *et al.*, 2004).

The major hookworm-related injury in humans occurs when the adult parasites cause morbidity in the host by producing intestinal hemorrhage. Because the adult hookworms ingest the blood, rupture the erythrocytes, and degrade the hemoglobin; the disease attributed to hookworm is

silent blood loss leading to iron deficiency anemia and protein malnutrition. During the feeding activity of the parasite, capillaries and arterioles are ruptured not only mechanically but also chemically, through the action of hydrolytic enzymes (Hotez and Pritchard, 1995). To ensure blood flow, the adult hookworms release anticlotting agents (Stanssens *et al.*, 1996) thereby farther increasing loss of blood from the intestine. The extent of iron deficiency anemia induced by hookworms varies with hookworm species. Infection with *A. duodenale* causes greater blood loss (0.2 ml blood /day) than does infection with *N. americanus* (0.03 ml blood /day) (Olsen *et al.*, 1998).

There is a correlation between parasite intensity and host intestinal blood loss. In children, women of reproductive age, and other populations with low iron stores, there is often a correlation between parasite intensity and reductions in host hemoglobin. In children, chronic heavy-intensity infections are associated with growth retardation, as well as intellectual and cognitive impairments; in pregnant women, they are associated with adverse maternal–fetal outcomes (Hotez *et al.*, 2005). Depending on the status of host iron, a hookworm burden (i.e., the intensity of infection, or number of worms per person) of 40 to 160 worms is associated with hemoglobin levels below 11 g per deciliter. However, other studies have shown that anemia may occur with a lighter hookworm burden (Olsen *et al.*, 1998).



**Figure 1.** The Life Cycles of *Necator americanus* and *Ancylostoma duodenale* (Hotez *et al.*, 2004).

#### **1.1.4. Hookworm prevention and control strategies**

Public education on the dangers associated with dirt contaminated with human faeces can help reduce the number of cases of hookworm infection. Education programs should highlight the importance of disposing of human faeces in a sanitary way so that it doesn't contaminate the soil, wearing covered footwear in areas where the disease is endemic and washing hands after using toilet. Therefore, sanitation and footwear are important means of reducing transmission of hookworm. Sanitation measures include the provision of latrines along with training in their use and the appropriate treatment of human waste before use in activities like agriculture (Stoltzfus *et al.*, 1997). Because of its high transmission potential in areas of poverty and poor sanitation, hookworm infection is difficult for eradication (Hotez *et al.*, 2005). In countries with less economic development, there is poor functioning of public health services in activities like sanitation, footwear and health education are minimal and therefore, control efforts have shifted to reduce morbidity through mass treatment (also known as "deworming") of affected populations with antihelminthic drugs (Brooker *et al.*, 2004). The most common control strategies through treatment for hookworm are Benzimidazoles (BZAs), specifically albendazole and mebendazole. BZAs kill adult worms by binding to the nematode's beta-tubulin and subsequently inhibiting microtubule polymerization within the parasite (Bethony *et al.*, 2006).

## **1.2. Podoconiosis**

Elephantiasis can be filarial or non-filarial in its origin. The filarial elephantiasis also known as lymphatic filariasis is caused by the parasitic filarial worms like *Wuchereria bancrofti*, *Brugia malayi* and *B. timori*, all of which are transmitted by mosquitoes (CDC, 2008). Lymphatic filariasis affects more than 120 million people in over 80 countries worldwide being most prevalent in Africa, India and South Asia, the Pacific, and the Americas. The other form of elephantiasis occurs in the absence of parasitic infection. This non-parasitic form of elephantiasis is known as podoconiosis, and has been reported from many parts of the world; but exists as an endemic disease in geographically circumscribed areas (Price, 1990).

Podoconiosis (endemic non-filarial elephantiasis) is widely distributed in the world with its highest prevalence in tropical or sub tropical latitudes. Highland areas of tropical and sub tropical regions are the main distribution foci of the disease. The distribution of the disease in this area is associated with the presence of irritant red clay soil of volcanic origin. The affected individuals are mainly bare footed farmers that work on red clay soils (Price, 1990). Characteristically, the soils are red clay loams, which are slippery and adhere to the skin when wet.

Most cases of podoconiosis have been reported from highlands of tropical Africa, Central America and Northwest India (Price, 1990). In Africa it is widely prevalent in countries such as Ethiopia, Kenya, Tanzania, Rwanda and Burundi, all of which are associated with the Rift Valley geological complex (Price, 1976; Price, 1990). Common features for affected areas are altitudes

above 1250 m, annual rainfall above 1000 mm, average annual temperature of 20°C, and soils of volcanic origin (Price, 1990).

The history of podoconiosis is better described in Ethiopia than anywhere else in the world (Davey, 2008). In Ethiopia alone, 13 million people (18% of the population) are at risk through exposure to irritant soil. An estimate based on prevalence data from an endemic area in southern Ethiopia suggested that between 500,000 and 1 million people are affected nationwide (Desta *et al.*, 2003). The worst part of the disease, besides health complication, is that it affects patients socio-economically. It has negative impact on economic activity in that it affects the productive age groups in the community. In most cases this disease occurs between the ages 16 and 45 years, the ages most responsible for agricultural and domestic productivity (Tekola *et al.*, 2006). It was found that about 80% of all patients and 86.7% of female patients were involved in economic and domestic activities, but worked less than those free of the condition. The time lost and the cost incurred through it is likely to recur day after day because of the chronic nature of the disease (Tekola *et al.*, 2006). Furthermore, social stigma against people with podoconiosis is a frequently observed situation. Patients are highly discriminated in the society and denied participation in local meetings, churches and mosques, and barred from marriage with unaffected individuals (Davey *et al.*, 2007; Yacob *et al.*, 2008).

### **1.2.1. Etiology, pathology and genetic predisposition**

The etiology, pathology and clinical features of podoconiosis have been well studied by Price (1990) where he showed that soil borne minerals are the primary causes of the disease. The minerals involved may include silica and alumino-silicate, and they are always found in the volcanic clays in areas where podoconiosis is endemic. These pathogenic soils are characterized by a high proportion of abrasive quartz crystals in the fine silts and of colloidal-size particles in clays. These soil particles are capable of penetrating through the skin of the bare foot. Micro-particles entering through the skin accumulate in different parts of body tissues. Accumulation of the particles is high in those segments of the lymph nodes, into which the lymphatics of the foot and the lower leg drain (Price, 1983). Soil particles causing the obstructive lymphopathy have been found in biopsy specimens from the dermis, lymphatic vessels and lymph glands (Price and Bailey, 1984).

The clinical state of patients with podoconiosis provides evidence that the condition is an obstructive lymphopathy. Epidemiological surveys indicate an association between the disease and the local soils. As demonstrated by Spooner and Davies (1986), the toxicity of the soil to cultures of macrophages depends on the concentration of soil particles entering the body. Changes are observed in the dermis, lymphatic channels and drainage lymph nodes, with long term deposition of silica and alumino-silicate thought to result in obstruction leading to lymphedema (Price, 1990).

Host genetic factors are important determinants of susceptibility to podoconiosis since not all individuals exposed to red clay soils develop podoconiosis (Davey *et al.*, 2007). The gene and environment interaction plays a role in development and progression of the disease. Price and Henderson (1978) had observed silicon and aluminium particles within lymph node macrophages of podoconiosis and non-podoconiosis individuals living on irritant soil. And their study suggested that there was abnormal reaction to mineral particles absorbed into the lymphatic system in the body of podoconiosis patients than in non-podoconiosis individuals. This implied the role of gene as predisposing factor for podoconiosis (Price and Henderson, 1978).



**Figure 2.** Soft 'water-bag' swelling in a 20-year-old man suffering from podoconiosis (Photo: Ewenet GebreHanna, with permission) (Davey *et al.*, 2007).

The early symptoms in podoconiosis are characterized by skin itching of the forefoot and a burning sensation in the lower leg, particularly the foot. Plantar edema, lymph fluid oozing from the area, increased skin markings and skin papillomas as well as rigid toes are early changes observed in early signs of podoconiosis. In the later stages of disease progression, the symptom of lower leg swelling may be noticed either as soft elephantoid (Figure 1) leg or hard and fibrotic type of swelling (Figure 2). The nature of progression of the disease from early to later clinical signs is very dormant and may take few to several years. Therefore, the victim suffers from the disease throughout life starting from the onset of the condition (Davey *et al.*, 2007; Price, 1990).



**Figure 3.** Hard and fibrotic type of swelling of the foot of a podoconiosis patient (Davey *et al.*, 2007).

### **1.2.2. Prevention and management of podoconiosis**

Prevention of contact with sticky red clay soils in the genetically vulnerable individuals will prevent the onset of the condition (Price, 1983). This can be achieved through use of footwear, education and raising public awareness of the disease within at risk communities and the improvement of farming techniques which protect the population from direct and prolonged skin contact with the offending soil.

Management of podoconiosis includes managing the early phase of the disease and the late (established) lymphoedematous presentation of the disease. Successful management depends on the understanding and co-operation of the patient and well informed community (Price, 1990). Early podoconiosis can be managed before persistent lymphedema develops (Price, 1983) and this involves educating individuals about the disease and its causes, encouraging shoe wearing at all times, especially when farming or in contact with the characteristic soil and prompt treatment of secondary infections with antibiotics. In podoconiosis endemic areas, sophisticated and expensive management techniques are unavailable and because of this the approach focuses on more practical, low-cost methodologies that are accessible in the resource poor settings of the majority of podoconiosis patients. These simple methods include cleansing, daily moistening of the limb, elevation of the limb to above hip height for prolonged periods to improve venous and lymphatic return and reduce limb size, compression or bandaging, manual lymphatic drainage by superficial massaging and use of appropriate or protective footwear (Fuller, 2005). Management of the established lymphedematous patients in podoconiosis shares many similarities with the

management of any lymphedematous diseases like diseases of lymphatic filariasis (Vagas and Ryan, 2003).

### **1.3. Podoconiosis and hookworm**

#### **1.3.1. Geographical overlap**

Both hookworm and podoconiosis have more or less similar geographical distribution in that they are highly prevalent in tropical and sub-tropical areas of developing world. One reason for the high prevalence of these diseases in these areas is that the majority of the people in the area are bare footed farmers (Price, 1990) who are actively working on the fertile soils of the area. Most of these farmers do not wear protective shoes throughout their life during farming. The fertile soils of tropical and sub-tropical areas conceal causative agents of both diseases. Silica and alumino-silicates, the etiologic agents of podoconiosis, are abundantly found in red clay soils of tropical and sub-tropical highlands (Abrahams, 2002). In the same areas where sanitary practices are poor, the soils are infested with eggs and larvae of hookworm and other helminths. Lack of safe water, latrine and appropriate waste disposal accounts for the highest infestation of eggs and larvae of helminths in the area (Ekpo *et al.*, 2008). The warm and humid climate of tropical and sub-tropical regions is another factor for persistence of parasites in the soil (Mengistu *et al.*, 2007). In any case, the bare footed farmers are the most exposed group of people in the tropical and sub-tropical areas. Therefore, podoconiosis and hookworm could have overlapping distribution (Abrahams, 2002).

### **1.3.2. Occupational overlap**

The distribution of podoconiosis and hookworm infections has relationship with occupation. These diseases are commonly observed among farm workers who work on bare foot. Soil is a source of infection for both diseases. Survival of hookworm larvae is favored in damp, sandy or friable environments (Abrahams, 2002). Inadequate footwear increases foot trauma, podoconiosis, hookworm and other parasitic infections that enter the bare foot. In order to prevent infections occurring due to skin-soil contact, the primary step is reducing soil contact behavior within risk groups. In relation to podoconiosis, soil particles absorbed through the feet cause the disease (Price and Henderson, 1979).

### **1.4. Anemia**

Anemia is defined as a condition where there is less than the normal hemoglobin (Hb) level in the body, which decreases oxygen-carrying capacity. World Health Organization (WHO) definitions for anemia differ by age, sex, and pregnancy status as follows: for children 6 months to 5 years of age anemia is defined as a Hb level < 11g/dL, children 5–11 years of age Hb < 11.5 g/dL, adult males Hb < 13 g/dL; non-pregnant females Hb < 12g/dL; pregnant females Hb < 11g/dL and Severe anemia is defined as Hb < 7.0 g/dL (WHO, 2001).

Anemia affects about 2 billion people worldwide and is a serious public health problem (Roland *et al.*, 2007). The highest prevalence of anemia occurs in the developing world. Anemia is related to reduced work capacity, reduced ability to execute activities of daily living, poor pregnancy outcomes and reduced cognitive function. Assessing the causes of anemia is complex,

especially where many different etiologic agents are at play simultaneously, as is the case in much of the developing world (Friedman *et al.*, 2005).

Anemia can be caused by different factors. Nutritional deficiencies are regarded as the most important cause of anemia in the world and a major potential contributor to adolescent anemia in sub-Saharan Africa (ACC/SCN, 1997; Leenstra *et al.*, 2004). Iron deficiency is the main source of nutrition related anemia. According to Krivienė and Ragelienė (2006), half of all reasons for anemia are iron deficiency.

Besides specific nutrient deficiencies, general infections and chronic diseases, as well as blood loss, can cause anemia. The risk of anemia increases when individuals are exposed to malaria and helminth infections. There are also many other rarer causes of anemia, the most common being genetic disorders such as thalassemia. Malaria, especially due to the protozoan *Plasmodium falciparum*, causes anemia by rupturing red blood cells and by suppressing the production of new red blood cells (Krivienė and Ragelienė, 2006). Malaria does not, however, cause iron deficiency, because much of the iron in hemoglobin released from the ruptured cells stays in the body. Helminths such as hookworms can cause blood loss and therefore iron loss. Adult hookworms attach themselves to the gut wall, where the mature larvae and adult worms ingest both the gut wall cells and blood. Hookworms change feeding sites every 4–6 hours and during feeding secrete an anticoagulant, resulting in secondary blood loss from the damaged gut wall after the worms have stopped feeding. The number of adult hookworms and the fecal egg count, which is an indirect estimate of the number of worms, are strongly correlated with the amount of blood lost which, if chronic, can result in iron deficiency anemia. The nematode

*Trichuris trichiura* can cause anemia when the worm burden is heavy. Heavy infections also cause inflammation and dysentery, which in turn can cause further blood loss (Koukounari, *et al.*, 2008).

Anemia adversely affects people worldwide and occurs when there is inadequate number of red blood cells or inadequate amount of hemoglobin for the body to function properly. Hemoglobin is a protein in red blood cells that carries oxygen to the brain, muscular system, immune system, and other parts of the body. Without adequate oxygen, the physical and mental capacities of individuals are reduced (MOST, 2004). Strong evidence links anemia to health and development problems. Anemia in pregnant women results in lower birth weight babies who have a higher risk of death. Iron deficiency with or without anemia reduces work productivity in adults and limits cognitive development in children, thus limiting their achievement in school and ultimately reducing investment benefits in education. In addition, iron-deficiency leads to impaired gastrointestinal functions. Iron deficiency anemia also has adverse effects on the immune system because it results in reduced resistance to infections (Stoltzfus, 2001).

In Ethiopia, there is a high prevalence of anemia. However, the magnitude and importance of iron deficiency anemia as a public health problem is still under investigation because in several developing countries the intake of iron from diet is more than adequate. For example, in parts of Ethiopia, the daily intake of iron is estimated to be between 180 and 500 mg/day which is 10–20 times the suggested daily requirement. This presumed high intake is attributed to consumption of a staple cereal, teff (*Eragrostis tef*) (90 mg of iron per 100 g of teff), and partly due to its contamination with iron-rich clay soil (Gebre-Medhin *et al.*, 1981 as cited in Umeta *et al.*,

2008). In spite of the high intake of iron in teff-consuming communities, there is high prevalence of anemia. Therefore, the cause of iron deficiency in Ethiopia may not be the inadequate dietary intake of iron i.e other etiologic factors like iron mal-absorption and iron loss might play a role in iron deficiency anemia. In such communities with an already high intake of iron, the conventional supplementation of iron might not be an effective method of intervention or might even be harmful as iron overload in the body damages organs such as liver and heart (Kohgo *et al.*, 2008). Therefore, all important risk factors have to be identified and their role in causing anemia evaluated (Adish *et al.*, 1998).

The aim of the present study is to investigate the co-existence of two chronically debilitating diseases, podoconiosis and helminth infections particularly hookworm, in a population of podoconiosis patients. No studies in Ethiopia have investigated the co- occurrence of intestinal helminth infection on podoconiosis patients. Podoconiosis and hookworm have two things in common: they enter the human body through the skin and they chronically affect patients. Low socioeconomic conditions, poor hygienic habits, lack of sanitation and lack of health education are related to the wider prevalence of hookworm and other intestinal helminthic infections (Khanum *et al.* 1999 in Muznebin *et al.*, 2007). The present study will also assess the impact of hookworm infection in increasing the prevalence and severity of anemia among podoconiosis patients in Wolaita Zone.

## **2. OBJECTIVES**

### **2.1. General objective**

The overall objective of this study is to determine the health impact of intestinal helminth infections, particularly hookworm, among podoconiosis patients in Wolaita, Southern Ethiopia.

### **2.2. Specific objectives**

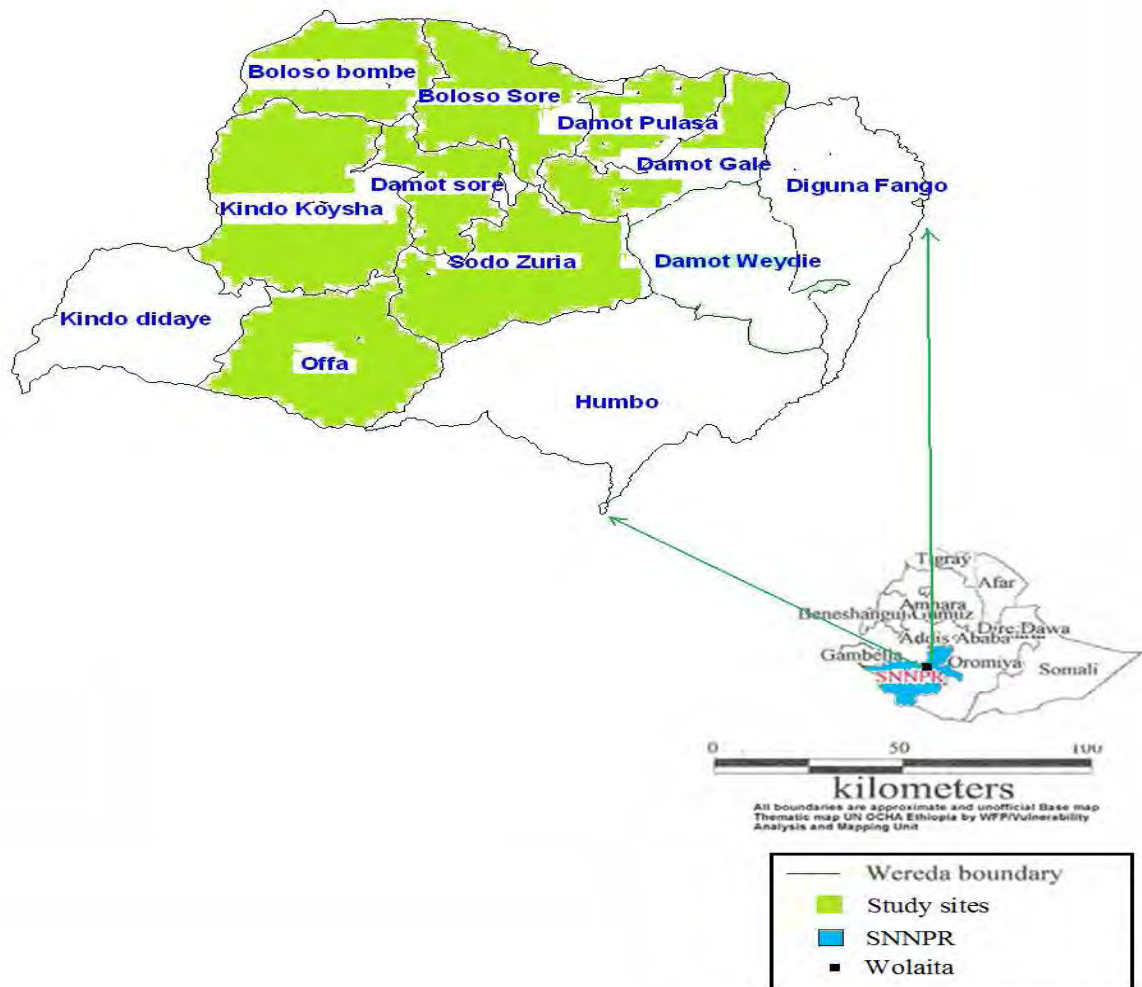
- To determine the prevalence of helminth infections, particularly hookworm among podoconiosis patients in selected localities in Wolaita.
- To determine the impact of intestinal helminth infections on hemoglobin level among podoconiosis patients.

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

#### **3.1. Study area**

The study was conducted in eight selected sites (out of 13 outreach sites supervised by Mossy Foot Treatment and Prevention Association/ MFTPA) in Wolaita Zone, Southern Ethiopia. The study sites were selected based on their accessibility for logistics. Wolaita is located about 380 kms south of Addis Ababa. The outreach sites, under MFTPA supervision, radiate from the Sodo town, the Zonal capital, at different distances. In Wolaita, the prevalence of podoconiosis is more than 5% (Desta *et al.*, 2003). The MFTPA works with the community at grass-roots level and many patients in the zone have become beneficiaries of the services provided. Clinical assessment in this endemic setting has proved the widespread nature of the non-filarial disease (Desta *et al.*, 2007). Control individuals were taken from one of the eight sites under study (Damot Gale *Woreda*). Preliminary observation made in some *woredas* of Wolaita Zone has pointed out that hookworm and other geo-helminth infections are prevalent in most of the clinics and health centers corresponding the outreach sites.

Agriculture is the main source of income in Wolaita, where the farming system is characterized by small scale production of mixed crops and livestock. The farmers of this area cannot afford to wear shoes when they work on farms. This exposes the farmers to podoconiosis and hookworm infection.



**Figure 4.** A map of Wolaita Zone, SNNPR (Finance and economic development bureau, 2009)

### **3.2. Study design**

The present study was a comparative cross-sectional study, comparing podoconiosis patients with non-podoconiosis individuals living in the same sub-administrative area. Both patients and non-patients were socio-culturally matching groups. Podoconiosis patients were registered according to their arrival at selected study sites and prepared for sample collection. Similar procedure was done for control individuals except that they were taken from one of the eight MFTPA sites under the study. Control individuals were healthy during data collection; they were screened for absence of podoconiosis by asking past history and conducting physical examination on their feet and legs.

### **3.3. Parasitological examination of stool**

All the study subjects were given relevant information at the beginning of data collection about the aim and significance of the study. In addition, an oral description and specific instruction for handling and avoidance of contamination of the stool specimen were given to all the subjects. Stool specimens were collected from every subject and immediately preserved in SAF solution (which is a mixture of sodium acetate (30gm), glacial acetic acid (40ml) and formalin (80ml) in water (1850ml) to prepare two liters of SAF solution) for transportation to the laboratory. Formol-ether concentration method was carried out in Biomedical Laboratory in Addis Ababa University, Biology Department. All stool specimens were examined microscopically for intestinal helminths in the laboratory and the findings were recorded using standard format. Double slides were prepared for each sample in order to increase the accuracy of diagnosis.

### **3.4. Blood film screening for malaria parasites**

Malarial infection was screened from thick and thin films of finger-prick blood fixed and stained with Giemsa. Thick films were considered negative if no parasite had been found in 100 high power fields (100 x objectives) (WHO, 1980). Thin films were used to identify plasmodium species.

### **3.5. Anemia determination**

Hemoglobin and hematocrit techniques were employed interchangeably depending on the availability of power source to determine anemia. Peripheral blood was collected by finger pricking by using a sterile lancet. The site for blood collection was cleaned with alcohol-soaked cotton and pricked with a blood lancet. One drop of blood was taken for hemoglobin (Hgb) measurement. Hemoglobin level was determined in finger prick blood by using a portable, battery-operated hemoglobinometer (HemoCue TM, Angelholm, Sweden) (Cohen and Seidi-Friedman, 1988). The first drop of blood was wiped away with dry cotton and the next drop was used to fill the cuvette by touching the cuvette tip in the middle of the drop of blood until completely filled. The filled cuvette was then put on the holder and pushed into the HemoCue instrument. The Hgb value displayed in g /dl after approximately 45 seconds was registered. For hematocrit technique, a free flow of blood from finger pricking was established to avoid dilution with tissue fluid. The first drop of blood was wiped away with dry cotton and the next drops were used to fill the heparinized blood micro capillary tubes. Every capillary tube was filled with blood from the red-banded end to about two-thirds capacity and the opposite end of the capillary tube was sealed with clay sealer. The blood in the capillary tubes was centrifuged for 5 minutes to separate RBCs from whole blood and the percentage (%) of red blood cells in a volume of

whole blood was registered for each subject after reading it with hematocrit reader. All hematocrit values were converted in to hemoglobin values by dividing the hematocrit value by three (i.e. hematocrit value is approximately three times the value of the hemoglobin) (Chernecky and Berger, 2008). Other anemia indicators in hematological indices like serum iron and ferritin level in the body were not done because of resource limitation.



**Figure 5.** Photographs showing: A/ sample collection in the field and B/ microscopic examination of preserved stool samples at biomedical research laboratory, Department of Biology, Addis Ababa University.

### 3.6. Sample size calculation

Sample size for this study was determined by using the following formula (Cochran, 1963 as cited by Kasiulevicius *et al*, 2006).

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

Where

$n$  = sample size,

$Z$  = Z statistic for a level of confidence,

$P$  = expected prevalence or proportion (0.5), and

$d$  = precision (0.05).

This calculation was conducted by using 95% confidence interval for Z statistics which is conventionally 1.96 and 5% precision. Since the prevalence of geo-helminth infection among podoconiosis patients was not well established in Wolaita, 50% prevalence was taken to determine the sample size. Therefore, according to the above formula the sample size ( $n$ ) was calculated to be 384. Taking  $\frac{1}{4}$  of test sample as control, the total sample size was determined to be 480

### 4. ETHICAL CONSIDERATION

Only volunteer individuals with informed consent were included in the study. The ethical consideration was addressed by treating positive individuals with albendazol. The study was reviewed and approved by ethical committee of Department of Biology, Addis Ababa University.

## **5. STATISTICAL ANALYSIS**

Statistical analysis was performed with SPSS software version 13. Chi-square was used to verify possible association between infection and exposure to different factors. Odds ratio was adjusted for influential variables and computed by using logistic regression to determine the magnitude of risk factors in causing anemia. Values were considered to be statistically significant for all tests when the obtained p-value was less than 0.05 i.e. 95% confidence level was used for all tests.

## 6. RESULTS

### 6.1. Overall prevalence of intestinal helminth infection

Three hundred eighty four podoconiosis patients (cases) and ninety six non-podoconiosis patients (controls) were involved in the study. All were screened for malaria and no one was positive. Podoconiosis patients aged 18-67 years with mean age of 38.5 years were included in the study from eight different MFTPA sites of Wolaita Zone. The male to female proportion was 43.5 % and 56.5%, respectively in patients and 58.3% and 41.7% in controls. The overall prevalence rate of helminth parasites was 57.8% among patients and 37.5% among non-patients. Of the study participants, 1 (0.3%) had quadruple infection, 14 (3.6%) triple infection, 49 (12.8%) double infection and 158 (41.1%) single infection among podoconiosis patients and 0 (0%) quadruple infection, 1 (1%) triple infection, 7 (7.3%) double infection and 28 (29.2%) single infection among controls (Table 3). Six different parasite species were identified among patients with the most dominant parasite being hookworm species 145 (37.8%) followed by *Ascaris lumbricoides* 79 (20.6%) and *Trichuris trichiura* 59 (15.4%). Among controls, four species were identified with hookworm the most frequent 27(28.1%) (Table 2). The result revealed that 28.8% of helminth positive podoconiosis patients harbor more than one parasite. Hookworm prevalence was significantly higher among podoconiosis patients of *woredas* with lowland areas 79.2% (42/53) than highland areas 60.9% (103/169) (Table 4). Shoe wearing behavior in relation to hookworm infection among podoconiosis patients was assessed (Table 8). 55.8% (24/43) of individuals who never wore shoe and the same proportion of those who wore shoes less than weekly were infected, but only 36.5% (46/126) of those wearing weekly but less than daily and 15.3% (17/111) of those wearing shoes daily were infected.

**Table 1.** The age and sex distribution of the study participants in Wolaita Zone, Southern Ethiopia (April and May, 2010).

Sex	Age group							
	Cases				Controls			
	18-35 n (%)	36-50 n (%)	>50 n (%)	Total n (%)	18-35 n (%)	36-50 n (%)	>50 n (%)	Total n (%)
Male	61 (15.9)	74 (19.3)	32 (8.3)	167 (43.5)	25 (26)	23 (24)	8 (8.3)	56 (58.3)
Female	92 (23.9)	116 (30.2)	9 (2.3)	217 (56.5)	20 (20.8)	14 (14.5)	6 (6.3)	40 (41.7)
Total	153 (39.8)	190 (49.5)	41 (10.7)	384 (100)	45 (46.9)	37 (38.5)	14 (14.6)	96 (100)

n represents number of study participants

**Table 2.** Prevalence of intestinal parasites among podoconiosis patients (cases) and non-podoconiosis individuals (controls) in Wolaita Zone, Southern Ethiopia (April and May, 2010).

Parasite species	Case n (%)	Control n (%)	$\chi^2$	P value
<b>Helminth</b>				
<i>Hookworm</i>	145 (37.8)	27 (28.1)	12.383	0.000**
<i>A. lumbricoides</i>	79 (20.6)	13 (13.5)	2.632	0.105
<i>T. trichiura</i>	59 (15.4)	4 (4.2)	8.446	0.004**
<i>Taenia Spp.</i>	8 (2.1)	1 (1.0)	0.453	0.501
<i>E. vermicularis</i>	9 (2.3)	0	2.293	0.130
<i>H. nana</i>	3 (0.8)	0	0.755	0.385
Total	222 (57.8)	36 (37.5)	12.747	0.000**
<b>Protozoan species</b>				
<i>E. histolytica/dispar</i>	8 (2.1)	1 (1.0)	0.453	0.501

n represents number of study participants

\*\* represents values significant at  $P < 0.05$ , 95% CL

**Table 3.** Prevalence of helminth polyparasitism among podoconiosis patients (cases) and non-podoconiosis individuals (controls) in Wolaita Zone, Southern Ethiopia (April and May, 2010).

Helminth Infections	Cases n (%)	Controls n (%)	X <sup>2</sup> (P value)
Single infections	158 (41.1)	28 (29.2)	4.643 (0.031)**
Double infections	49 (12.8)	7 (7.3)	40.510 (0.000)**
Triple infections and more	15(3.9)	1 (1)	7.654 (0.022)**

n represents number of study participants

\*\* represents values significant at P<0.05, 95% CL

**Table 4.** Prevalence of intestinal helminth infection among podoconiosis patients by highland and lowland *woredas* in Wolaita Zone, Southern Ethiopia (April and May, 2010).

Helminth parasite	Highland <sup>a</sup> n (%)	Lowland <sup>b</sup> n (%)	X <sup>2</sup> (P value)
Hookworm	103 (60.9)	42 (79.2)	4.384 (0.036)**
<i>A.lumbricoid</i>	66 (39.1)	13 (24.5)	2.524 (1.112)
<i>T. trichiura</i>	56 (33.1)	3 (5.7)	12.816 (0.000)**
<i>Taenia Spp.</i>	7 (4.1)	1 (1.9)	0.523 (0.470)
<i>E. vermicularis</i>	8 (4.7)	1 (1.9)	0.754 (0.385)
<i>H. nana</i>	3 (1.8)	0	0.912 (0.340)
Total	169	53	

n represents number of study participants

\*\* represents values significant at P<0.05, 95% CL

<sup>a</sup> *Woredas* with areas >1500m above sea level (Damot Sore (Gununo), Damot Gale (Boditi), Boloso Sore (Areka), Damot Pulasa (Shanto) and Sodo Zuria (Tome))

<sup>b</sup> *Woredas* with areas <1500m above sea level (Kindo Koisha (Bele), Ofa (Gesuba) and Boloso Bombe (Bombe))

## **6.2. Anemia prevalence and its relation to helminth infection**

The overall mean hemoglobin value of podoconiosis patients (case group) was 12.85g/dl (with range 8 to 17 g/dl) and that of control group was 14.71 g/dl (with range 10 to 17 g/dl). Anemia was present in 136 (35.4 %) of the cases and in 13 (13.5 %) of the controls. Among anemic podoconiosis patients, 105 (27.3%) were helminth-infected and 31 (8.1%) were helminth-free. Among anemic controls, 8 (8.3%) were helminth-infected and 5 (5.2%) were helminth-free. Podoconiosis patients were more anemic than non-podoconiosis controls and t-test comparison showed that their mean Hgb values were significantly different ( $P<0.05$ ) (Table 5). Mild and/or moderate anemia was observed among the study participants. Severe anemia (Hgb less than 7 g/dl) was observed neither in the cases nor in the control group. Individuals infected with hookworm or combination with hookworm had lower Hgb values than any other helminth infection or their combination (Table 9). Hookworm infected podoconiosis patients were at higher risk of being anemic when compared to podoconiosis patients who were not infected with hookworm (OR= 3.4,  $P=0.031$ , 95% CI=1.115, 10.388). Double infection with hookworm increased the risk of being anemic more than double infection without hookworm among podoconiosis patients (OR=3.9,  $P=0.032$ , 95% CI=1.124, 13.523). Helminth-positive podoconiosis patients were also at higher risk of being anemic as compared to helminth-free podoconiosis patients, though the association was not significant (OR=1.7, 95% CI=0.558,5.388) (Table 9). Hookworm infected podoconiosis patients were more likely to be anemic than hookworm infected non-podoconiosis individuals (OR=3.5,  $P=0.000$ , 95% CI= 1.833, 6.572) (Table 6).

**Table 5.** Comparison of mean Hgb level between podoconiosis patients (cases) and non-podoconiosis individuals (controls) without considering helminth infections in WolaitaZone, Southern Ethiopia (April and May, 2010).

Comparisons	Hgb(g/dl) Mean (SD)	T-test (P- value)
Average Hgb level		
Case (n=384)	12.85 (1.694)	9.614 (0.000)**
Control (n=96)	14.71 (1.698)	
Hgb level by sex		
Males		
Case (n=167)	13.09 (1.879) <sup>a</sup>	6.514 (0.000)**
Control (n=56)	14.90 (1.548) <sup>b</sup>	
Females		
Case (n=217)	12.66 (1.514) <sup>a</sup>	6.538 (0.000)**
Control (n=40)	14.44 (1.874) <sup>b</sup>	

n represents number of study participants

<sup>a</sup> represents mean Hgb values of male and female podoconiosis patients (t=2.464,p=0.014\*\*)

<sup>b</sup> represents mean Hgb values of male and female controls (t=1.338,p=0.184)

\*\* represents values significant at P<0.05, 95% CL

**Table 6.** Comparison of anemia prevalence between podoconiosis patients (cases) and non-Podoconiosis individuals (controls) in relation to helminth infections in Wolaita Zone, Southern Ethiopia (April and May, 2010).

Helminth infections	n (%)	Hgb(g/dl) mean(SD)	Anemic Vs Non-anemic	P - value
			OR(95% CI) adjusted	
Hookworm Cases Controls	106 (27.6) 20 (20.8)	12.37 (1.987) 13.76 (1.950)	3.471 (1.833,6.572) 1.00	0.000**
Double infection with hookworm Cases Controls	39 (10.2) 7 (7.3)	11.88 (1.771) 13.69 (1.442)	4.28 (1.832,9.849) 1.00	0.001**
Trichuris Cases Controls	33 (8.6) 2 (2.1)	12.56 (1.631) 13.80 (2.615)	0.982(0.436,2.213) 1.00	0.965
At least one helminth Cases Controls	222 (57.8) 36 (37.5)	12.53 (1.748) 14.55 (1.870)	1.946 (1.019,3.717) 1.00	0.044**

n represents number of study participants

\*\* represents values significant at P<0.05, 95% CL

OR: Odds ratio at 95% CL (adjusted for hookworm-positive, double infection with hookworm, trichuris-positive and at least one helminth infection)

**Table 7.** Comparison of helminth infections and anemia severity among podoconiosis patients in Wolaita Zone, Southern Ethiopia (April and May, 2010).

<b>Helminth infections</b>	<b>n (%)</b>	<b>No anemia n (%)</b>	<b>Mild anemia<sup>1</sup> n (%)</b>	<b>Moderate anemia<sup>2</sup> n (%)</b>	<b>X<sup>2</sup> (P-value) n (%)</b>
Hookworm	106 (100)	40 (37.7)	61 (57.5)	5 (4.7)	27.951 (0.000)**
Ascaris	51 (100)	33 (64.7)	18 (35.3)	0	0.684 (0.710)
Trichuris	33 (100)	17 (51.5)	15 (45.5)	1 (3.0)	0.214 (0.898)
Double infection with hookworm	39 (100)	12 (30.8)	22 (56.4)	5 (12.8)	13.636 (0.001)**
Double infection without hookworm	10 (100)	6 (60.0)	4 (40.0)	0	0.548 (0.760)

n represents number of study participants

\*\* represents values significant at P<0.05, 95% CL

<sup>1</sup> Hemoglobin level 10-11.9 g/dl non- pregnant women and 10-12.9 g/dl for adult men

<sup>2</sup> Hemoglobin level 7-9.9 g/dl

**Table 8.** Shoe wearing and hookworm infection between podoconiosis patients (cases) and non-podoconiosis individuals (controls) in Wolaita Zone, Southern Ethiopia (April and May, 2010).

Shoe wearing status	Hookworm Infection status n (%)					
	Cases			controls		
	Yes	No	X <sup>2</sup> (P value)	Yes	No	X <sup>2</sup> (P value)
Never wore	24 (55.8)	19 (44.2)	6.715 (0.010)**	2 (50)	2 (50)	0.988 (0.320)
Less than weekly	58 (55.8)	46 (44.2)	19.682 (0.000)**	7 (22.6)	24 (77.4)	0.696 (0.404)
Less than daily	46 (36.5)	80 (63.5)	6.125 (0.020)**	16 (30.2)	37 (69.8)	0.249 (0.618)
Every day wear	17 (15.3)	94 (84.7)	33.468 (0.000)**	2 (25)	6 (75)	0.042 (0.837)

n represents number of study participants

\*\* represents values significant at P<0.05, 95% CL

**Table 9.** Logistic regression analysis of helminth infection on hemoglobin values among podoconiosis patients in Wolaita Zone, Southern Ethiopia (April and May, 2010).

Helminth infections	n (%)	Hgb (g/dl) mean (SD)	Anemic Vs Non-anemic
			OR(95% CI) adjusted
Hookworm Yes No	106 (27.6) 278 (72.4)	12.37 (1.820) 13.03(1.609)	3.403 (1.115,10.388)** 1.00
Ascaris Yes No	51 (13.3) 333 (86.7)	13.16 (1.334) 12.80(1.739)	0.699 (0.213,2.293) 1.00
Trichuris Yes No	33 (8.6) 351 (91.4)	12.56 (1.631) 12.88 (1.693)	1.354 (0.356,5.157) 1.00
At least one helminth Free of any helminth	222 (57.8) 162 (42.2)	12.53 (1.748) 13.29 (1.516)	1.734 (0.558,5.388) 1.00
Double infection with hookworm without hookworm	39 (10.2) 10 (2.6)	11.88 (1.771) 12.88 (0.987)	3.898 (1.124,13.523)** 1.00

n represents number of study participants

\*\* represents values significant at P<0.05, 95% CL

OR: Odds ratio at 95% CL (adjusted for hookworm, ascaris, trichuris, at least one helminth infection, free of helminth infections double infection with hookworm and triple infection with hookworm)

### 6.3. Clinical stages of podoconiosis

The stage of podoconiosis disease was registered for each of the cases. It was done based on the staging system of Tekola *et al.* (2008). 51.6% (198/384) of podoconiosis patients were observed at stage 1. Only few cases were observed at late stages of the disease. 196 (51%) of these patients were suffered from podoconiosis for less than five years. The prevalence of hookworm infection was 56.6% (82/145) in stage 1, 30.3% (44/145) in stage 2, 13.1% (19/145) in stage 3 and above. The same trend of decrease was observed in the overall prevalence of helminth infections with increasing disease stages.

**Table 10.** Clinical staging of podoconiosis patients and prevalence of major intestinal helminth infections in Wolaita Zone, Southern Ethiopia (April and May, 2010).

Disease stages	Hookworm		<i>A.lumbricoides</i>		<i>T. trichiura</i>	
	n (%)	X <sup>2</sup> (P value)	n (%)	X <sup>2</sup> (P value)	n (%)	X <sup>2</sup> (P value)
Stage 1	82 (56.6)	82.219 (0.000) <sup>α**</sup>	44 (55.7)	45.979 (0.000) <sup>α**</sup>	28 (47.5)	31.686 (0.000) <sup>α**</sup>
Stage 2	44 (30.3)	8.953 (0.003) <sup>β**</sup>	25 (31.6)	5.301 (0.021) <sup>β**</sup>	22 (37.3)	6.315 (0.012) <sup>β**</sup>
Stage 3 and above	19 (13.1)		10 (12.7)		9 (15.3)	
Total	145 (100)		79 (100)		59 (100)	

n represents number of study participants

\*\* represents values significant at P<0.05, 95% CL

<sup>α</sup> P value of stage 1 and stage 2 comparison

<sup>β</sup> P value of stage 2 and stage 3 comparison

Disease stages refer to the clinical stage of podoconiosis (Tekola *et al.*, 2008).

Stage 1. Swelling reversible overnight.

Stage 2. Below-knee swelling that is not completely reversible overnight (bumps are below the ankle).

Stage 3. Below-knee swelling that is not completely reversible overnight (bumps above the ankle).

Stage 4. Above-knee swelling that is not completely reversible overnight.

Stage 5. Joint fixation; swelling at any place in the foot or leg.

**Table 11.** Clinical stages and duration of disease among podoconiosis patients in relation to hemoglobin level in Wolaita Zone, Southern Ethiopia (April and May, 2010).

Disease stages	Disease duration(in years)								
	<6			6-10			>10		
	n (%)	Hgb mean (g/dl)	P-value	n (%)	Hgb mean (g/dl)	P-value	n (%)	Hgb mean (g/dl)	P-value
Stage 1	107 (27.9)	12.67	0.187 <sup>α</sup>	59 (15.4)	13.06	0.890 <sup>α</sup>	32 (8.3)	12.90	0.162 <sup>α</sup>
Stage 2	68 (17.7)	13.00	0.154 <sup>β</sup>	37 (9.6)	13.01	0.047 <sup>**β</sup>	28 (7.3)	13.43	0.029 <sup>**β</sup>
Stage 3	21 (5.5)	12.44		9 (2.3)	11.69	0.666 <sup>γ</sup>	20 (5.2)	12.32	0.082 <sup>γ</sup>
>Stage 3	0	--		2 (0.5)	12.25		1 (0.3)	16.00	
Total	196 (51)			107 (27.7)			81 (20.8)		

n represents number of study participants

\*\* represents values significant at P<0.05, 95% CL

<sup>α</sup> P value of stage 1 and stage 2 comparison

<sup>β</sup> P value of stage 2 and stage 3 comparison

<sup>γ</sup> P value of stage 3 and > stage 3 comparison



**Figure 6.** Clinical manifestations of podoconiosis in Wolaita: Stage 1 (A), Stage 2 (B) and Stage 3 (C) (photograph taken in the field, April and May, 2010).

## 7. DISCUSSIONS

The high prevalence of helminthiasis in the present study is similar to the prevalence (55.7%) reported by Degarege *et al.* (2010) from the general population in Halaba Kulito, Southern Ethiopia. This might be an indication of similar epidemiological conditions in the two localities. On the other hand, the fact that helminth infection prevalence in the present study was lower than that reported (83.8%) by Legesse and Erko (2004) among school-children around Lake Langano is possibly due to the fact that children of school age are at a greater risk of helminth infections than the adult population (Hotez *et al.*, 2003), which makes up the present study participants. However, the present study showed higher helminth prevalence compared to the earlier (33.8%) report of Davey *et al.* (2005) from the general population in Butajira, Southern Ethiopia. This could be because, the burden of parasitic infections will be expected to be high in poor sanitary conditions such as in rural Wolaita environment compared to that of Butajira which consists of a relatively higher proportion of urban residents, who would have a better exposure to health education leading to a better hygienic living condition. In addition, it is a common knowledge that poverty plays a significant role in contributing to parasite prevalence (Hotez, 2008) in that people with low economic status, like podoconiosis patients (Tekola *et al.*, 2006), cannot afford even the basic hygienic practices. Thus, in an area like rural Wolaita where poverty and poor sanitation intersect with warm climate, more than one helminth parasite can co-infect an individual. Consistent with this, the present study determined 27.9% prevalence of polyparasitic helminth infections among the adult study participants. However, this is a much lower figure than that reported by Mengistu *et al.* (2007) from Jimma town, Southern Ethiopia, a wet tropical forest area, which is far more favorable to helminth parasites (Hotez *et al.*, 2003).

The finding that hookworm infections were more prevalent in the lowland areas in Wolaita is in agreement with the study of Jemaneh (1998) who showed a higher prevalence of hookworm infections in lowlands followed by temperate and highland areas of North and South Gondar. This can be explained by the fact that warm temperature (20-30 °C) and moisture in these areas will enhance embryonation of hookworm eggs in the soil (Komiya and Yasuraoka, 1966 cited in Hotez *et al.*, 2003). Thus the climate of Wolaita, which is mostly warm and wet (“woina-dega”) would favor persistence and development of hookworm and other helminth parasite ova and larvae in the soil.

Compared to a non-podoconiosis study conducted by Jemaneh and Lengeler (2001), which reported the prevalence of hookworm infection to be 16.3% in Gondar region, Northwest Ethiopia, hookworm infection in podoconiosis patients (37.8%) and non-podoconiosis study participants (28.1%) was significantly high in the present study. This may be accounted for by the fact that soil contact is invariably associated with podoconiosis, a situation that would guarantee exposure to geo-helminth infections, particularly hookworms. The study of Teklehaymanot (2009) which reported very low (3.5 %) prevalence of hookworm infection among Kara and Kwego semi-pastoralist tribes in lower Omo Valley, Southwestern Ethiopia, suggested the role of unfavorable arid climatic condition of the area that would affect the development of hookworm larvae in the soil and limited soil contact of the semi-pastoralists for the low hookworm prevalence. The overall prevalence of hookworm in non-podoconiosis study participants in this study is similar to the findings of Erosie *et al.* (2002) (26.7%) and Aragie (2006) (25.6%) from studies done in Boloso Sore *woreda* (Wolaita). This indicates that the

transmission dynamics of hookworm infection in Wolaita has remained the same over the past 4-8 years implying that no significant hookworm control intervention was taken in the area.

The finding that some (15.3%) podoconiosis patients, who wore shoes every day, were infected with hookworm possibly have resulted from an inappropriate wearing of shoes and by exposure of other body parts since hookworm larvae are able to penetrate all skin surfaces in contact, including the hands, and also *A. duodenale* can infect through the oral route (Bethony *et al.*, 2002; Hotez, 2008). The situation of podoconiosis patients who never wore shoes and were negative for hookworm infection may be explained by the exposure factor as helminth burdens are localized to specific 'niches' in the community or the fact that some individuals are differently predisposed to hookworm infection owing to genetic factors (Schad and Anderson, 1985).

Eventhough this study showed a significantly higher proportion of anemic individuals among helminth-infected podoconiosis patients, there also were anemic helminth-free counterparts. This is because, podoconiosis is a chronic inflammatory disease (Davey *et al.*, 2007) which can cause anemia on its own. This finding is supported by the results observed in the helminth-free controls, in whom the prevalence of anemia was lower than that in helminth-free podoconiosis patients. This shows that the causes of anemia are complex and multifactorial and other health factors such as nutritional deficiencies can play an important role (Antelman *et al.*, 2000; Tolentino and Friedman, 2007). Furthermore, body iron reserve and intensity of parasite infections are other attributes which determine the status of anemia in an individual (Tolentino and Friedman, 2007).

The present study has demonstrated the association of hookworm infection with anemia and reduced hemoglobin levels in podoconiosis patients as hookworm-positive podoconiosis patients were more anemic and had significantly lower mean hemoglobin values than hookworm-negative counterparts. This is due to the fact that hookworm results in intestinal blood loss and reduces the level of red blood cells and hemoglobin in the body (Geiger *et al.*, 2004; Nguyen *et al.*, 2006). In agreement with the reports of Belachew *et al.* (2006) and Brooker *et al.* (2008), the relationship between hookworm infection and reduced hemoglobin values in the study subjects has shown a significant association. However, the report of Erosie *et al.* (2002) showing the absence of significant association between hemoglobin levels and hookworm infection in school children may have to be taken with caution since hemoglobin levels in the body can be influenced by age, nutritional status, body iron requirement, body iron store, worm burden and the type of hookworm species (Pawlowski *et al.*, 1991).

Although the two species of human hookworm parasites were not differentiated in the present study, *N. americanus* would be expected to be the predominant species in Wolaita as can be deduced from the work of Tedla and Jemaneh (1985), which reported the prevalence and species distribution of hookworm in Ethiopia. Therefore, the absence of severe anemia in all hookworm infected study participants may suggest the infection to have been due to *N. americanus*, a less virulent hookworm species and causes mild or moderate anemia (Stoltzfus *et al.*, 1997).

The present study showed more reduced mean hemoglobin values in the female than in male podoconiosis patients ( $P < 0.05$ ). The lower mean hemoglobin values among female podoconiosis patients may be attributed to the fact that the women who participated in the study were of child

bearing age (menstruating) and would be expected to have higher demands for iron (Viteri, 1997; Brentlinger *et al.*, 2003; Umeta *et al.*, 2008).

Observations concerning the clinical staging of podoconiosis showed that most (51.6%) of the podoconiosis patients involved in the study were at the first stage of disease development and only limited cases were seen at late stages. The reason for this could be either most of the podoconiosis patients at late stages of clinical symptoms were treated by MFTPA (Davey and Burrige, 2009) or they could not come to the study sites because of the severity of the disease condition they were suffering from. It has also been reported that podoconiosis patients hide themselves from the community because of social stigma (Yakob *et al.*, 2008; Geshere, 2009).

Assessment of prevalence of geo-helminth infections in relation to the stages of podoconiosis showed that higher helminth prevalence was observed in patients at early disease stages than at the late stages. This may be explained by the observation that when podoconiosis progresses to the late stages, patients reduce farm work activities or the intensity of contact with the soil and hence are also less exposed to the geo-helminth infections.

Further assessment of hemoglobin level in relation to the disease stage and disease duration (how long a person had the clinical features of podoconiosis) among podoconiosis patients revealed that hemoglobin level decreases were better associated with increasing clinical stage of podoconiosis than its duration, though the associations were not consistent and significant. The absence of any clear association between body hemoglobin level and increasing disease duration was evident from the finding that some podoconiosis patients went into the severe form of the disease within a few years while some others stayed at earlier disease stages even after having

the disease for many years. This might partly be a reflection of variability in the genetic predisposition of the individuals. Such variability has been suggested as an important determinant of pathogenesis and pathology in podoconiosis (Davey *et al.*, 2007). In addition, it is possible that the measures taken by the patients to control disease progression or to treat podoconiosis through either modern or traditional means might have a role in influencing the progression of clinical picture of podoconiosis.

The reported prevalence of intestinal helminth infections in the present work was an indication of poor sanitary practices in the rural Wolaita. Moreover, podoconiosis patients were high risk groups to geo-helminth infections amongst the adult population in the study area due to the reason that most of them were uneducated and lived under poverty (Tekola *et al.*, 2006). Shoe wearing was so uncommon during farm work, mainly due to its unaffordability, that farmers were invariably exposed to podoconiosis and hookworm infections. Infections due to hookworm were clinically manifested by anemia in the observation that hookworm-infected podoconiosis patients were more anemic than non-infected counterparts, though anemia has multiple etiologies (Tolentino and Friedman, 2007).

## 8. CONCLUSIONS

- There was a significantly higher prevalence of intestinal helminth infection among podoconiosis patients than among non-podoconiosis individuals.
- Many study participants were either not using footwear appropriately or had never worn shoes, leaving them exposed not only to non-filarial elephantiasis but also to hookworm infections.
- A higher prevalence of geo-helminth infection was observed at earlier stages of clinical disease in podoconiosis patients implying the fact that podoconiosis patients during earlier disease stages have higher soil contact as they are capable of performing farming activities.
- Although three helminths (hookworm, *Ascaris* and *Trichuris*) were highly prevalent in the population studied, only hookworm was associated with anemia.

## **9. RECOMMENDATIONS**

The following recommendations are made based on the study findings:

- Since the study has provided a strong evidence for the use of footwear as protection both from hookworm infection and podoconiosis, efforts must be made to provide shoes to the population that work on loamy and clay soils in Wolaita.
- To improve the wellbeing of podoconiosis patients, priority must be given for deworming and improvement of hygienic conditions in the community.
- Supplementation of food with iron rich dietary items should be included in podoconiosis patient care to reduce anemia.
- Further investigation must be done on podoconiosis patients to determine other factors (eg. nutrition, malaria infection, etc) that might exacerbate anemia.

## 10. REFERENCES

- Abrahams, P.W. (2002). Soils: their implications to human health. *Sci. Tota. Environ.* **291**: 1–32.
- ACC/SCN (1997). Third Report on the World Nutrition Situation. Geneva.
- Adish, A.A., Esrey, S.A., Gyorkos, T.W. and Johns, T. (1998). Risk factors for iron deficiency anemia in preschool children in northern Ethiopia. *Public Health. Nutr.* **2**: 243–252.
- Antelman, G., Msamanga, G. I., Spiegelman, D., Urassa, E. J. N., Narh, R., Hunter, D. J. and Fawzi, W.W. (2000). Nutritional factors and infectious disease contribute to anemia among pregnant women with human immunodeficiency virus in Tanzania. *J. Nutr.* **130**: 1950–1957.
- Aragie, S. (2006). Intestinal helminths and anemia in a malaria endemic area, Areka, Wolyta Zone. *MSc thesis Addis Ababa University*, P. 28.
- Belachew, T. and Yosef, L. (2006). Risk factors for anemia among pregnant women attending antenatal clinic at Jimma University Hospital. *Ethiop. Med. J.* **44**: 3.
- Bethony, J., Chen, J. and Lin, S. (2002). Emerging patterns of hookworm infection: Influence of aging on the intensity of *Necator* infection in Hainan Province, People’s Republic of China. *Clin. Infec. Dis.* **35**: 1336–1344.
- Bethony, J., Brooker, S., Albonico, M., Geiger, S.M., Loukas, A., Diemert, D. and Hotez, P.J. (2006). Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *Lancet*, **367**:1521–1532.
- Brentlinger, P.E., Capps, L. and Denson, M. (2003). Hookworm infection and anemia in adult women in rural Chiapas, Mexico. *Salud Publica Mex.* **45**: 117-119.

- Brooker, S., Bethony, J. and Hotez, P.J. (2004). Human hookworm infection in the 21<sup>st</sup> century. *Adv. Parasitol.* **58**: 197–288.
- Brooker, S., Hotez, P.J. and Bundy, D.A.P. (2008). Hookworm-related anemia among pregnant women: A systematic review. *PLoS Negl. Trop. Dis.* **2**: e291.
- Bundy, D.A. (1995). Epidemiology and transmission of intestinal helminths. **In**: Enteric infection 2: intestinal helminths. Farthing, M.J.G., Keusch, G.T. and Wakelin, D., eds. New York: Chapman & Hall Medical, 5-24.
- CDC (2008). "*Lymphatic filariasis*". *Centers for disease control and prevention*.
- Chernecky, C.C. and Berger, B.J. (2008). Laboratory tests and diagnostic procedures, 5<sup>th</sup> ed. St. Louis: Saunders. p.410
- Cohen, A.R. and Seidi-Friedman, J. (1988). HemoCue system for hemoglobin measurement: evaluation in anemic and non-anemic children. *Am. J. clin. pathol.* **90**:302-305.
- Cooper, E.S. and Bundy, D.A.P. (1989). *Trichuris* is not trivial. *Parasitol. Today* **4**: 301–306.
- Davey, G., Venn, A., Belete, H., Berhane, Y. and Britton, J. (2005). Wheeze allergic sensitization and geohelminth in Butajira, Ethiopia. *Clin. Exp. Allergy*, **35**:301-307.
- Davey, G., GebreHanna, E., Adeyemo, A., Rotimi, C., Newport, M. and Destad, K. (2007). Podoconiosis: A tropical model for gene-environment interactions? *Trans. R. Soc. Trop. Med. Hyg.* **101**: 91–96.
- Davey, G., Tekola, F. and Newport, M.J. (2007). "Podoconiosis: non-infectious geochemical elephantiasis". *Trans. R. Soc. Trop. Med. Hyg.* **101**: 1175–1180.
- Davey, G. (2008). Podoconiosis: let Ethiopia lead the way. *Ethiop. J. Health. Dev.* **22**:1-2.

- Davey, G. and Burridge, E. (2009). Community-based control of a neglected tropical disease: the mossy foot treatment and prevention association. *PLoS Negl. Trop. Dis.* **3**: e424.
- De Silva, N.R., Brooker, S., Hotez, P.J., Montresor, A., Engels, D. and Savioli, L. (2003). Soil-transmitted helminth infections: updating the global picture. *Trends Parasitol.***19**: 547-551
- Degarege, A., Animut, A., Legesse, M. and Erko, B. (2010). Malaria and helminth co-infections in outpatients of Alaba Kulito health center, Southern Ethiopia: a cross-sectional study. *BMC Research Notes*, **3**:143-149.
- Desta, K., Ashine, M. and Davey, G. (2003). "Prevalence of podoconiosis (endemic non-filarial elephantiasis) in Wolaita, Southern Ethiopia". *Trop. Doct.* **32**: 217- 220.
- Ekpo, U.F., Odoemene, S.N., Mafiana, C.F. and Sam-Wobo, S.O. (2008). Helminthiasis and hygiene conditions of schools in Ikenne, Ogun state, Nigeria. *PLoS Negl. Trop. Dis.* **2**: e146.
- Erosie, L., Merid, Y., Ashiko, A., Ayine, M., Baliyu, A., Muzeyin, S., Teklemariam, S. and Sorsa, S. (2002). Prevalence of hookworm infection and hemoglobin status among rural elementary school children in Southern Ethiopia. *Ethiop. J. Health. Dev.* **16**:113-115.
- Finance and economic development bureau, (2009). Map of Wolaita Zone, SNNPR.
- Friedman, J. F., Kanzaria, H.K. and Mc Garvey, S.T. (2005). Human schistosomiasis and anemia: the relationship and potential mechanisms. *Trends Parasitol.* **21**: 386-392.
- Fuller, L. C. (2005). Podoconiosis: endemic non-filarial elephantiasis. *Curr. Opin. Infect. Dis.* **18**:119–122.

- Fyfe, N.C.M. and Price, E. W. (1985). The effects of silica on lymph nodes and vessels: a possible mechanism in the pathogenesis of non-filarial endemic elephantiasis. *Trans. R. Soc. Trop. Med. Hyg.* **79**: 645- 651.
- Geiger, S.M., Massara, C.L., Bethony, J., Soboslay, P.T. and Correa-Oliveira, R. (2004). Cellular responses and cytokine production in post-treatment hookworm patients from an endemic area in Brazil. *Clin. Exp. Immunol.* **136**: 334–340.
- Geshere, G. (2009). Identification of etiology of elephantiasis in Midakegni district, Western Shoa Zone. *MSc thesis Addis Ababa University*, P. 37.
- Horton, J. (2003). Human gastrointestinal helminth infections: are they now neglected diseases? *Trends Parasitol.* **19**: 527-531.
- Hotez, P.J. and Pritchard, D.I. (1995). Hookworm infection. *Sci. Am.* **272**: 68-74.
- Hotez, P. J., de Silva, N., Brooker, S. and Bethony, J. (2003). Soil transmitted helminth infections: The nature, causes and burden of the condition. *Maryland Nation. Inst. Health.* **3**: 1-79.
- Hotez, P.J., Brooker, S., Bethony, J., Bottazzi, M.E. and Loukas, A. (2004). Current concepts: Hookworm infection. *N. Engl. J. Med.* **351**: 799–807.
- Hotez, P.J., Bethony, J., Bottazzi, M.E., Brooker, S. and Buss, P. (2005). Hookworm: “The great infection of mankind.” *PLoS Med.* **2**: e67.
- Hotez, P. (2008). Hookworm and Poverty. *Ann. N.Y. Acad. Sci.* **1136**: 38–44.
- Hotez, P. J., Brindley, P. J., Bethony, J.M., King, C. H., Pearce, E. J., and Jacobson, J. (2008). Helminth infections: the great neglected tropical diseases. *J. Clin. Invest.* **118**:1311–1321.

- Jemaneh, L. (1998). Comparative prevalences of some common intestinal helminth infections in different altitudinal regions in Ethiopia. *Ethiop. Med. J.* **36**:1-8.
- Jemaneh, L. and Lengeler, C. (2001). The use of morbidity questionnaires to identify communities with high prevalence of geohelminth infections in Gondar region, Ethiopia. *Ethiop. Med. J.* **39**:213-28.
- Kloos, H., Lemma, A., Kibru, B., Gebre, A., Mazengia, B., Feleke, G. and DeSole, G. (1980). Intestinal parasitism in migrant farm labour populations in irrigation schemes in the Awash valley, Ethiopian, and in major labour source areas. *Ethiop. Med. J.* **18**:52-61.
- Kohgo, Y., Ikuta, K., Ohtake, T., Torimoto, Y. and Kato, J. (2008). Body iron metabolism and pathophysiology of iron overload. *Int. J. Hematol.* **88**:7–15.
- Koski, K. G. and Scott, M. E. (2003). Gastrointestinal nematodes, trace elements, and immunity. *J. Trace Elem. Exp. Med.* **16**: 237–251.
- Koukounari, A., Estambale, B.B.A., Njagi, J. K., Cundill, B., Ajanga, A., Crudder, C., Otido, J., Jukes, M. C.H., Clarke, S. E. and Brooker, S. (2008). Relationships between anemia and parasitic infections in Kenyan schoolchildren: a Bayesian hierarchical modeling approach. *Int. J. Parasitol.* **38**:1663- 1671.
- Krivienė, I. and Ragelienė, L. (2006). The prevalence of anemia among schoolchildren in Šiauliai region of Lithuania. *Acta Med. Lituan.* **13**: 56–59.
- Kumie, A. and Ali, A. (2005). An overview of environmental health status in Ethiopia with particular emphasis to its organization, drinking water and sanitation: a literature survey. *Ethiop. J. Health. Dev.* **19**: 89-103.

- Leenstra, T., Kariuki, S.K., Kurtis, J.D., Oloo, A.J., Kager, P.A. and Kuile, F.O. (2004). Prevalence and severity of anemia and iron deficiency: cross-sectional studies in adolescent schoolgirls in western Kenya. *Eur. J. Clin. Nutr.* **58**: 681–691.
- Legesse, M. and Erko, B. (2004). Prevalence of intestinal parasites among schoolchildren in a rural area close to the southeast of Lake Langano, Ethiopia. *Ethiop.J.Health. Dev.* **18**:16-120.
- Leykun, J. and Tedla, S. (1984). The distribution of *Nectar americanus* and *Ancylostoma duodenale* in school populations, Gojam and Gonder administrative regions. *Ethiop. Med. J.* **22**:87-91.
- Loukas, A. and Prociv, P. (2001). Immune responses in hookworm infections. *Clin. Microbiol. Rev.* **14**: 689-703.
- Mengistu, A., Gebre-Selassie, S. and Kassa, T. (2007). Prevalence of intestinal parasitic infections among urban dwellers in southwest Ethiopia. *Ethiop.J.Health. Dev.* **21**:12-17.
- MOST, USAID Micronutrient Program (2004). *A Strategic Approach to Anemia Control Programs*. Arlington, Virginia, USA.
- Mupfasoni, D., Karibushi, B., Koukounari, A., Ruberanziza, E. and Kaberuka, T. (2009). Polyparasite helminth Infections and their association to anaemia and undernutrition in Northern Rwanda. *PLoS Negl. Trop. Dis.* **3**: e517.
- Nguyen, P.H., Nguyen, K.C., Le, M.B., Nguyen, T.V., Ha, K.H., Bern, C., Flores, R. and Martorell, R. (2006). Risk factors for anemia in Vietnam. *Southeast Asian J. Trop. Med. Public Health.* **37**: 1213-1223.

- Olsen, A., Magnussen, P., Ouma, J.H., Andreassen, J. and Friis, H. (1998). The contribution of hookworm and other parasitic infections to hemoglobin and iron status among children and adults in western Kenya. *Trans. R. Soc. Trop. Med. Hyg.* **92**: 643- 649.
- Pawlowski, ZS., Schad, GA. and Stott, GJ. (1991). Hookworm infection and anemia – approaches to prevention and control. *WHO, Geneva*.
- Petney, T.N. and Andrews, R.H. (1998). Multiparasite communities in animals and humans: frequency, structure and pathogenic significance. *Int. J. Parasitol.* **28**:377-93.
- Price, E.W. (1976). The association of endemic elephantiasis of the lower legs in East Africa with soil derived from volcanic rocks. *Trans. R. Soc. Trop. Med. Hyg.* **70**: 288- 295.
- Price, E.W. and Henderson, W.J. (1978). The elemental content of lymphatic tissues of barefooted people in Ethiopia with reference to endemic elephantiasis of the lower legs. *Trans. R. Soc. Trop. Med. Hyg.* **72**: 132-6.
- Price, E.W. (1983). Endemic elephantiasis: early signs and symptoms and control. *Ethiop. Med. J.* **21**:243- 253.
- Price, E.W. and Bailey, D. (1984). Environmental factors in the etiology of endemic elephantiasis of the lower legs in tropical Africa. *Trop. Geogr. Med.* **36**: 1- 5.
- Price, E.W. (1990). Podoconiosis. Non-filarial elephantiasis. Oxford University Press, Oxford.
- Roland, K., Gernard, I. M., Ferdinand, M., Paul, P., David, J. H. and Wafaie, W. F. (2007). Iron status is an important cause of anemia in HIV-Infected Tanzanian women but is not related to accelerated HIV disease progression. *J. Nutr.* **137**: 2317–2323.
- Schad, G.A. and Anderson, R.M. (1985). Predisposition to hookworm infection in humans. *Science*, **228**:1537–40.

- Spooner, N.T. and Davies, J.E. (1986). Possible role of soil particles in the etiology of non-filarial (endemic) elephantiasis: a macrophage cytotoxicity assay. *Trans. Roy. Soc. Trop. Med. Hyg.* **80**: 222- 225.
- Stanssens, P., Bergum, P.W. and Gansemans, Y. (1996). Anticoagulant repertoire of the hookworm *Ancylostoma caninum*. *Proc. Natl. Acad. Sci. U S A.* **93**: 2149-2154.
- Stoltzfus, R. J., Dreyfuss, M. L., Chwaya, H. M. and Albonico, M. (1997). Hookworm control as a strategy to prevent iron deficiency. *Nutr. Rev.* **55**: 223-232.
- Stoltzfus, R.J. (2001). Iron-deficiency anemia: reexamining the nature and magnitude of the public health problem. Summary: implications for research and programs. *J. Nutr.* **131**: 697–701.
- Tedla, S. and Jemaneh, L. (1985). Distribution of *Ancylostoma duodenale* and *Nectar americanus* in Ethiopia. *Ethiop. Med. J.* **23**:149-158.
- Teklehaymanot, T. (2009). Intestinal parasitosis among Kara and Kwego semipastoralist tribes in lower Omo Valley, Southwestern Ethiopia. *Ethiop.J.Health. Dev.* **23**:57-62.
- Tekola, F., Damen, H.M. and Davey, G. (2006). Economic costs of endemic non-filarial elephantiasis in Wolaita Zone, Ethiopia. *Trop. Med. Int. Health.* **11**: 1136–1144.
- Tekola, F., Ayele, Z., Haile Mariam, D., Fuller, C. and Davey, G. (2008). Development and testing of a de novo clinical staging system for podoconiosis (endemic non-filarial elephantiasis). *Trop. Med. Int. Health.* **13**:1277–1283.
- Tesfa-Yohannes, T.M. and Kloos, H. (1988). Intestinal parasitism. **In**: The ecology of health and disease in Ethiopia (Zein, A.Z and Kloos, H. eds). Ministry of Health, Addis Ababa. p214-230.

- Tolentino, K. and Friedman, J. F. (2007). An update on anemia in less developed countries. *Am. J. Trop. Med. Hyg.* **77**: 44–51.
- Umeta, M., Haidar, J., Demissie, T., Akalu, G. and Ayana, G. (2008). Iron deficiency anemia among women of reproductive age in nine administrative regions of Ethiopia. *Ethiop. J. Health. Dev.* **22**: 252-258.
- Vagas, B. and Ryan, T. (2003). Lymphoedema: pathophysiology and management in resource-poor settings: relevance for lymphatic filariasis control programmes. *Filaria J.* **2**: 4.
- Viteri, FE. (1997). Effective iron supplementation does not happen in isolation. *Am. J. Clin. Nutr.* **65**:889-890.
- WHO (2001). Iron deficiency anemia: assessment, prevention and control. Geneva.
- WHO/UNICEF (2006). Meeting the MDG drinking water and sanitation target : the urban and rural challenge of the decade. Switzerland, Geneva 27.
- WHO (2007). *Report of the global partners meeting on neglected tropical diseases*, Geneva 1.
- Yakob, B., Deribe, K. and Davey, G. (2008). High levels of misconceptions and stigma in a community highly endemic for podoconiosis in southern Ethiopia. *Trans. R. Soc. Trop. Med. Hyg.* **102**: 439-444.

## 11. APPENDIXES

### I. Consent form

I am conducting a study on THE HEALTH IMPACT OF INTESTINAL HELMINTH INFECTIONS AMONG PODOCONIOSIS PATIENTS IN WOLAITA ZONE, SOUTHERN ETHIOPIA. I would like to obtain stool sample and a drop of blood by finger pricking to look for the presence of any intestinal helminths and malaria parasite, respectively. In addition, I will also take a drop of blood by pricking a finger using sterile lancet to look on the level of hemoglobin for checking anemia. There is no any health related risk in participating in this study. When you are found to be positive for intestinal helminthiasis and malaria, you will receive standard drugs free of charge. The information in your records is strictly confidential.

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

I am informed to my satisfaction the purpose of this study and its nature of laboratory investigation. I am also aware of my right to opt out of the study at any time during the course of the study without having to give reasons for doing so. This consent form has been readout to me in my own language, and I understood the content and I am voluntarily agreed to participate in the study.

Study Code No \_\_\_\_\_ Study area \_\_\_\_\_

Name \_\_\_\_\_ Signature \_\_\_\_\_ Date \_\_\_\_\_

Wittiness

Name \_\_\_\_\_ Signature \_\_\_\_\_ Date \_\_\_\_\_

Investigator

Name \_\_\_\_\_ Signature \_\_\_\_\_ Date \_\_\_\_\_

**II. Checklist of information on the study participants in Wolaita Zone, Southern Ethiopia, 2010.**

1. Code No. \_\_\_\_\_
2. Full name of respondent \_\_\_\_\_
3. Age \_\_\_\_\_
4. Sex: Male  Female
5. Residence: *Woreda* \_\_\_\_\_ Kebele \_\_\_\_\_
6. Study/clinic site \_\_\_\_\_
7. Work/Occupation: Farmer  Non-farmer  /specify/ \_\_\_\_\_
8. Educational status: Illiterate  Literate  /specify grade attained/ \_\_\_\_\_
9. Use of latrine: Do you have latrine? Yes  No
10. Drinking water source: Tap water  Non-tap water  /specify/ \_\_\_\_\_
11. If you use non-tap water source, do you undertake any water purification/cleaning means? Yes  /specify/ \_\_\_\_\_ No
12. Shoe wearing behavior: Never wear shoe  Less than weekly   
Less than daily  Every day
13. Foot/leg condition
  - Swollen (elephantoid)
    - One leg  Two legs
  - Not swollen
14. Stage of the disease/Podoconiosis
  - Stage 1  Stage 2  Stage 3  Stage 4  Stage 5
15. How long have you had this condition since the onset of foot/ leg swelling (in years)?  
\_\_\_\_\_ (in months)? \_\_\_\_\_
16. Did you receive treatment for podoconiosis? Yes  No   
If yes,
  - a. Traditional treatment? /specify/ \_\_\_\_\_
  - b. Modern treatment? /specify/ \_\_\_\_\_

17. Have you ever gone to clinics/Health care services for treatment (for diseases other than podocniosis)? Yes  No

If yes,

- a. For which disease? ( e.g. Malaria) \_\_\_\_\_
- b. How many times within one year? \_\_\_\_\_

**For Researcher Use Only**

**Result of Lab. Examination:**

1. Stool test and parasites detected:

- a. Consistency (formed, soft, loose, watery) \_\_\_\_\_
- b. Formol-Ether concentration: \_\_\_\_\_

2. Blood test

- a. Thick smear \_\_\_\_\_
- b. Thin smear \_\_\_\_\_
- c. Hemoglobin level (in g/dl) \_\_\_\_\_

### **III. Declaration**

I, the undersigned, declare that this thesis is my own original work and has not been presented for a degree in any University and that all sources of materials used for this thesis have been correctly acknowledged.

**Name of the candidate:** Bereket Alemayehu

**Signature** \_\_\_\_\_

**Date** \_\_\_\_\_

This thesis has been done under my supervision

**Name of the advisor:** Beyene Petros (Professor)

**Signature** \_\_\_\_\_

**Date** \_\_\_\_\_