# Malaria-Schistosomiasis mansoni Co-morbidities and the Possible Reciprocal Effects on the Parasitological, Clinical and Immunological Outcomes in Finchaa Sugar Estate, Western Ethiopia

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## ABSTRACT

In Ethiopia where malaria parasite and *Schistosoma mansoni* infections are coendemic, the general population is quite vulnerable both to malaria and *Schistosoma mansoni* infections singly and concomitantly. However, data about the prevalence of malaria-*Schistosoma mansoni* co-infection and their reciprocal parasitological, clinical and immunological effects are lacking.The aim of this study was to assess the reciprocal effects of malaria- *Schistosoma mansoni* co-infections with emphasis on parasitological, clinical and immunological interactions.A community based cross sectional study was conducted in Finchaa Sugar Estate, western Ethiopia. Blood samples were collected by finger pricking and thick and thin smears stained with Giemsa. Fresh stool samples were collected and processed by the Kato-Katz method. Haemoglobin level was determined using a portable spectrophotometer. Plasma was collected for cytokine assay and measurements of selected cytokines were performed using luminex IS 100 instrument. Schistosomal periportal and portal liver fibroses were determined by using the ultrasound assessment method. SPSS statistical software version 20 was used and P-value <0.05 was reported as statistically significant.The overall prevalence of parasite infections were: malaria (28.15%), *Schistosoma mansoni* (27.90%), malaria-*Schistosoma mansoni* co-infections (12.10%) and other intestinal helminths (11.85%).Among the total of 810 study participants, 452 (55.81%) harbored at least one parasitic infection and 358 (44.20%) had none of the investigated parasitic infections. Malaria parasite density increased with increasing infection intensity of *S. mansoni* and also *S. mansoni* parasite densities increased with increasing malaria parasite infection intensities. Malaria-*Schistosoma mansoni* co-infection was a significant factor for decrease in haemoglobin levels when compared with mono-infections.Malaria related anaemia was higher in children (6.22%) followed by adult women (4.42%) and adult men (3.27%), with a significant difference (P<0.05).Increased risk of anaemia was significantly associated with malaria (P=0.001), *Schistosoma mansoni* (P=0.002) and malaria- *Schistosoma mansoni* co-infection (P=0.000).Among the three groups of infections, the levels of IL-10 and IL-4 were higher in the co-infected individuals than in

the mono-infected.The overall prevalence of PPF was 17.71% and it was detected in 107 schistosomiasis mansoni patients of whom 54.21%, 42.06% and 3.74% had mild, moderate and severe PPF, respectively. PPF was associated with intensity of *S. mansoni* infection, being higher in individuals with moderate to heavy infection compared to those with light infections.The prevalence of PPF was higher in males than in females and as the age of infected individuals increased, PPF prevalence also increased.*S.mansoni* infection (P=0.000) and malaria- *Schistosoma mansoni* co-infection (P=0.002) were identified as significant risk factors for PPF among the study participants.Malaria-*Schistosoma mansoni* co-infected individuals with definite PPF had relatively higher levels of IL-10 and IL-4 when compared to co-infected individuals without PPF.In conclusion, the findings of this study showed that *P. falciparum* and *S. mansoni* co-infection reciprocally increase parasite densities, infection intensities, anaemia, anti-inflammatory cytokine (IL-10) and the degree of PPF.The study has provided an additional parasitological, clinical and immunological data on the adverse reciprocal effects of *P. falciparum* and *S. mansoni* co-infection , and could serve as a guide in designing, developing and implementing intervention strategies to mitigate co-morbidity due to co-infection among the high risk groups, under the Ethiopian health services condition and other endemic areas in Africa.

Key words: Anaemia, co-infections, co-morbidity, cytokine, Finchaa Sugar Estate, haemoglobin, periportal fibrosis, *P.falciparum*, *P. vivax, Schistosoma mansoni,* Ethiopia

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## LIST OF ABBREVIATIONS AND ACRONYMS

| | |
|---|---|
| CDC | Centers for Disease Control and Prevention |
| ECMP | Extracellular matrix proteins |
| Epg | Eggs per gram |
| FMoH | Federal ministry of health |
| Hb | Haemoglobin |
| IFN | Interferon |
| Ig | Immunoglobulin |
| IL | Interleukin |
| IRS | Indoor residual spraying |
| ITN | Insecticide treated nets |
| K3-EDTA | Ethylenediaminetetraacetic acid |
| MOP | Malaria operational plan |
| NSP | National strategic plan |
| Pg | Pico gram |
| PPT/F | Periportal thickening/fibrosis |
| PZQ | Praziquantel |
| SSA | Sub-Saharan Africa |
| STH | Soil transmitted helminth |

Th1 T-helper cells type I

Th2 T-helper cells type II

TNF Tumor necrosis factor

UNICEF The United Nations Children's Fund

WBC White blood cells

WHO World Health Organization

# 1. INTRODUCTION

## 1.1. General

Malaria, schistosomiasis and intestinal helminth infections are causes of high morbidity in most tropical parts of the world (Mazigo *et al.*, 2010). Helminths are among the most common chronic infections in the tropics and malaria parasite infections are the most deadly. These two groups of parasites have similar geographical distribution and co-infection is commonplace (Mwangi *et al*., 2006). It is estimated that over a third of the world's population, mainly those individuals living in the tropics and sub-tropics, are infected by parasitic helminths or one or more of the species of malaria parasite. In reality, passive detection of disease events in most resource-poor countries is incomplete, even outside Africa (Snow *et al.*, 2005).

Although multiple parasitic infections are common in tropical areas, it is often common to separately investigate the epidemiologic, clinical, or biologic outcomes of each human and animal parasite. However, studies on animal models have shown that concurrent infections by two or more parasite species could reciprocally affect the pathogenesis of each parasite (Christensen *et al*., 1988). Similar phenomenon in human parasites has also been suggested and in both cases, the possibility of antagonistic or synergistic interactions between parasites appears to be the mechanism in play (Whittle *et al*., 1969; Sokhna *et al*., 2004; Booth *et al*., 2004c; Faye *et al*., 2008; Wilson *et al*., 2009).

Malaria parasite and helminth infections are the major parasitic diseases in developing countries and their epidemiologic coexistence is frequently observed, particularly in

Africa. The implications of concomitant malaria and helminth infections have been mainly explored in animals under laboratory conditions (Yoshida *et al*., 2000).

In human populations, only few studies have been conducted, with contradictory results. The main finding is that there is a trend toward a protective effect of *A. lumbricoides* and *S. hematobium,* and worsening effect of hookworm and *S. mansoni* on the pathogenesis and incidence of malaria, respectively (Adegnika and Kremsner, 2012).Malaria and intestinal helminthiasis are sources of significant morbidity worldwide. Given the nature of shared endemicity, these diseases often co-exist in the same populations. Therefore, much attention is now being given to the interaction between helminths and malaria parasite in the situation of co-infection (Basavaraju and Schantz, 2006).

In Sub-Saharan Africa (SSA), malaria, schistosomiasis and soil transmitted helminth infections (STH) are the most important parasitic infections, contributing to the biggest share of clinical disease burden and have become major public health concern resulting in a major cause of mortality and morbidity (WHO, 2002).

Anaemia is a condition in which the number of red blood cells (and consequently their oxygen-carrying capacity) is insufficient to meet the body's physiologic needs. Specific physiologic needs vary with a person's age, gender, residential elevation above sea level (altitude), and different stages of pregnancy. Parasitic infections (such as malaria, hookworm infections and schistosomiasis), and inherited or acquired disorders that affect haemoglobin synthesis, red blood cell production or red blood cell survival, can all cause anaemia. Anaemia is an important health indicator and when it is used with

other measurements of iron status the haemoglobin concentration can provide information about the severity of iron deficiency (WHO, 2007). The level of anaemia is defined as stipulated by the World Health Organization (Cheesbrough, 2009): children less than five years of age, Hb < 11 g/dL; and children aged 5 to 10 years, Hb < 11.5 g/dL. Further classification was done to determine severe, moderate and mild anaemia cases, which produced values of <6 g/dL, 6.1–8 g/dL and 8.1–10.9 g/dL, respectively (Novelli *et al*., 2010).

Cytokines are vital intercellular communication molecules. These proteins carry signals or messages when they are released from one cell and are subsequently sensed by another cell. According to Maizels and Yazdanbakhsh (2003) there is increasing evidence that immune mechanisms are involved in the pathogenesis of many parasitic infections. In most cases a state of immunosuppression can be evidenced in chronic parasitic infections. This hyporesponsiveness to antigen-specific could be related to immunosuppressive cytokines (IL-10 and TGF-$\beta$) secreted by antigen presenting cells and regulatory T cells.

For this study, systemic cytokines indicative of CD4+Tcell-mediated Thelper (Th)1 (IFN-γ and TNF-α) and Th2-type (IL-4 and IL-10) responses were selected for investigation, as these are amongst the main immunological correlates of pathology and protective immunity to malaria parasite and schistosome infections (Pearce and MacDonald, 2002; Maizels and Yazdanbakhsh,2003; Booth *et al*.,2004a; Awandare *et al.*,2006).

Ultrasonography is currently the diagnostic tool of choice for detecting pathologic conditions associated with *S. mansoni* such as liver fibrosis and enlargement and dilatation of the portal vein (Richter *et al*., 2003).

## 1.2. Malaria

Malaria is a disease of the blood resulting from infection by protozoan parasites of the genus *Plasmodium*, transmitted by female Anopheline mosquitoes. Five species of *Plasmodium* infect humans, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae* and *Plasmodium knowlesi* (Bannister and Sherman, 2013).

Malaria is a major human health threat in tropical and subtropical regions of the world. It kills about 1 million people each year (Pierce and Miller, 2009). About 90% of all malaria deaths in the world occur in Africa, south of the Sahara (Guerra *et al.,* 2008). Severe anaemia and cerebral malaria constitute the major cause of death, mostly in children under the age of 5 years (Malaguarnera *et al,* 2002).

In Africa, over a quarter of school aged children are at high risk of coincidence infection and consequently at enhanced risk of clinical diseases (Brooker *et al.,* 2006). According to Eggena *et al.* (2006), there are three principal ways in which malaria can contribute to death in young children. First, an overwhelming acute infection, which frequently presents as seizures or coma (cerebral malaria), may kill a child directly and quickly. Second, repeated *Plasmodium* infections contribute to the development of severe anaemia, which substantially increases the risk of death. Third, low birth weight

frequently the consequence of *Plasmodium* infection in pregnant women is the major risk factor for death in the first month of life.

Some of the world's poorest countries in terms of national income have made the strongest gains in child survival. Seven high-mortality countries (Bangladesh, Ethiopia, Liberia, Malawi, Nepal, Timor-Leste, and United Republic of Tanzania) have already reduced their under-five mortality rates by two-thirds or more since 1990; six of these are low-income countries, proving that low national income is not a barrier to making faster and deeper gains in child survival. A further eighteen high-mortality countries have also managed to reduce their under-five mortality rates by half or more over the same period (UNICEF, 2013).

### 1.2.1. Malaria situation in Ethiopia

Malaria is endemic in Ethiopia with differing intensity of transmission, except in the central highlands which are malaria-free. According to reports of FMoH (2004) in Ethiopia in 2002/03 the disease has been reported as the first cause of morbidity and mortality accounting for 15.5% outpatient consultations, 20.4% admissions and 27% in-patient deaths. Furthermore in 2004/05, it was reported to be the leading infectious disease followed by helminthiasis and tuberculosis (FMoH, 2005).

Malaria distribution under normal condition depends largely upon topographic and climatic features and is endemic in warm and moist lowlands. The epidemiological pattern of malaria transmission in Ethiopia is generally unstable and seasonal, the level of transmission varying from place to place because of altitude and rainfall patterns.

The major transmission of malaria occurs during the months of September to December, while the minor transmission season occurs between April to May (FMoH, 2007).

Climatological changes such as increased temperatures, humidity, and unusually prolonged heavy rainfall is considered to aggravate malaria. The so-called ‘man-made malaria’ that refers to the creation of breeding places of malaria vectors as a result of human activities contributes considerably to spread of malaria (Martens and Hall, 2000).

Parasitological studies conducted during the two peak malaria seasons of 2005/06 among the residents of Finchaa Sugar Estate, in western Ethiopia revealed an infection prevalence of 1.43% in November 2005 and 3.86% in April/May 2006, from a random sample of 700 individuals (Chala and Petros,2011).On the other hand, retrospective clinical reports of Finchaa Sugar Estate Health Center during the past five years (2001-2005) showed that malaria was among the major infectious diseases constituting significant public health problem, accounting for over 30% average annual prevalence in the area (Chala and Petros,2011).

Malaria is ranked as the leading communicable disease in Ethiopia where an estimated 68% of the population lives in malarious areas and three-quarters of the total land mass is regarded as malarious (FMoH,2006) with two-thirds of the country’s population at risk (Kassahun,2004).This makes malaria the number one health problem in Ethiopia with an average of 5 million cases per year (Gabriel and James, 2005).The disease causes 70,000 deaths each year and accounts for 17% of outpatient visits to health institutions (MOP,2008).

All four species of *Plasmodium* are known to occur in Ethiopia (Krafsur and Armstrong, 1982). In Ethiopia, *Plasmodium falciparum* and *Plasmodium vivax* are the major parasites accounting for about 70% and 30% of infections respectively, during peak transmission periods (Tulu, 1993).A malaria trend analysis in northwest Ethiopia revealed 40 % prevalence; the species to cause the disease, as confirmed microscopically, were *P. falciparum* (75 %) and *P. vivax* (25 %) (Alemu *et al*., 2012); *P. falciparum* (64%), *P. vivax* (36%) (WHO, 2014).

Malaria appears to be on decline in Ethiopia and Zambia which have greatly increased ITN and IRS coverage and expanded programmes for diagnostic testing and treatment of malaria. In each of these countries, the number of cases reported annually fell by at least a quarter and, in some instances, by more than a half, between 2000 and 2010 (WHO, 2011c).

The main malaria control strategies in Ethiopia include: early diagnosis and prompt treatment, selective vector control, epidemic management and control, environmental management and personal protection through the use of insecticide-treated bed nets (ITN) (FMoH, 2004).

Ethiopia's malaria operational plan strategy MOP (2015) indicated that under the framework of health sector development program IV (HSDP IV), Ethiopia developed a five-year National Strategic Plan (NSP) for Malaria Prevention, Control and Elimination (2011–2015), subsequently truncated to 2011–2014.The following goals and objectives were set out in the new NSP: by 2020 (1) to achieve near zero malaria deaths (2) to

reduce malaria cases by 75% from baseline of 2013 and (3) to eliminate malaria in selected low transmission areas.

The malaria parasite has a complex; multistage life cycle that involves a sexual phase (sporogony) in the vector female *Anopheles* mosquito and an asexual phase (schizogony) in the human host. The survival and development of the parasite within the invertebrate and vertebrate hosts, in intracellular and extracellular environments, is made possible by a toolkit of more than 5,000 genes and their specialized proteins that help the parasite to invade and grow within multiple cell types and to evade host immune responses (Florens *et al*., 2002). The *Plasmodium* life cycle comprises numerous transitions and stages, and any of these can be targeted by host immune responses.

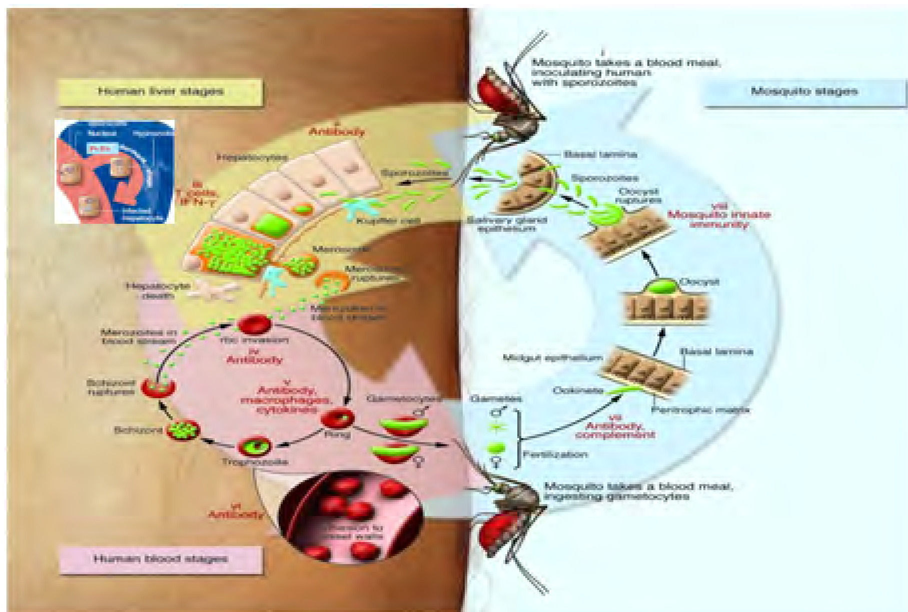



Figure 1. Life cycle of malaria parasite (Modified from Greenwood *et al*., 2008).

The surface proteins and metabolic pathways keep changing during these different stages and help the parasite to evade the immune clearance, while also creating problems for the development of drugs and vaccines (Florens *et al*., 2002).

### 1.2.2. Clinical manifestations of malaria

The clinical manifestations of malaria vary with geography, epidemiology, immunity, and age. Individuals are asymptomatic for 12 to 35 days (depending on parasite species), until the erythrocytic stage of the parasite life cycle. Release of merozoites from infected red cells, when they rupture, causes fever and other manifestations of malaria.In most cases, the incubation period for *P. falciparum* infection is about 12 to 14 days (range 7 to 30 days); most infections due to *P. falciparum* become clinically apparent within one month after exposure (WHO,2015).

CDC (2015) explained clinical manifestations of malaria as uncomplicated malaria and severe malaria based on their respective symptoms. Uncomplicated or classical (but rarely observed) malaria attack lasts 6-10 hours. It consists of a cold stage (sensation of cold, shivering); a hot stage (fever, headaches, vomiting; seizures in young children) and finally a sweating stage (sweats, return to normal temperature, tiredness).Severe or complicated malaria occurs when infections are complicated by serious organ failures or abnormalities in the patient's blood or metabolism. The manifestations of severe malaria include: Cerebral malaria, with coma, or other neurologic abnormalities; Severe anaemia due to hemolysis (destruction of the red blood cells); Haemoglobinuria (haemoglobin in the urine) due to hemolysis; Acute kidney failure; Hyperparasitemia, where more than 5% of the red blood cells are infected by malaria; Hypoglycemia (low blood glucose);

reduced tissue perfusion, hypoxia, pulmonary pathology and placental infection during pregnancy (CDC, 2015).

### 1.2.3. Malaria and Haemoglobin

Malaria contributes to reduced haemoglobin concentrations through a number of mechanisms, principally by destruction and removal of parasitized red cells and the shortening of the life span of non-parasitized red cells, and decreasing the rate of erythrocyte production in the bone marrow (McDevitt *et al*., 2004). Some of the mechanisms that cause anaemia during malaria are associated more with the acute clinical states (e.g. hemolysis or cytokine disturbances), whereas chronic or repeated infections are more likely to involve dyserythropoiesis (Menendez *et al*., 2000).

Recent meta-analyses of malaria intervention trials among African children provide compelling evidence that both symptomatic and asymptomatic malaria contributes substantially to anaemia in endemic regions (Korenromp *et al*., 2004).Asymptomatic *Plasmodium* infections, if untreated, persist and maintain malaria-induced inflammation that is commonly associated with iron deficiency anaemia (IDA) due to impaired intestinal iron absorption, impaired iron release from hepatocytes, and impairment of the recycling of iron derived from phagocytosis of parasitized red blood cells (Verhoef, 2010).

Anaemia in falciparum malaria is mainly due to the destruction of parasitized red cells. Parasitized cells also lose their deformability and are rapidly phagocytosed and destroyed in the spleen. The production of red cells in the bone marrow is also reduced

and there is a slow reticulocyte response. In young children with severe anaemia, tissue anoxia can develop, leading to acidosis (Cheesbrough, 2009).

### 1.2.4. Malaria and Immune responses/ Cytokines

The immune response to the malaria parasite is complex and still incompletely understood. Immunity to malaria develops slowly and protection against the parasite occurs later than protection against disease symptoms (Plebanski and Hill, 2000).

In endemic malaria areas, the prolonged carriage of *P.falciparum* triggers the development of acquired immunity that controls blood-stage parasitaemia, thereby reducing clinical symptoms and life-threatening complications in older children and adults (Smith *et al*., 1999).

The development of pathology in *Plasmodium* infection is associated with the imbalance of cytokines involved in the regulation of inflammatory responses (Clark *et al*., 2008). Although pro-inflammatory responses are associated with protective immunity to malaria during the early phases of infection, overproduction of Interferon-γ (IFN-ϒ) or tumor necrosis factor alpha (TNF- α) predisposes a subject to severe malarial pathology (Maitl and Marsh,2004). The regulatory responses that, as indicated above, seem to suppress immune responses and thereby allow parasite growth, might also contribute to the control of inflammatory responses and prevent the onset of severe malaria. Indeed, the anti-inflammatory cytokines Interleukin-10 (IL-10) and transforming groeth factor- β (TGF- β) and the ratio of IL-10/TNF- α could have a protective effect against pathology, as suggested by May *et al*.(2000).

Acute *Plasmodium falciparum* infection is usually associated with an increase of INFγ and TNF, regarded as the markers of the T helper 1 (Th1) and pro-inflammatory response (Clark *et al*., 2006). This pro-inflammatory response is thought to be needed to impede the multiplication of the parasite and favour its clearance, both in human and animal models (Perlaza *et al*., 2011). It has also been suggested that high levels of TNF induces marked dyserythropoietic changes in the red cell precursors and increased erythrophagocytosis (Faquin *et al*., 1992).

## 1.3. Schistosomiasis

Human schistosomiasis is caused by infection with blood flukes of the genus *Schistosoma*. There are five major species of schistosomes namely *S. mansoni, S. haematobium, S. japonicum, S. mekongi* and *S. intercalatum* (Rollingson and Southgate, 1987).

Three species: *Schistosoma haematobium*, *S. japonicum*, and *S. mansoni* cause the bulk of an estimated global burden of 4.5 million disability-adjusted life years (DALYs), and approximately 85% of them are concentrated in sub-Saharan Africa where the bulk of the at-risk population resides (Hotez and Kamath, 2009).The distribution of schistosomiasis is linked to the distribution of the snail intermediate hosts.

Schistosomiasis is the second most common NTD; 90 % of all these infections affect children, adolescents, and young adults in sub-Saharan Africa (Johnston *et al*., 2015).Typically, schistosomiasis is a disease affecting rural communities; particularly those dependent upon irrigation to support their agriculture (Steinmann *et al*., 2006).

*Schistosoma mansoni* is the most prevalent species of the *Schistosoma* genus infecting human beings. Infection with this organism causes intestinal and hepatic schistosomiasis in more than 100 million individuals that primarily live in sub-Saharan Africa, the Caribbean and South American areas, including Brazil (Gryseels, 2012).

### 1.3.1. Schistosomiasis in Ethiopia

Schistosomiasis is one of the most common parasitic diseases in Ethiopia and it is widespread in many parts of the country, usually at an altitude between 1,200 and 2,000 meters above sea level (Lo *et al*., 1988). Two forms of human schistosomiasis occur in Ethiopia, intestinal schistosomiasis caused by *Schistosoma mansoni* and transmitted by *Biomphalaria pfeifferi* and *B. sudanica,* and urinary schistosomiasis caused by *Schistosoma haematobium* and transmitted by *Bulinus abyssinicus* and *Bu. africanus*. The distribution of schistosomiasis is highly focal and varies from region to region because of several environmental, social and geographical factors (Erko *et al*., 1997). *S. mansoni* is widely distributed and covering most of the places between 1300-2200m altitudes where as the distribution of *S. haematobium* is restricted to some lowlands below 800m in Awash, Kurmuk (near the Sudan border) and Wabe Shebele areas (Kloos *et al*., 1988; Erko *et al*., 1997). In the western part of Ethiopia, most of the endemic places are located along Nile valley, in Finchaa sugar estate, Agallu-Meti and Dalati-Sirba areas (between Benishangul-Gumuz and Oromia) (Gunderson *et al*., 1998; Erko *et al*., 2003).

A community-based study from four endemic sites in Ethiopia by Berhe *et al*. (2007) indicated an overall prevalence of 65.9% *Schistosoma mansoni* infection. Studies conducted in Ethiopia in Southern and Central Zones of Tigray, by Dejenie and Petros

(2009) showed that the prevalence of *S. mansoni* was 27% in longstanding irrigated, 10.8% in recently constructed irrigation schemes and 1.8% in the non-irrigated rural localities. This indicates that the risk of *S. mansoni* in the irrigation sites is high which also holds true for the present study area, Fincaa Sugar Estate, western Ethiopia.

In Finchaa Sugar Estate the prevalence of *S.mansoni* was 30% among the residents in 1994 (Birrie *et al*., 1997) and 78% among school children in Finchaa Valley Elementary School (Erko *et al*., 1997). After schistosomiasis pilot control trial was initiated in the area, the prevalence of the disease decreased to 26% among the residents and to 56% among the school children of camp 7 after four years (Erko *et al*., 2003), indicating school-aged children are the most affected group and also male children are more affected than females (Assefa *et al*., 2013) which is attributed to high exposure to infected water bodies.

On the other hand, feasibility study conducted at Finchaa Sugar Estate,western Ethiopia by Dufera *et al.* (2014) based on clinical records showed that among the 7 camps, village A (camp 7) is the most schistosomiasis mansoni affected area with a prevalence of 37.5% followed by Kuyisa 25% which is related to the presence of permanent stream (Fekerie stream) near village A (Camp 7).The increase in prevalence and spread of the disease in Ethiopia, as in other developing countries, is due to increasing water related projects (irrigation), population migration and human water contact behavior (Tadesse, 2005).

All *Schistosoma* infections follow direct contact with fresh water that harbors free-swimming larval forms of the parasite known as cercariae. Cercariae penetrate the skin, shed their bifurcated tails, and the resulting schistosomula enter capillaries and lymphatic vessels route to the lungs and the portal venous system. The prepatent period between penetrations of cercariae to egg laying is about 30 – 40 days for *S. mansoni* (Davis, 2009).

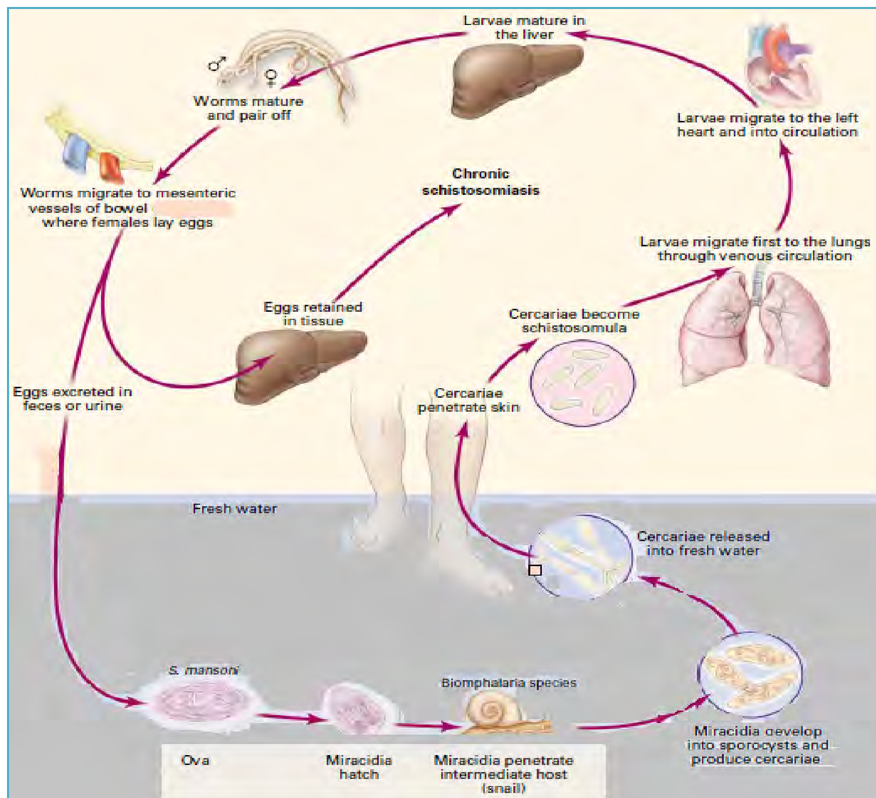



Figure 2. Life cycle of *S. mansoni* (Modified from Ross *et al*., 2002).

In Ethiopia, though there are challenges on the control of schistosomiasis due to change in climate, human migration, construction of new dams and irrigation schemes, mollusciciding is one of the control mechanisms (Erko *et al.* 2003).According to Erko *et al.* (2003) in 1995 *Phytolacca dodecandra* (Endod-type 44) was applied to transmission

sites of Finchaa Sugar Estate along Fekere stream on quarterly basis whenever *Biomphalaria pfefferi* was detected. After four years in 1998 a significant decrease in magnitude was observed on both the prevalence of schistosomiasis and the intensity of infection. However the sustainability of this snail control measure was not as expected.

Schistosomes are known to survive in the host for long periods, despite the development of concomitant immunity.A key characteristic of schistosomiasis promoting this longevity is the development of several mechanisms by which the parasites evade or modulate the host's immunological attack.Excreted or secreted (ES) products contribute to immune evasion strategies through mechanisms including: shedding of surface-bound ligands and cells, alteration of lymphocyte, macrophage and granulocyte functions and modulation of complement and other host inflammatory responses. ES promote Th2 differentiation (Cass *et al.,* 2007).IL-10 is the most critical in dampening pathology and is produced by both Tregs and Th2 cells during infection (Dewals *et al*., 2010). It is because of the longevity of infection that the disease causes serious chronic morbidity rather than acute mortality (Loukas *et al*., 2001).

Praziquantel is the drug of choice in the treatment of active infection by any species, with a cure rate of 80%. Other antischistosomal drugs include metrifonate for *S. haematobium*, oxamniquine for *S. mansoni* and Artemether and, possibly, Mirazid for both. Surgical treatment may be needed for patients with sever portal hypertention and oesophagial varices (Barsoum *et al*., 2013).

Recent policy and advocacy efforts have focused on the need to move toward programmatic integration of NTD and WASH activities in order to achieve long-term elimination of NTDs and diarrheal diseases. Research conveys that gains made from Mass Drug Administrations (MDAs) cannot be sustained without some level of investment in water, sanitation, and hygiene (Johnston *et al*., 2015).

### 1.3.2. Clinical features of schistosomiasis

Schistosomiasis causes a range of morbidities. Two main clinical conditions are recognized in *S. mansoni* infected individuals: Acute schistosomiasis and chronic schistosomiasis. The most common acute case of schistosomiasis is 'Katayama fever' which is a hypersensitivity reaction against the migrating schistosomule. It starts with fever, malaise, and eosinophilia (Gryseels *et al*., 2006). Schistosomiasis affects human host by slow damage of the host organs due to granuloma formation around the eggs in the tissues which leads to the development of fibrosis and chronic inflammation in the liver (Harrison, 2005).Unlike acute, chronic schistosomiasis is caused by repeated immunological reactions against the eggs and their antigens trapped in tissues (Utizinger *et al.,* 2007).

Several factors might influence both the development and level of morbidity in an exposed population, among them the degree and length of exposure, the intensity of the infection, concurrent pathologies, host and parasite genetics and nutritional status, which have all been associated with disease severity (Abath *et al*., 2006).

In school-aged children, *S. mansoni* associated hepatosplenomegaly is often seen in the absence of gross, ultrasound detectable periportal fibrosis (Vennervald *et al*., 2004), a type of morbidity more common in adults as it results from prolonged pro-fibrotic responses to infection (Booth *et al*.,2004a).

### 1.3.3. *Schistosoma mansoni* infection and Haemoglobin

There is a strong association between heavy intensity of *S. mansoni* infection, anaemia and Hb (Koukounari *et al*., 2008). *S. mansoni* causes severe anaemia at the community level, and haemoglobin levels drop as intensity increases (Sturrock *et al*., 1996). Children heavily infected with *S. mansoni* have lower haemoglobin concentration and also more likely to be anaemic compared with uninfected children (Koukounari *et al*., 2008).

Potential mechanisms through which *S. mansoni* may contribute to anaemia include: (1) chronic blood loss, as the spined eggs penetrate the wall of the bowl (Friedman *et al*., 2005); (2) like malaria destruction of red blood cells and /or dyserythropoiesis (Stephenson *et al*.,2000);(3) consumption of red blood cells by adult schistosomes (Sturrock *et al*., 1996) and (4) anaemia of inflammation which is typically characterised by decreased RBC production induced by pro-inflammatory cytokines (Tolentino and Friedman, 2007).

The effects of infection with a single helminth species on the risk of anaemia are well documented, with risk correlated with infection intensity (Friedman *et al*., 2005). Like malaria, anaemia due to schistosomes can additionally arise from destruction of red blood cells and/or dyserythropoiesis (Stephenson *et al*., 2000).

### 1.3.4. *Schistosoma mansoni* infection and cytokines

The cytokine environment created during the development of helminth-specific immune responses is thought to have effects on unrelated antigens by promoting regulatory effector responses (Araujo and de Carvalho, 2006).

In the murine model, *Schistosoma* egg deposition induces a type-2 immune response, which is characterized by the production of IL- 4, IL-5 and IL-13 cytokines that, in addition to IL-10, has been associated with the down-modulation of the initial type-1 immune response and granuloma formation (Cheever *et al.,* 1998; Pearce and MacDonald, 2002).

Studies conducted by Corrêa-Oliveira *et al*.(1998) suggested that IL-10 is an important cytokine in regulating the immune response and possibly controlling morbidity in human schistosomiasis mansoni, and that the production of IFN-ϒ may be associated with resistance to infection.

Egg-positive people had significantly higher levels of specific antibodies, IL-2, IFN-γ and IL-23. In contrast, egg-negative individuals had significantly higher circulating IL-10, IL-4, IL-13 and IL-21 that were detected with high frequency in all participants. When analyzed by age, IL-4 and IL-10 increased significantly, as did schistosome-specific antibodies (Milner *et al*., 2010).Also*,* Imai *et al*. (2011) additionally indicated that systemic cytokine levels rose with age as well as with schistosome infection and exposure.

### 1.3.5. *Schistosoma mansoni* infections and periportal fibrosis (PPF)

The clinical manifestations of schistosomiasis include acute, sub acute and chronic stages that mirror the immune response to infection. Chronic hepatic schistosomiasis is far more serious. It affects immunogenetically predisposed young and middle-aged adults (Dessein *et al*., 1999). Its severity correlates with the intensity of infection (Barsoum, 1987).

Fibrosis is the formation of excess fibrous connective tissue in an organ or tissue in a reparative or reactive process (Birbrair *et al*., 2013).The disease is the consequence of the excessive accumulation of Extracellular matrix proteins (ECMP) part of the normal repair process in the periportal space (Grimaud and Borojevic, 1977). Small percentage of infected subjects, especially in those with high infections, extended PPF leads to portal hypertension, varices, and ascites. As hepatoesplenic disease is a long-term complication of *Schistosoma mansoni* and is considered to be indicative of severe hepatic and periportal fibrosis (Ribeiro de Jesus *et al.,* 2004).

There are two forms of fibrosis; portal fibrosis and periportal fibrosis. In portal fibrosis, excess connective tissue forms within the portal tracts, which consequently become densily staining and expanded, but there is no extension into the adjacent parenchyma, while in periportal fibrosis, fibrous tissue occupies the periportal region and may extend into the neighboring parenchyma. Periportal fibrosis can represent the first stage in the evolution to bridging fibrosis, and it therefore often connotes an aggressive or progressive process (Baptista *et al*., 1988).In several community epidemiology studies conducted in different parts of the world, and among different ethnic groups, prevalence

of hepatomegaly peaked in older children and adolescents, the age group in which *S. mansoni* infection intensity peaks (Siongok *et al.*, 1976). Periportal fibrosis was linked to infection intensity, as indicated by worm recovery on blood perfusion (Cheever, 1968).

Ultrasonography is now the method of choice for detecting periportal fibrosis in epidemiological studies, as it is distinctive and clearly visible, and sensitivity is such that mild cases can be detected (Hatz, 2001).

### 1.3.6. Cytokines and PPF

Both type 1 and type 2 cytokine profiles are involved in granulomatous inflammatory responses. However type 1 (IFN-γ) cytokines and type 2 (IL-4 and IL-13) cytokines exhibit opposing roles in human schistosomiasis mansoni (Abath *et al.*, 2006).

Although Th2 responses seem to have a crucial role in modulating potentially life-threatening disease during the initial stages of schistosomiasis, prolonged Th2 responses contribute to the development of hepatic fibrosis and chronic morbidity (Cheever *et al.*, 2000). The formation of granulomas around schistosome eggs is mediated by CD4 T cells and more recent studies have furnished numerous insights into the cytokine cascade that controls the development of these lesions (Chiaramonte *et al.*, 2001).

In humans, the regulation of liver fibrosis during schistosomiasis is more complex, with multiple mediators regulating disease progression. Epidemiologic studies have indicated that *S. mansoni* infected patients presenting with severe fibrosis have elevated levels of tumor necrosis factor (TNF)-alpha, IL-5 and IL-13 (Henri *et al.*, 2002;Alves-Oliveira *et*

*al*.,2006),whereas patients with low levels of fibrosis present with high levels of IFN-ϒ and IL-10 (Henri *et al*., 2002; Booth *et al.*,2004a).

TGF-β, IL-1, and IL-4 are fibrogenic; they stimulate the differentiation of stellate cells into myofibroblasts and they exert effects opposite to those of IFN-ϒ on the synthesis of ECMP (Tiggelman *et al*., 1995). Observations indicate that IL-10 could have the key regulatory role of controlling excessive Th1 and T helper 2 (Th2) polarization of the granulomatous response (Hoffmann *et al*., 2000).

In humans, the anti-fibrogenic cytokine IFN-γ inhibits the production of extracellular matrix proteins by stellate cells and increases collagenase activity in the liver (Booth *et al*., 2004a).Towards the late stage of granuloma formation, the fibroblasts are stimulated by egg products and by T lymphocyte cytokines to proliferate, replacing most of the cellular elements, and mediating fibrotic collagenous material deposition around the portal vein tributaries (Olveda *et al*., 2014).

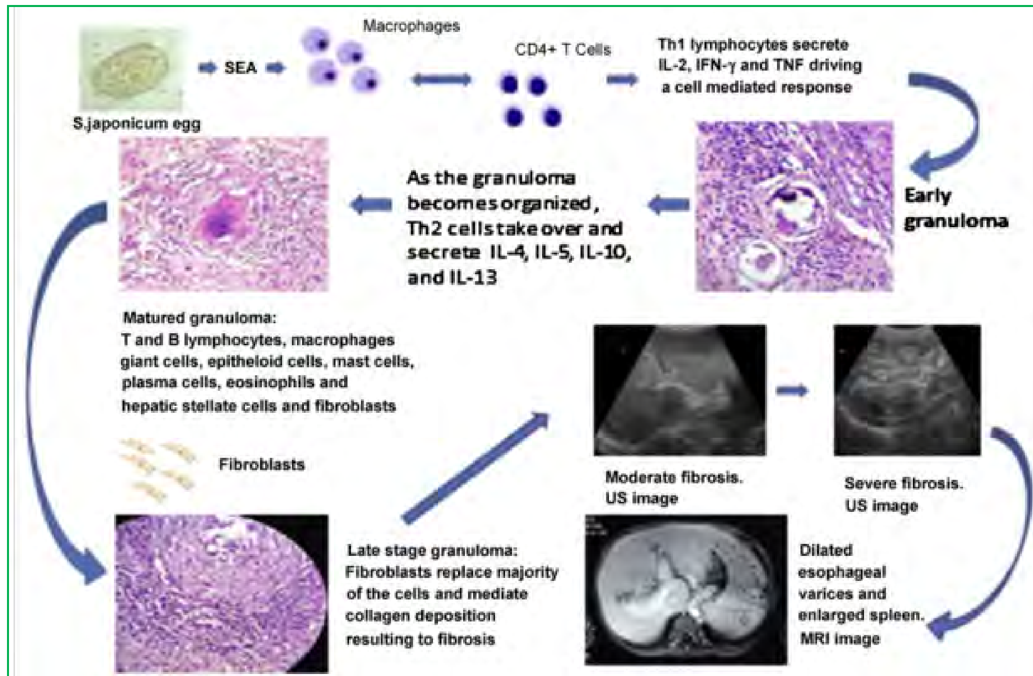



Figure 3. Pathogenesis of hepatic fibrosis leading to hepatosplenic schistosomiasis (Source: Olveda *et al*., 2014).

As the definitive site of *Schistosoma mansoni* is the mesenteric veins, it is necessary for eggs to transverse the gut wall, causing gut pathology associated with diarrhoea, sometimes bloody, and abdominal pain (King *et al.,* 2005).However, released eggs can be swept by the host's circulation into the liver, becoming trapped. The immune response to trapped eggs, characterised by Th2 - mediated granulomatous reactions can, over years, cause periportal fibrosis (Dunne and Pearce, 1999).

## 1.4. Malaria-*Schistosoma mansoni* co-infection and co-morbidity

Malaria parasites and soil-transmitted helminths (STH) are widely co-endemic and are largely a burden of the poor. The biology of the parasite and the host, climate, socio-economic status (SES) of the population and the like in the area are the major factors

that influence the epidemiological and geographical patterns of infections and co-infections. The extent to which SES is associated with malaria and helminth infection is not clear, with studies yielding contrasting results (Brooker *et al*., 2004).Climate determines the survival of the mosquito vector of the malaria parasite and the free living and infective stage of the helminth (Hay *et al*., 2000).

Overlap of schistosomes, STH and *P. falciparum* malaria depends on the conditions that favor multiple parasitic species survival and transmission.These conditions include poverty, environmental contamination and lack of effective preventive measures (Booth, 2006). In absolute numbers, most infections with *plasmodia* and helminth species occur in Asia (Snow *et al*. 2005). However, the largest clinical disease burden due to infections with both *P. falcipraum* and helminth species is carried by populations living in sub-Saharan Africa (Snow *et al*. 2005).The higher prevalence of these parasitic infections in this region and their overlap in geographical distribution result in high rates of co-infections in humans (Brooker *et al*, 2006).

Age patterns of helminth infections and malaria are likely to be affected by exposure to infection and acquisition of immunity or a combination of both (Brooker *et al*., 2007). Much of the morbidity due to malaria is generally concentrated among young children. However, age patterns of malaria morbidity are dependent on the level of transmission within a community, which affects the age at which adequate immunity is acquired (Snow *et al*., 1997). In areas of high malaria transmission, severe malaria is restricted to children under two years of age, whereas in areas of moderate transmission, it is restricted to those <5 years of age. At such young ages, helminth infections are generally

infrequent and of relatively light intensity (Brooker *et al*., 1999). In areas of low malaria transmission, mild clinical malaria episodes occur among school aged children at a time when helminth infections are most prevalent and intense (Mwangi *et al*., 2005).*S. mansoni*, *S. haematobium, A. lumbricoides* and *T. trichiura* are common in children aged 5-14 years. Severe malaria is uncommon in this age group but clinical malaria episodes and asymptomatic infections are common (Mwangi *et al*, 2006). It is evident that the occurrence of malaria and helminth co-infections and the possible clinical consequences are more important in school aged children than in other population groups (Mwangi *et al*, 2006).

Malaria and helminth co-infections may have considerable health consequences, leading to more severe clinical symptoms and pathology than for infection with single parasite species. Interactions of malaria and helminth infections increase the severity of anaemia and organomegaly (Nacher *et al*., 2001).

### 1.4.1. Malaria-*Schistosoma mansoni* co-infection in Ethiopia

In many areas of the world, malaria and STH are co-endemic; therefore co-infections are common, particularly in Africa (Briand *et al*., 2005). In Ethiopia some studies have been conducted to assess the prevalence and distribution of malaria-schistosomiasis co-infection.The rate of urinary schistosomiasis and *P. falciparum* malaria co-infection as reported by Deribew *et al.* (2013) was 2.8%; by Degarege *et al*. (2012), the prevalence of *P. falciparum* infection with *S. mansoni* was 23.1%; by Mulu *et al*., (2013) the rate of *S. mansoni* co-infection among the malaria patients was 22.6% and recently by Getie *et al*. (2015) the prevalence of *S. mansoni* co-infection among malaria infected patients

was 19.5%. According to Getie *et al.* (2015) the age group of 16–20 years old was significantly associated with co-infection. On the other hand, age group of 6–10 years old and moderate-heavy *S. mansoni* co-infection was significantly associated with severe malaria.

### 1.4.2. Clinical features of malaria-*Schistosoma mansoni* co-infection

In Africa different studies have been investigated the effect of helminths on clinical malaria. Studies conducted in Senegal by Sokhna *et al.* (2004) suggested that *S. haematobium* infected cases were predisposed to severe malaria and heavy *S.mansoni* infection increases risk of clinical malaria respectively.

However, how concurrent infections affect the epidemiology and/or the pathogenesis of each other (malaria and helminth) remains controversial (Sokhna *et al*., 2004) ranging from increased severity of malaria to reduced severity and incidence of malaria during helminths co-infection (Helmby and Bickle, 2006). The underlying reason for such different outcomes might be due to numerous factors, including differences in study design (hospital or community based) and population (adults or children), data analysis and interpretation, the demonstration of antigen cross-reactivity between co-infecting organisms and host factors (Helmby and Bickle, 2006).

Due to these conflicting findings, there is increasing interest in investigating the consequences of co-infection.The first evidence for this observation was obtained through a retrospective case-control study of individuals with and without cerebral malaria (Nacher *et al.*, 2000). It has been suggested that treatment of helminth infections

could, theoretically, increase the risk of cerebral malaria or complicate the interpretation of the vaccine effects on malaria (Nacher, 2001c). In Thai adults, infection with helminths appears to lead to an increased risk of non-severe malaria but protect against severe malaria. Also, people with helminths (without specifying the species) were protected against renal failure and jaundice caused by severe malaria (Nacher *et al.*, 2001a). It is suggested that helminth mediated Th2 shift may have complex consequences on malaria, decreasing anti-sporozoite immunity, but protecting against severe malaria (Nacher, 2002).

Studies conducted in Senegal by Diallo *et al.* (2004) suggest that *S. haematobium* co-infection could favor the development of malaria morbidity in children. On the other hand, Diallo *et al.* (2010) also described that in children, schistosomiasis co-infection favors anti-malarial protective antibody responses. In contrast, studies conducted in Ethiopia by Degarege *et al.* (2009) had indicated that STHs have very little contribution to malaria severity in co-infected children. The findings also suggest that STHs do not have significant impact on clearance rate of *P. falciparum* and *P. vivax* when treated with anti-malarial drugs.

An interaction between helminths and malaria could work in either direction. Helminth infection may alter susceptibility to clinical malaria or malaria may influence the clinical consequences of helminth infection. Most studies have investigated the effect of helminth infections on clinical malaria; fewer studies have investigated the effect of malaria in exacerbating helminth-related morbidity (Mwangi *et al.*, 2006). Splenomegally associated with *S. mansoni* infection is found to be exacerbated by

chronic malaria in children (Booth *et al*., 2004c). On the other hand, studies have shown that *S. mansoni* infections are found to increase incidence of malaria fever and malaria attack (Sokhna *et al*., 2004; Roussilhon *et al*., 2010).

### 1.4.3. Parasite density in malaria, *Schistosoma mansoni* mono infection and malaria-*Schistosoma mansoni* co-infection

Studies conducted among humans by Florey *et al.* (2012) revealed the association between heavy malaria parasitemia and heavy intensity of *Schistosoma mansoni* in co-infected individuals.

Studies that examined the effects of co-infection on the host found that malaria-schistosomiasis hematobium co-infected hosts had lower malaria parasitaemia (Lyke *et al*., 2005). Also, studies conducted in Senegal by Briand *et al.* (2005) indicated that children infected with *S. haematobium* had lower *P.falciparum* densities, hence no association between STH and *P.falciparum*. Furthermore, Lyke *et al*. (2005) reported that *S. haematobium* may be protective against *P.falciparum.*On the contrary,malaria-schistosomiasis co-infection (without specifying schistosome spp.) also resulted in higher overall prevalence of malaria parasite with greater incidence and densities of gametocytes than *P. falciparum* single infected children (Sangweme *et al*., 2010).

An age effect was also observed, in which children under the age of five had higher levels of parasitaemia than older children (Faye *et al*., 2008). Increased egg load of *S. mansoni* (Sokhna *et al*., 2004) was also associated with co-infection.By comparing malaria prevalence in the presence and absence of *S. mansoni* co-endemicity, studies conducted by Ndeffo-Mbah *et.al*. (2014) showed that the impact of schistosomiasis co-

infection on increasing malaria prevalence was higher in areas of low malaria transmission than areas of high malaria transmission.

Recently studies conducted in Northwest Ethiopia by Getie *et al.* (2015) indicated that co-infected patients with a moderate-heavy egg burden of *S. mansoni* had significantly high mean *P.* parasitemia.In contrary, a cross-sectional study conducted in northern Senegal by Diallo *et al*. (2004) and a prospective studies conducted in Mali by Lyke *et al*. (2005) showed that *S.hematobium* co-infection result in delayed clinical malaria and lower parasite densities in 4-8 years age groups and increased plasma levels of INF-Y in children age 7-15 and IL-10, TGFβ in adults age >30 years without influencing parasitaemia, respectively.

### 1.4.4. Malaria-*Schistosoma mansoni* co-infection and Haemoglobin

So far, the emphasis has been on how helminths may affect the epidemiological and clinical patterns of malaria. However, malaria may also exacerbate the consequences of helminth infection. An important consequence of both malaria and helminth infection is anaemia, an important public health problem in the tropics (Hotez *et al*., 2004).

The distribution of both malaria and schistosomiasis exhibits a large geographical overlap in tropical environments, particularly in sub-Saharan Africa. This part of the world harbours more than 85% of the estimated global burden of these diseases (Abay *et al*., 2013).

Like malaria, anaemia due to schistosomes can additionally arise from destruction of red blood cells and/or dyserythropoiesis. *Ascaris lumbricoides* and *Trichuris trichuria* typically have little impact on iron status (Koukounari *et al*., 2008). It can be

hypothesized that the combined presence of malaria and schistosomes might enhance the risk of anaemia. Studies conducted by Koukounari *et al*.(2008) found evidence that malaria parasitaemia, heavy intensity of *S. mansoni* infection and being stunted were significantly associated with lower mean Hb, although only heavy intensity of *S. mansoni* infection was significantly associated with the risk of anaemia among school children over 10 years of age.

Studies that examined the effects of malaria- schistosomes hematobium co-infections on the host found that co-infected hosts had lower haemoglobin level and high prevalence of anaemia (Okafor and Elenwo, 2007) and higher level of IL-10 (Courtin *et al*., 2011).

### 1.4.5. Cytokine Response in malaria-*Schistosoma mansoni* co-infection

Helminth infections, common in developing countries, can result in a chronic state of immune activation that is characterized by a dominant Th2 type of cytokine profile, high IgE levels, and eosinophilia (Robinson *et al*., 2003).

Regarding host immunity co-infection by malaria and schistosomiasis may have an important influence on the regulation of immune response associated with the development of these infections and their respective morbidity (Diallo *et al*., 2004).

Most studies that examined naturally occurring co-infection in humans indicated that co-infection with malaria and schistosomiasis has an effect on the host, both in terms of pathology and in terms of immunological response (Abruzzi and Fried, 2011). Little is known about the consequence of helminth co-infections on malaria antigen specific immune responses (Ateba-Ngoa *et al*., 2014).The co-endemicity of these two tropical

diseases has prompted investigation into the mechanisms of co-infection, particularly the competing immunological responses associated with each disease (Ndeffo-Mbah *et.al*. 2014).

The higher levels of regulatory modulators amongst *S. mansoni* infected children, compared to those without detectable *S. mansoni* and malarial infections, but exposed to malaria; suggest that *S. mansoni* infection may augment the underlying anti-inflammatory reaction (Wilson *et al*., 2009).

Cytokines contribute both to infection-related pathological processes and the development of protective immunity to malaria and schistosome parasites (Booth *et al*., 2004a). IL-10 and IFN-γ are involved in isotype switching to protective IgG sub-classes in *Plasmodium* parasite infections (Garraud *et al*., 2003), while IL-4, IL-5 and IL-10 appear to be important for the development of resistance to schistosome infection (Pearce and MacDonald, 2002). There is a growing body of evidence suggesting that there is a significant interaction in the development of protective immunity and pathology in individuals co-infected with these parasites (Remoue *et al*., 2003; Wilson *et al*., 2008; Diallo *et al*., 2010).

Hartgers and Yazdanbakhsh (2006) reported that the successful resolution of *P. falciparum* and *P. vivax* infection requires a coordinated succession from a Th1 to a Th2 type response, and anything that upsets the timing or balance of this process can lead to chronic or severe disease.This indicates that the Th2-skewed immune profile and profound cellular hypo responsiveness induced by chronic helminth infection might therefore be expected to affect the course of infection.

Hartgers and Yazdanbakhsh (2006) also further explained that during the early phase of *Plasmodium* parasite infection, Th1 and inflammatory cytokines such as IFN- γ, IL-12 and TNF-α are important to control the first cycles of parasitaemia and, overproduction of IFN-γ or TNF-α predisposes a subject to severe malarial pathology, whereas the anti-inflammatory cytokines IL-10 and TGF-β could have a protective effect against pathology. For example, acute infection with schistosomes increases the levels of Th1 cytokines and may help to control parasitaemia, while it may enhance the risk for severe malaria. In contrast, chronic infection with schistosomes will induce Th2 as well as regulatory cytokines, and may decrease the risk for severe malaria but increase the risk for early clinical malaria (Figure 4).

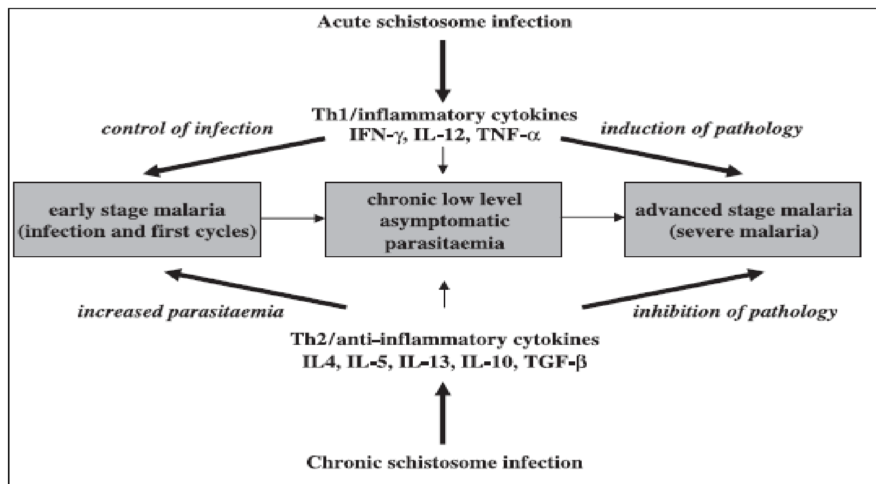



Figure 4. Immune consequences of malaria and schistosomiasis co-infection (Source: Hartgers and Yazdanbakhsh, 2006).

### 1.4.6. Malaria-*Schistosoma mansoni* co-infection and PPF

Hepatosplenomegaly can be caused by long-term exposure to malaria, or by schistosomiasis mansoni, and it is exacerbated when these two occur together (Wilson *et al*., 2009).These two causes of hepatosplenomegaly were originally thought to have a confounding relationship (Smith *et al*., 1979), however, there is an additive or synergistic effect, and that chronic co-exposure to the two parasites can result in both greater prevalence of hepatosplenomegaly (Whittle *et al*.,1969).In adtion to malaria and schistosomiasis,Leishmaniasis has been also reported as the cauasative agents of hepatosplenomegaly(Mabrook and Mohanna.,2015).

A retrospective study conducted in Kenya by Booth *et al*. (2004b) and a prospective study conducted in northern Senegal by Sokhna *et al*. (2004) suggested that malaria-*Schistosoma mansoni* co-infection increased splenomegaly in 6-16 years age groups and increase in clinical malaria (fever above $38^{0}_{c}$ and >5000 parasites /µL.blood) in 6-15 years age groups with highest helminth load respectively.In terms of pathological outcomes co-infections with malaria and schistosome parasites may also have synergistic effects on the organ pathology and increased hepatosplenomegaly has been reported in intestinal schistosomiasis malaria co-infected individuals (Booth *et al*., 2004c). Furthermore, Wilson *et al.* (2008) reported that hepatosplenomegaly among Kenyan school children has been shown to be exacerbated where there is transmission of both *S.mansoni* and *P.falciparum.*

Integration of disease control interventions is a strategy of choice when the diseases under consideration share a common geographical distribution and population at risk

have the same technical approach to control and if collectively they exert a huge disease burden to the affected population (Utzinger and de Savigny, 2006). Integrated control of the major tropical diseases such as malaria and schistosomiasis mansoni has the potential to contribute to global poverty reduction and attainment of the millennium development goals (Hotez *et al*, 2007).Abay *et al*. (2013) reported that artemether-lumefantrine (artemisinin derivative) may make possible the use of a single regimen to treat patients with *S. mansoni* and falciparum malaria co-infection.

**Research Question and Hypothesis**

In Ethiopia several studies have been conducted to assess the prevalence and risk factors of malaria (Kassahun, 2004; Gabriel and James, 2005; FMoH, 2006; Chala and Petros, 2011) and schistosomiasis mansoni (Erko *et al*., 1997; Erko *et al*., 2003; Berhe *et al*., 2007; Dejenie and Petros, 2009; Dufera *et al.*, 2014) in different parts of the country. However, concerning malaria-*Schistosoma mansoni* co-infection, limited studies have been conducted (Degarege *et al*., 2012; Mulu *et al*., 2013; Getie *et al*., 2015) focusing mainly on the study of co-prevalence and co-distribution.On the otherhand, there is no study conducted to assess the parasitological, clinical and immunological outcomes of malaria-*Schistosoma mansoni* co-infection in human populations with regard to parasite density, anaemia and /or level of Hb, level of related cytokines and periportal fibrosis (PPF) in malaria,*Schistosoma mansoni* mono-infection and malaria-*Schistosoma mansoni* co-infections in Ethiopia.Hence, it has been hypothesized that malaria-*Schistosoma mansoni* co-infections and co-morbidities would have increased reciprocal effects on parasite density, anaemia, IL-10 and IL-4 concentrations and severity of periportal fibrosis (PPF), a phenomenon critical when planning disease control programs in areas where the two parasites co-exist.

# 2. OBJECTIVE

## 2.1. General objective

The study was designed to assess the possible reciprocal effects of malaria-*Schistosoma mansoni* co-infection and co-morbidity on the parasitological, clinical and immunological outcomes in Finchaa Sugar Estate, western Ethiopia.

## 2.2. Specific objectives

1. To determine whether or not infection with *S. mansoni* increases malaria parasitaemia.
2. To assess whether or not infection with malaria parasite increases *S.mansoni* parasite density.
3. To determine the levels of Hb and/or anaemia in mono-infected and co-infected groups.
4. To assess infection-related patterns of cytokine production in malaria, *Schistosoma mansoni* and co-infected individuals in comparison with non-infected controls.
5. To evaluate the status of liver periportal fibrosis (PPF) in *Schistosoma mansoni* and malaria- *Schistosoma mansoni* co-infected groups.
6. To assess the association of Th1 cytokines (IFN-ϒ, TNF- α) and Th2 cytokines (IL-10, IL-4) with developments of periportal fibrosis (PPF).

# 3. MATERIALS AND METHODS

## 3.1. Descriptions of the study area

This study was undertaken in Finchaa Sugar Estate. Finchaa Sugar Estate is located in Finchaa valley, Oromia Regional state, western Ethiopia (Figure 5).The area is about 325 km west of Addis Ababa and is situated between $9^0$ 30'N to $9^0$ 60' N latitudes and $37^0$ 10' to $37^0$ 30' E longitudes and at an altitude of about 1,350-1,600 m above sea level. The area is stretched in most part of the Finchaa River valley with a population of more than 42,000 of which 12,000 permanent and 30,000 seasonal workers. Currently the Sugar Estate is cultivating more than 18,000 hectares of irrigated land using sprinkle irrigation system with a production of 10,000 kg of sugar per day. The scheme has a semi-arid climate with average maximum and minimum monthly temperatures of $30.7^OC$ and $14.8^OC$, respectively. The average monthly temperature is generally above $18^OC$. Average annual rainfall reaches 1,300 mm. There are rivers and streams known as Agemsa River, Bedessa Tino and Bedessa Guda River and Fekerie stream that flow throughout the year. The Sugar Estate has about seven camps in which each camp has one elementary school and one community health agent. The plantation area named as camps alphabetically from A to E and two villages called Kuyisa and Agemsa which are located around the sugar Estate plant. Finchaa sugar Estate health center which is staffed with medical doctors and auxiliary staff. The elementary school, high school, and other main services are located in Agemsa village.

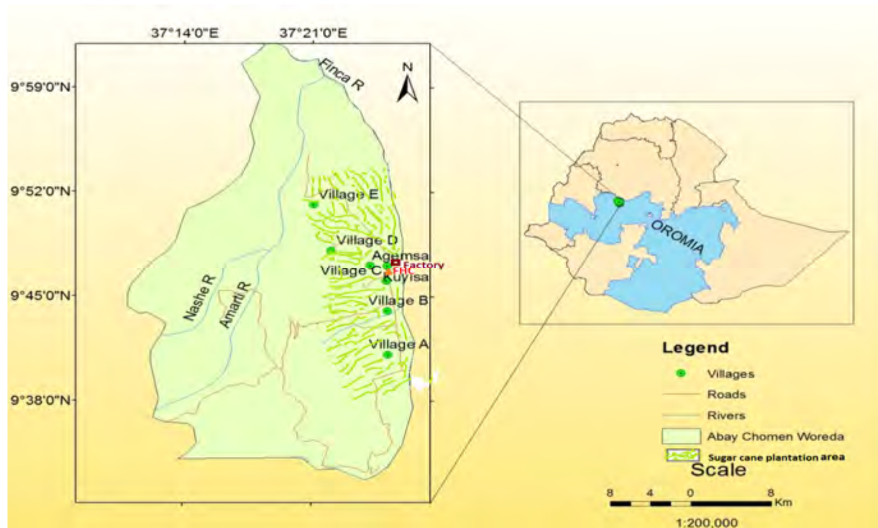



Figure 5. Sketch-map of the study area -Finchaa Sugar Estate, western Ethiopia (Source: Garmin 72 GPS)

## 3.2. Study Population, Sampling and Sample Size Determination

The study populations were residents of the three purposively selected villages. In the study area even though single infection prevalence were known for malaria: from actual observation 2.6% and from clinical records (30.9%) (Chala and Petros, 2011) and for schistosomisis mansoni: 26% (Birrie *et al*., 1997), 37.5% (Dufera *et al*., 2014), co-infection prevalence was unknown in the study area. However, previous studies conducted in Ethiopia by Mulu *et al*. (2013) showed a 22.6% (23%) prevalence of malaria and *Schistosoma mansoni* co-infection. Since there were heterogeneity in prevalence of schistosomisis mansoni in the three selected study villages.Then sample size was estimated for each study villages using Daniel's formula $n=Z^2$ P (1-P)/d2, Daniel, 1999 (cited in Zaied *et al*., 2014). Where n is sample size, Z is statistic for level of confidence (5% level (1. 96)), P is expected prevalence or proportion 23% (known

prevalence of malaria and *Schistosoma mansoni* co-infection), q is 1-p=77% (none observed) and d is precision 5% (tolerance or worst acceptable result). Based on these entities 273 study subjects were drawn from each village.Then using proportional allocations 364, 273 and 182 individuals were included in the study from Camp 7, Kuyissa and Agemsa respectively with a sum of 819 study subjects (Figure 6).

The study participants were selected from the community lists using stratified random sampling methods from the three villages after informed consent/assent. In addition for cytokine assays about 50 individuals (non-endemic healthy controls) were selected randomly from Holleta town 25 Km away from Addis Ababa to the west. A structured format was used to collect socio-demographic data including age, sex, village and relevant parasitological and clinical data of the study participants.

**Eligibility**

Prior to conducting the study, meetings were held with parents or guardians and community leaders to explain the aims and procedures to be used to collect data. Informed written consents were obtained from the study participants, children's parents or guardians and in addition assent was subsequently obtained from children.

Inclusion criteria to participate in the study were (1) >5 years of age of both sexes; (2) Adult populations, parents or guardians gave written informed consent; (3) in addition to the written consent from caretakers, children also were supposed to agree and provide informed assent; (4) Individuals lived in the study area for more than two years were included in the study.

Exclusion criteria were (1) Individuals with history of anti-malarial/anti-helminthic medication use within 2 weeks prior to enrollment and (2) pregnant women and children under 5 years were excluded from the study.

## 3.3. Study design

A cross-sectional community based study was conducted during a major malaria transmission season from October to December and a minor transmission season on April and May 2012-2014 in Finchaa Sugar Estate, western Ethiopia.

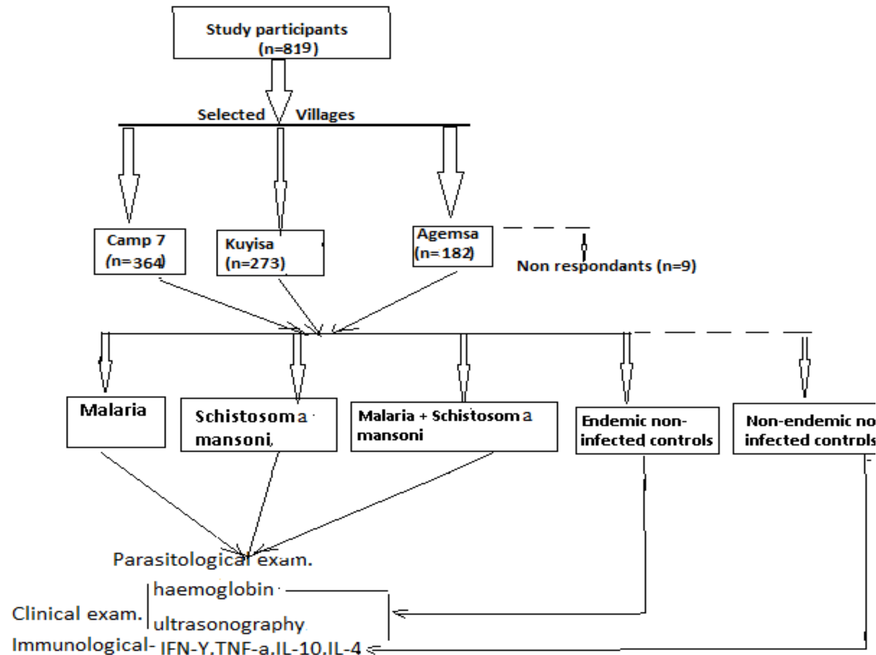



Figure 6. Study scheme of malaria-*Schistosoma mansoni* co-infection in Finchaa Sugar Estate showing the five arms (three infected and two non-infected controls) of the study participants, western Ethiopia, 2012-2014.

## 3.4. Blood sample collection and parasitological examination for malaria parasites

A finger prick blood sample was collected after cleaning the finger surface using sterile cotton wool soaked in methylated spirit. Both thick and thin smears were prepared in a single slide labeled with identification number and stained with 10% Giemsa (Sigma, Aldrich, Nairobi) in phosphate buffer (WHO, 1993). Species specific and parasite densities were estimated under a light microscope at high magnification power by counting the number of parasites per 200 white blood cells (WBC). Parasitaemia was calculated per 200 white blood cells (WBC) assuming as standard a WBC count of 8 000 WBC/μL of blood (Cheesebrough, 1998).

## 3.5. Determination of Haemoglobin (Hb) Concentration and Anaemia

After rubbing the finger tip using sterile cotton, finger-prick blood samples were collected and used to fill the microcuvette by touching the cuvette tip on the middle until completely filled with the drop of blood. The loaded microcuvette was then inserted into the holder of a portable, digital counter and/or battery-operated HemoCue Hb 201 analyzer (HemoCue AB, Angel Holm, Sweden) and analysed. The concentration of the haemoglobin was read from the digital counter in g/dL.

Haemoglobin concentrations was defined based on the reference ranges for haemoglobin concentration in adults as men: 14.0-17.5 (mean 15.7) g/dL; women: 12.3-15.3(mean 13.8)g/dL (Vajpayee *et al*., 2011) and in children as 2-6 years: mean 12.5 g/dL; 6-12 years: mean 13.5 g/dL; 12-18 years male: mean 14.5 g/dL and 12-18 years female: mean 14.0 g/dL (Marks and Glader, 2009).

Then individuals were classified as anemic or non-anemic according to the WHO age/gender cut-off limits (WHO, 2011a). Anaemia was defined as Hb concentrations <12.0g/dL and severe anaemia was defined as haemoglobin concentrations <8.0g/dL based on normal range of Hb concentrations for school age children in Africa (WHO, 1996).

## 3.6. Stool sample collection and parasitological examination for *S. mansoni*

Malaria patients and their counterpart controls were informed to bring sizable stool sample of their own. Those who volunteered to deliver the specimen were given a clean, dry, leak-proof plastic container and clean wooden applicator stick. Stool containers were then collected at the health stations and labeled with identification numbers. These samples were brought to the health center on the same day, and duplicate Kato-Katz cellophane thick smears were prepared from each specimen (WHO, 1993).

Coarse quantification of eggs was obtained by counting the number of eggs on a smear of 41.7mg of stool (Martin and Beaver,1968), and a quantitative variable scoring (light infection/low worm burden, moderate infection/medium worm burden and heavy infection/massive worm burden) was created.Then the mean number of eggs from each Kato Katz thick smear was multiplied by a factor of 24 in order to express infection intensities as the number of eggs per gram of faeces (Epg) (WHO, 1993) following the standard procedure used by World Health Organization (WHO, 2002).

**Quality Control**

As a quality control measure, for both parasitological examination of malaria parasites and *S. mansoni* infection all the necessary reagents, chemicals, and the performance of kits were checked by the principal investigator at Finchaa health center before processing and examination of samples. The slides were examined by two laboratory technologists independently. The results of laboratory examination were recorded on well-prepared format. At the end 10% each of the total slides were randomly selected for malaria parasite and *S. mansoni* infection and re-examined by an experienced laboratory technologist, who was blinded for the previous results, at Aklilu Lemma Institute of Pathobiology, Addis Ababa University.

## 3.7. Blood collection and plasma preparation for Cytokine measurement

For cytokine assays three to four ml of venous blood was collected into vacuum K3-EDTA tubes following the standard procedures. Plasma was separated from the peripheral blood mononuclear cells by Ficoll-Paque Plus density gradient centrifugation and transferred into two crovial tubes and kept at $-20^{o\ C}$ in Finchaa Sugar health center further cytokine assay.At the end of the day, every sample was packed in liquid nitrogen storage container and transported from the field to the Biomedical Sciences Research Laboratory at the Department of Microbial, Cellular and Molecular Biology, Addis Ababa University, and stored frozen at $-70^{o}{}_{C.}$

A total of 250 plasma samples (malaria positive (n=50); *Schistosoma mansoni* positive (n=50); malaria-*Schistosoma mansoni* co-infection (n=50); endemic non-infected

controls (n=50) and non endemic non-infected controls (n=50) were packed in an ice box and sent to Oslo University Hospital, Ulleval, Norway through a research collaboration arrangement. Four cytokines namely, IFN -γ , TNF-α, IL-10 and IL-4 were selected to be analyzed for all the five arms of the study groups (Figure 6).

## 3.8. Determination of cytokine concentration

Plasma levels of the cytokines IFN-γ, TNF-α, IL-10 and IL-4 were measured using a Luminex IS 100 instrument (Bio-Rad, Hercules, CA, USA) powered by the Bio-Plex Manager (version 6.0.1) software.

The plasma samples were thawed on ice, vortexed and spun at 10; 000xg for 10 min at $4^{O}$ C and the supernatant subsequently diluted three fold of the Bio-plex sample diluent). Fifty µL of the gently vortexed diluted samples was mixed in the well together with IFN-γ, TNF-α, IL-10 and IL-4 conjugated beads and incubated for 1 hour. The magnetic beads were then washed three times using a Bio-Plex Pro Wash Station (Bio Rad, Hercules, CA, USA) and 25 µL Biotin labelled reporter antibody was added and incubated on a IKA micro titre shaker (MTS) (IKA -Werke, GMBH and Co, Staufen, Germany) at 1,400 rpm for 30 sec and then 900 rpm for 30 min. The plate was washed and then the beads were incubated for 10 min with streptavidin bound phycoetrhythrin. The beads were then washed again prior to plate reading on the Luminex IS 100.

## 3.9. Ultrasound examination

Ultrasonographic assessments were performed to detect pathological changes associated with *Schistosoma mansoni* and malaria-*Schistosoma mansoni* co-infection using a EUB

405 portable ultrasound apparatus (Hitachi Tokyo, Japan) fitted with 3.5-MHz convex abdominal probe.

**Treatment**

Individuals positive for malaria were treated with anti-malarial drugs, chloroquine for *P.vivax* and coartem (artemether-lumefantrine) for *P.falciparum*. Also individuals found positive for *S. mansoni* and STH infections were treated with a single dose of praziquantel 40 mg/kg and mebendazole 500 mg, respectively. In addition, based on ultrasound determinations, patients who had definite schistosomal liver periportal fibrosis were treated with praziquantel every four weeks for 4-6 months. All treatments were given free of charge under the supervision of a clinician sonographer and physicians at the health center according to the national treatment protocols (FMoH, 2004). Individuals with severe anaemia were referred to the nearby health clinic for treatment and further follow up.

**Ethical considerations**

The study was approved by the Research Ethics Review Committee of Collage of Natural Sciences, Addis Ababa University and by the National Research Ethics Review Committee.To participate in the research project and to treate infected patients, permission was obtained from local administration and written consent/assent from the study participants.

## 3.10. Data Analysis

Data were entered into a computer and validation was performed in Microsoft Excel 2007 spreadsheets, and transferred into SPSS version 20.0 software for statistical analysis. Descriptive statistics was used to provide a clear picture of background variables. The frequency distribution of both dependent and independent variables was determined.Hb levels, intensity of *S.mansoni* infection (Epg), and malaria parasite intensity (parasites/µL) were expressed as means among study participants. All graphs were drawn using MS-Excel and all box-plots were drawn using SPSS version 20.0.

Chi-square tests were used to test for differences in proportions and means. Multiple linear regression analysis was performed to determine relationships where continuous outcome (dependent variable) and predictor variables (independent variables) were involved and have been used to compare mono-infection, co-infection, parasite intensities and haemoglobin levels.Univariate logistic regression analysis was performed to determine relationships where binary outcome (dependent variable) and categorical predictor variables (independent variables) were involved and have been used to identify predictors of anaemia and PPF. A significant level of $p<0.05$ was used for all tests. Independent sample t-test was used to compare the mean score between two independent groups on some continuous variable.

To show the relationship between median and range of a given continuous variables, box and whisker plot was used in which the horizontal bars within each box represent the median value. The lower and upper edge of each box indicates the 25$^{th}$ and 75$^{th}$

percentiles, respectively and the whisker represents the minimum and maximum values (range), excluding outliers.

To calculate sample cytokine concentrations of a total of 250 plasma samples (50 samples from each group) were analyzed for respective cytokines: IFN-γ, TNF-α IL-10 and IL-4 Cytokine distributions were log transformed for analysis and the geometric means were used for comparisons among the five study groups.

To determine ultrasonographic measurements, ultrasound image patterns suggestive of periportal fibrosis were compared with standard images and the corresponding image pattern scores were recorded using the WHO-Niamey protocol (Richter *et al.,* 2000). All examinations were performed by the same expert radiographer who was blinded to the schistosome infection status of the individuals.

# 4. RESULTS

## 4.1. Socio-demographic characteristics

A total of 810 study participants, male 415 (51.23%) and female 395 (48.77%), were included in the study. Their mean age, haemoglobin, *S.mansoni* Epg and malaria parasites/μL were 23, 13.84g/dL, 241 and 574, respectively. Of the 810 study participants, 364 (44.94%): male 187 (23.09%) and female 177 (21.85%) were from Village A (Camp 7); 273 (33.70%): male 143 (17.65%) and female 130 (16.05%) were from Kuyisa and 173 (21.36%): male 85 (10.49 %) and female 88 (10.86%) were from Agemsa.

Among the total of 810 study participants, 452 (55.81 %) harbored at least one parasitic infection and 358 (44.20%) have none of the investigated parasitic infections. Among mono-infections, the most prevalent parasitic infection was *S. mansoni* 117 (14.44%), followed by malaria 104 (12.84%), malaria-*Schistosoma mansoni* co-infection 98 (12.10%) and other intestinal helminth parasites such as, Hookworm, *T.trichiura*, *A.lumbricoides*, *S.stercoralis* andTaenia spp. 96 (11.85%).Males were more infected (32.72%) than females (23.09%). As age increased infection prevalence was decreased and individuals within 5-9 and 10-14 age ranges were more affected than other age groups. Of 810 study participants the overall (sum of only malaria and malaria-helminth co-infections; sum of only *S. mansoni* and *S. mansoni* -helminth co-infections) prevalence of malaria and *Schistosoma mansoni* was 28.15% and 27.9% respectively (Table 1).

Table 1. Prevalence of investigated parasitic diseases stratified by sex and age among study participants (n=810) in Finchaa Sugar Estate, Western Ethiopia, 2012-2014.

| **Sex** | Uninfected | Parasite | | | | | | | Over all total n (%) |
|---|---|---|---|---|---|---|---|---|---|
| | | Malaria n (%) | mal +Sm n (%) | Mal+OIHP n (%) | Sm n (%) | Sm+ OIHP n (%) | OIHP n (%) | Total infected n (%) | |
| Male | 164(20.25) | 55 (6.79) | 71(8.77) | 17(2.10) | 73(9.01) | 5(0.62) | 44(5.43) | 265(32.72) | 415(51.23) |
| Female | 194(23.95 ) | 49(6.05) | 27(3.33) | 9(1.11) | 44(5.43) | 6(0.74) | 52(6.42) | 187 (23.09) | 395(48.77) |
| Total | 358(44.20) | 104(12.84) | 98(12.10) | 26(3.21) | 117(14.44) | 11(1.36) | 96(11.85) | 452(55.81) | 810(100) |
| **Age(Years)** | | | | | | | | | |
| 5-9 | 6(0.74) | 3(0.37) | 14(1.73) | 5(0.62) | 48(5.93) | 8(0.99) | 49(6.05) | 127(15.68) | 133(16.42) |
| 10-14 | 31(3.83) | 7(0.86) | 19(2.35) | 9(1.11) | 35(4.32) | 3(0.37) | 37(4.57) | 110(12.35) | 141(17.41) |
| 15-19 | 22(2.72) | 18(2.22) | 14(1.73) | 8(0.99) | 5(0.62) | 0(0) | 2(0.25) | 47(5.80) | 69(8.52) |
| 20-24 | 56(6.91) | 25(3.09) | 23(2.84) | 2(0.25) | 12(1.48) | 0(0) | 4(0.49) | 66(8.15) | 122(15.06) |
| 25-29 | 86(10.62) | 15(1.85) | 10(1.23) | 2(0.25) | 5(0.62) | 0(0) | 2(0.25) | 34(4.20) | 120(14.81) |
| ≥30 | 157(19.38) | 36(4.44) | 18(2.22) | 0(0) | 12(1.48) | 0(0) | 2(0.25) | 68(8.40) | 225(27.78) |

mal=malaria, Sm=*S. mansoni*, OIHP-Other intestinal helminth parasites (Hookworm, *T.trichiura*, *A.lumbricoides*, *S.stercoralis* andTaenia spp).

## 4.2. Malaria parasite infection

Malaria positive individuals were more anemic than malaria negative individuals with a significant difference (P<0.05). Also, malaria positive children (6.22%) were more anemic than malaria negative children (2.62%) followed by malaria positive adult women (4.42%) and malaria positive adult men (3.27%) (Table 2).

Table 2. Anaemia prevalence by population group and malaria infection status among study participants (n=611) in Finchaa Sugar Estate, Western Ethiopia, 2012-2014.

| Population group | Malaria Infection status | Anaemia status | | | |
|---|---|---|---|---|---|
| | | Normal n (%) | Anemic n (%) | Total n (%) | P |
| Adult men | Malaria positive | 55(9.00) | 20(3.27) | 75(12.27) | 0.002 |
| | Malaria negative | 69(11.29) | 2(0.33) | 71(11.62) | |
| Adult women | Malaria positive | 35(5.73) | 27(4.42) | 62(10.15) | 0.000 |
| | Malaria negative | 93(15.22) | 15(2.45) | 108(17.67) | |
| Children | Malaria positive | 39(6.38) | 38(6.22) | 77(12.60) | 0.000 |
| | Malaria negative | 202(33.06) | 16(2.62) | 218(35.68) | |

### 4.2.1. Malaria parasite infection density

The over all malaria prevalence was 228 (28.15%) among which the majority 143 (62.72%) of the infection was due to *P. falciparum* and *P. vivax* accounted for 66 (28.94%) of the cases.The study also showed the youngest (≤24yrs) age groups were more infected than the older age groups (≥25 yrs).Although the differences were not statistically significant, malaria was more prevalent in males (64.04%) than in females (35.96%) (Table 3).

Table 3. Prevalence of malaria parasites by sex and age among study participants (n=228) in Finchaa Sugar Estate, Western Ethiopia, 2012-2014.

| Age (year) | *P.vivax* n (%) | *P.falciparum* n (%) | Mixed n (%) | Total n (%) | P |
|---|---|---|---|---|---|
| **Age (year)** | | | | | |
| 5-9 | 23 (10.09) | 33 (14.47) | 5 (2.19) | 61(26.75) | 0.840 |
| 10-14 | 18 (7.89) | 29 (12.72) | 1 (0.44) | 48 (21.05) | 0.770 |
| 15-19 | 9 (3.95) | 25 (10.96) | 3 (1.32) | 37 (16.23) | 0.207 |
| 20-24 | 5 (2.19) | 26 (11.40) | 4 (1.75) | 35 (15.35) | 0.721 |
| 25-29 | 8 (3.51) | 13 (5.70) | 4 (1.75) | 25 (10.96) | 0.532 |
| ≥30 | 3 (1.32) | 17 (7.46) | 2 (0.88) | 22 (9.65) | 0.224 |
| **Sex** | | | | | |
| Male | 40 (17.54) | 95 (41.67) | 11 (4.82) | 146 (64.04) | 0.110 |
| Female | 26 (11.40) | 48 (21.05) | 8 (3.51) | 82 (35.96) | |
| Total | 66 (28.94) | 143 (62.72) | 19 (8.33) | 228 (100.00) | |

Based on the level of *P.falciparum* parasite intensity (expressed as number of parasites/µL of blood), the total of 143 patients can be categorized as low (11.96%), moderate (33.97%), high (20.10%) and very high (2.40%). Individuals infected with *P.falciparum* with age ranges 5-14 and ≥30 years have highest parasite densities with high mean parasites/µL than other age groups (Table 4).

Table 4. Intensity of *P.falciparum* infection, stratified by sex and age among study participants (n=143) in Finchaa Sugar Estate, Western Ethiopia, 2012-2014.

| | Intensity of *P.falciparum* (parasites/µL) | | | | | |
|---|---|---|---|---|---|---|
| **Sex** | Low (1-50) n (%) | Moderate (51-500) n (%) | High (501-5000) n (%) | V.high (>5000) n (%) | Total n (%) | Mean parasites/µL |
| Male | 15(7.18) | 49(23.44) | 28(13.40) | 3(1.44) | 95(45.45) | 704 |
| Female | 10(4.78) | 22(10.53) | 14(6.70) | 2(0.96) | 48(22.97) | 720 |
| Total | 25(11.96) | 71(33.97) | 42(20.10) | 5(2.40) | 143(68.42) | 709 |
| **Age (Years)** | | | | | | |
| 5-9 | 3(1.44) | 6(2.87) | 8(3.83) | 2(0.96) | 19(9.09) | 1188 |
| 10-14 | 4(1.91) | 14(6.70) | 10(4.78) | 1(0.48) | 29(13.88) | 847 |
| 15-19 | 2(0.96) | 17(8.13) | 5(2.39) | 0(0) | 24(11.48) | 662 |
| 20-24 | 5(2.39) | 12(5.74) | 9(4.31) | 0(0) | 26(12.44) | 471 |
| 25-29 | 1(0.48) | 10(4.78) | 3(1.44) | 0(0) | 14(6.70) | 406 |
| ≥30 | 3(1.44) | 20(9.57) | 6(2.87) | 2(0.96) | 31(14.83) | 694 |

### 4.2.2. Anaemia in *P.falciparum* Infected individuals

Anaemia status was significantly associated with parasite intensity (parasetimia) (P=0.000).Severely anemic (6.29%) individuals had very high parasetimia (>5000 parasites/μL) (Table 5).

Table 5. Intensity of *P.falciparum* and anaemia status among study participants (n=143) in Finchaa Sugar Estate, Western Ethiopia, 2012-2014.

| *P.falciparum* Intensity (parasites/μL)* | Anaemia status | | | Total n (%) | P |
|---|---|---|---|---|---|
| | Normal n (%) | Anemic n (%) | Severely anemic n (%) | | |
| Low(1-50) | 10 (6.99) | 1 (0.70) | 0 (0) | 11 (7.69) | |
| Mod(51-500) | 76(53.15) | 17 (11.88) | 2 (1.40) | 95 (66.43) | |
| High(501-5000) | 0 (0) | 2 (1.40) | 6 (4.19) | 8 (5.59) | 0.000 |
| V. high(>5000) | 2 (1.40) | 18 (12.59) | 9 (6.29) | 29 (20.28) | |

*WHO category

### 4.2.3. Cytokine levels in malaria positive and negative individuals

Elevated levels of both IFN-γ and TNF-α and low IL-10 and IL-4 cytokine levels were observed in malaria positive study participants (Table 6).

Table 6. Mean cytokine expression in malaria positive and negative individuals among study participants in Finchaa Sugar Estate, Western Ethiopia, 2012-2014.

| Cytokines (pg/ml)* | Malaria status | n | Mean | P |
|---|---|---|---|---|
| logIFN_ϒ | Positive | 96 | 0.7034 | 0.000 |
| | Negative | 56 | 0.0813 | |
| logTNF_α | Positive | 99 | 1.2029 | 0.000 |
| | negative | 60 | 0.9448 | |
| logIL_10 | Positive | 100 | 1.1997 | 0.000 |
| | negative | 60 | 1.9206 | |
| logIL_4 | Positive | 93 | 0.8893 | 0.024 |
| | negative | 55 | 1.0151 | |

*log transformed

### 4.3. *Schistosoma mansoni* Infection

Among the three selected villages, Village A (Camp 7) was the most schistosomiasis affected area followed by Kuyisa and Agemsa. The overall infection prevalence of *S.mansoni* was 27.9 % (226/810). Males were more affected than females and *S.mansoni* infection was high among individuals age under 15 years when compared to individuals age above 15 years. Among which, 96 (42.47%); 76 (33.62%) and 54 (23.89%) were lightly, moderately and heavily infected respectively.

#### 4.3.1. *Schistosoma mansoni* infection density

Even though significant differences were not observed both in sexes and ages, higher mean Epg (266) was observed in males than in females (204).Regarding age categories, highest mean Epg (265) was observed in age ranges 5-9 years followed by 10-14 years (246) and mean Epg decreased as age increased.Overall, most people (42.47%) were infected lightly. However, moderate and high infection groups were over 50 % (Table 7).

Table 7. Intensity of *S.mansoni* infection, stratified by sex and age among study participants (n=226) in Finchaa Sugar Estate, Western Ethiopia, 2012-2014.

| Risk factors | Intensity of *S.mansoni* Infection* | | | Total | Mean Epg | P |
|---|---|---|---|---|---|---|
| | Light n (%) | Moderate n (%) | Heavy n (%) | | | |
| **Sex** | | | | | | |
| Male | 52 (23.00) | 46(20.35) | 37(16.37) | 135(59.73) | 266 | 0.372 |
| Female | 44(19.47) | 30(13.27) | 17(7.52) | 91(40.27) | 204 | |
| Total | 96 (42.47) | 76 (33.62) | 54 (23.89) | 226 (100) | 235 | |
| **Age (years)** | | | | | | |
| 5-9 | 33(14.60) | 24(10.62) | 13(5.75) | 70(30.97) | 265 | 0.246 |
| 10-14 | 21(9.29) | 22(9.73) | 14(6.19) | 57(25.22) | 246 | 0.240 |
| 15-19 | 7(3.10) | 4(1.77) | 8(3.54) | 19(8.41) | 242 | 0.215 |
| 20-24 | 14(6.19) | 12(5.31) | 9(3.98) | 35(15.49) | 240 | 0.400 |
| 25-29 | 7(3.10) | 6(2.65) | 2(0.88) | 15(6.64) | 181 | 0.574 |
| ≥30 | 14(6.19) | 8(3.54) | 8(3.54) | 30(13.27) | 163 | 0.662 |

* Infection intensity was defined according to classes of intensity used by WHO (2002).

### 4.3.2. Haemoglobin levels in *Schistosoma mansoni* positive and negative individuals

There was a clear association between infection intensity and Hb levels in that light infection was less anemic than heavy ones with a significant difference (P=0.000).The probability of becoming anemic was significantly increased as infection intensity increased, in which 51 (22.57%) were anemic in heavy infection while, 34 (15.04%) were anemic in light to moderate infections (Table 8).

Table 8. Intensity of *S.mansoni* infection and anaemia among study participants (n=226) in Finchaa Sugar Estate, Western Ethiopia, 2012-2014.

| Intensity (Epg) | Anaemia status | | | | |
|---|---|---|---|---|---|
| | Normal n (%) | Anemic n (%) | Severely-anemic n (%) | Total n (%) | P |
| light (1-100) | 90(39.82) | 6(2.65) | 0(0.00) | 96(42.48) | |
| Mod(101-400) | 47(20.80) | 26(11.50) | 2(0.88) | 75(33.19) | 0.000 |
| Heavy (>400) | 4(1.77) | 29(12.83) | 22(9.73) | 55(24.34) | |

### 4.3.3. Cytokine levels in *Schistosoma mansoni* positive and negative individuals

The expressions of the cytokines, INF_ϒ and TNF-α were significantly (P<0.005) higher among *Schistosoma mansoni* positives compared to *Schistosoma mansoni* negatives.Whereas, IL-10 expression was higher among *Schistosoma mansoni* negatives compared to *Schistosoma mansoni* positives (Table 9).

Table 9. Expression of selected cytokines in *Schistosoma mansoni* positive and negative individuals among study participants in Finchaa Sugar Estate, Western Ethiopia, 2012-2014.

| Cytokines (pg/ml)* | *Schistosoma mansoni* Infection status | n | Mean | P |
|---|---|---|---|---|
| logIFN_ϒ | positive | 83 | 0.744 | 0.001 |
| | negative | 69 | 0.250 | |
| logTNF_α | positive | 88 | 1.208 | 0.003 |
| | negative | 71 | 1.023 | |
| logIL_10 | positive | 89 | 1.420 | 0.000 |
| | negative | 71 | 1.939 | |
| logIL_4 | positive | 82 | 0.926 | 0.664 |
| | negative | 66 | 0.949 | |

*Log transformed

### 4.3.4. PPF status in *Schistosoma mansoni* positive individuals

Among 604 individuals who had ultrasonography for detection of schistosomal periportal fibrosis about 17.71% individuals had definite PPF and *S.mansoni* egg positive individuals (40.54%) were more affected than *S.mansoni* egg negative ones (10.31%) with a significant difference (P=0.000) (Table 10).

Table 10. Categories of PPF by parasitological characteristics of *S.mansoni* infection among study participants in Finchaa Sugar Estate, Western Ethiopia, 2012-2014.

| *Schistosoma mansoni* status | Category of PPF based on image pattern* | | | Total n (%) | P |
|---|---|---|---|---|---|
| | No PPF n (%) | Indeterminate n (%) | Definite PPF n (%) | | |
| Positive | 46 (31.08) | 42 (28.38 ) | 60 (40.54 ) | 148 (100 ) | 0.000 |
| Negative | 335 (73.46 ) | 74 (16.23) | 47(10.31 ) | 456 (100) | |
| Total | 381(63.08) | 116 (19.21) | 107(17.71) | 604 (100) | |

*Richter *et al*., 2000.

Among study subjects with periportal fibrosis, males were more affected by periportal fibrosis (68.23%) than females (31.77%) and children age 5-10 years old presented with the lowest overall definite periportal fibrosis (9.35%) (Table 11).

Table 11. Prevalence and distribution of definite PPF by sex and age among study participants (n=107) in Finchaa Sugar Estate, Western Ethiopia, 2012-2014.

| **Sex** | Definite PPF | | | |
|---|---|---|---|---|
| | Mild n (%) | Moderate n (%) | Severe n (%) | Total n (%) |
| Male | 48(44.86) | 17(15.89) | 8(7.48) | 73 (68.23) |
| Female | 22(20.56) | 7(6.54) | 5(4.67) | 34 (31.77) |
| Total | 70(65.42) | 24(22.43) | 13(12.15) | 107 (100.00) |
| **Age(years)** | | | | |
| 5-10 | 8(7.48) | 2(1.87) | 0(0) | 10 (9.35) |
| 11-20 | 13(12.15) | 7(6.54) | 0(0) | 20 (18.69) |
| 21-30 | 27(25.23) | 4(3.74) | 5(4.67) | 36 (33.64) |
| >30 | 22(20.56) | 11(10.28) | 8(7.48) | 41 (38.32) |
| Total | 70(65.42) | 24(22.43) | 13(12.15) | 107(100.00) |

Individuals with moderate to severe PPF had significantly higher *S.mansoni* egg counts compared to individuals with mild or no PPF (Figure 7).

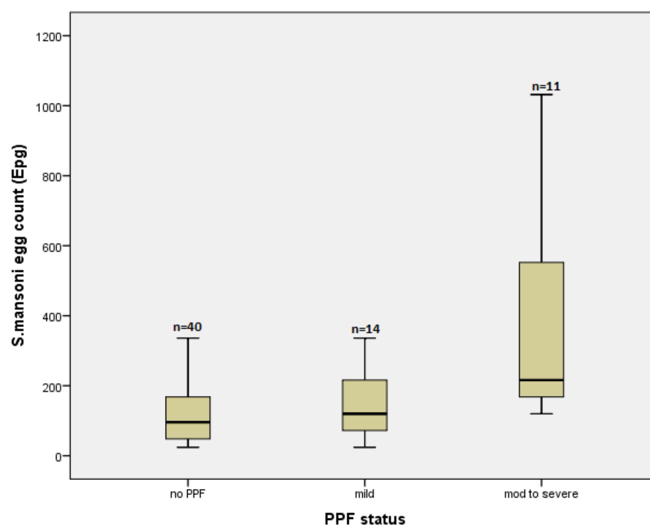
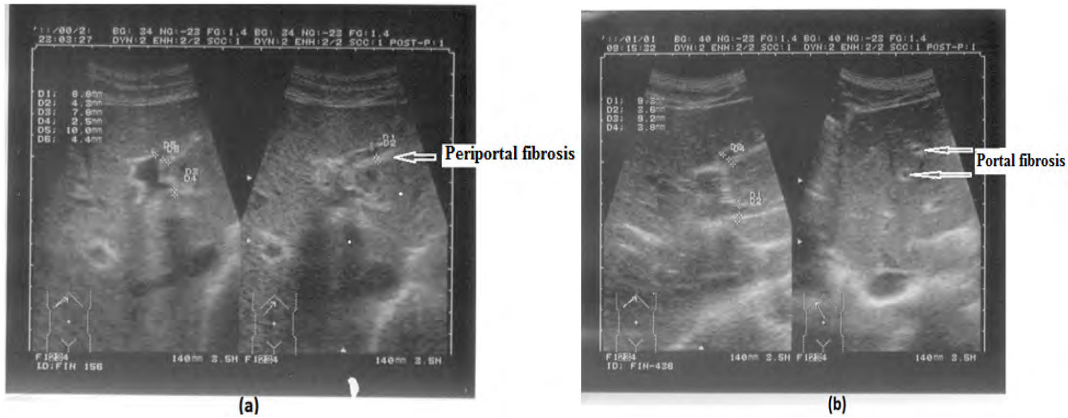



Figure 7. The expression of *S.mansoni* egg counts and PPF status, among study participants in Finchaa Sugar Estate, Western Ethiopia, 2012-2014.

**Note:** ***The thick line inside each box represents the median value. The lower and upper edge of each box indicates the 25$^{th}$ and 75$^{th}$ percentiles, respectively. The lower and upper whiskers represent the lower and upper values (range), respectively, excluding outliers.***




Figiure 8. Ultrasonographic images in hepatic schistosomiasis: (a) Periportal fibrosis (Grade C Mild fibrosis) (b) Portal fibrosis (Grade D moderate fibrosis) among study participants in Finchaa Sugar Estate, Western Ethiopia, 2012-2014.

## 4.4. Malaria-*Schistosoma mansoni* co- infections

Out of all 810 study participants about 98 (12.10%) were infected both with malaria and *Schistosoma mansoni.*

### 4.4.1. Malaria-*Schistosoma mansoni* co- infection prevalence and parasite density

Mean parasites/µL in malaria, and malaria-*Schistosoma mansoni* co-infected individuals is 574 and 1059, respectively.On the other hand, mean egg count in *Schistosoma mansoni*, and malaria-*Schistosoma mansoni* co-infected individuals were 241 and 389, respectively.Higher means of parasites/µL and Epg were detected in malaria-*Schistosoma mansoni* co-infected individuals than mono infected ones (Table 12).

Table 12. Prevalence of mean parasite density of malaria and schistosome parasites (n=552), among malaria-*Schistosoma mansoni* co-infection study participants in Finchaa Sugar Estate, Western Ethiopia, 2012-2014.

| Infection type | Parasite density | | |
|---|---|---|---|
| | Minimum | Maximum | Mean±Std. |
| Malaria (parasites /µL) (n=228) | 40 | 5320 | 574±1024.21 |
| *Schistosoma mansoni* (Epg) (n=226) | 24 | 960 | 241±250.75 |
| Malaria + *Schistosoma mansoni* (n=98): | | | |
| Parasites /µL | 160 | 5440 | 1059±1410.69 |
| Egg count (Epg) | 70 | 1080 | 389±300.98 |

Of 228 study participants about 42 (18.42%) malaria-*Schistosoma mansoni* co-infected individuals had higher mean parasites/µL (> 574 parasites/µL) compared to only malaria positive individuals  8 (3.51%). On the other hand, of 203 study participants about 60 (29.56%) malaria-*Schistosoma mansoni* co-infected individuals had higher mean Epg (> 254 Epg) compared to only *Schistosoma mansoni* positive individuals 13 (6.40%).

Intensity of *S.mansoni* infection (Mean Epg) in malaria-*Schistosoma mansoni* co-infected females was slightly greater than males with mean Hb of 399 and 385 respectively. Regarding age, mean Epg was high among age groups between 5-9 (665) and 10-14 (455) years than other age groups (Table 13).

Table 13. Intensity of *S.mansoni* infection in malaria-*Schistosoma mansoni* co-infected individuals, stratified by sex and age (n=98) among malaria-*Schistosoma mansoni* co-infection study participants in Finchaa Sugar Estate, Western Ethiopia, 2012-2014.

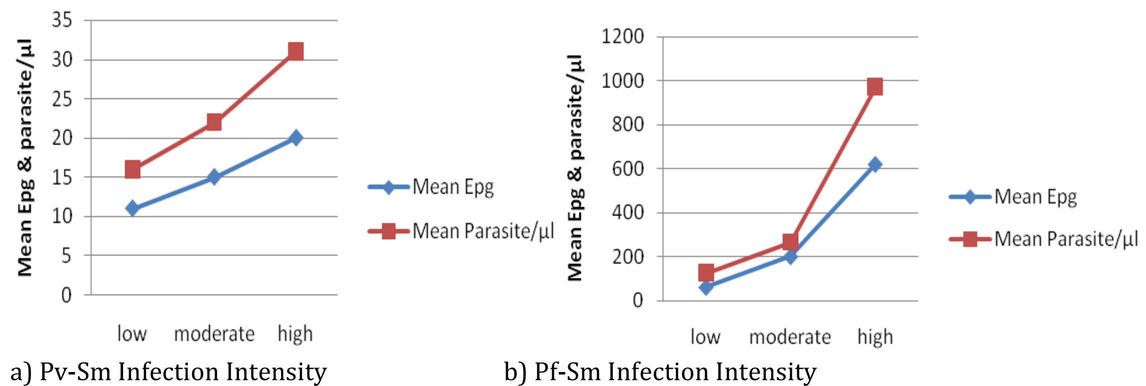
| | Intensity of *S.mansoni* infection (Epg) in malaria-*Schistosoma mansoni* co-infected individuals | | | Total | Mean Epg |
|---|---|---|---|---|---|
| **Sex** | Light (1-100) n (%) | Moderate (101-400) n (%) | Heavy (>400) n (%) | n (%) | |
| Male | 18(18.38) | 21(21.43) | 32(32.65) | 71(72.45) | 399 |
| Female | 4(4.08) | 8(8.16) | 15(15.31) | 27(27.55) | 385 |
| **Age (year)** | | | | | |
| 5-9 | 1(1.02) | 3(3.06) | 11(11.22) | 15(15.31) | 665 |
| 10-14 | 3(3.06) | 5(5.10) | 11(11.22) | 19(19.39) | 455 |
| 15-19 | 4(4.08) | 2(2.04) | 7(7.14) | 13(12.27) | 317 |
| 20-24 | 8(8.16) | 10(10.20) | 5(5.10) | 23(23.47) | 300 |
| 25-29 | 3(3.06) | 5(5.10) | 2(2.04) | 10(10.20) | 235 |
| ≥30 | 5(5.10) | 9(1.11) | 4(4.08) | 18(18.34) | 184 |

About 29 (29.59 %) of malaria-*Schistosoma mansoni* co-infected individuals were within 501-5000 parasites/µL and with heavy intensity of *S.mansoni* infection (> 400 Epg). Mean (parasites/µL) had been also increased as intensity of *S.mansoni* infection increased (Table 14).

Table 14. Intensity of *S.mansoni* and malaria parasite infection in malaria-*Schistosoma mansoni* co-infected individuals (n=98) among malaria-*Schistosoma mansoni* co-infection study participants in Finchaa Sugar Estate, Western Ethiopia, 2012-2014.

| Intensity of malaria parasite infection (parasites/µL) | Intensity of *S.mansoni* infection (Epg) | | | | Mean (parasites/µL) |
|---|---|---|---|---|---|
| | Light (1-100) n (%) | Moderate (101-400) n (%) | Heavy (>400) n (%) | Total n (%) | |
| 1-50 | 1(1.02) | 0(0) | 1(1.02) | 2(2.04) | 80 |
| 51-500 | 21(21.43) | 16(16.33) | 10(10.20) | 47(47.96) | 200 |
| 501-5000 | 0(0) | 10(10.20) | 29(29.59) | 39(39.80) | 1342 |
| >5000 | 0(0) | 0(0) | 10(10.20) | 10(10.20) | 4180 |

As infection intensity of malaria parasite increased, there was also an increase in intensity of *S.mansoni* infection.However, infection intensity was higher in *P.falciparum-S.mansoni* co-infected individuals compared to *P.vivax-S.mansoni* co-infected ones (Figure 9a, b).



a) Pv-Sm Infection Intensity b) Pf-Sm Infection Intensity

Figure 9. Synergistic effects of malaria parasites and *S.mansoni* infection intensity among malaria-*Schistosoma mansoni* co-infection study participants in Finchaa Sugar Estate, Western Ethiopia, 2012-2014.

In co-infection, more intensity of infection 72 (73.45%) was due to *P.falciparum*. High mean Epg (752) was due to mixed infection followed by *P.falciparum* with a significant difference between malaria parasite species (P=0.000) (Table 15).

Table 15. Intensity of *S.mansoni* infection in malaria parasite infected individuals (n=98) stratified by malaria parasite species among malaria-*Schistosoma mansoni* co-infection study participants in Finchaa Sugar Estate, Western Ethiopia, 2012-2014.

| Malaria parasite | Intensity of *S.mansoni* infection (Epg) | | | Total n (%) | Mean Epg | P |
|---|---|---|---|---|---|---|
| | Light (1-100) n (%) | Moderate (101-400) n (%) | Heavy (>400) n (%) | | | |
| *P.vivax* | 11(11.22) | 6(6.12) | 3(3.06) | 20(20.41) | 189 | |
| *P.falciparum* | 7(7.14) | 24(24.49) | 41(41.84) | 72(73.47) | 414 | 0.000 |
| Mixed (Pf+Pv) | 0(0) | 0(0) | 6(6.12) | 6(6.12) | 752 | |

### 4.4.2. Haemoglobin levels in malaria-*Schistosoma mansoni* co-infected individuals

The independent sample t-test mean comparision showed that except for malaria positive and *Schistosomiasi mansoni* positive cases (P=0.093),there were significant differences between both singly and co-infected and the negative cases ($p<0.05$) (Table 16).

Table 16. Gender and infection status based comparison of haemoglobin levels among malaria-*Schistosoma mansoni* co-infection study participants (n=810) in Finchaa Sugar Estate, Western Ethiopia, 2012-2014.

| | | | t-test for Equality of Means | | | |
|---|---|---|---|---|---|---|
| | | | | 95% Confidence Interval of the Difference | | |
| Independent variables | N | Mean Hb | t | Lower | Upper | P |
| **Sex** | | | | | | |
| Male | 415 | 14.06 | 3.189 | 0.175 | 0.737 | 0.001 |
| Female | 395 | 13.61 | | | | |
| **Infection status** | | | | | | |
| *Schistosoma mansoni* positive | 226 | 12.57 | -9.462 | -2.126 | -1.394 | 0.000 |
| *Schistosoma mansoni* negative | 584 | 14.33 | | | | |
| Malaria positive | 228 | 13.00 | -5.865 | -1.371 | -0.683 | 0.000 |
| Malaria negative | 383 | 14.03 | | | | |
| Malaria positive | 228 | 12.57 | 1.683 | -0.073 | 0.936 | 0.093 |
| *Schistosoma mansoni* positive | 226 | 13.00 | | | | |
| Malaria + *Schistosoma mansoni* | 98 | 11.71 | -7.190 | -3.100 | -1.760 | 0.000 |
| Uninfected | 712 | 14.14 | | | | |
| *Schistosoma mansoni* positive | 226 | 12.57 | 2.309 | 0.125 | 1.609 | 0.022 |
| Malaria + *Schistosoma mansoni* | 98 | 11.71 | | | | |
| Malaria positive | 228 | 13.00 | 3.608 | 0.591 | 2.007 | 0.000 |
| Malaria + *Schistosoma mansoni* | 98 | 11.71 | | | | |

Multiple linear regression analysis of haemoglobin level as dependent variable and infections and co-infections as Independent variables showed that malaria-*Schistosoma mansoni* co-infection was a strong significant factor for decrease in haemoglobin levels when compared to mono-infections. Malaria parasites /µL and *S.mansoni* Epg were significant negative predictors of haemoglobin levels (Table 17).

Table 17. Haemoglobin levels in relation to sex, age and infection with malaria, *Schistosoma mansoni* and malaria-*Schistosoma mansoni* co-infections, among malaria-*Schistosoma mansoni* co-infection study participants (n=611) in Finchaa Sugar Estate, Western Ethiopia, 2012-2014.

| | | 95.0% Confidence Interval for B | | |
|---|---|---|---|---|
| Independent variable | B* | Lower Bound | Upper Bound | P value |
| Sex | 0.540 | 0.270 | 0.809 | 0.000 |
| Age | 0.345 | 0.273 | 0.418 | 0.000 |
| Malaria | 0.611 | -0.961 | -0.260 | 0.001 |
| *Schistosoma mansoni* | 0.931 | -1.513 | -0.348 | 0.002 |
| Malaria + *Schistosoma mansoni* | 1.214 | -1.648 | -0.780 | 0.000 |
| Malaria parasite /µL | -2.069 | -2.393 | -1.744 | 0.000 |
| *Schistosoma mansoni* EPG | -1.282 | -1.575 | -0.989 | 0.000 |

*Coefficient of regression

Univariate logistic regression analysis of anaemia status (normal vs anaemia/severe anaemia) as dependent variable and infections and co-infections as risk factors showed that *Schistosoma mansoni* was a miled (P=0.041) risk factor whereas malaria alone (P=0.016) and malaria-*Schistosoma mansoni* co-infections were strongly (P=0.000) associated with increased risk for anaemia (Table 18).

Table 18. Malaria, *Schistosoma mansoni* and malaria-*Schistosoma mansoni* co-infections as risk factors for anaemia among malaria-*Schistosoma mansoni* co-infection study participants in Finchaa Sugar Estate, Western Ethiopia, 2012-2014.

| Risk factors | | | 95% C.I.for EXP(B) | | P value |
|---|---|---|---|---|---|
| | B* | EXP(B)** | Lower | Upper | |
| Malaria | 0.775 | 0.461 | 0.245 | 0.864 | 0.016 |
| *Schistosoma mansoni* | 0.523 | 0.593 | 0.359 | 0.979 | 0.041 |
| Malaria + *Schistosoma mansoni* | 2.243 | 0.106 | 0.052 | 0.217 | 0.000 |

*Coefficient of regression

**Odds Ratio

Individuals co-infected with *P.falciparum* and *S.mansoni* had lower mean Hb concentration (11.29) compared to mono- infected individuals (Figure 10).

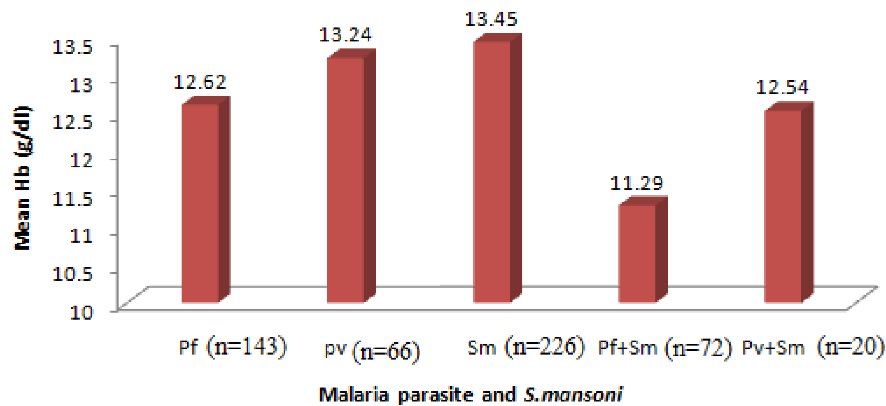



Figure 10. Mean Hb levels in *S.mansoni* alone, *S.mansoni* and *P.falciparum / P. vivax* co-infection among malaria-*Schistosoma mansoni* co-infection study participants in Finchaa Sugar Estate, Western Ethiopia, 2012-2014.

The highest percentage of anemic cases was due to *P.falciparum- S.mansoni* co-infection (10.53%) followed by *P.vivax-S.mansoni* co-infection (8.30%) compared to malaria or *S.mansoni* mono infections (Figure 11).

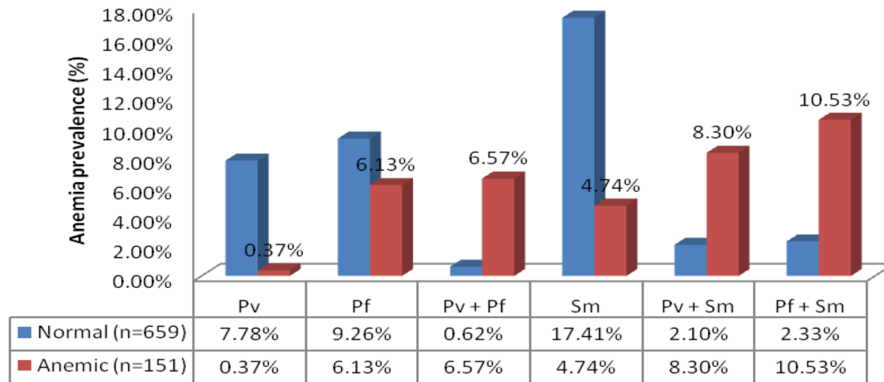



Figure 11. Anaemia prevalence in Pv/Pf/Sm mono and co-infected individuals among malaria-*Schistosoma mansoni* co-infection study participants in Finchaa Sugar Estate, Western Ethiopia, 2012-2014.

### 4.4.3. Cytokine levels in malaria-*Schistosoma mansoni* co-Infected individuals and non-infected controls

Malaria-*Schistosoma mansoni* co-infected individuals had relatively higher Th2 anti-inflammatory cytokines (IL-10 and IL-4) and lower Th1 inflammatory cytokines (IFN-ϒ and TNF-a) when compared to mono-infected groups (Figure 12).

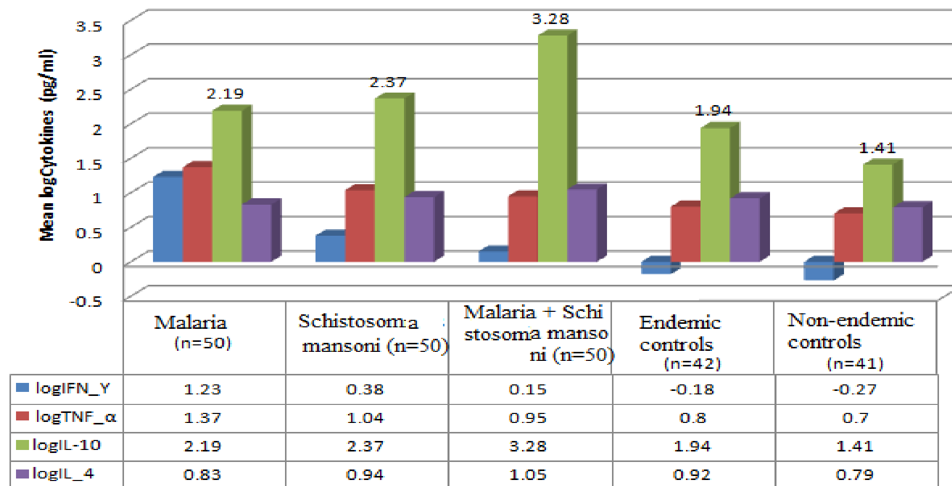



Figure 12. Expression of cytokines among the five arms of the study groups, among malaria-*Schistosoma mansoni* co-infection study participants in Finchaa Sugar Estate, Western Ethiopia, 2012-2014.

#### 4.4.4. PPF status in malaria-*Schistosoma mansoni* co-Infected individuals

Of total 107 fibrotic individuals, more males were fibrotic (61.68 %) than females (38.32%). As age increased, the prevalence of liver fibrosis also increased in moderate cases (Table 19).

Table 19. Definite PPF category in liver fibrotic patients (n =107) stratified by sex and age among malaria-*Schistosoma mansoni* co-infection study participants in Finchaa Sugar Estate, Western Ethiopia, 2012-2014.

| | Definite PPF Category | | | |
|---|---|---|---|---|
| Study group | Mild n (%) | Moderate n (%) | Severe n (%) | Total n (%) |
| **Sex** | | | | |
| Male | 37(34.58) | 27(25.24) | 2(1.87) | 66 (61.68) |
| Female | 21(19.63) | 18(16.82) | 2(1.87) | 41 (38.32) |
| Total | 58(54.21) | 45(42.06) | 4(3.74) | 107(100.00) |
| **Age (years)** | | | | |
| 5-9 | 4(3.74) | 0(0) | 0(0) | 4 (3.74) |
| 10-14 | 7(6.54) | 0(0) | 0(0) | 7 (6.54) |
| 15-19 | 13(12.15) | 4(3.74) | 0(0) | 17(15.89) |
| 20-24 | 16(14.95) | 5(4.67) | 0(0) | 21(19.63) |
| 25-29 | 10(9.35) | 16(14.95) | 0(0) | 26(24.30) |
| ≥30 | 8(7.48) | 20(18.69) | 4(3.74) | 32(29.91) |

Univariate logistic regression analysis of PPF status (no PPF vs mild/severe PPF) as dependent variable and infections and co-infections as risk factors showed that malaria, *Schistosoma mansoni* and malaria-*Schistosoma mansoni* co-infections were identified as risk factors for PPF.However, malaria was not identified as risk factors for PPF (Table 20).

Table 20. Malaria, *Schistosoma mansoni* and malaria-*Schistosoma mansoni* co-infections as risk factors for PPF (n=107) among malaria-*Schistosoma mansoni* co-infection study participants in Finchaa Sugar Estate, Western Ethiopia, 2012-2014.

| Risk factors | | | 95% C.I.for EXP(B) | | P value |
|---|---|---|---|---|---|
| | B* | EXP(B)** | Lower | Upper | |
| Malaria | 0.373 | 0.688 | 0.374 | 1.267 | 0.230 |
| *Schistosoma mansoni* | 1.985 | 0.137 | 0.077 | 0.246 | 0.000 |
| Malaria + *Schistosoma mansoni* | 1.360 | 0.257 | 0.106 | 0.620 | 0.002 |

*Coefficient of regression

**Odds Ratio

### 4.4.5. Cytokines and PPF

In the relationship between PPF and cytokine expression in malaria-*Schistosoma mansoni* co-infected individuals, the degree of fibrosis increased as the levels of Th1 inflamatory cytokines IFN_ϒ and TNF_α decreased (Figure 13).

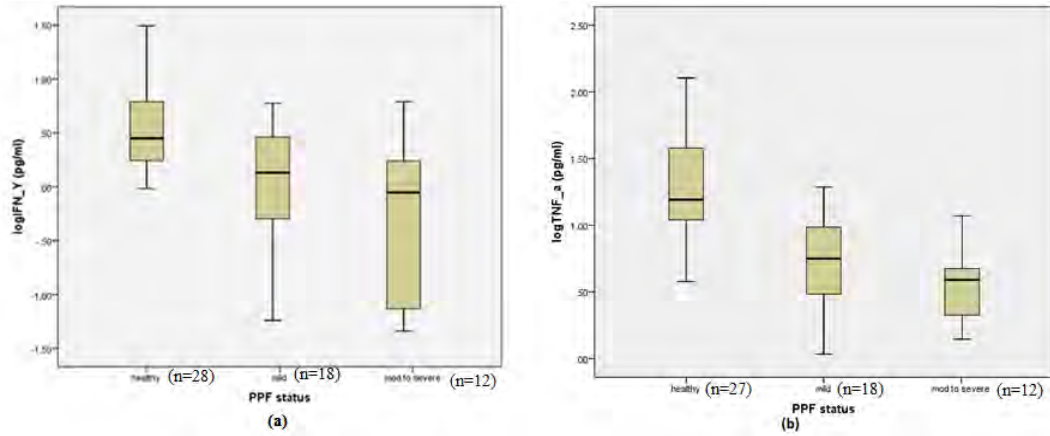



Figure 13. The expression of inflammatory cytokines and PPF status: a) logIFN_ϒ and b) log TNF_α (pg/ml), among malaria-*Schistosoma mansoni* co-infection study participants in Finchaa Sugar Estate, Western Ethiopia, 2012-2014.

On the otherhand, the levels of Th2 anti-inflamatory cytokines, IL_10 and IL_4 increased as the degree of fibrosis increased (Figure 14).

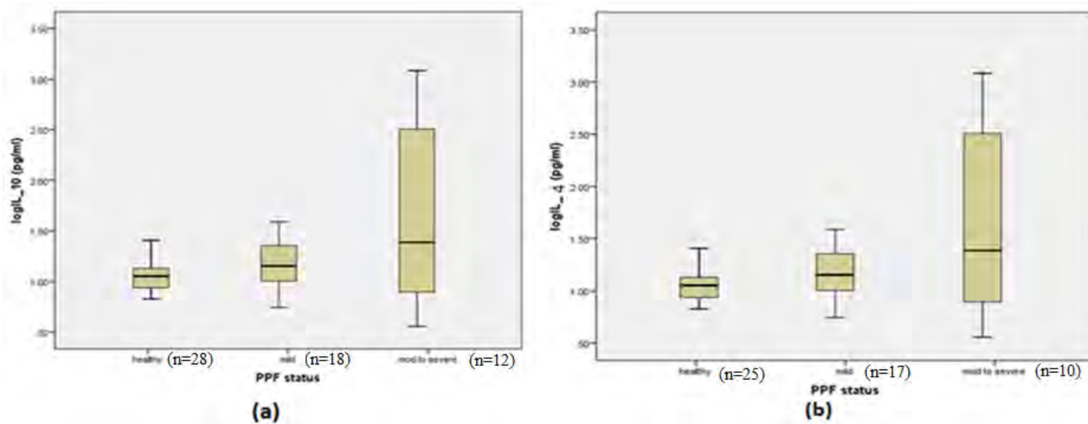



Figure 14. The expression of anti-inflammatory cytokines and PPF status: a) logIL_10 and b) logIL_4 (pg/ml), among malaria-*Schistosoma mansoni* co-infection study participants in Finchaa Sugar Estate, Western Ethiopia, 2012-2014.

Malaria-*Schistosoma mansoni* co-infected individuals with definite PPF had relatively higher levels of IL-10 and IL-4 when compared to co-infected individuals with out PPF. On the other hand, non-infected individuals with definite PPF had higher levels of IFN-Y and TNF-a when compared to non-infected individuals without PPF (Figure 15).

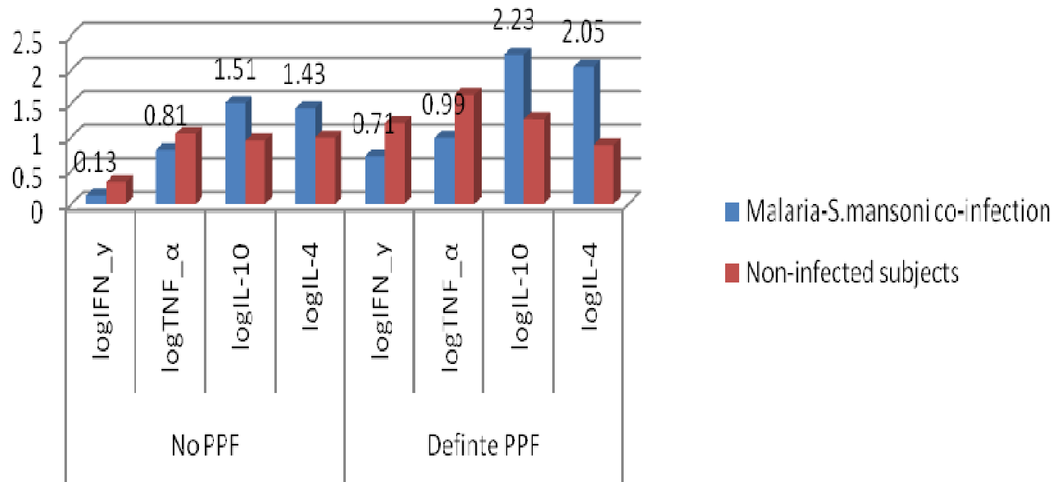



Figure 15. Plasma cytokine levels between malaria-*Schistosoma mansoni* co-infected (n=24) and non-infected individuals (n=61), stratified by PPF status, among malaria-*Schistosoma mansoni* co-infection study participants in Finchaa Sugar Estate, Western Ethiopia, 2012-2014.

# 5. DISCUSSION

The findings of this study showed that malaria and *S. mansoni* co-infection and co-morbidity reciprocally increase parasite densities, infection intensities, anaemia, IL-10 and the degree of PPF.

Despite the continuous efforts made to control malaria during the past decade, infections with malaria remained high in the country due to water resource developments (FMoH, 2006).Hence, according to the World Malaria Report in 2011 (WHO, 2011b), Ethiopia was categorized among the four countries with a 25 to 50% reduction in new malaria cases. However,

The overall malaria prevalence in the study population was lower than what was reported by Chala and Petros (2011) which could be a positive reflection of the malaria control efforts (WHO, 2011c).

Anaemia is a major risk factor associated with greatly increased morbidity and mortality for young children and pregnant women (WHO, 2007). This is because children are the highest at risk groups for parasitic infections and pregnant women are highly affected by blood lose that leads to anaemia through the reduction of the levels of Hb.

In the present study, among 228 (28.15%) subjects with malaria infection, *P. falciparum* and *P. vivax* accounted for 62.72% and 28.94%, respectively.Indicating that *P. falciparum* is the predominant species.The higher *P. falciparum* prevalence rate could be attributed to problems with emerging drug resistance (MOP, 2015),which unfortunately was not part of the present study.

The present study showed that malaria parasite prevalence decreased with increasing age which is a normal trend in malaria endemic areas and is related to the development of anti-malarial specific partial immunity.This is consistent with previous studies conducted by McGregor (1972), who reported that immunity builds up progressively in children as age increases. Similarly malaria was observed to be associated with gender in which, males were more affected than females, which conforms to the report by Alemu *et al*. (2012) in Gonder,northern Ethiopia, in which the prevalence of malaria was observed to be higher in males than in females. The higher prevalence of malaria observed in males than in females may be attributed to the activities of most males in the study area, such as guards that stay outside of their houses in the evening. This would expose them to the outdoor bite of mosquitoes and increase the probability of getting infected with the malaria parasites (Killeen *et al.,* 2016).

In the current work, it was shown that there was a statistically significant correlation between high parasitemia and anaemia which is a well established feature of increased prevalence of anaemia with increase in malaria parasite density. The observation that low Hb level is associated with higher intensity of malaria parasites is in line with a number of previous studies (Chang and Stevenson, 2004; Helleberg *et al.,* 2005) which demonstrated that malaria is the major cause of reduced haemoglobin concentration and anaemia due to rapture of parasitized red blood cells as well as non-specific immune (Apoptosis) destruction of non-parasitized red blood cells (Totino *et al*., 2010). Expectedly, mixed infections with *P. falciparum* and *P. vivax* were associated with more

severe anaemia compared with single infections; a phenomenon that may be ascribed to the synergistic effect of the two malaria parasites on aggravating anaemia (Mayxay *et al.*,2004).

The study also showed that malaria positive patients had significantly higher mean levels of INF-ϒ and TNF-α when compared with the uninfected, indicating that parasite induced inflammatory response results in the production of higher IFN-ϒ and TNF-α during acute malaria infections as shown by Kurtzhals *et al.* (1998) that the low IL-10 plasma levels associated with acute malaria could be attributed to the modulatory role of IL-10 during early infection of malaria parasites.This is in agreement with the findings by Awandare *et al*.(2006) who reported that in human malaria infections elevated levels of both IFN-γ and TNF-α were associated with acute malaria.

In the present study, the overall prevalence of *S. mansoni* was 27.9%, which indicates relatively similar infection rate that was reported from previous studies conducted in the same area by Erko *et al*. (2003) who reported a prevalence rate of 26%. In the present study, significant variations in the prevalence of *S. mansoni* infection was observed among the villages, with Village A (Camp 7) with highest *Schistosoma mansoni* infection similar to the report by Dufera *et al*. (2014).This shows poor control measures since the report 2 years ago. The fact that significantly more males were infected by *S.mansoni* than females indicates that the infection is an occupational hazard as more males work on the irrigated farms than females and get more exposure to infected water bodies than females. Such difference in male and female exposure to schistosome parasite has been reported by Assefa *et al*. (2013) and Wolde-Michael *et al*. (1999), from other community studies. The high *S.mansoni* infection among individuals age under 15

years in Finchaa is typical of schistosomiasis prevalence in school-aged children in all schistosomiasis endemic localities (Brooker *et al*., 2006; Mboera *et al*., 2006).The highest intensity of *S. mansoni* infection in the school-age group detected in the present study is also similar to the earlier reports from Jimma town, southwestern Ethiopia (Mengistu *et al*., 2011) and from Kenya (Handzel *et al*., 2003) as schistosomiasis prevalence and infection intensity are generally age dependent on water contact patterns and duration of exposure to infection (Hotez *et al*, 2004; Briand *et al*, 2005).The current finding reflects the general pattern of *Schistosoma mansoni* in that only a small fraction (23.89%) of the infected individuals were heavily infected but most cases (42.47%) had light burden of infection and the effects on the patients can be considered minimal.

Lower Hb levels were observed in *Schistosoma mansoni* positive individuals compared to the uninfected ones.This study also revealed that, there is a clear association between *Schistosoma mansoni* infection intensity and Hb levels in that, light infections were associated with higher mean Hb levels than heavy ones and the probability of becoming anemic is increased as infection intensity increases, with a significant difference. This is in agreement with the work of Friedman *et al*. (2005) who reported the risk of anaemia to be correlated with human *Schistosoma mansoni* infection intensity.

The high levels of IL-10 and IL-4 in *S.mansoni* egg-negative individuals and higher mean levels of IFN-ϒ and TNF-α in *S.mansoni* egg-positive individuals, detected in the present study is similar to the report by Abebe *et al*. (2014) from a study in Northern Ethiopia.In this regared, Sánchez-Arcila *et al*.(2014) reported that low levels of inflammatory mediators were associated predominantly with uninfected individuals.

In the present study, image pattern and portal vein wall thickness based ultrasound examination detected the two types of hepatic schistosomiasis: periportal fibrosis and portal fibrosis which is in line with studies conducted by Barsoum *et al*. (2013) and Baptista et *al*. (1988) who reported that periportal fibrosis can represent the first stage in the evolution of portal fibrosis, and often connotes an aggressive or progressive process.

The prevalence of definite PPF among *S.mansoni* egg positive and egg negative individuals were 40.54 % and 10.31%, respectively with a significant difference indicating that *S.mansoni* egg positive individuals to be more fibrotic than *S.mansoni* egg negative individuals. The 10.31% PPF among egg-negative could be attributed to HCV (Abdel-Kader *et al*., 1997; Blanton *et al.,* 2002) or is a miss diagnosis.

The finding that children age 5-10 years old had the lowest overall risk of definite periportal fibrosis (9.35%) is consistent with the report of Booth *et al*. (2004a) where they concluded that children presented the lowest overall risk of fibrosis. They suggested that this was probably because children are not exposed long enough for schistomal lesions to produce periportal fibrotic effect. The basis for the higher prevalence of PPF in males than in females, a finding that has also been reported by other researchers (Henri *et al*., 2002; Booth *et al*., 2004a), may be explained by the relatively higher burden of infection in the males, a factor which is correlated with the intensity of exposure of the males to the infection as they work in the irrigated farms.

On the other hand, the relationship between *S.mansoni* egg count and PPF that showed individuals with moderate to severe PPF to have significantly higher *S.mansoni* egg

counts compared to individuals with mild PPF, was contrary to what Abebe *et al*. (2014) reported from Gonder northern Ethiopia.These contradictory results could be attributed to differences in PPF severity, schistosomal infection stage (acute or chronic), in which acute infection could lead to high egg counts. However, this was not part of the present study). Furthermore, in the present study a positive association between intensities of *S.mansoni* infection and degrees of fibrosis was observed, which is in line with studies done elsewhere (Cheever and Andrade, 1967; Mohamed-Ali *et al*., 1999; van der Werf *et al*., 2003; Berhe *et al*., 2007), but contrary to Booth *et al*. (2004a) and Ribeiro de Jesus *et al*. (2004), indicating that the intensities of infection and degrees of fibrosis apparently fluctuate considerably between different endemic localities. The factors for such variations in the clinical outcomes could be the difference in the genetic variabilities of the parasite and the host (Aemro *et al*., 2015).

Concomitant parasitic infections are common events in different regions of the world. Malaria and schistosomiasis are among the parasitic infections that share common geographical areas in various tropical regions, especially in sub-Saharan African countries (Mwangi *et al*., 2006). Understanding the associations or interactions and reciprocal effects of these infections and their contribution in increasing morbidity is important as findings may guide the design of integrated disease control strategies.

The overall prevalence of malaria co-infection with *Schistosoma mansoni* was 12.10% an addition to very few malaria and *Schistosoma mansoni* co-infection studies in Ethiopia.However, the co-prevalence determined was lower compared to the report from different parts of the Country: southern Ethiopia, 22.6 % (Mulu *et al*., 2013) and 23.1%

(Degarege *et al*., 2012); and northwestern Ethiopia, 19.5% (Getie *et al*., 2015). The low co-infection prevalence in the present study could be attributed to chemotherapy and the repeated mass PZQ administrations taken in the community twice a year as it is an Agro-industrial enterprise.

The present study has shown that co-infection due to malaria and *Schistosoma mansoni* infections reciprocally leads to an increase in parasite density. This is similar to the report of Getie *et al*. (2015), a study conducted in northwest Ethiopia that indicated co-infected patients with a moderate to heavy egg burden of *S. mansoni* also had significantly higher mean malaria parasitemia.

Most studies that examined naturally occurring co-infection in humans indicated that co-infection with malaria parasites and schistosome has an increased detrimental effect on the host, both in terms of pathology and in terms of immunological response (Abruzzi and Fried, 2011). The direction of this response seems to depend on the worm burden, the level of malaria parasitaemia, the *Plasmodium* spp. responsible and host age.In addition, reports from Oswald *et al*. (1992) showed that chronic schistosomiasis may lead to the increase in parasitemia, suggesting during chronic schistosomiasis infection aTh2 cytokine, IL-10, is produced and then suppresses macrophages, that kill pRBC, hence indirectly increases malaria parasitaemia. In the present study higher parasitaemia and schistosome Epg were detected in malaria-*Schistosoma mansoni* co-infected individuals compared to the mono- infected ones. Contrary to the present findings, Sokhna *et al*. (2004) from Senegal,on *S.mansoni* in children reported that co-infected children had lower malaria parasitaemia than singly infected children.This discrepancy

in the present findings may have been caused by difference in the population group, acute schistosomiasis and schistosome spp.

In the current work an age effect was observed in malaria-*Schistosoma mansoni* co-infection, in which lower age groups had higher levels of mean Epg than older age groups, showing that school-aged children are the most affected group. This is similar to the report of Brooker *et al*. (2006) and Mboera *et al*. (2006).

The present study also indicated that in malaria-*Schistosoma mansoni* co-infected study participants malaria parasitaemia and heavy intensity of *S. mansoni* infection were significantly associated with lower mean Hb, which is in line with the findings of Koukounari *et al*. (2008).

The observed increase in malaria parasite density in *P.falciparum*-*S.mansoni* co-infected individuals compared to *P.vivax*-*S.mansoni* co-infected ones can be explained by the fact that *P.falciparum* infect both young and old RBCs.This would result in aggravation of infection intensities in malaria-*Schistosoma mansoni* co-infected individuals leading to a lower mean Hb level when compared to malaria or *Schistosoma mansoni* mono-infected individuals.Hence, the basis for malaria mediated anaemia (Menendez *et al*., 2000; McDevitt *et al*., 2004; Chang and Stevenson, 2004;Verhoef, 2010) and that for *S.mansoni* (Sturrock *et al*., 1996; Stephenson *et al*.,2000; Friedman *et al*., 2005) which has been elucidated by several investigators can be considered relevant to the findings of the present study.Given the distinct mechanisms by which malaria parasites and *S.mansoni* reduce haemoglobin concentrations, it is probable that malaria and

*Schistosoma mansoni* are predictors of anaemia and their co-infection would be synergistic in their ability to cause anaemia.Therefore, their combined presence and interaction to enhance the risk of anaemia may be responsible for the low haemoglobin concentration in concurrently infected individuals than those infected with either malaria or *Schistosoma mansoni* alone.

The present study showed that, in all cases there were significant differences in Hb levels between male and female patients infected singly or co-infected.Thus, malaria - *Schistosoma mansoni* co-infection was a strong significant factor for decrease in haemoglobin levels when compared to mono-infections and this was dependent on malaria intensity and *S.mansoni* burden.Therfore, *Schistosoma mansoni* and malaria-*Schistosoma mansoni* co-infections were significant risk factors of anaemia in the community, since co-infection with malaria and *Schistosoma mansoni* are significantly associated with increased risk for anaemia. Comparisions of the haemoglobin levels among the five categories of infections (*P.falciparum*, *P. vivax, S.mansoni, P.falciparum -S.mansoni* and *P.vivax-S.mansoni*), the mean Hb concentration in *P.falciparum-S.mansoni* co-infection to be greatly lower than the other categories which further provided clear evidence that concurrent *P. falciparum* and *S. mansoni* infection highly enhances the risk of lower Hb levels and anaemia. Similar findings have been reported by other studies (Nacher, 2002; Basavaraju and Schwantz, 2006). The observed association of malaria-*Schistosoma mansoni* co-infection with lower haemoglobin levels as compared to infections with only malaria and *Schistosoma mansoni* alone has been explained by Nacher *et al*. (2001b), that during malaria parasitic infection, pre-existing

helminth infections are associated with decreased haemoglobin concentration and reticulocytosis, which may aggravate malarial anaemia. Nacher *et al*. (2001d), also presented data from Thailand that concurrent helminth infections among malaria patients are associated with increased malaria parasite gametocyte carriage, which was again negatively correlated with haemoglobin concentration.These findings demonstrate a synergistic interaction of *P. falciparum* and *S. mansoni* infections as the etiology of anaemia with increased *P. falciparum* parasite density being responsible for a more severe outcome.However,it is understandable that *P.vivax* as a less severe pathogen did not have such a consequence in co-infection with schistosomiasis.

Overall, the present work has presented evidence that *S. mansoni* infection could aggravate malaria parasitemia in *P.falciparum* co-infection leading to malaria related anaemia and likewise *P.falciparum* infection in *Schistosoma mansoni* patients would enhance infection intensity and anaemia. This finding is similar to what was reported by Lalloo *et al.* (2007).Thus, such understanding of the interactions of malaria and *Schistosoma mansoni* and their effect on anaemia is a necessary factor in planninig community-based interventions in endemic areas where co-infection with the two parasites is common.

However, the shortcoming of the present study is that it did not adjust for the effect of other determinants such as nutritional deficiencies (including folate, vitamin B12 and vitamin A) that might play key roles in affecting the level of anaemia.

Regarding immune consequences of malaria and *Schistosoma mansoni* co-infection, it has been hypothesized that helminth infections interfere with immune responses to *P. falciparum* infection by inducing production of Th2, characterised by production of cytokines such as IL-4, IL-5, IL-10, IL-13 as well as IgE (Graham 2001; Spiegel *et al*, 2003; Maizels *et al*, 2004) and cytokines contribute to the development of protective immunity to both malaria and *Schistosoma mansoni* (Booth *et al*., 2004a). In the present study, comparisons of cytokine profiles among the five arms of the study categories (malaria only, *Schistosoma mansoni* only, malaria- *Schistosoma mansoni* co-infection, endemic and non endemic controls) showed that malaria- *Schistosoma mansoni* co-infected individuals had higher Th2 anti-inflammatory cytokines (IL-10 and IL-4) and lower Th1 inflammatory cytokines (IFN-ϒ and TNF-a) when compared to mono-infected groups.This is supported by the findings of Hartgers and Yazdanbakhsh (2006) who reported that chronic infection with *S.mansoni* and *Schistosoma mansoni* dominated malaria co-infection induces Th2 cytokines.

As age increased, the prevalence of liver fibrosis was increased. This is in line with Abebe *et al.* (2014), who reported that prevalence of PPF had a sharp rise in the age range of 10.1 to 20 years and reached its peak in the age range of 20.1 to 30 years.The present analysis has shown that *S.mansoni* infection and co-infections are risk factors for PPF in the community.

The assessment of cytokine responses in the study patients with hepatic fibrosis showed its association with a type 2 cytokine profile (the production of IL-10) and lower values of IFN-ϒ and TNF-α in individuals with moderate/ severe PPF. This is in line with the

report of Henri *et al*.(2002) who showed that schistosomiasis patients that have low production of IFN-ϒ have severe fibrosis and the reduction in IFN-ϒ might account for the higher risk of disease, in heavy infections.Also, in malaria-*Schistosoma mansoni* co-infection the levels of Th1 proinflammatory cytokines, IFN-ϒ and TNF-α, were decreased as the degree of fibrosis increased as opposed to the levels of Th2 anti-inflammatory cytokines IL-10 and IL-4, which increased as the degree of fibrosis increased, indicating that IL-10 and IL-4 are fibrogenic while IFN-ϒ and TNF-α are anti-fibrogenic. This is supported by report of Tiggelman *et al*. (1995) and Booth *et al*.(2004a) who reported that IL-4 is fibrogenic and stimulate the differentiation of stellate cells into myofibroblasts while IFN-ϒ is anti-fibrogenic and inhibits the production of extracellular matrix proteins by stellate cells and increases collagenase activity in the liver and is associated with protection against severe portal fibrosis.

On the other hand, malaria- *Schistosoma mansoni* co-infected individuals with definite PPF had relatively lower levels of INF-ϒ and TNF-a and higher levels of IL-10 and IL-4 when compared to malaria-*Schistosoma mansoni* co-infected individuals without definite PPF.The two antagonistic mediators (IL-4 and IFN-ϒ) (Abath *et al.,* 2006) in the presence of IL-10 therefore appear to be associated with the regulations of PPF or disease severity. This is supported by report of Hoffmann *et al*. (2000) who suggested that IL-10 could have the key regulatory role of controlling excessive Th1 and Th2 polarization responses. Therefore, the increase of IL-10 which was observed in the present study in malaria-*Schistosoma mansoni* co-infected individuals, compared to mono-infected individuals, could be an indication of a possible protective effect of IL-10 in co-infected individuals.

# 6. CONCLUSIONS

1. Malaria and schistosomiasis mansoni are common in Fincha Sugar Estate and were associated with varying degrees of morbidities which correlate well with both prevalence and intensity of infections.

2. The association of *S.mansoni* infection alone and co-infection with *P.falciparum* with anaemia and PPF dispels the opinion that, compared to the situation in Egypt and other endemic countries, schistosomiasis mansoni is not a serieous public health problem in Ethiopia.

3. The observed increased reciprocal effects on malaria parasite and *S.mansoni* parasite densities, reduced haemoglobin levels, IL-10 and IL-4 concentrations and severity of periportal fibrosis (PPF) have shed light on the possible reciprocal interactions between malaria and schistosomiasis mansoni. This finding provides evidence for the synergistic interactions causing disease severity in *P.falciparum-S.mansoni* co-infected individuals.

4. A strong association between worm burden as expressed by *S.mansoni* egg intensity and *P.falciparum* parasite density was observed which could be a dose-dependent risk factor for intensities of infections.

5. The low prevalence for malaria-*Schistosoma mansoni* co-infection, exclusion of micronutrient analysis, children under the age of 5 years and pregnant women limits interpretation of the present findings.

# 7. RECOMMENDATIONS

- Malaria- *Schistosoma mansoni* co-infection and schistosomal periportal fibrosis observed in the study area calls for a periodic deworming program to reduce transmission, worm burden and morbidity due to *S.mansoni* infection.
- Preventive chemotherapy complemented with environmental vector control measures is required for an integrated control of malaria and schistosomiasis mansoni in the study area.The malaria drug resisstance and vector insecticide resisstance must be evaluated.
- Health education, mass deworming programs proper disposal of wastes, avoiding unsafe water-contact behavior, using latrines and control of snails are must be considered.
- The presence or absence of a pre-existing helminth infection in patients from endemic areas should be carefully noted in understanding the effects of malaria-*Schistosoma mansoni* co-infections and development of standard integrated treatment protocols for both malaria and schistosomiasis mansoni.
- The influence of *Schistosoma mansoni* on the course of malaria needs more careful investigation in large populations and each species of malaria parasite infection should be studied separately, since they might have different effects on the course of *S.mansoni* infection.
- Further study need to be conducted to elucidate possible mechanisms underlying parasitological, clinical and immunological consequences of malaria-*Schistosoma mansoni* co-infection in different epidemiological settings which includes children under the age of 5 years and pregnant women with possible confounders such as micronutrient analysis.
- The possibility of synergistic interactions needs to be taken into account in planning and implementing interventions, so as to adjust control priorities and strategies accordingly.

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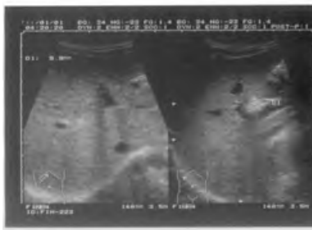
# APPENDICIES

**Appendix I**. Ultrasound image of healthy liver and definite periportal fibrosis among malaria-schistosomiasis mansoni co-infection study participants in Finchaa Sugar Estate, Western Ethiopia, 2012-2014.

The following images were taken during ultrasound examination of a cross sectional study in Finchaa Sugar Estate: Village A (camp 7), Kuyisa and Agemsa and illustrate different liver texture patterns.



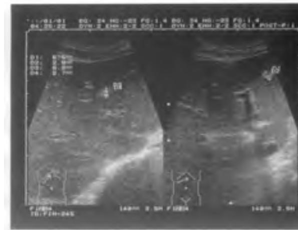
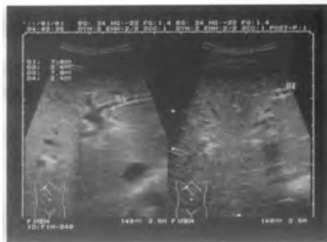


Figure 1. Healthy liver, image pattern A.

Figure 2. Intermediate PPF, image pattern B.



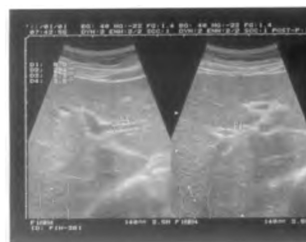
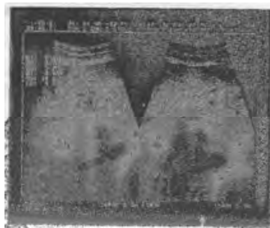


Figure 3a. Mild PPF, image pattern C.

Figure 3b. Mild PPF + Fatty Liver, image pattern C



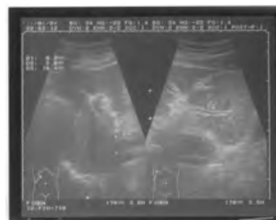


Figure 4. Moderate PPF, image pattern D.

Figure 5. Moderately PPF, image pattern E.

ID: **FIN** refers to **Finchaa**

## **Appendix II.** Material transfer agreement letter

This Material Transfer Agreement CMTA) has been prepared for use by The Department of Microbial Cellular and Molecular Biology (MCMB), Addis Ababa University (AAU) and Oslo university Hospital (OUS), Ullevaal (Oslo, Norway), in all transfer of *Research Material* (Plasma samples) related to the research project title:

***"Malaria-Schistosoma mansoni*** **co-infection and possible reciprocal effects on disease Severity in school-age children at** Finchaa **Sugar Estate, western Ethiopia".**

**Provider:** Addis Ababa University,Department of MCMB,P.O. Box 1176Phone +251-0118959216Addis Ababa, Ethiopia

**Recipient:** Oslo university Hospital (OUS), Ullevaal phone +4722119483 fax +4722118189 Oslo, Norway.

Agreed by:

**RECIPIENT**

*Institution:* **Department of Medical Biochemistry,** Oslo university Hospital (OUS), Ullevaal (Oslo, Norway)

*Name of Authorized Institution Representative:* *Hans Christian Dalsbotten Aass*

*Position of Authorized Institution Representative:* *Leader of the Flow Cytometry Core Facility*

*Signature of Authorized Institution Representative:* Hans Christian D. Aass

*Date:* *20.03.2014*

**PROVIDER**

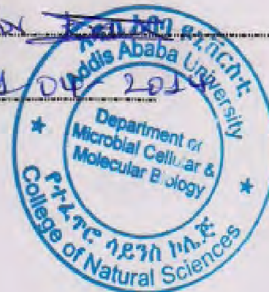
*Institution: DEPARTMENT OF MCMB, COLLEGE OF SCIENCES, AAU*

*Name of Authorized Institution Representative:* *Dr.Fassil Assefa*

*Position of Authorized Institution Representative:* *Chairman of MCMB Department*

*Signature of Authorized Institution Representative:*

*Date:* 01/04/2014



DECLERATION

I, the under signed, declare that this thesis is my original work and has not been Presented for a degree in any other university.

Mebrate Dufera, candidate Signature