Jimma University

College of natural sciences

Department of Biology

Malaria-Geohelminths co-infection and their association with severe malaria complications in Darimu health center, South West Ethiopia

By: Shamble Alie

MSc thesis submitted to Department of Biology, College of Natural Sciences, Jimma University in partial fulfillment of the requirement for the degree of master of science in Applied Microbiology

JUNE, 2017

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Advisor: Ketema Bacha (PhD)

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Declaration

I, the under signed, declare that this is my bona fida original work, has never been presented in this or other University, and that all the resources and materials used for the thesis have been dully acknowledged.

Name: Shambel Alie

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Date _____

Place: Jimma University

Date of submission_____

This thesis has been submitted for examination with my approval as the candidate's thesis supervisor

Name: Ketema Bacha (PhD)

Signature_____

Date _____

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ACRONYMS

CDC-center for diseases control and prevention

G6PD- glucose-6-phosphate dehydrogenase

IDA- Iron deficiency anemia

INDC-International Neuro infectious Disease Conference

IRS- Indoor residual spraying

ITNs- insecticide treated nets

LF- lymphatic filariasis

LICs -Low Income Countries

MEF- mosquito-derived exflagellation factor

*Pf*EMP- erythrocyte membrane protein

RDT-rapid diagnostic tests

STH- Soil-transmitted helminthes

WHO-World Health Organization

WWARN- worldwide antimalarial resistance net work

ABSTRACT

Malaria and helmnthic parasitic infections are determinant factors for incidence of severe anemia. Different scholars reported the association of malaria-helmnthic co-infection and severe malaria symptoms differently. Thus, the current study is designed to assess association between the two infectious diseases and their association with severe symptoms in Ethiopia, where the two infections co-exist and endemic. Accordingly, a total of 2758 febrile patients seeking medication in Darimu health center were tested for malaria infection. Malaria positive patients were further assessed for co-infection with helmnthic parasites using direct wet mount and formalin-ether sedimentation concentration methods. Accordingly, among 351 malaria positive patients (12.7%, 95%CI, 11.56-14.08, P<0.001), about 216 (61.54%, 58.26-68.58, P<0.001) were co-infected with single or more than one helmthic parasites. Anemic cases were significantly higher among patients mono-infected with P. falciparum (χ^2 = 15.32, P<0001), and P. vivax (χ^2= 13.61, P= 0.0002) but not during mixed infection of both plasmodium parasite (χ^2 =0.074, P=0.78). Likewise, significant increment of anemia symptom was observed in patients malaria co-infected with hookworm (χ^2 =9.37, P=0.002) and A. lumbricoides (χ^2 =4.39, P=0.036). However, significant association between anemia and malaria co-infection with T. trichiura (χ^2 = 2.24, P=0.134) was not observed. On the other hand although the frequency of severe malaria symptoms seems higher among malaria-helmnth co-infected patients than malaria mono-infected, their relative risk (RR) to jaundice, severe malaria, hemoglobunuria, hypoglycemia, hyperparasitemia and impaired consciousness was not significantly different (P>0.05) from malaria mono-infected cases. The study revealed lack of strong association between malaria-helmths co-infection and severe malaria symptoms among malaria patients.

Key word: anemia, malaria, helminth, co-infections, Darimu. co-infection,

1. INTRODUCTION

1.1. Background of the study

Malaria and Intestinal helminth infections are among the most common infections worldwide (Mengistu *et al*., 2010). Human malaria is caused by five species of *Plasmodium: Plasmodium falciparum, P .vivax, P.ovale* and *P. malariae*. *P. knowlesia,* a fifth species previously confine to monkeys, has also been implicated in human disease (Shaikh *et al*., 2011). Asymptomatic *Plasmodium* infections, if untreated, persist and maintain malaria-induced inflammation that is commonly associated with iron deficiency anemia (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 (Matangila *et al*., 2014). 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 (Matangila *et al*., 2014). *Plasmodium vivax* is the second important parasite of human malaria widely perceived as causing mild and self-limited illness. Unlike *P. falciparum*, it has wider geographical distribution (Ketema and Bacha, 2013).

On the other hand, Intestinal helminths are widely distributed mostly in tropical and subtropical regions of Asia, especially China, India and South East Asia as well as Sub-Saharan Africa. It was estimated that almost 2 billion people are infected by one or more of intestinal helminthes (Hotez *et al*., 2004). Soil-transmitted helminths (STH) are a group of common parasites that infect more than a billion people worldwide. In Ethiopia, like other developing countries, infections with the major STH, including *Ascaris lumbricoides* and hookworm, are widely spread with variable prevalences (Andargachew *et al*., 2013).

In many areas of the world, including Ethiopia, malaria and helminths are co-endemic, hence co-infections are common (Andargachew *et al*., 2013). Accordingly, overlapping distribution of intestinal helminths and malaria results in a high rate of co-infection, which result both in

synergism and antagonistic interaction between helminths and malaria parasites (Alemu *et al*., 2012). One of the main impacts of malaria and helminth infections is anemia. Malaria causes anemia, among other mechanisms, through haemolysis and increased spleenic clearance of infected and uninfected red blood cells. Similarly, intestinal helminths are significant causes of anemia as a result of direct blood loss and impairment of the appetite due to immunological factor (Abraham *et al*., 2010). Malaria and helminths reduce haemoglobin levels, it can be speculated that their combined presence might interact to enhance the risk of anemia. Helminths cause and/or aggravate malnutrition through worm-induced gastrointestinal tract physiopathology and reduced food intake, chronic blood-loss and intestinal inflammation (Abraham *et al*., 2010). The affected populations often endure infections with a number of different species, and individuals are commonly co-infected with combinations of helminths and malaria parasites. Such 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 anemia and organomegaly and thus may potentially create a great challenge for disease control in the tropics. There is also evidence that co-infection with multiple parasites may alter the immune responses (Matangila *et al*., 2014). Overlap of soil-transmitted helminth and *P. falciparum* malaria depends on the conditions that favour multiple parasitic species survival and transmission. These conditions include poverty, environmental contamination, water bodies and lack of effective preventive measures (Matangila *et al*., 2014). In Low Income Countries (LICs), more than half of the school-aged population suffers from anemia and in Sub Saharan Africa; approximately 85 million school-aged children are affected. Anemia reduces their cognitive potential, retards their growth, and predisposes them to other diseases. Moreover, malaria accounts for about for 13–50% of all annual school absenteeism and consequently impairs educational achievements of children (Matangila *et al*., 2014).

1.2. Statement of the problem

Malaria is commonly associated with poverty and has a major negative effect on economic development. In Africa, it is estimated to cause losses of 12 billion US$ a year due to increased healthcare costs, lost ability to work, and negative effects on tourism (WHO, 2015). The World Health Organization reports there were 198 million cases of malaria worldwide in 2013. This resulted in an estimated 584,000 to 855,000 deaths, the majority (90%) of which occurred in Africa (WHO, 2015).

The major clinical features of malaria include, severe anemia due to reduced production and increased destruction of RBCs, cerebral complications due to micro-vascular obstruction in the brain, which causes impaired consciousness, convulsions and long term neurological deficits metabolic acidosis, reduced tissue perfusion, hypoglycemia, hypoxia due to respiratory distress and pulmonary pathology and placental infection during pregnancy (Mackintosh *et al*., 2004).

Malaria is endemic in ninety one countries consisting 40% of the world population (Shaik *et al*., 2011). Half the world's population lives in malaria-endemic areas, with an estimated 500 million clinical cases and over one million deaths annually (Andargachew *et al*., 2013). Similarly, malaria is ranked as the leading communicable disease in Ethiopia, accounting for about 30% of the overall disability adjusted life years lost. Approximately 68% of the total population lives in areas at risk of malaria. In Ethiopia, *Plasmodium falciparum* and *Plasmodium vivax* are the major parasites accounting for about 70% and 30% of infections, respectively (Andargachew *et al*., 2013).

Children under 5 years of age and pregnant women are the worst affected by malaria. It is one of the leading causes of death among young children. Together with pneumonia, diarrhea, measles and malnutrition, malaria is responsible for over 70% of deaths in young children especially in developing countries (UNICEF, 2000). Malaria during pregnancy causes severe maternal illness and anemia, and is also associated with low birth weight among newborn infants, a leading risk factor for infant mortality (UNICEF, 2000). Approximately 500 million people are affected annually, and about three million, mostly children, die of *falciparum* malaria each year (Banzal *et al*., 1999). In addition an additive effect of *P. falciparum* and/or STH infection on childhood

anemia has been reported. Increased severity and incidence of malaria during helminths co-infection (Matangila *et al*., 2014).

Helminthes are parasitic worms. They are the most common infectious agents of humans in developing countries and produce a global burden of disease that exceeds better-known conditions, including malaria and tuberculosis (Hotez *et al.,* 2008). Approximately one third of the world's population is infected with helminths (Woodburn *et al.,* 2009). The contribution of intestinal helminth infections as well as malaria in the development of anemia and low weight status, the concomitant occurrence of malaria and intestinal helminth infections and their clinical manifestations among all age groups, in malaria endemic areas like Ethiopia is largely unreported(Abraham *et al.,* 2010).

Studies in same areas of our country have demonstrated the malaria helminth co-infection associated with severe malaria complication based on evidences of its occurrence in different setting. However, there are only few studies conducted with malaria helminth co-infection association with sever malaria complication and also the absence of research on this topic in the study area, thus, it is rational to assess the current status of malaria helminth co-infection association with sever malaria complication.

This finding is comparable to another report; it shows the occurrence of low haemoglobin concentration, impaired consciousness, splenomegaly, and hepatomegaly in patients co-infected with Plasmodium parasites and helminths in Ethiopia. This study also indicates that anemia prevalence and haemoglobin concentration difference between malaria/helminth co-infections and malaria infection was show an age and sex related pattern.

1.3. Objective

1.3.1. General objective

The general objective of this study is:

To assess malaria-helminthes co-infection and the associated severe malaria complications in patients visiting health center in Darimu District, Oromia Regional State, south western Ethiopia.

1.3.2. Specific objectives

- ✓ To assess demographic and clinical characteristics of the study populations in relation to malaria/helminth co-infection and the associated severe malaria complication.
- ✓ To determine the prevalence of malaria/helminth co-infection and the associated severe malaria complication in all age groups.
- ✓ To examine the effect of malaria and helminthes co-infection on hemoglobin level and incidence of anemia

1.4. Significance of the study

This study will provide substantial information on current situation of malaria in Darimu especially Malaria-helminthes co-infection and their associated severe malaria complication, Likewise; it provide evidences for the prevalence of Malaria-helminthes co-infection and their associated severe malaria complication in Ethiopia. In addition the result of this study could be used as base line data for other researcher.

2. LITERATURE REVIEW

2.1. Malaria

2.1.1. Epidemiology

Malaria is presently endemic in a broad band around the equator, in areas of the Americas, many parts of Asia, and much of Africa; in Sub-Saharan Africa, 85–90% of malaria fatalities occur. A 2010 estimate indicated the deadliest countries per population were Burkina Faso, Mozambique and Mali. As of 2010, about 100 countries have endemic malaria (WHO, 2010).

The geographic distribution of malaria within large regions is complex, and malaria-afflicted and malaria-free areas are often found close to each other. Malaria is prevalent in tropical and subtropical regions because of rainfall, consistent high temperatures and high humidity, along with stagnant waters in which mosquito larvae readily mature, providing them with the environment they need for continuous breeding (WHO, 2010).

The epidemiological pattern of malaria transmission in Ethiopia is generally seasonal and highly unstable due to variations in topography and rainfall patterns. Marked variations in the level of transmission from place to place or seasonal fluctuations in the number of cases are the main features of malaria transmission in Ethiopia (Kassahun, 2004). Malaria in Ethiopia often occurs below 2000 meters, with short-lived transmission following the rains. However, malaria epidemics have been recorded up to 2400 meters during periods when increased temperature and adequate precipitation are conducive for both vector survival and parasite development within the vector (Gebreyesus *et al* ., 2006).

2.1.2. Life cycle of malaria

The life cycle of Plasmodium, a female Anopheles mosquito (the definitive host) transmits a motile infective form (called the sporozoite) to a vertebrate host such as a human (the secondary host), thus acting as a transmission vector. Only female mosquitoes feed on blood; male mosquitoes feed on plant nectar, and do not transmit the disease. The females of the Anopheles genus of mosquito prefer to feed at night. They usually start searching for a meal at dusk, and will continue throughout the night until taking a meal. Malaria parasites can also be transmitted

by blood transfusions, although this is rare (WHO, 2010). Malaria infection may be acquired congenitally from mother to baby across the placenta, from platelet or blood transfusions and from the use of shared needles; however it is most frequently initiated with the bite of an infected, female *Anopheles* mosquito, which injects the sporozoite stage of the parasite with its bite (Satoskar *et al.,* 2009).

The malaria parasite is a multi-stage protozoan with a complex life cycle requiring an insect vector and a human host. There are three phases in its life cycle: the pre-erythrocitic cycle, the erythrocytic cycle, and the sporogonic cycle. The infective stages, which are injected by a mosquito are carried around the body until they invade liver hepatocytes where they undergo a phase of asexual multiplication (exoerythrocytic schizogony) resulting in the production of many uninucleate merozoites (Satoskar *et al.,* 2009). At the end of the hepatic stage of development, a single sporozoite can develop into a schizont that contains thousands of daughter parasites that fill the hepatocyte. Infected hepatocytes burst and release numerous merozoites into the bloodstream. *P. falciparum* can complete this liver stage within 7 days and each of its sporozoites produces about 40,000 daughter parasites (Satoskar *et al.,* 2009). For *P. vivax*, these values are 6-8 days and 10,000 merozoites; for *P. malariae*, 12-16 days and 2000 merozoites; and for *P. ovale*, 9 days and 15,000 merozoites.

In the case of *P. vivax* and *P. ovale*, some sporozoites transform to the dormant hypnozoite, remaining viable for up to 50 years This stage is responsible for relapses when it re-enters its developmental cycle. Inside the host's liver cell it undergoes asexual replication. Next stage of development, called the erythrocytic or blood stage, is initiated when exo-erythrocytic merozoites from the liver invade red blood cells (RBCs) (James *et al*., 2004). Merozoites of *P. falciparum* can infect RBCs of all ages, whereas those of *P. vivax* and *P. ovale* infect reticulocytes and those of *P. malariae* invade only older RBCs (James *et al*., 2004).

Shortly after merozoites are released from hepatocytes, they invade RBCs and over a period of 2 or 3 days, develop asexually. The time from invasion to exit varies with species, 48 h for *P. falciparum and P. vivax* and 72 h for *P.malariae* and *P. ovale*, and the synchronous release of merozoites coinciding with fever peaks. The stages of asexual development include the ring (early trophozoite), trophozoite and schizont stages (Satoskar *et al.,* 2009). The ring stage derives its name from its signet ring-like appearance, with a blue-stained nucleus and a pink-stained ring

of cytoplasm. The trophozoite is the feeding stage of the parasite and contains a single nucleus with pigment granules, called hemozoin (a product of hemoglobin digestion), located within the cytoplasm of the parasite (Satoskar *et al.*, 2009).

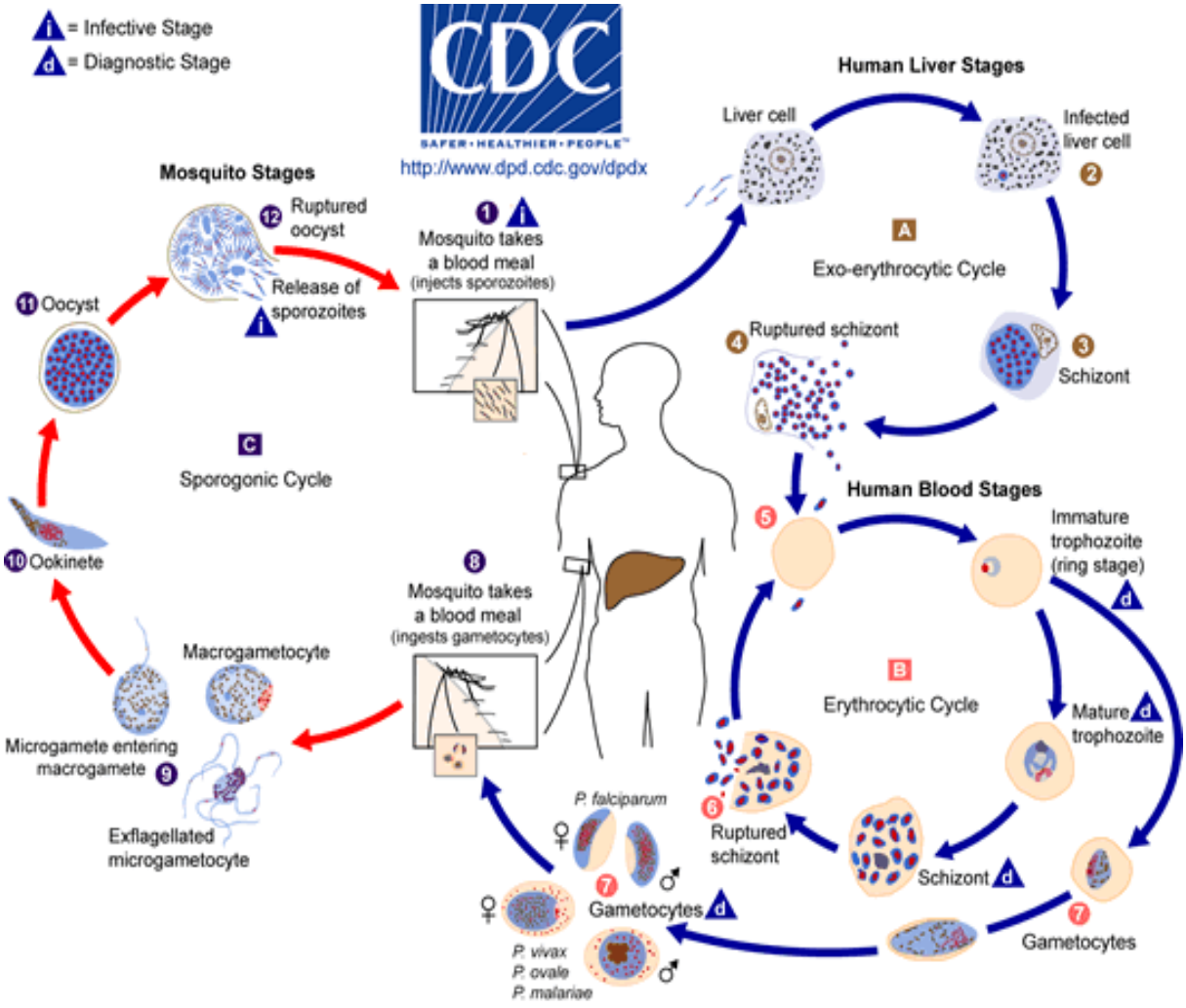

Figure 1 Plasmodium's life cycle (WWARN, 2015)

The schizont stage is initiated by the division of the trophozoite nucleus. Further nuclear division leads to enlargement of the parasite (Satoskar *et al.*, 2009). Each individual nucleus then becomes surrounded by parasite cytoplasm to form a merozoite. At maturation, the schizont bursts and releases merozoites into the blood circulation. Most of the released merozoites re-invade a new erythrocyte, thereby repeating their asexual life cycle (blood stage cycle). In some instances, however, invasion of an erythrocyte by a merozoite initiates sexual development

instead of asexual development (James *et al*., 2004). Thus, merozoites may develop into male gametocytes (microgametocytes) or female gametocytes (macrogametocytes). These gametocytes can develop further only when they are taken up by an appropriate species of *Anopheles* mosquito during a blood meal. They subsequently mate within the gut of the mosquito, the definitive host. The parasites eventually become sporozoites, which reach the salivary gland of the mosquito. With the next bite, the infected mosquito releases sporozoites into the host, thereby completing the life cycle (Satoskar *et al.*, 2009).

2.1.3. Nature of the disease

Malaria is an acute febrile illness with incubation period of 7 days or longer. The most severe form is caused by *P. falciparum*; variable clinical features include fever, chills, headache, muscular aching and weakness, vomiting, cough, diarrhea and abdominal pain. Other symptoms related to organ failure may supervene, such as acute renal failure, pulmonary edema, generalized convulsions, circulatory collapse, followed by coma and death. The initial symptoms, which may be mild, may not be easy to recognize as being due to malaria (WHO, 2010). Malaria infections may cause vital organ dysfunction and death. Severe malaria is defined by clinical or laboratory evidence of vital organ dysfunction. Nearly all deaths from severe malaria result from infections with *P. falciparum* (WHO, 2012). *Falciparum* malaria may be fatal if treatment is delayed beyond 24 hours after the onset of clinical symptoms (WHO, 2010). In areas of endemic malaria, the most common clinical presentation is that of uncomplicated infection with prompt recovery after treatment. However, in non immune individuals, malaria may present in its most severe forms (Banzal *et al*., 1999). Young children, pregnant women, people who are immunosuppressed and elderly travellers are particularly at risk of severe disease. Malaria, particularly *P. falciparum*, in non-immune pregnant travellers increases the risk of maternal death, miscarriage, stillbirth and neonatal death (Limaye *et al*., 2012).

Human malaria caused by other *Plasmodium* species results in significant morbidity but is rarely life-threatening. Cases of severe *P. vivax* malaria have been reported among populations living in (sub) tropical countries with malaria risk. *P. vivax* can remain dormant in the liver; relapses caused by these persistent liver forms ("hypnozoites") may appear months and rarely, several years after exposure. Relapses are not prevented by current chemoprophylactic regimens, with the exception of primaquine (WHO, 2010). *Vivax* malaria is long considered to have a benign

course. It is known for multiple relapses; but the typical complications seen with *falciparum* malaria are not found with *vivax* mono infection (Limaye *et al*., 2012). In areas of high malaria transmission, severe malaria mainly affects children under five years of age. In other areas, all age groups are at risk of developing severe malaria (Njuguna and Newton, 2004).

2.1.4. Pathogenesis and Severe Malaria

Key features of malaria are the adherence of infected red blood cells to the endothelium of small blood vessels compromising blood flow through tissues, and the production of pro-inflammatory cytokines (Parise and Lewis, 2005).

The malaria parasites that invade red blood cells are known as *merozoites*, and within the cell they replicate again, bursting out once they have completed a set number of divisions. It is this periodic rupturing of the red blood cells that causes most of the symptoms associated with malaria, as the host's immune system responds to the waste products produced by the malaria parasites and the debris from the destroyed red blood cells. Different species of malaria rupture the red blood cells at different intervals, which lead to the diagnostic cycles of fever which characterise malaria; *P. vivax*, for example, tends to produce cycles of fever every two days (WWARN, 2015). Malaria infections may cause vital organ dysfunction and death. Severe malaria is defined by clinical or laboratory evidence of vital organ dysfunction. Nearly all deaths from severe malaria result from infections with *P. falciparum* (WHO, 2012).

Plasmodium falciparum produces unique pathological effects, due to its manipulation of the host's physiology. When it infects red blood cells, it makes them stick to the walls of tiny blood vessels deep within major organs, such as the kidneys, lungs, heart and brain. This is called "sequestration", and results in reduced blood flow to these organs, causing the severe clinical symptoms associated with this infection, such as cerebral malaria (WWARN, 2015). Compared with *P. falciparum*, *P. vivax* has a slightly longer incubation period and produces fewer merozoites per schizont. It is generally believed that *P. vivax*merozoites require a single cell receptor, the Duffy antigen, to invade host erythrocytes. Humans lacking this antigen are not susceptible to infection, explaining why *P. vivax* is largely absent from West Africa, a highly malarious region where the Duffy negative blood group is ubiquitous (Ric *et al*., 2014).

Severe 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 abnormal behavior, impairment of consciousness, seizures, coma, or other neurologic abnormalities, Severe anemia due to hemolysis (destruction of the red blood cells), Hemoglobinuria (hemoglobin in the urine) due to hemolysis, Acute respiratory distress syndrome (ARDS), an inflammatory reaction in the lungs that inhibits oxygen exchange, which may occur even after the parasite counts have decreased in response to treatment, Abnormalities in blood coagulation, Low blood pressure caused by cardiovascular collapse, Acute kidney failure, Hyperparasitemia, where more than 5% of the red blood cells are infected by malaria parasites, Metabolic acidosis (excessive acidity in the blood and tissue fluids), often in association with hypoglycemia, Hypoglycemia (low blood glucose). Hypoglycemia may also occur in pregnant women with uncomplicated malaria, or after treatment with quinine. Severe malaria is a medical emergency and should be treated urgently and aggressively (CDC, 2015).

2.1.5. The global malaria burden

Around the World the malaria situation is serious and getting worse. Ninety percent of malaria cases in the world occur in Africa south of the Sahara. Children under 5 years of age and pregnant women are the worst affected by malaria (UNICEF, 2000). Approximately 500 million people are affected annually, and about three million mostly children die of *falciparum* malaria each year (Banzal *et al.*, 1999). Malaria during pregnancy causes severe maternal illness and anemia, and also associated with low birth weight among newborn infants, a leading risk factor for infant mortality (UNICEF, 2000).Malaria is a major problem in the developing countries, with an estimated 300-500 million cases and more than 1 million deaths each year (Zaki *et al.*, 2013).

The economic loss from malaria was estimated at US$2 billion in Africa alone in 1997. Malaria is a major cause of poverty, and poverty exacerbates the malaria situation (UNICEF, 2000).Malaria is mostly a disease of hot climate. The Anopheles mosquito, which transmits the malaria parasite from one human being to another, thrives in warm, humid climates where pools of water provide perfect breeding grounds. It proliferates in conditions where awareness is low and where health care systems are weak. Widespread drug resistance against commonly used antimalarial drugs such as chloroquine and pyrimethamine/ sulfadoxine (Fansidar) has been

reported all over the world (UNICEF, 2000).Refugees and people who are internally displaced as a result of civil war and natural disasters are particularly vulnerable to epidemics of malaria. Afghanistan recorded over 300,000 cases in a year, as a result of interruption of malaria control activities and the displacement of populations due to war. Where health facilities have been destroyed and health staff displaced because of the war, almost half the patients seen at referral hospitals are suffering from malaria (UNICEF, 2000).

2.1.6. Economic impact

malaria is one of the major public health challenges facing the poorest countries in the world, not only causing sickness and death, but also hinder economic development; as a result of lost working hours and the high costs of treating those affected, and of vector control (Bekele *et al.,* 2013). Cause significant economic and financial burden to affected countries (Ric *et al.,* 2014).The disease has been associated with major negative economic effects on regions where it is widespread. During the late 19th and early 20th centuries, it was a major factor in the slow economic development of the American southern states. Poverty can increase the risk of malaria, since those in poverty do not have the financial capacities to prevent or treat the disease. The economic impact includes costs of health care, working days lost due to sickness, days lost in education, decreased productivity due to brain damage from cerebral malaria, and loss of investment and tourism. Severe and cerebral malaria have far-reaching socioeconomic consequences that extend beyond the immediate effects of the disease (WHO, 2010).

2.2. Intestinal Helminths

Helminth is a general term meaning worm. The helminths are invertebrates characterized by elongated, flat or round bodies. The definitive classification is based on the external and internal morphology of egg, larval, and adult stages (Castro, 1996). There are two major phyla of helminths. The nematodes (also known as roundworms) include the major intestinal worms (also known as soil-transmitted helminths) and the filarial worms that cause lymphatic filariasis (LF) and onchocerciasis, whereas the platyhelminths (also known as flatworms) include the flukes (also known as trematodes), such as the schistosomes, and the tapeworms (also known as the cestodes), such as the pork tapeworm that causes cysticercosis (Hotez *et al*., 2016).

Gastrointestinal parasitic infections are amongst the most common infections worldwide. These cases are attributed to three common intestinal parasites: *Ascaris lumbricoides*, hookworm, and *Trichuris trichiura*. The global prevalence of parasitic diseases is estimated to be 478 million children for *A. lumbricoides*; 280 million for hookworms and 347 million children for *T. trichiura*. These infections are most prevalent in populations with low household income, poor handling of personal and environmental sanitation, overcrowding, and limited access to clean water (Nguyen *et al.*, 2013). Soil-transmitted helminth (STH) infections are well-known accompaniments of poverty in the developing world (Silva *et al.*, 2011). In Ethiopia, intestinal parasitic infections are serious public health concern. According to a report by the Ministry of Health, helminthiasis is the third leading cause of outpatient visits in health institutions in 2005–2006 (Nguyen *et al.*, 2013).

Helminths develop through egg, larval (juvenile), and adult stages. Knowledge of the different stages in relation to their growth and development is the basis for understanding the epidemiology and pathogenesis of helminth diseases, as well as for the diagnosis and treatment of patients harboring these parasites (Castro, 1996).

Over the last 60 years, several estimates have confirmed Stoll's initial observation that hundreds of millions of people harbor parasitic worms. The most common helminthiases are those caused by infection with intestinal helminths, *ascariasis*, *trichuriasis*, and hookworm, followed by schistosomiasis and LF. Practically speaking, this means that the inhabitants of thousands of rural, impoverished villages throughout the tropics and subtropics are often chronically infected

with several different species of parasitic worm; that is they are polyparasitized (Hotez *et al*., 2016).

2.2.1. Epidemiology of helminth infections

A large part of the world's population is infected with 1 or more of these helminths, but the prevalence is highest in tropical and subtropical countries where water supplies and sanitation are poor (CDC, 2015). There are several key determinants underlying the epidemiology of helminth infections. Environment, Climate and topography are crucial determinants of the distribution of helminth infections. Helminths transmitted by vectors are limited to landscapes in which host and vector come together in the same habitat, resulting highly focal distribution. For example, the distribution of schistosomiasis reflects the biotic and abiotic features (i.e., climatic, physical, and chemical factors) that affect the survival and development of the snail vector. In the case of onchocerciasis, the distribution and incidence of the disease are limited by biogeographic variations favorable to exposure to the blackfly vectors. Soil-transmitted helminths are highly affected by surface temperature, altitude, soil type, and rainfall (Hotez *et al.*, 2016)

2.2.2. Transmission

Adult worms live in the intestine where they produce thousands of eggs each day. Eggs are passed in feces from an infected person. Infection with roundworm and whipworm occurs when eggs in soil have become infective and are ingested (CDC, 2015). In areas that lack adequate sanitation, these eggs contaminate the soil. This can happen in several ways: eggs that are attached to vegetables are ingested when the vegetables are not carefully cooked, washed or peeled;eggs are ingested from contaminated water sources;eggs are ingested by children who play in the contaminated soil and then put their hands in their mouths without washing them(WHO, 2016).In addition, hookworm eggs hatch in the soil, releasing larvae that mature into a form that can actively penetrate the skin. People become infected with hookworm primarily by walking barefoot on the contaminated soil. There is no direct person-to-person transmission, or infection from fresh faeces, because eggs passed in faeces need about 3 weeks to mature in the soil before they become infective. Since these worms do not multiply in the human

host, re-infection occurs only as a result of contact with infective stages in the environment (WHO, 2016).

2.2.3. Global distribution and prevalence

More than 1.5 billion people, or 24% of the world's population, are infected with soil-transmitted helminth infections worldwide (WHO, 2016). Infections are widely distributed in tropical and subtropical areas, with the greatest numbers occurring in sub-Saharan Africa, the Americas, China and East Asia (Hotez *et al*., 2004). Over 270 million preschool-age children and over 600 million school-age children live in areas where these parasites are intensively transmitted (WHO, 2016).

Hookworm, Ascaris, and whipworm are known as soil-transmitted helminths (parasitic worms). Together, they account for a major burden of disease worldwide. An estimated 576-740 million people in the world are infected with hookworm. Hookworm was widespread in the southeastern United States until the early 20th century. 807-1,221 million people in the world are infected with *Ascaris lumbricoides*. Ascariasis is now uncommon in the United States and 604-795 million people in the world are infected with whipworm (CDC, 2013).

2.2.4. Burden of helminthiases

They are the most common infectious agents of humans in developing countries and produce a global burden of disease that exceeds better-known conditions, including malaria and tuberculosis (Hotez *et al.,* 2016).Helminth infections are acquired through contact with soil contaminated with infected feces. STH infections were once common throughout the world, including the United States, and some cases are still seen in the U.S. each year. With better sanitation, these infections are now common only in poorer countries. More than four billion people are at high risk throughout the world, with over one billion individuals already infected. STH infections can contribute to anemia, vitamin A deficiency, malnutrition and impaired growth, delayed development, and intestinal blockages (CDC,2011).Most helminth infections, if left untreated, result in multi-year chronic inflammatory disorders that cause both concurrent and delayed-onset pathology to the afflicted human host(Hotez *et al.,* 2016). In addition to the overt and dramatic effects of blindness and elephantiasis in individuals with onchocerciasis and LF, respectively, it is now appreciated that chronic helminth infections are also linked to more

insidious persistent health conditions such as anemia, growth stunting, protein-calorie undernutrition, fatigue, and poor cognitive development (Hotez *et al.,* 2016). These seemingly subtle and often overlooked morbidities are very important because of the high prevalence of helminthiases in the rural developing world, in which any health impairment is substantially magnified in terms of degradation of individual patient performance status (Hotez *et al.*, 2016).The temporal lag between initial high-intensity childhood infection and the delayed onset of "classical" parasite-associated pathologic findings have led to a serious underappreciation of the day-to-day burden of helminthic diseases (Hotez *et al*., 2016).

2.3. Malaria and Intestinal Helminth Co-infections

Even though most common co-infections of malaria are with intestinal helminths (Mwangi *et al.,* 2006), intestinal parasites infections are amongst the most common infections worldwide. It is estimated that some 3.5 billion people are affected, and that 450 million are ill as a result of these infections, the majority being children in the poor community (Mengistu *et al*., 2007). Recent studies show that malaria and hookworm infections are widespread throughout tropical and subtropical countries. Both hookworm and malaria are significant causes of anemia in pregnant women (Basavaraju and Schantz, 2006). Intestinal helminth and malaria co-infections occur in the tropical and subtropical countries. The prevalence of hookworm infection among reproductive age females in sub-Saharan Africa is estimated to be as high as 32% (Basavaraju and Schantz, 2006). 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).

P. falciparum and helminth co-infections result in lower birth weight than *P. falciparum* infections alone (Egwunyenga *et al,* 2001). Malaria and soil-transmitted helminthes (STH) are widely co-endemic and are largely a burden of the poor. Preliminary data suggest a relationship between helminth infection and malaria incidence in addition to helminth-associated reduction in the severity of *Plasmodium* spp infections (Basavaraju and Schantz, 2006).

The biology of the parasite and the host, climate, socio-economic status 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. Climate determines the survival of the mosquito vector of the malaria and the free living and infective stage of the helminth (Brooker and Michael, 2000; Hay *et al*., 2000). Low level of education and poverty associated with poor malaria prevention and poor access to effective anti-malarial drugs enhance the chance to be infective (Asaulo and Ofoezie, 2003). Such household related risk factors may partially explain the empirical observation that malaria as well as helminth infections tend to cluster within certain households (Shapiro *et al*., 2005).

Infections with intestinal helminthes may alter susceptibility to clinical malaria and malaria may influences the clinical consequences of helminth infection by exacerbating their morbidity. Splenomegally associated with *S. mansoni* infection is found to be exacerbated by chronic malaria in children (Booth *et al*., 2004). However recent studies have shown that *S. mansoni* and *S. haematobium* infections are found to increase incidence of malaria fever (Booth *et al*., 2004).

This study was conducted in the area since no such previous study has been reported. Knowledge about the prevalence of malaria and intestinal parasites in Darimu Health Center will be essential for the initiation and implementation of parasite control programs in the region.

3. METHODS AND MATERIALS

3.1. Description of the study area

The study was conducted at Darimu health center of Illu Abba Bora Zone, Oromia Regional State, located 662 km south west of Addis Ababa. Annual temperature of the study area ranges from 18.04 to 20.03°C and mean annual rainfall ranges from 756.7 to 1839.5mm. Malaria transmission pattern in Darimu district is unstable and seasonal which depends on the existing altitude and rainfall. The study population was all patients seeking medication at the health center during the study period and had symptoms of malaria infection. This district has an estimated total population of 68,512 of whom 34,023 were males and 34,489 were females; 11,416 of its population are urban dwellers.

3.2. Study population

A total of 351consent study participants were examined for malaria/helminthes co- infections in two seasons (April/May and November/December).

Inclusion and Exclusion Criteria

Cases positive for *Plasmodium* species and older than one year, had no history of anti-malarial drug administration in the last two months prior to screening, absence of any other serious chronic infection, had volunteer to give blood and stool samples were included in the study. However, pregnant women, children younger than 1 year and individuals with known concomitant chronic infection were excluded from the study.

3.3. Laboratory methods

The choice of diagnostic techniques usually depends on availability of standard equipment and reagents, experienced personnel, and considerations of time and cost. The techniques used in this study are: Direct Wet Mount Microscopic Examination***s*** and Formalin-Ether concentration techniques. Assessment of clinical history and clinical diagnosis of the sampled study patients were made by trained physicians working in the health center. All blood samples were collected

and processed by experienced laboratory technicians to ensure data quality besides ethical consideration.

3.3.1. Diagnosis for malaria parasites

Blood Film determination for malaria parasites:

Blood samples were separately collected on clean glass slides from lancet pricked finger and thin and thick blood smears prepared in duplicate per patient for microscopic examination. Thick and thin films were made on the same slide. After air-dried in a horizontal position, the thin blood films were fixed in methanol for 30 s. Then, smears were stained with 10% Giemsa solution for 20 min. Each slide was examined under oil immersion objective of a microscope by experienced laboratory technicians. Several fields were examined before negative result was reported. The thin smear was used to identify the type of *Plasmodium* species while the Thick smear was used to detect malaria infection and parasite quantification. The number of gametocytes per microliter of blood was calculated from the thick blood smear. For each participant only one slide was read to detect malaria infection and identify the type of *Plasmodium* species.

$$\frac{\text{Number of parasites counted x 8.000 WBC/UL blood}}{\text{Number of leucocytes (WBC)}}$$

Determination of haemoglobin concentration

Haemoglobin concentration was determined using a portable haemoglobin spectrophotometer, Hemocue Hb 201 analyzer (HemoCue, Angelholm, Sweden) and specially designed microcuvette (the Hemocue Hb 201 Microcuvette, Hemocue, Angelholm, Sweden). Then, the haemoglobin values were used to assess the status of anemia. Anemia was defined based on the observed hemoglobin concentration as: severe anemia (Hb <5 g/dl), moderate anemia (Hb: 5–8 g/dl) and mild anemia) Hb: 8–11 g/dl).

3.3.2. Diagnosis for intestinal parasites

Direct Wet Mount Microscopic Examinations for Detection of Intestinal parasites

Clean stool cap and stick applicator were provided to malaria positive subjects volunteered to participate in the study to provide stool specimen. Similarly, for each participant only one slide was read to detect helminth infection and identify the type of species.

A drop of saline and a small amount of faeces were placed on the microscope slide using applicator stick, mixed and covered by cover slip. Finally, the samples were examined microscopically at under high power magnification. A small portion of the stool samples (about 1 g) were also preserved in 10% formalin to repeat the tests by concentration technique when the result of direct wet mount turned negatives.

Formalin-Ether Concentration technique for stool Examination

The formalin preserved specimen was thoroughly stirred and a sufficient quantity was strained through gauze in to a 15ml pointed centrifuge tube to get the desired amount of sediment. Then saline was mixed and centrifuged at 2000 rpm for 1 minute. The supernatant was decanted and washed again with tape water. About 10ml of 10 percent formalin was added to the sediment and mixed thoroughly. Then, 3ml of ether was added and shacked vigorously in an inverted position for 30 seconds. The resulting solution was centrifuged at 1500 rpm for about 1 minute, and four layers were produced. The three top layers were decanted carefully, and adhering debris were removed from the top with a cotton swab. Finally, the samples were examined microscopically at under high power magnification.

3.4. Data analysis

Data were entered into an Excel spreadsheet and analyzed using SPSS statistical packages software (version 20). Like chi square test and Relative risk. Statistical significance was set at $p < 0.05$. The categorical parameters like sex, history, presentation, and results of clinical examination were presented by their frequencies and percentage. Statistical significances were considered at $p<0.05$

3.5. Ethical considerations

The study was ethically approved by Research and Ethical Review Board of Jimma University, College of Natural sciences. Letter of permission was handed to concerned officials at Darimu Health center, the study area. From all the study participants, either oral consent (for adults) or signed agreement (guardian) was obtaining prior to data collection. Participation was voluntary and the objectives of the study were explained to all the study participants (parents of children). Parents or guardians signed consent forms on behalf of their children. Diagnosis was done using sterile and disposable materials. Only trained laboratory technician took the blood samples and handled all activities in clinical examination.

4. RESULTS

4.1. Socio-demographic characteristics of study subjects and prevalence of malaria

A total of 2758 febrile patients, including 1212 (43.9%) males and 1546 (56.1%) females) were recruited for determination of presence or absence of malaria parasite. The age range of the study subjects fall within 1 to 70 years. Majority of the study subjects [1634 (59.2%)] were aged ≥15 years, followed by those aged between 5-14 years [728 (26.4%)] and <5 years [396(14.4%)]. Concerning their settlement, about 2370 (85.9%) of study subjects were rural dwellers.

Table 1 Socio-demographic characteristics of the study participants and proportion of malaria positive cases at Darimu Health Center, Oromia Regional State, Southwest Ethiopia, 2015/2016 (n=2758)

Characteristics		Number Examined (%)	Malaria positive (%)	P-value
Sex	Male	1212 (43.9)	209 (7.57)	P =0.135
	Female	1546 (56.1)	142(5.14)	
Age	1-4	396 (14.4)	54(1.96)	P <0.001
	5-14	728 (26.4)	92 (3.34)	
	≥15	1634 (59.2)	205(7.43)	
Residence	Urban	388 (14.1)	83(3.01)	P <0.001
	Rural	2370 (85.9)	270(9.78)	

The overall prevalence of malaria in the study area was 12.7% (351/2758). The prevalence was relatively higher among males, individuals aged more than 15 years and the rural community with prevalence rate of 7.57%, 7.43%, and 9.78%, respectively. The prevalence of malaria in different age groups, settlement, ethnicity and religion had showed statistically significant difference ($P < 0.05$). No significant association was observed between malaria prevalence and sex (P=0.135).

4.2. Mono- and Mixed Infection with Malaria parasites

Furthermore, of the total 351 malaria positive cases, malaria due to *P. falciparum* accounted for 59 % (n=207) while 36.2% (n=127) were due to *P. vivax* mono-infection. Only 4.8% (n=17) cases were malaria due to mixed infection involving both *P. falciparum* and *P. vivax*. As already stated in the preceding sections, the overall prevalence of malaria was higher among males (7.57%, n=209) as compared to the same in females (5.14%, n=142).

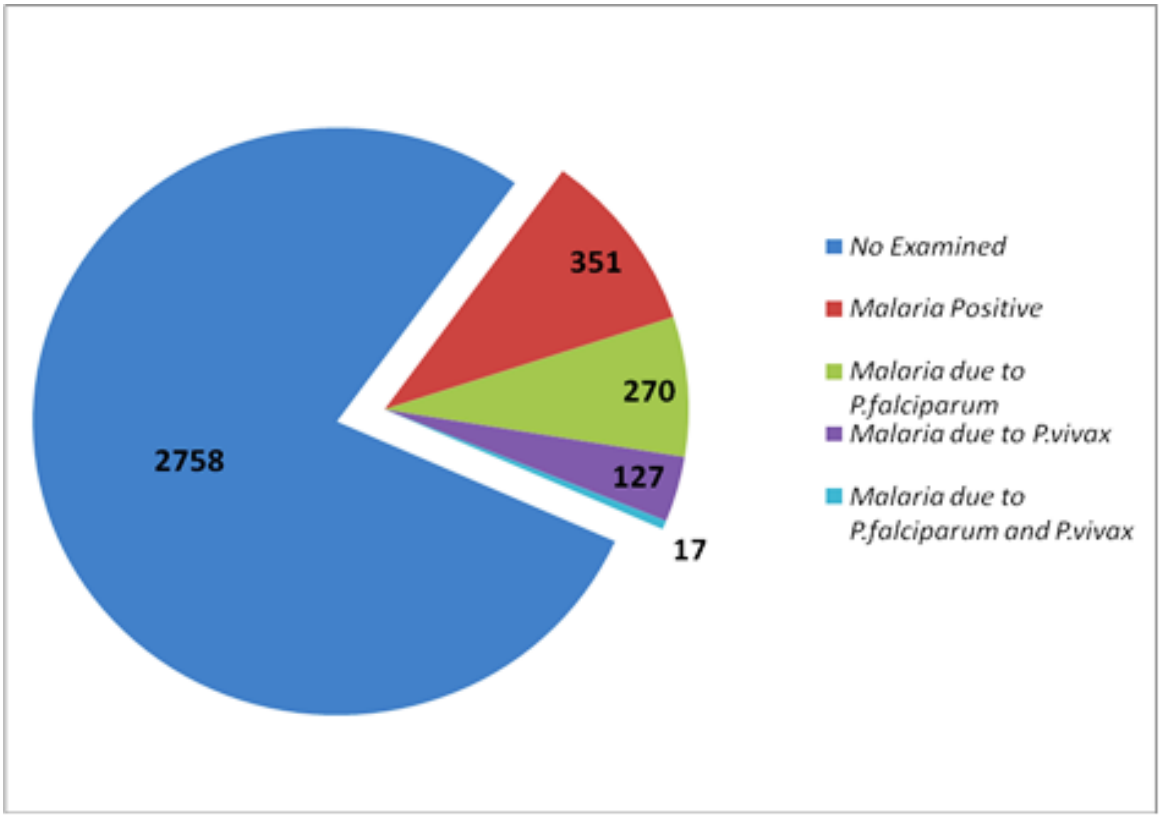

Figure 2 The prevalence of malaria species in Darimu District, Illuabba bora zone, Oromia Regional State.

4.3. Clinical presentations (Complicated and uncomplicated symptoms) of malaria patients

Regarding the clinical presentations at the time of admission of the 351 malaria positive patients, all the cases showed fever (100%), followed by headache 220(62.7%), vomiting 155 (44.2%). cough 104(29.6%), anemia 142 (40.5%), and abdominal pain 78(22.2%). The other less frequently observed clinical features were shortness of breath 61(17.4%), hepatomegaly 51 (14.5%), and splenomegaly, 36(10.3%) (Table 2).

Table 2. Clinical presentations of uncomplicated malaria symptoms among malaria positive patients attending Darimu health center, Oromia National Regional State, southwest Ethiopia (n = 351)

No	Clinical presentations	Frequency (%)
1	Fever	351 (100)
2	Headache	220 (62.7)
3	Vomiting	155 (44.2)
4	Anemia	142 (40.5)
5	Cough	104 (29.6)
6	Abdominal pain	78 (22.2)
7	Shortness of breath	61 (17.4)
8	Hepatomegaly	51 (14.5)
9	Splenomegaly	36 (10.3)
10	Epitaxis	16 (4.6)

In addition, severe malaria symptoms such as severe anemia, hyperpyrexia, jaundice, hyperparasitemia, hypoglycemia, and impaired consciousness were observed among significant number of malaria patients (Table 3). There were cases when a patient presents more than one severe malaria symptoms.

Table 3 Incidence of severe malaria symptoms among malaria positive patients attending Darimu health center, Oromia Regional State, southwest Ethiopia (n = 351)

No	Severe malaria symptoms	Frequency (%)
1	Severe anemia (Hb level <5mg/DL)	13 (3.7)
2	Jaundice	57 (16.2)
3	Hemoglobinuria	25 (7.1)
4	Hyperpyrexia	13 (3.7)
5	Hyperparasitemia (parasite load >10,000 parasite/UL)	29 (8.26)
6	Hypoglycemia (Blood Glucose level 40mg/dL)	13 (3.7)
7	Impaired consciousness	37 (10.5)

4.4. Malaria Helminths Co-infections

Hookworm, *Ascaris lumbricoides* and *T. trichiura* were the major helminthic diseases that have been associated with malaria infection in the study area while *T. trichiura* was the parasite least associated with malaria co-infection (Fig. 2).

There were variations in prevalence of most parasitic diseases among the different age groups of the study population. Accordingly, the prevalence of *Plasmodium spp,* Hookworm, *A. lumbricoides* and *T. trichiura* showed significant differences in all age groups of the study population with high prevalence of some of these parasites among young population as compared to the adults. The overall magnitude of intestinal parasitizes in all age groups was 61.5% (216/351). The most frequently diagnosed intestinal helminths were *A. lumbricoides,* hookworm and *T. trichiura,* with prevalence rate of 40.3% (87/216), 39.3% (85/216) and 20.4% (44/216), respectively. (Fig. 3)

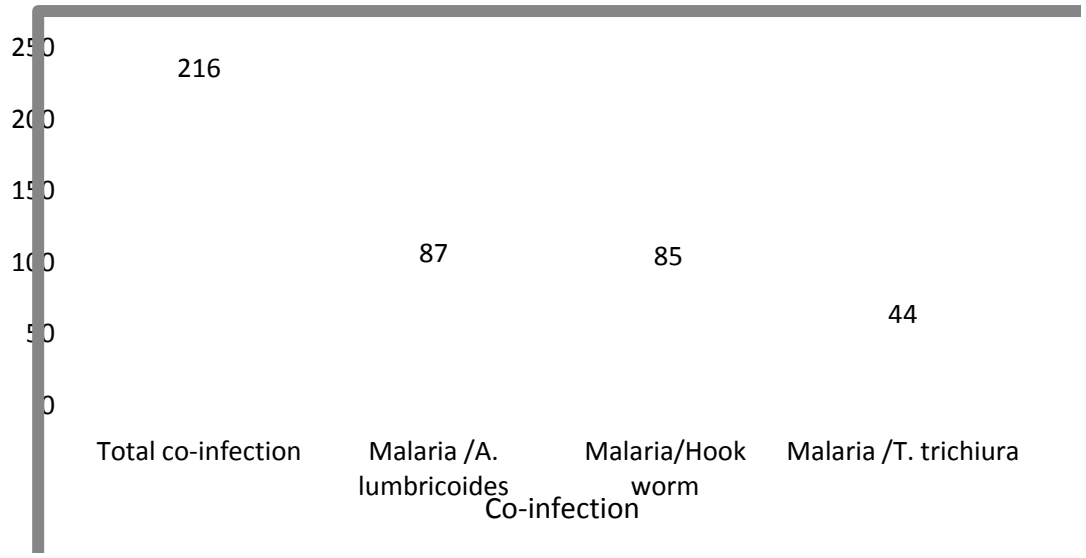

Figure 3 The prevalence of malaria helminths co-infection in Darimu Health Center, Illu abba bora zone, Oromia Regional State, southwest Ethiopia

4.5. Prevalence of Anemia and Malaria/Helminthe co-infection

Analysis of the prevalence of anemia among malaria patients indicated that about 9.9%, 3.5%, and 2.8% of *P. falciparum*, *P. vivax*, and mixed infection cases were anemic, respectively. Similarly, the prevalence of anemia was significantly higher in malaria/intestinal helminth parasite co-infected patients with prevalence rate of 35.2%, 32.4% and16.2% for hookworm, *A. lumbricoides* and *T. trichiura* co- infected subjects, respectively (Table 4). Malaria infection (P. falciparum or P. vivax) alone as well as with co-infection with helmnths were found to be a risk factor for occurance of anemia. This was observed in the chi-square analysis test employed….

Table 4 Prevalence of anemia by infection types among the study participants at Darimu Health Center, in Illu abba bora zone, Oromia Regional State,

Variable (Mono/co-infection)	**Anemia (n=142) No (%)**	**None anemia (n=209) No (%)**	**P.value**
Plasmodium falciparum	14(9.9)	67(32.1)	χ^2=15.32, P=0.0001
Plasmodium vivax	5(3.5)	38(18.2)	χ^2=13.6, P=0002
P. falciparum and *P.vivax*	4(2.8)	7(3.3)	χ^2=0.07, P=78
Malaria/Hook worm	50(35.2)	35(16.7)	χ^2= 9.36, P=0.0022
Malaria/*A. lumbricoides*	46(32.4)	41(6.7)	χ^2= 4.39, P=0.036
Malaria/*T. trichiura*	23(16.2)	21(10.0)	χ^2= 2.24, P=0.134

In general, the current data indicate that co-infection with *Plasmodium* and helminth parasites are associated with higher prevalence of anemia than mono-infection with *Plasmodium* parasites. Furthermore, patients co-infected with *Plasmodium* and helminth parasites showed lower mean haemoglobin concentration and were anemic (Table 5).

Based on the WHO cut off values of hemoglobin concentration, a total of 142 (40.5%) anemic cases were found among the malaria positive subjects. Severe anemia (hemoglobin concentration ≤ 5g/dl) was observed in 13(3.7%) patient with almost similar pattern among all age groups.

Table 5 Incidence of parasitemia, anemia and hypoglycemia among co-infected with helmnths or due to mono-infection patients attending Darimu health center in Illu abba bora zone, Oromia Regional State,

	Parasite loud/µl				Hemoglobin level (mg/dl)			Glucose level (mg/dl)	
Age (year)	light	moderate	heavy	very heavy	Severe	moderate	mild	< 40	>40
<5	11	29	9	5	5	6	15	3	51
5-14	31	45	9	7	3	21	25	4	88
≥15	68	74	46	17	5	18	44	6	199
Total	110	148	64	29	13	45	84	13	338

Although the overall parasite load was dominantly moderate (42 %, 148/351) among the patients, heavy (n=46) and very heavy (n=17) categories of parasite loud/µl were recorded among cases aged greater than 15 years. Similar pattern was observed for Glucose level (mg/dl) in that the highest number of patients (n=199) were found to have glucose level >40mg/dl. [The degree of parasitaemia was graded as light, moderate, heavy, or very heavy infections , respectively, when there is 1–499 parasites/µL, 500–1,999 parasites/µL, 2,000–9,999 parasites/µL and ≥10,000 parasites/µL. The level of anemia was also classified as severe, moderate and mild, when Hb concentration is <5 g/dl, between 5 and 8 g/dl, and between 8 and11 g/dl, respectively. And Hypoglycemia was considered when blood glucose concentration was < 40 mg/dl.

4.6 Association of malaria/helminth co-infection with complicated severe malaria

The prevalence of Jaundice was 18/135(13.3%) due to malaria infected individuals, while 39/216(18%) due to malaria/helminthes co-infection. Similarly in the other pattern the prevalence of *Severe* anemia and Hypoglycemia were 2/135(1.5%) due to plasmodium infection alone, while 11/216(5%) due to both plasmodium/helminthes co-infection. Plasmodium/helminth infected individual had lower haemoglobin concentration than plasmodium alone. There was no Hyperparasitemia prevalence difference between plasmodium/helminthes co-infection and plasmodium alone (Table 6)

Table 6 Association of malaria-helmnth co-infection and severe malaria symptoms among malaria positive patients, Darimu Health Center, Oromia Regional State, Southwest Ethiopia

Variables	Items	*P. falciparum*	*P. vivax*	*P. falciparum* & *P. vivax*	Malaria/ Helment	Malaria/ Hook worm	Malaria/*T. trichiura*	*Malaria/A. lumbricoides*
Jaundice	Yes	13	4	1	39	19	7	13
	No	68	39	10	177	66	37	74
	Total	81	43	11	216	85	44	87
Severe anemia	Yes	1	-	1	11	8	1	2
	No	80	43	10	205	77	43	85
	Total	81	43	11	216	85	44	87
Hemoglobinuria	Yes	6	1	2	16	7	3	6
	No	75	42	9	200	78	41	81
	Total	81	43	11	216	85	44	87
Impaired consciousness	Yes	9	3	2	23	8	4	11
	No	72	40	9	193	77	40	76
	Total	81	43	11	216	85	44	87
Hypoglycemia	Yes	1	-	1	11	8	1	2
	No	80	43	10	205	77	43	85
	Total	81	43	11	216	85	44	87
Hyperparasitemia	Yes	6	3	2	18	7	3	8
	No	75	40	9	198	78	41	79
	Total	81	43	11	216	85	44	87

In the current study relative risk of malaria-helmnths co-infection was compared against *P. falciparum*. Although the frequency of severe malaria symptoms seems higher during the co-infection, for almost all severe malaria symptoms such as jaundices, severe anemia, hypoglycemia, hyperparasitemia, hemoglubuniria and impaired conscious significant differences ($P>0.05$) were not observed during malaria-helmnth co-infection (Table 7).

Table 7 Relative risks of malaria-helmnths co-infected patients to the incidence of severe malaria complication in Darimu health center, Southwest Ethiopia

Type of Infections	Severe malaria symptoms											
	Jaundice		Severe malaria		Hemoglobunuria		Impaired consciousness		Hypoglycemia		Hyperparasitemia	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
P. falciparum mono-infection *(PfM)*	13	68	1	80	6	75	9	72	1	80	6	75
Malaria-helmnth co-infection (MHM)	39	177	11	205	16	200	23	193	11	205	18	198
Relative risk (RR)	RR= 0.88 (95%CI, 0.5-1.57), P=0.68		RR=0.24 (95%CI, 0.03-1.84), P=0.17		RR=1.0 (95%CI 0.4-2.46), P=1.0		RR=0.95 (95%CI, 0.46-1.98), P=0.9		RR=0.24 (95%CI, 0.03-1.84), P=0.17		RR=0.88 (95%CI, 0.36-2.1), P=0.79	
Malaria – hookworm co-infection (MHC)	19	66	8	77	7	78	8	77	8	77	7	78
Relative risk (RR) of MHC with reference to *PfM*	RR=0.71 (95%CI, 0.34-1.35), P=0.3		RR=0.13 (95%CI, 0.017-1.02), P=0.053		RR=0.89 (95%CI, 0.31-2.56), P=0.84		RR=1.18 (95%CI, 0.48-2.9), P=0.72		RR=0.13 (95%CI, 0.017-1.02), P=0.053		RR= 0.89 (95%CI, 0.31-2.56), P=0.84	
Malaria-Ascaris co-infection(MAC)	13	74	2	85	6	81	11	76	2	85	8	79
Relative risk (RR) of MAC with reference to *PfM*	RR=1.07 (95%CI, 0.53-2.17), P=0.82		RR= 0.53 (95%CI, 0.05-5.81), P=0.61		RR=0.53 (95%CI, 0.36-3.2), P=0.89		RR=0.87 (95%CI, 0.38-2.01), P=75		RR=0.53 (95%CI, 0.049-5.8), P=609		RR=0.805 (95%CI, 0.29-2.22), P=0.67	
Malaria- Trichuris co-infection (MTC)	7	37	1	43	3	41	4	40	1	43	3	41
Relative risk (RR) of compared to *PfM*	RR=1.01 (95%CI, 0.43-2.34), P=0.98		RR=0.54 (95%CI, 0.035-8.47), P=0.66		RR=1.08 (95%CI, 0.28-4.13), P=0.9		RR=1.22 (95%CI, 0.4-3.74), P = 0.7253		RR=0.54 (95%CI, 0.035-8.47), P=0.66		RR=1.08 (95%CI, 0.28-4.13), P=0.9	

5. DISCUSSION

Darimu has suitable climate and topography conducive for transmission of malaria and helminth infections as climate and soil type are known to determine the distribution of intestinal worm infections (Mabaso *et al*., 2003). Furthermore, the infection rate of malaria and helminths could vary from place to place based on differences in the socio-economic and educational status of the population (Zhou *et al.*, 2004). Such variations and its impacts are likely in the study area as there are apparent variations in these conditions among the study population (Data not given)

Peak transmission of malaria occurs following the main rainy season and a minor transmission peak occurs following light rainy season in the tropics (Hay *et al*., 2000). Likewise, relatively low peak transmission was the observed cases in the study area during light rainy seasons. Sex wise, the prevalence of malaria was higher in males than in females in Darimu District. This observation could be accounted to the fact that males spend early part of the night working in their farm place where they might be easily infected by anopheles mosquito bites, while most females do not experience such risk. In addition, when there is no much rain, the male may move far from their residences in search of potable water further contributing to possible exposure to outdoor infection in area where irrigation is practiced (the potential reproductive site of malaria vectors). In support of this justification, Sharma *et al* (2015) stated that agricultural laborers are known to be at a higher risk through increased risk of contact with malaria vector at field, outdoor sleeping, frequent movement and inadequate treatment.

Indoor residual spraying (IRS) and insecticide treated nets (ITNs) is the main stay in malaria prevention. IRS is a powerful method of vector control and is especially effective for the prevention and the control of malaria (WHO, 2012). In the study area, (IRS) was applied to 6500 houses and a total of 34000 insecticide treated nets (ITNs) were distributed (personal communication: Darimu Natural Disaster Management and Control Office).Thus, the relatively low malaria prevalence in the study area could be accounted to the intervention being practiced although still many remains to handle the challenge.

Prompt and effective treatment of malaria remains a key intervention in reducing the burden of disease and death from malaria (WHO, 2012). For all those confirmed to have uncomplicated

malaria, the treatment with first line drugs are recommended while those with complicated malaria are usually treated initially with quinine. In the present study, the frequently observed complicated malaria among the study participants were severe anemia (Hb < 5 g/dl, 3.7%), Hypoglycemia (Glu< 40 mg/dl, 3.7%), hyper parasitemia (>10,000 Para/µl, 26.5%), respiratory distress (17.4%), and vomiting (44.2%). In related study, the major malaria associated sever complicated recorded among the malaria patients assessed in Halaba and kersa districts, southwest Ethiopia, were severe anemia (5.8%), Hypoglycemia (2.4%, severe parasitemia (20.9%), respiratory distress (2.2%), hyperpyrexia (2.9%), and vomiting (35.3%), reported, Ethiopia (Ketema and Bacha, 2013).

Poor personal hygiene favors high prevalence of intestinal helminth infections (Mengstu *et al*., 2014). In Darimu, most of the people use unsanitary drinking water and live under inadequate hygienic conditions. Socio-demographic data of the study population shows that more than 85% were inhabitant of rural community with limited access to hygienic water. Furthermore, report shows that wearing shoes regularly has a significant contribution in lowering the prevalence rate of hookworm infections (Tadesse *et al*., 2005). To the contrary, most children in the study area do not wear shoes on regular basis. *T. trichiura* and *A. lumbricoides* were the parasites that showed higher prevalence among the age groups below 15 years. This could be associated to the facts that children have frequent contact with soil as their behavior (playing games) exposes them to soil irrespective of their gender. In the current study site, children were particularly at high risk acquiring infection with parasites as they were defecating in open sites that are already polluted by faeces and therefore can be exposed to more frequent and heavy infection than adults. As the age group below 15 years old is known to actively make soil contact as they play around, it is expected that the prevalence of helminths could be significantly higher in this age group (Mengstu and Mekdes, 2010).

Overlapping distributions of intestinal helminths and malaria result in high rate of malaria and helminth co-infections (Adrienne *et al*., 2005). In many regions of Sub-Saharan Africa, intestinal helminth infections particularly hookworm disease overlaps geographically with *falciparum* malaria (Guyatt and Snow, 2001). In the current study, the co-infection of malaria and helminths were common in Darimu District. Poor environmental sanitation and climatic conditions (hot, wet and humid) could favor the persistence of mosquito vectors, hence malaria transmission, and

the free living infective stage of hookworm larva (Brooker and Michael, 2000; Hay *et al*., 2000). Hookworms have a wider thermal tolerance than *A. lumbricoides* and *T. trichiuria,* adapting them to the tropical temperature that increases the chances of co-infection with malaria (Hotez *et al*., 2004). However, the prevalence of malaria-hookworm co-infection was lower than the malaria-*A. lumbricoides* co-infection in the study area. In the current study area, the prevalence of hookworm was relatively low in the age group above 14 years mainly because most adults of this age group were wearing shoes on regular basis.

In the present study, the overall prevalence of malaria among the study participants was 12.7%, which is very low compared to the prevalence reported from other areas of Ethiopia including the 27.9% prevalence reported from Halaba Kulito (Degarege *et al*., 2010) and 31.9% report from both Kersa district and Halaba Kulito (Ketema and Bacha, 2013). Compared to similar studies done in other countries, the prevalence of malaria in the current study was relatively very low: prevalence as high as 98.5% was reported recently from Cameroon (Njunda *et al*., 2015). The observed low prevalence in the study area might be due to the intense and diverse malaria control strategies undertaken at Darimu. The intervention made so far has significantly reduced prevalence of malaria in the study area.

This study has demonstrated that cases with malaria/helminth co-infections entails significantly higher prevalence of anemia and lower mean haemoglobin concentration when compared with malaria infection cases only. Our observation was in agreement with previous report (Nacher *et al*., 2001; Degarege *et al.,* 2010), which recorded a significant difference in haemoglobin concentration in malaria/helminth co-infected study patients and patients with malaria infection alone.

High rate of co-infection due to overlapping distribution of intestinal helminths and malaria parasites might results in synergism and antagonistic interaction between helminths and malaria parasites (Alemu *et al*., 2012). One of the main impacts of malaria and helminth infections is anemia. Malaria causes anemia, among other mechanisms, through hemolysis and increased splenic clearance of infected and uninfected red blood cells (Abraham *et al*., 2010). In the current study, 61.5% (216/351) of the study population were positive for malaria-helminth infection. Variation between the findings could be due to seasonal variation where the different studies

were conducted and other factors. In Ethiopia, epidemiological pattern of malaria transmission is generally unstable and seasonal (Abraham *et al*., 2010).

Different types of intestinal helminthes infection may affect the nutritional status, RBC (red blood cell) numbers and hemoglobin level in different ways. As reported earlier, hookworm, *T. trichuira* and *A. lumbricoides* infections might alter the hosts nutritional status, reduce RBC number and Hgb levels through various mechanisms (including nutrient absorption and damage to mucosa) (Alemu *et al*., 2012). Although the detailed mechanisms were not evaluated in the current study, reduction in hemoglobin levels and anemia were encountered under various combinations of infection (malaria and/or helminthes). In this study, the prevalence of anemia was recorded as 40.5%, value higher than the 31.4% prevalence reported from Halaba kulito health center, Southwest Ethiopia (Degarege *et al*., 2010), but lower than the 50% prevalence recorded in Azzezo health center of Gonder, Northern Ethiopia (Alemu *et al*., 2012), and 44.8% reported from Cameroon (Njunda *et al*., 2015). Children younger than 10 years had the lowest mean haemoglobin concentration compared to the older age groups. From 351 individuals tested for anemia, 142(40.5%) individuals were found anemic according to WHO classifications.

Patients co-infected with *Plasmodium* and helminth parasites were found to have lower mean haemoglobin concentration and anemic. The prevalence of anemia was 35.2%, 32.4% and 16.2% in malaria patient co-infected with hookworm, *A. lumbricoides*, and *T. trichiura*, respectively. The increased prevalence of anemia in co-infected cases may be attributed to chronic blood and iron loss due to worm infections in addition to the loss due to malaria. Accordingly, co-infection with *Plasmodium* and helminth parasites are associated with higher anemia prevalence compared to individuals infected only with malaria parasite. This finding is in agreement with earlier report made from other part of Ethiopia, namely Halaba Kulito (Degarege *et al*., 2010). In the present study, among the frequently encountered clinical manifestation/ complications of malaria were high temperature (all febrile patients), vomiting and headache (44.2% and 62.7%, respectively), and shortness of breath (17.4% cases) besides splenomegaly (10.3%) and hepatomegaly (14.5%) Likewise, a study conducted in Liaquat University Hospital of Hydrabad (sheikh *et al*., 2011) reported a relatively higher incidence of splenomegaly (28%) and hepatomegaly (40%) among patients of similar problem as that of our study.

In general, although the prevalence of malaria seems relatively low in the current study area, most malaria cases were co-infected with helminthes and are associated with complicated and uncomplicated severe malaria symptoms.

6. LIMITATION OF THE STUDY

- ✓ Due to time and budget constraints the kato-katz technique was not used for helminthes diagnosis as the result of which the prevalence of helminthes may have been under estimated.
- ✓ This study tried to determine malaria infection from all febrile patients and intestinal helminthic infection from all malaria positive patients attending health center by standard laboratory techniques. But it did not considered other factors like diet which may have impact on haemoglobin levels and nutrition status of the study participants.
- ✓ For each blood specimens collected from the study participants, only one slide was read to detect malaria infection and to identify the type of species.
- ✓ Furthermore, prevalence of soil transmitted helminths was high and the diseases were still major health problem in the study area. However, this study did not assess association between helminth intensity with anemia.

7. CONCLUSIONS

- The findings of the present study showed that malaria helminthes co-infection were more prevalent in children than in adults in the study area (Darimu health center),
- **M**alaria prevalence was relatively higher among males, those individuals aged more than 15 years, and the rural community.
- Multiple infections with malaria and helminths were very common in the study area.
- Patients co-infected with *Plasmodium* and helminth parasites showed lower mean haemoglobin concentration and were anemic.
- Anemia was more prevalent among malaria-helminthes co-infected subjects than mono-infection with malaria alone.

8. RECOMMENDATIONS

Based on the findings of this study, the following recommendations could be made:

1. The malaria burden in the study area calls for designing of additional intervention strategies besides the use of Indoor residual spraying (IRS) and insecticide treated nets (ITNs)
2. Improving personal hygiene and environmental sanitation through awareness development training in addition to wise use of ant-helminthic medication to minimize complication due to malaria –helminthe co-infection,
3. Further detailed investigation on the prevalence of helminthes using the Kato-katz method to determine the intensity of helminthes burden in the study area, including schistosomiasis
4. Complementing the current study with data on nutritional status of population in the study area.

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APPENDICES-1, Written consent form

I am conducting a study to malaria and helminth infections and co-infections. You are being to participate in this study if you agree. I would like to obtain finger prick blood sample in filter paper and stool samples in a plastic sheet from you or your children by using sterile and disposable materials, which would be used only to detect the presence of malaria and the mentioned intestinal parasites. Only a laboratory technician takes the blood sample and all activities in clinical examination as well as diagnosis by trained physicians of the health center. There is no serious risk in participating, but you may experience a small pain during finger pricking. When you or your children are found positive for malaria and helminths, you will receive drugs. The information in your records is strictly confidential.

Your Participation in this study is completely voluntary and you can refuse to participate or free to withdraw yourself from the study at any time. Refusal to participate will not result in loss of medical care or any other benefits.

Do you understand what has been said to you? If you have any question you have the right to get proper explanation. I am informed to my satisfaction the purpose of this study and the nature of laboratory investigations. I am also aware of my right to opt out of the study at any time during the course of the study without having to give reasons for doing so.

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

Study code no----------

Name----------

Signature----------Date----------

Witness Name----------Signature----------Date----------

Investigator Name----------Signature----------Date----------

APPENDICES -2**, Registration form**

ID	sex	Age	Residence	Religion	Ethnicity	malaria	helminths	Others