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SCHOOL OF GRADUATE STUDIES, DEPARTMENT OF ZOOLOGICAL SCINCES

Clinical Malaria Prevalence and the Associated Risk Factors in Abe Dongoro District, Oromia Region, West Ethiopia

A thesis submitted to the School of Graduate Studies, Addis Ababa University in partial fulfillment of the requirements for the degree of Masters of Science in Biology

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LIST OF ABBREVIATIONS

ABBREVIATIONS

AOI	Africa Orientale Italiana
AOR	Adjusted Odds Ratio
CI	Confidence Interval
CL	Confidence Level
CSA	Central Statistical Agency
DDT	Dichlorodiphenyltrichloroethane
FMoH	Federal Ministry of Health
HH	Household
IFNγ	Interferon gamma
IL	Interleukin
IRS	indoor residual spraying
LLIN	long-lasting insecticidal treated nets
mASL	Meter above sea level
OR	Odds Ratio
PCR	Poly chain reaction
QBC	quantitative buffy coat
RBC	red blood cell
RDT	rapid diagnostic test
TH	T-helper
TNFα	tumor necrosis factor alpha
SPSS	Statistical package for social sciences
WHO	World Health Organization

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ABSTRACT

Malaria is a disease caused by Plasmodium spp. The infections occur mainly in tropical countries with about 90% of these occurring in sub-Saharan Africa. In Ethiopia, approximately 4-5 million cases of malaria are reported annually and it is prevalent in 75% of the country, putting over 50 million people at risk. This study aimed at assessing the current situation of the disease in relation to relevant socio-demographic and environmental factors in Abe Dongoro district, Oromia Region, west Ethiopia. Descriptive community based cross-sectional study was conducted from September 2018 to May 2019 in three selected kebeles randomly. The samples have been collected in two ways. Data related with assessment of the risk factors associated with prevalence of malaria were collected by interviewing household's (HHs) age ≥18 years old from the selected kebeles that came to local health post as patient or brought other house hold (HH) members to health posts using structured and pretested questions. A total of 770 individuals belonging to the 385 randomly selected house hold (HHs) in the three kebeles, participated in the study. Of the total, 385 mRDT from the patients brought to the health centers malaria signs were blood tested for the presence of Plasmodium parasites in their blood were tested for malaria by mRDT using finger-prick blood samples, 131 (34%) were found to be mRDT positive. The association of malaria prevalence with a variety of the risk factors obtained by questionnaire and field observation of environmental factors were determined in the study area in the analysis of univ and multiv logistic regression in explanatory factors including the proximity of the house to mosquito breeding site, current use of long-lasting insecticidal treated nets (LLINs), types of material from which the roof of the house was made, the sources of drinking water, and educational status of the head of the households (HHs) have been determined that they were significantly associated ($p < 0.05$) with malaria. However, locality (kebeles) sex, age group and marital status of the patient, number of family size, presence of hole on the wall of the house and spray of insecticidal chemicals in the house were not significantly associated, with mRDT in the study area. The age group of 11 to 20 years was found significantly infected than those in reference group (0-10) years ($P<0.05$).

Key-words: Indoor residual spraying, long-lasting net, mRDT, malaria, prevalence, Abe Dongoro.

1. NTRODUCTION

1.1. Background

Malaria is a disease caused by *Plasmodium spp.*, of which *Plasmodium vivax, P. falciparum, P. ovale,* and *P. malariae* are the four species of *Plasmodium* known to cause malaria infections in humans. The first two species cause the most infections worldwide. The most widespread disease is caused by *P. vivax* and the most serious disease is the one caused by *P. falciparum*. *P. falciparum* is the agent that most commonly causes severe and potentially fatal malaria. *P. vivax* and *P. ovale* may have dormant liver stage parasites, which can reactivate and cause malaria several months or years after the infecting mosquito bite. *P.malariae* can result in long-lasting infections and if untreated can persist asymptomatically in the human host for years, even a life time. The first symptoms of malaria (most often fever, chills, sweats, headaches, muscle pains, nausea and vomiting) are often not specific and are also found in other diseases (such as influenza and other common viral infections). Likewise, the physical findings are often not specific (elevated temperature, perspiration, tiredness). In severe malaria (caused by *P.* falciparum), clinical findings (confusion, coma, neurologic focal signs, severe anemia, respiratory difficulties) are more striking and may increase the suspicion index for malaria (NNDSS, 2014). Humans are the only reservoirs for these four species. These protists pass part of their life cycle in the human reservoir and part in female *Anopheles* mosquito, the vector that transmits Plasmodium spp. The vector is distributed predominantly in the tropics and subtropics (Madigan *et al.,* 2012).

Malaria is important human disease which has played an important role in the development and spread of human culture and has even expected to affected human evolution. Despite several effective treatments are available, it remains still a significant human disease (Madigan *et al.,* 2012). It is currently endemic in more than 100 countries or territories, mainly in Central and South America, Asia, Oceania, the Caribbean and in sub-Saharan Africa (Kayser *et al.*, 2005).

The infections annually occur in 350 to 500 million people worldwide with about 90% of these occurring in sub-Saharan Africa and nearly 1 million of these will die (Madigan *et al.,* 2012), of which about 660,000 are mostly children under 5 years of age in sub-saharan Africa (SSA) (Menberu, 2013). In Ethiopia, approximately 4-5 million cases of malaria are reported annually and it is prevalent in75% of the country, putting over 50 million people at risk (Menberu, 2013).

The two plasmodium species known to cause malaria in Ethiopia are *Plasmodium falciparum and Plasmodium vivax* comprising 60% and 40% respectively (FMH, 2004, WHO/USAID, 1999, 2000)

1.2 Statement t of the Problem

Studying the prevalence of malaria is necessary to implement effective control measures. It is obvious that different control and prevention methods including long-lasting insecticidal treated nets (LLIN), indoor residual spraying (IRS) and prompt diagnosis and treatment of cases have been carried out by various bodies to combat the disease in Ethiopia. The efficacy of these measures could, in turn, be evaluated by determination of the clinical prevalence of the disease in different locals of the country.

Beside to this, the prevalence of malaria has been expected to be associated with different risk factors which could vary from an area to other (National Malaria Control Team, 2014). Studying these factors is also important in taking steps against the disease to control and/or prevent the disease in a local. However, as far as the knowledge of the researcher is concerned, both the prevalence and associated risk factors of malaria have not been studied in Abe Dongoro district so far.

1.3 0bjective

1.3.1 General Objective

The general objective of the current study is:

- To determine the prevalence and factors associated with Malaria in human

1.3 .2 Specific Objectives

- To determine clinical prevalence of malaria in Abe Dongoro District.
- To identify the risk factors associated with prevalence of malaria in the study area
- To assess possible measures of combating malaria in the study area.

1.4 Significance of the study

This study will provide significant information on the level of the current malaria prevalence, distribution, influencing local factors; evaluate the existing malaria control measures and the associated risk factors in the study area. Moreover, the result of this study may provide valuable information for further improvement on malaria control program in practices.

2. REVIEWS OF LITERATURES

2.1. Etiology

Malaria is one of the most harmful infections and happening over a large area disease found in tropical and subtropical regions. Every years this vector-borne disease causes worldwide about 273 million clinical causes and more than one million death (Toure` and Oduola 2004). Particularly in sub-Saharan Africa where *p. falciparum* causes high morbidity and mortality rate (Snow *et al*., 1999a, b). Malaria is caused by protozoan parasites of the genus *palasmodium* (Clark, 2010; Madigan *et al*., 2012).The four species of *Plasmodium* known to cause malaria infections in humans are Plasmodium vivax, *P. falciparum*, *P. ovale,* and *P. malariae* of which the two most serious forms of this genus are *p. falciparum* and *p. vivax*.). Humans are the only reservoirs for these four species. *Plasmodium spp*. can also cause malaria-like diseases in other warm-blooded animals. The protists cause malaria in human carry out part of their life cycle in the human reservoir and part in the female *Anopheles* mosquito, the only vector that transmits *Plasmodium spp*. The vector spreads the protist from person to person and distributed predominantly in the tropics and subtropics (Clark, 2010, Madigan *et al*., 2012; WHO, 2014). People suffering from malaria may experience fever, headache, malaise, severe anemia, comma, impaired consciousness, convulsions, hypoglycemia, and high parasitemia (Colwell and Patz 1998; Gay-Andrieu *et al*.2005). Deaths predominantly occur in young children and progressive comma, pulmonary edema, kidney failure, or shock caused by the collapse of the vascular system (Colwell and Patz 1998).

2.2. Malaria Transmission

The malaria parasite typically is transmitted to people by mosquitoes belonging to the genus *Anopheles* (NIH-b, 2009). The female *Anopheles* mosquito is the chief vector and the most common means for transmitting malaria to humans. Some 60 species of this mosquito have been identified as vector for malaria. The infection is transmitted by the bite of an infected female *Anopheles* mosquito (Ridley, 2012). The mosquito most frequently bites at dawn and at dusk, as this is the most active feeding times for mosquitoes. The mosquito is infected by biting a patient infected with malaria, where it aspirates the sexual forms of the parasite, the gametocyte continue the sexual phase of the cycle and the sporozoites fill the salivary glands of the infested mosquito (Ridley, 2012). In rare cases, a person may contract malaria through contaminated

blood. Malaria also may be transmitted from a mother to her fetus before or during delivery ("congenital" malaria). Because the malaria parasite is found in red blood cells, malaria can also be transmitted through blood transfusion, organ transplant, or the shared use of needles or syringes contaminated with blood (NIH-b, 2009).

Figure 1: Illustration drawn by Laveran of various stages of malaria parasites as seen on fresh blood. Dark pigment granules are present in most stages (CDC-a, 2012).

2.3. The parasite cycle

The cycle of the malaria parasite starts when the parasite within the insect is transmitted by a female mosquito to the human host whilst feeding (Price *et al*. 1996; Phillips 2001). A blood meal is required by the insect to produce eggs which are laid and then develop in the standing water. During the blood meal of female *Anopheles* mosquito sporozoites, the infective form of the malaria parasite within the mosquitoes, are injected into the human host. The human host is infected by plasmodia sporozoites, small, elongated cells produced in the mosquito that localize in the salivary gland of the insect. Sporozoites subsequently mature into schizont, which rupture and release numerous merozoites after 5-6 days (price *et al*. 1996; Madigan *et al.* 2012). When these merozoites enter the blood stream the so-called erythrocyte stage starts, beginning of an asexual cycle. In the erythrocyte stage merozoites attach themselves to specific red blood cell receptors where the asexual reproduction of the parasite (schizogony) leads to the development of immature trophozoites. This stage is the so-called ring stage as parasitized red blood cells of an infected host are identifiable under the microscope. Mature trophozoites finally evolve again into schizont. The erythrocytic cycle take about 48 hours to complete (Rosenberg *et al*. 1990a; Madigan *et al*., 2012,) and results in rupture of schizont, which liberate on average 16 merozoites (Eichner *et al*. 2001). The malaria parasite therefore cannot be identified before schizont has ruptured after about 7 day (Schneider *et al*. 2005).

The blood stage of parasite is responsible for clinical manifestations of the disease (Talman *et al.* 2004). Mature, asexual stages of *P. falciparum* are mostly absent from the peripheral circulation and are detectable under microscope. This is due to the sequestration of asexual parasites that is the adherence of infected erythrocytes to the micro vascular endothelia of many organs and tissues such as heart, lung, liver, skin, and brain (MacPherson *et al*.1985). the process often leads to a severe affection of the body(e.g. cerebral malaria) on the other hand bursting erythrocytes cause malaise and fever. Prolonged infection sometimes leads to severe anemia.

Sexual stages of the parasite play a minor role in terms of morbidity, but essential for the transmission of the parasite. Production of sexual form of the parasite also begins in the erythrocyte stage when merozoites either go into another round of schizogony or develop male and female gametocytes are preferentially sequestered in the bone morrow and spleen (Thomson and Robertson 1935; Smalley *et al*. 1981). Gametocyte maturation last about 8-11 days and after initial infection this form is detectable after approximately 11-28 days in blood. Male and female

gametocytes are finally released into blood stream and might be picked up by an anopheles vector.

The development of malaria parasite within the mosquito denotes the so-called sporogonic cycle or extrinsic incubation period (Price *et al*. 1996; Kayser *et al*, 2005; Madigan *et al.*, 2012). This cycle starts when gametocytes are ingested by biting mosquito vector. Male and female gametocytes fuse to form a zygote. These become mobile and transform into elongated ookinetes, which invade the mid-gut wall and turn into oocytes. Those oocytes increase in size, rupture, and finally release sporozoites. In the end, these sporozoites reach the salivary glands of mosquitoes and the life cycle of malaria parasite is perpetuated (Kayser *et al*., 2005; Madigan *et al*., 2012).

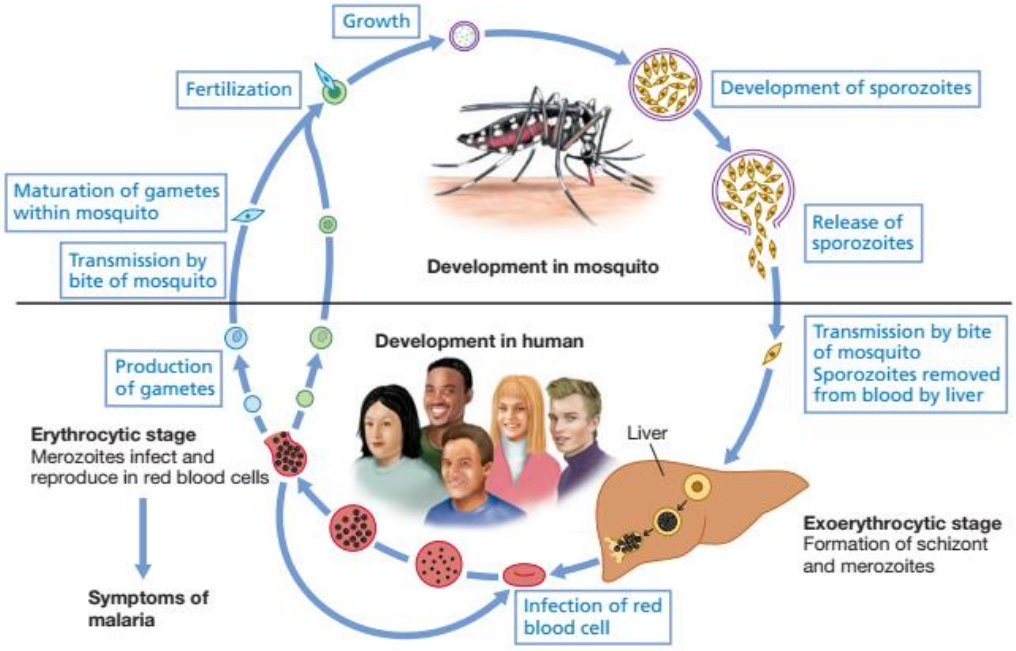

Figure .2: The Complex life cycle of *Plasmodium* (From: Madigan *et al.*, 2012)

2.4. Epidemiology of Malaria

2.4.1. The global picture

The term malaria is derived from the Italian words 'mal'=bad 'aria'=air is caused by a parasitic protozoa of genus plasmodium (p.) and is one of the world's most serious health problems (De Savigny and Binka 2004; CDC-a, 2012).Malaria is important human disease which has played an important role in the development and spread of human culture and has even expected to

affected human evolution. It remains still a significant human disease even though several effective treatments are available (Madigan *et al.,* 2012). The World Health Organization (WHO) estimated that about two billion people that are more than 40% of the total population are exposed to this mosquito-borne disease. Estimates revealed that malaria causes about 273 million clinical cases and 1.12 million deaths annually. At least 90% of worldwide malaria deaths occur in sub-Saharan Africa (Greenwood and Mutabingwa 2002; Greenwood *et al*. 2005; Madigan *et al.,* 2012).Of which about 660,000 deaths are mostly children under 5 years of age in sub-saharan Africa (SSA) (Menberu, 2013). This life-threatening disease is mostly restricted to young children as immunity to severe malaria is later developed (Gupta *et al.* 1999a). Pregnant women are especially prone to malaria causing an increased risk of infant low birth weight and infant mortality (Menendez 1999; Steketee and Mutabingwa 1999; D' Alessandro 1999). Making malaria one of the most common causes of death due to infectious disease worldwide (Kayser *et al.,* 2005, Clark, 2010; Madigan *et al.,* 2012, Glazer &Nikaido, 2007). Hence malaria is currently the leading global health problem, especially in the poor nations of Africa and Asia (Menberu, 2013). Malaria is currently endemic in more than 100 countries or territories, mainly in Central and South America, Asia, Oceania, the Caribbean and in sub-Saharan Africa (Kayser *et al.,* 2005). Endemic region are characterized by warm temperature high humidity, both suitable for mosquito breeding, and where human population and where human population malaria co-exist. Altitude and seasons are the two primaries descriptive for the epidemiology of malaria (Tulu, 1993).

2.4.2 Malaria in Ethiopia

In Ethiopia the earliest surveys of malaria were conducted between 1936 and 1941 during the Italian occupation, by malariologists from the Instituto di Malariologia, Rome. These investigations provided important geographic reconnaissance of dominant vectors, infection prevalence and the general health status of communities from the coast in Somalia to the then northern Eritrean territories under the *Africa Orientale Italian* (AOI). These studies laid the foundation for understanding the basic malaria epidemiology across the country related to altitude, seasonality, factors related to agriculture and vectors (Lega *et al.,* 1937). Studies indicated that the malaria prevalence in Ethiopia ranges from 0 to 37% (Yohannes *et al.,* 2005).

Malaria risks in Ethiopia have been profiled by identifying three eco-climatic zones to determine the endemicity of the disease. These are the cold zone (dega) above 2500 mASL, the temperate

zone (weyna dega) between 1500 and 2500 mASL with mean annual rainfall between 400 and 2400 mm and the warm zone (kolla) below 1500 mASL with between 100 and 400 mm of annual rainfall. Accordingly, the most important malaria zone was the warm zone, where intense, seasonal transmission occurs not characterized by epidemics except as a result of the introduction of non-immune labourers, soldiers, settlers and refugees (Anon, 1991). However, for the sake of control of the disease, malaria has been classified as highland malaria (1800-2000 mASL), urban malaria, settlement malaria, traditional rural agricultural malaria, development related malaria, coastal malaria, and desert fringe malaria) (FMoH, 1993).

In Ethiopia, approximately 4-5 million cases of malaria are reported annually and it is prevalent in75% of the country, putting over 50 million people at risk (Menberu, 2013). Among the four plasmodium species known to cause malaria in Ethiopia, the two epidemiologically important species are *Plasmodium falciparum* and *Plasmodium vivax* comprising 60% and 40% respectively (FMH, 2004).

2.5 Pathogenicity and Pathogenesis of Malaria

The pathogenic effect of a malarial infection have been considered to be directly related to hemolysis of infected red cell and uninfected cell, liberation of the metabolites of the parasite and the immunologic response of the host to this antigenic material and the formation of malarial pigment, additionally, in falciparum malaria the phenomenon of cytoadherence is basic to the locally diminished tissue perfusion seen in it is more severe complications, cytoadherence is the result of the expression on the surface of the parasitized red cell of strain and stage- specific parasite-derived ligands, which adhere to a specific receptor complex on the endothelial cells. In persons subjected to repeat attack of malaria anaemia is disproportional to the number of red blood cells infected, and indicates that non infected red blood cells may become sensitized and be destroyed (John and Petri, 2006)

An infection initially presents in nonspecific symptoms (headache, fatigue, nausea, fever) (Kayser*et al.,* 2005).

The clinical symptoms of malaria are primarily due to schizont rupture and destruction of erythrocytes (Trampuz *et al*., 2003). Malaria typically produces a string of recurrent attacks, or paroxysms, each of which has three stages-chills, followed by fever and then sweating. Along with chills, the person is likely to have headache, malaise, fatigue, muscular pains, occasional

nausea, vomiting and diarrhea. Within an hour or two, the body temperature rises, and the skin feels hot and dry. Then, as the body temperature falls, a drenching sweat begins. The person, feeling tired and weak, is likely to fall asleep. The symptoms first appear some 10 to 16 days after the infectious mosquito bite and coincide with the bursting of infected red blood cells (RBCs). When many RBCs are infected and break at the same time, malaria attacks can recur at regular time periods-every two days for *P.vivax* malaria and *P.ovale* and every three days for *P.malariae* (NIH-c, 2009).

Malaria can quickly become life-threatening as the vital organs are deprived of oxygen and nutrients due to disruptions in the blood supply (WHO, 2014). Untreated malaria particularly the one caused by P. falciparum can quickly develop to a lethal outcome (Kayser*et al.*, 2005).

The clinical manifestations of malaria are caused by the erythrocytic stages ("blood stages") of the plasmodia and reflect multifactorial pathogenic process affecting many different organs (Kayser*et al.*, 2005).

As a result of erythrocytic schizogony and the attendant rupture of erythrocytes or red blood cells (RBCs), malarial antigens (phospholipids and glycolipids) are released that stimulate macrophages and monocytes to produce tumor necrosis factor alpha (TNFα) and other cytokines Interleukin (IL-1, IL-6, IL-8, etc.). Also associated with this process are bouts of fever, to which hemozoin presumably contributes as well. Cytokine production is also initiated by IFNγ produced in the immunological TH1 response. TNFα plays a special role in pathogenicity, since the concentration of this cytokine in the blood correlates with the severity of a *P. falciparum* infection. This substance also, at higher concentrations, induces fever, inhibits erythropoiesis, stimulates erythrophagocytosis, and causes various nonspecific symptoms such as nausea, vomiting, and diarrhea. At lower concentrations, TNFα can contribute to the killing of the intracellular parasites. Various other cytokines either synergize with TNFα or induce different reactions. An important factor in malarial pathogenesis, especially in malaria tropica, is anemia, caused by destruction of RBCs in schizogony, increased elimination of both infected and no infected RBCs in the spleen, inhibition of erythropoiesis by TNFα, and other factors (Kayser*et al.*, 2005).

2.6 Diagnosis

Malaria presents a diagnostic challenge to laboratories in most countries (Singh *et al.,* 2010). Malaria should be considered a potential medical emergency and should be treated accordingly (CDC-d, 2012).

2.6.1 Clinical Signs

Clinical diagnosisis based on the patient's symptoms and on physical findings at examination. The first symptoms of malaria (most often fever, chills, sweats, headaches, muscle pains, nausea and vomiting) are often not specific and are also found in other diseases (such as the "flu" and common viral infections). Likewise, the physical findings are often not specific (elevated temperature, perspiration, tiredness). In severe malaria (caused by *P. falciparum*), clinical findings (confusion, coma, neurologic focal signs, severe anemia, respiratory difficulties) are more striking and may increase the index of suspicion for malaria. If possible, clinical findings should always be confirmed by a laboratory test for malaria (CDC-d, 2012).

The destruction of the host red blood cells by the *P. vivax* results in clinical symptoms of malaria, characterized by chills followed by fever of up to 40°C. The chill–fever pattern coincides with the release of P. vivax merozoites from the erythrocytes during the synchronized asexual reproduction cycle. Vomiting and severe headache may accompany the chill–fever cycles, and over the longer term, characteristic symptomatic malaria generally alternates with asymptomatic periods. Because of the destruction of red blood cells, malaria generally causes anemia and some enlargement of the spleen (splenomegaly) (Madigan *et al.,* 2012).

The clinical manifestations of malaria are caused by the asexual erythrocytic stages of the plasmodia and therefore commence shortly after parasitemia at the earliest. The incubation periods vary, depending on the Plasmodium species involved, from seven to 35 days after infection. These periods can, however, be extended by weeks or even months, particularly if the infection is suppressed by prophylactic medication. The clinical manifestations of malaria depend on a number of different factors, above all the Plasmodium species and immune status of the patient. The Plasmodium species with the most pronounced pathogenicity is *P. falciparum,* which causes “malignant tertian malaria” (malaria tropica), whereas the other Plasmodium species cause milder forms (“benign malaria”). Children and no immune adults from non

malarious areas, as well as children in endemic regions aged six months to three years, are most susceptible to infection (Kayser *et al.*, 2005).

Malaria begins with nonspecific initial symptoms that last several days, including for instance headache, pain in limbs, general fatigue, chills, and occasionally nausea as well as intermittent fever, either continuous or at irregular intervals. Several days to a week after onset of parasitemia, the schizogonic cycle synchronizes: in infections with *p. vivax*, *p. ovale*, and *p. falciparum*, a cycle is completed within 48 hours, in infections with *p. malariae* within 72 hours. Bouts of fever occur at the same intervals, i.e., on day 1, then 48 hours later on day 3 (hence "malaria tertian") or on day 1 and then again after 72 hours on day 4 (hence "malaria quartana"). After an initial rise in temperature to about 39 8°C, peripheral vasoconstriction causes a period of chills (lasting for about 10 minutes to one hour), then the temperature once again rises to 40–41 8°C (febrile stage two to six hours), whereupon peripheral vasodilatation and an outbreak of sweating follow. These bouts occur mainly in the afternoon and evening hours. Once the paroxysm has abated and the fever has fallen, the patient feels well again until the next one begins. In severe malaria tropica, however, circulatory disturbances, collapse, or delirium may occur without fever (algid malaria) (Kayser *et al.*, 2005).

2.6.2 Diagnostic Tests

The accepted laboratory practice for the diagnosis of malaria is the preparation and microscopic examination of blood films stained with Giemsa, Wright's, or Field's stain. Blood obtained by pricking a finger or earlobe is the ideal sample because the density of developed trophozoites or schizont is greater in blood from this capillary-rich area. Blood obtained by venipuncture collected in heparin or sequester (EDTA) an anticoagulant-coated tube is acceptable if used shortly after being drawn to prevent alteration in the morphology of white blood cells (WBC) and malaria parasites. Both thick and thin blood films should be prepared. The thick blood film concentrates the layers of red blood cells (RBC) on a small surface by a factor of 20 to 30 and is stained as an unfixed preparation using Field's stain or diluted Wright's or Giemsa stain. The thick blood film provides enhanced sensitivity of the blood film technique and is much better than the thin film for detection of low levels of parasitemia and reappearance of circulating parasites during infection recrudescence or relapse. The lyses of the RBC during the staining process can make the process of scanning for parasites more difficult until experience is gained

in finding the parasites among the WBC and platelets. The thin blood film is methanol fixed and stained with diluted Giemsa or Wright's stain using buffered water at pH 7.2 to emphasize the parasite inclusions in the RBC. Because of the fixed monolayer of RBC available in this procedure the morphological identification of the parasite to the species level is much easier and provides greater specificity than the thick-film examination. The thin blood film is often preferred for routine estimation of the parasitemia because the organisms are easier to see and count. The ability to count parasites in sequential blood films enables the response to therapy to be monitored, particularly for *P .falciparum* infections (Moody, 2002).

Microscopy remains the gold standard for diagnosing malaria infections in clinical practice and research. However, microscopy is labor intensive, requires significant skills and time, which causes therapeutic delays. The objective of obtaining result quickly from the examination of blood samples from patients with suspected malaria is now made possible with the introduction of rapid malaria diagnostic tests (RDTs) (Singh *et al.*, 2010

Conclusive diagnosis of malaria in humans requires the identification of Plasmodium-infected erythrocyte in blood smears. Fluorescent nucleic acid stains, nucleic acid probes, PCR assays, and antigen-detection methods rapid diagnostic tests (RDTs) may all be used to verify Plasmodium infections or to differentiate between infections with various Plasmodium species (Madigan *et al.*, 2012).

Furthermore, as malaria can develop life threatening outcomes, it is important to obtain an etiological diagnosis as quickly as possible by microscopic detection of the parasites in the blood, and to initiate effective treatment. These Plasmodium species can be identified and differentiated from each other by light microscopy in stained blood smears during the erythrocytic phase of the infection in humans. Capillary blood is sampled before chemotherapy is started, if possible before the onset of fever, and examined microscopically in both thick and thin blood smears following Giemsa staining. The quantitative buffy coat (QBC) method can be used to concentrate the plasmodia. Rapid tests (ParaSight, MalaQuick) have also been available for some years to diagnose *P. falciparum* infections. Using a monoclonal antibody, these tests can detect a specific Plasmodium antigen (HRP2) in whole blood with a very high level of sensitivity and specificity. Another rapid test (OptiMAL) for diagnosis of all Plasmodium species is based on detection of specific lactate dehydrogenase (Kayser*et al.*, 2005).

2.7 Prevention and Control

Vector control is the main way to reduce malaria transmission at the community level. It is the only intervention that can reduce malaria transmission from very high levels to close to zero. For individuals, personal protection against mosquito bites represents the first line of defiance for malaria prevention. Two forms of vector control are effective in a wide range of circumstances: insecticide-treated mosquito nets (ITNs) and: long-lasting insecticidal nets (LLINs) are the preferred form for public health distribution programmers. WHO recommends coverage for all at-risk area and in most setting. The most cost effective way to achieve this is through provision of free LLINs, so that everyone sleeps under a LLIN every night. Indoor residual spraying (IRS) is a power full way to rapidly reduce malaria transmission. Its full potential is realized when at least 80% of houses in targeted areas are sprayed. Indoor spraying is effective for 3-6 months, depending on the insecticide used and the type of surface on which it is sprayed. DicholoroDiphenylTrichloroethane (DDT) can be effective for 9-12 months in some cases. Longer-lasting insecticides, as well as new classes of insecticides for use in IRS programmers are under development Antimalarial medicines can also be used to prevent malaria (WHO-a, 2015). Patients who have severe *P. falciparum* malaria or who cannot take oral medications should be given the treatment by continuous intravenous infusion. Most drugs used in treatment are active against the parasite forms in the blood (CDC-e, 2012). Early and effective treatment of malaria is a cornerstone of malaria control programmers (WHO, 2014).Malaria is an entirely preventable and treatable disease. The primary objective of treatment is to ensure a rapid and complete elimination of the *Plasmodium* parasite from the patient's blood in order to prevent progression of uncomplicated malaria to severe disease or death, and to chronic infection that leads to malaria-related anemia. From a public health perspective, treatment is meant to reduce transmission of the infection too theirs, by reducing the infectious reservoir and to prevent the emergence and spread of resistance to antimalarial medicines (WHO-b, 2015).

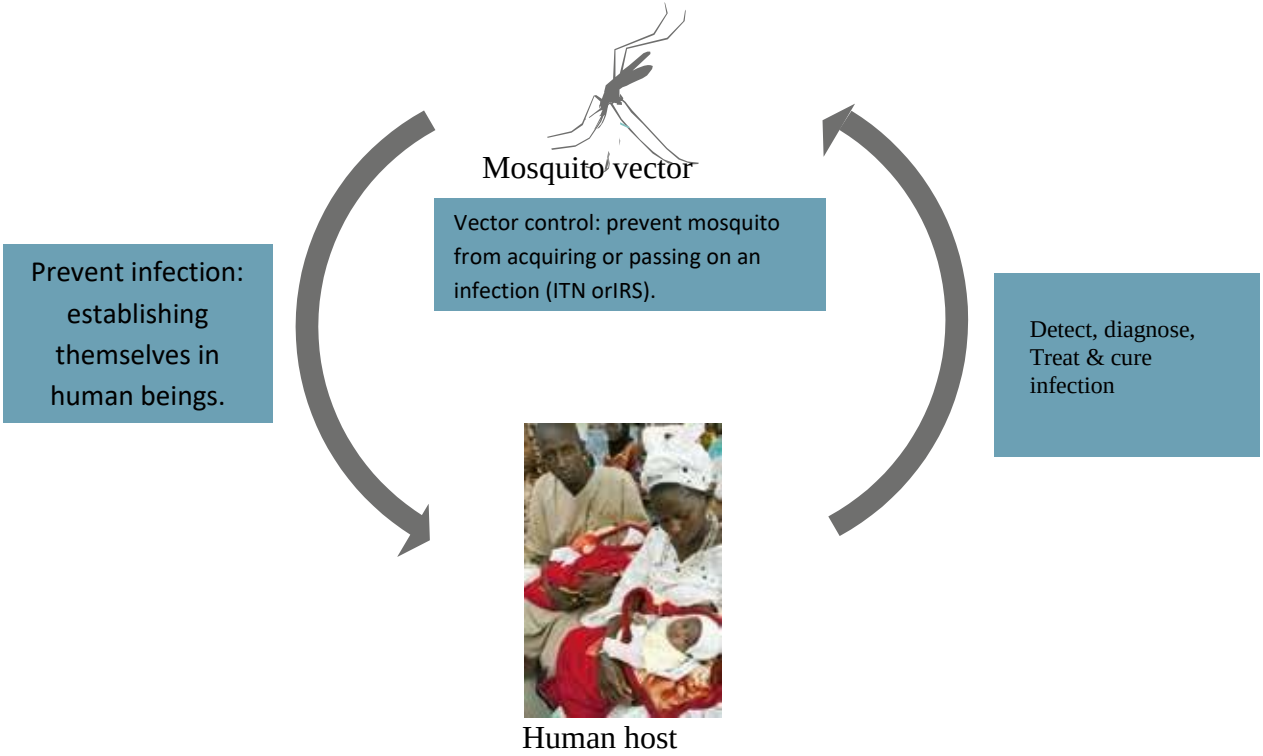

Figure 3: Main strategies to prevent and treat malaria (WHO, .2010).

There are three main ways to fight malaria (Fig.3):

●*vector control*, which focuses on blocking the transmission of parasites by the mosquito vector from humans to mosquitoes and then back to humans, thereby reducing the disease burden; the main interventions are vector control using insecticide-treated mosquito nets (ITNs) or indoor residual spraying (IRS) and, in some settings, mosquito larval control;

●chemoprevention, which suppresses the blood- stage infection in humans and prevents malaria disease; and

●case management, which involves prompt diagnosis and treatment of malaria infections with appropriate antimalarial medicines; this reduces the likelihood of progression to severe disease and death from malaria.

Chemoprophylaxis for travel to endemic areas and treatment of malaria is usually accomplished with chloroquine. Chloroquine is the drug of choice for treating merozoites within red cells, but does not kill sporozoites. The closely related drug primaquine, however, eliminates sporozoites of *P. vivax* and *P. ovale* that may remain in liver cells. Treatment with both chloroquine and primaquine produces a radical cure. Even in individuals who have undergone radical drug treatment, however, malaria may recur years after the primary infection. Apparently not all of the

sporozoites in the liver are eliminated; they reinitiate malaria months or years later by undergoing asexual reproduction (schizogony) and releasing a new generation of merozoites. In many parts of the world Plasmodium strains have developed resistance to chloroquine or primaquine or both and some strains have developed resistance to other drugs as well. In areas with known drug-resistant strains, mefloquin or doxycycline is prescribed for prophylaxis; a combination of atovaquone and proguanil (Malarone) is recommended for both treatment and prophylaxis. A new category of antimalarial drugs is comprised of synthetic derivatives of artemisinin, a natural compound containing reactive peroxide groups that form free radicals. These compounds are active in vivo. Even in the case of this relatively new drug, however, there are reports of artemisinin-resistant Plasmodium strains. A new experimental drug, NITD609, is unique in that it targets a parasite membrane transport protein. A single dose of this experimental compound is curative for Plasmodium infections in mice. Clinical trials in humans are in progress (Madigan *et al.,* 2012). *Plasmodium* has also been inhibited by chloramphenicol, rifamycin, macrolides and quinolones (Clark, 2010). Antimalarial drug treatment is an inexpensive but short-term solution to malaria prevention and control, and drug-resistant strains of Plasmodium spp. further complicate matters. The most effective control measure is to interrupt the life cycle of the protist by eliminating one of the obligate hosts, the *Anopheles* mosquito. Several approaches to mosquito control are possible. The first method requires elimination of habitat by drainage of swamps and similar breeding areas. Oil can also be spread on swamps to reduce the oxygen supply to mosquito larvae. The second method of mosquito control requires elimination of the mosquito by insecticides, followed by treatment of patients with antimalarial drugs, thereby breaking the Plasmodium life cycle. The insecticides dichlorodiphenyltrichloroethane (DDT), have been used to control larvae and adult mosquitoes (Madigan *et al.,* 2012).

3. MATERIAL AND METHODS

3.1. Description of the Study Area

The study was conducted in Abe Dongoro District of Oromia Regional state, West Ethiopia. Abe Dongoro is one of the eleven Districts of Horo Guduru Wollega Zone, in Oromia Region located in the western part of the Ethiopia. Its center named Tulu Wayu is 361 km from Finfine. It is geographically located between a latitudes of 9° 30'N and 9° 45'N and longitudes of 36°45'E and 37°, 00'E. The District shares common borders with Amuru *and* Jarte Jardaga Districtin the north, Horo Buluk district in the North East and with East Wollega Zone in the south, and in the west. The District is estimated to have a total area of 1092 sq. km.

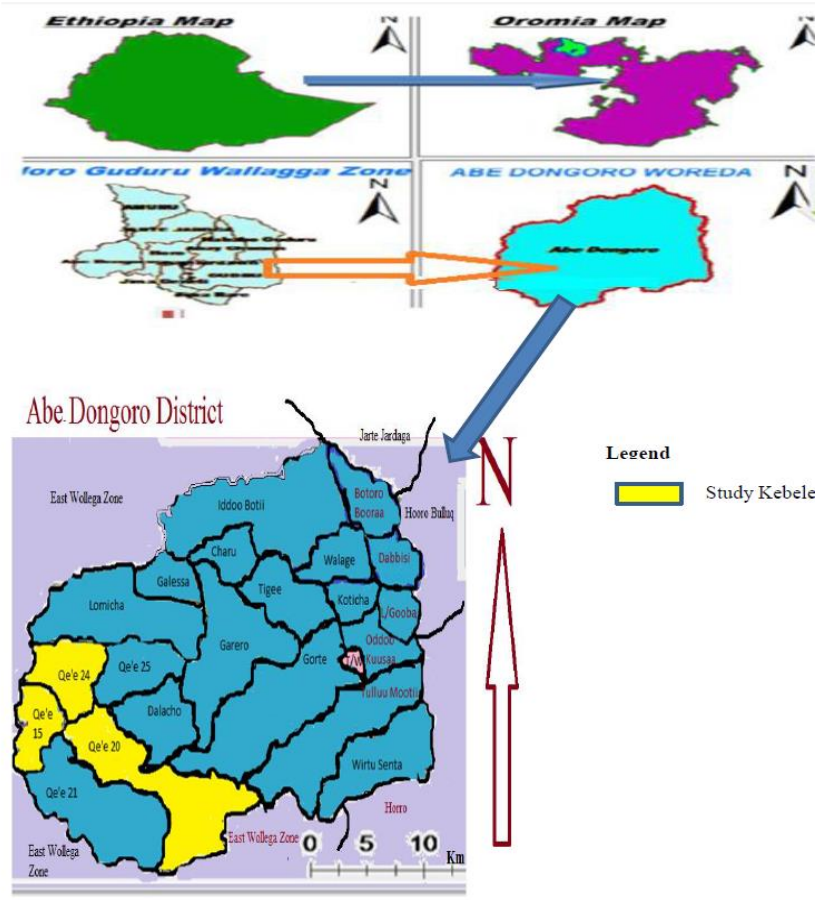

Figure 4: Map of the Study Area**.**

The altitude of Abe Dongoro District ranges between 1300 to 2800 meters above sea level (m.a.s.l.). Ecologically, places less than 1500m.a.s.l. are classified as kolla (low land) and those

between 1500 and 2000 m.a.s.l. as Wiona Dega (sub-tropical) and above 2000 m.a.s.l. 86.33% of the study areas belong to lowlands and 13.67% midlands and highlands.

The Average total rainfall of the area is 2125mm which varies from about 1750 mm to 2500 mm. locally. The four seasons in the District are autumn (from September through November), winter (from December through February), spring (from March through May) and summer (from June through August). The rainy seasons starts in the first month of Arfasa (spring) and lasts to the end of the last month of Birra (autumn) with highest rainfall time from mid-June to beginning of September i.e. during *Gann*a (summer).

The average temperature of the area is estimated to be 22°C which may vary with altitude and seasons. In the highlands of the District, during the months of November to January, the temperature may drop to the lower value. In the lowlands of the area the average temperature increases from 20°C to 32 °C during summer.

According to 2007 national census reports, Abe Dongoro District has a total population of 67,017, of which 50.93% were men and 49.07% were women; 2,519 (3.76%) of its population were urban dwellers (CSA, 2007).

Agriculture is the main economic activities of the District which involves mixed farming, with both crop production and livestock rearing of animals that about 96 percent of the total population depends on the activity. The area is known in producing cash crops such as coffee, pulse seed sesames, and others spices as red pepper, and ginger. Food crops such as maize, wheat, teff and barely and food animals such as cattle, goat, sheep and honey bee products are produced. The District constitutes 1 hospital, 21 clinics and 7 health centers.

As is estimated currently there are 74124 total populations living in Abe Dongoro District and are considered as study population. Of these, about 52 percent of the population is below the age of 25.

3.2. Study Design, Population and Sample Size Determination

The study was a community-based cross-sectional study was conducted among subjects attending the health centers from September 2018 to May 2019 of the study period to assess the clinical prevalence and associated risk factors of malaria in Abe Dongoro District. As there is no Kebele which is free of malaria, three kebeles were randomly selected.

The samples have been collected in two ways. Data related with assessment of the risk factors associated with prevalence of malaria were collected by interviewing randomly Individuals house hold members, who were came to health centers, from the selected kebeles, for any kind of health service during the data collections period, were selected randomly. All individual members who were permanent residents in the randomly selected households age ≥18 years old from selected kebeles that came to local health posts as patient or brought other household members to health posts using structured and pretested questions. A questionnaire was developed as a modification of the Malaria Indicator Survey Household (MIS) questionnaire (WHO 2005). The questionnaire had two parts; the household interview and malaria parasite form. The selected individuals members were volunteered to participate in this study were included in the study for questionnaire.

The questionnaire was administered to gather information on markers of socioeconomic status, demographic and geographic variables that included sex, age, family size, incomes, state of pregnancy, marital status, level of education, main source of drinking water, house distance from nearby mosquito breeding site, main material of room's roof, wall and floor, presence of eaves and opening on the wall, presence of latrines, incidence of anti-malaria spraying in the past 12 months, possession and use, number and type of LLINs specifically developed and applied for data collection using the local language. Every head of the selected HHs either female or male that came to local health posts as patients or brought other household members to health post was interviewed. The information was collected from households or representatives above 18 years old who presented the patient to the health centers.

Data for determination of malaria prevalence in the study area during the study time were collected by detecting of plasmodium parasite in blood samples from patients presented to the local health posts (Tulu Gana, Angar and Qe'e Digdama). Diagnostic test available in the centers was malaria Rapid Diagnostic Test (mRDT) and carried out in the centers to identify the malaria infection. One laboratory technician having more than two years of work experience and each selected Kebele health care providers were given one day training study protocol as well as data recording format.

The minimum sample size for each group was estimated by using minimum sample size determination formula: n=z2p (1-p)/d2 (Daniel, 2004);

$$n = \frac{1.96^2 * 0.5(1-0.5)}{0.05^2} = 384.5$$

Where: n = Sample size, z =the table value of 95% confidence interval (CI) =1.96, p = the population proportion. Since there was no previous study conducted on prevalence of malaria in the area, the population proportion was assumed to be 0.5. (Getu and Fasil, 2003).

Then n is 385 (rounded from 384.5).

A small blood film collection was carried out by laboratory technician by pricking the finger with disposable blood lancet. Peripheral blood smears examination of well-prepared and well stained blood film is the gold standard in confirming the presence of malaria parasite (pyne, 1988). Two blood slides each composed of thick and thin blood smears was taken from each participants by a medical laboratory technician according to the standard operating procedure (SOP) protocol and standards WHO, 2009). Slides were labeled and air-dried horizontally in a slides tray, and thin film was fixed with methanol after drying. Slides were stained with 3% Giemsa solution for 30-45 minutes at each laboratory unit (WHO, 2010). Blood slides were read and cross-laboratory units as, either negative for blood parasites, *p. falciparum* positive and *p. vivax* positive. The staining technique and blood film examination was conducted to standard of World Health Organization protocol (cheesbrough, 1987; Garcia, 2001). The thin and thick films were examined by using 100X magnification (oil immersion). Parasite positivity was determined from thick and thin smear slides. The results were recorded and used as data for the study.

Inclusion Criterion: All patients from the selected Kebeles presented to local health posts (Tulu Gana and Anger health posts) during the study period have been included in malaria prevalence study.

Exclusion Criterion: Patients from unselected kebeles have been excluded from malaria prevalence study.

Patients' managements: participants with malaria parasite positive were offered treatement according to national malaria guidelines CoArten for *p. falciparum* infection and Chloroquine for *p. vivax.*

Sampling techniques and study variables: a convenient sampling technique was used to select all patients coming to local health center as patient for examination or brought other household

members to health center and interviewed using structured and pretested questions. Sampling was collected till the final sample size was reached.

Dependent variable: malaria prevalence and *plasmodium species.*

Independent variable: Socio-economic characteristics: Age, Sex, Marital status, Educational status, Occupational status and Income level.

Living style: Living near stagnant water, Housing condition, Toilet condition and ITNs utilization.

3.3. Data quality control, entry and analysis

Questionnaire data quality control was assured during and at the end of each day of data collection. During data collection in the field and at the end of each day, the questionnaires were reviewed and checked for errors, completeness, accuracy and consistency before entry into Microsoft Office Excel and corrective measures were taken. For the malaria test, the RDTs were checked for expiration date and batch before the blood screen was taking place. The availability, current use, condition and the type of ITNs were checked by observation during the survey and all the verifiable information was verified by the principal investigator.

3.4 Malaria Diagnosis

Data were coded and entered into Microsoft Office Excel 2007, cleaned and exported to statistical package for social sciences (SPSS) version 20.0(SPSS Inc., Chicago, IL, USA) software for analysis. Then, the study findings were explained in words, tables and figures. The response variable or the outcome 0f interest in the study was mRDT result. RDT result, age, sex, state of pregnancy and marital status, were collected at individual level. All other questionnaire data were collected at a HH level. Descriptive statistics were used to describe the characteristics of the sample. Differences between proportions of the malaria positive and negative results in patients were tested using the Chi-square test. Binary logistic regression and multivariate analysis were employed to examine the association between socio-demographic variables and other risk factors with malaria infection in the members of the households. In all of the analysis, a p-value of less than 0.05 was considered as statistically significant.

3.5 Ethical Considerations

Prior to the commencement of the study ethical clearance was obtained from ethical committee of Biology Department of Addis Ababa University. After permission is obtained support letters by the University was submitted to all concerned bodies in the study sites. After discussing the objectives and application of the study to Abe Dongoro District Health Office and the residents of the study area. Written informed consent was obtained from the study participants and/or parents /guardians/ caregivers, of age 18 years younger. The ethical review committee approved that study participations under the legal age could provide written informed consent on their own behalf as their parents were not around. Participation in this study was voluntary. The risk and benefits of participation in this study were explained to them. Blood test was carried out free of charge. All data and informed were maintained confidentially by a code rather than participants name. Positives were treated without a fee, and health education about prevention and control of malaria was given to study participants.

4. RESULTS

Accordingly to the study design and sample size proposed the study incorporated 385 study participants which make the response rate of the study 100%. The finding of the study is categorized in to: Socio-demographic and economic characteristic of respondent's blood film results of respondents and factors associated with malaria infection among respondents. It is represented by tables, figures and statements for the purpose of clarity and simplicity.

4.1 Socio-demographic Characteristics

A total of 770 individuals belonging to the 385purposively selected HHs in the three Kebeles (Qe'e 24, Qe'e 15 and Qe'e 20) were participated in the study. Of these, 385(50%) individuals that came to the health center from the selected kebeles were diagnosed for plasmodium parasite in their blood using thin and thick blood films and the remaining half, one were interviewed for assessment of malaria risk factors. Regarding their locality, 133 (34.5%) of the individuals were from Qe'e 24, 123(31.9%) were from Qe'15 and the rest 129 (33.5%) were from Qe'20. Female participants were 224(58.2%) and male were 161(41.2%). The age of the individuals diagnosed for plasmodium ranged from 6 months to 69 years with mean age of 25.11 years (95% CI: 23.49–26.74). Of the 385 diagnosed for malaria, fifty eight (15.1%) individuals were children below 10 years old, one hundred fifty (29.9%) were 11-20 years old, 115(29.9%) were 21-30 years old and 97(25.2%) were adults above or equal to 30 years old. Of the 224 women participants above or equal to 16 years old 15(7.4%) were pregnant

Table 1: Demographic characteristics of inhabitants diagnosed for malaria

Variables		Age				Total
		0-10 n (%)	11-20 n (%)	21-30 n (%)	>30 n (%)	
Locality	Qe'e 24	27 (46.6)	69 (60.0)	22 (19.1)	15(15.5)	133 (34.5)
	Qe'e 15	14 (24.1)	29 (25.2)	71(61.7)	9 (9.3)	123(31.9)
	Qe'e 20	17 (29.3)	17 (14.8)	22(19.1)	73(75.3)	129 (33.5)
Sex	Male	49(84.5)	96(83.5)	6 (5.2)	10 (10.3)	161 (41.8)
	Female	9(15.5)	19(16.5)	109(94.8)	87 (89.7)	224 (58.2)
Total		58 (15.1)	115 (29.9)	115(29.9)	97(25.2)	385(100)

A total of 385 participants' one member from each HH of 385 HHs individuals with age ≥18 years were also interviewed for assessment of risk-factors of malaria in the locality. The subpopulation was composed of 320(83.1%) of the interviewee were males and 65(16.9%) were females. The study determined that the family size of the interviewee ranged from 1 to 9 with mean of 4.54 (95% CI: 4.37-4.70) individuals per household. The educational levels of respondents were 145(37.7%) able to read and write, 140(36.4%) reported to have attained primary education, 26(6.8%) had secondary education and 74(19.2%) illiterate (unable to read and write). The age of the respondents ranges from 18 to 87 years with mean of 50.54 years. Ninety six (24.9%) were the age group 18-41 years, 90(23.4%), 42-51 years 97(25.5%), 52-61 years and 102(26.5%) were in the age group ≥62 years of the interviewer were with age range group. The marital status of the respondents indicated that 293(76%) of them were married, 29(7.5%) were singles, 45(11.7%), divorced and rest 18(4.7%) widowed.

Majority 364 (94.5%) of the respondents relied on the farming (private) and very few 21(5.5%) of them were public servants. However, because of the reason that the kebeles have been producing cash crops.

The 307 (79.7%) of the inhabitants lived in houses with corrugated iron sheet roof and the rest 78 (20.3%) have reported as were living in the house with thatch roof and all of the wall of the house of the 385 respondents were made from wood sticks. 212 (55.1%) of the participants lived in the houses that had opening on their walls, and 239(62.1%) live in houses that had opening on their eaves. Table 2 shows detailed socio-demographic/ economic characteristics of the respondents.

Table 2: Socio-demographic/economics of the respondents

Socio-demographics		Locality			
		Qe'e 24(n=133) No (%)	Qe'e 15 (n=123) No (%)	Qe'e 20 (n=129) No (%)	Total (n=385) No (%)
Sex	Male	109 (82.0)	112 (91.1)	99(76.7)	320 (83.1)
	Female	24 (18.0)	11(8.9)	30 (23.3)	65 (16.9)
Age Group	18-41	11 (8.3)	5 (4.1)	16(12.4)	32 (8.3)
	42-51	12(9.0)	11 (8.9)	21(16.3)	44 (11.4)
	52-61	18 (13.5)	17 (13.8)	25 (19.4)	60 (15.6)
	≥62	92 (69.2)	90 (73.2)	67 (51.9)	249(64.7)
Marital Status	Married	9 (6.8)	5 (4.1)	15 (11.6)	293(76.1)
	Single	104(78.2)	102(82.9)	87(67.4)	29(7.5)
	Divorced	12 (9.0)	13(10.6)	20(15.5)	45(11.7)
	Widowed	8 (6.0)	3 (2.4)	7(5.4)	18(4.7)
Education	Illiterate	25(18.8)	28(22.8)	21 (16.3)	74(19.2)
	Read and Write	50(37.6)	50 (40.7)	45 (34.9)	145(37.7)
	Primary School	48(36.1)	39(31.7)	53(41.1)	140(36.4)
	≥Secondary school	10 (7.5)	6 (4.9)	10 (7.8)	26(6.8)
Occupation	Civil servant	10 (7.5)	3 (2.4)	8 (6.2)	21(5.5)
	Farmer (Private)	123 (92.5)	120(97.6)	121(93.8)	364 (94.5)
House wall Opening	Absent	65 (48.9)	49(39.8)	59 (45.7)	173 (44.9)
	Present	68(51.1)	74 (60.2)	70 (54.3)	212 (55.1)
House Roof type	Thatch	19(14.3)	25(20.3)	34(26.4)	78(20.3)
	Corrugated Iron sheet	114(85.7)	98(79.7)	95(73.6)	307 (79.7)
House eave	Absent	53(39.8)	84(68.3)	102(79.1)	239(62.1)
	Present	80(60.2)	39(31.7)	27(20.9)	146(37.9)

4.1 LLIN and IRS Coverage

Long-lasting Insecticidal bed Nets had been distributed to most participants in the study area. Of the interviewee, a total of 116(30.1%) of which 19 (14.3%) from Qa'e-24, 50 (40.7%), from Qa'e-15 and 47 (36.4%) from Qa'e-20 participants lacked the bed nets. Specifically the bed net coverage in the study area was to be a total of 269 (69.9%) from which in Qa'e-24 one hundred forty (85.7%), in Qa'e-15 seventy three (59.3%) and eighty two (63.6%) in Qa'e-20 kebeles. However, the coverage differs with individual that 90 (33.5%) owned only one, 151(56.1%) owned two and 28(10.4%) owned more than two and more bed nets in their house. Generally, according to the reports of the respondents the mean number of bed net in a household was to be 1.62 (95% CI: 1.55– 1.70).Of the total of 269 who owned bed net, 230(85.5%) have received the net as recent as two months prior to this survey and the rest 39(14.5%) acquired the nets some three years back and were still using them (Table 3).

The reasons for dispossession of the nets were impossibility of getting substituent for worn out one. The respondents were also asked to grade their own LLIN according to WHO LLIN grading criterion. Accordingly, 230 (85.5%) of the participants graded the nets as good and 39 (14.5%) were not (have had holes that fit a torch battery based on WHO). Majority of the respondents with bed nets, 230(85.5%) responded that the whole family slept under the nets. However, 29(10.8%) were used husband and wives and 10 (3.7%) of them reported that the nets were used mother and children. Twenty of the respondents who had the bed net did not use the bed net. Three (15%) of these were not using the bed net due to the reason that they were believing as that the bed net do not prevent malaria and the rest 17(85%)of them have not used the net due to afraid of its toxicity.

In the study, IRS coverage and frequency were assessed and the findings in the three localities are presented in table 3 below. Accordingly, 215 (55.8) of the respondents of the survey reported that their houses have been sprayed with insecticide chemical and 170(44.2) of them responded as that their house had not been sprayed since one year. Of the respondents whose house had been sprayed, 173 (80.5%) of them reported as that their house had been sprayed since last 6 months and 42 (19.5%) were in the last 12 months. All of the participants reported that LLINs is the main malaria preventive measure.

Table 3: LLIN and IRS coverage in the three selected kebeles during the study period

Description		Locality			
		Qe'e 24 No (%)	Qe'e 15 No (%)	Qe'e 20 No (%)	Total (N=385) No (%)
LLIN owned	No	19(14.3)	50(40.7)	47(36.4)	116(30.1)
	Yes	114(85.7)	73(59.3)	82(63.6)	269(69.9)
Last night LLIN used	No	57 (42.9)	50(40.7)	47(36.4)	154 (40.0)
	Yes	76(57.1)	73()59.3	82(63.6)	231(60.0)
LLINs/HH	One	56 (49.1)	12 (16.4)	22 (26.8)	90(33.5)
	Two	49 (43.0)	52(71.2)	50(61.0)	151(56.1)
	More than two	9 (7.9)	9 (12.3)	10(12.2)	28 (10.4)
LLIN used by	Mother and Father	29 (25.4)	0 (0.0)	0(0.0)	29(10.8)
	Mother and Children	10 (8.8)	0 (0.0)	0(0.0)	10(3.7)
	Whole Family	75 (65.8)	73(100)	82(100)	230(85.5)
LLIN delivered	2monthsback	75 (65.8)	73(100)	82(100)	230(85.5)
	3years back	39(34.2)	0 (0.0)	0 (0.0)	39 (14.5)
LLIN condition	Not Good	39(34.2)	0(0.0)	0 (0.0)	39 (14.5)
	Good	75(65.8)	73(100)	82(100)	230 (85.5)
IRS practice since 12 months	Yes	73 (54.9)	70(56.9)	72(55.8)	215(55.8)
	No	60 (45.1)	53 (43.1)	57 (44.2)	170 (44.2)
Last IRS practice	Last 6 months`	58 (79.5)	60 (85.7)	55 (76.4)	173(80.5)
	Last 12 months	15 (20.5)	10 (14.3)	17(23.6)	42 19.5)

4.2 Malaria prevalence by Sex, Age and Locality

Of the total 385 patients brought to the health centers and showing malaria signs were blood tested for the presence of plasmodium parasites in their blood were 131 (34%) individuals of which 48 (36.6%) males and 83 (63.4%) females were mRDT positive. Out of the positive cases, 50 (36.6%) were from Qe'e 24, 44 (33.6%) from Qe'e 15 and 37 (28.2%) from Qe'e 20.Kebeles.

The $\chi 2$ test result and distribution of malaria prevalence by locality, age group and sex is indicted in table 4. Possible variation of malaria prevalence between sexes, age group and localities were tested using the chi-squared test. It was not significantly association with all of the tested variables (sex, age group and localities).

Table 4: $\chi 2$-test of malaria prevalence by age, sex and locality among diagnosed participants

Characteristics		Malarial case		Pearson Chi-square	d.f.	*p*-value
		Number of tested	Positive (%)			
Sex	Male	161	48 (36.6%)	2.187	1	0.139
	Female	224	83 (63.4%)			
Age Groups	0-10	58	13 (9.9%)	5.313	3	0.150
	11-20	115	46(35.1%)			
	21-30	115	39 (29.8%)			
	>30	97	33 (25.2%)			
Locality	Qe'e 24	133	50 (38.2%)	2.562	2	0.278
	Qe'e 15	123	44 (33.6%)			
	Qe'e 20	129	37 (28.2%)			

4.3 Species of Plasmodium Identified in the Study Area

A total of 385 study subject were examined. Malaria positive individuals were identified from three kebeles. The overall prevalence of malaria infection in the study area was 28(21.4%) *P.* falciparum accounted for 103(78.6%) of infections, *p. viva*x. Fifty communities from Qe'e 24 Kebele plasmodium positive, 20% (n=10) person had *p. falciparum* infection and the rest 80% (n=40) were positive for *p. vivax* infection. In Qe'e 15 Kebele of total 44 malaria positive individuals 20.5% (n=9) of the case were due to *P.falciparum* and 79.5% (n=35) were due to the *P.vivax*. In Qe'e 20 Kebele 37 individuals were malaria positive and 24.3% (n=9) of the plasmodium positive cases were infected by *P.falciparum* and the rest 75.7% (n=28) were by *p.vivax*

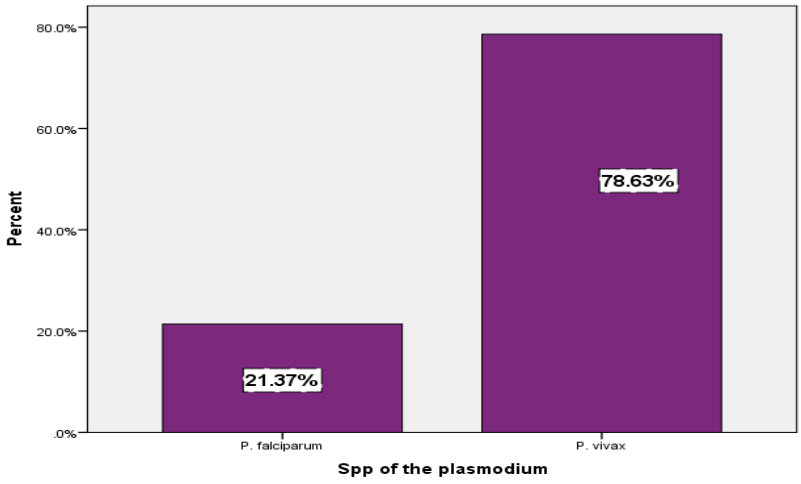

Figure .4: Plasmodium species identified in the study area

4.4 Univar analysis of malaria-risk- factors

The association of malaria prevalence with a variety of the risk factors obtained by questionnaire and field observation of environmental factors was determined in the study area. Table 5 shows the results of the binary logistic regression analysis for a list of independent variables tested for their potential association with risk of malaria infection. Accordingly, in the analysis of univariate logistic regression the explanatory factors including income level of the household, the proximity of the house to mosquito breeding sites, last night LLINs used, types of material from which the roof of the house was made, and the educational status of the head of the household have been determined that they were significantly associated ($p<0.05$) with malaria prevalence. However, the risk factors such as locality (kebeles), sex, age group and marital status of the patient, presence of hole on the wall of the house, and spray of insecticidal chemicals in the house were found not significantly associated ($p>0.05$) with malaria prevalence in the study area. In the case of the age group of the individuals tested for the plasmodium parasite in their blood, the overall chi-square analysis revealed that the factor did not show significant association with the prevalence of the infection. However, in univariate logistic regression, the age group of 11 to 20 years was found significantly highly infected than those in reference group (0-10 years) ($p<0.05$).

The family members with educational status of illiterate, read and write, and primary school head of household were about 15, 10 and 7 times respectively more affected by malaria than those whose head of the household finished at least secondary school. Moreover, individuals whose houses were less than 1km nearer to mosquito breeding site have been infected about 7 times as likelihood than those far above 1km from the breeding site of the insects ($p<0.05$).

Table 5: Univar logistic regression analysis for malaria prevalence versus associated risk factors in the study area

Variable	Category	N	mRDT Positive (%)	OR	95% CI		*p*-value
					Lower	Upper	
Sex	**Male**	161	48 (29.8)	**Ref.**			
	Female	224	83 (37.1)	1.386	0.899	2.137	0.140
Age	**0-10**	58	13 (22.4)	Ref.			
	11-20	115	46(40)	2.308	1.122	4.746	0.023
	21-30	115	39 (33.9)	1.776	0.858	3.678	0.122
	>30	97	33 (34)	1.785	0.846	3.765	0.128
Marital Status	Married	134	41 (30.6)	Ref.			
	Unmarried	251	90 (35.9)	1.268	0.809	1.986	0.300
Pregnant	Present	15	2 (13.3)	Ref			
	Absent	209	81 (38.8)	4.113	0.905	18.703	0.067
HH Educational status	**Illiterate**	74	34 (45.9)	4.675	1.467	14.903	0.009
	Read and Write	145	54 (37.2)	3.264	1.068	9.976	0.038
	Primary School	140	39 (27.9)	2.124	0.688	6.559	0.190
	≥2ndary	26	4 (15.4)	**Ref**			
Locality	**Qe'e 24**	133	50(37.6)	1.498	0.892	2.515	0.127
	Qe'e 15	123	44 (35.8)	1.385	0.815	2.354	0.229
	Qe'e 20	129	37 (28.7)	Ref			
House-Mosquito Breeding site	**>1km**	185	102 (55.1)	7.246	4.445	11.814	0.000
	<1km	200	29 (14.5)	Ref			
House Wall opening	**No**	173	50 (38.2)	Ref			
	Yes	212	81 (61.8)	1.521	0.990	2.338	0.056
House eaves	**No**	239	67 (51.1)	2.004	1.301	3.086	0.002
	Yes	146	64 (48.9)	Ref			
Latrine	**No**	162	67 (51.9)	1.837	1.199	2.816	0.005
	Yes	223	64 (48.1)	Ref			
House Roof	**Thatch**	78	43 (55.1)	3.057	1.836	5.093	0.000
	Corrugated Iron sheet	307	88 (28.7)	**Ref**			
Last night LLINs Used	**No**	154	128 (97.7)	94.447	77.0321	111.067	0.000
	Yes	231	3 (2.3)	Ref			

4.6 Multivar Analysis of Malaria Risk Factors

The risk factors which have significant association with malaria by using univariate logistic regressions have been checked by multivariate logistic regression model to determine the most important predictors among the variables. Table 6 below shows the result of multivariate logistic regression modeling for the potential risk factors of malaria in the study area. Accordingly, out of the six risk factors having shown significant association using univariate logistic regression, educational status of head of the household, distance of the home from mosquito breeding site, sources of drinking water, the types of material from which the roof of the house is made and current use of LLINs have remained showing strong significant association ($p<0.05$) with malaria infection in the locality.

Table 6: Multivariate logistic regression analysis for association between mRDT-positive and Socio-demographic and environmental risk factors

Variables		**N**	mRDT positive N_o (%)	**OR**	**AOR**	**95%CI**	**p-value**
Educational status of head of the HH	Illiterate	74	34 (45.9)	4.68	36.62	3.57-37.578	0.002
	Read and Write	145	54 (37.2)	3.26	59.815	3.214-111.3209	0.000
	Primary School	140	39 (27.9)	2.12	14.16	1.69-118.51	0.015
	≥Secondary school	26	4 (15.4)	**Ref**			
Distance from Mosquito Breeding site	<1km	185	102 (55.1)	7.25	15.172	3.69-62.3079	0.008
	>1km	200	29 (14.5)	Ref			
House eaves	Yes	239	67 (28.0)	Ref			
	No	146	64 (43.8)	0.49	8.11	0.09-74.630	0.364
House Roof	Thatch	78	43 (55.1)	3.06	4.51	1.05-19.34	0.043
	Corrugated Iron sheet	307	88 (28.7)	**Ref**			
Last night LLINs Used	No	154	128 (83.1)	374.15	18043 0.87	4164.34-7817630.83	0.00
	Yes	231	3 (1.3)	Ref			

Key: - OR (odds ratio) odds of response group to odds of reference group.

AOR (adjusted odds ratio) it indicates the contribution of a particular predictor when other predictors are controlled.

Ref (referent group)

LLINs (long-lasting insecticidal treated nets)

HH (household)

CI (confidence interval)

mRDT (malaria rapid diagnostic test)

*p-value <0.05 significantly associated

5 DISCUSSION

This study was aimed to evaluating the clinical prevalence of plasmodium infections malaria among patients brought to health center, and associated risk factors with malaria infection in Abe Dongoro district of Horo Guduru Wollega Zone, West Ethiopia. The prevalence of the blood parasite, plasmodium has been assessed among patients brought to the three health posts named Tulu Gana, Angar and Qe'e 20. Blood films from the patients were collected to detect the parasite in the blood by using rapid diagnostic tests. However, a rapid diagnostic test (RDT) is not very sensitive detecting parasites at low parasite densities (Florey, 2014).

A study conducted in Oromia, Amhara, and SNNP in Ethiopia found the overall prevalence of plasmodium parasite in randomly selected people to be 4.1% of 11601 sample size (Graves *et al.,* 2009). The current study found that the parasites have been detected in about 34% of the patients. In agreement with the current study, studies conducted in Sibu sire by (Deresse *et al.*, 2015, and in Serbo by Karunamoorthi and Bekele2009) reported that malaria prevalence as being 39.6% and 33.27% respectively. With less proportion to the current prevalence, Ararso (2017) have reported the malaria prevalence in Amuru district (near adjacent District to Abe Dongoro) to be 22.7%. Similarly, less prevalence of the infection has been reported from different part of the country. For example 11.45% in Arsi Negelle (Mangistu and Solomon, 2015), 11.53% in Motta (Tilahun, 2016), and 17% in Metema towns (Getachew *et al*., 2013) have been reported. This difference might be due to variation in sample size, altitude and climate.

The association of malaria prevalence, and socio-demographic and environmental factors were also determined in the current study. Accordingly, socio-demographic factors including sex, age, marital status and family size were not significantly associated with malaria infection in the study area. This study also showed that malaria parasite was higher in females (63.4%) than in males (36.6%). In the case of sex as risk factor, although the difference was not significant. In agreement similar studies, Tefera (2014), female subjects were more infected than males. Graves *et al.,* (2009), detected plasmodium parasite in high percentage of female (4%) than in males (3.7%). There was also study reporting equal prevalence of the infection in males and females. Bereka (2017) reported that the prevalence of malaria was equal, 7.7% each in males and females. In opposite to the current study, Ayele *et al.* (2013). And Ferede *et al.* (2013). Reported that the prevalence of malaria was higher among males than females. In majority of the studies in

the country, however, the difference in the prevalence between males and females were not significant.

The study has also found that the prevalence of malaria was insignificantly different among age groups of the patient. However, the age groups between 14 and 22 years old were the highest infected (42%) among the age groups. With the agreement to the current study Molla and Ayele (2015) have found that malaria prevalence was highest in age groups of 15-24 years old. Similarly Karunamoorthi and Bekele (2009) reported highest of positive semears observed in similar age group in Kersa Woreda of Jimma zone. It has been estimated that the reason behind the increased prevalence of malaria in this specific age group is regard to relatively high outdoor exposure to the mosquito vector bites and daily activities. Children less than 10 years old were the least infected age group in this study. The result is the same way with the result of Karunamoorthi and Bekele (2009). But it is to fail to agree with the result of Ferede *et al*., (2013) which reported children of 5-14 years old being highly infected than adults above 15 years old.

The household educational status is factors among socio-economic factors which showed statistically significance association with prevalence of malaria infection in the current study. Illiterates and who can at most read and write were found more infected than those finished at least secondary school (OR=4.675, 95% CI: 1.467-14.903), the association was significant ($p<0.05$). In consistent to this result (Seble, 2014) reported that the likelihood of getting malaria was lower with increase in education level in Jiga town even though the association was insignificant. Other study, Houmsou *et al*., (2011) reported that there have been higher malaria prevalence in individuals who completed primary school than those with no formal education but still less than those completed at least secondary schools. The importance of education, a direct and indirect influence on malaria control, which can never be overemphasized.The impact of education, can be considered from various dimensions. Education has been suggested as that it may be related to levels of awareness about malaria prevention and treatment strategies. In addition, the household head or members may possess knowledge regarding malaria transmission dynamics, than the uneducated individuals (Ricci, 2012; Sichande *et al.,* 2014).

The proximity of the house from mosquito breeding sites (swampy, river, ponds) was one of the highly determinants for malaria infection in the study area. Accordingly the study determined that household members living in a house nearer to vector breeding sites at distance less than

1km were infected about 7 times as likely to more than those living 1km far from swampy area. Living in the nearby stagnant water was also identified as a risk factor. The significantly higher parasite rate found among the individuals having stagnant water in their compound (OR=7.25; 95%CI: 4.445-11.814) ($p<0.05$). It can be explained from the fact that they are more exposed to mosquito bites, because these are suitable for breeding of mosquitoes around their homes. The result is agreed with studies which have suggested that the rate of parasite transmission may be higher closer to major mosquito breeding habitats; so that the risk of malaria is strongly associated with distance from breeding site (Trape *et al.,* 1992; Staedke *et al.,* 2013; Munyekenye *et al.,* 2005).Rivers and their floodplains provide great breeding grounds for mosquitoes in riverside urban communities, as demonstrated by the strong association between malaria risk and proximity to a floodplain (De Silva and Marshall, 2012).

The presence of eave on the house was also seen associated with malaria infection that individuals living in houses with eave were significantly more attacked by malaria than those living in eave lacking houses [OR: 2.004, 95% CI, 1.301-3.086]. In addition the material from which the roofs of the houses have been made was one of the risk factors for prevalence of malaria in the study. Accordingly family members living in a house with thatch roof were significantly more infected compared to those living in corrugated iron roofed house [OR: 3.057, 95% CI, 1.836-5.093].In agreement with the current findings Adiamah *et al.,* (1993) reviewed that houses with malaria-infected children are more likely to have mud walls, open eaves, and absent ceilings than those with uninfected children. Furthermore, similar finding was reported by Dawit *et al.,* (2013) that malaria infection rate was significantly higher when the roof of the house was thatched than when it was corrugated iron sheet. Studies have suggested that better-quality housing decreases the risk of malaria as it minimizes entry points for mosquitoes during the night (De Silva and Marshall, 2012).

The current study has also determined that individuals who were using mosquito nets were significantly less infected than those who were not using the nets ($p<0.05$). Similar findings have been reported by previous studies. Molla and Ayele (2015) reported that families owned mosquito nets or insecticide treated bed nets were more likely to be protected from malaria parasites hence less malaria cases compared to the families without mosquito nets. Hence it has been concluded that there is highly relationship between mosquito net ownership with malaria cases of the under five children.

The current study also identified the species wise distribution shows *plasmodium vivax* was the predominant species, accounting for 78.6% (n= 103), followed by *plasmodium falciparum* 21.4% (n=28). However, there were no mixed infections detected in the diagnosis. In agreement to the current study, Molla and Ayele (2015) identified high proportion of *P. vivax* (62.5%) than *P. falciparum* (26.8%) in RDT positive patients. But this result is contradicting with the national prevalence of *P. falciparum* and *P. vivax*, which is 65-75% and 25-35% respectively. In addition, the prevention and control activities are guided by National Strategic Plan (2006-2016) mainly focus on *P. falciparum* because it is assumed more prevalent and fatal malaria in the country. Other possible reason might be climate variability deviation between the finding of this study and the national figure of epidemic regarding *P. vivax* along with *P. falciparum* might be due to relapsing regarding *P. vivax.* As outlined by, the information obtained from communicable diseases prevention and control office of Abe Dongoro District, preventive and curative actions were taken particularly when there is *P. falciparum* epidemic. *P. vivax* might have developed resistance for the currently used drug Chloroquine has been reported in the current study Tefera *et al.* (2014).

However, the isolation rates of the species were inconsistent with the report of Ligabaw *et al.,* (2014) and Babamale and Ugbomoiko (2016) who identified high proportion of *P. falciparum* than *P. vivax* in RDT positive patients.

The absence of significance difference in malaria prevalence to conclude other environmental factors including locality (Kebeles), and spray of insecticidal chemicals as risk factor for malaria infection in the study area ($p>0.05$).

In summary, in Africa between 2000 and 2012, the scale-up of control interventions reduced malaria incidence rates by 31% and mortality by 49% (WHO 2013). In Ethiopia too latest reports testify a dramatic reduction in both malaria cases and deaths. But the situation in the present study area needs careful assessment to further scale-up control intervention in order to be able at least to plan for malaria pre-elimination program.

5. CONCLUSION AND RECOMMENDATION

From the above results one could conclude that prevalence of malaria was determined in the study area when compared to the previous findings in other parts of the world in general and in Ethiopia in particular. In addition socio economic factors including the being aged between 11 to 20 years, low educational status of the head of the households and/or the patient and low monthly income level were found increase the risk of being infected with malaria. Among the environmental and housing factors, absence of current use of mosquito nets, lack of toilet, presence of openings in the house such as eave and wall opening, proximity of the living house to mosquito breeding habitats, drinking unprotected water and living in house with thatch roof were found to increase the risk of malaria in Abe Dongoro District.

However the prevalence of malaria was not determined to be associated with socio-demographic factors such as sex, marital status, pregnancy and family size. Likewise, the infection rate of the disease did not reveal association with locality and insecticide spray in the house as environmental risk factors.

The above conclusion will lead us to forward the following recommendations

- Malaria prevention methods should focus on enhancing education and income generation for the family
- Mosquito nets should be distributed fairly considering family size and other risk factors
- Awareness about the mosquito breeding habitats and method of elimination should be given to the society
- Detail and epidemiological studies focusing on prevalence of asymptomatic malaria should be conducted in the study area

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7 APPENDICES

Annex 1: Consent/Assent form

Identification N_o ----------age ---------sex ----------

The primary objective of the study will be conducted to assess the current status of malaria in Abe Dongoro district area in relation to the ongoing control activities there to contribute towards informed decision making in the malaria control. For this purpose a blood sample will be taken by finger pricking.

To avoid infection with blood borne pathogens like HIV, one disposable lancet will be used for finger pricking for each study participants. For those who have bleeding problem, special care will be given.

All cost related to microscopic examination and anti-malaria drugs (if the study participants become positive) will be cover by the investigator.

I, who registered in ---------- identification number, clearly understood the above statement and agree to participate in the study.

Name and signature of the participant/parent/ care taker

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

Annex 2: English questionnaire

Title: The Clinical Prevalence of Malaria and the Associated Risk Factors in Abe Dongoro District Oromia region, West Ethiopia.

Malaria Indicator Survey Model Household Questionnaire

Questionnaire № _____

Date _____

SECTION I: HOUSEHOLD CHARACTERISTICS

District _____*Kebele*_____

1. Age _____Sex _____
2. Occupation: - A. government employee. B. Farmer. C. merchant. D other specify_____
3. Marital status: A. Single B. Married C. Divorced D. Widowed
4. Educational status: A. Illiterate B. read & write C. elementary school D. high school E. higher institutions
5. State of pregnancy A. pregnant B. delivery before 6 months C. delivery before 6 months back D other specify _____

SECTION II: HOUSING ENVIRONMENT

6. Type of the housing A. thatch top B. corrugated iron roof C. Other specify _____

6.2. Is eave present? A. yes B. no

6.3. Is hole present in the wall? A. yes B. no

8 Is there any mosquito breeding habitat around the village? A. yes B. no.

8.1 If yes, distance of house from mosquito breeding habitat? A. <1000m B. 1000m-2000m C >2000m D. other specify_____

SECTION III: INSECTICIDE SPRAYING AND MOSQUITO NETS

9 Is there chemical spraying to control mosquitoes? A. yes B. no

9.1 If yes how frequent? A. one/year B. twice/year C. other specify _____

9.2 Do you think that the spraying helps to decrease malaria? A. yes B. no

10 Do you use insecticide treated mosquito net? A. yes B. no

10.1 If yes, how many ITNs do you have in the HH/ A. 1 B. 2 C. 3 D. >3

10.2 Are they currently being used? A. yes B. no

10.3 Who usually sleeps under the net at night? A. mother B. children only C. mother & children D. husband & wife E. the whole family

10.4 When do you sleep under the net? A. all year B. only during the rains C. only during the winter

Annex 3: Laboratory results reporting format

S. №	Idenification HH №	Sex	Age	Malaria rapid diagnostic test result.		*Plasmodium* species			
				Positive	Negative	P.f	P. v	Mixed	Remark