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Prevalence of Malaria in out patients at Enticho Hospital, Ahferom Woreda, Central Tigray, Ethiopia

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A Thesis Submitted to the Department of Zoological Sciences in Partial Fulfillment of the Requirements for Master of Science Degree in Biology

August, 2024

Addis Ababa, Ethiopia

Declaration

I declare that this thesis is the result of my own work and all materials used fully acknowledged. This submitted for partial fulfillment of the requirements of MSc Degree in Biology within the College of Natural and Computational Sciences at Addis Ababa University, on the prevalence of malaria from 2015 to 2019 Ethiopian calendar.

Name _____ Signature _____ date _____

Approved by Advisors;

Name; _____ Signature _____ Date _____

Name; _____ Signature _____ Date _____

Acknowledgements

Primarily, my deepest gratitude extends to my advisors Dr. Tegenu Gelana and Dr. Araya Gebresilassie for their unreserved guidance and encouragement on every stage of this thesis work. I appreciated them for their nice approach, constructive comments, willingness to share experience and smooth treatments. Next, my special thanks go to Enticho Health Office and Woreda Health Center for providing me the necessary information, allowing the laboratory facilities and technical supports. I am very grateful to all those who directly or indirectly involved in my thesis work.

Moreover, my limitless thank goes to my Father Priest Gebrehiwot Gebre, my Mother Zewdi Gidey, all my sisters and brothers and especially my brother Ephrem who are always with me and encouraging me. Finally, my thanks go to the Almighty God, who did this entire thing.

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Acronyms and Abbreviations

ACT	Artemisinin-based combination therapies
AL	Artemether –Lumefantrine
AWSO	Ahferom woreda statistical office
CQ	Chloroquine
EHC	Enticho health center
HAD	Health Development Army
HEW	Health extension worker
IPTp	Intermittent preventive treatment in pregnancy
IRS	Indoor residual spraying of insecticide
ITNs	Insecticide treated bed net
LLINs	Long-lasting insecticidal nets
MOH	Ministry of health
P. f.	<i>Plasmodium falciparum</i>
P. v.	<i>Plasmodium vivax</i>
RDT	Rapid diagnostic test
SP	Sulphadoxine-Pyrimethamine
UNDP	United Nations Development program
WHO	World Health Organization

Abstract

*Malaria is a protozoan disease caused by parasites of the genus plasmodium and it is one of the leading causes of illness and death in the world. The objective of the present study was to show the prevalence of malaria infection among individuals who visited Enticho hospital. For this case descriptive cross-sectional study from September 2020 to June 2020 and retrospective data of the last five years (2015-2019) was collected to determine the prevalence of malaria in the study area. Three hundred eighty-four (384) malaria suspected participants were involved in this study by using single population proportion formula. Demographic and sanitation practice information's were gathered with pre-tested and semi-structured questionnaires and interview. Moreover, blood samples were collected and processed with professional laboratory technologists of the Enticho hospital. Statistical data were encoded to SPSS data sheet as well as Microsoft Excel and analyzed accordingly in considering $p < 0.05$ as statistically significant. The overall prevalence of malaria infections was 25% in the cross-sectional data and 21.14% in the retrospective data. Plasmodium species identified in the blood samples of the participants were *P. falciparum* and *P. vivax*. The prevalence of malaria infections were statistically associated with gender ($\chi^2 = 18.115, p=0.00$), having of indoor grass and plants ($P = 0.033, OR = 1.684$) having of stagnant water near house ($P = 0.000, OR = 26.253$) and frequency of participants to use bed nets in sleep time ($P = 0.000, OR = 5.117$). Malaria was more prevalent and one of the major health problems in population of the study area. Therefore, health professionals should give awareness on environmental hygiene and means of malaria prevention mechanisms to population of the study area.*

Keywords/phrases: Enticho; Prevalence; Malaria Parasite; Retrospective data

1. INTRODUCTION

1.1 Background

At the current time, Malaria is one of the most serious health problems facing the world today. It is caused by infection with parasitic protozoa belonging to the genus plasmodium. There are five species of plasmodium causing disease in humans. These are *Plasmodium falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*. More specifically, *P. falciparum* and *P. vivax* are the most common, accounting for about 60% and 40% of the infection, respectively (Woyissa *et al.*, 2012; Weidong and Robert, 2005; Adefioye *et al.*, 2015; Dawit *et al.*, 2012; Dadi *et al.*, 2010). The most known Mosquito that transmits the parasite to humans is called anopheles mosquito. Plasmodium parasites have a complex life cycle alternating between an insect vector (i.e. the genus Anopheles mosquitoes) and a human host. In order to complete the life cycle, they must move from mosquito to human and then back again. Malaria infection is principally transmitted by major malaria vectors known as *Anopheles arabiensis* followed by *Anopheles pharoensis* and other secondary vectors such as *Anopheles funestus* and *Anopheles nili* (Abebe *et al.*, 2011; CDC, 2012).

In Ethiopia, Ministry of Health (MoH) classified the country into three levels with respect to malaria transmission. These are non-malarias (25% of the country), highland with unstable malaria about two thirds of the country, and endemic transmission with seasonal peaks accounts remainder of the country. Malaria transmission is seasonal and unstable, causing frequent epidemics (Adhanom *et al.*, 2006; Abebe *et al.*, 2011; Newman *et al.*, 2003 and NSP, 2010). The two peak seasonal transmissions of malaria occur during the months of September to December and March to May (FMoH, 2004). However, prevalence varies among locations because of heterogeneous topography and weather variables (Adhanom *et al.*, 2006; Abeku *et al.*, 2003; Cheung *et al.*, 2008).

In areas above, 2000meters are believed malaria free environment, but because of irrigation and dams in the surroundings of Enticho city and many rural surroundings there is a malaria disease. Recently became under the administration of city and the people manage their life as the rural inhabitants and so people are being observed caught by malaria. Therefore, knowing prevalence of malaria is important to solve the problem of spreading disease and exposing to death. In this study the trend of malaria would be recorded Enticho Hospital.

The Tigray region, which is located in the northern part of Ethiopia, has a population of about 5 million, 90% of which is rural. It is divided into three major areas. The northwestern lowlands (1000-2000) meters above sea level) are fertile, but agricultural potential is under-exploited because of the high transmission of malaria and water-borne diseases. The central highlands (1500-3000 meters above sea level), where 65% of the population lives, are the most densely populated area. The eastern lowlands (100 meters below sea level to 1500 meters above sea level) are sparsely settled. The principal occupation of 84% of the rural people is subsistence agriculture and most of the remaining 16% are pastoralists and nomads. The major malaria vector is *Anopheles gambiae*, the major malaria vector. The two major malaria transmission seasons are September through November and March through April. The unstable nature of malaria in Tigray makes the area prone to outbreaks, and malaria is a major public health problem. In 1987 in western Tigray, 142,317 malaria cases and 349 deaths were recorded in a malaria epidemic (Tedros Adhanom *et al.*, 1996).

1.2 Statement of the Problem

The World Health Organization (WHO) has been reported in 2015 that there were 95 countries and territories in which malaria transmission increases frequently with about 3.2 billion people. The global burden of malaria is substantially declined, but Malaria causes nearly 250 times more deaths in the world's poorest countries than in the richest especially in children and pregnant women (WHO, 1999). In Africa the disease remains a major public health problem (WHO, 2013). High global malaria burden with 88% and 90% of malaria cases and deaths respectively occurs (Asrat, 2017). It is also the fourth leading cause of death in children under the age of five years in developing countries (Legesse *et al.*, 2007). In Ethiopia, also Malaria is one of the main public health problems. The bimodal type of malaria transmission is higher in the season of September to December, following the main rainy season from June to August and it is lower in April to May, following a short rainy season from February to March. The wet season in Tigray region extends from May to September, with most rains falling during June and July. In southeastern Tigray, an additional rainfall is happened during January and February. The mean annual temperature of Tigray region is 18°C. Almost 75% of the region is malarious and three million inhabitants live in this area. *Plasmodium falciparum* causes 60-70% of infections, *Plasmodium vivax* accounts for most of the remainder, although *Plasmodium malariae* is also present (Tedros Adhanom *et al.*, 1996).

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deaths have been reported (Adhanom *et al.*, 1996). Malaria is a major cause of morbidity and mortality in Tigray like the other joining regions. The scale up of malaria control activities in the past decade has resulted in significant reduction in malaria prevalence in Tigray. The proportion of total OPD visits, admissions and deaths due to malaria decreased from 20.5%, 10.5% and 5.1% in 2011/2012 to 11.6%, 4.4% and 1.9% in 2014/15, respectively, in the region. Despite, some reductions in prevalence, malaria remain the second causes of morbidity in 2014/15 with 296,785 (11.55%) cases as outpatient and 5417 inpatients (Kebede *et al.*, 2018).

In Enticho town for malaria control strategies such as the use of Artemethrine based Combination therapy (ACT), the use of insecticide treated bed net (ITNs) and indoor residual spraying of insecticide (IRS) have been implemented. Despite all the efforts made on control of malaria is still the major public health problem in this area. The reason for this problem could be the deficiencies in the Health system that leads to lack of access to malaria control interventions and low effectiveness of these interventions. Thus, the researcher wanted to assess the prevalence and trends of malaria cases, compare the prevalence of malaria at different age groups, determine at which season of the year malaria prevalence is higher and identify which Plasmodium species have higher rate of prevalence in the study area.

1.3 Objectives of the Study

1.3.1 General objective

- The overall objective of this study was to determine the prevalence of malaria infection in Enticho Hospital.

1.3.2 Specific objectives

- ✓ To determine the trend of malaria from 2015 to 2019 in Enticho, Tigray, Ethiopia
- ✓ To estimate the prevalence of malaria based on age and sex in Enticho Tigray, Ethiopia
- ✓ To identify species of Plasmodium in Enticho Tigray, Ethiopia,
- ✓ To determine in which season of the year malaria is higher in Enticho Tigray, Ethiopia
- ✓ To identify factors associated with malaria infections of the study area.

2. LITERATURE REVIEW

2.1 History of Malaria Disease

Malaria parasites were discovered in 1880 by French army surgeon in Algeria notices parasites in blood. Later in 1886, Italian neurophysiologist as two forms of the disease specified differentiation of malaria species. Naming of human malaria parasites appeared in 1890 and 1897. For the first time *P. vivax* and *P. malariae* was introduced since 1890. The malignant tertian malaria parasites *P. falciparum* named since 1897. The first documented human infection with *P. knowlesi* was in 1965 (www.cdc.gov/malaria/about/history/). Malaria is one of the most serious health problems facing the world. Most deaths of malaria are caused by *P. falciparum* because *P. vivax*, *P. ovale* and *P. malaria* generally cause a milder form of malaria. The *P. knowlesi* species rarely causes disease in humans (WHO, 2014). Ethiopia shows progress in the fight against malaria to attain MDG 6c: halt and begin to reverse the incidence of malaria by 2015. Recently the country achieved remarkable result through strong preventive and case management interventions with involvement of health extension workers (HEWs), Health development army (HAD) and volunteers providing community-based cares at the household level (Banteyarga, 2011). In Ethiopia, Malaria admissions and deaths decrease from 81% in 2001 to 73% in 2011 for children <five (Worku, 2015). In humans, malaria is caused by five parasite species that belong to the genus *Plasmodium*. Four of these are *P. falciparum*; *P. vivax*, *P. malaria* and *P. ovale* are human malaria species that transmit from one person to another by the bite of the genus female Anopheles mosquitoes (WHO, 2015). There are around 400 different Anopheles mosquitos' species, of these only 30 are vectors of major importance. Recently, human malaria cases due to *P. knowlesi* have been recorded this species cause's malaria among monkeys in certain forested areas of South-East Asia. Current information suggests that *P. knowlesi* malaria is not spread from person to person, but rather occurs in people when an Anopheles mosquito infected by a monkey then bites and infects humans (zoonotic transmission) (WHO,2015). *Plasmodium knowlesi* is a malaria parasite found in wild monkey populations and transmitted from this animal reservoir to humans *via* infected mosquitoes. It causes severe and fatal disease in humans, and is the most common cause of malaria in parts of Malaysia. The geographical distribution of this disease is largely unknown because it is often misdiagnosed as one of the human malarias. Human malaria parasites are primarily transmitted between humans *via* mosquitoes and are not frequently transmitted from other animals to humans. Many countries in Southeast Asia, where *P. knowlesi* infections have been reported, are making progress towards eliminating the human malarias (Shearer *et al.*, 2016).

2.2 The Malaria Parasites

The current distribution of malaria parasite in the world was highly variable from country to country. As World Health Organization (WHO), reported, 97 countries and territories had ongoing malaria transmission. There is usually less risk at altitudes above 1500m above sea level (WHO/UNICEF, 2015). Malaria parasites with their characteristic behavior are explained as follows. These are: -

Plasmodium falciparum; It found worldwide in tropical and subtropical areas responsible for the majority of malaria deaths globally and is the most prevalent species in sub-Saharan Africa. Approximately one million estimated people killed every year. Most highly virulent species and is responsible for almost all of 1.7 –2.5 million deaths worldwide. This was the dominant malaria parasite in Ethiopia accounts about 65-75% among total reported cases (Dadi *et al.*, 2010).

Plasmodium vivax; Occur mainly in areas with seasonal transmission that represent normal seasonal peaks in temperate and subtropical areas. The second most significant species are prevalent in South East Asia and Latin America. Add complication of a dormant liver stage, which can be reactivated in absence of a mosquito bite, leading to clinical symptoms. It is rare in most part of the African continent (Tsige *et al.*, 2009).

Plasmodium ovale; It is found mostly in Africa (especially West Africa) and the islands of the western Pacific (WHO, 2015). Infect individuals who are negative for the Duffy blood group, which is the case for many residents of sub-Saharan Africa. That explains the greater prevalence of *P. ovale* in most of Africa and causes tertian malaria in humans (Mohapatra *et al.*, 2008).

Plasmodium malariae; Found worldwide and wide spread throughout sub-Saharan Africa. In endemic regions, prevalence ranges from less than 4% to more than 20 % (Bruce *et al.*, 2006). However, evidence from different study shows that its infections under reported. The only human malaria parasite species that has a quartan cycle (Mohapatra *et al.*, 2008).

Plasmodium knowlesi; It is found throughout Southeast Asia as a natural pathogen of long-tailed and pig-tailed macaques. It has been recently shown to be a significant cause of zoonotic malaria in that region, particularly in Malaysia (CDC, 2012).

2.3 Burden of Malaria Disease

Hundred seven (107) countries and territories had areas at risk of malaria transmission at the end of 2004. Some 3.2 billion people lived in areas at risk of malaria transmission and an estimated 350–500 million clinical malaria episodes occur annually; most of these are caused by infection with *P. falciparum* and *P. vivax*. More than 1 million deaths are caused by *P. falciparum* malaria each year. It contributes indirectly for many additional deaths, especially in young children, via synergy with other infections and illnesses (WHO and UNICEF, 2005).

Patterns of malaria disease and transmission vary markedly between regions and even within individual countries. This diversity results from variations between mosquito vectors and malaria parasites, ecological conditions that affect malaria transmission and socioeconomic factors, such as poverty and access to effective health care and prevention services. Around 60% of the cases of malaria globally, about 75% of global *P. falciparum* malaria cases and greater than 80% of malaria deaths occur in Africa south of the Sahara. *P. falciparum* causes the bulk of infections in this region and around 18% of the deaths are in children under 5 years of age. Malaria is also a major cause of anemia, low birth weight, premature birth and infant mortality in children and pregnant women (WHO and UNICEF, 2005).

Malaria contributes synergistically with HIV/AIDS to morbidity and mortality in areas where both infections are highly prevalent, such as in Africa south of the Sahara. In addition to providing immediate health benefits, prevention and treatment of malaria may lessen transient increases in HIV viral load during malaria episodes and thus help limit the progression and transmission of HIV (WHO and UNICEF, 2005). Climatic conditions determine the presence or absence of anopheline's vectors. Malaria is especially dangerous in pregnant women and in children under 5 year's age. If a pregnant woman or young child gets malaria, severe illness may rapidly develop and may result in death. Such patients need special care in addition to standard malaria. In areas where malaria is very common, people may get the disease several times during their lives and gives them some resistance to the disease, so the attacks of malaria often become less severe as they get older. However adults who come from malaria free areas can become very ill with malaria just like children (WHO, 1996). Pregnant woman and their unborn child are exposable to malaria which is a major cause of low birth weight, maternal anemia and prenatal death (Taura, and Oyeyi, 2009). Malaria is one of the main public health problems in Ethiopia. Bimodal type of transmission: Major: September to December, following the

main rainy season from June to August and Minor: April to May, following a short rainy season from February to March. Focal outbreaks are common and the distribution varies from place to place depending on climate and altitude (Barofsky *et al.*, 2011 and Deressa *et al.*, 2004). Due to the existence of various environmental and physical factors, the nature of malaria in Ethiopia is variable (Ministry of Health, 2002). Malaria is pervasive to Ethiopia; 75% of the landscape areas below 2000 m above sea level is malarias which is fertile low land areas and suitable for agriculture. More than 54 million populations live in these areas and are at risk of malaria. The transmission is unstable and seasonal from September to December and April to May which is coincide with major planting and harvesting season for farmers - aggravate economic loss (Legesse, 2007 and Betemariam and Yayeh, 2002). In Ethiopia, major epidemics occur every 5-8 years, but focal epidemics were occurring every year. *P. falciparum* and *P. vivax* are the two species commonly known to cause malaria in Ethiopia accounting for 60% and 40% proportion, respectively (Ketema *et al* 2011). In most parts of Ethiopia, malaria is unstable and seasonal because of the altitude and climatic factors (Adhanom *et al.*, 2006). Because malaria is one of the leading causes of morbidity and mortality, it is a major concern in the country. Despite the current efforts to control malaria in Ethiopia, the situation has not improved mainly due to the increasing problems of parasite resistance to the relatively cheaper anti-malarial drugs, vector resistance to insecticides, low coverage of malaria preventive services, poor access to health care, rudimentary health service infrastructure, large population movements and limited financial and human resources (Nigatu *et al.*, 2014).

2.4. Life Cycle of Malaria Parasite Species

Malaria parasites have a complex life cycle. All species exhibit a similar life cycle with only minor variations. The life Cycle of Plasmodium can be divided into two distinct phases. These are, asexual cycle in humans and sexual cycle in mosquito (NIAID, 2012). Asexual Cycle begins as infected female Anopheles mosquito-carrying malaria causing parasites feeds on a human and injects the parasites in the form of sporozoites into the bloodstream. The sporozoites travel to the liver within 30 - 40 minutes and invade liver cells. This begins the exoerythrocytic stage of the life cycle asexual multiplication occur (<https://en.wikipedia.org/wiki/>). Over 5-16 days the sporozoites grow, divide and produce tens of thousands of haploid forms called merozoites per liver cell. Some parasite species remain dormant for extended periods in the liver, causing relapses weeks or months later. The merozoites exit the liver cells and re-enter the bloodstream, beginning a cycle of invasion of red blood cells, asexual replication and release of newly formed merozoites from red blood cells repeatedly over

1-3 days. This multiplication can result in thousands of parasite infected cells in the host bloodstream, leading to illness and complications of malaria that can last for months if not treated.

Cycle in mosquito

Cycle in human

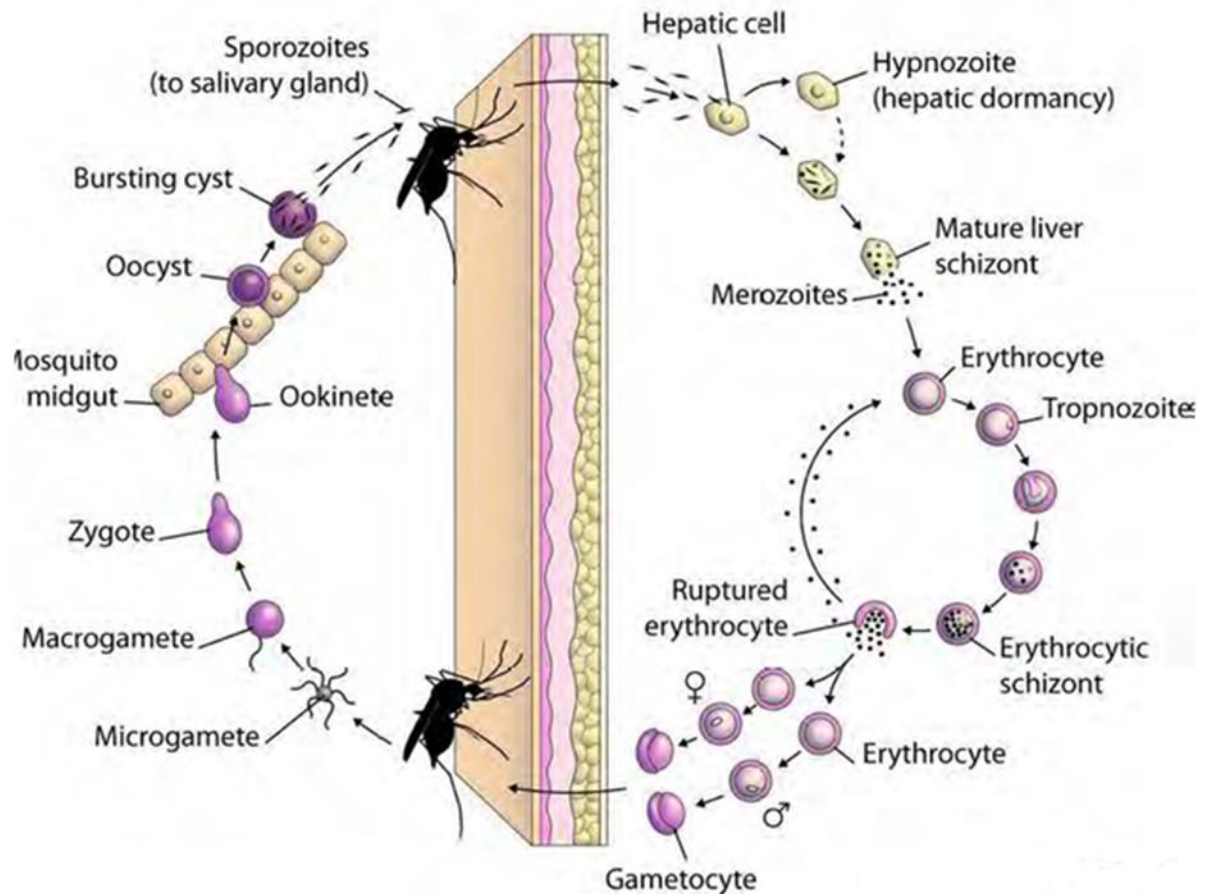


Figure 1. The life cycle of malaria parasites (Adapted from: <http://ocw.jhsph.edu>).

Some of the merozoites infected blood cells leave the cycle of asexual multiplication. Instead of replicating, the merozoites in these cells develop into sexual forms of the parasites called male and female gametocytes, which circulate in the bloodstream. It is blood stage parasites responsible for the clinical symptoms of malaria (John *et al.*, 2006). When a mosquito bites an infected human, it ingests the gametocytes. In mosquito gut the infected human blood cells burst, releasing the gametocytes, which develop further into mature sex cells called gametes. Male and female gametes fuse to form diploid zygotes, which develop into actively moving ookinetes that burrow into the mosquito mid gut

wall and form oocytes. Growth and division of each oocyst produces thousands of active haploid forms called sporozoites.

After 8-15 days the oocyst bursts, releasing sporozoites into the body cavity of the mosquito, from which they travel to and invade the mosquito salivary glands. The cycle of human infection re-starts when the mosquito takes a blood meal and injecting the sporozoites from its salivary glands into the human blood stream.

2.5. The Malaria Vector Mosquitoes

African countries have scaled-up insecticide treated mosquito nets, indoor residual spraying (IRS), improved diagnosis of malaria and treatment with Artemisinin-based combination therapy (ACT). Moreover, mortality in children under five years of age has fallen dramatically. Reductions in all-cause mortality rates of children fewer than five years in Ethiopia reached 28% (PMI, 2015). In Sub-Saharan Africa proportion of the population protected by at least one vector control method has increased in recent years and reached 48% in 2013 which range 44-51% (WHO, 2014). There are three most important African malaria vectors. These are *An. gambiaes.s*, *An. arabiensis* and *An.funestus* (Braack *et al.*, 2015). *Anopheles gambiaegiles* is the most efficient vector of human malaria in the Afro tropical Region. Thus, commonly called African malaria mosquito and considered to be the world's most important human malaria vectors (CDC, 2010). Members of *Anopheles gambiae* complex is found throughout tropical Africa (WHO, 1989). However, across the horn of Africa, the dominant vector is *Anopheles arabiensis* (Noor *et al.*, 2008). Seasonal abundance of *Anopheles gambiae* varies depending on location, but generally the population decreases during the dry season and increases in the wet season (Yaro *et al.*, 2012).

2.6. Pathogenesis and Clinical Manifestation of Malaria Infection

Clinical manifestations of malaria vary with geography, epidemiology, immunity and age. Initial manifestations (symptoms) of malaria disease are common to all malaria species. These are high fever, headache, severe chills, profuse sweating and general body pains (www.wikipedia.com). Still some patients may develop vomiting, cough, diarrhea and anemia.

Clinical manifestations of malaria were affected by anti-malarial medicines used, because it reduces the parasite density to very low levels. So, care must be needed to avoid over dose when diagnosed and self-prescription (WMR, 2010). The signs and symptoms of malaria typically begin 8-25 days

following infection. Based on World Health Organization (WHO) guidelines the disease is reasonably easy to recognize in people who have not had malaria before (WMR, 2010). All the clinical symptoms associated with malaria are caused by the asexual blood stage parasites (CDC, 2010).

In a non-immune individual, symptoms may appear up to seven days or more (usually 10-15 days) after the infective mosquito bite. In malaria endemic areas, persons may develop partial immunity that develop asymptomatic infections to occur. For both *P. vivax* and *P. ovale* clinical relapses may occur weeks to months after the first infection, even if the patient has left the malarias area. These new episodes arise from dormant liver forms known as hypnozoites (absent in *P. falciparum* and *P. malariae*), special treatment targeted at liver stages required for a complete cure (WHO, 2015).

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2.7 Anti-malarial drug resistance

Plasmodium falciparum resistance to Artemisinin has now been detected in five countries in the Greater Mekong sub region: Cambodia, Lao People's Democratic Republic, Myanmar, Thailand and Viet Nam. Despite the observed changes in parasite sensitivity, which manifest in the form of delayed parasite clearance, patients continue to respond to combination treatment, provided the partner drug remains effective. The efficacy of artemether-lumefantrine (AL) in Africa and South America remains high, with treatment failure rates generally below 10%. Failure rates of less than 10% have also been reported for artesunate-amodiaquine (ASAQ) in the 25 countries in Africa in which ASAQ is the first-line or second-line treatment. High treatment failure rates with artesunate-SP (ASSP) have been reported in northeast India (19–25.9%), Somalia (22%) and the Sudan (9.4%).

In Somalia, treatment failures are related to resistance to SP, in the absence of artemisinin resistance. For *P. vivax* malaria, at least one true case of chloroquine resistance (with whole blood concentrations of chloroquine plus desethylchloroquine >100 ng/ml on the day of failure) has been confirmed in countries: Bolivia, Brazil, Ethiopia, Indonesia, Malaysia, Myanmar, Papua New Guinea, Peru, the Solomon Islands and Thailand (WHO, 2015).

Malaria imposes a major global health burden in large part because the *Plasmodium* parasite readily evolves drug resistance. In *P. falciparum* resistance has arisen against all classes of first line antimalarial drugs and several have had to be withdrawn from use in many countries. CQ, for instance, is no longer recommended for treatment of *P. falciparum* in India, and has been replaced with the more expensive artemisinin-based combination therapy (ACT). It is now accepted as inevitable by the World Health Organization that all drugs will eventually fail in the face of resistance evolution. The only solution to this failure is to shift to new therapies or reformulations of old ones when resistance becomes too widespread (Das *et al.*, 2012). Resistance to anti-malarial drug in *P. falciparum* is well known since its first report on chloroquine in 1998. The resistance to chloroquine necessitated a change to Sulphadoxine-Pyrimethamine as first-line antimalarial in Ethiopia. However, recent data has showed high mean sulphadoxine-pyrimethamine treatment failure with 72% rate in some areas. Consequently, making another change to artemether-lumefantrine was suggested in 2004. Until now, there has been no report on the parasite's resistance to the last drug (Legesse, 2007).

2.8. Malaria in Ethiopia

About 75% of the land and 60% of the population is exposed to malaria in Ethiopia. Ethiopia is generally considered as a low-to-moderate malaria transmission intensity country. However, the health sector in Ethiopia is greatly affected by climate change which has profound consequences on the transmission cycles of vector-borne infection diseases like malaria. Due to the unstable and seasonal transmission of malaria in the country, protective immunity of the population is generally low and all age groups are at risk. Prevalence of malaria is currently estimated to be 1.3 (FMoH, 2007). Ethiopia is among the countries with a large burden of malaria with the peak transmission rate in the world. Researches addressing prevalence helps to assess malaria status within a given locality and has an important indication to value the overall effectiveness of prevention strategies being implemented in the area. About half of a population living in areas of an altitudinal range of 1500 and 2500 m above sea level are more likely to get malaria and these areas experience high malaria outbreak in Ethiopia.

Some studies from high altitude zones identified age, the proximity of households to potential mosquito breeding sites, sharing of houses with cattle, presence of windows and open attics as malaria risk factors. In addition to this, malaria is also related to factors like altitude, rainfall, and temperature. Thus, interventions focus on both the households and the surrounding environment (Mebrate *et al.*, 2020).

Ethiopia has achieved remarkable progress in the fight against malaria during the most recent decade through strong preventive and case management interventions with large engagement of the health extension workers (HEWs) and the health development army (HAD) volunteers providing community-based care at the household level (MoH, 2011). In children under five years of age, malaria admissions and death fell by 81% and 73% between 2001 and 2011 respectively.

2.9. Epidemiology of Malaria in Ethiopia

The epidemiological pattern of malaria transmission is generally unstable and seasonal in Ethiopia. The transmission level varies from place to place because of differences in altitude and rainfall patterns. Although, malaria distribution largely determined by altitude through its effect on temperature (MIS, 2007; 2011). About 75% of the landmass of Ethiopia is malarial and 68% of population at risk. These are 54 million people lives in malaria risk areas (Abebe *et al.*, 2011). As world Health Organization (WHO, 2013) estimates, that 198 million clinical cases and 500,000 deaths appeared by malaria. Malaria epidemics have been documented since the 1930s and 1940s in Ethiopia. The most devastating epidemic was 1958 which resulted in an estimated three million cases and 150,000 deaths (Lelisa *et al.*, 2014). Comparatively fewer epidemics since 2003 and 2004 in Ethiopia with reduced severity and mortality (WMR, 2008). Until recently, malaria has been the leading cause for morbidity and mortality in Ethiopia (FMoH, 2004 and MIS, 2007). Epidemics of malaria are relatively frequent as World Health Organization (WHO), 2006 in highland or highland fringe areas of Ethiopia, mainly areas 1,000 to 2,000 m above sea level. But, exist everywhere except the central highlands (FMoH, 2006). Areas less than 2,000m altitude considered malarial endemic in Ethiopia. Additionally, explosive epidemics, in highland areas of Africa have shown a trend of increasing malaria transmission in recent years (MIS, 2011). On the other hand, malaria epidemics can be categorized on the basis of their main causal factors. These are climate anomalies (rainfall in Semi-arid areas warm climates, causes increases in vector breeding and survival), inter-annual variation

caused by normal variations in climatology and sudden population movements, war and political instability (Abeku, 2007).

In line with causal factors in endemic areas, malaria is usually diagnosed clinically and only rarely confirmed by presence of the parasite in the peripheral blood. There are many more asymptomatic carriers of the parasite. Recent work has provided a quantitative framework for the analysis estimating probabilities that fever episodes are indeed of malaria etiology as a function of parasite density (Smith *et al.*, 1994; 1995). The current methods for epidemic detection in Ethiopia rely on passive case detection of clinically diagnosed cases at health posts and health centers (PMI, 2015).

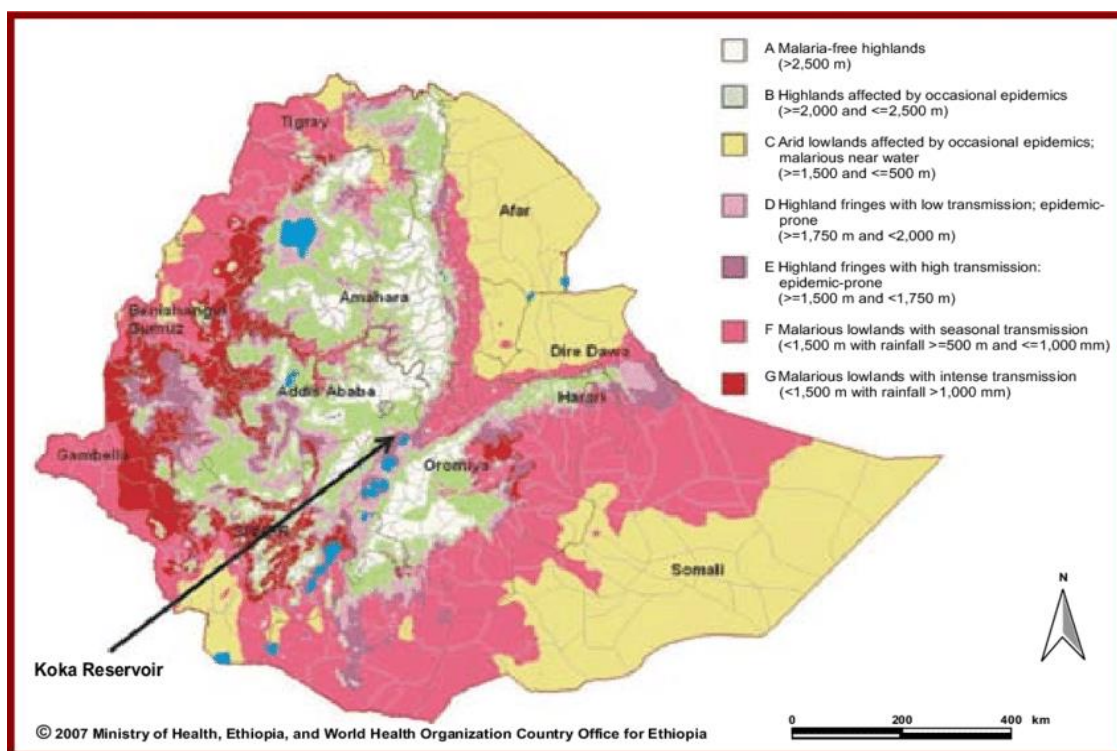


Figure 2. Malaria endemic areas of Ethiopia (MoH,2007)

2.10. Factors that Affect the Epidemiology of Malaria in Ethiopia

Ethiopia is a tropical country, which is located in the horn of Africa. The Carter Centre reported, about 55.7 million people faced the risk of malaria and approximately 80% of 736 districts considered malarias (Molla and Ayele, 2015). Factors that may cause outbreaks of malaria was an increase in vector breeding sites, migration of infected people into vector rich area populated with susceptible individuals, arrival of new efficient vectors, breakdown of vector control measures, resistance of the parasites to treatment and resistance of the vectors to insecticides (Gemechu Beffa Defi *et al.*, 2015).

2.10.1. Climatic Factors

For transmission of malaria parasite climatic factors are important determinants. Climatic factors greatly influence the pattern and level of malaria transmission in Ethiopia, Africa and the world. The most important climatic factors that directly affect malaria transmission are temperature, rainfall and humidity (MIS, 2007 and 2011). The distribution of malaria in Ethiopia is largely determined by altitude. Altitude also affects the pattern of malaria distribution in Ethiopia through its effect on temperature (MIS, 2007 and 2011). Temperature is a determining factor, malaria transmission often occurs in areas below 2,000m below sea level. While areas between 2000 and 2500m may become affected during epidemics, hot land malaria intensity and duration mainly is influenced by the amount and duration of rainfall (Aynalem, 2009). Temperature to a lesser extent with rainfall and humidity varies as a function of altitude (NSP, 2010). Changes in temperature, rainfall and relative humidity due to climate change are expected to influence malaria directly by modifying the behavior and geographical distribution of malaria vectors and by changing the length of the life cycle of the parasite (Abebe *et al.*, 2011).

Climate has three main elements. These are temperature, rainfall and humidity strongly associated with altitude and relief. Moreover, the location contributes to seasonal variation of rainfall and temperature in the country (Ashenafi, 2008). Climatic factors are important determinants for transmission of malaria parasite and greatly influence its pattern and level. Climate change is also expected to affect malaria indirectly by changing ecological relationships important to organisms involved in malaria transmission (the vector, parasite and host) (Abebe *et al.*, 2011).

2.10.2. Non-Climatic Factors

Factors that affect malaria transmission not related to climate. These are type of vector and parasite, environmental development and urbanization, population movement and migration, irrigation, agricultural practices, deforestation, the level of immunity to malaria in human hosts, insecticide resistance in mosquitoes, drug resistance in parasites and malaria control efforts, all have a role in affecting the severity and incidence of malaria (Abebe *et al.*, 2011).

The city conditions, such as stagnation of water facilitates, open wells and uncovered water tanks, and water logging at construction sites can create new places for mosquito larvae to develop (WHO, 2010). In Ethiopia man made breeding places including quarries, blocking natural drainage systems,

road building, irrigation in agriculture, water conservation or storages are among activities that enhance the incidence of malaria (MoH, 2002b).

2.11. Diagnosis of Malaria Parasite Infection

The clinical diagnosis where malaria is suspected based on the history, symptoms and laboratory technicians must confirm clinical findings. The microscopic examinations were done in Enticho health center by laboratory technician assisted by investigator. Microscopy remains gold standard for laboratory confirmation of malaria (WHO, 2014) a drop of patient's blood collected by finger prick from a larger venous blood. The specimen spread on glass slide (blood smear), dipped in a reagent that stains the malaria parasites (Giemsa stain), and examined under a microscope at 1000-fold magnification (WHO, 2014). Then microscopic examination of blood film is used for determination of malaria infection prevalence and malaria parasite prevalence in the study area.

2.12. Prevention and Control Strategies of Malaria

2.12.1 Treatment

Malaria can be severe, potentially fatal disease especially when caused by *P. falciparum*; the treatment should be initiated as soon as possible. Patients take oral medications should be given the treatment by continuous intravenous infusion. Most drugs used in treatment were active against the parasite in the blood. These are chloroquine, atovaquoneproguanil (Malarone), artemether-lumefantrine, mefloquine, quinine, quinidine, doxycycline, clindamycin and others (Ferede *et al.*, 2013). Ethiopia's treatment guidelines recommend artemetherlumefantrine as the first line treatment for *P. falciparum* and chloroquine for *P. vivax* malaria (Ferede *et al.*, 2013).

The malaria parasite is capable of becoming resistant to the action of anti-malaria drugs. This is due to small changes in the parasite DNA (point mutations), over prescription of anti-malaria (due to confusion with other febrile diseases) and uncontrolled selling of poor-quality drugs contribute to the increase in drug resistant parasites (CDC, 2006). As a result, the widespread and increasing occurrence of *P. falciparum* resists against affordable anti-malarial drugs. Chloroquine and sulphadoxine-pyrimethamine increasingly hampers the fight against malaria. This is the most widely used drugs for treatment in most of Africa, because of low cost and availability (CDC, 2006). The drug resistant problem of malaria has dual faces. These are resistance of the Plasmodium and resistance of the Anopheles mosquito. According to World Health Organization (WHO), origins of drug resistance to Plasmodium's are inadequate regimens, poor drug supply, poor quality and misuse of drugs (WHO,

2012). This imposes great difficulty in treatment and control of malaria (Ashenafi, 2008). Recently, Ethiopia reported resistance to all four classes of insecticides. These are DDT, pyrethroids, Malathion and bendio-carb (WHO, 2012).

2.12.2. Vector Control

Vector control is an essential component of malaria control programs and a fundamental element of existing global strategy to fight malaria (WHO, 2008). Vector control, if well planned, targeted and timely, can contribute to reducing the risk of malaria infection and saving lives (WHO, 2004a). Vector control used to reduce level of mortality and morbidity by reducing transmission of the disease (Toure, 2001). The most frequent type of vector control is mosquito control using a variety of strategies (WHO, 2012). Malaria control efforts involve a combination of prevention and treatment strategies and tools. These are insecticide-treated bed nets (ITN), Indoor Residual Spraying (IRS) with insecticides, diagnosis and treatment with anti-malarial drugs. Artemisinin-based combination therapy (ACTs) and intermittent preventive treatment in pregnancy (IPTp) are limited, although access to prevention and treatment services has grown over time (WHO, 2015). However, many challenges continue to complicate malaria control efforts in countries with ongoing malaria transmission. These are poverty, poor sanitation, weak health systems, natural disasters, armed conflict, migration, climate change, and the presence of counterfeit and/or sub-standard antimalarial drugs (WHO, 2014).

2.12.3. Insecticide Treated Nets

Insecticide treated Nets and LLINs are now essentially synonymous in Ethiopia. Insecticide-treated mosquito and other materials (ITMNs) were options combining one aspect of personal protection (mosquito nets) with insecticides (Jacklyn *et al.*, 2015). The use of insecticide-treated nets (ITNs) is effective in reducing malaria related morbidity and mortality (MoH, 2002a). The distribution of insecticide treated bed nets has been a major factor in reducing malaria deaths, where deaths have fallen by over 50% since 2007/08 (Banteyarga, 2011). The demonstrated impact of ITNs on reducing the risk for low birth weight and maternal anemia is important (RBMP, 2008). The major effort to scale-up the availability of ITNs in Africa was yielding impressive results (PMI, 2015).

In World Health Organization (WHO), African region, ITMNs application is recognized as one of the three key elements. These are the appropriate case management, use of insecticide-impregnated bed nets and other materials and strengthening local and national capabilities, which form the basis for intensified malaria control (WHO, 1992). As reported by MoH, Ethiopia has seen a major

improvement in LLIN coverage (MIS 2007; MIS, 2011). However, there are many challenges to ITN distribution, acceptance and correct utilization when trying to implement large-scale ITN programs (Kelly-Hope and McKenzie, 2009).

2.12.4. Indoor residual house spraying

Indoor residual house spraying was the process of spraying the inside of dwellings with an insecticide to kill mosquitoes that spread malaria (WHO, 2009). A dilute solution of insecticide was sprayed on the inside walls of certain types of dwellings those with walls made from porous materials such as mud or wood but not plaster as in city dwellings. Mosquitoes are killed or repelled by the spray, preventing transmission of the disease. As world health organization (WHO), malaria report shows, 44 countries employed IRS as a malaria control strategy. Several pesticides have historically been used for IRS, the first and most well-known being DDT (WHO, 2008).

The World Health Organization (WHO), recommends IRS as one of three primary way to control malaria (WHO, 2008). Previously, the WHO had recommended IRS only in areas of sporadic malaria transmission. Since 2006 it began recommending IRS in areas of endemic, stable transmission as well (Van den Berg, 2008).

2.12.5. Biological Control of Malaria

Vector control is any methods used to limit or eradicate the mammals, birds, insects or other arthropods, which transmit disease as pathogens. Mosquito control is the most frequent using a variety of strategies. Using fish that eat mosquito larvae or reducing breeding rates by introducing sterilized male tsetse flies had been shown to control vector populations and reduce infection risks (WHO, 2006; 2015). Biological control is an approach kills mosquitoes or their larvae in a more targeted manner than chemicals averting certain impacts on health and local environments. The best known of those controls include various species of larvivorous fish and biolarvicides, such as *Bacillus thuringiensis israelensis* and *Bacillus sphaericus* (Walshe *et al.*, 2013). Maintenance of larvivorous fish stocks and repeated applications of biolarvicides require substantial community awareness and involvement (Van den Berg, 2009; Walshe *et al.*, 2013).

2.12.6. Environmental Sanitation

Globally 2.4 billion people live under highly unsanitary conditions and have poor hygiene behaviours that their exposure to risks of incidence and spread of infectious diseases are enormous (WHO, 2015).

Malaria kills over 1.2 million people annually, mostly African children under the age of five. Poorly designed irrigation and water systems, inadequate housing, poor waste, disposal and water storage, deforestation and loss of biodiversity, all may be contributing factors to the most common vector borne diseases, including malaria, dengue and leishmaniasis (WHO, 2016).

2.12.7. Larval Breeding Management

The larval breeding management of aquatic habitats that are potential larval habitats for mosquitoes into prevent the completion of development of the immature stages (eggs, larvae and pupae) and hence the production of adult mosquitoes. There are four types: Habitat modification: a permanent alteration to the environment, e.g. land reclamation; Habitat manipulation: a recurrent activity, e.g. flushing of streams; Larviciding: the regular application of biological or chemical insecticides to water bodies; In general, environmental management (habitat modification and manipulation) should, where feasible, be the primary strategy to reduce the availability of larval habitats. However, no systematic reviews have so far been conducted to inform the development of WHO guidance in this area, and the Guidelines (WHO, 2019).

2.12.8 Traditional malaria prevention

These are eating garlic with green paper, drink gourd juice and avoiding eating vegetables such as tomato during rainy season to prevent malaria. These have both curative and preventive effect. There is also a home remedy called “haregres”, this is a root of a small plant, they drink its juice. There are also local plants such as “Dumuga” and “Endode” used to prevent malaria (Alelign and Dejene, 2016).

3. MATERIALS AND METHODS

3.1 Study Area

Enticho Town is located in Ahferom Woreda, Central Zone of Tigray at a distance of 917 km from Addis Ababa, the capital city of Ethiopia, while 167km far from Mekelle, capital city of Tigray region, Ethiopia. It is located with 14 ° 06'30" and 14°38'30" north latitude and 38°56' 30"and 39° 18'00" east covering an area 1881km².The mean annual rainfall is 540 to 650mL and the mean annual temperature ranges between 22°C to 27°C. Enticho has six urban kebele administrations in the district. According to the Ahferom woreda statistical office (AWSO, 2020) the total population of the District is 224, 924 with 108, 800 and 116,124 male and female, respectively. The total population of Enticho town district is 32, 470 with 14, 450 male and 18, 020 female. Enticho is a malarious endemic area, due to the suitable place for mosquito breeding, some cases of malaria every year occur mainly among Enticho residents who work and live outside of the city, and but come to seek medical care in Enticho. During the summer months or particularly wet rainy seasons; though, numerous cases can develop within Enticho itself. The Enticho health office, occasionally participates in community malaria awareness and plant clearing activities and evaluates the health clinics to ensure that they are administering fever and malaria rapid diagnostic tests during malaria season Enticho city, Woreda outside the city and health extension workers in rural areas often distribute bed nets, but in some case people cannot use properly.

Vegetation type consists of different herbs, shrubs and some common plant species in this area includes *Olea europaea*, *Acacia* species, *Eucalyptus* species, *Justicia chiperiana*, *Premnas chimperi*, *Podocarpus falcatus*, *Juniperus procera*. Some commonly cultivated crop plants in the study area includes Teff (*Eragrotis teff*), Bean (*Vicia faba*), *Sorghum bicolor*, Wheat (*Triticum* species), and Barley (*Hordeum vulgare*, Pea (*Pisum sativum*) and *Zea mays*.Source (Ahferom Agricultural and Rural Developme Office, 2020).

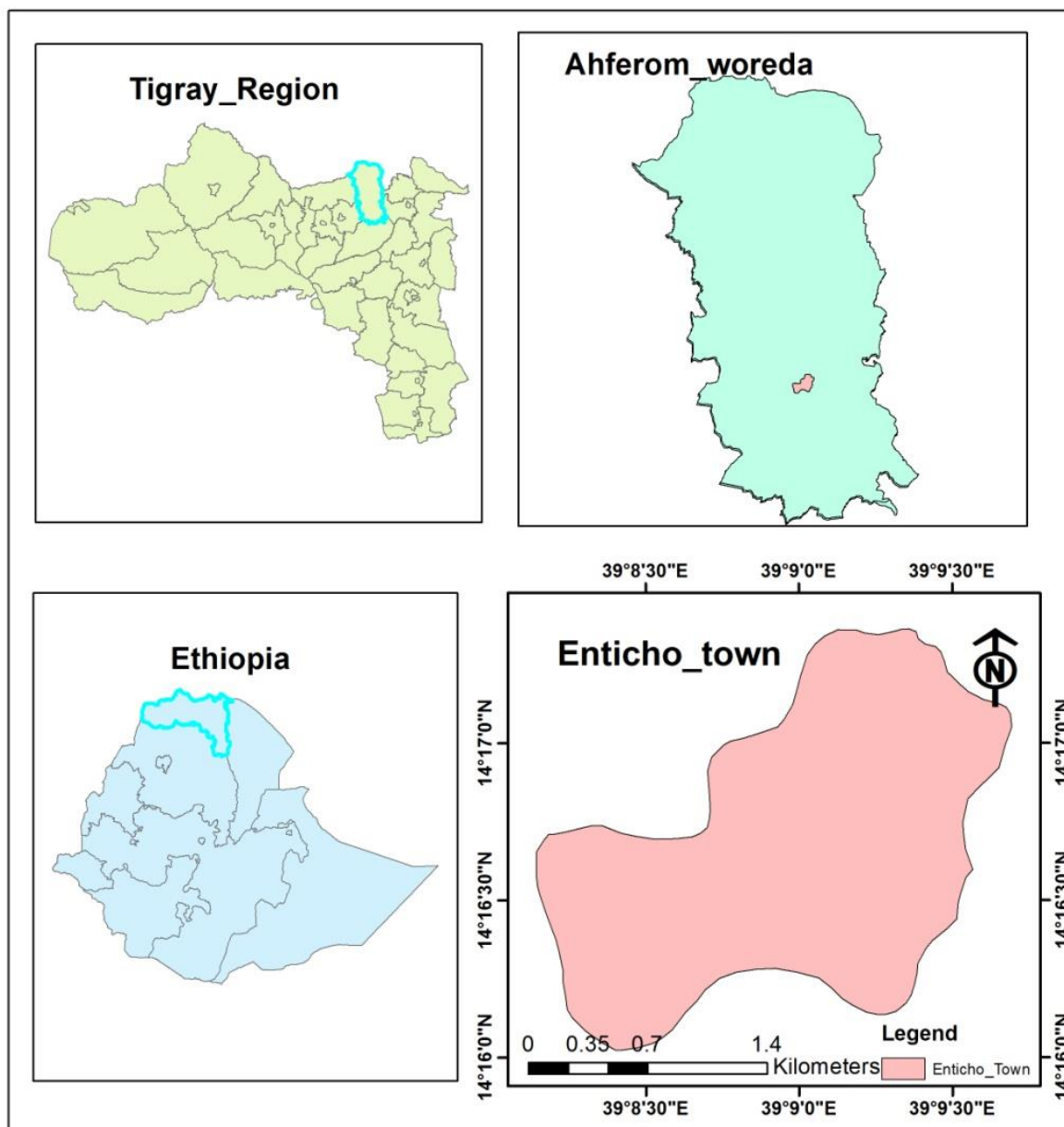


Figure 3 Location map of the study Area, (Source: Google earth map, 2019).

3.2 Study design and population

Descriptive cross-sectional study was conducted to determine the prevalence of malaria in the study area from September 2020 to June 2020. Moreover, retrospective data of the last five years (2015-2019) was reviewed from the medical records of the Enticho general hospital.

3.3 Population and Sample Size Determination

3.3.1 Study Population

The population for this study was all malaria suspected patients who visited Enticho hospital within the study period.

3.3.2 Sample Size Determination

The required sample size for the prospective study was determined by using single population proportion formula (Daniel, 2009) at margin of error (d) 5% (standard value of 0.05) and Prevalence of malaria (50%).

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

$$n = (1.96)^2 \times 0.5(1-0.5) / (0.05)^2$$

$$\mathbf{n=384}$$

Where:

- ✓ n – sample size
- ✓ P – Prevalence of malaria (50%)
- ✓ d – Error value (5%)
- ✓ Z – Confidence interval, Z (95%) = 1.96

Therefore, the sample size of the study area was 384, which were selected by the above formula. All clients visiting the Enticho hospital for malaria diagnosis until the allocated proportion was reached

3.4 Data Gathering Tools and Procedures

3.4.1 Questionnaire and interview

The researcher employed structured questionnaire and interview to assess demographic, personal and environmental sanitation practice of the information of participants'. Data were collected using interviewer administered structured questionnaires. To ensure reliability and validity the tool pretesting were done on 5% of the sample size other than the study area before actual data collection period and amendments were taken from the pretested questionnaire. Questionnaire were prepared in English then translated to Tigrigna (local language of the study area) and back translated to English for consistency.

3.4.2. Blood sample collection

Blood sample was collected by laboratory technician worked at Enticho hospital by cutting finger-tips of blood donor. First the finger cleansed with an alcohol-moistened swab then dried with a piece of dry cotton and penetrated with a disposable blood lancet.

3.4.3. Blood films preparation and staining techniques

The blood smear was prepared by laboratory technician from blood collected by finger prick. Both thin and thick smears had been made on the same slides side by side and properly labeled per individual with its code. Small drop of blood was placed near the frosted end of a clean glass slide and blood streaked over slides by clean spreader slide held at a 45° angle, toward the drop of blood on specimen slide, which then allowed to air dry. Thin films were fixed with 100% methanol allowed to dry for 30 seconds. However, thick film not fixed (<http://www.dpd.cdc.>). Following this, smear were stained, using standard guidelines of (WHO,1991), both thick and thin films stained with 3% Giemsa stain at pH 7.0 for 30min as recommended by WHO (Woyissa *et al.*, 2012).

Microscopic examination remains the gold standard" for laboratory confirmation of malaria (www.malariasite.com/microscopic-tests/). Thick and thin blood smear becomes the most reliable and preferred for diagnosis of malaria (WHO, 1991; Warhurst and Williams, 1996; Krafts *et al.*, 2011). Thick films were used to determine the parasite densities while thin films had been used to identify the parasite species and infective stages. A stained slide was examined under the light microscope using x100 objective lens (Meeusen *et al.*, 2001). One hundred microscopic fields should be examined on

thick film before smears were considered negative for parasites. The thin film was used for species identification when thick smears most likely to demonstrate infection (Krafts *et al.*, 2011).

3.4.4. Identification of Malaria Parasites

The presence of malaria parasites on thick blood smear was examined by using high power magnification objective (40x) and the identification of *Plasmodium* species from the thin blood smear was made through oil immersed objective (100x). Thick blood smears were most useful for detecting the presence of parasites, because they examined a larger sample of blood and thin blood smears used to identify type of *Plasmodium species*. Their different species distinguished their morphological Characteristics on Giemsa-stained thin blood smear.

3.5 Source of data

For this study, the researcher was used both primary and secondary source of data. Primary data was administered by questioner, interviewing and laboratory examinations from the study participants and secondary data were from document record 2015-2019 back five years in Enticho hospital.

3.6 Inclusion criteria and Exclusion criteria

3.6.1 Inclusion

⇒ All patients who visited Enticho hospital and suspected with malaria.

3.6.2 Exclusion

⇒ Unconscious clients, terminally ill clients, mentally ill clients, and deaf clients and all patients visited Enticho hospital but suspected with no malaria disease.

3.7 Data collection techniques

A format was prepared on a computer spreadsheet (Excel) to collect the secondary data from logbooks of local government primary health care units and from primary data by interviewing of the health professionals of the study area. Individual level data on malaria morbidity such as diagnosis results (*Plasmodium species* for positives) and available demographic data (age, sex) will be registered on the computer spreadsheet. All records of malaria patients who visited the health institutions during the timeframe considered and treated as malaria patients are including in the study. Dates of the health service visit and address of the malaria patients will exclude from the study.

3.8. Data quality control

To minimize error, questionnaires were pretested with 10% of the participants and all the laboratory procedures including collection and handling of specimens as well as microscopic procedures were carried out according to standard microbiological test (Monica, 2009). In addition to this, stool sample of participants were examined in duplicates. Finally, among the total positive samples, 10% were selected randomly for re-examination with laboratory technologist who did not have any information about the previous result. Staining and blood film examination was performed by following the standard protocol of World Health Organization (Garcia, 2001). The peripheral blood smear provides comprehensive information on the species, the stages and the density of parasitemia. The efficiency of the test depends on the quality of the equipment and reagents, the type and quality of the smear, skill of the technician, the parasite density and the time spent on reading the smear. Examination of the peripheral blood smear is an inexpensive but powerful diagnostic tool in both children and adults. It provides rapid reliable access to information about a variety of hematologic disorders (Bain, 2005).

3.9. Ethics consideration

The study was approved by the Ahferom Meles hospital (Pro.No 00127112). Supportive letter was obtained from the district health office before data collection, and written informed consent was obtained from study participants. Before conducting this study, the aim of the study was explained in details and the respondents were assured of the confidentiality of their information.

3.10. Data Analysis

Data were checked for consistency and completeness, and entered into SPSS-version 20 and Microsoft Excel. Specifically; socio-demographic characters, sanitation practice of participants and frequency of malaria per samples were summarized by descriptive statistics. Associations of malaria infection with socio-demographic profiles and potential risk factors of participants were tested by Pearson Chi-Square. Here, the associations were considered statistically significant if $P \leq 0.05$ and strongly significant if $P < 0.001$ (Semin, 2011).

4 RESULTS

4.1 Socio demographic characteristics of participants

For this study, socio-demographic characteristics of participants i.e. sex, age group, marital status, occupation, income status, family size and education level were assessed (Table-1). Among these 384 participants, 114(29.7%) were male and 270(70.3%) were females in gender. Their age distribution was ranged from 2-14 years; specifically, 97(25.3%) were in the age less than 18 years, 91(23.7%) were in 18-30 years, 129(33.6%) were in 31-40 years and 67(17.4%) were in above 40 years old.

Table 1. Socio demographic characteristics of participants

Characteristics	Categories	Frequency
Sex	Male	114(29.7%)
	Female	270(70.3%)
Age	< 18	97(25.3%)
	18-30	91(23.7%)
	31-40	129(33.6%)
	>40	67(17.4%)
Marital status	Single	19(4.9%)
	Married	156(40.9%)
	Widowed	117(30.5%)
	Divorced	92(24%)
Occupation	Private	103(26.8%)
	GO employed	87(22.7%)
	daily labor	129(33.6%)
	Student	65(16.9%)
In come status	>2000	209(54.4%)
	1000-2000	171(44.5%)
	<1000	4(1%)
Family size	1-3	133(34.6%)
	4-6	122(31.8%)
	>6	129(33.6%)
Educational status	Illiterate	13(3.4%)
	Read and write	123932%
	1-4	108(28.1%)
	5-8	120(31.3%)
	high school and above	20(5.2%)

4.2 Prevalence of malaria infections by age group and sex of participants

In this case, 384 patients 'was taken for malaria examination. Accordingly, the smear of the participants was showed 96(25%) total malaria prevalence rate (Table-2). Among these, 45(39.5%) were males and 51(18.9%) were females.

In case of age group, 33(34%) malaria infection were seen in less than 18 ages, 35(38.5%) was seen in 18-30 ages, 26(20.2%) was seen in 31-40 and 2(3%) were seen in above 40 age groups.

Table 2. Prevalence of malaria infections with age group and sex

Item	Categories	N ^o of examined (n= 384)	Malaria status
			Positive (%)
Age group	<18	97 (25.3%)	33 (34%)
	18-30	91 (23.7%)	35 (38.5%)
	31-40	129 (33.6%)	26 (20.2%)
	>40	67 (17.4%)	2 (3%)
	Total	384 (100%)	96 (25%)
Sex	Male	114 (29.7%)	45 (39.5%)
	Female	270 (70.3%)	51 (18.9%)
	Total	384 (100%)	96 (25%)

4.3 *Plasmodium* species identified in the examined participants

The malaria rapid diagnostic test (RDT) in the current study was also trying to examine types of plasmodium species in the patients (Figure-2). Accordingly, from the total 96 malaria positives, 71(74%) infection were with *P. falciparum* and the 25(26%) infection rate was with *P. vivax*.

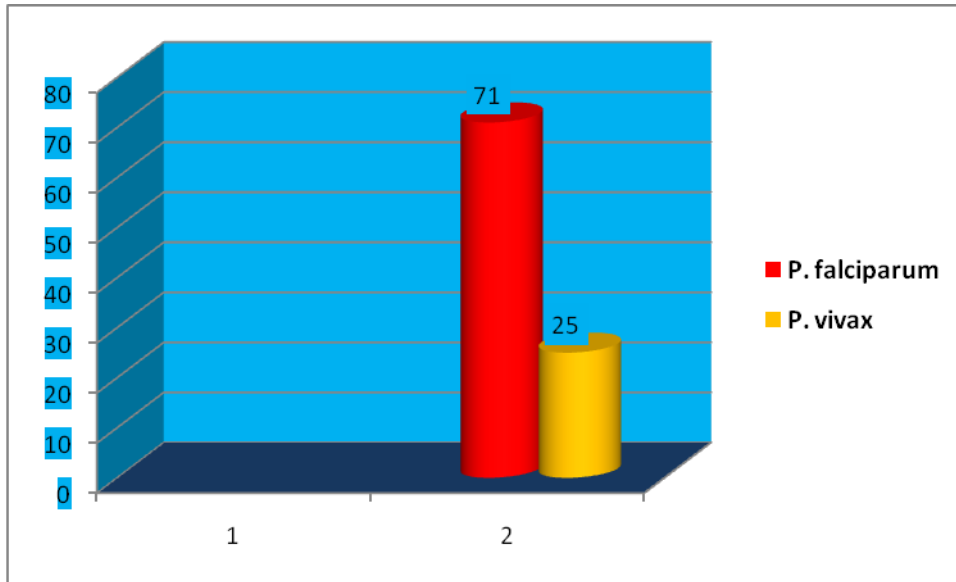


Figure 4 Plasmodium species identified in the examined participants

4.4 Association malaria infections with socio-demographic characteristics

Table-3 presents the overall prevalence malaria diagnosed in the study subjects and their association with some socio-demographic factors of the participants during the study period. The variables assessed in the present study were gender, age group, residence, marital status, educational level, family size, income, occupation, time stay of the participants in that area. Among these, infections the prevalence of malaria in the study area were statistically associated with the gender ($P = 0.000$, $OR = 2.801$), age group ($P = 0.000$, $OR = 2.91$), residence ($P = 0.000$, $OR = 3.71$), family size ($P = 0.001$, $OR = 3.03$) and income ($P = 0.004$, $OR = 2.438$) of the participants

Table 3. Association of malaria with socio-demographic profiles of participants

Variables		N ^o of examined	Malaria status	OR)	P- value
			N ^o +ve (n=96)		
Sex	Male	114 (29.7%)	45 (39.5%)	2.801	.000
	Female	270 (70.3%)	51(18.9%)		
Age group	<18	97 (25.3%)	33 (34%)	2.91	.000
	18-30	91 (23.7%)	35 (38.5%)		
	31-40	129 (33.6%)	26 (20.2%)		
	>40	67 (17.4%)	2 (3%)		
Marital status	Married	156 (40.6%)	38 (24.4%)	0.53	.720
	Single	19 (4.9%)	6 (31.6%)		
	Divorced	117 (30.5%)	32 (27.4%)		
	Widowed	92 (24%)	20 (21.7%)		
Educational level	Illiterate	13 (3.4%)	4 (30.8%)	1.06	.348
	Read and write	123 (32%)	33 (26.8%)		
	1-4	108 (28.1%)	27 (25%)		
	5-8	120 (31.3%)	24 (20%)		
	High school & above	20 (5.2%)	8 (40%)		
Family size	1-3	133 (34.6%)	42 (31.6%)	3.03	.071
	4-6	122 (31.8%)	37 (30.3%)		
	>6	129 (33.6%)	17 (13.2%)		
Income	<1000	4 (1%)	3 (75%)	2.438	.123
	1001-2000	171 (44.5%)	52 (30.4%)		
	>2000	209 (54.4%)	41 (19.6%)		
Occupation	Private	103 (26.8%)	25 (24.3%)	1.8	.609
	daily labor	87 (22.7%)	18 (20.7%)		
	Student	65 (16.9%)	37 (28.7%)		
	Gov. employed	129 (33.6%)	16 (24.6%)		
Time stay	<3 years	170 (44.3%)	51 (30%)	1.4	.234
	4-6 years	120 (31.3%)	24 (20%)		
	7-10 years	89 (23.2%)	20 (22.5%)		
	>11 years	3 (.8%)	1 (33.3%)		

4.5 Risk factors associated for malaria infection in participants of the study area

Table-4 presents the overall prevalence malaria diagnosed in the study subjects and their association with risk factors of the participants during the study period. The variables assessed as risk factors of the present study were having of screen in windows and door, posting of posters/ papers on the wall of bed room, having of indoor grass and plants, having of stagnant water near house, switching on habit

of light in sleeping time, frequency to use bed nets in sleep time, quality of the bed net used, knowledge on malaria epidemiology of the participants. Among these, infections the prevalence of malaria in the study area were statistically associated with having of indoor grass and plants ($P = 0.033$, $OR = 1.684$) having of stagnant water near house ($P = 0.000$, $OR = 26.253$) and frequency of participants to use bed nets in sleep time ($P = 0.000$, $OR = 5.117$). Other risk factors were not showing statistical significance for the prevalence of malaria infection in participants of the study area ($P > 0.05$).

Table 4. Risk factors associated with malaria infection in participants of the study area

Variables		N ^o of examined	Malaria status	OR)	P- value
			N ^o +ve (n=96)		
Having of screen in windows and door	Yes	58 (15.1 %)	20 (34.5%)	1.731	.070
	No	326 (84.9%)	76 (23.3%)		
Posting of posters/papers on the wall of bed room	Yes	383 (99.7%)	96 (25.1%)	.749	.563
	No	1 (.3%)	0 (0.0%)		
Having of indoor grass and plants	Yes	216 (56.3%)	63 (29.2%)	1.684	.033*
	No	168 (43.8%)	33 (19.6%)		
Having of stagnant water near house	Yes	60 (15.6%)	49 (81.7%)	26.253	.000**
	No	324 (84.4%)	47 (14.5%)		
Switching on habit of light in sleeping time	yes, always	80 (20.8%)	14 (17.5%)	.981	.066
	yes some times	109 (28.4%)	35 (32.1%)		
	No	195 (50.8%)	47 (24.1%)		
Frequency to use bed nets in sleep time	Yes, regularly	48 (12.5%)	2 (4.1%)	5.117	.000**
	Yes, some times	254 (66.1%)	38 (14.96%)		
	No	82 (21.35%)	56 (68.29%)		
Quality of the bed net used	Well	215 (56%)	49 (22.8%)	1.437	.266
	Deprived	88 (22.9%)	15 (17.0%)		
Knowledge on malaria epidemiology	Yes	343 (89.3%)	81 (23.6%)	1.77	.070
	No	41 (10.7%)	15 (36.6%)		

4.5.1 Prevalence of malaria in the study area 2015-2019

To assess the prevalence of malaria in the study area, backdated study was performed in five years i.e. 2015-2019 of the study area (Table-5). Then the overall prevalence of malaria was 2892(21.14%) in specific to 13.5%, 10.7%, 51.3%, 12.5% and 11.8% respectively for 2015, 2016, 2017, 2018 and 2019. In case of plasmodium species, two species were specified for infection of the patients' namely *P. falciparum* and *P. vivax* with 2029(70.16%) and 863(29.84%) in their average range respectively.

Table 5. Prevalence of malaria in outpatient at Enticho Hospital in different seasons 2015-2019

Year	Parameters	Sep-Nov.	Dec-Feb.	Mar –May	Jun-Aug.	Total
2015	No. examined	1321	459	619	323	2722
	Positive	166(12.56%)	95(20.69%)	64(10.3%)	29(8.97%)	390(14.3%)
	<i>P. falciparum</i>	111	62	36	22	259
	<i>P. vivax</i>	55	33	100	7	131
2016	No. examined	497	258	237	248	1240
	Positive	111(22.33%)	76(29.11%)	69(29.11%)	55(22.17%)	311(25%)
	<i>P. falciparum</i>	68	59	34	41	202
	<i>P. vivax</i>	43	17	35	14	109
2017	No. examined	1473	1503	1324	1288	5588
	Positive	373(25.32%)	390(25.94%)	302(22.8%)	228(17.7%)	1484(26.55%)
	<i>P. falciparum</i>	191	250	205	163	991
	<i>P. vivax</i>	182	140	97	65	493
2018	No. examined	629	364	390	245	1628
	Positive	160(25.43%)	70(19.23%)	86(22.05%)	48(19.59%)	364(22.35%)
	<i>P. falciparum</i>	105	46	56	36	243
	<i>P. vivax</i>	55	24	30	12	121
2019	No. examined	603	574	581	742	2500
	Positive	96(15.92%)	93(16.2%)	56(9.06%)	96(12.9%)	343(13.72%)
	<i>P. falciparum</i>	96	88	53	96	333
	<i>P. vivax</i>	0	5	5	0	10
Total	No. examined	4424	3520	3171	2563	13678
	Positive	1097(24.8%)	724(20.56%)	615(19.3%)	456(17.79%)	2892(21.14%)
	<i>P. falciparum</i>	754	505	412	358	2029(70.16%)
	<i>P. vivax</i>	343	219	203	98	863(29.84%)

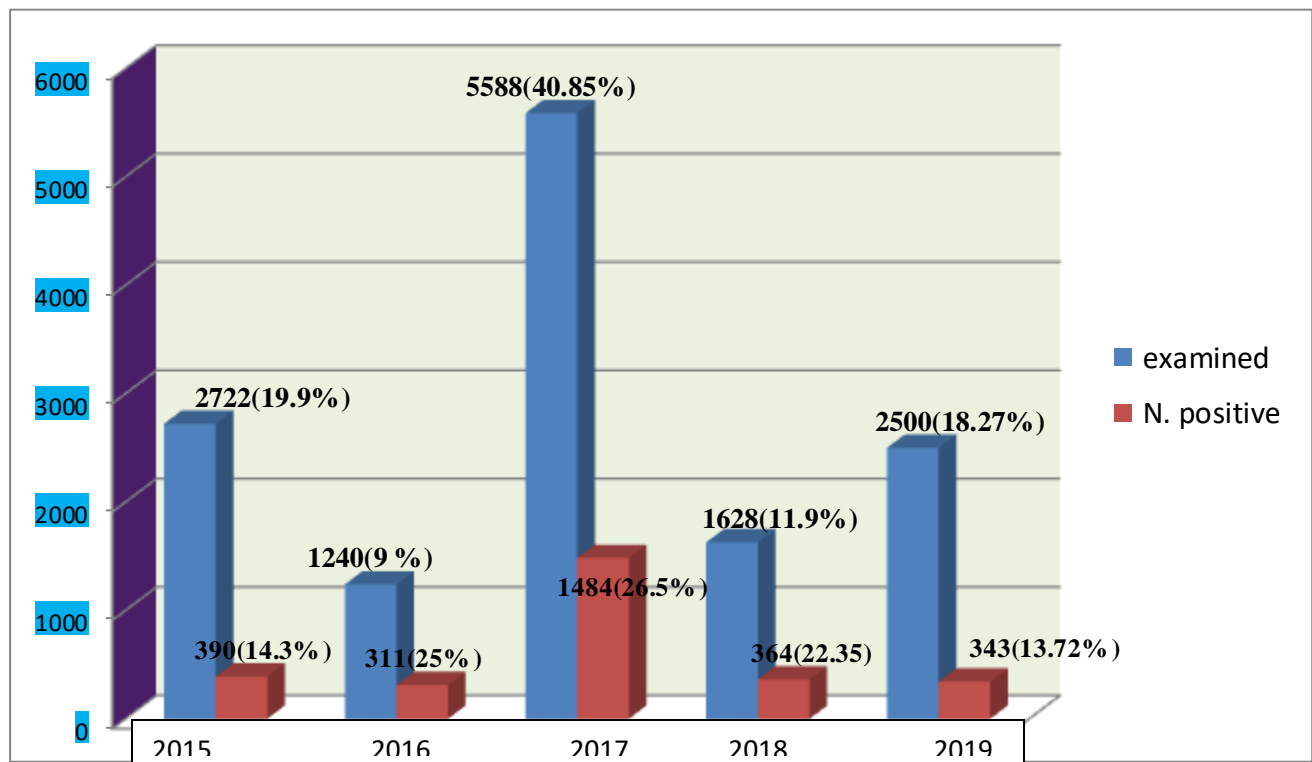


Figure 5. Five years retrospective data of malaria infections from 2015--2019

4.6.2 Trend of malaria cases per season specification

The seasonal distribution of malaria cases is summarized in Fig.3. Accordingly, the highest peaks of malaria were seen in autumn (1097) following by winter (724), spring (615) and summer (456) respectively.

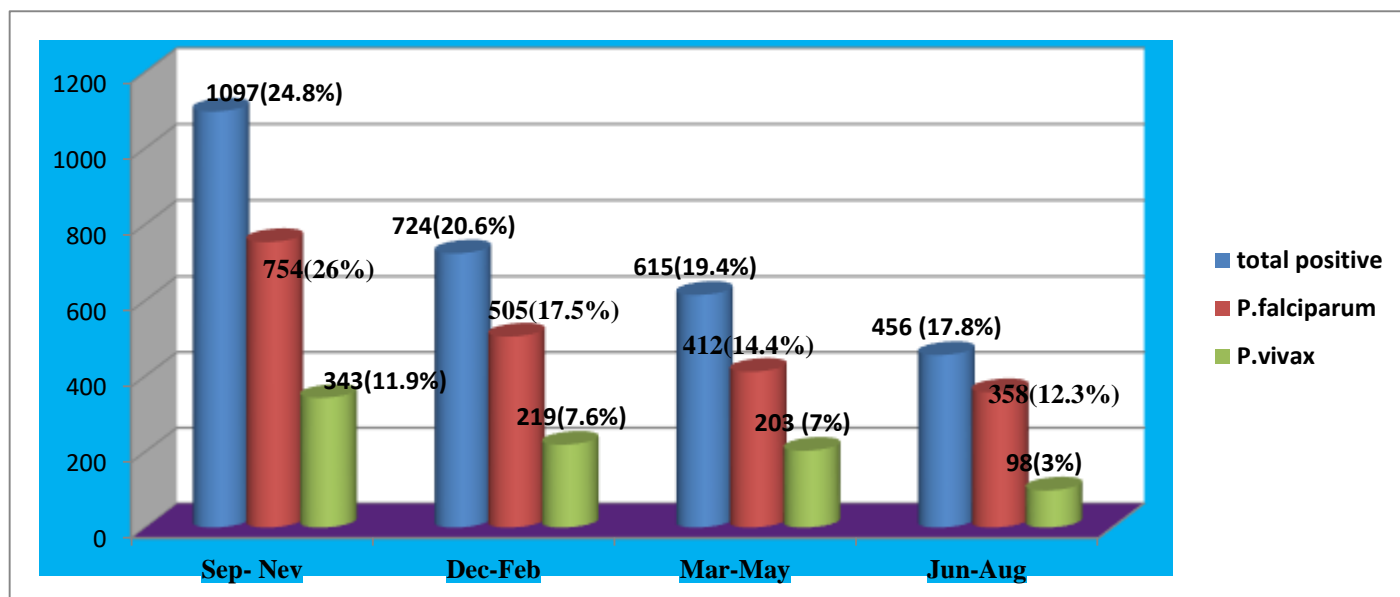


Figure6. Retrospective data of malaria infection per season

4.6 Prevalence of malaria in the study area by age group and sex specification

In case of age group specification, the overall trends of malaria were higher seen in above 15 age groups following to 5-15 and less than five with the average rang of 1883(65.1%), 573(19.8%) and 436 (15.07%) respectively (Table-6). Besides, the total prevalence of malaria was seen in 1764(60.9%) males and in1128 (39.1%) females (Table-7).

Table 6. Prevalence of malaria in outpatients at Enticho Hospital (2015-2019)

Age group	2015	year 2016	2017	2018	2019	Total
0-4	39(1.34%)	35(1.21%)	296(10.23%)	23(0.79%)	43(1.48%)	436(15.07%)
5-14year	78(2.69%)	54(1.86%)	335(11.58%)	51(1.76%)	55(1.9%)	573(19.8%)
>15	273(9.43%)	222(7.67%)	853(29.49%)	290(10.02%)	245(8.4%)	1883(65.1%)
Total	390(13.4%)	311(10.75%)	1484(51.3%)	364(12.58%)	343(11.86%)	2892(100%)

Table 7. Prevalence of malaria in outpatients at Enticho Hospital the (2015-2019)

Sex	2015	2016	2017	2018	2019	Total
Male	268(9.26%)	208(7.1%)	767(26.5%)	292(10.5%)	229(10.5%)	1764(60.9%)
Female	122(4.21%)	103(3.56%)	717(24.71%)	72(2.48%)	114(3.94%)	1128(39.1%)
Total	390	311	1484	364	343	2892

4.6.1 Trends of plasmodium species in the study area 2015-2016

P. falciparum were more prevalent than *P. vivax* in with their prevalence rate were 2029(70.16%) and 863(29.84%) (Table-8).

Table 8. Retrospective data of plasmodium species distribution in the study area

Spp.	2015	2016	2017	2018	2019	Total
<i>P. falciparum</i>	259(8.9%)	202(6.98%)	991(34.26%)	243(8.4%)	333(11.5%)	2029(70.16%)
<i>P. vivax</i>	81(2.8%)	109(3.76%)	493(17.04%)	121(4.18%)	10(0.3%)	863(29.84%)
Total	390	311	1484	364	343	2892 (100%)

4.6.4. Distribution of ITNS in Enticho Town 2015 to 2019

As Table 9 in Enticho Town, about 48,227 ITNS were distributed beginning from 7640 in 2015 up to 11341 in 2019 that indicated an increase of awareness of ITNS distribution. The distribution of ITNS in 2017 was the highest but also more malaria patients were recorded. This indicated that rain fall (collected water) is suitable for mosquito breeding.

Table 9. The distribution of ITNS & metrological information in Enticho Town (2015 to 2019)

Year	mean rainfall	mean annual Temperature (°c)	ITN distribute
2015	701.8 mm	21.2	7640
2016	859.3 mm	20.5	8244
2017	1646 mm	19.41	10460
2018	825 mm	20.1	10542
2019	415 mm	20.34	11341

Total ITN
48,227

5 DISCUSSION

Epidemiological studies on the prevalence of malaria infection and predisposing risk are essential to design its appropriate intervention strategies (Kebede *et al.*, 2018). The present study was there for aimed to show the prevalence of malaria infection and its associated risk factors among study participants who visited Enticho hospital. Based on the prospective data, 25% prevalence rate of malaria was found in the study area. Besides, 21.14% prevalence rate was found in population of the study area based on the retrospective data. This finding is higher than the study done in Shire town which was reported as 17.31% (Gehebreyesus Alemu *et al.*, 2015). This high prevalence rate of malaria in the study area shows that, population of the study area shows less health care emphasis (Thuilliez *et al.*, 2017). This difference might be due to health care accessibility and knowledge difference on the prevention of malaria (Amir *et al.*, 2017).

Nationwide, autumn is explained as the main risk of malaria infection following by spring. In line to this, the highest peak malaria infection in study area was in September to November (autumn). This might probably due to relapsing behaviour of some malaria parasite and irregular rain-fall in the area. Probably many factors might influence the seasonal distribution of malaria, e.g. climatic variables, behavioural and biological variation across mosquito species, environmental factors and economic factors (Kebede *et al.*, 2018) and this finding is consistent to the study done in Dansha-Tigray (Ahmed, 2018).

In case of the current both retrospective and prospective study, males show higher infection with malaria than females. This finding is consistent to the study done in Arsi Negelle health center of Southern Ethiopia (Hailemariam *et al.*, 2015) and in Dansha (Ahmed, 2018). So, it might be due the fact that in the present study area males were often engaged in early night outdoor agricultural activities. Hence, they have higher chance of exposure to be infected by the anopheles malaria vector (Brooker *et al.*, 2004).

In case of age group, it was reported that less than five years aged children have less malaria infection than the other higher groups (Amir *et al.*, 2017). In line to this the 93.5% malaria prevalence of the current study was seen in age group of greater than 5 years old. This finding is consistent to the reported done in Mekelle town (Tigabu, 2014). This might be due to the partially acquired immunity developed during childhood in such high malaria transmission area might have a protective role in this age group. In high malaria, transmission settings, partial immunity to the

disease can be acquired during childhood. In such areas, the majority of malarial disease, and particularly severe disease with rapid progression to death, occurs in young children without acquired immunity (WHO, 2015).

In spite of this, the retrospective study showed the infection of malaria was specified only for the two-plasmodium species. In comparison of the two species, *P. falciparum* was more prevalent. This difference might be due to differences in topographic adaptations and intrinsic factors of the two parasites (Amir *et al.*, 2017) and inconsistency of malaria preventing methods in the areas and increasing the drug resistance behavior of *P. falciparum* might be a cause of difference (Kebede *et al.*, 2018). This finding differs from the study done in Dansha which *P. vivax* was significantly higher prevalent as compared to *P. falciparum* (Ahmed Abafita, 2018).

Presence of mosquito spreading site near home site is a significant factor for malaria transmission (Couch *et al.*, 2013). In line with this, most of the participants responded that there is a mosquito spreading site near their home site and was the significant factor for malaria infection. This finding is consistent with the study done in Shire town which reported that a mosquito spreading site near home site is a significant factor for malaria transmission in addition to housing conditions, such as, tethering livestock inside house, presence of window, open eaves, absence of separate kitchen and presence of a single sleeping room (Abebe, 2013). This might be due to less knowledge about the breeding sites of mosquitoes and methods of mosquito preventive mechanism (Guzman, 2013).

In case of the five-year trend of malaria, the overall malaria infection was being reported as 2892 (21.14%). This finding is significantly lower than the five-year trend analysis of malaria prevalence in Guba district, Benishangul-Gumuz regional state done (Shemsia *et al.*, 2020). Raya Azebo district (Kebede *et al.*, 2018) which 8658 (51.04%) and 23,018 (76.9%) were confirmed positive malaria cases respectively. However, markedly higher overall malaria positivity rates were detected in the current study compared to other previous retrospective studies done in Abeshge, south-central Ethiopia which was reported as 21.8% prevalence rate (Yimer *et al.*, 2015). The observed variations may be due to differences in microclimate, altitude, expansion of development projects like dams or irrigation, community awareness about malaria transmission and control, and malaria intervention practices (Shemsia *et al.*, 2020). Besides, the difference among the findings might be due to environmental variations and type of study populations, differences in the scope of these studies, variations in the methods of malaria diagnosis, differences in the skills and overall competence of the microscopists, differences in the coverage

and utilization of intervention tools, community awareness (KAP), presence of co-infection (Ouma *et al.*,2017).

Comparison of all the five years data indicated that malaria prevalence fluctuated significantly annually, with the minimum and maximum number of cases recorded in 2016 and 2017, respectively in the study area. The maximum number of malaria cases recorded in 2017 may be due to community misconception surrounding malaria prevention (Ahmed 2018). Besides, the reduction of malaria cases in 2016 might be attributed to the combined effort of the society and other stakeholders. In 2017, the district health office yearly plan to distribute ITNs to all people who were at risk was successful. There was also a campaign to create awareness and environmental protection. In addition, due to the devastating problem of malaria before 2017, all stakeholders exerted maximal effort to control the disease. Government and non-government organizations participated in human capacity development.

In case of ITNs, about 48,227 ITNs were distributed beginning from 7640 in 2015 up to 11341 in 2019 that indicated an increase of awareness of ITNs distribution. The distribution of ITNs in 2017 was the highest but also more malaria patients were recorded. This indicated that rainfall (collected water) is suitable for mosquito breeding. Besides, improper using of bed nets might be the factor in the study area.

In general, the highest cases of malaria were observed during autumn (September, October and November). The seasonality observed in the current study was in agreement with studies in Raya azebo (Kebede *et al.*, 2018). This might be due the fact that variability of rainfall and temperature in each season affects the availability of breeding habitats for mosquito vectors, the length of mosquito larvae development, and the rate of growth of the malaria parasites inside the vector (Kebede *et al.*, 2018).

6 CONCLUSION AND RECOMMENDATIONS

6.1 conclusions

This finding shows that malaria is still a health problem in the study area with *P. falciparum* and *P. vivax* predominance. The prevalence of malaria in the study area was due to having indoor grass and plants, having of stagnant water near house and irregularity use bed nets in sleep time of the population. In this study area, out of all the age groups, populations ≥ 15 years were much more affected by malaria when compared to the other age groups (0-4 and 5-14 years old). And from these age groups male were particularly more seriously affected than females. This indicated that, the productive age groups are (≥ 15 years) particularly males are more exposable to malaria because their work is outside the residence area but females are mostly limited to the resident area.

Throughout the five years study period (2015 to 2019), the highest prevalence of malaria were observed in 2017. This indicated that the highest mean annual rainfall cause *Anopheles mosquito* to have an opportunity to lay eggs and multiply soon and then the prevalence of malaria increases. Although malaria infections were observed in almost every season throughout the five years. It predominantly during the rainy season from September-November and after the rainy season from December-February this showed that collected water and wet condition is suitable for mosquito breeding. Generally, in all the five years the *P. falciparum* (70.16%) displayed higher prevalence from 2015 to 2019 than *P. vivax* (29.84%) indicating that the study area is better suitable for *P. falciparum* being the study area is ≥ 2000 metres.

6.2. Recommendations

Based on the findings, the following points were forwarded to reduce the high prevalence rate of malaria infections in population of the study area

- Awareness on environmental hygiene and means of malaria prevention mechanisms should be given by health professionals to population of the study area.
- The woreda health bureau should regularly assessed and manage the utility of bed net usage on the community
- There should be provision of continuous diagnosis and mass-treatment with appropriate malaria drugs done by the woreda health bureau
- Over all, Enticho community needs to educate continuously before the occurrence of malaria parasite seasons.

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Appendix

Questionnaire to be answered by study individuals

Dear participants, the information that I ask you is crucial to assess the prevalence of malaria infection and its association with its risk factors then to put remedial solution accordingly. So, I kindly request you to tell the real information.

Thank you!!

Personal information

- 1) Age: -----
- 2) Sex : -----
- 3) Marital status : -----
- 4) Number of family size: -----
- 5) Educational status: -----
- 6) Occupation: -----
- 7) Monthly income In Birr.....

- 1) Duration of stay in years in the area
A) ≤ 3 B) 4-6 C) 7-10 D) ≥ 11 years

- 1) Do you use screens on doors and windows?
A) Yes B) No

- 2) Are posters or papers posted on the wall of your bed room?
A) Yes B) No

- 3) Have you caught by malaria before?
A) Yes B) No

- 4) Is there any indoor grass and plants of your home?

A) Yes B) No

6. Is there any stagnant water near of your home?

A) Yes B) No

7. Do you switching on light in sleeping time

A) Yes B) No

8. Did you use insecticide treated bed net in night sleeping?

A) Yes B) No

12. If your answer for question number-8 is yes, how much its frequency

A) Always B) same times C) rarely

13. do hou have knowledg on the transmision and prevention mechanisms of malaraiia

A) Yes B) No

Appendix-2: Tigrigna questionnaire and interview

ዩኒቨርሲቲ ኣዲስ ኣባባ

ቀመርተፈጥሮ ሳይንስ ኮሌጅ

ክፍሊ ትምህርቲ ስነ ሂደት

መጠይቅ ሑቶታት

ዝተከበርኩም ተሳተፍቲ፡

ናይዚ መጠይቕ ቀንዲ ዕለማ ኣብ እንቲ ጮሆ ሰጥቲ ታልዝርገሐሕማ ምዓሶን ተዛማዲ መንቀሊ ኣምን እንታይ ከምዝመስልብ ምፍታሽ መፍትሒ ምቅማጥ እዩ። ስለዚ ንሕድሕድ መጠይቕ ብፅሕን ብኣገባቡን ምምላእ ኹም ኣቐዲመዮ መስግን።

ኮድ: ____ ምታ: ____ ዕድመ: ____ ኩነታት ሓዳር: ____ በዝሒ ስድራ: ____ ኩነታት ት/ቲ: ____ ስራሕ: ____

ታ.ቁ	ሑቶታት	መማረፂ	መልሲ መውሃቢ
1	ኣብዚ ከባቢ ንክንደይ ዓመት ተቀሚጥካ/ኪ?	U. ≤3	
		ለ. 4-6	
		ሐ. 7-10	
		መ. ≥11	
2	ሞሰኮት ኩም/ማዕዳኩም ብቢት ሮዝ ተገጠመድዩ?	U. እወ	
		ለ. ኣይፋሉን	
3	ኣብ ግድግዳ ስድቀት ኹም ዝተለጠፉ ወረቀት/ፖስት ራት ኣለዉዶ?	U. እወ	
		ለ. ኣይፋሉን	
4	ቅድመ ሒዚ ብዓሶተ ሚም ኩም ደትፈልጡ?	U. እወ	
		ለ. ኣይፋሉን	
5	ኣብ ውሽጢ መረባኩም ዝበቆሉ ሰዓባት/ተክልታት ኣለዉዶ?	U. እወ	
		ለ. ኣይፋሉን	
6	ኣብ ከባቡም/መንደር ኩም ዝዓቆሩ ጀቅ ጀቅ ቦታታት ኣለዉዶ?	U. እወ	
		ለ. ኣይፋሉን	
7	ኣብ ናይ ለይቲ ድቃሰኩም መብራሀቲ ወሊዕኩም ደትድቕሱ?	U. እወ	
		ለ. ኣይፋሉን	
8	ኣብ ናይ ለይቲ ድቃሰኩም ዛንኬራት ጥቀሙዶ?	U. እወ	
		ለ. ኣይፋሉን	
9	ንቁፅሪ -8- መልስ ኹም እወ እንተ ከይኣኩነታት ምድግ ጋሙ ከመይ እዩ?	U. ኩሉ ግዜ	
		ሐ. ተድሓደግዜ	
10	ኣብ ኣገባብ ምትሕልላ ፍንም ክልኻል ንዓሶ እኩል ኣፈልጦ ኣለኩምዶ?	U. እወ	
		ለ. ኣይፋሉን	