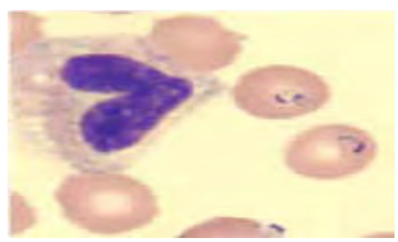
ADDIS ABABA UNIVERSITY COLLEGE OF NATURAL AND

COMPETATIONAL SCIENCE DEPARTMENT

OF ZOOLOGY.

THE RELATION OF MALARIA INCIDENCE AND CLIMATE OUT OF POPULATION IN AMURU WOREDA , HORO GUDURU WOLLEGA ZONE, WEST ETHIOPIA

FROM 2007-2016 YEAR.



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Acronyms and abbreviations

ACTs	Artemsinin Based combination therapy.
FDREMOH	Federal Democratic Republic Of Ethiopia Ministry Of Health
ELISA	Enzyme linked immunosorbent
FMOH	Federal ministry of health.
IFA	Immunofluorescences.
IRS	Indoor-residuals spraying.
LLINS	Long lasting insecticidal nets.
MIS	Malaria Indicator Survey.
IPT	Intermittent preventive therapy.
MDG	Millennium development goal.
MOP	Malaria Operation Plan.
NSP	National strategic plans.
PCR	Polymerase Chain Reaction.
PGLUDH	Plasmodium glutamate dehydrogenase.
PLDH	Plasmodium *L*actate Dehydrogenase.
RBC	Red Blood Cell.
RBMP	Roll Back Malaria partnership.
RDT	Rapid diagnostic tests.
SPSS	Statically package for social science.
SSA	Sub-Saharan Africa
UNICEF	Unit nation international children serving fund
USA	Unit state of America.
WHO	World Health Organization.

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Abstract

Malaria is one of the most important diseases in the world that is caused by a protozoan parasite of genus Plasmodium. It mostly affects (90%), Sub-Saharan African countries including Ethiopia. It was estimated that about 75% of the land and 68% of the population is exposed to malaria in Ethiopia. Although the severity of the problem at the regional and national level in the country is well known, there is limited information about the disease in Amuru district. To that end, a retrospective study was undertaken in the area based on ten years clinical and meteorological secondary data collected from three Amuru Health Centers and Shambu Metrological Station from 2007-2016. Accordingly, a total of 49,871 blood film samples were examined of which 11,335 (22.7%) of the samples were microscopically confirmed malaria case; and males were more infected than females with a ratio of 1.5:1(statically significantly(x^2=46.335, df =9, p < 0.0001associated with sex. Malaria was reported in all age groups but more than 60% of the infection occurred in the active age group 0f 16 - 45 years which was significantly associated (X^2= 12.255, P = 0.031, df = 5) with age group. The data also showed that the disease was caused mostly by Plasmodium falciparum (67.6%), Plasmodium vivax (24.8%) and mixed infection (7.6%). Despite the apparent fluctuation of malaria trend in the study area, the highest peak of malaria cases (34.9%) was recorded during September-November, followed by 32% in the trimester of June-August indicating that months at the beginning and immediately after rainy seasons increased the vulnerability to malaria infection. There was a steady reduction of malaria by 11% from 2007 to 2009 and increased by the same margin in 2010, remained stable up to 2012. However, it was reduced almost by half in 2013, and further decreased to 14.2% by 2016. All taken together, despite the fluctuations over the years, the prevalence of malaria decreased over 50% in 2016 compared to 2007. Although metrological factors such as relative humidity statically significantly correlated (r = 0.657, p = 0.020) with annual prevalence of malaria in study area, the most significant contribution over the years was national malaria control programs at the national and regional level through the Roll Back Malaria (RBM) partnership and three other consecutive strategic plans that increased access to availabilities of strong preventive and controlling methods that included distribution of long lasting insecticide-treated mosquito nets (LLITNs) and indoor residual spraying (IRS).

Key words: Diagnosis, Malaria, Morbidity, Mortality, *P falciparum,* Roll back malaria

1. Introduction

Malaria is an infectious disease caused by the protozoan parasite known as *plasmodium* and transmitted by *Anopheles* mosquitoes. It is still one of the major public health and medical concerns in many parts of the world, particularly in many tropical and sub- tropical countries in Africa, South East Asia, Indian subcontinent, the Middle East, Oceania and Latin America (WHO,2012) .

The most important *plasmodium* species are: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae*. In addition, there have been some reports of humans naturally infected with the simian parasites *Plasmodium simiovale* and *Plasmodium knowlesi* (WHO, 2013).

Malaria is one of the most severe public health problems worldwide with 300 to 500 million cases and about one million deaths reported to date, 90% of which were reported from sub Saharan African countries. It is the fourth leading cause of death of children under the age of five years in developing countries (Legesse *et al.*,2007).

A recent estimate in 2010 showed that 4 billion people were at risk to malaria infection in the different parts of the world by the two main causative agents namely; *Plasmodium falciparum* and *Plasmodium vivax* (Gething *et al.*, 2011).The highest level of *Plasmodium falciparum* transmission occurred in Africa, which contributed to 99% of the global and 95% of the African *falciparum* malaria cases.

According to the latest estimates from WHO, there were 214 million (range 149–303 million), new cases of malaria, and 438 000 malaria deaths (range 236 000–635 000) worldwide. Most of these deaths occurred in the African Region (90%), followed by the South-East Asia Region (7%) and the Eastern Mediterranean Region (2%) (WHO, 2016).

Malaria is an entirely preventable and treatable disease, provided the currently recommended interventions are properly implemented. To this end several strategic plan have been initiated to eradicate and control malaria. The global malaria eradication program during 1950s and 1960s suffered serious setbacks and the disease increased slowly in areas where it had been reduced to low level (WHO, 2011). In an effort to combat the growing threat of malaria, the Roll Back Malaria (RBM) partnership was launched in 1998 for the developing countries, by the UN with the goal to reduce the burden of malaria by half up to 2010 and halving again by 2015 (Sena, 2015).

In 2011, the world health Assembly, Roll Back malaria partnership, set the objectives: to reduce malaria case by 75% and malaria deaths to near zero from 200 levels by 2015. The objectives were linked to targets of achieving universal access to case management and universal access to and utilization of malaria prevention measure (WHO, 2012). Progress reports there after showed globally, malaria case decreased from227 million in 2000 to 198 million in 2013 (WHO, 2014). The incidence was projected to fall by 35% globally and by 40% in the African region by2015. Likewise, the proportion of population protected by at least one vector control method in sub-Saharan Africa, has increased in recent years; and reached 48% in 2013, and the number scaled up in 2014. Consequently, malaria incidence fell by 21% and mortality by 31% in Africa region between2010-2015 (WHO, 2016).

In Ethiopia, malaria is one of the leading causes of morbidity and mortality. It is estimated that about 75% of the total area of the country and 68% of the population (54million) were exposed to malarial infection (MOH 2007). Accordingly, malaria becomes the number one health problem in the country with average of 5 million cases per year and contributing to 70,000 deaths each year and 17% of the outpatient visits. The important infectious agents of malaria in the country are; *Plasmodium falciparum* and *Plasmodium vivax, that* account for 60% and 40% of malaria cases, respectively (WHO, 2013).

Malaria infection is incriminated with climate, and low altitude that determine the survival of the mosquito vector. Apart from that low level of education and poverty, and lack of effective anti-malarial drugs and long lasting insecticide treated bed nets aggravate the chance of the spread of the disease (Tilahun *et al*., 2009).

Malaria is seasonal in most parts of Ethiopia and its transmission picks biannually from September to December and June – august(major) from April to May (minor), coinciding with the major harvesting seasons. Due to the difference in altitude and rainfall, the country has a varied pattern of malaria transmission , with transmission season ranging from less than 3 months to more than 6 months (MOH, 2010).The prevalence of the disease is variable influenced by the large diversity in altitude and rainfall with a long time varying from a few weeks before the beginning of the rain season to more than a month after the end of the rainy seasons (Aynalem, 2008).

Malaria control programs have been introduced for many years now to control and prevent malaria in the country. Quite recently, prevention and control activities of malaria in Ethiopia have been initiated according to national strategic plan in line with the WHO recommendations. The national strategic plan for malaria control and prevention in Ethiopia, NSP (2011 -2015), aimed at strengthening and scale-up of malaria control interventions though prompt and effective diagnosis and treatment, case management though roll out of the highly efficacious anti-malarial drugs (MOH, 2010).

The strategic plan has projected goals to achieve malaria elimination in areas with historically low malaria transmission and near zero malaria deaths in all the remaining parts of the country by2015 by improving diagnosis of malaria cases using microscopy or using multi species rapid diagnostic test (RDT) and providing prompt and effect malaria case management at all health facilities (MOH, 2010).

The recent WHO report showed a success story of 50–75% decline in incidence and mortality rates of malaria between 2000 and 2013 (WHO, 2016). Accordingly, between 2010 and 2015, malaria incidence and mortality rates, particularly due to *Plasmodium falciparum*, have declined by more than 50% in Ethiopia (WHO, 2016). However, Ethiopia still accounts for 6% of malaria cases and about 12% of the global cases and deaths due to malaria.

Malaria remains a major public health problem in Oromiya regional State. Several researches indicated that it was one of the malaria prone regions in the country where 75% of the administrated weredas, and 64% of the kebeles are malareous (MOH, 2010). Accordingly, 17 million people were at risk with annual clinical cases of 1.5-2 million people in the Region. This accounts for 20-35% of outpatient visits, 16% of hospital admissions, and 18-30% of annual death.

Amuru Woreda is one the largest woreda in Horo Gudru Wollega Zone in the oromia regional State where the population is vulnerable to malaria. This is due to favorable environmental conditions such as low altitude, hot weather, and high humidity that are conducive for both vector and parasite development (Amuru Wereda Health Bureau, 2015).

This study was intended to estimate prevalence of malaria over ten years period (2007-2016) recorded from three Amuru Woreda Health Centers. The study will provide base line information on the status of malaria and the impact of the hitherto different national and regional control programs on control of malaria in the area for the last ten years.

1.1. Research Questions

The following research questions were formulated to conduct research on the prevalence of malaria in the study area.

- What was the prevalence of malaria in the Amuru Wereda for last 10 years (2007-2016)?
- Is there any variation in prevalence of malaria between sexes and ages group in the study area?
- Which *plasmodium* species were dominant in the study area for the last ten years?
- What are metrological factors influencing prevalence of malaria in Amuru Woreda?

1.2. General objectives.

To assess the prevalence of malaria infection among patients attending three health centers (Agamsa, Obora and Samo) in Amuru woreda from 2007-2016.

1.2.1. Specific objectives

The specific objectives of this study were;

- To determine the prevalence of malaria with regard to sex and age from 2007-2016 in study area.
- To find out recorded predominant species of *Plasmodium* from 2007-2016 in the study area.
- To identify prevalence of malaria with respect to demography and climatic factors and associated risk factors in the study area.

1.2. Limitation of the study

It is based on secondary data that could be prone to routine diagnostic errors and lack of serious considerations compared to duty-bound primary data collection. Sufficient and complete recorded data may not be available at health centers that ensures the data quality of the research.

1.3. Expected Outcomes

The study was designed to indicate the prevalence of malaria in relation to predominant *plasmodium* species, age and sex as well as demographic and metrological factors in study area. The research was expected to give a base line data for future research and intervention. It also gives a clue whether the hitherto national strategic plan for malaria control was successfully implemented in the area.

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2. Literature review

2.1. Epidemiology of malaria

2.1.1 *Plasmodium* parasite

Malaria is one of the most widely distributed parasitic disease in the tropics. However, on the globe it extends up to 60^0 north and 40^0 south of latitudes. Its distribution in the world is not uniform (Tayler *etal*., 1997). It is caused by the protozoan *Plasmodium* and transmitted to humans through bites from female Anopheles mosquitoes (Silver, 2008; Sathe and Tingare, 2010; Choumet, 2012). In order to produce eggs the female mosquito are in need of blood meals and it is therefore only the female mosquito that bites (Igweh, 2012). The protozoan Plasmodium has a development cycle that involves both the *Anopheles* mosquito and human host (Ovajde and Nriagu, 2011).

The major *plasmodium* species are *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae*, and occasionally humans can be affected by *Plasmodium knowlesi*, which commonly affect the wild animals such as monkey, so it is a zoonotic disease (WHO, 2012). Different species of *plasmodium* are found in different countries.

Plasmodium vivax is commonly found in Asia and South America. *Plasmodium vivax* is widely distributed because it can develop in most mosquitoes at lower temperature, and is the cause of the most prevalent form of malaria. Sometime referred to as **benign malaria**, the cycle of paroxysm occur every 3days, and the patients generally survive even without treatment (Tortora, 2010). About 43% of malaria in the world is caused by *Plasmodium vivax* and the **merozoites** invade only young erythrocytes, the reticulocytes are unable to mature in red blood cells. Merozoites can only penetrate erythrocyte with mediated receptors known as Duffy blood groups, two co dominant alleles FY^a and FY^b (Tortora, 2010).

Plasmodium falciparum is predominantly found in the warm and moist parts of Africa. It is the most deadly species found though out Africa with unique crescent shaped gametocyte. The most dangerous malaria is the one caused by *Plasmodium falciparum* and is referred as **malignant** or **sub tertian** malaria (Tortora, 2010). It accounts for about 50% of all malaria cases and true relapses does not occur. However, recrudescence of the disease may follow remissions of up to a year. The **Merozoite** of *Plasmodium falciparum* can invade erythrocytes of any age, more red blood cell are infected than other forms of malaria, and accounts to highest mortality rates in young children (Tortora, 2010).

Plasmodium malaria generally develops into mild clinical cases with lower prevalence than *Plasmodium falciparum,* and *Plasmodium vivax* found in South America, Asia and Africa. Also relatively causes **quartan** malaria, with paroxysms every 72hrs .The ring forms are less amoeboid than those of *Plasmodium vivax*, and the cytoplasm is somewhat thicker. *Plasmodium malariae* is only species of human malaria that is regularly found in wild animals, and can only invade **aging erythrocytes**.

Plasmodium ovale is the second most common species in Africa (after *Plasmodium falciparum*).It is biologically and morphologically similar to *Plasmodium vivax*. This species cause ovale or mild infection and is rarest of four malaria parasites of humans. *Plasmodium ovale* is difficult to diagnose because of its similarity to *Plasmodium vivax* and eight merozoites are usually formed, but there is range of4 to16.

The variation of malaria epidemiology is not limited by continents or between countries. There is also variation in the distribution of *plasmodium* in the single country. The epidemiology malaria in given country is determined by different factors(Brain and MeGregor,1989). These are conducive environmental for the transmission, the presence of suitable anopheles mosquitoes, the presence of *plasmodium*, and the presence of reservoir of the parasite. Also some time when the parasite it taken by travelers and immigrants while they are traveling from endemic areas.

2.1.2 Vectors

Vectors of malaria: *Anopheles* mosquito

Malaria is transmitted by the bite of female mosquitoes of the genus *Anopheles*. Globally, there are over 537 species of anopheles , most (78%) of which have been formally named (HerbachRE,2013) .Among these 70 species can transmit human malaria parasites, and 41 are dominated vectors globally. In Africa, there are over 140 anopheles species ,of which at least eight are effectively vectors of malaria (Choumet, 2012).

Malaria is mostly a disease of hot climate. The *Anopheles* mosquito, which transmits the malaria parasite from one human being to another, thrives in warm, humid climates where pools of water provide perfect breeding grounds. It proliferates in conditions where awareness is low and where health care systems are weak (UNICEF, 2000).

Figure 1. Vector - *Anopheles* mosquito **(**WHO, 2011 **)**

The three most dominant vectors in the continent are *Anopheles Gambiae* and *Anopheles arebiensis* (in the *Anopheles gambiae* complex) and *Anopheles funestus. Anopheles gambiae* complex contains the most wide spread species in the sub Saharan Africa (Harbach, 2013).

The malaria vector requires water to complete its life cycle: egg, larva, pupa, and the adult. ―While between 200-1000 eggs can be laid, the quantity is influenced by the amount of blood taken in". Blood-feeding usually starts at dusk and continues until dawn (Aynalem, 2008).

2.2. Malaria in Africa.

Malaria is one of the most severe public health problems worldwide with 300 to 500 million cases and about one million deaths reported to date, 90% of which were reported from sub Saharan African countries. It is the fourth leading cause of death of children under the age of five years in developing countries (Legesse *et al.,*2007).

It is one of the major disease of poor people in developing countries and the leading cause of available death , especially in children and pregnant women. Sub Saharan Africa carries the bulk of the global malaria burden, with 71% of causes and 86% of global deaths. A person in Africa dies of malaria every10 seconds (Tortora, 2010). African regions continue to shoulder the heaviest malaria burden (WHO, 2015). Women and young children are most at risk affects five times as many people as AIDS, leprosy, measles and tuberculosis combined. Currently 30 million African women are pregnant yearly malaria is more frequent and complicated during pregnancy. Malaria is one major tropical disease adversely affecting the health of people and economic development of many developing countries, particularly is sub-Saharan Africa (Legesse *et al*.2007).

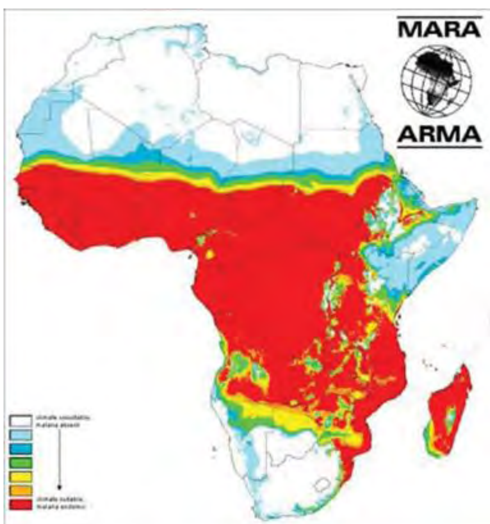


Figure 2. The distribution of malaria in Africa (WHO, 2013)

Malaria accounts for up to 60% of all health facility visits in the eastern African region. However, due to poor health care coverage and other factors, much of malaria related illness and death actually occurs in the home, therefore, going unreported. The disease epidemics affect non-immune populations in many high land and semiarid areas of the continent . It frequently occurrence of drug resistance parasites and insecticide resistant vectors, change in the re sting behavior of mosquitoes(from in door to outdoor as a result of frequent indoor insecticide sprays, lack of infrastructure, shortage of trained manpower, lack of appropriate management and inability to integrate several methods of control).(Howard *et al*.,2007).

The World Health Organization and the World Bank rank malaria as the largest single component of the disease burden in Africa, causing an annual loss of 35 million future life-years from disability and premature mortality. In Africa, malaria is responsible for about 20-30% of hospital admissions and about 30-50% of outpatient consultations (WHO, 2012).

2.3. Climate determinants of *Plasmodium falciparum* Transmission in Africa.

The most significant determinant of the intensity of the parasite transmission is climate. The development of both the vectors and the parasite is temperature dependent. The optimum temperature range for parasite development in the female Anopheles mosquito (sporogony) is between 25^{O}cand 30^{o}C, and development cease below 16^{o}C. Intermittent low temperatures delay sporogony, and the period immediately after the infective bite by the mosquito on an infected human host is the most sensitive to drops in temperature. Above 35^{o} C sporogony slows down considerably. Extremely high temperatures are associated with the development of smaller and less fecund adult mosquitoes .Thermal death of mosquito occurs at 40^{o}C to 42^{O}C. Altitude and temperature are strongly correlated: with every 100 meter increase in altitude, the temperature drops by 0.5^{0} C(Reiter, 2001).

Over all, the use of altitude as a marker of endemicity or disease risk is vague. Yet there is a tendency within the literature to refer to high land malaria in East Africa and Horn of Africa. The highest proportion of vectors surviving the incubation period is observed at temperatures between 28 ^{0}c -32 ^{0}c (Craig *et al,* 1999 and Jonathan *et al,* 2006). Numerous studies have demonstrated the association between *Anopheles gambiaes* (the most important vector of *Plasmodium falciparum* in Africa) abundance and rainfall. Without the surface of water the female Anopheles mosquito cannot lay eggs. Rainfall is also related in to humidity and saturation deficit, both affecting mosquito survival. Therefore, rainfall provides breeding sites for mosquitoes to lay their eggs, and ensures a suitable relative humidity of at least 50 to 60% to prolong mosquito survival (Reiter, 2001).

In Ethiopia, epidemiological pattern of malaria transmission is generally unstable and seasonal, the level of transmission varying from place to place because of differences in altitude and rainfall patterns (MOH, 2010). Changes have been observed in the epidemiology of malaria through time. Previously, malaria was known to occur in areas below 2000 m but currently it has been documented to occur even in areas above 2400 m, such as Addis Ababa and Akaki (MOH, 2010).

In addition to climate local ecology and active control affect the ability of malaria parasites and their anopheles mosquito vectors to coexist long enough to enable transmission. The frequency of transmission or endemicity depends on density and infectivity of anopheles vectors. These features depend on range of climate, physical and population characteristics. For example rain fall, location of human settlement near or at the river or other mosquito breeding sites, and the density of human populations in the village (Reiter, 2001).

Changes in the environment of the mosquito habitat, such as those taking place in Ethiopia, whether natural or man-made, ―…. rearranges the ecological landscape in which these vectors breed". Every Anopheles spp. occupies a specific ―…ecological niche that is genetically determined". Changes in temperature, humidity, altitude, population density of humans, and deforestation are just a few ecological factors that play essential roles in the transmission of malaria (Aynalem, 2008). Species of anopheles mosquitoes with a strong antropophilic behavior, i.e. preferring blood meal from human, are the most potent vectors and therefore associated with stable transmission of the disease. However, most species of Anopheles do not feed exclusively on either humans or animals (Choumet, 2012). Studies have therefore suggested that keeping cattle in the vicinity of human dwellings may have a distractive effect on the feeding behavior of Anopheles mosquitos. This is especially relevant for *Anapholes arabiensis,* which have been showing a high tendency of resting and feeding outside (Hadis *et al*., 1997).

2.4. Malaria in Ethiopia.

2.4.1 Trends of malaria prevalence in Ethiopia.

Malaria is the number of one of public health problem in which and accounts for the cause of illness and hospitalization (MOH,2010). 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 (Deresa *et al*.,2004) .

Malaria is a major concern in the country since it is one of the leading causes of morbidity and mortality. In Ethiopia, more than 75% of the landmass (altitude <2000m) of the country is malarious, and about 68% (greater than 54 million people) of the total population residing in areas of risk of malaria infections (Berhane Hailseasssie and Ahmed Ali, 2008). In 2002/03 the disease has been reported as the first cause of morbidity and mortality accounting for 15.5% outpatient consultations, 20.4% admission and 27% deaths (FMOH,2007).

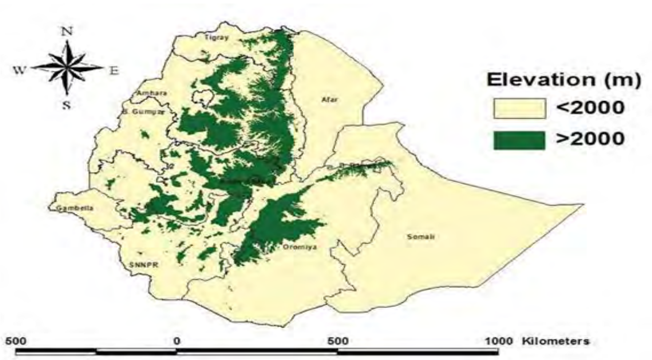


Figure 3. Malaria Distribution in Ethiopia (MOH, 2010).

The epidemiology of malaria may vary considerably with in relatively small geographical areas.The transmission of malaria in Ethiopia is generally unstable and seasonal. Frequently malaria epidemics in high land areas of Ethiopia where previously had not known have been currently occurring (MOH, 2010). *Plasmodium falciparum* is the dominate species during peak malaria transmission seasonal while *plasmodium vivax* tends to dominate during the dry season in Ethiopia (FMOH, 2007). The distribution and transmission of malaria in Ethiopia varies from place to place. Ethiopia can, in regard to malaria, be divided into four epidemiological strata. The first stratum is the highland areas above 2500 meter which are considered to be free of malaria. Highland fringe areas between 1500-2500 meters constitute the second strata and are frequently affected by the disease. The third and the fourth strata consists of lowland areas below 1500 meters where the malaria transmission is either seasonal or occurs all year round (WHO, 2014).

Altitude affects the pattern of malaria distribution in Ethiopia through its effect on temperature (Betemariam *et al.*, 2002). The central high lands of Ethiopia are free of malaria due to low temperature, which slows the development of the vector parasite (MOH, 2010). Transmission is limited by the lack of water collections for mosquitoes breeding an low humidity due to low rainfall and sparse vegetation. Mean annual precipitation, in general, ranges from 800 to 2,200mm in the highlands (>1,500m) to less than 200 to 800mm in the lowlands (<1,500m). Rainfall decreases northwards and eastwards from the high rainfall pocket area in the southwest, and seasonality is not uniform (Adhanom, 2010).

Risk of malaria is highest in the western lowlands of Oromia, Amhara ,Tigray and almost the entire regions of Gambella and Benishangul Gumuz regions. The midlands of Ethiopia between 1000 and 2200 meters attitude experiences seasonal transmission of malaria with sporadic epidemics for a very few years. In the eastern lowlands of Ethiopia (Primarily Afar and Somalia), malaria is endemic only along rivers, as this part of the country is largely dry away from rivers.

Malaria transmission intensity, along with its temporal and spatial distribution in Ethiopia, is mainly determined by the diverse eco-climatic conditions. Climatic factors including rainfall, temperature and humidity show high variability. Temperature, and to a lesser extent rainfall and humidity, varies as a function of altitude. In general terms, 75% of the landmass of Ethiopia is considered at risk of malaria, which corresponds to areas below 2,000m altitude. However, this estimate has not recently been revised to account for possible changes such as urbanization or land use of irrigation or dams (Adhanom, 2010).

As it is stated above the spatial variation of malaria in the country is largely governed by the topography of the country which affects the climate of the particular area (Tulu, 1993, FMOH,1997). In the cold zone (Dega), since the temperature does not support the breeding of anopheles mosquito and the development of the malaria parasite in the anopheles, there is no malaria at all.

In the Weyena dega zone, where the major portion of the country lies, due to Temperature variation malaria occurred most often in areas below 2000m above sea level. However, ion isolated pockets a few indigenous malaria cases have been seen during extensive epidemics up to 2500m altitude. This is due to the climate and local physical characteristics' that are conducive to breeding and survival of the vector. Generally speaking when malaria occurs in this zone it leads to high morbidity and mortality due to the absence of immunity in the high land population. The risk of epidemics in high and all age groups are equally vulnerable to infection (MOH,2007).

In the Kolla zone malaria is moderately to highly endemics. High malarious areas of Metena, Metekel, SettitHumera and Gambella are found in the western lowlands of Ethiopia due to low rainfall, high evapo-transpiration and absence of standing water bodies some of the warm zone of the country are characterized by short lived transmission (MOH,2007)

.2.4.2.The Role of Human Factors in the Spread of Malaria in Ethiopia.

Human factors in Ethiopia contributing to the spread of malaria include population growth and movements, urbanization, water development schemes, agricultural development, conflicts, and improper use of drugs and the attendant consequences of the emerging drug-resistant malaria parasites (Aynalem, 2008).

Ecological disturbances due to human actions such as deforestation and establishment of new settlements in previously unsettled areas ―…allow for the proliferation of mosquitoes that prefer human habitation to natural settings" as does the construction of dams (Aynalem, 2008). The burden of malaria has been increasing due to a combination of large population movements, increasing large- scale epidemics, mixed infections of *Plasmodium vivax and Plasmodium falciparum* , increasing parasite resistance to malaria drugs, vector resistance to insecticides, low coverage of malaria prevention services, and general poverty (Aynalem ,2008).

Also construction of water control projects can also lead to shifts in vector mosquito populations. Reservoirs, irrigation canals, and dams are closely associated with the increase of a variety of parasitic diseases that are water dependent. For most countries, dams are a crucial part of economic and social development, and represent a double-edged sword. The potential for dams to alleviate poverty significantly contributes to the enhancement of human health, and simultaneously increases the likelihood of human infection due to schistosomiasis, malaria, dysentery, and river blindness. During the construction of dams and canals, excavation pits provide temporary breeding sites for mosquitoes (Aynalem, 2008).

A study in Tigray investigating the possible impacts of small dams on malaria transmission found an unmistakable link. The rate of infection among children near dams was seven times greater than in communities with no dams. The study, thus, concluded that —…micro dams close to villages have the potential to increase the incidence of malaria substantially among children living nearby (Aynalem, 2008).

2.4.3 Vector species and their distribution in Ethiopia.

In Ethiopia, the identification of the anopheles species and their distribution was carried out for the first time in a few accessible areas by the Italian and British malariogist in the 1930s and 1940s. According to GebreMariam *et al.* (1988), there are 42 species of anopheles, however, small proportion of them are important as a vector (FMOH, 1997). These are *Anopheles arabiensis, Anopheles funustus, Anopheles pharaonsis and Anopheles nili*. In addition to this, *Anopheles coastaini, Anopheles pauraludis, Anopheles zeinmanni,* and *Anopheles thalis* that have been recorded to possess vector capacity (Gabre-Mariam *et al*.,1988; Tulu, 1993;MOH, 2007).

In Ethiopia *Anopheles arabiensis* is known to play a crucial role in malaria transmission in the country. Others such as *Anopheles funestus* and *Anopheles pharaones is* playing secondary role while *Anopheles nile* involves transmission in localized areas (Abose *et al*., 1997). *Anopheles gambiae* and *Anopheles arabiensis*, the main vectors for malaria transmission in SSA including Ethiopia, are also the most important *anopheles* species to maintain urban malaria transmission (Robert *et al*., 2003; Keizer *et al*., 2004).

2.5. Distribution

Malaria is one of most devastating disease that is transmitted in more than 97 countries in the world and territories with ongoing on malaria transmission, and seven countries in the prevention of reintroduction phase, making total of 104 countries and territories in which malaria is presently considered endemic (WHO, 2013). Globally, an estimated 3.4 billion people are at risk of malaria. The disease remains one of the most important causes of human mortality and morbidity with enormous medical, economical and emotional impact in the world (WHO, 2011), with 2-3 million deaths occurred each year (Snow *et al*., 2005). It was estimated that 207 million cases of malaria occurred globally in 2012 (WHO, 2013). It is leading cause of death in the children under the age 5 years and pregnant women in the developing countries.

During the period from 1990-2002, the global population has grown from 1 to 6 billion, with the malaria risk population increasing from 0.9 to 3 billion (Hay *et al*.,2004).It is estimated that 2 billion people were at risk of *Plasmodium falciparum* malaria infection in the tropical countries of which 1.13 and 1.44 billion people were at risk of unstable and stable infection, respectively (Gething *et al.*, 2011).The highest level of *Plasmodium falciparum* infection occurs in Africa, which contributes to 99% of the global transmission

In the same year, approximately 2.5 billion people were at risk of *Plasmodium vivax* infection. Among these, 1.5 billion and 1 billion were at risk of unstable and stable *Plasmodium vivax* malaria infection, respectively (Gething *et al*.,2011).The highest population at risk of *Plasmodium vivax* infection lived in central Asia (2.05 billion), which was followed by South east Asia (215 million) and South and Central America (137.4 million). Africa has a population of 74.4 million which is at risk to *Plasmodium vivax*.

Declining health services and increasing poverty in parts of Africa and South Asia are also playing an important part in malaria's comeback. The improvements in the malaria were largely due to the development of the health services and socio- economic development taking place at the same time. The more people are aware of the risks of malaria and can afford to be treated promptly and take necessary personal protection measures, the less malaria is able to spread (UNICEF, 2000).

A substantial reduction in malaria transmission has been achieved globally, particularly in endemic countries between 2000 and 2012 (Anoor Am *et al.*,2014).Over this period, the malaria mortality rate was reduced by 42% in all age groups and by 48% in children under five years of age. Approximately 3.3 million death were prevented between 2001 and 2012, of which 91% were children under five years of age in Africa. The reduction was mainly associated with scaled up support by international donors, socio economic developments, the deployment of a artermisinin based combination treatment, wider courage of long lasting insecticidal nets (LLISNS) and indoor residual spraying in malaria's areas(WHO, 2013).

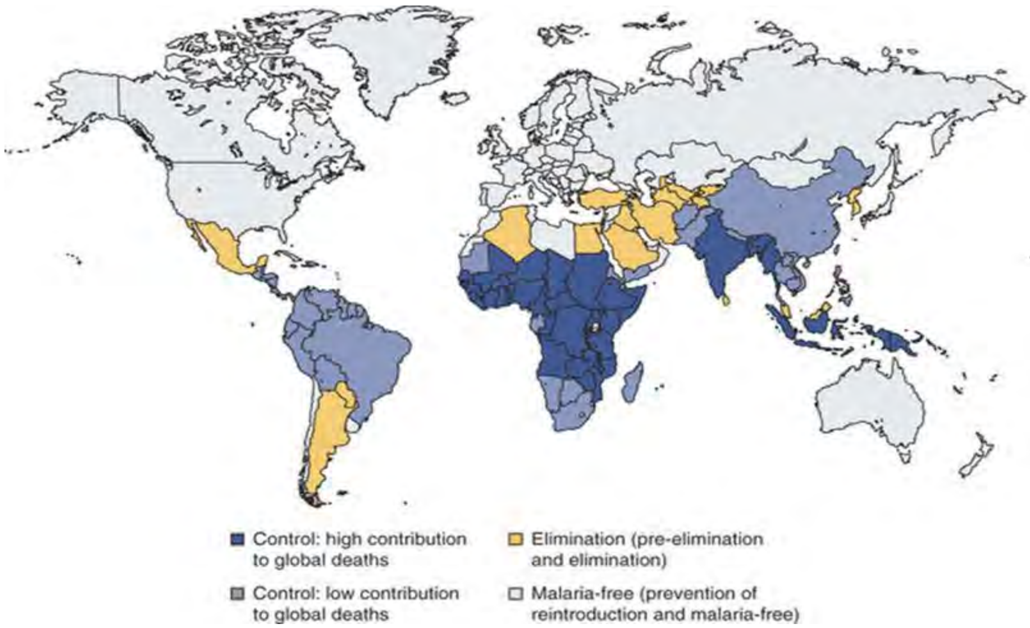


Figure 4. The distribution of malaria in the world (WHO, 2015).

Despite the scaled up intervention efforts and positive gains, malaria continues to be major public health problem (WHO,2013).In 2013, there were still 104 malaria endemic countries and territories where an estimated 3.4 billion people lived. Approximately 207 million cases and 627,000 deaths documented in 2012. Most cases (80%) and deaths (90%) occurred in Africa, where children under the age of five years contributed to 77% of the total deaths (WHO,2013).

WHO (2015) reported that 95 countries and territories with ongoing malaria transmission, and a further 6 countries that have recently eliminated malaria. According to the latest estimates from WHO, there were 214 million new cases of malaria worldwide in 2015 (range 149–303 million). In 2015, there were an estimated 438 000 malaria deaths (range 236 000–635 000) worldwide. Most of these deaths occurred in the African Region (90%), followed by the South-East Asia Region (7%) and the Eastern Mediterranean Region (2% (WHO, 2015). Between 2000 and 2015, malaria incidence rates (new malaria cases) fell by 37% globally, and by 42% in Africa. During this same period, malaria mortality rates fell by 60% globally and by 66% in the African Region (WHO, 2015).

2.6. Pathogenesis of malaria

Symptoms of malaria generally begin from 7 to 15 days after a bite from an infectious mosquito about the time when the red blood cells burst. The bursting cells release wastes and *toxins* (poisonous substances) along with merozoites. Fever develops as the immune system responds to the toxins in the blood (Tortora, 2010).

The symptoms and fever coincide with the multiplication of the parasite. That is when the red blood cells burst open and the parasites are released. The fevers occurs every third day in the *Plasmodium vivax* and *Plasmodium falciparum* malarias. The attacks can be complicated, however, as a result of successive infection by mosquitoes (Taylor *et al.,* 1997). People who have been exposed to infection since birth develop a certain amount of tolerance to the disease. People with no history of previous infection also develop serious disease very rapidly. Ten days after infection fever develops and the body temperature increases rapidly to 40.6-41.7•c.The fever may last as long as 12hrs accompanied by headache, generalized aches and nausea. After the fever, sweating starts and then temperature falls. The area of the abdomen over the spleen is tender. In addition anemia results of RBC destruction by parasite and autoimmune lyses' is one of the main symptoms of malaria (Moody, 2002).

The infection by *Plasmodium falciparum* cause malignant malaria in which the fever is accompanied by other complications. The parasite tends to accumulate in the blood vessels in the brain, causing convulsions or coma. Other common complication includes kidney failure and pneumonia. Malaria caused by *Plasmodium falciparum* can be fatal within two or three days (Tortora, 2010).

In general fever that characterizes malaria usually occurs in periodic attacks—every 72 hours from infection with *Plasmodium malariae* and every 48 hours from infection with the others. An attack begins with chills and shivering, soon followed by a high fever. Sweating then brings the temperature down. These attacks usually leave an individual exhausted. Headaches, nausea and vomiting, and achiness may also accompany the fever and chills. Aneamia may occur as a result of the bursting and destruction of red blood cells (Tortora, 2010). In *Plasmodium* *vivax* and *Plasmodium ovale* infections, some merozoites can remain dormant (inactive) in the liver. These merozoites periodically enter the bloodstream, triggering malaria relapse.

The disease of malaria and its symptoms are intimately related to its complex reproductive cycle. Infection is initiated by the bite of mosquito species of *Anopheles* mosquitoes which are the most potent vectors and therefore associated with stable transmission of the disease. However, most Species of Anopheles do not feed exclusively on either humans or animals (Choumet, 2012). Studies have therefore suggested that keeping cattle in the vicinity of human dwellings may have which carries the sporozoite stage of the plasmodium protozoa in its saliva (.Tortora, 2010).

The complete life cycle of human malaria parasite consists of a period of development within the mosquito, and a period of infection in man. After ingestion of human infected blood, a period of development (10-14days) takes a place in the mosquito resulting in the production of sporozoites. A bite of mosquito infects human host with sporozoites, which remain in circulation for 30 minutes or less and this phase is called sporozoite phase. Sporozoites enter the tissue cells of liver where pre-erythrocyticschzogony of a sexual reproduction takes places (Moody, 2002).

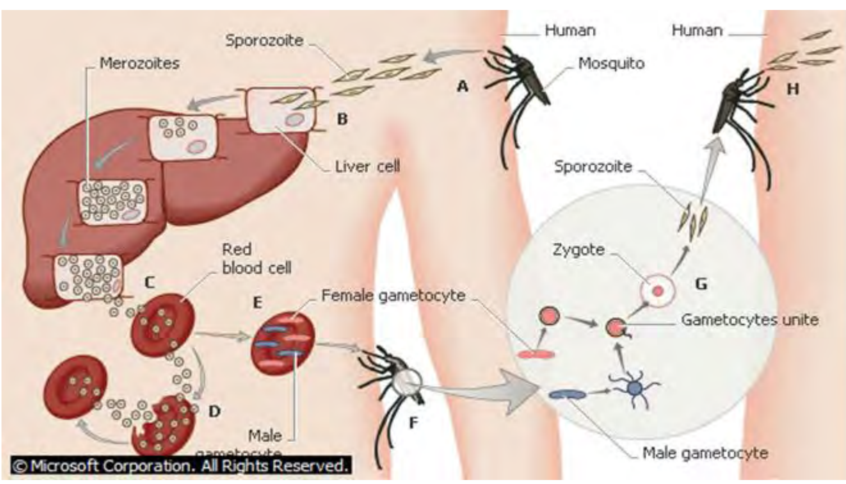


Figure 5. How *Anopheles* mosquito infect humans (Moody, 2002).

From the liver merozoites are released in to the blood stream and infect the red blood cells where a sexual reproduction (erythrocytic schizogony) takes place. In red blood cells (RBCS) they pass through several stages of development, namely tropozoite, schizonts, and finally merozoites. The parasitized RBC now burst, releasing merozoites. In *Plasmodium falciparum* the erythrocytes cycle takes places 36 to 48 hrs (subterian); in *Plasmodium vivax* and *Plasmodium ovale* infection takes a place in 48 hrs (terian); *Plasmodium malariae*72 hrs (quart an) (Moody, 2002).

The merozoits released after erthrocytic schizogony develop into male and female forms known as Gametocyte (the phase is called gametogony). Gametocytes are the only form; that can be transmitted to mosquito. Thus, they provide the a reservoir of infection enabling mosquitoes to maintain the malaria cycle, remaining with RBC for duration of their survival, i.e. up to 120 days (Moody, 2002).Many of released merozoit infect other RBCS within few seconds to renew the cycle in the blood stream. It only 1% of the RBC contain parasite with an estimated 100 billion parasite will be in circulation at one time in typical malaria patient (Tortora, 2010).

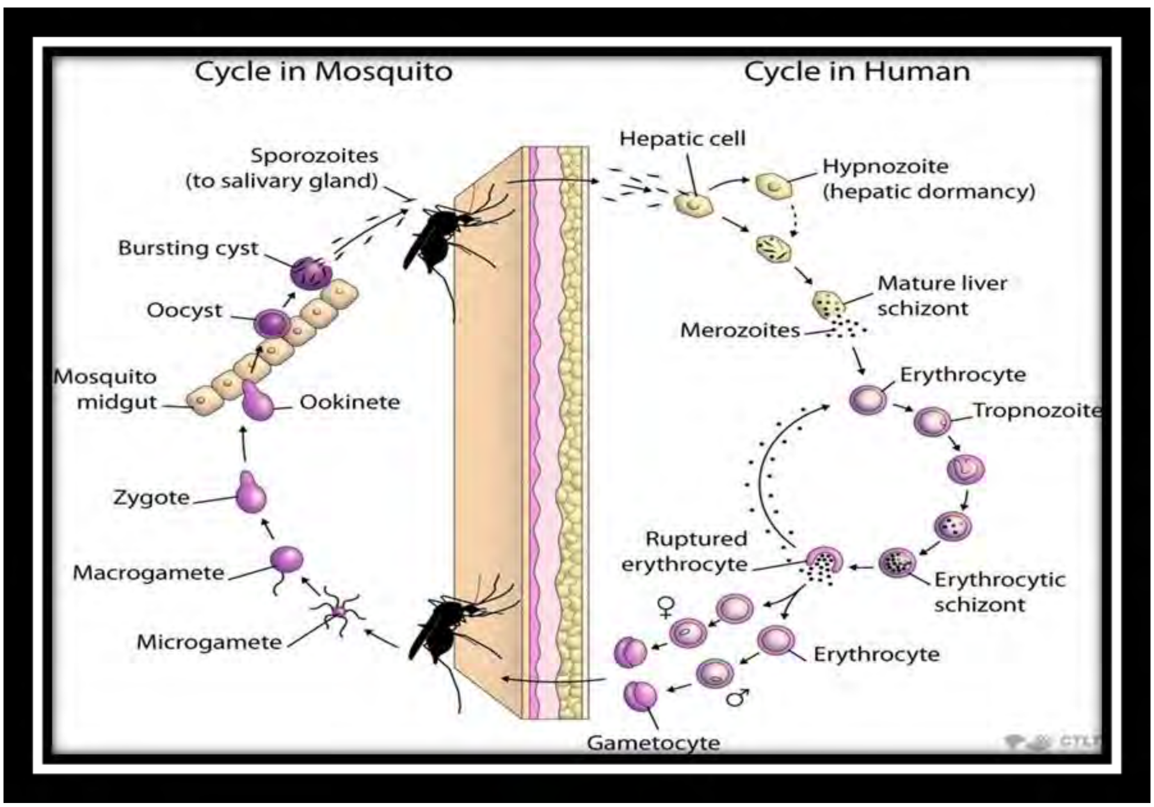


Figure 6.**The life cycle of malaria parasite in human and mosquito (source: WHO, 2012).**

2.7. Prevention and Control Malaria

2.7.1Malaria Prevention

Malaria is an entirely preventable and treatable disease, provided the currently recommended interventions are properly implemented. These include vector control though the use of long-lasting insecticide treated nets(LLINS), indoor residual spraying(IRS), and in some specific setting larval control, chemoprevention for the most vulnerable populations, particularly pregnant women and infants, conformation of malaria diagnosis though microscopy or rapid diagnosis test (RDTs) for every suspected case, and timely treatment with appropriate antimalarial medicine.

According to Word health organization (WHO), the strategic approaches to malaria control come within two major domains: prevention and case management. Accordingly, World health organization recommends universal access to and utilization at house level of long lasting insect

side treated nets (LLITNs) and indoor residual spraying(IRS) as most powerful and effective strategies of vector control, to rapidly control malaria transmission, hence reducing the burden of malaria morbidity and mortality (WHO ,2012).

In 2011, the world health Assembly, Roll Back malaria partnership, set the objectives: to reduce malaria case by 75% and malaria deaths to near zero from 200 levels by2015. And these objectives of mortality and morbidity reduction are linked to targets of achieving universal access to case management and universal access to and utilization of malaria prevention measure (WHO, 2012). According to world malaria report 2014, globally, malaria case decreased from227 million in 2000 to 198 million in 2013 and the incidence is projected to fall by 35%globally and by 40% in the who African region by 2015.In sub-Saharan Africa, the proportion of population protected by at least one vector control method has increased in recent years; it reached 48% in 2013, and the number scaled up in 2014. The prevalence of malaria parasite infection including both symptomatic and asymptomatic infection, has decreased significantly with average infection prevalence in children aged 2-10 years falling from 26% in 2000 to14% in2013, a relative decline of 46% (WHO, 2014).

The global malaria eradication program during 1950s and 1960s suffered serious setbacks and the disease increased slowly in areas where it had been reduced to low level. In an effort to combat the growing threat of malaria, the Roll Back Malaria (RBM) partnership was launched in 1998, with the goal to reduce the burden of malaria by half up to 2010 and halving again by 2015. The United Nations (UN) had also declared the Decade to Roll Back Malaria (2001–2010) in developing countries. Moreover, African heads of States met in Abuja, in 2000 to express their commitment to combating malaria (Sena, 2015).

According to WHO (2016) report Vector control is the main way to prevent and reduce malaria transmission. Two forms of vector control are effective in a wide range of circumstances: insecticide-treated mosquito nets (ITNs) and indoor residual spraying (IRS). ITNs are the cornerstone of malaria prevention efforts, particularly in sub-Saharan Africa. Over the last 5 years, the use of treated nets in the region has increased significantly: in 2015, an estimated 53% of the population at risk slept under a treated net compared to 30% in 2010.

Indoor residual spraying of insecticides (IRS) is used by national malaria programmers in targeted areas. In 2015, 106 million people globally were protected by IRS, including 49 million people in Africa. The proportion of the population at risk of malaria protected by IRS declined from a peak of 5.7% globally in 2010 to 3.1% in 2015 (WHO, 2016).

2.7.2 Malaria prevention in Ethiopia

Prevention and control activities of malaria in Ethiopia are implemented according to national strategic plan (NSP) for malaria control and prevention that operates in line with the WHO recommendation. The national strategic plan for malaria control and prevention in Ethiopia, NSP 2011 -2015, aimed at strengthening and scale-up of malaria control inter venations though prompt and effective diagnosis and treatment , case management though roll out of the highly efficacious antimalarial drugs(MOH, 2010). Artemisinin- based combination therapies (ACTS), and selective vector control with special emphasis to scaling up LLITNs coverage and ensuring its utilization at house hold level, and targeted and timely application of IRS of households with insect side and environmental management. The strategic plan has set goals to achieve malaria elimination in areas with historically low malaria transmission and near zero malaria deaths in all the remaining parts of the country by2015. To attain these goals it has set out the following specific targets.

- 100% of households in malarias areas own one LLINS per sleeping space.
- At least 80% of people at risk of malaria use LLINs, IRS coverage increased and maintained to 90% of households in IRS-targeted areas.
- 100% have access to effective and affordable malaria treatment (FMOH, 2012).

Studies show that sleeping under a bed net can reduce child mortality from malaria by as much as 20%. The repellent in the nets can also reduce the number of mosquitoes in the surrounding area (Mohammed *et al.,* 2015). When 80% of households use bed nets in a community, studies suggest that mortality from malaria for those living within 300 meters is significantly reduced (ACIPH, 2009).As outlined in the NSP 2011-2015, Ethiopia has a target of 1oo% access to effective and affordable malaria treatment. This requires improving diagnosis of malaria cases using microscopy or using multi species rapid diagnostic test (RDT) and providing prompt and effect malaria case management at all health facilities.

The national malaria indicator survey (MIS) 2011 that was conducted following the scale-up of malaria control inter venation in Ethiopia showed achievement in coverage of some malaria control interventions and malaria indicators between 2007 and2011(MOH, 2012). Malaria showed a decline in Ethiopia over last ten years as result of high coverage key malaria control interventions. This is attributable to the introduction of Artemisinin- based combination therapies (ACTS), the use of rapid diagnostic test(RDTs) at peripheral health facilities, Wide scale distribution of long lasting insect side nets (LLINs), High coverage of sprayed households though targeted indoor residual spraying (T. Degefa *et al.,* 2015).On the other hand, recent studies conducted across different parts of the country revealed that, despite the decrease in malaria mortality and morbidity attributed to the introduction of the current malaria control strategies, malaria still remains to be a major health problem of the country with unstable occurrences and fluctuating trends.

Ethiopia has registered remarkable progress in reducing the burden of malaria and other major communicable diseases over the last two decades (FMOH, 2016). Over the last decade, the burden of malaria has declined significantly, which could be the result of improved coverage of high impact interventions, such as prompt treatment of cases using artemisinin-based combination therapy (ACT), prevention and control of malaria among pregnant women using intermittent preventive therapy (IPT), use vector control methods including insecticide-treated bed nets (ITNs), and indoor residual spray (IRS) (Abeku *et al*., 2015). As result , malaria deaths and admissions in children age under-5 fell by 81 and 73%, respectively, after the scale-up of ITNs, IRS and ACT interventions between 2006 and 2011 (Aregawi *et al.*, 2014). However, malaria remains a major health problem for Ethiopia where only 25% of the population lives in areas that are free from malaria (WHO, 2014). It is still among the ten top leading causes of morbidity and mortality in children under-5 years (Deribew, 2013).

The MDG targets of halving mortality rate from malaria by 2015 and efforts to reverse the incidence of this disease have been encouraging globally although there were variations among regions and countries (WHO, 2015). Ethiopia has shown remarkable progress in reversing the burden and epidemics of malaria in the last two decades. Mortality and incidence rates of malaria declined by 96 and 89%, respectively, between 1990 and 2015. Other reports also show that Ethiopia has achieved the MDG targets of malaria (FMOH, 2016).

2.8. Drug Resistant Of Malaria

Drug resistance is the degree to which a disease or disease- causing organism remains unaffected by a drug which was previously able to eliminate it. In the case of malaria, it is the resistance of the malaria parasite, *Plasmodium falciparum,* to chloroquine or other anti-malaria's drugs (Taylor *etal* , 1997).Widespread drug resistance against commonly used anti- malaria drugs such as chloroquine and pyrimethamine/ sulfadoxine (Fansidar) has been reported all over the world. Epidemics are increasing in highland areas where malaria was uncommon, partly due to climatic changes including high rainfall patterns (WHO, 2012).

The drug resistant problem of malaria has dual faces. Those are resistant of the *Plasmodium* and resistances of *Anopheles mosquito*. According to, WHO (1996a), the origin of drug resistant is inadequate regimens, poor drug supply, and poor quality and misuse of drugs. This problem is particularly great in the treatment and control of falciparum malaria almost in all endemic countries, resistances to chloroquine has been found. Besides, a resistance to multiple drugs is common in the South East Asia (Abose *et al.,* 1997).

2.8.1 Factors responsible for the generation of drug resistance

Natural selection: The process of selection will depend upon variety of factors, including the size of the infecting biomass, the immunity of the host, the pharmacokinetic profile of the drug susceptibility and fitness of the mutant (White, 1999). Selection can occur either when a primary infection consists, in part, of resistant parasites capable of surviving treatment or when a sub set of parasites with spontaneous mutation encounter residual concentration of a slowly eliminated antimalarial drug. The number of parasite exposed to selective pressure will be far greater in the first case than the second and thus will provide the greater opportunity for resistant mutant to arise and spread (Price and Nosten, 2001).

Substandard drugs: Wide spread use of sub therapeutic antimalarial regiments is also likely to play a major role in facilitating the emergence of drug resistance. Substandard drugs have been widely available in the private sector like, pharmacies, clinics, drug shops and markets stall. Fake drugs with inadequate amount of active ingredient may kill off some susceptible parasites but leave those more likely to develop tolerance to multiply (WHO, 2011).

Mono-therapies: Mono-therapies are perceived as having fewer side effects and often cheaper than the ACTs. However, it is easier for a parasite to develop resistance to a single drug treatment as it only needs to adapt to the characteristics of one drug (Krishna *et al,* 2001).If a treatment involving two or more drugs is used, it is likely to kill the parasite even if it has develop resistance, to one of the drugs (Elbashira and Adam, 2008).

Lack of compliance: Patients often stop therapy as soon as their acute symptoms have resolved. This habit of poor compliance may arise because of the occurrence of adverse side effects, the cost of medication or because therapies are prolonged and complicated (White, 1997). Failure to take the full course of the drug means that while some susceptible parasites are killed, resilient ones live on, leading to resistance, to the drugs to which they were initially exposed (WHO, 2011).

2.9 Diagnosis of Malaria.

Malaria diagnosis must be recognized promptly in order to treat the patient and prevent further spread of infection in community via local mosquitoes. Malaria should be considered a potential medical emergency and should be treated accordingly. Delay in diagnosis and treatment is a leading cause of death in the world. Malaria can be suspected based on the patients travel history, symptoms, and physical finding at examination. However, for definitive diagnosis to be made, laboratory test must be under taken to demonstrate the presence of the malaria parasite and there components. Malaria diagnosis is difficult where malaria is not endemic any more for health care providers may not be familiar with disease (WHO, 2013)

It is particularly important to make an early diagnosis of malaria in young children and in pregnant women. These two groups may rapidly become very ill and may die within a few days. Pregnancy reduces the immune status of individuals and hence makes them more susceptible to malaria infection. Malaria during pregnancy is more difficult to treat, because the parasites tend to hide in the placenta, making diagnosis and treatment difficult (WHO, 2012)

2.9.1 Microscopic diagnosis

Microscopic diagnosis malaria parasite can be identified by examining under ―blood smear" on a microscope slide. Prior to examination, the specimen is stained (most often with the Giemsa stain) to give parasites a distinctive appearance. This technique remains the gold standard of laboratory confirmation of malaria. However, it depends on the quality of the reagent, of the microscope, and on the experience of the laboratorian. Blood smear stained with Giemsa, showing a white blood cell (on left side) and several red blood cells, two of which are infected with *Plasmodium falciparum* on right side (WHO, 2O11).

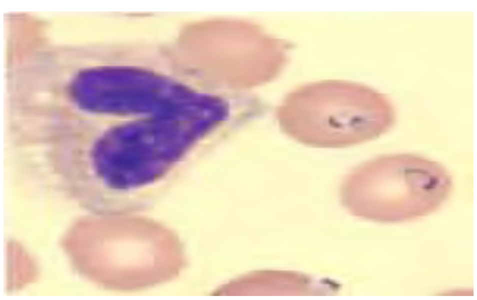


Figure 7. Giemsa staining of blood cells showing infected two red blood cells (WHO, 2011).

The most economic, preferred, and reliable diagnosis of malaria is microscopic examination of blood films because each of the four major parasite species has distinguishing characteristics. Two sorts of blood film are traditionally used. Thin films are similar to usual blood films and allow species identification because the parasite’s appearance is best preserved in this preparation. Thick films allow the microscopes to screen a larger volume of blood and are about eleven times more sensitive than the thin film. Picking up low levels of infection is easier on the thick film, but the appearance of the parasite is much more distorted and therefore distinguishing between the different species can be much more difficult (Warhurst and Williams, 1996). From the thick film an experienced microscopist can detect parasite levels (or parasitemia) as few as fire parasites/μL blood. Diagnosis of species can be difficult because the early trophozoites (ring form) of all four species look similar and it is never possible to diagnose species on the basis of a single ring form; species identification is always based on several trophozoites.(Richard *et al*., 2006*)*.

2.9.2 Clinical Diagnosis

Another patient diagnosis is physical diagnosis (subjective diagnosis) that is based on the patient’s symptoms and from physical diagnosis. The first symptoms of malaria (most often fever chills, sweats. headaches, muscles pains, nausea and vomiting) are not specific and are also found in other disease such as flu common viral infection. Likewise, the physical findings are 0ften not specific (elevated temperate, perspiration, tiredness). In sever malaria caused by *Plasmodium falciparum*, clinical finding (confusion, coma, neurological focal signs, severe anemia, respiratory difficulty) are more striking and increasing the index of suspicion for malaria. It possible, clinical findings should always be confirmed by laboratory test for malaria (moody, 2002).

2.9.3 Antigen Detection

Various test kits are available to detect antigens derived from malaria parasite. Such immunologic (immune chromatographic) test most often use dipstick or cassette format, provides results in 2-15 minutes. The rapid diagnostic tests (RDTs) offer a useful alternative to microscope in situation where reliable microscopic diagnosis not available.

Malaria RDTs are currently used in some clinical settings and programs. However, before malaria RDTs can be widely adopted, several issues remain to be addressed. The use of this RDT may decrease the amount of times that it takes to determine that patient is infected with malaria.

The first rapid diagnostic tests were using plasmodium glutamate dehydrogenase as antigen. PGLUDH was soon replaced by plasmodium lactose dehydrogenase (PLDH). Depending on which monoclonal anti bodies are used this type of assay can distinguish between different species of human malaria parasites, because of antigenic difference between their PLDH isoenzymes. Antibody tests can also be directed against other malarial antigens such as the *Plasmodium falciparum* specific HPRz. (Ling *et al.*, 1986).

2.9.4. Molecular Diagnosis.

Parasite nucleic acids are detected using polymerase chain reaction (PCR). Although this technique may be slightly more sensitive than smear microscopy, it is often limited utility for diagnosis of acutely ill patients in standard health care setting. PCR results are not available quickly enough to be of a value establishing the diagnosis of malaria infection. PCR is most powerful for confirming the species of malarial parasite after the diagnosis has been established by either microscopy or RDT (Moody, 2002).

2.9.5. Serology.

Serology detects antibodies against malaria parasites, using either immunofluorescence (IFA) or enzyme-linked immunosorbent (ELISA) .Serology does not detect current infection but measures past exposure (Moody, 2002).

2.10. Treatment

Early diagnosis and treatment of malaria can prevent it developing into a severe condition which could be fatal. In view of widespread drug resistance, treatment should follow national recommended protocols (UNICEF, 2000).If chloroquine fails to clear the malaria infection; an alternative drug needs to be used. If resistance to chloroquine is known to exist, other treatment is recommended. For example, pyrimethamine/sulfadoxine (Fansidar) or mefloquine may be used as first line drugs in areas of chloroquine resistance. Mefloquine is effective in the treatment of many cases of drug-resistant malaria, though resistance to mefloquine is growing in South East Asia. In addition, adverse reactions have been reported. Artemisinin is a natural product developed by Chinese scientists from the wormwood plant, Artemisia annua. Artemisinin clears the parasite from the body more quickly than chloroquine or quinine. It is also considered to be less toxic than quinine. Combination drug therapies are being advocated for treatment of malaria, such as mefloquine plus artemisinin (WHO, 2012).

The recommended treatment of severe complicated malaria is intravenous quinine or artemisinin derivatives. Intravenous infusion of quinine should be given slowly over 8 hours to avoid cardiac complications. This should be followed by oral quinine tablets for a total of 7 days once the patient is conscious and can drink. Although treatment may start at the health center, the patient should then be immediately referred to a hospital (UNICEF, 2000).

Chloroquine is the recommended treatment for uncomplicated cases in areas where resistance is low or non-existent. Fansidar is the recommended treatment in areas of high chloroquine resistance where Fansidar is still effective. To mask the bitter `taste of chloroquine, crushed tablets can be given to the child with banana or other local food. Quinine is the standard treatment for children with severe malaria (WHO, 2012).

Pregnant women in malaria endemic areas are more susceptible to malaria infections because of their reduced natural immunity and may therefore develop complications such as fever and severe aneamia. In some countries, national policies recommend routine use of anti-malarial drugs during pregnancy. Difficulties arise in providing pregnant women with prophylaxis in areas where there is resistance tochloroquine. Pyrimethamine/sulfadoxine (Fansidar) has been used as prophylaxis/intermittent treatment in Malawi and in Kenya with good preliminary results. In addition, all pregnant women should attend routine pre-natal clinic and should be protected from malaria by sleeping under treated mosquito nets. They should also receive ferrous sulphate and folic acid daily, to treat and prevent anaemia. When pregnant women become ill with malaria, treatment depends on national guidelines. Chloroquine, amodiaquine and quinine can all be safely given during pregnancy (UNICEF, 2000).

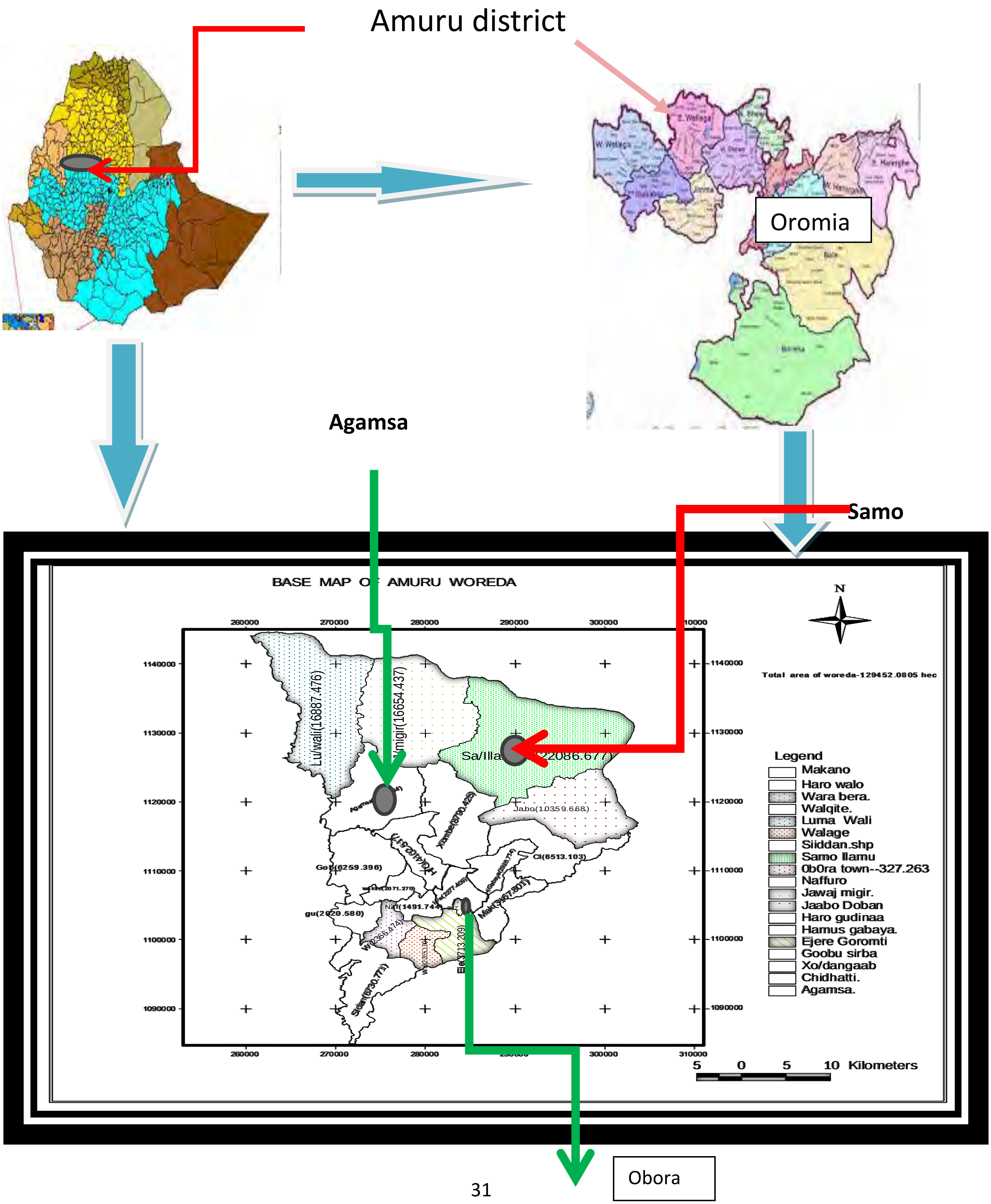
3. Materials and Methods

3.1 Study area Descriptions

The study was conducted on secondary data collected from health centers serving Amuru woreda which is located in Horo Guduru wollega zone, Oromia Regional State, Western Ethiopia, which was far away 382 km from Addis Ababa. The Wereda has various altitudes ranging from lowland of 760 to midland of 2,002 m above sea level. The woreda has an estimated total population of 70,501, of whom 29,774 (49%) were males and 30,984 (51%) were females, and of which 9,738 are urban dwellers and the rest are rural inhabitants; The majority of the population are farmers ,while the rest are merchants and government employers (Amuru Woreda Administrative office, 2017).

The Woreda has moist and hot climate with average annual temperature of 11.1^0c to 23.6^0c and rainfall of 1167mm to 1737.9mm to, respectively (Shambu metrological office, 2017). In the Amuru Woreda there are 21 kebeles and three health centers (Obora, Agamsa and Amuru).

Figure 8. Base map of Amuru District (source: Amuru Administrative office, 2016).



3.2. Study Design

A retrospective correctional study was conducted to determine the prevalence of malaria by reviewed a ten year recorded clinical data from the three Amuru health centers (Agamsa, Samo and Obora) collected from 2007 up to 2016. Consequently, the data sources were essentially secondary data from blood film identification of malaria reports at the health centers.

3.3. Study population and data collection.

The target population for the study was all population malaria cases attended three health centers. The selection of these health center facilities was done by using purposive sampling technique, because malaria patients were registered in an organized manner in these health centers compared to many private health facilities available in the district. During the study period Socio demographic and laboratory data were collected from patient's registered book. To assess the climatic factors such as temperature, humidity (rainfall), metrological data were collected from nearby office of metrological agency (Shambu Metrological agency, personal communication).

3.4 Data Analysis

Pearson chi square test was used to give clear picture of prevalence of malaria, socio demographic characteristics such as age and sex by the formula:

$X^2 = \Sigma \frac{(O - E)2}{E}$, Where, X^2= Chi square

O = observed value (malaria case in each sex or age groups)

E= expected value (obtained from calculation by using formula) (Calder, 1996).

Statically significance was defined in P-values less than 0.05 ($p<0.05$).

The data were entered and analyzed by SPSS 20 software package. To observe the correlation between meteorological variables and malaria cases, the monthly malaria cases were regarded as the dependent variables, while meteorological variables such as monthly mean temperature, total monthly rainfall, and monthly relative humidity were independent variables. Average yearly means temperature, total rainfall and relative humidity were calculated for all years. All data from metrological and health centers records were checked for completes and cleaned for any in consistencies. Final Pearson's correlation analysis was conducted to examine the type and strength of relationship between meteorological variables and malaria cases. In addition to this relationship between dependent (annual malaria case) and independent variables (metrological variables) done by using formula (Calder, 1996).

$r = \frac{N\sum(Xy) - \sum X \sum Y}{\sqrt{[N\sum X^2 - (\sum X)2][N\sum Y^2 - (\sum Y)2]}}$ Where, r = Correlation coefficient.

X= value of the first variables (annual malaria case).

Y = Values in second variable (value of one of metrological factor)

N = Total number of year (ten).

Finally, the data was described and presented by using tables and figures.

4. Results and Discussion.

4.1 The prevalence of malaria in Amuru District from 2007-2016 years.

In this study a total of 49,871 blood samples at the three health centers (Obora, Agamsa and Samo) were submitted for malaria diagnosis for the last ten years (2007 -2016) in Amuru District. From these 29,941 (60 %) were males and 19,930 (40%) were females. From the blood film samples, 11,335 (22.7%) were microscopically confirmed as malaria cases (Table 1).

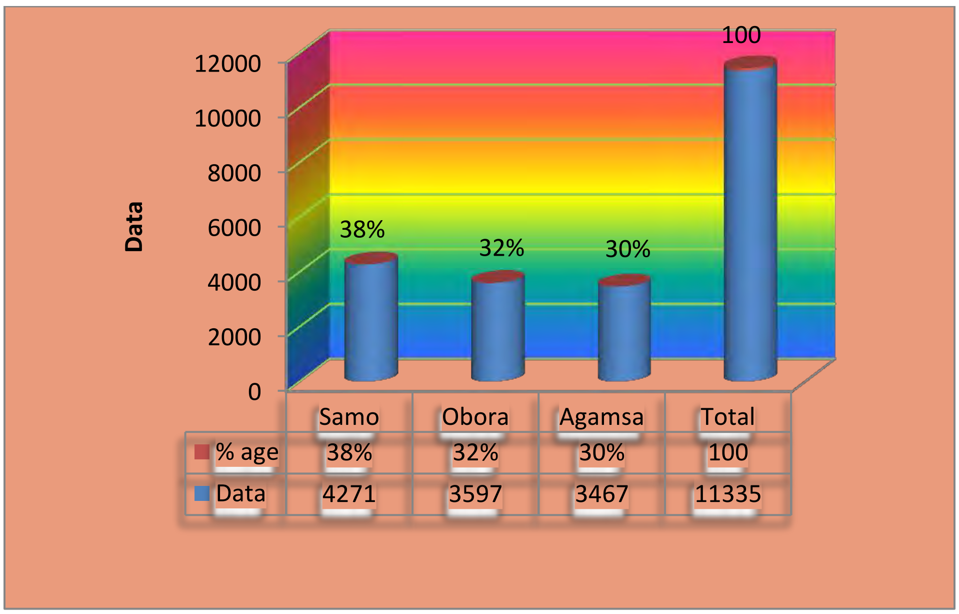
Table 1. Prevalence of malaria amongst out patients visiting the three health centers of Amuru district from 2007-2016year

Year	No of blood sample			No of positive sample		Total	% age	Trend	M : F	
	No	M	F	M(% age)	F(% age)					
2007	6700	3950	2750	1338(11.8	814(7.2)	2152	32.1 %	-	1.6:1	
2008	7016	4086	2930	976(8.6)	715(6.3)	1691	24.1%	8%	1.4:1	α <0.05
2009	5790	3641	2149	723(6.4)	496(4.4)	1219	21%	11%	1.5:1	df=9,
2010	3555	2176	1379	674(5.9)	407(3.6)	1081	30.4%	6.0%	1.7:1	x^2 =
2011	3522	2043	1479	596(5.3)	395(3.50	991	28%	13%	1.5:1	46.335
2012	3575	2208	1367	659(5.8)	453(4)	1112	31.1%	3.0%	1.5:1	P<o.ooo1
2013	5000	3158	1842	527(4.6)	412(3.6)	939	18.7%	41%	1.3:1	
2014	5171	3150	2021	424(3.7)	360(3.2)	784	15.1%	53%	1.2:1	
2015	5280	3177	2103	449(4)	309(2.7)	758	14.3%	56%	1.5:1	
2016	4262	2352	1910	397(3.5)	211(1.9)	608	14.2%	56%	1.9:1	
Total	49871	29941	19930	6763(60)	4572(40)	11335	22.7%		1.5:1	

The prevalence of malaria in the district showed variations from year to year ranging from the highest record of 32.1% in 2007 to that of 14.2% in 2016. The data showed a steady decrease by 8-11% between2007-2009 (from 32.1% to 21%). However, there was a significant increase to 30.4% v in 2010 and followed the same trend up to 2012 (Table 1), Thereafter, the prevalence of the disease progressively declined to 14.2% showing 41-56% decrease compared to the prevalence of malaria in 2007 (Table 1). However, it showed a fluctuating pattern with in the category of the high prevalence of (>30%) in the years 2007, 2010, 2012, medium prevalence of (20 - 30%) in the years of 2008, 2009 and 2011) and lower prevalence of (< 20%) in the years 2013, 2014, 2015 and 2016 (Table1).

The data collected from the three Health Centers in the Wereda showed variations in the prevalence of malaria (Fig.9). The highest prevalence of 38% was recorded from Samo Health Center followed by prevalence of 32% and 30% from Obora Health and Agamsa Health Centers, respectively.

Figure 9. Total prevalence of malaria in studies sites from 2007-2016 years.



4.2. Pattern of *plasmodium* infections in Amuru District From 2007-2016.

The prevalence of malaria in this study showed significant variations compared to the status of malaria infections in different parts of the country for the last ten years (Table 2). The prevalence of malaria in this study over the years (22.7%) was similar to that of 20.07% recorded from Sibu Sire (Temesgen Gemechu *et al*.,2015), but significantly higher than the prevalence of 11.45% in Arsi Negelle, 11.53% in Motta, and 17% in Metema towns where studies almost covered the same period between 2006-2016) (Table 2).

On the contrary, the disease intensity in Amuru district was much lower than the infection rate of 39.6% from Kola Diba, North and Central Ethiopia (Abebe *et al.*, 2012), and 33.27% from (Serbo Health Centre (Karunamoorthi and Bekele, 2009) ((Table2). This was because the study sites included study periods (2002-2011; 2003-2008) that may not have been covered by prevention and control activities of malaria in Ethiopia implemented according to national strategic plan (NSP) for malaria control and prevention that operated between 2011 -2015 in the country.

Table 2. Summary of total prevalence of malaria disease compared to other studies.

Study site	Prevalence	Study period	Referances
Amuru	22.7 %	2007-2016	This study
Metema	17%	2006-2012	Getachew *et al*. (2013).
Sibu sire	20.07%	2005-2013	Temesgen G. *et al*. (2015)
Arsi Negelle	11.45%	2009- 2013	(Solomon and Mangistu, 2015).
Kola Diba	39.6%	2002-2011	Alemu *et al.* (2012).
Motta	11.53%	2006-2015	Tilahun (2016)
Arbaminch	7%	2010	(Balayneh, 2014).
Dilla	16%	2015	Molla *et al.* (2015)
Welaita	39.6	2015	(Deresse *et al.*, 2015)
Serbo	33.27	2003-2008	Karunamoorthi and Bekele (2009)

The prevalence of 30.4% of malaria in the Amuru Wereda in 2010 was much higher than the prevalence of 7% recorded from Arbaminch at the same period (Balayneh, 2014), but a malaria infection of 14.3% recorded in the Wereda was slightly lower than the ones detected from Dilla town (16%) (Molla *et al,* 2016).

The differences in the prevalence of malaria in the country might be due to variations in methodological differences (sample size and years of study, primary data, secondary data, and vulnerability (location) of the study sites, and presence and absence of strong concerted national intervention plan and activities in the areas to control malaria.

The rate of infection differed between males and females with a male to female infection ratio of 1.2:1 (2014) to 1.9:1 (2016) with average ratio of 1.5:1 (60:40) significant (x^2 = 46.335, df = 9, P< 0.05), showing similar pattern of male and female outpatients visiting the health centers and the infection rate between the sexes (Table 1). The data also showed the same pattern amongst the different age groups, except the 16-30 age group, and >60 years with M:F ratios of 1.3:1, and 1.7:1, respectively (Table 4).

Table 3. Summery prevalence of malaria disease with respect to sex in Amuru District compared to other studies.

Study site	Sex		Years of study	M:F (ratio)	Reference
	Male	Female			
Amuru	59.7%	40.3%	2007-2016	1.5:1	This study
Arbaminch	57%	43%	2010	1.3:1	(Balayneh,2014)
Motta	57.47 %	42.57%	2016	1.3;1	(Tilahun, 2016)
Sibu sire	53.6%	46.4%	2004-2013	1.2:1	(Temesgen *et al.,* 2015)
Chuchu	56%	43.96%	2016	1.3:1	(Belete ,2016)
Metema	58.7%	42 %	2006-2012	1.4:1	(Getachew *et al.,* 2013)
Arsi Negelle	55%	45%	2009-2013	1.2:1	(Mangistu and Solomon, 2015).
Kola Diba	52.6%	47.3%	2002-2011	1.1:1	(Alemu *et al*., 2012)
Wolaita zone	50.74%	49%	2008-2013	1:1	(Deresse *et al*., 2015)
Dilla	58.9	41.1%	2015	1.4:1	(Molla *et al.,* 2015)
Serbo	56.1%	43.9%	2012	1.3:1	(Kurnamoothi and Bekele, 2012)
Nigeria (Aba)	46.5	53.5	2012	1:1.2	(Kalu *et al.,* 2012)
Nigeria	42	58	2002-2004	1:1.4	(Okonko *et al.,* 2009)

The male: female ratio in this study (1.5:1) was slightly higher than the 1.4: 1 recorded from both Metema (Getachew *et al*., 2013), and Dilla (Molla *et al*., 2015); but significantly higher than the others, and 1:1 ratio from Kola Diba (Abeba *et al.,* 2012) and Welaita Zone (Deresse *et al.*, 2013). Although malaria infection occurred more in males than females in most of the local studies, reports from Nigeria showed the contrary where females were more infected with ratio (F:M) of 1.2:1 (Kalu *et al.,* 2012), and 1.4:1 (Okonko *et al.,* 2005).

The higher prevalence might be due to males were engaged in outdoor activities to keep agricultural products, livestock such as cattle and they often migrated in to low land areas for seasonal works that are infested with malaria vectors and parasites.

In this study, malaria infection was recorded among all age groups (Table 4). The highest infection occurred on the age group of 31-45 years, with prevalence of 35.4 %, followed by the prevalence of 27.3% on the age group of 16-30 years. Similar pattern of infection of 11% was recorded from each age group of 46-60 years and the age group of 1-5 years, respectively. The least affected one was the age group of >60 years with a prevalence of 3.5 %. The male to female ratio also showed variation ranging from 1.3:1 (16-30 age group) to that of 1.7:1 (age group of >60).

Table 4. Prevalence of *Plasmodium* species in respect to sex and age groups in Amuru District from 2007-2016.

Parasite	Age groups							Total	
	Sex	1-5	6-15	16-30	31-45	46-60	>60		D f=5
Total	**M**	**798**	**726**	**1768**	**2412**	**801**	**258**	**6763**	$x^2 = 12.255$
	F	**512**	**482**	**1324**	**1572**	**531**	**151**	**4572**	α= 0.05
	T	**1310**	**1208**	**3092**	**3984**	**1332**	**409**	**11335**	P=0.031456
% age		**11.6%**	**10.7%**	**27.3%**	**35.1%**	**11.8%**	**3.5%**	**22.7%**	
Ratio (M: F		**1.5:1**	**1.5:1**	**1.3:1**	**1.5:1**	**1.5:1**	**1.7:1**	**1.5:1**	

The pattern of infection indicated that the disease affected largely productive age groups of 16-45 years (df =5, X^2=12.255, P=0.031) that contributed to more than 60% of the infection (Table 4). The cumulative prevalence of malaria in the active age groups of 31-45 and 16-30 was 62.4% which was much higher than the prevalence of 48.1% in Sibu sire (15-44age group) (Temesgen *et al.*, 2015) and 50% in Kola Diba (15-44age group) (Alemu *et al.,* 2012). This might be associated with their daily activities. Farming is extensive in Amuru district due to the fact that young daily laborers move to low land area from different areas for application of farming and harvesting of crops. Because of the area was conducive for breeding of mosquitoes and survival of the parasite, this may exposed them to the bite of mosquitoes.

4.3. The trend of malaria infection by the *Plasmodium* species in Amuru District From 2007 – 2016 years.

The data also showed that the prevalence of *Plasmodium falciparum* and *Plasmodium vivax* as causative agents of a disease of which *Plasmodium falciparum* was being the predominant parasite 7659 (67.6%), followed by *Plasmodium vivax* 2810 (24.8%) malaria morbidity respectively, whereas the mixed infection was 866 (7.6%) in study area (Table 5).

Table 5. The distribution of *Plasmodium* species in Amuru District from 2007-20016 year.

years	*Plasmodium* species			Total
	Pf (% age)	Pv (% age)	Mi (% age)	
2007	1431(12.6)	567 (5)	154(1.4)	2152
2008	1238 (10.9)	326 (2.9)	127 (1.1)	1691
2009	898 (7.9)	228 (2)	98 (0.9)	1219
2010	554 (4.9)	442 (3.9)	85 (0.9)	1081
2011	658 (5.8)	247 (2.2)	86 (0.9)	991
2012	740 (6.5)	292 (2.6)	80 (0.7)	1112
2013	614 (5.4)	246 (2.2)	79 (0.7)	939
2014	554 (4.9)	167 (1.5)	63 (0.6)	784
2015	553 (4.9)	151(1.3)	54 (0.6)	758
2016	424 (3.7)	144 (1.3)	40 (0.4)	608
Total	7659(67.6)	2810(24.8)	866(7.6)	11335

Note: Pf = *Plasmodium falciparum*, Pv = *Plasmodium vivax*, Mi= Mixed infection.

Plasmodium falciparum was the predominant parasite in the study area and accounted for 67.6% of malaria mortality and morbidity. This finding coincides with the malaria parasite distribution in Ethiopia which indicates *Plasmodium falciparum* and *Plasmodium vivax* was the two predominant malaria parasite, distributed all over the nation and accounting for 60% and 40% of malaria cases respectively (MOH, 2010).

The pattern of infection over the years showed a drastic reduction of 70% and 74% *Plasmodium falciparum* and *Plasmodium vivax* infections from 2007 to 2016 years respectively (Table5). This was significantly higher than 50 % of decline due to *Plasmodium falciparum* infection in Ethiopia (WHO, 2016).

Similar pattern of distribution of the two *Plasmodium* species to the malaria infection recorded at the three health centers. Accordingly, the highest *Plasmodium falciparum* distribution of 28.5% was recorded from Samo Health Center. Likewise, more infections by *Plasmodium falciparum* was recorded from Samo (28.5%) followed by Agamsa (20%) and Obora (19%) Health centers, respectively (Table 6).

Table 6. The distribution of *plasmodium* species in study location in Amuru district from 2007- 2016.

year	Study site	*Plasmodium* species				Ratio
		Pf (%)	Pv (%)	Mi (%)	Total (%)	(Pf: Pv)
2007 - 2016	Samo	3229(28.5)	636(5.6)	406(3.6)	4271(38)	5:1
	Obora	2170(19)	1182(10.4)	245(2.3)	3597(32)	2:1
	Agamsa	2260(20)	992(8.8)	237(2.1)	3467(30)	2.3:1
	Total	7659(67.6)	2810(24.8)	866(7.6)	11335(100)	2.7:1

However, the highest infection of 10.2% was detected by *Plasmodium vivax* from Obora Health Center (10.2%), followed by the 8.5% and 5.4% infections by the same parasite at Agamsa and Samo Health Centers. Interestingly *Plasmodium falciparum* was the most predominant parasite in study site and months with nearly 2:1 and 5:1 ratio over for *Plasmodium vivax* infections recorded from Obora and Samo Health Centers, respectively. This variation might be due to the presence of high malaria breeding sites attributed to relative agro ecological and altitude and relative differences amongst the population living and attending their respective health centers. This indicates location had impact on the distribution of the disease.

Table 7. Summary of predominant malaria parasites in Amuru District compared to other studies.

Study site	Malaria parasite			Years of study	References
	Pf	Pv	Mi		
Amuru	67.6%	24.6%	7.6%	2007-2016	This study
Ethiopia	60%	40%	-	2010	(MOH,2010)
- Arbaminich	64.3%	25%	10.7%	2010	(Balayneh, 2014)
Serbo	62.4%	37.3%	0.3%	2012	(Karnamorthi and Bekele, 2012)
-Sibu sire	66.1%	30.5%	3.4%	2004-2013	(Tamasgen *et al.*, 2015)
-Metema	90.7%	9%	0.3%	2006-2012	(Getachew *et al.*, 2013)
-Kola Diba	75%	25%	-	2002-2011	(Alemu *et al.*, 2012)
-Buta jira	12.4%	86.5%	1.1%	2012	(Woyesa *et al.*, 2012)
-Arsi Nagalle	19.8%	74%	6.2%	2009-2013	(Manigistu and Solomon, 2015)
-Butajira	37.5%	62.5	-	2000-2009	(Solomon *et al.*, 2012)
-Assendabo	45.7%	54.3%	--	2000	(Gebreyesus *et al.*,2000)
-Dilla town	26.8%	62.5%	10.7%	2014	(Molla *et al.*, 2015)
Global	92%	4%	-	2016	*WHO* (2016)

Note: Pf = *Plasmodium falciparum*, Pv =*Plasmodium vivax*, mi= mixed infection

The dominance of *Plasmodium falciparum* infection over the *Plasmodium vivax* infection at the national level was 60:40% (MOH, 2010). Although several studies in Ethiopia showed the predominance of *Plasmodium falciparum* with pattern of 60-66.1% in Arbaminch, Sibu Sire, and Serbo, 75% in Kola Diba, and 90.7% distribution in Metema (Table 7), few studies showed the reverse in that *Plasmodium vivax* dominated the infection ranging from 54% (Assenadabo), 86.5% (Butajira). It is tempting to assume that, with few exceptions, the two parasites have a geographical distribution in that *Plasmodium falciparum* dominates northern western and southern parts of the country; whereas *Plasmodium vivax* is widespread in the south central Rift valley areas (Table7).

4.4. Seasonal distribution of malaria infection in Amuru Wereda from 2007-2016.

The distribution of the parasite for malaria infection showed that 67.6% of the infections were caused by *Plasmodium falciparum,* followed by 24.8% infection with *Plasmodium vivax* with a ratio of 3.2:1(March-May) to that of 2.5:1 (June-August) with an average of 2.7:1 showing variations amongst the seasons (Table 8). It was also shown that males were slightly more infected (2.9:1) by *Plasmodium falciparum* compared to females (2.5:1). The data also showed 7.6% of the patients suffered by mixed infections.

Despite the apparent fluctuation of prevalence of the disease in the study area; malaria infection occurred in almost every season of the years. The high peak of malaria infection was observed during spring (September- November) with prevalence of 34.9% followed by summer season (June – August) and autumn (March – May) with prevalence of 32% and 21.6% respectively (Table 8).

Table 8. Seasonal variations of *plasmodium* infection in Amuru district from 2007- 2016 year.

Seasonal infection	No	*Plasmodium* species					% age
		Pf	Pv	Pf :Pv	Mi	Total	
December -February	9288	1069	416	2.6:1	102	1587	14 %
March-May	10164	1529	477	3.2:1	154	2160	21.6 %
June- August	13704	2387	940	2.5:1	302	3629	32 %
September- November	16715	2674	977	2.7:1	308	3959	34.9 %
Male Total	29941	4639	1625	2.9:1	499	6763	59.7 %
Female total	19930	3020	1185	2.5:1	367	4572	40.3 %
Total/average	49871	7659	2810	2.7:1	866	11335	22. 7 %

Note: NO = Number of screened, Pf= *P.falciparum*, Pv = *P. vivax,* Mi = Mixed

The two seasons (spring and summer) accounted for more than 2/3rd of the annual infection in the Wereda (Table 8). The lowest prevalence of malaria was observed during winter (December – February) which accounted for 1587(14%) in the study area.

Table 9. Summary of seasonal variation prevalence of malaria in Amuru district from 2007- 2016year.

Study site	Major and minor malaria cases in season		Time of study	References
	Major	Minor		
Amuru	Sep-Nov (34.9%)	Dec-Feb (14%)	2007-2016	This study
Ethiopia (National)	Sep-dec(Jun-sep) (56.2%)	April - May (28%)	2010	(MOH,2010)
Metema	Sep-Nov (38.6%)	Dec-Feb (18.7%)	2006-2012	(Getachew et al.,2013)
Motta	Sep-Nov (38.6%)	Dec-Feb (18.7%)	2006- 2015	(Tilahun, 2016)
Arsi Negele	Sep-Nov (32.3%)	Dec-Feb (16.2%)	2010-2014	(Manigistu-and Solomon,2015)
Kola Diba	Sep-Nov (53 %)	De- Feb (18%)	2002-2012	(Alemu et al.,2012)

In Ethiopia the major transmission of malaria follows the June to September rains and September to December but the minor transmission of malaria occurred from April to May following the February to march rains. Generally in Ethiopia, altitude and climate are the most important determinants for malaria transmission is seasonal and predominately unstable number of malaria cases (MOH, 2010).

4.5. Prevalence of malaria with respect to months in Amuru District from 2007-2016 year.

Table 10. Prevalence of *plasmodium* species with respect to month in Amuru district from 2007-2016 year.

year	Month	*Plasmodium* species			Total	% age
		P f	Pv	Mi		
2007---2016	Jan	327	134	42	503	4.4%
	Feb	322	116	39	477	4.2%
	Mar	445	135	46	626	5.5%
	Apr	465	147	49	661	5.8%
	May	619	212	67	898	7.9%
	Jun	870	354	127	1351	11.9%
	Jul	794	329	83	1206	10.6%
	Aug	723	281	74	1078	9.5%
	Sep	989	343	136	1468	12.9%
	Oct	902	297	85	1284	11.3%
	Nov	783	295	69	*1147*	10.1%
	Dec	420	167	49	636	5.6%
Total		7659	2810	866	11335	100%

Note: Pv =*Plasmodium vivax*, Pf = *Plasmodium falciparum*, Mi = Mixed infection

The occurrence of malaria also showed significant difference amongst months within seasons. Accordingly, the highest peak of 12.9% was recorded from September (with in September-November trimester followed by 11.9% in June, (June-November trimester), and 11.3% in October in the September- November trimester (Table 10). This indicates that most of the infections were recorded at the start of and immediately after the rainy seasons.

4.6. The prevalence of malaria in relation to metrological factors in Amuru District from 2007- 2016 years

The study area was generally characterized by moderate climate with a mean annual maximum and minimum temperature of 23.6°c (2016) and 11.1° c (2015) respectively. The annual rainfall ranged from 1,167 mm to 1,737.9 mm in 2015 and 2014 years respectively. The mean maximum rain fall which was accounted for 144.8 mm in 2014 followed by143.5 mm in 2012 year and minimum rain fall of 97.3 mm recorded in 2015 (Table 11). The relative humidity was in the range of 52.3 % (2016) to 69.4% (2007).

Table11. Mean annual metrological factors in Amuru district from 2007-2016 years.

Year	Metrological variables(factors)				Total Malaria case	% age
	Annual mean rainfall (mm)	Annual mean rel. humidity (% age)	Annual mean min. T°C	Annual mean max. T°C		
2007	138.05	69.4	11.5	23	2152	32.1%
2008	137.97	63.8	11.2	23.1	1691	24.1%
2009	121.34	61.3	11.5	23.3	1219	21%
2010	119.3	60.6	11.8	18.6	1081	30.4%
2011	118.5	67.7	11.4	23.1	991	28%
2012	143.5	65.4	12	23.4	1112	31.1%
2013	128.6	64.6	12.3	22.9	939	18.7%
2014	144.8	63.8	11.8	22.6	784	15.1%
2015	97.3	54.9	11.1	21.4	758	14.3%
2016	130.3	52.3	12.3	23.6	608	14.2%

4.7. Correlation between annual malaria case and metrological Factors

It is established that meteorological factors have been considered as important drivers of malaria transmission by affecting both malaria parasites and vectors directly or indirectly (Pemola and

Jauhari, 2013). The data showed a certain difference in rainfall, relative humidity, and maximum temperature amongst seasons. Accordingly, higher values for these parameters were recorded in 2007 compared to the lower values obtained in 2015 and 2016 seasons.

The decrease in relative humidity and rainfall also concurred with a significant reduction of malaria infection in the latter years. Although the SPSS correlation coefficient data analysis indicated that relative humidity and rainfall were positively correlated ($r = 0.657$, $p = 0.020$) ($p < 0.05$), and ($r = 0.391$, $p = 0.132$) ($p > 0.05$) with malaria transmission in the study area, respectively. Whereas other metrological facts such as maximum temperature and minimum temperature statically insignificant ($p > 0.05$). They were fluctuations in the different parameters in relation to the prevalence of the disease over the years.

Although the meteorological factors partly contributed to the significant reduction of malaria from 2007-2016, the most important factor to the drastic reduction of the disease over the years might be due to the increased attention to malaria control and preventive activities in line with global and national malaria eradication program which was designed to combat the growing threat of malaria (WHO, 2016). The Roll Back Malaria (RBM) partnership and three other consecutive strategic plans increased access to availabilities of strong preventive and controlling measure globally as well as at national level with the goal to reduce the burden of malaria by half up to 2010 and halving again by 2015 (Sena, 2015).

These include distribution of insecticide-treated mosquito nets (ITNs) and indoor residual spraying (IRS) which were are the cornerstone of malaria prevention efforts, particularly in sub-Saharan Africa (WHO, 2016). From 2010-2015, the use of treated nets in the region increased significantly, and consequently, an estimated 53% of the population at risk slept under a treated net compared to 30% in 2010.In 2015, 106 million people globally were protected by IRS, including 49 million people in Africa. The proportion of the population at risk of malaria protected by IRS declined from a peak of 5.7% globally in 2010 to 3.1% in 2015 (WHO, 2016).The 56% reduction of malaria between 2007 and 2016 in this study concurred with the 50–75% decline in incidence and mortality rates of malaria recorded between 2000 and 2013 in Ethiopia (WHO, 2016). Another study also showed a 29% and 31% decrease mortality in the world and Africa from 2010-2015, respectively (WHO, 2016). Similarly, in Ethiopia WHO report showed that between 2010 and 2015, malaria incidence and mortality rates, particularly due to *Plasmodium falciparum*, have declined by more than 50% in Ethiopia (WHO, 2016)

Ethiopia was one of the first countries to embrace the scaling up for impact concept of malaria control. The 2006- 2010 national strategic plan aimed to rapidly scale up malaria control interventions to achieve a 50 % reduction of the malaria burden in line with roll back malaria prevention slogan objectives (FMOH, 2010). The 2011 -2015 national strategic plan was up dated version of the 2006 -2010 national malaria strategic plan. It was based in strong collaboration between all actors that contribute in the fight against malaria in Ethiopia with resources secured to support universal coverage of key malaria interventions by the end of 2010. All taken together, the reduction of malaria in Amuru Wereda was due to the benefits that the Wereda may have obtained from such programs at a national level. Since the country is moving to scaling up from impact to sustained control, as key steps in the process forwards malaria elimination by 2020, it will build on the achievement of the strategic plans, and through sustained control will move towards malaria elimination through an integrated Community health approach, especially in areas of unable malaria transmission. Under the circumstances, Amuru Wereda will go further in reducing, if not eliminating the scourge of malaria in the coming years.

5. Conclusion and Recommendation

5.1. Conclusion

The study showed that the prevalence of malaria was variable ranging from out of clinical cases 32.1% in 2007 to that of 14.2% in 2016 with average prevalence of 22.7%. Although it showed a downward trend over the years, It showed fluctuations constituting a prevalence of high category (>30%) in the years 2007, 2010, 2012, medium prevalence of (20- 30%) in the years of 2008, 2009 and 2011; and lowest prevalence of (< 20%) in the years 2014, 2015 and 2016 indicating that the area is on the track, with implementation of national plan of controlling malaria.

The study shows that males were more affected (59%) than females. (41%) with a M : F ratio of 1.5:1;and younger age groups (16-45 year) were infected (60%) compared to the other age groups indicating that men and the active age groups were more vulnerable to malaria infection because of their mobility to malareous regions and their activity outside and during the night compared to other groups.

The predominant malaria parasite in the study area was *Plasmodium falciparum compared to P vivax with a ratio of 67.6%:24.8 %* comparable to the overall national distribution of the two parasites.

The data also showed seasonal peaks in malaria infection with the highest infection rate (34.9 %) during the months of (September - November) and followed by infection rate (32 %) during summer (June- August). However, severe malaria infections occurred in months at the beginning and immediately after rainy seasons. Moreover, its transmission peaks from September to November, coinciding with the major harvesting seasons. This has serious consequences for Ethiopia's subsistence economy as well as in the study area.

Metrological factors such as annual mean rain fall, relative humidity and maximum temperature partly played a role in the high prevalence of malaria in 2007/2008, and low prevalence in 2015/2016 in the study area. However, given the fluctuations of these parameters with prevalence of the disease in the middle of the years suggests that access to availabilities of strong preventive and controlling measures at national level may have been contributed more to the reduction of malaria in the study areas over the years

5.2. Recommendation

Based on this finding, the following recommendation were forwarded

- It is very crucial if patient records at health centers include family identity (ID) so that spatial analysis of diseases can be carried out by linking cases history to the family.
- Further studies should be conducted to determine the status of malaria based on year round primary data to circumvent the problem of documenting and collecting data from secondary sources.

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Appendix 1 The Prevalence of plasmodium species in respect to sexes in each years during the study periods (2007 -2016 years) in health center.

Year	sex	NOS	Malaria Positive									Total		
			Oboro health center			Agamsa health center			Samo health center			Positive		
			Pf	PV	MI	Pf	PV	MI	Pf	PV	MI	Pf	PV	MI
2007	M	4456	170	82	20	247	113	27	484	148	47	901	343	94
	F	3257	112	65	18	146	72	22	272	87	20	530	224	60
	T	7713	282	147	38	393	185	49	756	235	67	1431	567	154
2008	M	4091	196	81	30	67	20	2	469	78	33	732	179	65
	F	2935	161	75	29	48	14	6	297	58	27	506	147	62
	T	7026	357	156	59	115	34	8	766	136	60	1238	326	127
2009	M	3653	105	55	20	124	46	12	315	26	20	544	127	52
	F	2161	72	56	18	75	26	9	202	19	19	349	101	46
	T	5814	177	111	38	199	72	21	517	45	39	893	228	98
2010	M	2176	110	78	23	113	58	22	139	40	16	362	176	61
	F	1379	82	46	20	23	40	21	87	31	16	192	117	24
	T	3555	192	124	43	136	98	43	226	71	32	554	293	085
2012	M	2054	119	75	20	138	55	17	137	17	18	394	147	55
	F	1490	92	55	14	101	33	10	71	12	7	264	100	31
	T	3544	211	130	34	239	58	27	208	29	25	658	247	66
2013	M	2208	124	62	5	195	84	11	114	26	26	441	172	46
	F	1367	96	46	4	124	60	7	71	14	12	299	120	34

Appendix 2 The prevalence Plasmodium species in respect to sex and age groups in Amuru district from 2007 -2016 with respect to studies site.

Study sits	Sex	Nos	Age groups																		Total
			0 -5			6-15			16-30			31-45			46-60			>60			
			Pf	PV	MI	Pf	PV	MI	Pf	PV	MI	Pf	PV	MI	Pf	PV	MI				
obora	M	7832	148	23	-	103	87	2	328	177	40	388	190	89	164	105	19	10	40	1	1965
	F	5192	156	19	-	82	75	1	236	145	37	314	176	81	122	95	16	7	20	1	1583
	T	13024	354	42	-	185	162	3	564	322	77	702	366	170	286	20	35	17	60	2	3548
Aga…	M	8553	226	56	3	64	29	-	230	212	27	642	251	94	57	49	7	10	40	2	1999
	F	6650	140	36	1	29	17	-	279	132	23	377	148	66	33	29	5	6	20	2	1344
	T	15203	366	92	4	93	46	-	509	344	50	1019	399	160	90	78	12	16	60	4	3343
samo	M	13084	195	53	35	297	91	44	571	94	84	601	91	57	259	69	67	129	10	5	2752
	F	8560	119	35	15	209	54	24	384	60	37	320	57	42	167	41	32	89	8	3	1692
	T	21644	314	88	50	506	145	68	955	154	121	921	148	99	426	110	99	218	18	8	4444
Total	M	29469	619	132	38	464	207	46	1129	483	151	163	532	240	480	223	93	149	90	7	6714
	F	20402	415	90	16	320	146	25	899	327	97	1011	381	189	322	165	53	102	48	5	4621
	T	49871	1034	222	54	784	353	71	2028	820	248	2642	913	429	802	388	146	251	138	12	11335
Rates infection % age			9.1	1.9	0.5	6.9	3.2	0.6	17.9	3.2	2.2	23.3	8.1	3.8	7.1	3.4	1.3	2.2	1.2	0.2	100%

Appendix 3 Seasonal variation of prevalence of malaria in Amuru district in ten years from 2007- 2016

Study sits	sex	Nos	Seasons															Total malarial cases
			Winter			Automan				Sammer				spring				
			PT	PV	MI	Nos	PT	PV	MI	Nos	PT	PV	MI	Nos	PT	PV	MI	
obora	M	1158	175	100	14	1125	195	82	17	2728	467	231	52	2948	382	220	61	1996
	F	758	115	83	16	672	119	68	17	1912	384	192	51	2333	333	182	49	1611
	T	1916	290	183	30	1797	314	150	34	4640	851	425	103	5281	715	402	110	3597
Aga…	M	1750	123	70	15	2066	235	77	22	2415	488	234	49	3758	539	205	59	2116
	F	1179	78	48	10	1140	173	50	14	1569	270	135	28	1917	354	151	40	1351
	T	2929	201	118	25	3206	408	127	36	3984	758	369	77	5675	893	356	99	3467
samo	M	2719	347	71	52	3162	509	116	82	3147	482	93	84	3455	656	93	68	2653
	F	1724	231	44	33	1999	298	84	40	1933	296	53	38	2304	410	60	31	1618
	T	4443	578	115	85	5161	807	200	112	5080	778	146	122	5759	1066	153	99	4271
Total		9288	1069	416	140	10164	1529	477	182	13704	2387	940	302	16715	2674	911	308	11335
% age		18.6	9.3	3.6	1.2	20.4	13.5	4.3	1.6	27.5	21.1	8.3	2.7	33.5	23.6	8.1	2.7	100

Appendix 4 The prevalence of plasmodium species with respect to study site and month in Amuru district from 2007 – 2010 years

Study sits	Plas	Months												Total	% age
		Dec	Tan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov		
obora	Pf	150	70	70	60	60	194	320	283	248	289	250	176	2170	19.1
	Pv	80	50	53	29	32	89	160	150	115	150	120	132	1160	10.2
	Mi	15	8	7	8	7	19	53	26	24	62	25	23	277	2.4
Sub total		245	128	130	97	99	302	533	459	387	501	395	331	3607	31.8
Aga…	Pf	70	65	66	122	136	150	270	252	236	320	297	276	2260	20
	Pv	40	39	39	35	42	50	130	123	116	125	118	113	970	8.5
	Mi	10	13	12	10	12	14	30	26	21	40	33	26	247	2.2
Sub total		120	117	117	167	190	214	430	401	373	489	448	415	3477	30.7
samo	Pf	200	192	186	263	269	275	280	259	239	380	355	331	3229	28.5
	Pv	40	38	37	64	66	70	56	48	42	60	51	42	614	5.4
	Mi	30	28	27	35	37	40	50	37	35	40	33	26	418	3.7
Sub total		270	258	250	362	372	385	386	344	316	480	439	399	4261	37.5
Total	Pf	635	503	497	626	661	901	1379	1204	1076	1466	1282	1145	11335	100
% age		5.6	4.4	4.4	5.5	5.8	7.9	11.9	10.6	9.5	12.9	11.3	10.2	100%	

Appendix 5 Correlations metrological variables with total malaria cases in Amuru district from 2007-2016.

Metrological variable	Annual Total malaria case	Std.deviation	r	p- value
Mean Annual Rain Fall (mm)	ATMC	14.46	0.391	O.132
Mean Annual Rel. Humidity	ATMC	5.34	0.657	0.020
Mean Annual Minimum T^0c	ATMC	0.42	-0.376	0.142
Mean Annual Maximum T^Oc	ATMC	1.5	0.128	0.363

Note: STD= Standard deviation, ATMC=Annual total malaria case, r= correlation coefficient

Appendix 6 The correlation between metrological variables and prevalence of malaria with high, medium and low categories in Amuru district from 2007- 2016 years.

Metrological variables	Annual Prevalence of malaria cases	r	p-value
R/ humidity	High prevalence	0.764	o.446
	Medium prevalence	- 0.513	0.657
	Low prevalence	0.850	0.0015
Rain fall	High prevalence	0.327	0.788
	Medium prevalence	0.982	0.121
	Low prevalence	0.044	0.956
Ma/Temperature	High prevalence	0.456	0.698
	Medium prevalence	- 0.197	0.874
	Low prevalence	- 0.260	0.740
Mi/Temperature	High prevalence	- 0.907	0.276
	Medium prevalence	-0.791	0.419
	Low prevalence	-0.049	0.951

Appendix 7 Amuru district nearly recorded monthly rain fall from 200-2016

Mon	Years									
	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
Jan	14.9	0.5	13	29.1	5.9	14.8	2.5	11.5	0.3	0.0
Feb	80.9	2.0	17.1	2.2	1.2	81.2	1.2	-	7.4	7.4
Mar	25.4	0.0	55.1	29	45.9	24	12.8	50.6	37.9	17.6
Apr	98.2	81.7	95.5	80.3	23.5	96.8	29	157.0	11.6	44.6
May	20.5	293.1	17	290.6	229	206	270.9	314.2	-	354.7
Jun	246.4	259.9	229.4	185.6	197	271.5	291.9	205.9	159.1	203.7
Jul	358.6	379.3	339.9	299.3	362.9	397.4	369.5	217.1	325.4	318
Aug	281.1	344.9	350	289.4	210.6	280	222.5	363.1	347.1	342.6
Sep	257.7	191.2	126.1	189.4	292.1	258	188.2	226	200.4	230.6
Oct	88.4	-	179.3	12.6	7.3	88.2	85.1	85.8	21.3	34.2
Nov	0.0	92.8	22.7	10.4	44.6	1.5	69.8	85.5	40.7	0.4
Dec	0.0	10.3	11	13.8	1.9	2.2	0.0	21.2	15.8	10.1
Total	1656.6	1655.7	1456.1	1431.7	1421.	1721.6	1543.4	1737.9	1167	1563.9
Mean	138.05	137.97	121.34	119.3	118.5	143.5	128.6	144.8	97.3	130.3

Appendix 8 Amuru district nearly recorded monthly relative humidity from 200-2016

Months	Years									
	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
Jan	62.4	55	52	48	59.8	62	47.8	54.2	51	40
Feb	56.2	52	50	42	56	40.5	50.2	49	47	-
Mar	48	41	40	41	54.4	52	45.8	56.8	-	-
Apr	60.8	52	45	38	52.4	48.4	41.4	63.8	47.4	40
May	68.8	60.8	55.5	46	68	57.8	67	72.6	-	60
Jun	81.2	80.2	80.2	78.2	81	79	79.2	74.6	76.8	76
Jul	86.8	84.6	78.6	80.6	86.1	85.8	84.2	83.4	79	82
Aug	86	84.2	80.2	83	85	87	83.6	83.8	80.8	82
Sep	82.2	80.1	80	80	82	84	76.8	80.8	77.2	76
Oct	65.4	80.3	79.5	65.4	64	86	72.6	70.8	67.2	66
Nov	59.6	50	48.1	64.2	69.6	50	69.6	63.8	66.4	64
Dec	54.8	45	47	60.2	54	52.2	57	60.8	66.2	41
Total	812.2	765.2	736.1	726.6	812.3	784.7	775.2	765.4	659	627
Mean	67.68	63.8	61.3	60.6	67.7	65.4	64.6	63.8	54.9	52.3

Appendix 9 Amuru district nearly recorded monthly minimum Temperature from 200-2016

Months	Years									
	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
Jan	11.4	10.8	11.2	12.8	10.6	11.7	12.4	11.9	10.8	12.1
Feb	12.3	11.5	12	13.1	11.5	12.8	13.3	12	13.2	13.2
Mar	12.9	13.7	13	12.9	12.3	12.7	14.5	12.8	13.3	14.8
Apr	12.7	12.6	12.9	12.9	13.1	13.5	14.7	12.5	14.4	15
May	12.7	11.7	12.9	11.9	12.2	13.7	12.8	12.5	-	12.9
Jun	11.7	11.3	11.4	10.9	11.7	12.1	11.9	12.6	12.7	12.6
Jul	11.4	11.7	11	11.4	11.3	11.6	11.3	12.1	11.9	12
Aug	10.8	10.8	11.1	11.4	11.2	11.8	11.7	11.1	11.4	11.9
Sep	11.2	10.8	11.2	11.5	11.1	11.7	11.7	11.3	11.3	11.7
Oct	10.4	10.4	11	11.6	9.9	10.2	11.5	11.3	11.3	11.1
Nov	10.3	10.3	9.9	11	10.3	10.5	11.2	11.2	11.6	10.6
Dec	9.8	9.7	10.3	10.5	11.8	11.6	10.3	10.7	11.7	9.6
Total	137.6	134.3	137.9	141.9	137	143.9	147.3	142	133.6	147.5
Mean	11.5	11.2	11.5	11.8	11.4	12	12.3	11.8	11.1	12.3

Appendix 10 Amuru district nearly recorded monthly maximum Temperature from 2007-2016.

Months	Years									
	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
Jan	24.2	25.3	24.1	-	23.7	25.1	24.7	23.9	23.7	24.5
Feb	24.8	25.8	25.2	25.4	26.3	26.4	26.2	24.7	26.1	26.2
Mar	26	27	25.8	26.4	25	25.9	26.2	25.2	25.7	27.4
Apr	24.9	24.8	25.3	-	26.4	25.8	26.8	24.7	26	26.5
May	23.8	23	24.8	24.1	24.2	24.9	23.8	22.8	-	23
Jun	21.3	21.8	23.4	21.4	21.8	21.3	21.4	22.5	22.1	22.3
Jul	29.6	20.3	20.1	20	20.6	21.5	19.5	20.2	21.5	20.6
Aug	19.9	20.3	20.5	19.1	20	19.5	19	19.7	20.9	20.4
Sep	20.9	21.9	22.2	20.6	21.1	21.6	21.2	20.8	21.7	21.9
Oct	22.2	21.8	21.4	21.6	**22.7**	22.8	21.5	21.5	22.8	22.6
Nov	23.9	22.1	22.8	22.1	22.6	22.8	22.7	22.6	23	23.6
Dec	24.4	23.1	23.6	22.1	22.6	22.7	22.9	22.4	23.2	23.9
Total	275.9	277.1	279.2	222.8	277	280.3	275.9	271	256.7	283
Mea	23	23.1	23.3	18.6	23.1	23.4	22.9	22.6	21.4	23.6