



**Trends in prevalence of malaria infection and associated factors in Loka Abaya District,  
Sidama regional state, Ethiopia**

**MSc Thesis**

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**Trends in prevalence of malaria infection and associated factors in Loka Abaya**

**District, Sidama Regional State, Ethiopia**

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**A THESIS SUBMITTED TO THE DEPARTMENT OF BIOLOGY,  
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## **LIST OF ABBREVIATIONS/ACRONYMS**

ACTs	Artemisinin based Combination Therapies
AOR	Adjusted Odds Ratio
CDC	Centers for Disease Control and Prevention
COR	Crude Odds Ratio
FDRE	Federal Democratic Republic of Ethiopia
IRS	Indoor Residual Spray
ITNs	Insecticide Treated Net
LLINs	Long Lasting Insecticidal Nets
MDA	Mass Drug Administration
MOH	Ministry of Health
RBCs	The Red Blood Cells
RDTs	Rapid Diagnosis Tests
USAID	United States Agency for International Development

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## ABSTRACT

*Malaria is deadly vector-borne disease which is caused by Plasmodium species. It is essential to determine the pattern and prevalence of the disease, to evaluate the effectiveness of control and prevention measures. So, this study was aimed to assess the trends in prevalence and associated risk factors of malaria in Loka Abaya district. A retrospective and community based cross-sectional studies were conducted during April to June 2023. The population  $\geq 18$  years were included as study participants and the total sample obtained was 186. The study participants were selected by using systematic random sampling method. Retrospective data was collected from one primary hospital and five health centers in the district. Blood samples were collected from participants through structured questionnaire. The blood samples were examined in Hantate primary hospital. Descriptive statistics were used to summarize data. The association of dependent and independent variables was evaluated using chi-square test, binary and multiple logistic regressions. In the last five years, 34,613 blood films were examined for malaria and 9070 (26.2 %) were found positive. The minimum rate was reported in 2018 (20.3%) and the maximum was in 2020 (30.1%). The males were 52.6% and females were 47.4%. The adults  $\geq 15$  years were (57.2%), 5-14 were (21.6%) and children  $< 5$  (21.2%). The *P. falciparum* infection was (58.8%), *vivax* was (38.8%) and mixed infection was (2.4%). The highest infection rate of malaria was occurred from September up to November. The prevalence rate of malaria from parasitological survey was 18.3%. Sex, occupation and family size were shown significant association with the infection. The presence of stagnant water, habit of using net, window availability and type were associated risk factors. Malaria is still health problem of the study area which needs the strength and effectiveness of prevention and control programs.*

*Key words, Loka Abaya, Malaria, Plasmodium, Retrospective, Risk Factors, Trend,*

# 1. INTRODUCTION

## 1.1 Background of the study

Malaria is one of the deadliest life-threatening vector-borne disease which is caused by a single-celled protozoan species called. It is a serious health problem in many developing countries and one of the major diseases for people living in tropical and sub-tropical areas It is the world's most deadly parasitic disease and is caused by infection with single-celled parasites of the genus *Plasmodium* belonging to the apicomplexan phylum (Workineh Lemma *et al.*, 2021).

In 87 malaria-endemic countries, there were estimated 229 million malaria cases and 409,000 malaria fatalities in 2019. With estimated 215 million malaria infections and 384,000 malaria deaths in 2019, the WHO Africa region accounts for roughly 94% of the worldwide malaria burden. The WHO African region is home to 27 of the 29 nations that account for 95% of all malaria cases worldwide. The global change in malaria cases has not been consistent over the last two decades, and malaria incidence has been dropping at a slower rate in recent years, by only 2% between 2015 and 2019, compared to 27% between 2000 and 2015 (Tamiru Afework *et al.*, 2022).

Malaria continues to be serious public health issue that causes disease and fatalities. According to reports from 85 malaria-endemic countries, the number of cases of malaria worldwide increased from 227 million in 2019 to 241 million in 2020 (WHO, 2020). Due to disruptions caused by the coronavirus pandemic, there have been 14 million more instances of malaria worldwide in the past two years, and 47,000 fewer fatalities from the disease (WHO, 2021). Globally, there were an estimated 247 million malaria cases in 2021 in 84 malaria endemic countries which is increased from 245 million in 2020, with most of this increase coming from

countries in the WHO African regions (WHO, 2022). Humans are infected through the bite of infected female *Anopheles* mosquitoes, which transmit the parasites from one person to another while they are feeding blood (Workineh Lemma *et al.*, 2021). During the past few decades, (LLINs) have significantly contributed to the decline in malaria morbidity and mortality across sub-Saharan Africa (SSA) (Accrombessi *et al.*, 2022). However, the progress achieved so far has stalled in many countries, with some seeing an increased burden in the last few years. COVID-19-related disruptions in the provision of malaria prevention, diagnosis, and treatment appear to have contributed to an increase in the number of cases in 2020 (Accrombessi *et al.*, 2022).

Malaria is one of the major diseases affecting people of low socio-economic status in developing countries. The majority of the global malaria burden is in sub-Saharan Africa, with the highest global cases and deaths that can be observed from these regions, which accounted for the majority (95%) of these cases. Nigeria (27%), the Democratic Republic of the Congo (12%), Uganda (5%), Mozambique (4%) and Niger (3%) accounted for 51% of all cases globally (Bademos *et al.*, 2021). It hurts people's health as well as economic development of many developing countries particularly, in Sub sahara Africa (Bademos *et al.*, 2021).

The most common *Plasmodium* species which malaria infection in human are, *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium ovale*, and sometimes a fifth species, *Plasmodium knowlesi*, which is zoonotic (Duguma Tadesse *et al.*, 2022). *P. falciparum* is the most common pathogenic malaria parasite, responsible for 99.7% of malaria cases in the WHO African area, 70.9% of cases from Central America, 20.8% from South America, and 4.8% from Asia. It is also the parasite most frequently linked to severe illness and

mortality (Mace *et al.*, 2018). The majority of human infections are caused by *P. falciparum*, whereas *P. vivax* has the greatest geographic distribution (WHO, 2018). The World Health Organization (WHO) 2021 malaria report indicates that the WHO African Region continues to bear the largest burden of malaria. In 2020, the African Region was accounted for 95% of all malaria cases (228 million) and 96% of all malaria deaths (Debash Habtu *et al.*, 2022).

Malaria is one of the most distributed human parasitic diseases ranking first in terms of its socioeconomic and public health importance in the tropical and subtropical regions of the world. It is common in tropical and subtropical regions and poses a threat to Ethiopia and other highly endemic areas of Africa. The topography and ecology of Ethiopia vary greatly, which contributes to the highly variable malaria transmission (Getahun Biruk and Abebe Ayalewu Bekel, 2022).

Ethiopia is one of the countries which suffers from malaria disease, which is having a negative impact on the nation's economy and health. The distribution of malaria in the country varies depending on climate, rainfall patterns, and altitude. The large peak in malaria case occurs during the major transmission season from September to December, following the main rains June to August. The second transmission seasons in April and May following short rains are a second but less pronounced peak occurs (Berhe Brhane *et al.*, 2019).

In the country malaria transmission varies from one season to another due to variation in the altitude of areas with a relatively longer duration of transmission in lowland areas, river basins, and valleys. Due to variations in the altitude of places, malaria transmission fluctuates seasonally throughout the nation, with lowland areas, river basins, and valleys experiencing transmission for a comparatively longer period of time. In some highland or highland fringe areas of Ethiopia,

primarily those situated 1,000–2,000 meters above sea level, malaria epidemics are frequent (Berhe Brhane *et al.*, 2019). The *Anopheles mosquito* serves as the primary vector for malaria transmission in the country. *Anopheles arabiensis* is the main vector, followed by *Anopheles phronesis*, *Anopheles funestus*, and *Anopheles nili*, which play a role as secondary vectors. The risk of infection is determined by the number and species of mosquitoes present in a given area and the climate and geography (MoH, 2017).

In Ethiopia, malaria is one of top ten leading causes of morbidity and mortality among children. It is common in children, which ranges from 16% to 54% in the country (Aychiluhm Setognal *et al.*, 2020). It remains a public health problem in the country due to low coverage of insecticide-treated bed nets (ITN), low coverage of indoor residual spraying, drug resistance to malaria, insecticide resistance of malaria vectors, poor access to health care, migration of people from malaria endemic areas to non-endemic areas, and false microscopic results (Aychiluhm Setognal *et al.*, 2020).

Ethiopia is still one of the countries with a high malaria burden. In the country malaria is one of the most important public health issues by causing both mortality and morbidity. Around 75% of the country's land mass is favorable to malaria transmission, with an estimated 68 million individuals at risk for getting the disease. The Ethiopian federal ministry of health reported that out of 1,620,885 suspected cases of malaria from 2015 to 2019, 25% (410,409) tested positive for the disease; *P. falciparum* accounted for 65% of these positive cases, while *P. vivax* for the remaining cases (Workineh Lemma *et al.*, 2021). A 2021 systematic review showed that the highest malaria prevalence was occurred in the Southern Nations, Nationalities and Peoples'

Region (SNNPR) (16.17%), followed by Oromia and Amhara Regional State, (13.11%) and (12.41%) respectively (Kendie Fasil *et al.*, 2021).

Ethiopia is among the nations which are often susceptible to the cyclical epidemics because of the unpredictability of transmission patterns and alterations in the environment (FDREMH report, 2016). For this reason, monitoring and assessing the efficacy of therapies requires looking at the prevalence, course and potential risk factors of malaria infection in endemic locations. However, such useful data remain scarce in several endemic areas of Ethiopia, particularly in parts of Sidama Regional State. So, this study was aimed to assess the malaria trend and its current status with the possible risk factors for transmission in case of Loka Abaya District in Sidama Regional state, Ethiopia.

## **1.2. Statement of the problem**

Malaria continues to be a serious public health issue despite significant advancements and reductions in the disease burden. For a significant portion of the population, including adults and children under the age of five, it is one among the top ten causes of sickness and mortality (Kendie Fasil *et al.*, 2021). In the current study area, malaria continues to pose a serious threat to human health despite various preventive measures being implemented. This is because malaria lowers adult productivity during malaria season, kills children under age five, sickens pregnant women, and lowers the number of children attending school (Workineh Lemma *et al.*, 2021).

Evaluating the pattern and prevalence of malaria and comprehending how it fluctuates in the community due to seasonal, environmental, geographical or annual changes is essential in assessing the efficacy of proven disease control and intervention strategies in a given area

(Alealign Amir *et al.*, 2018). It also assists in locating malaria hotspots and provides crucial information regarding the accomplishments of national malaria programs. It also provides valuable insight into how the malaria situation is evolving, which may help to modify the malaria control initiatives and operations (Alealign Amiret *al.*, 2018). However, such useful information remains scarce in several endemic areas of Ethiopia, particularly in parts of Sidama Regional State (most notably the current study area). So, this study was aimed to assess the trends and prevalence of malaria infection and the associated factors contributing the transmission of disease in Loka Abaya Districts of Sidama region. Hence, the retrospective and community based cross-sectional were carried out among population in the district.

### **1.3. Objectives**

#### **1.3.1. General objective**

The general objective of current study was to assess the trend and prevalence of malaria infection and associated risk factors among population in Loka Abaya district of Sidama Regional state, Ethiopia.

#### **1.3.2. Specific Objectives**

- To assess the five-year (2018-2022) trend of malaria distribution in Loka Abaya District, in terms of year, season, age, sex and *Plasmodium* species
- To determine the current status of malaria infection in the study area.
- To investigate the associated factors of malaria transmission in the study area.

### **1.4 Research questions**

1. What is the trend and current status of malaria prevalence in the study area?

2. What is the malaria distribution in terms of season, age, sex and *Plasmodium* species in the study area?

3. What are the factors contributing the malaria distribution in the study area?

### **1.5 Significance of the study**

- ✓ This study will play a significant role in identifying the risk factors and prevalence of malaria infection in order to design efficient interventions to reduce the spread of the disease.
- ✓ It will provide public information on malaria infection and create awareness among study population on ways of malaria transmission.
- ✓ The results of the study will also provide a foundation for future research on the prevalence and risk factors of malaria parasite infection in the studied area by other researchers.

### **1.6 Scope of the study**

The present study was conducted in the Loka Abaya district of Sidama Regional state Ethiopia, which is well known for its malaria endemicity. The district is also known for its plain land and proximity to the Abaya Lake of Ethiopia. The study assessed malaria trends and prevalence in the district, with possible associated factors. The three localities have been included for the investigation of active cases.

### **1.7 Organization of the study**

The study was organized in seven chapters which are, the Introduction part which contained the (Background of the study, Objectives, Significance and Scope of the study), Literature review,

Materials and Methods which included (Study area description and Design, Study population and Sampling methods), the Result sections, Discussion, Conclusion and Recommendation.

### **1.8 Operational definitions**

**Trend:** The general direction in which something is developing or changing over time.

**Malaria hotspots:** the major reservoir for residual malaria transmission, with higher malaria incidence than neighboring areas, and therefore, have the potential to form the cornerstone for successful intervention strategies.

**Malaria relapse:** the recurrence of asexual parasitemia after drug treatment and the finding of its negativity in a given period of time due to the variety of factors.

**Febrile patients:** Febrile is an adjective that means related to fever. It is a condition in which someone is sick and running a temperature due to the symptoms of the infection.

**Active case detection:** is the operation carried out by surveillance agents who visit every locality in a defined area at regular intervals (usually monthly during the transmission season), in order to test for malaria (and treat if positive) and inquire about fever cases through individual house visits.

**Severe malaria:** develops when infections are made worse by significant organ failures or anomalies in the patient's blood or metabolism.

## 2. LITERATURE REVIEW

### 2.1 Malaria Parasites

Malaria is caused by protozoan parasites of the genus *Plasmodium*, single-celled organisms that cannot survive outside of their host. *Plasmodium falciparum* is responsible for the majority of malaria deaths globally and it is the most prevalent species in sub-Saharan Africa. It poses much of threat to human life than other *Plasmodium* species (Meerstein-Kessel *et al.*, 2018). *P. vivax* is the second most significant species and is prevalent in Southeast Asia and Latin America. *P. vivax* and *P. ovale* have the added complication of a dormant liver stage, which can be reactivated in the absence of a mosquito bite leading to clinical symptoms. *P. ovale* and *P. malariae* represent only a small percentage of infections. Human malaria has been caused by a fifth species of *P. knowlesia* which infects primates however, the precise route of transmission is yet unknown (Komaki-Yasuda *et al.*, 2018).

### 2.2 Malaria Vector

The main vector for malaria transmission are female mosquitoes of the genus *Anopheles*. Female mosquitoes take blood meals for egg production, and these blood meals are the link between the human and the mosquito hosts in the parasite life cycle (Carlos *et al.*, 2019). *Anopheles* are insects that are medically important because of their association with malaria, filariasis and arbovirus infections. *Anopheles*, like any other flying insects, are efficient vectors and by their biting the infection can be easily transferred. There are approximately 3500 species of mosquitoes grouped into 41 genera, but of the approximately 530 *Anopheles* species, only 30–40 transmit malaria in nature (Nicoletti, 2020). They can easily be present in rural and urban habitats, needing only small quantities of water to survive and being able to reproduce in large numbers.

Malaria is transmitted by different *Anopheles* species in several geographic regions, since different environments support different species. Male mosquitoes do not bite, so cannot transmit malaria or other diseases. The small percentage of biting adult females survive long enough to transmit malaria (more than 10 days in tropical climates). In general, these females have limited lifespans. These restrictions are insufficient to stop the spread of malaria anywhere (Nicoletti, 2020). *Anopheles* is found everywhere, with the exception of Antarctica. It is well known that malaria exists in temperate zones as well, albeit the vector is only active during the hot season (Brant *et al.*, 2016).

## **2.3 The life cycle and transmission of malaria**

### **2.3.1 The life cycle of malaria**

The *Plasmodium* species have the same life cycle with only slightly differences from each other. They need a vertebrate host (human) as well as an insect vector (mosquito) to successfully complete their life cycle. During feeding by an infected mosquito, the parasite, *Plasmodium* takes the chance for its successful transmission from vertebrate host to insect vector (Mala *et al.*, 2016). The malaria parasite develops both in humans and in the female *Anopheles* mosquitoes. The size and genetic complexity of the parasite mean that each infection presents thousands of antigens (proteins) to the human immune system. The parasite also changes through several life stages even while in the human host, presenting different antigens at different stages of its life cycle. In addition, the parasite can develop a series of strategies that allow it to confuse, hide, and misdirect the human immune system (Howick *et al.*, 2019).

Malaria infection starts when female *Anopheles* mosquito carrying the *Plasmodium* parasite injects sporozoites into a person's bloodstream through bite. The sporozoites enter the human

liver soon. Over the following seven to ten days, the sporozoites multiply asexually in the liver cells without producing any symptoms. In an animal model, the parasites travel via the heart and lung capillaries after being released from the liver cells in the form of vesicles. There, they settle inside the lung capillaries (Cowman *et al.*, 2016).

In the bloodstream, the merozoites invade red blood cells (erythrocytes) and multiply again until the cells burst. Then they invade more erythrocytes. This cycle is repeated, causing fever each time parasites break free and invade blood cells. Some of the infected blood cells leave the cycle of asexual multiplication. Instead of replicating, the merozoites in these cells develop into sexual forms of the parasite, called gametocytes, that circulate in the blood stream. When a mosquito bites an infected human, it ingests the gametocytes, which develop further into mature sex cells called gametes (Nurye Assefa and Solomon Dejene, 2020).

The fertilized female gametes develop into actively moving ookinetes that burrow through the mosquito's midgut wall and form oocysts on the exterior surface. Inside the oocyst, thousands of active sporozoites develop. The oocyst eventually bursts, releasing sporozoites into the body cavity that travel to the mosquito's salivary glands. The cycle of human infection begins again when the mosquito bites another person. In contrast to the human host, the mosquito vector does not suffer from the presence of the parasites (Zambare *et al.*, 2019).

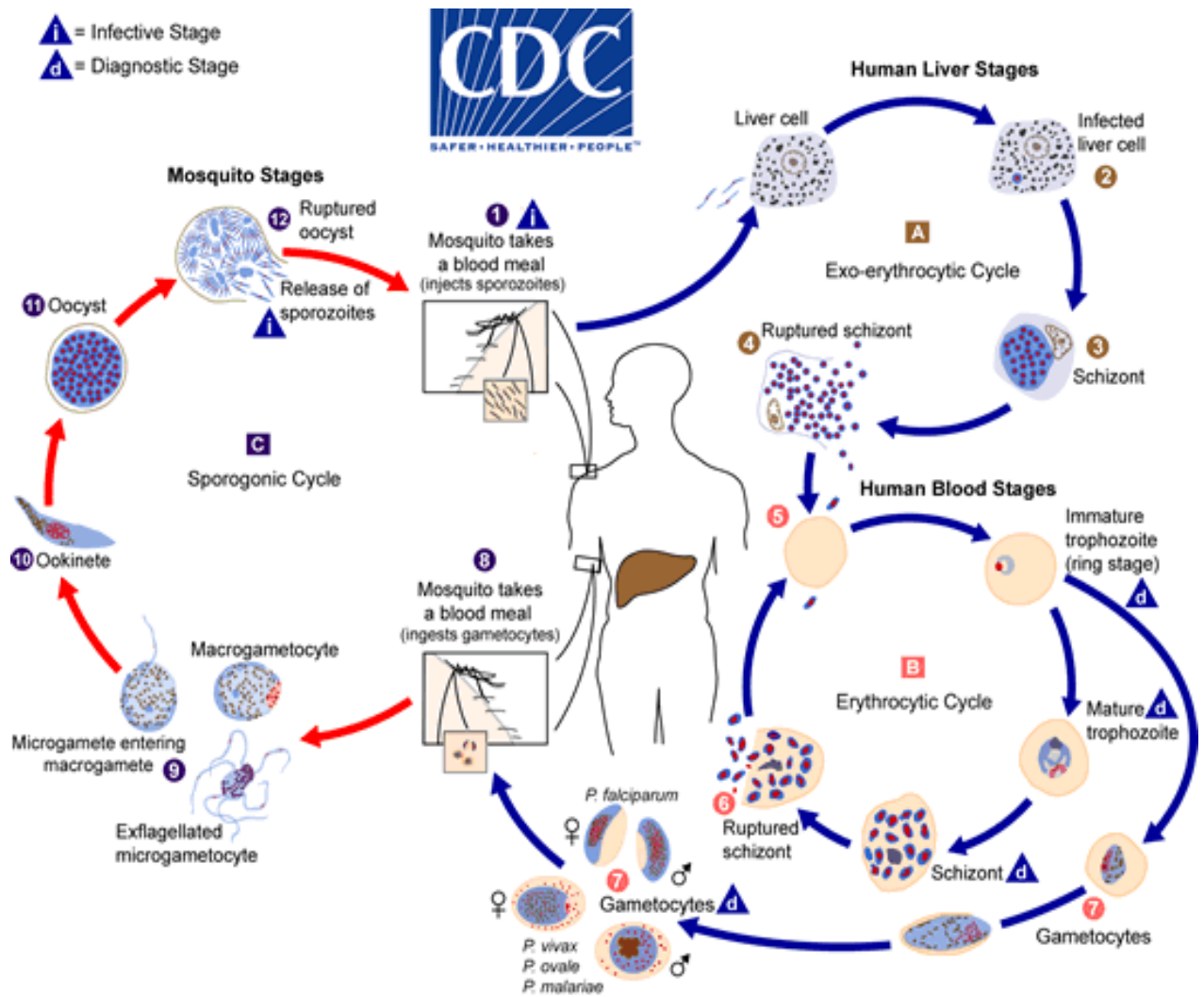


Figure 1: The life cycle of malaria parasite

source: [https://en.wikipedia.org/wiki/File:Life\\_Cycle\\_of\\_the\\_Malaria\\_Parasite.jpg](https://en.wikipedia.org/wiki/File:Life_Cycle_of_the_Malaria_Parasite.jpg)

### 2.3.2 Transmission of malaria

Malaria cannot transfer from one person to another and is not communicable. The disease can only be spread by female *Anopheles* mosquito bites. Certain locations have a higher risk of infection than others due to a variety of variables, including the kind of mosquitoes that live there. Seasonal variations are also possible, with the risk being highest in tropical nations during

the rainy season (Aremu *et al.*, 2022). When a mosquito bites an individual who has malaria, it contracts the disease and subsequently bites a person who is not affected. This is how malaria spreads. Once in the bloodstream of that individual, the malaria parasites proceed to the liver. Red blood cells become infected by the adult parasites as they exit the liver (Okkuneye *et al.*, 2019).

#### **2.4 Pathophysiology and clinical manifestations of malaria**

All the manifestations of malarial illness are caused by the infection of the red blood cells by the asexual forms of the malaria parasite and the involvement of the red cells makes malaria a potentially multisystem disease, as every organ of the body is reached by the blood. All types of malaria manifest with common symptoms such as fever, some patients may progress into severe malaria. Although severe malaria is more often seen in cases of *Plasmodium falciparum* infection, complications and even deaths have been reported in non-*falciparum* malaria as well (Dayanand *et al.*, 2018).

At the completion of the schizogony within the red cells, each cycle lasting 24-72 hours depending on the species of the infecting parasite, newly developed merozoites are released by the lysis of infected erythrocytes and along with them, numerous known and unknown waste substances, such as red cell membrane products, hemozoin pigment, and other toxic factors are also released into the blood. These products activate macrophages and endothelial cells to secrete cytokines and inflammatory mediators such as tumor necrosis factor, interferon- $\gamma$  and interleukins (Beeson *et al.*, 2016). The systemic manifestations of malaria such as headache, fever and rigors, nausea and vomiting, diarrhea, anorexia, tiredness, aching joints and muscles, coagulopathy, and central nervous system manifestations have been largely attributed to the

various cytokines released in response to the parasite and red cell membrane products (Dieye *et al.*, 2016). The infection of the red cells by malaria parasites, particularly *P. falciparum*, results in progressive and dramatic structural, biochemical, and mechanical modifications of the red cells that can worsen into life-threatening complications of malaria. While the vast majority of severe malaria and related mortality are caused by *P. falciparum* infection, complications can occur in non-*falciparum* infections as well. In recent years, several cases of severe infection and even deaths have been reported following infections with *P. vivax* and *P. knowlesi* infection (Hwang *et al.*, 2016).

Several pathophysiological factors such as an inflammatory response, cytoadherence, resetting and sequestration, altered deformability and fragility of parasitized erythrocytes and dysfunction have been found to be involved in the development of severe malaria. All these phenomena are more profound and wide spread in *P. falciparum* infection compared to non-*falciparum* infections (Wassmer *et al.*, 2015).

Asymptomatic malaria can be caused by all *Plasmodium* species in which the patient has circulating parasites but no symptoms (Cheaveau *et al.*, 2019). Uncomplicated malaria can be caused by all *Plasmodium* species. Generally, symptoms occur within 7-10 days after the initial mosquito bite. There are no clinical or biochemical indications of significant organ dysfunction, and the symptoms are non-specific and can include fever, moderate to severe shaking chills, profuse perspiration, headache, nausea, vomiting, diarrhea, and anemia (Mandala *et al.*, 2017). Severe malaria complication includes severe anemia and end-organ damage (cerebral malaria), pulmonary complications (for example, oedema and hyperpnoeic syndrome) and hypoglycaemia

or acute kidney injury. Severe malaria is often associated with hyper parasitemia and is associated with increased mortality (Conroy *et al.*, 2019).

## **2.5 Diagnosis and Treatment of malaria**

### **2.5.1 Diagnosis of malaria**

An accurate and timely diagnosis is essential to managing malaria effectively. The widespread effects of malaria have sparked interest in creating efficient diagnostic techniques, both in industrialized nations where malaria diagnostic proficiency is frequently deficient and in resource-constrained locations where malaria poses a serious threat to civilization. Diagnosing malaria involves determining whether a patient's blood contains malaria parasites or antigens/products. Despite the apparent simplicity, a number of factors can affect the diagnostic efficacy (Zimmerman and Howes, 2015).

Diagnosis can be difficult where the malaria is no longer endemic for healthcare providers unfamiliar with the disease. Physicians may neglect to request the required diagnostic tests and fail to recognize malaria as a possible diagnosis for some patients. When looking at blood smears under a microscope, technicians might not be familiar with or have experience with malaria and miss parasites (Tangpukdee *et al.*, 2009).

The primary method of diagnosing malaria has been via microscopic analysis of blood using blood films. While blood is the most common sample used for diagnosis, less intrusive alternatives such as saliva and urine have also been studied. Though they are not frequently used in malaria endemic areas, more contemporary methods that use polymerase chain reaction or antigen testing have been identified. The only way to diagnose malaria in areas without access to laboratory testing is frequently based on a patient's subjective fever history (Kang *et al.*,

2017). The most economic, preferred and reliable diagnosis of malaria is the microscopic examination of blood films because each of the four major parasite species has distinguishing characteristics. The two sorts of blood film are traditionally used. Thin films resemble standard blood films and enable species identification due to the best preservation of the parasite's appearance during preparation. Thick films are roughly eleven times more sensitive than thin films, enabling the microscopist to screen a greater volume of blood (Makhija *et al.*, 2015). For areas where microscopy is not available or where laboratory staff are not experienced at malaria diagnosis, there are commercial antigen detection tests that require only a drop of blood (Plucinski *et al.*, 2021).

### **2.5.2 Treatment of malaria**

Malaria is treated with prescription drugs to kill the parasite. The types of drugs and the length of treatment will vary, depending on, which type of malaria parasite a person have, the severity of symptoms, age and the pregnancy status (WHO, 2015). Each year, more than 600 000 people die of malaria a treatable disease. The primary objective of treatment is to ensure the rapid and complete elimination of the parasites causing the disease from a patient's bloodstream in order to prevent an uncomplicated case of malaria from progressing to severe disease or death. A successful course of therapy inhibits the infection from spreading to other people and stops the development and spread of resistance to antimalarial drugs (WHO, 2019).

Treatment for malaria must begin as soon as possible. Medicines for malaria don't work on certain parasites. Certain medications are administered with other medications. What kind of medication you take and how long you take it depends on the type of parasite. Artemisinin medications, such as artesunate and artemether, are antimalarial medications (Saito *et al.*, 2018).

If treatment for *Plasmodium falciparum* infection is available, combined therapy with artemisinin is the most effective. Atovaquone (Mepron), Chloroquine (certain parasites are resistant to this therapy), Doxycycline, Mefloquine, Quinine, and Primaquine are some further anti-malarial medications. Malaria can be cured with medication (Naing *et al.*, 2019).

Malaria can result in major health issues, including death and irreversible organ damage, if it is not treated appropriately. If you believe you have malaria, or if you have traveled to a region where malaria is prevalent, you must get treatment immediately. Early initiation of treatment yields greater efficacy. Malaria can be treated and infections removed from the body with the right treatment and dosage. If an infected mosquito bites you, you could get reinfected (Brunner *et al.*, 2021).

## **2.6 Factors affecting the distribution of malaria**

### **2.6.1 Climatic factors**

The pattern and severity of malaria transmission in Ethiopia, Africa, and the rest of the world are significantly influenced by climate conditions. Temperature, precipitation, and humidity are the main meteorological variables that have a direct impact on the spread of malaria (Panzi *et al.*, 2022). The development of the malaria parasite and its mosquito vector, which controls malaria transmission, is significantly influenced by the ranges of minimum and maximum temperatures. The malaria parasite's life cycle is impacted by temperature. About ten days are needed for the parasite to finish developing in the mosquito's gut, though this can vary according to the temperature (Shapiro *et al.*, 2017). Anopheline mosquitoes reproduce in water. Thus, for them to reproduce, the proper quantity of rainfall is frequently necessary. Different kinds of water bodies are preferred by different anopheline mosquito species for breeding. Malaria

transmission peaks during the rainy season in Ethiopia because water collections that facilitate vector breeding mostly emerge after rainfall (Dabaro Desalegn *et al.*, 2021). Higher humidity levels are more conducive to mosquito survival. They also become more active when humidity rises. This is why they are more active and prefer feeding during the night the relative humidity of the environment is higher at night. If the average monthly relative humidity is below 60%, it is believed that the life of the mosquito is so short that very little or no malaria transmission is possible (Mathew, 2020).

### **2.6.2 Non climatic factors**

Non-climatic factors are those that influence the spread of malaria but have nothing to do with the weather. The degree of immunity that human hosts have to malaria, the type of vector, the parasites, environmental development and urbanization, population movement and migration, insecticide resistance in mosquitoes, and drug resistance in parasites are all factors that affect the severity and incidence of malaria (Chirombo *et al.*, 2020).

Different species of anopheles mosquitoes have different capacity of malaria transmission. This depends on the biology and behaviour of the mosquitoes. Mosquitoes in the *Anopheles gambiae* group which includes (*A. arabiensis*), are the most efficient malaria vectors in the world. These mosquitoes are found only in Africa. In fact, the higher incidence of malaria in Africa compared to the other parts of the world is mainly due the efficiency of these mosquitoes in transmitting the parasites (Sinka *et al.*, 2020).

In some areas malaria infection caused by *P. falciparum* is more common while, in other areas *Vivax* malaria is more common. *Falciparum* malaria is more common in Africa than in other parts of the world, and this is one reason why there are more deaths from malaria in Africa

than elsewhere (Seyoum Dinberu *et al.*, 2017). Increased malaria transmission results from the growth of agriculture, especially when irrigation is used. This produces breeding grounds for malaria mosquitoes. For instance, flooding agricultural area with irrigation during rice cultivation has long been linked to an increase in the number of malaria vectors and an increase in the disease load. In Ethiopia's lowlands, irrigated cultivation and rice agriculture are becoming more widespread (Haileselassie Werisawu *et al.*, 2021).

The incidence of malaria is generally lower in urban areas than in rural areas. There are a number of reasons for this. When there is plenty of space for vector breeding in rural villages, mosquito breeding sites in urban areas are limited because more space is covered by houses. The main vectors of malaria in Ethiopia and elsewhere in Africa, are mosquitoes in the *A. gambiae* group, which breed in clean water; most water collections in urban settlements are polluted and unfavorable for mosquito breeding (Abebaw Abtie *et al.*, 202

Differences in human hosts also affect the pattern of malaria transmission and the severity of the disease. When it comes to malaria, people are either immune, or non-immune. Immune people often have a better chance of tolerating the effects of malaria and surviving the disease than non-immune people. In highly endemic areas, children under five years of age and pregnant women are the most at risk, because they have weak immunity to malaria infection (Acharya *et al.*, 2017). The mosquitoes develop insecticide resistance, which means that they are no longer killed by the insecticides. This means a large number of mosquitoes will survive in the community, and the risk of malaria infections rises and many people can be affected (Ochomo *et al.*, 2017).

If drug-resistant malaria parasites are not cleared by treatment from infected individuals, they are easily picked up by vector mosquitoes, and transmitted to new susceptible individuals who then develop drug-resistant malaria. Moreover, more people who are not getting cured through the drug treatment means that more will die of malaria (Blasco *et al.*, 2017).

## **2.7 Epidemiology of malaria infection**

### **2.7.1 Global distribution of malaria**

According to the latest world malaria report, the global cases of malaria reached 247 million in 2021 compared to 245 million in 2020 and 232 million in 2019 (WHO, 2022). The estimated number of malaria deaths stood at 619 000 in 2021 compared to 625 000 in 2020. Over the two peak years of the pandemic (2020–2021), disruptions related to COVID-19 caused an estimated 13 million additional cases of malaria and 63 000 additional deaths from the disease (WHO, 2022). The WHO African region continues to bear an excessively large portion of the global malaria burden. In 2021 the region was home to about 95% of all malaria cases and 96% of deaths. Children under 5 years of age accounted for about 80% of all malaria deaths in the region. Four African countries accounted for just over half of all malaria deaths worldwide, Nigeria (31.3%), the Democratic Republic of the Congo (12.6%), United Republic of Tanzania (4.1%) and Niger (3.9%) (WHO, 2022).

The 2022 edition of the report finds that despite disruptions to prevention, diagnostic and treatment services during the pandemic, countries around the world have largely held the line against further setbacks to malaria control (WHO, 2022). Even with extensive international and national efforts being made to control and eradicate the disease, malaria remains the most serious parasitic infection in the world. The worldwide effort to eradicate malaria, which began in 1969,

proved to be unsuccessful as hundreds of millions of people contracted the disease, tens of millions of people died from malaria-related causes (primarily in sub-Saharan Africa), thousands of pregnant women died from complications related to the disease during childbirth, and millions of children were born underweight, leading to early death or disability. However, the first two decades of the current century in the new millennium represent a golden era in history of malaria control (Al-Awadhi *et al.*, 2021). The risk of vector-borne illnesses like malaria has also grown as a result of changes in the temperature and ecology brought on by global warming. The epidemiological features of imported malaria have evolved in many malaria-free and non-endemic nations as a result of increased travel for business or pleasure, as well as other migratory movements brought on by employment opportunities or geopolitical conflicts (Mischlinger *et al.*, 2020).

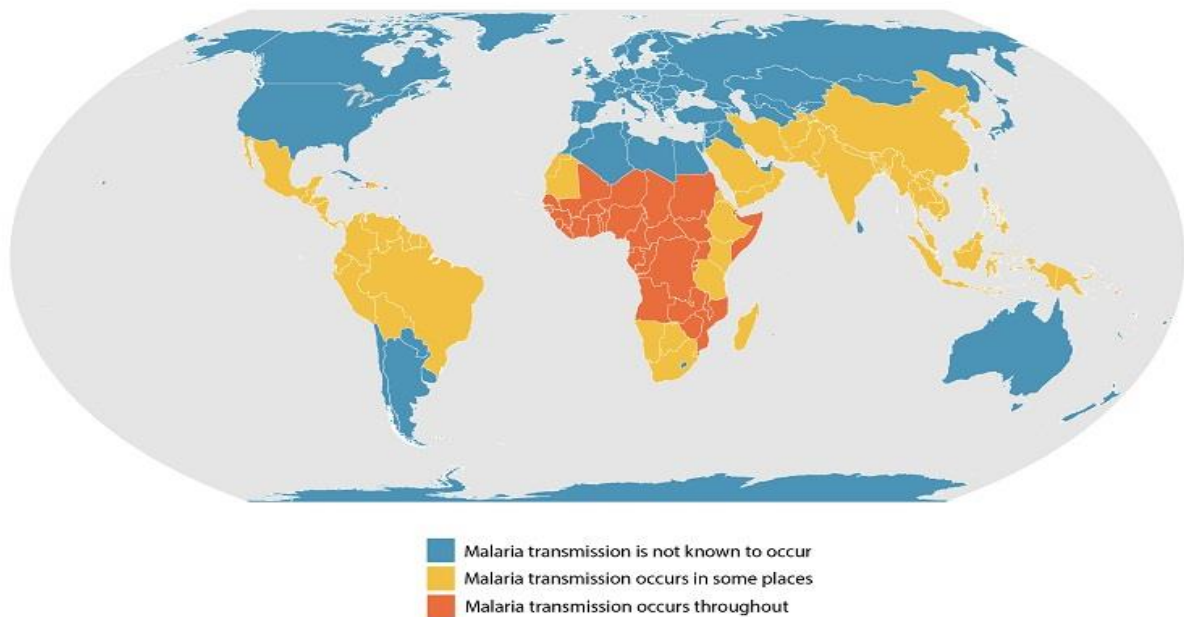


Figure 2: Global distribution of malaria and risk.

Source: [Malaria-world Map](#)

### **2.7.2 Prevalence of malaria in Ethiopia**

Malaria is a major public health and economic issue in Ethiopia. Location-specific factors such as altitude, rainfall patterns, and climate affect the distribution. As one of the main causes of illness and death in the nation, it is a serious problem. Malaria cases afflict 75% of Ethiopia's landscape areas below 2000 meters above sea level (Kendie Fasil *et al.*, 2021). The major epidemics occur cyclically in every 5–8 years in Ethiopia, but focal epidemics are occurring every year. Ethiopia has a population of above 100 million, of which approximately 68% of the population is at risk of the disease. About 2.9 million and 4782 related deaths of malaria have been reported annually and the rate of morbidity and mortality dramatically increases during epidemics (Kendie Adugna *et al.*, 2022).

Ethiopia is one of the countries that have implemented the revised strategies to control malaria. Among these, indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) are the most important in malaria prevention and control strategy. Additionally, introduction of rapid diagnostic tests at community level and adaptation of artemisinin-based combination therapies (ACTs) are also practiced in Ethiopia (Kenea Oljira *et al.*, 2019). Despite the massive efforts to implement these strategies, malaria continues to cause significant morbidities and mortalities in the endemic foci of Ethiopia. On the other hand, the developments of insecticide resistance on IRS and LLINS in different parts of the country have its own impact to control the main malaria vectors (Kendie Fasil *et al.*, 2021).

In Ethiopia, the burden of malaria infection continues to cause the substantial number of morbidity and mortality which accounts for most outpatient visits. Malaria has been one of the main causes of hospitalization and deaths in the country. About 60% of the population are living

in the malaria risky areas, mainly areas that lies below 2000 m above the sea level (Esayas Endashaw *et al.*, 2020). However, several pockets with micro-epidemiological conditions supporting malaria transmission occur in areas above this altitude. The *P. falciparum* and *P. vivax* are among the dominant parasites responsible for the majority of malaria cases in the country. The contribution of other *Plasmodium* species in the country is negligible (Esayas Endashaw *et al.*, 2020).

The distribution and transmission pattern of malaria in Ethiopia has a variation from place to place, which mainly depends on climate, rainfall patterns, and altitude. The transmission of malaria is generally unstable and seasonal (Alemu Abebe *et al.*, 2012). The transmission and incidence rate of malaria infection is determined by different factors such as environmental condition, human host, parasite and vector. Malaria intervention requires the proper identification of factors influencing malaria risk in the community (Esayas Endashaw *et al.*, 2020).

In Ethiopia, several factors such as, socio-demographic, place of study, house conditions, breeding site of mosquitoes, malaria control and prevention practices, human sleeping behavior and knowledge and practice of people about malaria have been identified as risk factors for malaria infection (Tadesse Frewu *et al.*, 2018). A Systematic Review and Meta-Analysis conducted in Ethiopia, based on the collected studies of different areas revealed, the pooled prevalence of malaria among adults in Ethiopia was 13.61%. Subgroup analysis based on types of malaria cases showed that the prevalence of malaria among symptomatic and asymptomatic adults was 15.34% and 11.99%, respectively (Kendie Fasilet *et al.*, 2021). Similarly, regional subgroup analysis showed that the highest malaria prevalence was recorded in Southern Nations,

Nationalities, and Peoples' Region (SNNPR) (16.17%) followed by Oromia Regional State (13.11%) and Amhara Regional State (12.41%). Adults had a pooled prevalence of malaria that was higher than that of the general population and almost as high as that of pregnant women. Thus, it is necessary to reinforce the current vector- and parasite-related prevention and control efforts (Kendie Fasil *et al.*, 2021).

## **2.8 Prevention and controlling methods of malaria**

### **2.8.1 Vector control**

Vector control is a highly effective way to reduce malaria transmission and is a vital component of malaria control and elimination strategies. WHO currently recommends deployment of either insecticide-treated nets (ITNs) or indoor residual spraying (IRS) for malaria vector control in most areas at risk of malaria (WHO, 2019). Malaria vector control requires periodic collection and interpretation of data based on the local vector species, the potential invasion by vectors from other geographical areas, their susceptibility to insecticides and vector and human behaviours. Ongoing monitoring of the coverage, usage, quality and durability of vector-control interventions is also vital (WHO, 2019).

### **2.8.2 Insecticide treated net**

Mosquito nets help keep mosquitoes away from people and reduce infection rates and transmission of malaria. Nets are not a perfect barrier and are often treated with an insecticide designed to kill the mosquito before it has time to find a way past the net. Insecticide-treated nets (ITNs) are estimated to be twice as effective as untreated nets and offer greater than 70% protection compared with no net. Most nets are impregnated with pyrethroids a class of insecticides with low toxicity (Pryce *et al.*, 2018).

### **2.8.3 Indoor residual spraying**

Indoor residual spraying is the spraying of insecticides on the walls inside a home. Most of the insecticides having residual effect are sprayed indoors, so that mosquitoes after having bite on an infective person will rest in the house and will pick up sufficient insecticide particles sprayed on the walls and other indoor surfaces of the house and its longevity will be reduced so much so that it does not survive to become infective (Bath *et al.*, 2021). In areas where the vectors are strongly endophilic, they tend to rest indoors, indoor residual spraying of human dwellings can give very effective control. Vectors that are exophilic which tend to rest outdoor but, feed or rest indoors briefly, can be effectively controlled by indoor residual spraying with insecticides that have good airborne effect (Bath *et al.*, 2021).

### **2.8.4 Housing modification**

House modification methods, such as screening (covering or closing potential house entry points for mosquitoes with mesh or other materials) or the use of specific house materials or designs, such as metals roofs instead of thatched roofs, or elevated rooms, may contribute to reducing the burden of malaria (Fox *et al.*, 2022). They work by preventing mosquitoes from entering houses, and reducing the number of bites householders receive indoors. Some house modifications under consideration additionally aim to kill any mosquitoes that attempt to enter houses by incorporating insecticide into the modification (Fox *et al.*, 2022).

### **2.8.5 Public health education**

The practice of malaria prevention is mainly related to the level of knowledge and belief of people. The understanding of the possible causes, modes of transmission, and decision about adoption of preventive and control measures may vary from community to community and

among individual households (Diema Konlan *et al.*, 2019). An adequate knowledge of mothers of under-fives about malaria has a great correlation with reduced morbidity and mortality among children less than five years. However, a lot of misconceptions concerning malaria still exist. Thus, local knowledge and practices related to malaria are important for the implementation of the culturally appropriate, sustainable, and effective interventions (Diema Konlan *et al.*, 2019).

## **2.9 Challenges for the management of malaria infection**

### **2.9.1 Resistance of *Plasmodium falciparum* to anti-malaria drugs**

Resistance to first-line treatments for *Plasmodium falciparum* malaria and to the insecticides used for Anopheles vector control is threatening malaria elimination efforts (Rahmassari *et al.*, 2022). Artemisinin and its derivatives provide the fastest parasite clearance among available antimalarial drugs and have been combined with an antimalarial drug of a different class in order to enhance complete cure rates, shorten the duration of therapy for artemisinin monotherapies, and delay the selection and spread of resistant parasites (Rahmassari *et al.*, 2022). Artemisinin based combination treatments, are currently recommended for the management of uncomplicated malaria cases.

### **2.9.2 Resistance of Anopheles mosquito vectors to insecticides**

The resistance of malaria vectors to the four insecticide classes of (pyrethroids, organochlorines, organophosphates, and carbamates) used for vector control interventions, threatens the prevention and control efforts (Carrasco *et al.*, 2019). Behavioural resistance for the insecticides may be an important factor restraining the efficacy of vector control. Understanding the mechanisms of insecticide resistance is a key to predicting how resistance may emerge, spread and hinder control interventions (Carrasco *et al.*, 2019).

### **2.9.3 Asymptomatic malaria infections and mass drug administration (MDA)**

One of the major problems to achieve the malaria elimination is represented by the hidden parasite reservoir in the human host. Microscopy (and rapid diagnostic tests (RDTs)) underestimates by about half the prevalence of *Plasmodium falciparum* infection and this difference is greatest in low transmission settings. Many asymptomatic infections persist for significant periods of time. The presence of *Plasmodium falciparum* gametocytes is positively associated with the absence of clinical symptoms and low parasite densities (Cheaveau, *et al.*, 2019). Mass drug administration aims to provide the full post-treatment courses to the whole population to clear asymptomatic infections and provide post treatment prophylaxis to prevent reinfection (Brady *et al.*, 2017).

### 3. MATERIALS AND METHODS

#### 3.1 Description of the study area

The study was conducted in Loka-Abaya district, which is located in South western part of Sidama regional state. It is located in South of Addis Ababa about 345 km away and 72 km from the regional capital, Hawassa. It is bordered to the South by Aleta Chuko district of Sidama region, on the West by the Bilate river, which separates it from Wolayita zone of South Ethiopia, on the South west by Lake Abaya, on the North by Dale district and on the East by the Boricha district of Sidama region.

The district is geographically situated between  $6^{\circ}39'06.75''\text{N}$  and  $38^{\circ}11'22.39''\text{E}$  with the altitude of 1633 m above sea level. It has bimodal rainfall, July to September (heavy rains) and February to April (light rains), the remaining months of the year are fairly dry. The mean annual rainfall and temperature in the area are 1,001-1,400 mm and 17.6-25 °C, respectively. The district has the total population of 114,306 from which males are about 53, 953 and females are about 60, 353 (The districts Office of Finance and Economic Development).

The district has 23 Kebele administrations and from which 11 of them are known by their malaria endemicity. There are five Health centers and one primary Hospital in the district. According to the 2014 official report of the district Office of Agriculture Development (OAD) the district has a total area of 119,000 ha and the crude density is estimated 90 persons per km square. According to traditional classification systems, the district is characterized by mid-lowland and low land agro-ecologies (The district OAD). These are lowland livestock dominant livelihood system and midland/lowland cereal crop and livestock mixed livelihood System. Malaria transmission occurs on annual basis in the district although the severity of the transmission typically limited to September up to November (The Districts Health Office).

Concerning the malaria control program, priority is given to the lowland malarious localities for spraying of indoor residual spray (IRS) and long-lasting insecticide-treated nets (LLINs) are distributed to all rural and urban localities of the district's (Personal communication, Head of the districts health offices). There is lack of integration between the district's health and agricultural office, in conducting developmental activities in communities that are at high risk of malaria transmission. For instance, terracing activates conducted by community members have been creating suitable mosquito breeding sites near the farm.

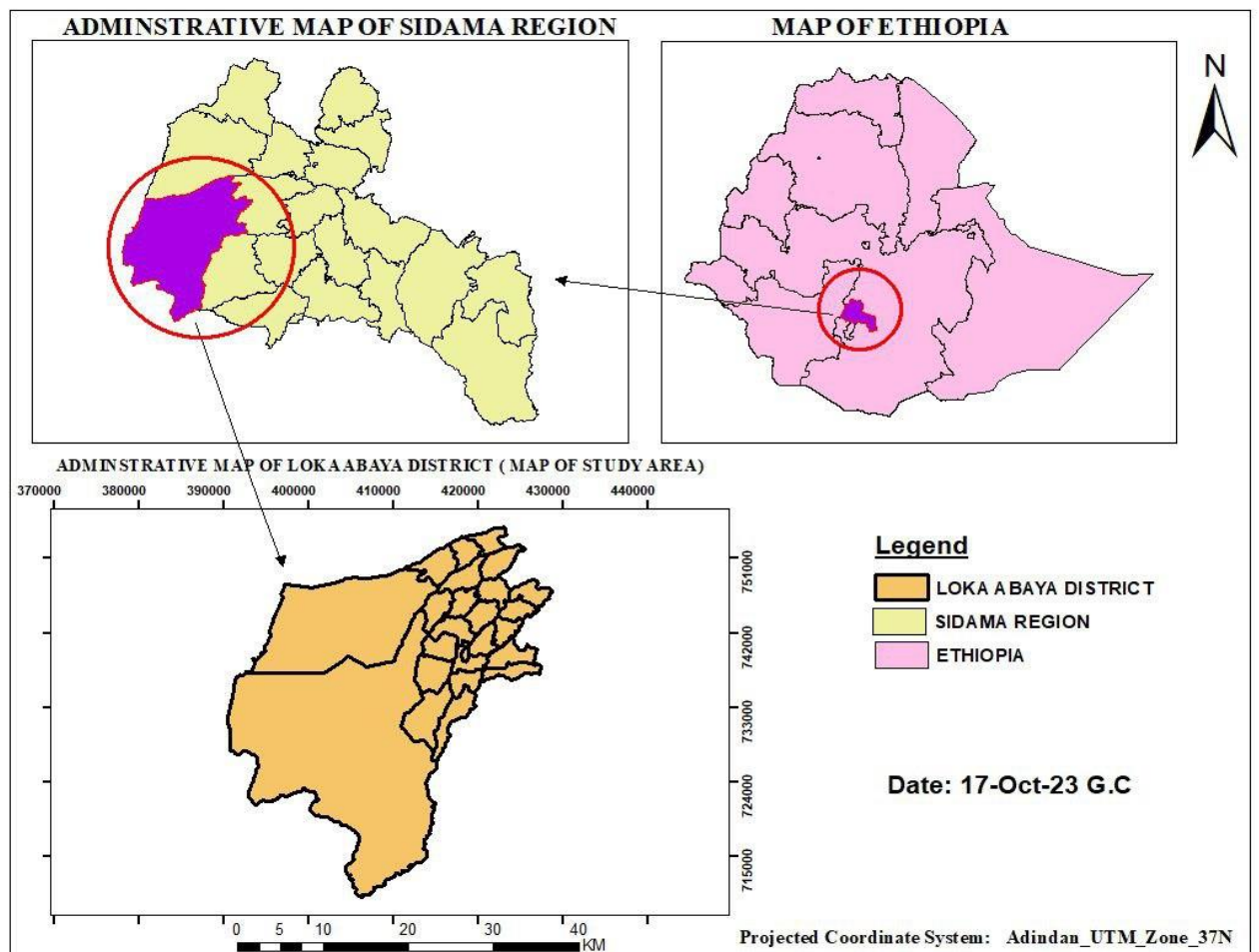


Figure 3: Map of the study area.

Source: GIS (2023)

### **3.2 Study design and period**

The health facilities and community based cross-sectional study were conducted during April to June 2023. A retrospective study was conducted to analyze the malaria trend by using the malaria data over five years (2018–2022) recorded in the five health centers and one primary hospital. The parasitological survey was conducted to assess the current status of malaria and household related questionnaire was collected to assess the associated factors, in selected localities of the study area.

### **3.3 Study population**

The study participants were populations of age  $\geq 18$  years, with the symptom of febrile case in purposely selected kebeles of the district.

### **3.4 Sample size calculation for parasitological survey**

Estimation of the sample size for the assessment of malaria prevalence was based on the values from the previous cross-sectional study conducted in other part of Ethiopia. It was calculated from similar study in conducted in Bahirdar zuriya district (Dejazmach Zelalem *et al.*, 2021). Employing assumptions of expected prevalence = 14.0%, margin of error  $d = 5\%$ ,  $\alpha = 5\%$  (95% confidence level) and 10% non-response rate, was applied to get final sample size (Andrade, 2020). The sample size was determined by using the formula for estimating single population. Based on this, the total sample will be calculated as follows:

$$n = \frac{Z^2 * p (1-p)}{d^2}$$

Whereas:

$n$  = sample size

$Z = 1.96$  ( $Z$ -score corresponds to 95% confidence interval),

p= (Prevalence from previous studies) and

d= 0.05 (margin error). Then,

$$n= \frac{(1.96)^2 * (0.14) (0.86)}{(0.05)^2} = 174$$

By adding 10% of non-response rate, the final sample size calculated was 190.

### **3.5 Sampling method**

The study population (households) were identified by using the systematic random sampling technique through proportionally from three localities. First the source population was stratified in to rural localities and urban localities). The rural localities in different altitudes (IRS is not sprayed in relatively high-altitude localities) and urban localities were selected purposively from the total rural and urban localities. The morbidity of malaria record for last months before the study begun, proximity to the laboratory center and moderate population density were considered during the selection of the localities.

Based on this, three localities (Kura, Argeda Harodinto and Danshe Gambela) were selected among the total of 11 malaria hotspot kebeles. The number of households to be taken from the three localities were determined by proportionally based on population density of the localities (85, 55 and 50 from each). The households for each locality were determined through systematic random sampling method (by using the household lists of selected kebeles). After the households were identified, individuals above the age of 18 with the symptoms for febrile case (febrile patients) were selected purposively for the parasitological survey. The same study participants were used for the questionnaire survey during the study period.

### **3.6 Inclusion Criteria & Exclusion Criteria**

#### **3.6.1. Inclusion criteria**

The study was included the participants that were permanent member in the community and able to give informed consent through their voluntary participation. For malaria parasitological survey, members of the selected households with febrile cases were included except those who were on anti-malaria therapy during the study period.

#### **3.6.2 Exclusion Criteria**

For questionnaire survey collection, children < age 18 and the community members who were unable to communicate and mentally impaired were not included the study. Individuals who were taking anti-malarial therapy or who had been treated with anti-malarial drugs within the past 2 weeks prior to the study were excluded from the study.

### **3.7 Methods of Data collection**

#### **3.7.1 Retrospective data collection**

To assess the trend of malaria prevalence in the study area, a five-year (2018-2022) data on the malaria was carefully reviewed from the institutional malaria laboratory registry book of the health facilities. All public health facilities (five health centers and one primary hospital) that provide malaria diagnosis and treatment service were included in the study. All facilities use the same kind of malaria laboratory register, so that the variables captured were similar. A well-prepared checklist (data collection format) was applied to collect data for the malaria trend in the study area from the health facilities. All laboratory registers of malaria morbidity were collected from the health facilities. Then the malaria morbidity data were transferred from the registers on the prepared data collection format and then finally to the Excel spreadsheet. The

parameters such as date of examination, total clinically treated and confirmed cases in months and years, types of malaria species, tools of diagnosis and socio-demographic data such as age and sex were collected. The records which missed one or more of these variables were excluded from the study.

### **3.7.2 Blood sample collection and examination**

The current malaria prevalence survey was carried out in the study population. Blood sample was collected from a participant who were voluntary participated in study by giving informed consent. Finger prick blood collection was done by trained professionals, from the purposively selected participants (febrile patients) through house-to-house visit. The finger of patients was cleaned up with 70 % alcohol before pricking. Then prick site was exposed to dry and the first drop of blood was removed with the dry cotton to prevent the external contamination.

The thick and thin blood smears were prepared on the same frosted slide side by side. The smears were air dried and the thin blood smear was fixed with methanol at the sites. The samples were packed in the slide box and carefully transported to the central laboratory (Hantate primary Hospital) where parasitological examination by microscopy was performed. All the parasitological examination of blood sample was conducted by following the protocols established (Appendix 1).

### **3.7.3 Questionnaire Survey**

The participants completed the structured questionnaire while a blood sample was being taken. It covered the participants' sociodemographic details as well as potential risk factors for the spread of malaria. The questionnaire was developed first in English language and then translated to the local language (Sidamu Afo) and administered to the study participants. After the purpose of the study was clearly explained to the study participants, questionnaire was filled out while

the blood sample collection. The heads of households helped by providing the necessary details. The questionnaire translated back to English in order to check its validity. The survey data was collected by using Kobo tool box.

### **3.8 Data processing and analysis**

The lead investigator reviewed the questionnaire and any discrepancies were confirmed with the respondent. The Statistical Package for the Social Sciences (SPSS version 20.0) and Microsoft Excel were used for the statistical analysis. The patterns of malaria transmission by season, year, gender, age, and malaria parasite species were displayed using descriptive statistics. Tables, figures, and percentages were used to display the data. The Chi square test was used to describe the associations of malaria infection with independent variable (sex, age, seasons). The significant association between independent variables with dependent variable was analysed by using bivariate logistic regression analysis. The proportional impact of each independent variable to the dependent variable was examined by using multivariate logistic regression. Test of significance was estimated by assuming  $\alpha$  at 0.05 and a P-value less than 0.05 was considered as Statistically significant.

### **3.9 Ethical considerations**

The collage ethical review committee was reviewed the proposal in accordance with the rules and regulations of the Hawassa University. Official letter of cooperation was written to all responsible bodies. Permission to conduct the study was got approval from the concerned authorities. The objective of the study explained in detail to the participants and other responsible bodies. All the data and information were maintained confidentially by using a code rather than the participants' name. Positives had been treated without a fee, and health education about prevention and control of malaria was given to study participants.

## 4. RESULTS

### 4.1 The annual trend of malaria infection in Loka Abaya district from 2018-2022

During the last 5-years (2018 to 2022), a total of 34,613 blood films, from malaria-suspected patients were requested for malaria diagnosis in the districts health facilities and examined to detect the presence of *Plasmodium* parasite. Of the total suspected cases, 26,587 (76.6%) were diagnosed by microscope and the remaining 8026 (23.4%) by RDT (rapid diagnostic test). Out of the blood films that were diagnosed, 9070 were tested positive for malaria, yielding a total prevalence of 26.2%. There were fluctuating trends of malaria within the last five years with the, least diagnostically confirmed malaria positive cases, 1106 (20.3%) which reported in 2018 and the largest number of positive cases, 2188 (30.1%) which reported in 2020 in the study area (figure 4). The data has revealed that malaria infection increased from 20.3% in 2018 to 28% in 2019 and it increased to 30.1% in 2020. The infection was decreased to 21.6 % in 2021, but it risen to 29.2% in 2022 (figure 4).

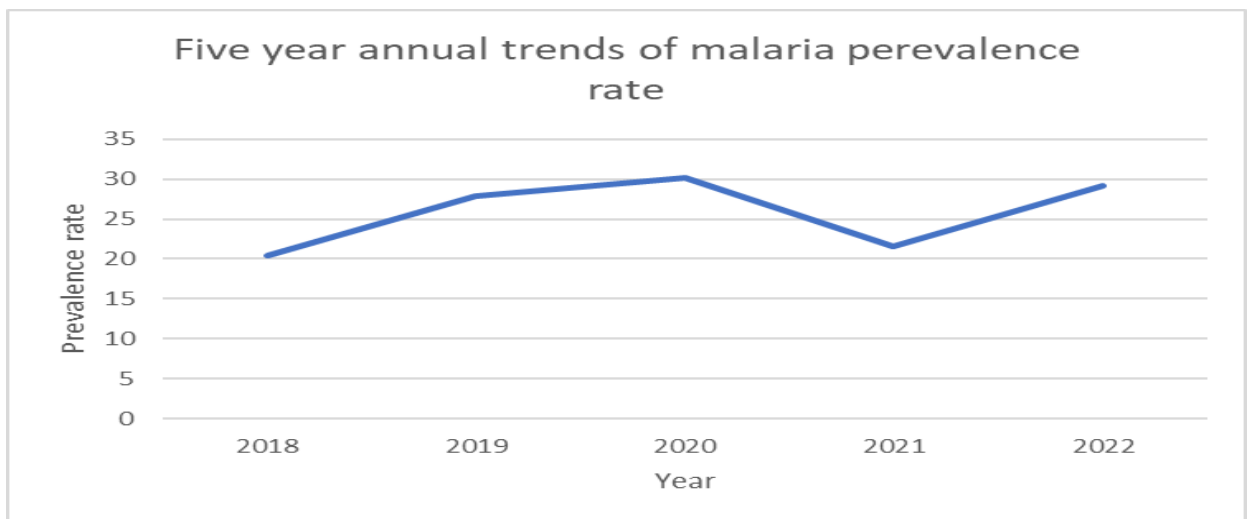


Figure 4: The blood film positivity rate for five years (2018-2022) in Loka Abaya District

## 4.2 Trends of malaria cases among sex and age groups

Out of 9070 positive outpatients, 4767 were males and 4303 were females. The infection rate of males was 52.6% and the females was 47.4%. Infection rates peaked in 2019 for males 57% and in 2018 for females 51.6%. The males were more infected than females in 2019, 2020, and 2022 with the prevalence rate of (57 %, 52.6% and 52.8%) respectively. The females were more infected than males in 2018 and 2021 with the prevalence rate of (51.6% and 50.2%). There was a minor overlap in the prevalence rate of malaria infection between the sex groups. The malaria infection was significantly associated with sex in the study area ( $\chi^2 = 362.301$ ,  $df = 3$ ,  $p < 0.041$ ) (Figure 5).

Regarding the age groups, malaria infection was recorded among all age groups. The age-specific prevalence rate of malaria was 21.4% for the age group < 5 years old, 21.6% for the age group between 5-14 years old, and 57% for the age group  $\geq 15$  years old. Although malaria infections were reported in all age groups, the most susceptible age groups were  $\geq 15$  years old with an overall prevalence of 57%, followed age group from 5 to 14 years old with a total prevalence of 21.6% and the least susceptible age groups were <5 years old with the total prevalence of 21.4%. The rates of malaria infection in children under age of 5 and those aged 5 to 14 were relatively similar (Figure 5).

In the age group <5 years old, the highest infection rate was occurred in 2021 (27.5%) and the least infection rate was occurred in 2019 (18.1%). For the age groups between 5 and 14 years old, the highest infection rate was occurred in 2020 (23.5%), and the least infection rate was occurred in 2018 that (19%), and for the age group  $\geq 15$  the highest infection rate was occurred in 2018 60.5% and the least infection rate occurred in 2021 53% in the study area.

Males of age group <5 were more infected in 2021 (29.5%) and females of the similar age were infected more in the same year (25.5%). The age group of 5-14 were infected more in 2020 (28.2%) for males and in 2019 (30%) for females. There was the same year when the prevalence rate of both sex in adult age group were reached high rate. For male age group >15 it was (56.3%) and for females >15 it was (64.5) in 2018 (Figure 5). The malaria infection was significantly associated with the age groups ( $\chi^2= 412.363$ ,  $df= 5$ ,  $p < 0.002$ ).

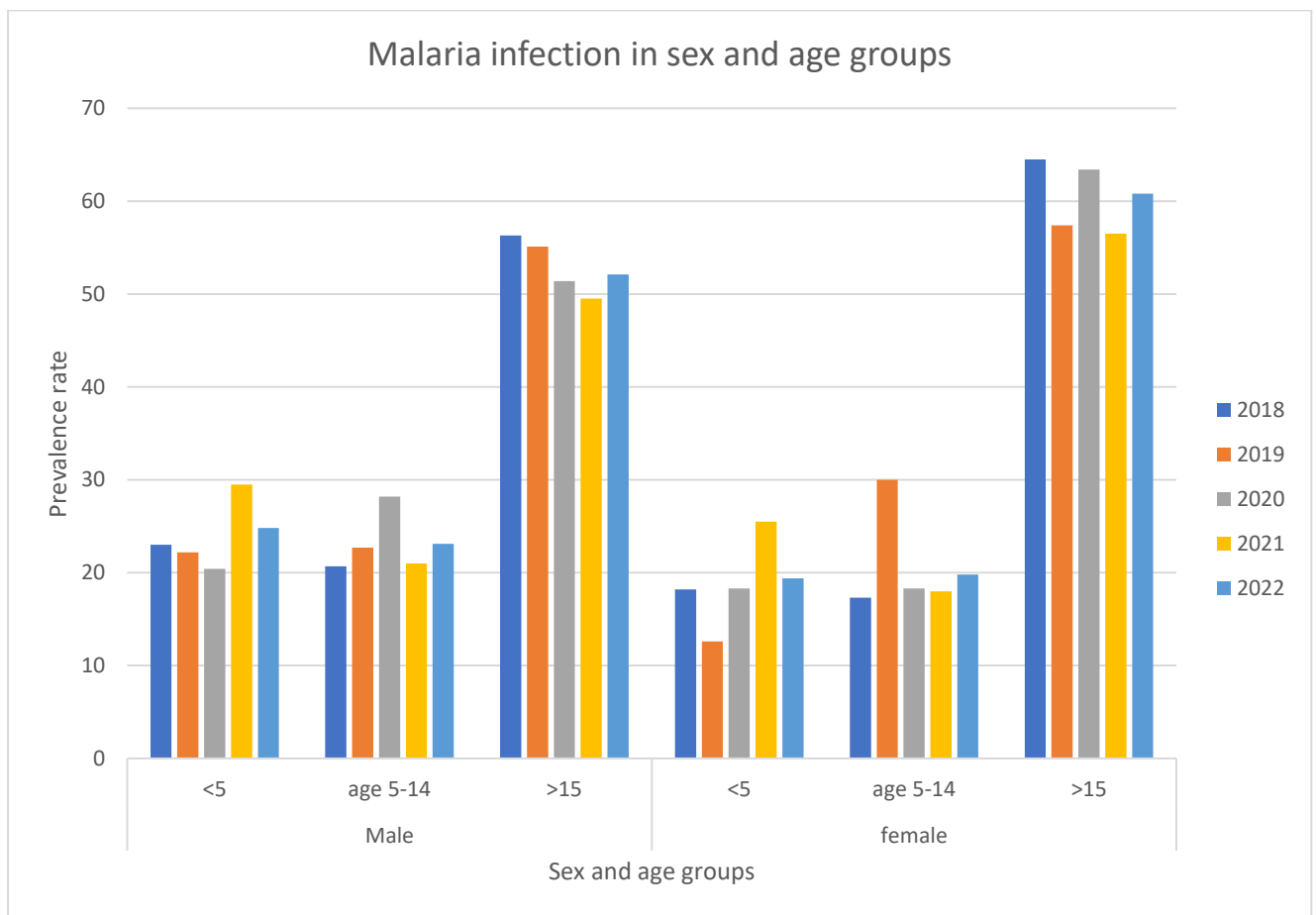


Figure 5: The trends of malaria positivity by sex and age groups in Loka Abaya District from (2018- 2022).

### 4.3. The yearly trends of *Plasmodium* species distribution.

*Plasmodium falciparum* was the dominant species in the study area which accounted for 5327 (58.8%) and followed by *Plasmodium vivax* with proportion of 3519 (38.8%) and the rest 224 (2.4) were the mixed infection. *P. falciparum* was found to be the predominant species over *P. vivax* except in the year 2021 when, the prevalence for *P. falciparum* was (46.7%) and that of *P. vivax* was 49.8% (Figure 6). *Plasmodium* species distribution has showed fluctuations within five-years. There were declining and increasing years of the species distribution. The highest prevalence rate for *P. falciparum* was in 2018 (65.6%) and the least was in 2021 (46.7%). The *P. vivax* also showed similar trends which has highest infection rate in 2021 (49.8%) and the least infection rate in 2018 (32.1%). The mixed infection was high in 2021 (3.5%) and it showed the least prevalence in 2019 (1.7%) (Figure 7). The difference in *Plasmodium* species distribution was statistically significant in the study area with ( $\chi^2 = 116.188$ ,  $df=6$ ,  $P<0.05$ )

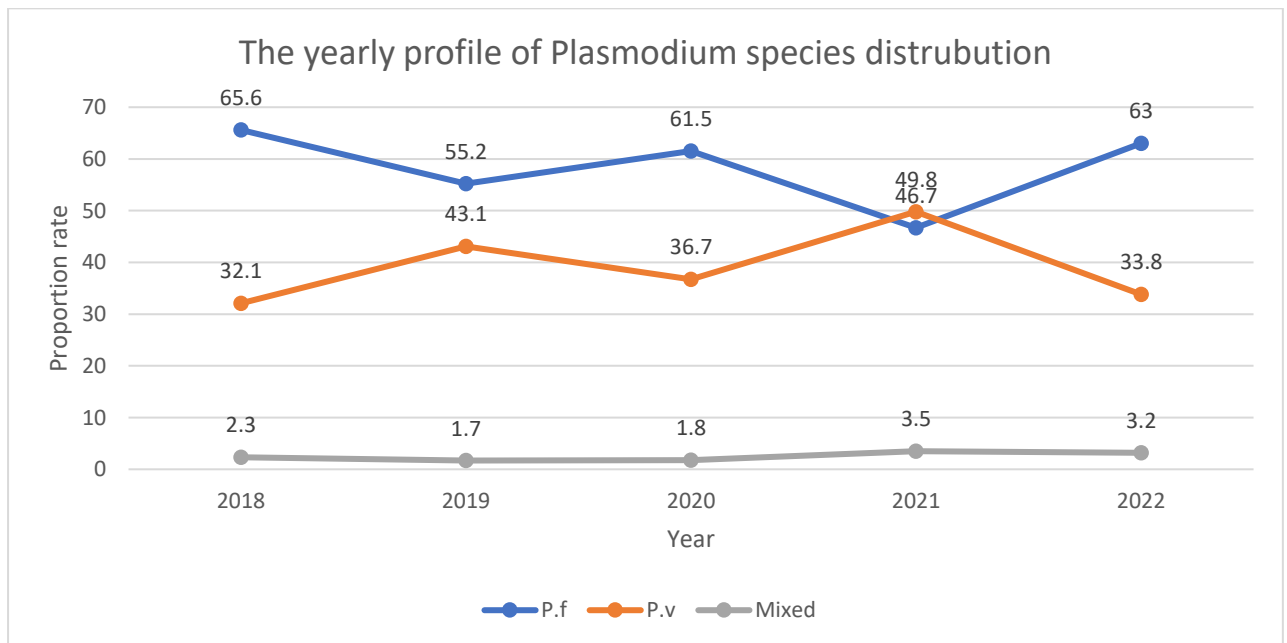


Figure 8: The yearly profile of *Plasmodium* species distribution.

#### 4.4 *Plasmodium* Species distribution by sex and age groups.

##### 4.4.1 *Plasmodium* Species distribution by sex group

The data showed that males were more affected by *P. falciparum* than females in all years except in 2020, where the prevalence of 62.1% and 63.3%) respectively. The outpatients were highly infected by *P. falciparum* in 2018 (65.6%), among these 360 (49.6%) were males and 366 (50.4%) were females. For *P. vivax* the outpatients were highly infected in 2021 (49.8%) and among these 358 (49.2%) were males and 369 (50.8%) were females. The females were highly infected by a mixed infection in the study area throughout the five years (2018-2022) with the total prevalence of 137 (61.2%) and the males were 87 (38.8%) which showed the high burden of mixed infection on females (Figure 9). The association of *Plasmodium* species with the sex group was not statistically significant ( $\chi^2= 215.321$ ,  $df=6$ ,  $P>0.05$ ).

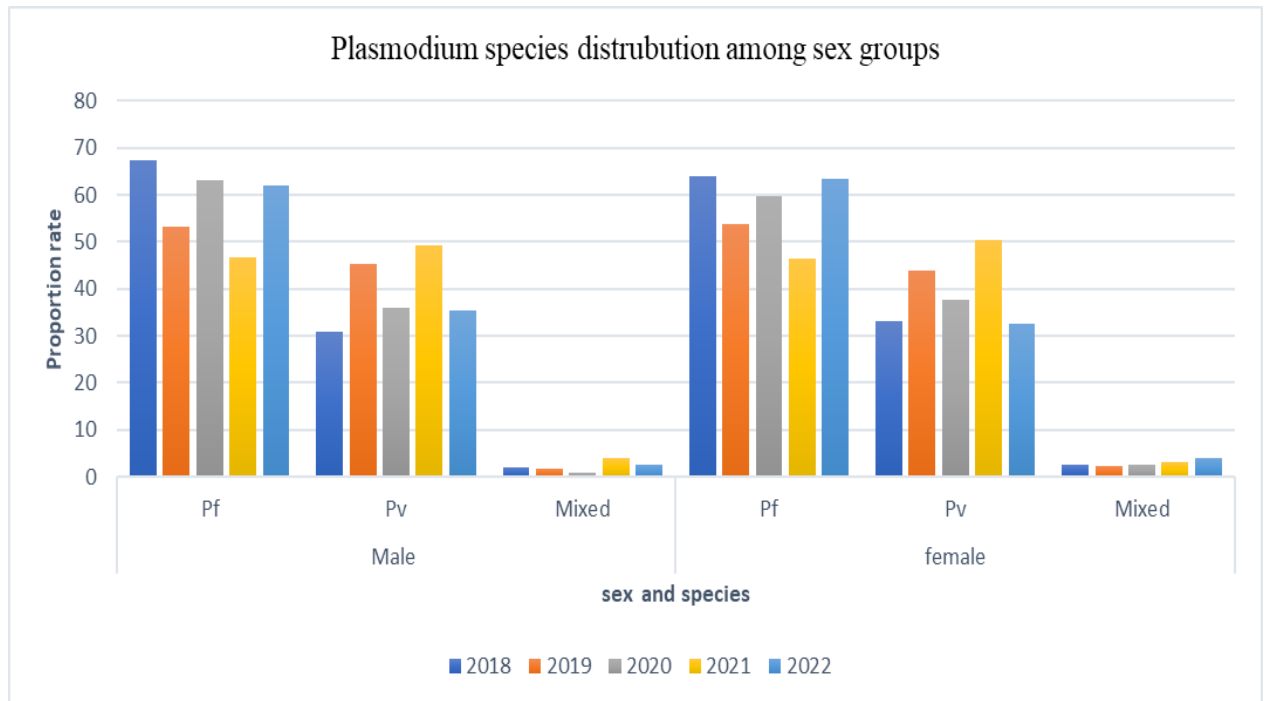


Figure 10: *Plasmodium* species distribution among sex groups.

#### 4.4.2 *Plasmodium* Species distribution among the age groups

In terms of the age-specific distribution of *Plasmodium* species, the age groups <5 years old were highly infected by *P. falciparum* in 2022 (19.3%), by *P. vivax* in 2018 (30.8%), and infected by mixed infection in 2020 (12%). The age groups <5 years old were mostly infected by *P. vivax* throughout five years in the study area. The age groups between 5 to 14 years old were highly infected by *P. falciparum*, *P. vivax* and mixed infection in 2020 (23.4%, 23% and 28%) respectively (Figure 11). The adults or the age groups  $\geq 15$  years old were highly infected by *P. falciparum* in 2021 (62.7%), by *P. vivax* in 2019 (54.3%). The mixed infection was dominated by the age group  $\geq 15$  throughout five years in the study area (Figure 12). There was difference in *Plasmodium* species distribution and age groups and but it was not statistically significant in the study area ( $P > 0.05$ ) (Figure 13).

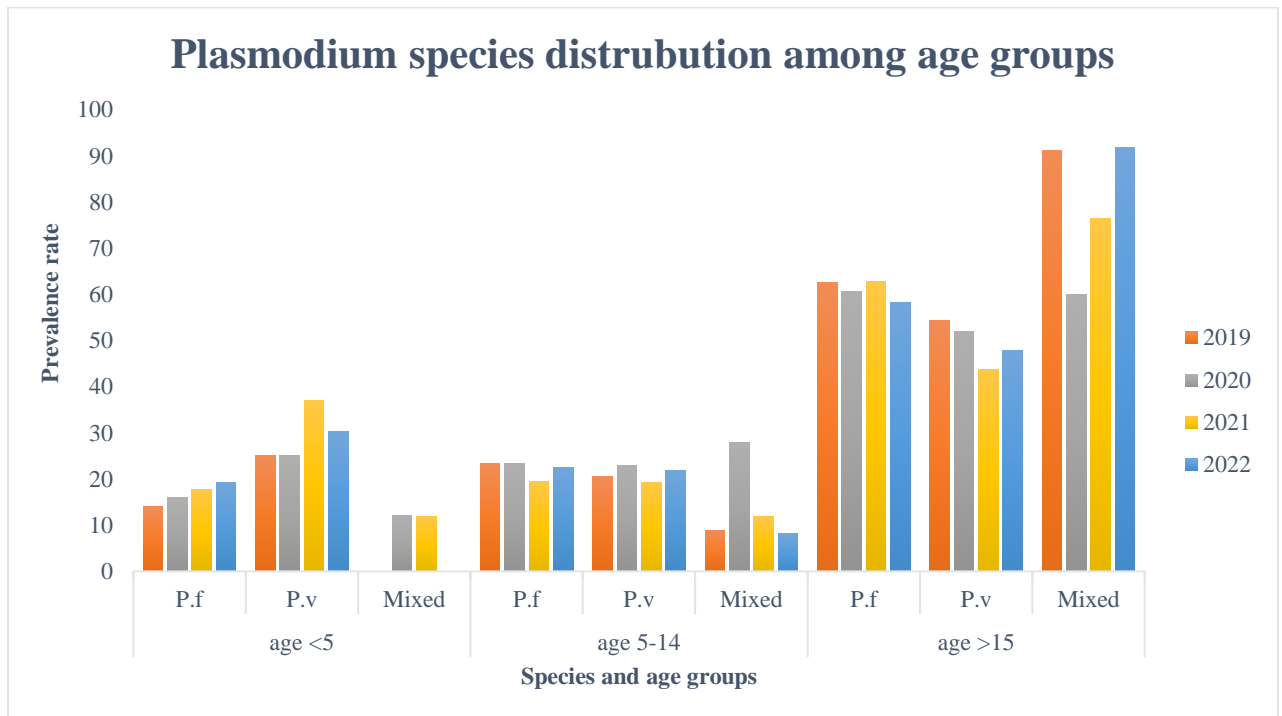


Figure 14: *Plasmodium* species prevalence with in different age groups

#### 4.5 Seasonal distribution of malaria cases in Loka Abaya District from 2018-2022

The five-year retrospective study showed that, malaria was occurred throughout the year in the current study area. Even though there were fluctuations in malaria infection, but the malaria cases occurred in almost every month of the year in the study area. The highest peak of malaria infection was mainly recorded from September up to November months (after long rainy seasons) throughout five years, followed by the months of May up to June (after short rainy seasons) and the minimum numbers of malaria cases were recorded during the months of December up to February (dry seasons) in the study area. The association of malaria transmission with the seasons was statistically significant with ( $\chi^2 = 315.248$ ,  $df = 14$ ,  $p < 0.002$ ) in the study area (Figure 15).

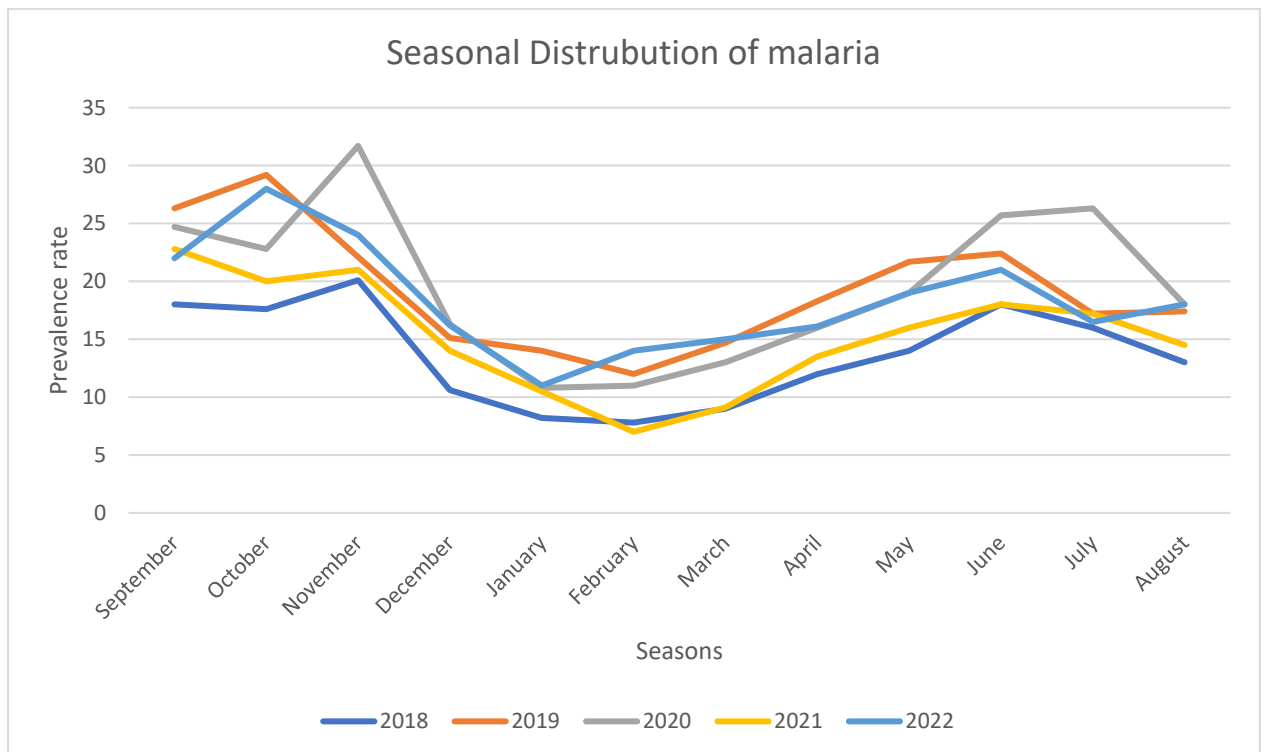


Figure 16 The seasonal prevalence rate of malaria in Loka Abaya District for five years (2018-2022).

#### **4.6. Socio-Demographic characteristics of respondents participated in parasitological survey**

A total of 190 study participants were recruited for this study, from which 186 were participated in the questionnaire interview and provided blood samples with non-response rate of 2.1%. From the total of study participants 89 (47.8%) were males and 97 (52.2%) were females. The age group 26-35 were covered highest amount (33.8) followed by age group 36-45 (27.5%) and age group 45 and above were (21.5%). The least amount was covered by participants with age group 18-25 (17.2%) (Table 1).

Majority of the respondents were rural residents (95.2%) and the urban were (4.8%). From the respondents 74.7% were married while 25.3% were unmarried. As shown in the (table 1) most of the respondents were housewives (37.1%), followed by farmers (33.9%), merchants (4.3%), government employees (1.6%), and unemployed (1.1%). About 14.0% of the respondents had no formal education and participants who can read and write were (15.6%). About (52.7%) had primary education, 14.0% had secondary education and 3.8% had diploma and above. Household size with 5-7 were covered about (56.4%) followed by 2-4 (35%) and household size 8 and above were (8.6%) (Table 1).

Table 1: The socio demographic characteristics of the study participants

Character	Categories	Frequency	Percent (%)
Sex	Male	89	47.8
	Female	97	52.2
Age	18-25	32	17.2
	26-35	63	33.8
	36-45	51	27.5
	45 and above	40	21.5
Marital status	Single	47	25.3
	Married	139	74.7
Main occupation	Farmer	63	33.9
	Housewife	69	37.1
	Student	41	22.0
	Merchant	8	4.3
	Government employed	3	1.6
	Unemployed	2	1.1
Educational status	illiterate	26	14.0
	read and write only	29	15.6
	primary school	98	52.7
	secondary school	26	14.0
	Diploma and above	7	3.8

Residence	Rural	177	95.2
	Urban	9	4.8
Household size	2-4	65	35
	5-7	105	56.4
	8 and above	16	8.6

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#### 4.7 Prevalence of malaria based on socio-demographic factors

Of the total febrile patients, 34 (18.3%) were infected with malaria parasites. Among the infected participants 21(61.8%) were males and 13 (38.2%) were females. Malaria was reported in all age groups, but the prevalence was highest in the 26–35 age group (38.3%) and the lowest infection rate was in age group 18-25 (14.7%), among the total participants. From the total of 34 malaria-infected individuals, 24 (70.5%) were infected by *Plasmodium falciparum* and 11 (29.5%) were infected by *Plasmodium vivax* and there were no mixed infections recorded among the participants during the study period. The *Plasmodium* species prevalence among the participants of active case investigation was statistically significant with (P= 0.002) (Table 2).

Table 2 Malaria prevalence from parasitological survey

Variables	Categories	Proportion in %
Sex	Male	61.8
	Female	38.2
Age group	18-25	14.7
	26-35	38.3

	36-45	31.4
	46 and above	15.6
Species	<i>P. falciparum</i>	70.5
	<i>P. vivax</i>	29.5

The prevalence of malaria was higher in males (23.6%) as compared to females (13.4%) in terms of totally examined blood films from each sex group and the variation was statistically significant ( $\chi^2 = 3.824$ ;  $P < 0.05$ ). Malaria was more prevalent in the age group 25-36 years old at this study site (38.3%) but the association was not statistically significant ( $P > 0.05$ ). Malaria infection based on the household size was statistically significant ( $\chi^2 = 6.238$ ,  $P < 0.05$ ). The household with 5-7 members were mostly infected by malaria in this study (64.8%) from total blood sample examined and also the infection rate was high (21%). According to the association between occupation and malaria infection, farmers made up the highest number 16/34 (47%) followed by the housewives 10/34 (29.4%) and the association was statistically significant ( $\chi^2 = 10.087$ ;  $P < 0.05$ ). The infection rate of malaria was high in illiterate group (23.1%), but the association was not statistically significant ( $P > 0.05$ ) (Table 3).

Table 3 : The association of malaria infection with the socio-demographic characteristics among the study participants.

Variables	Categories	Malaria	Malaria	X <sup>2</sup>	P-value
		positive (%)	negative (%)		
Sex	Male	21 (23.6)	68 (76.4)	3.824	0.032
	Female	13 (13.4)	84 (86.6)		
Age	18-25	5 (15.6)	27 (84.4)	5.234	0.192
	26-35	13 (20.6)	50 (79.4)		
	36-45	9 (17.6)	42 (82.4)		
	45 and above	7 (17.5)	33 (82.5)		
Occupation	Farmer	16 (25.4)	47 (74.6)	10.087	0.002
	Housewife	10 (14.5)	59 (85.5)		
	Student	6 (14.6)	35 (85.4)		
	Merchant	2 (25)	6 (75)		
Household size	2-4	10 (15.4)	55 (84.6)	6.238	0.047
	5-7	22 (21)	83 (79)		
	8 and above	2 (12.5)	14 (87.5)		
Education status	Illiterate	6 (23.1)	20 (76.9)	13.256	0.126
	Literate	28 (16.5)	142 (83.5)		

#### **4.8 The analysis of risk factors associated with malaria infection in the study area.**

The presence of stagnant water near the residential place, sleep under mosquito net last night, the way of using or hanging mosquito net during night time, the availability of window in house and the type of window available were among the possible risk factors determined that had shown significant association with malaria infection ( $P < 0.05$ ). These factors were highly associated with malaria infection, presence of stagnant water near residential (AOR = 13.296, 95% CI: 8.23, 26.96), sleep under mosquito net last night (AOR = 18.42, 95% CI :2.82, 32.85) and the travel history of participants, last season of LLIN received and the history of anti-malaria treatment of the participants were not associated with the malaria infection in the study area (Table 4).

According to the availability of mosquito net, 171 (92%), of the participants received mosquito net and it was not associated with the malaria infection ( $P = 0.478$ ). Almost 83 percent of the participants responded that their houses did not undergo IRS for more than twelve months, but the association with the malaria infection was not statistically significant ( $P > 0.05$ ) (Table 4).

The respondents who were not recently received information about malaria infection and not aware of its transmission and control were highly infected than those who had recent information and aware of the infection (AOR = 2.87, 95% CI :1.46–19.61) (Table 4).

Table 4 : The associated risk factors analysis of malaria infection in the study area.

Variables	Category	Malaria Negative	Malaria Positive	COR (Crude Odd ratio)	AOR (Adjusted Odd Ratio)	P-value
Presence of stagnant water near the residential place	Yes	94 (76.4)	28 (23.6)	0.83(0. 35- 0.109)	13.296(8.2 3-26.96)	0.001
	No	58 (92.1)	5 (7.9)	1	1	
Availability of mosquito net	Yes	141(82.4)	30 (17.6)	3.65 (0.36– 21.54)	1.81 (0.27– 15.48)	0.478
	No	11 (73.3)	4 (26.7)	0.82 (0.07– 9.26)	0.33 (0.06– 5.46)	
Sleep under mosquito net last night	Yes	86 (87.8)	12 (12.2)	1	1	0.021
	No	66 (75)	22 (25)	3.36 (1.41– 14.61)	18.42 (2.82– 32.85)	
The way using mosquito net at night	Hanging over the bed to the floor	50 (70.4)	21 (29.6)	1.23 (0.87– 2.68)	3.76 (1.88– 16.35)	

	Hanging over sleeping mat (matters)	29 (85.3)	5 (14.7)	0.46 (0.31–2.32)	0.85 (0.42–13.58)	0.032
	Hanging over the bed tucked in	58 (87.8)	8 (12.2)	1.56 (0.85–4.90)	2.13 (1.25–5.83)	
The last season of IRS	>12 months	152 (81.8)	28 (82.4)	1.27 (0.88–5.65)	1.05 (0.31–3.55)	0.134
	<12 months	34 (18.2)	6 (17.6)	1	1	
Availability of window	Yes	90 (81.1)	21 (18.9)	1.48 (0.65–3.21)	2.78 (1.32–11.34)	0.038
	No	62 (82.6)	13 (17.4)	1	1	
Type of window	Window without cover	66 (67.3)	32 (32.7)	0.93 (0.38–1.92)	2.83 (1.03–13.86)	0.022
	Windows with curtains or shutter	12 (85.7)	2 (14.3)	0.65 (0.38–2.31)	1.78 (0.91–4.28)	

History of anti-malaria treatment	Yes	72 (82.7)	15 (17.3)	1.37 (0.267–5.71)	1.06 (0.252–4.63)	0.621
	No	80 (80.8)	19 (19.2)	0.78 (0.36–2.86)	0.65 (0.31–2.54)	
The last period when LLIN received	<6 months	126 (79.7)	32 (20.3)	2.66 (1.56–9.51)	3.25 (1.77–11.52)	0.189
	6-12 month	8 (80)	2 (20)	0.43 (0.255–6.41)	0.213 (0.187–5.26)	
	>12 month	2 (66.6)	1 (33.4)	1	1	
Recently received malaria information	Yes	53 (85.5)	9 (14.5)	1.66 (0.78–14.61)	1.205 (1.46–19.61)	0.041
	No	99 (79.8)	25 (20.2)	2.62(1.13–18.21)	4.25(2.36–22.61)	
Travel history	Yes	71 (81.6)	16 (18.4)	1.11 (0.756–8.41)	0.89 (0.575–6.71)	0.831
	No	81 (81.8)	18 (18.2)	1	1	

## 5. DISCUSSION

Malaria affects the lives of almost all people living in sub-Saharan African countries. Ethiopia is still one of the countries with a high malaria burden. In the country malaria is one of the most important public health issues by causing both mortality and morbidity. The infection continues a major public health problem despite relatively low prevalence compared to some African countries (Workneh *et al.*,2021). Unstable malaria transmission patterns make the country prone to epidemics. The nation-wide elimination plan needs sub-national elimination. This is because the decrease in malaria burden is not uniform in the country which is imperative to understand the contextual diversity of malaria prevalence within each site (Tefera Selomon *et al.*, 2022)

The current study determined the 5 years trend (2018–2022) of malaria cases on basis of the retrospective study and assessed the current situation of malaria prevalence with its possible risk factors in the study area. The overall prevalence of microscopically confirmed malaria cases detected in the retrospective study were 26.2% (9070), with the minimum and maximum rate of prevalence reported in 2018 and 2020, (20.4%) and (30.1%) respectively. Even though there was fluctuating trends of malaria transmission from year to year, it has still great burden despite of the ongoing malaria control and prevention strategies implemented in the study area.

The prevalence of malaria infection from the retrospective finding of the current study was higher than the results of other studies conducted in different part of Ethiopia, which found 16.2% in Dembecha (Haile Desalegn *et al.*, 2020), 20.1% in Sibul Sire (Gemechu Temesgen *et al.*, 2015), 16.8% in Jardga jarte (Beyene Begna *et al.*, 2018), 5.4% in Wereta (Derbie Awoke and Megbaru Alemu Abebe ,2017), 11.5% in Arsi Negelle (Hailemariam Mengistu and Solomon Gebre, 2015), 8.6% in Wolkite town (Solomon Absra *et al.*, 2020), 7.5% in Kombolcha

(Gebretsadik Daniel *et al.*, 2018), 22.4% in Dembiya district (Tarekegn Mihretu *et al.*, 2021), 4.2% in Mojo town (Abate Andargie *et al.*, 2022), 7.0% in North west Tigray (Berhe Brhane *et al.*, 2019), 9.5% in Halaba (Shamebo Tsegay and Beyene Petros, 2019), and 7.6% in Chalia district (Asefa Tilahun and Befikadu Bogale, 2017). The current study's malaria prevalence was comparatively lower than that of previous research conducted in various regions of Ethiopia which found, 51.0% in Guba (Alkadir Shemsia *et al.*, 2020), 33.4% in Arjo-Didessa (Hawaria Dawit *et al.*, 2019), 33.8% in Abeshge (Yimer Fisseha *et al.*, 2015), 66.7% in Bale zone (Sanin Khalil *et al.*, 2020), 46.9% in Harari (Esayas Endashaw *et al.*, 2020), 39.6% in Kola diba (Alemu Abebe *et al.*, 2012), 41.5% in Hana and Keyafer health centers (Ergete Solomon *et al.*, 2018) and 31.1% in Ziqal District (Debash Habtu *et al.*, 2023 )

These differences might be due to, geographical variations, the type of malaria diagnosis applied, laboratory personnel performance difference in malaria parasite detection because the quality of microscopic diagnosis is highly influenced by slide preparation and staining methodology, microscopist skill, and quality of microscope (Essendi *et al.*, 2019). The finding of current study which showed the fluctuating trend of malaria transmission from year to year was supported by other documented records of many studies. The variations in the time of the studies might also bring the difference in the overall prevalence of malaria since malaria is being fluctuated from time to time as supported by the global and national report (Abate Andargie *et al.*, 2022).

Moreover, it could be due to that, the community awareness on malaria control methods and application of intervention activities, may varies from the area to area and contributes to the variation on pattern of malaria distribution. Variation in socio-demographic status and utilization

of intervention in the same locality also influences the prevalence of malaria infection. The difference in socio-demographic characteristics (sex, age), geographic location (altitude, temperature, rainfall) and economical activities, also had a great effect on the variation of malaria prevalence from one part of the country to the other. The population awareness about malaria controlling methods application, its transmission, and health seeking behavior had an also an impact on malaria distribution variations (Mulugeta Amanuel *et al.*,2022).

The highest peak of malaria distribution was occurred in 2020 with the annual prevalence rate of (30.1%). This finding was supported by others that, COVID-19-related disruptions in the provision of malaria prevention, diagnosis, and treatment appear to have contributed to an increase in the number of cases in 2020 (Accrombessi *et al.*,2022). It may due to that, the interventions and focuses were majorly given to COVID-19 pandemic, that influenced the controlling of malaria in the current study area.

According to the prevalence of malaria related with sex, the infection rates among males were (52.6%) and females were (47.4%). Males were (1.11) times infected than females in the study area. The result of present study was consistent with many other previous retrospective studies conducted in Boricha district in which males were 51.5% and females were 48.4% (Dabaro Desalegn *et al.*, 2020), in North west Tigray, males were 62.7% and females 37.3% (Berhe Brhane *et al.*, 2019), in Jarda Jarte, males were 60% and females were 40%(Beyene Begna *et al.*, 2018), in Shewarobit, males were 65.3% and females were 34.7% (Shiferawu Teshome, and Azene Tesfaye Desta, 2021), in Demebecha, males were 58.9% and females were 41.1% (Haile Dessalegne *et al.*, 2020), in Guba males were 58.1% and females were 41.9% (Alkadir Shemsia *et al.*,2020) and in Abergele District where, males were 57.2% and females 42.8% (Debash

Habtu *et al.*, 2022). The reason behind the high malaria cases in males might be, due to the fact that males are involved in outdoor activities in farm land, irrigation canals and frequently bitten by mosquitoes. They travel to malaria endemic areas for labor work and seeking temporary employment. Most of the time females do not perform these activities while they are house hold take cares and they stay in home which may reduce their risk of infection than males (Quarasemia *et al.*, 2021).

In current study are the are some pastoral societies, of which most are men and leave their homes to watch over livestock as they graze in field. These men are mostly vulnerable for mosquito bite in the field and have little protection from malarial mosquitoes and often far away from treatment facilities. These factors may increase the risk infection in males in current study area.

The age groups >15 years old were highly infected with malaria (57%) in present study area that aliens with finding of other studies, Arsi Negelle (Hailemariam Mengistu and Solomon Gebre, 2015), Dembiya district (Tarekegn Mihretu *et al.*, 2021), Wolkite town (Solomon Absra *et al.*, 2020), and Chalia district (Asefa Tilahun and Befikadu Bogale, 2017). Kombolcha (Gebretsadik Daniel *et al.*,2018), Mojo town (Abate Andargie *et al.*, 2022), Halaba (Shamebo Tsegaye and Beyene Petros, 2019),

The contributing factors for such highest burden of disease among adult age groups could be due to their frequent engagement in vary activities that may increase the exposure to infective mosquito bites like agriculture (Irrigation canals or Dams most of the time), trade (move to malaria endemic area most of the time) and other occupational risks.

The malaria infection in children less than 5 years old were low (21.2%) when compared to the rest age groups in current study area. This finding was consistent with the other studies conducted in, Shewarobit, (Shiferawu Teshome, and Azene Tesfaye Desta, 2021), in Halaba (Shamebo Tsegaye and Beyene Petros, 2019), in Boricha (Dabaro Desalegn *et al.*, 2020), Jardga jarte (Beyene Begna *et al.*, 2018) and in Mojo town (Abate Andargie *et al.*, 2022). This lower prevalence of malaria observed in children under age of 5 years might be because of their less likely exposure to a mosquito bite than adult age groups and sleeping under bed nets.

However, the finding of this study contradicts with the previous studies conducted in different parts of Ethiopia, in Arsi Negelle Health Center, reported that the age group under 5 years old were the most affected by the disease (Hailemariam Mengistu and Solomon Gebre, 2015), in Jimma town, that higher malaria prevalence rate was observed among under-five children (Alemu Abebe *et al.*, 2011) and other African countries, South east Gabon (Ledongo *et al.*, 2023) and Kampala Uganda (Clark *et al.*, 2008). This might due to the difference in applied preventive strategies and child care programs taken in order to reduce the risk of infection for children.

Concerning the distribution of *Plasmodium* species, the present study revealed that *P. falciparum* was the dominant species which accounted for (58.8%), followed by *P. vivax* (38.8%) and mixed infection (2.4%). This finding was in agreement with the national profile of *Plasmodium* species distribution in which, *P. falciparum* covered the major proportion, followed by *P. vivax* and mixed infection (Kendie Fasil *et al.*, 2021). Ethiopia is one of the few African countries where both *P. falciparum* and *P. vivax* species are co-endemic at substantial proportions and are accounted for in malaria diagnostic and treatment guidelines. *P. falciparum* accounts for 60% of all cases and *P. vivax* accounts the rest of the remaining cases in Ethiopia

(Leonard *et al.*,2022). The finding of current study result was in consistence with the studies conducted in other part of Ethiopia, in North west Tigray (Berhe Brhane *et al.*,2019), Boricha District (Dabaro Desalegn *et al.*, 2020), Kombolcha (Gebretsadik Daniel *et al.*, 2018), North west Ethiopia (Derbie Awoke and Megbaru Alemu, 2017), Hana and Keyafer (Ergete Solomon *et al.*, 2018) and Ataye (Feleke Daniel *et al.*, 2018) which found *P. falciparum* as the dominant species.

The dominance of *P. falciparum* over the *P. vivax* and mixed infection might be due to the topographic differences while higher prevalence of *falciparum* infection occurs mostly in lowland areas. It might also due to the fact that, out of about 460 species of Anopheles mosquito, more than 70 species transmit *falciparum* malaria. *Anopheles gambiae* is one of the most known and most prevalent vectors in Ethiopia which can influence the profile of *Plasmodium* species distribution (Abera Deriba *et al.*, 2021). This vector species is also dominant in the current study area (Districts health office report), which might cause the dominance of *P. falciparum* over the *P. vivax*.

This finding contradicts some studies conducted in different regions of Ethiopia which found, *P. vivax* as the dominant species, in Arsi negelle (Hailemariam Mengistu and Solomon Gebre, 2015), in Mojo town (Abate Andargie *et al.*, 2022) and in Wolkite town (Solomon Absra *et al.*, 2020). This might be the variation in vector control programs efficacy, lab personnel performance to identify species or the susceptibility of parasite to the drugs used and difference on geographical distribution of vectors.

The current study revealed that the adults were mostly affected by the *P. falciparum* when compared to other age groups. This finding was in agreement with the result of other studies

conducted in Kombolcha (Gebretsadik Daniel *et al.*, 2018), in Ataye (Feleke Daniel *et al.*, 2018), in Mojo town (Abate Andargie *et al.*, 2022) and in Harari town (Esayas Endashaw *et al.*, 2020). This might be due to the fact that productive age groups frequently engaged in outdoor activities like, agriculture associated with irrigation, staying with agricultural practices especially during the high transmission period. This may favor the situations being bite by *Anopheles* mosquito which mostly capable to transmit the severe *P. falciparum* (Buchwald *et al.*, 2019).

The children less than 5 years old were mostly infected *P. vivax* in the current study area. This might be due to that they do not perform activities like the adults. So, most of the time they may exposed to the relapsing malaria by *P. vivax* due to its dormant liver stage (the hypnozoite), which can activate weeks, months, or years after the primary infection to cause a relapse fever (Drysdale *et al.*, 2023).

Both males and females were mostly affected by *P. falciparum* with respect of its dominancy in the current study area, that aliens with the finding from other studies conducted in Shewarobit, (Shiferawu Teshome, and Azene Tesfaye, 2021), in Boricha district (Dabaro Desalegn *et al.*, 2020), in Ziqal District (Debash Habtu *et al.*, 2023) and in Guba (Alkadir Shemsia *et al.*, 2020) which found the dominancy of *falciparum* infection in both sexes. The adult females were highly infected by a mixed infection in the study area throughout five-year with the total prevalence of (61.2%). This may due to the fact that, females do not possess immunity to fight against the infection during their pregnancy period and more likely to be exposed for the mixed infection (Okiring *et al.*, 2022).

In the current study area, the highest peak of malaria infection was mainly occurred during September to November months (after long rainy seasons) throughout five years and followed

by May up to June. The minimum load of malaria cases was recorded during the months from January up to February (dry seasons). This result was in accordance with the findings from the other studies conducted in, Northwest Tigray (Berhe Brhane *et al.*, 2019), in Ataye (Feleke Daniel *et al.*, 2018), in Dembia district (Tarekegn Mihretu *et al.*, 2021), in Harari town (Esayas Endashaw *et al.*, 2020), in Dembecha (Haile Dessalegne *et al.*, 2020), in Guba (Alkadir Shemsia *et al.*, 2020) and in Jimma town (Jemal Abdurazak and Tsige Ketema, 2019) which found that, the highest pattern of malaria distribution mostly occurred after long rainy season and second transmission seasons were after short rainy periods.

This is due to that, the distribution and transmission of malaria largely unstable and seasonal that may depend on the altitude and climate. It mostly related to the formation of breeding sites for mosquito vector during and after the heavy rain season, favorable temperature, and high vegetation density for mosquito breeding. Malaria is more common in after long rainy seasons as most of the mosquitoes breed in waterlogged and damp places. The coinciding high prevalence of malaria with these cultivation months has a deleterious socio-economic effect on productivity and development in the country (Tigu Fitsum *et al.*, 2021).

The cross-sectional parasitological survey was conducted to determine the current status of malaria transmission and the responsible risk factors of transmission in the present study area. The overall prevalence of malaria from total blood sample was 18.3% (34/186), which indicated that malaria is still a health problem in the present study area. The males were more infected (61.8%) and the infection in females was (38.2%). The *P. falciparum* was found as predominant malaria parasite (70.5%) in current study area. This result was somewhat comparable to that of research done in Shewarobit health center that found the prevalence of 19% (Tesfaye Azene and

Tadegew Teshome 2022), Mizan-Aman town, 21.1% (Duguma Tadesse *et al.*, 2022) and 21.2% in Waghemira Zone of Northeast Ethiopia (Debash Habtu *et al.*, 2023).

The finding of current study was greater than the results of other cross-sectional studies conducted in different parts of Ethiopia, in Hawassa town reported prevalence of 3.9% (Fikrie Antenehl *et al.*, 2021), 8.7% in Wogera district (Tsegaye Adino *et al.*, 2021), 5.7% in North-shoa (Feleke Daniel *et al.*, 2020), 3.5% in Dembiya district (Tarekegn Mihretu *et al.*, 2021), 7.3% in Chagni town (Belay Bogale *et al.*, 2021) ,2.74% in East-shoa zone (Balcha Fufa *et al.*, 2023) and 6.7% in Kaffa zone (Duguma Tadesse *et al.*, 2023).

It was lower than other cross-sectional studies conducted in different parts of Ethiopia which reported, 41% in West Arsi Zone (Lakew Yosef *et al.*, 2023), 25% in East Shewa zone of Oromia (Tadesse Frew *et al.*, 2018), 39.8% in Jinella health center Harari (Asmerom Haftu *et al.*, 2023) and 28.4 % in North western Ethiopia (Getahun Biruk and Abebe Ayalewu Bekel, 2022).

This variation could be due to the habitat alterations, altitude, microclimate, variations in vector control and prevention programs efficacy, community understanding on malaria prevention and control measures, methodological capability, the type of diagnostic tools used and other socio demographic factors. It might also be due to differences of participants, different data collection periods and the performance laboratory personnel to detect malaria parasite. In this study data was collected during the second malaria transmission season.

The risk factors for transmission and distribution of malaria were analyzed in current study area. There was significant association between some socio-demographic characteristics of the participants and infection by malaria. The participants sex, occupation and household size were

the determined associated risk factors in current study area with ( $P < 0.05$ ). This finding was in consistent with other studies conducted in Dembiya district (Tarekegn Mihretu *et al.*, 2021), in North western Ethiopia (Getahun Biruk and Abebe Ayalewu Bekel, 2022), in West Arsi Zone (Lakew Yosef *et al.*, 2023) and in Waghemira Zone (Debash Habtu *et al.*, 2023) which found that, the occupation, house hold size and sex had shown significant effect on malaria prevalence among participants.

Males were infected more than females (61.8%) and this may due to they are being mostly engaged in outdoor activities like agricultural and irrigation activities and mostly travel to endemic areas which in turn increase the chance of infection (Quarasemia *et al.*, 2021). The farmers were more infected in the current study area (47.1%). This might be due to that, farming activities which take place mostly in the rainy season favour the breeding of mosquitoes and this makes the morbidity of infection apparently noticeable in rural areas due to their proximity to farmlands, particularly in areas where water management practice is pitiable. Thus, farming and malaria transmission are directly linked thereby, placing farm workers at significant risk of malaria infection (Babamale *et al.*, 2020).

The household size has shown significant association with malaria infection with ( $P < 0.05$ ) and it contradicts the study conducted in South central Ethiopia (Deressa, 2017) and Maygaba town of Wolkait district (Yhdego Tsegay *et al.*, 2022), which reported no statistically significant association of malaria infection was found with regard to the household size. It was in consistent with other studies conducted in Shewarobit health center (Tesfaye Azene and Tadegew Teshome, 2022), in Mumbai India (Mohan *et al.*, 2021) and Chenain India (Thomas *et al.*, 2018), which found significant association of household size with malaria infection, through declaring

that households with more than five members have a higher likelihood for malaria infection. The size of household contributes a significant risk to malaria prevalence. It may be due to that, family size or number of people living in the house per number of rooms is an important risk factor for malaria, as crowding attracts more mosquitoes due to their strong olfactory signals (Lutz *et al.*, 2017). Some households do not hang the mosquito net at night through the scarcity of hanging place and insufficient sleeping rooms because of their large family size. This might influence the risk of malaria infection on the population of the current study area.

The bivariate and multivariate logistic regression analysis were conducted to determine the effect of risk factors on malaria transmission. The presence of stagnant water near the residential place has shown a highly significant association with malaria infection, (AOR = 13.296, 95% CI: 8.23, 26.96) at ( $P < 0.05$ ). It increased the risk of malaria infection by 5.6 times more than respondents having no stagnant water in their residential area. This finding was in agreement with many other cross-sectional studies conducted in, West Arsi Zone (Lakew Yosef *et al.*, 2023), Wogera district (Tsegaye Adino *et al.*, 2021), North-shoa (Feleke Daniel *et al.*, 2020), Dembiya district (Tarekegn Mihretu *et al.*, 2021), East-shoa zone (Balcha Fufa *et al.*, 2023) and 6.7% in Kaffa zone (Duguma Tadesse *et al.*, 2023).

This is due to the fact that, stagnant water provides a convenient breeding site for the vectors which are able to transmit malaria. Mosquitoes need stagnant water in order to complete their lifecycle. It is the number one breeding spot for mosquitoes. They breed by laying eggs in stagnant water. Mosquito larvae live in stagnant water and require only minimal amounts of water, and even something as small as a puddle of water on a discarded plastic bag can house them. They lay eggs in still water and can go from egg to biting adult in as little as 5 days. This

is why it is important to check regularly for standing water in and around homes and to dump out or treat any water (Pinchoff *et al.*,2021).

The availability of window in house and its type were among the potential risk variables that were shown to have a significant association ( $P<0.05$ ) with malaria infection. The participants having windows in their household and without cover were infected more by malaria. It was in agreement with the study conducted in Siraro District of West Arsi Zone (Lakew Yosef *et al.*, 2023) and in Rural Tanzania (Kaindoa *et al.*, 2018), which stated that peoples living in the houses with windows without cover were higher likely of infecting by malaria.

This could be the result of the house's unimproved walls and windows creating, an environment that encourages mosquito entry and resting. As a result, it follows that enhancing home conditions must be promoted and stressed as one of the ways to defend mosquito attacks (as it acts as a physical barrier between the host and the vectors). Malaria can be prevented in homes by adding insecticide-impregnated materials to the eaves (the space between a wall's top and bottom) and screening windows, doors, and ceilings (Kayendeke *et al.*, 2023).

Another associated risk factor which has shown significant association ( $P<0.05$ ) with the malaria transmission was, the way of hanging mosquito net at night time. The participants hanging the net over the bed to the floor were infected more (AOR = 3.76, 95% CI: 1.88, 16.35) than those who use the net properly. Hanging the net above the sleeping space and tucking the net under the matters or mat prevent mosquito from getting inside. Keeping the net closed while sleeping can effectively prevent the mosquito biting (Perkins *et al.*, 2019). The current study was in agreement with the finding from Democratic republic Congo (Mankadi and Jin, 2021).

Another associated risk factor which has shown significant association with the malaria infection in current study area was, Sleeping under mosquito last night. The participants who did not sleep under mosquito net last time infected more by malaria (AOR = 18.42, 95% CI: 2.82, 32.85) than those who sleep under mosquito net regularly. This finding was in consistent with the study conducted in Illu Galan district of Oromia region (Mekuria Mulugeta *et al.*,2022), Arbaminch Zuriya district of South Ethiopia (Abossie Ashenafi *et al.*, 2020), Waghemira Zone of Northeast Ethiopia (Debash Habtu *et al.*, 2023) and Kaffa Zone South western Ethiopia (Duguma Tadesse *et al.*, 2023).

This is due to the fact that, sleeping under a mosquito net is one of the best ways to protect against mosquito bites that spread malaria. Mosquito nets works by creating a physical barrier which stops mosquitoes from reaching inside and chemical barrier through insecticides that are used for treating bed nets, kill mosquitoes, as well as other insects. Despite the distribution of free mosquito nets in malaria-endemic countries, new challenges to their use continue to emerge. The most common reasons for net non-use across all regions of malaria endemic countries were discomfort and perceived ineffectiveness of nets (Koenker *et al.*, 2018). In the current study area, many people got mosquito net recently, however it has been applied for another purpose in some households. This condition increased the risk of malaria infection in the current study area despite of recently distributed ITNs.

The participants recent information and awareness about malaria control and prevention methods were shown significant association ( $P < 0.05$ ) with malaria infection in current study area. The participants who recently did not get the awareness on malaria control programs were infected more by malaria (AOR = 4.25, 95% CI: 2.36, 22.61) than those who had taken recent

awareness on malaria. This finding was in agreement with the study conducted in other parts of Ethiopia, South Gondar (Flatie Tazebew and Abaineh Munshea, 2021), Halaba town (Menjjetta Tadesse, 2021), Chagni town (Belay Bogale *et al.*, 2021) and Eastern Indonesia (Guntur *et al.*, 2021). This is due to that, the community's awareness on means of malaria transmission, when mosquito bites and where it breeds and awareness on treatments and ways of prevention of malaria are critically reduce the risk of malaria infection. The community member who are not aware of malaria control methods and programs, the measurements recently taken and the current status of malaria can be under the risk of infection (Dejasmach Zelalem *et al.*, 2021).

## 6. CONCLUSION

The finding of current study from the five-year retrospective trend analysis of malaria and the current cross-sectional study indicated that, malaria is still a major health concern in Loka Abaya District of South Western Sidama region. The burden of malaria infection in the district is continued despite of the control programs and intervention strategies which have being implemented by the districts stake holders. Although the prevalence rate has varied, the malaria infection has not been effectively prevented and controlled in current study area. It suggested that the malaria prevention and control efforts in current study area should be strengthened.

The study revealed that, males were at higher risk of infection than females in the current study area, because they had a higher rate of malaria infection. The current cross-sectional study revealed the result that was in accordance with past retrospective trend. It had shown that adult age groups in the current study area a weighted chance of contracting malaria than other age groups.

In terms of the *Plasmodium* species, *P. falciparum* was found as the dominant species for the study area, followed by *P. vivax* and mixed infections. High levels of *P. falciparum* infection were present in both sexes. Infections caused by *P. falciparum* have declined in younger age groups, but they were more common in adults. The greater burden of mixed infection was occurred on females throughout the course of five years, due to their decreased immunity during pregnancy, it showed that adult females carried a disproportionately high burden of mixed infections. A five-year retrospective study revealed that malaria cases occurred all year long. Even though there were peaks and valleys in malaria infection, the current research area has shown cases of the disease practically every month of the year. There was statistically significant

association between malaria transmission and the seasons. In the study area, the months of December through February (dry seasons) were known by their lowest number of malaria cases, while the months of September through November (after long rainy seasons) were the highest peak of malaria infection over a five-year period. The second season of malaria transmission months were May and June (after short rainy seasons) in case of Loka Abaya District.

The distribution of malaria in the current study area has been found to be influenced by the Socio-demographic characteristics of the community. There was statistically significant association between sex, occupation, and household size with malaria infection ( $P < 0.05$ ). Members of the community in current study area were at risk of malaria infection due to their occupations, particularly farmers, who had a high infection rate. Malaria was more prevalent in the homes with large family sizes.

A number of possible risk factors were found to have exhibited a significant association ( $P < 0.05$ ) with the malaria infection, including the presence of stagnant water near residential areas, sleeping under mosquito nets the night before, how the net is used or hung at night, the type of windows available in homes and the community's recent awareness of malaria prevention measures. Stagnant water near residential areas was identified as a great risk factor for malaria infection in the current study location. In accordance with the availability of mosquito nets, however malaria infection has not been prevented. The use of mosquito nets varied greatly among community members, which raised the risk of malaria infection. This made it clear that, how little the community members awareness to prevent and manage malaria infection at the household level. The IRS had not been conducted in the current research location for the preceding 24 months, which revealed the shortcomings of malaria control programs of the

district. According to the results of the current study, there is a significant health issue with malaria in the Loka Abaya District of the South West Sidama region of Ethiopia. Therefore, in order to eliminate the infection and burden of a diseases, these findings demand quick evidence-based measures. Additional malaria prevention and control techniques that concentrate on outdoor malaria transmission should be included in the elimination program.

## 7. RECOMMENDATION

- The community of Loka Abaya District was discovered to have a serious need for health care about malaria infection. The following measures have therefore been suggested to the relevant authorities and for the community at risk based on the findings of this study.
- The district needs consistent intervention and control techniques that are successfully executed year-round due to the shifting patterns of malaria distribution that have been observed there.
- In particularly affected groups (males and adults), the relevant authorities and the district health office should pay special attention to sex- and age-based malaria burden.
- By implementing an efficient vector-control measures, the burden of *Plasmodium falciparum* infection (the prevalent species) should be reduced. It can be applied via indoor residual spraying, which involves applying an insecticide to surfaces where mosquitoes tend to rest, such as internal walls, eaves, and ceilings of homes and other domestic structures, to prevent bites while people sleep and to kill mosquitoes as they try to feed.
- In order to reduce the annual burden of the infection, special attention should be paid to the malaria high peak transmission seasons (September to November and May to June) in the current study area.
- Creating and successfully implementing measurements to prevent malaria infection connected to the socio-demographic characteristics of the community, particularly their occupation, which puts farmers in particular at high risk, and issues related to family size.

- The districts agricultural office, health office and other relevant authorities should cooperate together in order to limit the influence of stagnant water on malaria transmission. In order to permanently eliminate malaria infection in relation with standing water, it must be drained or filled, mosquito habitats must be altered, breeding grounds must be disrupted by cleaning drains to allow water to flow, or standing water must be larvicided by adding chemical or biological larvicides to destroy larvae.
- Increasing public knowledge of the controlling malaria at the household level by considering the materials used to construct their homes and the habit of using window in household.
- Providing the health educations that raises people's knowledge of the disease control methods and modifying community ITN using patterns may assist to halt the spread of the disease.
- Furthermore, a community health education campaign and instruction on malaria prevention and control techniques should be included in the research area's malaria elimination strategy. It is necessary to conduct additional research on the ecology, behavior, pesticide susceptibility, and varieties of breeding sites of malaria vectors in order to comprehend entomological risk factors.

## REFERENCES

- Abebaw Abtie, Yibeltal Aschale, Tadesse Kebede and Asrat Hailu. (2022). The prevalence of symptomatic and asymptomatic malaria and its associated factors in Debre Elias district communities, Northwest Ethiopia. *Malaria Journal*, **21**(1): 1-10.
- Accrombessi, M., Akogbeto, M. C., Dangbenon, E., Akpovi, H., Sovi, A., Yovogan, B. and Cook, J. (2022). Malaria burden and associated risk factors in an area of pyrethroid-resistant vectors in southern Benin. *The American journal of tropical medicine and hygiene*, **107**(3):681.
- Acharya, P., Garg, M., Kumar, P., Munjal, A. and Raja, K. D. (2017). Host–parasite interactions in human malaria: clinical implications of basic research. *Frontiers in microbiology*, **8**, 889.
- Abate Andargie, Mesfin Assefa and Lemu Golassa (2022). Five-year trend of malaria prevalence in Mojo town, Central Ethiopia: shifting burden of the disease and its implication for malaria elimination: a retrospective study. *Infection and Drug Resistance*, 455-464.
- Abera Deriba, Caleb K. Kibet, Teshome Degefa, Lucas Amenga-Etego, Joel L. Bargul, and Lemu Golassa. (2021). Genomic analysis reveals independent evolution of *Plasmodium falciparum* populations in Ethiopia. *Malaria Journal*, **20**, 1-11.
- Abossie Ashenafi, Tsegaye Adino Yohanes, Adisu Nedu, Weynshet Tafesse and Mengistu Damitie. (2020). Prevalence of malaria and associated risk factors among febrile children under five years: a cross-sectional study in Arba Minch Zuria district, South Ethiopia. *Infection and drug resistance*, **13**, 363-372.

- Addisu Ayenew, Yalewayker Tegegne, Yenesew Mihret, Abebaw Abtie Setegn and Ayalew Jejaw Zeleke. (2020). A 7-year trend of malaria at primary health facilities in Northwest Ethiopia. *Journal of parasitology research*, **2020**: 4204987.
- Adugna Fasil, Melaku Wale and Endalkachew Nibret. (2022). Prevalence of malaria and its risk factors in Lake Tana and surrounding areas, northwest Ethiopia. *Malaria Journal* **21**(1): 313.
- Al-Awadhi, M., Ahmad, S. and Iqbal, J. (2021). Current status and the epidemiology of malaria in the Middle East Region and beyond. *Microorganisms*, **9**(2): 338.
- Alelign Amir, Zinaye Tekeste and Beyene BegnaPetros. (2018). Prevalence of malaria in Woreta town, Amhara region, Northwest Ethiopia over eight years. *BMC Public Health*, **18**(1): 1-6.
- Alemu Abebe, Dagnachew Muluye, Mikrie Mihret, Meaza Adugna and Melkamu Gebeyaw. (2012). Ten-year trend analysis of malaria prevalence in Kola Diba, North Gondar, Northwest Ethiopia. *Parasites & vectors*, **5**(1): 1-6.
- Alkadir Shemsia, Tegenu Gelana and Araya Gebresilassie. (2020). A five-year trend analysis of malaria prevalence in Guba district, Benishangul-Gumuz regional state, western Ethiopia: a retrospective study. *Tropical Diseases, Travel Medicine and Vaccines*, **6**, 1-7.
- Andrade, C. (2020). Sample size and its importance in research. *Indian journal of psychological medicine*, **42**(1): 102-103.

- Aremu, T. O., Singhal, C., Ajibola, O. A., Agyin-Frimpong, E., Appiah-Num Safo, A. A., Ihekoronye, M. R. and Okoro, O. N. (2022). Assessing Public Awareness of the Malaria Vaccine in Sub-Saharan Africa. *Tropical Medicine and Infectious Disease*, **7**(9): 215.
- Asefa Tilahun and Befikadu Bogale. (2017). Magnitude of Malaria Infection in Chalia District, West Shoa Zone, Oromia Regional State, Ethiopia. *Journal of Health, Medicine and Nursing* 35, 2422-8419
- Asmerom Haftu, Kabtamu Gemechu Temesgen, Tilahun Bete, Beza Sileshi, Berhe Brhane Gebremichael, Muluken Walle and Mesay Arkew. (2023). Platelet Parameters and Their Correlation with Parasitemia Levels Among Malaria Infected Adult Patients at Jinella Health Center, Harar, Eastern Ethiopia: Comparative Cross-Sectional Study. *Journal of Blood Medicine*, **14** (2023): 25-36.
- Aychiluhm Setognal Birara, Kassahun Alemu Abebe Gelaye, Dessie Abebaw Abtie Angaw, Getachew Asfaw Dagne, Abay Woday Tadesse, Adugna Fasil and Dereje Dillu. (2020). Determinants of malaria among under-five children in Ethiopia: Bayesian multilevel analysis. *BMC Public Health*, **20**(1): 1-10.
- Babamale, O. A., Opeyemi, O. A., Bukky, A. A., Musleem, A. I., Kelani, E. O., Okhian, B. J. and Abu-Bakar, N. (2020). Association between farming activities and *Plasmodium falciparum* transmission in rural communities in Nigeria. *The Malaysian journal of medical sciences: MJMS*, **27**(3), 105.
- Badmos, A. O., Alaran, A. J., Adebisi, Y. A., Bouaddi, O., Onibon, Z., Dada, A. and Lucero-Prisno D. E. (2021). What sub-Saharan African countries can learn from malaria elimination in China. *Tropical Medicine and Health*, **49**(1): 1-6.

- Balcha Fufa, Takele Menna and Fantu Lombamo. (2023). Prevalence of asymptomatic malaria and associated factors among pregnant women at Boset District in East Shoa Zone, Oromia Region, Ethiopia: a cross-sectional study. *Malaria Journal*, **22**(1): 28.
- Bath, D., Cook, J., Govere, J., Mathebula, P., Morris, N., Hlongwana, K. and Kleinschmidt, I. (2021). Effectiveness and cost-effectiveness of reactive, targeted indoor residual spraying for malaria control in low-transmission settings: a cluster-randomized, non-inferiority trial in South Africa. *The Lancet*, **397**(10276): 816-827.
- Beeson, J. G., Drew, D. R., Boyle, M. J., Feng, G., Fowkes, F. J. and Richards, J. S. (2016). Merozoite surface proteins in red blood cell invasion, immunity and vaccines against malaria. *FEMS microbiology reviews*, **40**(3): 343-372.
- Belay Bogale, Tegenu Gelana and Araya Gebresilassie. (2021). Malaria prevalence, knowledge, attitude, and practice among febrile patients attending Chagni health center, Northwest Ethiopia: a cross-sectional study. *Tropical Diseases, Travel Medicine and Vaccines*, **7**, 1-10.
- Berhe Brhane, Fitsum Mardu, Haftom Legese and Hadush Negash. (2019). Seasonal distribution and seven-year trend of malaria in North West Tigray: 2012–2018, Ethiopia. *Tropical diseases, travel medicine and vaccines*, **5**(1): 1-7.
- Beyene Begna, Tegenu Gelana and Araya Gebresilassie. (2018). Five years trend analysis of malaria prevalence in Jardga Jarte district, western Ethiopia. *SINET: Ethiopian Journal of Science*, **41**(2): 61-69.

- Blasco, B., Leroy, D. and Fidock, D. A. (2017). Antimalarial drug resistance: linking *Plasmodium falciparum* parasite biology to the clinic. *Nature medicine*, **23**(8): 917-928.
- Brady, O. J., Slater, H. C., Pemberton-Ross, P., Wenger, E., Maude, R. J., Ghani, A. C. and Okell, L. C. (2017). Role of mass drug administration in elimination of *Plasmodium falciparum* malaria: a consensus modelling study. *The Lancet Global Health*, **5**(7): e680-e687.
- Brant, H. L., Ewers, R. M., Vythilingam, I., Drakeley, C., Benedick, S. and Mumford, J. D. (2016). Vertical stratification of adult mosquitoes (Diptera: Culicidae) within a tropical rainforest in Sabah, Malaysia. *Malaria journal*, **15**, 1-9.
- Brunner, N. C., Karim, A., Athieno, P., Kimera, J., Tumukunde, G., Angiro, I. and Hetzel, M. W. (2021). Starting at the community: treatment seeking pathways of children with suspected severe malaria in Uganda. *medRxiv*, 2021-12.
- Buchwald, A. G., Sorkin, J. D., Sixpence, A., Chimenya, M., Damson, M., Wilson, M. L. and Laufer, M. K. (2019). Association between age and *Plasmodium falciparum* infection dynamics. *American journal of epidemiology*, **188**(1): 169-176.
- Carlos, B. C., Rona, L. D., Christophides, G. K. and Souza-Neto, J. A. (2019). A comprehensive analysis of malaria transmission in Brazil. *Pathogens and global health*, **113**(1): 1-13.
- Carrasco, D., Lefèvre, T., Moiroux, N., Pennetier, C., Chandre, F. and Cohuet, A. (2019). Behavioural adaptations of mosquito vectors to insecticide control. *Current opinion in insect science*, **34**, 48-54.

- Cheaveau, J., Mogollon, D. C., Mohon, M. A. N., Golassa, L., Yewhalaw, D. and Pillai, D. R. (2019). Asymptomatic malaria in the clinical and public health context. *Expert review of anti-infective therapy*, **17**(12): 997-1010.
- Chirombo, J., Ceccato, P., Lowe, R., Terlouw, D. J., Thomson, M. C., Gumbo, A. and Read, J. M. (2020). Childhood malaria case incidence in Malawi between 2004 and 2017: spatio-temporal modelling of climate and non-climate factors. *Malaria journal*, **19**, 1-13.
- Clark, T. D., Greenhouse, B., Njama-Meya, D., Nzarubara, B., Maiteki-Sebuguzi, C., Staedke, S. G. and Dorsey, G. (2008). Factors determining the heterogeneity of malaria incidence in children in Kampala, Uganda. *The Journal of infectious diseases*, **198**(3): 393-400.
- Conroy, A. L., Datta, D. and John, C. C. (2019). What causes severe malaria and its complication in children? Lessons learned over the past 15 years. *BMC medicine*, **17**(1): 1-4.
- Cowman, A. F., Healer, J., Marapana, D. and Marsh, K. (2016). Malaria: biology and disease. *Cell*, **167**(3): 610-624.
- Dabaro Desalegn, Zewdie Birhanu and Delenasaw Yewhalaw. (2020). Analysis of trends of malaria from 2010 to 2017 in Boricha District, Southern Ethiopia. *Malaria journal*, **19**: 1-10.
- Dabaro Desalegn, Zewdie Birhanu and Delenasaw Yewhalaw. (2021). Effects of rainfall, temperature and topography on malaria incidence in elimination targeted district of Ethiopia. *Malaria journal*, **20**(1): 1-10.

- Dayanand, K. K., Achur, R. N. and Gowda, D. C. (2018). Epidemiology, drug resistance, and pathophysiology of *Plasmodium vivax* malaria. *Journal of vector borne diseases*, **55**(1): 1.
- Debash Habtu, Habtye Bisetegn, Hussen Ebrahim, Daniel Getacher Feleke Daniel, Alemu Abebe Gedefie, Mihret Tilahun, Agumas Shibabaw, Endris Ebrahim, Mesfin Fiseha and Getu Abeje. (2022). Prevalence and associated risk factors of malaria among febrile under-five children visiting health facilities in Ziquala district, Northeast Ethiopia: A multicenter cross-sectional study. *Plos one*, **17**(10):
- Debash Habtu, Habtye Bisetegn, Hussen Ebrahim, Mihret Tilahun, Dejazmach Zelalem, Nigatu Getu and Daniel Getacher Feleke Daniel. (2023). Burden and seasonal distribution of malaria in Ziquala district, Northeast Ethiopia: a 5-year multi-center retrospective study. *BMJ open*, **13**(8): e067103.
- Debash Habtu, Yonas Erkihun, and Habtye Bisetegn. (2022). Malaria Threatens to Bounce Back in Abergele District, Northeast Ethiopia: Five-Year Retrospective Trend Analysis from 2016-2020 in Nirak Health Center. *BioMed Research International*, **2022** (6388979).
- Debash Habtu, Gebru Tesfaw, Hussen Ebrahim, Agumas Shibabaw, Yimer Fisseha Melese, Mihret Tilahun, Ermiyas Alemayehu, Ousman Mohammed, Melkam Tesfaye and Mengistu Abate. (2023). Symptomatic and asymptomatic malaria prevalence and its determinant factors in pastoral communities of Waghemira Zone, Northeast Ethiopia: A community-based cross-sectional study. *Health Science Reports*, **6**(6), e1336.

- Dejazmach Zelalem, Getaneh Alemu, Mulat Yimer Fisseha, Banchamlak Tegegne and Abel Getaneh. (2021). Prevalence of malaria and associated knowledge, attitude, and practice among suspected patients in Bahir Dar Zuria District, Northwest Ethiopia. *BioMed research international*, **2021** (3741413).
- Derbie Awoke and Megbaru Alemu. (2017). Five years malaria trend analysis in Woreta Health Center, Northwest Ethiopia. *Ethiopia Journal of Health Science*, **27** (5): 465–472.
- Diema Konlan, K., Amu, H., Konlan, K. D. and Japiong, M. (2019). Awareness and malaria prevention practices in a rural community in the Ho Municipality, Ghana. *Interdisciplinary Perspectives on Infectious Diseases*, **2019** (9365823).
- Dieye, Y., Mbengue, B., Dagamajalu, S., Fall, M. M., Loke, M. F., Nguer, C. M. and Dieye, A. (2016). Cytokine response during non-cerebral and cerebral malaria: evidence of a failure to control inflammation as a cause of death in African adults. *Peer Journal*, **4**, e1965.
- Drysdale, M., Tan, L., Martin, A., Fuhrer, I. B., Duparc, S. and Sharma, H. (2023). *Plasmodium vivax* in Children: Hidden Burden and Conspicuous Challenges, a Narrative Review. *Infectious Diseases and Therapy*, **12**(1): 33-51.
- Duguma Tadesse, Abdulrezak Nuri and Yayeh Melaku. (2022). Prevalence of malaria and associated risk factors among the community of Mizan-Aman Town and its catchment area in Southwest Ethiopia. *Journal of Parasitology Research*, **2022** (3503317).

- Duguma Tadesse, Eyob Tekalign and Mitiku Abera. (2023). Asymptomatic *Plasmodium* Infection and Associated Factors in Selected Districts of the Kaffa Zone, Southwest Ethiopia: A Cross-Sectional Study. *Journal of Tropical Medicine*, **2023** (4144834).
- Duguma Tadesse, Eyob Tekalign, Dassalegn Muleta and Asnake Simieneh. (2022). Malaria prevalence and risk factors among patients visiting Mizan Tepi University Teaching Hospital, Southwest Ethiopia. *Plos one*, **17**(7): e0271771.
- Ergete Solomon, Solomon Sorsa, Eskindir Loha and Solomon Asnake. (2018). Trend of malaria cases in Hana and Keyafer health centers, South Omo zone, southern Ethiopia. *Ethiopian Journal of health sciences*, **28**(3): 277-286.
- Esayas Endashaw, Asefa Tufa, Fekadu Massebo, Abdulhamid Ahemed, Ibssa Ibrahim, Dereje Dillu, Eyuel Asemahegn Bogale, Solomon Yared and Kebede Deribe. (2020). Malaria epidemiology and stratification of incidence in the malaria elimination setting in Harari Region, Eastern Ethiopia. *Infectious Diseases of Poverty*, **9**(1): 1-12.
- Essendi, W. M., Vardo-Zalik, A. M., Lo, E., Machani, M. G., Zhou, G., Githeko, A. K. and Afrane, Y. A. (2019). Epidemiological risk factors for clinical malaria infection in the highlands of Western Kenya. *Malaria journal*, **18**(1): 1-7.
- Ethiopia Ministry of Health, National Malaria Guidelines, 4th edition. Addis Ababa, Ethiopia: Ethiopian Federal Ministry of Health. 2017.
- Feleke Daniel Getacher, Aderaw Adamu, Angesom Gebreweld, Melkam Tesfaye, Wondmagegn Demisiss and Genet Molla. (2020). Asymptomatic malaria infection among pregnant

women attending antenatal care in malaria endemic areas of North-Shoa, Ethiopia: a cross-sectional study. *Malaria Journal*, **19**(1): 1-6.

Feleke Daniel Getacher, Gebretsadik Daniel and Angesom Gebreweld. (2018). Analysis of trend of malaria prevalence in Ataye, North Shoa, Ethiopia between 2013 and 2017. *Malaria journal*, **17**, 1-6.

Fikrie Anteneh, Mihiret Kayamo and Henok Bekele. (2021). Malaria prevention practices and associated factors among households of Hawassa City Administration, Southern Ethiopia, 2020. *Plos one*, **16**(5): 0250981.

Flatie Belaynesh Tazebew and Abaineh Munshea. (2021). Knowledge, attitude, and practice towards malaria among people attending Mekaneeyesus Primary Hospital, South Gondar, Northwestern Ethiopia: A cross-sectional study. *Journal of Parasitology Research*, **2021**: 5580715

Fox, T., Furnival-Adams, J., Chaplin, M., Napier, M. and Olanga, E. A. (2022). House modifications for preventing malaria. *Cochrane Database of Systematic Reviews*, (9).

Gebretsadik Daniel, Daniel Getacher Feleke and Mesfin Fiseha. (2018). Eight-year trend analysis of malaria prevalence in Kombolcha, South Wollo, north-central Ethiopia: a retrospective study. *Parasite Vectors*, **55** (2018).

Gemechu Temesgen, Abdi Samuel and Delensaw Yewhalaw. (2015). Ten years trend analysis of malaria prevalence and its correlation with climatic variables in Sibu Sire District, east Wollega zone, Oromia regional state, Western Ethiopia: a retrospective study. *Science, Technology and Arts Research Journal*, **4**(4): 99-105.

- Getahun Biruk and Abebe Ayalew Bekel. (2022). Prevalence and Associated Risk Factors of Malaria in North-Western Ethiopia. *African Journal of Health Sciences*, **35**(4): 446-454.
- Guntur, R. D., Kingsley, J. and Islam, F. M. A. (2021). Malaria awareness of adults in high, moderate and low transmission settings: A cross-sectional study in rural East Nusa Tenggara Province, Indonesia. *PLoS One*, **16**(11), 0259950.
- Haile Dessalegne, Aster Ferede, Bekalu Kassie, Abtie Abebaw and Yihenew Million. (2020). Five-year trend analysis of malaria prevalence in Dembecha Health Center, West Gojjam Zone, Northwest Ethiopia: a retrospective study. *Journal of parasitology research*, **2020** (8828670):1-7.
- Hailemariam Mengistu and Solomon Gebre. (2015). Trend analysis of malaria prevalence in Arsi Negelle health center, Southern Ethiopia. *Journal of Infectious Diseases and Immunity*, **7**(1): 1-6.
- Haileselassie Werissaw, Endalew Zemene, Ming-Chieh Lee, Daibin Zhong, Guofa Zhou, Behailu Taye and Alemayehu Dagne. (2021). The effect of irrigation on malaria vector bionomics and transmission intensity in western Ethiopia. *Parasites & vectors*, **14**, 1-11.
- Hawaria Dawit, Hallelujah Getachew, Guofa Zhong, Assalif Demissew, Kasahun Habitamu, Beka Raya, Ming-Chieh Lee, Delenasaw Yewhalaw and Guiyun Yan. (2019). Ten years malaria trend at Arjo-Didessa sugar development site and its vicinity, Southwest Ethiopia: a retrospective study. *Malaria journal*, **18**, 1-11.

- Howick, V. M., Russell, A. J., Andrews, T., Heaton, H., Reid, A. J., Natarajan, K. and Lawniczak, M. K. (2019). The Malaria Cell Atlas: Single parasite transcriptomes across the complete *Plasmodium* life cycle. *Science*, **365**(6455): eaaw2619.
- Hwang, J., Cullen, K. A., Kachur, S. P., Arguin, P. M. and Baird, J. K. (2014, March). Severe morbidity and mortality risk from malaria in the United States, 1985–2011. In *Open forum infectious diseases*, **1**(1). Oxford University Press.
- Jemal Abdurazak and Tsige Ketema. (2019). A declining pattern of malaria prevalence in Asendabo Health Center Jimma zone, Southwest Ethiopia. *BMC research notes*, **12**(1): 1-5.
- Kaindoa, E. W., Finda, M., Kiplagat, J. and Okumu, F. O. (2018). Housing gaps, mosquitoes and public viewpoints: a mixed methods assessment of relationships between house characteristics, malaria vector biting risk and community perspectives in rural Tanzania. *Malaria journal*, **17**, 1-16.
- Kang, J. M., Cho, P. Y., Moe, M., Lee, J., Jun, H., Lee, H. W. and Na, B. K. (2017). Comparison of the diagnostic performance of microscopic examination with nested polymerase chain reaction for optimum malaria diagnosis in Upper Myanmar. *Malaria journal*, **16**(1): 1-8.
- Kayendeke, M., Nabirye, C., Nayiga, S., Westercamp, N., Gonahasa, S., Katureebe, A. and Hutchinson, E. (2023). House modifications as a malaria control tool: How does local context shape participants experience and interpretation in Uganda. *Malaria Journal*, **22**(1):244.

- Kendie FasilTamirat Hailegebriel W/kiros, Endalkachew Nibret Semegn and Melaku Wale Ferede. (2021). Prevalence of malaria among adults in Ethiopia: a systematic review and meta-analysis. *Journal of tropical medicine*, **2021**, 1-9.
- Kenea Oljira, Meshesha Balkew, Habte Tekie, Wakgari Deressa, Eskindir Loha, Bernt Lindtjørn and Hans J. (2019). Impact of combining indoor residual spraying and long-lasting insecticidal nets on *Anopheles arabiensis* in Ethiopia: results from a cluster randomized controlled trial. *Malaria Journal*, **18**(1): 1-11.
- Koenker, H., Arnold, F., Ba, F., Cisse, M., Diouf, L., Eckert, E. and Kilian, A. (2018). Assessing whether universal coverage with insecticide-treated nets has been achieved: Is the right indicator being used. *Malaria journal*, **17**(1): 1-11.
- Komaki-Yasuda, K., Vincent, J. P., Nakatsu, M., Kato, Y., Ohmagari, N. and Kano, S. (2018). A novel PCR-based system for the detection of four species of human malaria parasites and *Plasmodium knowlesi*. *PLoS One*, **13**(1): 0191886.
- Lakew Yosef Yohanes, Anteneh Fikrie, Sisay Bedane Godana, Fatuma Wariyo and Wongelawit Seyoum. (2023). Magnitude of malaria and associated factors among febrile adults in Siraro District Public Health facilities, West Arsi Zone, Oromia, Ethiopia 2022: a facility-based cross-sectional study. *Malaria Journal*, **22**(1): 1-10.
- Lendongo Wombo, J. B., Ibinga, E., Oyegue-Liabagui, S. L., Imboumy Limoukou, R. K., Okouga, A. P., Mounioko, F. and Ngoungou, E. B. (2023). Severe malaria in children and adolescents in Southeast Gabon. *BMC Infectious Diseases*, **23**(1): 1-8.

- Leonard, C. M., Mohammed, H., Tadesse, M., McCaffery, J. N., Nace, D., Halsey, E. S. and Rogier, E. (2022). Missed *Plasmodium falciparum* and *Plasmodium vivax* mixed infections in Ethiopia threaten malaria elimination. *The American Journal of Tropical Medicine and Hygiene*, **106**(2): 667.
- Mace, K. E., Arguin, P. M. and Tan, K. R. (2018). Malaria surveillance-United States, 2015. *MMWR Surveillance Summaries*, **67**(7):1.
- Makhija, K. S., Maloney, S. and Norton, R. (2015). The utility of serial blood film testing for the diagnosis of malaria. *Pathology*, **47**(1): 68-70.
- Mala, M., Imam, M. and Hassan, K. (2016). Interaction between parasite and vector for Malaria disease transmission-a review on Malaria. *Progressive Agriculture*, **27**(2): 168-174.
- Mandala, W. L., Msefula, C. L., Gondwe, E. N., Drayson, M. T., Molyneux, M. E. and MacLennan, C. A. (2017). Cytokine profiles in Malawian children presenting with uncomplicated malaria, mala severe malarial anemia, and cerebral malaria. *Clinical and vaccine immunology*, **24**(4): e00533-16.
- Mankadi, P. M. and Jin, Y. (2021). Effects of Door-to-Door Hang-Up Visits on the Use of Long-Lasting Insecticide-Treated Mosquito Nets in the Democratic Republic of the Congo: A Cluster Randomized Controlled Trial. *International Journal of Environmental Research and Public Health*, **18**(17): 9048.
- Matthew, O. J. (2020). Investigating climate suitability conditions for malaria transmission and impacts of climate variability on mosquito survival in the humid tropical region: a case

- study of Obafemi Awolowo University Campus, Ile-Ife, south-western Nigeria. *International journal of biometeorology*, **64**(3): 355-365.
- Meerstein-Kessel, L., Andolina, C., Carrio, E., Mahamar, A., Sawa, P., Diawara, H. and Bousema, T. (2018). A multiplex assay for the sensitive detection and quantification of male and female *Plasmodium falciparum* gametocytes. *Malaria journal*, **17**, 1-11.
- Mekuria Mulugeta, Dereje Negasa Binegde, Jirenga Derega, Elias Teferi Bala, Bikila Tesfa and Berhanu Senbeta Deriba. (2022). Insecticide-Treated Bed Net Utilization and Associated Factors Among Households in Ilu Galan District, Oromia Region, Ethiopia. *Environmental Health Insights*, **16** (11786302221078122).
- Menjetta Tadesse. (2021). Assessment of Knowledge, Attitude, and Practice towards Prevention and Control of Malaria in Halaba Town, Southern Ethiopia. *Journal of Tropical Medicine*, **2021**(5665000).
- Mischlinger, J., Rönnerberg, C., Álvarez-Martínez, M. J., Bühler, S., Paul, M., Schlägenhauf, P. and Ramharter, M. (2020). Imported malaria in countries where malaria is not endemic: a comparison of semi-immune and nonimmune travelers. *Clinical microbiology reviews*, **33** (2): e00104-19.
- Mohan, I., Kodali, N. K., Chellappan, S., Karuppusamy, B., Behera, S. K., Natarajan, G. and Balabaskaran Nina, P. (2021). Socio-economic and household determinants of malaria in adults aged 45 and above: analysis of longitudinal ageing survey in India, 2017–2018. *Malaria journal*, **20**, 1-9.

- Mulugeta Amanuel, Atsede Assefa, Atsede Eshetie, Birhanie Asmare, Meseret Birhanie and Yemataw Gelaw. (2022). Six-year trend analysis of malaria prevalence at University of Gondar Specialized Referral Hospital, Northwest Ethiopia, from 2014 to 2019. *Scientific Reports*, **12**(1): 1411.
- Naing, C., Whittaker, M. A., Htet, N. H., Aye, S. N. and Mak, J. W. (2019). Efficacy of antimalarial drugs for treatment of uncomplicated *falciparum* malaria in Asian region: A network meta-analysis. *Plos one*, **14**(12): e0225882.
- Nureye Dejene and Solomon Assefa. (2020). Old and recent advances in life cycle, pathogenesis, diagnosis, prevention, and treatment of malaria including perspectives in Ethiopia. *The Scientific World Journal*, **2020**, 1-17.
- Ochomo, E., Chahilu, M., Cook, J., Kinyari, T., Bayoh, N. M., West, P. and Mbogo, C. (2017). Insecticide-treated nets and protection against insecticide-resistant malaria vectors in Western Kenya. *Emerging infectious diseases*, **23**(5): 758.
- Okiring, J., Epstein, A., Namuganga, J. F., Kanya, E. V., Nabende, I., Nassali, M. and Dorsey, G. (2022). Gender difference in the incidence of malaria diagnosed at public health facilities in Uganda. *Malaria Journal*, **21**(1): 1-12.
- Okuneye, K., Eikenberry, S. E. and Gumel, A. B. (2019). Weather-driven malaria transmission model with gonotrophic and sporogonic cycles. *Journal of biological dynamics*, **13**(1): 288-324.
- Panzi, E. K., Okenge, L. N., Kabali, E. H., Tshimungu, F., Dilu, A. K., Mulangu, F. and Kandala, N. B. (2022). Geo-Climatic Factors of Malaria Morbidity in the Democratic Republic of

- Congo from 2001 to 2019. *International Journal of Environmental Research and Public Health*, **19**(7): 3811.
- Perkins, J. M., Krezanoski, P., Takada, S., Kakuhikire, B., Batwala, V., Tsai, A. C. and Bangsberg, D. R. (2019). Social norms, misperceptions, and mosquito net use: a population-based, cross-sectional study in rural Uganda. *Malaria journal*, **18**, 1-13.
- Plucinski, M., Aidoo, M. and Rogier, E. (2021). Laboratory detection of malaria antigens: a strong tool for malaria research, diagnosis, and epidemiology. *Clinical Microbiology Reviews*, **34**(3): e00250-20.
- Pryce, J., Richardson, M. and Lengeler, C. (2018). Insecticide-treated nets for preventing malaria. *Cochrane Database of Systematic Reviews*, **6** (11).
- Quaresima, V., Agbenyega, T., Oppong, B., Awunyo, J. A. D., Adu Adomah, P., Enty, E. and Castelli, F. (2021). Are malaria risk factors based on gender? a mixed-methods survey in urban setting in Ghana. *Tropical Medicine and Infectious Disease*, **6**(3): 161.
- Rahmasari, F. V., Asih, P. B., Dewayanti, F. K., Rotejanaprasert, C., Charunwatthana, P., Imwong, M. and Syafruddin, D. (2022). Drug resistance of *Plasmodium falciparum* and *Plasmodium vivax* isolates in Indonesia. *Malaria Journal*, **21**(1): 354.
- Sani Kalil Falaho Falaho, Mohammed Hasen Bedaso, and Shukri Kabeta Wario. (2020). Trends of malaria morbidity and mortality from 2010 to 2017 in Bale Zone, Ethiopia: analysis of surveillance data. *Infection and Drug Resistance*, **13** (2020): 4379-4387.
- Seyoum Dinberu, Delenasaw Yewhalaw., Duchateau, L., Brandt, P., Rosas-Aguirre, A. and Speybroeck, N. (2017). Household level spatio-temporal analysis of *Plasmodium*

- falciparum* and *Plasmodium vivax* malaria in Ethiopia. *Parasites & Vectors*, **10**(1): 1-11.
- Shamebo Tsegaye Adino and Beyene Petros. (2019). Trend analysis of malaria prevalence in Halaba special district, Southern Ethiopia. *BMC research notes*, **12**(1): 1-6.
- Shapiro, L. L., Whitehead, S. A. and Thomas, M. B. (2017). Quantifying the effects of temperature on mosquito and parasite traits that determine the transmission potential of human malaria. *PLoS biology*, **15**(10): e2003489.
- Shiferawu Tadegew Teshome and Azene Tesfaye Desta. (2021). Five-year trend analysis of malaria prevalence in Shewarobit, Amhara regional state, North-central Ethiopia. *Pan African Medical Journal*, **40**(1).
- Sinka, M. E., Pironon, S., Massey, N. C., Longbottom, J., Hemingway, J., Moyes, C. L. and Willis, K. J. (2020). A new malaria vector in Africa: predicting the expansion range of *Anopheles stephensi* and identifying the urban populations at risk. *Proceedings of the National Academy of Sciences*, **117**(40): 24900-24908.
- Solomon Absra, Daniel Kahase and Mihret Alemayehu. (2020). Trend of malaria prevalence in Wolkite healthcenter: an implication towards the elimination of malaria in Ethiopia by 2030. *Malaria Journal*, **19**(1): 1-8.
- Tadesse Frew, Andrew W. Fogarty and Wakgari Deressa. (2018). Prevalence and associated risk factors of malaria among adults in East Shewa Zone of Oromia Regional State, Ethiopia: a cross-sectional study. *BMC public health*, **18**(1): 1-8.

- Tangpukdee, N., Duangdee, C., Wilairatana, P. and Krudsood, S. (2009). Malaria diagnosis: a brief review. *The Korean journal of parasitology*, **47**(2): 93.
- Tarekegn Mihretu, Habte Tekie, Sisay Dugassa and Yitbarek Wolde-Hawariat. (2021). Malaria prevalence and associated risk factors in Dembiya district, North-western Ethiopia. *Malaria Journal*, **20**, 1-11.
- Tefera Selomon, Temesgen Bekele, Kefelegn Getahun, Abiyot Negash and Tsige Ketema. (2022). The changing malaria trend and control efforts in Oromia Special zone, Amhara Regional State, North-East Ethiopia. *Malaria Journal*, **21** (1) :128.
- Tesfaye Azene and Tadegew Teshome. (2022). Prevalence and Associated Factors of Malaria Infection among Outpatients Visiting Shewa Robit Health Center, Northcentral Ethiopia. Ethiopia. *Journal of Tropical Medicine*, **2022** (1784012).
- Thomas, S., Ravishankaran, S., Asokan, A., Johnson Amala Justin, N. A., Maria Jusler Kalsingh, T., Mathai, M. T. and Eapen, A. (2018). Socio-demographic and household attributes may not not necessarily influence malaria: evidence from a cross sectional study of households in an urban slum setting of Chennai, India. *Malaria journal*, **17**, 1-11.
- Tigu Fitsum, Tsegay Gebremaryam, and Asnake Desalegn. (2021). Seasonal profile and five-year trend analysis of malaria prevalence in Maygaba Health Center, Welkait District, Northwest Ethiopia. *Journal of Parasitology Research*, **2021**, 1-7.
- Tsegaye Adino, Tesfahun, Andualem Ayele and Simon Birhanu. (2021). Prevalence and associated factors of malaria in children under the age of five years in Wogera district, northwest Ethiopia: A cross-sectional study. *Plos one*, **16**(10), e0257944.

Wassmer, S. C., Taylor, T. E., Rathod, P. K., Mishra, S. K., Mohanty, S., Arevalo-Herrera, M. and Smith, J. D. (2015). Investigating the pathogenesis of severe malaria: a multidisciplinary and cross-geographical approach. *The American journal of tropical medicine and hygiene*, **93** (3): 42

Workineh Lemma, Misganew Lakew, Shega Dires, Teklehaimanot Kiros, Shewaneh Damtie, Wasihun Hailemichael, Dassalegn Muleta and Tahir Eyayu. (2021). Prevalence of Malaria and Associated Factors Among Children Attending Health Institutions at South Gondar Zone, Northwest Ethiopia: A Cross-Sectional Study. *Global Pediatric Health*, **8** (2333794X211059107).

World Bank, Four Ethiopia: A Regional Characterization (accessed 23 March 2006).

World Health Organization (2015). Guidelines for the treatment of malaria. World Health Organization.

World Health Organization (2019). Guidelines for malaria vector control. World Health Organization.

World Health Organization, "World Malaria Report 2021".

World Health Organization. "World Malaria Report 2020". 20 years of global progress and challenges, 2020

World Health Organization. (2018). World Malaria Report, Geneva.

World Health Organization. (2019). Compendium of WHO malaria guidance: *prevention, diagnosis, treatment, surveillance and elimination* (No. WHO/CDS/GMP/2019.03).

World Health Organization.

- World Health Organization. (2022). World malaria report 2022. World Health Organization.
- World Health Organization. *World malaria report 2022*. World Health Organization, 2022.
- Yhdego Tsegay, Gebremaryam, Asnake Desalegn Gardew and Fitsum Tigu Yifat (2022). Malaria prevalence, knowledge and associated factors among household heads in Maygaba town, Ethiopia. *PLOS Global Public Health*, **2**(3): e0000071.
- Yimer Fisseha, Abebe Animut, Berhanu Erko and Hassen Mamo. (2015). Past five-year trend, current prevalence and household knowledge, attitude and practice of malaria in Abeshge, South-central Ethiopia. *Malaria Journal*, **14**(1): 1-11.
- Zambare, K. K., Thalkari, A. B. and Tour, N. S. (2019). A review on pathophysiology of malaria: The overview of etiology, life cycle of malarial parasite, clinical signs, diagnosis and complications. *Asian Journal of Research in Pharmaceutical Science*, **9**(3): 226-230.
- Zimmerman, P. A. and Howes, R. E. (2015). Malaria diagnosis for malaria elimination. *Current opinion in infectious diseases*, **28**(5): 446-454.
- Tamiru Afework, Tadesse Tolossa, Bikila Regasa and Getu Mosisa. (2022). Prevalence of asymptomatic malaria and associated factors in Ethiopia: Systematic review and meta-analysis. *SAGE Open Medicine*, 10, 20503121221088085.

## APPENDICES

### Appendix 1: Staining and microscopic examination of malaria blood films

#### A) Staining of blood films with Giemsa

The process of staining malaria blood films with Giemsa involves three steps. First, buffered water to a pH of 7.2 for use in the preparation of the stain solution, then prepare the working solution of the stain for routine staining of the malaria blood film and finally, stain the malaria blood films using Giemsa stain. Both thick and thin blood smears should be stained with 3% Giemsa solution for 30 minutes in staining jars in the laboratory. The stained slides should be rinsed with tap water and placed in an upright position to dry.

The components of the Giemsa solution are methylene blue (azure) and eosin. The parasite nucleus is stained red by the eosin component, while the cytoplasm stained blue by methylene component. Methanol is used to fix the thin blood film. Dehemoglobinization of the thick film and staining takes place at the same time during the process.

#### B) Detection and identification of the *Plasmodium* species and stages

While the malaria parasites are retained intact and concentrated in the thick film, the red blood cells (RBCs) are lysed and dehemoglobinized, enabling for their accurate detection and identification. The RBCs in the thin film maintain their original morphology after being fixed with 100% methanol, and if malaria parasites are present, they become visible inside the cells. To ensure precise speciation and an accurate calculation of parasite density, it is essential that the diagnosis of malaria is based on properly produced thick and thin malaria blood films.

1. Place the Giemsa-stained blood film to be examined on the microscope stage
2. Position the thick film in line with the objective lens

3. Switch on the microscope and adjust the light source optimally by looking through the ocular and the  $\times 10$  objective (low power)
4. Place a drop of immersion oil on the thick film, and allow it to spread
5. To avoid cross contamination, ensure that the immersion oil applicator never touches the slide
6. Scan the blood film for parasites and blood elements. Select part of the film that is well stained and has evenly distributed white blood cells
7. Switch to  $\times 100$  oil immersion objective over the selected portion of the thick film. Raise the mechanical stage until the objective lens gently touches the immersion oil but not the slide
8. Examine the slide in a systematic manner. Start at the top left of the film, and begin at the periphery of the field, then move horizontally to the right, field by field. When the other end of the film is reached, move the slide slightly downwards, then to the left, field by field, and so forth. For efficient examination, continuously focus and refocus with the fine adjustment throughout examination of each field.

Source: [WHO \(microscopy examination of thick and thin blood films for identification of malaria parasites\)](#).

## Appendix-2: The informed consent form English version

PIN\_\_\_\_\_

Name of study participant\_\_\_\_\_

Age\_\_\_\_\_ Sex\_\_\_\_\_

Laboratory technician Name\_\_\_\_\_

Site/Health center \_\_\_\_\_

Hello my name is \_\_\_\_\_ and I work in Wolaita Sodo university and currently the post graduate student of Hawassa university. I am here to collect information about the trends and prevalence of malaria infection and the associated risk factors in case of Loka Abaya district.

Malaria is one of serious health problem which caused the *Plasmodium* parasite through mosquito biting. The diseases killed many people through the world and it is one of the widespread diseases in developing countries. It is also one of significant health issue in our country, which affected many communities. The purpose of the study is to determine the prevalence of malaria parasite infection and to assess the risk factors for the infection among people living in the district.

Therefore, I will ask you some questionnaires related to malaria and you are requested to give one drop of blood from your finger to examine for malaria parasite. Giving blood sample does not harm you while simple pain during the injection of lancet. You will receive a medication if your slide test indicates that you have malaria, but otherwise you will not receive any further

benefits for taking part in this study. We shall make every effort to maintain the confidentiality of the study's records. The study will not use any individual identities in any reports or publications.

All of the information gathered will be coded and kept apart from participant names or other direct identifying information. All research data will always be stored in locked files. The files will only be accessible to research staff, and only those who absolutely need to see names or other personally identifiable information will be able to access that specific file. Your data and the sample you provided will only be used for the above-mentioned purposes, and both are disposed of right away upon the completion of the laboratory analysis and result analysis.

After the study is over, a report on the results will be written and submit it to a publication or another venue. The reports won't contain any personal information about you, such as your name or identity.

Please read, tick off each of the boxes and sign the form if you agree to take part in this study.

1. I understand what this study is about and know how to contact the investigators if I want to.
2. I understand a portion of the sample may be stored and used for this study only. If there is leftover after the completion of the study, it will be discarded safely.
3. I understand a portion of the sample stored would be shipped abroad for further analyses.
4. I understand that all the information given to the investigators and all test results will be kept private and confidential.

5. I understand that I will not get benefit financially from this study except for transport and a bottle of soft drink after blood sample collection.
6. I understand that I am free to withdraw myself from this study if I want to.
7. I understand that if I refuse to take part in this study, that my care will not be affected.

### Appendix- 3 Questionnaire survey (English version)

Hawassa University, College of Natural and Computational Sciences, Department of Biology.

A Questionnaire to investigate the prevalence of malaria infection and the associated risk factors in case of Loka Abaya District, South western Sidama region Ethiopia.

#### Instructions:

Please select the most appropriate answer you wish to give and circle it in respective place

#### Part I: Sociodemographic characteristics.

Date ----- Village ----- Household number -----

1. Sex        A) male        B) female

2. Age

A) 18-25        B) 26-35        C) 36-45        D) 45 and above

3. Marital status

A) single    B) married    C) widowed    D) divorced

4. Religion

A) protestant    B) catholic    C) orthodox    D) muslim

5. Occupation

A) farmer    B) merchant    C) house wife    D) government employed    E) unemployed  
F) student    G) casual labor

6. Residence

A) rural        B) urban

7. Education level

A) illiterate    B) read and write only    C) primary school    D) secondary school    E)  
college        diploma and above

8. House hold size

- A) 2-4    B) 5-7    C) 8 and above

**Part II. The possible risk factors for malaria infection**

1 The presence of stagnant water near your residential place.

- A) yes                      B) no

2. Availability of windows in your household

- A) yes                      B) no

3. If yes what type of window

- A) window without cover    B) windows with glass    C) window with screens  
D) window with curtains of shutter

4. History of anti-malaria treatment

- A) yes                      B) no

5. Did you recently receive education or information on malaria?

- A) yes                      B) no

6. At any time in the last 12 months, has anyone sprayed your dwelling against mosquito?

- A) yes                      B) no

7. How many months ago was the house sprayed against mosquito?

- A) < 6 months    B) 6-12 months    C) >12 months

8. At any time in the past 12 months, have the walls in your dwelling been plastered or painted?

A) yes                      B) no

9. Does your household have mosquito nets?

A) yes                      B) no

10. How long ago did your household obtain the mosquito?

A) < 6 months          B) 6-12 months          C) >12 months

11. Did you sleep under this net last night?

A) yes                      B) no

12. How do you use the net at night?

A) hanging over the bed to the floor    B) hanging over the bed tucked in

C) hanging over the sleeping matters or mat

13. Travels history

A) recent travel to malariaous rural area    B) recent travel to malariaous urban

area

C) regular travel to malariaous rural area    D) regular travel to malariaous urban

area

## Appendix 4 Data collection methods of the researcher

### I) House to house blood sampling

A)



**B**



C

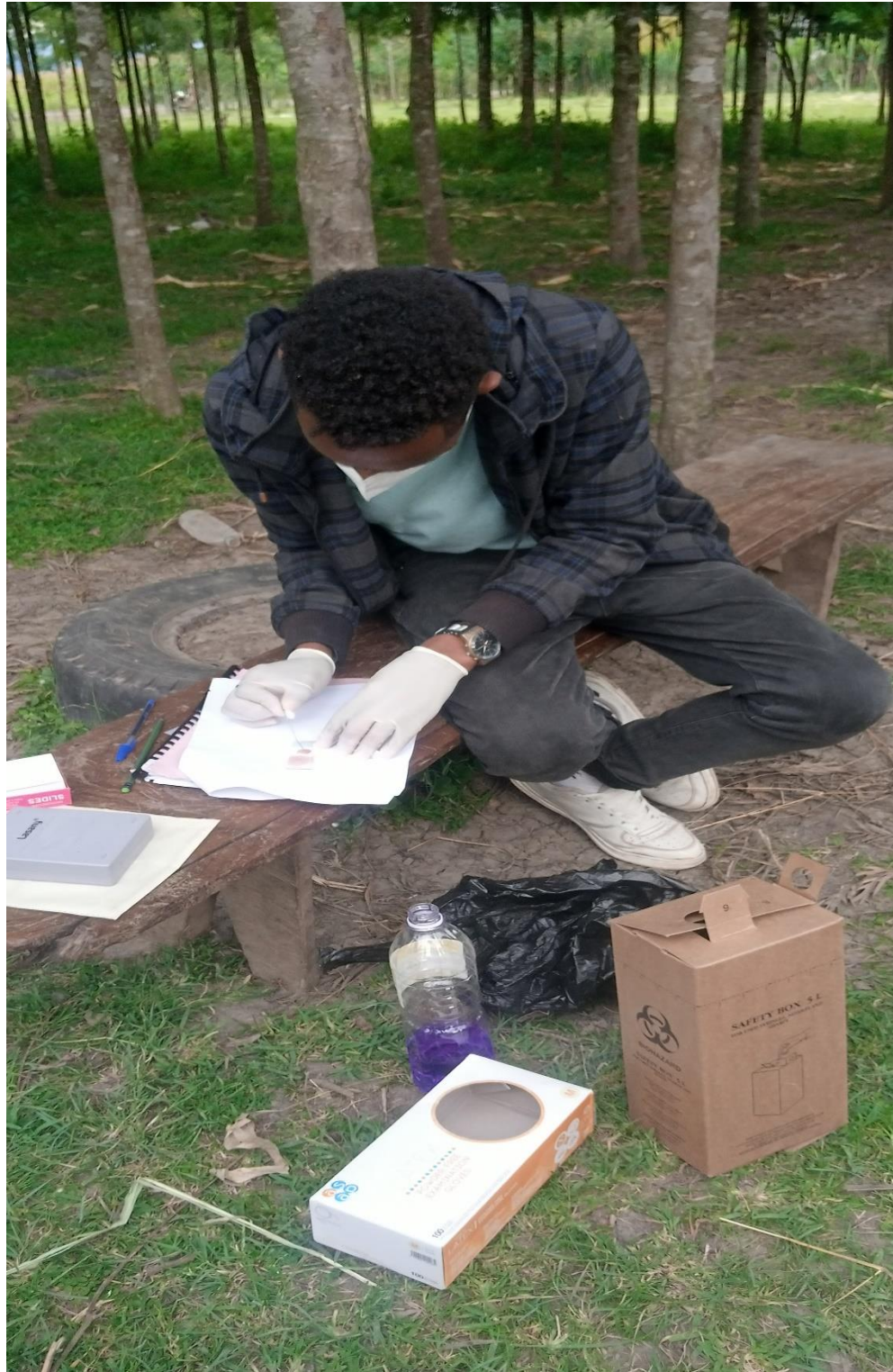


## II) Blood smears preparation in the field

A)



B)



C)



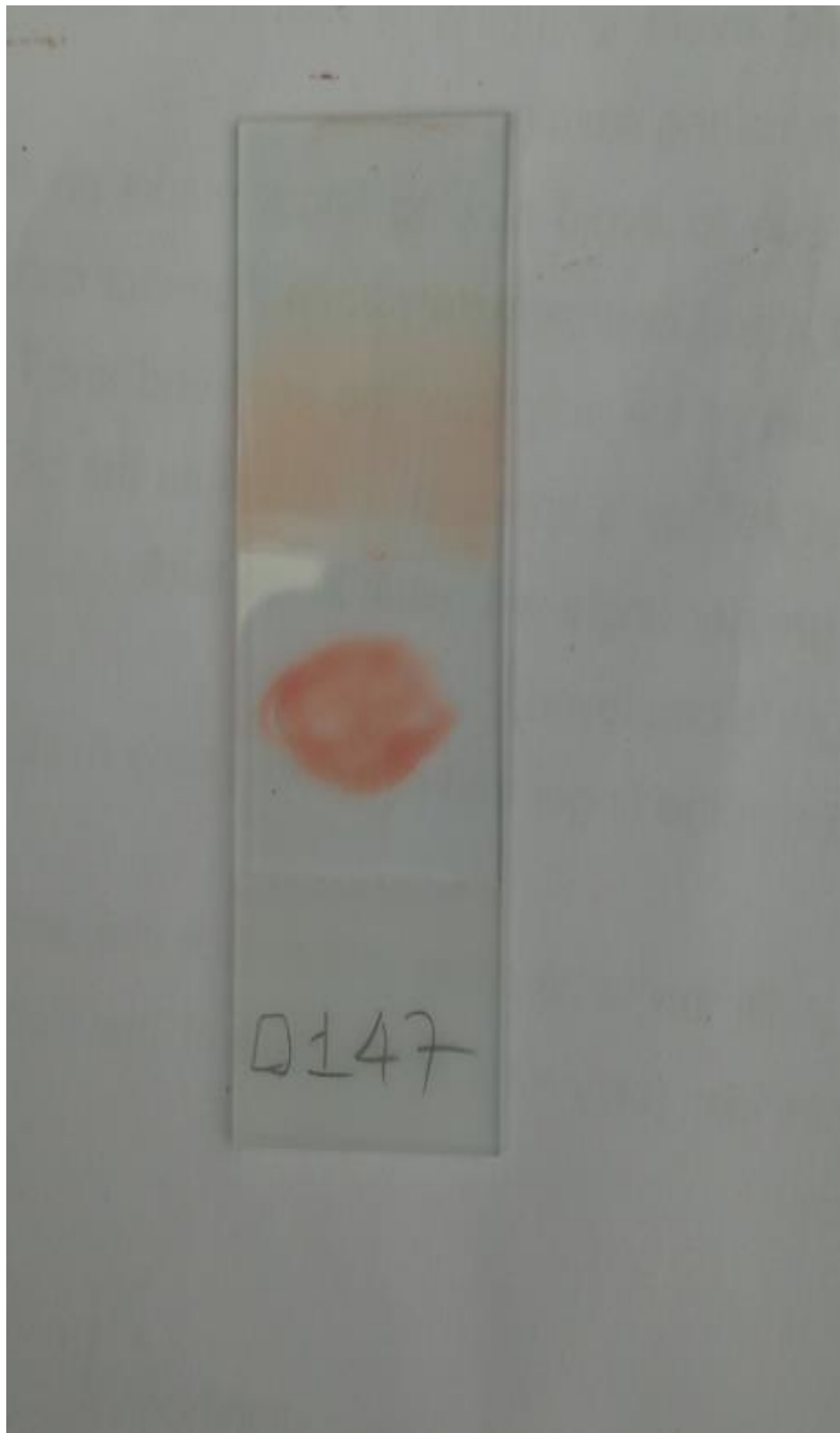
D)



E)



F)



### III) Staining and Examination of blood smears

A)



B)

