HAWASSA UNIVERSITY

COLLEGE OF MEDICINE AND HEALTH SCIENCES

SCHOOL OF MEDICAL LABORATORY SCIENCES

PREVALENCE OF MALARIA AND ASSOCIATED FACTORS AMONG UNDER-FIVE CHILDREN IN ILUGALAN DISTRICT, OROMIA REGION, WEST ETHIOPIA

BY: LELISA FIKADU(BSc, Medical Laboratory Technology)

PREVALENCE OF MALARIA AND ASSOCIATED FACTORS AMONG UNDER-FIVE CHILDREN IN ILU-GALAN DISTRICT, OROMIA REGION, WEST ETHIOPIA

BY: LELISA FIKADU(BSc, Medical Laboratory Technology)

Advisors

Main advisor: Dr. Solomon Asnake(PhD)

Co-advisor: Dr. Mengistu Hailemariam(PhD)

A thesis submitted to the School of Medical Laboratory Sciences, College of Medicine and Health Sciences, Hawassa University in partial fulfillment of the requirements for the Master of Science degree in Medical Parasitology

January 13, 2024

Hawassa, Ethiopia

Approval sheet

This is to certify that the thesis prepared by Lelisa FIkadu entitled "Prevalence of malaria and associated factors among under-five children in, Ilu Galan district, Oromia region, West Ethiopia, 2023." submitted in partial fulfillment of the requirements for the Degree of Masters of Sciences in Medical Parasitology with the regulations of the university and meets the accepted standards concerning originality and quality.

Signed by the Examining Committee:

Internal Examiner _____Signature _____ Date_____

External Examiner _____Signature _____ Date_____

Main advisor _____Signature _____ Date_____

Co-advisor _____Signature _____ Date_____

Chair of Department or Graduate Program Coordinator

Hawassa University examiners' approval sheet

We, the undersigned, members of the Board of Examiners of the final open defense by

Lelisa Fikadu Kedida has read and evaluated his thesis entitled **"Prevalence of malaria and associated factors among under-five children in, Ilu Galan district, Oromia region, West Ethiopia, 2023."**, and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirements for the **Masters of Medical Parasitology**.

Name of Major Advisor	Signature	Date
Name of Internal Examiner	Signature	Date
Name of Chairperson	Signature	Date
Name of External Examiner	Signature	Date
SGS Approval	Signature	Date

Stamp of SGS Date:_____

Declaration

I, the undersigned declare that this thesis is my original work in partial fulfillment of the requirement for the Master of Medical Parasitology. I also declare that it has never been presented in this or any other university and that all resources and materials in the proposal have been duly acknowledged.

Lelisa Fikadu (BSc)

Signature: _____ Date of submission: _____Hawassa, Ethiopia

Approval of the Advisors:

Dr. Solomon Asnake(PhD)

Signature: _____ Date: _____ Hawassa, Ethiopia

Dr. Mengistu Hailemariam (PhD)

Signature: _____ Date: _____ Hawassa, Ethiopia

Acknowledgments

First of all, I would like to thank the Almighty God, for His grace and providence. I would like to express my deepest gratitude to my advisors Dr. Solomon Asnake and Dr. Mengistu Hailemariam for their consistent valuable advice, comments, immediate response, and follow-up. Then I would like to acknowledge the Department of Medical Laboratory Science, Hawassa University for allowing and funding me to prepare my proposal and conduct this thesis work. I am very thankful to the Ethiopian Public Health Institute, Oromia Regional IBR, and the Ethiopian Pharmaceutical Supply Agency of Nekemte hub for supplying Malaria RDT kit tests for the study. I would also like to express my deepest gratitude to West Shewa Zonal Health Bureau, Ilu Galan District Health Office, and Ejaji Health Center for the provision of materials for the study. My deepest gratitude also goes to the Ejaji Health Center laboratory staff for examining the microscopic part of the study. Last but not least, I would like to acknowledge my family and friends, particularly my wife Ms. Almaz Abebe, Iranfachisa Gurmu, Gamechis Motuma, and Hailemariam Difabachew) for their moral support and I would like to express my gratitude to my study parents/participants for their willingness and patience during the sample collection.

Abstract

Background: Malaria continues to impact the health of children in Ethiopia. Ilu Galan is an area of intense and prolonged, seasonal malaria transmission, and malaria is still the leading cause of morbidity and mortality among children under five years. This study aimed to determine malaria prevalence and associated risk factors among under-five children in the Ilu Galan district.

Methods: A community-based cross-sectional study was conducted from April to June 2023 in the Ilu Galan district in West Shewa Zone, Ethiopia. A multi-stage sampling technique was carried out to select representative households. A systematic random sampling technique was applied to enroll 557 study participants. Malaria was determined using thick and thin blood film microscopy and a rapid diagnostic test kit. Furthermore, a pre-tested structured questionnaire was administered to collect the socio-demographic characteristics of the study participants who were associated with malaria infection. Data was cleaned and entered into Epi data version 3.1 and exported to SPSS version 26 for analysis. Bivariate and multivariable logistic regression analyses were done to identify risk factors associated with malaria. Adjusted odds ratios (AOR) with a 95% confidence interval (CI) were used to determine the strength of association of variables. P-value < 0.05 at 95% CI was considered statistically significant.

Results: The overall prevalence of malaria among under-five children in the study area was 5.2%(28/542). *P. falciparum*, *P. vivax*, and mixed infection (both species) accounted for 71.4%, 25%, and 3.6% of the cases, respectively. Low parasitemia15/28 (53.6%)was more common, and high parasitemia13/28(46.4%) with the proportion of parasite density respectively. Malaria infection was corelated to staying out door at night(AOR=3.09;95%CI:1.01-9.478),utilization of ITN(AOR=.261;95% CI:0.073-0.939), presence of eave on the house(AOR=4.081;95%CI:1.202-13.437),<1kmdistance of house hold from river(AOR=4.317;95%CI:1.194-15.618) and presence of stagnant water nearby house(AOR=11.399;95% CI:3.710-35.021).

Conclusion: Malaria is still a common health problem in the study area, so the local government and other concerned bodies should focus on malaria prevention and control to minimize the burden.

Keywords: Malaria infection, malaria control, mosquito (malaria vector), plasmodium species

Table of Contents

List of tables

List of Figures

Acronyms and abbreviation

ACT	Artemisinin-based combination therapy
AOR	Adjusted odd ratio
EDHS	Ethiopian Demographic and Health Surveys
ITN	Insecticide-Treated Net
IPTP	Intermittent preventive therapy in pregnancy
IRS	Indoor Residual Spraying
LLIN	Long-Lasting Insecticidal Net
LSM	Larval Source Management
MIS	Malaria Indicator Survey
SSA	Sub- Saharan Africa
OR	Odd ratio
RDT	Rapid diagnostic test
WHO	World Health Organization

Introduction

1.1.Background

Malaria is caused by the protozoa parasite of the genus *Plasmodium* and is considered a severe, infectious, parasitic disease. Malaria is transmitted by the bite of the female mosquito of the genus *Anopheles* infected with the protozoa, of which five species are responsible for infecting humans: *Plasmodium falciparum, P.vivax, P.malariae, P.ovale,* and *P.knowlesi*. However, *P. falciparum* and *P. vivax* are the most prevalent species in the world. *P. falciparum* is the most virulent species in terms of morbidity and mortality(White *et al.,* 2009). In humans, malaria parasites multiply and divide, initially in the liver cells and subsequently exponentially in the infected person's red blood cells. When the parasites grow and exit the liver to infect red blood cells, lifecycle produces the symptoms of malaria in humans(Warkaw *et al.*, 2022). Large plantations, swampy, poorly drained environments like those seen in tropical regions, as well as agricultural activities like irrigation, promote mosquito breeding. The amount of rainfall, the altitude, the local temperature, and other environmental conditions, such as poor drainage and unauthorised disposal sites, have an impact on mosquito activity and reproduction(Adebukola *et al.*, 2022).

Malaria is a global public health problem; however, the majority of the cases and deaths occur in the tropics and subtropics. Most of the malaria cases occur in the African region (95%) followed by Eastern Mediterranean regions (2.5%) and Southeast Asia (2%) (World Health Organization, 2022). Due to service disruptions during the coronavirus pandemic, African areas were accountable for about 95% of malaria cases, with 14 million more cases and 47 000 more deaths reported worldwide than the previous year(Monroe *et al.*, 2022). The majority of malaria cases and deaths worldwide occur in sub-Saharan Africa, where the disease is most prevalent. It hurts people's health as well as economic development in many developing nations, especially in sub-Saharan Africa(Sempungu *et al.*, 2023). Malaria is the most common cause of death in children under the age of five years and pregnant women in developing countries(Lee *et al.*, 2022). Due to their immune naivety to malaria parasites, children are one of the most vulnerable groups. Malaria may cause as many as 10% of all deaths in children globally. Severe anemia, respiratory distress, and cerebral malaria are common complications in children with severe malaria(Conroy *et al.*, 2019).

Ethiopia is one of the sub-Saharan African countries with the highest morbidity and fatality rates from malaria. The presence of malaria during harvest seasons, which lowers agricultural output and consequently causes food insecurity and poverty(Amenu, 2014)), is a major worry in the nation and has the potential to seriously harm the health and socioeconomic development of the country. The major epidemics occur cyclically every 5–8 years in Ethiopia, but focal epidemics are occurring every year (Shumbej *et al.*, 2019). In Ethiopia, malaria has been the main cause of disease and fatalities and is a very serious issue. It is estimated that 75% of the country's land is malarious with about 68%-70% of the total population living in areas at risk of malaria (Bugssa and Tedla, 2020). *Plasmodium falciparum* and *Plasmodium vivax* are dominant *Plasmodium* species accounting for almost 60% and 40% of all cases, respectively. Plasmodium *malariae* and *Plasmodium ovale* are rare; both for less than 1% of all confirmed malaria cases. The major malaria vector incriminated in Ethiopia is *Anopheles arabiensis* followed by *Anopheles pharoenesis*, *Anopheles funestus,* and *Anopheles nili* (Byrnes *et al*., 2010). In areas where mosquitoes live longer, transmission is more prevalent because the parasite has more time to mature inside the mosquito. The two transmission seasons are major (September to December) and minor (April to June), and the transmission pattern is seasonal and unstable. The other months (January–March) and (July–August) typically represent low malaria transmission seasons in most communities (Bugssa and Tedla, 2020). Since 2005, the Ethiopian government has significantly increased the scope of its efforts to combat malaria, including diagnostic testing, rapid case management using artemisinin-based combination therapy (ACT), insecticide-treated bed nets (ITNs), and indoor residual spraying (IRS)(Girum, Shumbej and Shewangizaw, 2019). However, among under-five children in Ethiopia, malaria remains one of the top major causes of illness and mortality. Moreover, the magnitude of malaria infection among under-five children is too high which ranges from 16 to 54% in Ethiopia(Aychiluhm *et al.*, 2020). These efforts notwithstanding, malaria remains a serious public health concern in endemic areas, among children under the age of five (WHO, 2021b) (Oxborough, 2016).

1.2.Statement of the problem

Globally, about half of the world population (3.3 billion people) was at risk of malaria infection and around 300 to 500 million cases occurred annually, leading to approximately 1.24 million deaths each year before 2010. Today malaria accounts for 2.6% of the total global disease burden. (Monroe *et al.*, 2022) . In 2021, the most vulnerable group hardest hit by malaria are children under-five who accounted for 80% of all malaria deaths globally(World Health Organization, 2022). With almost 90% of all malaria deaths occurring in children under the age of five, SSA has the highest malaria burden(Yankson, Anto and Chipeta, 2019) . More than half of the estimated 405 000 deaths from malaria each year are in children below five years of age and are caused by *Plasmodium falciparum*, with the vast majority taking place on the African continent(Pandey and Shingadia, 2022). Similarly, the highest malaria-related morbidity and mortality in Ethiopia is reported among children under 5 (Debo and Kassa, 2016)

The main clinical presentations of malaria in children are uncomplicated malaria and severe malaria (typically categorized as cerebral malaria or severe malarial anemia) (Pandey and Shingadia, 2022). As well as causing avoidable deaths in young children, malaria has other negative impacts on physical and neurocognitive health, with anemia, impaired school performance, and behavioral problems all associated with previous episodes of malaria (Nankabirwa *et al.*, 2014) (Crawley *et al.*, 2010). *P. falciparum* malaria is the most severe form of malaria, with fatality rates up to 15% in non-immune children with anemia and severe respiratory distress if appropriate therapy is not promptly, instituted(Modiano *et al.*, 2001). Many intervention initiatives, including the distribution of insecticide-treated nets (ITNs), indoor residual spraying (IRS), artemisinin-based combination treatment (ACT), and the dissemination of health information, has been implemented in Ethiopia to prevent and reduce malaria infections. Despite these initiatives, malaria remains a significant public health issue in places where it is prevalent, particularly for young children under the age of five (Oxborough, 2016).

According to the records available in the Ilu Galan district health office, the trend for the last three years indicated that high malaria prevalence was registered at both outpatient and inpatient. The overall pattern suggests that malaria remains a risk in the district. This verifies that there could be several reasons or factors contributing to the high prevalence of malaria in the Ilu Galan district despite all the efforts that the government has put in place to reduce the infections.

However, the epidemiology of community-based malaria in children under the age of five has not been documented, and it is also rare at the national level. So far, research conducted to ascertain malaria prevalence and associated factors were institution-based and are not sufficiently generalizable to under five communities. Hence, knowledge of the current prevalence of malaria in under five children and its associated risk factors in the communities has paramount importance to scale up intervention programs. But, to the best of our knowledge, no documented study was done in the Ilu Galan district. Therefore, this study aims to assess the prevalence of malaria and associated risk factors in under five children in the Ilu Galan district.

1.3.Significance of the study

The findings of this study will provide baseline information on the prevalence and associated risk factors of malaria among under five children in the study area, thus the findings will be utilized by interested non-governmental and governmental institutions that are concerned with the provision of health services related to malaria in the study area. The study findings would contribute to the understanding of the risk factors associated with malaria in the settlements. That would be very important for health professionals in the vicinity to design preventive strategies and create awareness in the community in the area, and it will also attempt to warrant policymakers to give more attention to the impact of malaria in under five children and upscaled intervention strategies accordingly. This study finding is also expected to fill gaps in this area of research by adding to the existing body of knowledge; it will help other researchers as a reference for their work.

2.Literature review

In 2020, the estimated number of malaria cases worldwide was 241 million (227 million in 2019) with an estimated 200 million malaria cases and 403,000 deaths in sub-Saharan Africa, of which 80% were children younger than 5 years(WHO, 2021b).

A community-based cross-sectional study, in Huye District, Southern Rwanda on a total of 222 under-five children indicates that the majority (54%) of the children were females. Most of the parents/guardians were married (95.9%), nearly all (99.5%) had attended primary school and most (97.3%) were peasants. The overall *Plasmodium falciparum* prevalence in children was 12.1%. Compared to children aged 13 to 59 months, children aged 1 to 12 months had a 3.5-fold higher risk of having malaria parasites. Children who were not sleeping under insecticide treated nets were 15 times more likely to be infected with malaria parasites compared to those who were sleeping under nets (Nyirakanani *et al.*, 2018).

A facility-based retrospective cross-sectional study done in Uganda, Kampala Fort Portal Regional Referral (Buhinga) Hospital on 122 under five children indicate that the prevalence of malaria was 41(36.6%). As the study was assessed the prevalence with the age group: 4-5 years was 21 (51.2%) followed by 2-3 years 12 (29.3%) and the lowest was < 2 years with 08 (19.5%). Children who live in rural were more infected than those who live in urban and Children who were not sleeping under insecticide-treated nets were more likely to be infected with malaria parasites compared to those who were sleeping under nets (Atanazio, 2019).

In Malawi, a study was done to compare the prevalence and associated factors with malaria parasitemia among children under the age of five years between 2012 and 2014 malaria indicator surveys. A multistage cluster sampling method was employed a total of 4040 children under age of five years were involved in the study. The 2112 (52%) were from the 2012 MIS(Malaria Indicator Surveys) and 1928 (48%) were from the 2014 Malaria Indicator Surveys (MIS) and these showed that the prevalence among the children under age of five years increased from 28% in 2012 to 33% in 2014 and these showed a high prevalence of malaria among children below the age of five years (Dunca *et al.*, 2007).

A cross sectional survey that was conducted among children attending the outpatient clinic at the Nyasa health center located in Nsenga district, Tabora region in Tanzania between august and

October 2010 to confirm malaria cases among children under five with fever and history of fever. A total of 300 children under five years a with fever or history of fever participated in the study and of the 300 children under five, 54.3 %were boys and 45.7 % were girls. In all the children, parents or guardians reported fever or history of fever as part of illness in all children although during physical examination only 25.3 % had an axillary temperature ≥37.5 degrees centigrade. The parasitological diagnosis of malaria revealed that out of the 300 children under five only 12 %(36/300 had positive slide readings and of these 52.7 % had fever and 42.7 %(17/36) had no fever on physical examination. The distribution of positive malaria slide readings between girls and boys was not statically significant. All the children who were positive, were positive for *P. falciparum* with 52.8%919/36 had 1-500 parasites per micro liter of blood and 22.2 % had between 501-1000parasite per micro liter and 25 % had≥1001 parasite per micro liter. There was no statistical significance on the distribution of positivity and parasite density by age on univariate and multivariate analysis, only children above one year of age were associated with malaria infection(Mazigo *et al.*, 2011)

An unmatched case-control study was conducted in Hohoe Municipality of Ghana in November 2015 involving 1697 children less than five years from 30 communities. Out of 1697 children screened, 676 (39.8%) tested positive with RDT (cases) and 1,021 tested negatives (controls). Older children aged 24-35 and 36-47, and 48 months and above were more likely to have malaria as compared to the younger age group 6-11 months . Current fever, History of fever within one week and antimalarial drug use at home were 3.07, 1.75 and 4.03 times more likely to occur among cases than in the controls, respectively. Anemia (HB<11.0g/dL) was 1.87 times more likely among the cases than in the controls. Children of parents/guardians who were farmers and traders were more likely to have malaria and, respectively(Kweku M1*et al.*, 2017).

In Ethiopia a systematic review and meta-analysis identified 12 studies with 34,842 under-five children were included. The pooled prevalence of under-five malaria was 22.03%. Lack of insecticide-treated mosquito net utilization , poor knowledge of child caretakers towards malaria transmission , and living near mosquito breeding sites were risk factors of under-five malaria. They also identified more than one in five children aged under five years were infected with malaria in Ethiopia (Biset *et al.*, 2022).

From January 2022 to December 2022, a facility-based cross-sectional study was carried out at Ziquala hospital, Tsitsika, Mishra, and Hamusit health clinics in Ziquala district, Northeast Ethiopia and the study enrolled a total of 633 under five children. The overall prevalence of malaria among children was 24.6% (156/633). *Plasmodium falciparum*, *P. vivax*, and mixed infection (both species) accounted for 57.1%, 38.5%, and 4.5% of the cases, respectively. load, moderate parasitemia was the most common, followed by low and high parasitemia with the proportion of 53.8%, 31.4% and 14.7% parasite density, respectively. Malaria infection was linked to irregular utilization of insecticide-treated bed nets, staying outside at night , and parents not receiving malaria health education in the past six months (Debash *et al.*, 2022).

A facility-based cross-sectional study was conducted in Sherkole refugee camp, Benishangul-Gumuz region from October to November 2019.The study enrolled a total of 356 under five children. The total prevalence of malaria was 3.9% .Outdoor stay at night, stagnant water near to house ,and the number of under-five children per household were found to increase the odds of getting malaria. Whereas, insecticide treated net (ITN)utilization and Health information about malaria reduce the odds of getting malaria(Ahmed, Mulatu and Elfu, 2021).

Another a facility based cross-sectional study was conducted in Arbaminch zuria district on 271 under five febrile children from April to May 2017.Microscopic blood film was used. The percentage of having a fever child who tested positive for malaria was 22.1% (60/271); the percentages of those who tested positive for *Plasmodium falciparum*, *Plasmodium vivax*, and mixed infections of both parasites were 50.0%, 48.33%, and 1.66%, respectively. Malaria infection was associated with nearby presence of stagnant water to resident areas Children who slept under insecticide-treated mosquito nets (ITNs) were more likely to be protected from malaria infection than those did not sleep under an ITNs (Abossie *et al.*, 2020).

A facility-based cross-sectional study was conducted among 585 under-five children in Wogera district North Gonder zone. 51 (8.7%) of the 585 children who gave blood samples had malaria. The predominant *Plasmodium s*pecies were *P. falciparum* 33 (65%) *and P. vivax* 18 (35%). Regularly sleeping under long-lasting insecticide treated nets (LLIN) was associated with decreased odds of malaria , and an increased odds of malaria was observed among children who live in households with stagnant water in the compound and children who stay outdoors during the night (Tsegaye, Ayele and Birhanu, 2021).

2.1.Conceptual Frame work

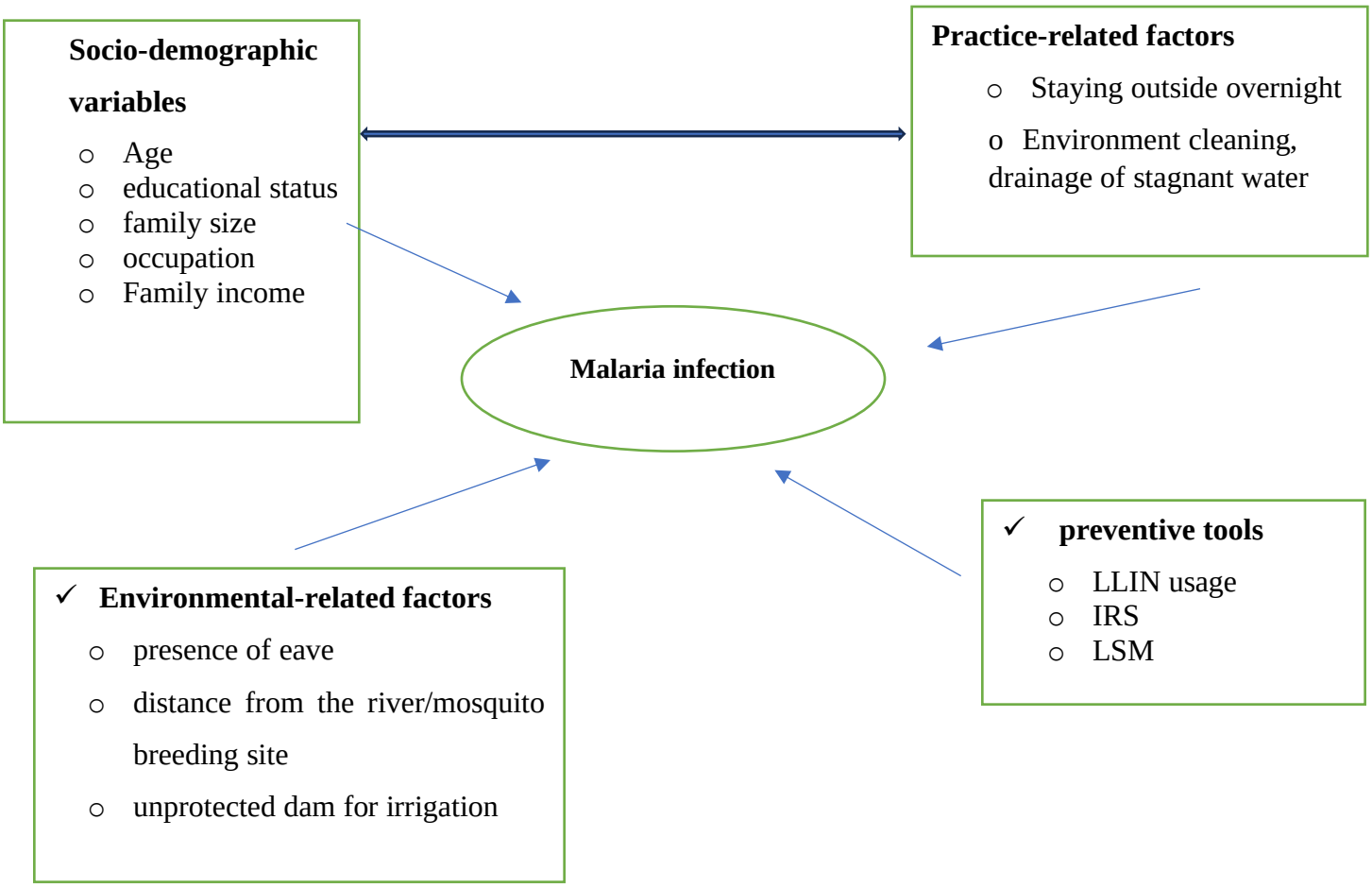


Figure 1 :Conceptual frame work from different articles(Solomon, Kahase and Alemayehu, 2020)(Biset et al., 2022)(Debash et al., 2022)(Abossie et al., 2020)

3. Objective of the study

3.1General objective

- To determine the prevalence of malaria and assess the associated risk factors among under five children in Ilu Galan district from April to June 2023.

3.2.Specific objectives

- To determine the prevalence of *Plasmodium* infection among under five children in the study area.
- To assess the risk factors associated with malaria infection in under five children.

4. Methods and Materials

4.1.Study area

Ilu Galan district is located in the West Shewa Zone and 215km of Addis Ababa which is the. It is located between 8°56′30″N and 8°59′30″N latitude and 37°47′30″E and 37°55′15″E longitude. The mean temperature of district is estimated to be around 18.87°C. The altitude of the district ranges from 1600 to 1900m above sea level with average rain fall from 1000 to 1200 mm3. Transmission of malaria is notably unstable and very seasonal. The main economic activities in the area are livestock breeding and subsistence farming. Major crops grown maize, sorghum and teff. Water contribute to mosquito breeding is available throughout the year because of some small rivers, stagnant water and wells in the district. The overall population of the district is 95923 (47002 males and 48921 females) in 2022(Woreda health office). The district has 9760 under-five children and 29840 pregnant women. There are 7218 households who have children less than five years and 18 kebeles in the district, with 95% of the district being malaria endemic(from West Shewa Zonal Health office third quarter report of Health Management Information System, Ambo Ethiopia, April, 2022).In the district, there are three health centers, and eighteen health posts. All health facilities give diagnostic and treatment services to the community.

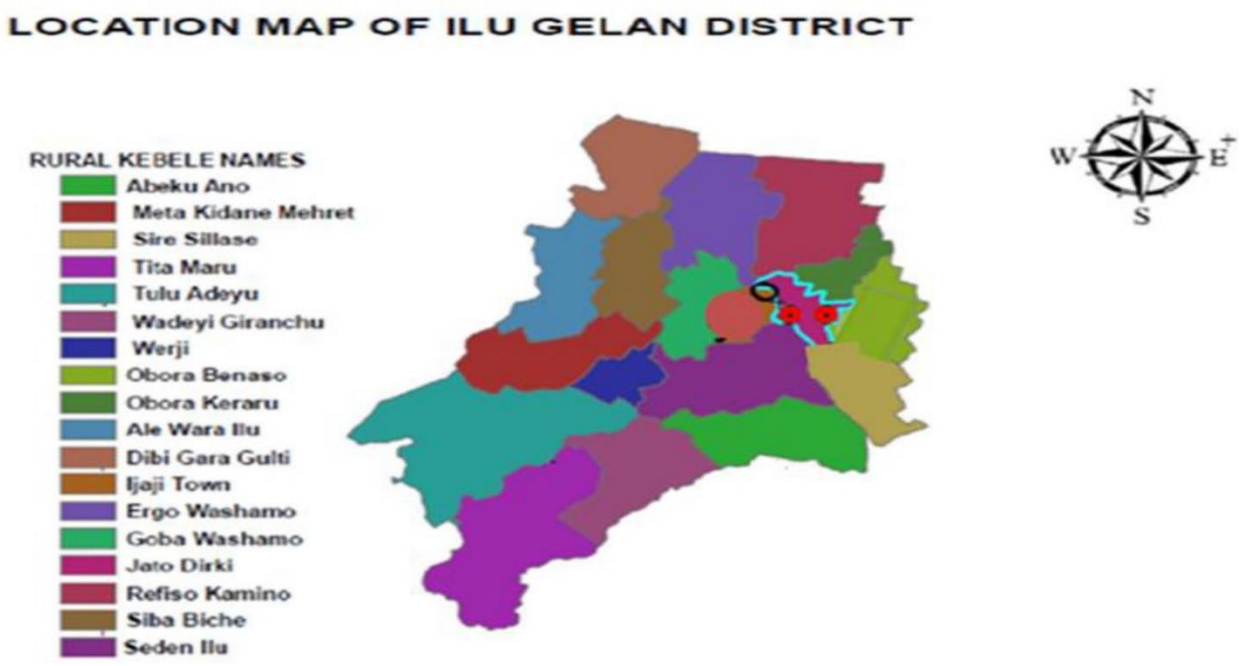


Figure 2: Map of the Ilu Galan district with sub-districts (kebeles), 2022.

4.2.Study design and period

Community based cross-sectional study was conducted from April to June 2023.

4.3.Population

Source population

All under five children in Ilu Galan district community

Study population

All under five children in selected kebeles of Ilu Galan district community who fulfills inclusion criteria.

4.4.Eligibility criteria

4.4.1.Inclusion criteria

All under five children whose parents/care givers were volunteered to give consent were included in the study.

4.4.2.Exclusion criteria

Under five children who were on anti-malarial treatment two weeks before data collection and those whose parents/care givers were not give assent were excluded from the study.

4.5.Sample size determination

The formula for single population proportion was used to determine the sample size.

$$\mathbf{n = (Z\ \alpha/2)^2\ p\ (1\text{-}p)/d^2 = 384}$$

n0 = the minimum sample size required for the study.

Z α/2= is the standard normal variable at 95 % confidence level (1.96).

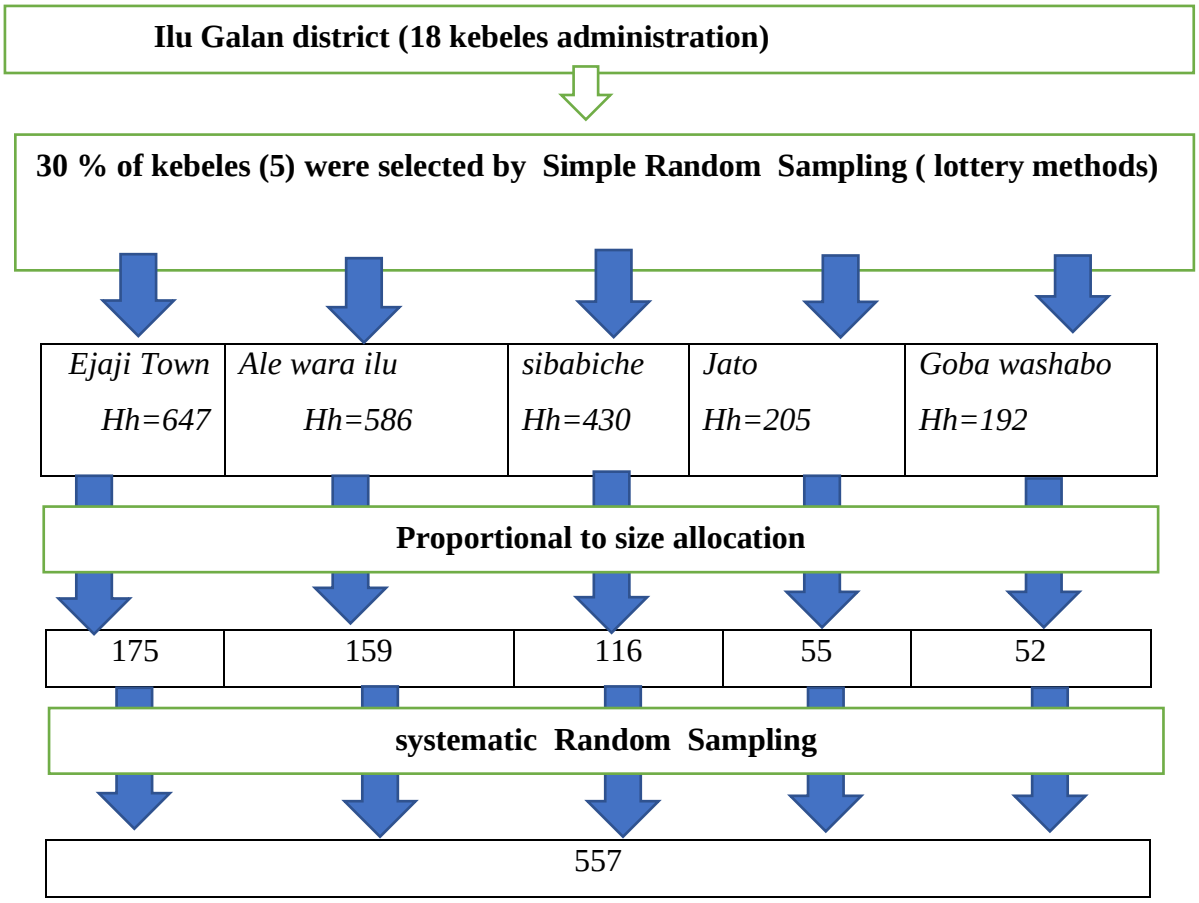
p = = estimation prevalence rate of malaria 50%.

d= margin of error which is taken as 0.05.

Accounting for a 10% non-response rate, and implementing correction formula and design effect of 1.5, the final sample size was 557.

4.6.Sampling technique

Ilu Galan district was purposely selected from West Shewa Zone districts as it has catchment of malaria burden compared to another districts. A multi-stage sampling approach were applied to select the study participants. In the first stage, 5 kebeles (Ejaji Town, Ale wara ilu, Siba biche,jato and Goba Washabo) were selected by lottery method from the total of 18 kebeles in the district. In the second stage, households with at least one under-five children were selected from selected kebeles using systematic random sampling techniques after proportional to size allocation based on the number of households in each kebele. The total number of under five children in selected five kebeles were 2735. The total number of households in each selected kebeles who has under-five children was obtained from the community health information system (CHIS) of the kebele at health post. The sampling intervals(Kth) value of each selected kebeles were determined by dividing the total number of households by the sample size which was 4. With the use of a lottery, the first household was chosen. In case of the house was closed at the time of data collection, a re-visit was attempted up to 2 times.



Hh=House hold

Figure 3 Schematic diagram of the study sampling technique

4.7.Study variables

4.7.1.Dependent variable

•Malaria infection

4.7.2.Independent variables

•Socio-demographic variables

Age, sex, educational status, family size occupation, and family income.

•Environmental-related factors

Presence of eaves, animals in the house, distance from the river/mosquito breeding site, unprotected dam for irrigation.

•Preventive tools-related factors

LLIN usage, IRS spray, and LSM

- **practice related factors**

staying out door during the night

Environment cleaning, drainage of stagnant water

4.8.Data collection method

4.8.1.socio-demographic and risk factors data

Socio-demographic information and data on risk factors associated with malaria were collected through face-to-face interviews with children's caregivers using a pretested structured questionnaire. The questionnaire contains information about socio-demographic characteristics; factors related to malaria preventive tools, and factors related to the environment and practice. The questionnaire was first prepared in English and translated to Afaan Oromoo which is the local language spoken by the residents of the site. The data was collected by trained BSc laboratory professionals and Health Extension Workers .

4.8.2.Blood sample collection and processing

After the interview, blood was drawn from a finger or heel prick to prepare thick and thin blood film smears in accordance with the Federal Democratic Republic of Ethiopia Ministry of Health National Malaria Guidelines (Federal Democratic Republic of Ethiopia Ministry of Health, 2017). Each child included in the sample, regardless of any indications or symptoms of malaria, had their finger pricked with a sterile lancet, and 5 micro liters of whole blood were taken. The blood smears were prepared on microscope slides and stained using 10% Giemsa to be examined under 100x microscopes by two experienced laboratory technologists at Jaji health center for the presence of malaria parasites. The presence or absence of malaria parasites was determined by the thick smear, while the species of *Plasmodium* was determined by the thin smear. Presence of one or more asexual *Plasmodium* stages (trophozoite, ring stage, merozoite, or gametocyte) was considered a positive result. Based on the evaluation of 200 fields of the thick smear, each film was assigned a positive or negative grade, depending on whether asexual malaria parasites were visible(Mahende et al., 2016).

When there were less than 1000 parasites per liter of blood, the level of parasitaemia or parasite density was considered low. When there were 1000–9999 parasites per liter of blood, it was considered high. In order to calculate parasite density, it was assumed that a typical person would have an average leucocyte count of 8000/L of blood.

$$\text{Parasites / } \mu\text{L blood} = \frac{\text{Number of parasites counted} \times 8000 \text{ white cells/}\mu\text{L}}{\text{No. of white cells counted}}$$ (WHO, 2016)

All the slides were transported to Ambo University Referral Hospital to be checked by a second experienced lab technologist. Any discordant result was resolved by a third technologist, who is blind to the earlier results. The RDT Care Start TM combo test (SD BIOLINE Malaria Ag *PF/PV* POCT test kits (Standard Diagnostic, Inc, Germany) was used in the study. The manufacturer's instructions must be followed during the test. The RDT detects *PF* histidine-rich protein II (PFHRP-II) and other *Plasmodium* species (Pan, pLDH) (PV or PO). (Mahende *et al.*, 2016).

4.9.Data quality assurance and Quality Control

Data collectors were trained for two days by the MSc parasitologist from Ambo University.

The tool of this study was piloted in a nearby kebele where it was administered to about 5% of respondents at the end of weeks of March. The pilot study was done to ensure that the questions were not ambiguous so as to generate the desired information with minimum bias.

All laboratory procedures were conducted based on standard operating procedures. Clean and grease-free frosted end slides were used for blood film preparation to avoid scratches on the slides. All Care Start TM malaria test kits and slides for blood film examination must be labeled with the participant ID number specifically given for this purpose and the test was done according to the manufacturer's instructions. The first drop of blood was swiped off to decrease the effect of tissue fluids. The Giemsa stain was tested on known positive and negative malaria slides and the Care Start TM malaria test kits were also tested on known positive and negative malaria samples. In all cases, the results of the Care Start test were determined earlier than microscopic results, with strict blinding to the microscopic examination of the thick and thin blood smears. During the microscopic examination, a slide was regarded as negative after 200 fields had been examined without finding of *Plasmodium* parasite by two laboratory technologists at each site. A colour atlas was used during microscopic examination. Two certified microscopists, who were blinded to the RDT results, carried out the microscopy for malaria parasites. Discordant slides between the microscopic readings and discordant results between RDT and microscopy were re-analyzed for the third time by the most experienced laboratory technologist from the Ambo university Referral Hospital laboratory. To assure quality of the microscopic examinations, all positive and 10% of the negative slides were re-examined by a third reader to resolve discrepant result.

4.10.Data processing and analysis

After data collection, data were entered using Epi data version 3.1. and then exported to SPSS version 26 for analysis. Bivariate and multivariable logistic regression models were used to find the correlates of malaria. Variables that have a P-value of <0.25 in the bivariable regression were included in the multivariable logistic regression analysis. A P-value <0.05 was considered to determine statistical significance. Finally, adjusted odds ratios (AOR) with a 95% confidence interval (CI) were used to determine the strength of the association of variables.

4.11.Ethical considerations

Ethical clearance was obtained from the School Research Ethics Review Committee of the School of Medical Laboratory Science, and the institutional reviewed board of the College of Medicine and Health Science, Hawassa University and Oromia Regional IBR. Permission was obtained from the West Shewa Zone Health Directive, Ilu Galan District Health Office, and the Chairman of kebele administrations. The parents/caregivers were given detailed explanations about the study's objectives, procedures, and potential risks and benefits, and written consent was obtained following that. Confidentiality was maintained by using codes instead of names for all participant-related data. All positive cases were linked to the nearest health institution for appropriate treatment as per the national treatment guideline.

4.12.Dissemination plan

The result of the study was presented and disseminated to Hawassa University College of Medicine and Health Science, Department of Medical Laboratory Science and Oromia Health Bureau with a soft and hard copy. It and also was communicated to West Shewa Zone Health Directives, Ilu Galan District Health Office, and other concerned bodies. Finally, the result will be published in reputable journals.

5.Results

5.1.Socio-demographic characteristics of study subjects

A total of 542 study participants were included in the study. From the total sample size of 557 children, parents of five children not present in the house during data collection, parents of nine children refused to participate and information collected from one child was incomplete that made the response rate of 97.3%. Among 542 study participants,229/542 (42.3 %) were female and 313/542 (57.6 %) were male. The study participants had age ranges between 2 and 59 months with a mean age of 27months (SD=16.183). The participants were primarily in the ages of<12months (143/542; 26.4 %).The majority of the children live in rural areas (68.50%), while the rest live in urban (Table 1). Regarding caregiver related socio-demographic characteristics, majority of them n = 514 (94.8%) were married and 114 (21%) were with no formal education by educational status (Table 1).

Table 1: Socio-demographic characteristics of under-five children in Ilu Galan district, Oromia regional state, West Ethiopia, 2023 (n = 542).

Socio-demographic variables	Category	n (%)
Sex	Female	229 (42.3)
	Male	313 (57.6)
Age (Months)	<12	143(26.4)
	12-23	105(19.4)
	24-35	108(19.9)
	36-47	85(15.7)
	48-59	101(18.6)
Residence	Urban	171(31.5)
	Rural	371(68.5)
Family size	<5	312(57.6)
	≥5	230(42.4)
Educational status of care giver	No formal education	114(21)
	Read and write only	153(28.2)
	Primary school	236(43.5)
	Secondary and above	39(7.2)
Occupation of care giver	Hose wife	79(14.6)
	Farmer	398(73.4)
	Daily laborer	26(4.8)
	Sivil servant	14(2.6)
	Business man	25(4.6)

5.2.Prevalence of malaria and its density

The overall prevalence of malaria among under-five children in Ilu Galan district was 5.2%(95%CI = 3.5-7.4).Of the total 542 under five children participated in this study,5.2%(28/542)and4.4% (24/542)were confirmed to be infected with *Plasmodium* species by microscopy and rapid diagnostic tests(RDTs), respectively(table2). Of these, 53.6% were males and 46.4% were females. There was no statistically significant variation in malaria prevalence in sex (P = 0.836). Likewise, the higher number of malaria cases was detected among the age group of 48-59 and 36-47 months with the prevalence of 15.8% and 5.9%, respectively(table 3). In this study, the proportion of *P.falciparum* and *P.vivax* was 3.7% and 1.3%, respectively, while that of mixed infection was 0.2%(figure 4).

Table 2:Prevalence of malaria among under-five children in Ilu Galan District; West Ethiopia, April to June 2023

Individuals	RDT		Microscopy		Total
	Pos n(%)	Total	Pos n(%)	Total	Pos, n(%)
Female	12(5.2%)	229	13(5.7)	229	13(5.2)
male	12(3.8%)	313	15(4.8)	313	15(4.8)
Over all	24(4.4%)	542	28(5.2)	542	28(5.2)

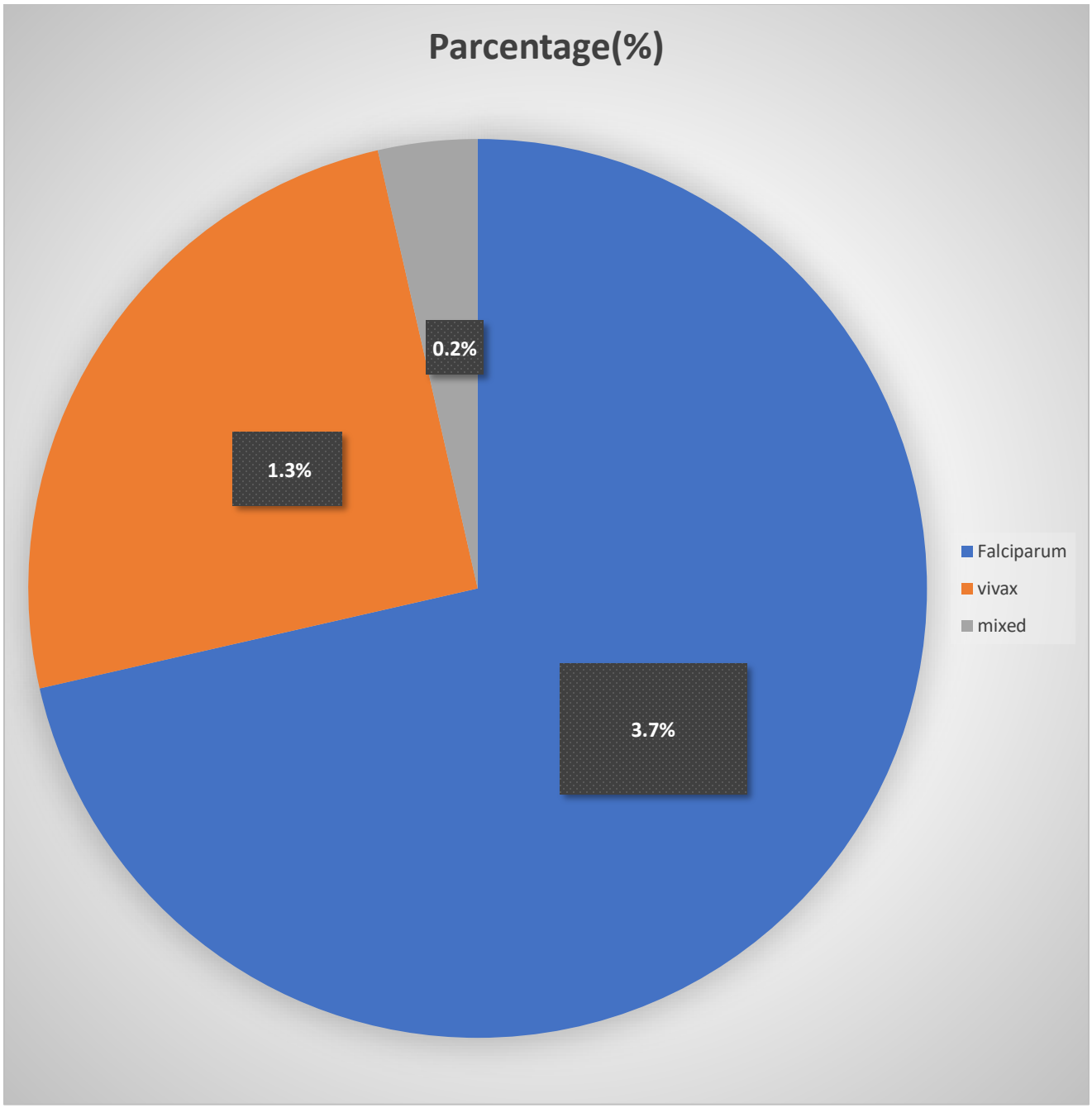


Figure 4 proportion of *Plasmodium* species

Table 3:Socio-demographic variables and malaria infection among children under five years in Ilu Galan district, West Ethiopia, 2023.

Variable	Categories	n	Positive n (%	*P.falciparum* n (%	*P.vivax* n (%	Mixed n (%	Negative n (%	P-value
Sex	Female	229	13(5.7)	11(84.6)	1(7.7)	1(7.7)	216(94.3)	0.52
	Male	313	15(4.8)	9(60)	6(40)	0(0)	298(95.2)	
Age in	<12	143	3(2.1)	2(66.7)	1(33.3)	0(0)	140(97.9)	0.99
months	12-23	105	0(0)	0(0)	0(0)	0(0)	102(100)	
	24-35	108	4(3.7)	4(100)	0(0)	0(0)	104(96.3)	
	36-47	85	5(5.9)	4(80)	1(20)	0(0)	80(94.1)	
	48-59	101	16(15.8)	10((62.5)	5(31.3)	1(6.2)	85(84.2)	
Residence	Urban	171	7(4.1)	3(42.9)	3(42.9)	1(14.3)	164(95.9)	0.15
	Rural	371	21(5.7)	17(81.0)	4(19.0)	0(0)	350(94.3)	
Total		542	28(5.2)	20(3.7)	7(1.3)	1(0.2)	514(94.8)	

5.3.Level of Parasitemia by Sex and Age Group

Regarding the level of parasitemia with respect to age and sex 15/28(53.6%)had less than 1000 parasites/μL(69.2%)in female and 40% in male). The highest parasitemia level (1000-9999 parasites/μL of blood) was observed in the age group 24-35months and the lowest high parasitemia level (1000-9999/μL of blood) was observed in individuals <12months old(table 4).

Table 4: Levels of parasite density by sex and age group among the parasitemic under five children.

Variable	Categories	Parasite density distribution per microliter of blood		
		Low(<1000)n(%)	High(1000-9999)n(%)	Total n
Sex	Female	9(69.2)	4(30.8)	13
	Male	6(40)	9(60)	15
Age in months	<12	2(66.7)	1(33.3)	3
	12-23	0	0	0
	24-35	1(25)	3(75)	4
	36-47	3(60)	2(40)	5
	48-59	9(56.2)	7(43.8)	16
Total		15(53.6)	13(46.4)	28

Multivariate analysis of malaria with associated factors

Potential malaria associated factors that showed a P-value < 0.25 in the bivariate analysis were entered for multivariate analysis to control confounding factors. Fifteen variables (sex, age group, occupation, family size, availability of ITN, ITN usage, presence of eave in the house, presence of any hole on the wall, outdoor activities at night, IRS service, having information about malaria, Household distance from river, Cleaning stagnant water in broken containers, Presence of stagnant water and Unprotected dam for irrigation) were selected and entered into multivariate logistic regression model .Six variables(Staying out door during night, Presence of ITN, IRS service, Presence of eave, Household distance from river and Presence of stagnant water) were significantly associated with malaria infection (P <0.05)(Table5)

Table 5 Multivariable logistic regression analysis of associated factors for malaria, in Ilu Galan district, Oromia regional state, Western Ethiopia, April to June 2023 (N = 542)

Variables	Category	Malaria		COR(95% CI)	AOR (95% CI)	p-value
		Yes n(%)	No n(%)			
Family size	≥5	19(8.3)	211(91.7)	3.032(1.345-6.831)	2.811(.894-8.834)	.077
	<5	9(2.9)	303(97.1)	1	1	
Staying out door during night	Yes	17(17.3)	81(82.7)	8.262(3.732-18.288)	3.094(1.010-9.478)	.0480
	No	11(2.5)	433(97.5)	1	1	
Presence of ITN	Yes	7(2.1)	332(97.9)	.183(.076-.438)	.261(.073-.939)	.040
	No	21(10.3)	182(89.7)	1	1	
IRS service	Yes	4(11.8)	30(88.25)	2.689(.877-8.248)	11.830(2.199-63.653)	.004
	No	24(4.8)	484(95.3)	1	1	
Presence of eave	Yes	21(9.8)	193(90.2)	4.990(2.082-11.956)	4.081(1.202-13.437)	. 024
	No	7(2.1)	321(97.9)	1	1	
information about malaria	Yes	11(2.5)	424(97.5)	.137(.062-.303)	.298(.080-1.103)	.070
	No	17(15.9)	90(84.1)	1	1	
Household distance from river	<1km	22(14.0)	135(86.0)	10.294(4.087-25.929)	4.317(1.194-15.618)	.026
	≥1km	6(1.6)	37(98.4)	1	1	
Cleaning stagnant water in broken containers	Yes	12(2.4)	484(97.6)	.048(.020-.107)	.339(.087-1.323)	.119
	No	16(34.8)	30(62.5)	1	1	
Presence of stagnant water	Yes	18(27.7)	47(65.2)	17.885(7.805-40.982)	11.399(3.710-35.021)	.000
	No	10(2.1)	467(97.9)	1	1	
Unprotected dam for irrigation	Yes	10(7.8)	118(92.2)	1.864(.838-4.149)	.632(.180-2.224)	.475
	No	18(4.3)	396(95.7)	1	1	

6.Discussion

The aim of this study was to determine the prevalence of malaria and associated risk factors among under five children in Ilu Galan district, the overall prevalence rate of malaria parasite infection was 5.2%. This prevalence is lower than the studies done in another countries like Uganda(19.04%)(Wanzira *et al.*, 2017),Malawi(33%)(Dunca *et al.*, 2007),Ghana(20.9%)(Nyarko and Cobblah, 2014), and in different parts of Ethiopia such as in Tselemt district north Ethiopia(20.5%)(Shiferaw *et al.*, 2018), Jima town(11%)(Alemu *et al.*, 2011), and in Benishangul Gumuz region which is 15.9%(Alkadir, Gelana and Gebresilassie, 2020). This difference may be related to the coverage of this study which was conducted in one malaria-endemic cell of Ilu Galan district whereas other studies were conducted on a larger scale. The discrepancy could possibly be a result of the different geographical variations and malaria prevention and control initiatives carried out in the studied locations. However, the prevalence of malaria in this study was higher than the prevalence determined by studies conducted in Sherkole refugee camp, Ethiopia (3.9%) (Ahmed, Mulatu and Elfu, 2021) and Malaria Indicator survey in Ethiopia which is the prevalence of malaria in under five is 0.9%(Jima *et al.*, 2010).

Both *P. falciparum* and *P. vivax* have been found in the area, however *P. falciparum* was found to be the most common type (3.7%), followed by *P. vivax* (1.3%) and mixed infections (0.2%). This almost similar to study conducted in Sanja Town, Northwest Ethiopia which was the prevalence of *P.falciparum* and *P.vivax* were5.2% and 1.6%respectively(Worku *et al.*, 2014). However, it was disagreed with the national malaria parasite distribution pattern of Ethiopia(Ministry of Health Ethiopia, 2021) , which showed that *P. falciparum* and *P. vivax* accounted for 60 and 40% of the malaria cases in the country, respectively. This discrepancy may be caused by the fact that this study was only conducted in a small portion of the country where malaria is endemic, varying the frequency of various species. Furthermore, the study area's relatively lowland climatic conditions, where *P. falciparum* is a common species in the lowlands, as well as the possibility of treatment failure or recrudescence for *P. falciparum* ,cannot be ruled out.

The prevalence of malaria was found to be greater in age groups between 48 -59months(15.8%) than in other age groups in the current study, which is consistent with studies conducted in Arba

Minch “Zuria” district that indicated the prevalence of malaria is more common among children of similar age(27.9%)(Abossie *et al.*, 2020),study conducted in Tanzania that indicate the prevalence of malaria in older age children(48-59months) was high(14.8%) and malaria Indicator Surveys (MISs) conducted in Malawi(Dunca *et al.*, 2007). It was observed that malaria cases increased with age and were lowest in infants under one year old. The fact that children under one year old had the fewest cases of malaria may be because they inherited antibodies from their mother during pregnancy, which allows them to fight the disease before their immunity wanes (Paul and Msengwa, 2018). This finding is also supported by (Nyarko and Cobblah, 2014) which was conducted in Ghana .Malaria prevalence was found to be greater in rural (5.7%) when compared to urban dwellers (4.1%). This may be due to availability of good vector condition to multiply, lower housing quality, and poor drainage systems(Oladeinde BH *et al.*, 2012). A similar result was obtained in Kenya by(Sultana *et al.*, 2017) and Uganda(Atanazio, 2019) which shows malaria cases were higher in rural areas than in urban setting.

The majority of infected children had a low(<1000 Parasites / μL blood)15(53.6%) and followed by high parasite density which accounts for 46.4% of malaria positive children. This study is in line with study conducted in Sanja Town, Northwest Ethiopia(Worku *et al.*, 2014). However, a high proportion of high parasite density was found in East Central Tanzania 69.4%(Chipwaza and Sumaye, 2020). The immunological conditions, age category, and dietary status of the study participants could all have an impact on parasite density. By decreasing parasite load, acquired or adaptive immunity protects against clinical disease, morbidity associated with parasite density, and new infection(Doolan, Doban and Baird, 2009).

Children who stayed out doors at night were 3.09 times more likely to be exposed from malaria infection than those that did not. Staying outside during the night showed a statistically significant association with malaria. This finding was in line with the previous studies done in Zimbabwe(Mutsigiri-Murewanhema *et al.*, 2017), Armachiho(Aschale *et al.*, 2018), in Dembia district(Agegnehu *et al.*, 2018) and Sherkole refugee camp in Benishangul-Gumuz(Ahmed, Mulatu and Elfu, 2021), Ethiopia. This could be explained by exophagic-exophilic mosquito biting behavior(Ali, Bashir and Elrahman, 2017). Children who utilize ITN had a reduced risk of malaria infection compared to those who do not utilize ITN. This finding is consistent with the previous study done in Nigeria(Kyu *et al.*, 2013), in Southern Ethiopia(Prevalence, Chichu and Centres,

2016) , and East Shewa zone of Oromia regional state(Haji, Fogarty and Deressa, 2016). When they use ITN consistently, the risk of getting mosquito bite might be avoided(WHO, 2021a). Therefore, ITN utilizations must be enforced by government.

Children who living in house with eave were 4.08 times more likely have a higher risk of acquiring malaria infection than those in the houses without eave. This is supported by the study conducted in some localities within Ethiopia(Ghebreyesus *et al.*, 2000) . The presence of eave(s) might enable mosquitoes to enter inside houses, and this increases the probability of indoor mosquito bites.

Presence of river in close proximity to house (<1 km) has shown a significant association. Children who lived proximity to river(<1km) were 4.3 times more likely have a higher risk of getting malaria infection than those who lived far from the river(≥1km). This is supported by the study conducted in Southwestern Nigeria(Awosolu, Yahaya and Haziqah, 2021). Studies also witness that the relationship between malaria vector density and the distance of settlement from a water body like river is an important indicator of malaria transmission(Alemu *et al.*, 2011).

Stagnant water around home was observed as a significant risk factor and was found to increase the odds of malaria disease among children by approximately eleven times compared with those without stagnant water around their homes. Previous studies have demonstrated that stagnant water is a favorable breeding site for mosquito development and proliferation, leading to increased malaria transmission(Hawaria *et al.*, 2020).

8.Strengths and limitations of the study

8.1 Strengths

The main strength of this study is that it is based on a systematic sampling technique and therefore, the findings can be generalized to all under-five children who lived in the Ilu Galan district. Another strength is also since the study conducted was community based, assessment of environmental factors ,observation of availability and utilization of ITN were assessed by data collectors in each selected house hold. Determination of malaria prevalence by both microscopy(Gold-standard) and RDT test kits is also one of the strengths of this study.

8.2. Limitations

Nevertheless, this study has some limitations. First, the study was conducted during the minor season, a period with significantly fewer mosquito-breeding sites compared to the major season. A suggestion is made that a longitudinal study should be conducted in order to provide a broader picture of malaria infection prevalence and risk factors in the study area. In addition, mosquito vector surveillance for identity, diversity, and abundance was not included in the study. Thus, no confirmation regarding the presence of Plasmodium infection in mosquito vector. This study also used a cross-sectional design and as such, no causal inference can be made regarding the identified determinants and child malaria infection. Despite these limitations, the study has identified potential risk factors for malaria infection among children under 5 in Ilu Galan district that can inform local programs.

9. Conclusion

In conclusion, the prevalence of malaria in under-five children in Ilu Galan district of selected 5 kebeles was 5.2%. The findings of this study have shown that malaria continues to be a major public health problem, particularly among children of under five years. The highest prevalence of malaria was found in those aged between 48-59 months old, and a higher proportion of low parasitemia was also observed in this study. Staying outside at night, utilization of ITN, Presence of eave on the house, IRS service in the last 12 months,<1km distance of house hold from river and Presence of stagnant water nearby house were the main correlates of malaria.

10. Recommendation

1.Oromia Health Bureau ,West Showa Zonal Health Office and Ilu Galan Woreda Health office should give focus on regular ITNs utilization, IRS service, infection associated with staying outdoors at night, environmental management to reduce mosquito breading and changing attitudes towards malaria prevention and control through health education to minimize the burden of malaria.

2. Further large-scale study should be performed by using different diagnostic techniques like polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP) which have higher detection capacity compared to light microscopy and RDT.

Also, mosquito vector surveillance for identity, diversity, and abundance should be performed.

References

Abossie, A. *et al.* (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, doi: 10.2147/IDR.S223873.

Adebukola, O. *et al.* (2022) 'Malaria prevention practices and malaria prevalence among children living in a rural community in Southwest Nigeria'. doi: 10.3855/jidc.14894.

Agegnehu, F. *et al.* (2018) 'Determinants of malaria infection in Dembia district, Northwest Ethiopia: A case-control study', BMC Public Health, doi: 10.1186/s12889-018-5370-4.

Ahmed, A., Mulatu, K. and Elfu, B. (2021) 'Prevalence of malaria and associated factors among under-five children in Sherkole refugee camp, Benishangul-Gumuz region, Ethiopia. A cross-sectional study', *PLoS ONE*, 16(2 February 2021), doi: 10.1371/journal.pone.0246895.

Al-Quhaiti, M. A. A. *et al.* (2022) 'Malaria among under-five children in rural communities of Al-Mahweet governorate, Yemen', Malaria Journal, 21(1), doi: 10.1186/s12936-022-04371-8.

Alemu, A. *et al.* (2011) 'Urban malaria and associated risk factors in Jimma town, south-west Ethiopia', *Malaria Journal*, 10, doi: 10.1186/1475-2875-10-173.

Ali, M. I. M., Bashir, N. H. H. and Elrahman, S. H. A. (2017) 'Mapping of anopheles mosquitoes (Diptera: Culicidae) in EnNuhud Town, West Kordofan state, Sudan', *International Journal of Mosquito Research*, 4(5),

Alkadir, S., Gelana, T. and Gebresilassie, A. (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), doi: 10.1186/s40794-020-00112-4.

Amenu, D. (2014) 'Prevalence of Malaria among Patients Visiting Nekemte Hospital', *Journal of Medical Microbiology & Diagnosis*, 03(02). doi: 10.4172/2161-0703.1000137.

Aschale, Y. *et al.* (2018) 'Prevalence of malaria and associated risk factors among asymptomatic migrant laborers in West Armachiho District, Northwest Ethiopia', *Research and Reports in Tropical Medicine*, Volume 9, doi: 10.2147/rrtm.s165260.

Atanazio, B. (2019) 'Prevalence and Factors Associated With Malaria Among Children'.

Awosolu, O. B., Yahaya, Z. S. and Haziqah, M. T. F. (2021) 'Prevalence, parasite density and determinants of falciparum malaria among febrile children in some peri-urban communities in southwestern nigeria: A cross-sectional study', *Infection and Drug Resistance*, 14(July), doi: 10.2147/IDR.S312519.

Aychiluhm, S. B. *et al.* (2020) 'Determinants of malaria among under-five children in Ethiopia: Bayesian multilevel analysis', *BMC Public Health*, 20(1), doi: 10.1186/s12889-020-09560-1.

Biset, G. *et al.* (2022) 'Malaria among under-five children in Ethiopia: a systematic review and meta-analysis', *Malaria Journal*, 21(1), doi: 10.1186/s12936-022-04370-9.

Bugssa, G. and Tedla, K. (2020) 'Feasibility of Malaria Elimination in Ethiopia', *Ethiopian journal of health sciences*, 30(4), doi: 10.4314/ejhs.v30i4.16.

Byrnes, H., Maxim, H. H. and Norris, J. M. (2010) 'Main Text*', *The Modern Language Journal*, 94, doi: 10.1111/j.1540-4781.2010.01136.x.

Chipwaza, B. and Sumaye, R. D. (2020) 'High malaria parasitemia among outpatient febrile children in low endemic area, East-Central Tanzania in 2013', *BMC Research Notes*, 13(1), pp. 4–9. doi: 10.1186/s13104-020-05092-4.

Conroy, A., Datta, D. and John, C. C. (2019) 'Causes Severe Malaria and Its Complications in Children', *BMC Medicine*, 17(1),

Crawley, J. *et al.* (2010) 'Malaria in children', *The Lancet*, 375(9724), doi: 10.1016/S0140-6736(10)60447-3.

Debash, H. *et al.* (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 October), doi: 10.1371/journal.pone.0276899.

Debo, G. W. and Kassa, D. H. (2016) 'Prevalence of malaria and associated factors in Benna Tsemay district of pastoralist community, Southern Ethiopia', *Tropical Diseases, Travel Medicine and Vaccines*, 2(1), doi: 10.1186/s40794-016-0033-x.

Doolan, D. L., Doban, C. and Baird, J. K. (2009) 'Acquired Immunity to Malaria', 22(1), doi: 10.1128/CMR.00025-08.

Dunca, S. *et al.* (2007) 'Tehnica examinării caracterelor morfologice și tinctoriale ale bacteriilor', *Microbiologie aplicată*,

Federal Democratic Republic of Ethiopia Ministry of Health (2017) 'National Malaria Guidelines, Fourth edition', *National Malaria Giuideline*, 3rd(November),

Ghebreyesus, T. A. *et al.* (2000) 'Household risk factors for malaria among children in the Ethiopian highlands', *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 94(1), doi: 10.1016/S0035-9203(00)90424-3.

Girum, T., Shumbej, T. and Shewangizaw, M. (2019) 'Burden of malaria in Ethiopia, 2000-2016: Findings from the Global Health Estimates 2016', *Tropical Diseases, Travel Medicine and Vaccines*, 5(1), doi: 10.1186/s40794-019-0090-z.

Haile, D. *et al.* (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. doi: 10.1155/2020/8828670.

Haji, Y., Fogarty, A. W. and Deressa, W. (2016) 'Prevalence and associated factors of malaria among febrile children in Ethiopia: A cross-sectional health facility-based study', *Acta Tropica*, 155, doi: 10.1016/j.actatropica.2015.12.009.

Hawaria, D. *et al.* (2020) 'Effects of environmental modification on the diversity and positivity of anopheline mosquito aquatic habitats at Arjo-Dedessa irrigation development site, Southwest Ethiopia', *Infectious Diseases of Poverty*, 9(1), doi: 10.1186/s40249-019-0620-y.

Jima, D. *et al.* (2010) 'Malaria indicator survey 2007, Ethiopia: Coverage and use of major malaria prevention and control interventions', *Malaria Journal*, 9(1), doi: 10.1186/1475-2875-9-58.

Kyu, H. H. *et al.* (2013) 'Evaluation of the association between long-lasting insecticidal nets mass distribution campaigns and child malaria in Nigeria', *Malaria Journal*, 12(1), doi: 10.1186/1475-2875-12-14.

Lee, E. H. *et al.* (2022) 'Quality and Integrated Service Delivery: A Cross-Sectional Study of the Effects of Malaria and Antenatal Service Quality on Malaria Intervention Use in Sub-Saharan Africa', *Tropical Medicine and Infectious Disease*, 7(11), doi: 10.3390/tropicalmed7110363.

Kweku M1. *et al.* (2017) 'Factors Associated with Malaria Prevalence among Children under Five Years in the Hohoe Municipality of Ghana', *Journal of Transmitted Diseases and Immunity*, 01(02), doi: 10.21767/2573-0320.100009.

Mahende, C. *et al.* (2016) 'Performance of rapid diagnostic test, blood-film microscopy and PCR for the diagnosis of malaria infection among febrile children from Korogwe District, Tanzania', *Malaria Journal*, 15(1), doi: 10.1186/s12936-016-1450-z.

Mazigo, H. D. *et al.* (2011) 'Confirmed malaria cases among children under five with fever and history of fever in rural western Tanzania', *BMC Research Notes*, 4, doi: 10.1186/1756-0500-4-359.

Ministry of Health Ethiopia (2021) 'Ethiopia malaria elimination strategic plan: 2021-2025', *Moh*, (August 2020)

Modiano, D. *et al.* (2001) 'Leucocytosis in severe malaria', *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 95(2), doi: 10.1016/S0035-9203(01)90152-X.

Monroe, A. *et al.* (2022) 'Reflections on the 2021 World Malaria Report and the future of malaria control', *Malaria Journal*, 21(1), doi: 10.1186/s12936-022-04178-7.

Mutsigiri-Murewanhema, F. *et al.* (2017) 'Factors associated with severe malaria among children below ten years in mutasa and nyanga districts, Zimbabwe, 2014-2015', *Pan African Medical Journal*, 27, doi: 10.11604/pamj.2017.27.23.10957.

Nankabirwa, J. *et al.* (2014) 'Malaria in school-age children in Africa: An increasingly important challenge', *Tropical Medicine and International Health*, 19(11), doi: 10.1111/tmi.12374.

Nyarko, S. H. and Cobblah, A. (2014) 'Sociodemographic determinants of malaria among under-five children in Ghana', *Malaria Research and Treatment*, 2014. doi: 10.1155/2014/304361.

Nyirakanani, C. *et al.* (2018) 'Prevalence and risk factors of asymptomatic malaria among under-five children in Huye District, Southern Rwanda', *Tanzania Journal of Health Research*, 20(1), doi: 10.4314/thrb.v20i1.6.

Oladeinde BH *et al.* (2012) 'Malaria and Anemia among Children in a Low Resource Setting In Nigeria.', *Iranian journal of parasitology*, 7(3), Available at: http://www.pubmedcentral.nih.gov/articlerender

Oxborough, R. M. (2016) 'Trends in US President's Malaria Initiative-funded indoor residual spray coverage and insecticide choice in sub-Saharan Africa (2008-2015): Urgent need for affordable, long-lasting insecticides', *Malaria Journal*, 15(1), doi: 10.1186/s12936-016-1201-1.

Pandey, A. and Shingadia, D. (2022) 'Treatment and prevention of malaria in children', *Paediatrics and Child Health (United Kingdom)*, 32(6), doi: 10.1016/j.paed.2022.03.001.

Paul, E. and Msengwa, A. S. (2018) 'Prevalence and socio-demographic factors associated with malaria infection among children under five years in Tanzania', *Journal of Public Health and Epidemiology*, 10(11), doi: 10.5897/jphe2018.1055.

Prevalence, M., Chichu, A. and Centres, W. H. (2016) 'Malaria Prevalence and Its Associated Risk Factors among Patients Attending Chichu and Wonago Health Centres, South Ethiopia', 16(4),

Ramos, J. M., Reyes, F. and Tesfamariam, A. (2005) 'Change in epidemiology of malaria infections in a rural area in Ethiopia', *Journal of Travel Medicine*, 12(3), doi: 10.2310/7060.2005.12304.

Sempungu, J. K. *et al.* (2023) 'The burden of malaria in sub-Saharan Africa and its association with development assistance for health and governance: An exploration of the GBD 2019 study', *Global Public Health*, 18(1). doi: 10.1080/17441692.2023.2229892.

Shiferaw, M. *et al.* (2018) 'The Prevalence of Malaria in Tselemti Wereda, North Ethiopia: A Retrospective Study', *Ethiopian journal of health sciences*, 28(5), doi: 10.4314/ejhs.v28i5.4.

Shumbej, T. *et al.* (2019) 'Therapeutic efficacy of chloroquine for treatment of Plasmodium vivax malaria cases in Guragae zone southern Central Ethiopia', *BMC Infectious Diseases*, 19(1), doi: 10.1186/s12879-019-4055-7.

Solomon, A., Kahase, D. and Alemayehu, M. (2020) 'Trend of malaria prevalence in Wolkite health center: An implication towards the elimination of malaria in Ethiopia by 2030', *Malaria Journal*, 19(1), doi: 10.1186/s12936-020-03182-z.

Sultana, M. *et al.* (2017) 'Prevalence and associated determinants of malaria parasites among Kenyan children', *Tropical Medicine and Health*, 45(1), doi: 10.1186/s41182-017-0066-5.

Tsegaye, A. T., Ayele, A. and Birhanu, S. (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 October), doi: 10.1371/journal.pone.0257944.

Wanzira, H. *et al.* (2017) 'Factors associated with malaria parasitaemia among children under 5 years in Uganda: a secondary data analysis of the 2014 Malaria Indicator Survey dataset', *Malaria Journal*, 16(1), doi: 10.1186/s12936-017-1847-3.

Warkaw, Y. M. *et al.* (2022) 'Spatial pattern and predictors of malaria in Ethiopia: Application of auto logistics regression', *PLoS ONE*, 17(5 May 2022), doi: 10.1371/journal.pone.0268186.

White, N. J. *et al.* (2009) 'Hyperparasitaemia and low dosing are an important source of anti-malarial drug resistance', *Malaria Journal*, 8(1), doi: 10.1186/1475-2875-8-253.

WHO (2016) 'Malaria Parasite Counting. World Health Organization.', Available at: http://www.wpro.who.int/mvp/lab_quality/2096_oms_gmp_sop_09_rev1.pdf.

WHO (2021a) *Global technical strategy for malaria 2016-2030, 2021 update, World Health Organization*. Available at: https://apps.who.int/iris/rest/bitstreams/1357541/retrieve.

WHO (2021b) *Word Malaria Report 2021, Word Malaria report Geneva: World Health Organization. (2021). Licence: CC.*

Worku, L. *et al.* (2014) 'Asymptomatic Malaria and Associated Risk Factors among School Children in Sanja Town, Northwest Ethiopia', *International Scholarly Research Notices*, 2014, doi: 10.1155/2014/303269.

World Health Organization (2022) 'Regional data and trends briefing kit World malaria', *World malaria report 2022*, (December)

Yankson, R., Anto, E. A. and Chipeta, M. G. (2019) 'Geostatistical analysis and mapping of malaria risk in children under 5 using point-referenced prevalence data in Ghana', *Malaria Journal*, 18(1), doi: 10.1186/s12936-019-2709-y.

Annexes

Annex I:Information sheet

Name of the Principal Investigator: Lelisa Fikadu

Name of the organization: Hawassa University

Introduction: This information sheet is prepared by groups of researchers whose main aim is to study malaria infections in children less than five years old.

Purpose: The purpose of this research is to determine malaria infection in under-five children and assess its associated factors. Malaria results in significant morbidity and socio-economic burden in Ethiopia, especially in under five children. Some epidemiological studies have been conducted in Ethiopia, however, no documented study on under five children in the study area. We therefore decided to conduct this investigation after taking this into account.

Procedure: We kindly invite your child to take part in this project which is aimed at determining the magnitude of malaria infection in under five children. If you are willing, for your children to participate in this project, you need to understand and sign the consent form. For laboratory examination, your children will provide a blood sample from the finger/heel. The laboratory examination results will be kept confidential using a coding system whereby no one will have access to your children's laboratory results. If the result of the laboratory examination shows positive, this will only be communicated to the health professional for management of the case.

Benefits: If your children participate in this research, he/she/they may get the direct benefit that the test result will be used for the management of his/her/their health. Besides, your participation will help us in studying the magnitude of the infection in the area, which is an input for national malaria and control activities being carried out.

Incentives: You and your children will not be provided any incentives to take part in this research.

Confidentiality: The information that we collect from this research project will be kept confidential. Information about you that will be collected from the study will be stored in a file, which will not have your name on it, but a code number assigned to it. It will be kept under lock and key, and it will not be revealed to anyone except the principal investigator and the concerned health professional.

Right to refuse or withdraw: You have full right to refuse from participating in this research if you do not wish to participate, and this will not compromise the health services you get at the health institutions in any way at any time.

Whom to Contact: If you have any questions, contact any of the following two individuals and you may ask at any time you want:

1. Dr. Solomon Asnake – Hawassa University, College of Medicine and Health Science, and department of Medical Laboratory Sciences, Hawassa, Ethiopia .Telephone no-0912088346

2. Dr. Mengistu Hailemariam -Hawassa University, College of Medicine and Health Science, Department of Medical Laboratory Science, Hawassa, Ethiopia. Telephone- 0913641103

3. Lelisa Fikadu- Principal Investigator. Telephone no-0913407031

Annex 2:Consent form

Introduction: We are asking you to take part in a research study on malaria infection among Children less than five years old in the Ilu Gelan district, West Shewa zone, West Ethiopia. We want to be sure that you understand the purpose and your responsibilities in the research before you decide if you want to your children be part of the study. Please ask us to explain any words or information that you may not understand.

Information about the Research

This is a research study that would involve the collection of about 0.25ml of blood from a peripheral by finger/heel prick for the detection of plasmodium parasitemia.

Possible Risks (explain risks –blood collection)

It is very unlikely that participation in this research will expose your children to any physical, social, or psychological risks.

Possible Benefits

Participation in this research may not benefit you and your children directly. But results from this study will be used to inform decisions in the implementation and strengthening of programs aimed at controlling and eradicating malaria in Ethiopia.

Confidentiality

We will protect information about you taking part in this research to the best of our ability. We will neither use your name in any reports nor discuss your participation with anyone outside the research team.

Leaving the Research: You may end your participation at any time with no negative consequence to you.

Respondent:

I consent to taking part in this study and agree to an oral interview in private with a research assistant who will record my answers. I also agree to let my child or children participate in blood collection procedures to test for the presence of malaria parasites.

Signed……. Date…….

Investigator…… Date….....

Annex 3: Guca walii galtee

Yeroo ammaa kana qorannoo dhibee busaa fi ka'umsa dhukkuba kanaa Ijoollee wagga shanii keessatti Dhiha Itiyoophiyaa, Godina Shawaa Lixaa, Aanaa Iluugelaanitti geggeessaa waan jirruuf isinis kana hubachuun daa'imni keessan kana keessatti akka qooda fudhatan kabajaan isin gaafanna. Qorannoo gaggeeffamu keessatti daa'ima osoo hin hirmaachisiin dura faayidaa qorannichaa fi ga`ee isin irraa eegamu irratti gaaffii fi yaada qabdan akkassumas dhimmoota isiniif hingalle irratti gaafattanii hubannoo keessan cimsachuu akka dandeessan isiniif mirkaneessuu barbaanna.

Annex III. I Odeeffannoo waliigalaa Qorannichaa

Qorannoon kun dhibee busaa adda baasuuf sakattainsi dhiigaa kan gaggeeffamu ta`a. Qorannicha keessatti daa'imman hirmaachuun miidhama qaamaa, hawaasumaafi sammuu kamiyyuu qaqqabsiisuu akka hin dandeenye isiniif mirkaneessuu barbaanna.

Bua`a qorannichaa irraa siniifi daa'imni kallattin fayyadamaa ta'uu baattanis sagantaawwan ittisaa fi to`annoo dhibee busaa akka biyyaatti hojiirra ooluu jajjabeessuuf fayyadamaa isin taasisa. Qorannicha keessatti daa'imni qooda akka fudhatuun fi ykn dhiisu mirga guutuu qabda.

Odeeffannoo dhunfaadaa'ima qorannicha keessatti hirmaatee qaama biraaf kennuu fi maqaa daa'ima sanaa gabaasa kamuu keessatti hindhiyaatu. Hirmaannaan taasifamu kaffaltii hin qabu. Gaaffii fi yaada yoo qabaattan teessoo fi bilbilli keenya kan armaan gaditi.

1.Dr.Salamon Asnaaqaa lakk.bilbilaa: 0912088346

2.0bboo Lalisaa Fiqaaduu Lakk.bilbilaa:0913407031

Annex IV: English questionnaire

Dear sir/madam

My name is_____

I am a Master's Degree student from Hawassa University. Thus, this questionnaire is prepared to get appropriate information on the prevalence of malaria in children less than five years old.

The information that I will obtain using this questionnaire will be used only for research purposes, and I need to assure you that confidentiality is our main quality.

Therefore; I respectfully request your cooperation to participate in this interview. You do have the right not to respond at all or to withdraw in the meantime, but your input has great value for the success of our objective. Thank you for being so cooperation!

Sign:_____

Code: _____ Kebele_____ Got_____

Child Age _____and Sex_____

I **Question related to background information**

	Questions	**Possible answers**	**Remark**

101	Caregiver Age and sex	_____and_____	
102	Ethnic group	1. Oromo 2.Amhara 3.Others_____	
103	Caregiver educational level	1. Illiterate 2. Read and write only 3. Primary school 4. Secondary school 5. Above Secondary school	
104	Occupation	1. Housewife 2.Farmer 3. Daily laborer 4 . Student 5.Civil servant 6. Businessman 7.Other specify_____	
105	Marital status	1. Single 2.Married 3. Divorced 4. Widowed	
106	Family monthly income(ETB)	1.<1727ETB 2.1727-3454ETB 3.>3454ETB	
107	Family size	_____	

II. Questions related to Household Conditions

201	The main material of the roof	1. Thatched 2. Corrugated iron sheet 3. Other, specify_____	
202	The main material of the wall	1. Wood planks 2. Grass 2. Mud 3. Cement 4.Other ,specify_____	
203	Presence of eave	1. Yes 2. No	
204	Are there animals living with the family inside the household?	1. Yes 2. No	

205	If yes, for Q 204 which animal?	1. Dog 2. Cat 3. Ruminant animal 4. Non-Ruminant animal	
206	Is there an Unprotected dam for irrigation?	1. Yes 2. No	
207	Is there stagnant water around your compound?	1. Yes 2. No	
208	Distance of house from the river?	_____meter	
209	Do you usually clean stagnant waters in broken pots, containers, and ditches around your house?	1. Yes 2. No	

III. Questions related to Practice and malaria information

301	Ever heard about malaria disease?	1. Yes 2. No	
302	If Question 301 is yes, from where you have heard?	1. Television 2. Radio 3. Other specify_____	
303	Do you use ITN to prevent mosquito bites?	1 Yes 2. No	
304	If yes to Qs 303, how many ITNs in house?	_____	

305	If yes to Q 303 Who is using the ITN currently?	1. Children under 5 years 2.Children above 5 years 3. pregnant women 4. Husband 5. All family members 6.No body	
306	Is the ITN impregnated on time?	1. Yes 2.No	
307	Does your child stay outside during the night?	1. Yes 2.No	
308	Is your house sprayed with insecticide in the last 12 months?	1. Yes 2.No	
309	What type of larval source management was performed in your locality? (More than one answer is possible)	1. permanent alteration to the environment 2. habitat manipulation 3. regular application of insecticide to water bodies 4. No measurements were taken	

IV Questions related to child

310	Child had fever in the last 2 weeks	1. Yes 2. No	
311	Which sign and symptoms have you seen on your child in the last 2 weeks?	1. Fever 2. Vomiting 3. Headache 4. Sweating 5. High temperature 6. Loss of appetite 7. Chilling	
312	Body temperature	_____	

(Al-Quhaiti *et al.*, 2022),(Kweku M., *et al.*, 2017),(Awosolu, Yahaya and Haziqah, 2021),(Al-Quhaiti *et al.*, 2022),(Abossie *et al.*, 2020),(Tsegaye, Ayele and Birhanu, 2021)

Annex V: Afaan Oromoo Questionnaire

Kabajamoo qooda fudhattoota qorannoo kanaa ani maqaan koo Lalisaa Fiqaaduun jedhama. Barataa barumsa laabooraatoorii digirii lammaffaa Yuuniversiitii Hawaasaati. Egaa gaafannoon kun kan qophaa'e odeeffannoo ga'aa fi qulqulluu ta'e dhukkuba busaa ijoollee wagga shanii gadii keessatti jiru adda baasuufi. Odeeffannon fudhadhu dhimma qorannoof qofa kan oolu yoo ta'u,iccitiiin kamiyyuu kan eegame ta'uu isin nan hubachiisa. Kanaafuu qorannoo kana keessatti nu waliin akka taataniif kabajaan isin gaafadha.Qorannoo kana irrattti hirmaachuu dhiisuus ta'e,yeroo barbaaddanitti keessaa ba'uuf mirga guutuu qabdu. Haa ta'u malee,hirmaannan keessan galma ga'iinsa qorannoo kanaaf bu'aa guddaa qaba.

Qorannichatti hirmaachuuf fedhii qabduu? A, Eyyee B, Lakki

Mallattoo : _____

koodii: _____ Ganda_____ Garee_____

I: Odeeffannoo waliigalaa

	Gaaffii	deebii	yaada
101	Umurii fi saala maatii	_____fi_____	
102	Qomoo	1.Oromoo 2.Amaaraa 3.kan biraa_____	
103	Sadarkaa barumsaa	1. Kan hin baratne 2. Dubbisuu fi barreessuu qofa	

		3. Barumsa sadarkaa tokkoffaa 4. Barumsa sadarkaa lammaffaa	
104	Hojii	1. Haadha warraa 2.Qotee bulaa 3.Hojjetaa guyyaa 4.Barataa 5.Hojjetaa mootumaa 6.Daldalaa 7.Kan biraa,Ibsi_____	
105	Haala gaa'elaa	1.Qeenxee 2.kan heerumte 3.Kan adda baate 4.Abbaan manaa kan irraa du'e	
106	Galii ji'aan(ETB)	1.<1727ET 2.1727-3454ETB 3.>3454ETB	

II. Odeeffannoo haala manaan walqabatan

201	Guutuun manaa kan ijaarame	1. Citaa 2. qorqorroo 3. kan biro yoo jirat_____	
202	Dhaabni manaa kan ijaarame	1. muka 2. Marga 3 .Dhoqqee 4.simmintoo 5.kan biraa Ibsi_____	
203	Irri keessi manaa qaawwa qabaa	1. Eyyee 2. Lakki	
204	Beeyladotni mana keessa ni jiraatuu?	1. Eyyee 2. Lakki	
205	Yoo deebiin gaaffii 204 eyyee ta'e maal fa'i?	1. Saree 2. Adurreet 3. Alaala kan guuran 4. Alaala kan hin guurre 5. kan biraa----------	
206	Bishaan jal'isiif fayyadamtan jiraa?	1. Eyyee 2. Lakki	
207	Bishaan kuufamaan mooraa keessa jiraa?	1. Eyyee 2. Lakki	
208	Fageenya iddoo wal hormaata bookee busaa irra qabu.	_____meter	
209	Yeroo hunduma bishaan okkoteecabaa, wantoota garaa garaa fi bo'ii keessatti kuufamee jiru maksitaa/ni dhabamsiiftaa?	1. Eyyee 2. Lakki	

III. Gaafannoo barsiifata/raawwiin wal qabatan

301	Waa'ee dhukkuba busaa dhageessanii beektuu?	1. Eyyee 2. Lakki	
302	Deebiin gaaffii 301 eeyyee yoo ta'e ,eessa dhageessan?	1. Televiziinii 2. Raadi'oo 3. Madda biroo ibsi_____	
303	Saaphana bookee busaa ittisu qabduu?	1. Eyyee 2. Lakki	
304	Yoo deebiin gaaffii 301, eeyyee ta'e, meeqatu jira?	_____	
305	Yoo deebiin gaaffii 301, eeyyee ta'e,eenyuutu fayyadamaa jira?	1. Miseensa maatii hunda 2. Abbaa fi haadha manaa qofa 3. Daaiimman waggaa shanii gadii 4. Daaiimman waggaa shanii olii 5.homtuu hin fayyadamu	
306	Saaphanichi yeroon keemikaala cuuphamee?	1. Eyyee 2. Lakki	
307	Ijoolleeen keessan halkan ala ni turuu?	1. Eyyee 2. Lakki	
308	Waggaa tokkoon asitti manni keessan keemikaala farra bookee buusaan biifamee?	1. Eyyee 2. Lakki	
309	Madda jiisaa Bookee busaa akkamiin to'atama? (Deebiin tokkoo ol ni danda'ama.)	1.Bakka wal hormaataa guutumaan guutuutti jijjiiruu 2. .Bakka wal hormaataa hanga tokko fooyyessuu 3.keemikaala jiisaa ajjeesu madda bishaanitti naquu 4.Homaa godhamee hin beeku	

IV .Gaafii Daa'ima ilaallatu

310	Daa'ima keessan irratii torbeewwan lamaan darban keessatti gubaan qaamaa mul'ateera?	1. Eyyee 2. Lakki	
311	Torbeewwan lamaan darban keessatti mallattoolee armaan gadii keessaa kan daa'ima keessan irratti mul'ate kami?	1. Qaama gubaa 2. Hoqqisiisuu 3. Bowwoo mataa 4. Dafqisiisuu 5. Hoo'inni qaamaa dabaluu 6. Fedhii nyaataa dhabuu 7. Dhaamochiisuu/hurgufuu	
312	Amma hoo'ina qaamaa	_____	

Annex V: Procedure for blood film preparation and examination

A thick and thin blood smear study is the gold standard method for malaria diagnosis. The steps in the process are as follows: a collection of peripheral blood, staining of smear with Giemsa stain, and examination of red blood cells for malaria parasites under the microscope.

Thick smear. It is not fixed in methanol; this allows the red blood cells to be hemolyzed, and leukocytes and any malaria parasites present will be the only detectable elements. However, the hemolysis may lead to distorted plasmodial morphology making plasmodium species differentiation difficult. Therefore, thick smears are mainly used to detect infection and estimate parasitemia.

Thin smear. It is fixed in methanol. Thin smears allow the examiner to identify malaria species, quantify parasitemia, and recognize parasite forms like schizonts and gametocytes.

Procedure

 Place the labelled slide on the template below.

 using a micropipette, place 6µl of blood for the thick film and 2–3µl for the thin film.

 using the ground edge of the spreader slide spread the blood for the thin film.

 using the corner of the spreader slide spread the blood for the thick film until the entire circle of 12 mm diameter is covered evenly.

 Dry the films on a flat surface, protected from dust and insects.

 Fix the thin film by dipping it in absolute methanol for a few seconds and then letting the slide air dry. Dry the thin film at an acute angle, with the film side of the slide facing up and the thin film downwards. This protects the thick film from being fixed by methanol fumes and run-off.

Examining the thick film

1. Place the Giemsa-stained blood film to be examined on the microscope stage, with the label to the left. Position the thick film in line with the 10x objective lens.

2. Switch on the microscope, adjust the light source optimally and find the focus by looking through the ocular and the 10x objective.

3. Scan the blood film for other parasites and blood elements. Select a part of the film that is well stained and has evenly distributed white blood cells.

4. Place a small drop of immersion oil on the thick film. To avoid cross-contamination, ensure that the immersion oil applicator never touches the slide. Do not allow the 40x objective to touch the oil.

5. Switch the 100x oil immersion objective over the selected portion of the thick film.

6. Examine the slide systematically. 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.

7. 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 the examination of each field.

Determining whether a thick film contains malaria parasites and identifying the species

1. Continue to examine the slide for 100 high-power or oil immersion fields. Move the blood film by one high-power field each time, following the pattern. Use the fine adjustment to focus.

2. A minimum of 200 high-power fields must be examined before a thick film can be declared as having “no malaria parasites seen”. If possible, the whole thick film should be scanned.

3. If parasites are observed, a further 100 fields must be examined before the final identification of the species, ensuring that a mixed infection is not overlooked.

4. The thin blood film should always be examined to identify parasite species definitively. The thin film allows visualization of parasite and red cell morphology, unlike the thick film. Examine the feathery end or edge of the thin film.

5. Identify and record all species and stages observed in the malaria microscopy blood register.

Note: Refer to the WHO bench aids for the diagnosis of malaria for identification of each species

Examining the thin film to confirm species and mixed infections

1. To confirm the parasite species or mixed infections after examining the thick film, examine the thin film.

2. Place a drop of immersion oil on the feathered edge of the thin film.

3. Move from the 10x lens to the 100x oil immersion lens.

4. Examine the feathery end or edge of the thin film where the red cells lay side by side

Performing a parasite count on a thick film and calculating parasite density

1. Place the glass slide on the microscope, with the label to the left
2. Determine the presence of malaria parasites and their species and stages, and record.
3. Starting at the top left of the smear, look for a typical field with bot parasites and white cells. Start counting.
4. Click the assigned key on the tally counter for each parasite or white cell observed.
5. After counting all the parasites and white cells in one field, move to the next, and repeat the counting procedure, and so on.
6. Depending on the number of parasites observed, stop counting after you have examined 200 or 500 white cells.
    - If you have counted ≥ 100 parasites in 200 white cells, stop counting, and record the results as the number of parasites per 200 white cells.
    - If you have counted ≤ 99 parasites in 500 white cells, stop counting, and record the results as the number of parasites per 500 white cells.
7. Count all parasites and white cells in the final field.
8. Record the actual numbers of parasites and white cells counted.
9. Calculate the parasite density from:

$$\text{Parasites / } \mu\text{L blood} = \frac{\text{Number of parasites counted} \times 8000 \text{ white cells/}\mu\text{L}}{\text{No. of white cells counted}}$$

Annex VII. RDTs Procedure

1. Make a gentle prick towards the pulp (ball) of the 4th finger with a sterile lancet at the disinfected site. Pricking at the tip or midline is more painful. Discard the used lancet in an appropriate sharp's container immediately after use. By applying gentle pressure to the finger express the first drop of blood and wipe it away with a dry piece of cotton wool. Make sure no strands of cotton remain on the finger to contaminate blood. Apply gentle pressure to the finger until a new blood drop appears. Emphasize the need for the right skills to ensure correct test performance and accurate results. The

reason for wiping out the first drop is that it contains too much tissue fluid which might dilute the antigens and it might be contaminated with the alcohol used for wiping the finger.

2. Using the blood collection device (pipette, inverted cup, or capillary tube) provided in the RDT kit, gently immerse the open end in the blood drop. Collect the required volume of blood as per the manufacturer's instructions. Good blood collection and an adequate amount of blood are fundamental to ensure good results. After pricking and collecting blood, apply dry cotton wool at the puncture site to stop the bleeding. Discard the blood collection device in the box for infectious waste.

3. Transfer the collected blood to the sample well (as indicated on the RDT cassette by the manufacturer). It's important to put the sample in the right well as indicated by the manufacturer. Different manufacturers may have different labelling for the different wells. Discard the blood collection device in the box for infectious waste.

4. Hold the buffer bottle vertically, and add the recommended number of drops of buffer into the buffer well. Put the exact amount of buffer as indicated by the manufacturer at the correct well of the test device and don't use any other buffer apart from the one provided and specified. Some test kits will come with a bottle of buffer for many tests and others will have enough buffers packed for a single test.

5. Keep a stopwatch for the test as recommended by the manufacturer. View the result window of the cassette for colour band(s).

a. **Negative** – The presence of only a control band indicates a negative result for *P. falciparum* malaria. If the RDT result is negative, alternative causes of fever should be investigated and treated appropriately. Note: Do not read the results before or after the set time. Don't treat any fever as malaria despite a negative result.

b. **Positive** – The presence of both a control band and a test band indicate a positive result.

Refer to the manufacturer's instructions to read positive results.

c. **Invalid** — If the test does not show the control band, even if there is a test band, the test is invalid. Perform another RDT.

d. Refer to the "RDT Provider job-aid" for pictures of negative, positive, and invalid results.

6. Report the results as “RDT Negative” “RDT Positive” or “RDT Invalid” (in the last case the RDT should be repeated. Record patient's information and RDT result in the appropriate register.'

7. Discard the cotton wool, RDT cassette, and gloves into the box for infectious waste. Discard empty bottles/ampulla of buffer, instructions, and RDT packaging into the box for non-infectious waste.