

PREVALENCE OF MALARIA AND ASSOCIATED FACTORS AMONG UNDER FIVE CHILDERN ATTENDING WOLKITE HEALTH CENTER, GURAGE ZONE, SNNPR, ETHIOPIA, 2022.

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LIST OF ABBREVIATION/acronym

EDHS Ethiopia demographic and health survey

FMOH Federal ministry of health

ID Identification

IPT Intermittent preventive therapy

IRS Indoor-residual spraying

ITNs Insecticide-treated mosquito nets

MOP Malaria Operational Plan

NGO non-governmental organization

RDTs Rapid Diagnostic Tests

RBM Roll Back Malaria

SNNPR South nation nationality and people region

WHO World Health Organization

UN United Nation

KAP Knowledge Attitude Practice

WHC Wolkite Health Center

FMH Federal Minster of Health

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Abstract

Background: Malaria is caused by protozoan parasites that belong to the Genus *Plasmodium,* which are transmitted to human via the bite of infected female anopheles mosquito. Malaria is usually restricted to tropical and subtropical areas with climatic conditions suitable for the development of the vector and parasite. Ethiopia is one of the malaria endemic countries with an average 60% *P. falciparum* and 40% *P. vivax* prevalence during peak malaria transmission time.

Objective*:* To determine the prevalence of malaria and associated factors among under five years of age children in Gurage zone, Wolkite town, Attending Wolkite Health centere.

Method: A cross-sectional study was carried out on the prevalence of malaria and associated risk factors among under five children patients who was visited Wolkite Health Centre from March to June 2022 G.C. Convenience sampling technique was used and data on socio-demographic characteristics were collected by interviewing the study subjects using pre-tested structured questionnaire. Blood samples were collected from the study subjects. Thick and thin smears were prepared and stained using Giemsa staining technique. The specimens were examined microscopically for the detection and identification of *plasmodium* species.

Result: Out of 356 patients whose blood film was examined, 174 and 182 were male and females, respectively. The prevalence of malaria was 35 (9.8%) and from this 16(9.2%) and 19(10.4%) were male and female patients, respectively. *P. vivax* had the highest prevalence with 77.1%, while *P. falciparum* takes the remaining portion with mixed infection among the patients.

Conclusion and recommendation: The prevalence is low compared with previous study due to several factors as explained in this study. But, effective malaria control measures should be implemented in order to reduce the prevalence of malaria among the under -fives more than this.

CHAPTER ONE: INTRODUCTION

1.1 Background

Malaria is a major public health problem in worldwide and causes high morbidity and mortality. It is a vector borne infectious disease caused by a eukaryotic Protista of the genus Plasmodium. The disease is transmitted by female Anopheles mosquitoes which carry infective sporozoite stage of Plasmodium Parasite in their salivary glands. It is transmitted from person to person through the bite of a female Anopheles mosquito that is infected with one of the five species of Plasmodium: Plasmodium *ovale*, Plasmodium *falciparum*, Plasmodium *vivax*, Plasmodium *malariae* and Plasmodium *knowlesi*. Children under five years are particularly vulnerable to the disease due to their weaker immune systems (1).

The transmission of malaria involves a complex interplay among the parasite, vector and host. It is influenced by behaviour, socioeconomic status and demographic factors of the human host and public health control measures (2). The presence and abundance of the vectors are usually affected in turn by seasonal variations and micro-ecology of the mosquitoes (3). Biting and resting behaviours of mosquitoes play also decisive role in the dynamics of malaria transmission (4). Climatic factors can determine both the parasite and the vectors of malaria in many ways: the extrinsic development of Plasmodium species are highly affected by temperature and optimum climatic variables mainly temperature, humidity and precipitation are needed for the proliferation of Anopheles mosquitoes to sustain malaria transmission (5). Additionally, human prevention and control behaviour, socioeconomic and demographic conditions can significantly affect the dynamics of the disease (6).

In Ethiopia, altitude and climate are the most important determinants for malaria transmission. Areas at altitudes between 1,600 and 2,000 meters above sea level (masl) are epidemic-prone hypo endemic zones of malaria. Malaria epidemics are thought to occur among populations settling as high as 2,500 masl. The major transmission of malaria occurs in the period from September – December follows the June – September rains while the minor transmission season occurs in April – May following the February – March rains.

Malaria killed 437,000 children before their fifth birthday in 2013, the majority in sub-Saharan Africa (7). According to the latest estimates, malaria mortality rates were reduced by about 47% globally and by 54% in the WHO African Region between 2000 and 2003.The incidence rates declined by 30% around the world and by 34% in the African region. Despite

enormous control efforts, increases in the drug resistance of the parasite, the insecticide resistance of its vectors, and human travel and migration have contributed to this resurgence.

1.2 Statement of the problem

Malaria affects an estimated 250 million people each year and is the most wide-spread parasitic disease encountered. The majority of these deaths are children under the age of 5 years. Thus, one child dies of malaria in Africa every 30 seconds, which translates into a tragic 3000 children each day (8).

Malaria is one of the major health threats in Ethiopia, particularly in the SNNP region in the South of the country. Despite the aforementioned study at different part of the country, malaria situation and its associated factor with regard to its prevalence, complication remain unknown in the study area, however it remains as commonest compliant in study area despite of maximum effort in control program.

Wolkite geography and climate influence (seasonal) outbreaks of malaria epidemics. Peculiarities of this area (seasonal heavy rainfall) lead to a high prevalence of mosquito breeding sites. Almost all parts in Wolkite are malaria prone, causing frequent outbreaks which in turn highly impacts on the immunity levels of the population. Over the years, commonly available drugs have become less effective as people have grown resistant to them. In addition, many are unaware of how to protect themselves against the disease, or lack the financial means for preventative measures (Wolkite town health office). The rationale behind the study is to assess the prevalence of malaria and associated factors (9).

1.3 Significance of the Study

Malaria is common problem in our country.Ttherefore, will primarily be important for the study is to know the magnitude of prevalence of malaria among under five children and, accordingly, to design strategies for intervention of the problem for those responsible sector. More ever, it will also be helpful for non-governmental organization to support financially and training, for health professionals to give health education about how to prevent and control prevalence of malaria, and for police makers to revise prevention and control of malaria at the country level working to decrease chilled mortality and morbidity. Finally, the finding from this study will serve as base line information for other researchers.

Chapter Two: Literature Review

Malaria is a major cause of morbidity and mortality worldwide especially in under five of age children. There are a number of factors that contribute to the high prevalence of malaria (9). Hence, the burden of malaria and control efforts, the roles socioeconomic factors, climatic variables, and intervention tools such as: insecticide treated bed nets in relation to malaria prevalence.

Globally, malaria affects the health more than three billion people and causes more than a million deaths annually (10). High burden of malaria is the cause and result of poor socioeconomic conditions resulting in vicious cycle of the impact (11). Pregnant women and children under 5 years of age are the most vulnerable groups. In adult's recurrent bouts of fever as the result of re infection and super infection reduce labour productivity (12); direct and indirect economic costs of malaria have been estimated to be high (13).

It also predisposes people, especially infants and disease increased slowly in areas where it had been reduced to low level (14). In an effort to combat the growing threat of malaria, the Roll Back Malaria (RBM) partnership was launched in 1998, with the goal to reduce the burden of malaria by half up to 2010 (15) and halving again by 2015(16). The United Nations (UN) had also declared the Decade to Roll Back Malaria (2001–2010) in developing countries. Moreover, African heads of States met in Abuja, in 2000 to express their commitment to combating malaria (17).

As the result, there has been substantial reduction of malaria in some countries (18). According to the WHO malaria report of 2010, in the decade from 2000 to 2009, out of 98 malaria endemic countries 42 (42.9%) of them reduced malaria cases by more than 50%; however, in 49 (50%) countries, including Ethiopia, there was no evidence of malaria cases reduction since 2005 (19). On the other hand, some studies have indicated substantial decline of malaria cases from sub-Saharan Africa, including Ethiopia due to intensive interventions, particularly scaled-up distribution of ITNs, IRS and use of rapid diagnostic kits at lower level health facilities and prompt treatment of cases (20).

According to the World Malaria Report of 2014, there have been substantial progresses both in coverage of intervention tools and, consequently, reduction in malaria related morbidity and mortality globally and, especially in the WHO African Region (21). In Ethiopia, the occurrences of epidemics of malaria have been documented since the 1930s and 1940s. The most devastating epidemic was that of 1958 which resulted in an estimated three million cases and 150,000 deaths (22). Until recently, malaria has been the leading cause of

morbidity and mortality in Ethiopia, accounting for over five million cases and thousands of deaths annually (23). The two peak seasonal transmissions of malaria occur during September to December and March to May (24). Study was done in malaria occurrence in Wolaita zone, SNNPR, Ethiopia revealed prevalence of malaria was 33% the predominantly reported species was *P. falciparum*, accounting for (71.80%) of the total occurrence, followed by *P. vivax* and mix of both species infections with *P. falciparum* and *P. vivax*, each constituting (23.95%) and (4.25%), respectively. The disease affected both males and female almost equally, accounting for (50.74%) and (49.26%) of cases, respectively (25).

2.1 Socio-economic and behavioral determinants

Socio-economic and behavioural factors can substantially affect malaria dynamics (26). At a macro level, there is clear evidence that the burden of malaria is greatest among the poorest countries of the world, especially those in sub-Saharan African. A study done in Central Vietnam involving complete community-based cross-sectional showed that the most important risk factor for malaria was the wealth category, the wealthiest group being much less infected. Another study done in India using five years' records of primary health care found that magnitude of malaria was three times more in the underdeveloped community compared to developed community (27). There were different studies conducted to determine the burden of malaria in urban area like Gondar town the prevalence was accounted (5.3%). Another study conducted in Addis Ababa showed that prevalence was accounted (3.7%).So the prevalence of malaria in this study is higher in rural area than in the urban area, this could be due to lack of bed net for use(28).

In case of occupation study done wolaita zone the highest infection with malaria parasites was seen in farmers, which accounted 30.7% this could be due to lack of proper use of bed net as well as presence of stagnant water near their home because the prevalence of malaria was also very high among the study population with stagnant water near their home which accounted (26.6%) (29).

2.2 Knowledge, attitude and perceptions of care givers

Study on knowledge of malaria and its association with malaria related behaviours in Ethiopia reported a wide gap between awareness of the study subjects to associate mode of malaria prevention by ITNs via protection from mosquito bites and knowledge of the symptoms of malaria (30). For instance, majority of women had heard of malaria in this survey and about one third of them (31.5%) reported that nets or ITNs could prevent malaria.

According to this study, the proportion of women who answered correctly to the knowledge of the prevention of malaria is almost similar among the overall women and women with under-five children.

Similarly, analysis of malaria indicator survey 2007 revealed that of 4,438 surveyed women, more than three fourth (79.5%) had heard of malaria (31). However, only half of them recognized fever as a sign of malaria, 1,763 (41.2%) mentioned mosquito bites as the cause of malaria, and 1,792 (38.2%) cited mosquito nets as a prevention method for malaria. In addition, this study shows that even though there is awareness in the community, the knowledge towards malaria prevention using insecticide treated nets remained low, which needs further study.

Since ITN is an effective mechanism of malaria prevention in Ethiopia, it is important to assess knowledge and perception of mothers towards ITN utilization. A study about ITNs usage against malaria in Tigray Region found that three fourth of the respondents claimed that children under-five years of age should be given priority to sleep under ITN in the household (32). Even though the attitude of the respondents is high concerning priority group who have to use ITN most, the actual utilization is questionable because, out of 260 nets observed, only 12.3% of the nets observed were found to be hanging and ready for use.

Furthermore, slightly less than two third (63.04%) of the interviewees had positive attitude towards buying ITNs if supplied by some body or organization with an affordable price while the rest of the respondents, about one third preferred free distribution by the government. A cross sectional study of the relationship between care-givers 'misconceptions and non-use of ITNs by under-five Nigerian children found that the level of misconception about the cause of malaria, prevention of malaria and knowledge of ITN had a significant effect on ITN utilization among under five children (33). For example, caregivers with misconceptions about prevention of malaria were about 20 percent less likely to own and use a net. According to this study, caregivers with correct knowledge of prevention of malaria were about 18 percent more likely to own and use a net compared with their counterparts who did not own a net. Even though, the perception of the majority of caregivers about the cause of malaria is mosquito bite**,** majority of them had misconceptions.

Study done in KAP of the society about malaria in Jiren Kebele, Jimma Town showed About (90.1%) of the respondent had heard and know some information about malaria and responded well, about (90.58%) respondents believed that malaria is preventable. They

mentioned different methods of preventing malaria transmission. Such as; burning wastes (88.2%) and the regular use of bed net (85.49%) was mentioned frequently of total respondents, most of them practice burning wastes and elimination of breeding site.

2.3 Dynamics of malaria and climatic variables

Malaria is considered to be an environmental disease. Environmental factors such as changing rainfall patterns and unusual temperature increase can play great rolls in malaria transmission dynamics (34). Brooker et al have indicated that the distribution of malaria is determined by climatic and other geographic factors, which affect mosquito and Plasmodium species reproduction. Warm temperatures, heavy rainfall, and high humidity are known to encourage mosquito breeding, longevity and shorten parasite sporogony. Changes in temperature, precipitation and humidity that are expected to occur under different climate change scenarios affect the biology and ecology of vectors, which consequently affect the risk of malaria transmission (35).

If water temperature rises, the larvae take a shorter time to mature and hence, there is a greater capacity to produce more offspring during the transmission period. Increased precipitation has the potential to increase the number and quality of breeding sites for vector mosquitoes and the density of vegetation, affecting the availability of resting sites. Cold temperatures induce mortality or slow developmental rates, or reduce host-seeking activity. Similarly, rainfall creates habitat for breeding for mosquitoes, and high humidity is necessary for survival of mosquitoes (36).

The change of temperature favours mosquitoes' development depending on the prevailing local weather conditions (37). In warmer climates, adult female mosquitoes digest blood faster and feed more frequently, thus increasing transmission intensity (38). Similarly, malaria parasites complete extrinsic incubation within the female mosquito in a shorter time as temperature rises there by increasing the proportion of infective vectors (39).

A study conducted in Zimbabwe, indicated that annual average temperature, rainfall and vapour pressure were strong positive predictors of increased annual malaria incidence, whereas maximum and minimum temperature had the opposite effects (40). Reid and colleagues documented also that malaria incidence rate was highly correlated with temperature and humidity, but inversely associated with precipitation (41).

They explained the negative correlation between malaria incidence and rainfall by the fact that a heavy rainfall can wash the ground and kill the eggs. Additionally, positive correlation

between malaria cases and maximum temperature and rainfall was highlighted by a study conducted in Bangladesh and elevation was also noted as an influential factor (42). Again, ten-year retrospective trend study done in Jimma Town, Ethiopia and showed not only fluctuation of number of malaria cases, but also changes in species of Plasmodium parasites following climatic variables (43). Thus, understanding how malaria varies in the community as a result of seasonal or year-to-year changes in environmental factors is important for the planning of national malaria control programs (44).

2.4 Insecticide treated bed net and Indoor residual spraying as main malaria prevention tools

Current methods of malaria vector control are highly dependent on pyrethroids, which are the most commonly used compounds for indoor residual spraying (IRS) and the only insecticide class used for insecticide treated nets (ITNs). The widespread use of a single class of insecticides increases the risk that mosquitoes will develop resistance, which could rapidly lead to a major public health problem (45). Apart from resistance, bed nets as a tool for malaria control can have other challenges such as coverage, proper use, and replacement of old and torn nets (46).

Further, consideration of the possible shift of the local malaria epidemiology prompts the need for evaluation of its proper use and effectiveness in protecting malaria to ensure the long-term benefit of this control method (47). Studies conducted in different settings pertaining to ITNs reported different findings regarding its utilization and effectiveness to reduce malaria risks due to various factors. For instance, a study conducted in Bangladeshi Highland indicated that households with less than three bed nets are two times at risk of malaria infection compared to households that had three or more bed nets (48). The study has identified also that 41.4% households with malaria positive cases had more than five household sizes.

This implies that distribution of ITNs one or two per households, especially in a rural setting where the household size is large, may not give guarantee to protect family members. It is known that children less than five years of age and pregnant women deserve priority to sleep under bed net.

A study done in Tanzania, revealed likelihood of having malaria in those ITN non users is two times higher than user (prevalence of malaria in ITN users and non-user is 22.5%, 41.2% respectively) or 83.68% 0f BF negative uses ITN, whereas 69.9% of BF positive are ITN user

and the likelihood of getting malaria among the families whom their houses were not sprayed with IRS was 3.011 times higher than the houses sprayed with IRS (49).

Change of mosquitoes‟ biting, behaviour is another challenge that make as questionable the protection efficiency of ITNs (50). For instance, a recent study conducted in northern part of Ethiopia revealed that the biting of mosquito shifted to early evening (51). According to that study, 70% of biting was between 19:00to 22:00. Similar study done in central Ethiopian Rift Valley also indicated that the peak biting activities of the vectors occurred before 22:00 (52).

The study was done on **insecticide** treated bed net utilizations among uunder five children and household bed net ownership in Adami Tulu District, Oromia Regional State, Ethiopia showed that ITNs were observed in 90% of those households reported to have ITN. About 47% of the total observed ITNs were in a good condition, without holes. Among ITN owning households, the coverage for one and two mosquito nets was (83.5%) and (16.5%) respectively (53).

Generally, malaria is a complex disease and its determinants vary with local conditions. Analysis of malaria relevant data and regular updating of the information is a useful method for prediction of the disease status and preparedness for malaria control (54). It is important to assess and analyse the local contexts that affect the transmission dynamics so that effective control measures can be targeted accordingly. The refined analysis of spatial and temporal distribution of malaria together with socioeconomic and demographic status of the population is thought to improve understanding of the factors that influence the dynamics of malaria in an area (55). Although factors linked to the dynamics of malaria are complex, the main factors that determine the dynamics of malaria are environmental factors, which limit the survival and longevity of malaria vectors; socioeconomic conditions that affect both exposure levels to mosquitoes as well as health-seeking behaviour after infection (56).

2.5 Conceptual Framework

The conceptual framework represents a relationship between factors that influence the prevalence of malaria among the under-fives.

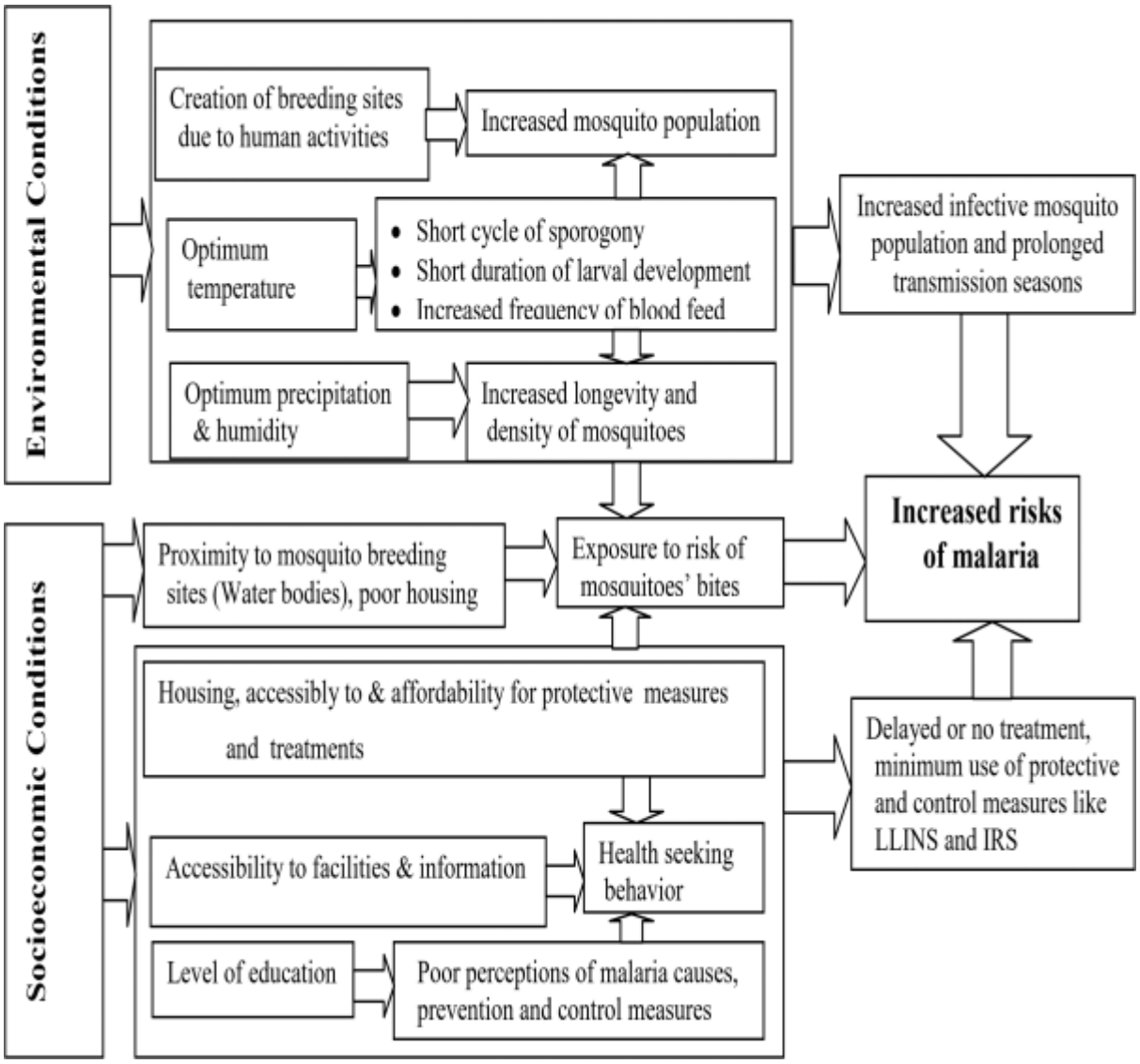


Figure 1 Conceptual framework indicating linkage of factors associated with increased risk of malaria Adapted (57)

Chapter THREE: Objective

3.1. General objective

- To assess the prevalence of malaria and its associated factors among under five children attending wolkite health centre, Gurage Zone SNNPR, Ethiopia

3.2. Specific objectives

- To assess the prevalence of malaria among under five children attending wolkite health centre.
- To identify the associated factors of malaria among under five children attending wolkite health centere.

Chapter 4: Method and Materials

4.1 Study area

Wolkite is administrative centre of the Gurage Zone of the Southern Nations, Nationalities and Peoples Region (SNNPR), this town has a latitude and longitude of 8°17′N 37°47′E/ 8.283°N 37.783°E and an elevation between 1910 and 1935 meters above sea level. Wolkite is a small city found in the southern side of Addis Ababa 158km. The structural plan of Wolkite town is set up from 5 kebeles and 3 sub towns. The three sub towns are Bekure, Addis birhan and Gubrea and the corresponding kebeles (the smallest administrative unit of Ethiopia) are Selamber, Edgetber, Menaheria, Addis birhan and Addis hiwot. There is one hospital, two health centers and six health posts. It is surrounded by Kebena woreda and it was part of former Goro woreda. Wolkite town has a total population of 66410, of whom 53% are men and 47% are women. The plurality of the inhabitants practiced Ethiopian Orthodox Christianity, with 48.17% of the population reporting that belief, while 42.31% were Muslim, 7.86% were Protestants, and 1.34% were Catholic. [Gurage zone socio-economic profile, accessed 4 September 2007]

4.2 Study period

- The survey was conducted from march-June /2022

4.3 Study design

Institution based cross sectional study was employed

4.4 Population

4.4.1 Source population

✓ All under five year children patient who came to wolkite health center.

4.4.2 Study population

✓ The study population all selected under five children who came to wolkite health center requested for laboratory examination during study period.

4.5 Study unit

❖ All under five children in which the data was collected and BF examination was done.

4.6 Inclusion and exclusion criteria

4.6.1 Inclusion criteria

- All under five Children Patients who have blood film requested and willing to participate in the study.

4.6.2 Exclusion criteria

- Those children whose mother could not respond and children who recived anti-malaria drug during study period.

4.7 Sample size determination and sampling technique

4.7.1 Sample size determination

The required sample size of the study was determined using a formula to estimate single population proportion with the following assumption;

Confidence Interval assumed 95%, margin of error 5%, and prevalence of malaria in under 5 children is 15.89% it was taken from similar study conducted previously from wolita.

So, $n = z^2 p(1-p)/w^2$ where, n= required sample size

Z= critical value for normal distribution at 95% confidence interval which is equal to 1.96(at alpha 0.05) P= 33.3%

W= margin of error which is 5%

$$n = \frac{z^2 p(1-p)}{w^2}$$

$(1.96)2(0.33)(1-0.33)/(0.05)2$

= 3.8416*0.2211/(0.05)2= 0.8493777/0.0025= 339

Considering the non-response rate we add 5% on the final sample size.

i.e. $n = nf + 5\%(nf)$

$n = 339 + 5\%(339)$

$n = 356$

Therefore, total sample size was 356 children.

4.7.2 Sampling technique

- Convenience sampling technique was used.

4.8 Data collection

4.8.1 Data collection process

Socio-demography data and risk factor related information were collected from her care giver using structured questionnaire by face to face interview. Capillary blood sample from finger prick of children was taken by the principal investigator after fulfilment of the consent form.

4.8.2 Collection, Preparation and Processing of Blood Sample

Capillary blood Specimen was collected using disposable sterile blood lancet from the finger under aseptic technique. A small drop (2 micro liter) of blood was added to the center of slide for thin blood film and larger drop (6 micro liter) of blood at the top end for thick blood film. After smearing, air drying ,labeling and Fixing the thin smears using concentrated methanol was made after the smear being dried and staining with 10% Giemsa for 10 minute, a drop of oil immersion was added and the smear was examined systematically under 1000 x magnification searching for at least 100 fields for the presence of parasite(2).

4.8.3 Data quality control assurance

To ensure the reliability and validity of the study the following activities were done just before, during and after the actual laboratory performance. Quality of the stain was checked before actual work by running positive and negative slide. Questionnaires were pre-tested and conducted before data collection. Quality of smear was checked .The microscope examination of full smear was done systematically to examine the whole slide. Standard reporting procedure (SOP) was followed throughout the study period. Questionnaire and slide was kept for re-checking.

4.9 Ethical Consideration

Official letter of permission was obtained from WKU University, department of Medical laboratory. Care giver of Children who participated on the study asked for their willingness, and were informed as they can skip question or questions that they do not wanted to answer fully or partly and also to stoped at any time if they wanted to do so. Confidentiality of the information was assured by not asked their name.Ethical issues were considered in all steps of research process. The purpose, benefit of the study, the procedure involved and the risks

that might encounter while conducting in the study was thoroughly explained to the study subjects. Confidentiality of the data was maintained.

4.10 Data entry and analysis

The collected data was cleand, checked for completeness and analysed using scientific calculator. The result of the study were presented using different graphs and tables. Prevalence of malaria was determined as the proportion positive blood film. Association between prevalence of malaria and associated risk factors was assessed using different test by using dependent and independent variable.

4.11 Variables

4.11.1 Independent variable

- Age
- Sex
- Residence
- Educational status
- Occupational status
- Usage of bed net
- Presence of stagnant water
- Knowledge about malaria transmission
- Religion
- Knowledge about malaria prevent

4.11.2 Dependent variable

- Prevalence of malaria

4.12 Operational definition

Malaria-free**:** An area in which there is no continuing local mosquito-borne malaria transmission, and the risk for acquiring malaria is limited to introduced cases only.

Malaria incidence**:** The number of newly diagnosed malaria cases during a specified time in a specified population

Malaria prevalence: The number of malaria cases at any given time in a specified population, measured as positive laboratory test results.

Parasite prevalence**:** Proportion of the population in whom Plasmodium infection is detected at a particular time with a diagnostic test (usually microscopy or a rapid diagnostic test).

Population at risk: Population living in a geographical area in which locally acquired malaria cases occurred in the current and/or previous years.

Endemic: Applied to malaria when there is an ongoing, measurable incidence of cases and mosquito-borne transmission in an area over a succession of years.

Epidemic**:** Occurrence of cases in excess of the number expected in a given place and time**.**

Incubation period: The time between infection (by inoculation or otherwise) and the first appearance of clinical signs, of which fever is the commonest.

Intervention (public health): Activity undertaken to prevent or reduce the occurrence of a health condition in a population

Surveillance (control programmes): Ongoing, systematic collection, analysis and interpretation of disease-specific data for use in planning, implementing and evaluating public health practice.

Transmission season: Period of the year during which mosquito-borne transmission of malaria infection usually takes place.

Vector control: Measures of any kind against malaria-transmitting mosquitoes intended to limit their ability to transmit the disease.

Vulnerability: Either proximity to a malarias' area or frequent influx of infected individuals or groups and/or infective anopheles**.**

4.13 Dissemination of the study finding

The finding of the study was presented to Wolkite University College of health science & medicine, department of Medical Lab, disseminated to Wolkite town administrative health unit & for others who needed it for further investigation.

CHAPTER FIVE: RESULT

This study was basically characterizing malaria prevalence and associated factor among the under-fives in WHC. The main findings of the study are summarized under sub-headings. Namely: demographic data, socio Analysis of Malaria Prevalence with dynamics of *P. falciparum* and *P. vivax* in a Micro Ecological Setting, Factors associated with the prevalence of malaria prevalence of malaria among under five children, Awareness about malaria, ITN and IRS coverage and Utilization and Housing environment of respondent.

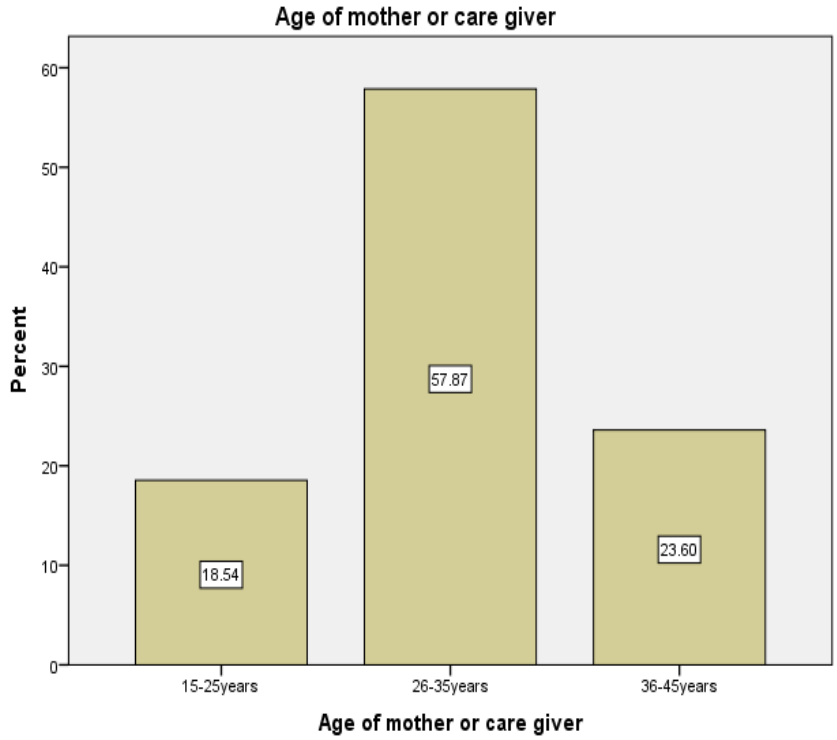
5.1 Socio demographic data

Three hundred fifty-six under-fives were recruited in the study and their age ranged from 0-5 Years whereby females were 182 (51.1%) and males were 174(48.9%). Out of these 92 (25.8%) children were in the age group of 0-11 month, 162 (45.5%) children in the age group 12-36 month, and 102(28.7%) in the age group 36-59 month. Regarding ethnicity Out of 356 participants, 316(88.8%) were Gurage, 25(7%) were Amhara, 13(3.7%) were Oromo and 2(0.6%) was other.

Majority of them were orthodox religion followers 177(49.7%). The percentages of Muslim, Protestant & Catholic were 21.1%, 14.9% & 13.8% respectively. Out of the 356 participants 206 (57.9%) were in the age group 26-35 years, 66 (18.5%) in the age group 15-25 years, and 84(23.6%) were found in the age group greater than or equal to 36 years.

Table;2 Social demographic characteristics by division of sex and age of children,ethinicity,age of mother, religion.

Variables	Frequency (n=356)	Percentage (%)
Sex of children		
Male	174	48.9
Female	182	51.1
age of the children		
< 1 year	92	25.8
1-5years	264	74.2
Ethnicity		
Gurage	316	88.8
Amhara	25	7
Oromo	13	3.7
Other	2	0.6
Religion		
Orthodox	177	49.7
Protestant	53	14.9
Muslim	75	21.1
Catholic	49	13.8
Other	2	0.6
Age of mother		
15-25year	66	18.5
26-35year	206	57.9
36-45year	84	23.6
>45year	0	0



Figer 2: distribution of age of the mother/care giver

5.2 Prevalence of malaria in Socio-Demographic characters

The overall prevalence of malaria was 35 (9.8%) from these males were 16 (9.2%), females were 19(10.4%). And age 9(9.8%) were in age less than one year, 26 (9.8%) were in between age of 1-5 years.

Majority of participant 158 (44.4%) reported to have attained primary education, and the rest 45(12.6%) had secondary, and preparatory and 71(19.9%) had some college or university education, 82 (23.0%) reported to have never been to school. And out of those 177(49.7%) were house wife, while the rest Merchant, governmental worker, Daily labour, and farmer were 22.8%, 20.5%,3.4%,and 1.1%, respectively. Depending on educational level highest prevalence seen in illiterate care giver (13.4%).Depending on occupation highest prevalence seen in farmer care giver (30.7%). Depending on residence highest prevalence occurred in rural area (22.7%).

Regarding in income estimated monthly income of the majority of them were in between 6000-10000birr followed by between2000-4000birr. 0.56% of the population had an income of <800 birr.

Table 2: Prevalence of malaria with age and sex of the children, Educational back ground of mothers, Occupation of care giver and monthly income in 2022GC

		Prevalence of malaria		Total	Percent(%)
		-ve	+ve		
Sex of children	Male	158(90.8%)	16 (9.2%)	174	(48.8%)
	Female	163 (89.6%)	19 (10.4)	182	(51.2%)
	Total	263	35	356	100
Age of children	<1 year	83 (90.2%)	9(9.8%)	92	25.8
	1-5 year	238 (90.2%)	26 (9.8%)	264	74.1
	Total	321	35	356	100
Educational level of mothers	Illiterate	71 (86.6%)	11 (13.4%)	82	23.0
	Grade(1-8)	139 (87.98%)	19 (12.02%)	158	44.4
	Grade (9-12)	42 (93.3%)	3 (6.7%)	45	12.6
	>12	69(97.2%)	2 (2.8%)	71	19.9
	Total	263(73.9%)	35 (9.8%)	356	100
occupation of mother	governmental worker	68 (93.2%)	5(6.8%)	73	20.5
	Merchant	75 (92.6%)	6 (7.4%)	81	22.8
	Daily labor	9(75%)	3 (25%)	12	3.4
	house wife	160 (90.4%)	17(9.6%)	177	49.7
	farmer	9 (69.3%)	4 (30.7%)	13	3.65
	Total	321 (90.2%)	35(9.8%)	356	100
Monthly income	<800birr	2 (100%)	0 (0%)	2	0.56
	800-2000birr	22 (84.7%)	4 (15.3%)	26	7.3
	2000-4000birr	60 (84.6%)	11 (15.4%)	71	20
	4000-6000birr	89 (87.3%)	13 (12.7%)	102	26.7
	6000-10000birr	141 (95.3%)	7 (4.7%)	148	41.6
	>10000birr	7 (0%)	0 (0%)	7	2
	Total	321(91.2)	35(9.8)	356	100
Residence	urban	314(90.5%)	33(9.5%)	347	97.5
	Rural	7(77.8%)	2(22.2%)	9	2.5

5.3 Awareness on Malaria

350(98.3%) of the respondents were heard about malaria from these prevalence of malaria was 32(9.1%). 333(93.5%) of respondents were know about transmission from these prevalence of malaria was 29(8.7%).Among a total of 349 (98.0%) knew prevention method of malaria from these prevalence was 33(9.5%).

Table 3: Mother/caregiver ever heard about malaria, knew about transmission and knew about prevention Vs prevalence of malaria in U5 children in WHC, 2022GC

Variable		prevalence of malaria		Total	Percent (%)
		-ve	+ve		
heard about malaria	Yes	318(89.3%)	32 (9.1%)	350	98.3
	No	3 (50%)	3 (50%)	6	1.7
knew about transmission	Yes	304 (92.3%)	29 (8.7%)	333	93.5
	No	17(73.9%)	6(26.1%)	23	6.5
	Total	321 (90.2%)	35(9.8%)	356	100
Knew about prevention	Yes	316(90.5%)	33(9.5%)	349	98
	No	5(71.5%)	2(28.5%)	7	1.9

5.4 ITN and IRS coverage and utilization

339 participants had bed nets from those 335(98.8%) reported bed net were in good order, from these prevalence was 30(8.9%). And also they mentioned 307 (90.6%) were currently used from these prevalence was 23(7.5%).Whereas, 32(9.4%) were not currently used from these prevalence was 8(25%).Among 356 participants 339(95.2%) were used bed net from these prevalence was 31(9.1%).Among 356 participants 20(5.6%) insecticide sprays from these prevalence was 1(5%).

Table 4: Usage of ITN, IRS, Coverage and utilization ITN Vs prevalence of malaria in U5 children in WHC, 2022GC.

current situation ITN	Good	305(91.1%)	30(8.9%)	335	98.8
	Torned	3 (75%)	1(25%)	4	1.2
	Total	308(90.9%)	31(9.1%)	339	100
currently used ITN	Yes	284(92.5%)	23(7.5%)	307	90.6
	No	24(75%)	8 (25%)	32	9.4
	Total	308 (90.9%)	31(9.1%)	339	100
had ITN	Yes	308 (90.9%)	31(9.1%)	339	95.2
	No	9 (52%)	8 (47%)	17	5.0
Used insecticide sprays	Yes	19 (95%)	1 (5%)	20	5.6
	No	302 (89.9%)	34 (10.1%)	336	94.4
	Total	32	35	356	100

5.5 Time of using ITN and its Prevalence

In this study it was observed that 264 (77.9%) families frequently mentioned that ITNs were used throughout from those prevalence was 15(5.7%). 37 (10.9%) mentioned that they were using the ITNs during the rainy season from these prevalence was 8(21.6%) and 38(11.2%) mentioned that they were using during dry season from these prevalence was 8(21.05%).

Table 5: Frequency and season family use ITN, Vs prevalence of malaria in U5 children in WHC, 2022GC.

		prevalence_of_malaria		Total	Percentage(%)
		-ve	+ve		
season of the year in which family use ITN	Rain	29 (78.4%)	8 (21.6%)	37	10.9
	Dry	30 (78.95%)	8 (21.05%)	38	11.2
	Throughout	249 (94.3%)	15 (5.7%)	264	77.9
Frequency of ITN use	Always	250(95.8%)	11(4.2%)	261	77.0
	Not always	58(74.5%)	20(25.5%)	78	23.0
	Total	307	31	339	100

5.6 Housing environment

Out of 356 participants 146 (41.0%) were reside in clean environment among these prevalence was 12(8.2%); 31(8.7%) in proximal to breeding site from these prevalence was 7(22.6%); 209(58.7%) in house farming activity, 285 (80.1%) in mud wall of the house and 15(4.2%) had stagnant water around house.

Table 6: housing environment Vs prevalence of malaria in U5 children in WHC, 2022GC

Variable		Prevalence of malaria		Total	Percent (%)
		-ve	+ve		
clean housing environment	Yes	115(81.5%)	12(8.2%)	146	41.0
	No	148(70.5%)	23(6.5%)	210	59.0
Proximal to breading site	Yes	26(83.8%)	7 (22.6%)	31	8.7
	No	237 (72.9%)	28 (8.6%)	325	91.3
wall of house	Mud	255(89.5%)	30(10.5%)	285	80.1
	Cement	66(92.96%)	5(7.04%)	71	19.9
	Total	263(73.9%)	93(26.1%)	356	100
	Yes	11(73.4%)	4(26.6%)	15	4.2
Presence of Stagnant water	No	310(90.9%)	31(9.09%)	341	95.8
	Total	321	35	356	100

5.7 The prevalence of malaria parasite among the under-fives children in WHC

We take total number of 356 under-five children we done BF examination.From that the majority of them who tested malaria were positive- for P. vivax 29 (82.8%) while those who tested malaria positive P. falciparum were 6(17.2%).

$$\text{Prevalence of malaria} = \frac{\text{total BF positive}}{\text{BF} - \text{Ve} + \text{BF} + \text{ve}} \times 100$$

$$= \frac{35}{356} \times 100$$

$$= 9.8\%$$

So, regard to our study prevalence of malaria among under five children in WHC was 9.8%.

Table 7: BF result

	Total	Percentage (%)
laboratory result		
Total positive	35	9.8
Negative	321	90.2
Total	356	100
Plasmodium species		
P .vivax	27	77.1
P .falciparum	6	17.1
P .falciparum + p .vivax	2	5.8
Total	35	100

5.8 Factors associated with the prevalence of malaria among under-fives in WHC

Malaria was found to be associated with variety of factors. Table 8 shows the results from bivariate analysis of the factors that are associated with the prevalence of Malaria. From the analysis the education status of the parents/guardians was statistically significant (p=0.001). The likelihood of getting malaria was found to increase 4.187 times more as compared to those who were educated [OR= 4.187 (1.827, 9.598). (P=0.005) It was further observed that the chances for the under-fives to get malaria of those who were in low monthly income 7.286 times higher than those who were get >800 birr monthly [OR=7.286 (1.827, 28.35)].

The likelihood of getting malaria among the under-fives whom their parents were never heard about malaria was 14.00 times higher than as compared to who knows about malaria (p=.046). [OR=14.00 (1.66, 12.96)].

It was observed that the association between the prevalence of malaria and presence of stagnant water around house was statistically significant (P=0.014). The likelihood of getting malaria was 3.224times higher than from no stagnant water around house [OR=3.224 (1.262, 8.235)). Prevalence of malaria in those reside in mud wall house 2.977 higher than those reside in cement wall (p=0.004). [OR=2.977 (1.416, 6.262)].

Table 8 Bivariate analysis of the factors associated with the prevalence of malaria.

Factor		Malaria prevalence		p-value	COR	CI -95%		Total
Education back ground of mother		-ve	+ve			lower	Upper	
	Illiterate	71 (81.6%)	11 (13.4%)	0.001	4.187	1.827	9.598	82
	Grade(1-8)	139(87.98%)	19(12.02)	0.004	3.097	1.425	6.730	158
	Grade(9-12)	42 (93.3%)	3 (6.7%)	0.916	1.062	0.350	3.268	45
	>12	69(97.2%)	2(2.8%)	0.00	0.176			71
Monthly income	<800birr	2 (100%)	0 (0%)	0.999	0.00	0.00	.000	2
	800-2000birr	22 (84.7%)	4 (15.3%)	0.005	7.286	1.915	28.35	26
	2000-4000birr	60 (84.6%)	11 (15.4%)	0.010	3.262	1.926	8.022	71
	4000-6000birr	89 (87.3%)	13 (12.7%)	0.03	4.371	1.636	11.67	102
	6000-10000birr	141 (95.3%)	7 (4.7%)	0.496	1.242	0.453	4.916	148
	>10000	7 (100%)	0 (0%)	0.157	1.962	0.772	6.609	104
heard on malaria	Yes	318(89.3%)	32 (9.1%)	.000	.346			350
	No	3 (50%)	3(50%)	.46	14.00	1.66	12.96	6
Have bed net	Yes	308 (90.9%)	31 (9.1%)	0.01	.174	.32	.485	339
	No	9 (52%)	8(47%)	.232	1.833			17
Currently ITN used	Yes	284 (92.5%)	23(7.5%)	.000	.258			307
	No	24 (75%)	8 (25%)	.000	7.39	3.388	16.13	32
ITN use Frequency	Always	250(95.8%)	11(4.2%)	.000	.120			261
	Not always	68(74.5%)	20(25.5%)	.000	21.06	11.28	39.77	78
Wall of the house	Mud	255(89.5%)	30(10.5%)	.004	2.977	1.416	6.262	285
	Cement	66(92.96%)	5(7.04%)	.00	.145			71
Presence of stagnant water around house	Yes	11(73.4%)	4(26.6%)	0.014	3.224	1.262	8.235	15
	No	310(90.9%)	31(9.1%)	.000	1.976			341

In table 9: shows the result from multivariate analysis the association between the prevalence of malaria and have ITN remained to be highly significant (P=0.00). The likelihood of getting malaria among the families who did not have ITN 2.73 times higher than have ITN [OR=2.73 (6.075, 14.551)].

Table 9: Adjusted (multivirant) association of the factors and the prevalence of malaria

Factor		Malaria prevalence		p-value	AOR	CI -95%		Total
		-ve	+ve			Lower	Upper	
Have bed net	Yes	308(90.8%)	31 (9.1%)					339
	No	9(52%)	8 (47%)	0.000	2.73	6.075	14.551	17
Currently used of ITN	Yes	284 (92.5%)	23(7.5%)					307
	No	24 (75%)	8 (25%)	.010	3.22	1.329	7.801	32
frequency of ITN use	Always	250(95.8%)	11(4.2%)	.000	.054	.027	.106	261
	Not always	68(74.5%)	20(25.5%)					78
Presence of stagnant water around house	Yes	11 (73.4%)	4(26.6%)	.026	2.118	1.073	4.18	15
	No	310 (90.9%)	31 (9.1%)	.000				341

CHAPTER SIX

6.1 DISCUSSION

This study was carried out in WHC where the primary objective of the study was to determine the prevalence, of malaria among under -fives and the associated factors. Overall prevalence of malaria among the under-fives in the study area was (9.8 %). The prevalence of specific species (P. vivax, P. falciparum,) were also being observed 27 (77.1%) and 6(17.1%)) respectively and mixed infection 2(5.8%). The prevalence of malaria infections found in this study was low as compared to a previous study conducted in the same region (Wolaita zone).

Study was done in Wolaita Zone revealed the overall prevalence of malaria in the study area was (33 %). And predominantly reported species was *P. falciparum*, accounting for (71.80%) of the total occurrence, followed by *P. vivax* and mix of both species infections with *P. falciparum* and *P. vivax*, each constituting (23.95%) and (4.25%), respectively. However, in our result the predominantly reported species was *P. vivax* accounting for (77.1%) of the total occurrence, followed by *P. falciparum*, and mix of both species infections with *P. falciparum* and *P. vivax*, each constituting (17.1%) and (5.8%), respectively. The disease affected both males and females almost equally, accounting for (9.2%) and (10.4%) of cases respectively.

Several factors were observed to be associated with the high prevalence, these includes: socioeconomic factors, physical/environmental factors of which favours the mosquito breeding sites as well as ineffective implementation of malaria control measures such as the use of ITNs and IRS. For example, this concurred with the analysis of a frequency of ITN utilization of this study where by the multivariate models revealed that the irregularly use of ITN was significantly associated with increased risk of the under-fives getting malaria. Furthermore, according to the study findings housing and environmental factors such as the presence of stagnant water around house were found to be associated with the prevalence of malaria.

Socio-economic and behavioural factors can substantially affect malaria dynamics. At a macro level, there is clear evidence that the burden of malaria is greatest among the poorest countries of the world, especially those in sub-Saharan African. A study done in Central Vietnam involving complete community-based cross-sectional showed that the most important risk factor for malaria was the wealth category, the wealthiest group being much less infected. Another study done in India using five years' records of primary health care

found that magnitude of malaria was three times more in the underdeveloped community compared to developed community. Also our study showed that similar character that means care givers who got >10,000 birr in month less infected than who got less than 10,000 birr.

According to the findings our study, the concept of using of ITNs and IRS was considered as the protective factor against the mosquito bite; hence reduce the prevalence of malaria among the under-fives. This was supported by the a study done in Tanzania, report likelihood of having malaria in those ITN non users is two times higher than user(i.e. 41.2% and 22.5% respectively).And in our study done in WHC likelihood of having malaria ITN non user is 3 times higher than users (25% and 7.5% respectively). And the likelihood of getting malaria among the families whom their houses were not sprayed with IRS was 3.011 times higher than the houses sprayed with IRS also our study showed that house not sprayed with IRS was 2 times higher than sprayed with IRS.

The study was done on insecticides treated bed net utilization among under five children and household bed net ownership in Adami Tulu District, Oromia Regional State, Ethiopia showed that ITNs were observed in 90% of those households reported to have ITN. About 47% of the total observed ITNs were in a good condition, without holes.Therefore, which stated that Insecticide-treated mosquito nets (ITNs) used for protection against mosquito bites have proven to be a practical, highly effective, and cost-effective intervention against malaria and Indoor residual spraying with insecticide has been shown to be highly effective as a malaria control measure in reducing the incidence of malaria infections.

The study also showed that the distribution of malaria was high on the population who live in rural area which accounted 22.2% compared to 9.5% in urban area. There were different studies conducted to determine the burden of malaria in urban area like Gondar town the prevalence was accounted (5.3%). Another study conducted in Addis Ababa showed that prevalence was accounted (3.7%).So the prevalence of malaria in this study is higher in rural area than in the urban area, this could be due to lack of bed net for use(19).

In case of occupation the highest infection with malaria parasites was seen in farmers, which accounted 30.7% this could be due to lack of proper use of bed net as well as presence of stagnant water near their home because the prevalence of malaria was also very high among the study population with stagnant water near their home which accounted 26.6%. Therefore,

breeding of mosquitoes in the stagnant water bodies could be accounted as reason for the high malaria incidence in these groups.

Taken together, the results presented here illustrates that: ITNs use, IRS, parents' /guardian's education status, economic status; physical/environmental factors are the predictive factors of the prevalence of malaria among the under-fives.

CHAPTER SEVEN: CONCLUSION AND RECOMMENDATIONS.

7.1 CONCLUSION

It can be concluded the prevalence of malaria among the under-fives children in WHC some what low compared previous study. Results of this study revealed that prevalence of malaria among under-five children is 9.8%, for this prevalence could be the result of several factors as explained in this study among those educational status of mother/caregiver, income, awareness about malaria, housing condition and high coverage and regular utilization of ITN are major factor for decrement of prevalence compared to previous study.

7.2 RECOMANDATION

- ✓ The use of personal protective methods like mosquito bed nets should be encouraged.
- ✓ Further study on the bed net utilization rate for malaria risk groups, especially under five-year age should be studied.
- ✓ Health education related to malaria should be encouraged by regional health workers and by FMH.
- ✓ For Health Centers: ensuring availability of printed materials like leaflets that help to increase awareness about malaria and understand the benefits of ITN use.
- ✓ For Service providers: should provide counselling on malaria and prevention method like avoid breading site, keep cleanness of environment ITN use & how they use.

7.3 Limitation of the study

1. Time of data collection might affect the prevalence due to seasonal variation of malaria transmission.
2. The study was not included qualitative method, particularly observation of housing conditions.
3. Since we used a Convenience sampling technique.

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ANNEXE-1

Questionary

WOLKITE UNIVERICITY

COLLEGE OF HEALTH & MEDICINE

DEPARTEMENT OF MEDICAL LABORATORY

Prevalence of malaria and associated factors among under five children in Wolkite health center, SNNPR, Ethiopia.

Good morning/afternoon?

My name is----------. Currently am a graduate student at wolkite university college department of health education

I am conducted research on prevalence of malaria among under five children in wolkite health center

To attain this purpose your honest and genuine participation is very important and highly appreciable. I, therefore, kindly request you to answer this questionnaire as accurately and carefully as possible.

Your name will not be asked and unique identification is not required. If you want to withdraw from the study at any time along the discussion process, you are not obliged to continue.

Are you willing to participate in this study?

1. Yes-----please turn to the page
2. No------please state your reason----------

Thank you, have a nice day

Data Collector

Name _____ Signature _____ Date _____

Supervisor

Name _____Signature _____ Date

Demographic data

1. Age of children in year

   A. <1yr B. 1-5yr

2. Sex of children

A. male B. female

3. Religion

   A. Orthodox B. Protestant

   C. Muslim D. Catholic E. Others specify---------

4. Address(Keble)----------

6. Ethnicity

   A. Gurage B. Amhara

   C. Oromo D. Other specify---------

7. Age of Mother/Care Giver:

   A. (10-20yrs) B. (21-30)

   C. (31-40yrs) D. (41-60yrs)

8. Educational Background of mother/care givers

   A. Primary B. Secondary

   C. Tertiary D. None

9. Do you know about Malaria?

   A. Yes B. No

10. If yes, what do you know about it?

11. Where did you get the knowledge?

A. mass media B. from printed media

C. from health workers D. If the other specify? ----------

12. Do you know how the malaria is transmitted?

A. Yes B. No

13. If yes for the above question, how it is transmitted?

14-Do you think that malaria can be prevented?

A. Yes B. No

15-If you say yes, how? ----------

Associated factors

16. Do you have bed net in your house?

A. Yes B. No

17. If yes, did the family use it regularly and appropriately?

A. Yes B. No

18. If no, for question number 6 what is the reason?

A. lack of awareness B. Discomfort (side effect C. Other (specify)

19. Is there any stagnant water around home?

A. Yes B. No

20. Use bed net currently?

A.yes B.no

21. Currently status of bed net?

A.good B.torned

22. When you used your bed net?

A.always B.not always

22. Monthly income of care givers?

A.800-2000 B.2000-4000 C.4000-6000 D.6000-10000 E.>10000

23.In which season used your bed net?

A.dry B.rain C.throught

ANNEXE-2

Health facility level report format

District _____ Name of Health Facility _____ _____

Name	Sex	Age	Address (Keble/Got)	Date of diagnosis (DD/MM/YYYY)	Results*	
					p.vivax	p.falciparium

ANNEXE –3

Laboratory procedure

Materials and reagents

Material

- Lead pencil
- Microscope
- Microscope slide
- Blood lancet
- Cotton
- Stain rack

Reagents

- ✓ Giemsa stock solution
- ✓ Distilled water
- ✓ Absolute methanol
- ✓ Oil immersion
- ✓ 70% of alcohol

Procedure for capillary blood specimen collection.

1. Clean the finger using gauze or cotton moistened with 70% alcohol.
2. Allow the area to dry.
3. Using sterile disposable lancet prick the finger and squeeze gentling to obtain adequate amount blood for both thick and thin blood smears.
4. Place a large drop of the blood at the top of the slide and smaller drop at the in front of the large one for thick and thin blood smaller preparation respectively.
5. Spread the smaller drop of blood using sterile smooth edged slide to produce regular and Iuse the edge of the spreads slide to make thick blood smaller.
6. Label the slides with code number using lead pencil.
7. Allow the smears to dry and place in dust free area.

Giemsa Staining procedure

1. Place the smeared slide horizontally on staining rack.
2. Fix the thin blood smear with methanol for 10-20 seconds and keep carefully for fixative not to touch the thick blood smear.
3. Cover the smear with diluted 10% Giemsa staining solution for 10-15 minutes.
4. Wash the slides with distilled water and let them air dry.
5. Clean the back of the slides and add a drop of oil immersion to examine the smear under oil immersion objective