PREVALENCE AND PREDICTORS OF ASYMPTOMATIC MALARIA AMONG PREGNANT WOMEN IN MERTI WOREDA, ARSI ZONE, OROMIA, ETHIOPIA

BY: BEREKET WAKE

A RESEARCH THESIS SUBMITTED TO JIMMA UNIVERSITY, INSTITUTE OF HEALTH, FACULTY OF HEALTH SCIENCES, SCHOOL OF MEDICAL LABORATORY SCIENCES, FOR THE PARTIAL FULFILLMENT OF DEGREE OF MASTERS IN MEDICAL PARASITOLOGY

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JIMMA, ETHIOPIA

JIMMA UNIVERSITY

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ABSTACT

Background: Asymptomatic malaria increases the risk of anemia, stillbirths, spontaneous abortion, premature delivery and low birth weight in pregnant women. The asymptomatic malaria makes it difficult to easily identify the cases for prompt intervention. It is also a major hurdle for malaria elimination, as infected hosts serve as silent reservoirs for transmission of malaria. There are different data on prevalence of malaria and risk factors at institutional level but there is scarce data on asymptomatic malaria among pregnant women at community level in general and particularly in the current study area.

Objectives: The aim of this study was to determine the prevalence and predictors of asymptomatic malaria among pregnant women in Merti Woreda.

Methods: A community based cross-sectional study was conducted in Merti Woreda among pregnant women from March to September, 2018. Study Kebeles were selected using simple random sampling techniques. 364 pregnant women fulfilling the study criteria residing in the study Kebeles were enrolled and Data on socio- demographic characteristics and malaria prevention practices were obtained using a structured questionnaire. About 2ml of peripheral venous blood was obtained from each subject for Rapid diagnostic test (RDT) and microscopy to determine *Plasmodium* species, gametocyte carriage rate, parasite density and anemia. Anemia status was assessed by packed cell volume (PCV) micro-centrifuge method and comparison was made between women with and without parasitaemia.

Results: The prevalence of asymptomatic *Plasmodium* infection among pregnant women was 3.6% (13/364) based on both RDT and microscopy. *Plasmodium falciparum* and *P. vivax* accounted for 46.2% and 53.8% of the cases, respectively. Gametocyte carriage rate was 30.7% among *Plasmodium* infected individuals. The sexual and asexual parasite density ranged from 160 to 600 and 320 to 2200 parasites /ul, respectively. Multivariate analysis showed that previously infection by *Plasmodium* (AOR= 5.42; 95% CI: 1.19-29.03, p = 0.047), Lack of ITN use (AOR=6.52; 95% CI:1.17-36.44, p = 0.032) and living close to stagnant water (AOR= 4.18; 95% CI (1.12-17.36, p = 0.049) were significantly associated with asymptomatic malaria in the study area. The overall prevalence of anemia was 102(28.0%) and it was significantly higher among *Plasmodium* infected than non-infected pregnant women (χ^2 = 27.62, p = <0.001).

Conclusion: Treatment of asymptomatic carriers is very important and persistent malaria prevention and control strategies should be enhanced to achieve the elimination program in malaria endemic areas.

Keywords: Asymptomatic malaria, Pregnancy, Predictors, Insecticide spray, maternal age

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LIST OF ACRONYMS/ ABBREVATIONS

ACT	Artemisinin- based combination therapy
ANC	Antenatal care
APIs	Asymptomatic *Plasmodium* infections
BF	Blood firm
CSA	Circumsporozoite antigen
DRC	Democratic Republic of Congo
EDTA	Ethylene diamine-tetra acetic acid
FMOH	Federal Ministry of Health
HRP-II	Histidine rich protein two
IPTP-SP	Intermittent preventive treatment with sulfadoxine-pyrimethamine
IRS	Indoor residual spray
ITN	Insecticide treated net
LLINs	Long lasting insecticidal nets
IUGR	Intrauterine growth retardation
PCR	Polymerase chain reaction
PLDH	*Plasmodium* lactate dehydrogenase
RDT	Rapid diagnostic test
SD	Standard diagnostic
SOP	Standard operating procedures
VAR2CSA	Variant 2 chondroitin sulfate A
WHO	World Health Organization

1. INTRODUCTION

1.1. Background

Malaria is an infectious disease caused by a protozoan parasite of the genus *Plasmodium* comprising five species: *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale*, *Plasmodium malariae* and *Plasmodium knowlesi* which infect humans (1). *P. falciparum* is the most prevalent and causes most of the fatal infections in Africa (2). Infections occurring outside of Africa are mostly due to *P.vivax*, as it is capable to develop in a wider range of temperatures(2). The disease is transferred between humans by the bites of female mosquitoes belonging to the genus *Anopheles*. The *Anopheles* genus comprises of close to 480 species of which over 70 are known to be able to transmit human malaria. However, only about 30 – 40 species are considered to play any important roles in the transmission of malaria (3, 4). *Anopheles arabiensis*, member of the *Anopheles gambiae* complex, is the primary vector of malaria in Ethiopia.

Malaria during pregnancy causes an enormous risk to the mother, fetus and neonates (5). It increases the risk of stillbirths, spontaneous abortion, premature delivery and low birth weight (6). In the African continent, where the greatest burden of malaria occurs, about 30 million women living in malaria endemic regions become expectant each year. Malaria is a threat to these women and their babies, with up to 200,000 new born deaths occurring each year as a result of malaria in pregnancy (6). Although patients with subclinical infections do not present with malaria symptoms, they still contribute to the cycle of transmission in a population. The relative contribution of sub-clinical infections has considerable implications for the design and use of elimination diagnostics (7).

In malaria endemic areas, a significant proportion of individuals have asymptomatic infection with *Plasmodium* species among whom pregnant women are at higher risk (8). In asymptomatic parasitemia, the person carries *Plasmodium* parasites in their bloodstream, but due to partial immunity the parasites are incapable of inducing symptoms to the affected individual. Nevertheless, the infected person could transfer the parasites to the mosquitoes biting them again to other individuals and the person might then serve as a reservoir of parasites in the population, even though all the symptomatic persons would have been treated (9).

According to Laishram *et al* (8), two classes of immune response develop in the asymptomatic *Plasmodium* infections (APIs): 1) an antidisease immunity that allows one to carry parasite loads without symptoms, and 2) an antiparasite immunity that may be responsible for the suppression of parasite loads after a certain age, which is likely a factor of exposure-related clinical immunity. In addition, exposure-related immunity may be achieved much earlier in life for individuals who live in low transmission regions due to predictably low parasite genetic diversity and few overlapping infections (8).

In areas where the transmission of malaria is seasonal due to the presence of wet and dry seasons and their effect to the incidence of *Anopheles* mosquitoes and makes it possible for malaria parasites to survive through the dry season in the bloodstream of the asymptomatic persons. This allows malaria to resume to the population during the wet season (10). During the following pregnancies, however, anti-VAR2CSA(Variant 2 chondroitin sulfate A) antibodies can be found in pregnant women's circulation, giving them protective immunity against these variant parasites(10).

These antibodies efficiently reduce the adhesion of infected erythrocytes to syncytiotrophoblasts in the intervillous spaces and protect the mother and the fetus from the most severe effects of placental infection. The level of antibodies can be measured and it makes it possible to evaluate the level of immunity towards placental malaria (11, 12). It is worth mentioning that primigravidae are also able to produce these antibodies from the first quarter of the pregnancy after encountering VAR2CSA expressing parasites but not in such quantities that it would provide them sufficient protection against malaria (11, 13). Therefore it is likely that malaria susceptibility in primigravidae is at least partly related to the low levels of anti-VAR2CSA antibodies (14).

Placental malaria may also lead to congenital malaria, meaning infection of the fetus or the new-born either during pregnancy or during the course of the delivery(15). It can be defined as having asexual *Plasmodium* parasites in cord blood or peripheral blood during the first week of life with or without symptoms. Earlier it was thought that congenital malaria occurs rather rarely but according to new estimates, it is likely that almost 30 % of new-borns in moderate to high transmission areas may be born with having *Plasmodium* parasites in their bloodstream (15-18).

The factors associated with asymptomatic *Plasmodium* infections are still poorly understood and therefore remain a challenge to malaria control programs as it significantly influences transmission dynamics.

1.2. Statement of the problem

Malaria is one of the most widespread human parasitic diseases ranking first in terms of its socio-economic and public health importance in tropical and subtropical region of the world. According to 2018 World Health Organization (WHO) report, about 3.2 billion people remain at risk of malaria and there were 219 million malaria cases worldwide, accounting for nearly 4,35,000 deaths, of which 93% were in WHO African region, 5% were in WHO South-East Asian region, and 2% were in WHO Eastern Mediterranean region (19).

The frequency and severity of malaria are greater in pregnant than in the non-pregnant women and malaria in pregnancy causes serious adverse effects including abortion, low birth weight and maternal anemia (20), because, the pregnancy-associated anti-parasite immunity have been identified to play important roles in *P. falciparum* infection (5). Malaria in pregnancy usually manifests as placental malaria and up to one fourth of all pregnant women in sub-Saharan Africa have evidence of placental infection at delivery (21). Placental malaria is often associated with asymptomatic parasitemia (22).

Most malaria disease among pregnant women in areas of high or moderate transmission are asymptomatic and infected women may not seek treatment with a consequent outcome of maternal anemia and severe malaria during pregnancy (23). Some studies have also shown that asymptomatic malaria is associated with anemia (24-26), a global public health problem with serious consequences for human health, as well as social and economic development(27). Although asymptomatic malaria infections can last up to one year (24, 28), they may seriously affect the host by causing an iron deficiency (29). This is due to the fact that they act as silent reservoir of gametocytes for transmission by mosquito vector (30).

Ethiopia is at a high risk of epidemics of malaria due to climate and topography. Broad range of epidemics happen every 5–8 years in some areas due to climatic fluctuations and drought-related nutritional emergency (31, 32), an estimated 75% of the landmass is malarious and 68% of the population live in malaria risk areas (29, 33). *P.falciparium* and *P.vivax* are the two most dominant malaria parasites. They are prevalent in all malarious areas in the country with *P.falciparium* representing about 65 to 75% of the total reported malaria cases and 30%–40% *P.vivax* (29). Malaria burden is higher in populations that are poor and malnourished. Migrant laborers traveling to endemic areas, children <5 years old and pregnant women are high-risk groups that are affected by the high burden of malaria (34).

Asymptomatic malaria can be observed in both stable endemic areas and unstable transmission areas(35, 36). However, much attention has been given to symptomatic *Plasmodium* infections, relatively little attention have been paid to asymptomatic malaria. Although it is difficult to define asymptomatic malaria because of lack of standard diagnostic criteria, the most widely used criteria include the presence of parasites in peripheral thick blood smears, an axillary temperature below 37.5 °C and absence of malaria-related symptoms(37).

There are different data on prevalence of asymptomatic malaria and risk factors at institutional level, but there is scarce data on asymptomatic malaria among pregnant women at community level in general and particularly in the current study area. However, for communities, asymptomatic hosts serve as a reservoir for the malaria parasite. Therefore, asymptomatic malaria is recognized as an important obstacle to malaria elimination.

National Malaria Control and Elimination Programmes are geared towards the protection of pregnant women living in malaria-endemic zones because of their reduced immunity (21). Therefore, the present study was designed to determine the prevalence and predictors of asymptomatic malaria among pregnant women in the community of Merti Woreda, Arsi Zone, Oromia, Ethiopia.

1.3. Significance of the study

Malaria is responsible for an increased risk of adverse pregnancy outcomes including miscarriage, anemia, stillbirth, abortion, prematurity and the delivery of a low birth-weight baby. Currently, the prevalence of malaria is declining even in high transmission areas with different prevention and control strategies. So, for a successful malaria elimination program study of parasite carriers, especially asymptomatic malaria is an issue to interrupt the transmission in a population.

This study therefore sought to determine the prevalence and predictors of asymptomatic malaria among pregnant women living Merti Woreda, Arsi zone, Oromia, Ethiopia. It is expected that the findings of this study is useful in instigating active surveys to identify asymptomatic carriers and treatment of the infectious parasite reservoirs and this will assist in policy decisions in the implementation of malaria eradication program and effective interventions in child and maternal morbidity and mortality in Ethiopia.

2. LITERATURE REVIEW

Malaria is the most deadly tropical infectious disease affecting disproportionately pregnant women, children under five and the poor. Most (93%) of the infections occur in sub-Saharan Africa. The South-east Asia region contributes the second largest number of cases in the world and India alone is estimated to have between 10 and 26 million cases (38). In Ethiopia, an estimated 55.7 million people (68% of the population) are at risk of malaria, and three fourth of the land mass is considered malarious (39).

Every year, millions of women become pregnant in malaria endemic regions of Africa and a significant proportion of individuals are asymptomatic with *Plasmodium* species (40, 41). Massive sequestration of *P.falciparum* parasites in the placenta, with or without detectable parasites in the peripheral circulation, is a distinct feature of pregnancy-associated malaria (42, 43) and is believed to be responsible for an increased risk of adverse pregnancy outcomes including miscarriage, anemia, stillbirth, abortion, prematurity and the delivery of a low birth-weight baby (44).

It is an important threat to pregnant women, with increased risk for both the mother and the newborn, especially in the first and second pregnancies and when caused by *P. falciparum* (45). The recent report from a low endemicity (non-Amazon) Southeastern area showed cases of malaria in pregnancy 1.6% and 5.6% by microscopy and PCR respectively, with most cases from a rural area near the forest (46).

A cross-sectional survey conducted in India showed that the prevalence of *Plasmodium* infections among pregnant women was 4.3 % and 5.4% by microscopy and RDT respectively. The study indicated that; *P. falciparum, P.vivax* and mixed infections was reported, which accounts; 5.4%, 86.8% and 8.1% respectively. Peripheral parasitemia was more likely higher among women living in rural areas when compared with those women from urban or semi urban areas (47).

A longitudinal active surveillance study done in Bangladesh on prevalence of asymptomatic *Plasmodium* infections among pregnant women was 2.3%, compared to 0.5% in non-pregnant women. Malaria-positive asymptomatic pregnant women, had statistically lower hemoglobin

than those women without malaria (48). Similar study done in Colombia showed that the prevalence of asymptomatic malaria among pregnant women was 10.8% (49).

Another similar study in northwest Colombia from 2008 to 2011 found that the prevalence of gestational malaria among pregnant women was 9.1% and 14.0% by microscopy and PCR respectively. 65% of cases was *P. vivax* and 16.5% for placental malaria mostly caused by *P. falciparum* (50, 51). This study showed that almost 20% of *P .falciparum* infections being afebrile (asymptomatic), at risk of being undetected (51).

Malaria is endemic only in the tropical and sub-tropical areas of the Earth, and most of the infections appear in sub-Saharan Africa, as an estimated 88 % of all the reported cases of malaria and 90 % of all deaths caused by malaria are recorded there, all the deaths caused by malaria, ranging from 219 000 to 421 000 are children under five of age living mostly in Africa (2, 52). These numbers are equal to one child dying every two minutes. The prevalence of asymptomatic malaria in endemic regions present a serious challenge in the sustained efforts to curtail, control and eliminate malaria. This is due to the fact that, they act as silent reservoir of gametocytes for transmission by mosquito vector(30).

Pregnant women are at high risk of *P. falciparum* infection with serious and multiple complications, including placental malaria, fever, maternal anemia, termination of pregnancy, fetal exposure to the parasite Congenital infection, low birth weight, intrauterine growth retardation (IUGR) and infant mortality with anemia(53, 54). The major risk factors associated with malaria in pregnancy include gestational age in pregnancy, lack of indoor residual spray (IRS), inadequate use of insecticide-treated bed nets, socio-economic factors (55). The severity of malaria during pregnancy has been reported to occur more in younger pregnant women from studies undertaken in Nigeria and Cameroon (56, 57).

The study conducted in Democratic Republic of the Congo (DRC) revealed that the prevalence of asymptomatic *P.falciparum* infection in pregnant women was, 27.4% and 29.5% by microscopy and RDTs, respectively (58). Another study done on children, showed that a prevalence of asymptomatic *Plasmodium* infection was 30.9% and 14.3% in two areas of Kinshasa, based on microscopic identification of thick blood smears (59).

A cross-sectional Study conducted at Ghana, the prevalence of asymptomatic maternal peripheral malaria by microscopy was 5.5% with *P. falciparum* and *P. malariae* which accounts; 95.5% and 4.5% respectively. Age, gravidity, education level, gestation, IPT-sp use and ITN use were not significantly associated with *Plasmodium* infections (60). Another study done in Burkina Faso showed that the prevalence of placental *P. falciparum* among maternal and newborn outcome was 17.2% and 9.1% by peripheral blood films and placental blood films. *P.falciparum* was the sole species found in all cases in their work that RDT shave some limits in detecting asymptomatic carriers of *P.falciparum* (61).

A descriptive cross-sectional study done in a tertiary Hospital, North central Nigeria found that the prevalence of asymptomatic malaria among pregnant women was 38.8%, the prevalence of malaria parasitaemia is usually higher in the rural area than urban and 38.85 were anemic (56) and similar study done in Nasarawa-Eggon in the same country showed that a prevalence of asymptomatic *Plasmodium* parasitaemia among pregnant women attending antenatal clinic was 22.7% by smear microscopy. Among these, 65.5% were anemic. The low level of education and lack of use of the insecticide-treated net (ITN) was significantly associated with malaria during pregnancy (62).

In Ethiopia, the Federal Ministry of Health (FMOH) estimates annual cases of clinical malaria 5–10 million accounting for 12% of outpatient consultations and 10% of hospital admissions (63). In most of the areas malaria transmission is unstable leading to epidemics and *P.falciparum* and *P.vivax* are the species accounting for roughly 60 and 40% of malaria cases, respectively (64) though recent reports indicate the shift of dominance from *falciparum* to *vivax* in highland areas (65, 66).

A community-based cross-sectional study design conducted at rural surrounding Arba Minch town, Southern Ethiopia showed that the prevalence of asymptomatic malaria among pregnant women was 9.1% and 9.7% by microscopy and RDTs, respectively and the species identified from thin blood smear include: *P.falciparum*, *P.vivax* and mixed infections; which accounts; 12(38.71%), 15(48.38%) and 4(12.9%) respectively. Gravidity, lack of ITN usage, and age groups showed significant association with *Plasmodium* infection (67).

A cross-sectional study design conducted at Mirab Abaya District Southern Ethiopia shows that, the prevalence of asymptomatic *Plasmodium* carriage in school children was 1.2 and 3.6% with light microscopy and RDT, respectively (68). Similar study done on Prevalence of febrile symptomatic and asymptomatic malaria in School children was 6.8%. Level of education, age, bed net usage, and frequent exposure to malaria infection were associated with risk of asymptomatic malaria were reported among study groups from Sanja in, 2014 (69) and >2 years 20.5% East Shewa (70).

A community-based cross-sectional study was conducted at 2016 on 385 migrant laborers in the West Armachiho district, showed that the prevalence of asymptomatic malaria was 18.4% detected by microscopic, thin and thick blood Giemsa stain (71). Another similar study done on population in West Arsi Zone, Oromia Region, showed that, the prevalence of asymptomatic *Plasmodium* carriage (*P. falciparum, P. vivax* and mixed species) in whole population was 5.0 % and 8.2 % as determined by microscopy and RDT respectively(72).

World Health Organization and roll back malaria partners promote the use of LLINs as a sustainable and cost effective form of protection against malaria in endemic regions in the world (73). Asymptomatic infections can be associated with high levels of gametocytes, likely serve as an important parasite reservoir and it has a significant contribution by maintaining parasite for the transmission (74). Various studies have assessed the prevalence of asymptomatic infections in the control and elimination phase of malaria, because detection and treatment of all sources of infection is very critical at this stage (72, 73).

As per the National Strategic Plan, the four major intervention strategies that are being applied in the country to combat malaria include early diagnosis and prompt treatment, selective vector control that involves the use of IRS and ITNs and environmental management (75). Now a days, early diagnosis and prompt treatment is one of the key strategies in controlling malaria. Blood smear microscopy and malaria RDT represent the two diagnostics most widely used (68). Ethiopia includes regions of differing malaria endemicity and malaria transmission and multispecies RDT are used at health posts and malaria microscopy is carried out at district-level health centers and regional-level hospitals for all suspected malaria cases (72).

3. OBJECTIVES OF THE STUDY

3.1. General Objective

To assess the prevalence and predictors of asymptomatic malaria among pregnant women in Merti Woreda, Arsi zone, Oromia, Ethiopia

3.2. Specific Objective

- To determine the prevalence and Plasmodium species of asymptomatic malaria among pregnant women in the study area
- To determine gametocyte carriage rate among pregnant women with asymptomatic malaria in the study area
- To determine the associated risk factors and preventive practices among pregnant women with asymptomatic malaria in the study area
- To assess anemia among pregnant women with asymptomatic malaria in the study area

4. MATERIALS AND METHODS

4.1. Study Area

The study was conducted in Merti Woreda. Merti woreda is located at 203km southeast of Addis Ababa. It is bordered on the south by Sude Woreda, on the west by Jeju Woreda, on the northwest by East Shewa Zone, on the north by Afar Region, on the east by Aseko and on the southeast by Chole Woreda. This woreda has 1 distinct Hospital, 5 Health centers and 17 health posts. It has 22 kebeles with a total population of 127,792 (62,618 males and 65,174 females) according to 2011 population census conducted by the Central Statistical Agency of Ethiopia (76). It is tropical and subtropical in weather condition. Out of the 22 kebeles, 17 kebeles are known to be malarious with intense transmission pattern. Its altitude ranges from 1,000 to 3280 meter ASL, with annual average temperature of 26°C and rainfall of 600mm3.

4.2. Study period

The study was conducted in Merti Woreda, Arsi Zone, Oromia, Ethiopia from March to September, 2018.

4.3. Study Design

A community based cross-sectional study was conducted to determine the prevalence and predictors of asymptomatic malaria among pregnant women in Merti Woreda.

4.4. Population

4.4.1. Source population

The source population was all pregnant women living in Merti Woreda

4.4.2. Study subject

All pregnant women living in selected Kebeles of Merti Woreda during study period and fulfill the inclusion criteria were the study population.

4.4.3. Inclusion criteria

All consenting asymptomatic pregnant women with absence of disease symptoms/signs of malaria within the past 48 hours, axillary temperature ≤ 37°c, permanent residents in the study area and those willing to participate in the study and signed the informed consent were included in this study.

4.4.4. Exclusion criteria

Exclusion criteria included women who had taken antimalarial drugs in the last 2 weeks of the study period and presentation with clinical symptoms of malaria (fever, chills, rigor, nausea, vomiting, headache, joint/muscle pains and anorexia).

4.4.5. Sample size determination

Sample size was determined using single population proportion formula using the following assumptions; 9.4% prevalence of asymptomatic malaria among pregnant women from the study done in southern Ethiopia, Arba Minch (67), 95% confidence interval and 3% margin of error.

Therefore; the sample size $n= z (\alpha/_2)^2 p (1-p)/d^2$

Where

n = Sample size

z = at 95% confidence interval Z value ($\alpha = 0.05$) =>$Z \alpha/2 = 1.9664$

α = level of significance

p = prevalence of previous study found from literature review=9.4%, 0.094

d = Margin of error at (3%), (0.03)

$n = ((1.9664)^2 x\ 0.094(1-0.094))/ (0.03)^2$

n = 364

10% non-response rate=36, so the total sample size (n) was

n=364 + 36 = **400**

4.4.6. Sampling technique

Merti woreda has 22 kebeles, of which 11 kebeles were randomly selected for this study by using simple random sampling method. These include: Worsha kona, Woticha dole, Abomsa 01, Ashe, Wataro dino, Shemo, Hella kiya, Abomsa 02, Gologota, Dembeka Iftu and Dembeka Gadjele. Since the total number of asymptomatic pregnant women found in selected kebeles during study period was less than the calculated sample size before, all asymptomatic pregnant women from the selected kebeles were included in the study.

4.5. Measurement and study variables

4.5.1. Dependent Variable

- Asymptomatic malaria in pregnancy

4.5.2. Independent Variable

- Age
- Educational level
- Marital status
- Occupation
- Residency
- Stage of pregnancy
- Gravidity/parity of pregnancy
- Antenatal services
- Knowledge of malaria prevention
- Insecticide-treated nets
- Indoor residual spray
- Live close to stagnant water
- Previous infection by *Plasmodium*
- Gametocyte carriage

4.5.3. Conceptual Framework

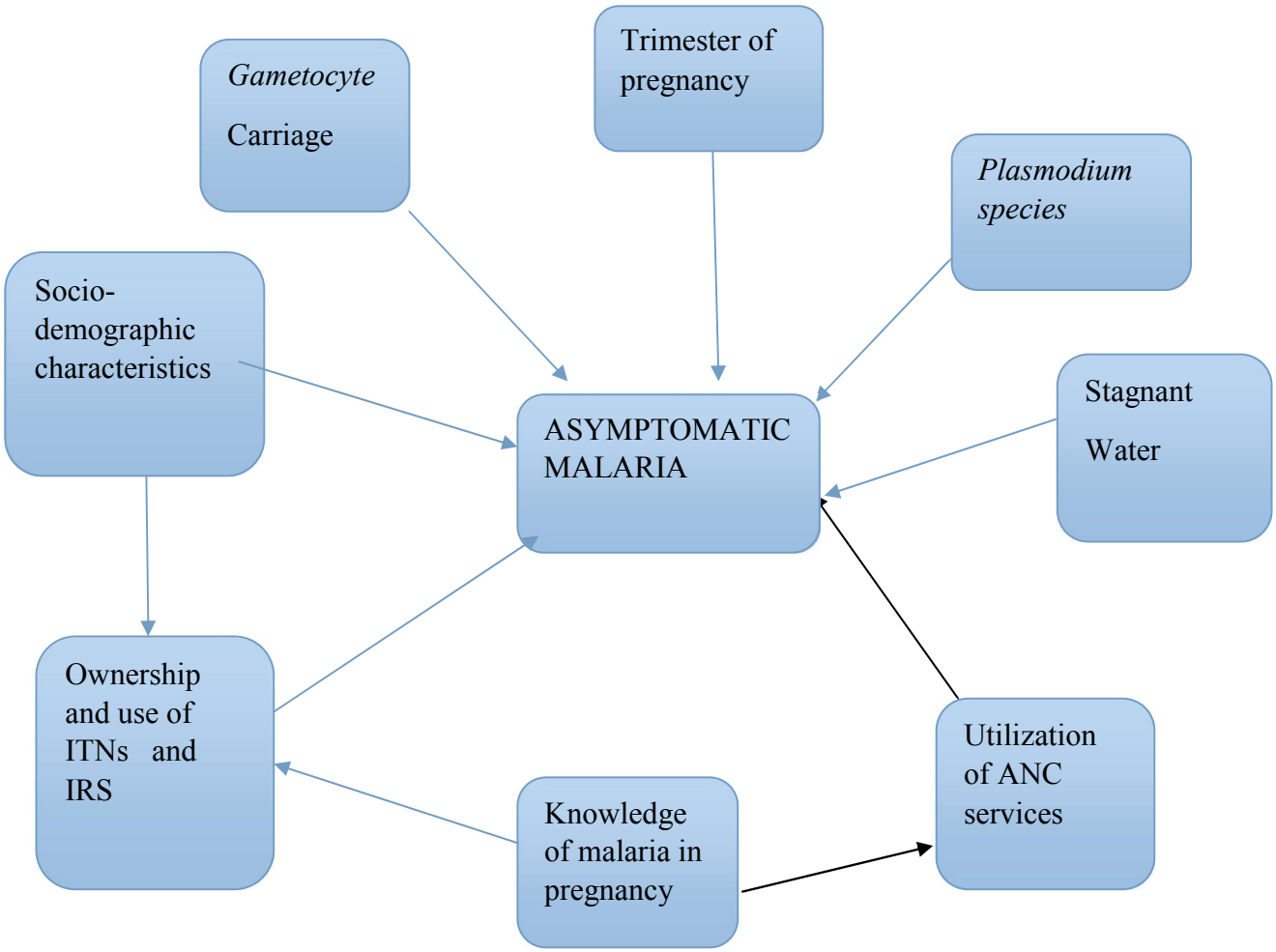

Figure 1: Conceptual framework of prevalence and factors associated with asymptomatic malaria in pregnant women

4.6. Data collection techniques and tools/instruments

4.6.1. Data collection

Trained data collectors visited community of Merti woreda and recruited study subjects fulfilling the eligibility criteria and provided a brief overview of the study. Individuals who could be recruited as per the healthcare worker's assessment were then referred for an informed consent session. The interviewer administered the informed consent, subjects who consented were re-assessed by the study and data on age, number of children, education, knowledge of malaria prevention, ownership of ITNs, sleeping under the ITN and use of IRS were obtained through closed ended personal interviews with structured questionnaire.

4.6.2. Blood sample collection and processing

About 2ml of blood sample was obtained by a phlebotomist from each study participant from a peripheral vein into an ethamine diaminetetraaceticacid (EDTA) tube for preparation of thick and thin blood film. RDTs and microscopy were employed for the diagnosis of asymptomatic malaria parasites. Thick and thin blood smears were made on the same slide, fixed by methanol, air dried and transported to Abomsa and Gologota Health Centers. The slides were then stained with 10 % Giemsa for 15 minutes and screened for the presence of *Plasmodium* infections. Two experienced Parasitologist read the slides blinded to individual's RDT results and to each other.

Asexual parasite density per microliter (µl) of blood was determined by counting the number of parasites per 200 white blood cells on a thick blood film assuming a total standard white blood cell (WBC) count of 8000/µl (77). The degree of parasite density were graded as mild, moderate, and severe when the counts were between 1–999 parasites/µl, 1000–9999/µl, and >10,000/µl, respectively, following the method described elsewhere (60).

$$\text{Parasitemia / ul} = \frac{\text{No. asexual stage x 8000 Leukocytes}}{\text{200 Leukocytes}}$$

Gametocyte density was quantified against 500 leukocytes. This was converted to the number of gametocytes per microliter of blood, assuming a standard approximation of leukocyte count of 8,000/ µl. A smear was considered negative if no parasites were seen after review of 100 high-powered fields.

4.6.3. Rapid Diagnostic Tests

The SD BIOLINE Malaria Ag P.f/P.v POCT test kit (Standard diagnostic, Inc, Germany, Lot No. 145021) was used to capture malaria antigens and was performed in accordance with the manufacturer's instructions. The kit targeted malaria antigens HRP-2 specific for *P. falciparum* and *Plasmodium* lactate dehydrogenase (pLDH) specific for *P. vivax.*

4.6.4. Packed cell volume (PCV)

Packed cell volume estimation using two heparinized capillary tubes, 4-5cm column of blood was obtained from blood already collected. This was to ensure that the average of the two values obtained is used for calculation. One end of the capillary tube was sealed with plasticin, several samples were assembled in the Centrifuge (hematocrit machine) and spinned at 5000 revolution per minute for 5 minutes. The PCV was read using Hawksleys micro hematocrit reader. A study participant was considered anemic if the value of PCV was below 33%, according to WHO recommendation (26, 78).

4.6.5. Data quality assurance

The professionals involved in sample collections, RDT and light microscopy examination were trained. Completed questionnaires were checked for consistency and completeness by the principal investigator before the data were entered. The specimens were also checked for serial number, quality and procedures of collection. Blood smear microscopy readers were blinded to the result of RDTs. Parasite density with discrepancies ≥20% were resolved by averaging the third microscopist's results and the closer of the two first results. In addition, to minimize missed parasite identification and discrepancy each microscopic slide was examined by the two trained professionals in Arsi University College of Health Sciences, Medical laboratory center. Species discrepancies while reading the microscope were resolved by a third external Parasitologist

4.6.6. Quality control

All laboratory materials such as rapid test kits, slides, thermometers, EDTA tube and sample transporting system were checked for expiration date, correct collection procedures and samples as well as in built control appearances by experienced laboratory professionals. The manufacturer's instruction was strictly followed for the RDTs. Double entry of all data was done to reduce the chances of data entry errors. Eligibility criteria was assessed and confirmed by the investigator administering the informed consent.

4.6.7. Training of interviewers consent to the study.

Research assistants recruited were trained to administer the questionnaires in a friendly and professional manner. They were particularly coached on ways of conducting the interviews efficiently for quality data collection.

4.6.8. Pre-testing and review of instruments

The questionnaire pre-testing was done at community of Merti Woreda. The questioners were administered by trained interviewer to obtain data on socio-demographic characteristics and factors associated with asymptomatic malaria.

4.6.9. Data quality management

Cross-checking and data cleaning were done on daily basis. Missing information obtained was checked by going back to the questionnaire and corrective measures were taken accordingly. All laboratory and clinical data were recorded on appropriate record during the study period and the data were stored on a CD, external memory flash and hard copy as back up.

4.6.10. Data processing and analysis

Data were coded, entered into Epidata version 3.1, cleaned and analyzed using SPSS version 20.0. Both descriptive and inferential statistics were employed for the analysis of data. Frequency was used to determine the prevalence of asymptomatic *Plasmodium* infections among pregnant women. Bivariate and multivariate logistic regression was employed to assess factors associated with asymptomatic malaria. Chi-square test was used to determine the association between malaria and anemia among pregnant women. Prevalence figures were calculated for the total study population and the association between variables was calculated. P value less than 0.05 was considered statistically significant.

4.6.11. Ethical considerations

The study was conducted after it was ethically reviewed and approved. Ethical approval was obtained from Research and Ethical Review Committee (RERC) of the school, followed by approval by Institutional Review Board (IRB), Institute of Health, Jimma University. Permission was also obtained from Arsi Zone Health Bureau and Merti Woreda Health Bureau. Written informed consent was obtained from each individual after the purpose of the study was explained using the common language they speak and hear. For individuals under the age of 18, consent was obtained from the Husbands.

Pregnant women who was found to be positive with *Plasmodium* infection were referred for treatment and medical consultation in the ANCs of nearby health facilities and followed up to ensure appropriate treatment.

Voluntary consent

All study procedures were clearly explained to participants while obtaining informed consent. Health center staff and study participants were assured of the confidentiality, data safety and Appropriate data usage.

4.6.12. Dissemination of results

The results will be presented to the school of Medical laboratory sciences, Institute of Health, Jimma University and other concerned bodies. Manuscript will be prepared and submitted to peer reviewed journals for publication.

5. RESULTS

5.1. Socio-demographic and obstetric characteristics of the study participants

A total of 364 pregnant women were participated in the study (Table 1). The age of study participants ranged from 15 to 40 years with a mean age of 28 years. About one-third (33.2%) of the study participants had no formal education (illiterate), whereas 48.6% and 18.1% of the participants had attended primary and secondary education, respectively. The vast majority (97.3%) of study participants were married. The majority (55.8%) of the study participants were full-time housewives. Most of the study participants were in their second trimester of pregnancy 160(44.0%). Multigravidae formed the lowest percentage of study participants 102(28.1%) while Secondigravidae constituted the highest of the study participants 145(39.8%). The majority (73.0%) of study participants attended ANC services. Most of study participants (71.7%) were from rural areas, while 28.3% were from urban areas.

Table 1: Socio-demographic and obstetric characteristics of the study participants

Variables	**Frequency (n, %)**	**Variables**	**Frequency (n, %)**
Age (years)		Gestation	
15-24	132(36.3)	First trimester	63(17.3)
25-34	165 (45.3)	Second trimester	160 (44.0)
≥35	67(18.4)	Third trimester	141 (38.7)
Education level		Gravidity	
Illiterate	121(33.2)	Primigravidae	117(32.1)
Primary	177(48.6)	Secondigravidae	145(39.8)
Above primary	66(18.1)	Multigravidae	102(28.1)
Marital status		ANC Attendance	
Married	354(97.3)	Yes	266(73.0)
Single	6(1.6)	No	98(27.0)
Divorced	4(1.1)		
Occupation		Residency	
Farmer	152(41.8)	Urban	103(28.3)
Government employee	9(2.5)	Rural	261(71.7)
Housewife	203(55.8)		

5.2. Prevalence of asymptomatic malaria.

The prevalence of asymptomatic malaria among pregnant women in this study was 3.6% (Table 2). *P. falciparum* and *P.vivax* accounted for 46.2% and 53.8% of the total positive cases, respectively. Similar results were obtained by both RDT and microscope. Of the microscopically confirmed cases, 30.7% (4/13) had gametocyte stage. *P. falciparum* gametocyte was detected in 50% (3/6) of the *P. falciparum*-positive cases, whereas *P. vivax* gametocyte was detected in 14.3% (1/7) of the *P. vivax*-positive cases.

Geometric mean density of asexual stage of the parasites was 994.7(interquartile [IQR], 320 to 2200) parasites/ul, whereas the geometric mean gametocyte density was 303.3 (interquartile range [IQR], 160 to 600). The majority of the study participants had mild parasitaemia 11(84.6%), while 2(15.4%) had moderate parasitaemia.

Table 2: Prevalence, *Plasmodium* species, gametocyte carriage rate and parasitemia of study participants

Variable	**Frequency (%)**	**Variable**	**Frequency (%)**
Prevalence		Gametocyte stages	
Total Positive	13(3.6)	Yes	4(30.7)
		No	9(69.3)
Parasite species		Parasitemia	
P.falciparum	6(46.2)	Mild	11(84.6)
P.vivax	7(53.8)	Moderate	2(15.4)

5.3. Risk factors for asymptomatic malaria

Table 3 shows bivariate and multivariate logistic regression analysis of predictors for asymptomatic *Plasmodium* infections among pregnant women. Lack of ITN use, previous infection by *Plasmodium* and living close to stagnant water were the main predictors of asymptomatic malaria in the study area. Pregnant women who did not use ITNs were 6.5 times more likely to have asymptomatic *Plasmodium* infection as compared those who used ITNs (AOR = 6.52, 95% CI 1.17-36.44, p = 0.032). Individuals who had previous history of *Plasmodium* infection were five times more like to have asymptomatic *Plasmodium* infection as compared to those with no previous history of malaria (AOR = 5.42, 95% CI 1.19-29.03, p = 0.047). The risk of asymptomatic *Plasmodium* infections among pregnant women who lived close to stagnant water (less than 1 km from vector breeding sites) was four times higher as compared to those who lived away from the vector-breeding site (AOR = 4.18, 95% CI 1.12-17.36, p = 0.049).Other covariates assessed did not show a significant effect on asymptomatic *Plasmodium* infection ($P > 0.05$).

Table 3: Bivariate and multivariate logistic regression analysis of predictors associated with asymptomatic malaria among pregnant women in the Community of Merti Woreda, between March-September 2018

Variables		Frequency (N=364)	Positive *Plasmodium* (%) (N=13)	COR (95% CI)	P-value	AOR (95% CI)	P-value
Age in year	15-24	132	5(38.5)	2.59(0.29-22.77)	0.388	4.05(0.38-42.58)	0.243
	25-34	165	7(53.8)	2.92(0.35-24.32)	0.224	5.44(0.57-51.81)	0.140
	>35	67	1(7.7)	1		1	
Residence	Rural	261	8(61.6)	1.50(0.48-4.68)	0.183	1.52(0.38-5.88)	0.562
	Urban	103	5(38.4)	1		1	
Gestational Age of pregnancy	1st trimester	63	3(23.0)	3.47(0.56-21.33)	0.179	2.95(0.41-21.33)	0.284
	2nd trimester	160	8(61.5))	3.65 (0.76-17.5)	0.105	3.69(0.67-20.22)	0.132
	3rd trimester	141	2(15.5)	1		1	
Previously Infected by *Plasmodium*	Yes	188	11(84.6)	5.43(1.18-24.8)	0.029	5.42(1.19-29.03)	**0.047***
	No	176	2(15.4)	1		1	
Close to stagnant water	Yes	129	10(76.9)	6.41(1.73-23.7)	0.005	4.18(1.12-17.36)	**0.049***
	No	235	3(23.1)	1		1	
ITN	Yes	171	2(15.4)	1		1	
	No	193	11(84.6)	5.1(1.16-23.37)	0.036	6.52(1.17-36.44)	**0.032***
IRS	Yes	176	5(38.5)	1		1	
	No	188	8(61.5)	1.52(0.48-4.73)	0.170	1. 75(0.44-6.57)	0.429
Gravidity of pregnancy	Primigravidae	117	4(30.7)	1.16(0.25-5.43)	0.482	1.61(0.25-10.26)	0.312
	Secondigravidae	145	6(46.1)	1.42(0.34-5.83)	0.250	3.15(0.53-18.44)	0.203
	Multigravidae	102	3(23.1)	1		1	
ANC attendance	Yes	266	5(38.5)	1		1	
	No	98	8(61.5)	4.61(1.14-15.57)	0.231	4.06(0.98-16.74)	0.252

Key: COR= Crude odds ratio; CI= Confidence interval; AOR= Adjusted odds ratio; N= Number; * Significant at P value < 0.05

5.4. The association between malaria and anemia

The overall prevalence of anemia among pregnant women in this study was 102(28.0%). There was statistically significant association between asymptomatic malaria and anemia prevalence among the pregnant women (χ^2 = 27.62, p = <0.001). The prevalence of anemia among *Plasmodium* infected and non-infected pregnant women was 92.3% and 7.7%, respectively (Table 4). The majority of the women with mild parasitaemia 10(90.9%) were anemic while 1(9.1%) was normal. From moderate parasitaemia all positive women with *Plasmodium* were anemic 2(100%).

Table 4: Association between malaria and anemia among study participants

Malaria status	**Anemia**		**χ^2**	**p value**
	Anemic	Normal		
Positive	12 (92.3)	1 (7.7)	27.62	0
Negative	90 (25.6)	261 (74.4)		
Total	102 (28.0)	262 (72.0)		

6. DISCUSSION

Malaria during pregnancy is still a major public health problem in sub-Saharan Africa. Indeed in this region, approximately 25 million pregnant women are at risk of *P. falciparum* infection every year, and about 25% of women carry placental *P. falciparum* infection at the time of delivery (21). The study of asymptomatic malaria cases has been given little attention in the prevention and control program. Understanding of the burden of asymptomatic malaria has great implication in the interruption of malaria transmission. As evidence has indicated that asymptomatic parasitemia can impair cognitive and cause anemia in the host, especially in pregnant women and children (79). The aim of this study was to determine the prevalence and predictors of asymptomatic malaria among pregnant women in the community of Merti woreda, Arsi Zone, Oromia, Ethiopia.

In this study, the overall prevalence of asymptomatic *Plasmodium* infections among pregnant women was 3.6% based on both microscopy and RDT. This finding is lower compared to the results of similar studies conducted in South Ethiopia, Republic of Congo, Nigeria, and Columbia, which reported asymptomatic malaria prevalence of 9.1%, 7.0%, 22-38.8% and 10.8% among pregnant women, respectively (49, 56, 62, 67, 80). The lower prevalence of asymptomatic *Plasmodium* infections among pregnant women in this study could be due to increased malaria control interventions in Ethiopia (81) or difference in malaria epidemiology between the study areas. The present study was conducted during minor malaria transmission season which could also contribute to the lower asymptomatic malaria prevalence among pregnant women reported in this study. On the other hand, the prevalence of asymptomatic *Plasmodium* infections documented in this study is similar with the findings of a study done in Bangladesh, which reported a prevalence of 2.3% among pregnant women (48).

In the present study, the prevalence of *P. vivax* was slightly higher than *P. falciparum*, in contrast to the national malaria parasite species composition which shows higher proportion of *P. falciparum* than *P. vivax* (64). Recent studies conducted in different parts of Ethiopia have also showed similar shift in malaria parasite species composition from *P. falciparum* predominance to *P. vivax* predominance (39, 66, 67, 72). This could be explained by the fact that the prevention and control activities of malaria in Ethiopia mainly focus on *P. falciparum* (82), other possible

reasons may be climate variability or that *P.vivax* might have developed resistance for Chloroquine (83).

Malaria transmission depends largely on the presence of viable gametocytes in peripheral blood, which are picked up by *Anopheles* mosquitoes during a blood meal (84, 85). Gametocyte carriers are reservoirs of infection that play a key role in sustaining malaria transmission (86); thus, the gametocytes could serve as a source of infection to larger populations. In our study the gametocyte carriage was 30.7% among *Plasmodium* infected pregnant women. The transmission of *Plasmodium* species from humans to mosquitoes requires the presence of infectious gametocytes in the human peripheral blood. Thus, the detection of asymptomatic cases with circulating gametocytes signals their importance in maintaining sustained transmission in the study area. Indeed, the presence of asymptomatic cases is a big challenge for the management of elimination programs in any malaria endemic area.

In present study, ITN use, previous history of *Plasmodium* infection and living close to vector breeding sites were the main predictors of asymptomatic malaria in the study area. Pregnant women who did not use ITN were 6.5 times more likely to have asymptomatic *Plasmodium* infection as compared those who used ITN. This result is in agreement with similar studies done in Southern Ethiopia (67) and Nigeria (62). Educating pregnant women on the role of ITNs in preventing malaria will impact positively in reducing the prevalence of malaria. In the present study, we observed some individuals who used ITNs for different purpose, for instance for temporary storage of maize rather than using at night for mosquito control.

Pregnant women who lived close to vector-breeding site (< 1km from stagnant water) were four times more likely to have asymptomatic *Plasmodium* infections as compared to those who lived away from the vector-breeding site. This is in agreement with studies done on prevalence of asymptomatic *P. falciparum* and *P. vivax* infections in West Arsi Zone (72) and Southwestern part of Ethiopia (87). There was also statistically significant association between asymptomatic parasitaemia and previous infection by *Plasmodium*. Individuals who had previous history of *Plasmodium* infections were five times more like to have asymptomatic P*lasmodium* infections as compared to those with no previous history of malaria. These results underscore the need to further evaluate ways to optimize management of asymptomatic P*lasmodium* infection in pregnant women living in malaria endemic areas.

It is important to note that asymptomatic malaria parasitaemia is one of the major causes of anemia in malaria endemic settings. Our study also suggested that *Plasmodium* infections in pregnant women contribute to maternal anemia. Other studies in Ethiopia (88) and elsewhere (58, 89, 90) have also reported significant association between asymptomatic malaria and anemia among pregnant women. The overall prevalence of anemia in our study was 28.0%; the result of this study was less than the findings of similar studies reported from; North central Nigeria 38.85% (56), but greater than study reported in India, accounted 23% (91). However, it might be difficult to attribute the prevalence of anemia solely to the effect of malaria parasitaemia as other causes such as helminthiasis (92), malnutrition, HIV infection (93) and sickle cell anemia, which might also cause anemia, were not assessed in our study.

7. LIMITATION

Asymptomatic infection is common in areas of endemic transmission; therefore, one would expect a higher prevalence in Merti woreda. It can be argued that the prevalence of asymptomatic *Plasmodium* infection may have somewhat lower because our study was carried out by RDT and microscopy, instead of the more sensitive PCR to determine the prevalence of parasitaemia which could have underestimated the real prevalence of parasitaemia.

8. CONCLUSION AND RECOMMENDATIONS

8.1. Conclusions

Malaria in pregnancy is a common and serious public health problem in our environment as some proportion of the asymptomatic pregnant women had malaria parasitaemia. Findings of this study indicate that asymptomatic malaria is an important health problem among pregnant women in Merti Woreda, with the predominance of *P. vivax* and *P. falciparum.* Anemia is also a serious problem among pregnant women with asymptomatic parasitaemia in study area. Previous history of malaria, living close to stagnant water (vector breeding sites) and lack of ITN use were the main predictors of asymptomatic *Plasmodium* infections among pregnant women in the study area.

Therefore, treatment of asymptomatic carriers is very important and persistent malaria prevention and control strategies, should be enhanced to achieve the elimination program, in malaria endemic areas.

8.2. Recommendations

Based on the findings, there is need to:

- Promote the use of ITNs and indoor residual spraying in the study area.
- Further studies for a better understanding of the asymptomatic *Plasmodium* infections and their contribution to the dynamics of malaria transmission and to the incidence of symptomatic infections.
- As part of ANC service package; educating on the appropriate usage and benefits of the bed nets, and encouraging early ANC attendance among pregnant women could enhance benefits for the women's health.
- Diagnosis of malaria at various stages of pregnancy as part of antenatal care package.

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APPENDICES

Appendix I: Consent form

Introduction

I am asking you to take part in a research study on Prevalence and factors associated with asymptomatic malaria among pregnant women at Merti Woreda, Arsi Zone, Oromia, Ethiopia

I want to be sure that you understand the purpose and your responsibilities in the research before you decide if you want to 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 collection of about 2ml of blood from a peripheral vein for the detection of plasmodium parasitaemia.

Possible Risks (explain risks –blood collection)

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

Possible Benefits

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

If You Decide Not to Be in the Research

You are free to decide if you want to be part of this research or not.

Confidentiality

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

Payment

No payments will be made for participation.

Leaving the Research

You may end your participation at any time with no negative consequence to you.

If You Have Questions about the Study

If you have any questions about the research, call 0913186061

Your rights as a Participant

Participants were notified about the purpose of the study, their right to refuse to participate in the study, and anonymity and confidentiality of the information gathered. Study participants were given detailed information concerning the study and for those who were literate the information sheet that had full information about the study was given and they were asked about the study to check whether they have understood it correctly or not and their questions were cleared.

This research has been reviewed and approved by the Jimma University ethical review board and permission from the Arsi Zone Health Biro. If you have any questions about how you are being treated by the study or your rights as a participant you may contact me by; (berwaklab4@gmail.com) Telephone number: 0913186061; Bereket Wake

Participant Code Number_____

Participant full name _____

I am informed fully in the language I understand about the aim of above mentioned research. I understood the purpose of the study entitled with "prevalence and predictors of asymptomatic malaria among pregnant at community of Merti Woreda, Arsi zone, Oromia, Ethiopia. I have been informed this study which involves collecting 2ml of blood sample from vein. During collection of the specimen I have been told that there is no harm except little discomfort and I have also read the information sheet or it has been read to me. In addition I have been told all the information collected throughout the research process will be kept confidential. I understood my current and future medical services will not be affected if I refused to participate or with draw from the study. I _____, after being fully informed about the detail of this study, hereby give my consent to participate in this study and approve my agreement with signature.

Patient Name _____ signature _____ Date_____

Investigator name _____signature _____Date_____

A. Garagalcha Afaan Oromiffaa

Baayina dhukkuba busaa fi sababoota dhukkubni irraa nama qabuu, dubartoota ulfaa mallattoo dhukkuba kanaa hinqabne irratti qorannoo waan adeemsisuu barbaadeef qaama qorannoon irratti godhamuu akka taatuuf sii gaafadhaa. Duraan dursee faayidaa qorannoo kanaa fi dhirqama kee akka beektuu barbaada. Gaaffii isiniif hin gallee na gaafachuu hin dandeessa.

Yaada Qorannichaa

Qorannoon kuni dhiiga milliliter lama hidda dhiigaa harkaa irraa fudhachuun, Dhiiga keessaa parasaayitii dhukuba busaa fidan qorachuuf**.**

Miidhaa Qorannichaa (yeroo dhiiga kennitan)

Dhukkubbiin xiqqaan qaama keessanitti dhagahamuu mala, soda isinitti dhagahamuu mala.

Faayidaa Qorannichaa

Qorannoon kuni qajjeelummaan faayidaa isinii kennuu dhabuu danda'a, garuu bu'aan qorannicha irraa argamuu dhubee fi du'aa Haadholii dhukkuba busaatiin dhufuu balleessuu danda'a**.**

Yoo qaama qoranichaa tahuu yookan dhiisuu barbaadde

Qaama qoranichaa tahuus yookan dhiisuuu ni dandeessa.

Iccitiin Eeguu

Ichiitii keessan eeguun dhirqama keennaa. Qaama qorattoootin alatti maqaa keessan barreeffama kamiinuu irratti in fayyadamnuu.

Kafaltii

Kafaltiin qaama qoratamaaf akkaa hin kennamne isiin beeksifnaa

Qorannoo keessaa bahuu

Yeroo qorannoon dhumatee keessaa bahuu ni dandeessaa

Yoo qorannoo irratti gaaffi qabaattee

Bilbila kanaan naaf bilbilaa: 0913186061

Mirga akka qaama hirmaataatti

Qorannoon kuni Universitii Jimmaa, boordii seera qorannoo ilaaluu irraa ilaalamee, mirkanaayee biiroo eegumsa fayyaa godinaa Arsii irraa heeyyamameera. Yoo gaaffii faayidaa qorannichaafi mirgaa akka qaama hirmaataati qabaattee; maqaan koo Barakati Waaqee jedhamaa…imeelii koo berwaklab4@gmail.com... bilbila: 0913186061 naaf bilbiluu dandeessuu.

Lakkoofsa hirmaataaf kenname ……

Maqaa hirmaataa……..

Yommuun qorannoo kana irratti hirmaadhu afaan naaf galuun natti himameera ykn naaf ibsameera.Faayidaa qorannoo kanaatis ''Baayina dhukuba busaa fi sababoota dhukkubni busaa irraa nama qabuu dubartoota ulfaa mallattoo busaa hin muldhifne irratti qorannoo gochuu'' naaf galeera.Waa'ee dhukkubbii busaa akkan gaafatamuu fi saamuda Hidda dhiigaa irraa akka kennamu naaf himameera. Odeeffannoo qorannoo kana irraa argamu hunduu iccitiin akka kaa'amus irratti walii galleerra.Qorannoo kana hirmaachuu yoon hin barbaadne ykn yoon addaan kute, ammas ta'ee fulduraaf fayyadamummaa kiyyarratti rakkoo tokkoollee akka hin uumnee naaf himameera. Ani _____ erga naaf gale booda mallattoo kootin nan mirkaneessa.

Maqaa dhukkubsataa……. mallattoo …… guyyaa…….

Maqaa qo'ataa…….. mallattoo…… guyyaa……

Appendix II: Questionnaire

Prevalence and Risk Factors associated with asymptomatic malaria among pregnant women at community of Merti Woreda, Arsi Zone

Introduction

Hello thanks so much for your permission. I am Bereket Wake, a student pursuing a Master of Medical Parasitology Program at the University of Jimma School of Medical laboratory sciences, Jimma Ethiopia. This interview is being conducted as part of a research into the Prevalence and Risk factors associated with asymptomatic malaria for Malaria control in pregnancy. I would be very much grateful if you would kindly find some time to answer these questions. Your views, opinions and contributions are very valuable and important and would go a long way to help me determine the prevalence and risk factors associated with asymptomatic malaria in pregnancy. This study is strictly for academic purposes and I can assure you of the confidentiality on any information that you would provide.

Thanks for your cooperation.

Name of village/ kebele:_____-

House hold ID:_____

Participant ID NO:_____

Participant name:_____

Name of Interviewer_____

Name of Investigator_____

Please tick [√] where appropriate and give the appropriate response to each item as presented.

Section A	**Socio-Demographic information**	**Selected items**
1	• age	_____
2	• Educational level	1. Illiterate 2. Primary 3. Secondary 4. College 5. Higher 6. Graduated

3	• Marital status	1. Single 2. Married 3. Divorced 4. Separated 5. Widowed
4	• Occupation	1. Daily labour 2. Government 3. NGO 4. Farmer 5. Others
5	• Residency	1.Urban 2.Rural

Section B	**Obstetric characteristics**	
6	• Gravidity	1. primigravidae 2. secondgravidae 3. multigravidae
7	• Current gestational age of pregnancy	1. First trimester 2. Second trimester 3. Third trimester
8	• Number of still birth/Abortion	1.once 2.twice 3.above two 4. not aborted
9	• Did you follow the ANC, before delivery? 1. Yes 2. No	

Section C	**Possession/ownership of ITN.**	
10	• Do you have a mosquito bed net?	1. Yes 2. No
11	• If yes, how many mosquito bed nets do you have?	1. One 2. Two 3. more than three

12	• Did you sleep under a mosquito net (ITN) last night?	1. Yes 2. No
13	• Which periods do you use the net?	1. All year round 2. During rainy season 3. During dry season 4. Others (specify).....
14	• What are the benefits of ITN use?	1. To prevent malaria 2. To sleep soundly 3. To provide warmth 4. To prevent insects bites 5. Other (specify).....
Section D.	**Use of indoor residual spray.**	
15	• Have you ever heard about IRS?	1. Yes 2. No
16	• Did spray indoor residual spray on your home?	1. Yes 2. No
17	• If yes to the above question, how often do you spray?	1. All year round 2. During rainy season 3. During dry season 4. Others (specify).....
18	• Is there stagnant water around your home? If yes, • Distance of home from the stagnant water	1. yes 2. no 1. <1km 2. 5km 3. > 10km
Section-E	**About *plasmodium* parasite infections**	
19	• Have you been infected with malaria in the last one year?	1.yes 2.no
20	• Where did you get diagnosed?	1.Hospital 2.Health center 3.Health post 4.Not tested all
21	• Did you utilize correctly all prescribed dose of antimalarial drugs?	1.yes 2.no

Date: _____/_____/_____

Name of village/ kebele:_____-

House hold ID:_____

Participant ID NO:_____

Participant name:_____

Investigator name:_____

<u>Laboratory results</u>

1. RDT results
   A. Histidine rich protein 2_____
   B. Lactate dehydrogenase_____
2. Microscopic results
   A. Parasite species_____
   B. Stages of *plasmodium*_____
   C. Parasite density_____
      1. Sexual_____
      2. Asexual_____
3. PCV value_____

Appendix; III. Amharic questionnaire (Amharic version)

ርዕስ; ምልክት ያላሳዩ የዎባ በሽታና መንስኤዎች በመርቲ ወረዳ አርሲ ዞን ነብሰጡር እናቶች ላይ ምርምር ለማድረግ

1. **ቀን**: _____/_____/_____

2. **የ መዝገብ ቁጥር/** _____

3.**ቃለመጠይቁን የሚያካሂደው ሰው ስም** _____ **ፊርማ**_____

4. **የተቆጣጣሪው ስም/**_____ **ፊርማ**_____

ነ.የስነማህበራዊሁኔታጥያቄዎች

ክፍልአንድ:የስነ-ማህበራዊሁኔታ			
#	**ጥያቄ**	**መለያቁጥር**	
1	**ዕድሜ**	……..	
2	**የትምህርትደረጃ**	ነ. **ማንበብናመጻፍየማይችል** 2. **አንደኛደረጃ** 3. **ሁለተኛደረጃ** 4.**ኮሌጅ(ዲፕሎማ)** 5. **ዩኒቨርሲቲ**	
3	**የጋብቻሁኔታ**	ነ. **ያላገባ/ች** 2. **ያገባ/ች** 3.**የፈታ/ች** 4. **የተለያዩ** 5. **የሞተችበት/ባት**	
4	**የመኖሪያቦታ**	1. **ከተማ** 2. **ገጠር**	

5	ስራዎትምንድነው?	1. አርሶዓደር 2. ተቀጣሪ 3.የግልስራ 4. ስራአጥ 5.ሌላ(ይገለፅ)	
6	የቤተሰብብዛት	1.አንድ 2.ሁለት 3.ሶስትናከዚያበላይ	
ክፍልሁለት: ስለእርግዝና			
7	ስንተኛእርግዝናሽነው	1.አንድ 2.ሁለት 3.ሶስትናከዚያበላይ	
8	ከአሁንበፊትውርጃአጋጥሞሽያዉቃል	1.አዎ 2.አይደለም	
9	እርግዝናሽሰንትወሩነው	1.አንደኛሶስትወራት 2.ሁለተኛሶስትወራት 3.ሶስተኛሶስትወራት	

ክፍል ሶስት: የአጎበርና የጸረወባ ኬሚካል አጠቃቀም

10	የአልጋ አጎበርአለዎት	1.አዎ 2.አይደለም	
11	አዎን ካሉ ምን ያህልነዉ	1.አንድ 2.ሁለት 3.ሶስትናከዚያበላይ	
12	በ ስንት ሰኣት ወደ መኝታ ይሄዳሉ		
13	የአልጋአጎበርበመኝታሰኣትይጠቀማሉወይ	1.አዎ 2.አይደለም	
14	በየትኛዉወክትነዉየምትጠቀሙት	1.ሁልጊዜ 2.ክረምት 3.በጋ 4.ሌላ…	
15	የአልጋአጎበርመጠቀሞትምንጥቅምያስገኛሎታል	1.ወባንለመከላከል 2.መዉቀትለማግኘት 3.በራሪነብሳትንለመከላከል 4.ሌላ…….	
16	ስለጸረወባኬሚካልርጪትሰምቶያዉቃሉ	1.አዎ 2.አይደለም	
17	ቤቶዎትንየጸረወባኬሚካልአስረጪቶያዉቃሉ	1.አዎ 2.አይደለም	
	ክፍል: አራትስለወባበሽታ		
18	ባለፉትአንድዓመትዉስጥበወባተይዞያዉቃሉ	1.አዎ 2.አይደለም	
19	የትነዉየተመረመሩት	1.ሆስፒታል 2.ጤናጣብያ 3..ጤናኬላ 4.አልታከምኩም	
20	የተዘዘሎትንመዳንትበአግባቡተጠቅሞዋል	1.አዎ 2.አይደለም	
21	ያቆረ /የተኛዉሃበአከባቢአለ	1.አዎ 2.አይደለም	

Appendix IV: Garagalcha Gaafillee Afaan oromoo (Afaan oromoo Version).

Baayyinna fi sababoota dhukkuba busaa mallattoo busaa hin qabnee dubartoota ulfaa uummata Aanaa Merti, Godina Arsi keesatti qoratamuuf.

Guyyaa: _____/_____/_____

Maqaa gandaa:_____-

Lakkofsa manaa:_____

Lakkofsa hirmaataaf kennamu:_____

Maqaa hirmaataa:_____

Maqaa dura bu'aa qoranichaa:_____

Seensa

Hello Eeyyamamaa waan taatanif baayyee galattoomaa. Ani Barakati Waaqee jedhama. Barataa Universitii Jimmaa Damee saayinsii fayyaa kutaa laboratorii keessaa Medical parasitology sadarkaa Masters barachaa jiraa. Gaaffileen isiniif qophaa'e qaama qorannoo; baayyina fi sababoota dhukkubni busaa, mallattoo osoo hin mul'isin dubartoota ulfaa miidhan Ittisuuf. Gaaffii isin gaafatamuu seeran waan deebiftaniif baayyee isin galatteefanna. Illaalchi, fedha fi hirmaannan keessan qoranichaaf gatii qaba. Qorannoon baruumsaa fi faayidaa dubartootaaf waan ta'eef iccitiin isaa seeran eegamaadha.

Gargaarsa keesaniif Galattoomaa.

Deebii keessan mallattoo kanaan agarsiisaa [√]

kutaa A	**Oddeeffannoo qaama hirmaataa**	**Iddoo filannoo**
1	• Waggaa meeqa?	----------
2	• Sadarkaa baruumsaa keessan?	1. Kan hin baratin 2. Sadarkaa 1ffaa 3. Sadarkaa 2ffaa 4. Kolleejjii 5. kan eebbiffamtee
3	• Haala gaa'elaa yeroo ammaa?	1. Kan hin heerumin 2. Kan heerumte 3. Kan hiiktee

		4. Kan irraa du'e
4	• Hojjaan keesan?	1. Dafqaan bulaa 2.Hojjata mootummaa 3. Mit-mootummaa 4. Qonnaan bulaa
5	• Iddoon jireenyaa kee eessa?	1. magaalaa 2. baadiyaa

Kutaaa B	**Amalootaa ulfaa**	
6	• Ulfi keesan meeqaffaadha?	1. jalqaba 2. lamaffaa 3. sadaffaa
7	• Yeroo meeqa ulfi sirraa deebi'ee?	1.tokko 2.lama 3.lama oli 4. hin deebinee
8	• Ulfi keessan ji'a meeqa?	1. marsaa tokkoffaa 2. marsaa lammaffaa 3. marsaa sadaffaa
9	• Hordoffii da'umsa dura gootaa?	1.ni hordofa 2. hin hordofu

kutaa C	**Waa'ee Fayyadammummaa Aggobaraa.**	
10	• Aggoobara qabduu?	1. hin qaba 2. hin qabuu
11	• Yoo qabaatte, meeqa qabdaa?	1. tokko 2. lama 3. sadi oli
12	• Yeroo raftuu agoobara keessa raftee?	1. eeyyu 2. hin rafnee
13	• Yeroo kami agoobara fayyadamtaa?	1. yeroo hundaa 2. yeroo roobaa 3. yeroo bonaa

14	• Faayidaan Agoobaraa maalii?	1.dhukkuba-busaa ittisuuf 2. sagalee dhoorkuuf 3. hoo'aa argachuuf 4.bookeen offirraa dhoowwuuf
Kutaa D.	**Fayyadammummaa keemikaala bookee busaa.**	
15	• Waa'ee keemikaala bookee balleesuuf biifamuu beektuu?	1. hin beeka 2. hin beekuu
16	• Itti fayyadamtee beektaa?	1. hin beekaa 2. hin beekuu
17	• Yoo beektan ta'ee yeroo meeqaaf?	1.yeroo hundaa 2. yeroo gannaa 3. yeroo bonaa
18	• Bishaan kuufame naannoo keessan jiraa? Yoo jiraate hangam fagaata?	1.jiraa 2. hin jiruu 1. 1km 2. 5Km 3. 10Km oli.
Kutaa- E	**Waa'ee dhukuba busaa**	
19	• Kana dura dhukuba busaatiin qabamtee beektaa?	1. eeyyu 2. hin beekuu
20	• Eessatti yaalamtee?	1.Hospital 2. buufata fayyaa 3.keellaa fayyaa 4.hin yaalamnee
21	• Qoricha siif kenname seeran fayyadamtee?	1.eeyyee 2. hin fayyadamnee

Appendix IV: SOP for preparation of Blood film, Rapid diagnostic test (RDT), collection and processing of specimens, staining and Identification of Plasmodium parasites by microscopy

1. General information on laboratory procedures

This section outlines the list of tasks required to complete this procedure. These tasks should be assigned to individual(s) capable of their execution and their name entered beside the task listed in the table below.

1.1. Tasks

	Study personnel
Label slides	
Prepare buffer solutions	
Take blood and make a blood smear	
Stain smear	
Mount slides	

1.2. Materials and Equipment

One or two glass slides (thick smear, +/- thin smear)

Pencil

Methanol

DPX (a mixture of distyrene (a polystyrene), a plasticizer (tricresyl phosphate), and xylene,) mounting solution

Giemsa stain

Giemsa buffer [Na_2HPO_4 (dibasic anhydrous), Na_2HPO_4*H2O (monobasic monohydrate), distilled water).

2. Procedure

2.1. Labeling Slides

Glass slides should be clean, grease and scratch free and have smooth edges without any cuts. Label the slides appropriately with a pencil. Write neatly and firmly so that the information can be easily read.

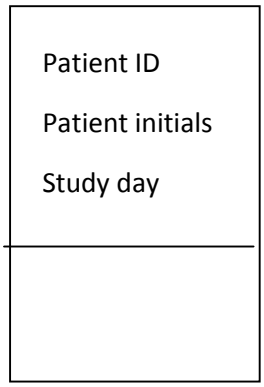

Figure 1. Labeling slides for blood smears

2.2 Making Smears

For preparation of a thick smear, between one and three drops of blood should be placed in the Centre of the slide and spread around evenly with a wooden stick or the corner of another slide to make a circle or square about 1cm.

For preparation of a thin smear, a smaller drop of blood should be placed at the end of the slide. Using another slide, the blood can be spread to create a feathered edge that reaches the other end of the slide.

The smears must be allowed to air dry free from flies and dust. Do not heat the slides as this will damage the parasites.

The thin smear can be fixed by submerging in 100% methanol for 30 seconds and then letting the slide air dry.

Since methanol fixation would prevent hemolysis, thick smears should not be fixed with methanol. This allows cell lyses necessary for accurate malaria diagnosis, parasite density calculation and identification of gametocytes.

2.3 .Preparation of Alkaline and Acid buffers

Alkaline buffer (one litre)

Weigh out 9.5 g of Na_2HPO_4 (dibasic anhydrous)

Dissolve in 900 mL of distilled water

Fill to a total volume of 1 L.

Acid buffer (one litre)

Weigh out 9.2 g of Na_2HPO_4*H20 (monobasic monohydrate)

Dissolve in 900 mL of distilled water

Fill to a total volume of 1 L.

2.4 .Preparation of Giemsa Staining Buffer

Mix together the proportions below to achieve a buffer of pH = 6.8:

Desired pH	Acid buffer (mL)	Alkaline buffer (mL)	Water (mL)
6.8	50	50	900

Giemsa staining buffer should be prepared every 1-2 weeks as needed.

2.5 .Staining Thick Smears

Prepare 3 % Giemsa staining solution daily (can be kept for approximately 8 hours).

Add Giemsa buffer to stain using the following mixture to achieve 3% Giemsa:

5 mL of buffer plus 100 µL of Giemsa

10 mL of buffer plus 200 µL of Giemsa

20 mL of buffer plus 400 µL of Giemsa.

Stain slides for 10-15 minutes with 3% Giemsa staining solution.

Rinse slide carefully with distilled water.

Allow slide to completely dry (time will vary dependent on ambient temperature, but average is 15 minutes).

2.6. Staining Thin Smears

After fixing the slide with methanol, allow to dry for 1-2 minutes.

Stain smears with 3 % Giemsa for 10-15minutes.

Rinse slide carefully with distilled water.

Allow slide to completely dry (time will vary depending on ambient temperature, but average is 15 minutes).

2.7. Mounting slides

After slide is dry, place 1-2 drops of DPX mounting solution directly on top of dried blood.

Place a large cover slip on top and then carefully press flat

3. MATERIALS

1 Giemsa stain (LabChem Inc.)

2 100% methanol

3 Bibulous paper (VWR)

4 Microscope with 100 oil immersion lens and 10x10 grid eyepiece

5 Microscope immersion oil

4. PROCEDURE

4.1 Giemsa staining of blood smeared slides

Fix slides in 100% methanol for ~30 s and rinse off in tap water;

Make up a fresh solution of 10% Giemsa stain in distilled water;

Stain ~30 min;

Rinse off slide in tap water and dry thoroughly using bibulous paper to dab.

4.2 Estimation of parasitemia

View slide under oil immersion with a 100X objective; Estimate parasitemia by counting the number of infected cells. A grid square in the eyepiece of the microscope facilitates the procedure as an even blood smear yields ~100 red blood cells per grid. Thus, for example, 8 infected blood cells in a grid is 8% parasitemia. Several fields (~10) should be counted and the average taken to obtain a representative estimate of the total parasitemia.

4.3. Cares tart ™ Malaria Pf/Pv (HRP2/PLDH) Ag Combo RDT

Immunochromatography used for Rapid qualitative detection of *Plasmodium* species HRP2 (Histamine rich protein 2) of *Plasmodium falciparum* and PLDH (*Plasmodium* Lactate dehydrogenase) of *Plasmodium vivax* in human whole blood as an aid in the diagnosis of malaria infection. The CareStart ™ malaria Pf/Pv (HRP2/PLDH) Ag combo RDT is designed for the differentiated diagnosis of P.falciparum and *P.vivax* infection.

Principles

It contains a membrane strip, which is pre-coated with two monoclonal antibodies as two separate lines across the test strip. One monoclonal antibody (test line PV) is *P.vivax* specific to PLDH and the other line (test line pf) consists of monoclonal antibody specific to HRP2 of *P. falciparum*. The conjugated pad is dispersed with antibodies adsorbed on gold particles.

Test procedures

1. Put on a new pair of gloves.
2. Write the patients name on the cassette.
3. Clean the area to be pierced using an alcohol swap.
4. Squeeze the end of a fingertip and pierce the cleaned area of the fingertip using a lancet provided. Discard the lancet in the sharps box.
5. Wipe out the first drop of blood with sterile gauze or cotton.
6. Collect the blood sample (5ul) using a provided specimen transfer device or a micropipette.
7. Add 5ul of whole blood into S well.
8. Add 2 drops (60ul) of buffer solution into a well. start a timer
9. Read result at 20 minutes.

Interpretation of the test result

- ✓ The test is valid when a line does not appear next to CON. If this occurs, the test should be repeated using a new cassette.
- ✓ The presence of a line next to CON indicates a negative results.
- ✓ The presence of two lines (one line in the result window next to CON and another line in the result window next to pf) indicates a positive result for *P. falciparum.*
- ✓ The presence of two lines (one line in the result window next to CON and another line in the result window next to pf) indicates a positive result for *P.vivax*.
- ✓ The presence of three lines (3 line in the result window next to CON (P.f and P.v) indicates a positive result for *P.vivax* and p. falciparum.

Precision of the test

The sensitivity of the diagnostic test used in different studies play a major role in detection of APIs. Microscopy has been the gold standard in malaria research and remains as a point-of-care diagnostic in clinical and epidemiological settings. An expert microscopist can detect 50 parasites/μl, The rapid diagnostic tests (RDTs) detect 100–200 parasites/μl and Polymerase chain reaction (PCR)-based tests improved the detection limit for malaria infection >1 parasite/μ(94).

Precision was evaluated by testing 10 replicates of CareStart malaria pf/pv (HRP2/PLDH) Ag combo RDT with three specimens; a negative, a low positive and a strong positive specimen. The results showed 100% confirmation of the expected results.

Quality Control

The accuracy of results for the diagnosis of malaria and quantification of parasite density is highly dependent on the quality of the preparation of thin and thick smears. Care should always be taken to use clean, new slides and to follow the instructions outlined above.

Declaration Letter

I hereby certify that I have read and evaluated this thesis prepared under my guidance, by Bereket Wake entitled " Prevalence and predictors of asymptomatic malaria among pregnant women in merti woreda, Arsi zone, Oromia, Ethiopia" I recommend that it can be submitted as fulfilling of the thesis requirement.

Name of principal investigator

1.Mr. Bereket Wake (BSc) Signature _____Date_____

Approval of internal examiner

Mio Ayana (MSc, PhD scholar) Signature _____Date _____

Approval of the first advisor

1. Dr. Teferi Eshetu (PHD, MSc) Signature _____Date_____

Approval of the second advisor

2. Mr. Teshome Degefa (MSc, PhD scholar) Signature _____Date_____