
**ADDIS ABABA UNIVERSITY**

**SCHOOL OF BIOMEDICINE AND LABORATORY SCIENCES**

# Magnitude of Asymptomatic and Symptomatic Malaria and Associated Factors among Military Personnel in the Bilate Commando and Airborne Training Center, Southern Ethiopia

**By:**

**Efrem Geremew Kebede (*BSc*)**

***A thesis submitted to the School of Biomedicine and Laboratory Sciences, Addis Ababa University, College of Health Sciences, Department of Microbiology, Immunology, and Parasitology in Partial Fulfillment of the Requirements for the Degree of Master of Science in Medical Parasitology***

***May, 2025***
***Addis Ababa, Ethiopia***

**Addis Ababa University**

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# List of Abbreviations and Acronyms

| | |
|---|---|
| **AAU** | Addis Ababa University |
| **ACT** | Artemisinin-based Combination Therapy |
| **AHRI** | Armauer Hansen Research Institute |
| **CDC** | Centre for Disease Control and Prevention |
| **DBS** | Dried blood spot |
| **DRERC** | Department Research Ethics Review Committee |
| **EDU** | Ethiopian Defense University |
| **FMoH** | Federal Ministry of Health |
| **IRS** | Indoor-Residual Spraying |
| **ITN** | Insecticide Treated Net |
| **LLIN** | Long Lasting Insecticidal Net |
| **MIS** | Malaria Indicator Survey |
| **qPCR** | Quantitative Polymerized Chain Reaction |
| **PMI** | President's Malaria Initiative |
| **RDT** | Rapid Diagnostic Tests |
| **SOP** | Standard operating procedure |
| **SPSS** | Statistical Package for Social Sciences |
| **WHO** | World Health Organization |

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# Abstract

**Background:** Malaria remains a significant global challenge in terms of health and economic impact, particularly in Ethiopia, where it poses serious risks to military personnel. The disease significantly affects soldiers, leading to illness and fatalities that impede military operations, recognizing the role of both asymptomatic and symptomatic soldiers in malaria-prone military settings is essential for effective control and prevention of disease transmission.

**Objective:** To assess the magnitude of asymptomatic and symptomatic malaria and contributing factors among military personnel in the Bilate Commando and Airborne training center.

**Methods:** A facility-based cross-sectional study was conducted in May and June 2024, involving 403 military personnel. Data were gathered using semi-structured questionnaires, along with finger prick blood samples for parasite detection through microscopy, RDTs, and qPCR analysis. Data entry and analysis were performed using Epi Info 3.1 and SPSS version 27. Bivariate and multivariate logistic regression was utilized to explore the relationship between outcome and predictor variables, supported by descriptive statistics. A *p-value* of $< 0.05$ was deemed statistically significant.

**Result**: The study involved 403 soldiers, and identified an overall magnitude rate of 37% (149/403), with nearly equal distribution of *P. falciparum* at 45.6% (68/149) and *P. vivax* accounted for 44.3% (66/149). Among the 293 asymptomatic soldiers, prevalence rates were 18.1%, 17.7%, and 45.1% using microscopy, RDTs, and qPCR respectively. In contrast, the 110 symptomatic soldiers showed higher prevalence rates of 64.5%, 60.9%, and 87.2% by microscopy, RDTs, and qPCR respectively. The geometric mean parasite density was 101,531 copies/µl by qPCR and 24,378 asexual parasites/µl by microscopy. Significant risk factors for malaria infection included a previous history of malaria (AOR=0.508, 95% CI=0.317-0.813) and receiving educational messages regarding malaria (AOR=3.385, 95% CI: 1.433-7.995), both statistically significant at $P < 0.05$. Additionally, there was almost perfect concordance between RDT and microscopy results.

**Conclusion:** The study identified high malaria rates among soldiers in military camps, suggesting these sites as transmission hotspots. It recommends including military camps in the national surveillance system to enhance tracking of infection trends.



**Keyword:** *Asymptomatic malaria, Symptomatic malaria, Military personnel, Ethiopia, Barrack*

# 1. Introduction

## 1.1. Background

Malaria is a serious and potentially fatal mosquito-borne disease caused by protozoan parasites belonging to the genus *Plasmodium*. Female *Anopheles* mosquitoes transmitted the disease by biting human and injecting the parasites, which then invade liver cells and subsequently multiply within red blood cells (Crutcher & Hoffman, 1996; Sato, 2021). The main species infecting humans are *Plasmodium falciparum (P. falciparum), Plasmodium vivax (P. vivax), Plasmodium malariae (P. malariae),* and *Plasmodium ovale (P. ovale),* with *P. falciparum* being particularly dangerous and prevalent in tropical regions, significantly impacting sub-Saharan Africa (Snow & Gilles, 2017; Zekar & Sharman, 2023). Additionally, *Plasmodium knowlesi (P. knowlesi),* which primarily infects monkeys, has started to infect humans in specific areas of Southeast Asia and the Western Pacific (CDC, 2024; Lee *et al.*, 2022; WHO, 2023a).

Malaria continues to be a major global health concern, leading to numerous illnesses and fatalities despite ongoing intervention efforts. According to the latest World Malaria Report, released in 2024, the estimated number of malaria cases was approximately 263 million worldwide; resulting in 597,000 deaths across 83 affected countries. This represents a rise of 11 million cases compared to the previous year. The WHO African Region was responsible for 94% of cases and 95% of deaths, with approximately 76% of fatalities occurring in children under five (WHO, 2024b).

Ethiopia is currently grappling with a significant malaria problem that poses risks to public health and economic growth (Worku *et al.*, 2014). Malaria transmission affects about 75% of the country's land, especially in regions below 2,000 meters in elevation, putting nearly 60% of the population at risk (FMoH, 2017). Between 2022 and 2023, Ethiopia observed an 82% increase in malaria cases, with the estimated total rising to 6.9 million over the past five years (2019-2023). The country has faced multiple challenges in recent years, including the insufficient implementation of prevention measures in conflict zones, the emergence of the new malaria vector *Anopheles stephensi*, insecticide resistance among vectors, and the impacts of climate change (WHO, 2024b).

Malaria has had a profound impact on the history of humankind for thousands of years, influencing the rise and fall of nations and kingdoms, as well as the outcomes of wars and revolutions. Many kings and military leaders have tragically succumbed to malaria in their prime, while numerous formidable warriors have fallen ill after returning from battle (Dhiman *et al.*, 2010).

Military personnel face various vector-borne threats and diseases during operations in conflict zones, with malaria being a major concern, particularly in tropical and hyper-endemic regions (Najafipour *et al.*, 2015). As a highly mobile population, military forces often operate in forested and remotely located localities, including areas bordering neighboring countries where malaria transmission rates are higher. During conflicts, the disease can spread rapidly among soldiers living in temporary shelters and exposed to mosquito vectors (Vilay *et al.*, 2019). In addition, some day-to-day activities of armed forces and their members frequently create many breeding grounds for the mosquitoes that transmit malaria, thereby significantly increasing its transmission (Dhiman *et al.*, 2010).

Malaria poses a significant threat to the health and effectiveness of soldiers, often leading to illnesses and deaths that impede military operations. Surprisingly, historical records indicate that malaria has caused more deaths among US soldiers than actual combat (Mertens, 2024). In regions where malaria is prevalent, particularly those where UN peacekeepers and trainees are deployed, the increased risks of malaria, result in a surge of reported cases. To address this issue, it is essential to prioritize measures such as vector control and personal protection strategies to ensure military readiness and reduce the incidence of malaria (Fernando *et al.*, 2017; Kotwal RS *et al*., 2005; Msellemu *et al.*, 2021; Pagès *et al.*, 2010; Whitman *et al.*, 2010).

Infection with *Plasmodium* can manifest with symptoms ranging from none to severe, life-threatening malaria. Common symptoms include fever, chills, fatigue, headache, muscle pain, and weakness (CDC, 2024), particularly affecting those without immunity (Zekar & Sharman, 2023). Symptomatic malaria features these symptoms alongside parasites in the blood (Abebaw *et al.*, 2022). Uncomplicated malaria may involve headaches, vomiting, fever, anemia, and loss of appetite, while severe anemia and cerebral malaria are significant causes of mortality (Worku *et al.*, 2014).

Some infected individuals may remain asymptomatic due to partial immunity known as "premunition" gained through repeated exposure to *Plasmodium* parasites. These asymptomatic carriers can still harbor and transmit the parasites via gametocytes, contributing to ongoing malaria transmission (Worku *et al.*, 2014). This asymptomatic state may help maintain immunity and lessen the chances of severe illness (Chen *et al.*, 2016).

Asymptomatic parasitemia is when malaria parasites are present in the bloodstream of individuals who do not exhibit clinical symptoms for at least two days, excluding inactive liver infections. Research indicates that asymptomatic carriers may be more infectious than those showing symptoms because they are more often bitten by mosquitoes, which increases transmission risk (Clarisse E. Mbah et al., 2022; Lindblade *et al.*, 2013). Detecting these infections is challenging due to the lack of visible symptoms and difficulties in identifying parasites under microscopy. Consequently, asymptomatic carriers are often neglected in malaria interventions, which hamper efforts towards disease eradication (Clarisse E. Mbah et al., 2022).

The National Malaria Strategic Plan of Ethiopia for 2021-2025 aims to eradicate malaria by 2030, with the objective of considerably decreasing illness and mortality associated with the disease. The plan focuses on eradicating malaria by 2025 in areas with an annual parasite index (API) below 10 and achieving a 50% decrease in overall malaria morbidity and mortality compared to 2020. To facilitate this, a technical manual for elimination programs has been developed by the Federal Ministry of Health (PMI, 2022, 2023).

Despite notable progress in reducing malaria cases over the last twenty years, the disease remains a major public health and economic concern, prioritizing it within Ethiopia's healthcare system. In particular, assessing the impact of both asymptomatic and symptomatic malaria in military settings is crucial for controlling transmission and meeting elimination targets. However, there is a shortage of evidence regarding the magnitude of these malaria types among the military. This study seeks to evaluate the magnitude and factors linked to both asymptomatic and symptomatic malaria in the Bilate commando and Airborne training center.

## 1.2. Statement of the Problem

Malaria has been an ongoing problem for humanity throughout history and remains a significant global health concern today. Although it can be prevented and treated, it still severely affects an individual's well-being and their capacity to earn a living around the globe (WHO, 2021).

Although Ethiopia has a lower prevalence rate of malaria compared to other malaria-endemic countries in Africa, it remains a significant public health issue (FMoH, 2016). Malaria continues to cause sickness and fatalities, posing a significant threat to individuals' health over an extended period (Abossie et al., 2017; Gessessew B. et al., 2020; Nega *et al.*, 2015).

Malaria is a major concern for military personnel who frequently face the risk of encountering the disease when they are deployed in areas where it is prevalent (Whitman *et al.*, 2010). During military operations, non-combat injuries and illnesses, such as malaria, have resulted in more casualties than actual fighting (Mertens, 2024). It has been a significant problem for soldiers on active duty, causing illness and death, and has repeatedly hindered various military missions (Kotwal RS *et al*., 2005). Furthermore, during times of war, the outbreak of malaria has led to weakened armies, the suspension or cancellation of military operations, and general confusion and unrest among hostile and neutral populations (Pagès *et al.*, 2010).

The UN frequently deploys military personnel to countries where malaria is prevalent, and upon returning home, there is an increased risk of them introducing the disease to malaria-free countries. Due to the nature of their work in forested and border regions with high malaria transmission rates, military personnel are considered at risk for malaria infection. They often lack adequate access to malaria prevention, diagnosis, and treatment, and their untreated parasitaemia can contribute to malaria transmission to nearby communities (Fernando *et al.*, 2017).

Ethiopia has successfully met the GTS 2020 target of reducing malaria cases and deaths by 40% or more (WHO, 2022). However, the fight against malaria is faced with a range of interconnected challenges, including the existence of asymptomatic individuals, financial limitations, the rise of drug-resistant parasites and insecticide-resistant mosquitoes, the appearance of new vector species such as *Anopheles stephensi*, *Pfhrp2/3* deletions, and the emergence of zoonotic *plasmodia*, like *P. knowlesi* (Abebaw *et al.*, 2022; WHO, 2021, 2023c).

Ethiopia's malaria control strategies focus on prompt identification and treatment of infected individuals, as well as the implementation of insecticide-treated bed nets (ITNs) and indoor spraying. However, these methods often miss asymptomatic infections, which can harbor malaria parasites and contribute to ongoing transmission. Asymptomatic cases are linked to high levels of gametocytes, posing a significant challenge to effective malaria control (Legesse *et al.*, 2024; Worku *et al.*, 2014).

Additionally, military training centers receive recruits from various regions with varying malaria endemicity, potentially introducing malaria infections that can spread within the camps and surrounding areas. The large influx of recruits in military camps increases the likelihood of malaria transmission compared to the general population. Unfortunately, no studies have been conducted in Ethiopia's military settings assessing malaria prevalence. Therefore, a research was initiated to assess the risk factors and magnitude of both asymptomatic and symptomatic malaria in military personnel in the Bilate Commando and Airborne Training Center southern Ethiopia.

## 1.3. Significance of the study

To effectively eliminate malaria among military personnel, it is crucial for decision-makers to understand the proportion of asymptomatic and symptomatic malaria infections, and the contributing factors. Identifying the most common *Plasmodium* species in the area is also vital for developing suitable management strategies. The findings of this study will enable local health centers and the Defense Health main directorate's malaria control office to better understand the malaria burden and the prevalent species in the region, allowing for the implementation of a targeted malaria prevention and control program. Addressing both asymptomatic and symptomatic cases could significantly lower the risk of malaria transmission in military settings. Moreover, the outcomes of this study will help evaluate the effectiveness of existing malaria interventions in military environments. This research will lay the groundwork for further investigations into asymptomatic and symptomatic malaria among military personnel

## 1.4. Literature Review

### 1.4.1. Human malaria Parasite

Malaria is a highly destructive infectious disease that affects humans and various other vertebrates, caused by single-celled protozoan parasites from the genus *Plasmodium,* which are classified as parasitic protozoans within sporozoan subclass Coccidian. Currently, there have been more than 200 formally described species of *Plasmodium*, each infecting specific hosts. However, only five species (*P. falciparum, P. vivax, P. malariae P. ovale* and *P. knowlesi)* infect humans and are responsible for widespread malaria cases around the world. *P. knowlesi* mainly resides in macaque monkeys and is responsible for zoonotic malaria in Southeast Asia (Britannica, 2023; Sato, 2021).

These parasites are exclusively transmitted to humans via bites from infected female *Anopheles* mosquitoes, which serve as the primary vectors. Of the more than 400 *Anopheles species,* around 40 are known to transmit malaria (WHO, 2023b). Female mosquitoes need to consume blood in order to produce eggs, establishing an essential link between humans and mosquitoes in the life cycle of the parasite. Upon entering the human body, the parasites infiltrate the liver and red blood cells, resulting in the characteristic symptoms of malaria (CDC, 2020).

### 1.4.2. Life Cycle of *Plasmodium*

The life cycle of malaria parasite involves two hosts: humans and female *Anopheles* mosquitoes. The sexual reproduction starts when a mosquito carrying the infection bites a human, initiating the sporogonic cycle. Inside the mosquito, male parasites (microgametocytes) fertilize female parasites (macrogametocytes) to form a *zygote*, which develops into an *ookinete* and invades the stomach wall. Here, it becomes *oocysts*, which multiply and release sporozoites. These sporozoites move to the mosquito's salivary glands and are then transmitted to a new human host during a subsequent bite. This entire cycle takes about 7 to 21 days, influenced by mosquito species and environmental factors (Chelsea, 2023; WHO, 2010).

When a female *Anopheles* mosquito carrying the parasites bites a human, it introduces sporozoites into the bloodstream. These sporozoites then move to the liver, where they penetrate liver cells and multiply during the exoerythrocytic cycle, which lasts between 7 and 21 days.. A

*sporozoite* multiplies into many new parasites, forming a liver *schizont*. When the schizont bursts, it releases *merozoites* into the bloodstream. The *merozoites* then invade red blood cells, initiating the erythrocyte cycle. Inside the red blood cells, the parasites feed and develop into ring-stage *trophozoites*, some of which mature into *schizonts* that also burst to release more *merozoites.* Others develop into gametocytes, necessary for malaria transmission back to mosquitoes. In the case of the *P. vivax* and *P. ovale* species, some liver-invading parasites enter a dormant phase called *hypnozoites*, which can cause malaria to recur after the initial infection (Chelsea, 2023; WHO, 2010).

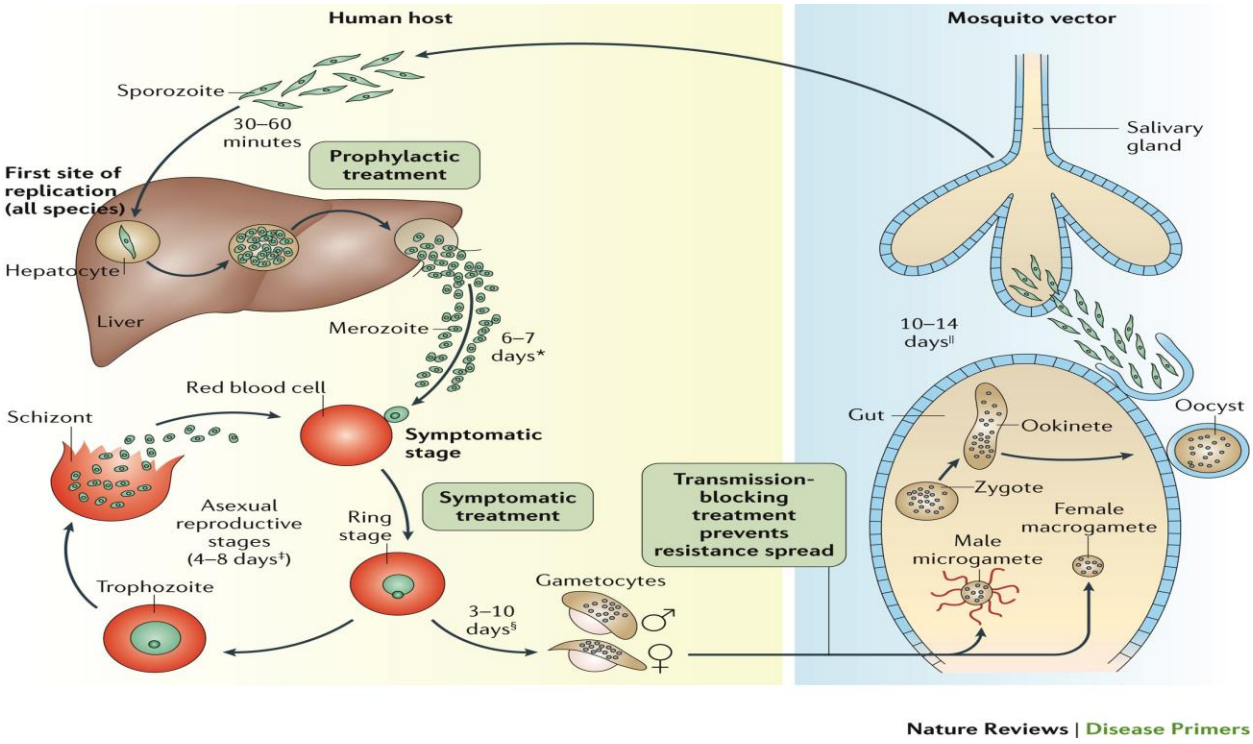


Figure 1: Life cycle of the malaria parasite (Phillips *et al.*, 2017).

### 1.4.3. Global Epidemiology of Malaria

In 2023, approximately 263 million malaria cases were reported across 83 endemic nations, marking an increase of 11 million cases since 2022. Since 2020, the estimated incidence of malaria has been consistently increasing. The incidence rate of malaria also rose slightly from 58.6 to 60.4 per 1,000 populations at risk between 2022 and 2023. In specific countries, the estimated incidence increased significantly: by 82% in Ethiopia, 71% in Madagascar, and 59% in

Pakistan. Since the year 2000, malaria deaths have consistently decreased from 861,000 to 567,000 in 2019. However, between 2021 and 2023, deaths increased again to 597,000. The proportion of total malaria deaths among children less than five years of age has declined from 86.7% in 2000 to 73.7% in 2023. Notably, 29 out of the 83 malaria-endemic countries accounted for nearly 95% of malaria cases and 96% of deaths globally in 2023. In the WHO African Region, there were 246 million reported cases and 569,000 fatalities. This area represented approximately 94% of the world's cases and 95% of the deaths. Notably, 76% of all deaths occurred in children under five years old in 2023, a decrease from 91% in 2000 (WHO, 2024b).

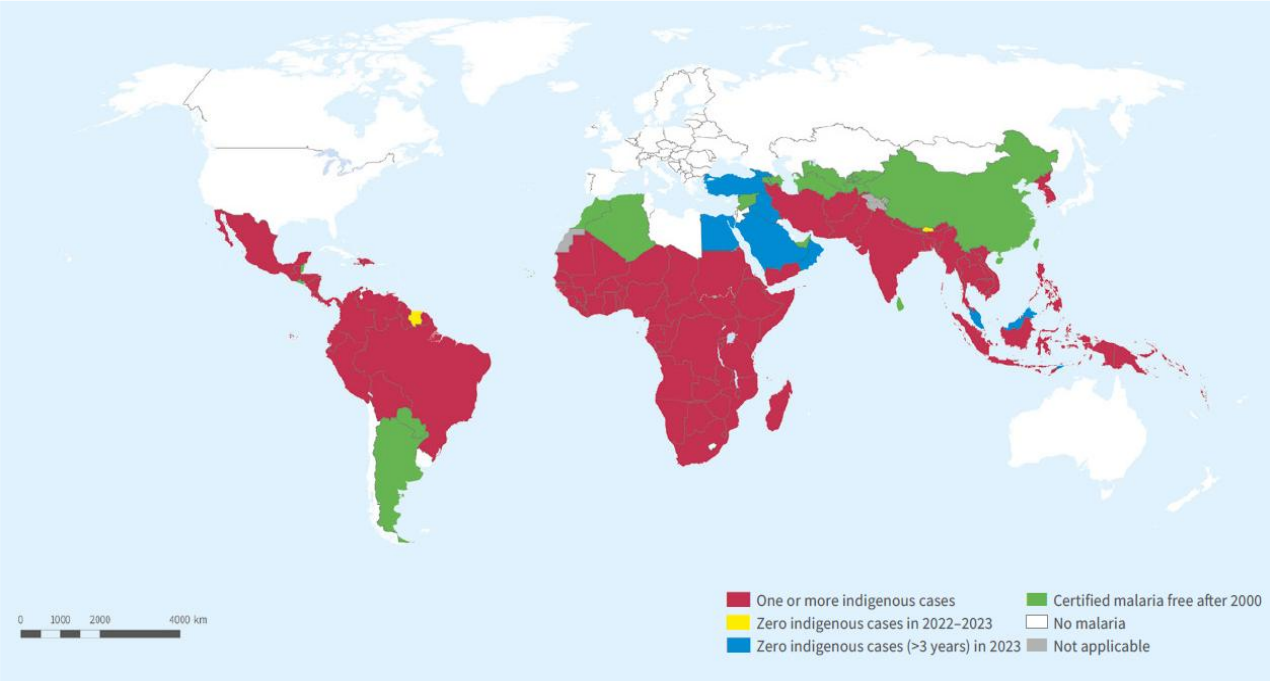


Figure 2: Countries with indigenous cases in 2000 and their status by 2023 (WHO, 2024b).

To be considered successful in eliminating malaria, a country must not have any cases of indigenous malaria for a continuous period of at least three years. In 2023, Malaysia celebrated its sixth consecutive year with no indigenous cases of human *Plasmodium* spp. Meanwhile, Saudi Arabia and Timor-Leste reported zero indigenous cases for three years in a row, indicating the end of malaria epidemic in both nations. In addition, Cabo Verde and Belize were recognized as malaria-free in 2023 after four years without any reported cases. Egypt received its malaria-free certification in 2024 (WHO, 2024b).

### 1.4.4. Epidemiology of Malaria in Ethiopia

Malaria presents a major public health issue in Ethiopia, with about 75% of the country's land area classified as malaria-endemic. Approximately 69% of the population lives in these regions, primarily in areas situated below 1,000 meters (WHO, 2024a). These regions include Gambela, Benishangul-Gumuz, Western Oromia, Amhara, parts of SNNP, and Tigray, where malaria transmission is highest (PMI, 2023). Malaria remains a major public health issue in Ethiopia due to the unstable and seasonal nature of transmission, leading to outbreaks in various parts of the country. Although there has been a decrease in cases and deaths over the past decade there was a 34% increase compared to 2019 data. The prevalence of malaria parasites in Ethiopia was found to be 0.5% when examined under a microscope and 1.2% when tested using RDT in areas below 2,000 meters, while it was less than 0.1% at altitudes higher than 2000m. More than 80% of the malaria burden in Ethiopia affects adults and children who are five years old or older (PMI, 2022, 2023).

In Ethiopia, the main malaria parasites are *P. falciparum* (~65%) and *P. vivax* (~35%). The transmission of the disease is primarily carried out by *Anopheles arabiensis* mosquitoes, with other species like *An. funestus*, *An. pharoensis*, *An. nili*, and potentially *An. coustani* is also playing a role. The presence of *Anopheles stephensi* in urban areas has also been detected (Carter *et al.*, 2018). Currently, areas below 2,000 are considered at risk for malaria, with the main transmission occurring from September to December and a low transmission period from April to June. This timing aligns with the planting and harvesting seasons, creating economic challenges for rural communities. In Ethiopia, both adults and children face an equal risk of infection and disease due to the unpredictable nature of malaria (PMI, 2022, 2023; WHO, 2010).

In 2023, Ethiopia accounted for 3.6% of global malaria cases and 3.1% of related deaths (WHO, 2024b). There was a 32% increase in estimated incidence from 46.3 to 60.9 cases per 1,000 populations at risk in 2022, followed by an 82% rise in 2023. From 2019 to 2023, Ethiopia reported a total of 6.9 million malaria cases, including an increase of 4.5 million cases between 2022 and 2023 (WHO, 2023c, 2024b).

Furthermore, in the first ten months of 2024, Ethiopia reported over 7.3 million malaria cases and 1,157 deaths, resulting in a 0.02% case fatality rate. This marked the highest annual

incidence in seven years. 95% of these cases were laboratory-confirmed, with *P. falciparum* accounting for more than two-thirds of the infections. In contrast, 2023 had 4.1 million malaria cases and 527 deaths, with *P. falciparum* representing about 70% of infections. Four regions were responsible for 81% of the cases and 89% of the deaths attributed to malaria: Oromia (44% of cases), Amhara (18%), Southwest (12%), and the South Ethiopia Regional State (7%) (WHO, 2024a).

Table 1: General Demographics and Malaria Situation (PMI, 2022).

| **Population** | **102,850,793 (Ethiopia NMSP, 2021)** |
|---|---|
| **Population at risk of malaria** | 53,577,865, (Ethiopia NMSP, 2021) |
| **Malaria prevalence** | 0.5 percent (Ethiopia National MIS, 2015) |
| **Malaria incidence/1,000 population at risk** | 23.4 (Federal Ministry of Health Annual Performance Report, FY 2021) |
| **Peak malaria transmission** | September to December |

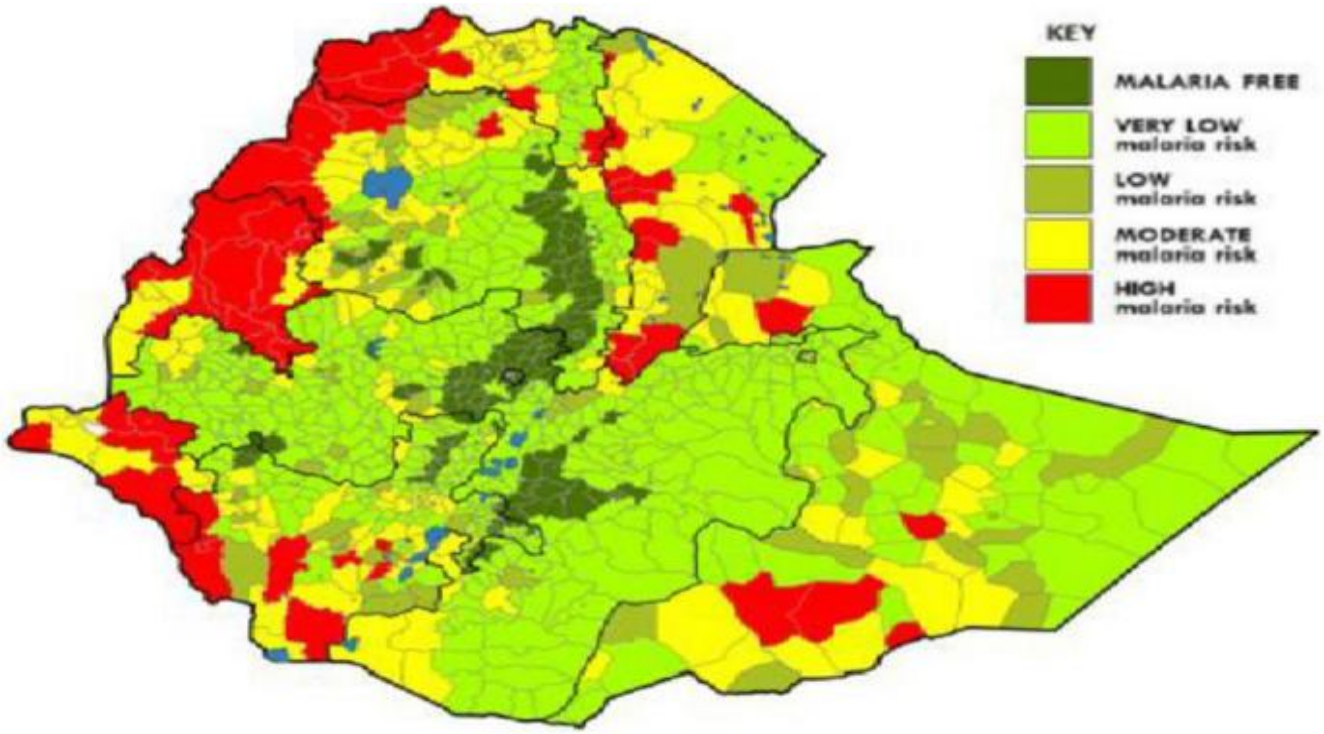


Figure 3: Malaria risk map of districts categorized by annual parasite index (API), Ethiopia, 2020 (PMI, 2022).

### 1.4.5. Studies on the Prevalence and Associated Factors of Malaria Globally

In a 2018 study conducted in Jazan, Saudi Arabia, 321 participants were tested for malaria using PCR, revealing a prevalence of 10.9% with 30 confirmed cases (Hawash *et al.*, 2019). Males and febrile individuals showed higher infection rates. Key risk factors included being aged 15-24, having a fever, and recent travel. The majority of infections were caused by *P. falciparum* (76.6%), followed by *P. vivax* (16.6%), with 6.6% being mixed infections. Nested PCR proved more accurate than microscopy and rapid diagnostic tests (RDT), demonstrating a sensitivity of 76.6% and perfect specificity of 100% (Hawash *et al.*, 2019).

Seasonal surveys conducted in 2015 along the China-Myanmar border found that the prevalence of asymptomatic *Plasmodium* infections was considerably greater detected by nRT-PCR (23.3%) compared to microscopy (1.5%) (Zhao *et al.*, 2018). The majority of infections were caused by *P. vivax* (89.6%), followed by *P. falciparum* (8.1%) and mixed-species infections (2.3%). Vector-based interventions, such as the use of bed nets and indoor residual spray, had a significant impact on reducing asymptomatic *Plasmodium* infections. Those who did not use these measures were at higher risk of infection. Furthermore, people residing in inadequately built homes or located farther from clinics were more susceptible to asymptomatic infections (Zhao *et al.*, 2018).

An additional cross-sectional study conducted in 2014 along the China-Myanmar border investigated malaria prevalence in Mong Pawk and Laiza. In Mong Pawk, microscopy detected a 0.3% prevalence of *P. falciparum*, while RDT showed 4.3%, cPCR 4.0%, and RT-PCR 7.8%. In Laiza, among 385 asymptomatic individuals, 2.3% tested positive for *P. vivax* via microscopy, 4.4% by cPCR, and 12.2% with RT-PCR, with 2.3% testing positive for *P. falciparum* only through RT-PCR. Among 34 symptomatic participants from Laiza, 32.4% were positive for *P. vivax* across all testing methods. The study also identified that males and children aged 5-17 had higher infection rates (Huang *et al.*, 2017).

In a 2016 cross-sectional study in Sabah, Malaysia, researchers analyzed data from 1,222 patients and found that 33.6% tested positive for malaria, with *P. knowlesi* being the most common species, accounting for 82.9% of cases. The study identified that males and rural

residents had a higher risk of infection, with rural dwellers having a notably increased likelihood of malaria (Ramdzan *et al.*, 2020).

A survey conducted in the Bandarban District of Bangladesh found that the prevalence of malaria was 30.7% as determined by PCR, and 14.2% as determined by microscopy. Among the positive cases, 58.9% were mono-infections of *P. falciparum*, 13.6% were mono-infections of *P. vivax*, and 1.8% was *P. malariae* and 1.4% was *P. Ovale*. Mixed infections were detected in 24.4% of the cases using PCR. Among the cases confirmed by PCR, 77.0% were asymptomatic, while 19.8% were oligosymptomatic, and only 3.2% were symptomatic. It was observed that individuals over 15 years old had a higher proportion of asymptomatic infections, while younger individuals had a higher prevalence and parasite density. The spleen rate among children aged two to nine was 18.6%, and the prevalence of malaria in this age group was 34.6% (Starzengruber *et al.*, 2014).

A comparative cross-sectional study conducted across rural and urban forest zone communities in Cameroon in 2020 found that, study detected asymptomatic malaria in 27.0% of participants, with 83 individuals testing positive for *P. falciparum* antigen. The prevalence of asymptomatic malaria in the urban community was 4.2% (5 positives), while in the rural community it was 41.5% (78 positives), indicating a significantly higher prevalence in the rural setting, around 9.9 times higher than in the urban setting. The study also found other significant associations between variables such as having a farm around the house and occupation, and *P. falciparum* antigen test results (Clarisse E. Mbah et al., 2022).

A survey conducted in five villages in the Ashanti region of Ghana in 2018 found that out of 400 participants, 73% had asymptomatic *Plasmodium* infection as determined by molecular testing. However, only 32% of these infections were detected by RDTs. Among those infected, 68% had *P. falciparum*, 8% had *P. malariae*, and 9% had *P. ovale*. Both *P. ovale curtisi* and *P. ovale wallikeri* were identified at similar rates of 3% and 4% respectively. *P. falciparum* was mainly found as a single infection (82%), while *P. malariae* and *P. ovale* were often found in combination with other infections (85% and 76% respectively). The sensitivity and specificity of the RDT for detecting *Plasmodium* infection compared to PCR testing were 43% and 96% respectively (Heinemann *et al.*, 2020).

Another study in Tanzania found that the prevalence of *P. falciparum* among school-aged children was 73.3% using RT-qPCR, 40.8% using RDT, and 36.3% using microscopy. Symptomatic children had higher prevalence rates, with RT-qPCR detecting 89% among them compared to 57.5% among asymptomatic children. RDT and microscope also showed higher prevalence rates in symptomatic children. Interestingly, asymptomatic individuals had higher rates of malaria gametocytes using microscope (2%) and RT-qPCR (14%) compared to symptomatic individuals (0.5% by microscope and 3% RT-qPCR). All three diagnostic methods detected significantly higher infection rates in symptomatic children attending health facilities compared to asymptomatic children in schools (Sumari *et al.,* 2017).

A cross-sectional study conducted in Kinshasa, DRC, in 2019 assessed school-age children and found the overall prevalence of *Plasmodium spp*. to be 33%, 42%, and 62% among asymptomatic children, depending on the detection method used: microscopy, RDTs, and PCR, respectively. In symptomatic children, the prevalence was even higher, recorded at 59%, 64%, and 94% for the same methods. In asymptomatic children, the predominant species identified was *P. falciparum*, which made up 58% of cases, followed by *P. malariae* at 20% and *P. ovale* at 11%. For symptomatic children, *P. falciparum* was again the most common at 93%, with *P. malariae* at 13% and *P. ovale* at 16%. The study noted a significantly greater prevalence of all *Plasmodium* species infections in rural areas compared to urban settings among asymptomatic cases (Nundu *et al.*, 2021).

### 1.4.6. Studies on the Prevalence and Risk factors Associated with Malaria in Ethiopia

In 2015, the most recent MIS conducted in Ethiopia showed that, the prevalence of parasites was 0.5% when observed through microscopy and 1.2% when using RDTs, in regions situated below 2000 meters. Above that elevation, the prevalence was less than 0.1%. Reports indicate that adults and children above the age of five bear the majority of the malaria burden in Ethiopia, accounting for over 80% of cases (PMI, 2022). *P. falciparum* was the most common species detected in positive cases, accounting for 82% of cases via RDT and 88% through microscopy, while *P. vivax* was found in 8% of cases by RDT and 9% by microscopy, across all age groups. The majority of positive cases were identified in Benshangul Gumuz and Gambella, where *P. falciparum* is the dominant species (FMoH, 2016).

A five-year (2016-2020) Cross-sectional study conducted in the Oromia Regional State, Ethiopia found that a total of 5,843,373 suspected cases of malaria were reported. Of these, 727,738 cases were suspected fever cases, with 15,655 clinically diagnosed cases (2.2%) and 712,083 parasitological confirmed cases (97.8%). *P. falciparum* was found to be more common than *P. vivax*, constituting 68.2% and 31.8% of the cases, respectively. The average annual parasite incidence (API) was recorded 4 cases per 1,000 individuals, with most cases occurring in spring (35%) and summer (28.8%). A total of 5,180 inpatient cases and 63 deaths were reported during this period (Olani *et al.*, 2023).

In two seasons cross-sectional surveys conducted in the Kaffa zone in 2021 revealed that among the 566 study participants, 16 cases of malaria were detected using microscopy and 22 were identified through RDT. Among the infections, 7 (1.2%) were found to be gametocyte stages of *P. falciparum*. The first survey found 11 asymptomatic cases, while the second, conducted during peak transmission, found 27 cases. Infections included 13 cases of *P. falciparum*, 11 of *P. vivax*, and 3 mixed infections. Significant factors linked with asymptomatic *plasmodium* infection included previous night use of long-lasting insecticidal nets (LLIN), presence of eave and recent house spraying (Duguma *et al.*, 2023).

In 2022, a cross-sectional study carried out in various districts of the Waghemra Zone in Northeast Ethiopia found that the overall prevalence of malaria was 21.2%. The *P. falciparum* parasite was responsible for the majority of malaria cases, making up 67.8% of the infections.

Among asymptomatic participants, 7.5% were diagnosed with malaria through RDT and 10.2% through microscopy. On the other hand, in symptomatic individuals, the malaria prevalence was significantly higher, with 44.5% diagnosed by RDT and 48.4% diagnosed by microscopy. The study also identified several factors that were positively associated with the prevalence of malaria, including the presence of stagnant water near homes, the utilization of insecticide-treated mosquito nets, the quantity of such nets, spending time outdoor at night (Debash *et al.*, 2023).

A study conducted in 2022 in the Boset District of Oromia, Ethiopia, assessed 328 pregnant women and found that the prevalence of asymptomatic *Plasmodium* infection was 2.74% when diagnosed via microscopy and 3.05% using RDTs. Among the women diagnosed with malaria, 71.4% exhibited moderate parasitaemia, while 28.6% had mild parasitaemia. The study identified several factors potentially linked to *Plasmodium* infection, including gravidity, the use and ownership of ITNs, discussions about malaria prevention during antenatal care visits, and residing near stagnant water sources (Balcha *et al.*, 2023).

A cross-sectional study conducted in Boricha district, Sidama, Ethiopia in 2021 revealed that among 573 asymptomatic participants tested for malaria, 6.1% tested positive using RDTs and 4.0% using microscopy. The study found a higher prevalence in males compared to females, with 54.3% of males testing positive with RDTs and 56.5% with microscopy. Among the RDT-positive cases, *P. falciparum* represented 88.6%, while *P. vivax* made up 11.4%. In microscopy-positive cases, *P. falciparum*, *P. vivax*, and mixed infections were identified in 82.6%, 13.0%, and 4.3% of cases, respectively. Several factors were identified as being associated with the prevalence of asymptomatic malaria, including age, use of ITNs, the presence of eaves and windows, previous travel history, educational background, and occupation (Dabaro *et al.*, 2023).

A 2019 cross-sectional study in Raya Kobo District, Northeast Ethiopia, found that 7.0% of the 270 participants had asymptomatic malaria. The prevalence rates using RDT, microscopy, and PCR were 3.0%, 5.2%, and 12.0% respectively. In terms of the species of malaria parasites identified, 57.9% were *P. falciparum* and 42.1% were *P. vivax*. Notably, PCR detected asymptomatic malaria cases approximately 2.7 times higher than RDT and 2.3 times higher than microscopy. Significant predictors of asymptomatic malaria included previous malaria history,

living with index cases, and having a family size greater than six members (Melese, Y. *et al.*, 2022).

Another community-based cross-sectional study carried out in the Debre Elias district in 2018 found that among 440 participants, the prevalence of malaria was 5% as diagnosed by microscopy and 5.5% by RDT. In asymptomatic individuals, the prevalence of malaria was found to be 4.8% using RDT and 4.2% with microscopy. The prevalence of symptomatic malaria was 7.5%, diagnosed by either RDT or microscopy. Factors such as the use of ITNs, availability of nets, having a house with eaves, previous malaria infection history, and family history of malaria infection were identified as significantly associated with malaria infection (Abebaw *et al.*, 2022).

In 2018, a study in the Dembiya District of North Gondar assessed malaria prevalence through a cross-sectional parasitological and retrospective survey among 2,157 individuals at two health facilities, finding an overall prevalence of 22.4%. Of the 735 slides examined, 3.5% (n=26) were found to be malaria-positive, predominantly caused by *P. falciparum* at 2.3%, followed by *P. vivax* at 0.7%, and mixed infections at 0.5 The research showed that males had a 2.6 times higher likelihood of contracting malaria compared to females and that those who frequently engaged in outdoor activities were 16.4 times more vulnerable to malaria. The findings also revealed a significant connection between awareness of malaria transmission and prevalence, emphasizing the importance of educational initiatives to combat the disease (Tarekegn *et al.*, 2021).

A 2019 community-based cross-sectional study in the Gondar Zuria district revealed a 12% (30/251) prevalence of asymptomatic *Plasmodium* infection, with 5.6% in males and 6.4% in females. The study identified cases of *P. falciparum* and *P. vivax*, with prevalence rates of 2% (5/251) and 10% (25/251), respectively. The highest rate of asymptomatic *Plasmodium* infections was observed in females (6.4%) and among individuals aged 15 to 29 years (4.4%). The research also highlighted significant associations between asymptomatic infections and various factors, including age, usage of bed nets, and history of previous malaria infections (Minwuyelet *et al.*, 2020).

Another community-based cross-sectional study carried out in 2017 involving the residents of Dembia district found that 6.7% of the 832 adults included in the study tested positive for

malaria parasite. The most common species was *P. falciparum*, accounting for 82% of cases, followed by *P. vivax* at 9%, and mixed infections at 9%. The study also revealed that factors such as being male, aged 15-19 years, having a history of travel, and having stagnant water around the home increased the likelihood of being infected with malaria. Conversely, the use of insecticidal treated nets (ITNs) decreased the probability of infection (Fekadu et al., 2018).

A cross-sectional study carried out in Mirab Abay district of Southern Ethiopia, (2014-2015) found that 1.2% of 422 surveyed school children had asymptomatic *Plasmodium* infections detected by microscopy, while RDTs identified a prevalence of 3.6%. Of the total cases, 8 (3.8%) were female and 7 (3.3%) were male. The total prevalence of asymptomatic *Plasmodium* carriage, encompassing both *P. falciparum* and *P. vivax*, was found to be 3.6% (15 cases). Of those with *Plasmodium* carriage, 11 individuals (73.4%) were infected with *P. flciparum*, while 4 individuals (26.6%) had P. vivax infections. Interestingly, the study did not find any correlation between the prevalence of asymptomatic *Plasmodium* carriage and the gender or age of the school children (Abossie et al., 2017).

Seasonal Cross-sectional surveys conducted from 2014 to 2015 in eight kebeles Jimma town, Oromia found that, out of the 1434 suspected cases examined, 428 cases were confirmed as clinical malaria, Among the confirmed cases, 327 (76.4%) were identified as *P. vivax*, 97 (22.7%) were *P. falciparum*, and 4 (0.9%) were mixed infections of both *P. vivax* and *P. falciparum*. The annual incidence rate was 1.7 cases per 1000 individuals at risk, with parasite prevalence in the community below 3%. *P. falciparum* was notably linked to being male and having traveled, whereas the risk factors associated with *P. vivax* malaria included a person's occupation and a recent history of malaria within the last 30 days (Zhou *et al.*, 2016).

A retrospective study at the Kola Diba Health Center in Ethiopia (2011) examined malaria prevalence from 2002 to 2011, analyzing 59,208 blood films which revealed 23,473 confirmed cases, at 39.6%. The main malaria species identified were *P. falciparum* (75% of cases) and *P. vivax* (25%). The study found that malaria affected all age groups and both sexes, with higher infection rates in males (52.6%) compared to females (47.3%), particularly among the 15-44 age group. The data indicated significant seasonal variation in malaria cases, peaking in spring, highlighting the ongoing challenges of malaria control in the region and the need for continuous monitoring and intervention strategies (Alemu *et al.*, 2012).

In 2014 health facility-based retrospective study in the Wolaita Zone reviewed laboratory-confirmed malaria cases over five years, revealing 105,755 (33.27%) total cases with an average of 21,151 cases annually. The study identified *P. falciparum* as the predominant species, responsible for 71.80% of cases. It also showed the highest slide positivity rates of 40.5% and 35.5% respectively in children aged 5–14 years and 1–4 years. Two peak seasons for malaria were noted: from September to December and April to June, underscoring the ongoing challenge of malaria and the necessity for continued surveillance and intervention efforts in the region (Legesse *et al.*, 2015).

Another retrospective study in Woreta, northwestern Ethiopia, from 2005 to 2012 examined 102,520 suspected malaria cases, confirming 33,431 cases (32.6% positivity by microscopy). Of these, 52.9% were males and 47.1% were females. The research found that children under five years were 1.3 times more likely to contract malaria than those aged 5 to 15 years. *P. falciparum* was the predominant species, making up 69.7% of cases compared to 26.5% for *P. vivax*, with a significant statistical difference. The study recorded a fluctuation in yearly malaria prevalence, ranging from 7% in 2008 to 47% in 2005, highlighting the persistent public health challenge posed by malaria in the region (Alelign *et al.*, 2018).

A 2013 cross-sectional study in Sanja Town, Northwest Ethiopia, involving 385 school children, found a 6.8% prevalence of asymptomatic malaria, with 65.5% of infected individuals showing low parasite density. The study identified two malaria species: *P. falciparum*, with a prevalence of 5.2%, and *P. vivax*, with a prevalence of 1.6%. The findings indicated that 9.8% of male participants had asymptomatic malaria, compared to 5.1% of female participants. There was a significant association between age and both *P. falciparum* and *P. vivax* infections. Additionally, factors such as school grade level, age, bed net usage, and regular exposure to malaria were linked to an increased risk of asymptomatic malaria (Worku *et al.*, 2014).

A community-based cross-sectional survey conducted in 2012 among pastoralist communities of Benna Tsemay district revealed an overall malaria prevalence of 6.1% (28/461). Among the cases of malaria, 18(64.3%) were caused by *P. falciparum*, 6 (21.4%) were caused by *P. vivax*, and 4 (14.3%) were mixed infections. Malaria prevalence was highest among infants (12.0%) and children under five years old (9.8%). Lactating women had an infection rate of 22.2% and

pregnant women had a rate of 17.6%, which was higher than other community groups. Traditional approaches to malaria prevention and treatment did not significantly correlate with the incidence of malaria. However, pregnancy status and the practice of reserving mosquito nets for future use were found to be independently linked to a greater prevalence of malaria infection (Debo & Kassa, 2016).

### 1.4.7. Malaria and the Military

Malaria has historically had a significant impact on the military, causing more deaths than combat in the 20th century (Mertens, 2024). It has influenced various wars, such as the Revolutionary War, Civil, World war I, World war II, Korean war, and Vietnam war, resulting in illness, fatalities, and impaired combat effectiveness (Brabin, 2014). During World War II, malaria caused significant troop casualties. General Douglas MacArthur of the U.S. Army was more concerned about controlling the *Anopheles* mosquito than defeating Japanese forces, as it was responsible for the deaths of 60,000 U.S. troops in Africa and the South Pacific (Dhiman *et al.*, 2010). In the 1942 campaign to stop the Japanese advance, approximately 24,000 of the 75,000 American and Filipino soldiers were affected by malaria (Paltzer, 2016).

Deployment to areas with high malaria prevalence is a concern, except in Bosnia. The disease continues to pose a threat to military personnel deployed in countries like Afghanistan, Iraq, and Liberia, causing casualties and hindering combat effectiveness. Even Mediterranean regions with milder climates are at risk of malaria outbreaks during transmission seasons, affecting military campaigns such as the Italian and Sicilian campaigns. A specific instance in 2006 involved a French military group observing 39 cases of malaria among personnel who had recently returned from a mission in Ivory Coast (Mayet *et al.*, 2010; Pagès *et al.*, 2010).

In 2017, a cross-sectional study was carried out on Soldiers in Zahedan, Iran. The study included 300 samples, and it was discovered that 4 samples (1.3%) tested positive for *P. vivax* and showed signs of asymptomatic malaria. Out of these 4 samples, 3 were from individuals native to Sistan and Baluchistan, while the remaining sample was from an individual native to Kerman. No other types of *Plasmodium* species were observed during the study (Sekandarpour & Shaddel, 2019)

A study conducted in 2017 in the Champasak and Attapeu provinces of the Lao Democratic Republic revealed that among 313 military personnel, the malaria infection prevalence was

11.2%. The specific types of infections were as follows: *Plasmodium falciparum* (1.3%), *Plasmodium vivax* (9.3%), and mixed infections (0.6%). PCR testing identified malaria parasites in 35 out of 313 blood samples, also reflecting an 11.2% rate. RDTs confirmed three cases of *P. vivax* mono-infection, six cases of *P. falciparum* mono-infection, and one case of a mixed infection involving both *P. falciparum* and *P. vivax.* Approximately one-third of the participants utilized long-lasting insecticidal nets, but their ability to use mosquito repellents and coils was limited due to supply issues (Vilay *et al.*, 2019).

A cross-sectional survey conducted in 2015 at four military camps in Tanzania found that the prevalence of malaria among asymptomatic recruits was 20.3% and 19.4% according to microscopic examination and mRDT tests respectively. The most common malaria species detected was *P. falciparum*, accounting for 99.2% of cases, while only 0.8% of samples had mixed infections of *P. falciparum* and *P. malariae*. There was a moderate level of agreement between the results of microscopy and mRDT tests. Interestingly, the prevalence of malaria parasitaemia, as determined by both microscopy and mRDT, was significantly higher among individuals aged ≤20 years and females (Kalinga *et al.*, 2019).

A 2015 study at the Nigerian Army 2 Amphibious Brigade barracks found that 52.5% of 1,461 participants tested positive for malaria. Most infections were among non-commissioned officers (80.44%), followed by commissioned officers (12.12%) and civilians (7.43%), with no significant differences in infection rates among these groups. Among infected females, commissioned personnel had the highest infection rate at 64.6%, while in males; children of commissioned officers had the highest parasitaemia at 56.5%. The study also found a significant difference in parasite load based on gender, indicating varying malaria impact across demographics (Egbom & Nzeako, 2017).

A case series study conducted within in the US Army healthcare system found that among 725 soldiers from a Ranger Task Force deployed to eastern Afghanistan from June to September 2002, there were thirty-eight confirmed cases of malaria. The attack rate was calculated to be 52.4 cases per 1000 soldiers, with all cases being caused by *P. vivax*. Two cases of relapse required treatment at 459 and 508 days after the deployment ended. An anonymous survey conducted after deployment revealed that self-reported compliance rates for various preventive measures included 52% for weekly chemoprophylaxis, 41% for terminal (post-deployment)

chemoprophylaxis, and 31% for both weekly and terminal chemoprophylaxis. Additionally, 82% reported treating their uniforms with permethrin, while only 29% reported using insect repellent (Kotwal RS *et al*., 2005).

In 2003, 44 U.S. Marines deployed in Liberia experienced an outbreak of *P. falciparum* malaria, prompting their evacuation. Of those affected, 14 (32%) were confirmed through blood smears, and 10 (31%) of the first 32 tested positive with the NOW ICT test. The affected Marines, all male and aged 19 to 43, had received preventive medicine education, but only 45% regularly used insect repellent, 12% wore permethrin-treated clothing, and none used bed nets. Additionally, only 55% adhered to weekly mefloquine medication. The outbreak was linked to insufficient personal protection and poor adherence to preventive measures, underscoring the need for better malaria prevention strategies for military personnel in high-risk areas (Whitman *et al.*, 2010).

## 1.5. Conceptual frame work

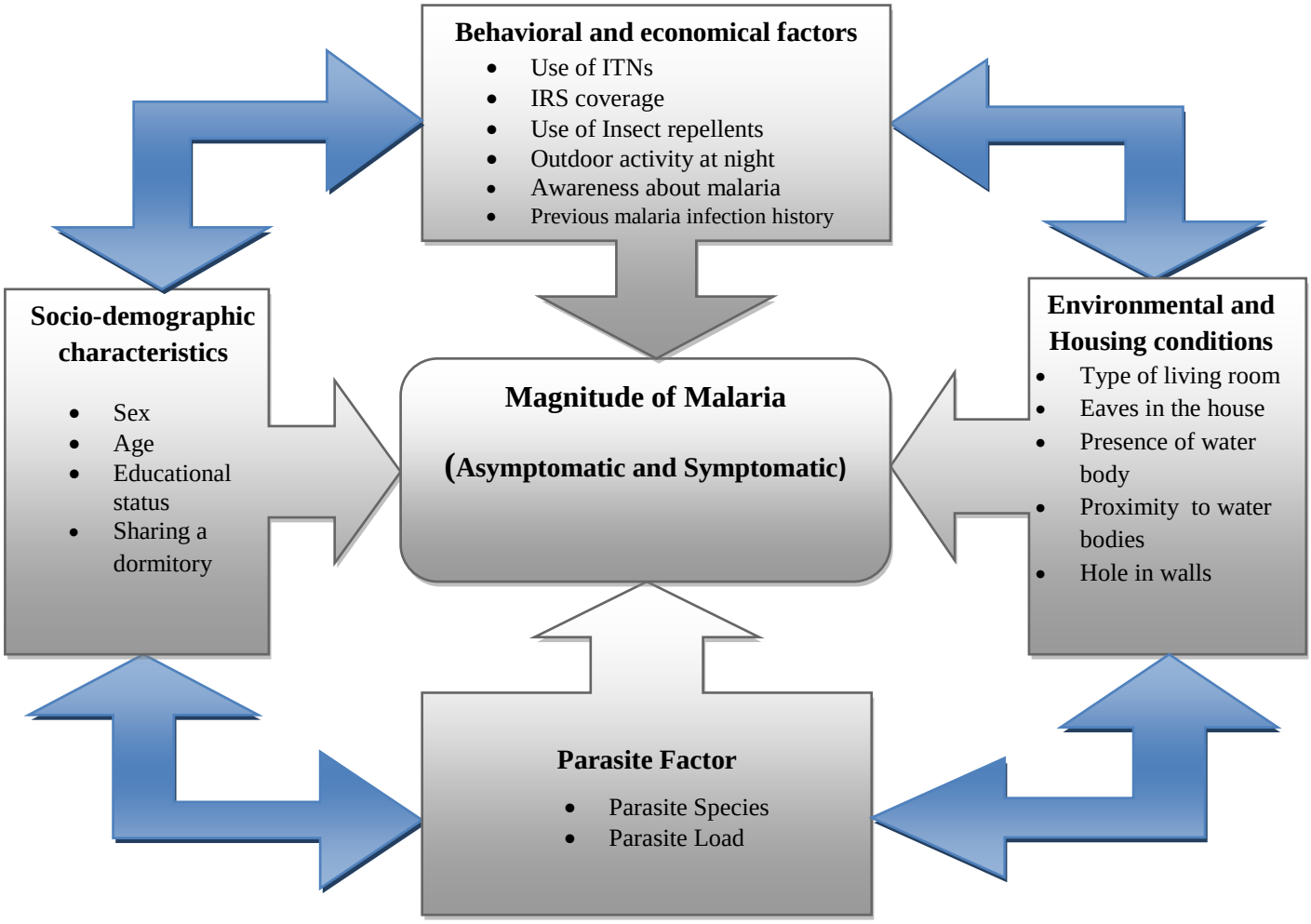


Figure 4: Conceptual framework, which is developed after a review of different literature (Abebaw *et al.*, 2022; Dabaro *et al.*, 2023; Debash *et al.*, 2023; Duguma *et al.*, 2023; Tarekegn *et al.*, 2021; Zhao *et al.*, 2018).

## 1.6. Research Questions

- What is the current burden of malaria among military personnel in the study area?
- Which species of *Plasmodium* is most prevalent among military personnel in the study area?
- What are the factors associated with malaria in military personnel?

# 2. .Objectives

## 2.1. General Objective

To assess the magnitude of asymptomatic and symptomatic malaria and associated factors among military personnel in the Bilate Commando and Airborne training center, Southern Ethiopia.

## 2.2. Specific Objectives

- To determine the magnitude of asymptomatic malaria among military personnel
- To determine the magnitude of symptomatic malaria among military personnel
- To identify factors associated with malaria infection among military personnel

# 3. Materials and Methods

## 3.1. Study Area

The study was conducted at the Bilate Commando and Airborne Training Center, located in the Diguna Fango district of Wolayita zone, South Ethiopia Regional State. The district is situated in the Great Rift Valley, spanning an area of 371 square kilometers, with an altitude ranging from 1,395 to 2,070 meters above sea level. It is approximately 350 kilometers south of Addis Ababa and 60-80 kilometers from Sodo, the capital of Wolaita Zone. The district shares borders with Damot Weyde to the southwest, Damot Gale to the west, Hadiya Zone to the north, Oromia Region to the northeast, and Sidama Region to the east, separated by the Bilate River. The area has a humid climate with an average annual rainfall of 700 mm and a temperature of 21°C. Malaria is a major health issue in the region, affecting 12 administrative districts, with cases reported consistently throughout the year, primarily caused by the *P. falciparum* and *P. vivax* parasites. Bitena Town is the administrative center of the woreda. The Bilate Commando and Airborne Training Center is located at coordinates 6°45'19.4"N 38°02'51.8"E, situated 305 kilometers south of Addis Ababa, 86 kilometers from Hawassa, and 25 kilometers from Wolaita Dimtu (also known as Bilate Tena) (Tadesse *et al.*, 2017; Wikipedia, 2023).

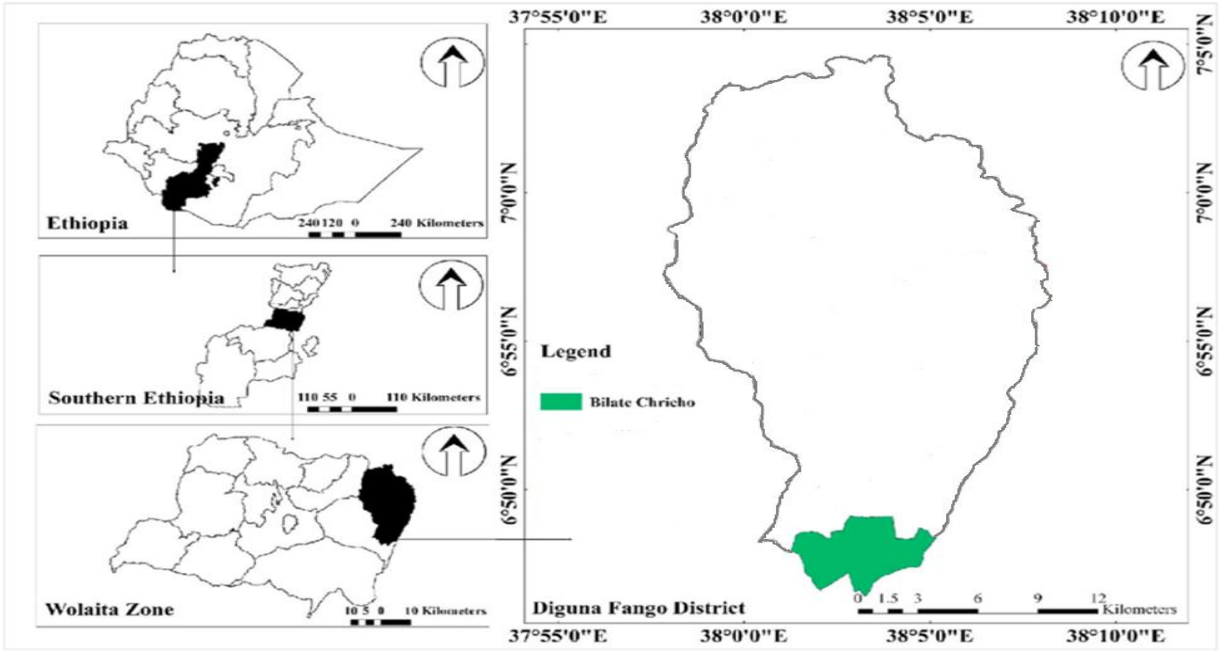


Figure 5: Map of Study area Diguna Fango District, Wolaita Zone, Southern Ethiopia (Wendimu & Tekalign, 2023)

## 3.2. Overview of malaria prevention activities in the study area

Malaria prevention efforts in the study area are essential due to its classification as the second most endemic region among Ethiopia's military training centers. This high prevalence is influenced by the influx of new recruits and commando trainers from various parts of the country, as well as the center's location in the Wolaita zone, which is known for its endemic malaria.

To combat malaria, the center employs several strategies. The primary treatment available for all *Plasmodium* species infections is artemether-lumefantrine (AL), also known as Coartem, for uncomplicated cases, while intravenous artesunate is used for severe malaria. However, chloroquine and primaquine are not available due to the high number of cases and limited supplies, making it difficult to effectively control malaria transmission with primaquine. For prevention, the center employs insecticide-treated nets (ITNs) and indoor residual spraying (IRS) to diminish mosquito populations. Additionally, insect repellents are used during fieldwork, and efforts are made to eliminate mosquito breeding sites as part of daily activities. These comprehensive measures aim to significantly reduce the risk of malaria transmission in the area.

## 3.3. Study Design and Period

A facility-based cross-sectional study was conducted at the Bilate commando and airborne training center in Southern Ethiopia from May to June, 2024. The study focused on active military personnel and recruits of the Ethiopian National Defense Force. The survey was conducted during a period of low malaria transmission.

## 3.4. Population

### 3.4.1. Source Population

All active military personnel serving in the Ethiopian National Defense Force, including those in the Ground Force, Air Force, Naval Force, and new recruits, during the specified study period.

### 3.4.2. Study Population

- All active military personnel and new recruits currently serving at the Bilate Commando and Airborne Training Center in the Ethiopian National Defense Force, who met the eligibility criteria during the study period, were the study population.

## 3.5. Eligibility criteria

### 3.5.1. Inclusion Criteria:

- All current military personnel who have resided in the selected military camp for at least a month.
- The study participants were individuals who were either afebrile (had a body temperature less than 37.5°C) or febrile (had a body temperature of 37.5°C or higher) within the past 48 hours. Their body temperatures were confirmed using digital axillary thermometers.
- The participants who provided both oral and written consent and were present during data collection were included in the study.

### 3.5.2. Exclusion Criteria:

- The study excluded soldiers who were currently taking anti-malarial medication or had used it in the month prior to data collection.
- Those individuals with severe illnesses were not included in the study.

## 3.6. Sample Size and Sampling Techniques

### 3.6.1. Sample Size Determination

The sample size was calculated using a formula intended for a single population proportion.

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

In this calculation, (n) represents the total sample size. The value of ($Z_{\alpha/2}$) is 1.96, which corresponds to a 95%, CI. The margin of error (d) is set at 5%, and the expected occurrence rate of malaria, (p), is assumed to be 50% due to the absence of prior studies conducted in military settings in Ethiopia. Additionally, a non-response rate of 5% is accounted for.

$$n = \frac{(1.96)^2 \times 0.5(1-0.5)}{0.0025} = \mathbf{384}$$

Total sample size = 384 + (384 × 0.05) =**403**

Table 2: Sample size determination for the third objective of the study.

The necessary sample size to address the third objective can be determined using OpenEpi version 7 statistical software.

| Variables | Significance level | Power | % in unexposed | AOR (95% CI) | Sample size | Literature |
|---|---|---|---|---|---|---|
| Outdoor sleeping | 95% | 80 | 8.3% | 2.76 | 310 | (Aschale *et al.*, 2018) |
| Previous malaria history | 95% | 80 | 4.3% | 3.39 | 306 | (Minwuyelet *et al.*, 2020) |
| Stagnant water around home | 95% | 80 | 5.5% | 3.7 | 266 | (Fekadu et al., 2018) |
| Bed net availability and usage | 95% | 80 | 11% | 0.16 | 272 | (Molla and Ayele, 2015) |

As a result, the sample size of 403 was selected from objective one, as it exceeded the calculated sample sizes resulting from objective two.

### 3.6.2. Sampling Techniques/ Procedure

Bilate Commando and Airborne Training Center has been selected purposefully based on the endemicity and reported magnitude of malaria infection in the area. To ensure a representative sample size, a systematic sampling technique was utilized. The overall sample size of the study was evenly allocated to each regiment, as each military unit consisted of approximately 600 soldiers and had an equal number of personnel. From four brigades in Bilate, two brigades were randomly selected using a lottery method, and four regiments were then chosen from each selected brigade, also through a lottery process. From these four regiments, a total of 403 active military members were selected from the register, amounting to about 101 individuals from each regiment.

To select participants, a predetermined interval, known as the sampling interval, was established. This interval was calculated by dividing the total number of soldiers (600) by the desired sample size (101), resulting in a sampling interval of 6. A random starting point between 1 and 6 was chosen, and participants were selected at regular intervals of 6 until the target sample size was achieved. Importantly, all active military members who met the eligibility criteria in the four regiments were included in the study. Once selected, participants remained at the study site, where data collection took place.

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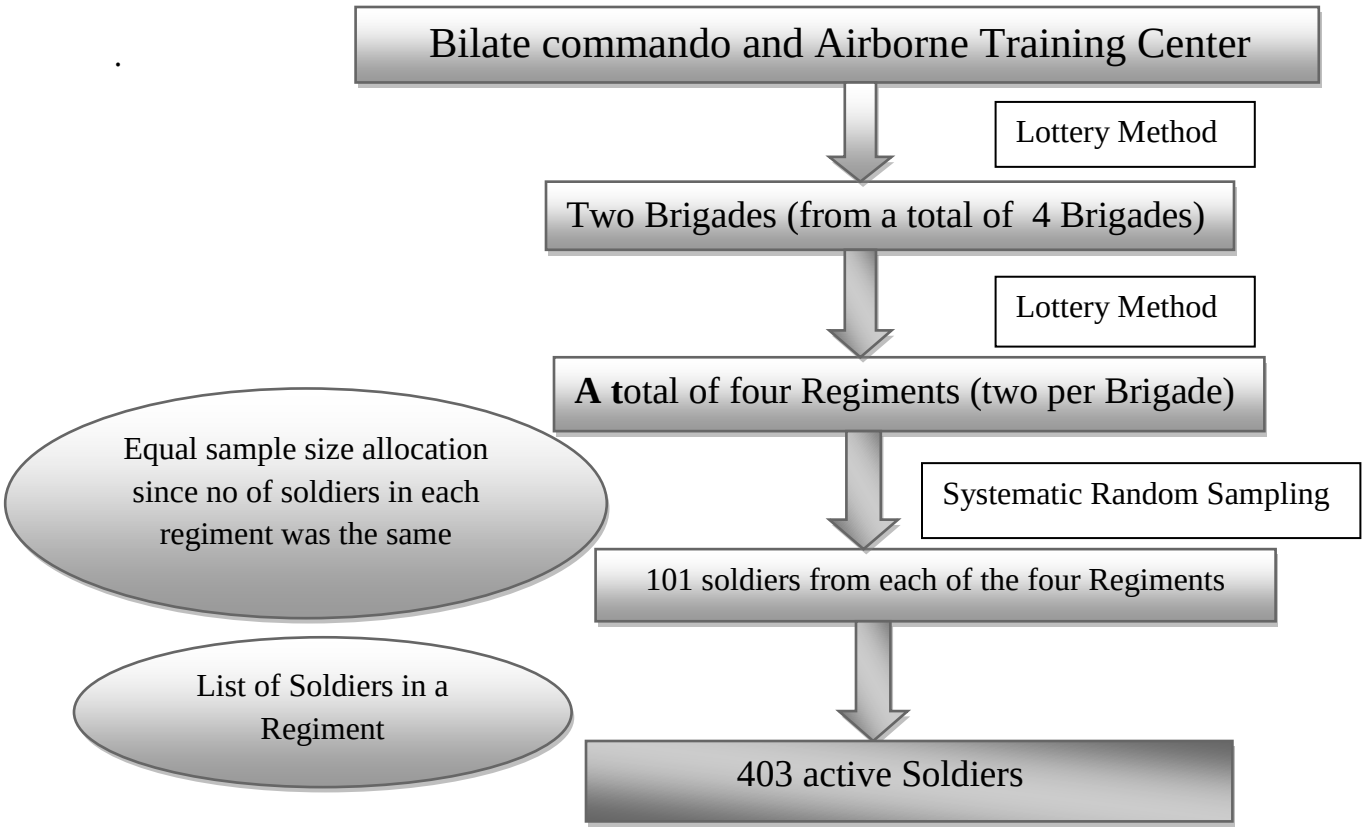


Figure 6: Schematic Presentation of Sampling Procedure

## 3.7. Study Variables

### 3.7.1. Dependent variable

- Magnitude of malaria (asymptomatic and symptomatic)

### 3.7.2. Independent Variables

- Socio demographic characteristics: age, sex, education, marital status.
- Housing condition: presence of eaves and presence of hole in the wall of house
- ITN coverage: availability and utilization of ITN
- IRS coverage: duration after spray
- Utilization of mosquito repellants
- Previous history of malaria infection
- Family history of malaria infection
- Distance of house to surface water bodies
- Health education
- Outdoor activities at night
- Environmental conditions

## 3.8. Operational Definitions

- **Asymptomatic malaria:** refers to the presence of asexual or sexual malaria parasites in the bloodstream without any noticeable symptoms particularly the absence of common symptoms like fever within a period of two days and at the time of testing. This condition does not include the dormant liver stages of the parasite.
- **Symptomatic malaria**: presence of malaria related symptoms (fever i.e., axillary temperature ≥37.5 °C, chills, headache, vomiting, joint pain etc.) within the past 2 days and at a time of examination and the presence of malaria parasites in blood.
- **Barracks**: Buildings designed to house military members and quasi-military personnel such as police officers.

## 3.9. Data Collection Instrument and Data management

### 3.9.1. Data Collection Team

The principal investigator provided training to data collectors on the study's objectives, data collection tools, and ethical considerations, and data collection was conducted in the Amharic language. A senior health officer was hired to evaluate clinical symptoms. The principal investigator and experienced lab technicians were in charge of collecting and examining blood samples using both rapid diagnostic tests and microscopic examination methods independently. In situations where the results varied, a third technician, unaware of the previous results, was brought in to address the discrepancy. The principal investigator oversaw all tasks and activities throughout the process.

### 3.9.2. Malaria Associated Factors

Socio-demographic information and malaria risk factor data were gathered using a pretested, self-administered questionnaire. The questionnaire was adapted from similar studies and covered various factors related to *Plasmodium* spp. infection at both the individual and household levels. These factors included family size, availability of mosquito nets, (IRS) coverage, type of walls, history of malaria, and housing conditions. The questionnaire was first prepared in English and subsequently translated into Amharic. A senior health officer used a digital thermometer to assess body temperature and differentiate symptomatic from asymptomatic cases among individuals selected from the study site.

### 3.9.3. Blood Sample Collection and processing

A disposable, sterilized lancet was used to obtain finger prick blood specimens for dried blood spot (DBS), rapid diagnostic test (RDT), and blood film. Approximately 2 μL and 6 μL of blood were collected on a glass slide to create thin and thick blood smears, respectively. In addition, three to four drops of blood were promptly placed on Whatman filter paper for molecular analysis. Thin smears were fixed in absolute methanol for 30 seconds, followed by staining with 10% Giemsa solution for 10 minutes. Afterward, they were air-dried and analyzed under a microscope by the investigator and other experienced lab technicians. The DBS samples were securely placed in individual double zip-lock plastic bags with desiccants and transported to the Armauer Hansen Research Institute (AHRI) in a cold box for molecular testing of malaria using

PCR analysis. Furthermore, five µL of finger prick blood was collected for the rapid diagnostic test (RDT).

### 3.9.4. Microscopic Examination

Blood sample were collected from each participant, and both thick and thin blood smears were prepared on the same slide. The slides were examined under a light microscope at high power magnification (100 xs) for parasites. Thick films were first assessed at the same magnification to check for parasites. If a positive result was found, the specific species was determined through the analysis of thin smears. The results were categorized as negative (no malaria parasites found), positive for a specific *Plasmodium* species, or indicative of a mixed infection. To measure parasite density in thick smears, asexual parasites were counted relative to 200 white blood cells (WBCs); if fewer than 99 parasites were identified in 200 WBCs, the count was extended to at least 500 WBCs. Calculations were based on an average WBC count of 8,000/µl, as per WHO recommendations. A thick smear was deemed negative if no parasites were seen in at least 100 high-power fields (WHO, 2010).

$$\text{No. parasites/µl of blood} = \left(\frac{\text{parasites counted}}{\text{200 WBCs counted}}\right) 8000 \text{ WBCs}$$

### 3.9.5. Rapid Diagnostic Tests (Bioline$^{TM}$ Malaria Ag Pf/Pv HRP2/pLDH Abbott)

For this study, the Bioline$^{TM}$ Malaria Ag Pf/Pv HRP2/pLDH Abbott test kits were employed. These kits are designed in a cassette format and utilize lateral flow immunochromatography. They enable the differential detection of histidine-rich protein-II (HPR2) specific to *P. falciparum* and *Plasmodium* lactate dehydrogenase (pLDH) specific to *P. vivax* in human whole blood. To conduct the test, the instructions provided by the manufacturer were followed. Upon opening the pouch, the test kits were labeled with the corresponding sample code. A volume of 5 µl of blood specimen was applied to the sample pad of the test kit. Four drops of buffer were then added to the buffer pad. This buffer served the purpose of lysing the cells, releasing the antigen, and facilitating the recognition of antibodies. After 15 minutes, the results of the rapid diagnostic test (RDT) were read and interpreted accordingly (Annex I, 2).

### 3.9.6. DNA Extraction from Dried Blood Spot

*Plasmodium* genomic DNA from DBS was extracted by heating the samples in a 10% Chelex®-100 solution. This heating process, which reaches temperatures close to 95-100 ºC in an alkaline medium, disrupts cell membranes and breaks down proteins, including those that are heat-sensitive. At the same time, Chelex®-100 serves as an ion chelator, protecting the DNA from damage by inactivating nucleases and binding heavy metals that could potentially harm the parasite's DNA. This method enables the effective extraction of parasite DNA suitable for PCR analysis (Archive, 2024; Simon *et al.*, 2020).

### 3.9.7. Real-Time PCR Assay (qPCR)

In this study, using DNA samples, extracted from DBS were then run with qPCR to detect and quantify *Plasmodium* species, particularly *P. falciparum* and *P. vivax*. Out of the 250 DBS samples, 124 were positive for malaria by RDT or microscopy, while 126 were negative. qPCR was run on Bio-Rad CFX Opus 96 qPCR machine. The limited number of samples analyzed was primarily due to economic constraints associated with the testing process.

Real-Time Polymerase Chain Reaction (qPCR) is a technique that enables the real-time monitoring of PCR reactions by detecting fluorescence emitted by a reporter molecule. In this study, qPCR amplification was performed in a total reaction volume of 11.9 µL, which included 2.1 µL of nuclease-free water, 6 µL of TaqMan Fast Advanced Master Mix, 0.4 µL of Pf18s forward and reverse primers, 0.2 µL of Pv18s forward and reverse primers, 0.1 µL of Pf18s probe, 0.1 µL of Pv18s probe, and 3 µL of extracted DNA. For *P. falciparum*, the reaction utilized 300 nM primers and 111 nM probes, while for *P. vivax*, 200 nM primers and 110 nM probes were used in a multiplex format. A serial dilution of the Pf/ Pv 18 plasmid was prepared, ranging from 10^7/mL to 10^3/mL. Additionally, both positive and negative controls were included to assess the quality of DNA extraction and the efficiency of the qPCR amplification.

The thermal cycling protocol starts with a 2-minute pre-incubation at 50°C to mix and stabilize the reagents, ensuring proper hydration of primers and even distribution of components. It then proceeds to a 10-minute denaturation at 95°C to eliminate residual DNA and activate certain DNA polymerases. This is followed by 45 amplification cycles, which include denaturing at 95°C for 15 seconds and annealing at 60°C for 1 minute, optimized for effective DNA

amplification and quantification (Lorenz, 2012). The target genes for *P. vivax* and *P. falciparum* were selected based on the sequences of the small subunit ribosomal RNA (18S rRNA), which features both conserved and variable regions. Additionally, this gene exists in at least five copies that are spread across different chromosomes within the Plasmodium genome (Gruenberg *et al.*, 2018; Kamau *et al.*, 2014; Mangold *et al.*, 2005).

In this assay, one of the most commonly used fluorescence markers, the TaqMan probe, was utilized. This hydrolysis probe features a reporter dye, commonly fluorescein (FAM), at its 5' end and a quencher, tetramethylrhodamine (TAMRA), at its 3' end. During the extension phase of the PCR, the Taq polymerase degrades the probe through its 5' nuclease activity, which separates the fluorescein from the quencher, generating a fluorescence signal. As the PCR cycles progress, the number of fluorescent molecules increases, leading to a rise in fluorescence intensity that is directly correlated with the amplification of the target DNA (Liu *et al.*, 2006). In this qPCR assay, the cut-off points were set at a Ct-value of 38.78 for *P. falciparum* and 36.52 for *P. vivax* to determine positive and negative results.

**Primers and probes sequences**

| | |
|---|---|
| **Pf18S_FW** | GTAATTGGAATGATAGGAATTTACAAGGT |
| **Pf18S_RV** | TCAACTACGAACGTTTTAACTGCAAC |
| **Pf18S_Probe** | FAM-AACAATTGGAGGGCAAG-MGBNFQ |
| **Pv18S_FW** | AGCAGCCGCGGTAATTCCA |
| **Pv18S_RV** | ATGCGCACAAAGTCGATACGAAG |
| **Pv18S_Probe** | TEXASRED-AGCAACGCTTCTAGCTTAATCCACAT-BHQ2 |

### 3.9.8. Quality Control

To ensure data quality, data collectors received training, and tools were discussed to guarantee complete and consistent data collection. A pre-test was carried out to assess the questionnaire's quality. Before data entry, returned questionnaires were carefully reviewed for completeness, and necessary corrections were made. All test procedures and result interpretations followed standard operating procedures. For proper use of a thermometer, we adhered to the manufacturer's guidelines, regularly cleaned it, waited for the audible signal to confirm temperature measurement, and adjusted the thermometer using an ice water bath or manual calibration methods. The expiry dates of reagents and materials were checked daily to ensure their

effectiveness. To control the quality of the Giemsa stain, we properly prepared it, followed recommended staining time and temperature, and used positive and negative blood films as controls. In a similar manner, positive and negative controls consisting of known human samples were utilized in PCR to verify the quality of the reagents and materials. The positive control confirmed the effectiveness of the PCR reaction in identifying the desired genetic material, while the negative control indicated the absence of the target genetic material. Any discrepancies in microscopic readings were reanalyzed by an experienced laboratory technologist. Additionally, another laboratory technician conducted internal quality control checks on a random sample of 5% of negative slides and all positive slides.

### 3.9.9. Pre-test

A pre-tested was conducted in a malarious area that was not included in the study. This pre-test involved 5% of the total sample size. The purpose was to evaluate the clarity, understandability, flow, and completeness of the questionnaire, as well as the time required to fill it out. Following the results of the pre-test, discussions took place with the data collectors, and adjustments were made to clarify any unclear questions.

## 3.10. Data Analysis

Data input and cleaning were carried out using Epi Data 3.1, while data analysis utilized the Statistical Package for Social Sciences (SPSS) version 27. Descriptive statistics, including frequency distributions and proportions, were employed to summarize the demographic characteristics of the study participants. Bivariate and multivariable logistic regression analyses were performed to explore the relationship between independent variables and the outcome/dependent variable. Variables that showed an association with the outcome in the bivariate regression analysis (with a P-value of $< 0.25$) were included in the multivariate analysis to account for potential confounders. A *p-value* of $< 0.05$ was deemed statistically significant. The data was reported using descriptive text, frequencies, odds ratios, and percentages, and illustrated in tables and charts. Additionally, the levels of parasitemia among individuals were analyzed to determine their correlation with clinical symptoms and disease severity. The Mann-Whitney U test was conducted to assess the differences in parasite density between symptomatic and asymptomatic soldiers.

## 3.11. Dissemination of Result

The research findings will be shared with the relevant institutions including Addis Ababa University, College of Health Sciences, Department of Microbiology, Immunology, and Parasitology, Ethiopian Defense University College of Health Sciences, and the study sites for future reference. Furthermore, a summary of the thesis will be submitted for publication in a peer-reviewed journal.

# 4. Ethical Consideration

Approval for ethical clearance was granted by the Department Research Ethics Review Committee (DRERC) within the Department of Microbiology, Immunology, and Parasitology at the College of Health Sciences, Addis Ababa University. In addition, permission to conduct the study was sought from the Ethiopian National Defense Force Commando and Airborne main division, as well as the Bilate commando and airborne training center. Before participating in the study, all potential participants were provided with comprehensive information regarding the purpose and objectives of the research. They were invited to participate on a voluntary basis, and written consent was collected from every participant. Once permission was granted, individuals responded to questionnaires and provided blood samples for analysis. To maintain confidentiality, participant-related data were coded rather than using their names. This ensured that the privacy and anonymity of the participants were protected throughout the study. It's important to mention that individuals diagnosed with either symptomatic or asymptomatic malaria were directed to the nearest healthcare facility for suitable treatment, following the national guidelines for malaria treatment guidelines.

# 5. Result

## 5.1. Socio- demographic Characteristics

A total of 403 participants were included in the study. The majority were male, n=372 (92.31%). The mean age of participants was 21.3 years (± 3.1 SD), with ages ranging from 18 to 50 years. Most participants fell within the age group of 18-25 years, totaling 374 (92.8%). Additionally, the majority of the participants held the rank of Junior Enlisted Ranks in the military, accounting for n=395 (98 %). Among the participants, 376 (93.3%) were single, and 224 (55.58%) had completed secondary school or higher. Most participants (n=355, 88.09%) resided in permanent living arrangements. On average, soldiers shared their barracks with 26.2 individuals, with a range of 1 to 92 individuals per barrack. Furthermore, most soldiers (n=205, 50.9%) lived in barracks with 15-30 other members (Table 3).

Table 3: Socio-demographic characteristics of participants, Bilate Training Center; May to June 2024 (N=403).

| Socio-demographic Variables | Category | Frequency (%) | (Mean ± SD) |
|---|---|---|---|
| **Sex** | Male | 372 (92.3) | |
| | Female | 31(7.7) | |
| **Age (Year)** | 18-25 | 374(92.8) | (21.3 ± 3.1) |
| | 26-33 | 25(6.2) | |
| | >=34 | 4(1) | |
| **Military rank** | Junior Enlisted Ranks | 395(98) | |
| | Officers | 7 (1.7) | |
| | Field Officers | 1(0.3) | |
| **Marital status** | Single | 376 (93.3) | |
| | Married | 19(4.7) | |
| | Widowed | 4(1) | |
| | Divorced | 4(1) | |
| **Educational Status** | Cannot read and write | 6(1.5) | |
| | Read and write | 4(1) | |
| | Primary school | 169(41.9) | |
| | Secondary school and above | 224(55.6) | |
| **Type of Living room** | Temporary | 48(11.9) | |
| | Permanent (Barrack) | 355(88.1) | |
| **Number of soldiers sharing the barrack** | <15 | 74(18.3) | (26.2 ± 12.2) |
| | 15-30 | 205(50.9) | |
| | >=31 | 124(30.8) | |
| **Malaria symptoms within the past 2 days** | Asymptomatic (<37.5 °c) | 293(72.7) | |
| | Symptomatic (≥ 37.5 °c) | 110(27.3) | |

## 5.2. Magnitude of Malaria

### 5.2.1. Magnitude of overall, symptomatic and asymptomatic malaria

The study assessed magnitude malaria infection using RDTs, microscopy, and qPCR. The overall magnitude of malaria was found to be 37% (149/403, 95% CI=32.3-41.7). The magnitude rates obtained through microscopy and RDT alone were 30.8% (124/403, 95% CI=26.3-35.3), and 29.5% (119/403, 95% CI=25.1-34), respectively. Molecular analysis using qPCR identified parasite DNA in 59.6% (149/250, 95% CI= 55.2-63.8) of DBS samples. Notably, qPCR confirmed the presence of parasite DNA in all samples that tested positive through microscopy and RDTs.

In addition, qPCR identified asymptomatic parasitemia in 21 samples that were negative by microscopy and 22 samples that were negative by RDTs, as well as detecting symptomatic parasitemia in 4 microscopy-negative and 8 RDT-negative cases. Ultimately, of the 149 positive qPCR results, 119 infections (79.9%) were identified through the combined use of all three diagnostic techniques by microscopy, RDTs, and qPCR.

All 403 participants were assessed for clinical manifestations related to malaria. Among these, 72.7% (293/403) were asymptomatic, while the remaining 27.3% (110/403) exhibited symptoms. The magnitude of malaria among asymptomatic individuals was 18.1% (53/293), 17.7% (52/293), and 45.1% (74/164) as measured by microscopy, RDTs, and PCR, respectively. On the other hand, the rate of malaria among symptomatic individuals was 64.5% (71/110), 60.9% (67/110), and 87.2% (75/86) by microscopy, RDTs, and PCR, respectively (Table 4).

In this study, 398 samples (98.8%) demonstrated perfect concordance between the two diagnostic methods microscopy and the Bioline™ RDTs assay for overall malaria diagnosis. Of these, 29.5% were positive, and 69.2% were negative. Asymptomatic individuals showed the highest rates of both positive results and perfect concordance between the two assays. As a result, we can conclude that there is excellent agreement between microscopy and the Bioline™ RDT for the diagnosis of malaria in this study.

Furthermore, the assessment of gametocyte rate through microscopy revealed that 33.9% (42/124) of both microscopy positive asymptomatic and symptomatic soldiers were gametocyte

carriers. Of these carriers, 78.6% (33/42) were asymptomatic, while the remaining 21.4% (9/42) were symptomatic soldiers.

Table 4: Magnitude of asymptomatic, symptomatic and overall malaria, Bilate Training Center; May to June 2024 (N=403).

| Health Status | RDT | | | Microscopic | | | PCR | | Total | Total Pos n(%) |
|---|---|---|---|---|---|---|---|---|---|---|
| | Pos n(%) | Neg n(%) | Total | Pos n(%) | Neg n(%) | Total | Pos n(%) | Neg (%) | | |
| **Asymptomatic** | 52 (17.7) | 241 (82.3) | 293 | 53 (18.1) | 240 (81.9) | 293 | 74 (45.1) | 90 (54.9) | 164 | 74 (45.1) |
| **Symptomatic** | 67 (60.9) | 43 (39.1) | 110 | 71 (64.5) | 39 (34.5) | 110 | 75 (87.2) | 11 (12.8) | 86 | 75 (87.2) |
| **Overall** | 119 (29.5) | 284 (70.5) | 403 | 124 (30.8) | 279 (69.2) | 403 | 149 (59.6) | 101 (40.4) | 250 | 149 (37) |

Neg: Negative, PCR: Polymerase Chain Reaction, Pos: Positive, RDT: Rapid Diagnostics Test

### 5.2.2. Proportion of *Plasmodium* Species in assymptomatic and symptomatic individuals Detected by microscopy, RDTs, qPCR.

Out of 403 soldiers examined for malaria, 149 were confirmed as malaria-positive by qPCR, which included all cases that tested positive via microscopy and RDTs. Among the 149 qPCR-confirmed malaria cases, the distribution of *Plasmodium* species was: 45.6% (68/149) *P. falciparum*, 44.3% (66/149) *P. vivax*, and 10.1% (15/149) had mixed infection of *P. falciparum* and *P. vivax*. Within the confirmed malaria cases, there were 83 cases of *P. falciparum* (50.6%) and 81 cases of *P. vivax* (49.4%).

Specifically, among the 74 qPCR-confirmed asymptomatic malaria cases, , the species distribution showed that 33.8% (25/74) were *P. falciparum*, 59.4% (44/74) were *P. vivax*, and 6.8% (5/74) had mixed infections. In contrast, for the 75 qPCR-confirmed symptomatic malaria cases, the proportions were significantly different, with 57.3% (43/75) being *P. falciparum*, 29.3% (22/75) being *P. vivax*, and 13.3% (10/75) having mixed infections.

Additionally, qPCR identified 6 further cases of *P. falciparum*, 11 cases of *P. vivax*, and 4 mixed infections among asymptomatic soldiers that were deemed negative by microscopy or RDT. In symptomatic individuals, qPCR found 1 case of *P. falciparum*, 2 cases of *P. vivax*, and 1 mixed infection that were also reported as negative by microscopy or RDT.

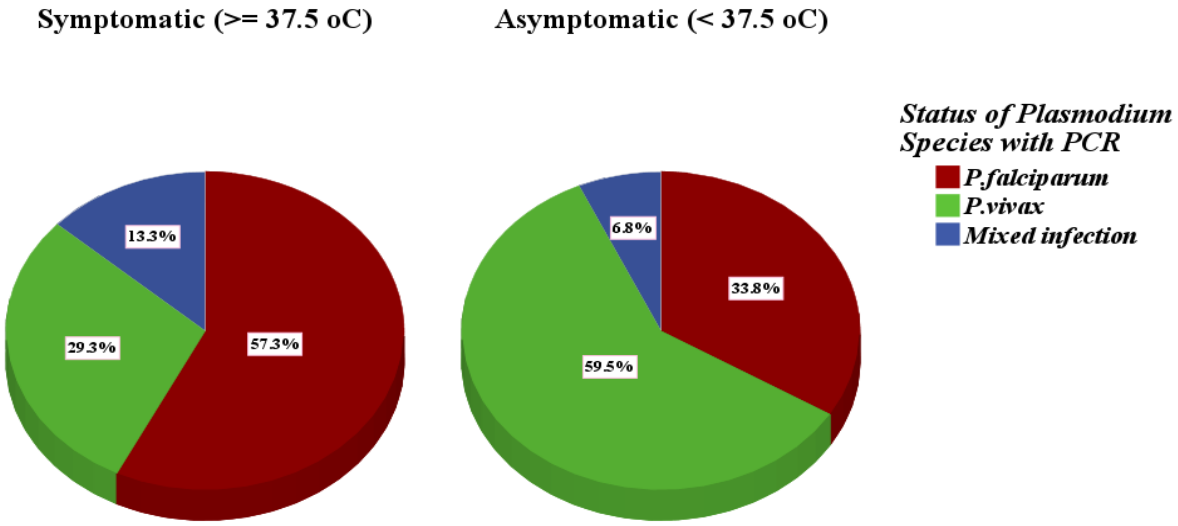


Figure 7: Relative Proportion of *Plasmodium* Species among symptomatic and asymptomatic soldiers confirmed by qPCR, Bilate Training Center; May to June 2024 (N=403).

### 5.2.3. Magnitude of symptomatic and asymptomatic malaria across different age groups and sex

The survey findings indicated a higher prevalence of both asymptomatic and symptomatic malaria among males compared to females. Specifically, the malaria prevalence was recorded at 37.4% (139/372) for males, while females exhibited a prevalence of 32.3% (10/31). Among the asymptomatic cases, the highest prevalence was observed in both male and female participants aged 18-25 years, which accounted for (69 cases), followed by the 26-33 age group, which comprised (4 cases) out of a total of 74 asymptomatic cases. Similarly, the majority of symptomatic malaria cases were also found in the 18-25 age groups, representing 71 out of 75 cases. Notably, there were no confirmed malaria cases among female participants aged 34 years or older (Table 6).

Table 5: Magnitude of symptomatic and asymptomatic malaria across different age groups and sex, Bilate Training Center; May to June 2024 (N=403).

| **Variables** | | **Asymptomatic soldiers** | | **Symptomatic soldiers** | | |
|---|---|---|---|---|---|---|
| **Sex** | Age group | Total | Pos (n, %) | Total | Pos (n, %) | Total Pos (n, %) |
| **Male** | 18-25 | 252 | 64(25.4) | 98 | 68(69.4) | 139(37.4%) |
| | 26-33 | 14 | 3(21.4) | 4 | 2(50) | |
| | >=34 | 3 | 1(33.3) | 1 | 1(100) | |
| | Total | 269 | 68(25.3) | 103 | 71(68.9) | |
| **Female** | 18-25 | 18 | 5(27.8) | 6 | 3(50) | 10(32.3%) |
| | 26-33 | 6 | 1(16.7) | 1 | 1(100) | |
| | >=34 | 0 | 0(0) | 0(0) | 0(0) | |
| | Total | 24 | 6(25) | 7 | 4(57.1) | |
| **Total** | | 293 | 74 | 110 | 75 | 149 (37%) |

Pos; Positive

### 5.2.4. Density of *Plasmodium* parasite

In this study, the density of parasites was quantified using microscope and quantitative PCR (qPCR). The results of qPCR indicated that parasite DNA levels exceeded 200 copies/µl, with only five infections showing copy numbers below 1,000 copies/µl. Among the participants who tested positive for infection via qPCR, 118(79.2%) individuals had densities ranging from 1,000 to 1,000,000 copies/µl, while 26(16.8%) individuals exhibited densities greater than 1,000,000 copies/µl.

The qPCR analysis indicated an overall parasite density with a geometric mean of 101,531 copies/µl (95% CI, 70,000-147,265 copies/µl) and an interquartile range (IQR) of 529,058 copies/µl (25,136-554,194 copies/µl). The parasite copy numbers ranged from a minimum of 200 copies/µl to a maximum of 9,264,243 copies/µl. Specifically for *P. falciparum*, the geometric mean was 222,998 copies/µl (95% CI, 129,062-385,212 copies/µl) with an IQR of

1,048,734 copies/µl (81,759-1,130,495 copies/µl). In contrast, *P. vivax* showed a geometric mean of 23,845 copies/µl and an IQR of 99,144 copies/µl (6,694-105,838 copies/µl).

The analysis also highlighted that symptomatic soldiers had significantly higher parasite densities, with a geometric mean of 335,583 copies/µl (95% CI, 221,462-508,394 copies/µl), compared to 30,227 copies/µl (95% CI, 18,557-49,238 copies/µl) in asymptomatic soldiers. Furthermore, it was observed that male participants had higher parasite copy numbers than their female counterparts (Table 7).

In addition to qPCR, microscopic quantification of parasite density was performed for all malaria parasites detected by microscopy (n=123). The parasite densities ranged from 240 to 557,920 asexual parasites/µl, with a geometric mean of 24,378 asexual parasites/µl (95% CI, 18,767-31,674 parasites/µl) and an interquartile range (IQR) of 61,320 asexual parasites/µl (10,840-72,160 asexual parasites/µl).

Among the study participants, symptomatic soldiers exhibited the highest parasite densities, with a geometric mean of 41,390 parasites/µl (95% CI, 30,374-56,390 parasites/µl), compared to asymptomatic soldiers, who had a geometric mean of 11,836 parasites/µl (95% CI, 8,084-17,326 parasites/µl) (Table 7).

For *P. falciparum*, the geometric mean was 43,793 asexual parasites/µl (95% CI, 31,218-61,433 parasites/µl) with an IQR of 83,760 parasites/µl (26,480-110,240 parasites/µl). In contrast, *P. vivax* showed a geometric mean of 8,734 copies/µl (95% CI, 6,298-12,112 parasites/µl) and an IQR of 24,540 parasites/µl (3,980-28,520 parasites/µl).

Additionally, it was noted that male participants had higher parasite densities, with a geometric mean of 25,206 asexual parasites/µl (95% CI, 19,134-33,205 parasites/µl), compared to their female counterparts, who had a geometric mean of 15,097 asexual parasites/µl (95% CI, 6,792-33,558 asexual parasites/µl).

Table 6: qPCR and microscopic quantification of parasite density in symptomatic and asymptomatic soldiers, Bilate Training Center; May to June 2024 (N=403).

| Symptom status | Parasite density (copies/asexual parasite/µl) | Methods of quantification | | Total n(%) | Geometric mean of parasite density by qPCR and microscopy |
|---|---|---|---|---|---|
| | | qPCR | Microscopy | | |
| **Symptomatic** | <1000 | 1 | 1 | 2(1.4) | 335,583 copies/µl (95% CI, 221,462-508,394) and 41,390 asexual parasites/µl (95% CI, 30,374-56,390) |
| | 1000-9999 | - | 7 | 7(4.8) | |
| | 10,000-99999 | 17 | 41 | 58(39.7) | |
| | 100,000-999999 | 34 | 22 | 56(38.4) | |
| | >=1,000,000 | 23 | - | 23(15.7) | |
| | Total | 75 | 71 | 146(100) | |
| **Asymptomatic** | <1000 | 4 | 3 | 7(5.5) | 30,227 copies/µl (95% CI, 18,557-49,238) and 11,836 asexual parasites/µl (95% CI, 8084-17,326) |
| | 1000-9999 | 16 | 17 | 33(26.2) | |
| | 10,000-99999 | 32 | 31 | 63(50) | |
| | 100,000-99999 | 19 | 1 | 20(15.9) | |
| | >=1,000,000 | 3 | - | 3(2.4) | |
| | Total | 74 | 52 | 126(100) | |
| **Overall** | | 149 | 123 | 272 | 101,531 copies/µl and 24,378 asexual parasites/µl |

qPCR: Quantitative Polymerase Chain Reaction, µl: Micro litter

To assess the significance of the findings, the Mann-Whitney U test was performed to determine if there is a statistically significant difference in parasite density between soldiers exhibiting symptoms and those who were asymptomatic. The results indicated a (U statistic of 1032.500 and a p-value of $< 0.001$), confirming a statistically significant difference in malaria parasite density between the two groups. Specifically, these results suggest that individuals with elevated body temperatures ($\geq 37.5$ °C) have higher parasite densities compared to those with normal temperatures. Overall, these findings highlight a potential link between fever and the severity of

malaria infections, suggesting that higher parasite loads may be associated with the presence of fever in infected individuals.

## 5.3. Analysis of Risk Factors for Malaria Infection

### 5.3.1. Bivariate analysis of malaria and its aassociated factors

Bivariate analysis was conducted at a significance level of $p< 0.05$, identifying seven variables significantly associated with malaria prevalence. These variables included the number of soldiers sharing a barrack (specifically those with 15-30 soldiers), type of living arrangement, presence of eaves in the house, use of insect repellent, previous malaria infection history, experiences of mosquito bites, and educational messages about malaria. In contrast, socio-demographic factors such as sex, age, and educational status did not show a significant correlation with malaria infections ($p > 0.05$). Additionally, factors such as proximity to water bodies, holes in house walls, outdoor activities at night, presence of artificial containers and man-made ditches, availability and use of insecticide-treated nets (ITNs), indoor residual spraying (IRS) coverage in the past 12 months, family history of malaria, and travel history to malaria-endemic areas were not associated with malaria infection.

The analysis indicated that soldiers sharing a barrack with other 15-30 soldiers were approximately 1.4 times more likely to contract malaria compared to those in barrack with fewer than 15 soldiers [COR=1.361 (0.906-2.043)]. Another factor significantly linked to malaria infection was the use of insect repellent; individuals who did not use insect repellent were about 1.3 times more likely to contract malaria than those who did use it [COR=1.314 (0.824-2.094)]. Additionally, soldiers with a history of malaria exposure had a 1.6 times increased likelihood of contracting malaria compared to those without such a history [COR=1.600 (1.034-2.478)].

Another variable that demonstrated significant associations was presence of eaves in the house. Soldiers residing in homes with eaves had about 1.6 times the likelihood of contracting malaria compared to those in homes without eaves [COR=1.567 (1.043-2.354)]. In terms of living arrangements, individuals residing in temporary accommodations faced a 1.7 times higher risk of malaria infection than those in permanent homes [COR=1.672 (0.912-3.067)]. Furthermore, individuals who experienced mosquito bites were 2.1 times more likely to become infected with malaria than those who did not experience bites, as indicated by a [COR=2.137 (0.900-5.075)].

Lastly, individuals who were unaware of malaria faced a 3.7 times greater risk of contracting the disease compared to those who were informed, with a [COR=3.740 (1.635-8.558)] (Table 8).

Table 7: Bivariate Analysis for factors associated with malaria infection among symptomatic and asymptomatic soldiers, Bilate Training Center; May to June 2024 (N=403).

| Variables | | Malaria | | Bivariate Analysis | |
|---|---|---|---|---|---|
| | | Positive n(%) | Negative n(%) | (COR 95% CI) | P-value |
| **Sex** | Male | 139(34.5) | 233(57.8) | 1.253 (0.253-2.738) | 0.572 |
| | Female | 10(2.5) | 21(5.2) | Ref | Ref |
| **Age** | 18-25 | 140 (34.7) | 234 (58.1) | 1.330 (0.589-3.001) | 0.493 |
| | 26-33 | 7 (1.7) | 18 (4.5) | 0.646 (0.263-1.586) | 0.341 |
| | >=34 | 2 (0.5) | 2 (0.5) | Ref | Ref |
| **Formal Education** | Cannot read and write | 3 (0.7) | 3 (0.7) | 1.732(0.342-8.779) | 0.507 |
| | Primary School | 64 (15.9) | 109 (27) | 1.017(0.674-1.534) | 0.937 |
| | Secondary school and above | 82 (20.3) | 142 (35.2) | Ref | Ref |
| **Number of soldiers sharing a dormitory** | <15 | 24 (6) | 50 (12.4) | Ref | Ref |
| | 15-30 | 83 (20.6) | 122 (30.3) | 1.361 (0.906-2.043) | 0.137* |
| | >=31 | 42 (10.4) | 82 (10.4) | 0.823 (0.528-1.283) | 0.390 |
| **Type of living room** | Temporary | 23 (5.7) | 25 (6.2) | 1.672 (0.912-3.067) | 0.097* |
| | Permanent | 126 (31.3) | 229 (56.8) | Ref | Ref |
| **Presence of water bodies near the house** | Present | 147(36.5) | 250 (62) | 1.176 (0.213-6.499) | 0.853 |
| | Absent | 2 (0.5) | 4 (1) | Ref | Ref |
| **Distance of water bodies from the house** | <=1000m | 35 (8.7) | 73 (18.2) | 0.768 (0.482-1.224) | 0.267 |
| | >1000m | 113 (28.1) | 181 (45) | Ref | Ref |
| **Presence of eave in the house** | Yes | 69 (17.1) | 146 (36.2) | 1.567 (1.043-2.354) | 0.030* |
| | No | 80 (19.9) | 108 (26.8) | Ref | Ref |
| **Hole in the wall of the house** | Yes | 50 (12.4) | 91 (22.6) | 0.905 (0.591-1.385) | 0.645 |
| | No | 99 (24.6) | 163 (40.4) | Ref | Ref |
| **Presence of grass around the house** | Present | 120 (29.8) | 198 (49.1) | 1.170 (0.708-1.934) | 0.540 |
| | Absent | 29 (7.2) | 56 (13.9) | Ref | Ref |
| **Artificial container and man-made** | Present | 60 (14.9) | 91 (22.6) | 1.208 (0.797-1.830) | 0.374 |
| | Absent | 89 (22.1) | 163 (40.4) | Ref | Ref |

| | | | | | |
|---|---|---|---|---|---|
| **ditches around home** | | | | | |
| **Outdoor activities overnight** | Yes | 142 (35.2) | 247 (61.3) | 0.575 (0.198-1.672) | 0.310 |
| | No | 7 (1.7) | 7 (1.7) | Ref | Ref |
| **Availability of ITN in the house** | Yes | 114 (28.3) | 200 (49.6) | Ref | Ref |
| | No | 35 (8.7) | 54 (13.4) | 0.879 (0.542-1.426) | 0.603 |
| **Utilization of ITN** | Daily | 103 (25.5) | 176 (43.7) | Ref | Ref |
| | Occasionally | 9 (2.2) | 16 (4) | 0.981 (0.610-1.576) | 0.936 |
| | Not Using | 37 (9.2) | 62 (15.4) | 0.943 (0.378-2.348) | 0.899 |
| **IRS coverage in the past 12 months** | Yes | 70 (17.4) | 124 (30.8) | Ref | Ref |
| | No | 79 (19.6) | 130 (32.2) | 1.076 (0.718-1.614) | 0.721 |
| **Taking chemoprophylaxis** | Yes | 11 (2.7) | 15 (3.7) | Ref | Ref |
| | No | 138 (34.2) | 239 (59.3) | 0.787 (0.352-1.762) | 0.561 |
| **Utilization of insect repellent** | Yes | 114 (28.3) | 181 (44.9) | Ref | Ref |
| | No | 35 (8.7) | 73 (18.1) | 1.314 (0.824-2.094) | 0.251* |
| **Previous malaria infection history** | Yes | 107 (26.6) | 156 (38.7) | Ref | Ref |
| | No | 42 (10.4) | 98 (24.3) | 0.625 (0.404-0.968) | 0.035* |
| **Family member prior exposure to malaria** | Yes | 56 (13.9) | 82 (20.3) | 1.263 (0.827-1.928) | 0.279 |
| | No | 93 (23.1) | 172 (42.7) | Ref | Ref |
| **Travel history to malaria- endemic area** | Yes | 51 (12.7) | 100 (24.8) | 0.801 (0.526-1.222) | 0.304 |
| | No | 98 (24.3) | 154 (32.8) | Ref | Ref |
| **Experiencing a mosquito bite** | Yes | 137 (34) | 244 (60.5) | 2.137 (0.900-5.075) | 0.085* |
| | No | 12 (3) | 10 (2.5) | Ref | Ref |
| **Educational message about malaria** | Yes | 131 (32,5) | 245 (60.8) | Ref | Ref |
| | No | 18 (4.5) | 9 (2.2) | 3.740 (1.635-8.559) | 0.002* |

COR: Crude Odds Ratio, (*): Statistical Significance at a *p-value* of less than 0.05, Ref: Reference Category.

### 5.3.2. Multivariate analysis of factors associated with malaria infection

In this study, potential malaria-associated factors that demonstrated a *P value* of < 0.25 in the bivariate analysis were selected for multivariate logistic regression to control for confounding variables. Seven variables were included in the backward stepwise multivariate logistic regression model, (including the number of soldiers sharing a dormitory (specifically those with

15-30 soldiers), type of living arrangement, presence of eaves in the house, use of insect repellent, Previous malaria infection history, experiences of mosquito bites, educational messages about malaria. Ultimately, only two variables emerged as significant in the multivariate model following the stepwise selection process, previous malaria infection history and educational messages about malaria ($p < 0.05$) (Table 10).

Of the 403 soldiers who participated in the study, 263 (65.3%) reported a history of previous malaria infection, while 140 (34.7%) had no such history. Additionally, 376 (93.3%) of participants indicated that they were aware of malaria, whereas 27 (6.7%) were completely unaware of the disease.

The analysis indicated that soldiers without a previous malaria infection history were 0.49 times less likely to contract malaria compared to those with such a history, with an AOR of 0.508 (95% CI= 0.317-0.813). Furthermore, individuals lacking awareness about malaria were approximately 3.4 times more likely to be infected than those who had received educational messages regarding malaria, with an AOR of 3.385 (95% CI = 1.433-7.995) (Table 9).

Table 8: Bivariate and multivariate analysis of factors associated with malaria among symptomatic and asymptomatic soldiers, Bilate Training Center; May to June 2024 (N=403).

| Variables | | Malaria | | Bivariate Analysis | | Multivariate Analysis | |
|---|---|---|---|---|---|---|---|
| | | **Pos n (%)** | **Neg n(%)** | **COR (95%, CI)** | ***P value*** | **AOR (95%, CI)** | ***P value*** |
| **Previous malaria infection history** | Yes | 107 (26.6) | 156 (38.7) | Ref | Ref | Ref | Ref |
| | No | 42 (10.4) | 98 (24.3) | 0.625 (0.404-0.968) | 0.035* | 0.508 (0.317-0.813) | 0.005* |
| **Educational message about malaria** | Yes | 131 (32.5) | 245 (60.8) | Ref | Ref | Ref | Ref |
| | No | 18 (4.5) | 9 (2.2) | 3.740 (1.635-8.559) | 0.002* | 3.385 (1.433-7.995) | 0.005* |

AOR: Adjusted Odds Ratio, COR: Crude Odds Ratio, Pos: Positive, Neg: Negative,

(*): Statistical Significance at a *p-value* of less than 0.05, Ref: Reference Category.

# 6. Discussion

Although previous malaria research among Ethiopian military personnel is lacking, both asymptomatic and symptomatic *Plasmodium* infections are prevalent in military communities throughout Ethiopia, especially in regions with unstable and seasonal transmission patterns. Detecting *Plasmodium* parasites among military personnel is vital for reducing transmission and advancing malaria control and elimination strategies. This study represents one of the first epidemiological assessments of malaria and its associated risk factors among soldiers in the Ethiopian military, particularly at the Bilate Commando and Airborne Training Center in southern Ethiopia. The study included 403 active military personnel (372 males and 31 females), with a mean age of 21.34 years (SD ± 3.192) and a median age of 20 years.

The present study identified a notably high prevalence of symptomatic and asymptomatic malaria within military communities, despite the widespread implementation of mosquito bed nets and other preventive strategies. This indicates that malaria remains to be a significant cause of morbidity among military personnel in our study area. This may be attributed to the location of military training centers, which provided conducive breeding habitats for *Anopheles* mosquitoes, thereby increasing the risk of malaria transmission. Additionally, military activities often take place outdoors during peak mosquito activity periods (dawn and dusk), resulting in greater exposure to mosquito bites. Furthermore, a significant number of asymptomatic carriers of malaria parasites, who exhibit no noticeable symptoms, may contribute to the ongoing transmission of the malaria parasite within the military population as mentioned in the previous studies (Kalinga *et al.*, 2019; Starzengruber *et al.*, 2014; Vilay *et al.*, 2019).

The analysis identified a high malaria positive rate of 37% within military communities. The results also highlighted positive rates of asymptomatic and symptomatic malaria at 25.3% and 68.1%, respectively. These findings align with other community-based studies conducted in different parts of the world and Ethiopia which demonstrated high prevalence rates, e.g., 33.6% in Malaysia (Ramdzan *et al.*, 2020), and 30.7% in Bangladesh (Starzengruber *et al.*, 2014). This finding is also comparable to the 36.1% Adi Arkay, North Gondar (Tesfa *et al.*, 2018), and 32.6% in Woreta, Northwestern Ethiopia (Alelign *et al.*, 2018). Additionally, studies conducted in the Abeshge District indicates prevalence of 33.8% (Yimer *et al.*, 2015), and in Wolaita Zone a prevalence of 33.3% (Legesse *et al.*, 2015), which are also comparable to our study.

Notably, the prevalence in this study was higher than that reported in several other studies, including 25% in East Shewa zone, Oromia (Tadesse *et al.*, 2018), 22.4% Dembia District, North Gonder (Tarekegn *et al.*, 2021). Additionally, it exceeds the prevalence rates of 21.1% in Waghemira Zone (Debash *et al.*, 2023), and 27% in Cameroon (Mbah *et al.*, 2022).

Furthermore, the findings are also considerably higher than those from military settings in other countries, such as 1.3% in Zahedan, Southeast Iran (Sekandarpour & Shaddel, 2019), 11.2% in Champasak and Attapeu, Lao PDR (Vilay *et al.*, 2019), and 20.3% in selected military camps in Tanzania (Kalinga *et al.*, 2019). In contrast, it was lower than that reported in several other areas. Specifically, an 49.4% in East Wollega (Bidu & Babure, 2019), and prevalence rates of 80.3%, 52.2%, and 78.3% were reported in various areas of Nigeria (Adepeju, 2017; Egbom & Nzeako, 2017; Ogomaka, 2020).

The observed difference in malaria prevalence may be influenced by several factors, including the type and risk profile of the study participants, particularly the focus on military populations in the current research. Variations in practices related to malaria prevention and control within the study area are also important. Moreover, differences in diagnostic techniques, geographical context, seasonal variations, timing of data collection, and climatic factors can all contribute to the differing prevalence rates.

The present study found that the rate of asymptomatic malaria was 25.3%. This finding is comparable to studies conducted in various regions of Ethiopia, such as 18.4% in West Armachiho, 21.5% in Gambella and 29.8% in Jimma (Aschale *et al.*, 2018; Girma *et al.*, 2019; Zhou *et al.*, 2016). Additionally, it aligns with findings from other countries, including 20.3% in Tanzania (Kalinga *et al.*, 2019), 23.3% along the China-Myanmar border (Zhao *et al.*, 2018). However, the magnitude rate of asymptomatic *P. falciparum* and *P. vivax* infections identified in this study was higher than that reported in several areas of Ethiopia, such as 7% in Raya Kobo (Melese, Yimer *et al.*, 2022), and 4.2% in Mojo (Abate *et al.*, 2022). It was also higher than 6.1% in Boricha Sidama (Dabaro *et al.*, 2023), but lower than the 34.4% observed in Tanzania (Chacha *et al.*, 2024).

Moreover, the rate of symptomatic malaria was significantly high at 68.2%. This result is consistent with findings from a study in Hallaba, which reported a rate of 82.8%(Tefera, 2014) ,

as well as from Afar, where the rate was 64% (Woday *et al.*, 2019). Similarly, the results were comparable to studies in Tanzania, which found a rate of 89% (Sumari *et al.*, 2017), , and in South Western Nigeria, where it reached 93.3% (Adepeju, 2017). However, this rate was higher than that recorded in a study conducted in Siraro Woreda, West Arsi Zone, which reported a rate of 41% (Lakew *et al.*, 2023).

The variations noted in this study can be attributed to several key factors. Differences in study design and sampling methods significantly contribute to these disparities. Moreover, the timeframe of the study is significant, as our research was conducted during a period of low malaria transmission. The demographics of the study participants also play a vital role; our sample primarily consisted of military personnel over the age of 18, with a majority being male. Furthermore, variations in malaria control and prevention strategies, as well as differences in sample size and diagnostic methods may further explain the observed differences.

In the current study, *P falciparum* and *P. vivax* were found to be co-endemic in the investigated regions, When considering the overall malaria infections, the distribution *plasmodium* species was nearly equal, with *P. falciparum*, accounting for 45.6% of cases, followed by *P. vivax* at 44.3% and mixed infections that comprised the remaining 10.1%. This was confirmed through various diagnostic methods, including microscopy, RDTs, and qPCR. Specifically, qPCR detected 68 cases of *P. falciparum* mono-infections, 66 cases of *P. vivax* mono-infections, and 15 mixed infections. In comparison, microscopy identified 61 *P. falciparum* mono-infections, 53 *P. vivax* mono-infections, and 10 mixed infections. Meanwhile, RDT results showed 56 *P. falciparum* mono-infections, 53 *P. vivax* mono-infections, and 10 mixed infections.

The unsurprising difference between microscopy and RDT results compared to qPCR findings may be attributed mainly to the low sensitivity and possible false-negative outcomes associated with microscopy and RDTs. In contrast, qPCR provides greater sensitivity and specificity for detecting both *P. falciparum* and *P. vivax,* highlighting its superiority over conventional diagnostic methods (Abebe *et al.*, 2023; Beyene *et al.*, 2022; Siwal *et al.*, 2018).

This finding differs from the national reports in Ethiopia (FMoH, 2012, 2016), which indicated a predominance of *P. falciparum* over *P. vivax*, as those assessments relied on microscopy and RDTs. Our study, which employed qPCR, identified a greater number of *P. vivax* cases that were

missed by conventional methods due to low parasitemia.. The predominance of *P. falciparum* among symptomatic cases is in line with findings from various studies conducted in different regions of Ethiopia, such as the Debre Elias District, East Wollega zone, Jawi District, and Kombolcha (Abebaw *et al.*, 2022; Bidu & Babure, 2019; Dagnew *et al.*, 2024; Gebretsadik *et al.*, 2018).

Additionally, high prevalence rates of *P. falciparum* have been reported in countries such as the Ghana and Zambia, report prevalence rates of 68% and 89%, respectively (Heinemann *et al.*, 2020; Sitali *et al.*, 2019). In Tanzania, this figure rises dramatically to 99.2% (Kalinga *et al.*, 2019), where *P. falciparum* accounts for a significant number of infections.

Conversely, studies conducted in Wolkite (Solomon *et al.*, 2020), Jimma town (Zhou *et al.*, 2016), and in Mojo (Abate *et al.*, 2022) reveal that *P. vivax* predominates over *P. falciparum infection*. Additionally, research in Arba Minch (Nega *et al.*, 2015), and the Gondar Zuria District (Minwuyelet *et al.*, 2020) also indicates a predominance of *P. vivax* in asymptomatic *infection* . Similar trends have been observed along the China–Myanmar border (Zhao *et al.*, 2018) and in Zahedan, Iran (Sekandarpour & Shaddel, 2019), where *P. vivax* predominates among asymptomatic individuals.

This variation may be attributed to differences in the epidemiological distribution of *Plasmodium* species in different regions of Ethiopia, along with *P. vivax's* ability to survive in colder climates compared to other species. Furthermore, many malaria control strategies and national treatment guidelines focus primarily on *P. falciparum* due to its higher mortality, which results in less attention being given to *P. vivax*, allowing it to remain stable within the population (Ketema *et al.*, 2021).

The higher prevalence of *P. vivax* in asymptomatic cases is largely due to its distinct biological characteristics. It typically shows lower blood-stage parasitemia with gametocytes emerging before symptoms manifest, enabling it to persist in the bloodstream without causing illness (Howes *et al.*, 2016; O'Meara *et al.*, 2023). Additionally, *P. vivax* can form dormant liver stages known as hypnozoites, which can reactivate later, complicating the clinical picture and increasing the number of asymptomatic carriers (Anwar *et al.*, 2024). Furthermore, immunity to *P. vivax* develops more rapidly than to *P. falciparum*, leading to higher morbidity rates at

younger ages in endemic regions, while adults are more frequently asymptomatic (Menkin-Smith & Winders, 2023).

In the current research, the density of parasites for both *P. falciparum* and *P. vivax* was significantly greater in symptomatic cases than in asymptomatic ones. Similar findings were observed in studies conducted in Tanzania, Bangladesh and India (Chourasia *et al.*, 2017; Starzengruber *et al.*, 2014; Sumari *et al.*, 2017), which noted a lower asexual parasite density among asymptomatic carriers. Additionally, the prevalence of gametocytes was observed to be 28.2% by microscopy, which is considerably higher than the 2% and 1.2% rates documented in other regions of Tanzania, (Mwingira *et al.*, 2014; Sumari *et al.*, 2017). This difference may be attributed to unavailability of treatments for the gametocyte stage in the study area.

The present study examined the relationship between malaria infections and various risk factors, including socio-demographic characteristics, the use ITNs, IRS coverage, housing conditions, history of malaria infection and awareness of malaria. The logistic regression analysis indicated that only two factors, i.e., a history of previous malaria infections and educational messages about malaria were statistically significant in their association with malaria infections. This finding contrasts with research conducted in various regions of Ethiopia and other countries, including: West Armachiho, Boset District, Oromia, Boricha District, Sidama, Dembia District, and East Shewa, Oromia (Aschale *et al.*, 2018; Balcha *et al.*, 2023; Dabaro *et al.*, 2023; Fekadu et al., 2018; Tadesse *et al.*, 2018). Additionally, studies from other parts of the world, such as Malaysia (Ramdzan *et al.*, 2020) and Saudi Arabia (Hawash *et al.*, 2019) have identified a broader range of risk factors associated with malaria infections.

Additionally, the analysis of demographic characteristics, including age, sex, and household size, aimed to explore their association with malaria infection. The study revealed a significantly higher prevalence of malaria among males (93.3%) compared to females (6.7%). Although males exhibited higher rates of both asymptomatic and symptomatic malaria (OR > 1), this difference was not statistically significant (P = 0.572). Consequently, military personnel were considered a mobile and susceptible group, which places them at greater risk for malaria infection.

These results are consistent with findings from previous studies in Kombolcha (Gebretsadik *et al.*, 2018), Boricha in Sidama (Dabaro *et al.*, 2023), Bahir Dar Zuria (Getahun & Bekel, 2022),

and Malaysia (Ramdzan *et al.*, 2020). On the other hand, some studies have reported a higher prevalence of malaria parasitemia in females, as described in research from Hallaba, Southern Ethiopia (Tefera, 2014), and Tanzania (Kalinga *et al.*, 2019; Nzobo *et al.*, 2015).

A high prevalence of malaria was observed in the 18-25 age groups, with 34.7%-testing positive, compared to only 1.7% in the 26-33 age groups and 0.5% in those aged 34 and older. This higher prevalence may be attributed to the fact that the majority of study participants were younger soldiers undergoing commando training in the military. But, there was no statistically significant association (COR= 1.3; P=0.493) on the prevalence of malaria and this age category. These findings are consistent with prior research conducted in Bahir dar Zuria (Getahun & Bekel, 2022), Tselemti (Shiferaw *et al.*, 2018), and Saudi Arabia (Hawash *et al.*, 2019).

Additionally, the study found that the use of ITNs had no significant impact on the prevalence of either asymptomatic or symptomatic malaria. Although ITNs are recognized as an effective vector control tool for reducing malaria transmission, the difference in prevalence between ITN users and non-users was not statistically significant ($p > 0.05$). In the study area, the ownership of ITNs was reported at 77.9%, with a utilization rate of 75.4%. These results align with studies conducted in Harari, Eastern Ethiopia, which reported a utilization rate of 73.3% (Teklemariam *et al.*, 2015), and Arbaminch, where the rate was 73% (Astatkie & Feleke, 2009). However, low ITN utilization has also been reported in Gamo Gofa, Ethiopia (37.2%) (Admasie *et al.*, 2018), and Jimma, Oromia (38%) (Zhou *et al.*, 2016). Very high utilization rates were also reported in Kola Diba, North Gondar (91.9%) (Alemu *et al.*, 2018), and Debre Elias District (94.3%) (Abebaw *et al.*, 2022).

The current study identified a statistically significant association between malaria parasite infection and previous malaria infection history. This finding aligns with earlier research conducted in Debre Elias District (Abebaw *et al.*, 2022), Raya Kobo District (Melese, Yimer *et al.*, 2022), and Gondar Zuria district (Minwuyelet *et al.*, 2020). Additionally, our study revealed a significant association between malaria parasite infection and awareness of malaria transmission and control methods. This is supported by studies conducted in Dembia District, North Gonder (Tarekegn *et al.*, 2021), Boset District, Oromia (Balcha *et al.*, 2023), and Jawi District (Dagnew *et al.*, 2024).

In our study, blood smear microscopy showed a positivity rate of 124 (30.8%), whereas the Bioline™ RDT assay indicated a rate of 119 (29.5%) for overall malaria diagnosis. A total of 398 samples (98.8%) showed perfect concordance between the two diagnostic techniques, with 29.5% of the cases yielding positive results and 69.2% being negative. No differences were observed in the detection rates of *P. vivax* and mixed infections between the two tests. However, the detection of *P. falciparum* varied when comparing microscopy to the Bioline™ Abbott RDT. The latter missed five cases of *P. falciparum* that were confirmed positive by microscopy, suggesting that the Bioline™ RDT may yield false negative results compared to microscopy.

These findings were align with studies conducted in Northwestern Tigray, Sheraro (Feleke *et al.*, 2017), Jimma (Mekonnen *et al.*, 2010), and various regions in Tanzania (Bwire *et al.*, 2019; Mahende *et al.*, 2016). These results highlight that, while microscopy is regarded as more accurate and reliable for detecting malaria parasites, the Bioline™ Abbott RDT also demonstrates excellent concordance with microscopy for diagnosing malaria. Therefore, we can conclude that there is a strong agreement between microscopy and the Bioline™ RDT, making it especially valuable in remote military settings (camps) where microscopy may not be accessible, as well as during malaria epidemics for identifying both asymptomatic and symptomatic cases.

# 7. Strengths and Limitations of the Study

## 7.1. Strengths

- This study was the first to assess the prevalence of malaria and its associated factors at the Bilate Commando and Airborne Training Center, along with the larger Ethiopian military community.
- The inclusion of PCR, alongside microscopy and RDT, enhances the accuracy and reliability of malaria diagnosis, allowing for a more thorough understanding of malaria prevalence and species distribution.
- The results can guide the development of customized malaria prevention and control strategies specifically aimed at the military community, which may vary from those intended for civilian populations.

## 7.2. Limitations

- The study only included military personnel and did not assess malaria prevalence among military families, civilian staff, or surrounding communities, potentially overlooking important transmission dynamics and risk factors present in these groups.
- Conducting the study during a low malaria transmission period may result in an underestimation of the true prevalence and risk factors associated with malaria.
- Being a cross-sectional study, it captures a snapshot of malaria prevalence at a single point in time, which may not reflect seasonal variations or long-term trends in malaria transmission.

# 8. Conclusion and Recommendations

## 8.1. Conclusion

The overall malaria positive rate among asymptomatic and symptomatic soldiers was 37% (149/403), indicating that malaria continues to be a significant health issue in military camps. Diagnostic methods revealed varying detection rates: microscopy identified 30.8% (124/403), RDTs found 29.5% (119/403), and PCR showed a higher rate of 59.6% (149/250). Both *P. falciparum* and *P. vivax* were co-endemic in the area, with the distribution of the species being nearly equal. Specifically, *P. falciparum* accounted for 45.6% of cases, followed closely by *P. vivax* at 44.3%, with mixed infections representing the remaining 10.1%. Additionally, the rate of gametocytes was significantly high at 33.9% (42/124), indicating that soldiers are significant reservoirs of gametocytes and potential sources of infection for the community. Factors such as a previous history of malaria infection and health education on malaria prevention and control were identified as associated factors influencing infection rates in the study area. To achieve effective malaria prevention and elimination within military and community settings, it is crucial to identify asymptomatic soldiers who serve as infectious reservoirs and to recognize military camps as significant transmission hotspots.

## 8.2. Recommendations

Based on the study's findings regarding the magnitude of asymptomatic and symptomatic malaria, as well as the associated risk factors among military personnel at the Bilate Commando and Airborne Training Center, the following recommendations are proposed to address the issue:

- Future studies should include military families, civilian staff, and surrounding communities to gain a comprehensive understanding of malaria transmission dynamics and risk factors.
- To better assess seasonal variations and long-term trends in malaria transmission, longitudinal studies should be undertaken in the study area.

- Given the high prevalence of gametocytes and the absence of effective treatment options in the study area, it is crucial to develop targeted therapies for the gametocyte stage of malaria to reduce the risk of transmission.
- It is advisable to incorporate military camps into the national malaria surveillance system to enhance the monitoring of malaria infection trends.
- Military leaders should collaborate with local health authorities and organizations to evaluate the effectiveness of specific malaria prevention and control strategies, while taking into account the unique characteristics of the population within military camps.
- Military leaders should initiate community engagement programs that educate both military personnel and local populations about malaria prevention, symptoms, and treatment options. Furthermore, control efforts should prioritize the identification and treatment of asymptomatic soldiers.

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# Annexes

## Annex I: Laboratory procedure

### 1. Giemsa staining of malaria blood films

Giemsa stain is an alcohol-based Romanowsky stain. Giemsa stain is a mixture of eosin, which stains parasite chromatin and stippling shades of red or pink, and methylene blue, which stains parasite cytoplasm blue. Giemsa stain (3%): This method is less appropriate when a quick result is needed but is excellent for staining large numbers (20 or more) of slides. It is ideal for staining blood films from surveys or research work or batches of slides for teaching. It performs best when slides have dried overnight. The method is economical because much less stain is used (WHO, 2010).

- Prepare a 10% solution of Giemsa stain by adding 1 ml of Giemsa stock solution to 9 ml of water buffered to pH 7.2, or multiples of this.

**Materials and reagents:**

- Giemsa stain (10% solution)
- Absolute methanol, acetone-free;
- Pasteur pipette;
- Staining rack;
- Timer
- Slide-drying rack;
- Protective latex gloves, powder-free, disposable;
- Distilled or deionized water buffered to pH 7.2;
- Clean water

**Procedure:**

1. Fix each thin film by methanol for a 30 seconds Place the slides on a tray or drying rack. Allow the methanol-fixed thin smear to dry completely in air (approximately 2 min) by placing the slides on a flat surface. Never let the slide dry in a vertical position with the thin film down, as this may lead to fixing of the thick film by methanol.

2. Place the slides back to back in a staining rack, making sure that the thick films are together at one end of the rack.

3. Pour the 10% Giemsa stain into the trough. Do not pour it directly onto the thick films, as it may float off the slides. Stain for 10 min; experience will indicate the correct time.

4. Wash the stain from the staining container using clean water

Important: Flushing the stain from the slide and staining container is necessary to avoid the film being covered with the fine deposit of stain.

5. Wipe the back of each slide clean and place it in a draining rack for the preparation to air-dry.

6. Carefully remove the slides one by one and wipe the back of each slide clean and placing them film side down in the drying rack to dry.

7. Then prepare the staining film for microscopic examination

**2. Rapid Diagnostic Tests (Bioline$^{TM}$ Malaria Ag Pf/Pv HRP2/pLDH Abbott RDT)**

For this study, the Bioline$^{TM}$ Malaria Ag Pf/Pv HRP2/pLDH Abbott test kits were employed. These kits are designed in a cassette format and utilize lateral flow immunochromatography. They enable the differential detection of histidine-rich protein-II (HPR2) specific to *P. falciparum* and *Plasmodium* lactate dehydrogenase (pLDH) specific to *P. vivax* in human whole blood.

To conduct the test, the instructions provided by the manufacturer were followed. Upon opening the pouch, the test kits were labeled with the corresponding sample code. A volume of 5 µl of blood specimen was applied to the sample pad of the test kit. Four drops of buffer were then added to the buffer pad. This buffer served the purpose of lysing the cells, releasing the antigen, and facilitating the recognition of antibodies. After 15 minutes, the results of the rapid diagnostic test (RDT) were read and interpreted accordingly.

**Negative result**: Only one band in the control line (‘’C’’) within the result window indicates a negative result.

**Positive result:**

- ***P. falciparum* positive**: Two colored lines (test line ‘*’P.f’’* and control line ‘’C’’) within the result window indicates a positive result for *P. falciparum.*
- ***P. vivax* positive*:*** Two colored line (test line ‘*’P.v’’* and control line ‘’C’’) within the result window indicates a positive result for *P. vivax*.
- **Mixed infection of *P.f* and *P.v*;** Three colored line (test lines ‘’ *P.f’’*, ‘*’P.v’’* and control line ‘’C’’) within the result window indicates mixed infection of *P.f* and *P.v*.

**Invalid results**: If the control line ‘’C’’ fails to appear within the result window the result is interpreted as invalid.

**3. Polymerase chain reaction (qPCR)**

Blood samples were collected using the finger-prick method directly onto filter paper. The extraction of DNA from these samples typically involves heating them in a 10% Chelex-100 suspension, which facilitates the breakdown of cell membranes and protects the DNA from degradation. This technique allows for the extraction of parasite DNA that is suitable for PCR. It is a rapid, inexpensive, and effective method for DNA extraction. Since this is the first step in the PCR process, it is essential to maintain sterile techniques to prevent contamination of the extracted DNA.

Quantitative Real time Polymerase Chain Reaction (qPCR) was utilized to detect and quantify Plasmodium DNA, making it particularly valuable for identifying Plasmodium falciparum and Plasmodium vivax in dried blood spot (DBS) samples. In the qPCR process, primers specific to pf and pv were employed to target unique genetic sequences of the parasites. The qPCR reaction mixture included TaqMan Fast Advanced Master Mix in a total volume of 12 µL, which comprised 3 µL of the qPCR input. For P. falciparum, 300 nM primers and 111 nM probes were used, while for P. vivax, 200 nM primers and 110 nM probes were used in a multiplex format.

During the amplification cycle, the DNA is exponentially replicated, allowing for the quantification of the initial amount of DNA present in the sample. The incorporation of fluorescent dyes or probes enables real-time monitoring of the amplification process, providing both qualitative and quantitative data. This method is highly sensitive and specific, making it ideal for detecting low levels of parasitic DNA in DBS samples.

**Required Materials and Equipment**

- 1.5 ml micro centrifuge tubes
- 1-20 ul single channel automatic pipettes
- 100-200 ul single channel automatic pipette
- 1000 ul single channel automatic pipette
- Filter pipette tips for the above pipettes
- Fine tip marker pens
- Ball point pen
- Paper towels or wipes
- Distilled water
- Phosphate Buffered Saline (PBS)
- Bleach (5 %) in a beaker or wash bottle
- TaqMan buffer

- MQ (DNase free water
- Eppendorf tubes (autoclaved)
- Rack (for the Eppendorf tubes)
- Centrifuge and vortex
- Dispenser
- BioRad qPCR machine
- Plate spinner
- Rack (for the plate
- Distilled water in a beaker or wash bottle
- Ethanol (70 %) in a beaker or wash bottle
- Chelex ®-100 Resin
- Scissors or 1/8 inch hole punch (plus spare filter paper if using a punch)
- Timer
- Micro centrifuge
- Heating block or water bath at 56 ºC
- Water bath at boiling temperature (96 ºC or above)
- Vortex
- Tips (10µl, 20µl, 100µl, 200µl, 1000µl)
- Primers (RV and FR
- Plate
- Probe (takeout at the end of mixing)
- Plate seal

**Master Mix preparation**

| MPX | | | | **96** | |
|---|---|---|---|---|---|
| 18S qPCR mix: | [stock] uM | [Final] uM | Volume Final 1X | | |
| TaqMan Fast advanced mix 2x | 2.0 | 1.000 | 6 | 633.6 | |
| Pf18S FW + Rev (10 uM) | 10.0 | 0.300 | 0.4 | 38.0 | |
| Pf18S probe (10 uM) | 10.0 | 0.110 | 0.1 | 13.9 | |
| Pv18S FW + Rev new (10 uM) | 10.00 | 0.200 | 0.2 | 25.3 | |
| Pv18S probe (10 uM) | 10.0 | 0.105 | 0.1 | 13.3 | |
| ddH2O | | | 2.1 | 226.2 | |
| Total: | | | 8.9 | 950.4 | |
| | | | | 9.9 | > 9 ul/well |
| | | | | 11.9 | > + 3 ul NA input |

**Procedure Summary**

1. Preparation of Filter Paper Discs:

- Cut 3 mm² pieces of filter paper containing blood spots using an automatic cutter.
- Place the discs into tubes with 820 µL of cool PBS-0.5% Tween-20 solution using a P1000 pipette. - Cap each tube and put them on a rack.

2. Incubation:

- Shake the tubes at 500 rpm and incubate overnight at 4°C using a heat block.

- The following morning, check that the filter paper discs have turned white or cream in color.
- Remove the brown solution and replace it with 1 mL of cooled (stored at 4°C) 1X PBS.
- Incubate for 30 minutes at 4°C in the heat block.

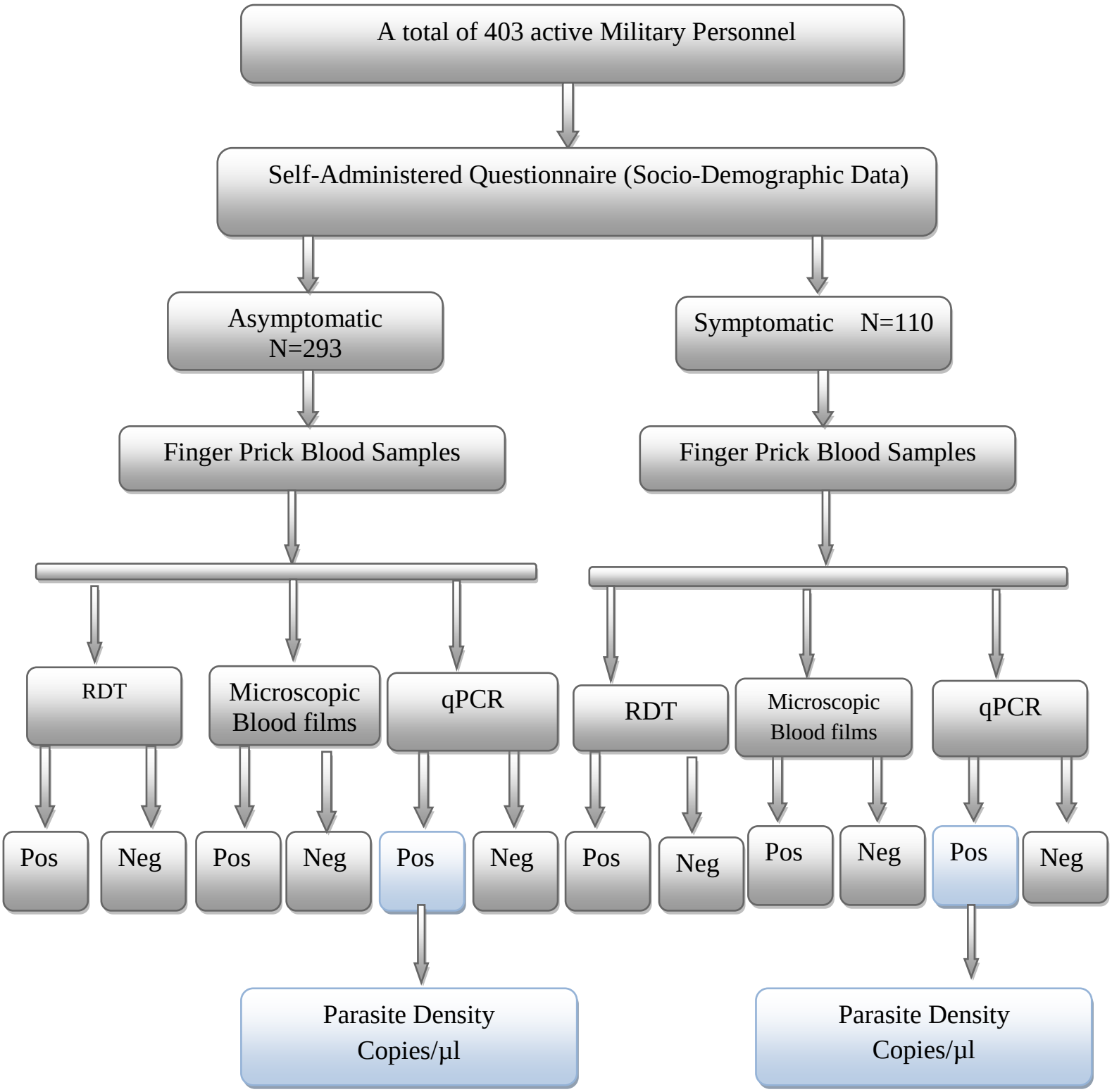
4. Centrifuge the tubes and carefully aspirate as much PBS as possible.

5. DNA Extraction: - Add 200 µL of 10% Chelex-100 resin to the fluid in the tray.

- Turn on the dry heat block and set it to 95°C.
- Extract the parasite DNA by incubating the tube at 95°C for 10 minutes, vortexing every two minutes. Repeat this step 2-3 times.

6. Centrifuge at 14,000 rpm for 5 minutes. - Transfer 150 µL of the supernatant containing the eluted DNA into a 2 mL Eppendorf tube for immediate PCR use or store at -20°C until needed (Archive, 2024).

**Annex II. The Flow chart utilized in this study**



## Annex III: Information sheet and consent form

**Title of the study**: **Magnitude** of Asymptomatic and Symptomatic Malaria and Associated Factors among Military Personnel in the Bilate Commando and Airborne Training Center, Southern Ethiopia**.**

**Principal Investigator: Cap. Efrem Geremew Kebede**

**Name of organization:** Addis Ababa University, Collage of Health Sciences, Department of Microbiology, Immunology and Parasitology.

**Introduction:** Hello, how are you? I am doing a research on the Magnitude of asymptomatic and symptomatic Malaria and associated factors among Military Personnel in the Bilate Commando and Airborne Training Center, Southern Ethiopia**.** You are invited to participate in a research study conducted by MSc candidate, from Addis Ababa University. Please read or listen as the investigator reads this text to you and take your time to decide to participate in this study.

**Purpose of the study:** Early and adequate diagnosis and prompt treatment is one of the main strategies in malaria prevention, control and effective disease management. So, in order to design and implement cost-effective malaria control interventions, up-to-date information on the prevalence and distribution; and identifying risk factors of the disease is very important. The aim of this study is to assess the current status of malaria among military members and to assess the risk factors. The result of this study will help the Health Centre and concerned Health Office for planning, implementing of malaria prevention and control program in the area.

**Procedure:** For this study to be successful we need your participation. If you agree to participate in the study, we will prick your finger and take a drop of blood. We use the sample to determine the presence of malaria parasites in the blood. We will also ask you some questions related to malaria (risk factors for the disease) and clinical manifestation by trained health professionals.

**Confidentiality:** All personal information you give and data obtained from laboratory analysis will be kept confidential. All the data will be coded with numbers without names.

**Discomforts and Risks:** you might feel a very small amount of discomfort during blood sample collection. The volume of the blood is less than half of a teaspoon. We will use sterile equipment to collect the blood sample and maximum care will be taken to minimize any risk during pricking and blood collection. In this study we expect that you will spend a maximum of 45 minutes for interview.

**Expected benefits:** You might not directly benefit from your participation in the study. You will not be paid for participation in this study but will be given treatment of the disease if you have the parasite in your blood. The result of this study will help the local health center to take appropriate action on control and prevention of malaria.

**Right to Refusal or Withdraw:** Your participation in the study is absolutely voluntary and your decision not to participate or to withdraw from the study will not affect the care you will receive at the clinic in any way. Even if you do agree to become a study participant, you can withdraw from the study at any time.

**Person to Contact:** If you have question regarding to this study, you can contact the study personnel or the principal investigator at any time using the following address: please do not hesitate to contact.

Cap. Efrem Geremew Kebede Mobile: +251910033374 /47665540
E-mail: delinaeph16@gmail.com
Department of Microbiology, Immunology and Parasitology, College of Health Sciences, Addis Ababa University.

## Consent form adult (for age >/= 18 years):

If you have understood the explanation well enough, the investigator requests to put your signature as illustrated below:

I the undersigned have been informed about the objectives and what is expected from me. I have had the opportunity to ask questions about the study and I have understood what I read or was explained. I have also been informed that the information on the study to be kept confidential that I have the right to decline from or cooperate in the study without affecting the care I normally receive at the health facility. Therefore, with full understanding of the study objective I agree to give the informed consent voluntarily to the investigator to participate on the study.

Participant name_____ Signature/thumbprint _____Date_____

**Investigator's statement** I, the undersigned, have defined and explained to the volunteer in a language he/she understands, the procedures of this study, aims and the risks and benefits associated with his/her participation. I have informed the volunteer that confidentiality will be preserved, that he/she is free to withdraw from the study at any time without affecting the care he/she will receive at the health facility.

Name (investigator) _____Signature_____ Date_____

## Thanks for your participation!!!

## Annex IV: Questionnaire

### 1. English Version of Questionnaire

**Date of collection _____/_____/____** **Questionnaire № _____**

**Name of Military setting/Division/ Regiment _____**

**Instruction: put the response in the blank space and for multiple-choice circle the answer.**

**Part I: Socio-demographic Characteristics**

| S.no | Questions | Alternatives | Skip |
|---|---|---|---|
| 101 | How old are you? | _____years | |
| 102 | Sex | 1. Male<br>2. Female | |
| 103 | Military rank | Specify_____ | |
| 104 | Marital status? | 1. Single 2. Married<br>3. Widowed 4. Divorced | |
| 105 | Educational status? | 1. Read and write<br>2. Cannot read and write<br>3. 5-8th 4. 9-12th<br>5. Diploma and above | |
| 106 | How many soldiers are currently residing with you in the military dormiraty? _____ | | |

**Part II: Household characteristics and environment**.

| S.no | Questions | Alternatives | Skip |
|---|---|---|---|
| 201 | Type of living room | 1. Temporary 2. Permanent | |
| 202 | Is eave present in your house? | 1. Yes 2. No | |
| 203 | Is there any hole present in the wall of your house? | 1. Yes 2. No | |
| 204 | The type of water body exists in the neighborhood?<br>(you can give more than one answer) | 1. River 2. Lake<br>3. Pond 4. Swamp<br>5. Stagnant water 6. None<br>7. Other ---------- | |
| 205 | How far is the nearby water body located from your house? | _____meters | |
| 206 | Do you sleep or work outdoor at your home overnight? | 1. Yes<br>2. No | |
| 207 | Grass around house | 1. Present 2. Absent | |

| | | | |
|---|---|---|---|
| 208 | Artificial containers, and man-made ditches around home | 1. Present<br>2. Absent | |

**Part III: Mosquito Nets and Insecticide Spraying**

| S.no | Questions | Alternatives | Skip |
|---|---|---|---|
| 301 | Is there insecticide treated bed net in the house? | 1. Yes<br>2. No | If No, Skip Q 302 &304 |
| 302 | If yes for Q=301, How many mosquito nets does your household have? | Specify exact number_____INT | |
| 303 | If no for Q 301, what is the reason for unavailability of ITNs? | 1. Not given at all<br>2. Lost/stolen<br>3. Used for other purposes<br>4. Old; then thrown away<br>5. Other specify _____ | |
| 304 | Where do you obtain the bed net? | 1. From health center free<br>2. From market /shop<br>3. From NGO<br>4. Other specify_____ | |
| 305 | Do you currently use a bed net while you are sleeping? | 1. Yes<br>2. No | If No, Skip Q 307 |
| 306 | If no for Q=305, Reasons for not using the available ITNs | 1. Nets do not prevent malaria<br>2. Very hot<br>3. Do not like the smell<br>4. Afraid of its toxicity<br>5. No malaria<br>6. Other specify _____ | |
| 307 | How often do you sleep under bed nets? | 1. Daily<br>2. Occasionally<br>3. During rainy season<br>4. Once a week<br>5. Other specify_____ | |
| 308 | Which type of bed net available in your house? | 1. Untreated Net<br>2. Locally Treated Net<br>3. Long-Lasting Insecticide Treated Net<br>4. Don't know | |
| 309 | Does your house sprayed against mosquitoes in the past 12 months? | 1. Yes 2. No<br>3. Don't know | |
| 310 | If yes for Q=309, How many months ago was the house sprayed against mosquitoes? | _____month/s | |
| 311 | Do you use insect repellant to protect mosquito bite? | 1. Yes 2. No | |
| 312 | Have you been taking Chemoprophylaxis in the last three weeks?? | 1. Yes<br>2. No | |

**Part IV: Living and travel to malaria endemic area**.

| S.no | Questions | Alternatives | Skip |
|---|---|---|---|
| 401 | Have you ever been malaria endemic area previously? | 1. Yes<br>2. No | |
| 402 | If yes for Q=401: where? | Specify_____ | |
| 403 | Do you travel malaria endemic area within the countries the last three week? | Yes<br>1. No | |
| 404 | Have you ever been previously exposed to malaria? | 1. Yes<br>2. No | |
| 405 | Have your family members ever been previously exposed to malaria? | 1. Yes<br>2. No | |
| 406 | Have you ever been mosquito bite experiencing | 1. Yes<br>2. No | |
| 407 | Have you heard any education (Messages) regarding malaria? | 1. Yes<br>2. No | If No, skip Q408 |
| 408 | By which source have you heard about malaria? (if Q 407=Yes) | 1. Radio 2. TV<br>3. Health facilities<br>4.Other, specify<br>_____ | |

## Laboratory Result

- Status of plasmodium parasite with microscopy

  1) Positive 2) Negative

### If the result is positive, which species are identified?

- Microscopic Result 1) *P.f* 2) *P.v* 3) Mixed
- RDT Result 1) *P.f* 2) *P.v* 3) Mixed
- PCR Results 1. *P.f* 2) *P.v* 3) Mixed

## የጥናቱ መረጃ ቅጽ

**የጥናቱ ርዕስ፡-** በኢትዮጵያ መከላከያ ሰራዊት በብላቴ ኮማንዶና አየር ወለድ ማሰልጠኛ ማዕከል ያለውን ምልክት የሚያሳይ እና ምልክት የማያሳይ የወባ በሽታ ስርጭት እና መጠን እንዲሁም ለበሽታው መስፋፋት ተፅዕኖ የሚያደርጉ ነግሮችን ማወቅ፡፡

**የዋና አጥኝው ስም፡-** ሻ/ል ኤፍሬም ገረመው ከበደ

**የድርጅት ስም:-**አዲስ አበባ ዩንቨርሲቲ ጤና ሳይንስ ኮሌጅ ማይክሮባዮሎጅ፣ ኢሚኖሎጅ እና ፓራስቶሎጅ ት/ት ክፍል

**ስለጥናቱ መግቢያ፡-**ጤና ይስጥልኝ፤ ደህና ነዎት? ይህ ጥናት በኢትዮጵያ መከላከያ ስራዊት በብላቴ ኮማንዶና አየር ወለድ ማሰልጠኛ ማዕከል ያለውን የወባ በሽታ ስርጭት እና መጠን እንዲሁም ለበሽታው መስፋፋት ተፅዕኖ የሚያደርጉ ነግሮችን ለማወቅ የሚካሄድ የማስተርስ ጥናት ነው፡፡ለጥናቱ መሳካት የእርስዎ ተሳትፎ ያስፈልጋል፡፡እባክዎ በጥናቱ ለመሳተፍ ከመወሰንዎ በፊት ይህንን ቅጽ በትክክል ያንብቡ ወይም ሲነበብልዎ በትክክል ያድምጡ፤ እንዱሁም ግልፅ ያልሆነልዎትን ነገር በሙሉ ነፃነት ይጠይቁ::

**የጥናቱ አላማ፡-** የወባ በሽታን በቀላሉ ለመከላከል እና ለመቆጣጠር በቶሎ መመርመርና ተገቢውን መድሃኒት መውሰድ በጣም አስፈላጊ ነው፡፡የዚህ ጥናት አላማም በዚህ አካባቢ እንዲሁም በሰራዊቱ ውስጥ ያለውን የወባ በሽታ ስርጭት እና ለበሽታው መስፋፋት የሚያመቹ ተፅዕኖዎችን ለይቶ በማወቅ በሽታውን በቀላሉ ለመከላከል እና ለመቆጣጠር ነው፡፡ይህም በሽታው በሰራዊቱ ላይ እያደረሰ ያለውን የጉዳት መጠን እና የስራዊቱ ተሳትፎ በሽታውን ለመከላከል እና ለመቆጣጠር ያለውን ጠቀሜታ ለማወቅ ይረዳል፡፡ በዚህም መሰረት ይህ ጥናት የሚመለከታቸው አካላት ወደፊት በሽታውን ለመከላከል እና ለመቆጣጠር የሚያግዙ መመሪያዎች እና እርምጃዎችን ለመቅረጽ ጠቃሚ የሆነ መረጃን ይሰጣል፡፡

**የአሰራር ሂደት፡-** በጥናቱ በሙሉ-ፈቃደኝነት እንዲሳተፉ እየጠቆምን በጥናቱ ለመሳተፍ ፈቃደኛ ከሆኑ በመጠይቁ እንዲሳተፉ እና በጥያቄዉ መሰረት መልስ እንዲመልሱ እንዲሁም ትንሽ ጠብታ የደም ናሙና እንዲሰጡ በአክብሮት እንጠይቃለን፡፡የደም ናሙናው በጤና ባለሙያ ከእርሰዎ ጣት ላይ ይወሰዳል፡፡የተወስደውን ናሙናም ለወባ በሽታ ምርመራ ብቻ እንጠቀምበታለን፡፡

**ሚስጥራዊነት**፡- የሚሰጡት የመጠይቅ እና የወላ ምርመራ ውጤትዎ መረጃ በጥናቱ ወቅትም ሆነ ከዛ በኃላ ባሉት ጊዜያት ሙሉ በሙሉ ሚስጥራዊነቱ ይጠበቃል፡፡ይህም መረጃውም የሚያዘው በስም ሳይሆን በመለያ ቁጥር ብቻ ይሆናል፡፡

**አለመመቸት ወይም አስጊ ሁኔታ**:-በተወሰነ መልኩ ትንሽ ያለመመቸት/ ህመም ሊኖር ይችላል፡፡ያም የሚሆነው የደም ናሙና ከጣትዎ ላይ ሲወሰድ ነው፡፡የምንወስድው የደም ናሙና በጣም ትንሽ ነው፡፡ለጥናቱ የምንጠቀማቸው መሳሪያዎች ለእርስዎ ብቻ እና ፍጹም ከጀርም የጸዱ ናቸው እንዲሁም ጉዳት እንዳይደርስብዎት ከፍተኛ ጥንቃቄ እናደርጋለን፡፡ ለ45 ደቂቃ ብቻ ከኛ ጋር ይቆያሉ፡፡

**የሚያገኙት ጥቅም**፡- በጥናቱ ለሚሳተፉ ፍቃደኛ ተሳታፊዎች ምንም አይነት የገንዘብ ክፍያ የለም:: በጥናቱ መሳተፍ ወዲያውኑ የሚያስገኘው ጥቅም ባይኖርም ወላ ከተገኘ አስፈላጊው ህክምና ባፋጣኝ እንድታገኙ ይደረጋል፡፡ይህ ጥናት የሚመለከታቸው አካላት ወደፊት በሽታውን ለመከላከል እና ለመቆጣጠር የሚያግዙ መመሪያዎች እና እርምጃዎችን ለመቅረጽ ጠቃሚ የሆነ መረጃን ይሰጣል፡፡

**ከጥናቱ ራስን የማግለል ነፃነት**፡- ጥናቱ ሙሉ በሙሉ በፈቃደኝነት ላይ የተመሰረተ ነው፡ ፡በማንኛውም ሰዓት ራስን ከጥናቱ ማግለል ይቻላል፡፡ይህም በምንም አይነት መልኩ የአሁን ወይም የወደፊት የህክምና አገልግሎት ተጠቃሚነትን አያስተጓጉልም፡፡በጥናቱ ላይ እያለ በፈለጉት ጊዜ የማቆም ወይም የማቋረጥ መብት አለዎት፡፡

**አድራሻ**:-ስለ ጥናቱ ምንም አይነት ጥያቄ ካለዎት የጥናት አባላትን ወይም ዋናው አጥኝውን መጠየቅ ይቻላል፡፡ጥያቄ ለመጠየቅ እንዳያመነቱ፣ ይህንን አድራሻ ይጠቀሙ፡-

ሻ/ል ኤፍሬም ገረመው ከበደ **ስልክ**፡-+251910033374/47665540

**ኢሜል**፡- delinaeph16@gmail.com

አዲስ አበባ ዩንቨርስቲ፤ የጤና ሳይንስ ኮሌጅ፤ ሜዲካል ማይክሮባዮሎጅ ኢሚኖሎጅ እና ፓራስቶሎጅ ት/ት ክፍል

## የስምምነት ቅፅ ከ18 አመት በላይ ለሆኑ ተሳታፊዎች:-

እኔ ከዚህ በታች የፈረምኩት ስለጥናቱ አላማ እንዲሁም ከእኔ ምን እንደሚጠበቅ በቃልም ሆነ በጽሁፍ የቀረበውን በሚገባ ተረድቻለው።።ተጨማሪ መረጃ ከፈለግኩም ማንን እንደማናግርም አውቂያለው።።የሰጠሁት መረጃም በአግባቡ እንደሚጠበቅ ተረድቻለው።። በማንኛውም ሁኔታ ከጥናቱ ራሴን ማግለል እንደምችል ይህም በምጠቀመው የጤና አገልግሎት ላይ ምንም አሉታዊ ተፅዕኖ እንደሌለው ተረድቻለው።። ስለዚህ በጥናቱ ላይ ለመሳትፍ በእራሴ ፍቃደኝነት ተስማምቻለሁ እንዲሁም እፈርማለሁ::

የተሳታፊው ስም_____

ፊርማ _____ቀን _____

**የአጭኛው አስተያየት፡-** እኔ ከታች የፈረምኩት፣ ስለጥናቱ ሂደት፣ ዓላማውን፣ጥቅሙን እንዲሁም ሊያስከትል የሚችለውን መጠነኛ ህመም /ጣታቸው ሲወጋ/፣ ለጥናቱ ፈቃደኛ ተሳታፊዎች በሚገባቸው ቋንቋ ገለፃ አድርጊያለሁ።።የሰጡት መረጃም በሚስጥር እንደሚጠበቅ እንዲሁም በማንኛውም ሰዓት ከጥናቱ ራሳቸውን ሊያገለሉ እንደሚችሉ ተነግሩዋቸዋል።።

የአጭኛው ሰም _____

ፊርማ _____ቀን _____

***ስለ ትብብርዎ እናመሰግናለን!!!***

## 2. Amharic Version Questionnaires

# መጠይቅ

ቀን____/___/_____ የመረጃ ማሰባሰቢያ ቅጽ ኮድ_____

የተሳታፊው ክፍል/ሬጅመንት_____

<u>መመሪያ</u>፡- ትክክለኛውን መልስ በማክበብ ወይም መልሱን በክፍት ቦታ ላይ በመፃፍ ይመለሱ፡፡

**መጠይቅ ክፍል አንድ፡- ስነ-ህዝባዊ መገለጫዎች**

| ተ.ቁ | ጥያቄዎች | አማራጮች | ዝለል/ሂድ |
|---|---|---|---|
| 101 | እድሜ(በዓመት) | _____ዓመት | |
| 102 | ጾታ | 1. ወንድ 2. ሴት | |
| 103 | ማዕረግ | _____ | |
| 104 | የጋብቻ ሁኔታ | 1. ያላገባ 2. ያገባ<br>3. የፈታ/ች<br>4. ባለቤቷ/ቱ በህይወት የሌለ/ች | |
| 105 | የትምህርት ደረጃ | 1.መጻፍና ማንበብ የማይችል<br>2. መጻፍና ማንበብ የሚችል<br>3. 1-4ኛክፍል 4. 5-8ኛክፍል<br>5. 9-12ኛክፍል 6. ዲፕሎማ እና ከዚያበላይ | |
| 106 | በቤት/ዶርም ውሰጥ ስንት ወታደሮች በጋራ ትኖራላችሁ? _____ | | |

**መጠይቅ ሁለት፡የቤት አሰራርን እና አካባቢን በተመለከተ**

| ተ.ቁ | ጥያቄዎች | አማረጮች | ዝለል/ሂድ |
|---|---|---|---|
| 201 | የመኖርያ ቤት ዓይነት | 1. ጊዜያዊ 2. ቋሚ | |
| 202 | በቤቱ ግድግዳ እና በጣራው መካከል ክፍተት (ቀዳዳ) አለ? | 1. አዎ 2. የለም | |
| 203 | በቤቱ ግድግዳ ላይ ስንጥቅ (ክፍተት) አለ? | 1. አዎ 2. የለም | |
| 204 | በቤትዎ ቅርብ ምን ዓይነት የውሃ አካል ይገኛል? (አንድና ከዛም በላይ መምረጥ ይችላሉ) | 1. ወራጅ ወንዝ 2. ሃይቅ<br>3. ኩሬ 4. ረግረግ<br>5. የተኛ ውሃ 6. ምንም የለም<br>7. ሌላከ ሆነ ይገለፁ_____ | |
| 205 | በቅርብ ያለው የውሃ አካል ከመኖሪያ ቤትዎ በምን ያህል ይርቃል? | _____ (በሜትር) | |
| 206 | በምሽት ከቤት ውጭ ተኝተው ወይም ስራ ሰርተው ያውቃሉ? | 1. አዎ<br>2. የለም | |
| 207 | በመኖርያ ቤትዎ ዙሪያ ያደገ ሣር አለ? | 1. አለ 2. የለም | |

| | | | |
|---|---|---|---|
| 208 | በመኖርያ ቤትዎ ዙሪያ ውሃ ሊያቁሩ የሚችሉ ቁሳቁሶች ወይም ሰው ሰራሽ ጉድጓዶች አሉ? | 1. አለ<br>2. የለም | |

**መጠይቅ ክፍል ሶስት፡-የአልጋ አጎበርን እና ፀረ-ወባ ትንኝ እ□ጭትን በተመለከተ**

| ተ.ቁ | ጥያቄዎች | አማረጮች | ዝለል/ሂድ |
|---|---|---|---|
| 301 | በቤትዎ/ታችሁ ውስጥ በጸረ□□-ወባ ኬሚካል የታከመ የአልጋ አጎበር አለ? | 1. አዎ<br>2. የለም | መልስዎ የለም ከሆነ ጥያቄ=302ን እና 304ን ይዝለሉ |
| 302 | ለጥያቄ 301 መልስዎ አዎ ከሆነ ምን ያህል አጎበር ይገኛል? | ትክክለኛውን ቁጥር ይግለጹ_____INT | |
| 303 | ለጥያቄ 301 መልስዎ የለም ከሆነ ምክንያቱ ምንድን ነው? | 1. አጎበር አልተሰጠንም<br>2. ጠፍቶ<br>3. ሌላ ጥቅም አለው<br>4. አሮጌ ስለሆነ ጣልነው<br>5. ሌላ ከሆነ ይገለፁ_____ | |
| 304 | አጎበሩን ከየት አገኙት? | 1. ከመንግስት በነፃ<br>2. ከገቢያ /ከሱቅ/በግዥ<br>3. መንግስታዊ ካልሆነ ድርጅት<br>4. ከሌላ ከሆነ ይገለፁ_____ | |
| 305 | በአሁኑ ጊዜ ሲተኙ የአልጋ አጎበር ይጠቀማሉ? | 1. አዎ 2. የለም | መልስዎ የለም ከሆነ ጥያቄ 307ን ይዝለሉ |
| 306 | ለጥያቄ 305 መልስዎ የለም ከሆነ አጎበሩን የማይጠቀሙበትን ምክንያት ምንድን ነው? | 1. አጎበር ወባን አይከላከልም<br>2. በጣም ስለሚሞቅ<br>3. ሽታውን አልወደውም<br>4. መርዙን ስለምንፈራ<br>5. ወባ ስለሌለ<br>6. ሌላ ከሆነ ይገለፁ_____ | |
| 307 | እርስዎ በአጎበር ውስጥ መቼ መቼ ነው የሚተኙት? | 1. በየቀኑ 2. አንዳንድ ጊዜ<br>3. በዝናብ ወቅት 4. ቢበዛ በሳምንት<br>5. ሌላ ከሆነ ይገለፁ_____ | |
| 308 | በአሁኑ ሰዓት እርስዎ የሚገለገሉበት የትንኝ መከላከያ አጎበር የትኛው ነው? | 1. ያልተነከረ<br>2. አገር ውስጥ የሚነከር<br>3. ፋብሪካ ውስጥ የሚነከር<br>4. አላውቅም | |
| 309 | ባለፉት 12 ወራት ውስጥ ቤትዎ በፀረ- ትንኝ ኬሚካል ተረጭቶ ያውቃል? | 1. አዎ 2. የለም<br>3. አላውቅም | |
| 310 | ለጥያቄ 309 መልስዎ አዎ ከሆነ ቤትዎ በፀረ-ትንኝ ኬሚካል የተረጨው መቼ ነው? | _____<br>ወራት ይገለጽ | |

| | | | |
|---|---|---|---|
| 311 | የወባ ትንኝ ንክሻን ለመከላከል የነፍሳት መከላከያ (ፀረ-ተባይ) ይጠቀማሉ? | 1. አዎ 2. የለም | |
| 312 | ባለፉት ሶስት ሳምንታት የጸረ ወባ መከላከያ መድሃኒት ወስደው ያውቃሉ? | 1. አዎ 2. የለም | |

**መጠይቅ ክፍል አራት፡-ወባማ አካባቢ መኖር ፤ መጓዝን እና ግንዛቤን በተመለከተ**

| ተ.ቁ | ጥያቄዎች | አማረጮች | ዝለል/ሂድ |
|---|---|---|---|
| 401 | ከዚህ በፊት የወባ በሽታ ያለበት አካባቢ ኖረው ያውቃሉ? | 1. አዎ 2. የለም | |
| 402 | ለጥያቄ 401 መልስዎ አዎ ከሆነ፡ የት? | ቦታውን ይግለጹ_____ | |
| 403 | ባለፉት ሶስት ሳምንታት የወባ በሽታ ያለበት አካባቢ ተጉዘው ያውቃሉ? | 1. አዎ<br>2. የለም | |
| 404 | ከዚህ ቀደም በወባ ተይዘው ያውቃሉ? | 1. አዎ 2. የለም | |
| 405 | ከቤተሰብዎ አባላት መካከል ከዚህ ቀደም በወባ ተይዞ የሚያውቅ አለ?\ | 1. አዎ 2. የለም | |
| 406 | የትንኝ ንክሻ አጋጥሞዎት ያውቃል? | 1. አዎ 2. የለም | |
| 407 | ከዚህ በፊት ስለወባ እና ስለሚያስከትለው ችግር ሰምተው ወይም ተምረው ያውቃሉ? | 1. አዎ 2. የለም | መልሰዎ የለም ከሆነ ጥያቄ 408ን ይዝለሉ |
| 408 | (ለጥያቄ 407 መልስዎ አዎ ከሆነ)ስለ ወባ በሽታ ከየትኛው የመረጃ ምንጭ ሰሙ? | 1. ከሬዲዮ 3. ከቴሌቪዥን<br>2. ከጤና ተቋማት 4. ሌላ ከሆነ ይገለፁ_____ | |

**ጥያቄው አልቋል!**

***ከልብ አመሰግናለው!!***

**የወባ ምርመራ ውጤት**

- RDT_____
- Microscopy _____
- PCR_____

**Annex V: Questions to assess the presence of clinical manifestations**.

**Participant's code**_____ Division/ Regiment_____

1. Do you have any of the following symptoms currently or in the previous two days?
   A. Fever YES _____NO_____
   B. Headache YES _____NO_____
   C. Joint pain YES _____NO_____
   D. Malaise YES _____NO_____
   E. chills YES _____NO_____
   F. Nausea and or vomiting YES _____NO_____
   G. Others _____

2. Axillary body temperature 1. < 37.5 oC. 2) ≥ 37.5 oC.

Name of examiner _____ Signature_____

**የወላ በሽታ ምልክቶች መኖር አለመኖራቸውን ማረጋገጫ ጥያቄዎች**

**የተሳታፊው ኮድ**_____

**ክፍል/ሬጅመንት**_____

1. **የሚከተሉት ምልክቶች በአሁኑ ሰዓት ወይም ባለፉት ሁለት ቀናት ውስጥ ከታዩ አዎ የሚለውን ይምረጡ፡**

**ሀ. ትኩሳት** **አዎ** ☐ **የለም** ☐

**ለ. ራስ ምታት** **አዎ** ☐ **የለም** ☐

**ሐ. የመገጣጠሚያ ቁርጥማት** **አዎ** ☐ **የለም** ☐

**መ. የሰውነት መድከም** **አዎ** ☐ **የለም** ☐

**ሠ. ማንቀጥቀጥ** **አዎ** ☐ **የለም** ☐

**ረ. ማቅለሽለሽ ወይም ማስመለስ** **አዎ** ☐ **የለም** ☐

**ሰ. ሌሎች**_____

2. **የሰውነት ሙቀት መጠን**

**ሀ.** ≤ 37.5 oC **ለ.** ≥ 37.5 oC

**የባለሙያው ስም**_____ **ፊርማ**_____

**Annex VII: Key Images from Various Laboratory Practices Conducted in the Field, EDU-CHS Lab, and AHRI**





FIRST AID

# DECLARATION

I the undersigned declare that, this MSc thesis report is my own work of that all sources of materials used for the thesis have been fully acknowledged.

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2. Dr. Gezahegn Solomon, (EDU) Signature_____Date___/___/_____
3. Mr. Yonas Alemu, (AAU) Signature_____ Date___/___/_____

Examiners:

1. Signature _____Date__/___/____
2. Signature _____Date___/___/___

Place of submission: Department of Microbiology, Immunology and Parasitology, School of Biomedicine and Laboratory Sciences, College of Health Sciences, Addis Ababa University.

Date of Submission: _____/____/_____

Department of Microbiology, Immunology andParasitology (DMIP)

Department Research Ethics Review Committee (DRERC)

Meeting No: DRERC/002/2024 Date: 07 Feb 2024

| Protocol Title: Prevalence of asymptomatic and symptomatic malaria and associated factors among military members at selected military setting in Ethiopia | | |
|---|---|---|
| **Principal Investigator** | Efrem Geremew | |
| **Institute/Department** | CHS-AAU/DMIP | |
| **Type of review** | [X] Initial Review: | [ ] Amendment [ ] Other (specify):_____ |
| **Elements Reviewed** | [ ] Attached | [ ] Not attached |
| **Decision of the meeting** | [X] Approved | [ ] Approved with Recommendation |
| | [ ] Revision requested | [ ] Disapproved |
| **Action Required** | [ ] Send to IRB | [X] Authorize Implementaion |

**Obligations of the PI:**

i. Should comply with the standard international and national scientific and ethical guidelines
ii. All amendments and changes made in protocol and consent form needs DREC approval
iii. The PI should report Serious Adverse Events (SAE) within 10 days of the event
iv. End of the study, including thesis work and manuscript should be reported to the DREC

**Follow up report expected in:**

3 Months_____ 6 Months __X__ 9 Months _____ one year _____

Asrat Hailu (Prof)

Chair, DRERC

Signature

Date: 07/02/2024

Department Stamp