ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
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THE ROLE OF SEROLOGICAL AND MOLECULAR TOOLS IN EVALUATING PERFORMANCE OF MALARIA CONTROL PROGRAMS IN ETHIOPIA

Migbaru Keffale

A Thesis submitted to the School of graduate studies of Addis Ababa University, Institute of Biotechnology, in partial fulfillment of the requirements for the Degree of Masters of Science in Biotechnology

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School of Graduate Studies

Institute of Biotechnology

This is to certify that the thesis prepared by Migbaru Keffale, entitled: "THE ROLE OF SEROLOGICAL AND MOLECULAR TOOLS IN EVALUATING PERFORMANCE OF MALARIA CONTROL PROGRAMS IN ETHIOPIA" and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Biotechnology complied with the regulations of the University and meets the accepted standards with respect to originality and quality.

Migbaru Keffale Bezabih: Signature: ----------Date: 08-11-2018

The work has been done under the supervision of:

Dr. Haileeyesus Adamu (PhD) ----------Date: Oct., 17, 2018

Dr. Endalamaw Gadisa (PhD) ----------Date: 10/17/18

Mr. Fitsum Girma (PhD fellow----------Date: 10/11/2018

Examiners

Dr. Hassen Mamo (PhD): ----------Date: Oct 17, 2018

Dr. Tekelehaimanot Haileselasie (PhD): ----------Date: Oct 17, 2018

Dr. Tesfaye Sisay (Director of Institute of Biotechnology)

Signature: ---------- Date: Oct 22, 2018

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LIST OF ABBREVIATIONS

ALMA	African Leaders Malaria Alliance
API	Annual Parasite Incidence
CQ	Chloroquine
DBS	Dried blood spot
EDTA	Ethylenediamine tetra-acetic acid
EIR	Entomological Inoculation Rate
ELISA	Enzyme-linked immunosorbent assay
FDREMOH	Federal Democratic Republic of Ethiopia Ministry of Health
G6PD	glucose-6-phosphate-dehydrogenase
IgG	Immunoglobulin G
IRS	indoor residual spraying
ITN	Insecticide-treated mosquito net
LLIN	long-lasting insecticidal net
MSP-1_{19}	19 Kilo Dalton Merozoite Surface Protein-1
MTI	Malaria Transmission Intensity
OR	Odds ratio
nPCR	Nested Polymerase chain reaction
Pf AMA-1	*Plasmodium falciparum* Apical Membrane Antigen I
PMI	President Malaria Initiative
PQ	Primaquine
*Pv*AMA-1	*Plasmodium vivax* Apical Membrane Antigen I
RCD	Reverse cumulative distribution
RDT	Rapid diagnostic tests
SCR	Seroconversion rate

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ABSTRACT

The global drop in malaria mortality (60%) and morbidity (37%) rates were recorded in 2015 from the 2000 baseline. But in 2016, approximately 5 million more new cases were recorded globally compared to 2015. The same trend was reported in Ethiopia during the same period. These facts underline the need to continuously monitor control programs as we move to malaria elimination, by using improved diagnostic tools than standard diagnostics, Microscopy and rapid diagnostic test (RDT). Thus, this study was designed with the objective; to evaluate seroconversion as readout for the effectiveness of malaria control program and determine the ongoing malaria infection prevalence based on nested polymerase reaction (nPCR) and microscopy. A cross-sectional study was done using a multistage sampling technique to select and enroll 1144 participants from Babile district. Retrospective malaria control program, socio-demographic data and finger pricks were collected. Serology, 18s nPCR and smear microscopy was done at Armauer Hansen research institute. Data were entered into MS-Excel and Statistical analysis was done using STATA 13 and Graph Pad Prism 5.0. Simple reverse catalytic model, profile likelihood test, reverse cumulative distribution **(**RCD), Kappa and receiver operating characteristic (ROC) tests were done and all the comparisons were considered as statistically significant at a *P* value <0.05. The 12 years retrospective data showed progressive decline in both malaria cases (33.4% to 2.9%) from 2005 to 2016. The obtained malaria-positivity was 1.84% (21/1144), 7.96% (91/1144) and 21.6% by microscopy, nPCR and serology. The serology showed changes in transmission occurred approximately 15.5 and 11.5 years ago for *PfAMA*-1 and *Pv*AMA-1 respectively. Serology and 18s targeted nPCR showed higher efficiency to detect submicroscopic and asymptomatic parasitemia in low transmission settings. Therefore, these tools should be used before the commencement of the elimination program than light microscopy.

Key words: Malaria elimination, Serology, Current infection, Malaria metrics

1. INTRODUCTION

1.1. Background

Malaria is one of the deadliest vector-borne diseases of mankind, approximately 3.2 billion people live in malarious countries among whom 1.2 billion are at higher risk of transmission (WHO, 2016a). As per the world health organization (WHO) report, 91 countries and territories have ongoing malaria transmission contributing for the 216 million cases of symptomatic malaria and 445 000 malaria associated deaths in 2016 (WHO, 2017a). Malaria occurs mostly in the tropical and the subtropical regions of the world such as Africa, Central and Southern America, Central, South and Southeast Asia, and Pacific Countries. However, developing countries are the most affected. For instances about 90% of malaria cases and deaths were reported from sub-Saharan African countries including Ethiopia (WHO, 2017c). According to the current stratification, about 60% of the Ethiopian population is at risk of malaria with 6.4% having high transmission (PMI, 2018). In Ethiopia malaria is common in both lowland and midland areas. Epidemics are commonly observed in the midlands, from 1600 to 2150 meters above sea level, during the months between September and December in most parts after the main rainy season from June to August (Behailu Taye *et al.*, 2016).

Malaria is caused by *Plasmodium*, a unicellular protozoan eukaryotic parasite. Human infection is mostly through the bite of an infected female Anopheles mosquito. There are four common *Plasmodium* species that infect and cause disease in humans. These are *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale* and the fifth spices *P. knowlesi*, a zoonotic parasite is also emerging (Manin *et al.*, 2016). Even though all the four human malaria parasites are found in Ethiopia, *P. falciparum and P. vivax* account for 57.1% and 30.5% respectively and mixed infection accounting to 12.4% of the disease burden. So far, there was no report of *P. ovale* and *P. malariae* from the routine health information system (PMI, 2018) except that *P. ovale* was detected in 3.03% (9/297) of malaria suspects from north Gondar in 2014 using 18sRNA gene targeted nested PCR (nPCR) (Abebe Alemu *et al.*, 2014).

Malaria has a major health system and economic impact in many developing parts of the world including Ethiopia due to the direct costs, expenditure on treatment and prevention (Cairns *et al.*, 2012; PATHMACEPA, 2016), as well as through the indirect consequences of working time lost due to illness and/or patient care (Gudo *et al.*, 2013). Therefore, malaria is one of the diseases that deserved intense prevention measures to reach the elimination phase by 2030 (WHO, 2017c). Malaria control includes methods to prevent infectious bites, and early diagnosis and adequate

medication (Ogutu *et al.*, 2010). Vector control through the application of indoor residual spraying (IRS) (Killeen *et al.*, 2014) and use of long-lasting insecticide treated nets (LLIN) (Sluydts *et al.*, 2016) are the most widely used infection prevention tools.

Over the last decades, several countries have made major progress in their fight against malaria. As a result, between 2000 and 2015 the global malaria incidence rate declined by 37% and mortality rates by 60% (Cibulskis *et al.*, 2016). In 2015, 10 countries and territories reported fewer than 150 indigenous cases, and additional nine countries reported between 150 and 1000 indigenous cases. During the same period following the major scale-up efforts on malaria prevention and controls began in 2004/ 2005, Ethiopia documented a reduction in confirmed malaria cases by 66% between 2001 and 2015 (MIS, 2016; Maru Aregawi *et al.*, 2014). This indicates many of the countries with ongoing transmission are on track to meet the 2020 target (WHO, 2014). As transmission declines the stumbling block in the move for elimination is identifying all malaria-infected individuals that could potentially contribute to the onward transmission (Fitsum Girma Tadesse *et al.*, 2015; Ouedraogo *et al.*, 2016).

Microscopy and the currently available RDTs may not be sufficiently sensitive to screen low parasitemia carriers as well as to evaluate elimination efforts (Lin *et al.*, 2014; Fitsum Girma Tadesse *et al.*, 2015). Repeated screening with RDTs and treatment of parasite positive individuals does not result in sustainable reduction in parasite prevalence in high endemic settings (Ogutu *et al.*, 2010), neither resulted in elimination in low endemic settings (Cook *et al.*, 2015a). Also, in areas of low transmission exposure to infection is markedly heterogeneous (Stresman *et al.*, 2014). In such scenario, traditional measures of malaria transmission intensity (MTI) such as the entomological and parasitological measures as well lack sensitivity because numbers of positive samples (mosquito and human) are low (Rosas-Aguirre *et al.*, 2013) and affected by seasonality (Drakeley and Cook, 2009; Lemu Golassa *et al.*, 2015; Behailu Taye *et al.*, 2016). As a result, these areas pose considerable challenges for monitoring and evaluation of the effectiveness of malaria control interventions to implement the elimination activities (Hsiang *et al.*, 2012; Wu *et al.*, 2015).

A potential alternative measure for MTI in low-endemic settings is serological assays targeted to antibody responses to parasite and vector antigens (Badu *et al.*, 2012b; Zakeri *et al.*, 2016). Combining molecular and serological tools are shown to considerably enhance the capacity of detecting current and past exposure to malaria infections in very low endemicity settings (Thongdee *et al.*, 2014; Dewasurendra *et al.*, 2017). Moreover, serological markers are shown to have greater sensitivity as a measure of cumulative exposures and are less affected by seasonality

(Ogutu *et al.*, 2010; Kerkhof *et al.*, 2016). These approaches have previously been used to assess MTI (Drakeley and Cook, 2009; D'Alessandro *et al.*, 2012; Cook *et al.*, 2015a), for the assessment of changes in prevalence following the implementation of parasite and vector control programs (Drakeley *et al.*, 2005; Ogutu *et al.*, 2010; Rizzo *et al.*, 2011a; Rizzo *et al.*, 2011b) and to measure malaria eradication successes (Snounou *et al.*, 1993; O'Meara *et al.*, 2008; Stone *et al.*, 2012). Also, serological markers can be used to determine levels of current infection, the history of infection in the past and determine the presence of any age- specific variations in infection rates (von Fricken *et al.*, 2014). And mathematical models to estimate anti-parasite antibody seroconversion rates are considered more sensitive transmission intensity estimation tools (Zakeri *et al.*, 2016).

1.2. Statement of the Problem

The Ethiopian malaria control program is one of the most successful activities; it had achieved a conspicuous cut in malaria morbidity and mortality in the past decades. Using this past two decades success as springboard, Ethiopia planned to achieve malaria elimination in selected low transmission areas by 2020. The countries plan for the stepwise elimination program to be successful, assessment tools for validation are crucial because the presence of low Parasitemia submicroscopic infection could potentially act as reservoir for onward malaria transmission (Das *et al.*, 2015) hindering the achievement of control programs during the elimination phase (Hemingway *et al.*, 2016). To effectively evaluate the malaria control efforts *Plasmodium* carriage and transmission intensity within target populations are inevitably important (Baidjoe *et al.*, 2013). However, currently applied malaria infection detection methods in Ethiopia; microscopy and RDT, are less likely to identify low parasitemia under elimination settings (Lemu Golassa *et al.*, 2015; Fitsum Girma Tadesse *et al.*, 2015). Morover, transmission intensity though can be determined by assessing human exposure to infectious bite of *Anopheles* mosquito by trapping techniques, it is unattractive in low endemic areas because vector populations may be sparingly infected and/or heterogeneously distributed (Smith *et al.*, 2010). Therefore, the combined serological and molecular tools would inevitably be important for monitoring low parasitemia infections under elimination settings (Wu *et al.*, 2015; Pothin *et al.*, 2016).

1.3. Significance of the study

To achieve the 2020 target, the Ethiopian national strategic plan for prevention, control and elimination (NMSP) set a phased approach. The program targeted 239 low transmission districts in preparation to rollout malaria elimination (PMI, 2018). According to the reports of WHO

(2016a), in Ethiopia the prevalence of malaria has been successfully cut but for a realistic stepwise elimination plan, detecting all potential infectious reservoirs might be a priority (Ouedraogo *et al.*, 2016). In this regard mathematical models that estimate anti-parasite antibody seroconverssion rates are gaining relevance as more sensitive transmission intensity estimation tools (Sepulveda *et al.*, 2015b). These kinds of models usually predict long-term changes in transmission occurring years before the time of sampling (Kusi *et al.*, 2016). Also, PCR based malaria infection detection techniques were shown to perform better to infer recent transmissions (Li *et al.*, 2014; Strom *et al.*, 2014; Thongdee *et al.*, 2014). Therefore, this study was planned to shed-light on the effectiveness of malaria control program in one of the malaria elimination targeted districts using seroconversion and assess the current infection prevalence with Microscopy and PCR based tools.

2. LITERATURE REVIEW

2.1. Malaria etiology

Malaria is a vector-borne disease caused by the protozoan parasites belonging to the Apicomplexan (Sub-phylum) and genus *Plasmodium*. Until recently, five different species from the *Plasmodium* genus are known to infect humans namely: *P. falciparum*, *P. vivax*, *P. ovale (*two sub species*: P. ovale curtisi* and *P. ovale wallikeri), P. malariae* and *P. knowlesi* (WHO, 2014). Globally, *P. falciparum* is the most common species identified (~75%) followed by *P. vivax* (4%). Although *P. falciparum* accounted for the majority of deaths, recent evidence indicated that *P. vivax* malaria is becoming a significant public health issue in 91 countries in the sub-tropical and temperate regions of the world (WHO, 2017c). Of the global reported *P. vivax* malaria in 2016, approximately 85% are from five countries: Afghanistan, Ethiopia, India, Indonesia and Pakistan (WHO, 2017). Moreover, human cases due to *P. knowlesi*, a zoonotic species that causes malaria in macaques, is (Manin *et al.*, 2016) reported mainly from Thailand, Mynmar and Malaysia (Brock *et al.*, 2016). Yet, *P. knowlesi's* importance as public health is still insignificant.

In Ethiopia, *P. falciparum (*57.1%) and *P. vivax (*30.5%) are the two dominant parasite species (Desalegn Nega *et al.*, 2015; Fitsum Girma Tadesse *et al.*, 2015). They are prevalent in all malaria-endemic areas of the country (PATH MACEPA, 2016; EFDRMoH, 2016). No report of *P. ovale* and *P. malariae* from the routine health information system (PMI, 2018) except 3.03% *P. ovale* report*ed* in north Gondar in 2014 through nested PCR (nPCR) (Abebe Alemu *et al.*, 2014).

2.2. Transmission of malaria

Malaria is transmitted through the bite of a female *Anopheles* mosquito, which occurs mainly between dusk and dawn (Cooke *et al.*, 2015). Other comparatively rare mechanisms for transmission include; congenitally (Falade *et al.*, 2007; Caroline *et al.*, 2013), blood transfusion (Owusu-Ofori *et al.*, 2010), sharing of contaminated needles (Johnny *et al.*, 2004), and organ transplantation (Owusu-Ofori *et al.*, 2010).

The habits of most of the anopheline mosquitoes have been characterized as anthropophilic, endophagic, and nocturnal. Their peak biting time is midnight, but ranged between 19:00 h and 6:00 h (Oljira Kenea *et al.*, 2016). From more than 515 species of *Anopheles* only 30-40 species are potential vectors for human malaria (WHO, 2017b). Each continent has its own mosquito species; *Anopheles gambiae* complex in Africa, *Anopheles freeborni* in North

America, *Anopheles culicifacies, Anopheles fluviatilis, Anopheles minimus, Anopheles philippinensis, Anopheles stephensi,* and *Anopheles sundaicus* in the Indian subcontinent. *An. leucosphyrus, Anopheles latens, Anopheles. cracens, Anopheles hackeri, and Anopheles dirus* vectors incriminated for the transmission of *P. knowlesi* (Eckhoff, 2011; Sinka *et al.*, 2012).

In Ethiopia, *An. arabiensis*, a member of the *An. gambiae complex* is the primary vector with *An. funestus, An. pharoensis,* and *An. nili* as secondary vectors. The sporozoite rate for *An. arabiensis* has been recorded to be as high as 5.4%. The host-seeking behavior of *An. arabiensis* varies with the human blood index collected from different areas ranging between 7.7% and 100%. *An. funestus,* a mosquito that prefers to feed exclusively on humans, can be found along the swamps of the Baro and Awash rivers and shores of lakes in Tana in the North and the Rift Valley areas (PMI, 2017). *An. pharoensis* is widely distributed in Ethiopia and has shown high levels of insecticide resistance, but its role in malaria transmission is unclear (PMI, 2018). *An. nili* can be an important vector for malaria, particularly in Gambella Regional State (PMI, 2017). However, according to the resent reports from south-central Ethiopia *A. zeimanni* was the predominant species (66.5 %) followed by *An. arabiensis* (24.8 %), *An. pharoensis* (6.8 %) and *An. funestus* (s.l.) (1.8 %) (Oljira Kenea *et al.*, 2016).

2.3. The life cycle of malaria

The life cycle of *Plasmodium* is divided into two (Richard, 2007); sexual (Fig 1A) and the asexual cycles (Fig 1B). The asexual cycle has two phases, the exo-erythrocytic or liver (Fig 1B, 1) and erythrocytic (Fig 1B, 2) phases (Cowman *et al.*, 2012). Human infection begins with the inoculation of sporozoites, a motile infective form, into the skin. Sporozoites are able to stay in the skin for up to six hours (Yamauchi *et al.*, 2007). About one third of the deposited sporozoites enter into the lymphatics, and the remaining into the bloodstream and get transported to the liver. Subsequently, they invade the hepatocytes and undergo asexual reproduction (tissue schizogony). A single sporozoite can multiply into thousands of merozoites that cause the rupturing of the infected liver cells. This exo-erythrocytic schizonts rip apart, and release merozoites into the bloodstream as membrane-bound merosomes that rupture and rapidly invade erythrocytes and multiply 6-20-fold every 48 to 72 hrs (Cowman *et al.*, 2012). This initiates the erythrocytic stage of the disease responsible for the development of clinical symptoms (Cowman and Crabb, 2006). In *P. vivax* and *P. ovale* infectious sporozoites are able to stay within hepatocytes in dormant forms (hypnozoites) for months to years and causes clinical relapses later (Eckhoff, 2011; Sutanto *et al.*, 2013).

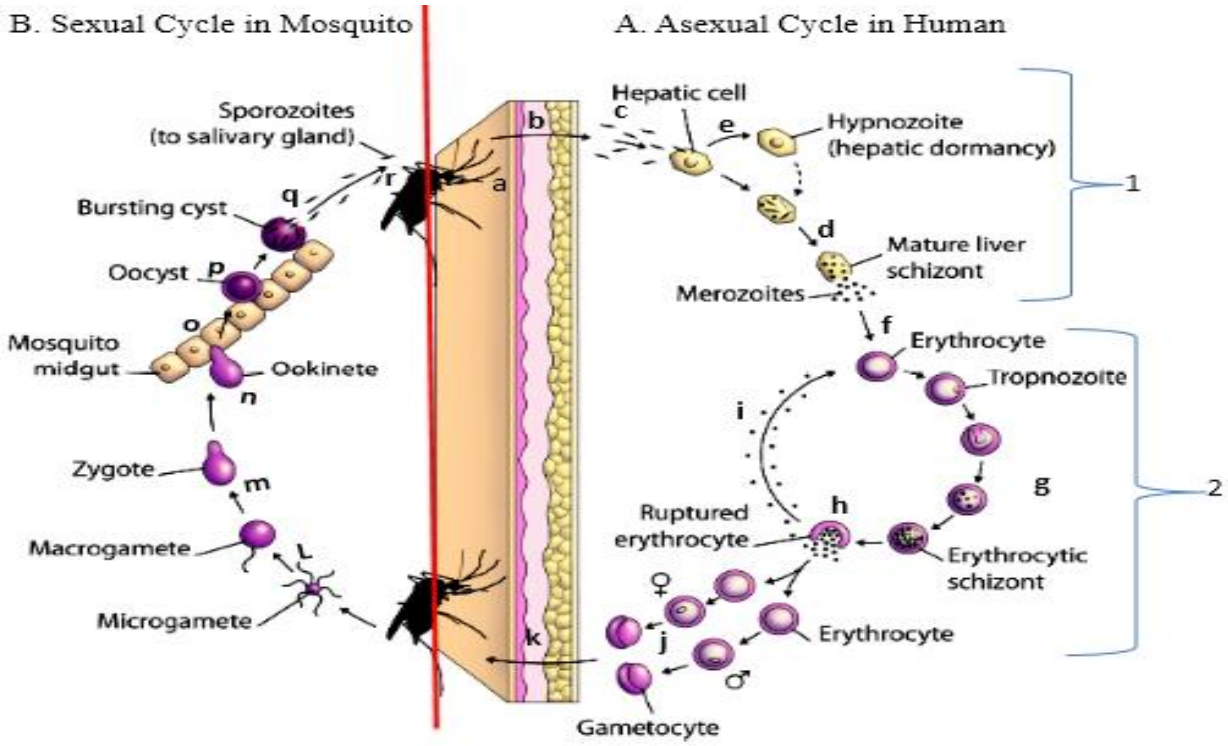


Figure 1: Life cycle of *Plasmodium* parasite (Source: Richard, 2007)

(A) Asexual cycle in human: Female *Anopheles* mosquito injects sporozoites into human host skin during a blood meal (a). A1). Exo -erythrocytic Phases: The sporozoites reach the bloodstream (b) and transported to the liver then infect liver cells (c). Sporozoites mature into schizonts, which rupture and release merozoites (d). Sporozoites of *P. vivax* can also differentiate into hypnozoites (e). Merozoites released into the vasculature and invade erythrocytes (f). A2). Erythrocytic phases: The merozoites undergo asexual multiplication in the erythrocytes and grow into ring stage trophozoites and mature into schizonts (g). The infected erythrocytes rupture and release merozoites (h). Some merozoites infect new red blood cells and repeat the cycle (i). Some merozoites undergo transformation into gametocytes which circulating in the vasculature (j). The micro (male) and macro (female) gametocytes can be ingested by an *Anopheles* mosquito during a blood meal (k) and sporogonic cycle starts.

(A) Sexual cycle in mosquito: The microgametes penetrate the macrogametes (L) and generating zygotes in the mosquito's midgut (m). zygotes transformed into motile and elongated ookinetes (n) that cross the mosquito midgut epithelium (o) and further develop into oocysts (p). The oocysts grow, burst and release thousands of sporozoites (q). The sporozoites migrate and invade the mosquito's salivary glands (r). Malaria life cycle perpetuates by inoculation of the sporozoites into a new human host (a).

The merozoites attach and then enter into the erythrocytes and initiate a series of asexual multiplication cycles (blood schizogony); where they develop into ring forms. Trophozoites and schizonts in turn produce 8 to 24 new infective merozoites at which point the cells burst and the infective cycle begins anew after they attached to uninfected erythrocytes. The remaining merozoites go through a serious of asexual cycles and differentiate into morphologically distinct sexual forms (gametocytes) that can circulate in the peripheral blood in about 7 to 15 days after the first invasion of erythrocytes (Drakeley *et al.*, 2006). During a blood meal, gametocytes are taken by the female anopheles' mosquito and continue the life cycle (Richard, 2007). The male and female gametocytes form zygotes in the insect's midgut, mature in to ookinetes then develop to oocystes and which divide to liberate several motile sporozoites (Angrisano *et al.*, 2012). The new sporozoites that migrate to the insect's salivary glands are ready to infect a new vertebrate host. The sporozoites in the saliva are injected into the skin when the mosquito takes a subsequent blood meal (Cowman *et al.*, 2012).

2.4. Epidemiology of malaria

2.4.1. Global epidemiology

Malaria threatens almost half of the world's population; 3.2 billion people in 91 tropical countries and territories are at risk (Fig 2). The over two decades of war against malaria, between 2000 and 2015, has reduced malaria cases and deaths by 37% and 60% respectively (WHO, 2017b). Yet, there is an indication of a stall in the battle in 2016, additional 5 million cases over the 2015 were reported. And of the total reported 90% of the cases and 91.2% of the deaths were from the WHO African Region (Cibulskis *et al.*, 2016; WHO, 2017b). Despite, the move by increasing number of countries towards elimination, this is unlikely to be achieved in the majority of African settings by further up scaling of conventional control measures (WHO, 2016). Several interconnected challenges are implicated; including lack of adequate financing (Doumbia *et al.*, 2012), political instability (Tsoka-Gwegweni and Okafor, 2014), development of drug resistant parasite (Doumbia *et al.*, 2012) and insecticides resistant mosquito vectors (Walker *et al.*, 2016), inadequate performance of health systems (Bastiaens *et al.*, 2014) and presence of asymptomatic malaria infection (Bousema *et al.*, 2014). Asymptomatic carriage is estimated to be the source for up to 20 to 50% of all human-to-mosquito transmissions (Mgbemena *et al.*, 2016, Okell *et al.*, 2012). In areas of very low transmission asymptomatic parasitaemia and submicroscopic carriers cannot be detected by the traditional diagnostic techniques on which the resource limited African countries are dependent (Bousema *et al.*, 2014).

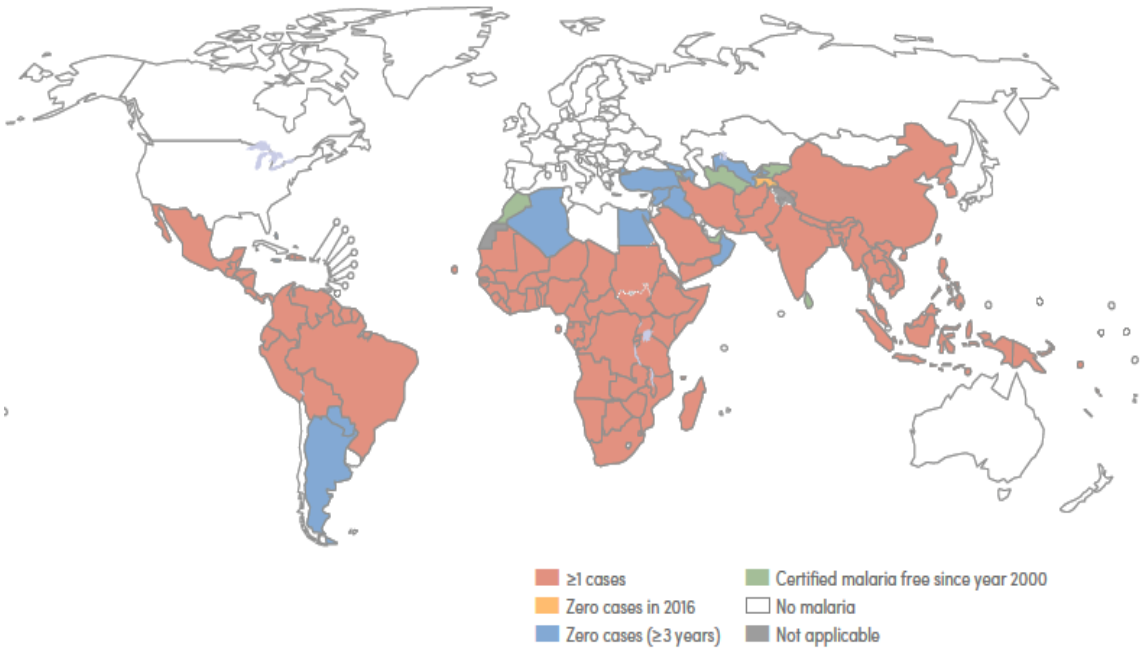


Figure 2: Global malaria distribution and trends of elimination (WHO, 2017)

2.4.2. Epidemiology of malaria in Ethiopia

In Ethiopia, the interaction of mountainous terrain with variable winds, seasonal rains, and ambient temperatures created diverse micro-climates for malaria transmission (Abebe Alemu *et al.*, 2011). The country`s weather is also influenced by tropical Indian Ocean conditions and global weather patterns, including El Niño and La Niña. When a micro-climate creates local puddles, flooding conditions, and warm ambient temperatures that persist for several weeks within a malarious area with low population immunity, the resulting *Anopheles* mosquito proliferation may cause focal malaria transmission to accelerate, sometimes explosively (PMI, 2018).

In most parts of Ethiopia, malaria is highly seasonal and peak malaria transmission occurs between September and December, after the main rainy season from June to August (Behailu Taye *et al.*, 2016). Certain areas experience minor pick transmission period from April to June, following the short rainy season from February to March (PATHMACEPA, 2016). January and July typically represent low malaria transmission seasons in most communities. Since, larval density increased after the rain ceased in both highland and lowland localities (Gone *et al.*, 2014; PMI, 2017).

As per the population-based surveys conducted in 2005, malaria was the leading communicable disease in Ethiopia. The survey indicated, 75% of the landmass and 68% of the total population were at risk of malaria. Approximately 9.5 million cases; 487,984 laboratory confirmed and about 70,000 associated deaths were reported annually from 2001-2005 (MIS, 2008). Major scale-up

efforts on prevention and controls began in 2004/2005. Artemisinin-based combination therapy (ACT) was rollout as the first line treatment; RDT as well as vector control and prevention through the wide distribution of LLINs; and targeted IRS were introduced and scaled up (MIS, 2008; PMI, 2009)

In 2015, the population at risk was reduced to 60% and the areas at risk shrank to 68% of the country's landmass. Moreover, the area with high transmission was 6.0% and the recorded parasite prevalence was 0.5% by microscopy and 1.2% by RDTs for areas below 2,000 meters and less than 0.1% above 2,000 meters (MIS, 2016). Many densely populated highland areas were declared malaria-free (API=0) and the districts with low transmission were targeted for elimination (PMI, 2015). Also based on annual parasite incidence (API), the program has stratified the country into high (≥100 cases/1,000 population/year), medium (≥5 - <100), low (>0 - <5), and malaria-Free (~0). Areas with the highest malaria transmission risk were largely in the western lowlands and midlands bordering South Sudan and Sudan, and also areas in or near the Rift Valley, which extends from the southwest to the northeast (PATHMACEPA, 2016) (Fig 3).

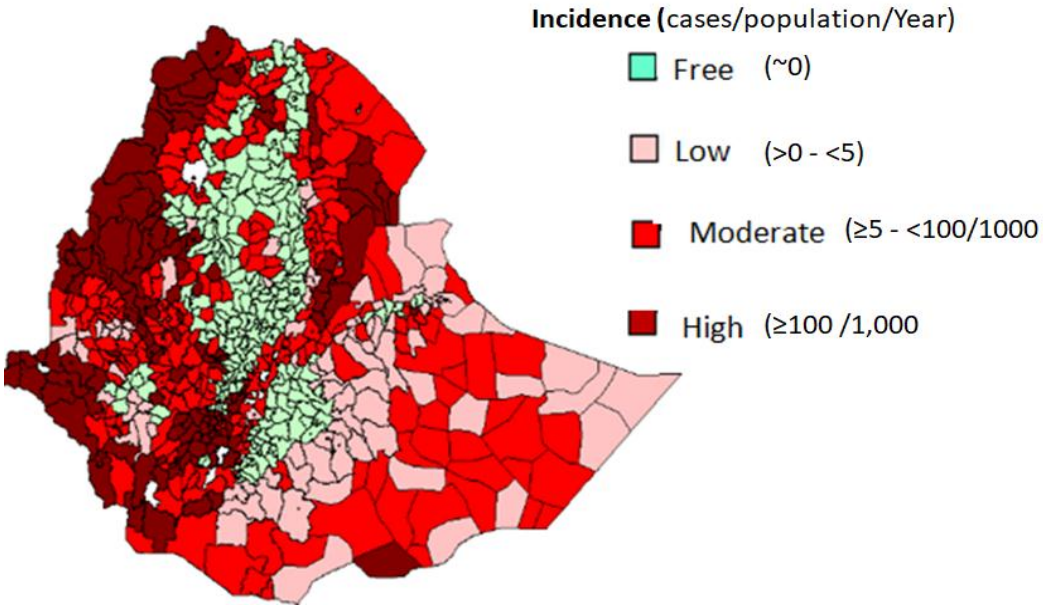


Figure 3: Malaria incidence by area, stratified based on API, 2015 (MIS, 2016).

2.5. Impacts of malaria

Malaria has a major burden on individuals, health systems and infrastructures in many parts of the developing world. The economic impacts and consequences on household's and beyond include: the direct expenditures on treatment and prevention (Cairns *et al.*, 2012), and the indirect consequences of time lost to illness or caring for sick household members, reduced productivity

(Emami *et al.*, 2013). In 2016, an estimated USD2.7 billion was invested in malaria control and elimination efforts globally (WHO, 2018). The African Leaders Malaria Alliance (ALMA) estimated that in Africa, as much as 40% of healthcare spending in endemic countries goes to malaria, costing the continent around USD12 billion a year (ALMA, 2015). On top of these, in the economic burden that malaria poses in Ethiopia is compounded for the peak transmission often coincides with the planting and harvesting season and the majority of malaria burden is among older children and working adults (Behailu Taye *et al.*, 2016).

2.6. Prevention and control

The outcome of malaria infection is the result of the interaction between the host immune system (Griffin *et al.*, 2015; Fowkes *et al.*, 2016; Griffin *et al.*, 2016), the virulence of the parasite strain and the environment (Bousema and Drakeley, 2011). Immunity that develops over years of exposure is partial and reduces occurrence of severe disease but never provide complete protection and there is no efficacious vaccine so far (Aaby *et al.*, 2009; Klein *et al.*, 2016). Therefore, the important components for reducing the burden; morbidity and mortality, mainly focus on diagnosis, adequate treatment and infection prevention (Killeen *et al.*, 2014; Slater *et al.*, 2014; Larsen *et al.*, 2015). However, current control options are threatened by the spread of drug resistant parasite strains (Witkowski *et al.*, 2013) and development of insecticide resistance of vectors (Hemingway *et al.*, 2016).

2.7. Malaria diagnosis and treatment

2.7.1. Diagnosis

The world health organization, recommends diagnostic testing for all people with suspected malaria before treatment. Malaria diagnosis is based on direct and indirect method. The direct methods are either based on detecting the whole parasite like in case of microscopy or the genetic material or antigens in case of molecular methods such as PCR and RDTs, respectively. The indirect methods depend on the detection of the host response against the parasite, such as Enzyme-linked immunosorbent assay (ELISA) (WHO, 2015a).

Direct parasite detection: Microscopy of Giemsa-stained blood smears remains the gold standard for confirmation of malaria diagnosis (Coleman *et al.*, 2006). It remained to be the cheapest and excellent in skilled manpower, and it allows identification and quantification of the causative species (Falade *et al.*, 2016). However, microscopy is not a feasible option in most parts of sub-Saharan Africa because of irregular electricity and, shortage of supply and skilled

laboratory professionals (Murungi *et al.*, 2017). Moreover, as transmission declines and most infections become low parasitemic, the possible false negative result increases (Nankabirwa *et al.*, 2015). For instances the prevalence of *P. falciparum* based on same sample was 8.7% and 50.8% when measured by light microscopy and PCR based techniques, respectively (Falade *et al.*, 2016). The sensitivity decline in microscopy could be witnessed below 500/µl parasite density (Coleman *et al.*, 2006). Also, lower negative predictive value (65.8%) was reported in Ethiopia, even if the positive predictive value was high (97.3%) (Abebe Alemu *et al.*, 2014).

The RDTs detect specific antigens (proteins) produced by malaria parasites that are present in the blood of infected individuals (Wu *et al.*, 2015) or detection of antibodies against malaria parasites (She *et al.*, 2007). Antigen-based RDTs have an important role at the periphery of health services capability because the rural clinics may not have the ability to diagnose malaria on-site due to a lack of microscopes and trained personnel to evaluate blood films (Oriero, 2015). The completed test takes a total of 15–20 minutes and the threshold of detection is in the range of 100-200 parasites/µl and >40 parasites/µl of blood in pLDH test and in *Pf*HRP2 tests respectively (Wongsrichanalai and Miller, 2002). The RDT tests has several limitations such as its sensitivity decreases markedly when the parasite density decreases below 100 parasites/µl blood (Reyburn *et al.*, 2007), Therefore, difficult to apply RDT in the malaria pre-elimination phase (Cook *et al.*, 2015a).

Numerous PCR based malaria diagnosis have been developed on genus or species-specific sequences of the Plasmodium small subunit 18S ribosomal RNA (18SrRNA) gene (Coleman *et al.*, 2006). An 18SrRNA gene amplification using a nested PCR technique is the most widely used molecular reference standard in malaria diagnostic and/or research use with a detection limit of ~0.2 parasites/µl of blood (Li *et al.*, 2014). Beyond identification and speciation, there are quantitative methods using more sensitive real-time PCR and nucleic acid sequence-based amplification assays (Mwacharo *et al.*, 2009). PCR-based malaria detection is very essential over microscopy and RDTs, particularly in cases with low-level parasitemia (Baum *et al.*, 2016).

Due to the limitations of RDTs and microscopy (Coleman *et al.*, 2006), PCR-based malaria detection techniques have been increased (Li *et al.*, 2014) for epidemiological research on malaria in endemic areas. PCR is used more in the evaluation of new strategies/interventions aiming at transmission reduction especially for detecting low parasitemia carriers (Wu *et al.*, 2015), for species identification (Mosha *et al.*, 2013; Daniels *et al.*, 2017), as well as in drug efficacy trials and drug resistance monitoring (Nankabirwa *et al.*, 2015; Mukherjee *et al.*, 2017). Reports showed that nPCR were 96% sensitive and 98% specific in asymptomatic malaria infection compared with

the microscopic method and other scholars reported its sensitivity and specificity for detection of *P. falciparum, P. vivax*, and their mixed infection were 71.4% and 100%, 100% and 98.7%, and 100% and 95.0%, respectively (Thongdee *et al.*, 2014). With its high sensitivity and specificity, PCR is increasingly being considered as gold standard method in research (Strom *et al.*, 2014). However, one limitation of this method is that the parasite DNA can remain in the blood long after infection has been cleared and therefore differentiating an active infection from a recently cleared infection could not be ascertained (Kamau *et al.*, 2011).

Indirect parasite detection: Serological methods of malaria diagnosis such as indirect ELISA and indirect immunofluorescence are usually based on detection of antibodies against asexual blood stage malaria parasites (She *et al.*, 2007). The ELISA-based methods are useful to detect antibodies of all Plasmodium species when applied as a screening tool for malaria infection in endemic areas particularly, for epidemiological investigation as well as for blood transfusion (Owusu-Ofori *et al.*, 2010). Antibodies to the asexual stage merozoite antigens like apical membrane antigen (AMA-1) and merozoite surface proteins (MSP1, MSP2, MSP3, MSP4, MSP5, MSP6, MSP7 and MSP10) (Beeson *et al.*, 2016), can be determined by using ELISA (Pothin *et al.*, 2016). Of these surface proteins, AMA-1, is highly immunogenic and tend to saturate detectable antibody responses in the population in low to moderate transmission settings (Badu *et al.*, 2012a; Dewasurendra *et al.*, 2017).

An ELISA, targeted to antibody responses to one or more malaria specific antigens are important to estimate past exposure to malaria and to monitor changes in transmission (Pothin *et al.*, 2016). ELISA has several advantages in lower transmission settings such as simple, quick and cheap to perform (Drakeley *et al.*, 2005). Antibodies persist for months or years after infection, therefore, the effect of seasonality in transmission is smoothed out and seroprevalence remains constant in low transmission settings (van den Hoogen *et al.*, 2015). According to the previous reports the sensitivity and specificity of the antibody-based ELISA test compared with the microscopic method for detection of Plasmodium genus were 96.6% and 100% respectively (Appawu *et al.*, 2003) and parasite detection limit was recorded as 1-5 parasites/µl of blood (Thongdee *et al.*, 2014).

2.7.2. Treatment of patients

Artemisinin-based combination therapies are highly effective against *P. falciparum*, the most prevalent and lethal malaria parasite affecting humans. Globally, the number of ACT treatment courses procured from manufacturers increased from 11 million in 2005 to 337 million in 2014.

The African Region accounted for most (98%) manufacturer deliveries of ACTs in 2014 (WHO, 2015). Currently, ACT is the first-line treatment for uncomplicated *P. falciparum* malaria. It rapidly clears asexual parasites and developing gametocytes but leaves mature *P. falciparum* gametocytes largely unaffected; a proportion of patients may transmit malaria after successful ACT treatment (Beshir *et al.*, 2013). Strategies to prevent malaria transmission after ACT have received a sense of urgency with the emergence of artemisinin resistance and have mainly focused on supplementing ACT with gametocytocidal and other alternative such as compounds that interfere with the mosquito and/or sporogenesis (White *et al.*, 2014). For instance, Ivermectin was shown to reduce the life span of Anopheles mosquitoes that feed on humans (Chaccour *et al.*, 2013) by activating glutamate-gated chloride channels in neuronal and neuromuscular tissues, thereby causing flaccid muscle paralysis (Chaccour *et al.*, 2015; Ouedraogo *et al.*, 2015).

Chloroquine (CQ) and Primaquine (PQ) are the two widely used antimalarials used not only for the treatment of malaria but also for the prevention and prophylaxis. Chloroquine acts by inhibiting hemozoin biocrystallization, which gives rise to toxic free heme accumulation that is responsible for the death of the parasites (Giovanella *et al.*, 2015). In general, *P. vivax* is sensitive to CQ but its susceptibility decreased from time to time in different parts of the world and effective only against blood-stage infections (Giovanella *et al.*, 2015). Therefore, radical cure (both blood-stage and liver-stage infections) of malaria caused by *P. vivax* and *P. ovale* is difficult with CQ only, thereby fail to prevent recurrence and relapse, respectively (WHO, 2015a).

The only drugs with significant activity against the hypnozoites are the 8-aminoquinolines (primaquine and tafenoquine). Primaquine was first synthesized six decades ago and remains effective treatment for the hypnozoites of *P. vivax* and *P. ovale* (Faucher *et al.*, 2013). To eradicate the hypnozoites, patients should be treated with a 14-day course of primaquine phosphate (WHO, 2015a). However, the aggravated hematotoxicity of primaquine in glucose-6-phosphate-dehydrogenase (G6PD) deficient patients as well as the low oral bioavailability of the compound have been obstacles to its widespread use (Faucher *et al.*, 2013) and G6PD deficiency testing is mandatory before any prescription (Giovanella *et al.*, 2015). To fill these gaps in PQ treatment, the scientific community search for alternative drug development like Tafenoquine (Yehenew Ebstie *et al.*, 2016).

Tafenoquine is an 8-aminoquinoline anti-malarial analog of primaquine having long–half-life being developed for malaria prophylaxis (Dow and Smith, 2017). Because of its long half-life (approximately 13 days), convenient dosing regimens, effectiveness against hypnozoites, broad

spectrum activity against all species of malaria, and the absence of documented drug resistance, tafenoquine represents a potential improvement over the standard-of-care in certain settings (Yehenew Ebstie *et al.*, 2016) without the need for the routine G6PD deficiency test (Dow and Smith, 2017). Tafenoquine is an efficacious drug for radical cure, terminal prophylaxis, and chemoprophylaxis of vivax malaria (Yehenew Ebstie *et al.*, 2016).

2.7.3. Infection prevention

To prevent malaria infection and reduce transmission, the WHO recommends vector control in addition to anti-malaria drugs. The two types of vector control recommended are insecticide-treated mosquito nets (ITNs) and IRS (WHO, 2016b).

Insecticide-treated bed nets: All mosquito nets act as a physical barrier, preventing access by vector mosquitoes and thus providing personal protection against malaria to the individual(s) using the nets. Pyrethroid insecticides, which are used to treat nets, have a repellent effect that adds a chemical barrier to the physical one, further reducing human–vector contact and increasing the protective efficacy of the mosquito nets. Insecticide-treated bed nets are one of the principal tools in the malaria prevention and control activities (Wilson *et al.*, 2014). Insecticide-treated mosquito nets work both at the individual and community level, as the insecticide kills the malaria vectors that come into contact with the ITN. By reducing the vector population in this way, ITNs, when used by a majority of the target population, provide protection for all people in the community, including those who do not themselves sleep under nets (Steinhardt *et al.*, 2017).

ITN utilization is ideal due to its low cost, low technology, longer durability, social acceptance, environment-friendliness and easy transportability but there are some factors that weaken the efficacy of these nets such as rise in pyrethroid resistance, low and irregular re-treatment rates, erratic dosages during treatment, differential loading of the insecticide on the surface of the nets, human errors and at times short supply (WHO, 2016b). To resolve most of these issues, the WHO advocates use of pre-treated, water-resistant LLINs, which require no further re-treatment and maintain effective levels of insecticide during its expected life span of at least three years (3-5 years) (Wilson *et al.*, 2014).

WHO recommends universal coverage of ITNs for at least every two people, an average of one LLIN for every 1.8 persons at risk of malaria and urges a switch to LLINs (WHO, 2016c). The ITNs are effective where vectors primarily bite indoors and late at night, but their effectiveness is uncertain where vectors bite outdoors and earlier in the evening (Steinhardt *et al.*, 2017). In most malaria-affected countries, sleeping under an ITN is the most common and most effective way to

prevent infection (Steinhardt *et al.*, 2017). According to the WHO, household ownership of at least one ITN increased from 50% in 2010 to 80% in 2016. However, the proportion of households with sufficient nets (one for every two people) in the region in 2016 remained insufficient at 43%. In 2016, an estimated 54% of people at risk of malaria in sub-Saharan Africa slept under an ITN compared to 30% in 2010 (WHO, 2017c). The LLINs provide a level of protection of about 50% in a range of settings, and to reduce malaria mortality rates by 55% in children aged under 5 years in sub-Saharan Africa (WHO, 2017a).

Indoor residual spraying: It involves application of insecticides to the inner surfaces of dwellings, targeting *anopheles* mosquitoes that rest on walls after having taken a blood-meal. indoor residual spraying may be effective for only two to six months, depending on the insecticide formulation and spray surface (WHO, 2017b). indoor residual spraying programmes can rapidly reduce local malaria incidence and mortality, provided that most of the houses and animal shelters in targeted communities are sprayed (Steinhardt *et al.*, 2017). WHO recommends spraying of at least 80% of houses, structures and units in the targeted area in any round of spraying (WHO, 2016c). However, fewer people are protected by use of IRS, in 2016 2.9% compared to 5.8% in 2010 (WHO, 2017a). The WHO argues that this decrease is due to countries changing insecticides to more expensive chemicals (WHO, 2018). The appropriate timing of interventions can greatly increase their effectiveness. As a general rule in the tropics, IRS with insecticides that are effective for only a few months should be conducted just before the start of the rainy season so that the population is protected throughout the period (WHO, 2016b).

WHO further recommends that all programmes in any transmission setting that invest in combined use of LLINs and IRS (WHO, 2016c). Indoor residual spraying may be used in areas where there are LLINs as part of an insecticide resistance management strategy and in LLIN coverage areas. If LLINs and IRS are to be deployed together in the same geographical location, IRS should be done with non-pyrethroid insecticides (WHO, 2017a). In a few settings and circumstances, the core interventions (LLINs and IRS) may be supplemented by larval source management, which includes four subcategories: vector habitat modification, habitat manipulation, larviciding and biological control (WHO, 2017b).

2.8. Malaria transmission intensity monitoring approaches and force of infection modeling

According to the reports of WHO (2015), an increasing number of malaria endemic countries are approaching the disease elimination phase that requires effective monitoring to evaluate and ensure the success of control efforts. Mathematical models that estimate anti-parasite antibody seroconversion rates are gaining relevance as more sensitive transmission intensity estimation tools (Kusi *et al.*, 2016). When countries progress towards elimination, finer scale malaria mapping and accurate stratification of transmission intensity are required, and stratification should be more specific, ideally at the level of the localities.

To monitor MTI and assessing the impact of anti-malaria interventions traditional measures such as entomological inoculation rate (EIR) and parasite prevalence were used (Fekadu Massebo *et al.*, 2013). An EIR is estimated by catching mosquitoes most commonly using light traps and then dissecting these mosquitoes to estimate the sporozoite rate which was used as a reference standard to determine MTI (Kilama *et al.*, 2014; Pothin *et al.*, 2016). Despite its usefulness in providing a direct estimate of the force of infection, it is time consuming, expensive and can lack precision, especially in low endemicity areas. An alternative measurement of MTI is parasite prevalence, which is the prevalence of individuals carrying the parasite, which can be estimated rapidly in populations in cross-sectional surveys using microscopy, RDT (Lemu Golassa *et al.*, 2015) or PCR (Baidjoe *et al.*, 2013) methods to detect infection in individuals. However, it is affected by seasonal variation, anti-malarial treatment levels, and lacks precision in low endemicity settings (Falade *et al.*, 2016).

The combination of molecular and serological tools allowed for an in-depth characterization of the current malaria transmission pattern as well as changes in species specific MTI of asymptomatic (Laoboonchai and Wongsrichanalai1, 2001) and sub-microscopic infections and RDT negative suspects (Hsiang *et al.*, 2012). For instance, Rosas-Aguirre *et al.*, (2013) reported the parasite prevalence found 0.3% *P. vivax only,* 1% both *P. vivax* and *P. falciparum,* and 13.6 *P. vivax* and 9.8% *P. falciparum* by microscopy, PCR and ELIISA respectively, as well as 68.0% and 88.0% seropositive were submicroscopic and asymptomatic respectively (Rosas-Aguirre *et al.*, 2013). Therefore, the combination of both serological and molecular tools can improve detection sensitivity and provided new insights into recent changes in transmission intensity for both *P. vivax* and *P. falciparum* species (Wang *et al.*, 2014). More importantly, serological data analysis using catalytic models is imperative to estimate the antibody SCR, the rate at which

seronegative individuals become seropositive which is proxy for the force of infection (van den Hoogen *et al.*, 2015).

The changes in MTI can be estimated with the profile likelihood analysis at which the time since the SCR changed (Cook *et al.*, 2011) in period of time for the change and the effects of different malaria control and prevention intervention activities on malaria seroprevalence change can be evaluated by the reverse cumulative distribution plot (George *et al.*, 1995).

2.9. Malaria control interventions in Ethiopia

The nationwide survey conducted in 2000 and 2005 showed low intervention coverage: 6.5% of households owned ITN, 17% of households had been sprayed with insecticide, and 4% of children under five years of age with a fever were taking an anti-malarial drug (CSA & Macro, 2006). In 2005 the Government developed a 5-year National Prevention and Control Strategy with an ambitious national goal on scaling-up interventions. The strategy aimed to provide 100% (with a mean of two ITNs per household) and 30% ITN and indoor IRS coverage respectively for households in malarious areas. Also, to scale-up the provision of case management with RDTs and ACT as Federal Democratic Republic of Ethiopia Ministry of Health (FDREMH, 2006).

According to the national malaria indicator survey (MIS, 2008), in malarious areas of Ethiopia (<2,000 m altitude) in 2007, 65.6% households owned at least one ITN and 53.2% of their family members had slept under an ITN the prior night. Also, 20.0% of the households were sprayed within the past 12 months and 16.3% household members that sought medical attention were given the service within 24 hours (Jima *et al.*, 2010). As per the 2015 report on key malaria indicators in 2015; the households with at least one ITN, children under five who slept under an ITN, pregnant women who slept under an ITN, households protected by at least one ITN or IRS were 63.6%, 45.3%, 44.3% and 70.5% respectively (PMI, 2017).

The mass distribution of ITNs, together with increased utilization of LLINs, IRS and adoption of ACT resulted in substantial declines in malaria related deaths in Ethiopia (Desalegn Nega *et al.*, 2015). The prevalence of microscopy detected malaria declined to 0.5% (PMI, 2017). Encouraged by the success, the national program has designed a phased elimination strategy and launched pre-elimination activities.

2.10. Malaria prevention at higher risk population

In malaria endemic areas, a significant proportion of individuals have asymptomatic infection with Plasmodium species among whom children under the age of 5 years (Tiono *et al.*, 2014; Roh *et al.*, 2016), and pregnant women are at higher risk (Khan *et al.*, 2014). Pregnant women are at greater risk of acquiring malaria infection and developing symptomatic and complicated malaria disease than their non-pregnant counterparts. The sequestration of Plasmodium species in placenta is believed to be associated with low birth weight, preterm delivery, miscarriage, and still birth (Roh *et al.*, 2016). Malaria causes significant mortality and morbidity during pregnancy and among children less than five years of age remains a major public health threat in sub-Saharan Africa including Ethiopia (Desalegn Nega *et al.*, 2015) where about 125 million pregnancies are at risk of malaria each year, and up to 200,000 babies die as a result (Sicuri *et al.*, 2013).

Use of long-lasting insecticidal nets, the administration of intermittent preventive treatment with sulfadoxine-pyrimethamine, and appropriate case management through rapid and effective therapy of malaria in pregnant women are the current strategies recommended by the World Health Organization (WHO, 2013). Malaria in pregnancy in Ethiopia is addressed through improving prompt access to diagnostics and treatment, prioritization of LLIN use by pregnant women, and enhanced social and behavior change communication (SBCC) activities targeting pregnant women in malaria endemic areas (PMI, 2017).

2.11. Application of biotechnology for malaria control and elimination

By definition Biotechnology is the use of living systems and organisms to develop or make products, or "any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use. Biotechnology is the integration of wide range of disciplines and components. From the components, “red biotechnology” encompasses applied to improve medical processes and health care. For instances, designing of organisms to produce pharmaceuticals and vaccines, Genetic testing and Bioinformatics, molecular and immunological based diseases diagnostics, and the engineering of genetic cures through genetic manipulation (Tanner *et al.*, 2015).

Regarding to malaria control and elimination, Biotechnology plays significant roles. Even though, many control strategies targeting either the *Plasmodium* parasite or the *Anopheles* vector were shown to be effective. Yet, the emergence of drug resistant parasites and insecticide resistant mosquito strains (Meredith and James, 1990), along with numerous health, environmental, and ecological side effects of many chemical agents, highlighted the need to develop alternative tools

that either complement or substitute conventional malaria control approaches (Chong *et al.*, 2013). Therefore, use of biotechnological techniques can play fundamental role for the recently launched malaria control and elimination program, although this approach is still in its infancy (Meredith and James, 1990). Several strategies can be applied through malaria vaccine development (Reis, 2012), genetic testing (De Niz *et al.*, 2013), vector genetic manipulation (Chong *et al.*, 2013), inheritable endosymbiotic bacteria transinfection (Walker and Moreira, 2011), production microbial insecticides through recombinant DNA technology, and implementations of the transgenic sterile insect technique (Gentile *et al.*, 2015). Genetic testing allows the genetic diagnosis of vulnerabilities to hemolysis during malaria therapeutics due to inherited variation like G6PD deficiency (De Niz *et al.*, 2013). Also PCR based diagnosis has the superior capacity for identifying all human malaria parasite species that facilitates prompt therapeutics to move to elimination (Zimmerman, 2015).

By using biotechnological techniques wide spectrum malaria vaccines were developed, including pre-erythrocytic vaccines, antibody-based subunit vaccines, vectored vaccines, whole sporozoite vaccines, genetically Attenuated parasites and sporozoite subunit vaccine, erythrocytic vaccines, sexual stage vaccine, transmission-blocking vaccine (Reis, 2012), synthetic peptides and conjugate vaccine (Mahmoudi and Keshavarz, 2018) radiation technology based vaccines from pathogens (Seo, 2015), as well as Plant-Made Malaria Antigens Vaccine (Clemente and Corigliano, 2012) has been introduced.

Vector control of malaria has predominantly focused on targeting the adult mosquito through insecticides and bed nets. However, current vector control methods are often not sustainable for long periods so alternative methods are needed such as inherited endosymbiotic Wolbachia bacteria transinfected into mosquitoes in order to interfere with pathogen transmission (Walker and Moreira, 2011). Genetic manipulation is one of the greatest breakthroughs for malaria elimination for instances, high titres of microbially sourced artemisinic acid were produced through cloning with increased expression of an artemisinin biosynthetic gene obtained in *Saccharomyces cerevisiae* and *Escherichia coli* recombinants (Chong *et al.*, 2013). Also, a specific, efficacious and environmentally friendly biopesticide employing genetically engineered fungus *Metarhizium anisopliae* was used to combat malaria transmission in areas where pyrethroid resistant mosquitoes found (oward *et al.*, 2010). Recombinant DNA technology was used to produce microbial insecticides using *Bacillus thuringiensis israelensis* and *Bacillus sphaericus* which produce protein toxins which are highly toxic to mosquitoes larvae after ingestion but harmless to other aquatic animals, and therefore it can be used even in ecologically sensitive areas (Chong *et al.*, 2013). Currently, transgenic implementations of the sterile insect

technique such as the release of insects with a dominant lethal, homing endonuclease genes, or flightless mosquitoes are another insight of vector control and in development. These implementations involve the release of transgenic male mosquitoes whose matings with wild females produce either no viable offspring or no female offspring (Gentile *et al.*, 2015).

2.12. Objectives

General objective

To evaluate the effectiveness of malaria control program through seroconversion rate and PCR-based molecular tools

Specific objectives

- To estimate the seroconversion and reversion rate across age groups in the study area
- To investigate the hidden submicroscopic malaria infection among the healthy population

2.13. Study hypothesis

Serology model and PCR can be used to verify the malaria control program's claim of decline in malaria transmission in elimination-targeted settings.

3. MATERIALS AND METHODS

3.1. Study area and population

In the country 239 low and moderate transmission selected districts located in 17 zones within 6 different regions were targeted for malaria elimination (PMI, 2018). From the targeted regions, Oromia was randomly selected by lottery method and East Hararghe was selected from the targeted zones in the region with the same pattern. From the 14 elimination targeted districts in this zone four districts (Goro Gutu, Kurfa Chele, Haramaya and Babile) were randomly selected and 8 years malaria control program data was collected and assessed. Then Babile district was selected for the record and it showed continuous reduction in incidence.

Babile is found in East Hararghe zone 31km from the town Harar and 557 km east from Addis Ababa. It lies between 8°, 9'- 9°, 23'N latitude and 42°, 15'- 42°, 53' E longitude and is characterized by semi-arid and arid climate with average annual rainfall of 410-800 mm and the annual temperature ranges from 24 to 28°C. Babile district has an altitude ranging from 950 to 1954 m.a.s.l. It borders with Gursum from the north, Harari Regional State from the northwest, Fedis from the west and Somalia Regional State in the southwest, south and east. The total landmass is 200,000 hectars with a human population of 121,970 (FDREMOH, 2016). The district is divided into 22 Kebeles (local term equivalent to a single peasant association) and all of the Kebeles were reported as malarious (Unpublished /progressive Babile district records, 2005-2016). For blood sample collection, eight kebeles approximately 1/3 of the total kebeles, found in the Erer valley, Tula (Sherqa), Erer Guda (Alikulo and Baha), Ifadin (Kufakasa and Abdi Nasir), Bisidimo (Bisidimo 01 and Bisidimo 02), Berkele (Seferdina), Ifa (Maya), Erer Ibada (Biftu, Ifa and Abdi) and Ibada Gemechu (Dughe and Elemo Dara) (Fig 4) were purposely selected based on availability of previous malaria records in the health post (Annex 6 (Fig 5) and presence of transport access.

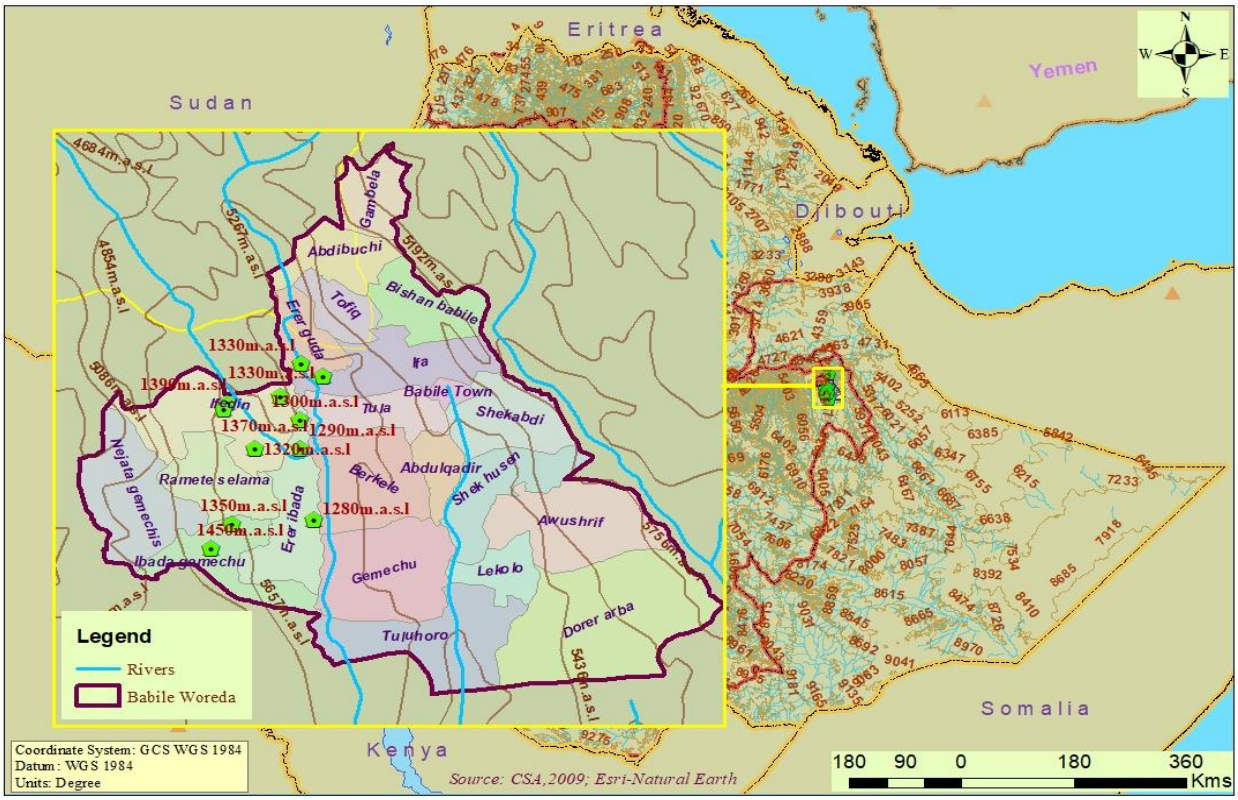


Figure 4: Map of study area

3.2. Sample size determination and sampling technique

The required sample size for this cross-sectional study was calculated using a formula for a single population proportion by taking 45.1% based on expected prevalence of antibodies to *P. falciparum* AMA-1 and/or 19 Kilo Dalton Merozoite Surface Protein-1 (MSP-1_{19}) (Fitsum Girma Tadesse *et al.*, 2017), 95% confidence level, 5% absolute desired precision, design effect of 3. Therefore, the total calculated sample size was 1142.

$$\frac{Z^2_{\alpha/2} Pexp(1 - Pexp) DEEF}{\delta^2}$$

Where

Pexp= Expected seroprevalence – 45.1%

δ= Desired precision – 5%

DEFF= Design effect – 3

Z= Significance level (α) – 5% (*Z = 1.96*)

On the other hand, the required sample size can be determined based on EIR of the disease. According to the reports of (Fekadu Massebo *et al.*, 2013) in southwestern Ethiopia the estimated EIR of *An. arabiensis* was 0.1, which is corresponding to the SCR of 0.0108 as described elsewhere (Sepúlveda and Drakeley, 2015). Also, their assumption links the estimated SCR to sample size determination for the most common situation of unknown seroreversion rate. For instances for the SCR of 0.0108 the recommended sample size is not less than 500 individuals to ensure that chance is smaller than 10%. Based on this hypothesis the calculated sample size (1142) is acceptable to conduct seroprevalence study where the estimated EIR of 0.1 is recorded. The calculated value beyond the recommonded size is imparative because according to (Sepulveda *et al.*, 2015a) explanation, increasing the sample size is an ideal strategy to increase the power calculated using the SCR assumption and to increase the chance of drawing the right conclusion.

3.3. Inclusion and exclusion criteria

3.3.1. Inclusion criteria

People who permanently lived in the study area, age ≥1 year and agreed to participate were included for participation.

3.3.2. Exclusion criteria

Individuals who haven't permanently lived in the study area and clinically sick were excluded from participation.

3.4. Study design

A cross sectional study design was used and the study was conducted during July 2017 to November 2017. Selected kebeles were subdivided in to several villages based on geographical location. From each kebele representative villages were selected randomly in proportional manner. Then the representative number of participants to be sampled was determined proportional to the village population. Sensitization was done by health extension workers and subsequent volunteers were included on arrival till the required number was attained. The selected participants were informed about the purpose of the study and requested to fill the consent form. All consented adult (>18 years) participants, children (≥1- 18 years) consented by their care takers/ guardians and old children (12-18) given asent in addition to their guardian concent, were sampled.

3.5. Ethical considerations

The study was approved by institutional ethics committees: The College of Natural and Computitional Sciences institutional Review Bord (CNS-IRB), Addis Ababa University (Ref. CNSDO/205/10/2017), Armauer Hansen Research Institute (Ref. PO24/17), and the Oromia Region Health Bureaus (Ref. BEFO/AHBIFH/1-8/2700). With official letter from the Oromia health bureau to the zonal health offices, the zone intern wrote a cooperation letter to each of the selected district. The districts delegated the malaria prevention and control focal person to provide the district`s malaria retrospective related data and facilitate community sensitization. With the malaria focal person, the kebele health extension workers give us access to the kebele based malaria program retrospective data, conducted community mobilization as well as witnessed informed consent process. Data was collected after getting written informed consent from each participant $\geq$18 years and parents/guardian of minors were requested to provide consent after the procedures were explained. In addition to parental informed consent, written ascent was taken from children between 12-17 years of age.

3.6. Data collection

3.6.1. Sociodemographic and retrospective data

Structured pre-tested forms were used to capture information on sociodemographic and malaria intervention utilization (Annex 1); such as on history of malaria episodes, LLIN and IRS use in their households, house construction material, and recent travel out of their district. Moreover, a retrospective malaria program data was retrieved from the four selected districts.

3.6.2. Blood sample collection and storage

Finger prick and occasionally venous blood were collected using sterile disposable automatic lancet (unistik 3, Owen Mumford Inc, Cat# 675-AT1007, UK) and needle. The Blood samples were used to prepare thin and thick blood films on frosted end microscopic slides (Thermo scientific, USA, cat# BS70W2 08) and dried blood spots (DBS) onto 3MM Whatman (Whatman, Maidstone, England, Cat# 2300916). The air-dried blood films were put in slide racks, transported and stored under ambient conditions until processing. The DBSs were air-dried and stored individually in sealed plastic bags with self-indicating silica gel beads (Loba Chemie, India, CAS: 112926-00-8) transported under ambient condition and stored at AHRI at -20^{o}C until further processing (Snounou *et al.*, 1993; Rizzo *et al.*, 2011b; Stone *et al.*, 2012).

3.7. Laboratory Procedures

3.7.1. Microscopic examination

Both thick and thin blood films of a single participant were prepared directly from finger prick blood on a single slide. After both thin and thick films completely dried, only the thin film was fixed with absolute methanol (100%) for 30 seconds and dried completely in a horizontal position before staining. After fully dried, both the thin and thick smears were stained with 10% Giemsa solution (VWR, Belgium cat # 350864X) for 30 minutes. The stain was washed from the slide by gentle dipping into clean water in the jar. The Microscopic examination of blood films were carried out by expert microscopists, without knowing neither the decision of other microscopists nor PCR and serology results.

The stained thick blood films were examined based on Basic Malaria Microscopy standard protocol (WHO, 2010), with a light microscope at x100 objective and x10 oculars lenses after addition of immersion oil (MERCK, Germany, cat # 56822-250M) to search for the presence of malaria parasites and the results were classified either negative or positive after careful examination of at least 100 power fields and also parasite speciation was done. The thin film was used to confirm the malaria parasite species, when this couldn`t be done in the thick film and the result was recorded as either negative, *P. falciparum*-positive, *P. vivax*-positive, or mixed infection.

3.7.2. Combined antibody elution and DNA extraction

The combined approach for DNA extraction and serum elution methodology was used (Baidjoe *et al.*, 2013). In brief, six-millimeter diameter filter paper discs were punched with DBS punches (Vaessen creative, Germany, Cat# 21438-004) and placed in deep well plates (Thermo Fisher Scientific, USA, cat# 3957). Then 820µl of a solution; 0.5% saponin (SIGMA ALDRICH, USA, Cat # 47036-50G-F) in phosphate buffered saline (PBS) (SIGMA-ALDRICH, USA, Cat# D8537) was added to each well and plates were incubated overnight on horizontal shaker set for gentle agitation (Heidolph, Germany, Cat# DSG-304). One hundred eighty micro liters of the elute that contain all soluble elements, was transferred to a new plate and stored at −20°C until used in ELISA. The remaining, after removal of the elute, was aspirated with vacuum aspirator (ESCHMAN, England, UK, cat# 605152) and then 1ml of PBS washing solution stored at 4°C was added to each well and incubated for 30 minutes at 4°C. Plates were subsequently incubated on horizontal shaker for 30 minutes as above followed by aspiration and discarding of PBS. One hundred fifty microliters of solution; 6% Chelex 100 Resin (Bio-Rad, USA, cat# 143-2832) in

DNase/ RNase free water was added to each sample. Then the plates were sealed using DNA, DNAse and RNAse free aluminum peeling foil (Simport Scientific, USA, Cat# T329-5) and was incubated in a water bath for 4 times for 7 minutes at 97°C. Between each of 7-minutes of incubation, plates were briefly centrifuged in order to relieve pressure and ensure optimal DNA elution. After this incubation, plates were spun down at maximum speed (4700g) for 5 minutes to allow the chelex to settle. Eighty micro liters of the DNA containing solution was taken then aliquoted into the new plates. Samples were stored at −20°C until further analyses.

3.7.3. Enzyme Linked Immunosorbent Assay

Indirect ELISA assay was used to detect Immunoglobulin G (IgG) antibody responses against *P. falciparum* (*Pf*AMA-1-3D7) and *P. vivax* antigens (*Pv*AMA-1) (Drakeley *et al.*, 2005; Cook *et al.*, 2010). Immulon-4, 96-well plates (Thermo Fisher Scientific, USA, Cat# DIS-950-090L) were coated with recombinant antigens; AMA-1-3D7 *and PvAMA-1 (*Sal-1*) (*obtained from London School of Hygiene and Tropical Medicine) diluted with coating buffer *(*1.59M Na2CO3 (Merck, Germany, cat# 011-005-00-2) and 2.93M NaHCO3 (VWR, Leuven, Belgium, cat# 144-55-8) pH 9.5±0.2)*)* in the concentration of 1/5836 and 1/1322 respectively. Test and negative control sera were analyzed in duplicate at 1:2000 dilution in 1% skimmed milk powder (Scharlau, Spain, Cat# 06-019-500)/0.05% PBST (PBS (5.7M NaH_2PO4 (VWR, Leuven, Belgium, cat# 7558 – 80-7) 16.7M Na_2HPO4 (VWR, Leuven, Belgium, cat# 7558 - 79-4), 85M NaCl (EMSURE®, Denmark, cat# 7647-14-5) Tween 20 (SIGMA, USA, cat# P1379)). On every plate, blank wells (PBS/T) were included to correct optical density (OD) for background antibody reactivity. Negative control serum was obtained from naive Europeans that never traveled to malarious area and WHO Reference positive control sera; 10/198 and 72/096 NIBSC, UK for P. *falciparum* and *P. vivax* respectively (Bryan *et al.*, 2017) were included in six, fourfold serial dilution starting at the concentration of 1/100 in 0.05% PBS/T to allow standardization of OD values for day-to-day and inter-plate variation as well as for test validation. Horseradish peroxidase-conjugated with polyclonal rabbit anti-human IgG secondary antibody (Dako, Glostrup, Denmark, cat# DK-2600) was used to detect the presence of antigen antibody interaction through blue color development after addition of 3,3′,5, 5′-tetramethylbenzidine (TMB) (Tebu Bio Laboratories, France, cat# TMBW-1000-01) which reacts with the hydrogen peroxide of secondary antibody. The enzymatic reaction is stopped by addition of 0.2M sulfuric acid (Sigma Aldrich, USA, cat# 339741) and OD Value was measured using ELISA reader (Multiskan EX 2004-02, Thermo Fisher, USA, cat# 51118170) with Ascent Software at 450 nm filter. The cut off for seropositivity among samples was determined as the mean OD of the negative control sera plus 3 standard deviations.

3.7.4. PCR based Parasite detection

Plasmodium species were identified using genus-specific and species-specific primers in nested PCR (nPCR) targeting the small subunit ribosomal RNA (18ssrRNA) gene (Snounou *et al.*, 1993). The first amplification reaction (Nest1) was performed in a 25µL reaction mixture containing 1X PCR buffer (Promega, USA, cat# M891A), 2mM MgCl2 (Promega, USA, cat# A351H), 0.25 mM of each dNTPs (Promega, USA, cat# UT240), 0 .25 µM of each Genus-specific oligonucleotide primers pairs (rPLU5; 5'- CCT GTT GCC TTA AAC TTC-3` and rPLU6; 5'-TTA AAA TTG TTG CAG TTA AAA CG-3'), 0.75U/reaction of Taq DNA polymerase (Promega, USA, cat# M7805) and diluents of nuclease free water (MQ) (Promega, Madison, USA, cat# P119E) and 5ng/µL genomic DNA were used (Oyedeji *et al.*, 2007). Similarly the second (nest 2) reaction was performed in a total of 25µL reaction mixture containing the same reagents except the 2µl of template in this case was the product of first reaction instead of 5µl (Bousema *et al.*, 2013) and the oligonucleotide primers pairs are species-specific: rFAL1; 5'-TTA AAC TGG TTT GGG AAA ACC AAA TAT ATT-3' and rFAL2; 5'-ACA CAA TGA ACT CAA TCA TGA CTA CCC GTC-3' for *P. falciparum*, (Oyedeji *et al.*, 2007) and rVIV1; 5'-CGC TTC TAG CTT AAT CCA CAT AAC TGA TAC-3' and rVIV2; 5'-ACT TCC AAG CCG AAG CAA AGA AAG TCC TTA-3' for *P. vivax* were used. Previously characterized (ref) DNA samples from clinical malaria cases were used in every PCR plate as a positive control, alongside a negative control (Nucleic acid free water) (Hsiang *et al.*, 2012).

The cycling condition for Nest1 was initial denaturation at 95°C for 10 min followed by 35 cycles of denaturation, annealing and extension; at 95°C for 1 min, 58°C for 1 min and 72°C for 1.5 min respectively with a final extension step at 72°C for 10 minutes. The conditions for Nest2 were identical to the Nest1 except for the number of cycles, 30 cycles instead of 35 (Oyedeji *et al.*, 2007). The nPCR products along with a 100 bp DNA ladder (NEB, USA, cat# N0467t)) were subjected to electrophoresis on a 2% Agarose gel (VWR, Leuven, Belgium, cat# 443666A) using 0.5X Tris-Borate EDTA buffer (Trizma® base, Sigma Aldrich, USA, cat# T1503; Boric acid, Sigma Aldrich, USA, cat# *B0394* and EDTA, Sigma Aldrich, USA, cat# E9884). Ethidium bromide (Promega, USA, cat# H5041), 10µl of 5mg/ml solution was used to visualize the DNA. The electrophoresis was run for about 60 minutes at 120 volts and visualized with Ultraviolet (UV) illuminator (Bio Rad, USA). Depending on the specific primer pairs, expected product size of *P. falciparum* (205bp) and *P. viax* (120bp) were determined by comparing with the 100 bp DNA ladder.

3.7.5. Estimation of seroconversion rates with cross-sectional data

The reversible catalytic model for incidence data can be fit to prevalence data with a simplification where exposure time is measured by age in years (Corran *et al.,* 2007). Estimates of transmission intensity will be derived from fitting reverse catalytic models to the age seroprevalence data (Oduro *et al.,* 2013). The Model is using the maximum likelihood method that assumes a binomial error distribution: $Pt = \lambda /(\lambda + \rho)[1\text{-}\exp^{(-(\lambda+\rho)t)}]$ where Pt is the proportion of individuals aged t (t) that is seropositive, λ is seroconversion rate and ρ is the seroreversion rate. The parameter λ is related to the force of infection (Corran *et al.,* 2007). This can be done to investigate the relationship between force of vector and parasite exposure with age.

3.8. Data management and analysis

Questionnaire data obtained from the study was entered into MS-Excel. The OD values for each test serum sample were entered into an Excel simple Macro data sheet to calculate the average and normalized OD value, to check the duplicates variation and the standard curve for each plate for test validation. The analysis was done using STATA 13 (Stata Corp, TX, USA) and Graph Pad Prism 5.0 (Graph Pad Software Inc., CA, USA). Kappa (K) test was used to assess the degree of agreement between nPCR and light microscopy: when K values 0.21–0.60, 0.61–0.80, and >= 0.80, means moderate, good and almost perfect agreement respectively (Osman *et al.,* 2010). The sensitivity, specificity and accuracy of light microscopy and Serology were evaluated by ROC curve analysis. The cut-off for seropositivity among samples was determined as the mean OD of the negative control sera plus 3 standard deviations. In SCR analysis a mixed model was considered; first assuming constant transmission intensity over time (Model 1) and then assuming a change of transmission intensity (Model 2). In Model 2, the past and present SCR and the time of change of transmission intensity were estimated using the profile likelihood method. A log-likelihood of a catalytic conversion model that allows for a change in transmission occurring at iterative years was plotted to evaluate the time at which seroconversion rates changed for each species (Cook *et al.,* 2010). The maximum log-likelihood that is the time point at which a change in transmission is most likely to have occurred was determined. Age specific seroconversion curves to determine the rate at which a population becomes seropositive for responses to AMA-1 antigen to both *P. falciparum* and *P. vivax* were modeled using a simple reversible catalytic model for all study villages combined as described before (Corran *et al.,* 2007). Reverse cumulative distribution plots were used to evaluate differences in the geometric mean antibody titres against age categories, each village and malaria prevention and control interventions. Wilcoxon rank-sum test with Bonferroni correlation was used to test between two continuous variables age and OD

values. Findings were related to (historical) malaria control program data from the district health office. For each measure listed above 95% confidence intervals (95% CI) were calculated. In all comparison's differences in which $p < 0.05$ were considered statistically significant (Tseroni *et al.*, 2015).

3.9. Quality control

Quality control measures were taken by strictly following Standard Laboratory Procedures (SOPs). Same unique study code was used for both sample and questionnaire for each participant. During sample processing serum elution, DNA extraction, PCR and ELISA known positive and negative controls were used.

As a quality control for DNA extraction, in each plate a clean DBS was included as extraction control. Moreover, the efficiency was evaluated by amplifying a housekeeping gene, human β tubulin, from randomly selected samples. For PCR; nucleic acid free water (Sigma, USA, cat# w4502) and known malaria DNA were used as negative and positive controls respectively.

During serological test (ELISA) SOPs and instructions for the ELISA MACRO of London School of Hygiene and Tropical Medicine (MACRO v01, Version: 003) were used. For every assay, Positive (standards), Negative controls and coating buffer only (blank wells) were included as described above. Known concentrations of serially diluted positive control (antibody) was used in each plate to produce a standard curve and then this data was used to measure the concentration of unknown samples by comparison to the linear proportion of the standard curve. This was done on the graph pad prism for each plate on each day with optical density versus the concentration of the serial dilutions to evaluate a sigmoidal curve, and by simple Macro data sheet for final analysis to determine the antibody concentration of unknown samples. Duplicate OD values agreement was determined based on the SOP mentioned above and were excluded from the analysis, if variation detected. And if such samples were >=5 and the average of OD value of blank wells exceeded 1.2, the result of the whole plate was dropped and the reaction repeated.

3.10. Limitations of the study

The retrospective data did not include the malaria incidence before 2005, and even after the IRS was from 2007 and ITN/LLIN distribution from 2010 onwards. The District HMIS and Health post level log book both for malaria incidence and confirmatory parasite detection, were not consistent and differed in the completeness of the data captured. Thus, the study depended on the more complete data, district level.

4. RESULTS

4.1. Retrospective data; Babile district 2005-2016

4.1.1. Annual trends of RDT and/or Microscopy confirmed malaria

Over the 12-year period 259, 963 suspects were diagnosed for malaria by either RDT or Microscopy out of which 25576 (9.84%) were found infected. Overall, a continuous decline in incidence rate was observed in the District (Table 1). The highest record incidence, 33.4% (7905/23670), was seen in the year 2005 and the lowest, 2.9 (326/11132), was in 2016. The trend of incidence rate by age group revealed that higher rates in the years 2005 and 2006 in the >5 years age group with 72.0% (6108/8484) and 30.9% (2873/9305) respectively compared to 11.8% (1797/15186) and 15.7% (939/5991) at the age groups ≤5 years. But in the years 2007 to 2009 higher incidence was recorded both in the ≤5 years, 19.9% (873/4382), 13.4% (739/5527) and 22.8% (952/4181), and >5 years, 14.0% (1878/13402), 12.2% (1360/11162) and 14.9% (2066/13864), age groups. Then the incidence rate showed a decline in the ≤5 years age group from 2013 to 2016, from 1.4% (154/10763) to 0.3% (18/5990) compared to 5.6% (1215/21787) to 2.9% (326/11132) in >5 years age groups respectively (Table 1).

4.1.2. Annual trends of microscopy confirmed malaria

In Babile district four Health Centers (Awsharif, Erer Valley, Wayu and Babile) and one Hospital (Bisidimo Hospital) were performing microscopic malaria detection and parasite species identification. The district health office maintained the monthly malaria morbidity and mortality registration logbook. For this study the last 12 years, from 2005 to 2016, malaria program data were retrieved. During the period, of the 24,861 blood films from febrile patients examined 4451 (17.9%) were malaria positive. In terms of causative species 12.1% (3000/24,861), 1.6% (388/24861) and 4.3% (1063/24861) were *P. falciparum*, *P. vivax* and mixed infection of *P. falciparum* and *P. vivax* respectively. The 12 years data showed an overall progressive decline from 2005 to 2016 at a proportion of 99.8% (1096/1098) to 7.3% (301/4128). Though the combined trend line (Figure 2, black solid line) had a sharply declining slope, there were miner pick humps were seen in the years 2009 (17.0%) and from 2012 (15.1%) to 2014 (18.3%) and 2013 (20.5%) (Table 1).

The highest proportions of *P. falciparum* and *P. vivax* mixed infections were recorded in the years 2005 (82.7%) and 2006 (43.9%), interestingly during the same years single parasite infection *P. falciparum* or *P. vivax* only were lower. After the year 2007 to 2016 *P. falciparum* revealed the

highest rate of infection at 18.2% and 7.0% respectively while *P. vivax* and mixed parasite infections decreased from 2006, 12.6% and 43.9% respectively to nearly zero from the years 2007 to 2016 (Table 1).

4.1.3. ITN/LLIN ownership ratio and IRS coverage

In the district ITN/LLIN was distributed in two rounds in 2010 and in 2013. In the first round only about 38.3% (42832/111690) of the population got ITN and the coverage during the second round was about 54.0% (65830/121970). These means on average 1.7 (0.5-2.1) and 2.6 (1.5-3.3) ITN/LLIN per household were distributed in the first and second rounds respectively. In the district areas and structures that need to be sprayed were identified in each year (Table 1). Indoor residual spraying coverage calculated as number of structures sprayed per those identified was 84.03% (77.1% to 100%) but 36.7% in 2007 was incomplete data (Table 1).

Table 1: Malaria infection prevention and the trend of annual incidence and microscopy confirmed malaria in Babile district; 2005 to 2016.

Year	IRS coverage %(y/Y)*	ITN/LLIN distribution ratio (N/n)*	% (Confirmed malaria cases (Microscopy/RDT)/ Total cases tested)			Microscopy confirmed incidence			
			<5years	>5years	Total	*P. falciparum*	*P. vivax*	Mixed	Total
2005	_	_	11.8(1797/15186)	72.0(6108/8484)	33.4(7905/23670)	14.85(163/1098)	2.3(25/1098)	82.7(908/1098)	99.8(1096/1098)
2006	_	_	15.7(939/5991)	30.9(2873/9305)	24.9(3812/15296)	43.5(107/246)	12.6(31/246)	43.9(108/246)	100(246/246)
2007	36.7(11174/30463)	_	19.9(873/4382)	14.0(1878/13402)	15.5(2751/17784)	18.2(215/1181)	0.5(6/1181)	0.25(3/1181)	19.0(224/1181)
2008	92.6(28202/30463)	_	13.4(739/5527)	12.2(1360/11162)	12.6(2099/16689)	7.6(78/1027)	0.2(2/1027)	0(0/1027)	7.8(80/1027)
2009	91.9(28202/30693)	-	22.8(952/4181)	14.9(2066/13864)	16.7(3018/18045)	16.0(209/1306)	0.7(9/1306)	0.3(4/1306)	17.0(222/1306)
2010	78.6(30670/39040)	38.3 (42832	7.6(248/3246)	7.9(766/9653)	7.9(1014/12899)	9.4(146/1553)	0.7(11/1553)	0(0/1553)	10.1(157/1553)
2011	97.1(28827/29703)	/111690)_	2.3(183/7911)	4.0(747/18638)	3.5(930/26549)	8.3(75/901)	1.0(9/901)	0(0/901)	9.3(84/901)
2012	96.8(28750/29703)	_	1.3(105/7969)	3.3(569/17473)	2.6(674/25442)	12.8(294/2304)	2.1(49/2304)	0.2(4/2304)	15.1(347/2304)
2013	77.1(22890/29703)	_	1.4(154/10763)	5.6(1215/21787)	4.5(1469/32550)	18.0(743/4117)	2.4(98/4117)	0.02(1/4117)	20.5(842/4117)
2014	100.0(22890/22890)	54.0 (65830	0.8(79/10214)	4.1(895/21722)	3.0(974/31936)	14.6(415/2835)	3.5(99/2835)	0.14(4/2835)	18.3(518/2835)
2015	89.6(22275/24870)	/121970)_	0.4(37/8988)	4.2(549/12993)	2.7(586/21981)	6.3(264/4165)	1.0(42/4165)	0.7(31/4165)	8.1(337/4165)
2016	90.3(13597/15056)	_	0.3(18/5990)	2.9(326/11132)	2.0(344/17122)	7.0(291/4128)	0.2(7/4128)	0.07(3/4128)	7.3(301/4128)
Total	84.03%		6.8(6,124/90,348)	10.2(17,286 /169,615)	9.0(23,410 /259963)	3000(12.1)	388(1.6)	1063(4.3)	17.9(4454/24861)
			P < 0.001		P < 0.001	P = 0.0001			

4.2. Cross-sectional survey result

4.2.1. Characteristics of study population

The study enrolled 1, 144 volunteers, 47.9% (548/1144) males and 52.10% (596/1144) females. We found a significantly (P= 0.001) higher history of exposure in males (29.9%, 162/542) than females (21.4%, 126/588). The participants' age ranged from 1 to 88 years with the median age of 12 years (IQR, 7 - 30). Thus, we categorized under four age groups with the interval of five, ≤5 years 19.8% (227/1144), >5-10 years 26.0% (297/1144), >10-15 years 18.7% (214/1144) and >15 years 35.5% (406/1144) having history of infection 20.7% (46/222), 21.1% (62/294), 17.0% (36/212), and 35.8% (144/402) respectively that showed higher report of infection was recorded at the age categories of above 15 years (P value = 0.0001) (Table 2). From the total participants 25.5% (288/1130) reported previous history of malaria infection, of which 56.9% (164/288), 29.17% (84/288) and 13.9% (40/288) recalled their infection episodes as once, twice, and three times and above respectively (Figure 5). But 1.22% (14/1144) didn't clarify their previous exposure. From all the participants, 69.0% of them owned at least one LLIN per household but only 63.1% of them used uninterruptedly and no significant difference was seen among different age groups and sex (P > 0.05). Most of the participant`s (82.2%) house was sprayed within past 12 months. Regarding their house construction, 46.7% (532/1140) had no eave openings (Table 2).

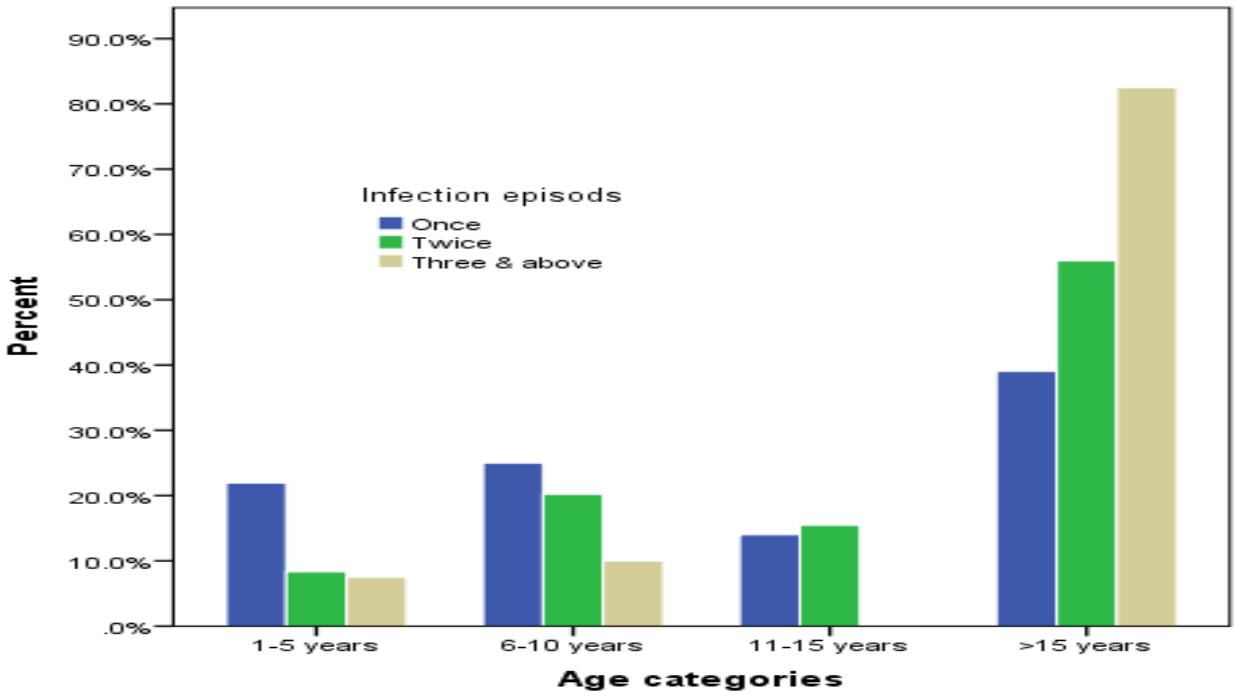


Figure 5: The chart showed the Malaria infection episodes recalled by the participants

Table 2:Characteristics of survey participants (n = 1144), sociodemographic and malariometric, Babile district, east Hararghe, Oromia 2018.

Sociodemographic		Total tested, % (n/N)	Malaria infection history, %	ITN/LLIN utilization		IRS spray	Eave opening present
				any use	Uninterrupted		
Sex	Male	47.9 (548/1144)	29.9 (162/542)	67.8 (371/547)	59.6 (221/371)	81.2 (440/542)	49.0 (267/545)
	Female	52.1 (596/1144)	21.4 (126/588)	70.3 (418/595)	66.2 (276/417)	83.2 (491/590)	44.5 (265/595)
	Total	100 (1144)	25.5 (288/1130)	69.1 (789/1142)	63.1 (497/788)	82.2 (931/1132)	46.7 (532/1140)
	χ^2 (p-value)		10.6 (0.001)	0.79 (0.375)	3.69 (0.055)	0.8 (0.37)	2.3 (0.132)
Age in years	<=5 years	19.8 (227/1144)	20.7 (46/222)	70.4 (159/226)	67.3 (107/159)	76.1 (172/226)	45.6 (103/226)
	>5-10	26.0 (297/1144)	21.1 (62/294)	72.3 (214/296)	60.8 (130/214)	87.3 (254/291)	42.3 (126/296)
	>10-15	18.7 (214/1144)	17.0 (36/212)	66.8 (143/214)	62.0 (88/142)	83.9 (177/211)	38.0 (81/213)
	>15 years	35.5 (406/1144)	35.8 (144/402)	67.2 (273/406)	63.0 (172/273)	81.2 (328/404)	54.8 (222/405)
	Total	100 (1144)	25.5 (288/1130)	69.1 (788/1142)	63.1 (497/788)	82.2 (931/1132)	46.7 (532/1140)
	χ^2 p-value		36.3 (< 0.001)	2.8 (0.43)	1.8 (0.62)	11.6 (0.009)	19.3 (<0.001)

4.2.2. Malaria infections detected by microscopy and PCR

In this study, among 1144 participants 1.84% were positive by microscopy; 1.2% (95% CI, 0.1 – 2.1) *P. falciparum* and 0.4% (95% CI, 0.1 – 1.0) *P. vivax*. The detected infection prevalence was significantly different between the study villages (Table 3). Using nPCR we detected 4.4% (95% CI, 3.3 – 5.7) and 3.0% (2.1 – 4.1) *P. falciparum* and *P. vivax* infections respectively. The level of agreement ($\kappa = 0.32$, 93.9%) between microscopy and nPCR (Table 4) was moderate. From the total nPCR detected infections, 76.9% (70/91) were microscopy-negative. Overall 6.2% (70/1123) of the infections were submicroscopic. Using PCR as gold standard, microscopy had a sensitivity of 23.1% (95% CI: 14.9-33.1) and a specificity of 99.9% (95% CI: 96.4-100) for either of the parasite species infection. The majority of nPCR detected *P. vivax* (94.1%, 32/34) infections were missed by microscopy compared to *P. falciparum* (68.0%, 34/50). Nested PCR also detected 0.6% (7/1144) of *P. falciparum* and *P. vivax* mixed parasite infection but not in microscopy. Taking mixed infections into consideration (counting a mixed infection for both species as positive) species specific ROC analysis was done. The sensitivity and specificity of microscopy relative to nPCR for *P. falciparum* were 33.3% (95% CI: 21.4-47.1%) and 99.8% (95% CI: 99.2-99.9%) respectively. The corresponding values for *P. vivax* were 4.9% (95% CI: 0.5-16.5%) for sensitivity, 99.7% (95% CI: 99.2-99.9%) for specificity (Table 4).

4.2.3. Seroprevalence versus nPCR for the detection of asymptomatic infection

Overall seroprevalence in the study villages was 21.6% (95% CI, 19.2 – 24.1): while the *PfAMA-1* was 11.2% (95% CI, 9.4 – 13.2), the *PvAMA-1* prevalence was 13.1% (95% CI, 11.1 – 15.1) (Table 2). Differences were observed for seropositivity to *PfAMA-1* between male and female participants ($P < 0.001$): females being at 3-fold lower risk than male participants (OR, 0.383; 95% CI, 0.247 – 0.594) after adjusting for village groups and age categories. Such difference was not observed for *PvAMA-1* (Table 3).

Table 3: Asymptomatic Malaria infections detected by microscopy, nPCR and ELISA, Babile, east Hararghe, 2018.

Village	Sex (F)	Microscopy			nested PCR				Indirect ELISA	
		P. falciparum	*P. vivax*	Total	*P. falciparum*	*P. vivax*	Mixed	Total	*Pf*AMA-1	*PvAMA-1*
1*	57.7 (49/85)	0 (0/85)	0 (0/85)	0 (0/85)	7.1 (6/85)	4.7 (4/85)	0 (0/85)	11.8 (10/85)	3.5 (3/3/85)	4.7 (4/85)
2*	54.7 (52/95)	6.3 (6/95)	0 (0/95)	6.3 (6/95)	17.9 (17/95)	3.2 (3/95)	2.1 (2/95)	23.7 (22/95)	24.2 (23/95)	4.2 (4/95)
3*	17.8 (13/73)	0 (0/73)	1.4 (1/73)	1.4 (1/73)	5.5 (4/73)	4.1 (3/73)	1.4 (1/73)	11.0 (8/73)	38.4 (28/73)	4.1 (3/73)
4	45.3 (58/128)	2.3 (3/128)	0.8 (1/128)	3.1 (4/128)	3.1 (4/128)	0 (0/128)	0 (0/128)	3.1 (4/128)	3.1 (4/128)	1.6 (2/128)
5	52.0 (53/102)	0.0 (0/102)	0 (0/102)	0 (0/102)	1.0 (1/102)	1.0 (1/102)	1.0 (1/102)	3.0 (3/102)	2.0 (2/102)	2.0 (2/102)
6*	52.2 (47/90)	5.6 (5/90)	2.2 (2/90)	7.8 (7/90)	8.9 (8/90))	6.7 (6/90)	1.1 (1/90)	16.7 (15/90)	14.4 (13/90)	15.6 (14/90)
7*	72.9 (51/70)	1.4 (1/70)	0 (0/70)	2.8 (2/70)	8.6 (6/70)	4.3 (3/70)	0 (0/70)	14.3 (10/70	4.3 (3/70)	17.4 (12/69)
8	48.9 (39/80)	0 (0/100)	0 (0/100)	0 (0/80)	0 (0/80)	1.3 (1/80)	1.3 (1/80)	2.6 (2/80)	6.3 (5/80)	38.8 (31/80)
9	41.4 (29/70)	0 (0/70)	0 (0/70)	0 (0/70)	1.4 (1/70)	1.4 (1/70)	0 (0/70)	2.8 (2/70)	8.6 (6/70)	75.7 (53/70)
10*	60.2 (56/93)	1.1 (1/93)	0 (0/93)	1.1 (1/93)	2.2 (2/93)	6.5 (6/93)	1.1 (1/93)	9.7 (9/93)	18.3 (17/93)	23.7 (22/93)
11	56.1 (37/66)	0.0 (0/66)	0 (0/66)	0 (0/66)	0 (0/66)	3.0 (2/66)	0 (0/66)	3.0 (2/66)	13.6 (9/66)	0 (0/66)
12	45.5 (40/88)	0 (0/88)	0 (0/88)	0 (0/88)	0 (0/88)	1.1 (1/88)	0 (0/88)	1.1 (1/88)	13.6 (13/88)	1.1 (1/88)
13	71.7 (33/46)	0 (0/46)	0 (0/46)	0 (0/46)	0 (0/46)	4.4 (2/46)	0 (0/46)	4.4 (2/46)	0 (0/46)	0 (0/46)
14	67.2 (39/58)	0 (0/58)	0 (0/58)	0 (0/58)	1.7 (1/58)	0 (0/58)	0 (0/58)	1.7 (1/58)	5.2 (3/58)	1.7 (1/58)
Total	**52.1 (596/1144)**	**1.4 (16/1144)**	**0.4 (5/1144)**	**1.8 (21/1144)**	**4.4 (50/1144)**	**2.9 (33/1144)**	**0.6 (7/1144)**	**7.96 (91/1144)**	**11.2 (128/1144)**	**13.1(149/1143)**
FET		P = 0.002			P < 0.001				Pr < 0.001	Pr < 0.001

Note: *= villages categorized under high nPCR prevalence; F (sex) = female; FET= Fischer's exact test; The village numbers (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and 14) represents the sampled villages Sherqa, Alikulo, Baha, Kufakasa, Abdi Nasir, Bisidimo 01, Bisidimo 02, Seferdina, Maya, Biftu, Ifa, Abdi, Dughe and Elemo Dara found in 8 kebeles of Babile Woreda.

Comparing serology and nPCR; of the 57 individuals *P. falciparum* positive and mixed infections by nPCR, 42 (73.7%) were antibody positive to *PfAMA-1* which showed 91.2% accuracy and area under curve of 0.83 (95% CI 0.81-0.85). Similarly of the 41 *P. vivax* and mixed infections nPCR positive individuals 29 (70.7%) were positive to *PvAMA-1* with 88.5% accuracy and area under curve of 0.8 (95% CI 0.77- 0.82) (Table 4). The serology showed 11.7% (29/248) cross-reactivity against *P. falciparum* and *P. vivax* antigens and from 7 mixed infections by nPCR 5 (71.4%) were also reactive for both *PfAMA-1* and *PvAMA-1*.

Seropositivity to *PfAMA-1* in the high-prevalence villages was 1.90-fold (95% CI of OR, 1.22 – 2.97) more compared to the lower prevalence villages after controlling for age and sex of participants. But, the risk of being *PvAMA-1* seropositive didn't reveal (OR, 0.553; 95% CI, 0.378 – 0.809; P = 0.002) when adjusted for sex and age groups. Seropositivity to *PfAMA-1* was high in individuals that had self-reported malaria in their life time (21.2%, 61/288) compared to those that did not report previous malaria (7.6%, 63/833, P < 0.001). Individuals with 3 or more episodes were 51.2% (21/40) seropositive for *PfAMA-1* compared to 23.8% (20/84) and 12.2% (20/164) for individuals with 2 and 1 episodes only, respectively. Regarding seropositivity to *PvAMA-1* statistically significant difference was not observed (ρ = 0.1022, P = 0.0833); while the seropositivity in individuals with 3 or more episodes was 22.5% (9/40), it was 19.1% (16/84) and 12.8% (21/164) in individuals with only 2 and 1 episodes respectively (Table 5).

Table 4: nPCR, Microscopy and serology agreement, sensitivity and specificity in detecting asymptomatic malaria, Babile, east Hararghe, 2018.

		PCR (Gold standard)				**ROC test**			**Kappa test**	
		Negative	*P. falciparum*	*P. vivax*	Total	Sensitivity (%)	Specificity (%)	Accuracy (%)	Agreement	K (P-value)
Microscopy										
Either species	Positive	0	21		21	23.1	100	93.9	93.9%	0.36
	Negative	1053	70		1123	(14.9-33.1)	(96.4-100)			(<0.0001)
	Total	1053	91		1144					
P. falciparum	Positive	0	16		16	28.1	100	96.4	96.4%	0.43
	Negative	1087	41	NA	1128	(17.0-42.0)	(96.4-100)			(<0.0001)
	Total	1087	57		1144					
P. vivax	Positive	0	3	2	5	4.9	99.7	96.3	96.3%	0.08
	Negative	1100	NA	39	1139	(0.5-16.5)	(99.2-99.9)			(<0.0001)
	Total	1100	3	41	1144					
Indirect ELISA									Area under curve	
*Pf*AMA	Negative	101	15		116	73.7	92.1	91.2	0.83 (0.81-0.85)	
	Positive	86	42		128					
	Total	187	57		1144					
*Pv*AMA	Negative	983		12	995	70.7	98.1	88.5	0.8 (0.77 - 0.82)	
	Positive	120		29	149					
	Total	1103		41	1144					

Table 5: Malaria seroprevalence versus probable risk factors

	Villages category based on PCR prevalence				**Sex**				
	<10%	**>10%**	**OR (95% CI)**	**P value**	**Male**	**Female**	**OR (95% CI)**		**P value**
PfAMA	6.58 (42/638)	16.7 (83/497)	1.9(1.22-2.97)	0.005	14.1(77/548)	8.6 (51/596)	0.383 (0.25-0.59)		0.003
***Pv*AMA**	13.98 (89/637)	11.7 (58/498)	0.55(0.38-0.8)	0.002	13.5 (74/548)	12.6 (75/596)	0.53 (0.427-0.63)		0.061
	History of malaria infection				**Number of malaria infection episodes**				
	Yes	**No**	**OR (95% CI)**	**P value**	**Once**	**Twice**	**≥Three**	**ρ**	**P value**
PfAMA	21.2 (61/288)	7.6 (63/833)	3.3 (2.2-4.8)	<0.001	12.2 (20/164)	23.8 (20/84)	52.5 (21/40)	0.297	<0.001
***Pv*AMA**	16.0 (46/288)	10.2 (85/832)	1.7 (1.1-2.5)	0.009	12.8 (21/164)	19.0 (16/84)	22.5 (9/40)	0.1022	0.0833

4.3. Seroconversion rates (SCR)

The likelihood of individuals testing seropositive increased with age for both *PfAMA-1* (OR, 1.07; 95% CI, 1.06 – 1.08; P < 0.001) and *PvAMA-1* (OR, 1.02; 95% CI, 1.01 – 1.03; P < 0.001). Overall, a very strong association was observed between age groups and seropositivity (P < 0.001) for both *PfAMA-1* and *PvAMA-1*. The highest seropositivity was detected in adults (> 15 years) for *PfAMA-1* (89.9%, 116/129, P < 0.001) and individuals older than 10 years for *PvAMA-1* (77.9%, 116/149, P < 0.001). The odds of being seropositive for *Pf*AMA-1 was 3.65 (95% CI, 1.80 – 7.39; P < 0.001) and 5.0-fold (95% CI, 2.63 – 9.52; P < 0.001) higher for children between >10 – 15 years and adults, respectively as compared to children ≤5 years (Table 6).

Age seroconversion curves for responses to AMA antigen to both *P. falciparum* and *P. vivax* were modeled. Profile likelihood analysis showed evidence of a change in transmission in all villages and in the sub-groups (Fig 6). A change in force of infection (FoI) was detected at 15.5 and 11.5 years for *PfAMA-1* and *PvAMA-1*, respectively. Thus, the maximum log-likelihood time point at which a change in transmission have occurred was approximately 15.5 and 11.5 years back (black dotted lines) for *P. falciparum* and *P. vivax* (gray dotted lines) respectively. The horizontal lines represent the 95th percentile of the Chi-Squared on 1 degree of freedom below the maximum. The two points at which this line crosses the log-likelihood profile are used to determine an approximate 95% confidence interval for the time since the change in SCR (Fig 6 and 7).

Table 6: Seroprevalence versus age for study villages

	PfAMA-1 Positivity				*PvAMA-1* positivity			
Village	≤5 Years	>5-10 Years	>10-15 Years	≥15 Years	≤5 Years	>5-10 Years	>10-15 Years	≥15 Years
1	0 (0/21)	0 (0/29)	0 (0/16)	15.8 (3/19)	0 (0/21)	3.4 (1/29)	12.5 (2/16)	5.3 (1/19)
2	12.5 (3/24)	17.6 (3/17)	18.2 (2/11)	32.6 (14/43)	0 (0/24)	0 (0/17)	0 (0/11)	9.3 (4/43)
3	0 (0/4)	0 (0/6)	0 (0/3)	46.7 (28/60)	0 (0/4)	16.6 (1/6)	0 (0/3)	3.3 (2/60)
4	0 (0/13)	2.1 (1/48)	2.1 (1/48)	10.5 (2/19)	0 (0/13)	0 (0/48)	0 (0/48)	10.5 (2/19)
5	3.8 (1/26)	2.0 (1/51)	0 (0/20)	0(0/5)	0 (0/26)	2.0 (1/51)	0 (0/20)	20.0 (1/5)
6	5.3 (1/19)	0 (0/23)	7.1 (1/14)	32.4 (11/34)	5.3 (1/19)	13.0 (3/23)	28.6 (4/14)	20.7 (6/34)
7	0 (0/6)	0 (0/18)	0 (0/10)	8.3 (3/36)	16.7 (1/6)	5.6 (1/18)	20.0 (2/10)	22.9 (8/35)
8	0 (0/17)	6.3 (1/16)	0 (0/20)	14.8 (4/27)	11.8 (2/17)	43.8 (7/16)	30.0 (6/20)	57.7 (15/27)
9	0 (0/15)	0 (0/9)	6.7 (1/15)	16.1 (5/31)	40.0 (6/15)	66.7 (6/9)	93.8 (15/16)	86.7 (26/30)
10	0 (0/11)	0 (0/18)	5.9 (1/17)	34.0 (16/47)	18.2 (2/11)	5.6 (1/18)	18.8 (3/16)	33.3 (16/48)
11	0 (0/14)	4.5 (1/22)	0 (0/14)	50.0 (8/16)	0 (0/14)	0 (0/22)	0 (0/14)	0 (0/16)
12	0 (0/10)	0 (0/19)	14.3 (2/14)	24.4 (11/45)	0 (0/10)	0 (0/19)	0 (0/14)	2.2 (1/45)
13	0 (0/27)	0 (0/9)	0 (0/7)	0 (0/3)	0 (0/27)	0 (0/9)	0 (0/7)	0 (0/3)
14	0 (0/20)	0 (0/12)	0 (0/5)	14.3 (3/21)	0 (0/20)	0 (0/12)	0 (0/5)	4.8(1/21)
Total	2.2 (5/227)	2.4 (7/297)	3.7 (8/214)	26.6 (108/406)	5.3 (12/227)	7.1 (21/297)	15.0 (32/214)	20.8 (84/405)
	OR (95%, CI)	1.07 (1.06 – 1.08); P < 0.001				1.02 (1.01 – 1.03); P < 0.001		

Note: The village numbers 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and 14 represents the sampled villages Sherqa, Alikulo, Baha, Kufakasa, Abdi Nasir, Bisidimo 01, Bisidimo 02, Seferdina, Maya, Biftu, Ifa, Abdi, Dughe and Elemo Dara found in 8 kebeles of Babile District.

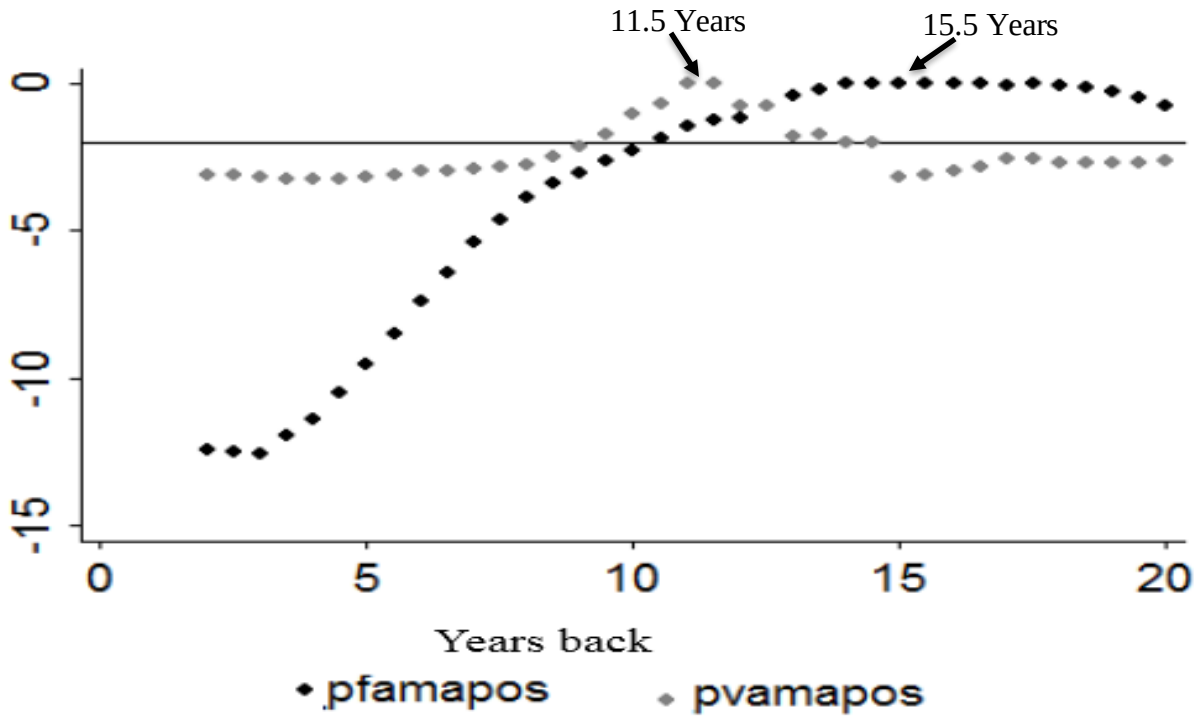


Figure 6: Profile likelihood plots to evaluate the time at which SCRs changed. *PfAMA-1* (black dotted lines) and b) *PvAMA-1* (gray dotted lines); showing the log-likelihood of a catalytic conversion model allowing for a change in transmission occurring at iterative years.

Seroprevalence plots assuming a change in SCR 15.5 (n = 1144, P < 0.001) and 11.5 (n = 1143, P = 0.010) years ago for *PfAMA-1* and *PvAMA-1* are shown in Fig. 7a and 7b, respectively. In case of *PfAMA-1* (λ_1 = 0.0179 (95% CI 0.0091 - 0.0352), λ_2 = was 0.0027 (95% CI 0.0017 - 0.0043), a 6.53-fold higher force of infection was estimated before a change in time point than post change. Whereas a more drastic change was seen in *PvAMA-1* SCR (λ_1 = 0.5463,95% CI 0.0371 - 9.0472), λ_2 = 0.0174,95% CI 0.0120 - 0.0254), which showed a 58.9-fold change in force of infection (Fig 7).

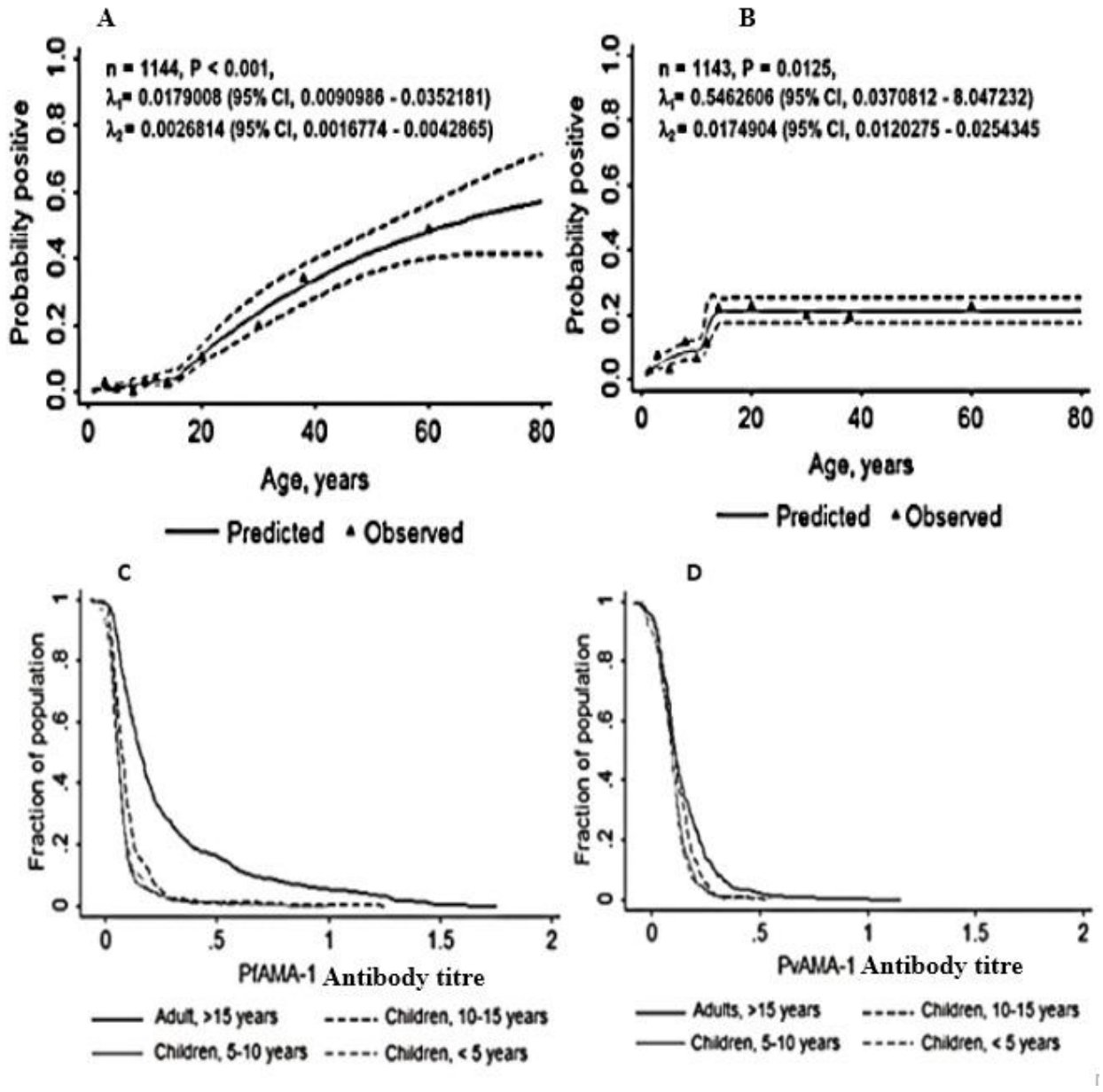


Figure 7: Age seroprevalence plots and trends in antibody titres.

Seroconverssion curves are presented for A) *PfAMA-1* (n=1144) and B) *PvAMA-1* (n=1143) using a simple reversible catalytic model fitted by maximum likelihood. Triangles represent observed data and black lines predicted values. Dotted black lines represent upper and lower 95% CI for the predicted seroprevalence by age. A likelihood ratio test was used to determine if a model with a change in transmission fitted the data best, associated P-values as well as SCR estimates pre- and post-change are shown on the plots. Numbers indicate pre (λ_1) and post (λ_2) change seroconversion values. Reverse cumulative distribution plots for age groups are

indicated for C) *PfAMA-1* and D) *PvAMA-1* for the four age groups (adults older than 15 years in black, children between 10 and 15 years black dotted lines, children between >5 and 10 years in grey lines and children ≤5 years in dotted grey lines. Shown in the X-axis in C and D are log_{10}-transformed antibody titre and on the Y-axis the percentage of individuals having the indicated antibody titre or higher on the X-axis.

Smaller sample size per village limited checking a change in SCR for each village but for categories. The plots for *PfAMA-1* suggest that a change in transmission occurred earlier for the low-prevalence village group (18 years ago) as compared to a more recent change in the high-prevalence village (15 years ago) while the same period was detected for *PvAMA-1* for the two village groups (12 years) (Fig. 8). The resulting SCR suggests a 1.7-fold decline in transmission in the low -prevalence village as compared to the high-prevalence village within the respective years (λ_{low} = 0.0021481 and λ_{high} = 0.0036215 for *PfAMA*-1) (Fig. 9). For *PfAMA-1* (P < 0.001, λ_{low} = 0.0052842 without assuming change, λ_1 = 0.0134322 & λ_2 = 0.0021481 after assuming change at 18 years for low-prevalence village group) and (P < 0.001, λ_{high} = 0.0090451 without assuming change, λ_1 = 0.0210213 and λ_2 = 0.0036215 after assuming change at 15 years for the high-prevalence village group). For *Pv*AMA-1 it was significant only in the low-prevalence village (P = 0.0032, λ_{low} = 0.0199206 without assuming change, λ_1 = 0.8318286 & λ_2 = 0.0194751 after assuming change at 12 years) and (P = 0.2516, λ_{high} = 0.0117683 without assuming change, λ_1 = 0.0022599 & λ_2 = 0.0101262 after assuming change at 12 years for low village group (Fig. 9).

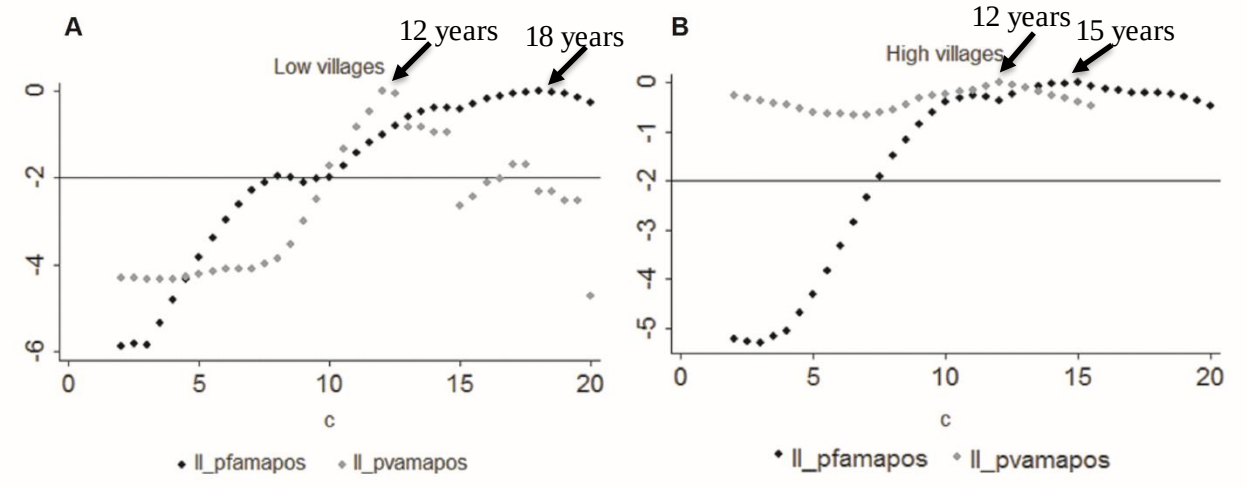


Figure 8: Profile likelihood plots to evaluate the time at which sero-conversion rates changed

For a) low and b) high-prevalence villages as determined by PCR for *Pf*AMA-1 (black dots) and *PvAMA-1* (grey diamond). The plots for *PfAMA-1* suggest a change in transmission occurred approximately 18 and 15 years ago (black dotted lines) while for *PvAMA-1* (gray diamonds) change occurred approximately 12 years ago. The horizontal lines represent the 95th percentile of the Chi-Squared on 1 degree of freedom below the maximum. The two points at which this line crosses the log-likelihood profile are used to determine an approximate 95% confidence interval for the time since the change in SCR.

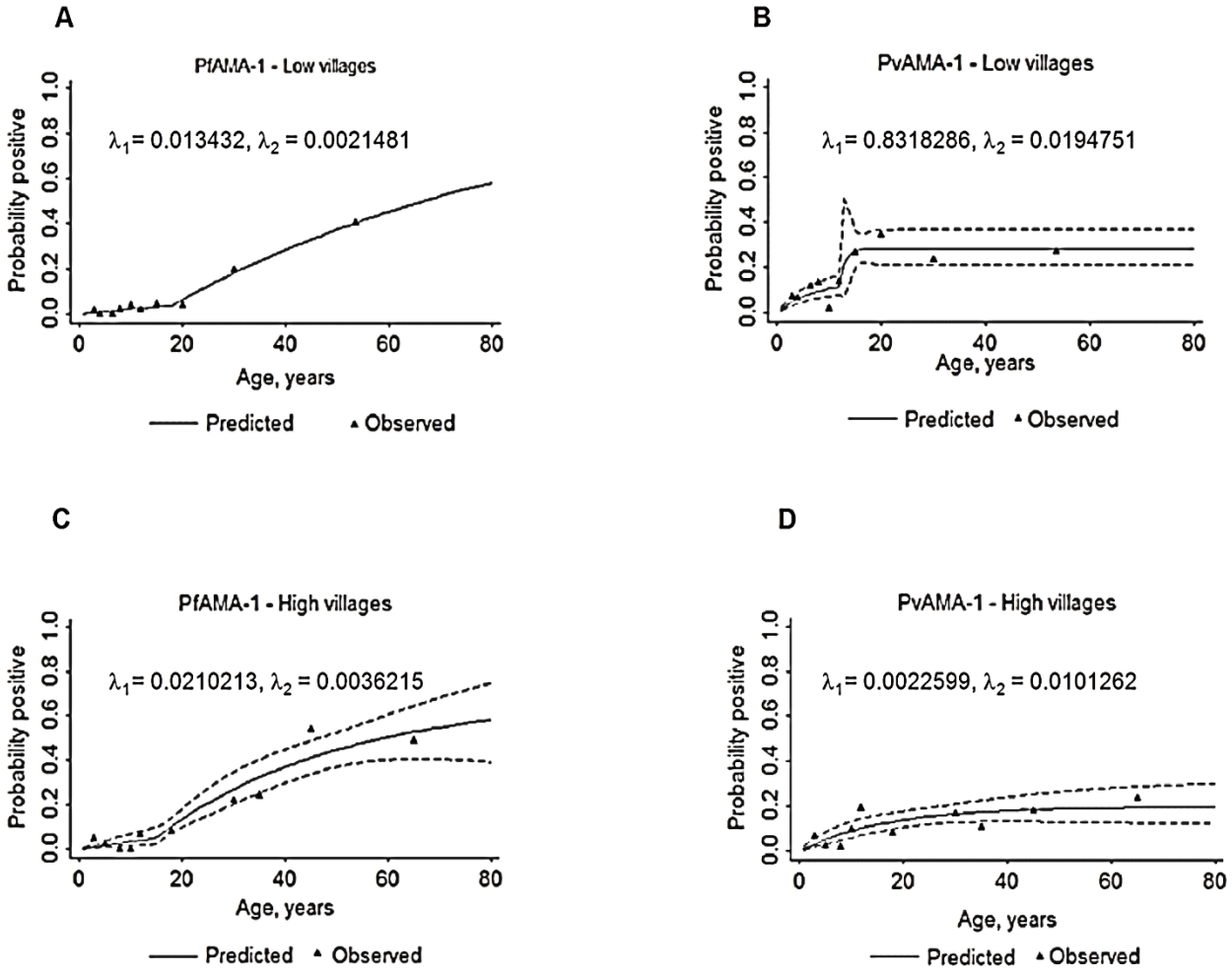


Figure 9: Age-seroprevalence plots for *PfAMA-1* and *Pv*AMA-1 in village groups

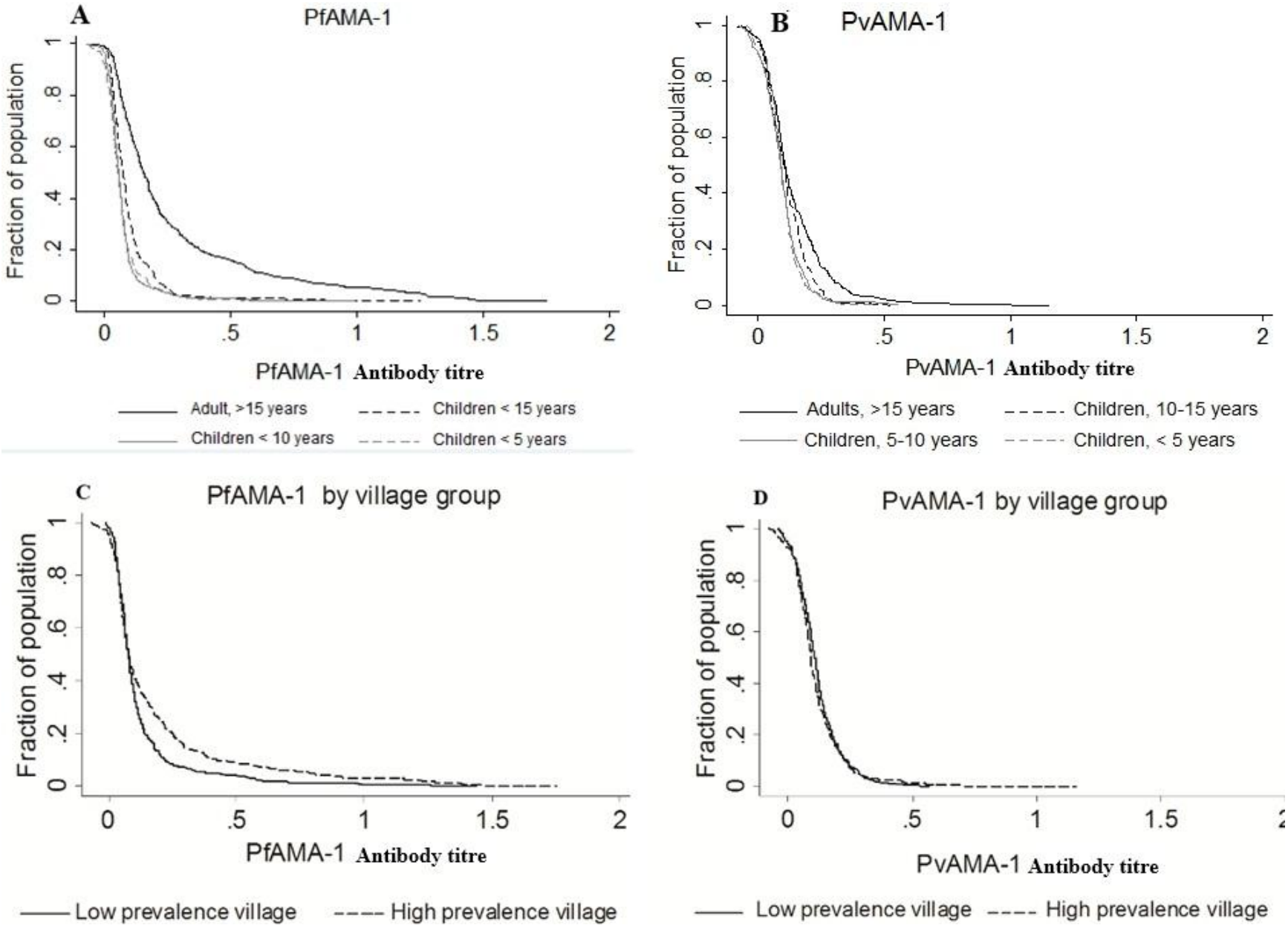
For *PfAMA-1* it was significant in both villages ($P < 0.001$, $\lambda_{low} = 0.0052842$ without assuming change, $\lambda_1 = 0.013432$ and $\lambda_2 = 0.0021481$ after assuming change at 18 years for low village group) (Fig 9a) and ($P < 0.001$, $\lambda_{high} = 0.0090451$ without assuming change, $\lambda_1 = 0.0210213$ and $\lambda_2 = 0.0036215$ after assuming change at 15 years for low village group) (Fig 9c) and for

*Pv*AMA-1 it was significant only in the low village (P = 0.0032, λ_{low} = 0.0199206 without assuming change, λ_1 = 0.8318286 & λ_2 = 0.0194751 after assuming change at 12 years) and (P < 0.2516, λ_{high} = 0.0117683 without assuming change, λ_1 = 0.0022599 & λ_2 = 0.0101262 after assuming change at 12 years for low village group)

4.4. Reverse cumulative distribution

To visualize distributions of antibody titres in more detail RCD was used to examine the difference between the magnitude of antibody responses between age and village groups and intervention utilization. The antibody levels for age groups older than 15 year showed significantly higher than that of children younger than 15 years for both *PfAMA-1* (Fig 10a) and *PvAMA-1* (Fig 10b). species (P < 0.001).

There was significant vitiation in antibody level (normalized OD) between high and low nPCR prevalence villages for *PfAMA-1* (p<0.001) (Fig 10c) and in *PvAMA-1* (P = 0.003) (Fig 10d). The antibody level against intervention types was evaluated; lower *PfAMA-1* antibody level was seen from participants using IRS (P = 0.005) but significant difference was not observed from participants using ITN only (P = 0.073) and using both interventions (P = 0.075) (Fig 10e), but antibody level against *PvAMA-1* did not differ between the intervention types; ITN only (P = 0.368), IRS only (P = 0.291) and both (P = 0.533) (Fig 10f).


A
PfAMA-1
Fraction of population
0
.2
.4
.6
.8
1
0
.5
1
1.5
2
PfAMA-1 Antibody titre
Adult, >15 years
Children < 15 years
Children < 10 years
Children < 5 years
B
PvAMA-1
Fraction of population
PvAMA-1 Antibody titre
Adults, >15 years
Children, 10-15 years
Children, 5-10 years
Children, < 5 years
C
PfAMA-1 by village group
Fraction of population
PfAMA-1 Antibody titre
Low prevalence village
High prevalence village
D
PvAMA-1 by village group
Fraction of population
PvAMA-1 Antibody titre
Low prevalence village
High prevalence village

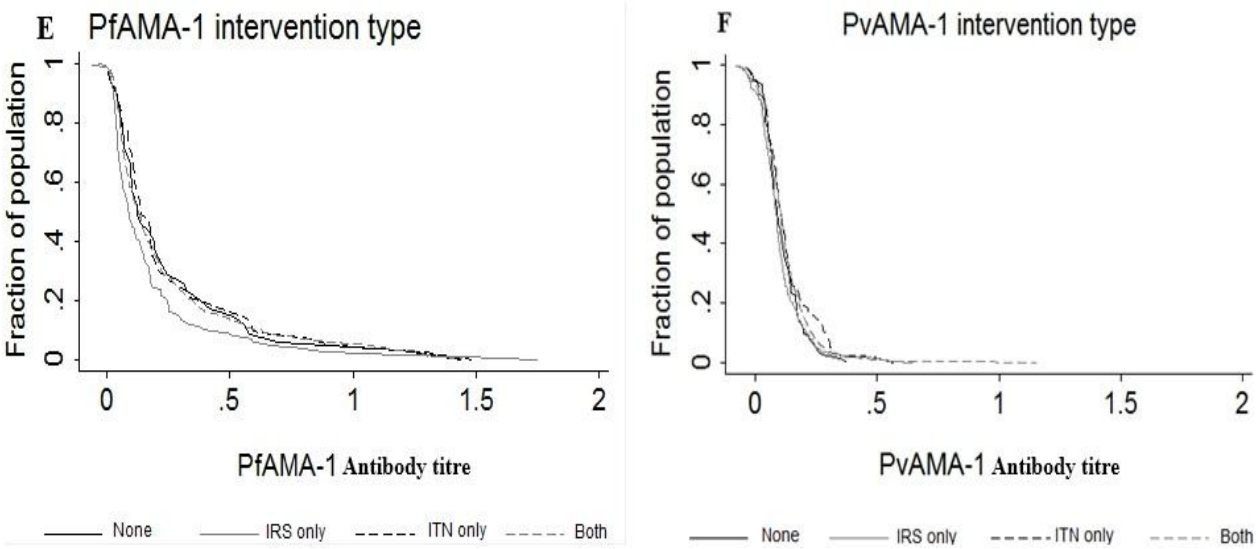


Figure 10: Reverse cumulative distribution plots

Reverse cumulative distribution plots for comparisons of antibody level (normalized OD) between age groups, villages and intervention types. a) Antibody level among different age categories for *PfAMA-1*. b) Antibody level among different age categories for *PvAMA-1*. For adults and children younger than 15 years, which are $\log_{10}$-transformed antibody titre on the x-axis and the percentage of individuals having the indicated antibody titre or higher on the y-axis. Wilcoxon rank-sum test with Bonferroni correlation used to test between two continuous variables. For: c). Antibody level for all ages in higher PCR prevalence villages and lower prevalence villages for *PfAMA-1*. d). Antibody level for all ages between high versus low PCR prevalence villages for *PvAMA-1*. e). Antibody level between participants using IRS and ITN or both for *PfAMA-1*. f). Antibody level against *PvAMA-1* between the intervention types they used; ITN only IRS only or both.

5. DISCUSSION

In Ethiopia, surveillances done nationwide documented that there is a decline in the annual incidence after the start of scaled up malaria prevention and control interventions since 2004/5 (MIS, 2008). Thus, in this study both retrospective program data and cross-sectional survey approach were used. Structured forms and questionnaires were used to collect retrospective data regarding the incidence of malaria, (suspected and confirmed) cases, distribution of LLIN/ITN, IRS campaigns, and causative malaria species and their proportion. Also, a cross sectional survey was done in selected villages (Kebeles) documenting on participant's malaria exposure history and utilization intervention measures; LLIN/ITN and IRS as well as their sociodemographic information: sex, age, and housing and household characteristics. The collected data was used to evaluate the changes in malaria infection force in the District and contribution of the utilized malaria prevention interventions.

The programmatic performance of the district was comparable to malaria prevention interventions elsewhere. For instance, in the Babile District about 38.3% of the population got ITNs in the year 2010, which is similar to the reported 37% in Amhara, Oromia and Southern nations nationalities and people's regions of Ethiopia (Graves *et al.*, 2009). Also, 54.0% of the residents of the malarious areas received LLIN in the second-round distribution which is comparable to the 57.9% reported in Harari region (Zelalem Teklemariam *et al.*, 2015) and 51% in different parts of Ethiopia below 2000m a. s. l. (Maru Aregawi *et al.*, 2014). However, ITN/LLIN distribution was started late, first distribution was only 2010 and the second round in 2014, which was up to 78% as early as in elsewhere 2007 (Maru Aregawi *et al.*, 2014).

Regarding IRS, in the District the targeted areas to be sprayed were identified each year and sprayed every year from year 2007 to 2016. On average spray coverage from targeted structures was 84.03%; ranging from 77.1% in 2010 to 100% in 2014 and during the years 2008, 2009, 2011, 2012, 2015 and 2016, the IRS coverage was above 90%. Which were comparable with the planned annual coverage of national strategic plan for malaria prevention and control 2011 – 2015, 85% coverage of houses in geographic areas targeted for IRS (PMI, 2009) and also in consistent with the WHO recommendation of spraying at least 80% (and ideally 100%) of houses, structures and units in the targeted area in any round of spraying (WHO, 2016b).

In this study the 12-year (2005-2016) retrospective data showed a substantial reduction in the ratio of the confirmed malaria incidence to the total tested (suspected cases) from time to time. Grossly the incidence of confirmed cases declined from 33.4% in 2005 to 2.9% in 2016. A dramatic decrease was recorded in 2007 (15.5%) compared to 2005 (33.4%) and 2006 (24.9%), which coincided with the scaled-up in malaria intervention activities (MIS, 2008). Also, comparable out-patient laboratory-confirmed cases reduction (85%) were reported from the year 2001 to 2005 in four major malarious regions of Ethiopia (Maru Aregawi *et al.*, 2014). This reduction in transmission might be the outcome of many factors, including utilization of ITN/LLIN, annual application of IRS, health facilities improvement on early parasite detection and effective treatment, as well as breeding site clearance and community health education done annually. Also, the success of the interventions was unquestionable I want to underline that, as retrospective intervention data before the year 2007 was difficult to obtain, attributing every change to the intervention need be cautiously viewed; like climate change could have also played a significant role on malaria transmission (Caminade *et al.*, 2014).

In this study asymptomatic infection was 1.8% and 4.4% by microscopy and PCR respectively. Moreover, 76.9% malaria infections were submicroscopic, detected by nPCR only. This finding is consistent with the reported 70% submicroscopic malaria infection in Peru (Rosas-Aguirre *et al.*, 2013). The observed low sensitivity (23.1%) of Microscopy in comparison to nPCR to detect asymptomatic carriage was in line with the reported of 26.4% sensitivity in Myanmar (Wang *et al.*, 2014). Microscopy had a better sensitivity for the detection of *P. falciparum* (71.9% missed) compared to *P. vivax* detection, which missed the majority of nPCR detected *P. vivax* 94.1% infections. This finding agreed with the reports of Cheng *et al.*, (2015) in which 54% of the *P. vivax* infections detected by nPCR were missed by microscopy. This might be because in *P. vivax* infection there is low-density blood stage per sample volume, explained by the strict host cell preference of this species infecting only reticulocytes that account for less than 1% of all erythrocytes (Gruenberg *et al.*, 2018). Most importantly a reticulocyte tropism of *P. vivax* is restricted to $CD71^{+}$ reticulocytes (Lim *et al.*, 2016). Changes in the membrane nanostructure of *P. vivax*–infected reticulocytes occurred, notably the rapid switch from a ridged immature reticulocyte to a deformable infected red blood cell. That would enable the parasitized red cell to escape splenic clearance and augment the delivery of mature parasites into the bone marrow compartments and the egress of infected reticulocytes into the peripheral blood system (Malleret

et al., 2015). This reduces the number of infected cells in the peripheral circulation even lower than the light microscopy detection limit, that decreases the sensitivity of the test. This poses major challenges on the malaria control and elimination because, the low-density blood stage parasite can infect the mosquito vector (Fontoura *et al.*, 2016) and also supported by its fast development within vectors compared to *P. falciparum* (Sattabongkot, *et al.*, 2004). Thus, accurate estimate of *P. vivax* prevalence is essential for the successful implementation of malaria control and elimination program (Moreira *et al.*, 2015). Therefore, more sensitive tools like molecular methods (Snounou *et al.*, 1993) and evaluation of accurate *p. vivax* prevalence using a reactive case detection strategy (Fontoura *et al.*, 2016) are imperative to detect such low density parasitemia in elimination target settings.

Overall seroprevalence in the study villages was 21.6%; 11.2% and 13.1% positivity to *PfAMA-1* and *PvAMA-1* respectively. This finding is comparable with the finding in Ahuri, Amhara region; 11.0% and 9% for *P. falciparum* and *P. vivax* respectively (Fitsum Girma Tadesse *et al.*, 2017) but far below the reported from different part of Amhara region, 39.4% for either species (Woyneshet G. Yalew *et al.*, 2017) and specifically around Bahir Dar, 45.1% and in Jawi, 44% for *P. falciparum* and 36.3% and 27.0 % around Bahir Dar and Jawi for *P. vivax* (Fitsum Girma Tadesse *et al.*, 2017). The variations on the total seroprevalence might probably be due their work was targeted more than one antibody and to the extensive drought and less natural water bodies availability in our study area; less likely conducive for larval development as described elsewhere (Moller-Jacobs *et al.*, 2014).

The seroprevalence was significantly higher in male participants ($p < 0.001$); females had 3-fold lower risk of being positive (OR, 0.383). This finding is similar with the reported in Kurunegala district of Sri Lanka (Mosha *et al.*, 2013; Dewasurendra *et al.*, 2017). This might be due to the differences in outdoor activities (farming) of males in the area which predispose males more than females for outdoor biting (Russell *et al.*, 2011).

With regard to low and high prevalence village sub-groups the change in SCR were at different time period for *PfAMA-1* (18 and 15years ago respectively) but this variation was not observed for *PvAMA-1* for the two village groups (12 years). For *PfAMA-1*, also 1.7-fold SCR decline was observed in the low prevalence village as compared to the high-prevalence village within the

respective years of change. The changes in *P. vivax* transmission coincided with the recorded declined in the district retrospective data in 2005 (2.3%). Yet, the time of change for *P. falciparum* was not verified because it was before 2005, unavailability of recorded retrospective data. The observed heterogeneity at micro-geographical and time of change of the species could possibly be due to the presence of more *P. vivax* than *P. falciparum* asymptomatic carriage which is supported by the nPCR result of this study. This might be due to the presence of reservoir of latent infection (hypnozoite) in *P. vivax* that can generate multiple recurrent blood-stage infections (Olliaro *et al.*, 2016). In such a case a single infectious bite, markedly increases the potential for onward transmission but remain undetectable at any time point (WHO, 2015b).

The study showed very strong association between age groups and seropositivity ($P < 0.001$) for both *PfAMA-1* and *PvAMA-1*. The highest seropositivity was detected in adults (> 15 years) for *PfAMA-1* and individuals older than 10 years before the study, while for *PvAMA-1* with 3.65 and 5.0-fold seropositivity respectively. The profile likelihood analysis showed evidence of a change in transmission in all villages at 15.5 with 6.63-fold difference (from $\lambda_1 = 0.0179$ to $\lambda_2 = 0.0027$) and 11.5 with 31.4-fold difference (from $\lambda_1 = 0.5463$ to $\lambda_2 = 0.0174$) years for *PfAMA-1* and *PvAMA-1*, respectively. The change point predicted for *P. falciparum* and *P. vivax* was about 2003 and 2005 respectively. The change point in *P. vivax* SCR coincided with the decline of malaria incidence in the district and with the started scaled up malaria control interventions in 2005 (MIS, 2008). These finding is in line with the report in other parts of Ethiopia, the predicted time point for the decline in case of *P. falciparum* based on SCR was around year 1998; which is 15 years back from 2013 (Woyneshet G. Yalew *et al.*, 2017).

Overall seroprevalence in the study villages was 21.6% which was about 5.0-fold and 12.0-fold higher than nPCR and microscopy respectively. Though the SCR had the same trend as in nPCR; both for *P. falciparum* ($\rho = 0.1809$, $P < 0.001$) and *P. vivax* ($\rho = 0.0585$, $P = 0.0489$) the infection prevalence detected by nPCR was lower, which is natural (serology is a cumulative history (Woyneshet G. Yalew *et al.*, 2017). Considering nPCR as a gold standard the ROC curve analysis showed serology had accuracy of 91.2%; 0.83 (95% CI 0.81-0.85) area under curve and 88.5%; 0.8 (0.77 - 0.82) area under curve for *PfAMA-1* and *PvAMA-1* respectively. The serology also showed 11.7% (29/149) cross-reactivity against *P. falciparum* and *P. vivax* antigens. Yet several scholars reported that the cross-reactivity between the two Plasmodia species (*PfAMA-1* and *PvAMA-1*) is either

unlikely or very low (Bai *et al.*, 2005; Igonet *et al.*, 2007; Amanfo *et al.*, 2016). Therefore, the observed high rate of cross reactivity against *Pf*AMA-1 and *PvAMA-1* might be due to the fact that the co-endemicity of the two-plasmodium species in our set up, which was also detected in the nPCR indicating high possibility for co-infection by both and 71.4% (5/7) of nPCR detected mixed infections were also detected in serology.

The asymptomatic malaria infection prevalence detected varied substantially between villages ($P < 0.001$) by all measures Microscopy, nPCR and serology. Based on nPCR the villages were sub-divided into two, high-prevalence (>10%) and low-prevalence villages (<10%). Individuals in the high-prevalence villages were 1.90-fold more likely to be *PfAMA-1* positive than individuals in the low-prevalence villages ($P = 0.005$). But the risk of being *PvAMA-1* seropositive didn't show significant variation between the two villages groups (OR, 0.56, $P = 0.002$). This might be due to the probability of high rate of previous infection, since anti-malarial antibodies are biological markers that thought to be a good indicator of previous malaria exposure (Drakeley *et al.*, 2005; Bousema *et al.*, 2010a).

Positivity to *PfAMA-1* was statistically higher in individuals that had self-reported malaria in their life time (21.2%) than individuals that did not (7.6%) ($P < 0.001$) and also showed a positive association with number of reported malaria episodes ($\rho = 0.2974$, $P < 0.001$); more than half (51.2%) of those *PfAMA-1* positives had 3 or more reported malaria episodes. In case of *PvAMA-1* 22.5%, 19.1% and 12.8% seropositivity was observed in individuals who reported 3 and more, two and one infection episodes respectively but no significant variation observed ($\rho = 0.1022$, $P = 0.0833$). The lack of association between the self-reported episode malaria and *Pv*AMA-1 positivity might be due to the presence of infection that does not reach a threshold to develop clinical symptoms (de Mast *et al.*, 2015) yet could induce adaptive immune response (Lindblade *et al.*, 2013).

The reverse cumulative distribution showed that the distributions of antibody titres had statistical difference between age groups; <15 and >15 years, between high and low nPCR prevalence villages for both *Pf*AMA-1 and *PvAMA-1* antigens which verified the SCR change time and nPCR result of this study. This could be due to the cumulative exposure; as older age groups had higher antibody level than the younger ages because of repeated exposure (Obiero *et al.*, 2015). From the

interventions applied, IRS showed significant difference compared to ITN or both against for *Pf*AMA-1 but neither of them showed significant variation for *PvAMA-1*. This can be as a result of several factors; first the effectiveness of the ITN might be deteriorated by its status, number of washing, uninterrupted use, (Steinhardt *et al.*, 2017) presence of outdoor bite before bed (Russell *et al.*, 2011) and the presence of ITN resistant mosquito vectors (WHO, 2015b). For instances, in Southwestern Ethiopia (Behailu Taye *et al.*, 2016) reported the highest peak biting activity of *An. gambiae s.l.* was between 18:00 and 21:00 hours and shown that no significant difference in mean indoor and outdoor density and the mean probability of surviving sporogony for *P. vivax* was higher than *P. falciparum*. This supports the absence of variation on antibody titre difference between the ITN users and none users. However, there was no research done about the behavior of the insect specifically in our setting.

7. CONCLUSIONS AND RECOMMENDATIONS

7.1. Conclusions

The study demonstrated the usefulness of serology in elimination move with our setups; both field and laboratory. Findings detectable serological responses in children, often accompanied by nPCR-positivity, suggest that there is still non-negligible ongoing local malaria transmission. We identified, significant difference between villages in their infection prevalence. The study revealed no significant variation in antibody level between participants utilizing IRS or ITN only and both intervention types from none users.

7.2. Recommendations

1. There is a need for continued efforts to further reduce malaria in the district, Babile.
2. The striking variations in malaria indicators between villages highlights the need for better understanding of the uptake of interventions and potentially tailor interventions to the local needs to accelerate elimination efforts in the region.
3. Serology could be integrated in our programs for it expedites both the collection and processing of large volume of samples for evaluation of performances of elimination efforts.
4. Research should be done on mosquito biting behavior and insecticide resistance

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9. APPENDICES

9.1. Appendex 1: Questionnaire-based interview

Name of PI: Migbaru Keffale

Title of the study: The role of serological and molecular tools in evaluating performance of malaria control programs in Ethiopia

Date: __ __/__ __/__ __ __ _,

Location:_____ Elivation_____

1. Participant's name: _____ Code : _____
   1.1. Parent/guardian name (<18 years old): _____
2. Age (in years)_____years
3. Gender: a. Male b. Female
4. Literacy level a. Illiterate b. Can read and write c. Primary school d. Secondary school/college
5. Does your household have any of the following?
   a. Electricity b. Radio c. Television d. All e. None
6. Household number:_____
7. What is the nearest health facility to your house?
   a. Hospital b. Health center c. Clinic d. Health post
8. The time it takes to arrive the nearest health institution on walking)?_____minutes
9. Have you (has the child) had malaria in the last time?
   a. Yes b. No c. Don't know
10. If yes, how many times you have been infected?
    a. Once b. Twice c. Three times d. Four times e. ≥5
11. Exact time first and last infections _____ &_____E.C.
12. Was treatment given? a. yes b. No C. Don`t know
13. What kind of water body exists in your neighborhood? (you can give >1 answer)
    a. None b. River c. Lake d. Pond e. Swamp f. Stagnant water
14. The time it takes to arrive to the nearest water body on walking from your house? _____(minutes)

15. What type of roof was used for the construction of the house?
    a. Grass thatch c. Wood and mud
    b. Iron sheet d. Other: _____
16. What type of wall was used for the construction of the house?
    a. Wooden plastered with clay or mud c. Iron sheets e. other_____
    b. Mud with cement plastering d. Brick or stone
17. What is the main material of the house's Floor?
    a. Earth c. Cement
    b. Local dung plaster d. Wood e. Other_____
18. Is there any eave either on the roof or the wall of the house?
    a. Yes b. No c. Don't know
19. Do you own a long-lasting insecticide treated mosquito nets (LLINs) for your household?
    a. Yes b. No c. Don't know
20. How many people permanently live in the house? _____
21. How many LLINs are available in the house?_____
22. For whom LLINs are prioritized?
    a. All household members b. Women c. Children
23. How long ago did you use the LLIN?
    a. Less than 1 year ago c. 6-10 years ago e. 16-20 years ago
    b. 1-5 years ago d. 11-15 years ago f. More than 20 years ago
24. Did you/your child interrupt using LLIN?
    a. Yes b. No c. Don't know
25. At any time in the past, have the interior walls of the house been sprayed against mosquitoes? A. Yes b. No c. Don't know
26. How long ago did your house sprayed for the first time? _____E.C.
    a. Less than 1 year ago c. 6-10 years ago .e 16-20 years ago
    b. 1-5 years ago d. 11-15 years ago f. More than 20 years ago
27. How many months ago was the house recently sprayed? _____
28. Have you (your child) made any overnight trips outside of the district in the past?
    a. Yes b. No c. Don't know
29. If yes, where did you (your child) spend the most time? _____

30. What time do you (does the child) enter to the house usually in the night?_____

31. What time did you (the child) enter to the sleeping space permanently? _____

32. What time do you (the child) leave the sleeping space in the morning? _____

33. What time do you (the child) leave the house in the morning?_____

Technician's Name: _____

Signature: _____ Date:_____/_____ (DD/MM/YR).

Name of Health Facility: _____

የመረጃ ማሰባሰቢያ መጠይቅ

የጥናቱ ርዕስ፡-በኢትዮጵያ እየተሰራ ያለውን የወባ ቁጥጥር ፕሮግራም ውጤታማነት ለመገምገም የሴሮሎጂ እና ሞለኪዩላር ባዮሎጅ ላቦራቶረ ሚና

ቀን፡-__ __/__ __/__ __

1. የተሳታፊው ስም፡-_____ ኮድ፡-_____
   a. የቤተሰብ ወይም የአሳዳጊ ስም (ከ18 ዓመት በታች ከሆነ) _____
2. የተሳታፊዉ ዕድሜ (በዓመት) _____ዓመት
3. ፆታ፡- ሀ. ወንድ ለ.ሴት
4. የትምህርት ሁኔታ

   ሀ. ያልተማሩ ሐ. 1ኛ ደረጃ የተማሩ መ. 2ኛ ደረጃ እና ኮሌጅ የተማሩ
   ለ. ማንበብና መፃፍ የሚችል ሠ. ዲግሪና ከዚያ በላይ የተማሩ
5. ከሚከተሉት ውስጥ የትኛው በቤትዎ ውስጥ ይገኛል?

   ሀ. መብራት ለ. ራዲዮ ሐ. ቴሌቪዚዮን መ. ሁሉም ሠ. ምንም
6. የቤተሰብ ብዛት _____
7. በቅርበት ያለው የጤና ተቋም ምንድን ነው?

   ሀ. ሆስፒታል ለ. ጤና ጣቢያ ሐ. ክሊኒክ መ. የጤናኬላ
8. በቅርብ ያለው የጤና ማዕከል ለመድረስ ምን ያህል ይፈጃል (የሚፈጀው ሰኣት በደቂቃ)፡-_____ደቂቃ
9. ልጅዎ (እርስዎ) ባለፉት ጊዜያት ወባ በሽታ ይዞት ያውቃል? ሀ. አዎ ለ. አያውቅም ሐ. አላውቅም
10. መልስዎ አዎን ከሆነ ስንት ጊዜ ታመው ያውቃሉ? ሀ. 1ጊዜ ለ. 2ጊዜ ሐ. 3ጊዜ መ. 4ጊዜ ሠ. ≥5
11. ለመጀመሪያ እና ለመጨረሻ ጊዜ በትክክል በቅደም ተከተል ----------እና ----------ዓ.ም.
12. ህክምና ተሰጥቶት ነበረ? ሀ. አዎ ለ. አልተሰጠውም (ኝም) ሐ. አላውቅም
13. በቤትዎ ቅርብ ምንዓይነት የውሃ አካል ይገኛል? (አንድና ከዛም በላይ መምረጥ ይችላሉ)

   ሀ. ምንም ለ. ፈሳሽ ወንዝ ሐ. ሃይቅ መ. ኩሬ ሠ. ረግረግ ረ. የተኛውሃ
14. በቅርብ ያለው የዉሃ አካል ከመኖሪያ ቤቱ በእግር ጉዞ ምን ያህል ደቂቃ ይርቃል? _____ (ደቂቃ)
15. መኖሪያ ቤታችሁ ከምን አይነት ጣራ ነው የተሰራው?

   ሀ. ከሳርከዳን ለ. ከቆርቆሮ ሐ. እንጨት እና ጭቃ መ. ሌላ (ይገለጽ)_____
16. ቤቱ ከምን አይነት ግባት ነው የተሰራው?

   ሀ. ከእንጨት እና ጭቃ ምርጊት ለ. ጭቃ በሲሚንቶ ምርጊት ሐ. ከቆርቆሮ

   መ.ከድንጋይ ወይም ብሎኬት ሠ. ሌላ (ይገለጽ)_____
17. የቤቱ ወለል ከምንድነው የተሰራው?

ሀ. ዓፈር ለ.እበት የተለቀለቀ ሐ. ሲሚንቶ መ. እንጨት (ጣውላ) ሠ.ሌላ (ይገለጽ)_____

19. ጣራው ወደ ዉጭ ክፍት ነው ወይስ ዝግ? ሀ. ክፍት ለ. ዝግ ሐ. መለስተኛ ክፍት

20. ለራስዎ (ለልጅዎ) አጎበር ይጠቀማሉ? ሀ. አዎ ለ. አልጠቀምም ሐ. አላውቅም

21. ለራስዎ (ለልጅዎ) ባለፈው ምሽት አጎበር ተጠቅመዋል? ሀ. አዎ ለ. አልተጠቀምኩም ሐ. አላውቅም

21. በቤቱ ውስጥ ስንት ሰዎች ይኖራሉ?_____

22. ስንት አጎበሮች አለዎት?_____

23. አጎበሩን ቅድሚያ ማን እንዲጠቀምበት ይድረጋል?

ሀ. ወላጅ እናት ለ. ህፃናት ሐ. ሁሉም ቤተሰብ

24. አጎበር መጠቀም ከጀመሩ ስንት ዓመት ሆነ? _____

ሀ. ከ1 ዓመት ወዲህ ሐ. 6-10 ዓመት ሠ. 16-20 ዓመት

ለ. 1-5 ዓመት መ. 11-15 ዓመት ረ. ከ20 ዓመት በላይ

25. እርስዎ /ልጅዎ አጎበር መጠቀሙን አቋርጦ ያውቃል(ሉ)? ሀ. አዎ ለ. አያውቅም ሐ. አላውቅም

26. የመኖሪያ ቤታችሁ ውስጡ የወባ መከላከያ ተረጭቶ ያዉቃል? ሀ. አዎ ለ. አያውቅም ሐ. አላውቅም

27. ለመጀመሪያ ጊዜ ቤትዎ ቤትዎ የወባ መከላከያ የተረጨው መቼ ነው?

ሀ. ከ1 ዓመት ወዲህ ሐ. 6-10 ዓመት ሠ. 16-20 ዓመት

ለ. 1-5 ዓመት መ. 11-15 ዓመት ረ. ከ20 ዓመት በላይ

28. ከስንት ወር በፊት ነው ቤትዎ የወባ መከላከያ የተረጨው?_____

29. ባለፉት ጊዜያት እርስዎ (ልጅዎ) የሌሊት ጉዞ አድርጎ(ገው) ያውቃል(ሉ)?

ሀ. አዎ ለ. አላደረግንም ሐ. አላውቅም

30. መልስዎ አዎን ከሆነ በሄዱበት ጊዜ አብዛኛውን ምሽት የት ነበር የሚያሳልፈው(ፉት)?_____

31. እርስዎ (ልጅዎ) በብዛት ወደ ቤት ስንት ሰዓት ላይ ነው የሚገባው(ቡት)?_____

32. እርስዎ (ልጅዎ) በብዛት ወደ መኝታ ቦታው ስንት ሰዓት ላይ ነው የሚገባው(ቡት)?_____

33. ልጅዎ (እርስዎ) ጠዋት ላይ ብዙ ጊዜ በስንት ሰዓት ከመኝታ ቦታዎ ይወጣል(ሉ)?_____

34. እርስዎ (ልጅዎ) ጠዋት ላይ ብዙ ጊዜ በስንት ሰዓት ከቤት ወጣ(ጡ)?_____

የቴክኒሺያኑ ስም፡----------ፊርማ፡ ----------

ቀን፡----/------/-------(ቀን/ወር/ዓም)

የጤና ተቋሙ ስም፡ ----------

Gaafannoo Yaada Guuruf Qopaha'e

Mat-duree Qorannichaa: Gahee meeshaan qorannoo dhiigaa fi Moleculaarii sagantaa to'annoo dhibee Busaa itoophiyaa madaaluu keessatti qabu.

Guyyaa:-__ __/__ __/__ __ __ __

1. Aanaa:_____Maqaa Magaalaa ykn gandaa:_____
2. Maqaa Hirmaataa: _____ Koodii _____
3. Maqaa maatii/Guddistuu(Ijoollee umurii<18):_____
4. Lakk. Bilbila Hirmaataa: __ __ __ __ __ __ __ __ __ __
5. Umurii (waggaan)_____
6. Saala: a. Dhiira b. Dhalaa
7. Lakk. Manaa:_____
8. Manni yaalaa mana keessanitti dhiyeenyan argamu kami?
   a. Hospitaala b. Buufata fayyaa c. Kiliinika fayyaa d.Kellaa Fayyaa
9. Mana yaalaa dhiyootti argamu ga"uuf yeroo hangam (daqiiqaan)fudhata? Daqiiqaa_____**(on walking)**
10. Yeroo darbe kana keessa isin ykn daa"imman keessan dhibee busaa dhukkubsattaii/dhubsatanii beektu? a. Eeyyee b. Lakki c. Hinbeeku
11. Yoo deebi"iin keessan eeyee ta"e, yoomi laata?_____
12. Yaalii argattaniittuu? a. Eeyyee b. Lakki c. Hinbeeku
13. Mana keessaniitti dhiyeenyaan qaamoleen bishaanii argamu kami? (deebi"ii tokkoo ol kenuun ni danda"ama)
    a. Hinjiru c. Garba bishanii e. Chaffee
    b. Laga d. Burqaa/yaatuu f. Bishaan chiisaa
    g. Kan biro (Haa ibsamu)_____
14. Bishaan mana keessaniitti dhiyeenyaan argamu kanaga"uuf deemsa miillaa hangamii (daqiiqan) ta"a? Daqiiqaa _____
15. Baaxii manni keessanii maal irraa ijaareame?
    a. Chitaa c. Mukaa fi dhoqqee
    b. Qorqoorroo d. Kanbiroo (haaibsamu)_____

16. Gidgi-ddaan mana maalirraa hojjetame?

a. Muka dhoqeen maragame c. Qorqoorroo

b. Simmintoon garafame d. Xuubii ykn dhagaa e. Kanbiroo(haa ibsamu)____

17. Baaxiin ykn xaaraan manaa garabakkeetti banaa moo cufaadha?

a. Banaadha b. Cufaadha c. Walakkan banaadha

18. Matta keessaniif ykn daa‟imman keessaniif agoberii ni fayyadamtuu?

a. Eeyyee b. Lakki c. Hin beeku

19. Kalessa galgala mataa keessaniif (daa‟ima keessaniif) agobera siree fayyadamtaniittuu?

a. Eeyyee b. Lakki c. Hinbeeku

20. Mana kessan keessa dhaabbataan nama meeqatu jirata? _____

21. Agoobera siree meeqa qabdu? _____

22. Agoobera siree hammamiif fayyadamtaniittu?_____

23. Isin yookan ijoolleen keessan agoobera fayyadamuu addaan kuttanii beektuu?_____

a. Eeyyee b.Lakki c. Hin beeku

24. Ji‟oota meeqan dura manni keessan qoricha farra bookee busaan kan biifame?

a. Eeyyee b. lakkii c. hin beeku

25. Manni keessan farra bookee busaa yeroo jalqabaaf erga biifamee hagamta‟a?_____

26. Manni keessan farra bookee busaa erga biifame Ji‟oota meeqa ta‟a?(Ji‟a tokkoo gadi yoo ta‟e “00”barreessi.

27. Farra bookee busaa biifsisuu addaan kuttanii beektuu?

a. Eeyyee b. Lakki c. Hin beeku

28. Yoo eeyyee ta‟e Maaliif addaan kuttan?_____

29. Isin ykn Ijoolleen keessan yeroo darbe deemsa halkanii aanaa kana ala gootanii beektuu?

a. Eeeyyee b. lakkii c. Hin beeku

30. Yoo eeyee ta‟e eessaa fi yeroo akkamii deemtani?_____

31. Isin ykn ijolleen keessan galgala yeroo baay‟ee sa‟a meeqatti manatti galtu/galu?_____

32. Isin ykn ijolleen keessan galgala sa‟a meeqatti gara iddoo chiisaa deemtu/deeman? _____

33. Isin ykn ijolleen keessan yeroo baay‟ee ganama sa‟a meeqatti ciisichaa kaatu/kaaatan?_____

34. Isin ykn ijolleen keessan ganama sa‟a meeqatti manaa batan ykn ba‟e/bate?

35. “Thick” fi “thin” film Fudhatameeraa? a. eeyyee b. Lakki c. Hin beekamu

36. Saamudni Maayikrootainer fudhatameeraa?

a. Eeyyee b. Lakki C. Hin beekamu

37. “Filter paper” Fudhatameeraa? a. Eeyyee b. Lakki c. Hin beekamu

Maq-aa teekniishaanii: ----------

Mallatoo: ----------

Bara: ---------/----------/---------- (DD/MM/YR)

Maqaa waajjiiraa Eeguumssaa fayyaa: ----------

9.2. Appendix 2: Participant information sheet

Study Title: **The role of serological and molecular tools in evaluating performance of malaria control programs in Ethiopia**

Purpose of the study

Ethiopia has enjoyed a remarkable decrease in malaria incidence and mortality in the last decade after the intensive parasite and vector control interventions. However, it is not clear if this success will continue and if malaria may disappear from Ethiopia or if malaria may persist. The biggest faltering block in elimination settings is about identifying all malaria-infected individuals that could potentially contribute to the infectious reservoir. Microscopy and RDT may be insufficiently sensitive to guide or evaluate these elimination efforts in low malaria transmission settings. As a result, these areas pose considerable challenges for monitoring and evaluation. The alternative measures are molecular and serological assays. Molecular assays have the advantage of increased sensitivity to detect *P. falciparum* and *P. vivax* infections but these are unrealistic in field conditions and affected by seasonal variations. More importantly, serological markers of transmission show greater sensitivity in low transmission areas, as seroprevalence reflects cumulative exposure and thus is less affected by seasonality due to the longer duration of specific antibody responses. Thus, this study aims to evaluate the effectiveness of malaria control program through estimation of seroconverssion and molecular assay. Since conducting serological and molecular assays in moderate and low prevalent areas of the country is inevitably reflective to evaluate the effect of malaria control activities for the success of malaria elimination from the country.

We therefore ask your permission to allow us to collect finger prick blood samples from you/ your child. These samples will only be used only for this research related to the spread of malaria. In case of further research on stored samples, all identifying information such as your name or address will be removed from the data and ethics approval will be sought.

1) Study procedures

Completion of this form requires a maximum of 20 minutes. We would like to thank you in advance for your patience and taking part in the study. You /your child will be asked to donate a small finger prick blood sample that we will use for this study and for further investigations. Sampling will happen once, only today and will be a total of 0.4mL (less than ¼ of a teaspoon) blood.

2) Voluntary participation

Your decision not to participate or to withdraw from participation will not affect the care you or your child will receive in any way. Even if you do agree to become a study participant, you or your child can withdraw from the study at any time. If you chose not to participate, you have access to the same level of clinical care.

3) Discomforts and Risks

You or your child might feel a small amount of discomfort during blood sampling and you or your child may have a small amount of bruising or bleeding where the blood sample will be taken. This is considered not to be harmful. We will use sterile equipment to collect the blood sample and the small wound that may arise from the procedure will be treated adequately. The volume of blood is too small to influence your or your child's health and the blood will quickly be replaced by your/your child's body.

4) Benefits

You or your child will not be paid for participation in this study, but will play an important role for future malaria elimination program from the area.

5) Confidentiality statement

The records concerning your/your child's participation are to be used only for the purpose of this research project. Your/your child's name will not be used on labels on laboratory specimens or in any report resulting from this study. At the beginning of the study, we will give you/your child a study identification number and this number will be used on the forms and on the laboratory specimens. Any information obtained in connection with this study will be kept strictly confidential and under lock and key. Only senior members of the study team will have access to information linking your/your child's name with your/your child's study number.

6) Long term storage of samples

We will make your samples anonymous by removing the name and any identifying information. Samples may be stored at AHRI, Ethiopia till processing for this study only. We will only test for the presence of malaria particles and body responses against malaria parasite antigens. If further studies will be planed on this sample, ethics approval will be sought.

Questions and freedom to withdraw from the study

If you have any question concerning this study, do not hesitate to contact the principal investigator, Migbaru Keffale Bezabih (0909604041/0910083862). Results from the study will be communicated to your community. In case you want to contact an independent person, about the research study itself and you or your child's rights as a research participant or any research-related injury, you can contact the AHRI/ALERT Ethics Review Committee, Addis Ababa, Ethiopia, (0118-962183) and College of Natural Sciences Research Ethics Review Board, Addis Ababa University, Addis Ababa, Ethiopia (+251116551901).

9). Circumstances under which participation may be terminated without your consent:

1. Health conditions under which participation would be dangerous.
2. Other conditions which might occur that would make continued participation detrimental to his/her own health.

10). INVESTIGATOR DETAILS:

Migbaru Keffale (MSc Candidate in Medical Biotechnology),

Dr. Haileeyesus Adamu and Fitsum Girma Tadesse (Addis Ababa University, Institute of Biotechnology),

Dr. Endalamaw Gadisa (Armauer Hansen Research Institute)

ለተሳታፎች የሚሰጥ መረጃ

የጥናቱ አርእስት፡ በኢትዮጵያ እየተሰራ ያለውን የወባ ቁጥጥር ፕሮግራም ውጤታማነት ለመገምገም የሴሮሎጂ እና ሞለኪዩላር ባዮሎጅ ላቦራቶረ ሚና

1) የጥንቱ አላማ

ባለፉት አስር ዓመታት በተደረገው የመከላከል ስራ ተጨባጭ የሆነ የወባ ስርጭትና በወባ ምክንያት የሚከሰት ሞት መቀነስ ታይቷል። ነገርግን፤ ይሕ ውጤት የህመም ምልክት የማይታይባቸው በዝቅተኛ ሁኔታ የወባ ተውሳክ በሰውነታቸው ያለባቸው ሰዎች ሊገኙ በመቻላቸውና እንደማይክሮስኮፕ ባሉ እለት ከለት በምንጠቀምባቸው የመመርመሪያ መሳሪያዎች መለየት ስለማይቻል በሽታውን ሙሉ በሙሉ የማጥፋት ዓላማውን ተግዳሮት ሊገጥመው ይችላል። በሚመጡት አስርት አመታት ውጤታማ የሆነ ወባን የማጥፋት አላማን ለማሳካት፤ የወባ ቁጥጥር ጥረት ስለ ህመም ምልክት የማይታይባቸው በዝቅተኛ ሁኔታ የወባ ተውሳክ በሰውነታቸው ያለባቸው ተሸካሚ ሰዎች ስርጭትና የወባን ስርጭት ለማስቀጠል ያላቸውን ሚና መረጃ ማግኘት አስፈላጊ ይሆናል። ስለዚህ ይህ ጥናት ዓላማው በዝቅተኛ ሁኔታ የወባ ተውሳክ በሰውነታቸው ያለባቸውን ሰዎች ሙሉ በሙሉ ማወቅ የሚያስችሉ የምርመራ ዘዴዎችን በመጠቀም እስካሁን የተሰራውን የቁጥጥር ስራ ውጤታማነት መገምገም ይሆናል።

እነዚህ ናሙናዎች ስለ ወባ ስርጭት ተዛማጅ ጥናቶችን ለማድረግ ብቻ እንጠቀምባቸዋለን። በናሙናዎቹ ላይ ተጨማሪ ጥናት በሚያስፈልግበት ሁኔታ ከሚመለከተው አካል ፍቃድ እንጠይቃለን፣ እንደ ስምና አድራሻ ያሉ ግለሰባዊ መረጃዎች ከመረጃው ይነጠላሉ፤ የጥናት የስነምግባር ይሁንታም ይጠየቅባቸዋል።

2) የጥናቱ ሂደት

እርስዎም ሆነ ልጅዎ ለአንድ ጊዜ ብቻ ማለትም ዛሬ ጣት በመብጣት የሚወሰድ 0.4 ሚሊ ሊትር (¼ የሻይ ማንኪያ ያነሰ) ደም እንዲሰጡን እንጠይቃለን።

3) በፈቃደኝነት ላይ የተመሰረተ ተሳትፎ

በጥናቱ ለመሳተፍ ሙሉ በሙሉ በእርስዎ ፈቃደኛነት ይወሰናል። ራስዎም ሆነ ልጅዎ በጥናቱ እንዳይሳተፍ በማንኛውም ጊዜ እንዲያቁዋረጥ ማድረግ ይችላሉ። እርስዎም ሆነ ልጅዎ በጥናቱ እንዳይሳተፉ/ፍ ወይም እንዲያቁዋርጡ/ጥ በመወሰንዎ ልጅዎም ሆነ እርሶ የሚያገኙት የጤና አገልግሎት ላይ ምንም ተፅእኖ አይኖረውም።

4) የምቾት መጓደልና ተጋላጭለት

ከእርስዎም ሆነ ከልጅዎት የደም ናሙና በሚወሰድበት ገዜ የተወሰነ የምቾት መጓደል ሊኖር ይችላል፤ ደም በተወሰደበትም ቦታ የተወሰነ የመቅላት ወይም መድማት ሊኖር የችላል። ይህ ክስተት ግን ጎጂ እንዳልሆነ ይታሰባል። ናሙናዎችን ለመውሰድ ከጀርም የጸዱ መገልገያዎችን እንጠቀማለን፤ ከናሙና መውሰድ ጋር በተገናኘ ሊከሰት የሚችልን ቁስለት በተገቢው እናከማለን። የሚወሰደው የደም መጠን በጤና ላይ ተጽኖ ለማስከትል በጣም ትንሽ ነው፤ በሰውነት በቶሎ ይተካል።

5) ጠቀሜታ

እርሶም ሆኑ ልጅዎት በጥናቱ በመሳተፋችሁ የምታገኙት ክፍያ አይኖርም ነገር ግን በሽታውን ከአካባቢው ለማጥፋት ወደፊት ለሚደረገው ስራ ጉልህ ሚና አለው።

6) ሚስጥርን ስለመጠበቅ

ከልጅዎና ከእርስዎ መሳተፍ የሚያገኙት መረጃዎች ለዚህ ጥናት ጠቀሜታ ብቻ ይውላሉ። የእርስዎም ሆነ የልጅዎት ስም ለናሙና መለያነት ወይም በማንኛውም የዚህ ጥናት ውጤት ሪፖረት ላይ አንጠቀምም። በጥናቱ መጀመሪያ ለተሳታፊዎች የመለያ የሚስጥር ቁጥር ይሰጣል፤ ይህም ለናሙናዎችና በጥናቱ በጥቅም ላይ ለሚውሉ ቅጾች መለያነት ይጠቅማል። ማንኛውም ከዚህ ጥናት በተዛመደ የሚገኝ መረጃ በሚስጥር ይያዛል፤ መረጃዎችም በቁልፍ ተቆልፎባቸው ይቀመጣሉ። የርስዎንም ሆነ የልጅዎን ስም ከጥናቱ የሚስጥር ቁጥር ጋር የሚያገናኘውን መረጃ የጥናቱ ዋና ተመራማሪዎች ብቻ ናቸው ማየት የሚችሉት።

7) ናሙናዎችን ረዘም ላለ ጊዜ ስለማስቀመጥ

ከርሶና ከልጅዎት የሚገኘውን ናሙና የምንጠቀመው ለዚህ ጥናት ብቻ ሲሆን መጠቀም እንድንችል ፈቃድዎትን እንጠይቃለን። ተጨማሪ ጥናት አስፈላጊ ከሆነ የስነምግባር ኮሚቴ ይሁንታን የሚጠየቅበት ይሆናል።

8) ጥያቄዎችን የመጠየቅና ከጥናቱ አቁዋርጦ የመውጣት ነጻነት

እርሶም ሆኑ ልጅዎት ጥናቱን በተመለከተ ጥያቄ ካላችሁ፤ ዋና ተመራማሪውን ምግባሩ ከፋለ በዛብህን (0910083862/ 0909604041) ለመጠየቅ አያመንቱ። ስለጥናቱ ከጥናቱ ጋር ያልተገናኘ ገለልተኛ ወገንን ማማከር ካስፈለጋችሁ እንደጥናቱ ተሳታፊነታችሁ እርሶም ሆኑ ልጅዎት የስነ-ምግባር ኮሚቴ ሰብሳቢውን በተመለከተው አድራሻ ማግኘት ይችላሉ። አርማዉር ሐንሰን የምርምር ተቋም የስነ-ምግባር ኮሚቴ (0118-962183) እና አዲስ አበባ ዩኒቨርሲቲ የተፈጥሮ እና ቀመር ሳይንስ የስነ-ምግባር ኮሚቴ (+251116551901).

9). በጥናቱ ለመሳተፍ ፈቃደኛነታቸውን ቢገልፀም ከጥናቱ አቁዋርጦ እነዲወጡ የሚያስችሉ ሁኔታዎች

1. በጥናቱ መሳተፍዎ በጤና ሁኔታዎ ላይ ጉዳት የሚያመጣ ከሆነ
2. በጥናቱ መሳተፍዎ ማነኛውም ዓይነት በጤና ላይ ጉዳት ሊያመጡ የሚችሉ ተያያዥ ሁኔታዎች ይከሰታሉ ተብሎ ሲታሰብ

10). የተመራማሪዎች ዝርዝር መረጃ: ምግባሩ ከፋለ በዛብህ (አዲስ አበባ ዩኒቨርሲቲ 2ኛ ዲግሪ ተማሪ)፣ ፍፁም ግርማ ታደሰ እና ዶ/ር ኃይለየሱስ አዳሙ (አዲስ አበባ ዩኒቨርሲቲ መምህር)፣ ዶ/ር እንዳላማው ጋዲሳ (አርማዉር ሐንሰን የምርምር ተቋም ተመራማሪ)

Guca odeffanoo

Matadureе Qoranichaa: Gahee meeshaan qorannoo dhiigaa fi Moleculaarii sagantaa to'annoo dhibee Busaa itoophiyaa madaaluu keessatti qabu.

Kaayoo Qoranichaa:

Woggota kurna darban kanatti biyya keenya kessatti tamsa'innii dhibee busaa fi sababa dhibee busan lubbuu ba'u hir'isaa dhufufuun isaa qabatamaan mul'ateera. Haata'uu malee bu,aan kun mallattoon maxxantuun dhukuba busaa qaama kessatti argamus dhibeen irratti argamuu dhabuun yemmuu microspitti fayyadamee ilallamus adda basuun ulfaata waan ta'eef tamsa inna dhibee kanaa gutumaan guututti dhabamsiisuu dadhabuun kayyoo qoranno kanaatti rakkoo ta'eera. Woggoota kurnan dhufan kana kessatti mikaa' inna dhabamsiisa dhukuba kanaa, kayyoo kana bakkaan basuuf too'annaan dhukkuba kanaa, waa, een namoota mallattoon dhukuba busaa gad' aanaa qaamaa isaanii kessatti baattoo ta'anii tamsasa dhibichaaf akkasumas tamsa'ina busaa akka itti fufuuf ga'een isaan qaban ragaa argachuun barbaachisaa ta'eera. kanaafuu qorannoon kayyoo dhibee busaa maxxantuu gad'aanaan qaama isaanii kessatti argamuu isaa guutumaan guututti beekuuf meshaalee qorranno kan hamma har 'aatti jirutti fayyadamuun dalagaa hamma har'aatti gaggeefame maddaluu ta'a.

Kannaafuu isinii fi maatii keessan irraa dhiigni akka saampliitti fudhatamee waa'ee dhukkuba busaa qorachuuf itti fayyadamuu barbaanna. Sampilii irraratti qorannoo dabalataa yoo nu barbaachise nama dhimmii ilaallatu irray eeyyamni ni gaafatama. Wontootni akka maqaafi eddo jireenyaa eenyummaa dhunfaa boodarratti guca kana irraa ni balleefama sababiinis iccitii namootaa eguuf jecha ta'a Garuu gucni kun eyyama nama dhimmi ilallatu irra argatuu qaba.

2) Adeemsa Qorannichaa

Unk gaafannoo kunii fi qorannicha irratti hirmaachuuf fedhii qabachuu keessan kan irratti mallatteessitan namoota qorannicha gaggeessan keessaa tokkon isiniif dhiyaata. Unka kana guutuf hanga daqiiqaa 20 isinitti fudhata. Qorannicha irratti hirmaachuuf fedha keessan waan nuuf laattanii fi obsa keesaniif dursinee isin galateeffana.

Qorannicha irratti odeeffannoo ragaa irratti hundaa'e akka nuuf lattan fedha keessan ni gaafanna. Kanaafis isiini fi ijoolleen keessan qorannoof akka nugargaaru quba keessan irraa dhiiga 0.4ml (qabiyyee ¼ fal'aana sha'ii) fudhachuun maxxantuun dhibee busaa qama keessan keessa jirachuu isaa qoranna.

3) Hirmannaa Fedha irratti hunda'e

Qorannaa kana irratti akka hin hirmmanne ykn addan kutuuf murteessuun keessan tajaajila fayyaa argachuu qabdan irratti gonkumaa dhiibbaa hin qabu. Qorannicha irratti hirmaachuu dhiisuu/

addan kutuu ni dandeessu. Qorannicha irratti hirmaachuu yoo dhiiftaniyyuu tajaajila argachuu qabdan ni argattu.

4) Hir'inoota fi Miidha'aa isaanii

Isiini fi Ijoollee keessaniraYeroo dhiigni fudatamu hamma tokko hir'inni mul'achuu ni danda'a. Fakkeenyaf iddoo dhiigni fudhatamee xiqqoo ishee dirmama'uu ykn dhiiguun jiraachuu ni danda'a. Kun garuu rakoo akka hinqabanne ni amanama. Dhiiga fudhachuuf meshaalee dhibee kamirraallee walaba ta'an ni fayyadamna. Saampilii fudhacuutiin walqabatee madaan mul'achuu danda'an yoo jirate hala gariin ni yaalla. Dhiigni fudhatamu xiqaa waan ta'eef qaama irratti dhiibbaa tokko hin qabu sababni isaas qamni dafee bakka waan buusuufi.

5) Faayidaa

Isiinis tahee ijoolleen keessan qoraanno kanarrati hirmachu keessaniif kafaltiin addaa isiinif kafaalamu hin jiru. but will play an important role for future malaria elimination program from the area.

6) Ichiitii Eeguu

Odeeffanoon isiin fi ijoollee keessan irraa argame faayidaa qorannoo kanatiif qofa ola. Maqaan keessanis tahee kan ijoollee keessanii gabaasa qorannaa kanaa keessatti tasumaa hin fayyadamnu. Jalqaba qoranichaa irratti koodiin hirmaattotaaf ni kennama. Kunis Saampilootaa fi qorannicha keessatti unkoota barbaachisan addan baasuuf ola. Odeeffanooni qorannaa kanaan walitti dhufeenya hunduu icciitin ni qabamu, odeeffanoon kunis sanduqa keessatti uguramanii (qollofamanii) ka'amu. Maqaa keessan lakkoofsa icciitii qorannichaa fana walqabsiisanii beekuu kan danda'an qorattoota qorannichaa qofa ta'u.

7) Saampiloota yeroo dheeratiif kaa'uu

Odeeffanoowwan maqaalee koodii samplii wajjin walqabate ni dhabamsiifama. Saampilootni dhaabbilee hirmaattota qorannichaa kessa hamma qoranichi turu qofa tura. Nut samplii kan barbaannu qoranno dhukuba busaaf qofa akka ta'e hubatamuu qaba. garuu yoo dabalataan qorannoon irratti adeemsifamuu barbaadame eyyamni abba dhimmi ilaallatu nigaafatama.

8) Mirga Gaaffilee Gaafachuu fi qorannicha addaan kutanii ba'uu

Qoranichaan walqabatee gaaffileen isinis ta'ee maatii keessan gaafachuu ni danda'a kanaafuu, akka hin nuuffineef akkasummas wonta rakkina qabuuf abbaa qoranicha qabaatamaan gaggeessu kan ta'e qorataa Migbaaruu Kaffalee Bezabih gaafachuuf ni danda'ama. Teessoon isaanii: Yuuniversitii Finfinnee Inistiitiyuutii baayootekinoolojii, garee Biootekinoolojii Fayyaa, Finfinnee, Itoophiyaa lakkofsa bilbilaa 0909604041/0910083862 tiin argachuu ni dandeesu. Waa'ee qoranichaa fi kannen waliin walqabatan odeeffannoo dabalataa ykn addaa yoo barbaadan Hospitaala Aleert akkasumas dhaabbata qoranna "Armauer Hansen Research Institute (AHRI)" dame qorannoo garee xin-samuu kan taate barreessituu karaa sarara bilbilaa 0118-962183tiin argachuu ni dandeessu and the secretariat of the ethics committee of Addis Ababa University CNS, (+251116551901).

9). Qorannicha irrati hirmaachuuf akkuma eeyyamama taatan wontotni akka qoranicha addaan kutan kan taasisuu danda'an

1. Yoo hirmachuun fayyaa isa/ishee irratti dhibaa kan fidu ta'e
2. Yoo hirmaachuun isa/ishee irratti miidhaa fi wontoota midhaan wolqabate kan fidu yoo ta'e.

10). Odeefannoo namoota qorannoo gaggeesanii:

Migbaruu Keffelee Bezabih (barataa universitii Addis ababaa kan digrii lammaffaa),

Fitsum Girma Tedelee fi Dr Haylayesus Addamuu (barsiisaa universitii addis ababaa kan ta'an): Dr Endaalammaaw Gaaddisaa (qorannaa gaggeessaa kan ta'e jaarmayaa Armar hansaniittii).

9.3. Appendix 3: Informed consent

Title: The role of serological and molecular tools in evaluating performance of malaria control programs in Ethiopia

I the participant, after reading the information sheets or listing to someone reading agreed to participate in this study by understanding the study procdures and my participation rights listed below

1. I ***acknowledge / do not acknowledge*;** the receipt of explanation on the study (underline one)
2. I ***understand / do not understand*** the practical consequences of this study (underline one)
3. I ***agree / disagree*** to be interviewed on my socieodemographic data (underline one)
4. I ***agree / disagree*** to give about 0.4 mL of blood by finger pricking (underline one)

Thumbprint of participant if he/she is illitrate

sign

Participant's name: _____

Participant's signature: _____ Date: _____

Impartial witness's name: _____

Impartial witness's signature: _____ Date: _____

Local investigator's name: _____

Local investigator's signature: _____ Date: _____

Informed consent agreement for parents/ Guardians

Title: The role of serological and molecular tools in evaluating performance of malaria control programs in Ethiopia

I the parent/legal gardian of the participant, after reading the information sheets or listing to someone reading agreed that my child to participate in this study by understanding the study procdures and my rights listed below

1. I ***acknowledge / do not acknowledge;*** the receipt of explanation on the study (underline one)
2. I ***understand / do not understand*** the practical consequences of this study (underline one)
3. I ***agree / disagree*** to be interviewed on my child's socieodemographic data (underline one)
4. I ***agree / disagree*** that my child gives about 0.04 mL of blood by finger pricking (underline one)

Thumbprint of participant/guardian if he/she is illitrate

Parent's/guarian's name: _____

Signature: _____

Date: _____

Impartial witness's name: _____

Impartial witness's signature: _____ Date: _____

Local investigator's name: _____

Local investigator's signature: _____ Date: _____

Informed assent agreement for minors

Title: **The role of serological and molecular tools in evaluating performance of malaria control programs in Ethiopia**

I the participant, after reading the information sheets or listing to someone reading agreed that my child to participate in this study by understanding the study procdures and my rights listed below

1. I ***acknowledge / do not acknowledge;*** the receipt of explanation the agreement of my parent/guardian (underline one)

2. I ***acknowledge / do not acknowledge;*** the receipt of explanation on the study (underline one)

3. I ***understand / do not understand*** the practical consequences of this study (underline one)

4. I ***agree / disagree*** to be interviewed on my child's socieodemographic data (underline one)

5. I ***agree / disagree*** that to give about 0.4 mL of blood by finger pricking (underline one)

Thumbprint of participant if he/she is illitrate

Participant's name: _____

Signature: _____Date: _____

Impartial witness's name: _____

Impartial witness's signature: _____ Date: _____

Local investigator's name: _____

Local investigator's signature: _____ Date: _____

የስምምነት መረጃ ቅፅ

ለአዋቂዎች የሚሰጥ የስምምነት መግለጫ ቅፅ

የጥናቱ አርእስት፡ በኢትዮጵያ እየተሰራ ያለውን የወባ ቁጥጥር ፕሮግራም ውጤታማነት ለመገምገም የሴሮሎጂ እና ሞለኪዩላር ባዮሎጅ ላቦራቶረ ሚና

እኔ በፈቃደኛነት በጥናት ተሳታፊ የሆንኩት የተሳታፊነት ማሳወቂያ ቅፁን ከመፈረሜ አስቀድሞ ሥለጥናቱ አካሄድ እና ከዚህ ቀጥሎ የተዘረዘሩትን የጥናቱ ተሳታፊ መብቶች ግልፅ በሆነ መልኩ ከመረጃ ቅፁ አንብቤ ተረድቻለሁ /ተነቦ በግልፅ ተብራርቶልኛል፡፡

1. ሥለጥናቱ የተሰጠውን ማበራሪያ ተገንዝቤ እውቅና **ሰጥቻለሁ/አልሰጠሁም** (አንዱን አስምር)
2. ይህ ጥናት በተግባር የሚያስከትለውን ተፅዕኖ በግልፅ **ተገንዝቤያለሁ/አልተገነዘብኩትም** (አንዱን አስምር)
3. ማነኛውንም ማህበራዊ ሆነ አካባቢያዊ መረጃ ለማግኘት ጥያቄ እንዲጠይቁኝ በሙሉ ፈቃደኛነት **ተስማምቻለሁ/አልተስማማሁም** (አንዱን አስምር)
4. ለአንድ ጊዜ ከጣት የሚወሰድ 0.4ሚሊ ሊትር የደም ናሙና ለመስጠት በፈቃደኛነት **ተስማምቻለሁ/አልተስማማሁም** (አንዱን አስምር)

Thumbprint if subject is unable to sign

የተሳታፊው ስም፡_____

የተሳታፊው ፊርማ: _____ ቀን: _____

የገለልተኛ ምስክር ስም_____

የገለልተኛ ምስክር ፊርማ: _____ ቀን: _____

በቅርብ የሚገኘው ተመራማሪ ስም: _____

በቅርብ የሚገኘው ተመራማሪ ፊርማ: _____ ቀን:_____

ለህፃናት ወላጆች/አሳዳጊዎች የሚሰጥ የስምምነት መግለጫ ቅፅ

የጥናቱ አርእስት፡ በኢትዮጵያ እየተሰራ ያለውን የወባ ቁጥጥር ፕሮግራም ውጤታማነት ለመገምገም የሴሮሎጂ እና ሞለኪዩላር ባዮሎጅ ላቦራቶረ ሚና

እኔ ልጄ በፈቃደኛነት በጥናት ተሳታፊ እንዲሆን የፈቀድኩት የተሳታፊነት ማሳወቂያ ቅፁን ከመፈረሜ አስቀድሞ ሥለጥናቱ አካሄድ እና ከዚህ ቀጥሎ የተዘረዘሩትን የጥናቱ ተሳታፊ መብቶች ግልፅ በሆነ መልኩ ከመረጃ ቅፁ አንብቤ ተረድቻለሁ /ተነቦ በግልፅ ተብራርቶልኛል፡፡

1. ሥለጥናቱ የተሰጠውን ማበራሪያ ተገንዝቤ እውቅና **ሰጥቻለሁ/አልሰጠሁም** (አንዱን አስምር)
2. ይህ ጥናት በተግባር የሚያስከትለውን ተፅዕኖ በግልፅ **ተገንዝቤያለሁ/አልተገነዘብኩትም** (አንዱን አስምር)
3. ማነኛውንም ሥለ ልጄ ማህበራዊም ሆነ አካባቢያዊ መረጃ ለማግኘት ጥያቄ እንዲጠይቁኝ በሙሉ ፈቃደኛነት **ተስማምቻለሁ/አልተስማማሁም** (አንዱን አስምር)
4. ለአንድ ጊዜ ከጣት የሚወሰድ 0.4ሚሊ ሊትር የደም ናሙና ልጄ እንዲሰጥ **ተስማምቻለሁ/አልተስማማሁም** (አንዱን አስምር)

የተሳታፊው ወላጅ/አሳዳጊ ስም: _____

Thumbprint if subject is unable to sign

የተሳታፊው ወላጅ/አሳዳጊ ፊርማ: _____ ቀን: _____

የገለልተኛ ምስክር ስም_____

የገለልተኛ ምስክር ፊርማ: _____ ቀን: _____

በቅርብ የሚገኘው ተመራማሪ ስም: _____

በቅርብ የሚገኘው ተመራማሪ ፊርማ: _____ ቀን:_____

ለአካለ መጠን ያልደረሱ ህፃናት የሚሰጥ የስምምነት መረጃ ቅፅ

የጥናቱ አርእስት፡ በኢትዮጵያ እየተሰራ ያለውን የወባ ቁጥጥር ፕሮግራም ውጤታማነት ለመገምገም የሴሮሎጂ እና ሞለኪዩላር ባዮሎጅ ላቦራቶረ ሚና

እኔ በፈቃደኛነት በጥናት ተሳታፊ የሆንኩት የተሳታፊነት ማሳወቂያ ቅፁን ከመፈረሜ አስቀድሞ ሥለጥናቱ አካሄድ እና ከዚህ ቀጥሎ የተዘረዘሩትን የጥናቱ ተሳታፊ መብቶች ግልፅ በሆነ መልኩ ከመረጃ ቅፁ አንብቤ ተረድቻለሁ /ተነቦ በግልፅ ተብራርቶልኛል፡፡

1. ሥለጥናቱ የተሰጠውን ማበራሪያ እና የቤተሰቦቸን ስምምነት እውቅና **ሰጥቻለሁ/አልሰጠሁም** (አንዱን አስምር)
2. ሥለጥናቱ የተሰጠውን ማበራሪያ ተገንዝቤ እውቅና **ሰጥቻለሁ/አልሰጠሁም** (አንዱን አስምር)
3. ይህ ጥናት በተግባር የሚያስከትለውን ተፅዕኖ በግልፅ **ተገንዝቤያለሁ/አልተገነዘብኩትም** (አንዱን አስምር)
4. ማነኛውንም ሥለራሴ ማህበራዊም ሆነ አካባቢያዊ መረጃ ለማግኘት ጥያቄ እንዲጠይቁኝ በሙሉ ፈቃደኛነት **ተስማምቻለሁ/አልተስማማሁም** (አንዱን አስምር)
5. ለአንድ ጊዜ ከጣት የሚወሰድ 0.4ሚሊ ሊትር የደም ናሙና ለመስጠት **ተስማምቻለሁ/አልተስማማሁም** (አንዱን አስምር)

የተሳታፊው ስም፡_____

Thumbprint if subject is unable to sign

የተሳታፊው ፊርማ: _____ ቀን: _____

የገለልተኛ ምስክር ስም_____

የገለልተኛ ምስክር ፊርማ: _____ ቀን: _____

በቅርብ የሚገኘው ተመራማሪ ስም: _____

በቅርብ የሚገኘው ተመራማሪ ፊርማ: _____ ቀን:_____

Unka Waliigaltee Hirmaattoota Qorannichaa

Formii waliigaltee kan Namootaa Guregudaa (umuriinisanii 18 oli)

Mata duree: Gahee meeshaan qorannoo dhiigaa fi Moleculaarii sagantaa to'annoo dhibee Busaa itoophiyaa madaaluu keessatti qabu.

Anii hirmaatanii qoraano'o kana Qabixillee armaan gadii ergaan dhubisee bodaa ykn na ibsaame bodaa qorannoo kana irratii hirmachufii waligale mallatoo koo arman gaditii maalatessaa.

1.Hirmaanaa qorannoo kanaaf ibsaa kenameefii **nangaletefedhaa/hin galetefadhu.**

2.Odeffanoo qorannoo kan fii hirmanaa qorannoo kan bua'an issa mal akka tahe'e **hubadheraa/hin hubane**.

3.Gaffilee waligala(socio demografic data)gafatamufii dhebii kenufii **waligaleraa/walihingale**.

4.Dhigaa 0.4ml qubaa koo irraa kenufii **waligaaleraa/wali hingale.**

Maqaa hirmaataa:_____

Mallattoo hirmaataa:_____

Guyyaa_____

Maqaa Shaidaa:_____

Mallattoo Shaidaa:_____

Guyyaa:_____

Maqaa Qorata'a:_____

Mallattoo Qorata'a:_____

Guyyaa:_____

Thumbprint if subject is unable to sign

Formii waliigaltee kan maatii (Guddistootaa)

Mata duree: Itiyoopiyaa keessatti Aanaalee tamsa'inni dhibee busaa gad-aanaa fi giddugaleessa ta'anii filatamanitti 'serological fi molecular techniques' fayyadamuun hojiirra oolmaa sagantaa dhibee busaa to'achuu gamaggamuu.

Anii matiin ijolee/gudistuu ykn gudisaa nii ijolee qorannoo kana Qabixillee armaan gadii ergaan dhubisee bodaa ykn na ibsaame bodaa qorannoo kana irratii muchan ykn muchiyoon koo akka hirmaatuu waligaale mallatoo koo arman gaditii maalatesseraa.

1.Hirmaanaa qorannoo kanaaf ibsaa matii koofii ykn gudistootaa koofii kenameefii **nan galetefedhaa/hin galetefadhu.**

2.Odeffanoo qorannoo kan fii hirmanaa qorannoo kan bua'an issa mal akka tahe'e hubachu koo tiif**nan galetefedhaa/hin galetefadhu.**

3.Gaffilee waligala(socio demografic data)gafaatamufii muchanii koo dhebii akka kenufii **waligaleraa/walihingale**.

4.Dhigaa 0.4ml qubaa irraa akkakenufii **waligaaleraa/wali hingale**.

Maqaa matii/gudisaa:_____

Mallattoo matii/gudisaa:_____

Thumbprint if subject is unable to sign

Guyyaa_____

Maqaa Shaidaa:_____

Mallattoo Shaidaa:_____

Guyyaa:_____

Maqaa Qorata'a:_____

Mallattoo Qorata'a:_____

Guyyaa:_____

Formii waliigaltee kan Ijoollee

Mataduree: Itiyoopiyaa keessatti Aanaalee tamsa'inni dhibee busaa gad-aanaa fi giddugaleessa ta'anii filatamanitti 'serological fi molecular techniques' fayyadamuun hojiirra oolmaa sagantaa dhibee busaa to'achuu gamaggamuu.

Anii hirmaatanii qoraano'o kana Qabixillee armaan gadii ergaan dhubisee bodaa ykn na ibsaame bodaa qorannoo kana irratii hirmachufii waligale mallatoo koo arman gaditii maalatessaa.

1. Hirmaannaa qorannoo kanaaf ibsaa maatiikoof/guddistuu koof kenameefii **nan galetefedhaa/hin galetefadhu**

2. Hirmaanaa qorannoo kanaaf ibsaa kenameefii **nan galetefedhaa/hin galetefadhu.**

3. Odeffanoo qorannoo kanaaf hirmanaa qorannoo kan bua'an issa mal akka tahe'e **hubadheraa/hin hubane**.

4. Gaffilee waligala(socio demografic data)gafatamufii muchanii koo dhebii akka kenufii **waligaleraa/walihingale**.

5. Dhigaa 0.4ml quba koo irraa kenufii **waligaaleraa/wali hingale**.

Maqaa hirmaataa:_____

Mallattoo hirmaataa:_____

Guyyaa_____

Maqaa Shaidaa:_____

Mallattoo Shaidaa:_____

Guyyaa:_____

Maqaa Qorata'a:_____

Mallattoo Qorata'a:_____

Guyyaa:_____

Thumbprint if subject is unable to sign

9.4. Appendix 4: Ethical approval from collaborating institutions

BIIROO EEGUMSA FAYYAA OROMIYAA

OROMIA HEALTH BUREAU
የኦሮሚያ ጤና ጥበቃ ቢሮ

Lakk/Ref. No. BEFO/AHBTH/1-8/27...
Guyyaa /Date 17 - 8 - 2007

Wajjira E/Fayyaa Godina Harargee Bahaa tiif
Haraar

Wajjira E/Fayyaa Godina Arsii tiif
Asallaa

Dhimmi: **Xalayaa Deeggarsaa Kennuu ilaala**

Akkuma beekamu Biiroon keenya ogeeyyii, dhaabbilee akkasumas namoota qorannoo gaggeessuuf piroppoozaala dhiyeeffatan piroppoozaala isaanii madaaluun akkanumas iddoo biraatti ilaalchisanii fudhatama argatee (approved) dhiyaateef, piroppoozaala isaanii ilaaludhaan waraqaa deeggarsaa ni-kenna. Haaluma kanaan mata-duree **" EVALUATION OF THE EFFECTIVENESS OF MALARIA CONTROL PROGRAM BY USING SEROLOGICAL AND MOLECULAR TECHNIQUES IN SELECTED LOW AND MODERATE PREVALENT DISTRICTS OF ETHIOPIA"** kan jedhu irratti **Godina Harargee** (Midhagaa Toolaa, Qumbii fi Malkaa Jabduu) **fi Godina Arsi** (Gololchaa, Lookoo Abbaayaa) **keessatti 'Barataa MSc Migbaaruu Kaffalee'** qorannoo geggeessuuf piroppoozaalii isaanii Koreen "Health Research Ethical Review Committee" Biiroo keenyaatti dhiyeeffataniiru. Haaluma kanaan Koreen **"Health Research Ethical Review Committee"** Biiroo keenyaas piroppoozaala kana ilaaluun mirkaneesse qorannoon kun akka hojiirra oolu murteessee jira. Waan kana ta'eef hojii qorannoo kana irratti deeggarsa barbaachisaa akka gootaniif jechaa. **'Barataa MSc Migbaaruu Kaffalee'** wayitii qorannoon kun qaacceffamee xumurame fiiriisaa Biiroo Eegumsa Fayyaa Oromiyaa fi iddoo qorannoon irratti adeemsifameef kooppii tokko tokko akka galii goodhan garagalchaa xalayaa kanaatiin isaan beeksifna.

Anis, 'Barataa MSc Migbaaruu Keffalee' wayitii qorannoon kun qaacceffamee xumurame fiiriisaa kooppii tokko tokko **Biiroo Eegumsa Fayyaa Oromiyaa** fi iddoo qorannoon irratti adeemsifameef akka galii godhu mallattoo kiyyaan mirkaneessa.

Nagaa Wajjin

Gammachuu Shuumu

Maqaa **Migbaaruu Kaffalee**
Mallattoo______
Guyyaa ______
Lakk. Bilbilaa; 0909604041
Email;migkafe@gmail.com

Teessoo: Tel:+251-011-369-06-49, orphem@gmail.com Fax: +251-011-361-01-27 P.O.Box.24341 E-mail: ohbhead@telecom.net.et Address: ADDIS ABABA/FINFINNE-ETHIOPIA

Aanaa Haramayaatiif

Aanaa Baabileetiif

Aanaa Goro Guutuutiif

Aanaa Kurfaa Calleettiif

Bakka jiranitti

Lakk EF D5/1650/09

Guyyaa 26/8/2009

Dhimmi:- Xaalayaa deeggarsaa kennu ilaala

Akkuma mata duree irratti ibsuuf yaalametti Obbo Migbaaruu Kaffalee Mata duree **"EVALUATION OF THE EFFECTIVENESS OF MALARIA CONTROL PROGRAM BY USING SEROLOGICAL AND MOLECULAR TECHNIQUES IN SELECTED LOW AND MODERATE PREVALENT DISTRICTS OF ETHIOPIA"** jedhu irratti akka hojjetaniif Biiroon Egumsa Fayyaa Oromiyaa Xaalayaa Guyyaa 17/8/2009 lakk.BEFO/AHBFH/1-8/2700 Barreessen hayyamuu isaa nu beeksiseera . Nutis haaluma kanaan Aanan keessanis kanuma beekuudhaan deeggarsa barbaachisu akka gootaniif xaalayaa deeggarsaa kanaan isin beeksifna

Nagayaa wajjin

Asaffaa Abdisaa

	AHRI/ALERT Ethics Review Committee	Date: July 25, 2017 No: _____

ANNEX 4
Form AF-10-015

AAERC approval letter

Protocol number PO24/17

Investigators: Migbaru Kiflale

Protocol Title: **Evaluation of the effectiveness of malaria control program by using serological and molecular technologies in selected districts classified as low and moderate prevalence by the Ethiopian malaria control program.**

Study Site(s): Hararghe Zone (Gorogut and Babile districts)

Application Type: Initial ■ Amendment ☐ Renewal ☐

Review Procedure: Full Board ■ Expedited ☐ Secretariat ☐

Review Date: July 4, 2017

Final Decision: ■ Approved **Approval Date:** July 24, 2017

Approval period: July 25, 2017 **to** July 24, 2018

I. Elements approved- Protocol Version 0.01, July 2017; Information sheet and consent forms (Amharic, English, and Oromifa versions).

II. Obligations of the Principal Investigator-

1. Should comply with standard international & national scientific and ethical guidelines.
2. All amendments and changes made in protocol and consent form need AAERC approval.
3. End of the study, including manuscripts and thesis works should be reported to the AAERC.

III. Does the protocol need to be reviewed by the National ERC (NRERC)? Yes ☐ No ■

Follow up report expected in:

3 Months ___ 6 Months ✓ 9 Months ___ One year___

Name:	Dr Martha Zewdie	Dr. Geremew Tarekegne	Dr. Taye Tolera
Signature:			
Date:	25/7/17		27 July 2017
	AAERC Secretary	**AAERC Chairperson**	**AHRI Director General**

Taye Tolera Balcha (Dr.)
Director General
Hansen Research Institute

COLLEGE OF NATURAL & COMPUTATIONAL SCIENCES
Addis Ababa University

የተፈጥሮና ኮምፒቲሽናል ሳይንስ ኮሌጅ
አዲስ አበባ ዩኒቨርሲቲ

OFFICE OF THE DEAN
የዲን ጽ/ቤት

Ref. No. ቁጥር CNSDO/205/10/2017
Date ቀን December 19, 2017

To Whom It May Concern

The College of Natural & Computational Science Institutional Review Board (CNS-IRB) Committee in its meeting held on 10/15/2017 Minute No. IRB/029/2017 has examined the project proposal entitled "**Evaluation of the effectiveness of Malaria control program by using serological and molecular techeniques in selected districts classified as low and moderate prevalent by the Ethiopian malaria control program**" by Migbaru Kefale.

The proposal is conditionally approved for implementation.

With regards,

Shibru Temesgen /Dr./
Dean, College of Natural & Computational Science
Addis Ababa University

ስልክ/Tel.+251-11-123-94-72
ፋክስ/Fax: +251-11-123-94-69
ፖ.ሣ.ቁ/POBox 1176 Addis Ababa, Ethiopia
ኢ.ሜይል/E-mail: dean_cns@aau.edu.et

Please Quote our reference number in you correspondence

"Examine all things; hold fast that which is good" "ሁሉን መርምሩ፡ መልካሙን ያዙ"

9.5. Appendix 5: Graphic representation of preliminary and retrospective data

According to the findings mentioned on the graphs below we decided to conduct the research in Babile district because the annual malaria case incidences verify the national malaria control program claim of reduction.

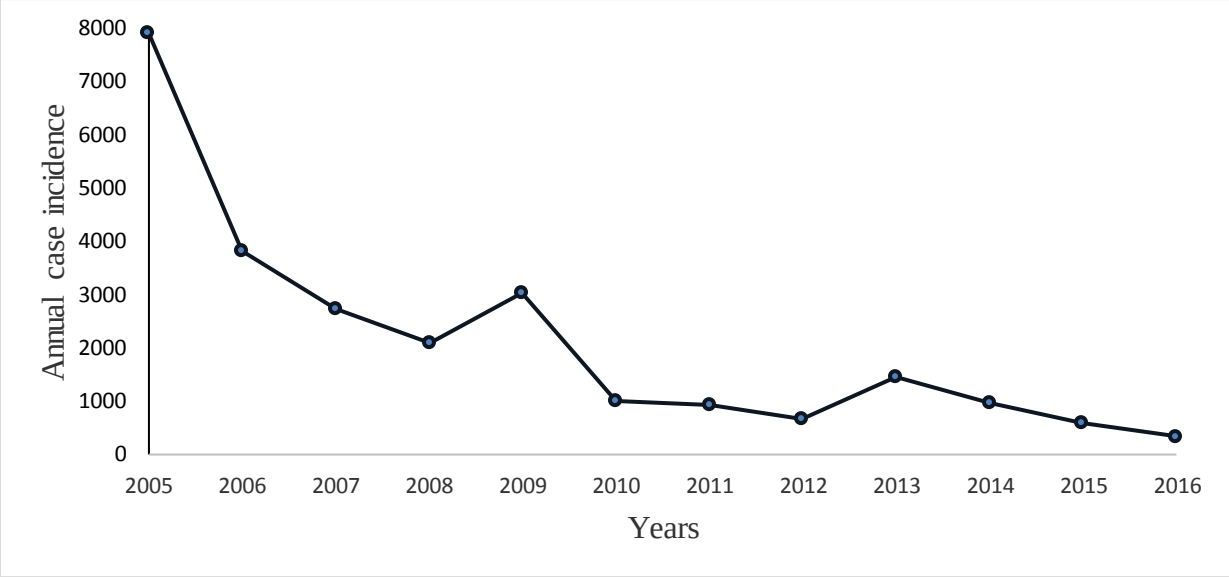

Figure 11: Annual malaria case number trends in Babile district (2007-2016)

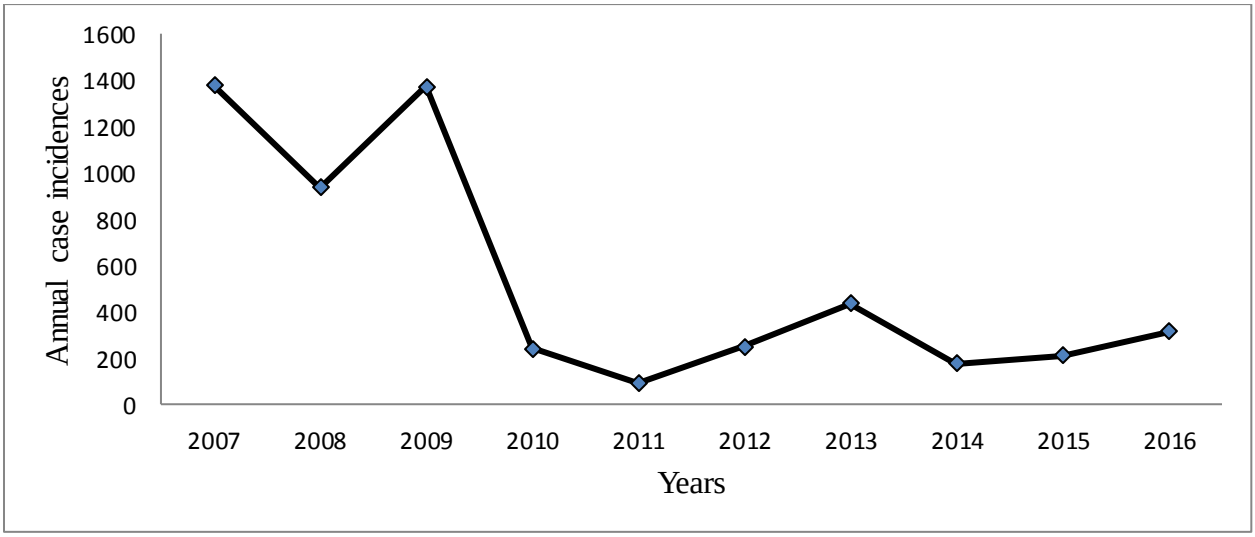

Figure 12: Annual malaria case number trends in Goro Gutu district (2007-2016)

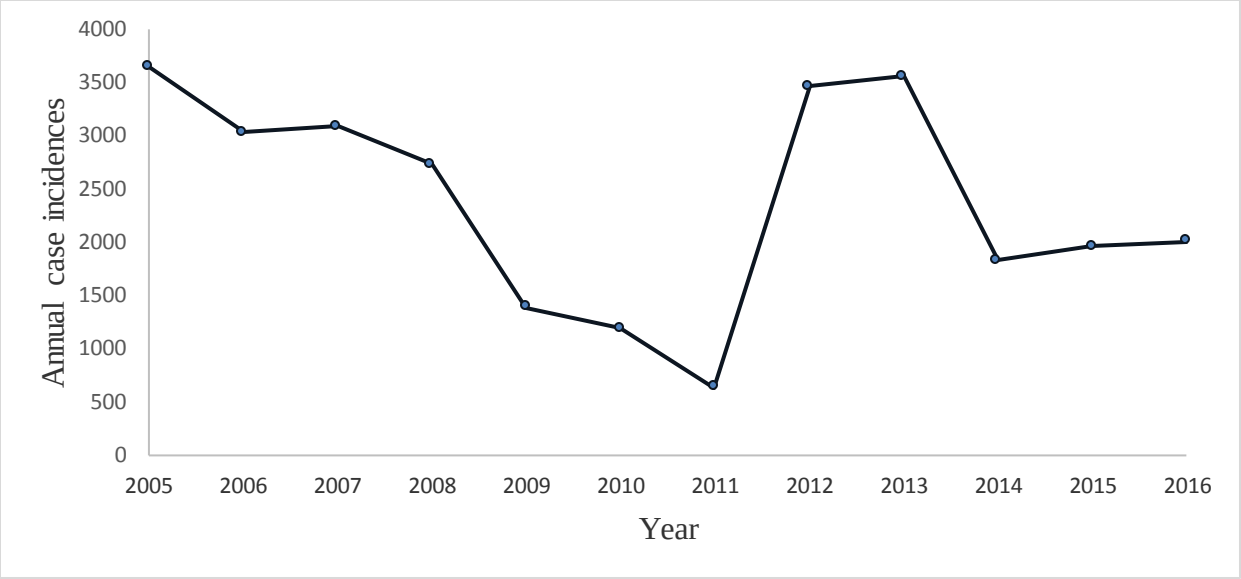

Figure 13: Annual malaria case number trends in Haramaya district (2005-2016)

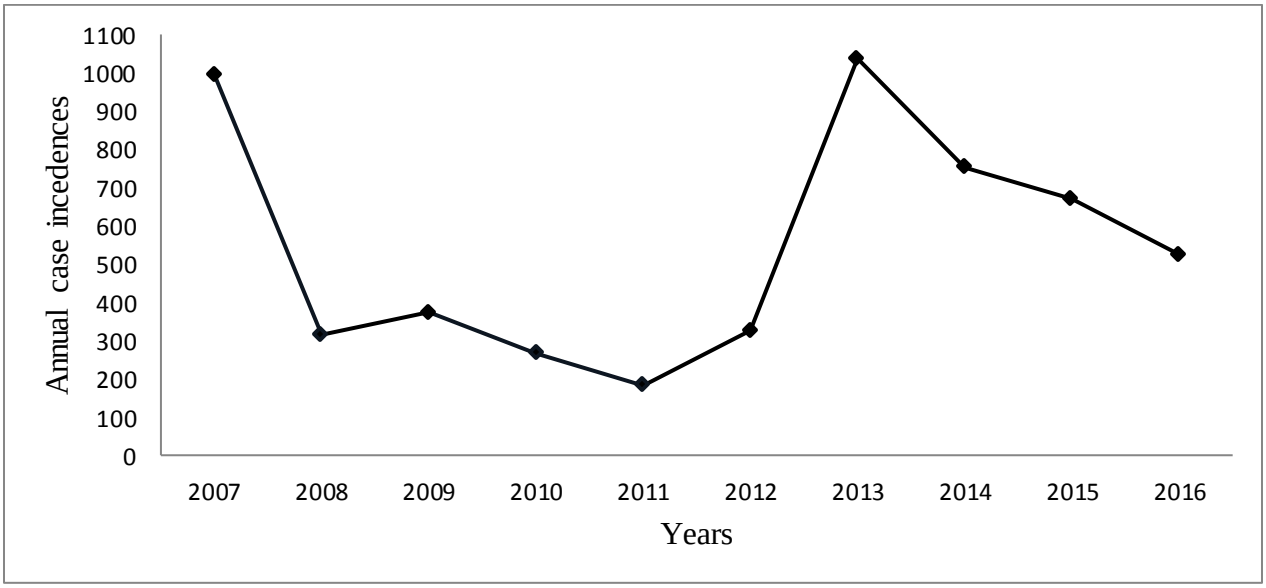

Figure 14: Annual malaria case number trends in Kurfa Chele district (2007-2016)

Incomplete Kebele based retrospective data

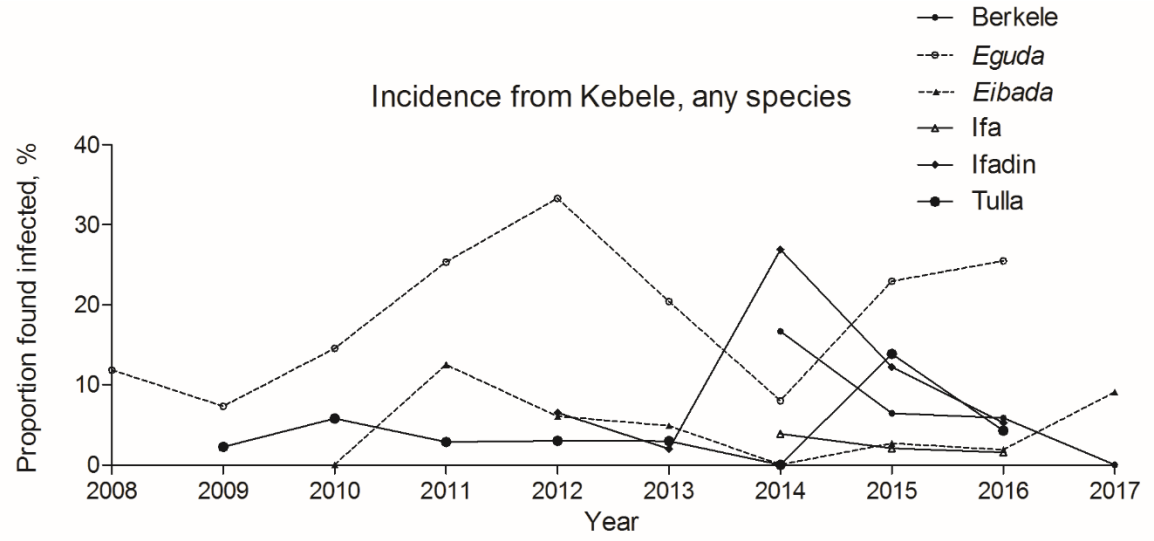

Figure 5: Trend in the incidence rate of malaria detected by microscopy and/or RDT from 2008 to 2016 in eight selected kebeles of Babile district.