ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF MEDICAL BIOCHEMISTRY

A HYBRID APPROACH TO SCREEN A SENTINEL POPULATION TO IDENTIFY CLUSTERS OF SUB-PATENT MALARIA INFECTIONS IN LOW ENDEMIC SETTING IN BATU DEGAGA KEBELLE, ADAMA WOREDA, OROMIA, ETHIOPIA

MULUALEM BELACHEW

A Master's thesis submitted to the department of Medical Biochemistry, school of Graduate Studies, Addis Ababa University in partial fulfillment of the requirements for the degree "Master of Science in Biochemistry" in the department of Medical Biochemistry.

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This is to certify that the dissertation prepared by Mulualem Belachew, entitled: "**A hybrid approach to screen a sentinel population to identify clusters of sub-patent malaria infections in Low endemic setting in Batu Degaga kebelle, Adama woreda, Oromia, Ethiopia"** and Submitted in partial fulfillment of the requirements for the degree "Master of Science in Biochemistry" in the department of medical Biochemistry Complies with regulations of the university and meets the accepted standards with respect to originality and quality.

Signed by the examining committee:

Head Department of Medical Biochemistry……signature……Date……...

Examiner: Dr/Mr. …. . ……Signature…… ……..Date……

Advisors:

1. Dr.ENDALAMAW GADISA**…….** ……..Signature…….Date……

2. Dr.SOLOMON GENET…… …Signature……..Date…….

3. Mr. FITSUM GIRMA……..Signature…….. Date…….

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List of abbreviation

ACD	Active case detection
AHRI	Armauer Hansen Research Institute
DBS	Dry Blood Spot
DNA	Deoxyribonucleic acid
gDNA	Genomic DNA
GIS	Geographical Information System
GPS	Geographical Positioning System
HC	Health Center
HH	Household
HP	Health post
IRS	Indoor Residual Spraying
ITN	Insecticide Treated Net
LLIN	Long Lasting Insecticide Net
LLR	Log Likelihood Ratio
NPCR	Nested Polymerase Chain Reaction
qPCR	Quantitative Polymerase Chain Reaction
RACD	Reactive active case detection
RDT	Rapid Diagnostic Test
RR	Relative Risk
RT-PCR	Real Time Polymerase Chain Reaction
WHO	World Health Organization

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Abstract

Background: As the incidence of malaria decreases the distribution of malaria becomes highly heterogeneous and concentrated in certain geographical areas and households. Due to this the risk of being infected by malaria becomes highly variable within the same locality and households. Identifying the distribution patterns of malaria is crucial in the control as well as elimination of malaria.

Methods: A cross sectional survey was carried out targeting a total of 18 rapid diagnostic test(RDT)-confirmed malaria infected and 18 individuals that visited the health-post for malaria unrelated cases between October and December 2016 including their immediate six neighbors and family members. Consenting individuals were screened for malaria using RDT and dried blood spots were collected for quantification of parasites using species specific 18S based quantitative polymerase chain reaction (qPCR). Spatial clustering of malaria infections was assessed using SaTScan Software.

Results: RDT-detected malaria (any species) was higher in the community around index cases compared to controls (P = 0.001). Asymptomatic qPCR-detected *P. falciparum* infections were higher in the community around index cases (13.9%) compared to controls (9.5%; P = 0.038) while the distribution of qPCR-detected *P. vivax* was similar (P = 0.926). Children had the highest burden of malaria and carry high density infections compared to adults. SaTScan detected four geographically non-overlapping significant hotspot of any malaria cases with relative risk of 2.11, 1.9, 1.89 and 1.86. Individuals who lived in households (HHs) within at risk areas were more likely to have previous malaria episodes (33.1%, 177/233) compared to individuals in HHs outside risk areas (1.5%, 3/203; odds ratio [OR], 32.9; 95% CI, 10.2 – 106.3). People in risk areas utilize malaria control interventions better than people in HHs outside of risk areas and live in iron sheet houses with eave openings and better HH facilities. HHs within the clusters of higher malaria incidence was closer to water bodies and farther from health posts. People who lived in HHs at risk areas walk in the night, enter their houses late and leave their houses early than people who lived in risk free areas.

Conclusion: The distribution of malaria was heterogeneous and clustered in the study district. Symptomatic and asymptomatic malaria distributed significantly around index cases compared to non-malaria control cases. Malaria control and elimination strategies of the country might benefit by targeting hotspots of malaria by following patients. Hot spot population carries the biggest burden of malaria and they might contribute disproportionately to the onward maintenance of malaria infections even outside of the risk areas.

Key words: Cluster, Asymptomatic, Heterogeneous, qPCR Sat Scan

Declaration by the candidate

I, Mulualem Belachew, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signed_____ date_____

(Mulualem Belachew)

Advisors:

1. Dr.ENDALAMAW GADISA

2. Dr.SOLOMON GENET

3. Mr. FITSUM GIRMA

1. Introduction

1.1 Background

In spite of substantial gains in malaria control in the past decade, malaria still continues to be a major public health problem; responsible for 262 million cases and 438,000 deaths globally in 2015 (WHO,2016). During the same period, cases and deaths attributable to malaria declined substantially in Ethiopia. The morbidity and mortality burden has decreased significantly; between 2010-2015 estimated change in malaria incidence and mortality rates decrease of more than 40% has been achieved (Aregawi *et al*., 2014b, Deribew *et al*., 2017). Encouraged by this result, in some endemic countries aggressive malaria control has decreased the burden of malaria to a point where malaria elimination is possible in the coming decades. This shifted the concern of policy makers and the concerned stake holders to identify and design appropriate intervention strategy to eliminate malaria (WHO, 2016).

The success in malaria control in endemic countries re-ignited elimination efforts. However, these efforts are challenged by the widespread presence of infections at parasite densities below the detection limit of conventional rapid diagnostic tests (RDT) and microscopy in settings with recent reduction in transmission, low endemic settings (Bousema *et al.*, 2014, Slater *et al*., 2015). Low density infections do not often elicit treatment seeking behavior and contribute substantially to onward malaria transmission to mosquitoes (Ouedraogo *et al*., 2016, Vallejo *et al*., 2016, Alves *et al*., 2005).

Despite the relevance of all infections, especially in pre-elimination settings with low endemic areas, people with mild symptoms and low density parasite infections cannot be detected by the routine health system. RDT able to detect 200 parasite/µl, microscopy around 100parasite/µl while molecular techniques able to detect around 5 or less than 5 parasite/µl. Reactive Active case detection(RACD), whereby individuals living in close proximity to passively detected cases are screened and treated, could play a central role in finding these infections (Sturrock *et al*., 2013). However, the heterogeneous distribution of malaria infections within villages in pre-elimination settings in low endemic areas further complicates targeting of all relevant infections (Bousema *et al*., 2012, Organization, 2017). There is growing evidence that as transmission intensity decreases malaria episodes cluster within villages owing to heterogeneity in malaria risk factors (Bousema *et al*., 2012). As a result only a small proportion of people and geographic areas carry the majority of the malaria burden and disproportionately contribute to the onward transmission (Mosha *et al*., 2014b). Identification of malaria distribution patterns in low endemic settings could be extremely

useful, as it would allow targeted malaria control, which would reduce costs of deploying interventions, as only a subset of the population would be targeted. One of the plausible approaches is to follow symptomatic malaria cases detected at the health facilities to identify additional symptomatic and asymptomatic cases living around or in close proximity to the index cases (Mosha *et al*., 2014a). In the present study, we assessed the prevalence and distribution of symptomatic and asymptomatic malaria infections and investigated the associated risk factors around self-presenting RDT-confirmed malaria cases in low endemic settings in Adama Woreda, Ethiopia in comparison with controls.

1.2 Statement of the problem

The distribution pattern of malaria is highly associated with socio economic, demographic and geographic factors; thus the distribution tend to be spatially clustered around certain area (Alemu *et al*., 2014). The prevalence varies from village to village as well as there could be difference among different households. Moreover, across all levels of transmission intensity, a substantial proportion of malaria infections are asymptomatic and often present at densities below the threshold for detection by microscopy or rapid diagnostic tests (RDTs) (Brooker *et al*., 2004).

A study conducted in Senegal revealed disparity in the prevalence of malaria within the same geographical areas in different villages; that ranged from less than 2% to 25% (Ndiath *et al*., 2014) . Similar study conducted in other setting by tracing confirmed malaria patients to their households identified more infected persons within the households or in the vicinity, of the cases (Baliraine *et al*., 2009a). Also in Zambia clinical cases of malaria that appeared during the low transmission season occurred in a cluster, behind malaria index cases, in the same household or village (Stresman *et al*., 2010) .

A study conducted in Kenya showed heterogeneous distribution of malaria among different localities; ranging from 0% to 51.5%, within the same geographical area. Most of the asymptomatic infections (≥75%) identified during the study clustered around the clinically confirmed malaria cases (Bousema *et al*., 2013a).

Among the factors implicated for the clustering were the distance between the households to nearest mosquito breeding site, the wind direction, vegetation around the households, house construction features, and human genetic and behavioral factors. Variations in these factors over a small area could result in spatially heterogeneous transmission and result in malaria

hotspots, in where transmission intensity is higher than its surrounding (Haque *et al*., 2011),(Tuyishimire *et al*., 2016).

The success of malaria control depend on a systematic understanding of the micro geographic risk factors of malaria transmission that would enable to identification at high-risk spots (Baliraine *et al*., 2009a). Thus, understanding the distribution as well as the prevalence and the associated risk factors of malaria, and determining the prevalence of the asymptomatic subpopulation provides an opportunity to estimate the epidemiological importance of this group in malaria control and elimination.

1.3 Significance of the study

Heterogeneity in malaria risk factors and malaria prevalence among different population and geographic area suggests that small proportions of people and geographic areas are not only carrying the majority of the malaria burden, but are also contributing disproportionately to the onward transmission. It has been suggested that individuals experiencing the majority of the malaria burden tend to cluster in space and form hotspots of infection.

Thus, identification of malaria distribution could be extremely useful, as it would allow targeted malaria control, which would reduce costs of deploying interventions, as only a subset of the population would be targeted. In addition, as hotspots might fuel malaria transmission in larger regions, it is conceivable that such a targeted approach could lead to a greater reduction in malaria transmission in areas surrounding the hotspots. And this study sought to identify the distribution and hotspot of malaria infection

The present study, therefore, aimed to determine the prevalence and distribution of symptomatic and asymptomatic malaria infections around confirmed malaria cases and the associated risk factors in Oromia regional state east Showa Zone, Adama Woreda, Ethiopia.

2. Literature review

In the eastern and southern Africa around 319 million people are at risk for malaria and among this 232 million people are at higher risk according to the WHO 2016 report and no country in this region eliminated malaria since 2010. Ethiopia accounts for 6% of the total malaria cases in this region (WHO, 2017).

In Ethiopia, Plasmodium falciparum accounts for 60-70% and P. vivax accounts for 30-40% of malaria cases, although these proportions might fluctuate on the spatial and temporal scale. In most endemic regions across Ethiopia both Plasmodium's co-exist and around 6 million suspected malaria cases occurred in 2015 and 1.1 million and 600, 000 Plasmodium falciparum and P. vivax cases occurred respectively(Woyessa *et al*., 2013).

Malaria is also among the leading communicable diseases in Ethiopia. It is estimated that 67 million (68%) of the population of Ethiopia live in areas at risk of malaria (Aregawi *et al*., 2014a).

In common with most infectious diseases, malaria distribution within certain geographical area is heterogeneous and can vary between villages and even households. Among the contributing factors for such distribution are; distance to the nearest mosquito breeding site, wind direction, vegetation, house construction features, and human genetic and behavioral factors. The variation over a small area results in spatially heterogeneous transmission and malaria hotspots, in where malaria incidence, parasite prevalence, and mosquito exposure would be higher inside the hotspots. Malaria hotspots may exist in all malaria endemic areas but are most readily identifiable in areas of low transmission intensity (Bousema *et al*., 2016a) .

In Zambia, a higher prevalence of malaria was found that the in households with a symptomatic case (8.0%) compared to in households without a symptomatic case (<1.0%) (Baliraine *et al*., 2009a, Stresman *et al*., 2010) . In another study using Polymerase chain reaction (PCR diagnosis), statistically higher cases were detection (8.0%) among homesteads compared to a control group of randomly selected households (0.7%). When RDT was used, only 2.3% of the RDTs positive were among the case population as compared to the controls (0.7%), but this difference was not statistically significant (Stresman *et al*., 2010).

Similarly in Senegal, it was found that the risk of being malaria positive was more than 3 times higher when residing in a household with a symptomatic case (Ndiath *et al*., 2014) and from a study done in Rwanda among health center attendee who presented with the main complaint of fever, 22.8% were microscopy confirmed malaria cases; from households with

index cases 17.3% had at least one symptomatic or asymptomatic case of malaria and the overall malaria prevalence in the community was 5.1% (Rulisa *et al*., 2013) . Also in Kenya it was found that the likely hood of being positive by nPCR increased with the number of RDT-positive individuals within a household (Baliraine *et al*., 2009a). Malaria positivity among health center attendee is significantly correlated with households (HH) having at least one malaria member but no spatial clustering for health center (HC) malaria cases was observed (Rulisa *et al*., 2013) .

Studies on risk factors micro-epidemiological scale variation in malaria transmission dynamics showed different factors in different set ups. As per the study done in Cambodia fever and history of malaria infection were significant predictor for being RDT and PCR positive for *P. falciparum* (Hustedt *et al*., 2016). An inverse relationship was observed between malaria infection prevalence and altitude in Kenya with significantly greater risk at the valley bottom compared with uphill sites. In the same study it was found that the risk of infection among children aged 5–9 years was ~6 times compared to older children, and girls had significantly less chance of being infected (Baliraine *et al*., 2009a). According to the study conducted in Bangladesh the risk of malaria infection in males was higher than in females. Children 0–14 years of age were more vulnerable than individuals 15–49 and >50 years of age (Haque *et al*., 2011).

From study conducted in northern Ethiopia, Tigray, the relative risk of malaria was higher for households with earthen type of roof, presence of window, absence of separate kitchen, keeping animal within the dwelling, ownership/working of/on irrigated land, and number of people slept in the same room (>1 person) had significant association with the risk of malaria positivity (Ghebreyesus *et al*., 2000a) . Another study also showed that with increase in family size, the odds of positive RDT test increases. Furthermore, the risk of malaria is lower for those individuals using a flushing toilet to those who have septic tanks or pit latrine slabs. And, for a unit increase in the number of total rooms, the number of nets in the house and the number of rooms in the household sprayed with anti-mosquito, the risk of malaria for an individual decreased (Ayele *et al*., 2013, Ayele *et al*., 2012) .

Malaria risk among health center attendee is associated with age and bed net owner ship. Compared to fewer than five children, malaria prevalence was 3 times higher among 6-15 years old. Owner ship of greater than 4 bed nets per household and sleeping under a mosquito net the night before the interview date was shown to have significant protective effect. Also having a measured fever greater than 37.5 ^{o}C at presentation, history of malaria was documented to have strong correlation with being malaria positive. Additionally household

members living in houses made up of wood/mud/tent, thatched roofs, opens eaves compared to those household members living in dwellings whose walls were made from stones or bricks, with smoky room, coils in window and an owner ship of an inner open house water vessel were associated with higher relative risk of malaria (Rulisa *et al*., 2013).

Similarly, from study conducted in Peruvians, Peru it was observed that distance to water drain, material in walls different than concrete or brick, lack of potable water, high bed net ownership, and having domestic (i.e. cats, dogs) or peri-domestic (i.e. fowl, pigs) animals around the household were significantly associated with malaria case. Among these factors; distance to water drain within 200 meters; household size (≥ 5 individuals per household), limited availability of potable water and having domestic and peridomestic animals around the household showed significantly association with malaria clustering (Rosas-Aguirre *et al*., 2015a) .

Generally, malaria risk decreased with altitude, with significantly fewer cases occurring at altitudes >1800 masl compared to altitude < 1750masl (Brooker *et al*., 2004). A study done in Ethiopia documented that with an increase in altitude, the relative risk of being positive by rapid diagnosis test decreased (Ayele *et al*., 2012, Ayele *et al*., 2013). Similar study which was conducted in Kenya shows an inverse relationship between infection prevalence and altitude. As altitude increase the prevalence of malaria declined significantly (Baliraine *et al*., 2009b). Similar study in Tanzania shows the prevalence of malaria infection related spleenomegaly decreased significantly as altitude increases (Balls *et al*., 2004).

3. Objective of the study

3.1 General objectives

To test: a hybrid approach to screen a sentinel population to identify clusters of sub-patent malaria infections in Batu Degage kebelle, Adama woreda, east shoa zone, the Oromia regional state, Ethiopia.

3.2. Specific objectives.

To investigate the burden of asymptomatic malaria infection in the kebelle

To quantify the asymptomatic parasite infections using quantitative PCR

To compare the distribution of asymptomatic malaria around index cases confirmed using RDT and controls

To assess risk factors for malaria around households (HHs) within and outside risk areas.

4. Materials and methods

4.1. Hypothesis

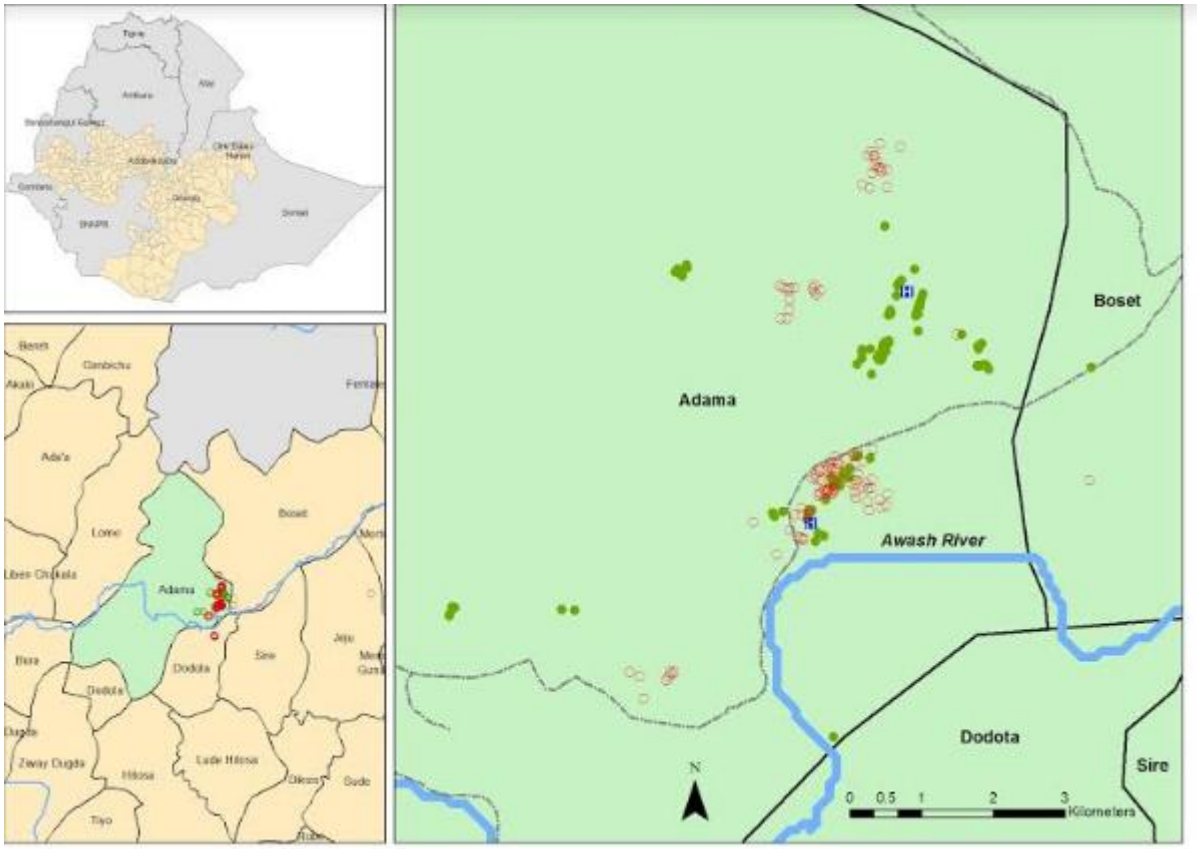
The distribution of malaria was random. (H_O)

The distribution of malaria was not random. (H_A)

4.2. Study site, population and period

This study was conducted in Batu Degaga kebelle (the smallest administrative division) that is located within Adama woreda (district), ~120km southeast of Addis Ababa. The total *land* area of Adama woreda is 48,556 hectares, with an altitude range of 1,400 – 2,300 meters above sea level, and has an estimated population of 183,502 within 38,230 households (Adama district health office report 2015 unpublished data). Awash River defines the south and eastern boundaries of the woreda and large scale irrigation by a sugar factory created suitable environment for malaria vector breeding as a result, most of the villages, kebelles and camps are malarious. Both *P. falciparum* and *P. vivax* are co-endemic in the district (Peterson et al., 2009) of which 60% due to *P. falciparum* and 40% due to *P. vivax* (Yohannes and Petros, 1996) with unstable transmission that peaks following the two rainy seasons: September to November (following the major rain from July – September) and April to May (following the short rain from March – April). There are two health posts (the lowest level governmental heath service providing institute) in the kebelle. Self-presenting RDT-confirmed uncomplicated malaria patients (herein after defined as index cases) and individuals who presented to the health-posts for non-malaria related cases (herein after defined as controls) participated in this study from October – December 2016. Family members of the index cases and controls who were permanently living in the study area and their immediate six neighbors were screened for malaria with RDT within 2 days of the case being identified. The likelihood of reactive active case detection (RACD) detecting additional infections is high when it is conducted within a week of the index presenting at the health posts (Sturrock *et al*., 2013). Geospatial coordinate data for each household (HH) were recorded during the surveys using handheld Garmin eTrex Global Positioning System devices.

A



B **C**

Figure 1 Study area.

Shown in (A) is a map of Ethiopia with boundaries indicating regions and woredas within Oromia region (Adama woreda green color). Indicated in (B) is Adama woreda in detail. (C) Circles indicate the location of surveyed households; the colors uniquely identify the categories, red colors are households around controls and green are households around index cases. The two health posts are indicated in blue colors (H).

4.3. Sample size

Based on recent works in the highlands of Ethiopia which assessed the prevalence of submicroscopic malaria we assumed 10% submicroscopic infections in the community in general and 20% in areas with index case (Tadesse et al., 2015, Golassa et al., 2013). Preliminary visits to the study area and population census indicated that the average family size would be 4.8 per HH. We would be able to sample 30 individuals, including neighbors and family members, around each index case. Thus, using cluster sample size calculation data collected from18 clinical cases and 18 controls and their immediate neighbors.

4.4. Data collection

4.4.1 Sample collection

Detailed clinical, anthropometric, socio-demographic and malaria control intervention utilization data were captured using a pre-tested questionnaire.

Upon receiving informed written consent, trained medical laboratory technologists collected finger prick (0.3ml) blood samples that was used to prepare dried blood spots (DBS) containing three drops of ~20µl each on 3MM Whatman filter paper (Whatman, Maidstone, UK). The blood spots on the DBS were air dried and stored in -20^{o}C freezer in zip-locked plastic bags containing self-indicating silica gel desiccant beads (Geejay Chemicals Ltd) until being processed for extraction of nucleic acids to determine submicroscopic parasite prevalence by qPCR (HERMSEN *et al*., 2004).

4.4.2. Household mapping and household survey

House of every index case enrolled in the study was visited and the location and the elevation of all households were determined using a hand held global positioning system which provides a positional accuracy with in 5m. After briefing the purpose of the study, consent was obtained from the head of the house hold and from all household residents. Then the head of the household or wife was interviewed using the local language to obtain data on household risk factors and the room where they slept was visited to record information on exposure factors that may affect human-mosquito contact.

All household members were screened and each member of the household provided a single finger-prick blood samples for preparation of rapid diagnostic test (RDT) and dried blood spots (DBS) on Whatmann 3MM filter papers for subsequent analysis by PCR for the presence of malaria. Index cases do not provide a blood sample at the household; only their original diagnosis from health center was available. If one of the household members was not at home during the time of survey, he/she was actively sought by the research team and subsequently screened by the team.

4.5. Laboratory procedures

4.5.1. Preparation of RDT and DBS

To identify malaria among health center attendee RDT was administered, blood specimen was obtained by a single finger prick and100 µL of blood was spotted on Whatmann 3MM filter paper for PCR analysis.

RDT findings were immediately reported to the study subjects and their parents or guardians. Positive malaria cases were referred to nearby health post and treated according to the national guideline for malaria by a nearby health center.

Blood spots were dried and packaged in to individual sealed plastic bags with desiccant.

Sample was stored at 4 °C (in a standard refrigerator) up to 24 hrs after collection until it was transferred to the Oromia regional lab in Adama and stored at -20^{o}C until the sample was transferred to Armauer Hansen research institute in Addis Ababa(AHRI) for PCR analysis. After sample was transferred to (AHRI), till extraction the DBS samples were stored at -20^{o}C.

4.5.2. Molecular Analysis

Total Genomic DNA (GDNA) was extracted from a punch of 6mm diameter as described in Hermsen et al. (Hermsen *et al*., 2001) and Wampfler et al. (Wampfler *et al*., 2013) and (Tadesse *et al*., 2017) respectively on a previously collected filter paper. Briefly, premixed 180µl tissue lysis buffer (Roche Applied Sciences) and 20 µl Proteinase K (QIAGEN) was put to each well that contained 6mm discs and incubated at 56^{o}C overnight on a shaker. Plate was centrifuged and put on a shaker for 20 minutes. Discs were squeezed out as much as possible by pressing the filter to the bottom of the plate with the pipettips. Once the discs lost the blood color gDNA was extracted from the eluate using the MagNaPure automated extraction machine and total nucleic acid high performance kit (Roche Applied Sciences). gDNA was eluted in 100µl and stored at -80^{o}C until further use. Parasite density was quantified using species specific quantitative real-time polymerase chain reaction (qPCR) that targeted the 18S small rRNA gene on DNA as described before (Tadesse *et al*., 2017).

A standard dilution series of mature, *in vitro* cultured NF54 ring stage parasite culture material was used to quantify the *P. falciparum* parasite carriage in duplicate. Gene copies of the Pv18S were quantified by running duplicates of a serial dilution of recombinant plasmids that contained the respective gene in each plate. All qPCR reaction plates contained duplicate of molecular grade water in place of sample to control for contamination. Assays were run

using TaqMan® Fast Advanced Master Mix (Applied Biosystems) and specific probes (SIGMA-ALDRICH) using the CFX96™ Real-Time PCR Detection System (BIO-RAD).

4.6. Data analysis

4.6.1. Statistical analysis

Statistical analyses were performed using STATA 13 (StataCorp., TX, USA) and Graph Pad Prism 5.0 (Graph Pad Software Inc., CA, USA). Two-sample Wilcoxon rank-sum (Mann-Whitney) test was used to compare non-parametric variables between groups. Proportions were compared by Pearson's χ^2 test or Fisher's exact test. Association between continuous variables was assessed using Spearman's correlation coefficient (ρ) and *P*-value. The QGIS software QGIS v.2.16 (QGIS developer team, Open Source Geospatial Foundation) was used to map all surveyed households and to classify them according to the number of household members with malaria infections identified by PCR. The SaTScan software v.9.3 (M Kulldorff and Information Management Services Inc, USA) was used to identify spatial clustering of households with malaria infections, using the following characteristics: pure spatial analysis, Bernolli probability model, latitude/longitude coordinates, report of most likely and low clusters with no geographical overlap, maximum spatial cluster size equal to 50% of total population. A 1 km window was optimal to find clusters of parasite prevalence and allowing hotspots to contain up to 50% of the population was a better predictor of infection in the future using spatial scan statistics than smaller maximum population sizes (Kulldorff and Nagarwalla, 1995).(Mosha *et al.,* 2014b). The analysis was first done without adjustment for covariates, and then done including the variables identified as fixed-effect risk factors by the mixed-effects logistic regression analyses. SaTScan applied multiple circular windows across the study area, each circle representing a possible cluster. Clusters were assessed based on 999 Monte Carlo simulations to determine the probability of observed frequency of infected individuals being due to chance relative to expected frequency under the null hypothesis of no clustering. The null hypothesis was rejected if any resulting p value of assessed clusters was <0.05 and the window with the maximum log likelihood ratio (LLR) were identified as the most likely cluster. The relative risk (RR) reported for each identified cluster was the estimated risk within the cluster divided by the estimated risk outside the cluster. Uni- and multivariate mixed-effects logistic regression models, with fixed effects and a random intercept that accounted for individual clustering at household, were used to multivariate model. Using manual backward, final models retained all factors that were

significantly associated with malaria infection (Wald p values <0.05). Interactions were systematically checked for up to order two. Likelihood ratio tests (LRTs) were used for univariate analysis were considered for inclusion to determine risk factors for species-specific malaria infection. Factors with p values <0.1 for the Wald test in the univariate analysis were included in the multivariate analysis.

4.7. Inclusion and exclusion criteria

In this study these Inclusion criteria were used to recruit patients in to the study

- ✓ Positive and negative for malaria
- ✓ History of fever within the past 24hrs
- ✓ Axillary temperature >=37.5 degree Celsius with chills, malaise, headache or vomiting at the time of examination
- ✓ Uncomplicated malaria cases
- ✓ Resident in the study area

Exclusion criteria

- ✓ Complicated malaria cases
- ✓ Infants younger than 6 months of age
- ✓ Resident out of the study area

4.8. Ethical consideration

This study was part of received ethics approval from the ethics review boards of Addis Ababa University (CNSDO/264/08/16), Jimma University (RPGC/395/06), Armauer Hansen Research Institute (PO52/14), The National Research Ethics Review Committee (310/109/2016) and the London School of Hygiene & Tropical Medicine (10628). This study was reviewed and approved by the institutional ethics review committee of the Department of Medical Biochemistry at the School of Health Sciences of Addis Ababa University (DRERC 19/16). Written Informed consent was obtained from all participants and parents/guardians who were permanently living in the study area.

4.9. Expected outputs

This study was conducted with the expected outcome of determining the distribution of malaria and the associated risk factor for malaria at the individual household and environmental level and assessing the heterogeneous distribution of malaria.

4.10. Beneficiary of the results

The result of this study benefits all stakeholders from the community to nationwide.

At the community level, based on their result to malaria and associated risk factor, they were provided with preventive guidance and treatment for malaria according to the national guideline for malaria by the research team and the local health center.

At higher level the ministry of health, policy makers, higher education institutions, and other interested researchers in the field will benefit from the information provided by this research work.

4.11. Dissemination of result

The result of this study was disseminated to AHRI, Addis Ababa University, ministry of health and to the study area health bureau and other concerned stakeholders. The scientific community was reached via a publication in an international peer-reviewed journal.

4.12. Study variables

4.12.1 Dependent variable

Malaria status, clustering of malaria, Parasite density

4.12.2 Independent variables

Age, sex, history of malaria, household feature, water body, bed net utilization and distribution, IRS, Health facility, electricity, Radio, TV,

4.13. Operational Definitions

Health post: primary health care delivery system that serve from 3000-4000 people.

***P. falciparum* positive**: detection of plasmodium falciparum species either by RDT or qPCR

***P. vivax* positive**: detection of plasmodium vivax species either by RDT or qPCR

Mixed infection: detection of plasmodium falciparum and plasmodium vivax species together either by RDT or qPCR

Invalid: failure of RDT or qPCR to rule out the presence or absence of malaria species

Hot spot: area in which the risk of malaria was higher based up on sat scan software

Cold spot: area in which the risk of malaria was lower based up on sat scan software

Symptomatic: those who are positive for any malaria species plus who show sign and symptom

Asymptomatic: those who are positive for any malaria species but not show sign and symptom

Index case: malaria positive and negative cases detected at the health post

Early morning: before 12:00 clock local time in the morning

Late in the night: after 2:00 clock in the night

5. Results

5.1 Demographic and intervention utilization results

A total of 18 RDT-confirmed self-presenting uncomplicated *P. falciparum* (10), *P. vivax* (6) mixed malaria species (2) infected individuals participated in this study as index cases. Within the same period 18 individuals that visited the health-post for malaria unrelated cases participated as control group. The family members of the index cases and controls and their immediate six neighbors including their family members were screened for malaria using RDT within 2 days of screening the index cases.

In total, 932 individuals in 240 HHs (130 around index cases and 110 around controls) participated in this study, with a mean of 6 HHs around index cases and 5 HHs around control. A median of 28 (IQR, 24 – 31) and 26 (IQR, 22 – 30) individuals were screened around index and control cases, respectively. The median age of the study participants was 10 years (interquartile range [IQR], 5-26) and females comprised 58.4% (544/932) of the participants with no difference between the two groups (Table 1). Self-reported malaria in the last three months was significantly higher in the community around index cases (24.7%, 120/485) compared to community around controls (3.6%, 16/439; odds ratio [OR], 8.7; 95% confidence interval [95% CI], 5.1, 14.9; $P < .001$). Intervention utilization that includes bednet ownership, reported bednet use in the previous night, and insecticide residual spraying were all higher in the community around index cases compared to community around controls ($P < .001$).

Household construction materials significantly differed between the two communities ($P < .001$): roof type, wall type, floor type, and eave opening. Facilities (presence of electricity, ownership of radio and television) were all significantly higher in the community around index cases ($P < .001$). Individuals around index cases walk in the night ($P = .002$), enter their houses ($P = .005$) and go to bed ($P < .001$) late in the night and leave their houses early morning ($P < .001$).

RDT-detected malaria (any species) was significantly higher among community members that lived around index cases compared to controls (OR, 2.4; 95% CI, 1.5-3.9; $P < .001$). qPCR-detected *P. falciparum* infection was higher around index cases 13.9% (66/474) compared to controls 9.5% (41/433; OR, 1.55; 95% CI, 1.02, 2.34; $P = .038$) but the *P. vivax* was the same between the two populations.

Table 1: Demographic and malariometric characteristics of the study population

CHARACTERISTICS	Control, %(n/N)	Case, %(n/N)	Total, % (n/N)	P-value
Age in years, median (25^{th} – 75^{th} percentile)	11(6 - 26)	10(5 - 26)	10(5 - 25.5)	
0 - 5 years, % (n/N)	26.6(118/444)	24.6(120/488)	25.5(238/932)	0.42
5 - 15 years, % (n/N)	39.6(176/444)	37.5(183/488)	38.5(359/335)	
Above 15 years, % (n/N)	33.8(150/444)	37.9(185/488)	35.9(335/932)	
Female sex	57.9(257/444)	58.8(287/488)	58.4(544/932)	
History of malaria in the last three months	3.6(16/439)	24.7(120/485)	14.7(136/924)	<0.001
Intervention utilization				
Ownership of bed nets	40.4(162/401)	58.8(271/461)	50.2(433/862)	<0.001
Bed net use last night	30.8(132/429)	45.9(212/462)	38.6(344/891)	<0.001
Bed net/person <1, median (IQR)	0.33(0.25 - 0.50)	0.33(0.25 - 0.43)	0.33(0.25 - 0.50)	
Insecticide residual spraying	16.3(68/350)	35.8(168/469)	26.6(236/887)	<0.001
Fever (temperature ≥37.5 ^{o}C)				
Water bodies				0.001
Water bodies within 15 minutes walk	0.9(4/435)	17.8(81/459)	9.5(85/894)	
Water bodies above 20 minutes walk	99.1(431/435)	82.4(378/459)	90.5(809/894)	
Distance to health facility				0.001
Within 5 minutes	48.0(207/431)	58.7(278/474)	53.6(485(905)	
Between 10 and 30 minutes	49.7(214/431)	21.9(104/474)	35.1(318/905)	
Above 30 minutes	2.3(10/431)	19.4(92/474)	11.3(102/905)	
Roof type				<0.001
Thatch	78.1(336/430)	66.6(323/485)	72.0(659/915)	
Iron sheet	21.9(94/430)	33.4(162/485)	28.0(256/915)	
Wall Type				<0.001
Wooden plastered with mud /clay	98.2(428/436)	88.7(431/486)	93.2(859/922)	
Others	1.8(8/436)	11.3(55/486)	6.8(63/922)	
Floor Type				<0.001
Soil, Earth or local dung	0.9(4/439)	8.4(41/486)	4.9(45/925)	
Cement	99.1(435/439)	91.6(445/486)	95.1(880/925)	
Eave opening	1.6(7/438)	12.4(60/486)	7.3(67/924)	0.001
Facilities				
Electricity	19.0(83/438)	43.9(209/476)	32.0(292/914)	<0.001
Radio	6.9(30/434)	17.0(78/460)	12.1(108/894)	<0.001
Television	6.4(28/437)	14.0(65/464)	10.3(93/901)	<0.001
Time				
Night trips	0.3(1/407)	2.9(13/445)	1.6(14/852)	<0.001
Late entry	0.2(1/434)	2.5(11/457)	1.4(12/891)	0.0022
Late sleeping	0(0/439)	6.6(32/483)	3.5(32/922)	<0.001
Early wakeup	1.8(8/443)	1.6(8/487)	1.7(16/930)	0.8485
Leaving time	0(0/265)	5.1(18/350)	2.9(18/615)	<0.001
***Plasmodium spp.* by RDT**				0.001
P.falciparum	2.9(13/444)	8.8(43/488)	6.0(56/932)	
P.vivax	1.8(8/444)	2.9(14/488)	2.4(22/932)	
Mixed spp. Infection	1.1(5/444)	1.4(7/488)	1.3(12/932)	
***Plasmodium spp*. Confirmation by Qpcr**				
P.falciparum	9.5(41/433)	13.9(66/474)	11.8(107/907)	0.038
P.vivax	14.6(63/433)	14.8(70/474)	14.7(133/907)	0.926

Mixed spp. Infection		1.6(7/429)	2.0(9/460)

5.2 Malaria Parasite carriage and density prevalence

In total there were 9.7% (90/931) RDT and 26.5% (240/907) qPCR-detected infections. There was a strong association between age and malaria positivity ($P < .001$): 29.4% (70/238) of under five children, 30.1% (108/359) children younger than 15 years, and 11.9% (40/335) adults were RDT and/or qPCR-detected malaria infected (Figure 2). The majority of the burden of malaria was on children younger than 15 years: 75.8% (69/91) *P. falciparum* and 88.3% (106/120) *P. vivax* infections by qPCR (Supplementary Figure 3). While the overall median age was 10 years (IQR: 5 – 26), in HHs with at least one malaria positive individual the median age was 7 years (IQR: 5 – 12). *P. vivax* is the dominating species in the early ages, while a pronounced increase in prevalence was documented for both species until the age of 5 (Figure 2). The *P. vivax* infection begins to decrease gradually afterwards while *P. falciparum* remains leveled-off between the age of 5 and 15. Afterwards *P. vivax* decreases substantially where *P. falciparum* follows this same trend in few years time. Both infections reach their lowest prevalence after the age of 18.

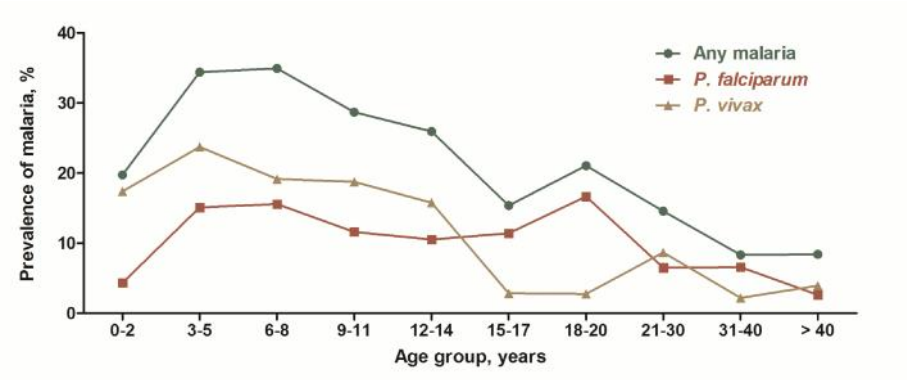


Figure 2 Trends in malaria positivity with age.

Indicated in the Y-axis is prevalence of *P. vivax* (yellow), *P. falciparum* (red) and either species (blue) in the different age groups indicated in X-axis.

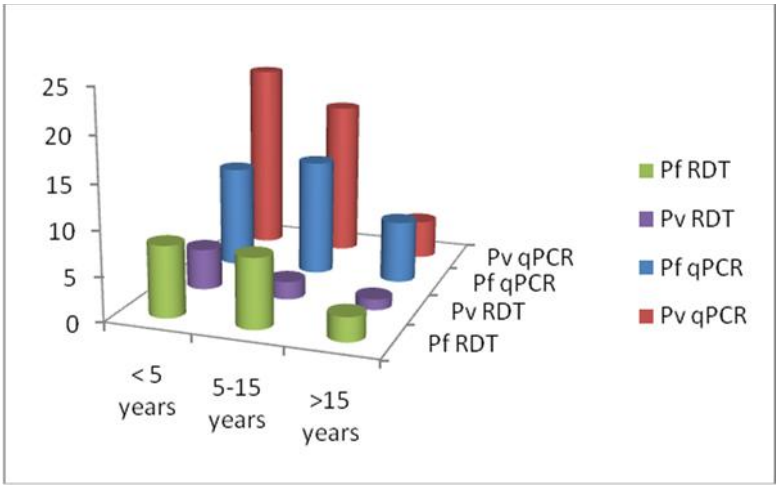


Figure 3 positivity of malaria by RDT and qPCR Vs age

Figure 3 supplementary to figure 2. Shown in the X-axis is age category and on the Y-axis is prevalence of *P. vivax*, *P. falciparum* by RDT and qPCR for respective age category.

Parasite density was negatively associated with age for both P. vivax (Spearman's correlation coefficient = -0.1788; $P < .001$) and for *P. falciparum* (Spearman's correlation coefficient = -0.0879; $P < .0096$). Adults harbored lower *P. vivax* parasite density (median, 22.6 copies/µl; IQR, 9.5 – 94.0; $P < .001$; Figure 4A) compared to both under five children (median, 96.4 copies/µl; IQR, 23.8 – 387.9) and children younger than 15 years (median, 32.1 copies/µl; IQR, 14.3 – 111.9). Similar trends were observed for *P. falciparum*: lower parasite density in adults (median, 83.3 parasites/µl; IQR, 52.4 – 357.0) compared to under five (median, 445.1 parasites/µl; IQR, 126.7 – 1218.0; $P = .0451$) and younger than 15 years children (median, 195.2 parasites/µl; IQR, 44.0 – 608.1, $P = .0059$; Figure 4B).

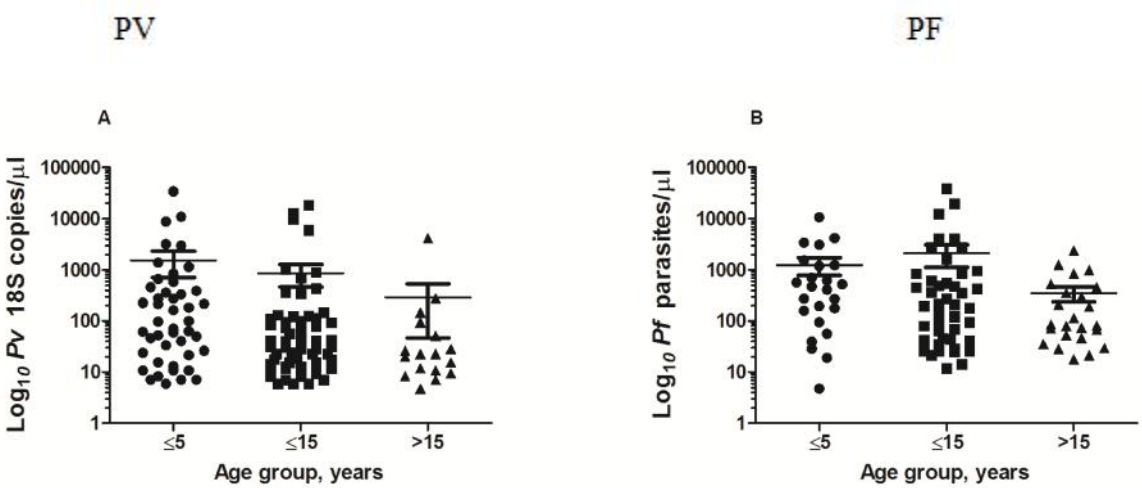


Figure 4 Age dependence of *P. vivax (A)* and *P. falciparum* (B) parasite densities.

Shown in the Y-axes are the Log_{10} transformed *P. vivax* 18S copy numbers/µl (A) and *P. falciparum* parasite densities/µl (B) with age group indicated on the X-axes. Lines refer to the median parasite density and the inter-quartile ranges.

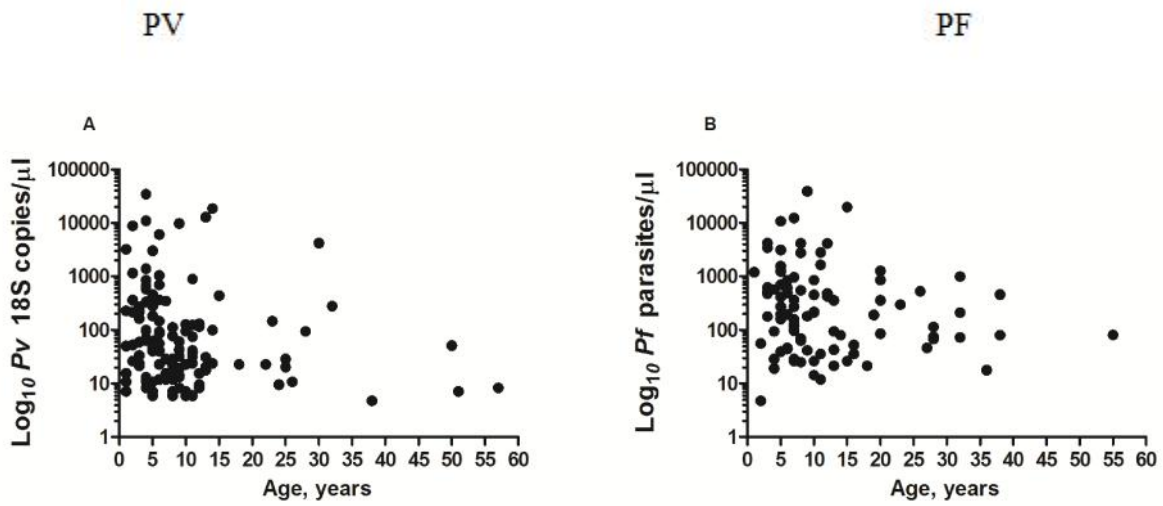


Figure 5 Age dependence of *P. vivax* (A) and *P. falciparum* (B) parasite densities.

Shown in the Y-axes are the Log10 transformed *P. vivax* 18S copy numbers/µl (A) and *P. falciparum* parasite densities/µl (B) with age indicated on the X-axes.

The parasite density in RDT positive individuals with *P. vivax* (median 320.2 copies/µl; IQR, 99.4 – 4,670.0) and *P. falciparum* (median, 534.9 parasites/µl; IQR, 191.1 – 1,917.0) infections was higher ($P < 0.001$) compared to RDT negative individuals (median 26.2 copies/µl; IQR, 11.9 – 94.6) and (median, 76.8 parasites/µl; IQR, 28.6 – 254.6) infections, respectively (Figure 4). RDT-detected *P. vivax* symptomatic infections were higher (median, 4,213.0 copies/µl; IQR, 894.9 – 18,588.0) than RDT-detected asymptomatic (median, 215.4 copies/µl; IQR, 61.9 – 1,395.0; $P = .0431$) and qPCR-detected (median, 26.2 copies/µl; IQR, 11.9 – 94.6; $P < .001$). RDT detectable asymptomatic infections compared to qPCR-detected ($P < .001$). *P. falciparum* parasite density was higher in RDT-detected symptomatic individuals (median, 1,076.0; IQR, 399.7 – 19,993.0) compared to qPCR-detected (median, 76.8 parasites/µl; IQR, 28.6 – 254.6; $P < .001$) individuals with a non-significant difference with RDT-detected asymptomatic (median, 477.2 parasites/µl; IQR, 185.1 – 1,453.0; $P = .1297$). RDT detectable asymptomatic infections were high density compared to qPCR-detected infections ($P < .001$).

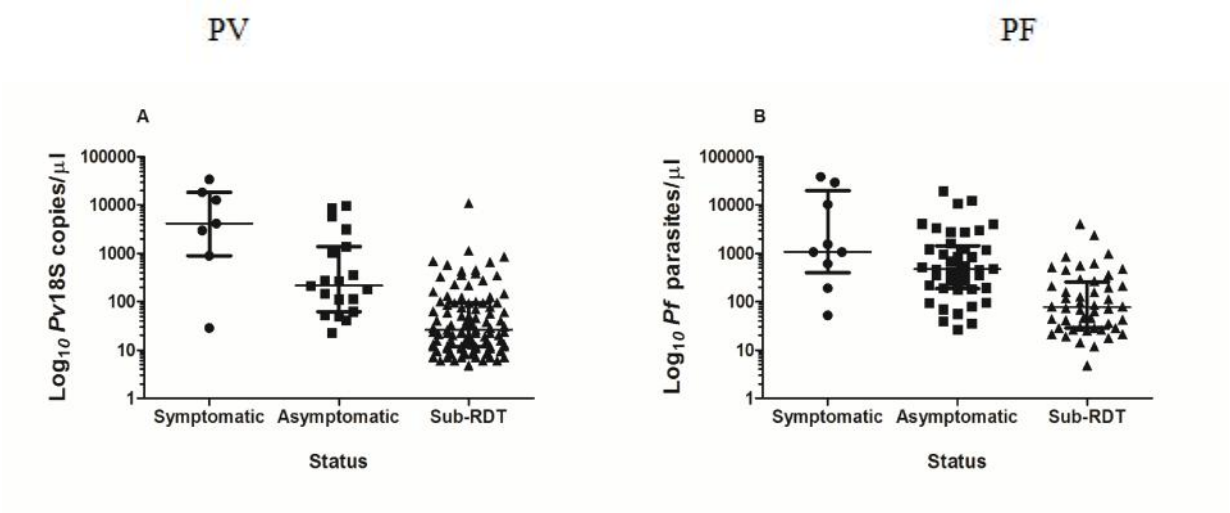


Figure 6 parasite density differences between symptomatic and asymptomatic infections.

Shown in the Y-axes are the Log_{10} transformed *P. vivax* 18S copy numbers/µl (A) and *P. falciparum* parasite densities/µl (B) with status indicated on the X-axes. Lines refer to the median parasite density and the inter-quartile ranges.

5.3 Clustering of infection

Out of the total HHs surveyed at least one individual was found infected in 27.9% (67/240) HHs by RDT and 57.1% (137/240) HHs by qPCR. RDT correctly identified 47.4% (65/137) of HHs that were detected by qPCR. At least one *P. falciparum* infected individual was found in 17.1% (41/240) HHs and in 22.5% (54/240) HHs ≥1 *P. vivax* infected individual was found while 16.7% (40/240) of the HHs had ≥1 *P. falciparum* and ≥1 *P. vivax* infected individuals in the same HH. The number of RDT-positive individuals per HH ranged from 1 – 2 for *P. vivax* and 1 – 4 for *P. falciparum* while it was 1 – 3 and 1 – 4 using qPCR, respectively.

Family members who live in HHs with ≥1 RDT-confirmed *P. falciparum* individual were more likely to have qPCR-confirmed *P. falciparum* infection (64.4%, 58/90) compared to HHs without RDT-detected *P. falciparum* infection (5.9%, 47/795; OR, 28.8; 95% CI, 17.1 – 48.6; $P < .001$). The qPCR-confirmed *P. vivax* infection was high in HHs with RDT positive *P. vivax* infection (40.0%, 36/90) compared to HHs without RDT-detected infection (11.8%, 94/795; OR, 5.0; 95% CI, 3.1 – 8.0; $P < .001$). More young people live in HHs with ≥1 malaria infected individual (81.7% younger than 15 years, 179/219) compared to those who live in HHs without malaria infected individual (58.6%, 418/713; $P < .001$). Risk factors and intervention utilization did not differ between the HHs with and without malaria infected

individuals except eave opening (13.0%, 28/216 vs 5.5%, 39/708; $P < .001$) and early leaving of houses (8.9%, 14/158 vs 0.9%, 4/457; $P < .001$).

Purely spatial analysis by SaTScan revealed that malaria episodes were heterogeneously distributed in the study area. Geographically non-overlapping significant clusters of higher incidence of any malaria species (hot spots) detected by RDT and/or qPCR. The cold spots were relatively closely located with each other. Four most likely significant spatial clusters of any malaria species were detected within a radius ranging from 280 – 590 meter composed of 62 HHs (25.8% of total HHs surveyed) in which 152 people live (16.32% of total population) accounting for 143 malaria episodes (15.35% of total population) with a relative risk that ranged from 1.83 – 1.90. In addition, three cold spots composed of 48 HHs (20.0% of total HHs surveyed) were detected. Two P. falciparum and 1 P. vivax hotspots and 1 cold spot were detected for each species with overlapping HHs, Table 2 blue color show cold spots and red color show hot spots.

Table 2 Characteristics of any malaria clusters detected

Cluster	Radius	Number of households	Log likelihood ratio	Ratio(observed cases/expected cases)	Relative risk	Number of population	Percent cases	P-value
Any 1	0.97	53	75.8	0.05(2/41.38)	0.033	72	2.8	1.00E-17
Any 2	0.46	16	24.62	0.00(0/14.94)	0	26	0	1.22E-09
Any 3	0.65	23	24.62	0.00(0/14.94)	0	26	0	1.22E-09
Any 4	0.31	9	11.67	0.00(0/7.47)	0	13	0	0.000724
Any 5	0.87	88	23.98	1.53(66/43.1)	2.11	75	88	2.35E-09
Any 6	0.44	12	13.72	1.74(23/13.22)	1.9	23	100	0.0000878
Any 7	0.28	20	13.08	1.74(22/12.64)	1.9	22	100	0.00017
Any 8	0.59	15	10.56	1.74(18/10.34)	1.86	18	100	0.00226
Any 9	0.37	15	8.1	1.74(14/8.05)	1.83	14	100	0.029
Pf 1	0.97	53	43.8	0.00(0/20.27)	0	32	0	1.00E-17
Pf 2	0.63	22	10.8	1.58(21/13.30)	1.8	21	100	0.000721
Pf 3	0.28	20	7.5	1.58(15/9.50)	1.72	15	100	0.03
Pf 4	0.67	80	12.9	1.44(40/27.87)	1.92	44	90.9	0.0000813
Pv 1	0.97	53	45.6	0.07(2/27)	0.049	50	4	1.00E-17
Pv 2	0.87	45	42.5	0.00(0/22.1)	0	41	0	1.00E-17
Pv 3	0.62	79	17.4	1.67(37/22.1)	2.33	41	90.2	0.00000218

Shown in table 2: the 1st column shows the most likely clusters around any malaria cases (Any), *P.falciparum* cases and *P. vivax* malaria cases, the 2nd column show radius of the cluster in kilometer, 3rd column show total number of households in the cluster, 4th column show the Log likelihood ratio of having or not having malaria in the cluster, 5th column show the ratio of observed malaria cases to expected malaria cases in the cluster, 6th column show the relative risk of malaria in the cluster, 7th column show the size of the total population in the cluster, 8th column show the prevalence of malaria in the cluster, 9th column show the p-value.

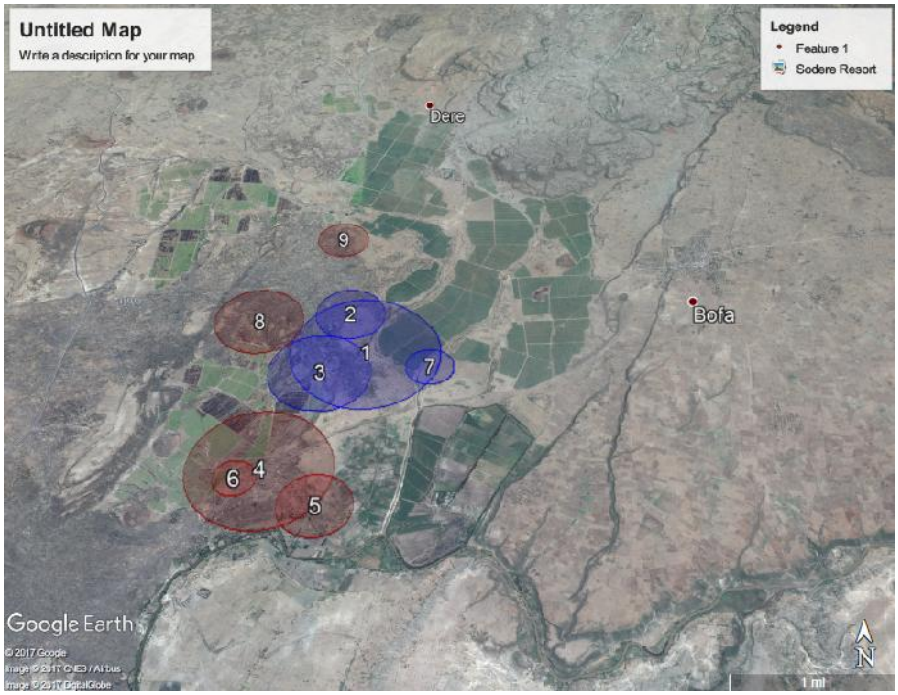


Figure 7 Clustering around any malaria cases.

The blue and red circles represent the most likely significant clusters around any malaria cases. The red circles represent hot-spot clusters while the blue circles represent cold-spot clusters.

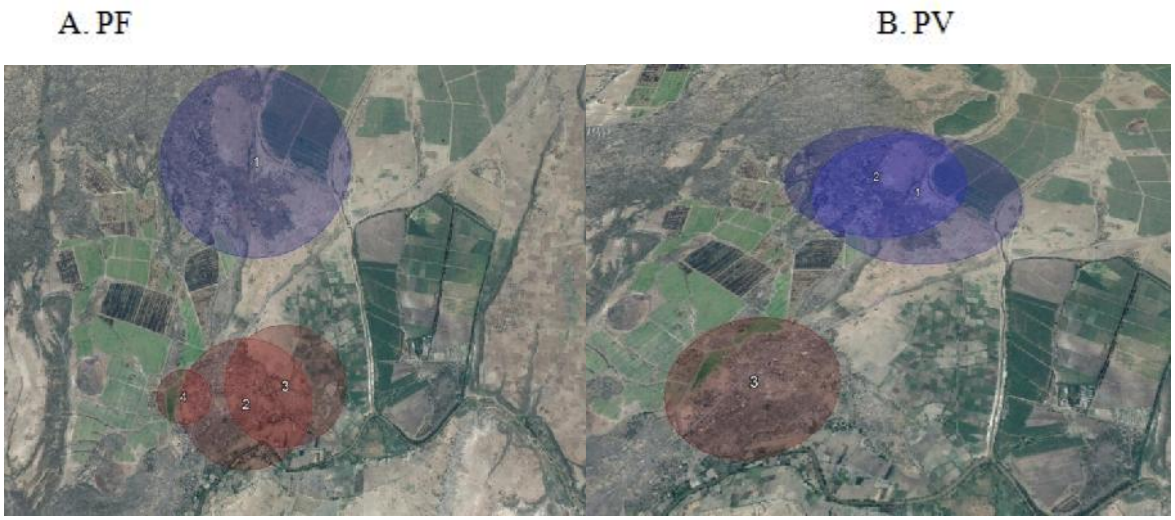


Figure 8 Hot-spots and cold-spots of *P. falciparum* (A) and *P. vivax* (B) malaria infections.

Most likely cluster around any *P.falciparum* and any *P.vivax* malaria cases respectively. The blue and red circles represent the most likely significant cold-spot and hot-spot clusters around any *p.falciparum* and *p.vivax* malaria cases respectively. The red circles represent hot-spot clusters while the blue circles represent cold-spot clusters.

5.4 Risk factors between HHs within high and low risk areas

Households were classified into two based on their location within clusters of malaria infected areas (HHs within risk) or outside of risk areas (HHs outside risk). Individuals who lived in HHs within risk areas were more likely to had previous malaria episodes (reported history of previous malaria, 33.1%, 177/233) compared to individuals in HHs outside risk areas (1.5%, 3/203; OR, 32.9; 95% CI, 10.2 – 106.3; Table 3). People in risk areas utilize malaria control interventions better than people in HHs outside of risk areas. The HHs in risk areas were characterized by iron sheet roof than thatch, opening in the eaves, and have better facilities such as television, radio and electricity. People who lived in HHs at risk areas walk in the night, enter their houses late and leave their houses early than people who lived in risk free areas.

Table 3 Factors associated with residing in a cluster of higher malaria incidence (any malaria species)

CHARACTERISTICS	HH outside risk, %(n/N)	HH within risk, %(n/N)	Total, % (n/N)	OR(95%, CI)	P-value
History of malaria in the last three months	1.5(3/203)	33.1(77/233)	18.4(80/436)	32.9(10.2,106.3)	<0.001
Intervention utilization					
Ownership of bed nets	9.8(18/184)	62.8(140/223)	38.8(158/407)	15.6(8.9,27.2)	<0.001
Bed net use last night	3.5(7/201)	48.2(105/218)	26.7(112/419)	25.8(11.6,57.3)	<0.001
Bed net/person <1, median (IQR)					
Insecticide residual spraying	10.1(20/199)	36.2(80/221)	23.8(100/420)	5.1(3.0,8.7)	<0.001
Fever (temperature ≥37.5 ^{o}C)					
Water bodies					
Water bodies within 15 minutes walk	1.5(3/201)	11.6(26/224)	6.8(29/425)		
Water bodies above 20 minutes walk	98.5(198/201)	88.4(198/224)	93.2(396/425)	0.12(0.03,0.39)	<0.001
Distance to health facility					<0.001
Within 5 minutes	33.8(68/201)	55.8(129/231)	45.6(197/432)		
Between 10 and 30 minutes	66.2(133/201)	10.4(24/231)	36.3(157/432)		
Above 30 minutes	0(0/201)	33.8(78/231)	18.1(78/432)		
Roof type					
Thatch	97.5(197/202)	66.5(155/233)	80.9(352/435)		
Iron sheet	2.5(5/202)	33.5(78/233)	19.1(83/435)	19.8(7.8,50.2)	<0.001
Wall Type					
Wooden plastered with mud /clay	100.0(203/203)	87.8(204/234)	93.1(407/437)		<0.001

Others	0(0/203)	12.8(30/234)	6.9(30/437)		
Floor Type					<0.001
Earth/Local dung	0(0/202)	11.5(27/234)	6.2(27/436)		
Cement	100.0(202/202)	88.5(207/234)	6.0(26/436)		
No eave opening	100.0(201/201)	84.6(197/233)	6.0(26/436)		<0.001
Facilities					
Electricity	0(0/202)	39.6(89/225)	20.8(89/427)		<0.001
Radio	4.4(9/203)	15.7(34/217)	10.2(43/420)	4.0(1.9,8.6)	<0.001
Television	0(0/202)	11.9(26/218)	6.2(26/420)		<0.001
Time					
Night trips	0(0/199)	3.8(8/209)	2.0(8/408)		0.005
Late entry	0(0/202)	2.4(5/206)	1.2(5/408)		0.026
Late sleeping	0(0/202)	5.6(13/232)	3.0(13/434)		0.001
Early wakeup	2.4(5/206)	1.7(4/234)	2.1(9/440)	1.4(0.4,5.4)	0.596
Early leaving	0(0/167)	2.7(5/187)	1.4(5/354)		0.033

6. Discussion

In the current study the clustering of malaria infections and associated risk factors around self-presenting RDT-detected *P. falciparum* and *P. vivax* mono and mixed species infected patients was evaluated compared to controls that presented at the health facilities for cases not related with malaria. Four hot spots and two cold spots of any malaria species were detected. Community members who lived in risk areas had 32.9 fold higher self-reported episodes of malaria in the previous three months. Individuals in HHs with RDT-detected *P. falciparum* and *P. vivax* infections were 28.8 and 5.0 fold more likely to have qPCR detected infections compared to individuals who lived in HHs without RDT detected infection, respectively. RDT correctly identified 47.4% of the HHs with qPCR detected infections. Closer vicinity to water bodies, farther distance from health posts, eave openings, and outdoor activities characterd the community in risk areas. Age was found to be a strong predictor of parasite carriage and density of infections, with children under 15 years carrying the majority of the malaria burden.

Current malaria infections (mainly *P. falciparum*) detected by RDT and 18S based qPCR and self-reported history of malaria in the last three months significantly higher around index cases compared to controls. Differences in human host factors such as red blood cell polymorphisms (Shekalaghe *et al*., 2009) and differential host attractiveness to *Anopheles* mosquitoes (de Boer *et al*., 2017) could partly explain the differences observed between communities. However, in a genetically homogenous area variation in exposure to infected mosquito bites is more likely to play significant role (Bousema *et al*., 2010b). Individuals bitten most often are more likely to get infected and maintain onward transmission to the nearby population during the subsequent bites (Smith *et al*., 2007). A more favorable environment for mosquito breeding, the presence of water bodies, in closer vicinity was detected in the present study around index cases in addition to more eave openings reflecting

micro-heterogeneity in risk factors (Stresman *et al*., 2010). In addition, reduced treatment seeking behavior in the community around index cases that is attributable to their farther location from the health posts, that could delay timely treatment, plays substantial role for the disproportionate risk of malaria in the specific community (Bousema *et al*., 2016b, Hustedt *et al*., 2016).

Indicators of higher socioeconomic status which are commonly linked with reduced risk of malaria (Deressa *et al*., 2007) existed around index cases; living in houses with iron sheet roof compared to thatched and better HH facilities (such as electricity, TV and radio). However, most of the HHs around index cases had eave openings that allow mosquitoes to enter and leave the houses easily. Although not evaluated in the current study, ownership of domestic animals such as cows, another indication of wealth status, is associated with increased rates of *Anopheline* house entry (Ghebreyesus *et al*., 2000b). The majority of the thatched houses in the study area are single room and used for the combined purposes of cooking with fire and sleeping. Domestic fire (mainly for cooking) repels mosquitoes from homes (Bockarie *et al*., 1994, Organization, 2008).

Furthermore, better malaria control intervention utilization characterized the community in risk areas in the current study. Due to the better access of electricity, radio and television and closer location to the major pavement road the community members around index cases might get adequate information and this may help them to make informed decision regarding utilization of intervention tools and they also become easily accessible during intervention deployment such as during times of spraying. In spite of better utilization of malaria control packages the community around index cases was still more vulnerable to malaria infections. this might be because in addition to their closer vicinity to water bodies and presence of eaves in their HHs the community around index cases are engaged in farming activities that need them walk in the night, enter their houses late and leave their houses early morning indicating

that outdoor biting might play significant role in the vicinity together with the possible presence of higher infectious reservoir from which the mosquitoes get infected in their prior blood feed. Increased investment and wider utilization of indoor intervention tools that contributed substantially to the malaria control strategies in recent decades is currently challenged by the increased insecticide resistance in *Anopheles* mosquitoes (Yewhalaw *et al*., 2011, Taye *et al*., 2016) and increased outdoor malaria transmission (Russell *et al*., 2011). In order to achieve/accelerate malaria elimination outdoor interventions need to be incorporated into integrated vector management especially in village setting with clustered houses (Zhu *et al*., 2017).

The majority of infections of both species (more than three-quarter) were detected in children younger than 15 years (Rogier and Trape, 1993, Mwangi *et al*., 2005) and age was found as a strong predictor of density of infection, with children younger than 15 years carrying higher density infections. A notable decrease in duration, prevalence and density of infection with increasing age was observed in similar studies (Baliraine *et al*., 2009b) which might be due to gradual acquisition of immunity through reinfection (Bereczky *et al*., 2007). Immune responses suppress parasitemia, or even clinical disease, in proportion to immune levels, which are a function of both age and level of exposure to infections (Bereczky *et al*., 2007). Furthermore, parasite densities in RDT-detected infections in symptomatic individuals were higher compared to RDT-detected and RDT-undetectable asymptomatic infections.

qPCR detected ~3-fold higher infections than RDT and infection was detected in 27.9% HHs by RDT and 57.1% HHs by qPCR. Family members who live in HHs with ≥1 RDT-confirmed *P. falciparum* and *P. vivax* infection were 28.8 and 5.5 fold at risk to develop qPCR-confirmed *P. falciparum* and *P. vivax* infection compared to HHs without RDT-detected infection, respectively consistent for example with a study from Swaziland (Sturrock

et al., 2013). This finally resulted in detection of four high malaria incidence hot spots (HHs at risk) and three malaria free clusters (HHs outside risk). Malaria infections are known to cluster at the HH level and it has been shown that asymptomatic parasite carriers are more likely to reside in HHs when a symptomatic case occurs in the same household (Baliraine *et al*., 2009a, Stresman *et al*., 2010, Bousema *et al*., 2013b). Reported malaria, eave opening, time of entry, going to bed and leaving houses, intervention utilization, distance to health post were all higher in the community at risk area compared to community outside risk area similar to the differences observed between members around index and control cases. This indicates that RACD precisely indicates the risk factors and clusters of malaria. The clusters of higher malaria incidence could be explained by closer vicinity to water bodies—that is, a higher likelihood that individuals whose HHs were located near water bodies were at higher risk of malaria (Oesterholt *et al*., 2006, Clark *et al*., 2008, Rosas-Aguirre *et al*., 2015b). Variations in risk factors over a small area could result in spatially heterogeneous transmission and result in malaria hotspots, where transmission intensity is higher than its surrounding (Haque *et al*., 2011) (Tuyishimire *et al*., 2016) and might be due to the presence of reservoir of the infection which perpetuate the transmission to HH members and their neighbors. These malaria hotspots may be present in all malaria endemic areas but are most readily identifiable in areas of low transmission intensity, where malaria incidence, parasite prevalence, and mosquito exposure may be higher inside hotspots owing to variations in risk factors at micro-epidemiological scale (Bousema *et al*., 2016a).

In the present study, RDT-predicted most of the infections at HH level, which make it a considerable potential tool to guide targeted intervention of hotspots. Furthermore, clusters of PCR-detected parasite prevalence can be accurate predictors of future infections (Mosha *et al*., 2014b). Identification and targeting of hotspots of malaria infections through both passive and active methods of case detection accelerates the stride towards successful malaria control and elimination. In addition, as hotspots might fuel malaria transmission in larger regions, it

is conceivable that a targeted intervention to eliminate hotspots could lead to a greater reduction in malaria transmission in areas surrounding the hotspots. Newly acquired malaria infections commonly lead to fever, even in the partially immune individuals. If the majority of asymptomatic infections are preceded by a symptomatic phase, enhanced approaches to case management to maximize accessibility of diagnosis and care may abrogate infections early on and, potentially, before individuals become infectious.

7. CONCLUSION

Despite a significant decline in malaria cases nationally the prevalence of malaria was higher in the study district. The distribution of malaria in the district was heterogeneous and clustered around index cases. Self-reported, RDT and qPCR-detected malaria were significantly more prevalent around index cases than around the control population. In HHs with RDT-positive individuals the presence of asymptomatic infection in the HH was significantly higher. Significant association was detected between age and malaria positivity and parasite density. Closer vicinity to water bodies, farther distance to health facility, household structural features, the presence of open eave and night time outdoor activity were significantly associated risk factors in the community around index cases.

8. Limitation of the study

The limitation of this study was a cross-sectional study always assesses the factor at a point in time but does not enable to see spatial-temporal association between different factors. Future studies might benefit from investigating the stability (dynamics) of hot and cold spots of infections over time. If other measures of clustering of infections, such as serological markers, were included the study would have added information. In another study serological markers of exposure to malaria showed a tight correlation with malaria incidence and predicted transmission hot spots with high precision (Bousema *et al*., 2010a). Investigation of the genetic network of the circulating clones might also add more information on the sources of infection at the level lower than clusters.

9. Recommendations

I recommend:

Tracking health post positive malaria cases to their household

Incorporate molecular methods (PCR) in the RACD of malaria.

The Provision of Health education especially to mothers and school children's regarding the prevention, importance of health seeking behavior, proper utilization of prevention tools and the impacts of malaria.

Intervention to be deployed around the hotspots of malaria

Further investigation should be carried out on spatial-temporal clustering of malaria in order to explore the distribution pattern deeply.

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11. Annexes

11.1 informed consent

Parental/guardian information and consent form for children between 6months -11 years

Parental/guardian information

Assessing the distribution of malaria in low endemic settings in Ethiopia

1) Purpose of the study

Ethiopia has enjoyed a remarkable decrease in malaria incidence and mortality in the last decade. The distribution of malarias was heterogeneous and can vary between villages and households. These patterns of malaria indicate a composite of heterogeneity in vector distribution, human-vector contact, and human host factors. Identified risk factor for malaria heterogeneous distribution include distance to mosquito breeding sites, household construction, household crowding, personal protection measures against mosquito biting and these factors intern influenced by differences in environmental landscape, and socio-economic status. In this study, we determine the distribution of malaria and associated risk factor for clustered distribution of malaria. Thus, this study assessed the distribution pattern of malaria in Ethiopia with low heterogeneous malaria transmission setting.

This study provides highly relevant information for national malaria control and elimination efforts; finding was likely to be of value outside Ethiopia. Every study comes with a discomfort for participants. We had done our best to minimize this discomfort and all procedures were adhered to Good Clinical Practice and the Declaration of Helsinki.

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

2) Study procedures

This information sheet was delivered by one of the members of the study team. 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.

We enrolled people who are member of the household of index case of malaria diagnosed at health center. We asked people who fulfill our enrolment criteria to donate a single finger

prick and a total of 2-3 drops of blood. Informed written consent was obtained from study participants by one of the members of the study team. Blood samples was collected by nurses.

3) Voluntary participation

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

4) Discomforts and Risks

Your child might feel a small amount of discomfort during blood sampling and your child may had a small amount of bruising or bleeding where the blood sample was taken. This is considered not to be harmful. We used sterile equipment to collect the blood sample and the small wound that may arise from the procedure was treated adequately. The volume of blood was too small to influence your Childs health and the blood was quickly replaced by your Childs body.

5) Benefits

If your child was diagnosed with clinical malaria, the children receive treatment free of charge. Cases that require special attention was linked to the nearby health service offering institution. You or your child was not be paid for participation in this study

6) Confidentiality statement

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

7) Long term storage of samples

We asked your consent for long term storage of your child's samples. New techniques may become available to study the research questions we want to answer in this study. We make your child's samples anonymous by removing the name and any identifying information. Samples may be stored at any of the collaborating institutions in Ethiopia. We only test for factors related to malaria parasite distribution and its associated risk factor. If further studies was conducted on stored study material, ethics approval would be sought.

8) Freedom to ask questions

If you or your child had any question concerning this study, do not hesitate to contact the investigator of the study, Mulualem Belachew Shuba, Medical Biochemistry department, school of medicine, Addis Ababa University, Addis Ababa, Ethiopia. Tel +251(0)920173791. Results from the study will be communicated to your community. In case you want to contact an independent person, not related to the study, about the research study itself, you or your child's rights as a research subject or any research-related injury, you can contact the secretariat of the Ethics Committee at and ALERT/AHRI (0118-962183).

Parental/guardian Informed consent form for children between 2-11 years

Assessing the distribution of malaria in low endemic settings in Ethiopia

I, _____ (Parent's/guardian's Name), having full capacity to, do hereby consent to participation of my child _____ (Child's name) in the research study entitled "Assessing the distribution of malaria in low endemic settings in Ethiopia", under the principal investigator Mulualem Belachew Shuba. The implications of my voluntary participation, the nature, duration and purpose; methods and means by which it is to be conducted; and the inconveniences and hazards which may reasonably be expected have been explained to me by _____, and are set forth in the Informed Consent Explanation. I have been given an opportunity to ask questions concerning this investigational study, and such questions have been answered to my full and complete satisfaction. If there are further questions that may arise, I may contact Mulualem Belachew Shuba (at +251920173791) or the secretariat of the Ethics Committee at ALERT and AHRI (0118-962183), an independent one. I understand that I may at any time, during the course of this study, revoke my consent and withdraw my child from the study without prejudice; however, my child may be requested to undergo further examinations if, in the opinion of the physician, such examinations are necessary for his or her wellbeing.

I **acknowledge** / **do not acknowledge** the receipt of explanation on this Informed Consent Form (circle)

I **understand** / **do not understand** the practical consequences of this study, asking my child to give a single prick blood sample (circle)

I **approve** / **disapprove** part of the sample to be analyzed outside Ethiopia (circle)

I **approve** / **disapprove** part of the sample to be stored for future analyses (circle). If studies are conducted using stored study material, approval from ethics committees will be sought.

I **agree** / **disagree** to be interviewed for the questionnaire (circle)

I **agree** / **disagree** to let my child participate in this study (circle)

Thumbprint if subject is unable to sign

Participant's name: _____

Parent's / guardian's name: _____

Parent's / guardian's signature: _____

Date: _____

Impartial witness's name: _____

Impartial witness's signature: _____ Date: _____

Local investigator's name: _____

Local investigator's signature: _____ Date: _____

Parental/guardian Informed consent form for children between 12-18 years

Participant information

Assessing the distribution of malaria in low endemic settings in Ethiopia

1) Purpose of the study

Ethiopia has enjoyed a remarkable decrease in malaria incidence and mortality in the last decade. The distribution of malarias is heterogeneous and can vary between villages and households. These patterns of malaria indicate a composite of heterogeneity in vector distribution, human-vector contact, and human host factors. Identified risk factor for malaria heterogeneous distribution include distance to mosquito breeding sites, household construction, household crowding, personal protection measures against mosquito biting and these factors intern influenced by differences in environmental landscape, and socio-economic status. In this study, we want to determine the distribution of malaria and associated risk factor for clustered distribution of malaria. For this, we perform studies in different areas in Ethiopia. Thus, this study aims to assess the distribution pattern of malaria in Ethiopia with low heterogeneous malaria transmission setting

This study provides highly relevant information for national malaria control and elimination efforts; findings are likely to be of value outside Ethiopia. Every study comes with a discomfort for participants. We have done our best to minimize this discomfort and all procedures will adhere to Good Clinical Practice and the Declaration of Helsinki.

We therefore ask your permission to allow us collect finger prick blood samples from you. These samples will only be used for future research related to the distribution 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.

2) Study procedures

This information sheet will be delivered by one of the members of the study team. 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. Your child will be asked to donate a small finger prick blood sample that we will use to determine malaria status. We are aiming to enroll people who are member of the household of index case of malaria diagnosed at health center. We will ask people who fulfill our enrolment criteria to donate a single finger prick and a total of 2-3 drops of blood.

Informed written consent will be obtained from study participants by one of the members of the study team. Blood samples will be collected by nurses.

3) Voluntary participation

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

4) Discomforts and Risks

You might feel a small amount of discomfort during blood sampling and you may have a small amount of bruising or bleeding where the blood sample was taken. The repeated blood sampling may cause a discomfort and pain. 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 health and the blood will quickly be replaced by your body.

5) Benefits

If you are diagnosed with clinical malaria, you will receive treatment free of charge. Cases that require special attention will be linked to the nearby health service offering institution. You will not be paid for participation in this study.

6) Confidentiality statement

The records concerning your participation are to be used only for the purpose of this research project. Your 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 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 name with your study number.

7) Long term storage of samples

We will ask your consent for long term storage of your samples. New techniques may become available to study the research questions we want to answer in this study. We will make your samples anonymous by removing the name and any identifying information. Samples may be stored at any of the collaborating institutions in Ethiopia If further studies are conducted on stored study material, ethics approval will be sought.

8) Freedom to ask questions

If you have any question concerning this study, do not hesitate to contact the investigator of the study Mulualem Belachew Shuba, Medical Biochemistry department, school of medicine, Addis Ababa University, Addis Ababa, Ethiopia. Tel +251(0)920173791,. Results from the study will be communicated to your community. In case you want to contact an independent person, not related to the study, about the research study itself, your rights as a research subject or any research-related injury, you can contact the secretariat of the AHRI Ethics Review Committee (0118-962183).

Informed assent form for children between 12-17

Assessing the human infectious reservoir for malaria in low endemic settings in Ethiopia

I, _____ (PARTICIPANT'S NAME), having full capacity to, do hereby consent to my participation in the research study entitled "Assessing the distribution of malaria in low endemic settings in Ethiopia", under the principal investigator Mulualem Belachew Shuba. The implications of my voluntary participation, the nature, duration and purpose; methods and means by which it is to be conducted; and the inconveniences and hazards which may reasonably be expected have been explained to me by _____, and are set forth in the Informed Consent Explanation. I have been given an opportunity to ask questions concerning this investigational study, and such questions have been answered to my full and complete satisfaction. If there are further questions that may arise, I may contact Mulualem Belachew Shuba (at +251920173791) or the secretariat of the Ethics Committee at AHRI (0118-962183), an independent one. I understand that I may at any time during the course of this study revoke my consent and withdraw myself from the study without prejudice; however, I may be requested to undergo further examinations if, in the opinion of the physician, such examinations are necessary for my wellbeing.

I **acknowledge** / **do not acknowledge** the receipt of explanation on this Informed Consent Form (circle)

I **understand** / **do not understand** the practical consequences of this study, asking me to give a single prick blood.(circle)

I **approve** / **disapprove** part of the sample to be analysed outside Ethiopia (circle).

I **approve** / **disapprove** part of the sample to be stored for future analyses (circle). If studies are conducted using stored study material, approval from ethics committees will be sought.

I **agree** / **disagree** to be interviewed for the questionnaire (circle)

I **agree** / **disagree** to take part in this study (circle)

Thumbprint if subject is unable to 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: _____

Participant information and consent form for adults (above 18 years)

Participant information

Assessing the distribution of malaria in low endemic settings in Ethiopia

1) Purpose of the study

Ethiopia has enjoyed a remarkable decrease in malaria incidence and mortality in the last decade. The distribution of malarias is heterogeneous and can vary between villages and households. These patterns of malaria indicate a composite of heterogeneity in vector distribution, human-vector contact, and human host factors. Identified risk factor for malaria heterogeneous distribution include distance to mosquito breeding sites, household construction, household crowding, personal protection measures against mosquito biting and these factors intern influenced by differences in environmental landscape, and socio-economic status. In this study, we want to determine the distribution of malaria and associated risk factor for clustered distribution of malaria. For this, we perform studies in different areas in Ethiopia. Thus, this study aims to assess the distribution of malaria and associated risk factors.

This study provides highly relevant information for national malaria control and elimination efforts; findings are likely to be of value outside Ethiopia as well to better understand the relevance of low density infections for malaria transmission. Every study comes with a discomfort for participants. We have done our best to minimize this discomfort and all procedures will adhere to Good Clinical Practice and the Declaration of Helsinki.

We therefore ask your permission to allow us collect finger prick blood samples from you. These samples will only be used for future 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.

2) Study procedures

This information sheet will be delivered by one of the members of the study team. 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 will be asked to donate a small finger prick blood sample that we will use to determine malaria status. We are aiming to enroll people who are member of the household of index case of malaria diagnosed at health center. We will ask people who fulfill our enrolment criteria to donate a single finger prick and a total of 2-3 drops of blood.. Informed written consent will be obtained from study participants by one of the members of the study team.

Blood samples will be collected by female and male nurses from men and women, respectively.

3) Voluntary participation

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

4) Discomforts and Risks

You might feel a small amount of discomfort during blood sampling and you may have a small amount of bruising or bleeding where the blood sample was taken. The repeated blood sampling may cause a discomfort and pain. 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 health and the blood will quickly be replaced by your body.

5) Benefits

If you are diagnosed with clinical malaria at any point during the follow-up, you will receive treatment free of charge. Cases that require special attention will be linked to the nearby health service offering institution. You will not be paid for participation in this study.

6) Confidentiality statement

The records concerning your participation are to be used only for the purpose of this research project. Your 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 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 name with your study number.

7) Long term storage of samples

We will ask your consent for long term storage of your samples. New techniques may become available to study the research questions we want to answer in this study. We will make your samples anonymous by removing the name and any identifying information. Samples may be stored at any of the collaborating institutions in Ethiopia we will only test for the presence of malaria. If further studies are conducted on stored study material, ethics approval will be sought.

8) Freedom to ask questions

If you have any question concerning this study, do not hesitate to contact the investigator of the study Mulualem Belachew Shuba, Medical Biochemistry department, school of medicine, Addis Ababa University, Addis Ababa, Ethiopia. Tel +251(0)920173791. Results from the study will be communicated to your community. In case you want to contact an independent person, not related to the study, about the research study itself, your rights as a research subject or any research-related injury, you can contact the secretariat of the Ethics AHRI Ethics Review Committee (0118-962183).

Informed consent form for adults (above 18 years)

Assessing the distribution of malaria in low endemic settings in Ethiopia

I, _____ (PARTICIPANT'S NAME), having full capacity to, do hereby consent to my participation in the research study entitled "Assessing the distribution of malaria in low endemic settings in Ethiopia", under the principal investigator Mulualem Belachew Shuba. The implications of my voluntary participation, the nature, duration and purpose; methods and means by which it is to be conducted; and the inconveniences and hazards which may reasonably be expected have been explained to me by _____, and are set forth in the Informed Consent Explanation. I have been given an opportunity to ask questions concerning this investigational study, and such questions have been answered to my full and complete satisfaction. If there are further questions that may arise, I may contact Mulualem Belachew Shuba (at +251920173791) or the secretariat of the Ethics Committee at AHRI (0118-962183), an independent one. I understand that I may at any time during the course of this study revoke my consent and withdraw myself from the study without prejudice; however, I may be requested to undergo further examinations if, in the opinion of the physician, such examinations are necessary for my wellbeing.

I **acknowledge** / **do not acknowledge** the receipt of explanation on this Informed Consent Form (circle)

I **understand** / **do not understand** the practical consequences of this study, asking me to give single prick blood sample (circle)

I **approve** / **disapprove** part of the sample to be analysed outside Ethiopia (circle).

I **approve** / **disapprove** part of the sample to be stored for future analyses (circle). If studies are conducted using stored study material, approval from ethics committees will be sought.

I **agree** / **disagree** to be interviewed for the questionnaire (circle)

I **agree** / **disagree** to take part in this study (circle)

Thumbprint if subject is unable to 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: _____

11.2 Questionnaire-based interview

Title of the study: Assessing the distribution of malaria in low endemic settings in Ethiopia

This questionnaire-based interview will be collected on paper by the interviewers. The interviewer begins by introducing himself and the purpose of the study to the participants, requesting the respondent's for participation in the survey, thanking the participant for giving consent to take part in the study.

Date: __ __/__ __/__ __ __ __ (dd/mm/yyyy)

1. Participant's name: _____
2. Participant's code : __ __/__ __ __ __/ __ __ __
3. Participant's telephone number: __ __ __ __ __ __ __ __ __ __
4. Date of Birth: __ __/__ __/__ __ __ __(dd/mm/yyyy)
5. Age (in years)_____years
6. Gender:
   a. Male b. Female
7. Wereda (District) name:_____
8. Name of town or village: _____
9. Household number:_____
10. What is the nearest health facility to your house?
    a. Hospital c. Clinic
    b. Health center d. Health post
11. How far is the nearest health institution located (time it takes, in minutes, to arrive there with the available transportation)?_____minutes
12. Have you (has the child) had malaria in the last three weeks?
    a. Yes b. No c. Don't know
13. If yes, when exactly?_____
14. Was treatment given?
    a. Yes, name of the drug if known_____
    b. No
    c. Don't know
15. Parent/guardian name (for children <18 years old): _____
16. What kind of water body exists in your neighborhood? (you can give more than one answer)
    a. None c. Lake e. Swamp
    b. River d. Pond f. Stagnant water
    g. Other (Specify) _____
17. How far (in minutes) is the nearest water body located from your house on walking? _____(minutes)
18. Does your household have any of the following?
    a. Electricity:
    i. Yes ii. No iii. Don't know
    b. Radio:
    i. Yes ii. No iii. Don't know
    c. Television:
    i. Yes ii. No iii. Don't know
19. What type of roof was used for the construction of the house?
    a. Grass thatch c. Wood and mud
    b. Iron sheet d. Other_____

20. What type of wall was used for the construction of the house?
    a. Wooden plastered with Clay or mud
    b. Mud with cement plastering
    c. Iron sheets
    d. Brick or stone
    e. Other_____
21. What is the main material of the house's Floor?
    a. Earth
    b. Local dung plaster
    c. Cement
    d. Wood
    e. Other_____
22. Are the eaves open or closed?
    A. Open
    b. Closed
    c. Partially open
    d. No eaves/not applicable
23. Do you own a bed net for yourself (the child in the study)?
    a. Yes b. No c. Don't know
24. Did you use the bed net (for the child in the study) during the last night?
    a. Yes b. No c. Don't know
25. How many people permanently live in the house? _____
26. How many bed nets are available in the house?_____
27. At any time in the past 12 months, have the interior walls of the house been sprayed against mosquitoes?
    a. Yes b. No c. Don't know
28. How many months ago was the house sprayed? (If less than one month, record '00' months ago)? _____
29. Have you (your child) made any overnight trips outside of (the DISTRICT) in the last 3 months?
    a. Yes b. No c. Don't know
30. If yes, where did you (your child) spend the most time during this overnight trip and when exactly? _____
31. What time do you (does the child) enter the house usually in the night?_____
32. What time did you (the child) enter the sleeping space last night? _____
33. What time do you (the child) leave the sleeping space in the morning? _____
34. What time do you (the child) leave the house in the morning?_____
35. Temperature (^{o}C):_____
    If ≥37.5^{o}C carry out RDT
    If <37.5 ^{o}C skip to Hb results
36. RDT taken?
    a. Yes b. No
37. If not, why not?_____
38. RDT results
    a. Positive b. Negative c. Invalid

If positive, refer to the nearest health institution for treatment to be given

39. Hb taken?
    a. Yes b. No
40. If not, why not?_____
41. Hb result: _____

If < 8 g/dL, refer to the nearest health institution for treatment to be given

42. Microscope slide film taken?
    a. Yes b. No

43. If not, why not?_____

44. Filter paper taken?

a. Yes
b. No

47. If not, why not?_____

Afan Oromo version of questionnaire-based interview

Gaafannoo Yaada Guuruf Qopaha'e

Mataduree Qoranichaa: Teesuma Lafaa Itoophiya iddoo tamsa'inni dhiben bussaa gad'aanaa ta'etti, dandeettii dhibee busaa dabarsuuu danda'uu namoota qaama isaaanii keessatti maxantuu dhibee busaa gad'anaa qabaatanii mallattolee busaa hin agarsiifnee hubachuudha.

Odeeffanoon kuni meeshaalee eleektiroonikaa gargaaramuun sassaabama. Namni odeeffanoo kana sassaabus mataa isaa fi kaayyoo qoranichaa ibsuun hirmaattoni qoranichas yaada isaanii fedhiiniin waan kennaniif dursee galateeffachuun eegala.

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

1. Maqaa Hirmaataa: _____
2. Koodii Hirmaataa : __ __/__ __ __ __/ __ __ __
3. Lakk. Bilbila Hirmaataa: __ __ __ __ __ __ __ __ __ __
4. Guyyaa dhalootaa: __ __/__ __/__ __ __ __
5. Umurii (waggaan)_____
6. Saala:
   a. Dhiira b. Dhalaa
7. Aanaa:_____
8. Maqaa Magaalaa ykn gandaa: _____
9. Lakk. Manaa:_____
10. Manni yaalaa mana keessanitti dhiyeenyan argamu kami?
    a. Hospitaala c. Kiliinika fayyaa
    b. Buufata fayyaa d. Kellaa Fayyaa
11. Mana yaalaa dhiyootti argamu ga'uuf yeroo hangam (daqiiqaan) fudhata? Daqiiqaa_____
12. Torban sadan darban kana keessa isin ykn daa'imman keessan dhibee busaa dhukkubsattaii/dhubsatanii beektu?
    a. Eeyyee b. Lakki c. Hin beeku
13. Yoo deebi'iin keessan eeyee ta'e, yoomi laata? _____
14. Yaalii argattaniittuu?
    a. Eeyyee yoo jettan, maqaa daawwaa yoo beektan_____
    b. Lakki
    c. Hin beeku
15. Maqaa maatii/Guddistuu (ijollee umurii waggaa 18 gad ta'aniif): _____
16. Mana keessaniitti dhiyeenyaan qaamoleen bishaanii argamu kami?(deebi'ii tokkoo ol kenuun ni danda'ama)
    a. Hinjiru
    b. Laga
    c. Garba bishanii
    d. Burqaa/yaatuu
    e. Chaffee
    f. Bishaan chiisaa

g. Kan biro (Haa ibsamu) _____

17. Bishaan mana keessaniitti dhiyeenyaan argamu kana ga'uuf deemsa miillaa hangamii (daqiiqan) ta'a? Daqiiqaa _____
18. Manni keessan kanneen armaan gadii ni qabaataa?
    a. Ibsaa eleektiriikaa:
    i. Eeyyee ii. Lakki iii. Hin beeku
    b. Radiyooo:
    i. Eeyyee ii. Lakki iii. Hin beeku

    c. Televizninii:
    i. Eeyyee ii. Lakki iii. Hinbeeku

19. Baaxii manni keessanii maal irraa ijaareame?
    a. Chitaa
    b. Qorqoorroo
    c. Mukaa fi dhoqqee
    d. Kanbiroo(haa ibsamu)_____
20. Gidgiddaan mana maalirraa hojjetame?
    a. Muka dhoqeen maragame
    b. Simmintoon garafame
    c. Qorqoorroo
    d. Xuubii ykn dhagaa
    e. Kanbiroo(haa ibsamu)_____

21. Lafti mana keesanii mal irraa tolfame?
    a. Biyee
    b. Dhoqee looniin lolla'amaa
    c. Simintoo
    d. Muka
    e. Kanbiroo(haa ibsamu)_____

22. Baaxiin ykn xaaraan manaa gara bakkeetti banaa moo cufaadha?
    A. Banaadha
    b. Cufaadha
    c. Walakkan banaadha
23. Matta keessaniif ykn daa'imman keessaniif agoberii ni fayyadamtuu?
    a. Eeyyee b. Lakki c. Hin beeku
24. Kalessa galgala mataa keessaniif (daa'ima keessaniif) agobera siree fayyadamtaniittuu?
    a. Eeyyee b. Lakki c. Hin beeku
25. Mana kessan keessa dhaabbataan nama meeqatu jirata? _____
26. Agoobera siree meeqa qabdu? _____
27. Ji'oota 12n darban kana keessatti manni kessan qoricha farra bookee busaatiin biifamee beekaa?
    a. Eeyyee b. Lakki c. Hin beeku
28. Ji'oota meeqan dura manni keessan qoricha farra bookee busaan kan biifame?(Yoo ji'a tokkoo gadi ta'e ,jia '00' jechuun galmmessi) _____
29. Ji'oota 3n darban kana keessa isinis ykn ijoolleen keessan adeemsa halkanii taasiftanii/taasisanii beektuu?
    d. Eeyyee e. Lakki f. Hin beeku
30. Deebi'iin keessan eeyyee yoo ta'e, galgala yeeroo hedduu eessatti dabarsitu/dabarsu? _____
31. Isin ykn ijolleen keessan galgala yeroo baay'ee sa'a meeqatti manatti galtu/galu?_____

32. Isin ykn ijolleen keessan Kaleesa galgala sa'a meeqatti gara iddoo chiisaa deemtan/deeman? _____
33. Isin ykn ijolleen keessan yeroo baay'ee ganama sa'a meeqatti mana baatu/ba'u?_____
34. Isin ykn ijolleen keessan kaleessa ganama sa'a meeqatti manaa batan ykn ba'e/bate?
35. Ho'a qaamaa (^{o}C):_____

yoo ≥37.5^{o}C ol ta'e RDTn haa hojjetamu

yoo <37.5 ^{o}C gadi ta'e gara qorannaa Hb tti haadarbu/tu

36. RDTn fudhatameeraa?

c. Eeyyee d. Lakki

37. Yoo lakki ta'e, maaliif?_____
38. Bu'aan qoranaa RDT?

d. Poozetiivii e. Nagatiivii f. Gatii hinqabu

Yoo poozetiivii ta'e, gara buufata fayyaa dhihootti yaliif haa ergamu/ttu

39. Hbn hojjetameeraa?

c. Eeyyee d. Lakki

40. Yoo lakki ta'e, maaliif?_____
41. Bu'aan qorannoo Hb :_____

yoo 8 g/dL gadi ta'e Ireenii kenniif

42. Hujumoo maayikirootiin dhiigni fudhatameeraa?

a. Eeyee b. Lakki

43. Yoo lakki ta'e, maliif?_____
44. Isliidii maayikirooskoppii irratti dhiigni fudhatameeraa?

c. Eeyyee d. Lakki

45. Yoo lakki ta'e, maaliif?_____
46. Waraqaa cophsa dhiigaa irratti fudhatameeraa?

c. Eeyyee b. Lakki 47. Yoo

48. hinfudhatamne ta'e, maaliif? _____

Declaration by the candidate

I, Mulualem Belachew, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signed_____ date_____

(Mulualem Belachew)

Advisors:

1. Dr.ENDALAMAW GADISA

2. Dr.SOLOMON GENET

3. Mr. FITSUM GIRMA