Reactive Case Detection of Malaria in Selected Health Centers of Jimma Zone, Southwest Ethiopia

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- **Mr. Endalew Zemene**

Abstract

Background*: Malaria is a significant public health problem in Ethiopia with an estimated 75% of the land and 60% of the population being at risk of infection. In the last decade, malaria burden has dramatically decreased in several areas of the country, likely due to scale up of vector control interventions and early treatment approach. However, when malaria incidence decreased, residual transmission may occur even in areas with scaled up control interventions. Passive case detection (PCD) at health facility level alone may not have a significant impact in interruption of malaria transmission under such circumstances. The objective of this study was, therefore, to identify malaria cases and determine risk factors of Plasmodium infection among the study participants using Reactive Case Detection (RACD).*

Methods*: A cross-sectional study was conducted from July to November 2016 in Kishe and Nada Health Center catchment kebeles, Jimma Zone, Southwest Ethiopia. Initially malaria cases (index cases) were identified at Kishe and Nada Health Centers by PCD. Following detection of the index cases, household members and residents in close proximity (200 meter radius) to the index households were screened for malaria infection. Microscopy and multi-species rapid diagnostic test (RDT) were used to detect Plasmodium species. Moreover, household and individual-level risk factors associated with Plasmodium infection were collected using semi-structured questionnaire. Data were analyzed using STATA12MP-1.*

Results: *A total of 726 individuals were screened for malaria using RDTs and microscopy following 39 index cases. From these study participants, malaria c*ases *detected by microscopy were 29(3.99) (3 P. vivax and 26 P. falciparum) and using multispecies RDTs were 33(4.55%) (3 P. vivax and 30 P. falciparum). The detection of malaria cases is higher among age groups of less than five years as compared to ≥15 age groups (AOR=3.1; 95%CI=1.20 - 8.17; p < 0.05). Fever (AOR=17; 95%CI=5.28 - 56.92; p < 0.05) and malaria history in the last one year (AOR= 4.3; 95%CI=1.90 - 9.91; p < 0.05) had also strong association with detecting malaria cases in the RACD method. Moreover, the odds of detecting malaria cases was higher in non sprayed houses than sprayed one. Presence of eaves and proximity to the index house were also strongly associatioted with the detection of malaria cases (AOR=4.5; 95%CI=1.49 - 13.67; p < 0.05and AOR=5.17; 95%CI=1.37-19.48;p < 0.05), respectively.*

Conclusion*: Reactive case detection of malaria is promising method to detect additional malaria cases in the community, and identify reservoir infections and hotspots. Children under five years, fever, the previous one year malaria history, IRS status, distance from the index house and presence of eaves were associated with detecting additional malaria cases. The implementation of RACD could enhance malaria elimination program in Ethiopia but further studies are recommended.*

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List of Acronyms and Abbreviations

- ACD - Active Case Detection
- ASL- Above Sea Level
- FMoH - Federal Ministry of Health
- HEWs – Health Extension Workers
- HH - Household
- IRS - Indoor Residual Spraying
- ITNs - Insecticide Treated Nets
- LLINs – Long Lasting Insecticide-treated nets
- PACD - Proactive Case Detection
- PCD - Passive Case Detection
- PMI - President's Malaria Initiative
- RACD – Reactive Case Detection
- RDTs - Rapid Diagnostic tests
- WHO - World Health Organization

Chapter One

1. Introduction

1.1 Background

Malaria remains a significant public health problem in Ethiopia with an estimated 75% of the land and 60% of the population being at risk of infection. *Plasmodium falciparum* (*P. falciparum*) and *Plasmodium vivax* (*P. vivax*) are the main parasite species causing almost all cases of malaria, and less than 1% of the cases are accounted by *Plasmodium malariae* and *Plasmodium ovale* [1,2]. Malaria transmission varies in different altitudes across the country. The transmission is high up to an altitude of 2,000m and it is rare beyond 2,400m Above Sea Level (ASL). Risk of malaria infection and transmission intensity also depends on differences in climate, rain fall, topography and human settlement patterns. In most parts of the country the transmission increases from September to December, whereas, in some Eastern and Western parts, minor transmission also occurs from April to June [3,4].

Several species of anopheline mosquitoes are responsible for the transmission of malaria parasites. In Ethiopia, *Anopheles arabiensis,* which is a member of the *An. gambiae* complex, is the primary malaria vector, while, *An. pharoensis, An. funestus* and *An. Nili* are considered secondary vectors of malaria [5]. *Anopheles arabiensis* has a dynamic behavior which helps it to adapt/evade vector control interventions. It feeds blood meal alternatively from human and other animals as well rests indoor and outdoor. These allow evading malaria control interventions particularly, Indoor Residual Spraying (IRS) and Long Lasting Insecticide-treated Nets (LLINs) [6,7,8]. Moreover, multiple blood feeding pattern (from different human per gonotrophic cycle) of *An. Arabiensis* increases its capacity to transmit malaria parasite [9].

History of malaria control in Ethiopia dates back to more than half a century. In the 1950's malaria control began as a pilot project which was then launched as National Malaria Eradication Campaign followed by Malaria Control strategy. Then National Organization for the Control of Malaria and Other Vector-borne Diseases (NOCMVD) was established in 1976 to control malaria and other vector-borne

diseases. However, malaria became endemic in several places due to *Plasmodium* anti-malarial drug resistance and insecticide resistance by the vector mosquito [10]. Meanwhile, Roll Back Malaria took effect mainly targeting malaria risk groups; children and pregnant women [11]. Currently, the National Malaria Control Program is taking effort to scale up prevention and control interventions for malaria elimination program [1].

Malaria cases visiting public health facilities in Ethiopia have remarkably declined in the last decade. This is possibly due to scale up of vector control interventions and early diagnosis and treatment of malaria in the country [12,13,14]. Depending on these milestones of malaria control efforts, Ethiopia set goals to achieve malaria elimination in low transmission areas and near zero deaths in all malarious areas by 2020 and embarking on malaria elimination by 2030 with integrated community health approach [15,16]. With this goal of malaria elimination in the near future, the usual way of Passive Case Detection (PCD) at health facilities alone may not be sufficient, necessitating Active Case Detection (ACD) and prompt treatment [14].

In the case of ACD, individuals in defined population are screened for malaria and treated accordingly. Active case detection targets both symptomatic and asymptomatic infections which can be divided into Reactive Case Detection (RACD) and Proactive Case Detection (PACD). Proactive case detection involves screening and treating of population at high risk. It is best suited for moderate transmission settings and enables to target population with poor access to health facilities and screening of migrants at country boarders [17]. Reactive case detection targets individuals in close proximity to passively detected malaria cases. It is best suited for low-transmission settings during pre-elimination, elimination, and prevention of re-introduction of malaria [18]. This study is aimed to apply RACD to detect additional malaria cases in the community following of index cases identified at health facilities, and assess associated risk factors.

1.2 Statement of the Problem

The global burden of malaria is still alarming, causing an estimated 212 million cases and 429,000 deaths in 2015. The burden is particularly high in sub-Sahara African countries and Ethiopia is among the World Health Organization (WHO) African region with high burden of malaria [13]. The transmission dynamics of malaria in the country varies depending on geographical and climatic variables. The weather of the country is mainly influenced by tropical Indian Ocean conditions and global weather patterns including *El Nino* and *La Nina*, which may have effect on *Anopheles* mosquito proliferation [19,20].

Over the past decade, malaria burden has dramatically decreased in Ethiopia due to introduction of artemisinin-based combination therapy (ACT), accessibility of Rapid Diagnostic Tests (RDTs) at health post level and scale-up of vector-control interventions particularly, LLINs and IRS [3]. Historically, malaria outbreaks usually occur every five to seven years, but no major malaria outbreaks has been recorded in the last decade, and morbidity and mortality due to malaria also sharply decreased [1]. Depending on previous achievements, Ethiopia planned to sustain malaria control in endemic areas, and seek elimination in areas with low transmission settings [16].

In situations of low transmission settings, residual transmission of malaria is common even in areas with scaled up control interventions [21,22]. Residual malaria may occur in geographically hotspots or demographically hotpops with some groups sustaining malaria transmission throughout the year at higher rates than their surroundings [23]. These malaria cases may become symptomatic or remain asymptomatic. Asymptomatic individuals do not seek diagnosis and treatment; as a result, they serve as reservoirs for long period and sustain malaria transmission [24,25].

Diagnosis of malaria in Ethiopia depends on multispecies RDTs at health post level and microscopy at health center and hospital levels. The sources of malaria data are usually also these health facility reports, which is restricted to people seeking treatment. Nevertheless, remarkable proportion of asymptomatic malaria cases also documented in community based studies [26], including among school children and pregnant women [27,28]. Therefore, it is important to implement programs targeting both symptomatic

and asymptomatic malaria cases, and understanding epidemiology of asymptomatic reservoirs is essential as malaria elimination is top on the agenda.

Passive case detection, detecting individuals with malaria who present to health facilities, alone is unlikely to have an impact on interruption of malaria transmission. To overcome the limitations of passive case detection and target asymptomatic infections or individuals who do not seek treatment, it should be accompanied with ACD in moderate-to-low transmission settings [17].

As Ethiopia has plan of eliminating malaria from selected low transmission settings, active surveillance is likely to be utilized as additional tool together with the usual passive surveillance [16]. However, RACD was not implemented as a routine activity in these settings and similar studies were not obtained in Ethiopia. Therefore, the aim of this study was to determine the prevalence of malaria using RACD in Kishe and Nada Health Centers catchment *kebeles*.

1.3 Significance of the Study

For effective malaria control and elimination, it is important to target all symptomatic and asymptomatic malaria cases. Asymptomatic/subclinical and symptomatic reservoirs, who may be unable to seek treatment, can sustain malaria transmission for longer periods. These cases may sustain malaria transmission and, may result in outbreaks. Currently, mass screening and treatment at community level to address these reservoirs in malaria endemic areas may not be feasible due to possible limitations related to logistics in Ethiopia. This necessitates development of other strategies to complement the PCD in the country. The goal of this study was to determine prevalence of malaria among family members of index cases diagnosed at health centers and neighbors residing in close proximity to the index cases (index households). Moreover, individual and household-level risk factors associated with malaria were also assessed. The findings of the study will help policy makers to develop evidence based intervention strategies in targeting asymptomatic carriers of malaria in low transmission settings.

Chapter Two

2. Literature Review

2.1 Malaria situation in Ethiopia

In Ethiopia, malaria is mainly caused by two of the *Plasmodium* species, *P. falciparum* and *P. vivax,* with *Anopheles arabiensis* being the primary vector. [29]. According to Federal Ministry of Health (FMoH) 2014/2015 malaria report, malaria was significant parasitic disease to cause morbidity and mortality in endemic areas of the country. The report indicates total (clinical and confirmed) cases were 2,174,707 among which, laboratory confirmed cases of malaria (microscopy and/or RDTs confirmed) account for 1,867,059 cases [12]. Diverse malaria transmission patterns exist in Ethiopia. Stable transmission occurs in western lowlands of Gambella and Benshangul Gumuz while seasonal transmission occurs in areas below 1500m ASL. Areas between 1500m and 2500m ASL are considered as malaria epidemic regions, whereas, highland areas > 2500m ASL are malaria-free [29].

Earlier reports documented predominance of *P. falciparum* over *P. vivax;* however, recent studies in some parts of the country indicate *P. vivax* is dominating cases of malaria [30,31,13]. According to the 2015 Ethiopia National Malaria Indicator Survey conducted in areas <2000m ASL, the overall malaria prevalence using RDTs was 1.2% [29]. The report of malaria indicator survey in 2011 also indicates high prevalence of malaria at altitude of <2,000m ASL (overall prevalence of 1.3%, with 1.0% *P. falciparum* and 0.3% *P. vivax*) and lower prevalence at altitude of >2,000m ASL (overall prevalence of 0.1%, all *P. vivax* cases) [3].

Since 2005, overall malaria prevalence exhibited decline likely due to scale up of vector control interventions and introduction of ACT and RDTs. Slide positivity of *Plasmodium* declined to 15% (2005-2011) from 23% (2001-2004) [32]. Moreover, inpatient admissions due to malaria decreased by 50-70% in 2013 [33]. Beside malaria control achievements in Ethiopia, different challenges associated with human, environment, parasite and the vectors transmitting the parasite is posing threats. Human behavior of not using or misusing LLINs [34,35] and resistance of the vector to the commonly used insecticides [5]

may sustain malaria transmission. On the other hand, parasite drug resistance may pose threats in the elimination efforts [36,37]. Moreover, asymptomatic reservoirs may have also an impact on sustaining malaria transmission in hotspots. As a result, when malaria transmission declines, focal transmission may become common and it is important to adapt approaches targeting the remaining asymptomatic/subclinical parasite reservoirs through ACD particularly, RACD [38].

2.2 Malaria control measures

Malaria control measures in Ethiopia include early diagnosis and treatment, and vector control interventions including IRS and LLINs [39]. Long lasting insecticide treated nets in combination with IRS are effective vector control measures for malaria [34]. Fifty-one million LLINs were distributed between 2005 and 2012 in mass campaign with provision of comprehensive health information [1]. In 2013, more than half of households in population at risk areas had LLINs and 42% of the population was protected from malaria using IRS [33]. Community ownership and involvement at all stages of malaria control measures is mediated by health extension workers (HEWs). Over 38, 000 HEWs are recruited and trained in integrated community case management including the use of RDTs to confirm malaria cases before treatment. Communities are encouraged to participate in environmental management for vector control, which includes draining and filling of communal mosquito breeding sites [40,41]. All these measures appear to have played a remarkable role in decreasing malaria cases in several places across the country.

Ethiopia also developed National Strategic Plan and passed through processing and achieving specified goals, and in the process towards malaria elimination. Community mobilization to enhance ownership of the strategy, case detection and access to treatment within 24 hours is a key component to sustain malaria prevention strategies [16].

2.3 Elimination strategies in low transmission settings

Over the past decade global burden of malaria has dramatically decreased, particularly in sub-Saharan Africa [13]. Further elimination of malaria in low transmission areas requires interruption of transmission between human and vector using additional strategies. Asymptomatic parasite carriers may contribute to the ongoing low-level transmission in low endemic countries. For instance, Sri Lanka was successful in downing the number of cases to only 17 in 1963. However, due to early withdrawal of malaria control measures, major epidemic was recorded from1967 to 1969 [42].

Ethiopia harbors different eco-epidemiological zones of malaria transmission, and as a result, elimination strategies will not be uniform throughout the country [4]. So far, PCD is the only means of detecting malaria cases, however, it is well documented that asymptomatic and sub-clinical individuals remains for long periods as substantial sources of infection possibly maintaining low-level malaria transmission [26]. New strategies including ACD and rolling cross-sectional surveys are needed to track asymptomatic parasite pool in low and unstable transmission settings [43]. Choosing when and what method of ACD to implement is critical which depends on transmission-level and logistic issues.

Reactive case detection is one of the ACD methods in which members of index households and neighbors within specified radius of the index households are screened. It is a valuable means of detecting additional malaria cases when implemented in low transmission settings. It is typically best suited method to low malaria transmission settings because it minimizes the cost of tracing and treating each case [44]. Individuals living in close proximity to passively detected cases are targeted to take advantage of spatially clustered malaria cases as hotspots in low transmission settings [45]. Reactive case detection is therefore a valuable tool for targeting and tracing these hidden cases in the community.

Reactive case detection is becoming popular in different countries and recommended by WHO for malaria elimination programs [46]. Among these countries, malaria endemic Asian Pacific countries, Africa (implemented in Swaziland and South Africa and under pilot in Zambia, Senegal and Namibia), and others in the elimination phase are employing RACD activity [47,44,48,49,50,51]. However, despite

the widespread use of RACD and WHO's recommendation, there is no standard guideline for what threshold of incidence and at which screening radius of index case to be used [52].

Studies done using RACD in Ethiopia have not been obtained for review. However, reports from other countries show valuable outcomes of the RACD in detecting additional malaria cases. For example, in a study conducted in Swaziland between December 2009 and June 2012, in which 250 index cases were included, further 74 malaria cases were detected with the probability of detecting the cases being higher in households within 100m radius of the index households. In the study, detection of additional malaria cases was not significantly different between households>100m radius. With respect to time, higher number of cases was detected within one week of index case presentation compared to more than 2 weeks [44].

Another study conducted in Zambia between June 2012 and June 2013 on 426 index cases, 735 additional malaria cases were detected from 1,621 households. According to the result of the study, the detection of malaria cases using RACD is significantly increased when an index case is less than 5 years old. Similarly distance from the index case and distance from the road are also significantly associated with the detection of malaria cases. The clustering of the index households significantly increased during rainy season as compared to dry season or malaria cases increased during rainy season [53]. However, this study involved only 14% of the index cases detected at the health facility, which affects its generalizability.

Even though RACD is popular in many countries, the impact on malaria transmission is not well studied and it needs development of monitoring and evaluation tools based on standardized indicators to assess the process and its impact. The other challenge posed on applicability of the RACD for *P. vivax* elimination due to very low parasite density and the fact that *P. vivax* has dormant liver stage, the hypnozoites, which are responsible for relapse [45].

Chapter Three

3. Objective

3.1. General Objective

- To assess *Plasmodium* infection and associated risk factors among household members of index cases and their neighbors in Kishe and Nada Health Centers catchment *kebeles* using RACD.

3.2. Specific Objectives

1. To determine prevalence of *Plasmodium* infection among household members and neighbors of the index cases using RACD
2. To determine the association of individual and household-level risk factors with *Plasmodium* infection among the study participants
3. To determine the parasitaemia and gametocyte carriage of *Plasmodium* infected individuals identified using RACD

Chapter Four

4. Methods

4.1 Study Setting

The study was conducted in Catchment *Kebeles* of Nada and Kishe Health Centers found in Jimma Zone, Oromia Regional state, Southwest Ethiopia (Figure 1.) Kishe Health Center is located in Shebe Sombo district. It is located 415kms from Addis Ababa, and 62kms from Jimma Town. The catchment *kebeles* of Kishe Health Center are Walla Kella, Hallo Sebeka, Kishe and Gasera Kekero with the total population of 26,415. The livelihood of the inhabitants mainly depends on subsistence farming, with maize being commonly produced cereal. Rice and nuts are also grown particularly in Kishe and Hallo Sebaka *kebeles* which may create favorable conditions for mosquito breeding. The average altitude of the *kebeles* is 1350m ASL, with average temperature ranging from 15^{o}C to 26^{o}C. The catchment area has one health center.

Nada Health Center is located in Omo Nada district, Jimma Zone, 285km from Addis Ababa and 70kms from Jimma Town. The catchment *kebeles* of Nada Health Center are Nada town, Nada Sokote, Nada Chala, Biso Gombo and Doyo Yaya with total population of 39,724. Teff, maize and pepper are commonly grown in the *kebeles* and there are swampy areas in all *kebeles* which may create favorable conditions for mosquito breeding. The average altitude of the catchment is 1811m ASL with average temperature of 19.5^{o}C. There is one health center and some private pharmacies in Nada Town in the catchment area.

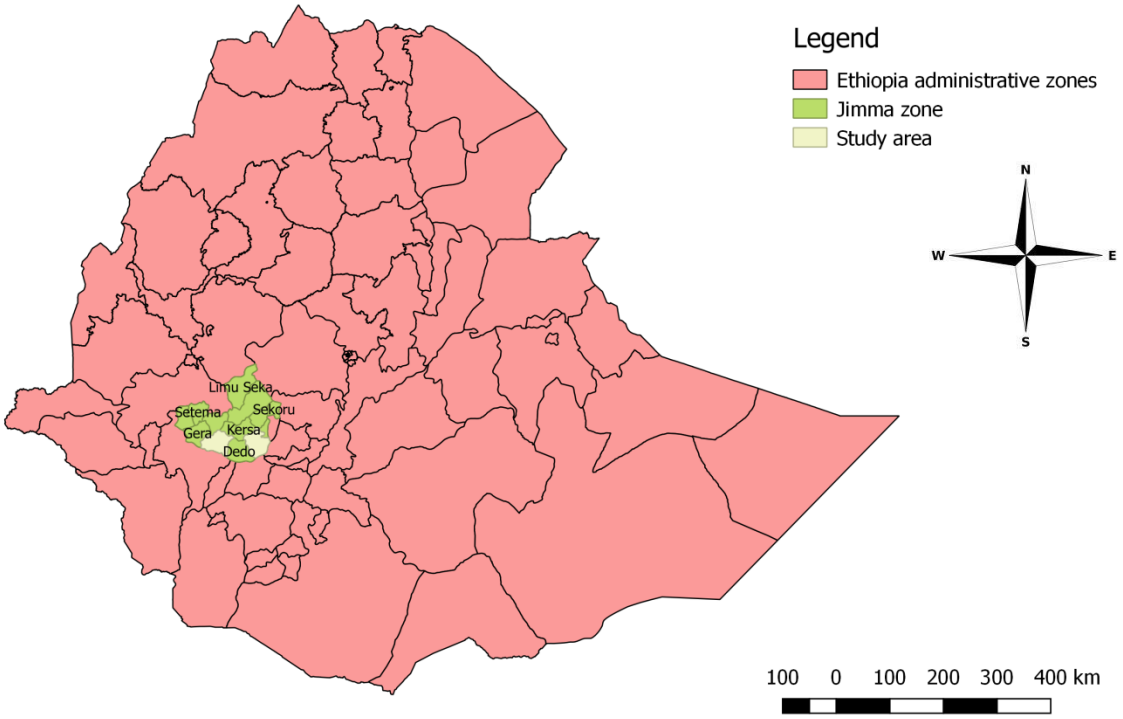

Figure I: Map of the study area

The annual relative humidity and rainfall of both Nada and Kishe Health centers catchment *kebeles* has relatively the same with Jimma town. The annual relative humidity and rainfall of Jimma town is 71% and 1494mm, respectively.

4.2 Study Design and Period

Community based cross-sectional study was conducted to determine prevalence of malaria cases from July to November 2016 using RACD method.

4.3 Population

4.3.1 Source Population

All residents in Nada and Kishe Health Centers catchment *kebeles* dwelling in the area from July to November, 2016.

4.3.2 Study Population

All individuals from the catchment area with malaria infection diagnosed in Kishe and Nada Health Centers by PCD as index cases, and household members of the index cases and neighbors located within 200m radius of index households were included in this study.

4.4 Sample Size and Sampling technique

4.4.1 Sample size

All individuals residing in the catchment *kebeles* of the two health centers with malaria infection diagnosed in Kishe and Nada Health Centers by PCD from July to November 2016 (index cases) and household members of the index cases and neighbors located within approximately 200m radius around index households were included in the study.

4.4.2 Sampling technique

Index cases from the catchment areas diagnosed with malaria from July to November 2016 in Nada and Kishe Health Centers and all family members of the index cases and their neighbors were prospectively included.

4.5 Eligibility

4.5.1 Inclusion criteria

- Index cases with microscopy-confirmed malaria residing in the catchment *kebeles* during the study period and willing to participate in the study.
- Family members of index cases and their neighbors who were willing to participate in the study and residing in the catchment *kebeles*.

4.5.2 Exclusion criteria

- Individuals living in catchment *kebeles* for less than two weeks
- Individuals missed after two visits at their homes

4.6 Study variables

4.6.1 Dependent variable

- Malaria infection

4.6.2 Independent Variables

- Age
- Sex
- Educational status
- Occupation
- Family size
- Travel history
- Pregnancy
- Membership of index household
- Distance from index household (for neighbors)
- Presence and utilization of LLINs in the household
- IRS application
- Housing conditions
- Presence of animals within/separated house
- Malaria species of the index case
- Presence of fever
- The previous one year malaria related fever history
- Choice of health facility for treatment of the previous one year malaria related fever

4.7 Data collection

4.7.1 Questionnaire

Address and contact information of the index cases were collected from individuals with confirmed malaria in Kishe and Nada Health Centers. Data from household members of the index cases and their neighbors were collected within one week after index case presentation to the Health Centers. These households were visited by laboratory technologist using the address provided by each index case. Appointment for missed household members and re-visit of closed houses were made to address all study participants. Socio-demographic information including age, sex, occupation, educational status and

relationship to the household head were collected. Moreover, individual and household-level malaria related risk factors including number and use of bed nets, travel history, fever, choice of health facility for treatment of the previous one year malaria related fever, housing conditions, IRS status and presence of animals within/separated houses were collected using pre-tested questionnaire. These data were collected by two laboratory technologists. Geographical coordinates of the households were also recorded by portable Geographical Positioning System (GPS) unit.

4.7.2 Laboratory analysis

Finger-prick samples were collected from each consenting individuals at their homes, following standard procedures. The samples were tested at field for malaria infection using multi-species RDT kits (CareStart^{TM}, *Manufacturer – Access Bio, Inc., Lot – MR14J62, Expiry date – FEB 2017*). The test kits were kindly provided by Jimma Zonal Health Office. Performance of this test kit was previously evaluated in Jimma area [54] and elsewhere in Ethiopia [55]. As the RDTs results are immediately ready, malaria positive individuals were immediately referred to the health centers for possible recheck using microscopy and treatment. Aliquot of the samples were used to prepare thin and thick smears on the same slide. The samples were transported to Jimma University; the thin films fixed with absolute methanol and stained using 10% Giemsa stain (P^H 7.2) on the same day the samples were collected. The stained slides were later examined using microscope (100x objective) by two independent experienced laboratory technologists. The personnel reading the slides were masked of the RDTs results of the samples. Owing to the fact that it is a low transmission setting and to minimize losing low density parasitaemia, a minimum of approximately 200 microscopic fields were examined before declaring a slide as negative. Any discordant results between the laboratory personnel were resolved by a third reader, who was blind about the results. Parasitaemia densities were estimated by counting the asexual forms per 200 WBCs.

4.8 Data Quality control

- The questionnaire, and sample collection and processing materials were tested prior to actual data collection during pre-test period.
- Completeness and consistency of the collected data were regularly checked.
- The working solution of Giemsa stain was prepared daily, and fresh working solution was used daily.
- 10% of randomly selected negative slides and all positive slides were rechecked by another laboratory technologist who was blinded for the previous readings.

4.9 Data management and analysis

The questionnaires were checked for completeness, coded and entered into excel and cleaned. Then it was exported to and analyzed using STATA12MP_1 statistical software. Descriptive statistics has been utilized to summarize the socio-demographic profile of the study participants. Bivariable and multivariable logistic regressions were utilized to determine the association of the dependent variable with the independent variables. Variables significantly associated with the independent variable and those with $p < 0.2$ were candidates for the multi-variable analysis. Odds ratio and 95% confidence intervals were used to show the degree of the association. $p < 0.05$ was considered statistically significant during the analysis.

4.10 Ethical considerations

Ethical approval was obtained from Ethical Review Board (IRB) of College of Health Sciences, Jimma University. Permission was sought from Zonal and district health offices. Prior to data collection, written consent was obtained from each study participant (guardian/parents in cases of children), and only consenting individuals and households were included in the study. Confidentiality of information from individuals and households was maintained and all individuals with RDT positive cases were referred to Kishe and Nada Health Centers for possible treatment.

4.11 Pre-test

Pre-test was conducted on four households with cases identified in Kishe Health Center. Adjustment of the tools and materials were made accordingly.

4.12 Operational Definitions

Index case: Confirmed malaria case detected in health facility by passive case detection

Index household: Household where a malaria index case resides

Additional Malaria cases: Malaria cases detected from the household members of the index households and their neighbors

Close proximity: households located within 200m radius of an index household

Reactive case detection: A method to detect malaria cases by screening individuals residing in index households and households around it within specified radius (in this case within 200m radius).

Catchment *kebeles*: Administrative areas assigned under one health center.

Eaves: Openings/gaps between wall and roof of the houses

Chapter Five

5. Results

5.1 Socio-demographic characteristics of the study participants

During the study period, a total of 40 individuals with microscopy-confirmed malaria were enrolled as index cases, 17 from Kishe Health Center (nine *P.vivax*, eight *P. falciparum*) and 23 from Nada Health Center (seven *P. vivax* and 16 *P. falciparum*). Thirty-nine of the 40 index cases were included in this study, with one of the index case was lost to follow up due to missed contact information. Following the 39 index cases, 155 households were visited and a total of 726 individuals were screened for malaria using RDTs and microscopy. From the total study participants 220 and 506 individuals were from Kishe and Nada Health Center catchment *kebeles*, respectively.

Table 1-Socio-demographic characteristics of the study participants, Jimma Zone, Southwest Ethiopia, 2016

Characteristics		**Frequency (%)**	**Total**
Sex	Female	404 (55.65)	726(100)
	Male	322 (44.35)	
Age group (years)	<5	144 (19.83)	726(100)
	5-15	221 (30.44)	
	>15	361 (49.72)	
Educational status	Illiterate	235 (42.57)	552 (100)
	Literate	317 (57.43)	
Occupation	Under age	174(23.97)	726(100)
	Farmer	155(21.35)	
	Spouse	143(19.70)	
	Student	216(29.75)	
	Others	38(5.23)	
Family size	≤ 5	98(63.23)	155(100)
	>5	57(36.77)	
House type	Iron sheet	103(66.45)	155(100)
	Tukul	52(33.55)	
Type of wall	Mud	152(98.06)	155(100)
	Cement	3(1.94)	

More than half of the study participants, 55.65% (404/726), were females and 44.35% (322/726) were males. Regarding the age group, 144 (19.8%) were < 5 years, 221 (30.50%) were 5-15 and 361 (49.70%) were >15 years. Of all the study participants, 174 (23.97%) were under age (not categorized with

educational status) and of the remaining 552 study participants, 317 (57.43%) were literate from grade one to college and 235 (42.57%) were classified as illiterate [Table 1]. The distribution of the study participants by occupation was 174(23.97%) under age, 155(21.35%) farmers, 143(19.70%) spouse (housewife), 216(29.75%) students and 38(5.23%) others. From 155 households in the study, the roof structure of 103(66.45%) were corrugated iron sheet and the rest 52(33.55%) were tukuls. Only 3(1.94%) houses had cemented walls and the rest 152(98.06%) were mud [Table 1].

5.2 Prevalence and associated risk factors of malaria

Out of the total 726 study participants screened, the number of additional malaria cases detected by microscopy were 29 (three *P. vivax* and twenty six *P. falciparum*) and 33 (three *P. vivax* and 30 *P. falciparum*) by multispecies RDTs. The results of RDTs were used for data analysis and malaria case treatment. Following the 15 *P. vivax* index cases, 245 individuals from 57(36.77%) households were screened and four additional malaria cases were identified. The other 481 (66.25%) individuals from 98(63.23%) households were screened following the 24 *P. falciparum* index cases and 29 additional malaria cases were identified. From these additional malaria cases, all the three *P. vivax* malaria cases were detected in July and August, and all 30 *P. falciparum* malaria cases were detected in October and November.

The prevalence of malaria among male and female study participants were 5.59% (18/322) and 3.71% (15/404) respectively. Age groups of <5 years, 5-15 years and >15 years had malaria prevalence of 6.94% (10/144), 5.88% (13/221) and 2.85% (10/361), respectively. The overall prevalence of malaria in the study was 4.55% [Table 1, Table 2]. The parasite density, calculated per 200WBC count, of the asexual stage ranges from 40-51,600/µl with geometric mean of 1278.23/µl. The gametocyte carriage of malaria infected study participants was 55.17% (16/29).

Table 2- Prevalence of malaria and associated risk factors in the RACD, Jimma Zone, Southwest Ethiopia, 2016

Characteristics		Malaria RDT result		
		Negative n (%)	Positive n (%)	Total n (%)
Age group in years	<5	134(93.06)	10(6.90)	144(19.83)
	5-15	208(94.12)	13(5.88)	221(30.44)
	>15	351(97.23)	10(2.77)	361(49.72)
Sex	Male	304(94.41)	18(5.59)	322(44.35)
	Female	389(96.29)	15(3.71)	404(55.65)
Last one year malaria treatment (n=726)	No	616 (96.55)	22 (3.45)	638 (87.88)
	Yes	77 (87.50)	11 (12.50)	88 (12.12)
Treatment sought in the last one year (n=88)	Public health facilities	48 (92.31)	4 (7.69)	52 (7.16)
	Private clinic/ Pharmacy	29 (80.56)	7 (19.44)	36 (4.96)
Fever during the survey (=726)	No	683(96.20)	27(3.80)	710(97.80)
	Yes	10(62.50)	6(17.50)	16(2.20)
ITN usage the previous night (n=726)	No	419 (94.80)	23 (5.20)	442 (60.88)
	Yes	274 (96.48)	10 (3.52)	284 (39.12)
Plasmodium spp. of the IC (n=726)	*P. vivax*	241 (98.37)	4 (1.63)	245 (33.75)
	P. falciparum	452 (93.97)	29 (6.03)	481 (66.25)
Travel last two weeks (n=726)	No	689 (95.43)	33 (4.57)	722 (99.45)
	Yes	4 (100.00)	0 (0.00)	4 (0.55)
Malaria Index and addressed households	*P. vivax*	53(92.98)	4(7.02)	57(36.77)
	P. falciparum	79(80.61)	19(19.39)	98(63.23)
Distance of the house from the index household (n=155)	Index	28(77.78)	8(22.62)	36(23.23)
	1-50m	36(76.60)	11(23.40)	47(30.32)
	51-200m	68(94.44)	4(5.56)	72(46.45)
House type (n=155)	Iron sheet	87(84.47)	16(15.53)	103(66.45)
	Tukul	45(86.54)	7(13.46)	52(33.55)
Type of wall (n=155)	Mud	129(84.87)	23(13.13)	152(98.06)
	Cement	3(100.00)	0(0.00)	3(1.94)
Presence of hole in the wall (n=155)	No	90(87.38)	13(12.62)	103(66.45)
	Yes	42(80.77)	10(19.13)	52(33.55)
Presence of window (155)	No	47(87.04)	7(12.96)	54(34.84)
	Yes	85(84.16)	16(15.84)	101(65.16)
Presence of Eaves (n=155)	No	97(88.99)	12(11.01)	109(70.32)
	Yes	35(76.09)	11(23.91)	46(29.68)
Spray status	No	64(77.11)	19(22.89)	83(53.55)
	Yes	68(94.44)	4(5.56)	72(46.45)
Animals within the house (n=155)	No	69(88.46)	9(11.54)	78(50.32)
	Yes	63(81.81)	14(18.19)	77(49.68)
Animals in separate house	No	53(85.48)	9(14.52)	62(40.00)
	Yes	79(84.95)	14(15.05)	93(60.00)
Family size (155)	≤ 5	90(91.84)	8(8.16)	98(63.23)
	>5	42(73.68)	15(26.32)	57(36.77)

Eighty eight (12.12%) study participants reported malaria treatment the previous one year and 11 (12.50%) of them were positive for malaria. Among these 88 individuals, 52 (59.10%) of them sought treatment from public health facilities and the rest 36 (40.90%) sought treatment from private clinics and pharmacies. Individual level utilization of LLINs was 39.12% (284/726) and the remaining 442 (60.88%) were not used LLINs the previous night. The prevalence of malaria among individuals using LLINs and not used LLINs the previous night was 3.65% and 5.45%, respectively. From 16 (2.20%) of the study participants having fever (axillary temperature >37.50C0) during the survey, 6 (37.50%) were malaria positive and 10 (62.50%) were negative by multispecies RDTs. Only 4 (0.55%) individuals reported travel in the last two weeks and the remaining 722 (99.45%) did not travelled anywhere in the last two weeks [Table 2].

From visited 155 households, 83(53.55%) were the sum of index households and households at approximate distance of 1-50 meters from the index households. Of these households 19(22.89%) were screened with one or more malaria cases. Out of 72 households located at approximate distance of 51-200m, 4(5.56%) households were identified with one or more malaria cases. Houses having hole in their walls were 52(33.55%) and 10 (19.23%) were with one or more malaria cases. The rest 103(66.45%) houses lacked hole, of which 13 (12.62) were with one or more malaria cases.

There were 46(29.68%) houses with eaves and 11(23.91%) were detected with one or more malaria cases. The remaining 109(70.32%) houses had no eaves and 12(11.00%) were with one or more malaria cases. From addressed 155 households, 83(53.55%) were not sprayed in the last one year, from which 19(22.89%) of them were identified with one or more malaria cases. Only 4(5.56%) households with one or more malaria cases were from 72(46.45%) not sprayed houses. Animals were kept within the same houses in 77(49.68%) of addressed households and the rest 78(50.32%) did not keep animals in their residence. Fifty seven (36.77%) of households surveyed had family size of greater than five and 98(63.23%) households had <5 family size. From these households with family size of >5 and <5, 15 (26.32%) and 8(8.16%) households were with one or more malaria cases, respectively [Table 2].

5.3 Bivariable analysis of factors associated with malaria

The assessed characteristics of individual level socio-demographic and associated risk factors were age, sex, education, occupation, the previous year malaria history, choice of health facility for treatment of the previous one year malaria, fever and LLINs usage. From these characteristics sex, educational status, occupation, choice of health facility for treatment of the previous one year malaria and the previous night LLINs usage were not significantly associated with detecting malaria case using RACD method ($p > 0.05$).

Table 3-Results of bivariable analysis of factors associated with malaria among the study participants, Jimma Zone, Southwest Ethiopia, 2016

Characteristics		RDT result			COR	p-value
		Negative n (%)	Positive n (%)	Total n (%)		
Sex	Male	304(94.41)	18(5.59)	322(44.35)	1.5	0.231
	Female	389(96.29)	15(3.71)	404(55.65)	Ref	Ref
Education	Literate	308(97.16)	9(2.84)	317(57.43)	1.5	0.370
	Illiterate	225(95.74)	10(4.26)	235(42.57)	Ref	Ref
Treatment sought for fever in the last one year (n=88)	Public health center	48 (92.31)	4 (7.69)	52(59.09)	Ref	Ref
	Private clinic/ Pharmacy	29 (80.56)	7 (19.44)	36(40.91)	2.9	0.112
ITN usage the previous night (n=726)	No	419 (94.80)	23 (5.20)	442(60.88)	1.5	0.291
	Yes	274 (96.48)	10 (3.52)	284(39.12)	Ref	Ref
House type (n=155)	Iron sheet	87(84.47)	16(15.53)	103(66.45)	1.2	0.732
	Tukul	45(86.54)	7(13.46)	52(33.55)	Ref	Ref
Type of wall (n=155)	Mud	129(84.87)	23(13.13)	152(98.06)		
	Cement	3(100.00)	0(0.00)	3(1.94)		
Presence of hole in the wall (n=155)	No	90(87.38)	13(12.62)	103(66.45)	Ref	Ref
	Yes	42(80.77)	10(19.13)	52(33.55)	1.6	0.278
Presence of window (n=155)	No	47(87.04)	7(12.96)	54(34.84)	Ref	Ref
	Yes	85(84.16)	16(15.84)	101(65.16)	1.3	0.632
Animals within the house (n=155)	No	69(88.46)	9(11.54)	78(50.32)	Ref	Ref
	Yes	63(81.81)	14(18.19)	77(49.68)	1.7	0.248
Animals in separate house (n=155)	No	53(85.48)	9(14.52)	62(40.00)	Ref	Ref
	Yes	79(84.95)	14(15.05)	93(60.00)	1.0	0.927

COR: Crude Odds Ratio

Other variables of age groups, the previous one year history of malaria treatment and presence of fever during the survey were individual level risk factors which had significant association with the detecting additional malaria cases. Moreover, household level risk factors of presence of eaves, IRS status, family size, *Plasmodium* species of the index cases and distance from the index household were significantly associated with detecting malaria cases. These variables were selected for multivariable analysis.

5.4 Multivariable analysis of factors associated with malaria

Age, the previous one year malaria history, choice of health facility for treatment of the previous one year malaria and fever were selected individual-level risk factors for multivariable analysis. Among these characteristics, choice of health facility had no statistical significance with malaria infection [Table 3].

Table 4-Result of multivariable analysis of factors associated with malaria among the study participants, Jimma Zone, Southwest Ethiopia, 2016

Characteristics		Malaria infection		COR (95%CI)	AOR(95%CI)
		Negative n (%)	Positive n (%)		
Age group in years	≤5	134(93.1)	10(6.9)	2.6 (1.06 - 6.43)	3.1(1.20 - 8.17)*
	5-15	208(94.1)	13(5.9)	2.1(0.94 - 5.09)	2.2(0.94 - 5.58)
	≥15	351(97.2)	10(2.8)	Ref	Ref
The last one year malaria history	Yes	77(87.5)	11(12.5)	4(1.86 - 8.56)	4.3(1.90 - 9.91)*
	No	616(96.6	22(3.4)	Ref	Ref
Fever at the time of survey	Yes	10(62.5)	6(37.5)	15(5.14 - 44.81)	17(5.28 - 56.92)*
	No	683 (96.2)	27(3.8)	Ref	Ref
Presence of eave	No	97(89.0)	12(11.0)	Ref	Ref
	Yes	35(76.0)	11(24.0)	2.5 (1.02- 6.27)	4.5(1.49 - 13.67)*
Spray status of the structure	Not sprayed	64(77.1)	19(22.9)	5(1.62 - 15.63)	7.1(1.58 - 31.52)*
	Sprayed	68(94.4)	4(5.6)	Ref	Ref
Plasmodium species of index HH	*P. vivax*	53(93.0)	4(7.0)	Ref	Ref
	P. vivax	79(80.6)	19(19.4)	3(1.02 - 9.89)	0.7(0.17 - 3.32)
Family size	<5	90(91.8)	8(8.2)	Ref	Ref
	≥5	42(73.7)	15(26.3)	4(1.58 - 10.21)	2.7(0.90 - 8.31)
Distance from the index HH	Index HH	28(21.21)	8(34.78)	4.86(1.35-17.44)	4.44(1.1- 18.07)*
	1-50m	36(27.27)	11(47.83)	5.19(1.54-17.48)	5.17(1.37-19.48)*
	51-200m	68(51.52)	4(17.39)	Ref	Ref

*significant at $p < 0.05$ AOR= Adjusted Odds Ratio

Age groups of < 5 years were 3 times more likely to be malaria positive as compared to age groups ≥ 15 years (AOR=3.1; 95%CI=1.20 - 8.17; p < 0.05). The previous one year malaria infection history had also significant association with detecting malaria cases in which individuals having history were four fold to be malaria positive as compared to individuals without malaria related fever history (AOR=4.3; 95%CI=1.90 - 9.91; p < 0.05). Febrile study participants had significantly higher association with malaria infection as compared to afebrile study participants. (AOR=17; 95%CI=5.28 - 56.92; p < 0.05).

Household level associated risk factors of family size, index *Plasmodium* species, IRS status, presence of eaves and approximate distance from the index household were selected for multivariable analysis. The odds of detecting malaria infection was higher for *P. falciparum* index cases as compared to *P. vivax* index cases (COR=3; 95%CI=1.02 - 9.89; p < 0.05), however, lacked statistical significance in detecting malaria cases in multivariable analysis (AOR=0.7; 95%CI=0.17 - 3.32; p > 0.05). Family size had also significant association with detecting malaria cases (COR=4; 95%CI=1.58 - 10.21; p < 0.05), but lacked association in multivariable analysis (AOR=2.7; 95%CI=0.90 - 8.31; p > 0.05) [Table 4].

Presence of eaves, IRS status and approximate distance from the index households had significant association with malaria infection in multivariable analysis. The odds of detecting malaria cases among individuals from houses of having eaves was higher as compared to houses without eaves in multivariable analysis (AOR=4.5; 95%CI=1.49 - 13.67; p < 0.05). Indoor residual spray status had strong association with detecting malaria cases in which individuals from non sprayed houses are more likely to be malaria positive (AOR=7.1; 95%CI=1.58 - 31.52; p 0.05). Study participants from index households and neighbor households located within approximate distance of 50m are more likely to be malaria positive as compared to households at 51-200m distance (AOR=4.44; 95%CI=1.1- 18.07; p < 0.05 and AOR=5.17; 95%CI=1.37-19.48; p < 0.05, respectively) [Table 4].

Chapter Six

6. Discussion

Reactive case detection is one of the malaria elimination strategies, targeting malaria infection left in the community following index cases detected in health facilities. *Plasmodium* parasite pool may remain in the community without diagnosis and treatment due to asymptomatic infection or having low severity of sign and symptoms of malaria [25]. There may be also delay for seeking treatment which may extend the period of parasite transmission from human to the anopheline vectors [56]. Active case detection of malaria, particularly the RACD, plays a pivotal role in targeting these parasite pools. This study, which is, to the best of our knowledge, the first in its kind to be conducted in Ethiopia, has attempted to determine the prevalence of malaria cases associated with index cases detected in two public health facilities, and identify factors associated with malaria cases.

The prevalence of malaria in the community obtained in this study was higher than the national prevalence of malaria in high risk areas (<2,000m ASL) of Ethiopia [29]. This may show the importance of RACD method in detecting further malaria cases left in the community. Similar studies done in other countries also indicated the value of RACD in identifying malaria cases. For example, in a study done in Swaziland, 74 further cases (2%) of malaria were identified following 250 index cases [44]. Similarly, a study conducted in Senegal identified 23 malaria cases (0.3%) following 110 index cases [47]. In this study a relatively higher prevalence of malaria cases (4.5%) was obtained. This could be due to difference in the level of malaria transmission in these settings. While the RACD proves to be a valuable tool in detecting malaria cases left in the community, large scale implementation of this method in our set up requires further feasibility study.

In this study, the odds of malaria infection among febrile study participants was significantly higher than those without fever. Other study done in Cambodia, using RACD, also reported fever as significant predictor of malaria cases [57]. Hence, as the febrile study participants did not seek treatment at the time of the survey, this may contribute to the ongoing transmission of malaria in the community [56]. Early

treatment seeking for fever remarkably contributes to early detection and prompt treatment of malaria cases, which may reduce the transmission [56]. Moreover, in this study, more than half of the individuals who were positive for malaria harbored gametocytes. This may sustain the transmission of malaria in the study area. Hence, a strategy should be in place to target such possible reservoirs of infection.

Indoor residual spraying and LLINs remain effective malaria control tools, which significantly contributed to the observed decline of malaria cases in Ethiopia [58]. Indeed, the ITNs contributed to about 68% of the observed decrease in *P. falciparum* in sub-Saharan Africa from 2000-2015 [59]. Although the prevalence of malaria among study participants who did not use the LLINs the previous night before the survey was higher than those who used, the difference was not significant. This could be due to possible outdoor transmission in such low transmission setting or shift in biting time of the vectors in these areas [59,60]. The ITNs are effective in control of malaria when the predominant malaria vectors are endophagic and bite late at night. Possibly inaccurate information may also be provided by the study participants. On the other hand, individuals residing in houses sprayed with insecticides were at lower risk of acquiring malaria infection as compared to those residing in unsprayed houses. Similarly, a study done in Swaziland documented the risk of malaria infection is significantly higher among individuals living in non sprayed houses as compared to those living in sprayed houses [44]. Similar protective role of IRS in decreasing malaria infection were reported previously in Ethiopia [61,62] and elsewhere in Africa [63,64]. In this study, there were households particularly from Nada Health Center catchment *kebeles* which were located in non-sprayed villages. These villages were not covered in the IRS program probably due to lack of information on the malaria transmission in the area. This indicates that the RACD could help to recognize such hotspots, in addition to detecting malaria cases remained in the community.

Good housing condition is also another important factor that plays role in deterring nocturnal human-vector contact [65]. Eaves are the main gates of *Anopheles* mosquitoes to the houses during the night. The results of this study also denoted that individuals residing in houses with eaves had a significantly higher prevalence of malaria as compared to those living in houses with no eaves. Study conducted in Kenya

indicated that eaves are the preferred entry sites for the mosquitoes. The risks of eaves on acquisition of malaria have also been highlighted in previous studies [66,67]. This calls for consideration of the house structure in the interventions to be undertaken for the control of malaria in low transmission settings.

In this study the odds of malaria infection was significantly higher among study participants with history of malaria infection the preceding one year as compared to those without malaria history. Similar study conducted in Cambodia also indicated a significantly higher prevalence of malaria among individuals with malaria history as compared to those lacked malaria infection history [57]. This could be due to persistence of individual and household-level risk factors of malaria in the individuals and the houses where they dwell. Moreover, the clustering nature of malaria in hotspots may also result in repeated infection of malaria in such low transmission settings [21].

In Ethiopia, children under five years and pregnant women are identified as high risk groups and they are the primary targets of malaria intervention programs [11]. The burden of malaria morbidity and mortality is higher in children under five years [68]. In this study, children less than five years of age had higher odds of malaria infection as compared to the adults. This requires special attention to minimize the burden of malaria in this age group.

Reactive case detection of malaria is resource intensive by its nature and is usually limited to certain radius around the index household, with no established standard of the distance. Previous studies conducted using RACD method screened the neighboring households located at different radius from the index household [44,47,69]. A minimum radius of 150m around the index case has been suggested by Parker et al [70]. In this study, households within approximately 200m radius around the index cases were included. The prevalence of malaria was significantly higher among individuals residing in index households and those living within 50m of the index household compared to those located farther. Similar studies conducted in Senegal and Swaziland showed the odds of malaria infection is higher among household members of index cases as compared to neighbors [44,47]. Spatial clustering of malaria is a

usual phenomenon, in which individuals residing in certain households or localities are disproportionately highly infected [67]. However, as this study was limited to screening the individuals residing in households located within approximately 200m radius only, the possibility of detecting the cases at farther distance needs to be investigated.

The findings of this study should be viewed considering the following limitations: first, individuals (index cases) from the study areas seeking treatment in other nearby public health institution, private clinics and possibly pharmacies were not included in this study. Nevertheless, the proportion constituting these groups of patients is expected to be very low as the study was mainly conducted in rural settings. Second, the diagnostic methods utilized in this study, the RDTs, have their own drawback. Hence, it is expected that molecular technique could have detected more cases of malaria among the secondary cases. Finally, the RACD utilized in this study did not include control group to compare the prevalence with individuals not related with index households.

Chapter Seven

7. Conclusion and Recommendation

7.1 Conclusion

The reactive cases detection method used in this study had valuable outcome in detecting additional malaria cases left in the community. It could also help to identify hotspots such as not sprayed areas. The prevalence of malaria was significantly higher among under-five children, those with fever and history of malaria in the preceding one year. Moreover, individuals living in insecticide unsprayed houses, houses with eaves and nearer to the index houses had significantly higher prevalence of malaria. More than half of malaria cases had gametocytemia.

7.2 Recommendation

Awareness should be created for the community to close eaves and consider this as a possible risk for malaria in the construction of new houses. Indoor residual spraying operations should be strengthened and coverage be improved. The RACD of malaria is promising approach in detecting malaria cases, hence may be applied with the existing malaria intervention programs in similar settings. However, the feasibility of large scale application of the RACD in different malaria transmission settings should be investigated further.

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Annex I- Materials

- Frosted edge Slide
- Staining jar
- Giemsa staining reagent
- Lancet
- Multi-species RDTs
- Absolute methanol (for fixation)
- Cotton
- Stationary
- Tissue paper
- 70% alcohol
- Timer
- Slide box
- Microscope
- Immersion oil
- Manual counter
- Safety box
- disposable gloves
- Dilution buffer
- Test Tube
- Soap
- Filter Paper
- GPS
- Thermometer
- Tap water

Annex II - Information sheet

Name of Principal Investigator: Abebaw Tiruneh

Name of Organization: Jimma University

Back ground: This information sheet is prepared by researchers with main objective of identifying determinants of *Plasmodium* species infection detected by using reactive case detection method. The group includes principal investigator (Msc (candidate) graduate in Medical Parasitology) and advisors from Jimma University, Institute of Health, School of Medical Laboratory Sciences. Data collectors and supervisors are also part of the team for collecting and reporting quality data for study group.

Purpose: The purpose of this study is to identify determinants of *Plasmodium* detection among residents in close proximity to index cases, identified in health facilities, using RACD. As malaria transmission declines, focal transmission becomes common, and it is important to adapt targeting the remaining parasite reservoirs through active case detection. RACD is one component of ACD which depends on passively detected malaria cases (index cases) and individuals around index case HH (200m in this study) will be tested for *Plasmodium* species. RACD will be among strategies for malaria elimination programs by target both symptomatic and asymptomatic malaria cases depending on the focal nature of malaria in low transmission settings.

RACD is implemented and being implemented in different countries to achieve their malaria control and elimination plan. WHO also recommend this RACD for successful countries in controlling malaria transmission, and Ethiopia is among these countries where malaria burden dramatically decreased. However, there is no report of RACD or other ACD method implementation in the country. This study will be conducted in Kishe and Nada Health Centers Catchment *Kebeles*, Southwest Ethiopia, to fill the gap and to identify factors associated with malaria case detection using RACD method.

Procedure: We kindly invite you to participate in this research with the aim to identify determinants of malaria detection among residents in close proximity to passively detected malaria cases in Kishe Kebele.

If you agree to participate in this study, you will need to understand and sign agreement form. You will be asked some questionnaires and only your answer will be filled on questionnaire sheet. Blood sample for laboratory diagnosis of malaria using both RDTs and microscope will be collected from your finger using sterile lancet. The result of RDTs will be diagnosed at the time of spot and microscopy sample will be collected and transported with the standard protocol. Your questionnaire data and sample will be used only for this study and coding will be used for your confidentiality. If the result of your sample will be positive for malaria, you will be treated according to the national drug guideline.

Risk and Discomfort: It is known that there is some pain during finger prick for collecting blood sample. There may be also some pain after finger prick when you will perform your own jobs for a day (few days). To reduce the pain we will select appropriate finger and we will also use sterile lancet to reduce the probability of microbe entrance to the body. Therefore you will not be worry about the pain and microbial infection during finger prick.

Benefits: If you participate in this study, you will get direct benefit for diagnosis and treatment of malaria. Your participation also has an input for identifying determinants malaria detection among residents in close proximity to index cases, identified by passive case detection.

Incentives: There are no incentives for participating in this study.

Confidentiality: The information that we will collect in this research will be kept confidential and stored in a file. Code numbers will be used instead of your name and the file will be kept in a key and lock, and will not be accessed by anyone except the principal investigator.

Wright to refuse and withdraw: You have full right to refuse or withdraw from participation in this study. Your refusal will not compromise your service in health facilities or other government sectors in any way at any time.

Whom to contact: If you have any questions or comments you can contact these individuals at any time.

1. **Abebaw Tiruneh**- Jimma University, Institute of Health, School of Medical Laboratory Sciences, **Jimma**

Annex III- Consent form

I have been informed about a study and have read (has been read to me) all the information stated in the introductory part and I have had an opportunity to ask any question about the study. I got satisfactory answers for all of my questions. For this study I have been requested to give blood sample from my finger and give information for the study. I have fully understood and gave my consent to give the blood specimen and to reply for questionnaires. It is therefore my willing to give my informed consent and cooperate at my will in the course of the conduct of the study.

Name of participant ----------Signature ----------Date ----------

Name of data collector ----------Signature ----------Date ---------

If cannot read and write: name of the independent witness,

Name and signature_____,_____/_____/_____(DD/MM/YY)

Annex IV - Questionnaire

A. For Index Cases*Date ___/___/2016*

Name_____ Kebele _____ Gere/gott _____

Phone No _____ HH leader name_____. Name of gere leader _____ Reg. number/code _____

Q.101	Age (in Month for children <5 and completed years for >5 years)	_____.
Q.102	Sex	(1) Male (2) Female
Q.103	For women 15-49 years old. Are you pregnant now?	(1) Yes (2) No
Q.104	Have you stayed/resided in the HH,you live now, for the last two weeks?	(1) Yes (2) No
Q.105	Have you slept last night under bed net?	(1) Yes (2) No
Q.106	Previous history of confirmed malaria disease prior to this presentation?	(1) Yes (2) No
Q.107	Days spent with fever?	_____(days).
Q.108	Travel history to malarious areas in the last two weeks?	(1) Yes (2) No
Q.109	*Plasmodium* species identified	(1) P. vivax (2) P. vivax (3) Others/specify _____.

Name of Lab. Personnel (species identified by)_____ sign _____ Date __/__/2016

B. For Residents in close proximity to Index Cases *Date ___/___/2016.*

Read the consent form! Is the participant agreed? (1). Yes. (2). No. If "No" **stop** the Questionnaire.

Name _____ Kebele name _____ Gare/gott _____

HH code_____. Reg. No._____. Reg. number/code of Index Case _____.

Socio-demographic

Q.201	**Age** (in Month for children **<5** and completed years for **>5** years)	_____.
Q.202	Sex	(1) Male (2) Female
Q.203	Number of HH members	Specify _____
Q.204	Membership to the HH	(1) Husband (2) Spouse (3) Son/daughter (4) Relative (5) Other/specify _____
Q.205	Occupation	(1) Farmer (2) Merchant (3) Daily labourer (4) Student (5) Government employed (6) Others/specify _____
Q.206	Educational status	(1) Illiterate (2) Grade 1-8 (3) Grade 9-12 (4) College and above

Risk Factors

Q.301	Are you pregnant? (only for women 15-49)	(1) Yes (2) No
Q.302	Travel history to malarious areas in the last two weeks	(1) Yes (2) No
Q.303	Have you malaria history the previous one year?	(1) Yes (2) No
Q.304	If "Yes" Have you visited health facilities for treatment?	(1) Yes (2) No
Q.305	Choice of health facility to seek treatment for malaria?	(1) Health center/hospital (2) Health post (3) Private clinic/hospital (4) Self prescribe (5) Other/Specify _____
Q.306	Have you ITNs	(1) Yes (2) No

Q.307	If "yes" How many ITNs in the HH?	Specify _____.
Q.308	Observe ITNs "Mark" write all type	- -
Q.309	Did you slept last night under ITN?	(1) Yes (2) No
Q.310	Have all houses in the compound, where human resides in, get IRS application?	(1) Yes (2) No
Q.311	If "No" how many of them does not sprayed?	Specify _____.
Q.312	In which house did you sleep the last two weeks?	(1) In sprayed house (2) In non-sprayed house
Q.313	For sprayed houses, when the spray applied?	(1) <6 month (2) 6-12 months (3) 12-24 months (4) >24 months.
Q.314	Housing condition (observe) - HH roof	(1) corrugated iron (2) Thatched roof (3) Others/specify _____
Q.315	Housing condition (observe) – HH floor	(1) Cemented (2) Mud (3) Others _____
Q.316	Housing condition (observe) –HH wall	(1) Mud (2) Cemented (3) Thatched (4) Others _____
Q.317	House ventilation (observe) - Window	(1) Yes (2) No
Q.318	House ventilation (observe) - Eaves	(1) Yes (2) No
Q.320	Have you Cattle/other animals?	(1) Yes (2) No
Q.321	If "yes" Where they live?	(1) Within the same house with human (2) Separated house (3) Others/specify _____
Q.322	Distance from the index HH?	(1) The same HH (2) < 10m (3) 10-20m (4) 20-50m (5) 50-100m

Axillary temperature _____. Microscopy result _____.

Name of Data collector _____ Sign _____ Date ___/___/2016.

Name of Supervisor _____ Sign _____ Date ___/___/2016.

Annex V – Consent form (Afaan Oromoo)

Waa'ee qorannoo kanaa ilaalchisee hubannoon gahaan naaf kennamee jira. Wanta naaf hin galles akkan gaafadhu carraan naaf laatamee deebiin quubsaan naaf kennamee jira. Kanaafuu odeeffannoo fi dhiiga quba irraa akkan laadhuuf gaafatameen jira. Anis guutummaan guutuutti waa'ee qorannoo kenaa hubadhee dhiiga quba irraa fi odeffannoo barbaachisu kenneen jira. Kunis kan ta'e fedha kootiin ta'uu isaa mallattoo kootiin nan mirkaneessa.

Maqaa Hirmaataa qorannoo _____ Mallattoo _____ Guyyaa _____

Maqaa Hirmaataa qorannoo _____ Mallattoo _____ Guyyaa _____

Annex VI - Questionnaire */Afaan Oromoo*

C. For Index Cases *Date ___/___/2016*

Name _____Name of health center_____ *Kebele* _____

Gere/gott _____ Phone No _____

HH leader name_____. Reg. number/code _____

Q. No.	**Question**	Response
101	Umurii	_____.
102	Saala	(1) Dhiira (2) Dubara
103	Ulfa dhaa? (Dubartoota umuriin jaraa waggaa 15-49 ta'aniif)	(1) Eeyye (2) Lakki
104	Mana amma ati dhufte keessa Jiraattuu/taa dha? (yoo xinnaate turban lamaaf)	(1) Eeyye (2) Lakki
105	Kaleessa halkan agoobara jala rafte?	(1) Eeyye (2) Lakki
106	Kanaan dura dhukkuba busaatiin qabamtee beekta?	(1) Eeyye (2) Lakki
107	Dhukkubni ammaa kun yoom si jalqabe?	Guyyaa_____.
108	Torban lamaan darban keessa bakka biraa deemtee jirta?	(1) Eeyye (2) Lakki
109	*Plasmodium* species identified	(1) P. falciparum (2) P. vivax (3) Kanbiroo/_____.

Name of Lab. Personnel (species identified by)_____ sign _____ Date __/__/2016

D. For Residents in close proximity to Index Cases *Date ___/___/2016.*

Read the consent form! Is the participant agreed? (1). Yes. (2). No. If "No" **stop** the Questionnaire.

Name _____ Name of health center _____

Kebele name _____ Gare/gott _____ HH code_____. Reg. No._____. Reg. number/code of Index Case _____.

Socio-demographic

Q. No.	Question	Response
201	**Umurii**	_____.
202	Saala	(1) Dhiira (2) Dubara
203	Lakkofsa maatii	_____
204	Firooma abbaa warraatiif qabdu	(1) Abbaa manaa (2) Haadha manaa (3) Ilma/intala (4) Fira (5) Kan biro/ibsi _____
205	Hojii	(1) Qonnaan bulaa (2) Daldalaa (3) Hojiii guyyaa (4) Hojjataa mootummaa (5) Kanbiroo/ibsi _____
206	Sadarkaa Barnootaa	(1) Hin baranne (2) Kutaa 1-8 (3) Kutaa 9-12 (4) Koolleejii fi sanaa ol
207	Ulfa Dhaa?	(1) Eeyye (2) Lakki

Risk Factors

Q. No.	Question	Response
301	Torban lamaan darban keessa bakka biraa deemtee beekta?	(1) Eeyye (2) Lakki
302	Qaamni kee ni guba? (ilaali)	(1) Eeyye (2) Lakki
303	Kanaan dura busaa dhukkubsattee beekta?	(1) Eeyye (2) Lakki
304	Waggaa kana hoo busaan dhukkubsattee beekta?	(1) Eeyye (2) Lakki
306	Deebiin kee "eeyyee" yoo ta'e, eessatti yaalamte?	(1) Buufata fayyaa/hospitaala (2) Kellaa fayyaa (3) Kilinika/faarmaasii dhunfaa (4) Kanbiroo/ibsi_____
307	Agoobara qabda?	(1) Eeyye (2) Lakki
308	Agoobara meeqa qabda?	_____.
309	Kaleessa halkan agoobara jala bulte?	(1) Eeyye (2) Lakki
310	Manni kemikaala bookee balleessu biifamee jira?	(1) Eeyye (2) Lakki
311	Housing condition (observe) – House type	(4) Rectangular corrugated iron (5) Rectangular thatched roof (6) Tukul with thatched roof (7) Tukul with iron roof (8) Others/specif_____
312	Housing condition (Presence of hole)	(4) Yes (5) No
313	Housing condition (observe) –HH wall	(5) Mud (6) Cemented (7) Thatched (8) Others _____
314	Housing condition (Presence of eave)	(3) Yes (4) No
	Housing condition (presence of window)	(1) Yes (2) No
315	Mana isin keessa jiraattan keessa horii/beeladni jiraatu jira?	(1) Lakki (2) Indaaqqoo _____ (3) Hoolaa/re'ee (4) Loon/kotte duudaa
316	Beeladootni mana qophaatti ni jiru?	(1) Eeyye (2) Lakki
317	Approximate distance in meter from index HH?	_____

Auxiliary temperature(^{0}C) _____RDT result_____. Microscopy result(species) _____. Stage _____.

Name of Data collector _____ Sign _____ Date ___/___/2016.

Name of Supervisor _____ Sign _____ Date ___/___/2016.

Annex VI-Giemsa staining procedure

Reagents and materials

- Giemsa Stock solution
- Buffered water (ph 7.2)
- Methanol
- Pasteur pipettes
- Graduated cylinder
- Drying Rack
- Staining trough
- Clean tap water
- Timing clock

Preparation of 10% Giemsa working solution

1. Pour 90 ml of buffered water (ph 7.2) into a 100 ml graduated cylinder
2. Using a pasteur pipette, draw 10 ml of Giemsa stock solution. Add the stain to the buffered water in the graduated cylinder
3. Cover the top of the graduated cylinder with parafilm and gently invert the cylinder several times until completely mixed;
4. Giemsa working solution stain must be discarded through the sink after 24 hours and prepare a fresh working solution again.
5. For individual slide staining, each slide needs approximately 3 ml of Giemsa working solution to cover it.

Blood film preparation

1. Puncture 3rd or 4th finger aseptically and touch the blood drop with clean slide for thick and thin smears
2. Using the cover of another slide, prepare thin blood film and air dry.
3. Fix thin film with 100% methanol for 15-30seconds and stain with Giemsa working solution for 15-30 minutes.
4. Rinse with tab water and air dry
5. Observe under microscope (100x objective)

DECLARATION

I declare that this research paper is my original work and has not been presented for degree in this or any other University. All sources of materials used in this thesis have been duly acknowledged.

Name of the student: **Abebaw Tiruneh** Signature _____ Date _____

Name of the institution: Jimma University

Approval of the advisors

This paper has been submitted with my approval.

Dr. Delenasaw Yewhalaw Signature _____ Date_____

Mr. Endalew Zemene Signature _____ Date_____

Name of institution: Jimma University

Approval of the internal examiner

Mr. Mitiku Bajiro Signature _____ Date _____

Name of the institution: Jimma University