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Editorial

Ethiopia: Moving towards evidence-based malaria elimination program

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As affirmed in the 2010 review (MOH 2010) and repeatedly underscored in articles in this special issue (Nigatu et al. 2019.1, 2019.2; Yohannes 2019; Mekuria et al. 2019; Waldetensai et al. 2019) and all major government (MOH 2010; MOH 2011; MOH 2014) and other review (Deressa et al. 2006; Alemu et al. 2012; WHO 2017; Deribe et al. 2017; Vajda and Webb 2017; EPHI 2016) documents, malaria remains a major challenge in Ethiopia. In spite of important gains in anti-malarial interventions “Almost three-fourths of the total landmass and above half of the total population are at malaria risk” (Nigatu et al. 2019.1). Major challenges remain and in the perpetual dance a duo [or is it a trio (parasite-vector-host)?] (McCann 2014) we should anticipate surprise moves such as new vectors (Kinfe et al. 2019); more outdoor transmission (Nigatu 2019.2; Gatton et al. 2013 citing cases from Ethiopia); peak biting activity shifting to the early part of the night or “… shifting behavior of malaria vectors following prolonged deployment of indoor based vector control interventions (IRS and LLINs)” (Nigatu 2019.2); potential for reintroduction in areas where it has been eliminated (Yukich et al. 2013); climate change induced malaria shifting to higher altitude areas (Vajda and Webb 2017; Alonso et al 2011; Siraj et al. 2014; Bouma et al. 2016); development related e.g. dams increases (Deribe et al. 2017; Vajda and Webb 2017; Lautze et al. 2007; Kibret et al. 2016); or malaria considered a predominantly rural disease becoming a considerable urban health problem (Donnelly et al. 2005). On the opportunities side, note the possible return of chloroquine-sensitive Plasmodium falciparum parasites (Mekonnen et al. 2014) and Ethiopians holding lead positions as CEO of the Roll Back Malaria (RBM) Partnership or director-general of the World Health Organization (PATH MACEPA 2017).

In this context of high burden of disease and major social and economic impact (Nkumama et al. 2017) and even famine (Mengesha et al. 1998), the lure of resolving the problem once for all – elimination/eradication - is compelling. Thus, Ethiopia decided, in 1958, probably prompted by the well documented and severe epidemic in that year (Fontane and Najjar 1961), to join the malaria eradication effort of the 1950s. A lot has been written about the motivations, outcomes and challenges and failures of that effort (Kitaw 1969, 1981; Farid 1980; Gish 1992; Kitaw et al. 1998). Now, some 60 years later, Ethiopia has once again joined the elimination (an euphemism for eradication?) band wagon – “at the cutting edge” (McCann 2014) - and was one of the first countries to embrace the Scaling Up for Impact (SUF) concept for malaria control and develop successive strategic plans to achieve malaria elimination within specific geographical areas with historically low malaria transmission by 2015 (MOH 2010 2014). The government has developed a national strategic plan (2014 – 2020) for the prevention, control and elimination of the disease (MOH 2014; Kinfe 2019). In spite of major gains - 39% of households with at least one LLIN for two people (Nigatu et al. 2019.2), 95% reduction in deaths over 25 years (Deribe et al. 2017) -, major challenges persist (MOH 2011; Deribe et al. 2017) and achieving ‘elimination’ will require sustained and evidence-based policies, strategies and implementation plans.

This is where research institutions such as EPHI could, as shown in the articles in this special issue, play a pivotal role. The government (MOH 2010; Nigatu et al. 2019.2) has called upon research institutions and universities to support its effort with operational research. As demonstrated by articles in this issue and others, the needs are extensive. Entomological information is still limited (Kinfe 2019). There are local evidences of widespread resistance to insecticides used for IRS (Nigatu et al. 2019.1; Mekuria et al. 2019) and LLINs (Nigatu et al. 2019.2; Waldetensai et al. 2019); the need therefore to develop and implement a national plan for insecticide resistance monitoring and management. For IRS, there is need to assess quality on different wall surfaces, determine the residual efficacy, estimate the decay rate, evaluate community acceptability etc. (Nigatu et al. 2019.1). Of note, community resistance was one of the major challenges of the previous malaria eradication program (Kitaw 1969) and present efforts (Deribe et al. 2017) in the highly ecologically, socially and economically diverse (Kitaw et al. 2017) Ethiopian context. Potential mosquito breeding sites should be identified and appropriate Mosquito Larval Source Management (LSM) undertaken (Yohannes
Studies on effective duration, retention and efficacy of LLINs are scarce (Mekuriaw et al. 2019; Waldeyesa et al. 2019). There are also signs of increasing parasite resistance (Mekonnen et al. 2014; Beyene et al. 2016). The potential impacts of development activities and accelerating urbanization; the assessment and adoption new technologies; e.g. remote sensing (Midekisa et al. 2012), malaria antibody (i.e. serological) test (Birhanu et al. 2018) require studies as “Conventional tools of malaria surveillance and response are likely not sufficient in many elimination settings (Jacobson et al. 2017) even though the Integrated Disease Surveillance and Response (IDS&R) for malaria in Ethiopia is considered reasonably functional (Jimma et al. 2012). Overall, combined environmental monitoring and epidemiological surveillance and spatially stratified approaches that reflect the altitudinal and climatic diversity of the country (Midekisa et al. 2015) are required. There is also an urgent need to harmonize information collection and reporting (MOH 2011).

The future of the malaria program holds promises and challenges. “Malaria is a multifactorial disease, because the etiological agent has a complex life cycle requiring an insect vector, and the factors that regulate its distribution and abundance are diverse and complex” (Siraj et al. 2014; see also McCann 2014). As indicated in the FMoH 2010 review, “During the last decade, there have been substantial changes in the interventions and the delivery strategies for essentially all of malaria control… We can expect that a similar or even faster degree of change will occur in the coming years. In the coming decade, we can anticipate the arrival of a malaria vaccine, new diagnostics, new drugs and insecticides, and new strategies, including enhanced surveillance, to interrupt transmission” (MOH 2011). On the other hand, there are indications of emerging behavioral and species changes but the data base is weak (Gatton et al. 2013).

Lessons from previous experiences, the eradication attempt in particular (Kitaw 1969; Kitaw et al. 1998), show that the challenges of the elimination effort should not be underestimated. Currently, the global fight against malaria has stalled (WHO 2018). The bottom line for success is anchoring programs on as evidence-based – “thoroughly evaluating societal and political issues” (Aylward et al. 2000) in particular -, adapted and sustainable program as possible.

For academic and research Institutions, the challenge is “To identify problems and to design and implement research activities that fit the local conditions (representativeness & standard of protocol) so that the findings will have the quality to be used to influence policy” (MOH 2010). This implies major efforts in defining/reviewing priorities, building capacity and fostering collaboration. The articles in this special issue seem to indicate that EPIH is on track as they indicate focus on priority issues, building capacity and collaboration as most articles are multiple authors involving multiple institutions (NMCP, FMoH; Arab Minch U; Jimma U; Mekelle U; AAU; PATH/MACEPA; UNICEF; CSA; USAID/PMI; ACIPH). It seems appropriate to conclude with the wise words of the Second General Report of the Malaria Commission of the League of Nations issued in 1927: “… the fight against malaria must be waged not as a separate and isolated task but as part of a general social, economic and sanitary campaign by an enlightened public health service which is able to obtain assistance from other Government departments and from unofficial agencies, and to secure continuity of action and unity of purpose” (as quoted in Aylward et al. 2000) and those of African malaria experts “…malaria can be beaten, but it will require significant political will as well as continued research on scientific breakthroughs and an emphasis on using both old and new tools” (Wernsdorfer et al. 2009; Kamwi et al. 2018). As McCann (2014) points out, “We have some tools, some hope, but no panacea”.

References


Performance evaluation of chemical insecticides used for indoor residual spraying against Anopheles arabiensis in Ethiopia


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Abstract

Introduction: Monitoring of the residual efficacy of insecticides is essential to determine the periods that they remain effective in interrupting malaria transmission and schedule when to re-spray. There is a critical need to establish evidence-based information for the insecticide(s) that would be used for IRS in Ethiopia.

Objectives: (1) To assess the Indoor Residual Spray quality on different wall surfaces. (2) To determine the residual efficacy of Benidocarb 80% WP, Propoxur 50% WP and Actellic 300 CS sprayed on different wall surfaces against An. arabiensis. (3) To estimate the decay rate of Benidocarb 80% WP, Propoxur 50% WP and Actellic 300 CS sprayed on different wall surfaces. (4) To evaluate the community acceptability of the Indoor Residual Spray.

Materials and Methods: The study employed both cross-sectional (assessment of quality of spray) and longitudinal monitoring of the residual efficacy of chemical insecticides on different wall surfaces of sprayed houses in purposively selected villages. In Wondo Genet 48 test houses and 4 control houses were selected for the study. Similarly, 36 test houses and 3 control houses were used for the Mirab Abaya study. Each insecticide treatment was carried out in 12 houses for each wall type on the condition that all wall types were present in each study village. This means that an insecticide was sprayed in 48 and 36 houses respectively where the wall types were rough, smooth, cement and painted. The concentration of the insecticides was assessed by High Performance Liquid Chromatography.

Results: The first, second, third and fourth rounds test results summarized as follows. The overall mosquito mortality exposed to wall surfaces sprayed with Propoxur 50% WP ranged from 82.6% - 100% and 91% - 100% for Wondo Genet and Mirab Abaya sites, respectively. The overall mosquito mortality exposed to wall surfaces sprayed with Benidocarb 80% WP ranged from 85.4% - 100% and 91.1% - 100% for Wondo Genet and Mirab Abaya sites, respectively. The overall mosquito mortality exposed to wall surfaces sprayed with Actellic 300 CS ranged from 96.9% - 100% and 94.8% - 100% for Wondo Genet and Mirab Abaya sites, respectively. At round five of the assessment, mosquito mortality from Actellic 300 CS sprayed wall surfaces was 84.4% and 87% for Mirab Abaya and Wondo sites, respectively. During the six round residual efficacies monitoring mosquito mortality was found to drop to 52.2% and 48.3% for the two sites, respectively, which is below the required WHO cutoff (point ≥ 80%). The effect of Benidocarb 80% WP on mosquitoes' mortality was found below the WHO cutoff value during the fifth and sixth round monitoring (51.5% for Mirab Abaya site in the sixth round, and 70.9% then 41.25% for Wondo Genet sites in the fifth and sixth round monitoring, respectively) with exception in the fifth round for Mirab Abaya sites (84.1%). The performance of Propoxur 50% WP was below the required WHO value for both sites in the fifth and sixth round monitoring (78.2% and 77.4% in the fifth round, and 50.3% and 51.4% in the sixth round for Mirab Abaya and Wondo Genet sites, respectively). The performance of the Propoxur 50% WP, Benidocarb 80% WP and Actellic 300 CS on rough, smooth, cement and painted wall surfaces was similar up to the third-round monitoring. Differences in performance were observed at fourth and fifth round monitoring. With regard to acceptability of Indoor Residual Spray, 90.5% of the respondents in Wondo Genet site agreed to allow house spraying in the future and .100% of the respondents in Mirab Abaya site agreed to allow IRS for malaria and other vector borne diseases protection.

Conclusion: The performances of Actellic 300 CS and Benidocarb 80% WP were better in smooth, cement and painted wall surfaces than the rough wall surface types which is found within the WHO recommendation limit. For drawing further conclusions similar studies are recommended in other sites by different partners in a collaborative and coordinated manner.

Key words: Malaria, insecticides, indoor residual spraying, performance, Anopheles arabiensis, Ethiopia

Introduction

In Ethiopia, malaria is among the top public health significant diseases. Almost three-fourths of the total landmass and above half of the total population are at malaria risk (Terefe et al. 2015). According to National Malaria Control Program recent report indicates that about 2,174,707 malaria cases and 662 deaths occur annually (Federal Ministry of Health
Generally, indoor residual spraying (IRS) is one of the key elements of malaria vector control and has been in use since the 1950s. IRS is the application of a long-lasting, residual insecticide to potential malaria vector resting surfaces such as internal walls, eaves and ceilings of all houses or structures (including domestic animal shelters) where such vectors might come into contact with the insecticide. IRS and long-lasting insecticidal nets (LLINs) are pillars in malaria prevention and control strategy in Ethiopia and are being used on a large scale. Since 2005, there has been a large-scale application of chemical-based malaria vector control in Ethiopia including indoor residual spray and long-lasting insecticidal nets (FMOH Report 2010). However, appearance and widespread of resistance in malaria vectors has become a potential threat to eliminating malaria from the country in the upcoming decade. There are local evidences showing the widespread of insecticide resistance to various chemicals used for IRS (Yewhalaw et al. 2010; Asale et al. 2014; AIRS 2014).

Monitoring of the residual efficacy of insecticides is essential to determine the periods that they remain effective in interrupting malaria transmission and schedule when to re-spray (WHO 2012). The efficacy of IRS could be influenced by different factors such as mosquito susceptibility to insecticide, mosquito behavior (endophilic and endophagic), type of sprayable surfaces, quality of IRS, residual efficacy of an insecticide and community acceptance (WHO/CDS/NTD/WHOPES/GCDPP/2006.1; Das 2007). In addition, other factors have been reported to contribute to the residual efficacy of an insecticide, such as: the type of insecticide; formulation; applied dose; physical and chemical properties of the sprayed surfaces; and weather conditions (Verrone 1962; de Arias et al. 2003, 2004). The insecticide should be sufficiently stable to maintain biological efficacy on treated surfaces over time in order to minimize the number of spraying cycles required to cover the targeted malaria transmission seasons (WHO/CDS/NTD/WHOPES/GCDPP/2006.3). An insecticide for IRS is considered to have adequate residual efficacy when mortality of the exposed mosquitoes is ≥ 80% 24 hours post-exposure (WHO/CDS/NTD/WHOPES/GCDPP/2006.1).

*Anopheles arabiensis* is the most important malaria vector in Ethiopia (White et al. 1980). It is found in all malaria endemic administrative regions of the country (O’Connor 1967). Other malaria vectors in the country include *An. pharoensis*, *An. funestus*, and *An. nili* (Gebre-Mariam 1988; Nigatu et al. 1995) recorded the association of DDT resistance with inversion polymorphism in *Anopheles arabiensis* from Ethiopia. Abose et al. (1998) also reported DDT resistance in the primary malaria vector (An. arabiensis) in Arbaminch and Gambella, of southern and southwestern Ethiopia. Similarly, Balkew et al. (2003) reported different resistance levels to Pemethrin and Propoxur against the same vector in eastern Ethiopia. Presently, unpublished reports also indicate that the country’s primary malaria vector has developed different levels of resistance to all classes of insecticides that are being used for vector control. This calls for generation of quality data on insecticides currently in use and new ones to be registered for use in the future.

Careful planning, monitoring, and evaluation processes are more important than ever to maximize the benefits of the limited resources. Accordingly, the Federal Ministry of Health, together with EPHI and other partners, has planned to conduct different surveys (Mosquito behavior and its dynamics, Decay rate study, Susceptibility study, LLINs longevity study) to assist policymakers, health care providers, and partners to have a source of important information that can be used in decision making in the areas of malaria prevention and control. Therefore, there is a critical need to establish evidence-based information for the insecticide (s) that would be used for IRS. The information generated from this study will be useful in planning the introduction of new insecticides, replacement of resistant insecticides and making decisions to procure insecticides to be used for IRS.

**Materials and Methods**

**Study area:** The study was carried out in two sites namely Wondo Genet and Mirab Abaya areas. Wondo Genet is found in Oromia Regional State, located on the western escarpment of the central rift valley of Ethiopia, about 270 km south of the capital city, Addis Ababa. Mirab Abaya Woreda is located about 520 km south of Addis Ababa in Gamo Gofa Zone, SNNP Regional States. The two sites are malaria endemic and selected as sentinel sites for monitoring performance of insecticides and anti-malarial drugs designed to represent the different eco-epidemiological settings in the country.

**Study design:** The study employed both cross-sectional (assessment of quality of spray) and longitudinal residual efficacy study in purposively selected villages. Up to two villages with a relatively easy access and characterized by higher malaria transmission were selected from each district. House selection were based on the availability of different wall types such as mud (rough mud, smooth mud, dung, cement or painted), and brick. In Wondo Genet 48 test houses and 4 as control houses were selected for the study. Similarly, 36 test houses and 3 control houses were used for the Mirab Abay study. Each insecticide was sprayed in 12 houses of each wall type on the condition that all wall types were present in each study village. This means that an insecticide was
sprayed in 48 houses where the wall types were rough, smooth, cement and painted.

**Setting the application of insecticides:** The IRS was conducted by well trained personnel. They were recruited from the local community trained and supervised by study team from EPHI and NMCP/FMOH. Preparations were undertaken in households prior to the spraying which include: removal of movable household utensils; covering of non-moveable contents with plastic sheets; and removal of wall coverings and curtains. Household occupants were instructed to stay outdoors during and for at least 2 hours of post spray.

**Conducting application of insecticides:** Bendiocarb 80% WP, Propoxur 50 % WP and Actellic 300 CS were sprayed on all wall surfaces at the recommended doses. Bendiocarb 80% WP was sprayed at a rate of 0.4 g active ingredient (a.i.)/ square meter (sq. m), Propoxur 50 % WP at 2 g a.i./sq. m and Actellic acid 300 CS at 1 g of a.i./sq. m (FMoH 2012). Spraying was done by using a Hudson® X-pert compression sprayer (Hudson Manufacturing Company) with a flat nozzle as recommended for IRS following the national spraying operation guidelines (FMoH 2012) and WHO recommendations (WHO 1989). A distance of 45 cm from the nozzle tip to the surface to be sprayed was maintained during spraying. At this distance, the width of the swath at the point of impact was 75 cm. A 5 cm overlap was maintained between the swathes to make sure that no wall surface was left unsprayed.

**Assessment of spray quality:** To assess the quality of spraying, three Whatman® filter paper No. 1 (size 10 cm x 10 cm) leveled properly were placed on the walls of each surface type (one at low, one at middle and one at upper part) of each wall surface type in each house, before spraying, and removed after complete drying. The papers were wrapped in aluminum foils and subjected to analysis for required insecticide concentration. The chemical analysis results were combined for each substrate to provide the average concentration of insecticide (in mg/m). Insecticide concentration in the Whatman® filter paper was analyzed using High Performance Liquid Chromatography (HPLC).

**Cone wall bioassay test:** Assessment of the bio-efficacy and residual performance of insecticides sprayed on wall surfaces was a longitudinal study aimed at collecting information on monthly basis for six or more month’s period. Bio-efficacy and decay rate monitoring of the insecticides on wall surfaces were measured using standard WHO cone tests (WHO/ CDS/NTD/WHOPEX/GCDEPP/2006.3) in houses on different wall surfaces and each surface was replicated four times and one unsprayed house served as control. To assess the quality of spraying, cone testes were carried out 1 – 2 days after spraying. Thereafter, performance of the residual efficacy of each insecticide on treated wall surfaces was monitored on monthly bases post IRS for 6 consecutive rounds.

On a single wall surface three cones were fixed using fine steel pins at the lower, middle and upper parts of the wall. Cones were not fixed on areas 60 cm above the ground and 60 cm below the roof. Three to five days-old unfed females of *An. arabiensis* of insectary colony were used. Ten mosquitoes were gently transferred into each cone and exposed for 30 minutes. At the end of exposure time, the mosquitoes were transferred back into insecticide free holding paper cup for 24 h holding period and were provided with 10% sugar solution. Paper cups were kept in wooden box or cartoon which was covered by damp towel to create favorable temperature (27±2 °C) and humidity (70±10).

Bioassays were terminated when the mortality was below 80% in two consecutive bioassays. Mortality counts were taken 24 h post exposure. Mosquitoes were classified as dead if they are immobile or unable to stand or fly in a coordinated way. When average mortality of the controls was between 5% and 20%, test mortality was corrected using Abbot’s formula. But if control mortality was above 20%, the results were discarded and the tests were repeated.

**Acceptability of the IRS by the community:** Data were collected from households that were selected at the beginning and end of the study using well-structured questionnaire. Together with the insecticides’ impact on the target vector species, data for factors that can limit acceptability of the IRS such as the visible insecticide stains on the walls, an unpleasant odor, skin and nasal irritation were collected. Ease of application (mixing and spraying) by the operators and impacts on other household insects including nuisances were also recorded.

**Results**

**Results from High Performance Liquid Chromatography (HPLC):** The quality of spraying was assessed by chemical analysis from three Whatman® filter papers that were stuck on the walls of each surface type (one at lower, one at middle and one at upper part) in each selected house for the study. Chemical analysis using HPLC showed that the concentration of Bendiocarb 80% WP, Propoxur 50 % WP and Actellic acid 300 CS sprayed on all wall surfaces was found above the WHO recommended except for Propoxur in Wondo Genet found close to the borderline limit (Table 1). The chemical analysis from Whatman® filter paper was determined by type
of wall surfaces. The results of the analysis showed that the concentration of active ingredient per type of wall surface was again found above the WHO recommended dose for each insecticide except for Propoxur in Wondo near to the lower border limit (Table 2).

### Table 1: Active content of insecticides determined by High Performance Liquid Chromatography (HPLC) from samples of Whatman® filter paper collected from different location on wall surfaces

<table>
<thead>
<tr>
<th>Insecticide Type</th>
<th>Study site</th>
<th>Location of Wall surface</th>
<th>Sample count</th>
<th>Active Ingredient, gm/m²</th>
<th>WHO recommended dose (gm/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propoxur 50% WP</td>
<td>Arbaminch</td>
<td>Upper</td>
<td>12</td>
<td>2.852</td>
<td>2.7434</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Middle</td>
<td>12</td>
<td>2.429</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lower</td>
<td>12</td>
<td>2.968</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wondo Genet</td>
<td>Upper</td>
<td>12</td>
<td>1.749</td>
<td>1.558</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Middle</td>
<td>12</td>
<td>1.515</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lower</td>
<td>12</td>
<td>1.4092</td>
<td></td>
</tr>
<tr>
<td>Bendiocarb 80% WP</td>
<td>Arbaminch</td>
<td>Upper</td>
<td>12</td>
<td>0.7542</td>
<td>0.8497</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Middle</td>
<td>12</td>
<td>0.9636</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lower</td>
<td>12</td>
<td>0.8401</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wondo Genet</td>
<td>Upper</td>
<td>12</td>
<td>0.855</td>
<td>0.8681</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Middle</td>
<td>12</td>
<td>0.8967</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lower</td>
<td>12</td>
<td>0.8525</td>
<td></td>
</tr>
<tr>
<td>Actellic 300 CS</td>
<td>Arbaminch</td>
<td>Upper</td>
<td>12</td>
<td>2.0758</td>
<td>2.2712</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Middle</td>
<td>12</td>
<td>1.965</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lower</td>
<td>12</td>
<td>2.774</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2: Active content of insecticides determined by High Performance Liquid Chromatography (HPLC) from samples of Whatman® filter paper collected from different types of wall surfaces

<table>
<thead>
<tr>
<th>Insecticide Type</th>
<th>Study site</th>
<th>Type of Wall surface</th>
<th>Sample count</th>
<th>Active Ingredient, gm/m²</th>
<th>Average per wall surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propoxur 50% WP</td>
<td>Arbaminch</td>
<td>Unpainted</td>
<td>15</td>
<td>60.56</td>
<td>40.373</td>
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<td></td>
<td>Painted</td>
<td>21</td>
<td>35.46</td>
<td>1.773</td>
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<tr>
<td>Wondo Genet</td>
<td>Unpainted</td>
<td>6</td>
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<tr>
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<td>0.8766</td>
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<td>1.9033</td>
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<tr>
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<td></td>
<td>Painted</td>
<td>24</td>
<td>64.26</td>
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</table>

**Results of wall cone bioassay tests:** The following results were recorded based on WHO’s wall-cone assay procedure for Propoxur 50% WP, Bendiocarb 80% WP and Actellic 300 CS for total six rounds after initial spray. Field results for KD after 30 minutes were not considered due to inconsistent records. Results for mortality rate 24 hours after application of each insecticide are summarized in the following manners.

**Results of wall cone bioassay from Wondo site, Oromia Regional State:** Mean mortality rates of *An. arabiensis* exposed to Propoxur 50% WP sprayed rough mud wall surfaces was 100%, 95%, 99%, 70%, 81% and 21.7% in the first, second, third, fourth, fifth and sixth rounds, respectively. Similar pattern was observed for cement wall surfaces with 100%, 92.5%, 100%, 60% and 57.5% mortality rates in the first, second, third, fourth and fifth round tests, respectively. For smooth and painted wall surfaces 100% mortality rates were recorded till the fourth round of the study. The mosquito mortality rate on the smooth surfaces fall to 80% and 33.3% in the fifth and sixth round monitoring, respectively. While in the sixth-round monitoring, results for painted surfaces were found to be 41.4%. The overall mortality rates in the first, second, third, fourth, fifth and sixth were found to be 100%, 97%, 98.8%, 82.5%, 77.4% and 41.4%, respectively.

The performance of Bendiocarb 80% WP in the first, second, third, fourth, fifth and sixth round monitoring on rough wall surface was 100%, 99.2%, 100%, 86.7%, 75% and 30% respectively. For smooth wall surface, a mortality rate of 100% was recorded for the first three rounds while mortality rates of 73.3%, 55% and 43.3% were recorded on the fourth, fifth and sixth round monitoring, respectively. The performance of Bendiocarb 80% WP on painted surface was similar to
that of the smooth surface in the first three rounds. Whereas, mortality rates on Bendiocarb 80% WP sprayed cement surface was 100%, 87.5%, 96.7%, 90%, 54.4% and 36.7% in the first, second, third, fourth, fifth and sixth round, respectively. The mosquito mortality rate on bendiocarb 80% WP sprayed painted wall bioassay results declined to 47.5% in the sixth round from the 91.7% in the fourth round of the study. Over all 100%, 97%, 97.1%, 85%, 71% and 41.25% mortality rates were documented in the first, second, third, fourth, fifth and sixth round of the study, respectively.

The results for Actellic 300 CS showed 100% mosquito mortality on smooth wall type in the first four rounds. The mortality rate dropped to 96.7% and 56.7% in the fifth and sixth rounds, respectively. For painted wall surface 100% mosquito mortality was registered in the first three rounds while 95.5%, 95% and 41% were recorded in the fourth, fifth and sixth rounds, respectively. On the rough wall surface 100% mosquito mortality was obtained on the first two rounds, while mortality rates decreased to 95%, 92.5%, 84.4% and 45% in the third, fourth, fifth and sixth round, respectively. This pattern was mostly similar for cement wall surface type. Overall mosquito mortality rate on Actelic 300CS sprayed wall surface was 100%, 98.3%, 98.1%, 96.9%, 87% and 48.3% in the first, second, third, fourth, fifth and sixth round, respectively.

**Results from Mirab Abaya, SNPP Region:** Mosquito mortality rate on Propoxur 50% WP sprayed smooth and painted wall surface was 100% in the first three rounds. The mosquito mortality rate declined to 95.8%, 71.7 and 56.7% in the fourth, fifth and sixth rounds of the study, respectively on smooth wall surfaces. In the painted wall surface, mosquito mortality declined to 94.2%, 84.2% and 50% in the fourth, fifth and sixth rounds of the study, respectively. Similar performance was observed for rough wall surface with 100% mosquito mortality on first and third rounds, and 98.3% and 82.2% on the second and fourth rounds, respectively. Mosquito mortality rate during the sixth round was 42.2%. Overall, 100%, 99.4%, 98.1%, 91.5%, 78.2% and 50.3% mortality rates were recorded in the first, second, third, fourth, fifth and six rounds, respectively. Due to the problem of availability of cement wall surfaces, this type of test was not conducted in this study site.

The results for Bendiocarb 80% WP showed 100% mosquito mortality on both rough and smooth wall surfaces in the first two rounds. Mosquito mortality rate (87.7%) declined in the third and fourth on both rough and smooth surfaces. Moreover, mortality rate declined to 73.3% and 35% for rough surfaces, and 84.4% and 52% for smooth surfaces, in the fifth and sixth round, respectively. For painted wall surface, 100% mosquito mortality was recorded for the first-round while 98.3%, 95.8% and 100% were recorded in second, third and fourth round, respectively. For the fifth and sixth rounds 94.4% and 52% mortality rates were recorded, respectively. Over all, 100%, 99.4%, 90.8%, 91.1%, 84.1% and 51.5% mortality rates were recorded in the first, second, third and fourth round tests after spray. The results for Actellic 300 CS showed 100% mortality on rough, smooth and painted wall surfaces in the first two rounds. Mosquito mortality declined during third, fourth, fifth and sixth rounds for smooth and painted surfaces. The performance Actellic 300 CS was high on smooth and painted surfaces compared to the rough surfaces. Over all 100% mosquito mortality was recorded for the first and second round tests, while 98.7%, 94.8%, 84.4% and 52.2% mortality rates were registered for the third, fourth, fifth and sixth round, respectively.

**Efficacy of the three IRS insecticides:** The performance of the three IRS insecticides in the two study sites is presented in figures1 and 2. Figure 1 shows that Propoxur 50% WP and Bendiocarb 80% WP did not meet the WHO cutoff value, i.e., ≥ 80% (WHO 2006;1; WHO 2016), after the fourth-round wall bioassay tests in Wondo sites. Actellic 300 CS performed well up to the fifth month.
Figure 1: Result of Propoxur 50% WP, Bendiocarb 80% WP and Actellic 300 CS wall bioassay test (Round 1-6) Wondo Genet site, Oromia Region, Ethiopia.

Figure 2 shows that Propoxur 50% WP did not meet the WHO cutoff value after the four months in Mirab Abaya site. Whereas, Bendiocarb 80% WP and Actellic 300 CS fulfill the criteria up to fifth month.

Figure 2: Result of Propoxur 50% WP, Bendiocarb 80% WP and Actellic 300 CS wall bioassay test (Round 1-6) Mirab Abaya site, SNNPR, Ethiopia.

The performance of three insecticides sprayed on different wall surfaces: The decay rates of Propoxur 50% WP on rough, smooth, cement and painted wall surfaces were the same up to the third month in all sites. An interesting observation was that this insecticide performed well up to the fifth round on painted wall surfaces in all sites (above the required cutoff value) while the results from other wall surfaces were not sufficient to draw valid conclusion (effectively performed up to third rounds). Moreover, its performance was good on smooth wall surface up to the fourth round in both sites (Figures 3 and 4).
Figure 3: The decay rate effects of Propoxur 50% WP on rough, smooth, cement and painted wall surfaces, Wondo Genet site, Oromia Region, Ethiopia

Figure 4: The decay rate effects of Propoxur 50% WP on rough, smooth and painted wall surfaces, Mirab Abaya site, SNNPR, Ethiopia

The performance of Bendiocarb 80% WP on rough, smooth, cement and painted wall surfaces up to the third round was similar to the performance of Propoxur 50% WP, no differences in performance was observed on different wall surfaces in both sites. It was also found that this insecticide worked up to the fourth round on all wall surfaces. It also worked up to the fifth round on painted wall surface. The performance was low after the fourth round on rough, smooth and cement wall surfaces (Figures 5 and 6).
The performance Actellic 300 CS on rough, smooth, cement and painted wall surfaces was found to be above the WHO required cutoff value up to the fifth round. However, performance declined at the fourth round on rough wall types in Mirab Abaya site (Figures 7 and 8).
Figure 7: The decay rate effect of Actellic 300 CS on rough, smooth, cement and painted wall surfaces, Wondo Genet site, Oromia Region, Ethiopia

Figure 8: The decay rate effect of Actellic 300 CS on rough, smooth, and painted wall surfaces, Mirab Abaya site, SNNPR, Ethiopia

Acceptability of indoor residual spraying: Summary results on the acceptability of indoor residual spraying at the beginning of the study for both Wondo Genet and Mirab Abaya sites was presented in Table 3.
Table 3: Summary results on the acceptability of indoor residual spraying at the beginning of the study

<table>
<thead>
<tr>
<th>Questions asked</th>
<th>Wondo Genet site</th>
<th>Mirab Abaya (Mole village)</th>
</tr>
</thead>
<tbody>
<tr>
<td>If they know how malaria is transmitted? Those who responded yes</td>
<td>90.5%</td>
<td>88.6% (31/35)</td>
</tr>
<tr>
<td>If they protect themselves and family against this disease? Those who responded yes</td>
<td>85.7%</td>
<td>77.1%</td>
</tr>
<tr>
<td>Allowed spraying in all rooms of their houses</td>
<td>97.6%</td>
<td>91.4%</td>
</tr>
<tr>
<td>If the insecticides leave stains on walls? Those who responded yes</td>
<td>45.2%</td>
<td>48.6%</td>
</tr>
<tr>
<td>Those perceived that the sprayed insecticides would have effects on mosquitoes and bedbugs</td>
<td>90.5%</td>
<td>91.4%</td>
</tr>
<tr>
<td>Those who agree to use insecticide spray in the future</td>
<td>90.5%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Wondo Genet site: Ninety-one percent (38/42) of respondents mentioned that malaria is transmitted by insects and 85.7% (36/42) of them protected themselves and family against the disease. Of the total respondents, 97.6% of them allowed spraying in all rooms of their houses. Regarding the question asked if the insecticides stained the walls, 45.2% responded yes and 47.6% said no. The majority (90.5%) of the respondents perceived that the sprayed insecticides had effects on mosquitoes and bedbugs. Generally, 90.5% of them agreed to allow insecticide spraying in the future.

Mirab Abaya (Mole village): About eighty-nine (31/35) respondents mentioned that malaria is transmitted by insects and 77.1% of them protected themselves and family against this disease. Of the total respondents, 91.4% of them accepted house spraying operation. Forty nine percent of the respondents mentioned that insecticides sprayed left stains on walls. The majority (91.4%) of the respondents perceived that the sprayed insecticides would have effects on mosquitoes and bedbugs. All (100%) agreed to use insecticide residual spraying in the future for malaria and other vector borne diseases protection.

Results on the acceptability of indoor residual spraying at the end of the study period: The general pattern of response on acceptability of IRS at the end of the study period was similar to the beginning. Generally, over 95% of the respondents perceived that the insecticides sprayed on walls of houses would have effects on mosquitoes and bedbugs. Similar proportions (95%) agreed to use insecticide residual spraying in the future in both sites.

Discussion

The concentration of the insecticide assessed by conducting High Performance Liquid Chromatography (HPLC) on different wall types (smooth, rough, painted, unpainted, cemented) as well as different heights of each wall surface type (low, middle and upper part) in each selected house showed that the three insecticides sprayed fulfilled the WHO recommended doses. Previous study conducted on the effect of pH and wall type on the residual life of the carbamates bendiocarb and propoxur in experimental huts of Ethiopia has the limitation that no attempt was made to quantify the amount of insecticide deposited on the wall during the spray through the use of filter papers and chromatography or other methods (Yemane et al. 2016). This study is the kind that tried to quantify the amount of insecticide deposited on different wall surfaces during the spray in the community houses.

The round one, two, three and four round residual efficacy monitoring results in both Wondo Genet and Mirab Abaya sites indicated that the performance of all the three insecticides were good. The overall mosquito mortality rate due to Propoxur 50% WP ranged from 82.5% - 100% and 91% - 100% for Wondo Genet and Mirab Abaya sites, respectively. The overall mosquito mortality rate due to Bendiocarb 80% WP ranged from 85.4% - 100% and 91.1% - 100% for Wondo Genet and Mirab Abaya sites, respectively. The overall mosquito mortality rate due to Actellic 300 CS ranged from 96.9% -100% and 94.8% - 100% for Wondo Genet and Mirab Abaya sites, respectively. Relatively the performance of Actellic 300 CS was better than the other two insecticides after four months of the first spray. At round five of the assessment, mortality rate due to Actellic 300 CS was 84.4% and 87% for Mirab Abaya and Wondo sites, respectively. During the sixth round of monitoring mosquito mortality rates dropped to 52.2% and 48.3% for Mirab Abaya and Wondo, respectively, which is below the required WHO cutoff point ≥80% (WHO 2016). The performance of Bendiocarb 80% WP was below WHO cutoff value in the fifth and sixth rounds of the study (51.5% for Mirab Abaya sites in the sixth round, and 70.9% and 41.2 5% for Wondo sites in the fifth and sixth rounds, respectively) with exception in the fifth round for Mirab Abaya sites (84.1%). The performance of Propoxur 50% WP was below the required WHO cutoff value for both sites in the fifth and sixth round assessments (78.2% and 77.4% in the fifth round, and 50.3% and 41.4% in the sixth round for Mirab Abaya and Wondo sites, respectively). Previous study conducted in Ethiopia shows that the mortality rate of mosquitoes exposed to painted, dung plastered and mud plastered wall surfaces with bendiocarb and propoxur remained above 80% for six consecutive months in experimental huts (PMI Africa IRS 2016). The performances of Actellic 300 CS and Bendiocarb 80% WP were better in smooth, cement and painted wall surfaces than the rough wall surface types. The acceptability of indoor residual spraying in the two
sites was high. In general, 90.5% of the respondents agreed to use insecticide residual spraying in the future in Wondo Genet site. Whereas, all of them (100%) agreed to use insecticide residual spraying for malaria and other vector borne diseases protection in Mirab Abaya site. For drawing valid conclusions similar studies are recommended in other sites by different partners in collaborative and coordinated manner.

This investigation has the following limitations. Primarily the study omits the KD results due to inconsistent recordings in the field. On the other hand, literatures show that this type of recording is important for pyrethroid formulated insecticides. But this study focuses on non-pyrethroid insecticides which do have formulations under organophosphates and carbamates classes (Yemane et al. 2016). Secondly, some of the houses enrolled at the beginning of the study were skipped during the process of the follow up investigations due to various reasons.

Conclusions and Recommendations

This study provides evidence on the performance of three IRS insecticides in different wall surfaces of two study sites in Ethiopia. The first, second, third and fourth round test results of wall bioassay in both Wondo Genet and Mirab Abaya sites indicated that the decay rates of all the three insecticides were good. Proxoxur 50% WP and Bendiocarb 80% WP did not meet the WHO cutoff value, i.e., ≥ 80%, after the fourth-round wall bioassay tests in Wondo sites. Actellic 300 CS achieved well up to the fifth month. Proxoxur 50% WP did not meet the WHO cutoff value after the four months in Mirab Abaya site. Whereas, Bendiocarb 80% WP and Actellic 300 CS fulfill the criteria up to fifth month. The performance of Bendiocarb 80% WP was found good up to the fourth-round monitoring on different wall surfaces. It worked up to the fifth round on painted wall type. Yet, its performance was not in the acceptable range after the fourth round on rough, smooth and cement wall surfaces. The performance of Actellic 300 CS on rough, smooth, cement and painted wall surfaces was found above the WHO required cutoff value up to the fifth round. However, its performance declined at the fourth round on rough wall types in Mirab Abaya sites. It appeared that the performances of Actellic 300 CS and Bendiocarb 80% WP were found better in the smooth, cement and painted wall surfaces types than on rough wall surface types. Generally, from this study drawing conclusions may not be enough on IRS insecticides utilization and choices in Ethiopia to make decisions. Future assessment studies are relevant in another geographical areas that consider local parameters such different house structures to draw conclusion and recommendations.

Acknowledgements

We thank the Federal Ministry of Health, Regional Health Bureaus, Zonal Health Departments, District Health Offices, Health Centers and Kebele Administration Offices for their supports in this study. We acknowledge all participants, surveillance teams and health personnel for their contribution to this work. Informed consent of the subjects involved in the study was obtained. All technical personnel involved in this survey from EPHI and NMCP:MOH are highly appreciated for their excellent technical support in field mosquito collection and for their assistance in laboratory processing of mosquitoes. This work obtained financial & logistic support from Global Fund through NMCP:FMOH.

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Characteristics of *Anopheles arabiensis* larval habitats in selected urban areas in Kafa Humera district of Tigray Region, northwestern Ethiopia

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**Abstract**

**Introduction:** Mosquito Larval Source Management could be a valuable additional tool for malaria vector control especially in small urban hotspots where the breeding sites are limited in scope and where indoor residual spraying is not applied.

**Objective:** This study aimed to assess the abundance of potential mosquito breeding sites and identify the most important larval habitats of *Anopheles arabiensis* in urban settings of the agriculturally important district of Kafa Humera in western Tigray, northwestern Ethiopia.

**Methods:** Cross sectional larval surveys were conducted in October 2016 and 2017 in Setit-Humera town and nearby semi-urban communities of Adebay and Rayyan, in western Tigray. All water bodies encountered were sampled using standard dippers and their physical features recorded. A total of 132 potential mosquito breeding sites were visited and six aquatic habitats containing *Anopheles* larvae were identified: rain puddles, riverbed pools, vehicle rims, leaking water pipes, dugsouts, and artificial containers. Anopheine larval stages collected were reared and the emerged adults identified by morphological means. Sub-samples of *Anopheles gambiae s.s.* were identified by PCR in a parallel insecticide susceptibility bioassay study. Logistic regression analysis was used to determine the key ecological factors explaining the different densities of mosquito larvae.

**Results:** Of the 132 habitats visited, 70% were positive for anopheine aquatic stages, of which 82% comprised of high larval density sites. Eighty eight percent of the larval positive habitats also had the pupal stages, indicating the high productivity of the habitats. Ninety nine percent of the 4,925 morphologically identified *Anopheles* species were *Anopheles gambiae s.s.*, a subsample of which was identified as *Anopheles arabiensis* by PCR. *Anopheles arabiensis* larvae were more likely to be found in small and fully sunlit water bodies located within 10-20 meters of human habitats. Vehicle rims were 12 times (OR = 12; P < 0.001) more likely to be colonized by anopheline larvae than puddles and 5 times (OR = 5.19; P = 0.001) more likely than riverbed pools. Overall, human made water bodies were 5 times (OR = 5.19; P = 0.001) more likely to be colonized by anopheline larvae than natural water bodies. Besides, water bodies encountered in Rayyan were nearly 6 times (OR = 5.64; P < 0.001) and those in Adebay 5 times (OR=5.23; P = 0.003) more likely to host *Anopheles arabiensis* stages than water bodies in Humera town.

**Conclusion:** The findings suggest that targeting smaller human-made aquatic habitats could result in effective larval control of *Anopheles arabiensis* in the study area. Frequent surveillance of mosquito breeding sites, especially during the rainy season, is considered necessary for effective control planning.

**Keywords:** *Anopheles arabiensis*, urban, breeding site, larval habitat, Humera, Ethiopia

**Introduction**

Vector control, especially large-scale distribution of long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS), is among the key strategies that have contributed to the significant reduction in malaria morbidity and mortality in Ethiopia in the past 15 years. In order to maintain the current gains and progress further, attention should be given to the emerging threat of insecticide resistance (Messenger et al. 2017) and changes in behavior of *Anopheles arabiensis*, the main vector of malaria in Ethiopia. There is also a growing need to understand and plan control around special risk settings, including urban areas, water development projects and challenges posed by refugee populations and seasonal migrants (NMCT et al. 2014). As the population of sub-Saharan Africa rapidly urbanizes, there are worries that this could lead to increased risk of malaria in cities and towns located in endemic and fringe areas of malaria transmission. Although urban areas are normally characterized by low malaria burden (Robert et al. 2003), unplanned urbanization can lead to the proliferation of larval habitats that can potentially contribute to malaria transmission (Dongus et al. 2009). On the other hand, as malaria transmission in urban settings is usually lower and more focal than in rural settings (Stuedke et al. 2003; Hay et al. 2005), urban areas hold promise for vector control and integrated vector management (WHO 2013). Although nearly 84% of the Ethiopian population is still rural (CSA 2007), in view of the rapidly growing number of small- and medium-sized towns, there is a pressing need to give utmost attention and improve our understanding of the epidemiology of malaria in these
settings. Setit-Humera is one of the populous towns in the country that could benefit from specialized municipal vector control strategies and surveillance (NMCT et al. 2014). Setit Humera is located in the high malaria endemic district of Kafa Humera in Tigray, northwestern Ethiopia. A number of other fast-growing towns in this area include Maikadra and Dansha.

The epidemiology of malaria in Kafa Humera is complex due to cross border trade, huge agricultural activity and seasonal migration of young workers. The towns in this area host tens of thousands of these non-immune migrant laborers during the weeding and harvesting season which overlaps with the main malaria transmission season. The migrant workers can not only increase the intensity of malaria transmission in the area, they can also serve as a source of infection back in their villages. To date, vector control in Kafa Humera area is limited to distribution of LLINs. Indoor residual spraying (IRS) operation has been discontinued in the area some 10 years back. Although larval control through environmental management and larviciding is an integral part of the malaria control initiatives in Ethiopia, in general, yet little has been done in the district due perhaps to limited expertise and the necessary basic resources. As a result, outbreaks are common, the most recent one being the one that has occurred in Humera in 2014. Mosquito Larval Source Management could thus be a valuable additional tool for malaria vector control in these areas. However, this requires knowledge of the distribution and characteristics of larval habitats for planning and implementing larval control strategies effectively. The purpose of the current study was to assess the abundance of potential mosquito breeding sites and identify particularly productive larval habitats of *An. arabiensis* in the town of Setti Humera and two semi-urban communities nearby.

**Materials and Methods**

**Study area:** The study was conducted in October 2016 and 2017 in the town of Setti-Humera (14°16’50”N, 36°37’03”E; at 580-603 meters above sea level-masI) and semi-urban communities of Adayeb and Rawyan in Kafa Humera district of Tigray, northwestern Ethiopia. Setti-Humera town is located about 991 km northwest of Addis Ababa and 585 km west of Mekelle, the regional capital. Rawyan town (14°16’50”N, 36°37’03”E; at 603masI) is just 3kms southeast of Humera while Adayeb town (14°16’50”N, 36°37’03”E; at 659masI) is 16kms northeast of the town. Kafa Humera district is bordered by Sudan to the west and Eritrea to the north. It is one of the most fertile agricultural zones in the region with large scale farming of cash crops such as sesame, sorghum, and cotton. The area is rich in fertile black cotton soil and clay loam. Setti Humera is the administrative center of the district which covers an area of around 7000 km². The area is a *kola* (lowland) zone with arid and semiarid climate and consisting of mainly wide-open plains. Temperatures rise to an average of 42°C between April and June and falls to between 25 and 35°C during the moderate months of June through February. The mean annual rainfall is 540.6 and varies from 357.8mm and 650mm minimum and maximum respectively. On average the main rainy season (June—September) contributes 85% to the annual rainfall totals (Niguse & Aleme 2015). This is the planting and weeding time and the harvesting season extends from October to December. During this period, over 600,000 young workers migrate into the Kafa Humera lowlands mainly from the whole of Tigray, northern Amhara, and Sudan.

As current population figures are not available (2017), the extrapolated population of Humera town from the 2007 census is about 36,074. According to the local administration, the population of Adayeb is about 12,000 while that of Rawyan is around 8000. The most important water course in the study area is Tekeze River. Tekeze River is perennial and borders the town of Humera in the north and west. Four kilometers southeast of Humera town is found another seasonal river crossing the town of Rawyan east-west. The availability of water for both human and animal consumption is a serious problem in Kafa Humera district. Malaria and diarrhea are the major health risks in the zone. Malaria is associated with the onset and offset of rains and is transmitted by *Anopheles arabiensis* as in most parts of the country.

**Survey of Anopheles arabiensis breeding sites:** Searches for potential *An. arabiensis* breeding sites were conducted in October 2016 and 2017. Standing waters and around the three towns were checked for the presence of anopheline aquatic stages with a standard dipper (350 mls) (BioQuip Products, Inc. California, USA). From each habitat up to 10 dips were made depending on its size. During the survey, a habitat was first investigated for the presence of anopheline larvae visually. If anopheline aquatic stages could be seen in large numbers without dipping or nearly every dip contained anopheline larvae, the aquatic habitat was defined as having a high *Anopheles* density. Sites where only one or two dips out of 10 contained anopheline larvae, they were defined as having a low *Anopheles* density. The presence of pupae was also noted. For each larval habitat type, physical characteristics, including origin of habitat or habitat type (natural or human made), habitat size, light exposure, turbidity, presence of emergent vegetation/ and proximity to human habitation were estimated and recorded visually.
Mosquito identification: All anopheline aquatic stages were collected in small wide-mouthed jerry cans and reared in white enamel plates in a field laboratory in Humera town. Upon pupation, they were transferred into glass beakers and placed in mosquito cages until emergence. Emerged adult mosquitoes were morphologically identified according to the key of Gillies and Coetzee (1987). Emerged female adults of *An. gambiae* s.l. were used for insecticide resistance tests. Sub-samples of the test mosquitoes were processed for molecular identification using PCR and all identified as *An. arabiensis*. Hence, the anopheline mosquitoes morphologically identified as *An. gambiae* s.l. in the present survey shall hereafter be referred to as *An. arabiensis*.

Results

Species composition: All the aquatic stages collected from the anopheline larval habitats sampled were reared. Of the emerged 4925 anopheline adults, 4922 (99.94%) were *An. gambiae* s.l. (henceforth *An. arabiensis*) and the remaining 3 were identified to be *An. maculipalpis* (0.06%). Culicines were encountered in a couple of polluted waters but not quantified for the purpose of the present study.

Description of *An. arabiensis* larval habitat types: A total of 132 potential anopheline mosquito breeding sites were visited and six larval habitat types were identified in the area. These were grouped into 4 different types of habitats for data analysis: rain puddles (28), riverbed pools (54), vehicle ruts (40) and “Others” (10). The category “Others” included, pools from leaking water pipes (5), water tanks (2) and dug outs / ditches – in construction sites (3). Rain puddles and riverbed pools were considered as natural habitats while the rest were classified as temporary human made habitats. Of the 132 potential anopheline breeding sites, the most abundant type was riverbed pools (41%) followed by vehicle ruts (30%) and puddles (21%). Nearly 82% of the riverbed pools were recovered from Rawyan while 68% of the vehicle ruts were from Adebay town (Table 1).

Table 1: Frequency of potential anopheline mosquito breeding habitat types and presence of *An. arabiensis* aquatic stages in Adebay, Humera and Rawyan towns in western zone of Tigray, northwestern Ethiopia, Oct. 2016/17

<table>
<thead>
<tr>
<th>Variables</th>
<th>Puddles</th>
<th>Riverbed pools</th>
<th>Vehicle ruts</th>
<th>Others</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of habitats visited n (%)</td>
<td>28 (21.2)</td>
<td>54 (40.9)</td>
<td>40 (30.3)</td>
<td>10 (7.6)</td>
<td>132</td>
</tr>
<tr>
<td>Proportion of <em>Anopheles</em> larvae</td>
<td>42.9 (12/28)</td>
<td>66.7 (36/54)</td>
<td>90 (36/40)</td>
<td>80 (8/10)</td>
<td>69.7 (92/132)</td>
</tr>
<tr>
<td>Proportion of <em>Anopheles</em> positive sites that had high larval density</td>
<td>75 (9/12)</td>
<td>44.4 (16/36)</td>
<td>83.3 (30/36)</td>
<td>87.5 (7/8)</td>
<td>81.5 (75/92)</td>
</tr>
<tr>
<td>Proportion of <em>Anopheles</em> pupae</td>
<td>100 (12/12)</td>
<td>69.4 (25/36)</td>
<td>100 (16/36)</td>
<td>87.5 (7/8)</td>
<td>87 (80/92)</td>
</tr>
<tr>
<td>Number of habitats visited n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adebay</td>
<td>1 (3.1)</td>
<td>4 (12.5)</td>
<td>27 (84.4)</td>
<td>0</td>
<td>32</td>
</tr>
<tr>
<td>Humera</td>
<td>13 (40.6)</td>
<td>6 (18.8)</td>
<td>8 (25)</td>
<td>5 (15.6)</td>
<td>32</td>
</tr>
<tr>
<td>Rawyan</td>
<td>14 (20.6)</td>
<td>44 (64.7)</td>
<td>5 (7.4)</td>
<td>5 (7.4)</td>
<td>68</td>
</tr>
<tr>
<td>Proportion with <em>Anopheles</em> larvae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adebay</td>
<td>0 (0/1)</td>
<td>0 (0/4)</td>
<td>92.6 (25/27)</td>
<td>0</td>
<td>78.1 (25/32)</td>
</tr>
<tr>
<td>Humera</td>
<td>23.1 (3/13)</td>
<td>16.7 (1/6)</td>
<td>75 (6/8)</td>
<td>60 (3/5)</td>
<td>40.6 (13/32)</td>
</tr>
<tr>
<td>Rawyan</td>
<td>64.3 (9/14)</td>
<td>79.5 (35/44)</td>
<td>100 (5/5)</td>
<td>100 (5/5)</td>
<td>79.4 (54/68)</td>
</tr>
</tbody>
</table>

Determination of positive and negative mosquito breeding sites: Among the 132 potential mosquito breeding sites inspected in the three study sites, 70% were found to be positive for *An. arabiensis* larval stages (Table 1). Of these, nearly 82% had high larval density and 87% contained the pupal stages. Nearly 67% of riverbed pools and over 80% of vehicle ruts and others were positive for *An. arabiensis* aquatic stages. On the other hand, over 50% of the puddles did not harbor *An. arabiensis* larval stages during the survey period. Among the sites positive for *An. arabiensis* larval stages, 69% of riverbed pools and over 87% of the puddles, vehicle ruts and other habitats harbored the pupal stages (Table 1).

Spatial distribution of anopheline larval habitats: The distribution of anopheline larval habitats was heterogeneous across the three study sites. The most common potential anopheline larval habitat in Adebay town was vehicle ruts (84%), of which 93% harbored *An. arabiensis* aquatic stages. The seasonal stream about 0.5km south of Adebay was already dry during the survey period except one big pool and 3 smaller ones nearby. In Humera, rain puddles (41%) and vehicle ruts (25%) accounted for 66% of the potential *An. arabiensis* breeding habitats. Three fourth of the vehicle ruts and nearly one fourth of the puddles were positive for *An. arabiensis* aquatic stages. The contribution of the residual pools left by the receding Tekeze River was minimal during the survey period. The same was true with the big puddles located at the southern periphery of the town. On the other hand, riverbed pools (65%) and rain puddles (21%) were the most widespread habitats in Rawyan town. Nearly 80% of the riverbed pools and 64% of the puddles were positive for *An. arabiensis* aquatic stages. Overall, in Rawyan 79% of the 68 habitats contained *An. arabiensis* aquatic stages whereas 78% of the 32
Characteristics of Anopheles arabiensis larval habitats in selected urban areas of Kafa Humeraworeda, northwestern Ethiopia

habitats in Adebay and 41% of the 32 habitats in Humera town were positive for *An. arabiensis* aquatic stages. All the positive breeding sites in Adebay were created by vehicle ruts. On the other hand, puddles and riverbed pools in Rawyan town constituted over 81% (44/54) of the *An. arabiensis* positive breeding sites (Figure 1).

**Figure 1:** The percentage of habitat types positive for *An. arabiensis* aquatic stages in Adebay, Humera, and Rawyan towns in Kafa Humeraworeda district of Tigray, northwestern Ethiopia, Oct. 2016/17

**Characterization of *An. arabiensis* larval habitats:**

The key parameters of the potential anopheline breeding sites are summarized in Table 2 below. Accordingly, logistic regression analysis showed that vehicle ruts were 12 times (OR = 12; P < 0.001) and riverbed pools nearly 3 times (OR = 2.67; P = 0.04) more likely to be colonized by *An. arabiensis* larvae than puddles. Overall, human made water bodies (vehicle ruts, leaking water pipes, dugouts and artificial containers) were 5 times (OR = 5.19; P = 0.001) more likely to be colonized by *An. arabiensis* aquatic stages than natural water bodies (riverbed pools and puddles). Overall, among the larval habitat types, vehicle ruts were found to be the most productive habitats in terms of the proportion that had harbored the larval and pupal stages of *An. arabiensis* as well as the level of larval density.

**Table 2:** Factors associated with the presence of *An. arabiensis* larvae in the three study towns in Kafa Humeraworeda district, western Tigray, northwestern Ethiopia, October 2016/2017

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Variables</th>
<th>Number of Water bodies n (%)</th>
<th>Anopheline habitats n (%)</th>
<th>Univariate analysis 95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Towns</td>
<td>Adebay</td>
<td>52 (24.2)</td>
<td>25 (78.1)</td>
<td>5.23 (1.75 - 15.61)</td>
<td>0.003^p</td>
</tr>
<tr>
<td></td>
<td>Rawyan</td>
<td>68 (51.5)</td>
<td>54 (79.4)</td>
<td>5.64 (2.29 - 14.12)</td>
<td>&lt;0.001^p</td>
</tr>
<tr>
<td></td>
<td>Humera</td>
<td>32 (24.2)</td>
<td>13 (40.6)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Water bodies types</td>
<td>Puddles</td>
<td>28 (12.3)</td>
<td>12 (42.9)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Riverbed pools</td>
<td>54 (26.7)</td>
<td>36 (66.7)</td>
<td>2.67 (1.04 - 6.82)</td>
<td>0.04^p</td>
</tr>
<tr>
<td></td>
<td>Vehicle ruts</td>
<td>40 (20.5)</td>
<td>36 (90)</td>
<td>12 (3.35 - 42.97)</td>
<td>&lt;0.001^p</td>
</tr>
<tr>
<td></td>
<td>Other**</td>
<td>10 (5.9)</td>
<td>8 (80)</td>
<td>5.33 (0.95 - 29.81)</td>
<td>0.05^p</td>
</tr>
<tr>
<td>Size (m²)</td>
<td>≤ 1</td>
<td>107 (51.2)</td>
<td>83 (77.6)</td>
<td>6.15 (2.42 - 15.65)</td>
<td>&lt;0.001^p</td>
</tr>
<tr>
<td></td>
<td>&gt; 1</td>
<td>25</td>
<td>9 (36)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Stability and size</td>
<td>Temporary &lt;1m²</td>
<td>52 (26.3)</td>
<td>46 (88.5)</td>
<td>3.73 (1.34 - 10.35)</td>
<td>0.01^p</td>
</tr>
<tr>
<td></td>
<td>Permanent ≥1m²</td>
<td>55 (28.7)</td>
<td>37 (67.3)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Proximity (meters)</td>
<td>&lt;200</td>
<td>69 (33.8)</td>
<td>56 (81.2)</td>
<td>3.83 (1.48 - 8.90)</td>
<td>0.005^p</td>
</tr>
<tr>
<td></td>
<td>20 - 1000m</td>
<td>28 (14.7)</td>
<td>17 (60.7)</td>
<td>1.30 (0.48 - 3.57)</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>&gt;1000m</td>
<td>35 (18.5)</td>
<td>19 (54.3)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Water turbidity</td>
<td>Turbid</td>
<td>79 (42.1)</td>
<td>48 (60.8)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clear</td>
<td>53 (28.3)</td>
<td>44 (83)</td>
<td>3.16 (1.35 - 7.37)</td>
<td>0.008</td>
</tr>
<tr>
<td>Source of breeding site</td>
<td>Human made</td>
<td>50 (26.3)</td>
<td>44 (88)</td>
<td>5.19 (1.99 - 13.56)</td>
<td>0.001^p</td>
</tr>
<tr>
<td></td>
<td>Natural</td>
<td>52 (28.7)</td>
<td>48 (55.8)</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

^p = Unadjusted Odds Ratio; 95% CI = Confidence Interval

The analysis also showed that proximity to human dwellings, size and turbidity of water bodies appeared to influence the presence of *An. arabiensis* aquatic stages. Water bodies within 20 meters of houses were positively associated with the presence of *An. arabiensis* larvae (OR = 3.63, P = 0.005). Over 50% (69/132) of the sampled water bodies were within 20 m of human dwellings and of these 81% (56/69) were positive for *An. arabiensis* larvae. Water bodies <1m² in size appeared to harbor *An. arabiensis* aquatic stages 6 times (OR = 6.15, P < 0.001) more frequently than water bodies of a relatively bigger size. The size of the majority (81%; 107/132) of the water bodies visited was less than 1m². Nearly 78% (83/107) of these water bodies harbored *An. arabiensis* aquatic stages. On the other hand, there was a negative association between turbidity of water bodies and presence of anopheline larvae. Clear water bodies were 3 times (OR = 3.16, P = 0.008) more likely to harbor *An. arabiensis* aquatic stages than turbid/semi-
turbid water bodies. Overall, water bodies encountered in Rawya and Adebay were on the average 5.4 times more likely to host An. arabiensis aquatic stages than water bodies in Humera town.

Discussion

The purpose of the current study was to assess the abundance of potential mosquito breeding sites and identify the particularly productive larval habitats of An. arabiensis in the towns for informed larval intervention. Accordingly, the findings of this study revealed that the main potential breeding sites of An. arabiensis in these urban settings are riverbed pools, rain puddles, vehicle ruts, leaking water pipes, dugouts and artificial container habitats which are characteristic of many urban areas in Africa (De Silva and Marshall 2012; Mahgoub et al. 2017). Of the 132 standing water bodies visited 70% were positive for An. arabiensis aquatic stages, 82% of which had high larval density. Nearly 88% of the larval positive sites also harbored the pupal stages reflecting the high productivity of the habitats. The findings also indicated that human made habitats, such as vehicle ruts, leaking water pipes, dug outs and artificial container habitats, were more likely to be colonized by An. arabiensis aquatic stages than the natural habitats. Eighty eight percent of the human-made habitats had An. arabiensis aquatic stages compared to 59% of the natural habitats. It should be noted that although riverbed pools were categorized under natural habitats, many of the larval positive side pools in Rawya were associated with anthropogenic activities, such as sand excavation from the riverbed. Overall, vehicle ruts were found to be the most productive / preferred in terms of occurrence, abundance (density) and maturity (presence of late instars and pupae) of the aquatic stages of An. arabiensis.

In this study, human made surface waters in close vicinity to human habitation were more likely to be colonized by An. arabiensis larval stages than those far away from human dwellings. Proximity of households to standing water is proved to be a significant risk factor for increased malaria infection in Nazareth / Adama town in Ethiopia (Yohannes & Petros 1996; Peterson et al. 2009) as well as other urban areas in sub-Saharan Africa (Diedhiou et al. 2016; Staedke et al. 2003). Geographical localization and mapping of breeding sites would thus be needed to spatially rank malaria risk in urban settings and focus control activities on a small scale.

The distribution of anopheline larval habitats was heterogeneous across the three study sites. In Rawya, riverbed pools were the highest contributors during the survey period, accounting for approximately 65% of the larval positive sites. During the rainy season (June to September) the contribution of Rawya River appears to be minimal as it remains full until mid October, depending on the amount of rainfall and temporal distribution. Its influence is expected to increase after October as the river recedes.

On the other hand, the majority of the productive breeding sites in Humera and Adebay towns were temporary rain - dependent habitats and their contribution would be limited to the rainy season. In Adebay, vehicle ruts, along the streets of the town, were the only productive breeding sites of An. arabiensis, whereas in Humera vehicle ruts and rain puddles, in that order, accounted nearly 70% of the positive An. arabiensis breeding habitats. In Humera, An. arabiensis was also found breeding in water tankers and dug outs in construction sites which accounted for 23% of the vector positive sites. This indicates that filling puddles, vehicle ruts and dugouts and dealing with container habitats can significantly reduce the population of An. arabiensis mosquitoes in these towns. In Humera, as searches for breeding sites were confined to the streets and other open areas, additional larval habitats are likely to exist inside private and public compounds.

As elsewhere in sub-Saharan Africa (Gillies and Coetsee 1987; Mereta et al. 2013), An. arabiensis breeding sites in the study area were characteristically small, simlit and without emergent vegetation. Smaller fully simlit water bodies are generally characterized by high water temperature that promotes microbial growth and rapid larval development time (Pannatier et al. 2010; Machorl et al. 2009). In this study, An. arabiensis showed a preference for breeding in clear water and this is consistent with many studies (Diedhiou et al. 2016; Robert et al. 1998; Shillitoe et al. 2003). However, nearly 61% of the semi-turbid/ turbid pools were also inhabited by An. arabiensis aquatic stages. This is consistent with the report of Ye-Ehio et al. (2003), where An. arabiensis was found in turbid water. It should be noted that turbidity here was not due to water pollution. Besides, it would be difficult to compare outcomes from different studies if the degree of turbidity is not measured.

Overall, the findings of this study suggest that targeting rain puddles and smaller human-made aquatic habitats could result in effective larval control of An. arabiensis in the study area. In view of the absence of indoor residual spraying in the district as a whole, measures targeted against the larval stage of An. arabiensis should be of utmost priority as a complement to treated nets and prompt access to treatment. Paving the side streets with selected soil material and/or cobble stones would dramatically reduce the problem in Adebay and Humera towns.
Acknowledgements

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Conflict of interest: No conflict of interests.

References


First report of African malaria vector *Anopheles gambiae* (Diptera: Culicidae) from Ethiopia

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Abstract
Introduction: Reports of different researches conducted in Ethiopia consistently implicated *An. arabiensis* as the primary vector and *An. pharoensis, An. funestus* and *An. nili* as secondary vectors. These facts prevailed until today as the vectorial roles of the rest of *Anopheles* are not yet well known due to several limitations. This indicates that there is a need to identify other potential or suspected *Anopheles* species. The aims of this study were to determine *Anopheles* mosquito fauna, abundance and characterize mosquito breeding habitats in selected study sites in Ethiopia.

Materials and methods: A longitudinal study design with purposive and multi-stage sampling technique was conducted in Southern Nations Nationalities People’s Region of Ethiopia. The study was carried out in Jolie and Gogete study sites from Meskan and Sodo districts, respectively. A total of 4,118 third and fourth instars larvae of *Anopheles* mosquitoes were collected. Moreover, a total of 4,461 indoor adult female *Anopheles* were caught from both study sites. Identifications of *Anopheles* species were done using morphological keys under a compound microscope and polymerase chain reaction test for sibing species detection.

Results: Of the 4,118 samples collected ten *Anopheles* species from Jolie study site and eleven from Gogete study site were identified. Twenty seven percent of the *Anopheles* larvae were collected from marshy breeding site. In both study sites the predominant *Anopheles* species was *An. gambiae* s.l which is accountable for malaria transmission. Seasonal variations (between dry and wet season) statistically significant differences (P=0.001) in Jolie and in Gogete (P=0.001) study sites were observed in *Anopheles* larval collection. In Jolie and Gogete study sites *An. gambiae* s.l. was found to be the predominant species making up 64% and 64%, respectively. Statistically significant differences were observed in mean adult *An. gambiae* s.l. (P=0.002) and *An. pharoensis* (P=0.001) collection among the study sites. Besides, statistically significant (P=0.001) variations between wet and dry seasons were observed in the number of adult *An. gambiae* s.l. populations in both study sites. Of 239 *An. gambiae* s.l. analyzed by PCR, 94.1% were identified as *An. arabiensis* and 5.9% were identified as *An. gambiae*.

Conclusion: In the present study *An. gambiae* was reported for the first time in Ethiopia indicating the presence of the highly efficient vector in Ethiopia, thus establishing appropriate control strategy for this species might be necessary. The breeding habitats of *Anopheles* mosquitoes in the study sites particularly in the dry season were due to anthropogenic activities rather than environmental factors that require larval control.

Key words: Malaria vectors, larval habitat, *An. arabiensis*, *Anopheles gambiae*, Ethiopia.

Introduction
Reports of different findings conducted in Ethiopia consistently incriminated that *An. arabiensis* is the primary vector responsible for the transmission of malaria where as *An. pharoensis, An. funestus* and *An. nili* are considered as secondary vectors (Abose et al. 1998; Ameneshewia 1995; Gone et al. 2014; Animut et al. 2012; Fettene et al. 2004). Recently, based on strong genetic evidence the *An. gambiae* s.s M and S molecular forms and *An. quadriannulatus* species B are assigned formal names: M form (*An. coluzzii*) and S form (*An. gambiae*) and *An. quadriannulatus* species B (*An. amharicus*) (Ethiopia) (Coetzee et al. 2013). As demonstrated in other studies the taxonomy of this complex species, no distinct characters are available for rapid, accurate, morphological identification of the species and thus identifications must be based on the use of molecular methods (Coetzee et al. 2013).

However, the above mentioned data are facts until now the vectorial roles of the rest of *Anopheles* were not yet completely known due to several limitations. Most of the currently available entomological information is emanated only from limited areas where there are easily accessible means thus the information are insufficient (Gebre-Mariam et al. 1988). Besides, communication problem to reach inaccessible areas has hindered the research on malaria and its vectors. For instance, *An. tenobrosus* Donitz was found positive for *P. falciparum* gametocyte in south Ethiopia with infection rates of 15.8% by dissection and 7% by DNA hybridization (Adugna et al. 1998). This indicates that the need to identify other potential *Anopheles* species in the epidemiology of malaria throughout the country is unquestionable. On the other hand, investigating the recent variations in the abundance and dynamics of malaria vector populations is also important (Animut et al. 2012). Since, the abundance and dynamics of *Anopheles* population have significant impact on the intensity of malaria transmission from place to place (Katrijn et al. 2010). For example, a study conducted by Brooker et al. (2004) showed that the existence of variation in the intensity of incidence of malaria within the same area.
Pertinently, the density of malaria vectors has shown a great variation in the same topography even at household level within the same area. For instance, in Ethiopia White et al. (1980) around Gilgil Ghibe River valley reported that a variation in the density of malaria vectors with a result of less than 1 in January-March where as it go greater than 100 in July-October. But, in Gambela areas of Ethiopia, Krafur (1970, 1978) reported that the average hut resting density was less than 30. Meanwhile, a collections made in the 1958 malaria epidemics revealed the average hut resting density were vary from 100 to 150 (Fontaine et al. 1961). However, Indoor Residual Spray (IRS) and LLINs are reliable and effective in a wide range of situations (Noor et al. 2009; MOH 2011; Shargie et al. 2008, 2009). Similarly, the impact of IRS in lowering indoor densities of An. gambiae s.l. was reported in study conducted in western foothill of Madagascar (Ratovonjato et al. 2014). Therefore the aim of this study was to identify species composition, abundance and breeding habitat contribution for Anopheles mosquitoes in the selected study sites.

<table>
<thead>
<tr>
<th>Zone</th>
<th>Districts/Werada</th>
<th>Study site</th>
<th>Latitude/Longitude</th>
<th>Elevation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gurage</td>
<td>Meskan</td>
<td>Jolie</td>
<td>N 08°13.217’; E 038° 28. 268’</td>
<td>1842 m</td>
</tr>
<tr>
<td></td>
<td>Sodo</td>
<td>Gogete</td>
<td>N 08°10.371’; E 038° 30. 449’</td>
<td>1846 m</td>
</tr>
</tbody>
</table>

**Study design:** longitudinal study design using purposive and multi-stage sampling technique Southern Nations Nationalities and Peoples region (SNNPR), Gurage Zone as well as the two districts were purposively selected to identify species composition, abundance and breeding habitat contribution in the study sites. This study used two study sites and it was done by random selection from the list of malarious Kebeles (localities) in the district health office. Thus, study was carried out in Jolie and Gogete study sites from Meskan and Sodo districts, respectively.

**Sampling procedures**

**Larva collection, species identification and investigation of their habitat:** Immature stage collections were carried out to supplement the data of adult collection to identify species complex and determine the principal Anopheles species accountable for malaria transmission in the study areas (WHO 2013). The collections of Anopheles larvae were conducted in a variety of aquatic habitats of the study areas once per month by two collectors using dippers, pipettes and containers from temporary and permanent breeding sites (ponds, pools of rain water, water from broken pipes, River pocket, stagnant water in irrigation canals and marsh) in the study areas and the number of larvae collected from each larval habitats were compared to identify the major contributor of Anopheles mosquitoes breeding. The collected Anopheles larvae were killed in hot water (about 50°C) and preserved in small vials containing 70% ethyl alcohol (WHO 2013). All the specimens collected from a particular breeding place deposited in a labeled bottle or vial. The label were written in pencil on a piece of paper and dropped into the specimen bottle and transported to Addis Ababa (EPHI or AAU laboratory) for identification. The preserved specimens were soaked for 12 hours in watch glasses containing distilled water. Each larva is then mounted on a glass slide separately in a drop of gum chloral mountant and covered with a piece of cover slip. Each cover slip measuring 22x22 mm is cut into four parts and one piece is used for a single specimen. The gum chloral mountant is prepared in the laboratory as described in Gordon and Lavoipierre (1969). The constituents are 25 ml distilled water, 160 gm crystal chloral hydrate, 15gm gum Arabic, 10 gm glucose syrup and 5ml glacial acetic acid which are then mixed in the above order in a water bath at a temperature of 80°C. At all times the mountant is kept in a dark and well-screwed bottle. The identifications of species on immature made by using third and fourth instars larvae based on Verrone (1962a) and Gillies and Coetze (1987) under a compound microscope.

**Adult Anopheles collections, identification and density determination:** Indoor female Anopheles mosquito collection using mouth suction aspirators, CDC- light traps and pyrethrum spray collection methods were carried out once every month for a total of 26 houses of the study sites (WHO...
The collected female *Anopheles* mosquitoes were sorted, counted, categorized as unfed, fed, half gravid and gravid, finally placed in a separate labeled paper cups thus all collected specimens transported to Addis Ababa (EPhI or AAU laboratory) for species identification. The procedure of collection by the three methods described below (Samples collected from the study sites were stored in Entomology laboratory of EPhI with voucher number NATESA 312).

**Pyrethrum spray collection:** The pyrethrum spray collection method as described by WHO (2013) was employed to collect indoor resting adult *Anopheles* mosquitoes. Pyrethrum spray collections of indoor resting mosquitoes were carried out once every month early in the morning beginning 6:00 am to 10:00 am. After permission is granted from the head of household or inhabitants, all human occupants, animals, domestic utensils, exposed food and water were voluntarily moved out of the dwellings prior to the spraying all household items that cannot be moved out were covered with plastic material. Then, the entire floor were covered with white cloth sheets cut to 1.2mX2.20m, and doors and windows were closed, openings and eaves also properly covered and all entire parts of the rooms were sprayed with aerosol (containing pyrethrum) after spraying the dwelling were left closed for 20-30 minutes to produce a knockdown effect.

**Mouth suction aspirator collection:** Mouth suction aspirator collections of *Anopheles* mosquitoes were carried out on a different day once every month, early in the morning beginning 6:00 am to 10:00 am. Mouth suction aspirator and flash light were used to collect indoor resting mosquitoes from walls, under roofs, hanging clothes, household utensils and dark corners.

**CDC-Light traps collection:** CDC light traps collections were used to collect *Anopheles* mosquitoes once every month; CDC light traps were positioned inside and outside of selected houses from 06:30 pm to 6:00 am at night to collect *Anopheles* mosquito. To identify the species of *Anopheles* mosquito two methods were employed. One of the methods is morphologically based on keys under a stereo dissecting microscope with a magnification power of 20x (Verrone 1962b, Gillies and Coetzee 1987), the second is by using polymerase chain reaction (Scott et al. 1993).

**Molecular identification of adult *An. gambiae* complex using Polymerase Chain Reaction (PCR)**

**DNA extraction, amplification and gel electrophoresis procedures:** Almost 10% of indoor collected and morphologically identified as *An. gambiae* s.l were selected randomly and identified to sibling species by polymerase chain reaction (PCR), using an adapted version developed by Scott et al. (1993) based on species specific single nucleotide polymorphism (SNPs) in the intergenic spacer region (IGS) following a minor modification by Wilkins et al. (2006) incorporating intentional mismatch primer (IMP) to increase specificity was used for the allele amplification. Genomic DNA was extracted from legs and/or abdomen of individual mosquitoes according to the method described by Collins et al. (1987). The *Anopheles gambiae* s.l. legs and/or abdomen were grinded in 100µl grinding buffer solution (0.2M sucrose, 0.5% SDS, 0.1 M tris-HCl pH 7.5, 0.1 NaCl, 0.05M EDTA pH 9.1) using electric motor pestle with a sterile blue loop on centrifuge tubes until all parts remain homogeneous. The products of the grinded solution were heated at 65°C for 30 minutes. The DNA were precipitated by adding 18µl of 5M ice-cold Potassium acetate (KAC) and incubated in a container filled with ice for 30-60 minutes, next the solutions were centrifuged at maximum speed of 13, 200 revolutions per minute at room temperature for 20 minutes. The DNA supernatant were gently transferred (let alone transferring the precipitate) to a new labeled centrifuge tubes followed by adding 200µl of 100% ethyl alcohol, after that the solution mixed by shaking inverting the centrifuge tube and incubated inside a negative 20°C deep-refrigerator for overnight. In the next day the DNA were precipitated by centrifuging at maximum speed of 13, 200 revolutions per minute for 30 minutes at 4°C and then the supernatants were gently discarded without disturbing the pellets. Then, 200µl of 70% ethyl alcohol added and re-centrifuged for 10 minutes at 13, 200 revolutions per minute. Finally, the 70% alcohol was disposed slowly without disturbing the pellets; the DNA sediments were allowed to dry for 30 minutes at room temperature. Lastly, the DNA pellets were dissolved in 100µl sterilized water with gentle tapping of the tube to allow the DNA to re-suspend for amplification process.

Four species-specific and a universal primer were used in DNA coping processes, species-specific primer for *An. arabiensis* (AR-3T-R, GTG TTA AGT GTC CTT CTC CGT C); for *An. gambiae*, (GA-3T-R, GCT TAC TGG TTT GT CGG CAT GT); for *An. quadriannulatus*, (QD-3T-R, GCA TGT CCA CCA ACAG TAA ATC C); for *An. merus/melas* (ME-3T-R, CAA CCC ACT CCC TTG ACG ATG) and one universal primer sequence (IMP-UN-F, GTC GCG AGT TGT AGA GAT GCC). Mopti and Savanna DNA were sequenced using PCR amplified rDNA by the methods of Fanello et al. (2002) and Favia et al. (1997) and furthermore by DNA sequencing. Targeting rDNA intergenic spacer (IGS) for the *Anopheles gambiae* complex species were amplified in a multiplex reaction as illustrated by Wilkins et al. (2006). PCR reaction was carried out using TaqDNA polymerase (AccuStart II PCR Supermix) and the manufacturer’s (Quanta Biosciences) recommended buffer at 1×concentration was used for all reactions.
PCR reactions buffer consisted of a 25pmol (3.25 DI water) or/and free water, 6.25 AccuStart II PCR Supermix, 0.5 IMP-UN-F and 0.5µl of each primer concentration and 0.5µl of DNA in a final 12µl reaction mix. The plate covers were fastened tightly and spine down in microcentrifuge at maximum speed for two to three minutes and then amplified.

The final reaction mix, thermal cycling for all analyses was performed in a Bio-Rad iCycler®. PCR cycling consisted of melting at PCR conditions in 1: 95°C for 4" followed by 34 cycles of: 95°C for 30"; 60°C for 30" and 72°C for 30" and a final elongation step at 72°C for 5." Agarose gel was prepared on the bases described by Wilkins et al. (2006) protocol. The prepared agarose gel was placed in to electrophoresis box and 1µl of molecular weight ladder was loaded into the first and the last lane of the agarose gel, then 1µl of PCR amplified DNAs (the experimental and control) were loaded into the rest wells of the agarose gel.

The electrophoresis box positive and negative ion electrodexes properly plugged to the gel electrophoresis machines and subsequently allowed to run at a voltage of 90, 400amper for 90 minutes. DNA fragments of An. gambiae complex species were visualized by using Benchtop UV Transilluminator machine. MultiDoc-ItTM Imaging System-Masterflex computer software was used to identify DNA fragments and capture photo of the DNA bands. The gel electrophoresis (DNA fragment) results were interpreted by using bands of the markers on the first and the last lane of the gel.

An. gambiae s.l. sibling species were identified by comparing the DNA band size with already known molecular weight ladder bands. DNA fragments having 463bp were determined to be An. gambiae Giles/An. coluzzii Coetsee and Wilkerson, 528bp An. melas Theobald/An. merus Donitz, 636bp An. quadrimaculatus Theobald/ An. anthoracicus Hunt, Wilkerson and Coetsee and 387bp An. arabiensis Patton. The bp numbering is as previously designated [Genbank: AY787486].

**Data analysis:** The collected data were computerized using Epi Info 7 and analyzed using Stata SE 11.0, percentage, mean, proportion and 95% CI was constructed to help in making inference towards the target population, for significance differences (p<0.05) two-sample t test with equal variance and two-samples t test of proportion were used. The proposal was presented and reviewed by Addis Ababa University and Ethiopian Public Health Institute (EPHI) of Scientific and Ethical Review Office (SERO). In addition verbal and signed consent were obtained from the study participants for permission to carry out indoor adult female Anopheles mosquito collections using mouth suction aspirators, CDC- light traps and space spray in their houses.

**Results**

**Species composition, monthly variation and breeding habitats preference of Anopheles larvae:** A total of 4638 mosquito larvae were collected from different types of breeding sites, of which 4253 were Anopheles mosquitoes and the rest 385 were Culex larvae. About 135 of the larvae of Anopheles could not be identified because of bad preparation and mechanical damage, this 4118 third and fourth instar larva were identified. The majority 27.51% of Anopheles mosquito larvae were collected from marshy breeding site which served as a major breeding habitat during the dry season, followed by irrigation canals 24.50%, rain pools 20.45%, river pools 17.63% and ponds 9.91% in the study areas (Figure 1).

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**Figure 1:** Breeding habitats and percentage of Anopheles larvae collected in the study areas (May 2013 to June 2015)
Table 2: Species composition and percentage of Anopheles mosquitoes identified from larval collections in the study areas Ethiopia (May 2013 to June 2015)

<table>
<thead>
<tr>
<th>Species</th>
<th># Mosquitoes larvae collected n(%)</th>
<th>Total n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>An. gambiae s.l.</td>
<td>763 (36.1)</td>
<td>1482 (36.0%)</td>
</tr>
<tr>
<td>An. cinereus</td>
<td>599 (24.1)</td>
<td>988 (24.0%)</td>
</tr>
<tr>
<td>An. christyi</td>
<td>488 (23.1)</td>
<td>947 (23.0%)</td>
</tr>
<tr>
<td>An. pharoensis</td>
<td>149 (7.1)</td>
<td>284 (7.0%)</td>
</tr>
<tr>
<td>An. demeaelii</td>
<td>113 (5.4)</td>
<td>214 (5.2%)</td>
</tr>
<tr>
<td>An. garnaehi</td>
<td>37 (1.8)</td>
<td>66 (1.6%)</td>
</tr>
<tr>
<td>An. longipalpis</td>
<td>28 (1.3)</td>
<td>49 (1.2)</td>
</tr>
<tr>
<td>An. marshalli</td>
<td>20 (0.9)</td>
<td>33 (0.8%)</td>
</tr>
<tr>
<td>An. pretoriensis</td>
<td>2 (0.1)</td>
<td>4 (0.1%)</td>
</tr>
<tr>
<td>An. sergenti</td>
<td>2 (0.1)</td>
<td>4 (0.1%)</td>
</tr>
<tr>
<td>An. squamosus</td>
<td>0 (0)</td>
<td>47 (1.1%)</td>
</tr>
<tr>
<td>Total</td>
<td>2111 (100)</td>
<td>4118 (100)</td>
</tr>
</tbody>
</table>

[Graph showing Anopheles larval collections by month for Jolie and Gogete]

Figure 2: Anopheles larva collected in different months in Jolie and Gogete study sites (May 2013 to June 2015)

Identification of Anopheles larvae: A total of 2111 and 2007 Anopheles mosquito larvae were collected from Jolie and Gogete study sites, respectively. In Jolie study site ten Anopheles mosquito species were identified. An. gambiae s.l. was the predominant species accounted for 36.1% (n=763) followed by An. cinereus 24.1% (n=599), An. christyi 23.1% (n=488) and An. pharoensis 7.1% (n=149) the rest were
collected in small amount. In Gogete study site eleven *Anopheles* mosquito species were identified, similarly *An. gambiae* s.l. was also the dominant species accounted for 35.8% (n=719), followed by *An. cinereus* 23.9% (n=479). *An. christyi* 22.9% (n=459) and *An. pharoensis* 6.7% (n=135) (Table 2).

**Monthly dynamics of Anopheles larvae in study sites:** Statistically significant differences in Jolie (P=0.001) and in Gogete (P=0.001) study sites (seasonal variations) in *Anopheles* larval collection were observed. The highest mean 129.25 collection were done in the months of July, August, September and March in Jolie study site and in similar months the highest mean 121.5 collection were done in Gogete. The peaks of *Anopheles* mosquitoes were collected in major and minor rainy seasons (Figure 2).

**Indoor Anopheles mosquito collection:** A total of 4461 indoor adult female *Anopheles* were caught in both study sites, of which 31.1% (n=1386) from Jolie and 68.9% (n=3075) from Gogete. Of 1386 female *Anopheles* mosquitoes caught from Jolie study site *An. gambiae* s.l. was the predominant species accounted for 63.9%, followed by *An. pharoensis* 19% (Figure 3). In Gogete study site, 3075 of female *Anopheles* caught *An. gambiae* s.l. was also the predominant species accounted for 64%, followed by *An. pharoensis* 19.1% (Figure 3). Statistically significant differences were observed in both *An. gambiae* s.l. (P=0.002) and *An. pharoensis* (P=0.001) collection among the study sites, i.e. higher number of *An. gambiae* s.l. and *An. pharoensis* were collected in Gogete than the Jolie study site.

![Figure 3: Percentages of adult Anopheles mosquito species collected indoor using different collection method in the two study sites (May 2013 to June 2015)](image)

There was significant difference (P=0.001) in mean percentage in *An. gambiae* s.l between wet and dry seasons, in both Jolie and Gogete study sites, i.e. higher number of *An. gambiae* s.l. collected during wet season than dry season presented in Figure 4.

**Molecular identification of Adult Anopheles gambiae complex:** About 272 indoor collected and morphologically identified as *An. gambiae* s.l. were subjected for molecular identification by PCR. of which due to technical problem the result of 33 specimens were rejected. Thus our result showed that, of the total (n=239) *An. gambiae* s.l. (Figure 5) tested 94.1% were identified as *An. arabiensis* and 5.9% as *An. gambiae* (Table 3).

**Table 3: The number and percentages of Anopheles arabiensis and Anopheles gambiae identified by polymerase chain reaction (PCR) from Jolie and Gogete study sites**

<table>
<thead>
<tr>
<th>Species</th>
<th>Jolie n (%)</th>
<th>Gogete n (%)</th>
<th>Total n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>An. arabiensis</em></td>
<td>117 (48.9)</td>
<td>108 (45.2)</td>
<td>225 (94.1)</td>
</tr>
<tr>
<td><em>An. gambiae</em></td>
<td>4 (1.7)</td>
<td>10 (4.2)</td>
<td>14 (5.9)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>221</strong></td>
<td><strong>218</strong></td>
<td><strong>439</strong></td>
</tr>
</tbody>
</table>

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Figure 4: Monthly indoor collections of *Anopheles gambiae* s.l. in Jolie and Gogete study sites (May 2013 to June 2015)

Figure 5: Image of PCR result of Gel electrophoresis of *Anopheles gambiae* s.l species identification comparing products run on a 2% agarose gel red. Lanes 1 and 24 1kb ladder, lanes 2-5, 6 not amplified, lanes 7, 8, 11, 13, 15 and 16 were *An. arabiensis* and lanes 9, 10, 12, 14, and 17-20 were *An. gambiae* and lanes 21-23 control.
Discussion
This study showed the presence of *Anopheles* larvae throughout the study period. The persistence of *Anopheles* mosquitoes throughout the year in Jolie and Gojete study sites were mainly associated with irrigation practices and river pocket. In addition, the existence of permanent vector breeding habitats created by poor management of water supported the survival of vector species throughout the year. Ten *Anopheles* species in Jolie and eleven in Gojete study sites were identified during data collection period. In both localities the predominant *Anopheles* species was *An. gambiae* s.l. which is the main vector in the country (Abose et al. 1998; Ameneshewa 1995; Animut et al. 2012; Gone et al. 2014). *An. cinereus* and *An. christyi* are the second predominant species in the study areas. Similar to the present study *An. cinereus* and *An. christyi* have been reported from the neighboring villages at about the same altitude (Animut et al. 2012). The occurrence of high number of *An. cinereus* and *An. christyi* in this study might be the presence of marsh habitat in the area which is ideal habitat for these two species (Woyessa et al. 2004; Tesfaye et al. 2011; Animut et al. 2012). Substantial numbers of *An. pharoensis* were collected in the present study similar to the study conducted by (Animut et al. 2012) in the neighboring village.

Irrigation activities in the study area might be associated with the existence of *An. pharoensis* throughout the year, similar to the study conducted in the central rift valley of Ethiopia (Kibret et al. 2010). However, higher numbers of *Anopheles* mosquitoes larvae were collected during the wet seasons both in major and minor rainy seasons. The occurrence of higher number of *Anopheles* larvae in this study during the wet season is not consistent with the findings of the study conducted in neighboring villages by Animut et al. (2012) where more larval collections were recorded during the dry season. Frequent larval sampling during the wet season might be the reason for encountering larval positives rain pools in the present study compared to a study conducted by Animut et al. (2012). Local meteorological and other environmental factors might explain for the dominance of *Anopheles* larvae during the wet season.

During our study both temporary and permanent breeding habitats were dominantly occupied by *An. gambiae* s.l throughout the wet seasons. Alike, the study conducted in Butajira area near to the present study sites conducted by Animut et al. (2012) *An. gambiae* s.l was the predominant species in every types of habitat during the wet season. The monthly variation in the number of *An. gambiae* s.l. in the present study might be due to climatic factors such as the amount of rain, temperature and relative humidity.

The breeding habitats of *An. gambiae* s.l. in the study localities particularly during the dry season are due to human activities rather than environmental causes. That is, the water accumulated from overflow of irrigation canals, interrupted marshy areas and River pockets remain the most important breeding sites created by human activities. Similarly, studies conducted in Butajira area and western Kenya, reported that marshes, irrigation canals and river pockets were served as breeding habitat for *An. gambiae* s.l. during months of low precipitation (Animut et al. 2012; Kenea et al. 2011; Imbahale et al. 2011; Kweca et al. 2015).

The result of adult *Anopheles* collection revealed the presence of five species throughout the study period. *An. gambiae* s.l., *An. christyi*, *An. pharoensis*, *An. demellion* and *An. cinerus* were collected as an adult stage whereas *An. gambiense*, *An. longipalpis*, *An. squamosus*, *An. marshali*, *An. pretoriensis* and *An. serventi* were only collected as larvae. The reason for this discrepancy might be the resting behaviour of *Anopheles* mosquitoes and the lack of efficient adult collection methods. This is supported by the higher number of *An. christyi*, *An. demellion* and *An. cinerus* in larval collection compared to lower indoor adult collection in the present study, the reason might be CDC-light trap may not capture enough mosquitoes outdoor in absence of host close to collection area since some species of *Anopheles* mosquitoes need CO2 as cue in addition to light (WHO 2013).

Of the five species identified in adult collections *An. gambiae* s.l. was also found in greater number, this species was collected indoor more frequently than the other. Looking at the evidences from the present data no other malaria vector is important than *An. gambiae* s.l. in both study areas. In agreement with other studies, this species is incriminated as the principal vector of malaria in Ethiopia (Abose et al. 1998; Ameneshewa 1995; Animut et al. 2012; Kibret et al. 2010) and elsewhere in East, West and South Africa (Abose et al. 1998; Ameneshewa 1995; Kenea et al. 2011; Imbahale et al. 2011; Kweca et al. 2015). The presence of this species in the study area during the dry months is an indication for the uninterrupted malaria transmission throughout the year. However, *An. pharoensis* was found in both study sites therefore this species can be considered as a secondary vector of malaria in the two study areas as in other parts of Ethiopia (Animut et al. 2012; Kibret et al. 2010).

In this study, seasonal and local variations in indoor mosquito collection were observed. For instance, higher indoor collections were observed in both study sites during the wet season. Similarly, the presence of higher indoor numbers of *An. gambiae* s.l. during the
wet seasons were observed in a studies conducted in Ethiopia and Kenya (Massebo et al. 2013; Gari et al. 2016; Amek et al. 2012) during the wet season. The possible explanation of higher *An. gambiae* s.l. during the wet season in the present study and other studies conducted in different parts of Ethiopia could be linked with the abundance of small temporary rain pools which is a suitable larval habitat especially for *An. gambiae* s.l. (Munga et al. 2013; Kweka et al. 2012). On the contrary, higher number of *An. gambiae* s.l. was reported during the dry season in a previous study conducted near to the present study area (Animut et al. 2013). The number of *An. gambiae* s.l. and *An. pharoensis* collected during the study period were greater in Gogete than Jolie study site. Similarly, Katrijn et al. (2010) indicated the abundance and dynamics of *Anopheles* population have significant impact on the intensity of malaria transmission from place to place. Besides, a study conducted by Broeker et al. (2004) showed that the existence of variation in the incidence of malaria within the same area. Pertinently, the density of malaria vectors has shown a great variation in the same topography and even at household level within the same area. In Ethiopia White et al. (1980) around Gilgil Ghibe River valley reported seasonal variation in the density of malaria vectors. But, in Gambela areas of Ethiopia, Krafsur (1970; 1978) reported that the mean mosquito resting density was less than 50 mosquitoes/house. Meanwhile, a collections made in the 1958 malaria epidemics revealed the mean mosquito density vary from 100 to 150 mosquitoes/house (Fontaine et al. 1961).

In the present study *An. gambiae* was reported for the first time in Ethiopia indicating the presence of more efficient vector of malaria in the *An. gambiae* complex in addition to *An. arabiensis*. Previous and recent studies conducted in different part of Ethiopia reported that *An. arabiensis* and *An. amharicus* are the only two member species of the *gambiae* complex identified in Ethiopia (Coetzee et al. 2013; Animut et al. 2012). The absence of *An. gambiae* in the previous study conducted in different parts of Ethiopia might be the lack of sensitivity and specificity of molecular assays coupled with technical and technological short comings.

**Conclusion**

The breeding habitats of *Anopheles* mosquitoes in the study sites particularly in the dry season were due to human activities rather than environmental factors that require larval control. In the present study *An. gambiae* was reported for the first time in Ethiopia indicating the presence of an efficient vector in Ethiopia, thus establishing appropriate control strategy for this species might be necessary.

**References**


First report of African malaria vector *Anopheles gambiae* from Ethiopia


A survey for long-lasting insecticidal net coverage and use in Ethiopia

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Abstract

**Introduction:** The 2015 Ethiopia Malaria Indicators Survey shows that 64% of households owned Long Lasting Insecticide Net (LLIN) in Ethiopia. During the period between 2014-2016, 59.5 million LLINs were distributed.

**Objective:** To assess the LLINs coverage, utilization and physical condition across the different regions of Ethiopia.

**Materials and Methods:** National survey on LLIN was carried out in 2017. Three domains of estimation were considered in order to make separate survey estimates. For sample size determination different assumptions were also considered. Sample weighting was applied for data analysis. Twenty households were selected from each enumeration area randomly from the census (fresh list of the households) for questionnaire and interview administrations.

**Results:** In the 2017 LLINs survey, a total of 5527 households were surveyed with a response rate of 96.3%. The mean age of the participants was 22.47 years (standard deviation [SD] +16.989). Of the 20,982 participants, there were nearly an equal proportion of males and females (50.5%, vs. 49.5%, respectively). The extrapolated national malaria area weighted result of this survey indicated that 3,746,752 (64.8%) of the households owned at least one long lasting insecticide net in Ethiopia. The proportions of households with at least one LLIN for two people and the proportion of population that sleep under net in the night before the survey were found to be 39.1% and 42.6%, respectively. Similarly, the proportion of under five children and pregnant women who slept under LLINs in the previous night before the survey were 51.4% and 59.1%, respectively. Nationally, the proportion of LLINs retention in the household was 54.3% (N = 2,033,658). But only 1,947,727 (33.7%) of the households can recall the key LLINs messages. Although the national LLINs coverage was 64.8%, there was significant difference in LLIN coverage among regional states (P-value < 0.001). There is a difference among urban and rural areas regarding receiving information about LLINs utilization (P-value < 0.001).

**Conclusions:** Although a progress has been made in addressing universal coverage of LLINs in the country (64.8%), still almost 35% of households did not have LLINs in malarious areas (<2000m elevation). So, the NMCP and stakeholders should work together to achieve universal coverage in malarious areas of the country. None of the Regional States fulfilled the national as well as the global target of LLINs coverage. Although almost 81% of the LLINs were distributed through the health extension workers, only 33.7% of the household participants had knowledge on mosquito net utilization. Hence, there is a need to scale up the distribution of LLINs to meet the national targets and enhance community awareness about net utilization.

**Key words:** Long-lasting insecticidal nets, net utilization, net coverage, Ethiopia

**Introduction**

Malaria is endemic in Ethiopia, with differing intensities of transmission. The disease is prevalent in areas below 2000 m altitude covering three quarters of the country’s land mass, with an estimated population of 56.6 million (EMIS 2007). An epidemic occurs every 5–8 years in these areas, with frequent outbreaks within short periods. With an average of more than 3 million clinical cases per year, malaria remains the biggest health problem in Ethiopia (EMIS 2015; FMOH 2014). The Government of Ethiopia has developed a national strategic plan, from the year 2014-2020 for prevention, control and elimination of the disease (FMOH 2014). Accordingly, the country has been implementing vector control activities. The main vector control measures are indoor residual spraying (IRS) and long-lasting insecticide-treated nets (LLINs) (EMIS 2015; FMOH 2014). IRS is mainly applied in high malaria transmission and malaria epidemic prone areas; whereas LLINs are distributed to all malarious areas of the country. The 2015 national MIS shows 64% of households owned LLINs in Ethiopia (EMIS 2015).
The use of insecticide treated nets (ITNs) is identified by WHO as one of the main interventions to reduce the burden of malaria (WHO 2010). In 2015, 45% of the children aged <5 years were sleeping under ITNs in Ethiopia (EMIS 2015). In areas of intense malaria transmission, malaria-related morbidity and mortality are concentrated in young children, and the use of ITNs for children under 5 has been demonstrated to considerably reduce malaria disease incidence, malaria-related anemia and all causes of under-five morality (WHO 2010, 2018; FMOH 2010.1; FMOH 2010.2). To enhance effective utilization of LLINs, social behavioral change and communication have been implemented such as information education communication using radio and training of media professionals and school teachers, who are expected to play a major role in educating the public and students (WHO 2018; FMOH 2010.1; FMOH 2010.2). Most recently, from 2014-2016 universal distribution of LLINs has been achieved in the country. Do people use the mosquito nets distributed? What factors are limiting use of LLINs? What is the current net coverage and status of nets in terms of wear and tear? To address these questions, this study was carried out to assess the LLINs coverage, use and physical condition in Ethiopia.

The general objective of this survey was to measure the post universal campaign LLIN ownership and utilization at country level. The specific objectives were (1) to determine LLINs ownership and utilization across different regions of the country, (2) to determine proportion of LLIN ownership at the country level, (3) to determine proportion of LLIN utilization, (4) to assess major behavioral factors for net care and repair, and (5) to assess LLINs retention in the community. Parameters that were measured by the assessment in this survey are:- proportion of households with at least one LLIN, proportion of households with at least one LLIN for every two people, proportion of population that slept under an LLIN the previous night, proportion of under-five-year that slept under an LLIN the previous night, proportion of pregnant women that slept under an LLIN the previous night, proportion of LLINs retained in the households and proportion of households that can recall the key LLIN message. The results of the survey are expected to be used in the following ways: (1) To provide the Regional and National Malaria Control Programmers and partners with valuable information on the success of current status of LLIN usage in addressing universal coverage and intervention utilization at national and regional state, and (2) To offer insights into behavioral factors influencing net use and retention and to use that information to inform the SBCC component of future campaigns.

Materials and Methods
Sample size determination
i. Domains: Assessment of LLIN domain is a population subgroup for which separate survey estimates were desired. All enumeration areas (EAs) were within mean altitude of below 2000 m based on accurate complete digital database for EAs (maps with accurate altitude). Explicit stratification was made by altitude: malaria endemic areas (EAs with altitude =2,000m above sea level)
The following domains of estimation were considered:
1. National (country): rural for mean altitude of ≤2,000m above sea level
2. National (country): urban for mean altitude of ≤2,000m above sea level
3. Sub-national for mean altitude of ≤2,000m above sea level: Tigray, Afar, Amhara, Oromia, Somali, Benishangul-Gumuz, SNNP, Gambella, Harari and Dire Dawa.

ii. Sample size determination assumptions and study settings
Proportion of households owned a LLIN in Ethiopia being 64% (EMIS, 2015), relative precision of 7%, 95% CI, design effect of 2, 45% of children under 5 years sleeping under LLINs in Ethiopia (EMIS, 2105), and 10% non-response rate, n=5660 households, EAs=283. EAs distribution in different regional states across urban and rural (Table 1).

Table 1: Enumeration areas (EAs) distribution or allocations in the different regional states across urban and rural levels

<table>
<thead>
<tr>
<th>REGION</th>
<th>Urban</th>
<th>Rural</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tigray</td>
<td>4</td>
<td>25</td>
<td>29</td>
</tr>
<tr>
<td>Afar</td>
<td>3</td>
<td>19</td>
<td>22</td>
</tr>
<tr>
<td>Amhara</td>
<td>6</td>
<td>40</td>
<td>46</td>
</tr>
<tr>
<td>Oromia</td>
<td>8</td>
<td>54</td>
<td>62</td>
</tr>
<tr>
<td>Somali</td>
<td>3</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td>Benishangul Gumuz</td>
<td>3</td>
<td>17</td>
<td>20</td>
</tr>
<tr>
<td>SNNP</td>
<td>6</td>
<td>44</td>
<td>50</td>
</tr>
<tr>
<td>Gambella</td>
<td>3</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>Harari</td>
<td>3</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Dire Dawa</td>
<td>3</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>42</td>
<td>241</td>
<td>283</td>
</tr>
</tbody>
</table>

* - EA selection: Central Statistics Authority (CSA) was involved in the selection of EAs

iii. Sampling weighting design for data analysis: The post campaign LLINs survey 2017 has 4 main different folders/part:
a) The census which helps us to calculate primary and final weighting,
b) The household folder which has the key indicators of the survey and we calculate its full data using sampling weighting design,
c) The net/LLINs part/file, that helps us to calculate its full data using sampling weighting design for the purpose of assuring country representativeness of the data, and
d) The family member data/file, used as it is without using sampling weighting design.
Household selection: Questionnaire and Interview:
A questionnaire adapted from the PMI Monitoring LLIN Durability Study standardized questionnaire was used. Twenty households were randomly selected from each enumeration area from the census (fresh list of the households). After getting the household consent questions were asked by using the field guide reference for the tools developed. Each household head was asked about net ownership, utilization and retention. During the household visit the data collectors observed the status condition of the nets. The questionnaires were administered in Amharic and translated into Tigrigna and Oromiffa.

Quality Control: At the end of each day of data collection, the team supervisors checked completeness and possible inconsistencies of data and incomplete informations were corrected while still in the field.

Fieldwork
Survey Teams and organization: In each region, there was field survey team(s) to carry out the survey. A total of 27 field survey teams were deployed for the survey. single EAs were assigned to each team during the fieldwork. A team was composed of one supervisor, four interviewers and four guides per team. One guide was accompanying one interviewer. Interviewers were selected from the pool of public health officers employed by the respective Regional Health Bureaus. In each enumeration area (EA) four interviewers were working as a team. Each person in the team was responsible to conduct interviews of 5 households per day in a chosen cluster. One supervisor was assigned per region to support and monitor field survey teams. The supervisors were staffs of the Regional/National-level Malaria Control Program. The fresh census of an EA and interviews for 20 household were completed within a one and half day.

Assessment of LLIN condition/status: The assessment of LLIN condition/status was conducted using a questionnaire.

Training: Data collection and supervision was done by health personnel identified from each region. Partners and academic institutions were involved in the provision of training. All data collectors and supervisors were given one-week training. The data collection instruments were pre-tested in a non-study Woreda with similar setting. All data collection sites were supervised in the first week of data collection to give feedback on the spot. Expert supervision was done by FMOH, EPHI and other partners closely working with the study team.

Data management: Ethiopian Public Health Institute (EPHI) had developed good experience of using Smart Phone for data collection and sending it online. Local server at EPHI already used for EMIS-2015 had helped in facilitating paperless data collection. Data were collected and recorded on tablets and stored at EPHI. The data were transferred via internet into EPHI local server every day. Data were accessed by the investigators and local staff only. No personal identifiers were asked during data collection. Descriptive statistics was employed to measure proportion of net ownership and utilization across the different regions.

Results
Socioeconomic characteristics: In this survey, a household was defined as a person or group of persons, related or not, living together in the same dwelling unit, under one household head, sharing a common cooking arrangement. Basic demographic and socioeconomic characteristics for each person who spent the night preceding the survey in the household, including usual residents and history of travel, as well as information on their household characteristics were collected. A total of 5,527 respondents participated in the study and the response rate was 96.3%. The mean age of the respondents was 22.47 (SD =16.989) and the range was 0-100. Table 1 shows that, of the 20,982 participants there were nearly an equal proportion of men and women (50.5%, vs. 49.5%, respectively). The result showed that 54% of the participants were less than 20 years old. Only 2.04% of the participants were aged 65 years and above. Figure 1 shows populations mortality rates. There was a wide base that rapidly shrinks as age increases. In addition, this pyramid showed gaps between men and women at different age levels. There were more women than men at the age group of 21-25 and 31-35. Contrary to this, there were more men than women at the ages 36-40 and 41-45 years. Almost 75.5% of the heads of households were men and 98.7% of the member of households lived there. Regarding travel history and spending the night there before the interview, 99% of them had no travel history and 98.3% spent the previous night there. The majority (88.6%) of the head of households had primary education and 42% of them could read and write. But 60.5% of them had only secondary education.
Ownership and coverage of LLINs: The result of the survey for long-lasting insecticidal net coverage and use in Ethiopia indicated that, 3,746,752 (64.8%) of the households owned at least one long lasting insecticide net. The proportions of households with at least one LLIN for two people and the proportion that slept under the net in the previous night were found to be 39.1% and 42.6% respectively. Similarly, proportion of under five years children and pregnant women who slept under LLINs the previous night preceding the survey was 51.4% and 59.1%, respectively. The proportion of households with one, two, three, and four nets were 54.3%, 36.3%, 8% and 1.3% respectively and 0.2% of the households owned five or more LLINs (Table 1). However, only 33.7% of the household had information about the use of LLINs.

Table 2: The national household LLINs Coverage of key malaria indicators survey of Ethiopia population (Ethiopia LLIN survey 2017)

<table>
<thead>
<tr>
<th>Study variables</th>
<th>Frequency</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion of households with at least one LLINs</td>
<td>Yes</td>
<td>3746752</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>2031622</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>5778374</td>
</tr>
<tr>
<td>Proportion of households with at least one LLINs for every two people</td>
<td>Yes</td>
<td>2459632</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>3318742</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>5778374</td>
</tr>
<tr>
<td>Proportion of population that sleep under net the previous night</td>
<td>Yes</td>
<td>1088</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>1029</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>2117</td>
</tr>
<tr>
<td>Proportion of sampled under five years children that sleep under LLINs the previous night</td>
<td>Yes</td>
<td>202</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>342</td>
</tr>
<tr>
<td>Proportion of LLINs retained in the house holds</td>
<td>one net</td>
<td>203658</td>
</tr>
<tr>
<td></td>
<td>two nets</td>
<td>1359196</td>
</tr>
<tr>
<td></td>
<td>three nets</td>
<td>298518</td>
</tr>
<tr>
<td></td>
<td>four nets</td>
<td>48711</td>
</tr>
<tr>
<td></td>
<td>five nets</td>
<td>4338</td>
</tr>
<tr>
<td></td>
<td>seven nets</td>
<td>2330</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>3746752</td>
</tr>
<tr>
<td>Proportion of households that can recall the key LLINs massages</td>
<td>Yes</td>
<td>1947727</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>3496966</td>
</tr>
<tr>
<td></td>
<td>I did not know</td>
<td>333680</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>5778374</td>
</tr>
</tbody>
</table>

National Distribution of households that owned at least one LLIN with respect to altitude: The distribution of long-lasting insecticidal nets is one of the central interventions for prevention and control of malaria infection. The national policy aims to provide one LLIN for every sleeping space in malaria endemic areas (with elevation <2000m). Proper use of LLINs protects the whole community from malaria for at least three years without treatment. Table 3 shows that, in Ethiopia, 64.8% of the households owned at least one
long lasting insecticidal net. Of the households located at an altitude <2000masl, 64.8% (3458912/538330) of them owned a mosquito net while of the households located at an altitude ≥2000masl, 287840/44044 (65.4%) of them owned a mosquito net. Whereas nationally, households located in areas <2000m and ≥

2000m that owned a mosquito net were 3458912/577834 (59.9%) and 287840/5778374 (49.8%), respectively. In all areas the mean number of nets per household was 1.35 (Table 3). There was a significant association between altitude and net ownership (p < 0.001).

Table 2: Distribution of households that owned at least one LLIN with respect to altitude (Ethiopia LLIN survey 2017)

<table>
<thead>
<tr>
<th>Altitude categories</th>
<th>Households that owned at least one LLIN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency</td>
</tr>
<tr>
<td>&lt;2000m</td>
<td>3,458,912</td>
</tr>
<tr>
<td>≥2000m</td>
<td>287,840</td>
</tr>
<tr>
<td>Both</td>
<td>3,746,752</td>
</tr>
</tbody>
</table>

In Ethiopia the households that had eight sleeping spaces scored the highest LLIN ownership (100%) followed by households with three sleeping spaces (72.4%) (Table 3). There is also significant association between the number of sleeping places and LLIN ownership at (p < 0.001).

Table 3: National percentage of household with at least one net with respect to number of sleeping spaces (Ethiopia LLIN survey 2017)

<table>
<thead>
<tr>
<th>Number of sleeping spaces</th>
<th>Households owned at least one LLIN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>1</td>
<td>1,638,531</td>
</tr>
<tr>
<td>2</td>
<td>1,415,922</td>
</tr>
<tr>
<td>3</td>
<td>498,090</td>
</tr>
<tr>
<td>4</td>
<td>145,426</td>
</tr>
<tr>
<td>5</td>
<td>29,910</td>
</tr>
<tr>
<td>6</td>
<td>9,722</td>
</tr>
<tr>
<td>7</td>
<td>5,562</td>
</tr>
<tr>
<td>8</td>
<td>1,518</td>
</tr>
</tbody>
</table>

This survey results revealed that, households in Gambella region had the highest coverage of LLIN (68.7%) followed by Harari (67.9%), Tigray (65.1%) and Benishangul Gumuz (63.9%). LLIN coverage in Amhara, SNNPR and Afar Regional States was 61.8%, 62.7% and 62.9%, respectively (Table 4). There was a significant association between the regional states and proportion of households who owned at least one LLIN (p-value is 9885.78 or P-value < 0.001).

Table 4: The regional household coverage of LLINs malaria indicators survey of Ethiopia, 2017

<table>
<thead>
<tr>
<th>Region</th>
<th>Proportion of households who owned at least one LLINs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency</td>
</tr>
<tr>
<td>Tigray</td>
<td>237,928</td>
</tr>
<tr>
<td>Afar</td>
<td>59,419</td>
</tr>
<tr>
<td>Amhara</td>
<td>387,695</td>
</tr>
<tr>
<td>Oromia</td>
<td>1,818,448</td>
</tr>
<tr>
<td>Somalia</td>
<td>66,537</td>
</tr>
<tr>
<td>Benshangul G</td>
<td>50,108</td>
</tr>
<tr>
<td>SNNPR</td>
<td>377,079</td>
</tr>
<tr>
<td>Gambela</td>
<td>256,528</td>
</tr>
<tr>
<td>Harari</td>
<td>141,538</td>
</tr>
<tr>
<td>Dire dawa</td>
<td>150,470</td>
</tr>
<tr>
<td>Total</td>
<td>374,6750</td>
</tr>
</tbody>
</table>

The result of this survey indicated that, in Ethiopia 53% of the households located 2000 meters below sea level owned one LLIN, 37.1% of the households two LLINs, 8.3% owned three LLINs, and 1.57% owned four or more LLINs while households located 2000 meters above sea level (none malarious areas), 69.8% owned at least one LLIN, 26.04% owned two LLINs, 4.2% of the households owned three or more LLINs (Table 5).
Table 5: National Distribution of households with at least one and more than one LLINs with respect to altitude, 2017

<table>
<thead>
<tr>
<th>Altitude categories</th>
<th>National number of households with at least one LLINs</th>
<th>% of households with at least one LLINs</th>
<th>% of households with that owned two LLINs</th>
<th>% of households with that owned three LLINs</th>
<th>% of households owned four LLINs</th>
<th>% of households owned five LLINs</th>
<th>% of households owned seven LLINs</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2000m</td>
<td>53</td>
<td>37.1</td>
<td>8.3</td>
<td>1.4</td>
<td>o.1</td>
<td>0.1</td>
<td>0.07</td>
</tr>
<tr>
<td>≥2000m</td>
<td>69.8</td>
<td>26.04</td>
<td>3.7</td>
<td>0.2</td>
<td>0.3</td>
<td>0.02</td>
<td>0.00</td>
</tr>
<tr>
<td>Total</td>
<td>54.3</td>
<td>36.3</td>
<td>8.3</td>
<td>1.3</td>
<td>0.12</td>
<td>0.12</td>
<td>0.07</td>
</tr>
</tbody>
</table>

The national distribution LLINs/bed nets/per household: Figure 2 presents the national distribution LLINs/bed nets/per households without considering altitude. Nationally, concerning the number of nets owned/retained in the households, 54.3% households had one net, 36.3% households had two nets, 8% households had three nets, 1.3% households had four nets, and 0.2% of the household had 5 and above LLIN.

![LLINs graph](image)

Figure 2: The national distribution LLINs/bed nets/per households, 2017

Duration of LLINs and knowledge on mosquito net utilization: The LLINs survey indicated that, 11.9%, 27.4%, 32.8%, 5.3% and 22.6% of the households received LLINs before 1, 2, 3, 4 and 5 years, respectively. Of the received LLINs, 17.3% of them had holes and 87.5% of the holes repaired by sawing. Of the total households, 49% of them washed their nets. Twenty five percent of the households had information about malaria. Of the total households, only 10.6% of them received LLINs from the last campaign.

The majority (97.3%) of the shape of the LLINs was rectangular and the source of net was mainly a government sector. The majority (80.9%) of the LLINs were distributed by the health extension workers followed by government health institution (13.5%) such as health centers and health posts. Regarding LLIN fixation inside houses, 38.6% of the LLINs were set on the ceiling, 20.4% of the LLINs were located openly from its package and placed in the bed room and 16.3% of the LLINs were located in bed rooms without ceiling. Regarding sleeping under LLINs, 55% of the total households were slept under LLINs. For the reason why, the rest population did not sleep under LLINs, 22.7% of them were respond due to living in a none malaria area, 21.7% due to absence of anopheles mosquitoes, 8.7% due to fear of LLINs suffocation and temperature and 7.5% of them were do not having a place to use the LLINs.

National distribution of LLINs by brand net: Overall, 5,913,981 LLINs were distributed in Ethiopia. Of these, PermaNet 2.0, Magnet and Olyset accounted 69%, 17.9% and 4.6%, respectively (Table 6).
Table 6: National distribution of LLINs by brand net, 2017

<table>
<thead>
<tr>
<th>Study variable</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Types of brand nets</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Permanent</td>
<td>4082771</td>
<td>69.0</td>
</tr>
<tr>
<td>Odyssey</td>
<td>270713</td>
<td>4.6</td>
</tr>
<tr>
<td>Magnet</td>
<td>1999591</td>
<td>17.9</td>
</tr>
<tr>
<td>Interceptor</td>
<td>133667</td>
<td>2.3</td>
</tr>
<tr>
<td>Yorkkol</td>
<td>115571</td>
<td>2.0</td>
</tr>
<tr>
<td>Dawa Net</td>
<td>57828</td>
<td>1.0</td>
</tr>
<tr>
<td>Other tried sign</td>
<td>28069</td>
<td>.5</td>
</tr>
<tr>
<td>I did not know</td>
<td>165770</td>
<td>2.8</td>
</tr>
<tr>
<td>Total</td>
<td>5,913,981</td>
<td>100.0</td>
</tr>
</tbody>
</table>

**LLIN utilization by urban and rural areas:** The result of the survey revealed that there is a difference between urban and rural areas on LLINs coverage and use in Ethiopia. Regarding receiving information about LLINs utilization, Somalia regional state urban areas participants had better information (45.48%) about net utilization followed by Harari Regional State Rural areas (44.67%) and Gambella Regional State urban areas (43.67%). Whereas, Gambella Regional State rural areas scored less (28.76%) followed by Benishangul Gumuz Regional State urban areas and Amhara Regional State urban areas (27.9%, and 28.76%), respectively (Table 7). There was significant difference (p < 0.001) between urban and rural areas regarding information on LLINs utilization.

Table 7: Percentage of regional information received about net utilization by urban and rural area, LLINs survey 2017

<table>
<thead>
<tr>
<th>Region</th>
<th>Receive information about net utilization</th>
<th>% of received Information</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Urban area</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tigray Urban area</td>
<td>37266</td>
<td>61533</td>
</tr>
<tr>
<td>Afar Urban area</td>
<td>9408</td>
<td>13328</td>
</tr>
<tr>
<td>Amhara Urban area</td>
<td>37387</td>
<td>88370</td>
</tr>
<tr>
<td>Oromia Urban area</td>
<td>107191</td>
<td>192981</td>
</tr>
<tr>
<td>Somali Urban area</td>
<td>26996</td>
<td>26744</td>
</tr>
<tr>
<td>Benshangul Gumuz Urban</td>
<td>4448</td>
<td>9987</td>
</tr>
<tr>
<td>SNNP Rural</td>
<td>3834</td>
<td>7230</td>
</tr>
<tr>
<td>Gambela Urban</td>
<td>46657</td>
<td>60655</td>
</tr>
<tr>
<td>Harari Urban</td>
<td>22793</td>
<td>48787</td>
</tr>
<tr>
<td>Dire Rural</td>
<td>22457</td>
<td>50957</td>
</tr>
<tr>
<td>Rural area</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tigray Rural area</td>
<td>98213</td>
<td>141547</td>
</tr>
<tr>
<td>Afar Rural area</td>
<td>26062</td>
<td>39892</td>
</tr>
<tr>
<td>Amhara Rural area</td>
<td>290647</td>
<td>473886</td>
</tr>
<tr>
<td>Oromia Rural area</td>
<td>789316</td>
<td>1514965</td>
</tr>
<tr>
<td>Somali Rural area</td>
<td>17061</td>
<td>24776</td>
</tr>
<tr>
<td>Benishangul Gumuz Rural</td>
<td>21361</td>
<td>37797</td>
</tr>
<tr>
<td>SNNP Rural</td>
<td>186869</td>
<td>370284</td>
</tr>
<tr>
<td>Gambela Rural</td>
<td>76530</td>
<td>174269</td>
</tr>
<tr>
<td>Harari Rural</td>
<td>57645</td>
<td>65774</td>
</tr>
<tr>
<td>Dire Rural</td>
<td>55387</td>
<td>93212</td>
</tr>
<tr>
<td>Total</td>
<td>1947728</td>
<td>3496065</td>
</tr>
</tbody>
</table>

Discussion

The results of the 2017 household malaria indicator survey in Ethiopia indicated that 3,746,752 (64.8%) of the households owned at least one long lasting insecticidal net. This finding was slightly higher than study conducted two years back in Ethiopia. Because the EMIS 2015 indicated that 64% of households owned at least one LLIN (EMIS 2015). Studies conducted in rural Southwestern Uganda, Central India and west of Yemen between 2016 and 2017 showed that the LLINs possession rates were 84.0%, 98% and 90.6%, respectively (Tatemwa et al. 2017; Raghavendra et al. 2017; Al-Eryami et al. 2017). Similarly, the result of the 2017 LLINs survey revealed that the proportion of households with at least one LLIN for two people was 59.1%. This was higher when compared to the EMIS 2015 which revealed that only 32% owned at least one LLIN for every two persons who stayed in the household the previous night before the survey. Moreover, this LLINs survey indicated that the proportion of individuals who slept under net the night before the survey was 42.6% in contrast to the proportion of individuals slept under a LLIN the previous night in the 2015 MIS which was 40% (EMIS 2015).

The result of 2017 MIS also showed that, proportion of under five years children and pregnant women that slept under LLINs in the night preceding the survey was 51.4% and 59.1%, respectively. This finding was higher than the findings of 2015 MIS in which the proportion of under five children and pregnant women that slept under LLINs the night preceding the survey was 45% and 44%, respectively. This may be due to behavioral change in the community. Related study carried out in northern Uganda on utilization of insecticide treated nets (ITNs) among pregnant women showed that 35% of pregnant women had utilized ITNs (Obol 2013). Nationally, 54.3% households had one net, 36.3% households had two,
8% households had three, 1.3% households had four, and 0.2% of the household had 5 or more LLINs. Although there is high ownership and coverage of nets in Ethiopia, only 33.7% of the households had information on LLIN utilization.

The results of this survey showed that, nationally, the overall LLINs coverage was 64.8%, despite differences among regional states. Households in Gambella region had the highest percentage of coverage of bed net ownership (68.7%) followed by Harari (67.9%), Tigray (65.1%) and Benishangul Gumuz (63.9%). Relatively Amhara region (61.8%), SNNPR (62.7%) and Afar region (62.9%) were found to be those with the lowest bed net ownership. A study conducted in Mirab Abaya District, in Southern Ethiopia in 2014 found a higher proportion of LLINs ownership and utilization by households than the present investigation. The latter study showed ownership of at least one LLIN among surveyed households was 89.9% and using at least one LLIN during the previous night from the survey among net owners was 85.1% (Tassew 2017). During the past three years (2014-2016) the country distributed 59.5 million LLINs (un published data, NMCP/FMoH). The LLIN coverage need to be increased in order to attain the national target (80%).

The result of this survey revealed that there was a difference among the urban and rural area of each region regarding information on LLINs utilization. In Somalia region urban area participants (45.48%) scored the highest percentage among those who received information about net utilization followed by Harari region rural area and Gambella region urban area, 44.67% and 43.47% respectively. Whereas, Amhara region urban area scored the lowest percentage (26.11%) followed by Benishangul Gumuz region urban area and Gambella region rural area (27.9% and 28.76%) respectively. Regarding to information utilization on LLINs utilization the difference between urban and rural areas was significant (p < 0.001).

Conclusion and Recommendation

Although there is a good progress in addressing universal coverage of LLINs in the country, still almost 35% of households did not have LLIN in the malarious area (<2000m elevation). So, the concerned bodies (FMoH and regional states) and stakeholders must work together to achieve a universal coverage (100%) in both malarious and none malarious areas of the country. The result of this survey revealed that, there is difference among regional states concerning bed net coverage. Even though households which are located in Gambella region have the highest percentage or coverage of bed net ownership (68.7%), Amhara region (61.8%), SNNPR (62.7%) and Afar region (62.9%) were found to have the lowest bed net ownership. Therefore, giving priority especially to the most malarious areas must be taken in to consideration in the time of LLINs distributions. Though almost 81% of the LLINs were distributed by the health extension workers, nationally, only 33.7 % of the household participants had knowledge on mosquito net utilization. Hence, all players must collaborate and enhance programs and strategies that can support community awareness about proper net utilization to narrow the existing gaps.

Acknowledgements

We acknowledge the technical groups which involved more than 21 members from EPHI/NMCP and partners such as WHO, UNICEF, PMI, PATH-MACEPA and ACIPH. We also would like to thank the representatives from Ministry of Health and Regional Health Bureaus who participated in this survey. Regional, Zonal, Woreda and Kebele officials who directly or indirectly rendered support for the success of this survey are also acknowledged. Finally, guides that participated in the survey are thanked for their contribution to this work. The survey was carried out primarily by the EPHI, and NMCP/MoH and its partners. The budget required to carry out this survey was obtained from Global Fund through NMCP/FMoH. The ethical approval for the study was obtained from EPHI institutional review board (IRB).

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Assessing longevity of long lasting insecticidal nets in some selected sentinel sites of Ethiopia

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Abstract

Introduction: Long-lasting insecticidal nets are conventional tools in the prevention and control of malaria. Survey information on monitoring longevity of long-lasting insecticidal nets under field conditions is important for insecticide resistance monitoring and management in the country.

Objective: The current study aimed at evaluating the effective life time of long lasting insecticidal treated nets under operational conditions.

Materials and Methods: A cross-sectional study was conducted from January 2016 to December 2017 in Oromia, Amhara, Tigray and Southern Ethiopia regions on a total of 1416 randomly selected households owing Magnet, PermaNet 2.0 and Interceptor nets. Two years old long-lasting insecticide nets were examined for their physical status, bio-efficacy and chemical analysis.

Results: Community net utilization was 75.7% and the proportional hole index of Magnet, PermaNet 2.0 and Interceptor nets were 24.2, 21.7 and 21.6, respectively. The mean mosquito knocks down and mortality rates for Magnet net were 93.5% and 95.1% respectively. Mean knock down and mortality rates for PermaNet 2.0 were 85% with 87% while mean mosquito knock down and mortality rates for Interceptor were found to be 96% with 92.3%, respectively. The mean knockdown for all different type of net was 91.8% and the mortality rate was 91.75%. The mean concentrations of deltamethrin on Magnet and PermaNet 2.0 were 362.41 mg/m2 and 377.2mg/m2 respectively. Concentration of Alphacypermethrine in the Interceptor was 2627 mg/m2.

Conclusion: The current study revealed that, community net utilization was low while, the physical integrity and insecticidal activities of all net types were found to have acceptable protection after two years of use. Additional study should be required after two years on durability of long-lasting insecticidal nets.

Key words: Net integrity, net utilization, durability, long lasting insecticidal nets, proportional hole index.

Introduction

Long-lasting insecticidal nets (LLINs) are important tools in the prevention and control of vector-borne diseases, especially malaria and recommended to be used by all population at risk of vector borne diseases (Massue et al. 2016). LLINs are designed to provide physical and chemical protection for those who sleep under it (Dutta et al. 2018). Personal protection is one of the good strategies in malaria control using bed nets and other vector control methods targeting adult mosquitoes. Though the mosquitoes find entry into the nets through holes, endemic areas with malaria need to use such preventive measures without interruption (Acharya and Acharya 2015). This physical barrier of the net was supplemented with modern synthetic pyrethroids lasting long deposits (Eng et al. 2015). In malaria endemic regions, especially in several African settings, malaria incidence and death has been reduced by ITN (Dutta et al. 2018). Large scale distribution of LLINs to achieve universal coverage has been associated with highly successful malaria control outcomes (Hakizimana et al. 2014). LLINs have a relatively uniform life span of about 3 years and retain its effective biological activity without retreatment. The link between the physical condition of an LLIN and the malaria protection provided by the tool is complex, not well understood because of many reasons (ALMA 2016). As to current WHO recommendation, the replacement cycles of LLINs should be planned every 3 years. Information on durability of LLINs is an important tool to understand whether they are functional in terms of physical integrity and biological activities as this will help for further net procurement decisions and replacement cycle. This confirms that the service life span of LLINs for national malaria control programs through improving available information. Thus, information is required to ensure the quality maintained with better informed decision about the most appropriate products for different settings, supporting more accurate planning for universal coverage and to increase the cost effectiveness. In Ethiopia, various studies have been conducted with respect to coverage and utilization of LLINs in different parts of the country (EPHI 2016). However, there is a scarcity of data on evaluation of the effective duration, retention and efficacy of LLINs which enables programs to optimize LLINs operations. Information is also needed to formulate a desired strategic framework for further scaling up the coverage and for evidence-based decision on replacement cycle/period of LLINs. Thus,
this study was conducted on three products of nets, alpha-cypermethrin incorporated into polyethylene, alpha-cypermethrin and deltamethrin coated on polyester under field and laboratory condition at different socio-demographic settings in Ethiopia.

Materials and Methods

Study setting: The study was conducted from January 2016 to December 2017 during major and minor malaria transmission seasons in four districts (Wondo Genet, Jabitehlan, Medebay Zana and Mirab Abaya) of Ethiopia. The study regions were Oromia, Amhara, Tigray and Southern Ethiopia (SNNPRs) which represents different environmental and cultural settings in the LLINs mass distributions. The study sites were selected based on epidemiology of malaria, LLIN distributed to local households and accessibility. Households or the study units and sampling frame in the four sentinel sites (districts) were mapped with the number of households per sentinel sites ranging from higher region to lower kebele. List of households with LLIN aged between 24 and 36 months were received. Systematic random sampling method was used to select the study units, households. The sample size was determined using 95% confidence interval which yielded 354 households for each region, totally 1416 households for all. Sample size calculation was based on the proportion of households in malaria endemic areas in Ethiopia who had at least one insecticide treated mosquito net (64%), (p = 0.64), according to the 2015 Ethiopia malaria indicator survey (EPIH 2016).

Procedures

Questionnaire: A total of 1416 heads of households willing to participate in the study were interviewed. A semi-structured questionnaire was administered to collect information on the household demographics, history of the net, use and care of LLINs, type of sleeping materials they used under the net and housing condition.

LLINs collection: LLINs were collected from selected households of each sentinel site in four regions for physical status inspection, bioassay and chemical analysis. During collection, new LLIN was provided to each selected household in exchange of the LLINs used for the study. The age and types of the net was recorded at the time of collection based on the information gained from the Health Center in the specific district.

LLINs physical status /Integrity Evaluation: The physical status inspection of LLINs was undertaken according to the WHO Guidelines (WHO 2011). Of the LLINs found in the selected households, a single net was randomly selected to count and measure the size of holes at each position. Each LLIN sampled was hung up from the four corner points and examined for the hole appearance and size. Hole categories were designed to be easily and accurately measured under field conditions and were weighted as 1, 2, 3, 196 and 578 respectively. Then number of holes in each category were multiplied by the category weight and expressed as appropriate hole index (PHI) following the WHO hole category (WHO 2013). The measurements of hole or tear height and width on the net were recorded in centimeters at different parts, torsion location (upper, middle, lower and seams) of the net.

Cone bioassay: Cone bioassays were performed according to World Health Organization guidelines (WHO 2011). 30 nets were randomly selected from each sentinel sites. From each type of LLINs collected, four pieces 25 cm by 25 cm in size with two replicates were cut from four sides of the net using sharp scissors. The test was conducted using susceptible *Anopheles arabiensis* strain. Five non-blood fed 2–5-day old female *An. arabiensis* were exposed to each net section for 3 minutes at temperature of 25±2°C and relative humidity of 70±10%. Testing was conducted over a series of days. Untreated net obtained from the local market was used as a control (FMHACA or market). The mosquitoes, from both the test and the control, were then transferred to holding cups for a period of 24 hours and mosquitoes were provided with 10% sugar solution in the form of soaked cotton. Mosquitoes knocked down and mortality was recorded at 60-minutes and 24h post exposure respectively.

Chemical analysis: Following the cone bioassay, sixteen subsamples per LLIN type were cut 30 cm by 30 cm according to WHO guideline (WHO 2011). The nets were rolled up with aluminium foil, labeled and stored at 4°C prior to chemical analysis. Chemical analysis was conducted in Adami Tulu pesticides processing share Company’s laboratory. The data were calculated for each insecticide that shows the chemical concentration available within 2 years of service period.

Data Analysis: Generalized Linear Model with a negative binomial distribution was employed to analyze the differences in the physical condition of nets over time. The net damages were compared through calculating Proportional Hole index (PHI). Hole size category was estimated at 1-4 and holes were counted from the roof, upper, middle and seams of each net. Total mean hole size was calculated to compare which position more vulnerable to tear or hole. LLIN condition was classified as “good” condition (PHI= 0-64), “acceptable” condition (PHI= 65-642), and “torn” (PHI= 643+). The bio-efficacy of nets were determined by cone test. All distributed nets in all districts were the same age category, i.e. two years. So that the
comparison was done between the regions considering different insecticide class and community settings. According to WHOPES guidelines, LLINs should remain effective if knock down is >95% after 60 minutes or if mortality is >80% 24 h post exposure for a minimum of three years under field condition (WHO 2005). All analyses were carried out using Statistical package for social sciences (SPSS) software (version 20) with 0.05 level of significance. The study protocol was reviewed and approved by the Ethiopian Public health Institute (EPHI) Institutional Review Board (IRB) and verbal or written consent was obtained from the study participants before the commencement of the study.

**Results**

**Physical or integrity status:** A total of 1416 LLINs with different ages confirmed by batch numbers and date of hanging were evaluated about their physical status and integrity. Of the LLINs samples, 120 LLINs were analyzed for residual concentration. Since the ages of all nets were the same, the median age was 2 years. The majority of LLINs (75.7%) were used for sleeping and 22.6% of LLINs had holes from all assessed.

From all physically evaluated, the total numbers of holes at each size with respective positions were calculated and presented in Figure 1. For all physical status evaluated nets, the summed holes count at size 1 ($p<0.001$) were 37 on roof, 37 on upper and 34 on lower; at size 2 ($p > 0.001$) were 21 on roof, 38 on upper and 26 on lower; at size 3 ($p > 0.01$) were 15 on roof, 16 on upper and 24 on lower and at size 4 ($p > 0.01$) were 10, 8 and 16 on roof, upper and lower position respectively. From all inspected physical status of the LLINs for the presence of hole, seams positions were found to be free of any hole/tears.

![Figure 1: Physical condition/status of the long lasting insecticide nets (LLINs)](image)

The summed physical status result of the nets by insecticide classes was presented. There was no significance difference of percentage at size category of all nets in different community settings (Table 1). The percent values of holes at each size category were found to be below 10% and had been decreased from size 1 to size 4 after two years of services. The total number of holes and median number at size 1, size 2, size 3 and size 4 (WHO size category) were 108 (7.8), 85 (7.22), 55 (7.52) and 34 (12) respectively. The number of holes counted in different brands of nets were summed with their proportional hole index (pH) weighed according to WHO guideline hole size category (WHO 2011) (Table 1).

### Table 1: Number and percentage of hole by net type

<table>
<thead>
<tr>
<th>Net type</th>
<th>Measurement</th>
<th>Size 1 n (%)</th>
<th>Size 2 n (%)</th>
<th>Size 3 n (%)</th>
<th>Size 4 n (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnet Net</td>
<td>n (%)</td>
<td>49 (6.92)</td>
<td>37 (5.22)</td>
<td>24 (3.39)</td>
<td>20 (2.82)</td>
<td>130 (18.36)</td>
</tr>
<tr>
<td></td>
<td>Weighed</td>
<td>49</td>
<td>851</td>
<td>4704</td>
<td>11560</td>
<td>17164</td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td>0.07</td>
<td>1.202</td>
<td>6.64</td>
<td>16.33</td>
<td>24.242</td>
</tr>
<tr>
<td>Permanet 2.0</td>
<td>n (%)</td>
<td>51 (14.41)</td>
<td>28 (7.91)</td>
<td>15 (4.44)</td>
<td>7 (1.98)</td>
<td>101 (28.53)</td>
</tr>
<tr>
<td></td>
<td>Weighed</td>
<td>51</td>
<td>644</td>
<td>2940</td>
<td>4046</td>
<td>7681</td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td>0.14</td>
<td>1.82</td>
<td>8.30</td>
<td>11.43</td>
<td>21.69</td>
</tr>
<tr>
<td>Interceptor</td>
<td>n (%)</td>
<td>8 (2.26)</td>
<td>20 (5.65)</td>
<td>16 (4.52)</td>
<td>7 (1.98)</td>
<td>51 (14.41)</td>
</tr>
<tr>
<td></td>
<td>Weighed</td>
<td>8</td>
<td>466</td>
<td>31.36</td>
<td>40.46</td>
<td>76.50</td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td>0.023</td>
<td>1.3</td>
<td>8.86</td>
<td>11.43</td>
<td>21.61</td>
</tr>
</tbody>
</table>
WHO pH formula is \( (\text{size 1 hole’s x 1}) + (\text{size 2 holes x 23}) + (\text{size 3 holes x 196}) + (\text{size 4 holes x 578}) \) to categorize the physical integrity of nets. Based on pH value, LLINs were assigned to one of the following WHO categories: “good” (pH ≤ 64), “damaged” (pH = 65–642) and “too torn” (pH ≥ 643). The pH calculated for Magnet, PermaNet 2.0 and interceptor were 24.24, 21.7, and 21.6 respectively (Table 5). The medians of each net class, Magnet, Permanet2.0 and Interceptor were 7, 5.5 and 3.5 respectively. The comparisons for each type of nets were also measured according to their size category (Figure 2). The numbers of holes for Magnet were 49, 40, 29 and 22; PermaNet 2.0, 51, 29, 21 and 8; Interceptor, 7, 19, 19 and 24 at size 1, 2, 3 and 4, respectively. The overall number of holes for Magnet, PermaNet 2.0 and Interceptor net appeared at any size was 141, 109 and 69, respectively. The significance and values difference interaction at each hole size category (Table 2) was significantly varied for overall LLINs. The reasons for appeared hole on the net were torn or split when caught on an object, burning and animals (Table 5).

| Cone bioassay: Three LLIN types: Magnet, PermaNet2.0 and Interceptor were subjected to WHO cone bio-assay against the primary malaria vectors (Anopheles arabiensis). The minimal residual bio-efficacy was observed by mosquito knock down and mortalities of the nets. The pooled bio-efficacy of all net types was observed in each position with two replicates. Knock down rate (KDR) after 1 hour at position 2 was found to be 91% with 94.3% mortality rate after 24 hour post exposure. Regarding position 3, the knock down result after 1 hr was 93% and 94.5% mortality after 24 hr post exposure. Position 4 and 5 knock down rates were found to be 94.33% and 95.67% respectively with the mortality rate 96.5% at position 4 and 94.83% at position 5. Knock down and mortality rate (MR) of each individual nets were tested at their respective position (P), 2, 3, 4 and 5 were 84.33%, 83.7%, 87% and 85% and 86.7%, 84%, 88.3% and 89% for PermaNet 2.0, 94%, 94.7%, 98.33% and 96.33% and 90%, 91.67%, 93.7% and 94% for interceptor net and the magnet net result of knock down and mortality were above 90% and 95% respectively at each position (Figure 3). Though the insecticide classes in each position indicated different results, the knock down and mortality rate of the different nets were not varied significantly \((p<0.001)\) when compared by regions. Bio-efficacy observation was not observed at position 1 (P1), because it is not used for protection rather than tacking under the bed and contagious because of contact while it is found at the lower part of the net. |
Figure 3: Pooled results in percent for Cone Bioassay for both Knockdown and mortality of each net type

The overall results of the insecticidal activity of all nets regarding knockdown after 60 min and mortality after 24 hours from the cone bio-assays against *An. arabiensis* (Figure 1) were observed. Mortality for the magnet was 95.1% while the mortality for PermaNet 2.0 was 87%, and for interceptor net was 92.3%. According to the result of two years used nets, the risk of mosquito death was increased significantly when compared to mosquito exposed in untreated nets as control. The knock down and mortality percent of PermaNet 2.0 was found to be less than other LLINs. PermaNet 2.0 passed WHO cut off point with mortality but, the knock result was less than 90% which was fail to pass the criteria of WHO with all significance values (Table 3). The overall mean ($df = 15, P\ value = 0.000$) difference knock down and mortality rates for all nets were 275.763 (91.825%) and 277.013 (92.304%) respectively.

Table 3: Bioactivity of insecticide on each net on the An. arabiensis

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>Mean Difference</th>
<th>F</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knock down rate</td>
<td>15</td>
<td>275.76</td>
<td>22.054</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Mortality rate</td>
<td>15</td>
<td>277.01</td>
<td>22.990</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Knock down %</td>
<td>15</td>
<td>91.82</td>
<td>22.251</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Mortality %</td>
<td>15</td>
<td>92.30</td>
<td>22.990</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

**Chemical Analysis:** Insecticide concentration of 2 years old 64 different nets from all study sites were measured using HPLC. The product types measured were alpha-cypermethrin incorporated into polyethylene and alpha-cypermethrin and Deltamethrin coated on polyester. The average insecticide concentration of Magnet net in Amhara region and SNNPRs was 202.41 mg/m2 and Permanet 2.0 from Oromia was 377.2mg/m2 at two replicate each position designation of the Piece net. An available chemical on interceptor net from Tigray region was 2627 mg/m2 (Table 4)).

Table 4: Concentration of insecticides on each LLINs surface area

<table>
<thead>
<tr>
<th>Net Type</th>
<th>Area of sample in (m2)</th>
<th>Available chemicals in mg/m2</th>
<th>Number of nets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnet</td>
<td>1*10^-2</td>
<td>202.41</td>
<td>32</td>
</tr>
<tr>
<td>Permanet 2.0</td>
<td>1*10^-2</td>
<td>377.2</td>
<td>16</td>
</tr>
<tr>
<td>Interceptor</td>
<td>1*10^-2</td>
<td>2627</td>
<td>16</td>
</tr>
</tbody>
</table>

Of tested net classes, only interceptor nets showed concentrations more than the 1000 mg/m2, indicating that the decrease of these other net’s chemicals were due not a lack of sufficient insecticide, but rather to other different factors with bio-availability of the insecticide on the surface of the net fibre.
### Table 5: Characteristics of factor associated with net durability by net type and use status by the community

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Net type</th>
<th>Magnet</th>
<th>Permanuel 2.0</th>
<th>Interceptor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>708(100.0%)</td>
<td>354(100.0%)</td>
<td>354(100.0%)</td>
</tr>
<tr>
<td>House type</td>
<td>House with separate bedroom and kitchen</td>
<td>671(94.1%)</td>
<td>297(83.9%)</td>
<td>249(70.3%)</td>
</tr>
<tr>
<td></td>
<td>Tukul with kitchen /bedroom together</td>
<td>37(5.2%)</td>
<td>57(16.1%)</td>
<td>105(29.7%)</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td>708(100.0%)</td>
<td>354(100.0%)</td>
<td>354(100.0%)</td>
</tr>
<tr>
<td>Use of the net in the House</td>
<td>was damaged and thrown away</td>
<td>570(80.5%)</td>
<td>253(71.5%)</td>
<td>274(77.4%)</td>
</tr>
<tr>
<td></td>
<td>was given away to others</td>
<td>101(14.3%)</td>
<td>82(23.2%)</td>
<td>53(15)</td>
</tr>
<tr>
<td></td>
<td>was stolen</td>
<td>6(0.8)</td>
<td>10(3.0)</td>
<td>7(2)</td>
</tr>
<tr>
<td></td>
<td>was old</td>
<td>0(0.1)</td>
<td>0(0.1)</td>
<td>1(0.3)</td>
</tr>
<tr>
<td></td>
<td>was being used in other location</td>
<td>11(1.6)</td>
<td>9(2.5)</td>
<td>2(0.6)</td>
</tr>
<tr>
<td></td>
<td>was being used for other purposes</td>
<td>17(2.4)</td>
<td>6(0.7)</td>
<td>13(3.7)</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td>136(19.4%)</td>
<td>99(28)</td>
<td>80(22.7)</td>
</tr>
<tr>
<td>The time duration of the net not available for sleeping</td>
<td>0-6 months</td>
<td>55(7.8)</td>
<td>42(11.9)</td>
<td>31(8.8)</td>
</tr>
<tr>
<td></td>
<td>0-6 months</td>
<td>82(11.6)</td>
<td>59(16.7)</td>
<td>49(13.8)</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td>137(19.4%)</td>
<td>101(28.6)</td>
<td>80(22.8)</td>
</tr>
<tr>
<td>Last night used net for sleeping</td>
<td></td>
<td>569(80.4%)</td>
<td>254(71.8)</td>
<td>273(77.1)</td>
</tr>
<tr>
<td>Reason why the net used last night</td>
<td>The net is Too hot</td>
<td>66(9.3)</td>
<td>47(13.3)</td>
<td>23(6.5)</td>
</tr>
<tr>
<td></td>
<td>because of the smell</td>
<td>7(1)</td>
<td>2(0.6)</td>
<td>1(0.3)</td>
</tr>
<tr>
<td></td>
<td>Feel closed in</td>
<td>8(1.1)</td>
<td>0(0.1)</td>
<td>3(0.8)</td>
</tr>
<tr>
<td></td>
<td>Absence of malaria</td>
<td>9(1.3)</td>
<td>9(2.5)</td>
<td>8(2.3)</td>
</tr>
<tr>
<td></td>
<td>Absence of mosquito</td>
<td>14(2)</td>
<td>5(1.4)</td>
<td>13(3.7)</td>
</tr>
<tr>
<td></td>
<td>The net is too torn/old</td>
<td>6(0.8)</td>
<td>3(0.8)</td>
<td>5(1.4)</td>
</tr>
<tr>
<td></td>
<td>The net is not available</td>
<td>38(5.4)</td>
<td>27(7.6)</td>
<td>26(7.3)</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td>148(20.9%)</td>
<td>93(26.7)</td>
<td>79(22.3)</td>
</tr>
<tr>
<td>Duration of the net used to sleep under</td>
<td>All the year</td>
<td>437(61.7%)</td>
<td>272(76.8)</td>
<td>230(65)</td>
</tr>
<tr>
<td></td>
<td>Only the rainy season</td>
<td>266(37.6)</td>
<td>78(22)</td>
<td>95(26.8)</td>
</tr>
<tr>
<td></td>
<td>Only the dry season</td>
<td>5(0.7)</td>
<td>4(1.1)</td>
<td>29(8.2)</td>
</tr>
<tr>
<td>Other places used for sleeping from the main houses</td>
<td>Field</td>
<td>12(1.7)</td>
<td>5(1.4)</td>
<td>1(0.3)</td>
</tr>
<tr>
<td></td>
<td>Beach</td>
<td>6(0.8)</td>
<td>6(1.7)</td>
<td>4(1.1)</td>
</tr>
<tr>
<td></td>
<td>Forest</td>
<td>35(5.7)</td>
<td>36(10.2)</td>
<td>10(2.8)</td>
</tr>
<tr>
<td></td>
<td>Farm hut</td>
<td>2(0.3)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Type of sleeping place with the net cover</td>
<td>Reed mat</td>
<td>232(32.8%)</td>
<td>116(32.8)</td>
<td>116(32.8)</td>
</tr>
<tr>
<td></td>
<td>Cut bamboo</td>
<td>23(3.2)</td>
<td>13(3.7)</td>
<td>12(3.4)</td>
</tr>
<tr>
<td></td>
<td>Grass</td>
<td>72(10.2%)</td>
<td>34(9.6)</td>
<td>34(9.6)</td>
</tr>
<tr>
<td></td>
<td>Foam mattress</td>
<td>217(30.6%)</td>
<td>106(29.9)</td>
<td>108(30.5)</td>
</tr>
<tr>
<td></td>
<td>Wooden bed frame finished</td>
<td>48(6.8)</td>
<td>29(8.2)</td>
<td>26(7.3)</td>
</tr>
<tr>
<td></td>
<td>Wooden bed frame sticks</td>
<td>23(3.2)</td>
<td>12(3.4)</td>
<td>12(3.4)</td>
</tr>
<tr>
<td></td>
<td>Metal bed frame</td>
<td>23(3.2)</td>
<td>11(3.1)</td>
<td>12(3.4)</td>
</tr>
<tr>
<td></td>
<td>Bare floor or ground</td>
<td>70(9.9)</td>
<td>33(9.3)</td>
<td>34(9.6)</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td>148(20.9%)</td>
<td>93(26.7)</td>
<td>79(22.3)</td>
</tr>
<tr>
<td>Type of washing soap</td>
<td>Water only</td>
<td>59(8.3)</td>
<td>19(5.4)</td>
<td>24(6.8)</td>
</tr>
<tr>
<td></td>
<td>Local bar soap</td>
<td>246(34.7%)</td>
<td>126(35.6)</td>
<td>155(43.8)</td>
</tr>
<tr>
<td></td>
<td>Detergent powder</td>
<td>232(32.8%)</td>
<td>113(31.9)</td>
<td>93(26.3)</td>
</tr>
<tr>
<td></td>
<td>Detergent powder with mix bar</td>
<td>154(21.8)</td>
<td>88(24.9)</td>
<td>80(22.6)</td>
</tr>
<tr>
<td></td>
<td>Bleach</td>
<td>17(2.4)</td>
<td>8(2.3)</td>
<td>2(0.6)</td>
</tr>
<tr>
<td>Scrubbing the net on the hard surfaces during wash</td>
<td>Water only</td>
<td>289(42.1%)</td>
<td>137(38.7)</td>
<td>104(29.4)</td>
</tr>
<tr>
<td></td>
<td>Local bar soap</td>
<td>379(53.5%)</td>
<td>212(59.9)</td>
<td>121(34.2)</td>
</tr>
<tr>
<td></td>
<td>Outside in the sun</td>
<td>319(45.1%)</td>
<td>137(38.7)</td>
<td>226(63.8)</td>
</tr>
<tr>
<td></td>
<td>Inside</td>
<td>8(1.1)</td>
<td>5(1.4)</td>
<td>7(2)</td>
</tr>
<tr>
<td>Water condition for wash</td>
<td>Cold</td>
<td>499(70.5%)</td>
<td>245(69.2)</td>
<td>308(87)</td>
</tr>
<tr>
<td></td>
<td>Warm</td>
<td>181(25.6%)</td>
<td>97(27.4)</td>
<td>42(11.9)</td>
</tr>
<tr>
<td></td>
<td>Hot</td>
<td>28(4)</td>
<td>12(3.4)</td>
<td>4(1.1)</td>
</tr>
<tr>
<td>Any hole appeared on the net</td>
<td>Tore or split</td>
<td>133(18.8%)</td>
<td>97(27.4)</td>
<td>52(14.7)</td>
</tr>
<tr>
<td></td>
<td>Burnt</td>
<td>23(3.2)</td>
<td>9(2.5)</td>
<td>3(0.8)</td>
</tr>
<tr>
<td></td>
<td>Animals</td>
<td>11(1.6)</td>
<td>4(1.1)</td>
<td>4(1.1)</td>
</tr>
</tbody>
</table>

## Discussion

In Ethiopia, focusing malaria endemic area, LLINs distribution/coverage was seems to be the same. The net durability is not clearly known after distribution. In the study communities, the nets were being used differently in different periods: all the years, only during rainy and dry seasons. All sampled households were owned at least one LLINs with two years old since the campaign net distribution is commonly in the same time. Net usage was found to be low with 76.5% of all the communities used a net every night in their house. This contradicted to the study conducted in Tafea Province, Vanuatu (Dutta et al. 2018). The reasons related to this were early net attrition, community perception (adverse effects) to ward
chemicals on the nets, absence or presence of mosquitoes and are either discarded, given away or used for something other than preventing mosquito bites at night (Table 5) which is in line with the study conducted in Zambia (Dev et al. 2016; Tan et al. 2016). The current study found that, about 24% of the nets hadn’t been used by the households which were distributed by the MoH before 24 months. It was because of associated factors; given away to others for use, stolen, being used in another location and other purpose, hotness, smell, feel closed in, absence of malaria and mosquitoes at the time (Table 5). LLIN loss, measured by survivorship/attrition was incomparable with the study conducted in Benin after three years use (Gnanguenon et al. 2014).

The physical status of all nets was found in a good condition in line with the study in Tanzania (Massue et al. 2016). There was no significant difference in the different types of LLINs regarding attrition rate. A low number of the used nets had holes (<20%), which contradicted/opposed previously published studies (Craig et al. 2015). Factors assessed in the current study could explain in a holistic manner net use by individuals in households owning at least a net are less contributed for the hole distribution. Factors that affect LLIN durability, act to a lesser extent, in different settings. The rate at which holes appear (loss of fabric integrity) in nets found to be lower in Ethiopia is compared to published studies in different countries (Hakizimana et al. 2014; Tan et al. 2016). The number of washed bed nets was high, almost all nets used in the study communities were found to be washed frequently with similar proportion in contrast of published studies (Dutta et al. 2018) which dried outside in the sun. The total hole number were also found to be changed depending on the position at different size category: roof, upper, lower and seams. The numbers of tears and holes was relatively high in PermaNet®2.0 when compared with the left LLINs. Contrary to other studies, the observed holes were in the roof and upper parts of the nets in all LLIN types (Dutta et al. 2018). This is because of a sleeping materials and house type in which the net is used and materials the net hanged over may affects at any time during its lifetime (Tan et al. 2016). The proportional hole index (PHI) that calculated for overall was 22.5 (Table 1) which indicated that the nets to be classified as “Good” according to WHO criteria or cut off. This is because, LLIN based intervention technology is appropriate if, the respondents appreciated and used their nets, adapting to the needs of their specific context for greater community acceptance, compliance and retention in malaria control are considered. According to the current study observation, the two-year service able life’ assumption, currently used in Ethiopia did not subject to program distribution/replacement of LLINs while the nets being used were found in a good condition regarding with physical integrity.

In current study based on WHO measures of optimal effectiveness and minimal effectiveness criteria, the insecticidal effectiveness of the LLINs in the study were evaluated. Bio-efficacy tests for each chemical class were compared based upon the results as a confirmed 24-hour mortality of susceptible An. arabiensis exposed to the nets for 3 min. We found that, the Magnet and Interceptor nets performed significantly better than PermaNets 2.0 nets. Approximately 2 years after nets were distributed, 95.1% of Magnet and 92.3% of Interceptor in the field remained effective in killing susceptible Anophelines. The Permanet 2.0 performed significantly worse (87% mortality), with no nets remaining effective after 2 years but, effectively passed the WHO criteria with knock down percent (Figure 4). This may be because of the wider range of mortalities produced by nets 2 years probably reflects difference in human related behavior with regard to net usage: general handling, amount of use, storage, washing, and attempts at insecticide regeneration (Lindblade et al. 2005). The Magnet and Interceptor net were observed to be more durable and robust than the PermaNet®2.0 LLINs in terms of Mortality rate (Bio-efficacy). In line with published studies, the residual bio-efficacies of nets varied between locations (P < 0.05) which suggest that the community practices for net care and maintenance varied (Dev et al. 2016). The presented mortality and knockdown results indicated that the efficacy of permaNet 2.0 begins to decrease during the second year of use under programmatic conditions; even though the nets were designed to last for 3 years. The available residual chemicals after two years in all nets were above 360 mg/m2. High insecticidal concentration was found on Interceptor and Permanet 2.0 where, low insecticidal concentration was found on magnet net (Table 4). Surprisingly, high concentration insecticide on permanent and low concentration insecticide on Magnet net was not associated with percent mortality. This is similar with study conducted on durability of LLINs in Zambia (Tan et al. 2016). There are some limitations of the current study that should be noted. In this study, the following possible limitations were considered: LLINs available may be a biased sample, as worn-out LLINs may no longer be present; a significant and unknown portion of the local population has moved into or out of the study area; the number of opportunities for follow-up are limited and labels on LLINs fade or are lost over time, making identification difficult.
Conclusions and Recommendations
The current study revealed that, the community net utilization is low while the physical status and insecticidal activities of all nets are in a good condition to give acceptable protection after two years services. Results of the current study using bed nets collected over two years in Ethiopia indicated some of the potential strengths of the pH including: underestimating the contribution of really large holes, overestimating the contribution of smaller holes and ignoring extra small holes and hole location. This information should be used to inform decisions on replacing LLINs through avoiding compromised efficacy of LLINs earlier than what expected. Though, the LLINs condition in Ethiopia is found to be in a good condition, special focus for some households in the communities need replacement before three years net use which could be important to achieve universal coverage standing from the current. Longitudinal studies are needed to determine the attrition rate, understand the reasons, utilization and insecticidal activities of LLINs.

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Competing interests
The authors declare that they have no competing interests.

References


Distribution and trends of insecticide resistance in malaria vectors in Ethiopia (1986 - 2017): a review

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Abstract
Introduction: Malaria vector control intervention relies mainly on long-lasting insecticidal nets and indoor residual spraying. The distribution and frequency of insecticide resistance have increased dramatically and are now threatening the global success of the control program. Resistance to the public health insecticides has been frequently reported in Ethiopia. This review aimed at mapping the spatial and temporal distribution, and the trend of insecticide resistance to different insecticides used for vector control in the Ethiopian populations of Anopheles arabiensis.

Objective: To assess the distribution and trends of insecticide resistance, which could help the implementation of Insecticide Resistant Management strategy in the country.

Methods: Relevant published articles and reports on malaria mosquito resistance management were reviewed. The obtained data were organized and the geographical distribution resistance was mapped. For those sites where resistance was reported, but had no GPS coordinates, the coordinates were obtained from Google Earth.

Results: Since 1986, resistance increased from time to time even after the country stopped DDT spraying. The Anopheles arabiensis population has developed resistance to pyrethroids and organochlorine class insecticides in most parts of the country. Few vector populations also developed resistance to carbamate insecticides. However, most populations surveyed were fully susceptible to primiphos–methyl and lindane.

Conclusions: Wild populations of An. arabiensis were found resistant to pyrethroid insecticides used for treating nets, therefore there is a need to consider new generation nets to improve the protective effect of nets. As the vector was fully susceptible to primiphos–methyl, it can be used as an alternative insecticide for indoor residual spraying. Moreover, insecticide resistance management strategies should be implemented in the country in such a way that a change in insecticide pattern is ensured before the resistance to a certain insecticide reaches its tipping point.

Key words: Anopheles arabiensis, resistance monitoring, insecticide resistance.

Introduction
Malaria is a complex disease caused by a single-celled protozoan parasite that belong to the genus Plasmodium and transmitted by the female Anopheles mosquito. The disease predominately occurs in tropical and sub-tropical parts of the globe and remained a major public health problem. In 2015, about 88% cases and 90% deaths are estimated to occur in the African Region (WHO 2015a). In fact, malaria shows a declining trend following the scale up of vector control tools and effective treatment as compared to the end of the 1990s. Globally, estimated malaria cases and deaths decreased by 18% and 48% respectively, and by 12% and 48% in Africa from 2000 to 2015 (WHO 2015a). Chemical-based malaria vector control has been a key component of malaria control intervention. With the high coverage of long-lasting insecticidal nets (LLINs), the use of chemicals has been escalated. The world health organization (WHO) recommends active ingredients of four classes of insecticides, including pyrethroid, organochlorine, organophosphate and carbamate to be used for public health interventions(WHO 2015b). Among those classes of insecticides, pyrethroids are recommended for the treatment of LLINs, due to their safety and effectiveness (WHO 2011a).

A recent review documented that the distribution and frequency of insecticide resistance increased dramatically in the malaria vector population and is now threatening the success of control programs (Ranson and Lissenden 2016). The emergence and the widespread range of insecticide resistance may compromise vector control in malaria endemic areas worldwide and this calls for developing an insecticide resistance management strategy in malaria endemic countries (WHO 2011b). The dependency of the current malaria control on pyrethroids and the development of resistance by malaria vectors to these products, puts the current global of control efforts at
higher risk. Pyrethroid insecticide resistance is spreading and intensifying, reaching levels that threaten the control programs in most malaria endemic African countries (Brogdon et al. 2014) Similarly, insecticide resistant populations of *An. arabiensis* are widespread in Ethiopia. This might also be associated with long standing use of chemical insecticides by the national malaria control program for IRS since the 1950s. Moreover, there has been an increase of indoor residual spraying (IRS) and LLINs since 2005. Subsequently, resistance has been documented in different parts of the country following this intensification of IRS and LLINs (PMI 2016; Abate and Hadis 2011; Yewhalaw et al. 2011). Hence, many studies recommend to monitor insecticide resistance and to implement insecticide resistance management strategies to minimize its impact on vector control (Najera and Zaïm 2003; WHO 2014).

**Malaria epidemiology:** Efforts to control malaria have been boosted in the past few years with increased international funding and greater political commitment. Consequently, the reported malaria burden is being reduced in a number of countries throughout the world, including in some countries in tropical Africa where the burden of malaria is greatest (Mendis et al. 2009). Four human malaria parasite species, namely *Plasmodium falciparum, P. vivax, P. malariae* and *P. ovale* have been reported in Ethiopia. However, most of the malaria cases are due to *P. falciparum* and *P. vivax* and the remaining two species have low epidemiological significance in the country. *P. falciparum* represents about 65-75% (WHO-Afro 2016) of the total reported malaria cases. Over 42 malaria vector species were recorded in Ethiopia (Authority 1988). However, *An. arabiensis* is the principal vector responsible for malaria transmission (Abeku et al. 2003) The other malaria vector *An. pharoensis* is playing a secondary role in transmission of malaria in the country (White 1982).

**Malaria control:** Malaria vector control methods focus on the use of insecticides, insecticide-treated materials, environmental management, and personal protection methods against mosquito vectors (WHO 1995). The concept of modifying vector habitat to discourage larval development and/or human–vector contact is generally referred to as environmental management (Singer et al. 2005). The techniques of environmental management are generally grouped into three main categories—environmental modification, environmental manipulation, and modification of human habitations/behaviors (Ault 1994; WHO 1982). DDT was the first synthetic organic insecticide used for effective vector control with reasonable success. The historic successful eradication of malaria in various parts of the world is achieved mainly by vector control (Harrison 1978). Application of insecticides remains the primary control tool in the majority of vector control programs throughout the world since early nineteenth century (Breman 2001). However, high coverage of breeding sites is required to achieve significant impact, which is a major operational and logistical challenge in many ecological settings (Pampanga 1969). In India, in urban areas, the major vector control strategy is larviciding of breeding habitats with organophosphate insecticides namely temephos and fenothion. However, fenothion has been withdrawn for use in vector control in India due to the recent development of insecticide resistance (Sharma et al. 1996).

Malaria control in Ethiopia started during the 1950s. It is mainly focused on case management and vector control as well as health education. For the treatment of malaria infection, chloroquine was used in the country for over five decades. However, *P. falciparum* developed resistance to chloroquine (Teklehaimanot 1986) and led to use sulphadoxine–pyrimethrin as first line treatment for *P. falciparum*. Currently the first line drug for treatment of uncomplicated malaria infection is artemether lumeferonine, which was introduced in 2004 (FMoH 2004).

The vector control intervention started in Ethiopia during the malaria eradication era in the 1959, and Dichlorodiphenyltrichloroethane (DDT) was used for IRS. It was the insecticide of choice for malaria vector control until 2008. According to FMoH, Malathion was used as an alternative insecticide in areas where vectors had become resistant to DDT. The insecticide was used for both malaria vector control and crop protection in agriculture (PMI 2008). In order to meet the annual needs of the country, Adami Tulu Pesticide Processing Plant located in East Shoa, Central Ethiopia has started formulating insecticides since 2001. In addition, this plant also formulates other insecticides for crop protection. According to Ethiopian FMoH data, DDT was replaced by pyrethroids in 2009, and bendiocarb and propoxur from the same class have been used for IRS in 2011 and 2012, respectively. Up to 2015, the amount of propoxur used in vector control intervention is 3.34 times higher than bendiocarb. The type and amount of insecticide used for IRS shown in Figure 1.
LLIN was introduced to Ethiopia as a vector control tool since 1998 (PMI 2008). Then, the NMCP scaled up the distribution of LLIN in 2005 to increase the ownership and utilization of LLINs. This resulted in the reduction of malaria cases and deaths. Unfortunately, the highest coverage of IRS and scaled use of LLINs is believed to enhance the appearance and development of resistance in vector species to various classes of insecticides (Yewhalaw et al. 2012). Before 2005, ITN were treated with conventional K-O tab® and it serves for one year in the absence of re-treatment. During large scale LLINs distribution, the purpose of global ITN distribution was to deliver two ITNs per household (PMI 2008). The amount of LLINs distributed from 2005 to 2013/14 in Ethiopia shown in Figure 2.

**Materials and Methods**

For this review, relevant published articles and reports on malaria resistance management were consulted. Graphs and maps were constructed after data on mosquito mortality obtained from published sources were organized in excel. The database incorporated variables like location, insecticide type, percent mortality, and resistance level, year of study.
and GPS location. For those publications that had no GPS coordinates, the coordinates obtained using the Google Earth application by tracing the name of the study site. Key words used to search the literature from Pub-Med and other journals for this review were: insecticide resistance, resistance management, vector control, history of insecticide utilization and impact of resistance. The inclusion criteria considered all manuscripts, reports and publications in English language that report on vector control, insecticide resistance in malaria vectors, the epidemiology of malaria, resistance management and integrated vector management (IVM).

Results

**Distribution and trends of An. gambiae s.l resistance to DDT:** Resistance to DDT was first detected in 1986 at middle wash, central Ethiopia (Abose et al. 1998). From 1986 to 1995, the susceptibility status of wild Anopheles arabiensis to DDT was assessed using a WHO tube test in six sites. During this time, mean percent mosquito mortality rates were 80% (range from 67% to 95%) (Abose et al. 1998). Moreover, populations of An. gambiae s.l developed resistance to DDT in Metahara and Melka Worer (Balkew et al. 2003). In 2005, Abate and Hadis reported DDT resistant An. arabiensis populations at three sites with mean percent mortality 86% (range 80.5% in Finchuwaha to 92.2% in Andassa). Balkew (2010) also reported DDT resistance in An. arabiensis with mean percent mortality of 44% (range 3.8% to 78.8%). In 2007, resistant to DDT by the same species has been reported in Wondogenet, but population of An. arabiensis was susceptible in Pawi (Abate and Hadis 2011).

In 2008, susceptibility status of DDT was assessed in 11 sites in north western, western and southern Ethiopia; six sites by Abate and his colleagues (Abate and Hadis 2011), four sites by Presidents Malaria Initiative (PMI) team (PMI 2010), and one site by Yewhalaw and his colleagues (Yewhalaw et al. 2010). In ten of the eleven sites, populations of An. arabiensis were resistant to DDT. The mean percent mortality of An. arabiensis to DDT was 20.8% (ranging between 1% in Assendabo to 100 % in Zabatison). In 2009, the susceptibility status of An. arabiensis to DDT was assessed at eight sites in northern, western and southern Ethiopia. The populations of An. arabiensis were highly resistant to DDT with the mean percent mortality of 12.86% (range 0.5% to 36.1%) (Yewhalaw et al. 2011; Abate and Hadis 2011). In 2010, the susceptibility status of populations was assessed at 25 sites (Balkew et al. 2012, Massebo et al. 2013). The An. arabiensis populations collected from the above sites developed resistance to DDT with mean percent of mortality of 22.5% (range 0 to 82%) (Unpublished-Data; PMI 2016a). In 2011, the susceptibility status of An. arabiensis to DDT was assessed in 28 sites. The populations of An. gambiae s.l developed resistance in all sites with mean percent of mortality 13.65% (Asale et al. 2014; Fettene et al. 2013; Unpublished-Data). In 2012, An. arabiensis population developed resistance to DDT in all eleven sites with mean percent of mortality 10.24 % (PMI 2014). In 2013, An. arabiensis populations were resistant to DDT in eleven sites with mean percent of mortality of 18.3% (PMI 2015) and in 2014 An. arabiensis population was resistant to DDT in seven sites with mean percent mortality of 10.5% in 2014 (PMI 2016). Though DDT resistance was first identified in 1986, the trend of insecticide resistance further increased from 2005 to 2015 as shown in Figure 3.

There has been a wide distribution of DDT resistance in the country since 1986 (Abose et al. 1998). However, An. arabiensis populations from certain sites of Benishangul Gumuz and Amhara regions was susceptible to DDT in 2007 and 2008 (Abate and Hadis 2011).

![Figure 3: Trends of resistance of Anopheles arabiensis populations to DDT, Deltamethrin and Permethrin in Ethiopia (1986 to 2017).](image-url)

**Legend**
- DDT
- Deltamethrin
- Permethrin

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Distribution and trends of *An. gambiae* s.l resistance to organophosphates: The susceptibility status of *An. gambiae* s.l to fenitrothion was assessed from 2011-2017 at 107 localities. Of these sites, *An. gambiae* s.l was susceptible to fenitrothion from 92 sites but possible resistance and resistance to this insecticide has been reported in 10 and 5 sites, respectively sites (Figure 4) (Balkew et al. 2012, Fettene et al. 2013, PMI 2016, Asale et al. 2014).

Moreover, susceptibility status of *An. gambiae* s.l to Malathion was monitored in 127 sites from 2005 to 2017. The populations of *An. gambiae* s.l was susceptible at 36 sites, possible resistance at 55 sites and resistance at 36 sites to Malathion. (Balkew et al. 2012; Fettene et al. 2013; PMI 2016; Asale et al. 2014; Yewhalaw et al. 2010; Abate and Hadis 2011). Susceptibility status of wild populations of *An. gambiae* s.l against pirimiphos-methyl assessed in six regions of the country (Figure 5). *Anopheles gambiae* was susceptible to pirimiphos-methyl in all assessed 51 sites. However, possible resistance and resistance also documented in 4 sites and 1 sites, respectively (Unpublished-Data; PMI 2014; PMI 2015; PMI 2016).

Distribution and trends of *An. gambiae* s.l resistance to pyrethroids: Susceptibility level of wild *An. gambiae* s.l population to alpha-cypermethrin assessed in 12 sites from 2015 to 2017. The population developed resistance to Alpha-cypermethrin in 8 sites. The population was susceptible in 2 sites and, possible resistance was recorded in 2 sites (PMI 2015; Unpublished-Data). In addition, Susceptibility level of wild *An. gambiae* s.l population to Alpha-cyhalothrin assessed in 17 sites from 2010-2014. The *An. gambiae* s.l population develops resistance at all monitored sites to the insecticide (Massebo et al. 2013; PMI 2014; PMI 2015). The susceptibility status of *An. gambiae* s.l population to lambda-cyhalothrin was assessed in 62 sites from 2010 to 2015. Of these, twenty-one sites were assessed in 2010. Population of *An. gambiae* s.l collected from five sites showed possible resistance, while mosquito populations from the remaming sites was resistant to lambda-cyhalothrin (Massebo et al. 2013; Asale et al. 2014; PMI 2015; PMI 2014). Interestingly populations with possible resistance to the insecticide in 2010 developed resistance the following years in all surveyed sites.

In 2005, populations of *An. gambiae* s.l from Sabure, Andassa and Finchwuda were susceptible to deltamethrin (Abate and Hadis 2011). Mosquito population was also susceptible from Sodere, but resistant to the insecticide from Ghibe and Gorgora (Balkew et al. 2010). Before 2007, among five sites assessed, Populations of *An. gambiae* s.l was susceptible to deltamethrin at two sites in the Amhara region. The resistance frequency of the populations to deltamethrin increased, since 2008. However mean percentage mortality decreased since 2015 (Figure 3).
In 2008, populations of *An. arabiensis* were susceptible at two sites; Gilge Ghibe (Yewhalaw et al. 2010), and Enddo-Kontole and Assendabo (PMI 2010). Later in 2009, *An. arabiensis* population from five sites developed resistance to deltamethrin. (One site from Amhara region, two sites from SNNPs and two sites from Oromia region) (Abate and Hadis 2011; Yewhalaw et al. 2010). In 2010, *An. arabiensis* populations from ten sites were resistant to deltamethrin However, possible resistance has been reported from this species from seven sites while susceptible populations has been reported at four sites (Massebo et al. 2013; Unpublished-Data).

The susceptibility status of *An. arabiensis* population to deltamethrin was assessed at 24 sites of six regions in 201 (Unpublished-Data; Fettene et al. 2013; Asale et al. 2014). In 2012, of the three regions, mosquito populations developed resistance to deltamethrin at 16 sites with a mean mortality rate of 27% (range 1% to 61%) (Unpublished-Data; PMI 2014). In 2013, populations from twelve sites of three regions, developed resistance to the same insecticide with a mean mortality of 26% (range 13%-51%) (PMI 2015; Unpublished-Data). Resistant populations were also reported from 40 sites of seven regions during the period 201-2017 with a mean percent of mortality of 43 (range11% - 83%) (PMI 2016). Resistance to etofenprox has been reported between 2012- 2015 from 22 surveyed sites: three sites (i.e. Bahirdar, Zenzlima Robit and Asendabo) in 2012, three sites (i.e. Abaya, Shemen and Zenzlima Robit) in 2013, and sixteen sites (i.e. Alamata, Sedi, Zenzlima Robit, Chewaka, Kurgen, Osso Billi, Asendabo, Burka and Shemen) in 2014 and 2015 (PMI,2013 - 2015). From 2000 to 2017, 69 sites surveyed to assess the susceptibility level of populations of *An. gambiae s.l* to permethrin. Insecticide resistance to permethrin has been reported since 2000 (Balkew et al. 2003), however, populations from some sites were susceptible to permethrin in 2008 (Abate and Hadis 2011). In 2008, resistance of this species to permethrin has been reported from 52 sites (Unpublished-Data; Abate and Hadis 2011; Yewhalaw et al. 2010; PMI 2014-2018). The resistance trend of this species to permethrin increased until 2014 as shown in Figure. Distribution and trends of *An. gambiae s.l* resistance to carbamates: The susceptibility status of the malaria vector to bendiocarb was first assessed in 2010 in Ethiopia. From 2010 to 2017, a total of 106 sites were surveyed and. In 2010, wild populations of *An. gambiae s.l* from 22 sites were susceptible to this insecticide (Balkew et al. 2012; PMI 2015). Of the fourteen localities surveyed later in 2011, only populations from five localities were susceptible to this insecticide (Fettene et al. 2013).
Populations of this species from seven sites in 2012, eight sites in 2013 and four sites in 2014 were susceptible to this insecticide (PMI 2013-2015; PMI Unpublished-Data). From 2015 to 2017, Mosquito populations from 35 sites was assessed for bendiocarb resistance during the period 2015-2017. Of this, populations from 27 sites were susceptible to the insecticide while, possible resistance and confirmed resistance were documented from two and 6 sites, respectively (PMI 2017-2018; PMI Unpublished-Data). Populations of An. arabiensis from the Amhara region developed resistance to this insecticide. The distribution of bendiocarb resistance in populations of An. arabiensis in Ethiopia is depicted in figure 7.
Populations of *An. gambiae* s.l. was assessed for their susceptibility status to propoxur in 106 localities in Ethiopia from 2010 to 2017. The populations from 91 sites was susceptible to this insecticide and resistance was only developed at populations from six sites (four from Amhara, one from SNNPRs and one from Tigray) were resistant to the same insecticide. Possible resistance to propoxur was documented from nine sites (Balkew et al. 2003; Balkew et al. 2012; Balkew et al. 2010; Yewhalaw et al. 2010; Fettene et al. 2013; PMI 2016). Spatial distribution *Anopheles arabiensis* resistance to propoxur shown in Figure 8.

**Figure 8:** Map showing the distribution resistance in *Anopheles arabiensis* to propoxur in Ethiopia (2000 -2017).

**Insecticide resistance management in malaria vector control:** Insecticide resistance monitoring and management (IRMM) strategic plan developed and endorsed by the Ethiopian FMOH in collaboration with stakeholders. For this purpose, 25 malaria sentinel sites identified to generate both entomological and epidemiological data for evidence-based decision making to sustain control and enhance elimination efforts. In high malaria transmission, two type of intervention (i.e. LINNs and IRS) from different class of insecticides will implemented. However, in moderate malaria transmission area rotate IRS insecticides from different classes recommended yearly. The remaining two insecticide resistance managements (i.e. mixture and mosaic) are not operationally feasible in Ethiopia (IRMM).

**Future prospects for malaria control:** For the success of the goal of malaria elimination in Ethiopia set by 2030, innovation of vector control tools to counteract the emergence of drug and insecticide resistance is crucial (WHO 2016a; Yewhalaw and Kweka 2016). For this, ivermectin became a potential tool receiving attention to be used as a malaria control tool (Carlos et al. 2013; Chaccour et al. 2015; WHO 2016b). The residual insecticides in insecticide-treated wall lining (ITWL) are durable and maintain control of insects significantly longer than IRS and may provide an effective alternative or additional vector control tool to ITNs and IRS (Munga et al. 2009).

Since the discovery of the mosquito larvicidal activity of *Bacillus thuringiensis* israelensis (serotype H-14) in 1977, several formulations of Bti have used against different mosquito species (Mittal 2003). Moreover, there is a growing interest in the use of very safe insect growth regulator (IGRs) that are emerging as promising vector control compounds for mosquito control with specific action and are relatively safe to non-target organisms (Mulla and Darwazah 1979; Morrison et al. 2008). Transmission blocking vaccines (TBVs) are being assessed as a way to control the spread of malaria (Wu et al. 2015). Genetic engineering of mosquito’s transgenic technology acts as an alternative strategy to the conventional vector control methods (Catteruccia et al. 2000).

**Discussion**

DDT were used in the country for four decay to malaria control. Since 1986, it is resistance increased from time to time even after the country stopped DDT spraying. This might be due to cross-resistance with other insecticides used to impregnate LLINs. In the country, DDT and pyrethroids resistance are
Widespread, which is constinance with study conducted in western Kenya (Mulamba et al. 2014). Similarly *An. gambiae* s.l. was developed resistant to DDT and pyrethroid in central and eastern Uganda (Verhaeghe et al. 2010). However, *An. arabiensis* was susceptible to DDT in Zanzibar (Haji et al. 2013). Resistance to pyrethroids by *An. gambiae* s.s. and *An. arabiensis* also reported from several districts of the mainland of Tanzania (Kabula et al. 2014; Matowo et al. 2010). *An. gambiae* s.l. showed different levels of resistance to deltamethrin, lambdacyhalothrin and bendiocarb at different study sites of Ethiopia, which is consistence with study conducted in Kilifi, Malindi and Taveta districts in coastal Kenya (Stump et al. 2004) and in Zanzibar (Haji et al. 2013). Moreover, pyrethroid resistance has been also reported in *An. gambiae* s.s and *An. arabiensis* from four districts of Western Kenya (Stump et al. 2004). In Ethiopia, *An. arabiensis* were fully susceptible to bendiocarb, fenitrothion and Pirimiphos-methyl until 2011. Similarly, *An. gambiae* s.s and *An. arabiensis* were fully susceptible to bendiocarb and fenitrothion from eastern Uganda (Mwesige et al. 2013) And In Zanzibar, *An. arabiensis* was susceptible to carbamates (bendiocarb). Moreover, in a similar *An. gambiae* s.s to was susceptible to bendiocarb, and malathion (Haji et al. 2013).

**Conclusion and recommendations**

The development of resistance in *An. arabiensis* to DDT substantially increased after 2006 following the large-scale distribution of LLINs. Even if utilization of DDT for IRS discontinued in 2009, the populations of *An. arabiensis* remains resistant to DDT in the country. This might be because of cross-resistance between organochlorines and pyrethroids. A large proportion of the *An. arabiensis* population developed resistance to pyrethroids class insecticides which are used to impregnate nets, and therefore the use of new generation bed nets might be important to enhance the killing effect of nets. Though the amount of bendiocarb used for vector control is lower than propoxur, a large proportion of the wild *An. arabiensis* population developed resistance for bendiocarb compared to propoxur. *Anopheles arabiensis* was susceptible to pirimiphos–methyl, and hence it may be used for IRS instead of carbamates in the country to limit the resistance from reaching its tipping point. In addition, considering non-insecticidal vector control method is important to regain vectors susceptibility for carbamate class, like screening of doors and windows and environmental managements.

**Acknowledgements**

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Of Anopheles Gambiae In Response To Insecticide-Treated Bed Net Trials. *The American Journal Of Tropical Medicine And Hygiene*, 70:591-596.


Anopheles mosquito species composition, density, longevity and malaria prevalence around Gilgel-Gibe area, Southwest Ethiopia

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Abstract

Introduction: Change in temperature, humidity, altitude, population of humans, deforestation and construction of reservoir (dam) are ecological factors that play essential roles in changing the dynamics of malaria transmission. Objective: This study was intended to assess the effect of dam on anopheles mosquito species composition, abundance, longevity, and density and malaria prevalence pattern at Gilgel-gibe hydro-electric dam I.

Methods: Monthly entomological study was conducted from June to December 2013 in two kebele (Koticha Gibe and Decha Nadi) of Tiro Afeta district, Jimma zone Southwest Ethiopia. Centers for Disease Control and Prevention (CDC) Light Trap (LT) and Pyrethrum Spray Collection (PSC) was employed for biting and resting Anopheles mosquitoes sampling respectively while retrospective parasitological records was taken from Logbook of health facilities. A total of 1521 adult anopheline mosquitoes were collected. Students t-test for mean density comparison, Chi-square for comparison of malaria prevalence and Pearson’s correlation to test the association between means were used. P-values less than 0.05 were considered as stastically significant.

Result: Overall, 1521 adult anopheleine mosquitoes belonging to two species were collected. An. gambiae senso lato was the predominant species (72.9%) followed by An. coustani senso lato (27.1%). The mean monthly An. gambiae senso lato density, collected by LTcS and PSCs, was 5.6 per trap/night and 3.51 per house/day, respectively. The density of An. gambiae senso lato in Koticha Gibe was higher (8.5 per trap/night and 5.6 per house/day) than that of Decha Nadi (2.71 per trap/night and 1.95 per house/day), respectively. There was a significant difference between mean indoor and outdoor An. gambiae senso lato density (P < 0.05). There was no significant difference between mean indoor and outdoor density of An. coustani senso lato in the two kebele (P > 0.05). Degree of exophily increased from 1.61 to 1.28 and 1.35 to 1.23 in Koticha Gibe and Decha Nadi kebele, respectively, post Indoor Residual Spray (IRS) operation and Long Lasting Insecticide Nets (LLITNs) distribution. Overall probability of daily survival of An. gambiae senso lato decreased from 0.70 to 0.56 during post IRS operation and LLITNs distribution. The prevalence of malaria in the study setting was 2.2%.

Conclusion: Despite the two kebele having identical ecotypes and weather conditions, the kebele located near to the dam had a relatively high mosquito density and malaria prevalence than the kebele located far away from the dam.

Key words: Anopheline, malaria, longevity, Gilgelgibe dam, Ethiopia.

Introduction

Despite remarkable achievements, the human toll of malaria and the global risk it still poses remains unacceptably high (WHO 2015; WHO 2017). In 2017. P. falciparum accounted for 99.7% of estimated malaria cases in the WHO African Region followed by WHO regions of South-East Asia (62.8%) (WHO 2017). In 2015, over two million malaria cases (confirmed plus clinical) were recorded, responsible for 662 deaths (MOHa 2015; MOHb 2015). Of these total cases, P. falciparum accounted for 63.7%, and the remaining were due to P. vivax (MOH 2015). Change in temperature, humidity, altitude, population of humans and deforestation are just a few ecological factors that play essential roles in changing the dynamics of malaria transmission (Shiuli 2003). Among ecological changes occurring in Ethiopia one is construction of dams (Abbink 2012; Franco et al. 2013). It is obvious that the construction of water storage reservoirs is critical for eradicating hunger, improving access to clean water (Millennium Development Goals 1 and 7, respectively), and generating electricity usually results in elevated malaria transmission in surrounding human communities and contributes to a disease burden that claims 1.5 and 2.5 million lives each year (Lautez 2007). The development, management and operation of water resources have a history of modifying the frequency and transmission dynamics of malaria (WHO 2005). According to Ledec and Quinitero (2003), Ethiopia has been constructed a large number of dams to produce electricity, irrigate farmlands and control flood. However, these development projects could have impact on ecology of vectors and malaria transmission dynamics (Norris 2004; WHO 2014). Therefore this study was intended to assess the effect of Ethiopian dams and metrological variables on
anopheles mosquito species composition, abundance, longevity, and density and malaria prevalence pattern at Gilgel-gibe hydro-electric dam I.

Materials and Methods

Description of the Study area and period: The study was conducted in two kebeles (sub districts/peasant associations) in Tiro-Afeta district Jimma zone southwestern Ethiopia located 260km away from Addis Ababa with an altitude of 7°42’00”N and longitude 37°18’00” E. The two kebeles were Koticha Gibe (near to dam) and (Decha Nadi) away from the dam. Koticha Gibe is located <1km away from the reservoir of Gilgel Gibe Hydroelectric dam while Decha Nadi is relatively far (5km) from the dam. (Figure 1).

![Map of the study area](https://example.com/map.png)

Figure 1: Map of the study area (modified from Tiro Afeta District Communication Office, 2013; Yewhalaw et al. 2013).

The study area district lies between at an altitude of 1,734–1,864 m a.s.l. The kebeles has a sub-humid, warm to hot climate, both cultivated and uncultivated land, is characterized by two rainy seasons, (June to September-the main rainy season and March to May-the short rainy season and receives annual minimum and maximum rain fall ranges from 1,300-1,800mm and has mean minimum and maximum annual temperatures of 16°C and 30°C respectively). The estimated total populations of the two kebeles (Koticha Gibe and Decha Nadi) were 3493 and 3240, respectively (Tiro Afeta District Communication Office 2013).

Study area sampling method: From 190 woreda located in Oromia regional state 172 of them were malarious which is, 65% of the total population of the Region residing in malarious areas (Deressa 2004). Among 13 woreda located in Jimma zone 10 of them were malarious of them three woreda (Omo Nada, Sokoru and Tiro-Afeta) share boundaries with Gilgel-Gibe hydro-electric dam 1 among them Tiro-afeta woreda was randomly selected. Among four kebele Koticha Gibe which is <1km from dam and whereas from those kebele located away (>5km) from the reservoir of Gilgel Gibe, Decha Nadi kebele was randomly selected. The census from the district administrative office 2006 showed that the population of Koticha Gibe and Decha Nedhi has 3493 and 3240, respectively (Tiro Afeta District Communication Office 2013).

Mosquito sampling and identification: Adult mosquitoes were collected from the two kebeles (four villages) using pyrethrum spray catches (PSCs) and Center for disease control and prevention (CDC) light trap catches (LTCs) (Model 512; John W. Hock Co., Gainesville, FL). Two villages were selected from each kebele for mosquito sampling. From the two kebele total of 16 houses for CDC LTCs (eight from Koticha Gibe and Eight from Decha Nadi) and twenty houses for PSCs (ten from Koticha Gibe and Decha Nadi kebele respectively) were randomly selected and mosquito collection was conducted monthly on each
of the selected houses. LTCs were placed indoor near the bed at 1.5 meters above the ground whereas outdoor mosquito collection was set in the radius of 8 m surrounding the house selected for outdoor collection from 6:00 PM in the evening to 6:00 AM in the morning.

For PSCs each selected house was sprayed between 6:00-7:00 AM following the standard procedure (WHO 1975). The residents were informed to leave the houses, remove food items and animals from each selected house, white cloth sheets were spread to cover the entire floor surface. Windows and doors were closed and other openings were covered to prevent the exit of mosquitoes. A commercially available insecticide (Byogon) containing pyrethroids (Tetramethrin 0.3%, Permethrin 0.25%, Perfurme 0.25%, Kerosine and propellant 99.2%, manufactured by Changzhou Zhongtian Aerosol product co.) was used. The room was kept closed for 15 minutes after the spray to ensure maximum knock-down of mosquitoes (WHO 1975). All mosquitoes were collected from sheets with a hand-held battery-powered with forceps. Specimens were killed with chloroform and placed in paper cup. The collected mosquito specimens were morphologically identified at Asendabo Field Vector Biology Laboratory of Jimma University; following standard key (Gillies and Coetze, 1987; Verrone 1962 b). Gravid and half gravid mosquito were labeled according to site, species, date and sampling techniques and preserved individually in eppendorf tubes over silica gel.

Parity rate determination: The freshly killed mosquito with chloroform was placed on a microscope slide with a drop of Phosphate Buffered Saline (PBS) solution. On the sixth or seventh abdominal segment, the contents were pulled out gently. The ovaries were then left to dry. The ovaries were then observed under the stereoscope dissecting microscope to determine parity based on ovarialtracheoles skeins (Detinova 1962; WHO 1975).

Parasite prevalence survey: A record and chart review of malaria cases were conducted in Dintu Health Center, Kothika Gibe and Decha Nedi health post of Tiro Afeta district from June to December, 2013. The prevalence of clinical malaria in the total population was calculated as the number of malaria cases diagnosed in the health facilities during study period divided by the total population of the communities (Koticha and Decha Nadi Kebele) that the individual health facilities serves. Where K is constant indicating the size of the populations to which the rate is applies (usually 100, 1000 or 10 000 but more generally 10⁶ (WHO 1986; Falgue et al. 2007)

\[ P = \frac{\text{Number of malaria cases}}{\text{Total number of population at risk}} * K \]

Meteorological data: Relative humidity (%), monthly rainfall (mm), maximum and minimum temperature (°C) of the study area were obtained from the southwestern branch regional office of the Ethiopian Meteorological Agency from June-December, 2013.

Data analysis: Data were entered and analyzed using SPSS version 16.0 statistical package. The abundances and percentage composition of anopheles mosquitoes were computed. Data was log transformed, Student t-test was used for mean density comparison of anopheles mosquitoes between Kebelle located near dam and far from the dam. Chi-square was used for comparison of malaria prevalence between Kebelle located near and away from the dam. An association between mean Anopheles mosquitoes density, monthly malaria prevalence and meteorological variables was assessed and further checked by Pearson’s correlation at zero, one, two months and three months lag periods. P-value less than 0.05 were considered statistically significant during the analysis. Mean Anopheles mosquitoes Density (D) = (number of females ÷ number of houses) ÷ number of nights. P = duration of indoor resting after blood feeding. This parameter is obtained from the analysis of the abdominal condition of resting females. P = 1 + (number of half-gravid and gravid females ÷ number of freshly fed females (WHO 2003). Parity rate (S) was obtained by

\[ S = \frac{\text{Number of parous mosquitoes}}{\text{Total number of dissected Anopheles mosquitoes}} * K \]

Olayemi and Ande, 2008)

The probability of surviving one day (denoted as p) can be estimated as: \[ p = \frac{m}{\text{Proportion parous and three day interval were assumed for An. gambiae}.1\text{. gonotrophic cycle (ge). Life expectancy was estimated using the formula } L = 1 - \log p \text{ (Davidson 1954; WHO 2003).} \]

Where \( L \) = life expectancy \( p \) = probability of daily survival.

Ethical consideration: Ethical approval for the study was obtained from Jimma University, College of Natural Science. Written informed consent was also sought from head of the selected house hold.

Results

Entomological survey
Composition and abundance of anopheles mosquitoes: A total of 1521 adult anopheles mosquitoes belonging two species were collected during longitudinal entomological survey. An. gambiae.s.l. was the predominant species in the study areas which accounted for 72.9%, followed by An. constans.s.l.(27.1%). The majority (70.41%) of anopheline mosquitoes was collected from Koticha Gibe (Table 1).
### Table 1: Species composition and abundance of anopheles mosquitoes in Tiro Afeta District, Jimma zone, Southwest Ethiopia.

<table>
<thead>
<tr>
<th>Kebele</th>
<th>Anopheles species</th>
<th>LTC (Indoor)</th>
<th>LTC (Outdoor)</th>
<th>LTC (Total)</th>
<th>PSC</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dacha Nadi</td>
<td>An. constani s.l.</td>
<td>54 (38.8)</td>
<td>63 (45.3)</td>
<td>117</td>
<td>22  (15.8)</td>
<td>139 (100.0)</td>
</tr>
<tr>
<td></td>
<td>An. gambiae s.l.</td>
<td>116 (37.3)</td>
<td>36 (11.6)</td>
<td>152</td>
<td>159 (51.1)</td>
<td>311 (100.0)</td>
</tr>
<tr>
<td>Koticha Gibe</td>
<td>An. constani s.l.</td>
<td>93 (34.1)</td>
<td>118 (43.2)</td>
<td>211</td>
<td>62  (22.7)</td>
<td>273 (100.0)</td>
</tr>
<tr>
<td></td>
<td>An. gambiae s.l.</td>
<td>305 (38.2)</td>
<td>152 (19.0)</td>
<td>457</td>
<td>341 (42.7)</td>
<td>798 (100.0)</td>
</tr>
<tr>
<td></td>
<td>An. constani s.l.</td>
<td>147 (35.7)</td>
<td>181 (43.9)</td>
<td>328</td>
<td>84  (20.4)</td>
<td>412 (100.0)</td>
</tr>
<tr>
<td></td>
<td>An. gambiae s.l.</td>
<td>421 (38.0)</td>
<td>188 (17.0)</td>
<td>609</td>
<td>500 (45.0)</td>
<td>1109 (100.0)</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td>568 (37.3)</td>
<td>369 (24.3)</td>
<td>937</td>
<td>584 (38.4)</td>
<td>1521 (100.0)</td>
</tr>
</tbody>
</table>

Key: LTC = Light trap catches, PSC = Pyrethrum spray catches, Number in parenthesis indicate percentage.

**Anopheles mosquito density:** Mean monthly *An. gambiae* s.l. density was 8.5 and 2.70 per trap/night with statistically significant difference (P=0.04) from near dam and far away from dam whereas 5.07 and 1.95 per house/day using PSC with statistically significant difference (P=0.048) was recorded kebele located near to dam and far away from dam respectively (Figure 2).

![Graph](image)

**Figure 2:** Mean *An. gambiae* s.l. density in villages near dam (Koticha Gibe) and away from dam (Decha Nadi), Tiro Afeta district, Jimma zone Southwest Ethiopia (June-December 2013).

Table 2 presents indoor and outdoor *An.gambiae* s.l. and *An. constanti* s.l. density in the near dam and far away from dam kebelle. The result shows that in both kebelles, mean indoor density of *An.gambiae* s.l. was significantly higher than the outdoor density (P=0.04; 0.03). On the other hand, *An.constanti* s.l. indoor/outdoor density didn’t show significant difference (P=0.49/0.79). Moreover, there was a significant difference in mean indoor density of *An. gambiae* s.l. (p=0.038) before (June-August 2013) and after IRS operation (September-December 2013) while there was no significant difference (P=0.81) in mean indoor density of *An.constanti* s.l.in both kebelles (Table 2).

### Table 2: Mean Indoor and outdoor Anopheline mosquitoes density per trap night in Tiro Afeta District, Jimma zone Southwest Ethiopia (June-December 2013).

<table>
<thead>
<tr>
<th>Kebele</th>
<th>Species</th>
<th>Density</th>
<th>M±SE</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decha Nadi</td>
<td><em>An. gambiae</em> s.l.</td>
<td>In</td>
<td>2.21±0.48</td>
<td>0.04*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Out</td>
<td>0.50±0.44</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>An. constanti</em> s.l.</td>
<td>In</td>
<td>0.96±0.38</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Out</td>
<td>0.38±0.26</td>
<td></td>
</tr>
<tr>
<td>Koticha Gibe</td>
<td><em>An. gambiae</em> s.l.</td>
<td>In</td>
<td>5.45±1.03</td>
<td>0.03*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Out</td>
<td>2.70±0.46</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>An. constanti</em> s.l.</td>
<td>In</td>
<td>1.66±0.29</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Out</td>
<td>2.11±0.29</td>
<td></td>
</tr>
</tbody>
</table>

*Significant at p < 0.05
The mean monthly *An. gambiae* s.l. density per trap night and its association with meteorological variables in the two kebellas is shown in figure 3. In both kebellas, the mean monthly density of *An. gambiae* s.l. was strongly correlated with two month lag of minimum temperature (r=0.74, p=0.058) and (r=0.92, p=0.004) whereas weak correlation was observed for RH (r = 0.034, p = 0.94 and (r =0.0026, p = 0.95) and RF (r=0.39, p=0.37 and r= 0.24, p=0.61) in kebele near to dam and far away from the dam respectively (Figure 3).

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**Figure 3: Correlation of mean *An. gambiae* s.l. density with meteorological variables in Tiro Afeta district, Jimma zone, Southwest Ethiopia (June-December 2013).**

**Duration of resting indoor after blood feeding**

Overall 500 *An. gambiae* s.l. were collected from the two kebellas by PSCs during the seven consecutive months (June-December 2013). Of these, 365 *An. gambiae* s.l. were collected during pre IRS operations (June - August 2013). Of these, 241 were fed mosquito specimens while 124 were half gravid and gravid. After IRS operation (September - December 2013) of the total 135 *An. gambiae* s.l. collected, 107 of them were fed and the rest 28 were half gravid and gravid (Table 3). In Koticha-Gibe duration of resting indoor after blood feeding decreases from 1.61 to 1.28 during Pre and post IRS operation respectively. Also in Decha-Nadi kebele duration of resting indoor after blood feeding decreases from 1.35 to 1.23 during Pre and post IRS operation and LLINs distributions respectively.

**Table 2: Fed to gravid ratio and degree of exophily of *An. gambiae* s.l. pre and post IRS operations and LLINs distributions in Tiro Afeta district, Jimma zone Southwest Ethiopia.**

<table>
<thead>
<tr>
<th>Intervention (IRS &amp; LLINs)</th>
<th>Kebele</th>
<th>F</th>
<th>HGG</th>
<th>F:G</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>Decha Nadi</td>
<td>90</td>
<td>32</td>
<td>2.80:1</td>
<td>1.35</td>
</tr>
<tr>
<td></td>
<td>Koticha Gibe</td>
<td>151</td>
<td>92</td>
<td>1.64:1</td>
<td>1.61</td>
</tr>
<tr>
<td>Post</td>
<td>Decha Nadi</td>
<td>30</td>
<td>7</td>
<td>4.29:1</td>
<td>1.23</td>
</tr>
<tr>
<td></td>
<td>Koticha Gibe</td>
<td>77</td>
<td>21</td>
<td>3.66:1</td>
<td>1.28</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>348</td>
<td>152</td>
<td>2.29:1</td>
<td>1.44</td>
</tr>
</tbody>
</table>

Key: F = fed, HGG = half gravid to gravid, DE = Degree of Exophily, IRS = Indoor Residual Spraying, LLINs = long- lasting insecticidal nets.

**Parity rate and longevity of *An. gambiae* s.l.:** The parous rate of *An. gambiae* s.l. was higher (34.69%) pre IRS operation than post operation (17.64%) (Table 4). Moreover, *An. gambiae* s.l. showed longer survival rate before control intervention as compared to post control operation.
Table 3: Parity rate, daily survival and longevity of An. gambiae s.l. before and after control intervention in Tiro Afeta District, Southwest Ethiopia (June-December 2013).

<table>
<thead>
<tr>
<th>Intervention (IRS &amp; LI.Ns)</th>
<th>Dissected</th>
<th>Nulliparous</th>
<th>Parous</th>
<th>Parity rate (%)</th>
<th>P(days)</th>
<th>L(E(days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>98</td>
<td>20</td>
<td>34</td>
<td>55.1</td>
<td>0.75</td>
<td>2.80</td>
</tr>
<tr>
<td>Post</td>
<td>68</td>
<td>35</td>
<td>12</td>
<td>0.21</td>
<td>0.56</td>
<td>1.72</td>
</tr>
</tbody>
</table>


Malaria prevalence: During the study period, 112 febrile patients visited the three health facilities from the two kebelles. Overall, 15 (13.14%) of the febrile cases were positive for malaria parasites. P. falciparum accounted for 73.3% of the positive cases and the remaining 26.7% cases was due to P. vivax. More than half (53.3%) of the positives cases were recorded from kebelle near to dam with prevalence of 0.31 while prevalence of 0.12 were recorded from kebelle away from dam with over all prevalence of 2.22. There was no significance difference in malaria cases between the two kebelles ($X^2 = 9.386$, $P = 0.052$). (Table 5).

Table 4: Malaria prevalence in Tiro Afeta district, Jimma zone southwest Ethiopia.

<table>
<thead>
<tr>
<th>Kebeles</th>
<th>Number of cases</th>
<th>Species</th>
<th>Pf</th>
<th>Pv</th>
<th>Total</th>
<th>$X^2$</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ducha Nadi</td>
<td>43(87.8)</td>
<td>5(8.2)</td>
<td>2(4.1)</td>
<td>50(100.0)</td>
<td>9.386</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Koticha Gibe</td>
<td>54(87.1)</td>
<td>6(9.7)</td>
<td>2(3.2)</td>
<td>62(100.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>97(86.6)</td>
<td>11(9.8)</td>
<td>4(3.6)</td>
<td>112(100.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


In kebelle near to dam monthly malaria cases was positively correlated with zero month lag of relative humidity, rain fall and minimum temperature and statistically there was no significant correlation with RH ($r=0.62$, $P=0.13$), Min-T ($r=0.30$, $P=0.51$) and RF ($r=0.26$, $P=0.56$).Similar trend was observed in kebelle away from dam. Malaria cases was positively correlated with zero month lag of relative humidity, rain fall and minimum temperature and there was no significant correlation between malaria cases and RH ($r=0.6$, $P=0.15$), between malaria cases and Min-T ($r=0.43$, $P=0.43$) and malaria cases and RF ($r=0.40$, $P=0.38$) and there was weak correlation between malaria cases and meteorological variables at one month, two month and three month lag time in both kebelles (Figure 4).

Figure 4: Correlation between malaria prevalence and metrological variables in Tiro Afeta district South west Ethiopia (June-December 2013).
Discussion
Malaria is a serious threat to human life in sub-Saharan Africa, claiming many lives and causing the greatest morbidity as compared to other infectious diseases (Obion et al. 2014). Ethiopia is one of the sub-Saharan countries which are suffering from this threat. Malaria control program in Ethiopia has a history of more than 40 years. There were a lot of obstacles which could hamper the successful malaria control program in Africa particularly in Ethiopia. Of these problems, occurrence of malaria parasite resistance to drugs (Ketema et al. 2009), insecticide resistance species of major malaria vectors (Asale et al. 2014; Yewhalaw et al. 2011 and 2010), climate change (Avanade et al. 2008; Hay et al. 2005) and man-made ecological transformation such as construction of dams for controlling flood, producing electricity and irrigated farm land (Guerra et al. 2008; WHO2005). The development, management and operation of water resources have a history of modifying the frequency and transmission dynamics of malaria in Ethiopia (Kibret et al. 2009; Yewhalaw et al. 2009; Lautze et al. 2007) and malaria vector density most importantly An. gambiae s.l. (Afrane et al. 2006; Tunon et al. 2005).

The species compositions of Anopheles mosquitoes in the two kebeles) showed that An. gambiae s.l. and An. coustani s.l. were found in sympathy. An. gambiae s.l. was predominant malaria vectors in the study sites which is similar with other parts of Ethiopia (Coetzee et al. 2000). Likewise, this study also found An. coustani s.l. where some of its sibling which was believed to be less anthropophilic with no epidemiological importance in malaria transmission (Adugna et al. 1996) was abundant next to An. gambiae s.l. in the study sites.

Significant differences in mean monthly An. gambiae s.l. density between the two kebele were observed. In our surveys, higher density of An. gambiae s.l. were collected from kebele which was found near to the dam than kebele which was found far from the dam during the period of long rainy seasons and after long rainy seasons. Thus, the presence of dam together with seasons may have contributed to the presence of higher adult mosquito density in the kebele near to the dam. This finding corroborate with Yewhalaw et al. (2013), higher densities of the major local malaria vector, An. arabiensis, which were recorded during the wet season in villages nearer to the dam reservoir.

An. gambiae s.l. showed significant difference in indoor over the outdoor density, this suggested that the species has higher tendency to bite indoor than outdoor. Gillies and Coetzee (1987) and White (1974) reported that An. gambiae s.l and An. funestus, primarily feed and rest indoors where they can be efficiently targeted with intra-domiciliary residual insecticides. The other study which complemented this observation was that An. gambiae s.l. showed endophagic behavior in Gambella region, Ethiopia (Krafur 1977). In contrast to this, other studies reported that An. gambiae s.l. had predominantly exophagic behavior than endophagic behavior in central highlands of Ethiopia (Taye et al. 2017; Woyessa et al. 2004). While, there was no significant difference in indoor and outdoor density of An. coustani s.l., this may be due to the inhabitant partially dwell with Cattles (domestic animals) mixed home. However studies conducted in this area showed this species more of exophagic and exophilic (Taye et al. 2016; Lelisa et al. 2017). In this study there was no significant difference in mean indoor and outdoor An. coustani s.l. density. Other studies also indicated that An. coustani s.l. is well known exophagic species in Ethiopia (Abose et al. 1998) and in Kenya (Mwangangi et al. 2013). Moreover there was no significant difference in mean indoor density of An. coustani s.l. before (June-August 2013) and after (September-December 2013) IRS operation and LLINs delivery while there was significant difference in mean indoor density of An. gambiae s.l. in both kebeles. This finding was in line with Woyessa et al. (2004).

The finding of the study further showed that the density of An. gambiae s.l. was more affected by meteorological variables as mean monthly density of An. gambiae s.l. was influenced by Rain fall, Relative humidity and Temperatures. Other previous studies had also indicated the effect of meteorological variables on mosquito density (Yewhalaw et al. 2013; Woyessa et al. 2004). Mean monthly An. gambiae s.l. density and monthly malaria prevalence was positively correlated with two month lag and zero month lag (a month where An. gambiae s.l. proliferate) respectively. This finding suggested that malaria prevalence in June was transmitted by presence of An. gambiae s.l. in previous two months. Previous studies had indicated the biology of the Anopheles mosquitoes and of the Plasmodium parasite, the effect of meteorological variables on malaria transmission is expected to be lagged in time. Other previous empirical studies suggested that the effect of rainfall on malaria transmission is lagged by approximately 8 weeks (Kefris, 2013; Deresa et al. 2003; Tull 1993).

Lower vector density was observed after IRS operation (September-2013). The results indicated that IRS operation that sprayed (propoxur) had an effect on the vector population which decreased vector density and longevity. This finding is consistent with Obion et al. (2014) and Patrica et al. (2014). Anopheles mosquito density began to buildup after IRS. This may
be due insecticide resistance (Asale et al. 2014; Yewhalaw et al. 2011) insecticide decay rate or operational problems.

Post IRS operation decrease in density of resting indoor after blood feeding was observed in both kebelles. This could be explained by behavioral resistance or a strong decrease in the proportion of gravid and half gravid mosquitoes. This finding was consistent with Padonou et al. (2012) and Mutuku et al. (2011) where strong decrease in proportion of gravid and half gravid mosquito was observed after IRS operation.

Before IRS operations June-August 2013 the observed parity rates were high. This indicates that older populations of mosquitoes tend to accumulate with time (Service 1976). This could be due to availability of potential breeding sites. This allows for increased feeding frequencies and thus, increased chances of the vectors becoming infected or even re-infected during subsequent feeding (Olayeni and Ande 2008; WHO 1975). Decrease in parity rate after interventions could be due to wide spread IRS in the locality. The current vector control strategies of IRS with primarily affect the daily probability of mosquito survivorship and/or reduce vector-host contact (Taye et al. 2016; Enayatiet al. 2010; Curtis et al. 2003).

The prevalence rate of malaria observed in this study was 0.22%, which is less with prevalence rate of 10.5% from similar studies around Gilgel Gibe area (Yewhalaw et al. 2009). This may be due to the intervention programs that have been took place in the locality during the study period. Similar finding in India and Thailand revealed that no increase of malaria incidence was observed near the in Uttaranchal dam India and Nong Wai dam and Ubol Ratana dam in Thailand because of IRS of all houses with DDT (Shukla et al. 2001; Bunnag et al. 1979). The study documented that relatively highest malaria prevalence with 0.31% were recorded from Koticha-Gibe (Near dam) while 0.12% prevalence of malaria were recorded from Decha-Nadi Kebele. Chi square test confirmed that there was no significant difference in malaria prevalence between the two keb elles. Whereas in Ethiopia; small dams constructed for irrigation (Ghebreyesus 1999, Koka dam (Kibret et al. 2009), and Gilgel-Gibe hydropower dam (Yewhalaw et al. 2009) indicated that dams are associated with an increased malaria risk where malaria control program were not under taken.

Generally it has been reported elsewhere that the decline in malaria prevalence in Ethiopia (PMI 2014; Ketema and Bacha 2013), in general, and in the study area, in particular, has been attributed to a combination of factors including improved access to effective malaria treatment with artemisinin combination therapy, interventions using IRS and LLINs decreases mosquitoes bite and decreasing daily porbability of survivorship which decreases the completion of parasite development into infectious sporozoites stages (Taye et al. 2016; Enayat et al. 2010). It may also be due to the sudden heavy rain fall recorded in September in the middle of the study which washed larval breeding sites.

Conclusion

In this study, the dam was found to be an important factor in variation in the density of Anopheles mosquitoes. That means higher density of anopheline mosquitoes and high prevalence of malaria parasites was observed in the villages nearby dam. An. gambiae s.l. was predominant malaria vector with significantly higher indoor biting behavior in the study area. Therefore this information lets every developmental activity such as dam and reservoir should consider the environmental impact analysis in relation to malaria and other infection and special emphasize must be given particularly for those community that dwell nearby water reserves or dam.

References


Entomological surveillance in the context of malaria elimination in some selected sentinel sites of Ethiopia

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Abstract

Introduction: Following the commendable progress made on malaria control programme so far, Ethiopia is persuading malaria elimination in 239 selected low transmission districts located in 6 different regions. Of these, 50 districts has been selected for elimination of local transmission of falciparum malaria based on standard WHO customized criteria.

Objective: The objective of this study was to generate evidence to allow policy making for optimal malaria vector control in the eight districts targeted for elimination by the national malaria control program (NMCP) of Ethiopia.

Method: Eight sentinel sites were selected from four regional states (Oromia, Amhara, SNNPR and Tigray) and one city administration (Diredawa). Entomological surveillance was conducted for two months (November and December 2017). Mosquitoes were collected using human landing collection (HLC), light trap catches (LTCs), pyrethrum spray catches (PSCs) and pitfall shelter (PFSSs). Larval collection were made using standard dip. Over all 14,471 mosquitoes were collected by all collection methods (HLCs, LTCs, PSCs, and PFS) from eight sentinel sites.

Results: Of the 14,471 mosquitoes, 946 were anopheline and 13,525 were culicine mosquitoes. Of the 946 Anopheline mosquitoes, An. gambiae s.l. comprised the majority (67.8%) followed by An. coustani (22.4%). The rest Anophelines (An. pharoensis, An. demilioni, An. squamosus, An. funestus, An. cences, An. pretoriensis and An.maculipalpis) comprised 11.8%. The proportion of anopheline mosquitoes collected outdoor by HLC was significantly higher (p < 0.001) compared to indoor collection. All anophelines showed early peak biting activity (18:00h to 23:00h) both indoor and outdoor in all study sites. There was significant difference in mosquito population density (p < 0.001) across the sentinel sites, between sites Kalu (0.48/night/person-hour), Legharem (0.38/night/person-hour) and Kaberna (0.16/night/person-hour). A total of 352 and 139 anopheline mosquito larvae were collected from Kebena and Merti, respectively. Of this, the mean density of An. gambiae s.l. larvae in Kebena and Merti site was documented as 41.9 ± 12.0, and 7 ± 6.2, respectively.

Conclusion: Higher anopheine mosquito densities were recorded outdoor than indoor. This may sustain outdoor or residual transmission and could be challenging for malaria elimination efforts in these sites. Given the evidence of peak biting activity in the early part of the night before people go to bed; existing interventions methods in particular use of LLINs alone may not be helpful for effective vector control. Thus, new or alternative vector control intervention tools which targets outdoor resting, outdoor and early biting malaria vectors should be developed and implemented as a component of integrated vector management (IVM) to sustain malaria control and enhance elimination efforts.

Key words: Entomology, surveillance, elimination, mosquito, long lasting insecticide

Introduction

It has been known that the global and national communities have done much towards malaria control in the last decades. In recent years malaria related deaths and sickness has been declined due to the huge vector control investments (WHO 2016). As a result many countries have shown a strong commitment toward malaria elimination. On the other hand, elimination phase is more of technical and problematic as cases occur sporadically or in distinct foci (Sturrock et al. 2013). Imported cases may comprise a significant proportion of all cases and may pose a risk for re-establishment of transmission in areas in which it had previously been interrupted (WHO 2012). Hence, transition from control to elimination requires re-orientation of the program and introduction of new strategies. Entomological surveillance is an important and essential aspect of malaria vector control as it provides information on vector species, distribution, density and bionomics used for malaria control and elimination (Manguin et al. 2008). It is also useful for monitoring potential vectors and the role they could play in disease transmission. Information collected through entomological surveillance will help to better understand the spatial and temporal changes in vector
species, efficacy and effectiveness of vector control interventions employed in malaria vector control and elimination (Sinka et al. 2010). It is also one of the critical activities in malaria elimination both to determine and target interventions to eliminate malaria transmission foci and to monitor the impact of interventions (Durnez & Coosemans 2013).

Implementation of interventions for malaria elimination needs more precision than the control phase because the aim is to eliminate existing pockets of transmission or transmission risk. Vector surveillance is therefore vital to guide the targeted interventions in specific foci and prevent re-establishment of malaria cases in elimination areas. Monitoring of vector biomarkers, including abundance, feeding and resting behavior, and insecticide resistance is relevant for effective planning of appropriate interventions (WHO 2012). Moreover, in elimination phase entomological surveillance is a core element and part of intervention. The amount of malaria in an operational unit (area) at a given time can be measured by several methods like transmission intensity measures such as the entomological inoculation rate (EIR) (WHO 2014). The EIR remains the most direct measurement for assessing the effect of anti-vector measures because it quantifies the parasite-infected mosquito pool and its propensity to transmit infectious parasites to the human population (Shaukat et. al. 2010). It also measures the intensity of malaria transmission in a particular area, therefore, used to quantify the potential level of human exposure to infected mosquitoes and to assess the impact of interventions on malaria transmission.

Based on the progress made so far and to achieve the National Malaria Strategic Plan’s (NMSP), strategic goal of eliminating malaria in selected low transmission areas by 2030, Ethiopia has recently set goals to eliminate malaria in 240 selected districts located in 6 different regions (MOH 2014). Of these 50 districts has been selected for elimination of local transmission of falciparum malaria based on standard WHO criteria customized to Ethiopia’s setting. To measure the progress of the elimination agenda in the years to come it is imperative to have a baseline data. Cognizant of this, the Ethiopian public health institute (EPHI) has decided to conduct malaria elimination entomological surveillance in 2017 form eight selected sentinel sites. Thus, the objective of this study was 1) to determine species composition, relative abundance and spatial distribution of malaria vectors, 2) to dermine resting, and mosquito biting rate(s) and pick biting time, 3) to characterize and map the breeding sites of malaria vectors and 4) to measure transmission intensity in spot areas of districts targeted for malaria elimination.

Methods and Materials

Study area and period: Of the total 240 districts selected for elimination, 8 districts were selected using both cluster sampling technique. Accordingly, Kobo and Harbu from Amahra, Kola Temben from Tigray, Dire Dawra from Dire Dawa, Seru and Merti from Oromia, Kebena and Badawacho from Southern Nations and Nationalities People’s Region (SNNPR) were selected for this study. The study was conducted in months of November and December 2017.

Adult Mosquito Collection

Human Landing catches (HLCs): In each of the eight sites selected for the entomological surveillance, four houses were selected as sentinel houses for HLCs. In each of the four sentinel houses indoor and outdoor mosquito collection were carried out from 18:00 pm to 6:00 am for 2 nights in each month. Collection was made by two teams of four people using a mouth aspirator and torch. Outdoor mosquito collection was carried out about 8-10 meters from each of the two sentinel houses. In the first half of the night (18:00 to 00:00 hours), one pair of collectors were assigned to outdoor set up and the other pair was assigned to indoor setting. The collectors were trained to collect probing mosquitoes form both their own exposed legs and from their partner when they find it more convenient. In the second half of the night (00:00 to 06:00 hours) the first four people were rested and replaced by another two pair. In the first night the collection teams were assigned randomly to the selected houses and then exchanged houses in the next collection night. Hourly exchange of indoor and outdoor collection was done. The collecting paper cups were changed hourly following mosquito capture. At the end of collection, mosquitoes were identified morphologically using taxonomic keys. The collectors were supervised during collection to collect those mosquitoes landing on their legs before biting. The volunteers were followed up for fever and no fever has occurred during the study period.

CDC Light trap Collection (LTC): In each of the eight study sites, ten houses were selected randomly for indoor and outdoor CDC light trap catches. These houses were served as sentinel stations for mosquito collection involving light trap catches. Mosquitoes were collected indoor and outdoor from 18.00 pm to 6:00 am from each house using standard CDC light traps (CDC, Atlanta, GA, USA). Traps were hanged from the ceiling or from roof supports at the foot end of the bed where a person slept inside untreated net. The trap was suspended about 1.5 meters above the ground (Mboera et al. 1998). A verbal consent was obtained from each household head. Traps were set by trained research team members. Collection bags were labeled marked with the date and the site number. The
number of people slept, and animals kept inside the house the previous night were also recorded. The following morning, collection bags were retrieved from traps in each house in the morning from 6:00 am to 9:00 am by research teams and field assistants. Collection was made once in each month of November and December 2017.

Pyrethrum Spray Collection (PSC): Twenty-five houses were selected randomly in each of the selected villages. Mosquitoes were collected from 6:00 am-8:00 am early in the morning. For this pyrethrum spray collection all the eaves, windows and other exit points were covered by clothes. White cloth sheet was spread on the floor. Pyrethrum based aerosol (Baygon, SC Johnson & Son Inc, USA) were sprayed in the entire space of the room and the house was closed for fifteen minutes after spraying. Prior to spraying, the heads of each household was informed about the purpose and the time of spray and were given clear instructions as to what they have to do before and after the spray. After fifteen minutes, all the knocked down mosquitoes lying on the white sheets were collected carefully with forceps and placed in paper cups. All anopheline mosquitoes were identified to species morphologically using taxonomic keys. Collections were made once from each house during November and December 2017.

Pit Shelter Collections: Five houses were selected from each study villages and outdoor resting mosquitoes were collected using mouth aspirators from artificially dug pit shelters. The dimension of each pit shelter was 1.5 m deep and had 1.2 m x 1.2 m size. About 0.5 m high from the bottom of each pit shelter, 30 cm deep horizontal cavity was prepared in all four sides. The mouth of each pit shelter was covered with untreated net during collection time to prevent mosquitoes from flying out of the pit shelter. Mosquitoes’ were sampled from 6:00 to 9:00 am twice per month from each pit shelter. Species of Anopheles mosquitoes were done morphologically using taxonomic keys.

Sporozoite infection detection using ELISA and estimation of EIR: The sporozoite ELISA test was conducted according to the protocol developed by Wirtz et al. (1987). Accordingly, 50 μL of grinding buffer (PBS-pH7.8) was added to each vial. Using sterile blade and forceps the head and thorax part of each mosquito was separated from the rest of the body, added to the vial with grinding buffer and homogenized using battery operated pistol. Separately each well of micro plate was coated with 50 μL of the capture MAb and the plate was incubated for 1 hr at room temperature. Plates were covered with aluminum sheet and kept under dark condition during the incubation period. The capture MAb was aspirated and the plates were filled with blocking buffer (BB) (IGEPAL CA-630) completely and incubated for 1 hr at room temperature. The blocking buffer was then completely aspirated. Then 50 μL of positive (SIGMA ALDRICH) and negative control (head and thorax region prepared from lab reared An. arabiensis) were added in to the first and second column. Then, 50 μL/well of mosquito sample was loaded to the remaining wells of the plate. The plate was covered and incubated for 2 hours at room temperature. Then the plate was aspirated and washed two times with PBS-Tween 20. Then 50 μL/well of Peroxidase-conjugated MAb was added and incubated for 1 hour at room temperature. The conjugate was then aspirated and washed three times using PBS-Tween 20. 100 μL/well ABTS substrate was added and incubated for 30 minutes. Finally, the result was read visually by color change (Awolola et al. 2002) or at 405-414 nm using an ELISA plate reader 30 and/or 60 minutes after the substrate had been added.

HLCs based EIR was determined using the formula indicated below:

\[
\text{EIR} = \frac{\text{Human biting rate} \times \text{sporozoites rate (%)}}{100}
\]

Larval collection (LC): Larval collections were done to determine the types and abundance of habitats where potential vectors exist. Larval collection was carried out during November and December 2017. All possible bodies of water within in the sentinel sites were surveyed. All potential habitats were inspected systematically for the presence of Anopheles larvae. When mosquito larvae are present, a standard mosquito 350 mL dipper was used to collect the larvae by lowering it gently into the water. Samplings was always done in the morning (09:00-12:00 h) or afternoon (14:00-17:00 h) for about 30 min at each larval habitat. The water was poured in 30 x 15 cm plastic tray and carefully observed for the presence of mosquito larvae. Then, larvae were collected alive by means of a pipette and transferred to a labeled plastic bag. The larvae collected were reared into adults in white plastic tray by providing diet and under optimum temperature and identified to species using standard taxonomic keys. The coordinates of habitat containing mosquito larvae were marked using a handheld Geographic Positioning System (GPS).

Data analysis: Mean Vector density and mean parity rates were calculated using excel spread sheet. Indoor and outdoor hourly biting activity of mosquitoes was presented using line graphs. Mean mosquito density between indoor and outdoor collection (CDC & HLC) were compared using independent t-test. The CSP rate was determined as the proportion of mosquitoes found
positive for *P. falciparum* and *P. vivax* CSP out of the total number of mosquitoes tested. Mean mosquito density by collection sites were analyzed using general linear model univariate analysis, using mosquito densities as dependant variable. Mosquito species and collection methods were considered as fixed factors while study sites of collections were considered as random factor effect. The post hoc univariate analysis was done on multi comparison of the observed means and significant levels were separated by HSD-tukey test for mean number of mosquitoes for method of collection, species across the sentinel sites. Means were considered significant different at p value <0.05. All analysis was done using SPSS software package version 20 (SPSS Inc., Chicago, IL).

**Ethical Consideration:** The study protocol was reviewed and approved by the institutional review board (IRB) of the Ethiopian Public Health Institute (EPHI). Consent was obtained from heads of selected households selected for HLC, LTC and PSC and from volunteers involved in human landing collection.

**Results**

**Species composition and abundance:** Over all 14,471 mosquitoes were collected by all collection methods (PSCs, LTCs, HLCs and PFS) during November and December 2017 from eight (Kebena, East-Badawacho, Merti, Kolo, Kolo-Temben and Legehare) sentinel sites, Ethiopia. Of these, the number of anopheline and culicine mosquitoes were 946 and 13,525, respectively (Annex 1). Of the 946 Anopheline mosquitoes, *An. gambiae* s.l. comprised the majority (67.8%) followed by *An. costani* (22.4%). The rest Anophelines (*An. pharoensis*, *An. demissus*, *An. squamosus*, *An. funestus*, *An. conenurus*, *An. pretoriensis* and *An. maculipalpis*) comprise 11.8%.

**Annex 1: Mosquito species composition, relative abundance and**

<table>
<thead>
<tr>
<th>Site</th>
<th>Collection method</th>
<th>An. gambiae s.l.</th>
<th>An. pharoensis</th>
<th>An. conanti</th>
<th>An. squamosus</th>
<th>Other anopheline species</th>
<th>Culix sp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Tot (mean ± SE)</td>
<td>Tot (mean ± SE)</td>
<td>Tot (mean ± SE)</td>
<td>Tot (mean ± SE)</td>
<td>Tot (mean ± SE)</td>
<td>Tot (mean ± SE)</td>
</tr>
<tr>
<td>East</td>
<td>HLC</td>
<td>14 (0.04 ± 0.01)</td>
<td>7 (0.02 ± 0.009)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.275 (3.32 ± 0.72)</td>
</tr>
<tr>
<td></td>
<td>LTC</td>
<td>7 (0.18 ± 0.06)</td>
<td>6 (0.15 ± 0.06)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.601 (6.02 ± 0.66)</td>
</tr>
<tr>
<td></td>
<td>PSC</td>
<td>6 (0.12 ± 0.05)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>119 (2.38 ± 0.45)</td>
</tr>
<tr>
<td></td>
<td>PFC</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>40 (2.00 ± 0.48)</td>
</tr>
<tr>
<td>Kebena</td>
<td>HLC</td>
<td>62 (0.16 ± 0.02)</td>
<td>0</td>
<td>183 (0.46 ± 0.06)</td>
<td>34 (0.08 ± 0.02)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>LTC</td>
<td>8 (0.2 ± 0.06)</td>
<td>0</td>
<td>12 (0.3 ± 0.09)</td>
<td>0</td>
<td>0</td>
<td>200 (5.70 ± 0.0)</td>
</tr>
<tr>
<td></td>
<td>PSC</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>25 (0.5 ± 0.12)</td>
</tr>
<tr>
<td>Merti</td>
<td>HLC</td>
<td>17 (0.04 ± 0.01)</td>
<td>0</td>
<td>12 (0.03 ± 0.01)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>LTC</td>
<td>4 (0.10 ± 0.05)</td>
<td>0</td>
<td>5 (0.12 ± 0.05)</td>
<td>0</td>
<td>0</td>
<td>15 (0.38 ± 0.04)</td>
</tr>
<tr>
<td></td>
<td>PSC</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>27 (0.54 ± 0.20)</td>
</tr>
<tr>
<td>Sire</td>
<td>HLC</td>
<td>2 (0.01 ± 0.004)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>299 (0.78 ± 0.17)</td>
</tr>
<tr>
<td></td>
<td>LTC</td>
<td>4 (0.10 ± 0.05)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3 (0.08 ± 0.04)</td>
</tr>
<tr>
<td></td>
<td>PSC</td>
<td>7 (0.17 ± 0.06)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (0.5 ± 0.16)</td>
</tr>
<tr>
<td>Kalo</td>
<td>HLC</td>
<td>145 (0.38 ± 0.04)</td>
<td>6 (0.02 ± 0.06)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3.449 ± (9.8 ± 0.57)</td>
</tr>
<tr>
<td></td>
<td>LTC</td>
<td>59 (1.47 ± 0.16)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>61 (1.22 ± 0.30)</td>
</tr>
<tr>
<td></td>
<td>PSC</td>
<td>17 (0.34 ± 0.10)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>601 (30.03 ± 4.85)</td>
</tr>
<tr>
<td></td>
<td>PFC</td>
<td>57 (2.85 ± 0.47)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Kolo</td>
<td>HLC</td>
<td>1 (0.00 ± 0.003)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>LTC</td>
<td>2 (0.55 ± 0.08)</td>
<td>0</td>
<td>24 (0.60 ± 1.92)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PSC</td>
<td>0 (0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Kolo Temben</td>
<td>HLC</td>
<td>1 (0.00 ± 0.003)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>LTC</td>
<td>0 (0.03 ± 0.03)</td>
<td>0</td>
<td>9 (0.31 ± 0.19)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PSC</td>
<td>0 (0.08 ± 0.04)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Legehare</td>
<td>HLC</td>
<td>110 (0.29 ± 0.03)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3.353 (8.73 ± 0.53)</td>
</tr>
<tr>
<td></td>
<td>LTC</td>
<td>50 (0.15 ± 0.19)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>236 (6.13 ± 0.83)</td>
</tr>
<tr>
<td></td>
<td>PSC</td>
<td>15 (0.30 ± 0.07)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>138 (2.76 ± 0.37)</td>
</tr>
<tr>
<td></td>
<td>PFC</td>
<td>34 (1.70 ± 0.34)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>371 (18.55 ± 2.79)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>642 (0.18 ± 0.04)</td>
<td>19 (0.02 ± 0.04)</td>
<td>212 (0.17 ± 0.02)</td>
<td>34 (0.04 ± 0.009)</td>
<td>39 (0.07 ± 0.01)</td>
<td>13,525 (4.57 ± 0.23)</td>
</tr>
</tbody>
</table>

**Human landing collections (HLCs):** Overall 593 anopheline mosquitoes belonging to nine species (*An. gambiae*, *An. pharoensis*, *An. conanti*, *An. demissus*, *An. squamosus*, *An. funestus*, *An. conenurus*, *An. pretoriensis* and *An. maculipalpis*) were collected from the eight sentinel sites during the study period by HLC. *An. gambiae* s.l. was the predominant species (59.2%) followed by *An. conanti* 32.8%. The proportion of anopheline mosquitoes collected outdoor by HLC was significantly higher (p<0.001) compared to indoor collection. Mosquito population density varied across the sentinel sites, with sites Kalo (0.48/night/person-hour), Legehare (0.38/night/person-hour) and Kebena (0.16/night/person-hour) showed significantly higher proportion (P<0.001) (Table 1).
Table 1: Mean Anopheline mosquito density collected from eight sentinel sites in Ethiopia (November to December 2017)

<table>
<thead>
<tr>
<th>Sentinel sites</th>
<th>Species</th>
<th>An. gambiae s.l.</th>
<th>An. pharoensis</th>
<th>An. coustani</th>
<th>Other anophelines</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SE</td>
<td>LB 95% CI</td>
<td>Mean ± SE</td>
<td>LB 95% CI</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>Kako</td>
<td>0.48±0.02²</td>
<td>.437 .521</td>
<td>0.16±0.02¹</td>
<td>-21 .52</td>
<td>00²</td>
</tr>
<tr>
<td>Legheare</td>
<td>0.38±0.02²</td>
<td>.357 .423</td>
<td>00</td>
<td>00 00</td>
<td>00²</td>
</tr>
<tr>
<td>Kebea</td>
<td>0.16±0.02¹</td>
<td>.119 .203</td>
<td>00</td>
<td>00 00</td>
<td>00²</td>
</tr>
<tr>
<td>East-Badawacho</td>
<td>0.05±0.02²</td>
<td>.007 .092</td>
<td>00</td>
<td>00 00</td>
<td>00²</td>
</tr>
<tr>
<td>Kobo</td>
<td>0.05±0.02²</td>
<td>.010 .095</td>
<td>00</td>
<td>00 00</td>
<td>00²</td>
</tr>
<tr>
<td>Merti</td>
<td>0.05±0.02²</td>
<td>.007 .092</td>
<td>00</td>
<td>00 00</td>
<td>00²</td>
</tr>
<tr>
<td>Sire</td>
<td>0.03±0.02²</td>
<td>.014 .071</td>
<td>00</td>
<td>00 00</td>
<td>00²</td>
</tr>
<tr>
<td>Kola-Temben</td>
<td>0.02±0.02¹</td>
<td>.040 .044</td>
<td>00</td>
<td>00 00</td>
<td>00²</td>
</tr>
</tbody>
</table>

*Biting activity: Figures 1 presents indoor and outdoor hourly biting activity of An. gambiae s.l, An. pharoensis, An. coustani complex and other anophelines across the sentinel sites during November and December 2017. All anophelines showed early peak biting activity (18:00h to 23:00h) both indoor and outdoor in all study sites. There was a significant difference (p = 0.008) in hourly mean An. gambiae s.l. density collected indoor and outdoor.

![Figure 1: Mean indoor and outdoor hourly biting activity of Anopheline mosquitoes collected from eight sentinel sites across Ethiopia (Nov-Dec, 2017)](image)

**Light trap catches (LTCs):** Overall 210 anopheline mosquitoes belonging to six species (An. gambiae s.l., An. coustani complex, An. cenerus, An. demillinii, An. pharoensis and An. pretoriensis) were collected from the eight sites during study period using LTCs. The highest proportion (73%) of the total LTC collection was An. gambiae s.l. followed by An. coustani 8% (Figure 2). Comparison of indoor and outdoor collections showed no statistical significance (p < 0.05).
**Pyrethrum spray collections (PSCs):** A total of 51 anopheline mosquitoes species (*An. gambiae* s.l., *An. gambiae s.s.*, and *An. demitoni*) were collected using PSC across the study sites over the two months study period. No Anopheline mosquito was collected from Merti and Kebena using PSC however both sites were positive for culicine mosquitoes.

**Pitfall shelter collection (PFS):** Pitfall shelters were mostly negative except for two sites Kalu and Legehare from which 91 *An. gambiae* s.l. mosquitoes were collected.

**Larval collections:** The total number of anopheline mosquito larvae collected from Kebena and Merti during the study period was 352 and 139, respectively. Of 352 anopheline mosquito larvae collected from Kebena site, 73.9% (260/352) were identified as *An. gambiae* s.l. and 21.5% (76/352) as *An. constani* complex. Moreover, of 139 anopheline mosquito larvae collected from Merti site, 31.7% (44/139) were identified as *An. gambiae* s.l. while 66.2% (92/139) as *An. constani* complex. The highest mean density of *An. gambiae* s.l and *An. constani* complex larvae in Kebena site was $41.9 \pm 12.0$, $23.2 \pm 7.8$, respectively while from Merti the highest mean density of *An. gambiae* s.l and *An. constani* complex larvae was $9.7 \pm 6.2$, $21 \pm 5.0$, respectively (Table 2).

![Bar Chart showing Mean mosquito density](chart.png)

**Table 2: Mean density of anopheline mosquito larvae in Kebena and Merti sites, Ethiopia (2017)**

<table>
<thead>
<tr>
<th>Sentinel site</th>
<th>Village</th>
<th>Mean density of anopheline larvae**</th>
<th>An. constani complex(M±SE)</th>
<th>Both species (M±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kebena</td>
<td>Kalajbe</td>
<td>$41.9\pm12.0$</td>
<td>$23.2\pm7.8$</td>
<td>$65.08\pm19.67$</td>
</tr>
<tr>
<td></td>
<td>Muzo</td>
<td>$31.6\pm7.8$</td>
<td>$1.7\pm1.0$</td>
<td>$33.27\pm7.57$</td>
</tr>
<tr>
<td></td>
<td>Rebu**</td>
<td>11</td>
<td>7</td>
<td>18</td>
</tr>
<tr>
<td>Merti</td>
<td>Kela</td>
<td>$9.7\pm6.2$</td>
<td>$21\pm5.0$</td>
<td>$30.67\pm9.24$</td>
</tr>
<tr>
<td></td>
<td>Tadiso</td>
<td>$6.4\pm3.7$</td>
<td>$12.3\pm3.9$</td>
<td>$18.69\pm7.63$</td>
</tr>
</tbody>
</table>

**Mean density of anopheline/10dips expressed as Mean ± Standard error, **Specimen obtained from single site
In Kebena both permanent and temporary larval breeding habitats were productive whereas in Merti only temporary larval breeding habitats were productive. *Anopheles gambiae* s.l. larvae were most abundant in Quarries followed by rivers in Kebena site while the larvae of this species were most abundant in swamps and farm ditches in Merti site. Unfortunately, searches for anopheline aquatic immature stages along the course of the Borkena River (Kalu district) were not successful except few collections positive and found to be 23 larvae emerged as adult *Anopheles gambiae* s.l. Potential breeding sites identified in Legehare/Gendegeber were found totally unproductive.

In Kola Tembeni site, the potential breeding sites encountered were surface waters associated with a small pond near the village. However, searches made in these waters were negative for *An. gambiae* s.l. aquatic stages. In Kobo sentinel sites, the potential breeding site identified was the Golina River, located just south of Aradom study village. However, as Golina is a fast flowing perennial river, searches for anopheline aquatic stages along the course of the river were not fruitful.

**Mosquito Infectivity and parity rate:** Overall, 348 anopheline mosquitoes (86 *An. gambiae* s.l., 214 *An. coustani* complex, 36 *An. squamosus*, 9 *An. pharoensis* and 2 *An. funestus* complex) collected from the two sites were tested for *Plasmodium* circumsporozoite protein (CSP). However, none of the samples collected from both sites were positive for *Plasmodium* circumsporozoite protein (Table 3). No blood meal ELISA test was conducted for vertebrate host blood source as the mosquito collection by PSC was not productive at all. Parity status of unfed mosquitoes collected from two sites was determined and mean parous rates of *An. gambiae* s.l. in Kebena and Merti were 50% and 53%, respectively.

<table>
<thead>
<tr>
<th>Site</th>
<th>Method</th>
<th>Anopheline species</th>
<th># tested</th>
<th>Ff</th>
<th>P210</th>
<th>P247</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kebena</td>
<td>HLC</td>
<td><em>An. gambiae</em> s.l.</td>
<td>60</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>An. coustani</em></td>
<td>189</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>An. pharoensis</em></td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>An. squamosus</em></td>
<td>33</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>An. funestus</em></td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CDC</td>
<td>HLC</td>
<td><em>An. gambiae</em> s.l.</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>An. coustani</em></td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>An. squamosus</em></td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Merti</td>
<td>Pit shelter</td>
<td><em>An. gambiae</em> s.l.</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>HLC</td>
<td><em>An. gambiae</em> s.l.</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>An. coustani</em></td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>An. pharoensis</em></td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>An. squamosus</em></td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CDC</td>
<td></td>
<td><em>An. gambiae</em> s.l.</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>An. coustani</em></td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Discussion**

Malaria disease continues to be one of major health threats in Ethiopia despite its sharp decline in years between 2000 to 2015 (WHO 2016). Ethiopia and Rwanda top African countries in combating malaria problem with remarkable disease reduction documented up to 50 to 75% when compared to its tally in year 2000 (WHO 2016; 2017). More recently, the Federal Ministry of Health in collaboration with national and international partner organizations launched the first phase of eliminating malaria from 239 districts selected from five different regional states (MoH 2014). To this end, entomological surveillance was conducted from selected eight sentinel sites in order to evaluate the ongoing process. This study documented over 14,471 mosquitoes from eight (Kebena, East-Badawacho, Merti, Sire, Kalo, Kobo, Kola-Temben and Legehare) sentinel sites, with nine anopheline species (*An. gambiae* s.l., *An. pharoensis*, *An. coustani*, *An. demilioni*, *An. squamosus*, *An. funestus*, *An. cenerus*, *An. pretoriensis* and *An. maculipalpis*) and culex collections, which is among most comprehensive and long listings of anopheline species in the country. Culex mosquito collections accounted 93.5% of the total collection followed by *An. gambiae* s.l. the primary malaria vector in Ethiopia. The predominance of *An. gambiae* s.l. was documented from different parts of the country including southern Ethiopia (Taye et al. 2006; Massebo et al. 2015) east central Ethiopia (Kibret et al. 2009; Amenshewa 1995), south central Ethiopia (Anun et al. 2013), southwestern Ethiopia (Taye et al. 2016; Lehsa et al. 2017); northern Ethiopia (Yohannes and Boelew 2012) and western Ethiopia (Krasfur 1977; Jaleta et al. 2016).

In this study malaria vector mosquitoes tend to bite both indoor and outdoor however significantly higher mosquitoes were caught biting outdoor. Moreover the
vector mosquitoes tend to bite in early part of the night between 18:00 to 23:00 hours. The early and outdoor biting tendency of malaria vectors can be considered as a serious threat for the control and elimination program. Similarly Taye et al. (2016), from southwestern ethiopia, Yohannes and Boelte (2012) from Northern Ethiopia, Fornadel et al. (2010) from southern Zambia and Russell et al. (2011) from Tanzania reported the shifting behavior of malaria vectors following prolonged deployment of indoor based vector control interventions (IRS and LLINs). This is in contrast to the findings from earlier study from southern Ethiopia by Taye et al. (2006) which reported 81% of the An. arabiensis collected through HLC was found to be indoor biter with pick biting time ranging between 23:00 to 03:00. CDC collections however, show no significant difference between indoor and outdoor collections. This could be due to lower density of mosquitoes collected per night-trap.

In this study, only three sentinel sites (Merti, Kebeba and Kalu) had positive breeding habitats whereas it was not possible to get productive habitats in the rest five sites. Culicine and Anopheline (An. gambiae s.l. & An. constanti) mosquitoes were collected from both Merti and Kebeba whereas only Anopheline larvae was found in Kalu. Anopheles gambiae s.l. larvae were found commonly in temporary breeding habitats such as footprints, roadside pools, quarries, pits dug for plastering and semi-permanent habitats such as riverbanks, interrupted streams, farm ditches and swamps. The preference of temporary breeding habitats by An. gambiae s.l. and other anopheline mosquitoes was further corroborated with similar findings reported from southwestern Ethiopia (Mereta et al. 2013) and south central Ethiopia (Kenea et al. 2011) which documented the preference of short lived, small, open and clear water bodies as mostly (90 to 95%) preferred productive larval habitats. One possible explanation for the association between temporary habitats and malaria vectors breeding behavior could be the nature of smaller water bodies which are generally characterized by high temperature which are also critical for rapid larval development (Paajimans et al. 2011).

In this study testing of Parity status of unfed mosquitoes collected from two sites (Kebeba and Merti) showed more than 50% parity rate of An. gambiae s.l. implying the presence of more aged adult mosquitoes which can be potentially infectious as they stay longer. The testing of infectivity rate of 384 anopheline mosquitoes using CSP-ELISA however, showed no positive specimens in direct contrast to the observed higher proportion of parous mosquitoes. Insecticides currently used in indoor residual sprays (PMI 2018) have short residual life span. Moreover, the quality of the spray (WHO 2007), the susceptibility status of local vector population (Hanson et al. 2004) and the degree of endemicity of the disease (WHO 2006) can all influence the efficacy of our interventions. One possible explanation for the presence of more parous mosquito population in the surroundings as it is evidenced in this study could be either the residual insecticides have started to exhaust since the spray operations are implemented in August and September or it could be combination of the above factors.

This study reflects the data collected from two months (November and December 2017) only however, it is recommended that the entomological surveillance should be planned to include at least one year full data in order to present the whole picture of vector population dynamics. Due to the aforementioned reasons the study has the limitation of determining EIR and other entomological indices necessary to quantify the level of malaria transmission and the impact of IRS application on these indices.

**Conclusion**

In conclusion nine anopheline mosquitoes species (An. gambiae s.l., An. pharoensis, An. constanti, An. deminoni, An. squamosus, An. funestus, An. census, An. pretoriensis and An.maculipalpis) were collected from eight sentinel sites, and An. gambiae s.l. the primary malaria vector in Ethiopia was found to be the predominant species. Vector population in this study showed the tendency of biting early evening and outdoor. This may sustain outdoor transmission or residual transmission which could be challenging for malaria elimination efforts in these sites. Given the evidence of peak biting activity in the early part of the night before people go to bed; existing interventions in particular use of LLINs alone may not be helpful for effective vector control. Thus, new or alternative vector control intervention tools which targets outdoor resting and outdoor biting malaria vectors should be developed and implemented as a component of integrated vector management (IVM) to sustain malaria control and enhance elimination efforts.

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field mosquito collection and for their assistance in laboratory processing of mosquitoes. This work obtained financial & logistic support from Global Fund through NMCP/FMOH.

Conflict of interest: All the authors declare that there is no conflict of interest.

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Instruction for Authors

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1. Research Articles: report the results of original public health research in up to 3500 words in the text, a structured abstract with up to five tables and/or figures, and no more than 35 references. The text must have an introduction and separate sections for Methods, Results, Discussion, and Conclusion.

2. Brief Articles: present preliminary findings or novel findings in up to 1200 words in the main text, a structured (except if justified otherwise in the cover letter) abstract, up to 1 table or figure, and no more than 12 references.

3. Research Brief: Articles must have an introduction and separate sections for the Methods, Results, Discussion, and Conclusion. Some policy-focused Brief Articles which are short essays and do not report study results do not require the “method, results, discussion, public health implications” format subheadings.

4. Systematic Reviews and meta-analyses: including quantitative, meta-narrative, and qualitative reviews, have clearly formulated questions and use systematic and explicit methods to identify, select, and critically appraise relevant research, and to collect and analyze data from the studies that are included in the reviews. The recommended text word limit is up to 4000 words. Statistical methods (meta-analysis) may or may not be used to analyze and summarize the results of the included studies. EJPHN recommends using these headings—Title, Abstract, Methods, Results, Discussion, Funding—in an expanded research article format, with flexibility when needed for clear assessment and presentation. References, tables, and figures ought to be pertinent to the topic at hand.

5. Rapid Communication: up to 1000-3000 words including tables, figures and references. Papers representing concise and original studies of scientific importance are considered. In the cover letter the author should justify the request for Rapid Communication. The review process is 10 days, authors are allowed one revision if accepted, and the final version of the paper appears in the next available issue of the journal.

6. Letters to the Editor: are encouraged if they directly concern articles previously published in this journal or subjects related to the matters discussed. The Editor reserves the right to submit copies of such letters to the authors of the articles concerned prior to publication in order to permit them to respond in the same issue of the journal (max. 500 words).

7. Case Report: up to 2500 words including tables, figures, and references. Case Reports include case studies of 4 or fewer patients that describe a novel situation or add important insights into mechanisms, diagnosis or treatment of diseases.

8. Editorials: are usually invited by the Editor (max. 1,000 words). Please send suggestions to the Editor.

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1) On a topic outside the scope of the Journal,
2) Lacking technical merit,
3) Plagiarized manuscript as evaluated based on international standards,
4) Did not pass through appropriate ethical review procedures, or
5) Fragmentary and provides marginally incremental results, or
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Please note that original articles must contain the following components:
1. Cover letter
2. Title page
3. Abstract (structured)
4. Introduction
5. Materials and Methods
6. Results
7. Discussion
8. Acknowledgements
9. Conflict of Interest
10. References
11. Tables
12. Figures
In-Text Citations and references
All in-text citations must be in name/date form. Place the citation immediately after the textual information cited, placing name and date within parentheses without a comma.

Example: Single author: (Wing 2002); (Wing and Wolf 2000).


Chapter in an edited book

Print Journal article: Examples:


Submissions: Original manuscripts in three hard copies and its electronic version must be submitted to the Editor-in-Chief by mail and should be accompanied by a covering letter signed by the author(s) provided that they have not been published previously and are not under consideration for publication elsewhere.

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Typescripts: Submissions should be typed on one side of an A4 paper, with double spacing and with a margin of 25 mm (1 inch).

Tables and Figures: These should be presented on separate sheets and should be numbered with Arabic numerals consecutively in the order of their citation in the text. Only up to five tables and or figures are accepted in one manuscript.

Abbreviations and symbols: Only standard abbreviations should be used. Abbreviations must be avoided in the title and abstract sections. Abbreviations must be preceded by their full description in the first use.

Ethics: All studies on human subjects must have been conducted in accordance with national and international ethical standards. Patients’ names, initials or hospital numbers must be removed when submitting a manuscript.

Conflicts of interest: Authors are responsible for declaring any conflict of interest related to the submitted research work. The source of funding for the research work should be acknowledged.

Authorship: Authorship credits should be based only on substantial contributions to the manuscript.

Acknowledgments: These should be limited to most important contributions and must be placed on a separate page.

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