

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 pursuing malaria elimination in 239 selected low transmission districts located in 6 different regions. Of these, 50 districts have 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 (Dire Dawa). 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 (PFSs). Larval collection was 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. demilloni*, *An. squamosus*, *An. funestus*, *An. cenerus*, *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), Legehare (0.38/night/person-hour) and Kebena (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 anopheline 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 bionomics, 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 determine 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 Amhara, Kola Temben from Tigray, Dire Dawa 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 from 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 pestle. Separately each well of micro plate was coated with 50 µL of the capture MAb and the plate was incubated for 1hr 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 1hr at room temperature. The blocking buffer was then completely aspirated. Then 50 µL of positive (SIGIMA 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:

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

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 30x 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 hand-held 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-tukeys 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, Sire, Kalo, Kobo, Kola-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. coustani* (22.4%). The rest Anophelines (*An. pharoensis*, *An. demilloni*, *An. squamosus*, *An. funestus*, *An. cenerus*, *An. pretoriensis* and *An. maculipalpis*) comprise 11.8%.

Annex 1: Mosquito species composition, relative abundance and

Site	Collection method	Species					
		<i>An. gambiae</i> s.l. Tot (mean ± SE)	<i>An. pharoensis</i> Tot (mean ± SE)	<i>An. coustani</i> Tot (mean ± SE)	<i>An. squamosus</i> Tot (mean ± SE)	Other anopheline Tot (mean ± SE)	<i>Culex</i> sp Tot (mean ± SE)
East	HLC	14 (0.04 ± 0.01)	7 (0.02±0.009)	0	0	0	1,275 (3.32 ±0.72)
Badawacho	LTC	7 (0.18±0.06)	6 (0.15±0.06)	0	0	0	2,601(65.02±5.66)
	PSC	6 (0.12±0.05)	0	0	0	0	119 (2.38±0.45)
	PFC	0	0	0	0	0	40 (2.00±0.48)
Kebena	HLC	62 (0.16±0.02)	0	183 (0.46±0.06)	34 (0.08±0.02)	0	0
	LTC	8 (0.2±0.06)	0	12 (0.3±0.09)	0	0	200 (5±0.70)
	PSC	0	0	0	0	0	25 (0.5±0.12)
Merti	HLC	17 (0.04 ±0.01)	0	12 (0.03 ±0.01)	0	0	0
	LTC	4 (0.10±0.05)	0	5(0.12±0.05)	0	0	275 (6.88±0.94)
	PSC	0	0	0	0	0	27(0.54 ±0.20)
Sire	HLC	2(0.01 ±0.004)	0	0	0	0	299(0.78 ±0.17)
	LTC	4(0.10 ±0.05)	0	0	0	0	3(0.08 ±0.04)
	PSC	7 (0.17±0.06)	0	0	0	0	25(0.50±0.16)
Kalo	HLC	145(0.38 ±0.04)	6(0.02 ±0.06)	0	0	0	3,449(8.98 ±0.57)
	LTC	59(1.47±0.16)	0	0	0	0	424(11.16±1.25)
	PSC	17(0.34±0.10)	0	0	0	0	61(1.22±0.30)
	PFC	57(2.85±0.47)	0	0	0	0	601(30.05 ±4.85)
Kobo	HLC	1(0.00 ±0.003)	0	0	0	0	0
	LTC	21(0.55 ±0.08)	0	0	0	24(0.60±1.92)	6(0.16±0.07)
	PSC	0(±)	0	0	0	2(0.04±0.03)	0
Kola Temben	HLC	1(0.00 ±0.003)	0	0	0	0	0
	LTC	1(0.03±0.03)	0	0	0	9(0.31±0.19)	0
	PSC	0(±)	0	0	0	4(0.08±0.04)	0
Legehare	HLC	110(0.29±0.03)	0	0	0	0	3353(8.73±0.53)
	LTC	50(1.35 ±0.19)	0	0	0	0	233(6.13 ±0.83)
	PSC	15(0.30±0.07)	0	0	0	0	138(2.76 ±0.37)
	PFC	34(1.70 ±0.34)	0	0	0	0	371(18.55 ±2.79)
Total		642(0.18±0.09)	19(0.02±0.04)	212(0.17±0.02)	34(0.04 ±0.009)	39 (0.07±0.01)	13,525(4.57±0.23)

Human landing collections (HLCs): Overall 593 anopheline mosquitoes belonging to nine species (*An. gambiae*s.l., *An. pharoensis*, *An. coustani*, *An. demilloni*, *An. squamosus*, *An. funestus*, *An. cenerus*, *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. coustani* 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)

Sentinel sites	Species		An. gambiae s.l.			An. Pharoensis			An. Coustani			Other anophelines		
	Mean ± SE		95% CI			Mean ± SE			Mean ± SE			Mean ± SE		
			LB	UB		LB	UB		LB	UB		LB	UB	
Kalo	0.48±0.022 ^a		.437	.521	0.16±0.02 ^a	-.21	.52	00 ^b	00	00	00 ^b	00	00	
Legehare	0.38±0.022 ^b		.337	.423	00 ^b	00	00	00 ^b	00	00	00 ^b	00	00	
Kebera	0.16±0.021 ^c		.119	.203	00 ^b	00	00	.46±.02 ^a	.43	0.50	.08±.02 ^a	.05	.12	
East-Badawacho	0.05±0.022 ^d		.007	.092	.02±.02 ^b	-.02	.05	00 ^b	00	00	00 ^b	00	00	
Kobo	0.05±0.022 ^d		.010	.095	00 ^b	00	00	00 ^b	00	00	00 ^b	00	00	
Merti	0.05±0.022 ^d		.007	.092	00 ^b	00	00	0.03±.02 ^b	-0.006	0.066	00 ^b	00	00	
Sire	0.03±0.022 ^d		-.014	.071	00 ^b	00	00	00 ^b	00	00	00 ^b	00	00	
Kola-Temben	0.02±0.021 ^d		-.040	.044	00 ^b	00	00	00 ^b	00	00	00 ^b	00	00	

*Means with the same letters in the same column are not significantly different at 0.05 level.

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.

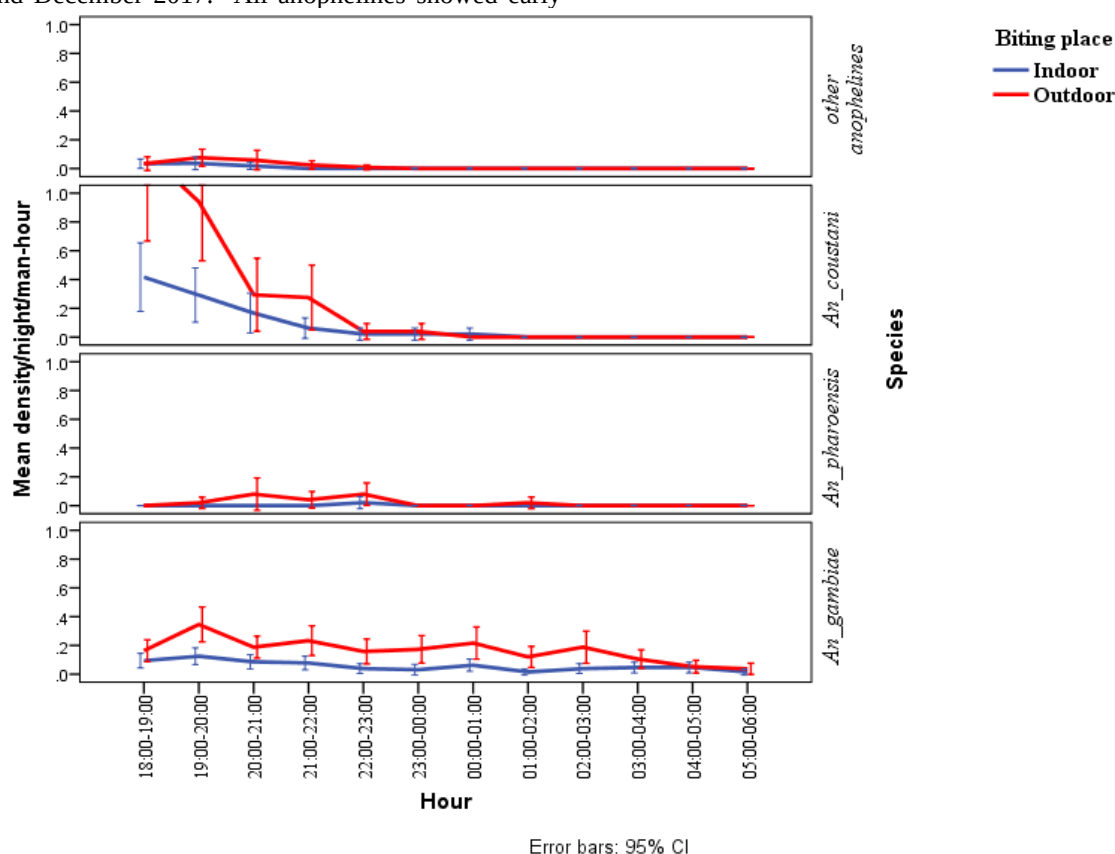


Figure 1: Mean indoor and outdoor hourly biting activity of Anopheline mosquitoes collected from eight sentinel sites across Ethiopia (Nov-Dec, 2017)

Light trap catches (LTCs): Overall 210 anopheline mosquitoes belonging to six species (*An. gambiae* s.l., *An. coustani* complex, *An. cenerus*, *An. demilloni*, *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 ($p < 0.05$).

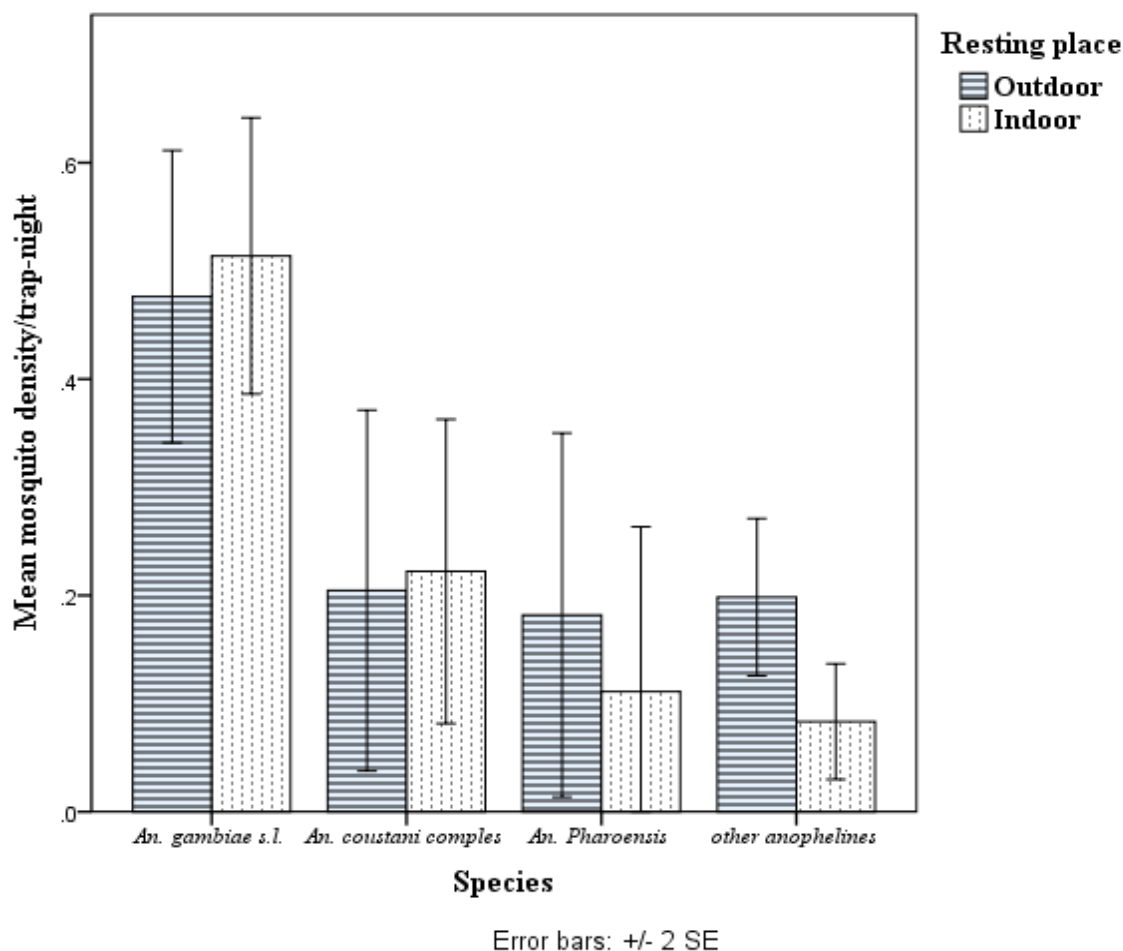


Figure 2: Mean anopheline mosquito density collected from eight sentinel sites in Ethiopia (Nov-Dec, 2017)

Pyrethrum spray collections (PSCs): A total of 51 anopheline mosquitoes species (*An. gambiae* s.l. *An. cenerus* and *An. demilloni*) 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. coustani* 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. coustani* complex, The highest mean density of *An. gambiae* s.l and *An. coustani* complex larvae in Kebena site was 41.9 ± 12.0 , 23.2 ± 7.8 , respectively while from Merti the highest mean density of *An. gambiae* s.l and *An. coustani* complex larvae was 9.7 ± 6.2 , 21 ± 5.0 , respectively (Table 2).

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

Sentinel site	Village	Mean density of anopheline larvae**		
		<i>An. gambiae</i> .l (M $\pm$ SE)	<i>An. coustani</i> complex(M $\pm$ SE)	Both species (M $\pm$ SE)
Kebena	Kalajabe	41.9 $\pm$ 12.0	23.2 $\pm$ 7.8	65.08 $\pm$ 19.67
	Muzo	31.6 $\pm$ 7.8	1.7 $\pm$ 1.0	33.27 $\pm$ 7.57
	Rebu***	11	7	18
Merti	Kela	9.7 $\pm$ 6.2	21 $\pm$ 5.0	30.67 $\pm$ 9.24
	Tadiso	6.4 $\pm$ 3.7	12.3 $\pm$ 3.9	18.69 $\pm$ 7.63

Mean density of anopheline/10dips expressed as Mean $\pm$ 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 Tembien 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 3: Sporozoite ELISA results for anopheline mosquitoes collected from Kebena and Merti sites, Ethiopia (November to December 2017)

Site	Method	Anopheline species	# tested	<i>Pf</i>	<i>Pv</i> 210	<i>Pv</i> 247		
Kebena	HLC	<i>An. gambiaes.l.</i>	60	0	0	0		
		<i>An. coustani</i>	189	0	0	0		
		<i>An. pharoensis</i>	8	0	0	0		
		<i>An. squamosus</i>	33	0	0	0		
		<i>An. funestus</i>	2	0	0	0		
	CDC	<i>An. gambiaes.l.</i>	8	0	0	0		
		<i>An. coustani</i>	13	0	0	0		
		<i>An. squamosus</i>	2	0	0	0		
		Merti	Pit shelter	<i>An. gambiaes.l.</i>	1	0	0	0
				HLC	<i>An. gambiaes.l.</i>	17	0	0
CDC	<i>An. coustani</i>		11	0	0	0		
	<i>An. pharoensis</i>		1	0	0	0		
	<i>An. squamosus</i>		1	0	0	0		
	<i>An. gambiaes.l.</i>	1	0	0	0			
	<i>An. coustani</i>	1	0	0	0			
Total			348	0	0	0		

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. demilloni*, *An. squamosus*, *An. funestus*, *An. cenerus*, *An. pretoriensis* and *An. maculipalpis*) and culex collections, which is among most comprehensive and long listings of anopheles 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; Ameneshewa 1995), south central Ethiopia (Animut et al. 2013), southwestern Ethiopia (Taye et al. 2016; Lelisa et al. 2017); northern Ethiopia (Yohannes and Boelee 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 Boelee (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 peak biting time ranging between 23:00 to 03:00. CDC collections however, showed 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, Kebena 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. coustani*) mosquitoes were collected from both Merti and Kebena whereas only Anopheline larvae were 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 (Kebena 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. coustani*, *An. demilloni*, *An. squamosus*, *An. funestus*, *An. cenerus*, *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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