Parasite Epidemiology and Control 2 (2017) 61–69

Contents lists available at ScienceDirect

Parasite Epidemiology and Control

journal homepage: www.elsevier.com/locate/parepi

High entomological inoculation rate of malaria vectors in area of high coverage of interventions in southwest Ethiopia: Implication for residual malaria transmission

Misrak Abraham[a], Fekadu Massebo[a,*], Bernt Lindtjørn[b]

[a] Department of Biology, Arba Minch University, Arba Minch, Ethiopia
[b] Centre for International Health, University of Bergen, Bergen, Norway

ARTICLE INFO

Keywords:
Anopheles arabiensis
Entomological inoculation rate
Insecticide resistance
Residual malaria
Sille village

ABSTRACT

In Ethiopia, vector control is the principal strategy to reduce the burden of malaria. The entomological indicators of malaria transmission such as density, sporozoite rate and entomological inoculation rate (EIR) are parameters used to assess the impact of the interventions and the intensity of malaria transmission. The susceptibility of malaria vectors also determines the effectiveness of insecticide based vector control tools. Hence, the aim of the study was to assess the species composition, sporozoite rate and EIR, and insecticide susceptibility status of malaria vectors.

33 houses (18 for Centre for Disease Control and Prevention (CDC) light traps and 15 for exit traps) were randomly selected to sample *Anopheles* mosquitoes from October 2015 to May 2016. *Plasmodium* circum-sporozoite proteins (CSPs) of *An. arabiensis* and *An. pharoensis* were determined using Enzyme-Linked Immuno-Sorbent Assay (ELISA).

Five *Anopheles* species were identified from CDC Light traps and exit traps. *An. arabiensis* (80.2%) was the predominant species, followed by *An. pharoensis* (18.5%). *An. pretoriensis*, *An. tenebrosus* and *An. rhodesiensis* were documented in small numbers. 1056 *Anopheles* mosquitoes were tested for CSPs. Of which nine (eight *An. arabiensis* and one *An. pharoensis*) were positive for CSPs with an overall CSP rate of 0.85% (95% CI: 0.3–1.4). Five *Anopheles* mosquitoes were positive for *P. falciparum* and four were positive for *P.vivax*_210. *P. falciparum* CSP rate of *An. arabiensis* was 0.46% (95% CI: 0.13–1.2) and it was 0.54% (95% CI: 0.01–2.9) for *An. pharoensis*. The overall EIR of *An. arabiensis* was 5.3 infectious bites per/person (ib/p)/eight months. *An. arabiensis* was resistant to dieldrin (mortality rate of 57%) and deltamethrin with mortality rates of 71% but was fully susceptible to propoxur and bendiocarb. Based on the EIR of *An. arabiensis*, indoor malaria transmission was high regardless of high coverage of indoor-based interventions.

Finally, there was an indoor residual malaria transmission in a village of high coverage of bed nets and where the principal malaria vector is susceptibility to propoxur and bendiocarb; insecticides currently in use for indoor residual spraying. The continuing indoor transmission of malaria in such village implies the need for new tools to supplement the existing interventions and to reduce indoor malaria transmission.

* Corresponding author.
E-mail addresses: fekadu.massebo@amu.edu.et (F. Massebo), bernt.lindtjorn@cih.uib.no (B. Lindtjørn).

http://dx.doi.org/10.1016/j.parepi.2017.04.003
Received 7 December 2016; Received in revised form 10 April 2017; Accepted 17 April 2017
Available online 02 May 2017

1. Introduction

Malaria is a severe disease resulting deaths, sickness and economic losses in tropics and subtropics (Gething et al., 2016; Murray et al., 2012). Until the mid-19th century, malaria was endemic in most countries in the world (Mendis et al., 2009). In 1955, a global malaria eradication campaign was launched to interrupt transmission using Dichloro-Diphenyl-Trichloroethane (DDT) (Najera et al., 2011). As a result of the campaign, several countries that were endemic in 1950s' were free from malaria by 1970s' (Najera et al., 2011). Currently, malaria has been declined in most malaria endemic countries due to the widespread distribution of public health interventions (Bhatt et al., 2015). Despite all efforts, it remains as a major public health problem of the world with annual estimate of 212 million cases and 429,000 deaths in 2015. About 92% of deaths occurred sub-Saharan Africa alone (WHO, 2016a).

In the last 10 years, malaria control efforts have been scaled up across Africa. Indoor residual spraying (IRS) and long lasting insecticidal nets (LLINs) are the two major interventions contributing for the current malaria reduction (Bhatt et al., 2015). These malaria control interventions have averted 663 million cases since 2000 of which 68% of cases were by long-lasting insecticidal nets (LLINs), 22% by artemisinin based combination therapy (ACT) and 10% by indoor residual spray (IRS) (Bhatt et al., 2015). But the control of malaria is being threatened by insecticide resistant malaria vectors (Ranson and Lissenden, 2016). Also, limited numbers of public-health insecticides are available for vector control (Hemingway et al., 2013). For example, pyrethroid insecticide are the only insecticides for treating bed nets, and only four classes of insecticides are available for IRS. Moreover, malaria vectors are rapidly developing resistance to all insecticides for public use (Ranson and Lissenden, 2016). Hence, implementation of effective insecticide resistance management strategies is vital to mitigate the development and spread of insecticide resistance.

Moreover, the entomological indicators such as density, species composition, sporozoite rate and entomologic inoculation rate (EIR) are important parameters to measure the impact of vector control interventions. EIR in particular is crucial to quantify the exposure of the human population to malaria vectors (Shaukat et al., 2010). Little information is available on EIR of the principal malaria vector *An. arabiensis* in Ethiopia (Animut et al., 2013; Massebo et al., 2013a). The *Plasmodium falciparum* sporozoite rate of 0.5% for *An. arabiensis* from human landing catches (HLC) was documented in Sille in 2006 (Taye et al., 2006). *An. arabiensis* was dominant species, and *An. coustani* was the second dominant species in the village (Taye et al., 2006). The village is still malaria endemic regardless of the implementation of massive vector control interventions such as IRS and LLINs. Hence, the current information on the species composition, vector density, sporozoite rate of *Anopheles* mosquitoes, and EIR and insecticide susceptibility status of *An. arabiensis* are relevant for evaluating the impact of existing vector control interventions and planning for appropriate supplementary interventions. Hence, the aims of the study were to investigate the species composition, sporozoite rate and EIR of *Anopheles* mosquitoes, and assess the susceptibility status of *An. arabiensis* in Sille village ten years after the previous survey in the same area.

2. Materials and methods

2.1. Description of the study area

The study was conducted in Sille (5°99′ N and 37°50′ E), one of malaria endemic villages of Gamo Gofa zone, southwest Ethiopia (Fig. 1). It is located 518 km from Addis Ababa, capital of Ethiopia, 308 km from Hawassa, capital of South Nations Nationalities and Peoples Regional State (SNNPRs) and 13 km from Arba Minch, capital of Gamo Gofa zone. The altitude ranges from 1120 to 1380 m above sea level. Its temperature range from 25 to 36 °C and mean annual rainfall ranges from 900 to 1300 mm (millimeter). The village has high potential of irrigation. The water sources for irrigation are Sille River and Lake Chamo. The primary economic source is agriculture, and banana is the main cash crop.

There is large number of animals in the village. During the night, animals kept either in separate animal houses (just to protect from rain), outdoors in the compound or in communal places. Hence, humans and animals do not share the same house. No seasonal or permanent movement of animals (live permanently in the village). Corrugated roofed and grass thatched houses are found in the village. The total human population of the village is 3452. There is one health post in the village to provide basic public health services mainly focusing on prevention. IRS and LLINs are the two major vector control interventions implemented by the District Health Office. IRS of either propoxur or bendiocarb conducted once a year. 88% (3048/3452) of the total population was protected by IRS in 2016, while LLINs distribution was based on the number of household occupants. Those households with 1–2 occupants had 1 bed net; those with 3–4 had 2 bed nets, 5–7 had 3 bed nets and ≥ 8 had 4 bed nets. The bed nets coverage was > 98% (Arba Minch area district health office unpublished data, 2016).

2.2. Adult Anopheles mosquito sampling by Centre for Disease Control and Prevention (CDC) light trap

Adult female *Anopheles* mosquito sampling was carried out in Sille village from October 2015 to May 2016 using CDC light traps (Model 512; John W. Hock Company, Gainesville, FL, USA). Sample collection was carried out three times each month in 18 randomly selected houses. CDC light traps were installed 45 cm above the floor at the foot end of a human sleeping under untreated mosquito nets in house (Lines et al., 1991). Traps were switched on at 18:00 PM and switch off at 6:00 AM. All mosquitoes collected in the traps were removed from the bags, counted, identified morphologically into species using a key (Gillies and Coetzee, 1987). Live female *Anopheles* mosquitoes were killed either by freezing or by suffocation with chloroform vapor. Abdominal conditions of female were examined under a dissecting microscope and classified into unfed, freshly fed, half-gravid or gravid (WHO, 2003). The female *Anopheles* mosquitoes were preserved individually in vials with silica gel for circum-sporozoite proteins (CSPs) analysis. The

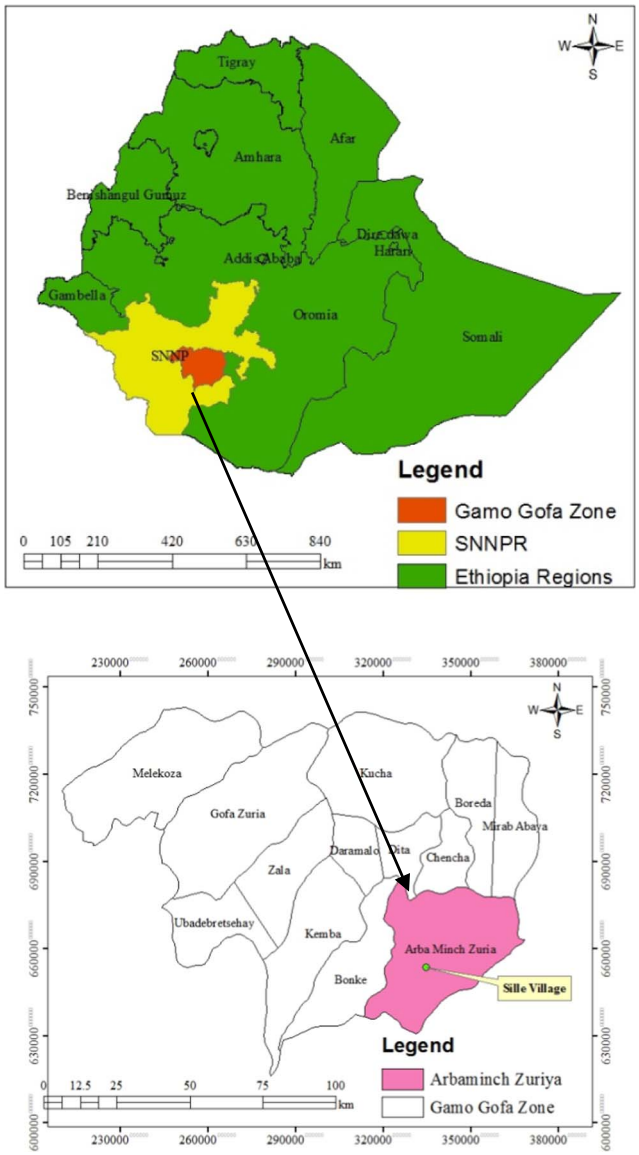

Fig. 1. Map of Ethiopia; location of Sille village in Arba Minch Zuria Woreda, south-west Ethiopia (2016).

same houses were visited throughout the study period.

2.3. Adult Anopheles collection by exit traps

Exit traps were placed externally on the windows to catch mosquitoes leaving the houses. The traps were installed before sunset and emptied after sunrise (Govella et al., 2011). It was designed to catch mosquitoes leaving houses after feeding indoors or for oviposition after blood meal digestion and egg production. Fifteen houses with single window were randomly selected for exit traps. Mosquitoes captured were removed from the traps using hand-held aspirator and anophelines were counted and identified using a key to species (Gillies and Coetzee, 1987). Live female *Anopheles* mosquitoes were killed either by freezing or by suffocation with chloroform vapor. Female *Anopheles* were examined under a dissecting microscope and classified on the basis of their abdominal condition as unfed, freshly fed, half-gravid and gravid (WHO, 2003) which was used to calculate exophillic index of the vectors. The same houses were also visited throughout the study period.

2.4. Rearing Anopheles larvae and pupae and insecticide susceptibility test

Anopheles larvae and pupae were collected from Sille River and other natural breeding habitats using dipper in December 2015. Once collected, the larvae and pupae were transported in well labeled plastic bottles to Arba Minch University, and maintained at

26 ± 2 °C and 65 ± 5% relative humidity. The pupae were transferred into plastic cups and placed in labeled cages for adult emergence. The emerged adults were provided with sterilised 10% sugar solution and morphologically identified using a key (Gillies and Coetzee, 1987). Insecticide impregnated papers (malathion 0.8%, deltamethrin 0.05%, bendiocarb 0.1%, permethrin 0.75%, propoxur 0.1% and dieldrin 4%) were used for the bioassay. Female *An. arabiensis* aged 3–5 days were exposed to the insecticide and oil impregnated papers. 25 sugar-fed female adult *An. arabiensis* were exposed to each replicate (four treatment and two control replicates). Observation of the number of knocked-down mosquitoes was made during one hour-long exposure period at regular intervals, after 10, 20, 30, 40, 50 and 60 min. At the end of the exposure period, the tested mosquitoes were transferred into recovery tubes and provided with a cotton wool soaked with 10% sugar solution. They were kept for 24 h under laboratory condition at a temperature of 27 °C and RH of 60–70%. Total mortality was recorded after 24 h exposure. When control mortality was between 5% and 20%, the observed percentage mortality was corrected by Abbott's formula (Abbott, 1925).

2.5. Processing female Anopheles mosquito for CSPs detection

The heads and thoraces of *An. arabiensis* and *An. pharoensis* were used to detect CSPs of *P. falciparum* and *P. vivax*_210 malaria using Enzyme-Linked Immuno-Sorbent Assay (ELISA) (Beier et al., 1988). The test was done on *An. arabiensis* and *An. pharoensis* from CDC light traps in Arba Minch University Medical Entomology Laboratory. Heads and thoraces were grinded in 50 µl blocking buffer (BB) by using plastic grinder. Then 100 µl BB was added twice to bring the final volume to 250 µl per mosquito. BB was removed from plates after 1 h and 50 µl of each homogenized mosquito was added per plate and *P. falciparum* and *P. vivax*_210 positive sample and laboratory-colony of *An. arabiensis* were used as negative controls, respectively. After 2 hr incubation, plates were washed twice with PBS-Tween 20. Horseradish peroxidase (HRP)-conjugated monoclonal antibody was then added to each plate and after hr. incubation, plates were washed 3 times with PBS-Tween 20. Finally, 100 µl of peroxidase substrate was added per well and incubated for 30 min. The plates were observed visually for green color and also their optical density determined at 414 nm in the microplate ELISA reader. Samples with green color and with optical density values of greater than two times the average optical density of the negative controls were considered sporozoite positive.

2.6. Data analysis

All data were entered and analyzed using IBM® SPSS® Statistics version 20 (Armonk, New York: IBM Corporation). The sporozoite rate (SR) and entomological inoculation rates were calculated. SR is the fraction of vector mosquitoes with *Plasmodium* sporozoite protein in their salivary glands. The EIR is calculated as the product of the human biting rate and the sporozoite rate (Shaukat et al., 2010). The SR and human biting rate (HBR) were determined for CDC light trap catches. For CDC based EIR, we treated CDC light trap catches as being approximately equivalent to true human exposure rates without using the conversion factor (Briët et al., 2015). Thus, the standard EIR was calculated as (number of sporozoite positive ELISAs/number of mosquitoes tested) x (number of mosquitoes collected by CDC/number of CDC catches). The alternative method was also used to calculate the 95% Confidence interval of EIR.

The monthly density of *Anopheles* mosquitoes were compared by Analysis of Variance (ANOVA). The relative degree of exophily was indicated by the index Fed/Gravid ratio (WHO, 1975). If the ratio of the fed to gravid is > 1, indicates that the blood fed mosquitoes were exiting (exophily of the species). If the index is constantly less than1, the species shows indoor rest tendency (endophily). If the index is closer to 1, it indicates smaller in differentiation in resting tendencies as exophily and endophily.

Probit analysis was used to determine the 50% (KT_{50}) and 95% (KT_{95}) knockdown time of the insecticide. The resistance status of mosquito samples was determined according to the WHO (WHO, 2013) criteria such as mortality rates ranging from 98 to 100%: the population was considered fully susceptible, when the mortality rate ranged between 90 and 97%: indicate the presence of resistant genes in the vector population but it need s confirmation by additional tests, and when mortality rates < 90%, the vector population was considered resistant to the tested insecticides.

2.7. Meteorological data

Monthly rainfall data of the study area were obtained from the south branch regional office of the Ethiopian Meteorological Agency.

2.8. Ethical approval

This study was reviewed and approved by Ethiopian Public Health Institute (EPHI) (SERO-042-12-2015). House hold owners, village and district authorities were informed prior to the study, and signed informed consent was obtained from the head of each household for both exit trap and CDC light trap collection.

3. Results

3.1. Anopheles species composition

1291 *Anopheles* mosquitoes comprising *An. gambiae* s. l (presumably *An. arabiensis* from previous study), *An. pharoensis*, *An.*

Table 1
Number of adult *Anopheles* mosquitoes collected by CDC and exit traps from Sille village, south-west Ethiopia (October 2015–May 2016).

Anopheles species	CDC light trap	Percentage	Exit trap	Percentage
An. arabiensis	1035	80.2	252	91
An. pharoensis	240	18.6	22	8
An. pretoriensis	11	0.8	3	1
An. tenebrosus	2	0.2	0	0
An. rhodsiensis	3	0.2	0	0
Total	1291	100.0	277	100.0

pretoriensis, *An. tenebrosus* and *An. rhodesiensis* were collected by CDC light traps (Table 1). *An. arabiensis* was dominant species which accounted for 80.2% (1035/1291), followed by *An. pharoensis* (18.5%; 240/1291). *An. pretoriensis* (0.9%; 11/1291), *An. tenebrosus* (0.2%; 2/1291) and *An. rhodesiensis* (0.2%; 3/1291) were present in small numbers.

277*Anopheles* mosquitoes of three species; *An. arabiensis*, *An. pharoensis* and *An. pretoriensis*, were collected by windows exit trap collection. *An. arabiensis* was also the dominant (91%; 252/277) species followed by *An. pharoensis* (9%; 22/277). Only a small number of *An. pretoriensis* (1%; 3/277) was sampled using the exit traps.

3.2. Monthly variation in mosquito population

The highest number of mosquito was collected in January with 7.8 *Anopheles* per CDC light trap/night and in December with 3 *Anopheles* per exit trap/night. There was statistically significant variation in the density of *Anopheles* between months ($F = 10.7$, $DF = 7$; $P < 0.001$). Maximum number of *Anopheles* was collected in January 2016 following the decline of rainfall (Fig. 2).

3.3. Fed/gravid ratio of Anopheles mosquitoes

Majority of house exiting *An. arabiensis* and *An. pharoensis* were freshly fed as the fed/gravid ratio was 2.6 for *An. arabiensis* and it was 4.3 for *An. pharoensis* (Table 2).

3.4. Plasmodium CSP rates of Anopheles mosquitoes

1056 female *Anopheles* mosquitoes (872 *An. arabiensis* and 184 *An. pharoensis*) were tested for CSPs. Of which nine (eight *An. arabiensis* and one *An. pharoensis*) were found to be positive for *Plasmodium* CSPs (Table 3). Overall CSP rate of *Anopheles* mosquito was 0.85% (9/1056: 95% CI: 0.3–1.4%). Four *Anopheles* were positive for *P.vivax*_210 and 5 were positive for *P. falciparum*. The overall CSP rate of *An. arabiensis* was 0.92% (95% CI: 0.39–1.8). *P. falciparum* CSP rate was 0.46% (95% CI: 0.13–1.2) for *An. arabiensis* and it was 0.54% (95% CI: 0.01–2.9) for *An. pharoensis*.

3.5. Entomological inoculation rate of An. arabiensis

The overall EIR of *An. arabiensis* was 5.3 infectious bites per/person (ib/p)/eight months (Table 4). The *P. falciparum* EIR of *An. arabiensis* was 2.67 ib/p/eight months. The highest EIR (2.5 ib/p/month) was in February following the highest density of *An. arabiensis* in January. The main malaria transmission occurred from December 2016–February 2016, following the short rainfall (Fig. 2).

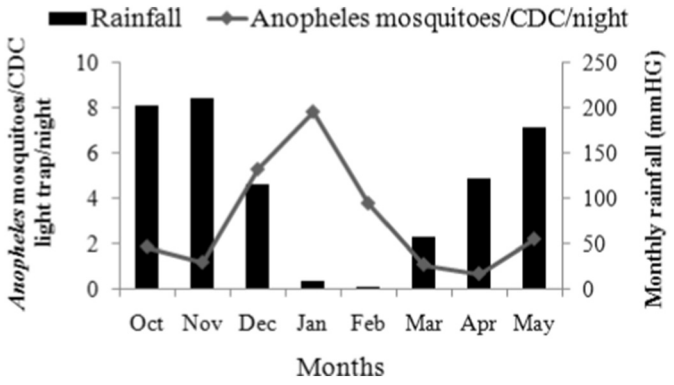

Fig. 2. Density of *Anopheles* mosquitoes/CDC light trap/night and monthly rainfall in Sille village, south-west Ethiopia (October 2015–May 2016).

Table 2
Fed/gravid ratio of *Anopheles* mosquitoes collected by exit trap in Sille village, south-west Ethiopia (December–May 2016).

Anopheles species	Abdominal stages				Fed/gravid ratio
	Unfed	Fresh fed	Half gravid	Gravid	
An. arabiensis	126	87	10	24	2.6
An. pharoensis	11	13	1	2	4.3
An. pretoriensis	3	–	–	–	–
Total	140	100	11	26	

3.6. Knockdown and mortality effects of insecticides against An. arabiensis

Only bendiocarb, permethrin and propoxur resulted in > 95% knockdown within 60 min of exposure time. Malathion and dieldrin took 100 and 149 min respectively, to bring 95% knockdown (Table 5). The highest KDT_{50} (88 min) value was detected for dieldrin.

An. arabiensis was resistant to dieldrin (4%) with mortality rates of 57% and the mortality rate due to deltamethrin (0.05%) was 71%. Permethrin (0.75%) and malathion (0.8%) showed possible resistance with mortality rates 90.4% and 92.5%, respectively. *An. arabiensis* was fully susceptible to propoxur (0.1%) and bendiocarb (0.1%) (Table 5). The control mortality was always < 5%, and hence the mortality due to insecticides was not corrected by Abbot's formula (**Abbott,** 1925).

4. Discussion

An. arabiensis, *An. pharoensis*, *An. pretoriensis*, *An. tenebrosus* and *An. rhodesiensis* were documented in Sille village, south-west Ethiopia. *An. arabiensis* and *An. pharoensis* were important malaria vectors as they were identified positive for *Plasmodium* CSPs. The indoor based EIR (5.3 ib/p/eight months) of *An. arabiensis* indicated the existence indoor malaria transmission in a village of high coverage of indoor based interventions. The principal malaria vector was fully susceptibility to propoxur and bendiocarb (the insecticides currently in use for IRS), while resistant to pyrethroid insecticides.

An. arabiensis and *An. pharoensis* were present throughout the study period, but the abundance varied from month to month. *An. arabiensis* was the major malaria vector and it was the only member of the *An. gambiae* complex in the study area (Taye et al., 2006). The finding is consistent with reports from other parts of Ethiopia (Abose et al., 1998; Massebo et al., 2013a). *An. pharoensis* is an important malaria vector in Sille village. A study from south-central Ethiopia documented its role in malaria transmission (Animut et al., 2013). Recently, the species was documented in large number in the same place in south-central Ethiopia, but none of them were positive for CSPs (Kenea et al., 2016). With regard to the feeding pattern, *An. arabiensis* showed variable feeding behaviours. Its tendency to feed on cattle was documented in village where cattle and other animals are usually kept outdoors (Massebo et al., 2013b). Tirados and his colleagues also reported indoors and outdoors anthropophagic behaviour in the Konso district in southern Ethiopia (Tirados et al., 2006). Zoophilic behaviour of the same species in another locality of the same region was reported (Habtewold et al., 2001). On the other hand, *An. pharoensis* showed more of outdoor biting behaviour (Turner, 1972; Nigatu et al., 1994). Low HBI was reported from south-central Ethiopia (Animut et al., 2013). These complex feeding patterns of the vectors may make them less susceptible to indoor based interventions and also may contribute for residual malaria transmission; maintaining transmission of malaria in area of high coverage of quality interventions and where the vectors are susceptible to the interventions (WHO, 2016b).

An. arabiensis collected by exit trap exhibited the tendency of exophily (the fed/gravid ratio was > 1). Exophilic tendency of the species was reported in south Ethiopia (Tirados et al., 2006). Also, a slight exophilic and exophagic behaviour of *An. arabiensis* was also documented in south-central Ethiopia (Abose et al., 1998). The high exit rate of malaria vector could be in response to a wide application indoor based intervention, and it may contribute for residual malaria transmission in area even with high intervention coverage and usage of quality interventions (WHO, 2014; Killeen, 2014). The insecticides used for bed nets impregnation are known for their exito-repellent effect to *Anopheles* mosquitoes.

An. arabiensis and *An. pharoensis* were positive for CSPs and hence are important vectors of malaria in the study area. The overall sporozoite rate was 0.85% (95% CI: 0.3–1.4%). *P. falciparum* CSP rate of *An. arabiensis* from CDC light traps was 0.46%; which is comparable with CSP rate of 0.5% from HLC reported in Sille village in 2006 (Taye et al., 2006). The *P. falciparum* CSP infection rate of *An. arabiensis* showed little change after 10 years of vector control interventions in the village. But, the overall CSP rate is < 2.3%

Table 3
CSP infection rate of *An. arabiensis* and *An. pharoensis* in Sille village, south-west Ethiopia (October 2015–May 2016).

Anopheles species	No. collected	No. assayed	Pf CSP positive (SR; %)	Pv CSP positive (SR; %)	Overall CSP positive	Overall CSP rate (%)
An. arabiensis	1035	872	4 (0.46)	4 (0.46)	8	0.92
An. pharoensis	240	184	1(0.54)	_	1	0.54
Total	1275	1056	5 (0.47)	4 (0.38)	9	0.85

Table 4
Monthly and eight months overall entomological inoculation rate (EIR) of *An. arabiensis* collected by CDC light traps in Sille village, south-west Ethiopia (October 2015–May 2016).

Months	No. collected	No. assayed	CSP positive	SR (%)	EIR (PfEIR)[a]	EIR (95% CI)[b]
October	91	73	1	1.37	0.72	0.58 (0.015–3.04)
November	59	47	0	0	0	0
December	251	204	1	0.49	0.71	0.58 (0.015–3.04)
January	350	314	2	0.64	1.29	1.15 (0.14–4.03)
February	139	119	4	3.36	2.5	2.15 (0.58–5.18)
March	28	23	0	0	0	0
April	32	23	0	0	0	0
May	85	79	0	0	0	0
Overall EIR	1035	872	8	0.92	5. 3 (2.67)	4.5 (1.7–8.7)

Alternative eight months EIR: no. ELISA positive/no. catches × no. days in eight months.
Overall EIR = *P. falciparum* and *P. vivax* entomological inoculation rate of eight months.
PfEIR = *P. falciparum* entomological inoculation rate of eight months.
SR = sporozoite rate; CSP = circum-sporozoite protein; 95% CI = confidence interval.
[a] Standard monthly EIR = no. ELISA positive from CDC light trap/no. ELISA tested × no. *An. arabiensis* collected from CDC light trap/no. of catches × no. days in a month. Standard eight months EIR = no. ELISA positive from CDC light trap/no. ELISA tested × no. *An. arabiensis* collected from CDC light trap/no. of catches × no. days in eight months.
[b] Alternative method: no. ELISA positive/no. catches × no. days in month.

Table 5
Mortality and KDT of *Anopheles arabiensis* collected from Sille village, south-west Ethiopia (December 2015).

Insecticides	Total no. tested	% mortality	KDT 50	KDT95
Bendiocarb 0.1%	150	100	20.5 (17.74–23.14)	32.6 (29.09–38.9)
Propoxur 0.1%	150	100	26.2 (23.54–28.95)	39.0(35.29–45.36)
Deltamethrin 0.05%	150	71	32.2 (27.22–36.77)	64.8 (56.75–78.93)
Permethrin 0.75%	150	90.4	23.6 (2.81–33.84)	55.6 (42.83–107.55)
Malathion 0.8%	150	92.5	69 (20.52–93.44)	99.6 (81.4–159.1)
Dieldrin 4%	150	57	88.5 (67.84–201.08)	149.2 (104.43–409.35)

Mortality measured after 24 h and KDT is knockdown time within 1 h exposure.

in 2006 (Taye et al., 2006). A recent study in south-west Ethiopia reported *P. falciparum* CSP rate of 0.32% for *An. arabiensis* from CDC light traps (Massebo et al., 2013a). Generally, the CSP rate of *An. arabiensis* varied from place to place and the method used to collect mosquitoes (Shaukat et al., 2010). The CSP rate of 0.54% in the current study implies that the species is an important vector of malaria at least as a secondary vector. *An. pharoensis* was not identified positive for *Plasmodium* sporozoite in previous study in the same place (Taye et al., 2006). It has also been incriminated as a vector of malaria in different parts of the country (Animut et al., 2013; Abose et al., 1998).

The overall EIR of *An. arabiensis* was 5.3 ib/p/eight months. This EIR may be overestimated due to the false ELISA positivity (Durnez et al., 2011) which may also be influenced by zoophilic tendency of the vector species (Massebo et al., 2015; Durnez et al., 2011). On the other hand, few studies have been attempted to estimate the EIR in Ethiopia followed similar mosquito sampling technique and used ELISA to estimate the CSP rate (Massebo et al., 2013a; Animut et al., 2013). Hence, it would be possible to compare and contrast the current result with other studies. The result of this study is > 3.66 ib/p/year in south-central Ethiopia (Animut et al., 2013) and lower than the 17.1 ib/p/year reported from nearby village in the same region (Massebo et al., 2013a). For interruption of malaria transmission, the EIR of principal malaria vector should be < 1 ib/p/year (Beier et al., 1999). LLINs and IRS are effective interventions that have reduced malaria incidence and mortality, and EIR of malaria vectors in many malaria endemic countries (Bhatt et al., 2015). In many malaria endemic countries, however, these interventions failed to stop the transmission mainly due to the residual malaria transmission (Killeen, 2014). On the other hand, the residual malaria transmission may be caused by either due to behavioural avoidance of house entry of malaria vectors, outdoor and early hours biting and animal feeding tendencies of the species (WHO, 2014). The early hours biting behaviour (both indoors and outdoors) of *An. arabiensis* has been documented in south-central and northern Ethiopia (Kenea et al., 2016; Abose et al., 1998; Yohannes and Boelee, 2012).

The EIR of *An. arabiensis* from indoor CDC light traps in a village of high intervention coverage may indicate the existence of indoor residual malaria transmission. In many settings, the impact of indoor based interventions is relatively low on *An. arabiensis* and its role is even increasing in maintaining residual malaria transmission. The principal malaria vector *An. arabiensis* was resistant to pyrethroid insecticides (immediate killing effect of bed nets may be compromised) and it is widespread in the population of malaria vectors in malaria endemic areas in Africa (Hemingway et al., 2016). The vector is fully susceptible to carbamate insecticides used for IRS in the study area as reported in different parts of Ethiopia (Balkew et al., 2012). Regardless of the widespread of resistance, those people sleeping under bed nets, however, can be protected from the infectious bites of mosquitoes because the nets acting as physical barriers (Bhatt et al., 2015). Hence, malaria control programme should promote consistent and proper use of bed

nets even in area with widespread of resistant malaria vectors. The time of feeding of endophagic vector populations may also be a critical importance if it occurs in the hours outside of LLINs use (Killeen, 2014; WHO, 2014). The need for the additional tools is therefore obvious to reduce and interrupt residual malaria transmission (WHO, 2014; Killeen, 2014). The existing intervention tools need to be supplemented by interventions that reduce house entry of vectors such as improving housing (Massebo and Lindtjørn, 2013c) and killing the adults attempt to enter houses or bite humans indoors (Durnez and Coosemans, 2013). The inclusion of insecticide zooprophylaxis (the use of animals to divert malaria vectors away from human hosts) into an integrated vector management package may be used to control residual malaria transmission by killing those malaria vectors feeding outdoor on animals (those avoid contact with insecticides applied indoors) (Njoroge et al., 2017; Rowland et al., 2001). But, the insecticides should be with no repellent effect to avoid diversion to humans (Franco et al., 2014). Finally, the EIR in the current study is high enough to maintain malaria transmission and may even result resurgence if the implementation of the existing interventions weakened or physiological resistance of malaria vectors to insecticides is happened (Killeen, 2014; Cohen et al., 2012). Hence, the use of propoxur and bendiocarb could be strengthened to reduce indoor human-vector contacts and hence malaria transmission and new tools need to be available to supplement the existing interventions.

5. Conclusions

The findings of this study reveal that *An. arabiensis* and *An. pharoensis* are vectors of malaria which are positive for *Plasmodium* CSPs. *P. falciparum* CSP rate of *An. arabiensis* showed little change after 10 years of vector control interventions in the village thought the collection methods was different. Based on the EIR of the vector, indoor malaria transmission was substantial high regardless of high coverage of bed nets and the susceptibility of malaria vector to carbamate insecticides (used for IRS); implies the existence of indoor residual malaria transmission. Hence, there is a need to new tools to interrupt the transmission and the use of propoxur and bendiocarb need to be strengthened to reduce the transmission and prevent malaria rebound.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgements

NORHED-Arba Minch University (QZA-0484 ETH-13/0025) project is highly acknowledged for funding this study. Arba Minch University, Department of Biology acknowledged for providing laboratory facilities. We thank Yohannes Negash from Aklilu Lemma Institute of Pathobiology for giving training on Enzyme-Linked Immuno-Sorbent Assay and analyzing mosquitoes for circum-sporozoite proteins. Nigatu Eligo and Gemechu Leta of Arba Minch University are acknowledged for their assistance in sporozoite proteins analysis.

References

Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18, 265–267.

Abose, T., Yeebiyo, Y., Olana, D., Alamirew, D., Beyene, Y., Regassa, L., Mengesha, A., 1998. Re-orientation and definition of the role of malaria vector control in Ethiopia. In: The Epidemiology and Control of Malaria With Special Emphasis on the Distribution, Behaviour and Susceptibility of Insecticides of Anopheline Vectors and Chloroquine Resistance in Zwai, Central Ethiopia and other Areas.

Animut, A., Balkew, M., Gebre-Michael, T., Lindtjørn, B., 2013. Blood meal sources and entomological inoculation rates of anophelines along a highland altitudinal transect in south-central Ethiopia. Malar. J. 12, 76.

Balkew, M., Getachew, A., Chibsa, S., Olana, D., Reithinger, R., Brogdon, W., 2012. Insecticide resistance: a challenge to malaria vector control in Ethiopia. Malar. J. 11, 139.

Beier, J., Perkins, P., Wirtz, R., Koros, J., Diggs, D., Gargan, T., Koech, D., 1988. Bloodmeal identification by direct-enzyme linked immunosorbent assay (ELISA), tested on *Anopheles* (Diptera: Culicidae) in Kenya. J. Med. Entomol. 25, 9–16.

Beier, J., Killeen, G., Githure, J., 1999. Short report: entomologic inoculation rates and *Plasmodium falciparum* malaria prevalence in Africa. Am. J. Trop. Med. Hyg. 61, 109–113.

Bhatt, S., Weiss, D.J., Cameron, E., Bisanzio, D., Mappin, B., Dalrymple, U., Battle, K.E., Moyes, C.L., Henry, A., Eckhoff, P.A., Wenger, E.A., Briët, O., Penny, M.A., Smith, T.A., Bennett, A., Yukich, J., EISELE, T.P., Griffin, J.T., Fergus, C.A., Lynch, M., Lindgren, F., Cohen, J.M., Murray, C.L., Smith, D.L., Hay, S.I., Cibulskis, R.E., Gething, P.W., 2015. The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. Nature 526, 207–211.

Briët, O., Huho, B.J., Gimnig, J.E., Bayoh, N., Seyoum, A., Sikaala, C.H., Govella, N., Diallo, D.A., Abdullah, S., Smith, T.A., Killeen, G.F., 2015. Applications and limitations of Centers for Disease Control and Prevention miniature light traps for measuring biting densities of African malaria vector populations: a pooled-analysis of 13 comparisons with human landing catches. Malar. J. 14, 247.

Cohen, J., Smith, D.L., Cotter, C., Ward, A., Yamey, G., Sabot, O.J., Moonen, B., 2012. Malaria resurgence: a systematic review and assessment of its causes. Malar. J. 11, 122.

Durnez, L., Coosemans, M., 2013. Residual transmission of malaria: an old issue for new approaches. In: Manguin, S. (Ed.), *Anopheles* Mosquitoes—New Insights Into Malaria Vectors. Intech, pp. 671–704 (DOI).

Durnez, L., Van Bortel, W., Denis, L., Roelants, P., Veracx, A., Trung, H.D., Sochantha, T., Coosemans, M., 2011. False positive circumsporozoite protein ELISA: a challenge for the estimation of the entomological inoculation rate of malaria and for vector incrimination. Malar. J. 10, 195.

Franco, A., Gomes, M.G.M., Rowland, M., Coleman, P.G., Davies, C.R., 2014. Controlling malaria using livestock-based interventions: a one health approach. PLoS One 9, e101699.

Gething, P., Casey, D.C., Weiss, D.J., Bisanzio, D., Bhatt, S., Cameron, E., Battle, K.E., Dalrymple, U., Rozier, J., Rao, P.C., Kutz, M.J., Barber, R.M., Huynh, C., Shakelford, K.A., Coates, M.M., Nguyen, G., Fraser, M.S., Kulikoff, R., Wang, H., Naghavi, M., Smith, D.L., Murray, C.J.L., Hay, S.I., Lim, S.S., 2016. Mapping *Plasmodium falciparum* mortality in Africa between 1990 and 2015. N. Engl. J. Med. http://dx.doi.org/10.1056/NEJMoa1606701.

Gillies, M.T., Coetzee, M., 1987. A Supplement to the Anophelinae of Africa South of the Sahara. 55 Publications of the South African Institute of Medical Research, Johannesburg.

Govella, N.J., Chaki, P.P., Mpangile, J.M., Killeen, G.F., 2011. Monitoring mosquitoes in urban Dar es Salaam: evaluation of resting boxes, window exit traps, CDC light traps, Ifakara tent traps and human landing catches. Parasit. Vectors 4, 40.
Habtewold, T., Walker, A.R., Curtis, C.F., Osir, E.O., Thapa, N., 2001. The feeding behaviour and *Plasmodium* infection of *Anopheles* mosquitoes in southern Ethiopia in relation to use of insecticide-treated livestock for malaria control. Trans. R. Soc. Trop. Med. Hyg. 95, 584–586.
Hemingway, J., Vontasb, J., Poupardina, R., Ramanc, J., Linesd, J., Schwabee, C., Matiase, A., Kleinschmidt, I., 2013. Country level operational implementation of the global plan for insecticide resistance management. PNAS 110, 9397–9402.
Hemingway, J., Ranson, H., Magill, A., Kolaczinski, J., Fornadel, C., Gimnig, J., Coetzee, M., Simard, F., Roch, D.K., Hinzoumbe, C.K., Pickett, J., Schellenberg, D., Gething, P., Hoppé, M., Hamon, N., 2016. Averting a malaria disaster: will insecticide resistance derail malaria control? Lancet 387, 1785–1788.
Kenea, O., Balkew, M., Tekie, H., Gebre-Michael, T., Deressa, W., Loha, E., Lindtjørn, B., Overgaard, H.J., 2016. Human-biting activities of *Anopheles* species in south-central Ethiopia. Parasit. Vectors 9, 527.
Killeen, G., 2014. Characterizing, controlling and eliminating residual malaria transmission. Malar. J. 13, 330.
Lines, J., Curtis, C.F., Wilkes, T.J., Njunwa, K.J., 1991. Monitoring human-biting mosquitoes (Diptera: Culicidae) in Tanzania with light-traps hung beside mosquito nets. Bull. Entomol. Res. 81, 77–84.
Massebo, F., Lindtjørn, B., 2013c. The effect of screening doors and windows on indoor density of Anopheles arabiensis in south-west Ethiopia: a randomized trial. Malar. J. 12, 319.
Massebo, F., Balkew, M., Gebre-Michael, T., Lindtjørn, B., 2013a. Entomologic inoculation rates of *Anopheles arabiensis* in southwestern Ethiopia. Am. J. Trop. Med. Hyg. 89, 466–473.
Massebo, F., Balkew, M., Gebre-Michael, T., Lindtjorn, B., 2013b. Blood meal origins and insecticide susceptibility of *Anopheles arabiensis* from Chano in south-west Ethiopia. Parasit. Vectors 6, 44.
Massebo, F., Balkew, M., Gebre-Michael, T., Lindtjørn, B., 2015. Zoophagic behaviour of anopheline mosquitoes in southwest Ethiopia: opportunity for malaria vector control. Parasit. Vectors 8, 645.
Mendis, K., Rietveld, A., Warsame, M., Bosman, A., Greenwood, B., Wernsdorfer, W.H., 2009. From malaria control to eradication: the WHO perspective. Tropical Med. Int. Health 14, 1–7.
Murray, C., Rosenfeld, L.C., Lim, S.S., Andrews, K.G., Foreman, K.J., Haring, D., Fullman, N., Naghavi, M., Lozano, R., Lopez, A.D., 2012. Global malaria mortality between 1980 and 2010: a systematic analysis. Lancet 379, 413–431.
Najera, J.A., Gonzalez-Silva, M., Alonso, P.L., 2011. Some lessons for the future from the global malaria eradication programme (1955–1969). PLoS Med. 8, 84–90.
Nigatu, W., Petros, B., Lulu, M., Adugna, N., Wirtz, R., 1994. Species composition, feeding and resting behaviour of the common anthropophilic anopheline mosquitoes in relation to malaria transmission in Gambella, south-west Ethiopia. Int. J. Trop. Insect. Sci. 15, 371–377.
Njoroge, M., Tirados, I., Lindsay, S.W., Vale, G.A., Torr, S.J., Fillinger, U., 2017. Exploring the potential of using cattle for malaria vector surveillance and control: a pilot study in western Kenya. Parasit. Vectors 10, 18.
Ranson, H., Lissenden, N., 2016. Insecticide resistance in African *Anopheles* mosquitoes: A worsening situation that needs urgent action to maintain malaria control. Trends Parasitol. 32, 187–196.
Rowland, M., Durrani, N., Kenward, M., Mohammed, N., Urahman, H., Hewitt, S., 2001. Control of malaria in Pakistan by applying deltamethrin insecticide to cattle: a community-randomised trial. Lancet 357, 1837–1841.
Shaukat, A., Breman, J., Mckenzie, E., 2010. Using the entomological inoculation rate to assess the impact of vector control on malaria parasite transmission and elimination. Malar. J. 9, 122.
Taye, A., Hadis, M., Adugna, N., Tilahun, D., Wirtz, R., 2006. Biting behavior and *Plasmodium* infection rates of *Anopheles arabiensis* from Sille, Ethiopia. Acta Trop. 97, 50–54.
Tirados, I., Costantini, C., Gibson, G., Torr, S.J., 2006. Blood feeding behaviour of the malaria mosquito *Anopheles arabiensis*: implications for vector control. Med. Vet. Entomol. 20, 425–437.
Turner, R., 1972. Malaria epidemiological observations from unsprayed study districts in Ethiopia. Mosq. News 32, 608–613.
WHO, 1975. Manual on Practical Entomology in Malaria. Part 2. (Switzerland, Geneva).
WHO, 2003. Malaria Entomology and Vector Control. World Health Organization, Geneva, Switzerland.
WHO, 2013. Test Procedures for Insecticide Resistance Monitoring in Malaria Vector Mosquitoes. World Health Organization, Geneva.
WHO, 2014. Control of Residual Malaria Parasite Transmission. WHO/HTM/GMP/MPAC/2014.5.
WHO, 2016a. World Malaria Report 2016. World Health Organization, Geneva.
WHO, 2016b. WHO Malaria Terminology. World Health Organization, Geneva.
Yohannes, M., Boelee, E., 2012. Early biting rhythm in the Afro-tropical vector of malaria, *Anopheles arabiensis*, and challenges for its control in Ethiopia. Med. Vet. Entomol. 26, 103–105.