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

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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.
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 insecticides 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°59′ 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 into unfed, freshly fed, half-gravid or gravid (WHO, 2003). The 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
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 sterilized 10% sugar solution and morphologically identified using a key (Gillies and Coetze, 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 exophilic 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 (exophilic of the species). If the index is constantly less than 1, the species shows indoor rest tendency (endophily). If the index is closer to 1, it indicates smaller in differentiation in resting tendencies as exophilic and endophily.

Probit analysis was used to determine the 50% (KT50) and 95% (KT95) 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. 1 (presumably An. arabiensis from previous study), An. pharoensis, An.
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).

**Table 1**

| 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. rhodesiensis  | 3              | 0.2        | 0         | 0          |
| Total             | 1291           | 100.0      | 277       | 100.0      |

Fig. 2. Density of Anopheles mosquitoes/CDC light trap/night and monthly rainfall in Sille village, south-west Ethiopia (October 2015–May 2016).
transmission of malaria in areas of high coverage of quality interventions and where the vectors are susceptible to the interventions make them less susceptible to indoor-based interventions and also may contribute to residual malaria transmission; maintaining species was reported in south Ethiopia (Tirados et al., 2006). Also, a slight exophilic and exophagic behaviour of coverage and usage of quality interventions (WHO, 2014; Killeen, 2014). The insecticides used for bed nets impregnation are known application indoor-based intervention, and it may contribute to residual malaria transmission in areas with high intervention also documented in south-central Ethiopia (Abose et al., 1998). The high exit rate of malaria vectors could be in response to a wide HBI was reported from south-central Ethiopia (Animut et al., 2013). These complex feeding patterns of the vectors may ending 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 numbers 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 Exophilic and exophagic 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 areas 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, 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 areas of high coverage of quality interventions and where the vectors are susceptible to the interventions (WHO, 2016b).

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 KDT50 (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).

### 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         | 10 (0.54)              | -                      | 1                    | 0.54                 |
| Total             | 1275          | 1056        | 5 (0.47)               | 4 (0.38)               | 9                    | 0.85                 |
Mortality measured after 24 h and KDT is knockdown time within 1 h exposure of the species (WHO, 2014). The early hours biting behaviour (both indoors and outdoors) of An. arabiensis has been documented 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.

The 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). 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 Boelte, 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 as physical barrier to prevent mosquito bites.

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) | EIR (95% CI) |
|-------------|---------------|-------------|--------------|--------|-------------|--------------|
| 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         | 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) |
| Propoxur 0.1%     | 150              | 100         | 26.2   | (23.54–28.95) |
| Deltamethrin 0.05%| 150              | 71          | 32.2   | (27.22–36.77) |
| Permethrin 0.75%  | 150              | 90.4        | 23.6   | (2.81–33.84)  |
| Malathion 0.8%    | 150              | 92.5        | 69     | (20.52–93.44) |
| Dieldrin 4%       | 150              | 57          | 88.5   | (67.84–201.08) |

Mortality measured after 24 h and KDT is knockdown time within 1 h exposure.
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 (Masebo and Lindtjorn, 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. pharaohi 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.

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