Chapter

Effect of Bendiocarb Indoor Residual Spraying on Entomological Inoculation Rate of *Anopheles arabiensis* in Northwestern Highlands of Ethiopia

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Abstract

Entomological inoculation rate (EIR) is a method to estimate the level of human exposure to infective mosquito bites and assess impacts of vector control measures. The objective is to assess the effect of indoor residual spray (IRS) on blood meal index (BMI), sporozoite infection rate (SR), and EIR in *An. arabiensis* under local ecological settings in Ethiopia. A total 1541 fresh fed (FF) female *An. arabiensis* collected by CDC light trap and PSC were processed at the Center for Disease Control and Prevention Laboratory, Atlanta, Georgia, USA, to determine their BMI and SR, using enzyme-linked immunosorbent assay (ELISA). IRS reduced the abundance of FF female *An. arabiensis* in sprayed villages (n=62) while the number remained high in non-sprayed villages (n=1,690). The relative adjusted reduction in human blood feeding index (HBI) due to IRS varied between 3 and 10% except in 2014 when no human blood was detected in any of the three mosquitoes tested. The relative adjusted reduction in *P. falciparum* infection and EIR in *An. arabiensis* was 100% after IRS. The results illustrated that IRS was strong enough to reduce EIR in *An. arabiensis*. IRS is recommended to control malaria transmission in areas of similar ecological set.

Keywords: *A. arabiensis*, Ethiopia, EIR, IRS, vector control

1. Introduction

Current malaria vector control strategies rely heavily on indoor residual spraying (IRS) and long-lasting insecticide-treated mosquito nets (LLINs). The impact of these intervention tools on entomological malaria transmission risk factors needs to be evaluated. The level of exposure to infective mosquito bites could be measured using entomological inoculation rate (EIR) in the vector [1, 2]. The EIR is defined as the number of infective bites received by an individual per unit time

(night, month, or year). It is the product of human-biting rate (HBR) and plasmodium sporozoite infection rate (SR) [3, 4].

The human landing catch (HLC) is the most commonly used method to determine the human-biting rate because it is the direct measure of human-vector contact [4]. However, due to ethical and logistic constraints associated with HLC, light trap catches (LTC), pyrethrum spray catches (PSC), and exit trap catches could be used as alternatives to human landing catches [3] to estimate the HBR. In this study, the Centers for Disease Control and Prevention (CDC) light trap and PSC mosquito sampling methods were used to estimate the HBR.

Malaria is a public health problem in Ethiopia. Indoor residual spraying and LLNs are the frontline pillars of malaria vector intervention tools that have been used in all malarious parts of the country. However, studies on the impact of these interventions on EIR are either limited or unavailable [5, 6]. Besides, EIR varies from region to region, even from locality to locality. Therefore, narrowing this knowledge gap would be valuable for vector control program. The present study was carried out to assess the impact of the current vector control strategy specifically IRS on BMI, SR, and EIR.

Dichlorodiphenyltrichloroethane (DDT) was the choice of insecticide for IRS operation that had been used for decades in many malarious areas of Ethiopia except at a few places where malathion was used for DDT-resistant vector populations. This was continued until 2007 when DDT was replaced by deltamethrin due to the development of DDT resistance in the major malaria vector populations [7]. Payable to the occurrences of deltamethrin resistance in different vector populations, in view of the possibility of cross-resistance between DDT and pyrethroid insecticides and the scaling up of the distributions of pyrethroid-treated LLINs, IRS control program again replaced deltamethrin by bendiocarb (carbamate group) in 2010 and still in use for IRS operations in different parts of the country.

The residual efficacy of bendiocarb with the recommended concentration could last between 2 and 6 months depending on the nature of sprayable surfaces [8]. Therefore, bendiocarb was the choice of insecticide used for IRS operation during the present study.

2. Materials and methods

2.1 Study site

The study was carried out in two adjacent villages, namely, Andassa (N11° 30′ 14.6″, 037° 29′ 27.8″) and Tikurit (11° 30′ 49.8″, 037° 28′ 02.8″), Bahir Dar Zuria District, North West Ethiopia. These villages were separated by Andassa River and buffered by about 2 km vegetable and fruit farms. The study villages were selected purposively by considering malaria endemicity and the history of IRS implementation. Indoor residual spraying and LLINs are the primary intervention tools that have been used for years against *A. arabiensis* (important vector of the study area). The vector has developed different levels of insecticide resistance to insecticides of different classes recommended for both LLIN treatment and IRS operation [9].

2.2 Design

A comparative study was carried out in Andassa and Tikurit villages. The study was conducted for 2 consecutive years. Andassa received two rounds of sprays

one in 2013 and another in 2014, while no spray was implemented in Tikurit. Participants in the unsprayed villagers were provided with treated bed nets free of charge, and individuals found infected received free treatment at the nearest health center. The susceptibility status of *A. arabiensis* to bendiocarb was confirmed before the application of IRS.

2.3 Mosquito sampling

Adult female *A. arabiensis* were collected from 24 residential houses (12 houses/village and 6 houses/sampling method) using pyrethroid spray catch (PSC) and CDC light trap sampling methods. PSC was applied by spraying pyrethroid insecticide under which a white muslin cloth was placed to facilitate knocked-down mosquito collection. Human baits, sleeping in beds covered with treated bed nets, were used to reinforce CDC light traps. Mosquitoes that were collected by each sampling method before and after IRS were then stored individually in tubes containing silica gel to process and determine their BMI and SR in the lab.

2.4 Blood meal host source and sporozoite rate determination

Enzyme-linked immunosorbent assay (ELISA) originally described by Beier et al. [10] and CS-ELISA [11] protocols were adopted and used for BMI and SR analyses, respectively. Blood-fed mosquitoes preserved individually in tubes containing silica gel were used to determine their BMI and SR. Heads-thoraxes of mosquitoes were separated from their abdomens, and each body part (abdomen/head-thorax) was given a corresponding ID number and kept individually in tubes for analyses.

2.5 Blood meal source determination

The mosquito abdomen, which was kept individually in tubes containing silica gel, was ground in a tube containing 100 μl of phosphate buffer saline (PBS) with a plastic pestle fitted with foot-operated grinder. The pestle was rinsed twice with 200 μl of PBS to achieve the final volume of 500 μl. The samples were either incubated at room temperature for 3 h and then stored at 4°C and tested the next day. Mosquitoes were tested to assess the blood meal origin of human and bovine only because these hosts were the predominant hosts of the vector during the study period. A 96-well ELISA plate was used, and 50 μl of the positive control for the blood meal host being tested was loaded. Wells A2–A5 had 50 μl of the negative controls, and wells A6–A8 were blanks containing 50 μl of blocking buffer. The plate was then covered and incubated for 3 h. The mosquito triturate was then aspirated by multichannel pipet, and the plate was washed three times with 200 μl PBS-Tween20 (5%). For a full 96-well plate, the peroxidase conjugate anti-host IgG antibody was prepared by adding 4800 μl of blocking buffer and 19.2 μl of anti-host and 1 μl of 1:100,000 of each of the negative control [10]. Fifty microliter of peroxidase conjugate was added to each well, and the plate was covered and incubated for 1 h at room temperature. The plate was then washed three times with 200 μl PBS-Tween20 (5%), and the one component ABTS peroxidase substrate was added to each well. PBS-Tween20 was aspirated by multichannel pipet, and plates were banged between washes. After 30 min of covered incubation at room temperature, the plate was read with the SpectraMax 340 plate reader (Molecular Devices) at 414 nm.

2.6 Sporozoite rate determination

The head-thorax of a mosquito, which was kept individually in step tubes containing silica gel, was ground in 1.5 μl microcentrifuge grinding tube containing 50 μl PBS with a plastic pestle fitted with foot-operated grinder. The pestle was rinsed twice with 100 μl of PBS and dried with tissue paper to prevent contamination between mosquito samples.

A 96-well ELISA PVC plate was coated with 50 μl of capture monoclonal antibodies (mAb) of each plasmodium sporozoite species (Pf, Pv-2010, and Pv-247) in each well of the ELISA plates (a separate plate used for each species), covered and incubated for half an hour. After the well contents were aspirated, plates were banged upside down on paper towel five times. The wells were then filled with 200 μl blocking buffer (BB), covered with lid and incubated for 1 h at room temperature. Well contents are aspirated and the plate is banged on paper towel five times. Samples and controls were loaded into the plate (well 1A, positive control; wells 1B–1H, negative control; and the rest of the wells with mosquito triturate) and covered and incubated for 2 h. Well contents were aspirated, and the plates were banged upside down on paper towel five times and washed two times with 200 μl of PBS-Tween20. The wells were aspirated, and plates were banged upside down five times with each wash. Then a 50 μl of peroxidase conjugate solution of each plasmodium sporozoite species (Pf, Pv-2010, and Pv-247) was added to each well and covered and incubated for 1 h. After aspirating the well contents and banging the plates, wells were washed three times with 200 μl of PBS-Tween20 and aspirated, and plates were banged five times with each wash. Finally, a 100 μl of the substrate solution was added per well, covered with cover plate and incubated for 30 min. The results were then read visually at the SpectraMax 340 plate reader (Molecular Devices) at 405–414 nm. All positive samples were retested for confirmation.

2.7 Determination of entomological inoculation rate

Plasmodium EIR of *A. arabiensis* was determined based on CDC light traps and PSC. The EIR was estimated from PSC samples as described by the World Health Organization [12] using the formula: number of fresh fed (FF) mosquitoes caught by PSC/ no. human occupants who spent the previous night in sprayed house) × (number of human fed mosquitoes/number of mosquitoes tested for human blood meal) × (number of sporozoite positive ELISAs/ number of mosquitoes tested, i.e., HBR × CSP rate. The human-biting rate was calculated by dividing the total number of freshly fed *A. arabiensis* caught in PSC by the total number of occupants who slept in the houses in the previous night of mosquito collection and multiplied by the HBI. The HBI was calculated as the proportion of *Anopheles* mosquitoes that fed on humans to the total *Anopheles* analyzed for blood meal origin [13, 14]. EIR from CDC light trap catches was estimated using the standard method, 1.605 (number of circumsporozoite-positive ELISA results from CDC light trap/ no. of mosquitoes tested) × (number of mosquitoes collected by CDC LT/ no. of CDC LT catches), and the alternative method, 1.605 (no. positive ELISA/no. catches) [15].

2.8 Data analyses

The relative adjusted reduction in human blood feeding index (HBI), sporozoite rate (SR), and the entomologic inoculation rate (EIR) of the vector after intervention was calculated using the formula [Ref]: PR = $100 - \frac{C1T2}{C2T1} \times 100$, where C1 and

C2 and T1 and T2 describe the either the number of *A. arabiensis* or percentages of BMI, SP, or EIR in sprayed (T) and non-sprayed villages (C) before IRS (subscript 1) and after IRS (subscript 2). This formula takes into account that changes in the mosquito population and parasite prevalence are taking place at the same level and rate in both sprayed and non-sprayed villages, i.e., the reductions were adjusted for the background differences. This formula was used only when the denominators were non-zero.

2.9 Ethical clearance

Ethical permission for the study was obtained from the Ethiopian Public Health Institute and Amhara Regional Health Bureau. Verbal consent was also obtained from the owner of each house sampled for mosquitoes. The study did not involve human or animal subjects.

3. Results

3.1 Effect of IRS on the abundance of *A. arabiensis*

Table 1 shows the abundance and abdominal status of *A. arabiensis* collected before and after spray. The abundance and abdominal status of *A. arabiensis* varied by sampling method, spray status, study village, and year. Among 5425 *A. arabiensis*, 3111 of them were collected by CDC light traps and 2314 of them by pyrethrum spray catches (PSC). The number of semi-gravid and gravid *A. arabiensis* was smaller in CDC light trap catches than in PSC collections. The proportions of unfed *A. arabiensis* were higher in CDC light trap catches than in PSC collections. Fresh fed *A. arabiensis* was dominant in PSC collections (>75%), while <54% of them were FF in CDC light trap catches. The abundance of these FF mosquitoes was declined after IRS in sprayed villages (n = 62), while the number of FF remained high in non-sprayed villages (n = 1690). The abundance of unfed, gravid, and semi-gravid mosquitoes also decreased after spray.

3.2 Effect of IRS on HBI

Among 3451 FF *A. arabiensis* collected, 1574 (45.61%) of them were tested to determine their blood meal sources and sporozoite infection rate. The relative adjusted reduction in *A. arabiensis* human blood feeding index (HBI) due to IRS implementation varied from 3 to 10% except in 2014 when no human blood was detected in any of the three mosquitoes that were collected and tested. Despite IRS implementation reduced HBI, a non-negligible proportion of *A. arabiensis* still fed on humans (**Table 2**).

3.3 Effect of IRS on SR

The estimated sporozoite rate in *A. arabiensis* was low in both sprayed and non-sprayed villages especially after IRS implementation. As indicated by ELISA test, *P. falciparum* was more prevalent than *P. vivax* in both sprayed and non-sprayed villages. *Pv*-247 was the only subspecies detected during the study period. There was no any mixed infection in the vector in both study villages during the study period. Neither *P. falciparum* nor *P. vivax* was not detected in *A. arabiensis* collected from sprayed villages after the implementation of

Year	Village	Before spray					After spray					Adjusted reduction (%)
		CDC light trap collection										
		Row total	UF	FF	SG	G	Row total	UF	FF	SG	G	
2013	Sprayed	103	46	57	0	0	12	6	6	0	0	8.6
	Non-sprayed	599	356	240	0	3	811	341	468	0	2	
	Column total	702	402	297	0	3	823	347	474	0	2	
2014	Sprayed	139	56	83	0	0	67	18	48	0	1	5.7
	Non-sprayed	146	69	71	0	6	1234	583	650	0	1	
	Column total	285	125	154	0	6	1301	601	698	0	2	
		987 pyrethrum spray collection 45.69% 2124 = 3111 53.65% AIRS										
2013	Sprayed	176	13	151	10	2	6	1	5	0	0	4.2
	Non-sprayed	769	33	666	49	21	624	19	543	48	14	
	Column total	945	46	817	59	23	630	20	548	48	14	
2014	Sprayed	471	16	302	86	67	3	0	3	0	0	3.9
	Non-sprayed	228	25	129	33	41	37	6	29	0	2	
	Column total	699	41	431	119	108	40	6	32	0	2	
1644		75.91 670 = 2314 86.57% AIRS 5425										

Table 1.
Abundance and abdominal status of A. arabiensis collected by PSC and CDC light traps from sprayed and non-sprayed villages in Bahir Dar Zuria District, North West Ethiopia, in 2013 and 2014.

Before spray		After spray				
Year	**Host**	**Sprayed (n)**	**Non-sprayed (n)**	**Sprayed (n)**	**Non-sprayed (n)**	**Adjusted reduction (%)**
CDC light trap collection						
2013	HBI	19.30 (57)	18.18 (176)	16.67 (6)	17.61 (176)	-10.83
	BBI	31.58 (57)	42.05 (176)	33.33 (6)	40.91 (176)	+8.48
	Mix	19.30 (57)	1.7 (176)	16.67(6)	0 (176)	
	Un	29.82 (57)	38.07 (176)	33.33 (6)	41.48 (176)	+2.58
2014	HBI	18.75 (80)	18.57 (70)	16.67 (48)	17.05 (176)	-3.17
	BBI	33.75 (80)	44.29 (70)	37.50 (48)	46.02 (176)	+6.93
	Mix	7.5 (80)	0 (70)	0 (48)	0 (176)	
	UN	40 (80)	31.14 (70)	45.83 (48)	36.93 (176)	-3.39
Pyrethrum spray sheet collection						
2013	HBI	20.71 (140)	25 (176)	20 (5)	25 (176)	-3.43
	BBI	36.43 (140)	51.70 (176)	40 (5)	55.11 (176)	+5.16
	Mix	20 (140)	0 (176)	20 (5)	0 (176)	
	UN	22.8 (140)	23.30 (176)	20 (5)	21.02 (176)	-2.76
2014	HBI	19.89 (176)	18.75 (80)	0 (3)	17.24 (29)	100
	BBI	32.95 (176)	48.75 (80)	33.33 (3)	48.28 (29)	+2.14
	Mix	24.43 (176)	21.25 (80)	66.67 (3)	0 (29)	
	UN	22.73 (176)	30.00 (80)	0 (3)	34.48 (29)	0
		453	502-955	62	557-619	

HBI, human blood index; BBI, bovine blood index; UN, unknown hosts; n, number of mosquitoes tested for their blood meal origin.

Table 2.
Effect of bendiocarb IRS on blood meal sources (BMS) of A. arabiensis in sprayed and non-sprayed villages, Bahir Dar Zuria District, North West Ethiopia, in 2013 and 2014.

IRS. Similar results were observed for *Pv*-247 in non-sprayed villages except in 2013 when SR was 0.57% in *A. arabiensis* caught by CDC light trap. The relative adjusted reduction in *P. falciparum* infection in *A. arabiensis* in sprayed villages was 100% after IRS. A similar result was observed for *Pv*-247 EIR in 2013 in *A. arabiensis* collected by CDC light traps (**Table 3**).

3.4 Effect of IRS on EIR

The reduction in EIR after the implementation of IRS had similar trends with the reduction in SR because EIR is the product of SR and HBI. Compared with CDC light trap catches, EIR was high in PSC catches, i.e., *Pf*-EIR in *A. arabiensis* was 452 infective bites/night/house in PSC catches, while it was 32.2 infective bites/night/house in CDC light trap catches. *Pv*-247 EIR was 226 and 16 infective bites/night/house in *A. arabiensis* collected by PSC and CDC light traps, respectively. The relative adjusted reduction in *Pf*-EIR in *A. arabiensis* was 100% after the implementation of IRS. A similar result was observed for *Pv*-247 EIR in 2013 in *A. arabiensis* caught by CDC light traps (**Table 4**).

Before spray				After spray		
Year	**Parasite**	**Sprayed (n)**	**Non-sprayed (n)**	**Sprayed (n)**	**Non-sprayed (n)**	**Adjusted reduction (%)**
CDC light trap collection						
2013	Pf	1.75 (57)	1.14 (176)	0 (6)	0.57 (176)	100
	Pv-247	1.75 (57)	0.57 (176)	0 (6)	0. 57 (176)	100
	Pv-210	0 (57)	0 (176)	0 (6)	0 (176)	
	Mixed	0 (57)	0 (176)	0 (6)	0 (176)	
2014	Pf	2.5 (80)	1.43 (70)	0 (48)	1.70 (176)	100
	Pv-247	0 (80)	0 (70)	0 (48)	0 (176)	
	Pv-210	0 (80)	0 (70)	0 (48)	0 (176)	
	Mixed	0 (80)	0 (70)	0 (48)	0 (176)	
Pyrethrum spray sheet collection						
2013	Pf	1.43 (140)	1.14 (176)	0 (5)	1.14 (176)	100
	Pv-247	0.71 (140)	0.57 (176)	0 (5)	0 (176)	
	Pv-210	0 (140)	0 (176)	0 (5)	0 (176)	
	Mixed	0 (140)	0 (176)	0 (5)	0 (176)	
2014	Pf	1.70 (176)	1.25 (80)	0 (3)	0 (29)	
	Pv-247	0 (176)	0 (80)	0 (3)	0 (29)	
	Pv-210	0 (176)	0 (80)	0 (3)	0 (29)	
	Mixed	0 (176)	0 (80)	0 (3)	0 (29)	

Pf, Plasmodium falciparum; Pv-247, Plasmodium vivax 247; Pv-2010, Plasmodium vivax 2010; n, number of mosquitoes tested for CSP ELISA.

Table 3.
Sporozoite rate of A. arabiensis (based on LTC).

Before spray				After spray		
Year	**EIR**	**Sprayed**	**Non-sprayed**	**Sprayed**	**Non-sprayed**	**Adjusted reduction (%)**
CDC light trap collection						
2013	Pf	16	32.2	0	4.47	100
	Pv-247	16	16.1	0	4.47	100
	Pv-210	0	0	0	0	
	Mixed	0	0	0	0	
2014	Pf	26.76	13.38	0	13.34	100
	Pv-247	0	0	0	0	
	Pv-210	0	0	0	0	
	Mixed	0	0	0	0	
Pyrethrum spray sheet collection						
2013	Pf	101.58	452.01	0	151.62	100
	Pv-247	50.44	226	0	0	
	Pv-210	0	0	0	0	

Before spray				After spray		
Year	**EIR**	**Sprayed**	**Non-sprayed**	**Sprayed**	**Non-sprayed**	**Adjusted reduction (%)**
	Mixed	0	0	0	0	
2014	Pf	249.2	88.83	0	0	
	Pv-247	0	0	0	0	
	Pv-210	0	0	0	0	
	Mixed	0	0	0	0	

EIR, entomological inoculation rate; Pf, Plasmodium falciparum; Pv-247, Plasmodium vivax 247; Pv-2010, Plasmodium vivax 2010.

Table 4.
Estimated the effect of IRS on EIR of A. arabiensis based on CDC light trap and pyrethrum spray sheet collection from sprayed and non-sprayed villages in Bahir Dar Zuria District, North West Ethiopia, in 2013 and 2014.

4. Discussion

The aim of vector control using IRS and LLIN interventions is to reduce vectors' abundance, survival, contact with human, and feeding frequency [16]. Vector abundance is an important determinant of malaria transmission [13, 14], and thus factors that increase or decrease vector abundance could have an impact on the intensity of disease transmission. The present study demonstrated that IRS implementation brought about 4–9% reduction in the abundance of *A. arabiensis* signifying that the abundance of this vector could not be reduced to non-detectable level by the implementation of IRS. Previous similar studies in Ethiopia are either missing or unavailable to compare and contrast with the present study. However, studies from Zambia [17] validated that the effect of IRS on the density of *A. arabiensis* was not as strong as on *A. gambiae* s.s and *A. funestus* due to its exophilic and wide-ranging feeding behavior. Alegana et al. [18] also confirmed that IRS intervention reduced the density of *A. funestus* and *A. gambiae* s.l disproportionally, twice as high on *A. funestus* compared with *A. gambiae* s.l. Thus, malaria transmission through the bites of *A. arabiensis* could not be intercepted entirely by the application of IRS so that the impact of IRS should be complemented by and integrated with other vector control interventions. Blood meal source analyses indicated that *A. arabiensis* was found to have strong preferences to bovine and other hosts over human hosts. Similar results from other parts of the country were published in previous studies [19–21] where *A. arabiensis* demonstrated strong blood meal preferences of bovine over human hosts. Similar results were also reported from neighboring Eritrea [22] and Kenya [23]. Contrary to zoophilic, strong athrophilic tendency was observed in *A. arabiensis* in Zambia [24–26]. The potential reason for the differences observed in the anthrophilic tendency of *A. arabiensis* between East and South African countries would be justified by the differences in their ecological setups and the impact of these ecological differences on the ecology and behavior of *A. arabiensis* populations in these two sub-African regions. The application of IRS in the present study further reduced the anthropophily of the vector signifying that zooprophylaxis could be considered as a potential malaria vector control strategy in areas having similar ecological setups with the present study site. On the contrary, a considerable proportion of *A. arabiensis* still fed on human hosts suggesting that zooprophylaxis alone

cannot intercept malaria transmission. Thus, zooprophylaxis would advance the effectiveness of malaria interventions if used in an integrated way with other vector control intervention measures.

Either data are unavailable or no previous attempts were made about the impact of IRS on SR in Ethiopia. However, studies from other African countries [27, 28] demonstrated that the implementation of IRS reduced SR to non-detectable level, which is consistent with the results of the present study. And these would substantiate the contribution of IRS implementation in reducing malaria transmission risks in general and SR in particular in the present study area and others having similar ecological setups.

In the present study, *P. falciparum* was more prevalent than *P. viva* in *A. arabiensis.* No *A. arabiensis* was found positive for either *P. falciparum* or *P. vivax* in sprayed villages after IRS. Although too few *A. arabiensis* were recorded in sprayed villages after IRS, it would have been necessary to process thousands of mosquitoes to find any of them were infected by malaria parasites. There was no any mixed infection detected. The proportion of plasmodium-infected *A. arabiensis* was also low in non-sprayed villages indicating that SR might be low in naturally occurring vector population. Contradictory results about the prevalence of *P. falciparum* and *P. vivax* in *A. arabiensis* have been reported from different parts of Ethiopia at different times. Massebo et al. [21] reported the dominance of *P. falciparum* over *P. vivax*, while [6] reported the dominance of *P. vivax* over *P. falciparum* in South West Ethiopia. Animute et al. [29] reported the dominance of *P. vivax* over *P. falciparum* in South Central Ethiopia. Differing from all these, [30] reported that no *A. arabiensis* was found positive either for *P. falciparum* or *P. vivax* in South West Ethiopia. Except Taye and his colleagues [30], other investigators used either CDC or PSC mosquito sampling method so that the differences observed in the prevalence of malaria parasites in *A. arabiensis* could be potentially justified by the differences in ecological setups of the study sites and time period in which the study was conducted. Otherwise, this would be a question of validation.

Malaria transmission intensity, which is normally expressed by EIR, is highly variable with annual EIRs ranging from < 1 to >1000 infective bites per person per year in Africa [31]. Variations in EIR in malaria vectors could be due to different factors such as ecological heterogeneity at continental, regional, and country level [29, 32, 33] and season (dry or wet) [29, 34, 35]. For example, the burden of malaria is high in tropical countries having warm temperature, heavy rainfall, high humidity, and efficient *Anopheles* vectors than nontropical countries [36]. Previous studies indicated that the impact of wet or dry season on EIR is inconsistent, i.e., published reports indicated that EIR is higher during wet season [15, 35, 37] or vice versa [38, 39].

In the present study, a very high *Pf*-EIR was observed in the vector in both years and study villages although SR and HBI were low. The trend was also similar for *Pv*-247 EIR in both study villages before IRS in 2013. These findings would be justified by the occurrences of high mosquito density during the study periods. The level of EIR of both parasites went to zero in sprayed villages after the implementation of IRS suggesting that IRS application is 100% effective to control disease transmission. In contrast, previous studies reported that EIR was 90% lower in the ITN community and 93% lower in the IRS community, relative to the community without intervention. The differences observed between the present and previous studies would be attributed to heterogeneity in the ecology and behavior of the vector.

Variation in EIR could also differ by mosquito collection methods [40]. [41] indicated that PSC might underestimate the HBR, which again underrates EIR. Previous studies also reported CDC light traps were more efficient than PSC to estimate EIR [21, 42–44]. Contrary to these, a study from Bioko Island, Equatorial Guinea, demonstrated that CDC light traps failed to determine the human-biting

rate of the anthropogenic *A. gambiae* s.s [45]. Different from all previous reports, the present study indicated that higher EIRs were recorded from PSC catches than CDC light trap catches. Both CDC and PSC are reported to have shortcomings in mosquito sampling. While CDC light traps attract fed indoor-resting mosquitoes [3, 46], PSC tends to miss mosquitoes that leave the house after feeding including those entering the house after feeding outdoor [47]. Therefore, estimating the HBR using either CDC light trap or PSC has limitations, and the need to develop standard HBR remains high. Thus, the differences observed between the present and previous studies might be associated with limitation stated for each sampling method.

5. Conclusion

This study was linked with IRS application to assess its effect on EIR and other entomological risk factors for malaria transmission. The results illustrated that IRS was strong enough to reduce mosquito abundance, sporozoite rate, and EIR in areas having similar ecological setup with the present study villages [48].

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References

[1] Bruce-Chwatt LJ et al. Chemotherapy of Malaria. Revised. 2nd ed. Geneva: World Health Organization; 1986

[2] MacDonald G. The Epidemiology and Control of Malaria. London: Oxford University Press; 1957

[3] Lines JD, Curtis CF, Wilkes TJ, Njunwa KJ. Monitoring human-biting mosquitoes (Diptera: Culicidae) in Tanzania with light-traps hung beside mosquito nets. Bulletin of Entomological Research. 1991;**81**:77-84

[4] World Health Organization. Manual on Practical Entomology in Malaria. Part 2. Methods and Techniques. Geneva: World Health Organization; 1975

[5] Abose T, Yeebiyo Y, Olana D, Alamirew D, Beyene Y, Regassa L, et al. Re-orientation and Definition of the Role of Malaria Vector Control in Ethiopia. Geneva: World Health Organization; 1998

[6] Taye A, Hadis M, Adugna N, Tilahun D, Wirtz RA. Biting behaviour and Plasmodium infection rates of *Anopheles arabiensis* from Sille, Ethiopia. Acta Tropica. 2006;**97**:50-54

[7] FDREOH. National Malaria Guidelines. 3rd ed. Addis Ababa, Ethiopia: The Federal Democratic Republic of Ethiopia, Ministry of Health; 2012

[8] Cullen JR, De Zulueta J. Observations on the effect of residual insecticides in experimental huts in Masaka District, Uganda. Bulletin of the World Health Organization. 1964;**30**:263-278

[9] Abate A, Hadis H. Susceptibility of *Anopheles gambiae* s.l to DDT, malathion, permethrin and deltamethrin in Ethiopia. Tropical Medicine and International Health. 2011;**16**:486-491

[10] Beier JC, Killeen GF, Githure JI. Short report: Entomologic inoculation rates and *Plasmodium falciparum* malaria prevalence in Africa. The American Journal of Tropical Medicine and Hygiene. 1999;**61**(1):109-113

[11] WHO. World Malaria Report. 2011. Available from: http//www.who.int/entity/malaria/world_malaria_report_2011/9789244564403_eng.pdf

[12] World Health Organization. Malaria Entomology and Vector Control. Geneva: World Health Organization; 2003

[13] Garrett-Jones C. Prognosis for interruption of malaria transmission through the assessment of mosquito vectorial capacity. Nature. 1964a;**204**:1173

[14] Garrett-Jones C. The human blood index of malaria vectors in relation to epidemiological assessment. Bulletin of the World Health Organization. 1964b;**30**:241-261

[15] Drakeley C, Schellenberg D, Kihondaj J, Sousa CA, Arez AP, Lopes D, et al. An estimation of the entomological inoculation rate for Ifakara: A semi-urban area in a region of intense malaria transmission. Tropical Medicine & International Health. 2003;**8**:767-774

[16] Wirtz RA, Zavala F, Charoenvit Y, Cambell GH, Burkot TR, Shneider I, et al. Comparative testing of *Plasmodium falciparum* sporozoite monoclonal antibodies for ELISA development. Bulletin of the World Health Organization. 1987;**65**:39-45

[17] Chanda E, Phiri FN, Chanda J, Ramdeen VR, Kamuliwo M, Baboo KS. Impact of entomological interventions on malaria vector bionomics in low transmission settings in Zambia. Journal of Public Health

and Epidemiology. 2012;**4**(7):89-196. DOI: 10.5897/JPHE12.038. Available from: http://www.academicjournals.org/JPHE

[18] Alegana VA, Kigozi SP, Nankabiraw J, et al. Spatio-temporal analysis of malaria vector density from baseline through intervention in a high transmission setting. Parasites & Vectors. 2016;**9**:637. DOI: 10.1186/s13071-016-1917-3

[19] Habtewold T, Walker AR, Curtis CF, Osir EO, Thapa N. The feeding behaviour and *Plasmodium* infection of Anopheles mosquitoes in southern Ethiopia in relation to use of insecticide-treated livestock for malaria control. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2001;**95**:584-586

[20] Hadis M, Lulu M, Makonnen Y, Asfaw T. Host choice by indoor resting *Anopheles arabiensis* in Ethiopia. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1997;**91**:376-378

[21] Massebo F, Balkew M, Gebre-MichaelT,LindtjørnB.Entomologic inoculation rates of *Anopheles arabiensis* in Southwestern Ethiopia. The American Journal of Tropical Medicine and Hygiene. 2013;**89**(3):466-473. DOI: 10.4269/ajtmh.12-0745

[22] Waka M, Hopkins RJ, Akinpelu O, Curtis C. Transmission of malaria in the Tesseney area of Eritrea: Parasite prevalence in children, and vector density, host preferences, and sporozoite rate. Journal of Vector Ecology. 2005;**30**:27-31

[23] Collins FH, Mendez MA, RasmussenMO,MehaffeyPC,BesanskyNJ, Finnerty V. A ribosomal RNA gene probe differentiates member species of the *Anopheles gambiae* complex. The American Journal of Tropical Medicine and Hygiene. 1987;**37**:37-41

[24] Fornadel CM, Norris DE. Increased endophily by the malaria vector *Anopheles arabiensis* in southern Zambia and identification of digested blood meals. The American Journal of Tropical Medicine and Hygiene. 2008;**79**:876-880

[25] Mzilahowa T, Hastings JM, Molyne ME, McCall PJ. Entomological indices of malaria transmission in Chikhwawa district, Southern Malawi. Malaria Journal. 2012;**11**:380. http://www.malariajournal.com/content/11/1/380; Accessed in December 2017

[26] Thompson R, Begtrup K, Cuamba N, Dgedge M, Mendis C, Gamage-Mendis A, et al. The Matola malaria project: A temporal and spatial study of malaria transmission and disease in a suburban area of Maputo, Mozambique. American Journal of Tropical Medicine and Hygiene. 1997;**57**:550-559

[27] Coleman S, Dadzie SK, Seyoum A, et al. A reduction in malaria transmission intensity in Northern Ghana after 7 years of indoor residual spraying A reduction in malaria transmission intensity in Northern Ghana after 7 years of indoor residual spraying. Malaria Journal. 2017;**16**:324. DOI: 10.1186/s12936-017-1971-0

[28] Ossè RA, Aïkpon R, Gbédjissi GL, et al. A shift from indoor residual spraying (IRS) with bendiocarb to long-lasting insecticidal (mosquito) nets (LLINs) associated with changes in malaria transmission indicators in pyrethroid resistance areas in Benin. Parasites & Vectors. 2013;**6**(73). Available from: http://www.parasitesandvectors.com/content/6/1/73

[29] Animute A, Balkew M, Gebermichale T, Lindtjørn B. Blood meal sources and entomological inoculation rates of anophelines along a highland altitudinal transect in south-central Ethiopia. Malaria

Journal. 2013;**12**(76). Available from: http://www.malariajournal.com/content/12/1/76

[30] Taye B, Lelisa K, Emana D, Asale A, Yewhalaw D. Seasonal dynamics, longevity, and biting activity of Anopheline mosquitoes in Southwestern Ethiopia. Journal of Insect Science. 2016;**16**(1):1-7

[31] Beier JC, Perkins PV, Wirtz RA, Koros J, Dlggs D, Gargan TP, et al. Blood meal identification by direct enzyme-linked immunosorbent assay (ELISA), tested on *Anopheles* (Diptera: Culicidae) in Kenya. Journal of Medical Entomology. 1988;**25**:9-16

[32] Hay SI, Rogers DJ, Toomer JF, Snow RW. Annual *Plasmodium falciparum* entomological inoculation rates (EIR) across Africa: Literature survey, internet access and review. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2000;**94**(2):113-127

[33] Ndenga B, Githeko A, Omukunda E, Munyekenye G, Atieli H, Wamai P, et al. Population dynamics of malaria vectors in Western Kenya highlands. Journal of Medical Entomology. 2006;**43**(2):200-206

[34] Mabaso MIH, Craig M, Ross A, Smith T. Environmental predictors of the seasonality of malaria transmission in Africa: The challenge. The American Journal of Tropical Medicine and Hygiene. 2007;**76**(1):33-38

[35] Shililu JI, Maier WA, Seitz HM, Orago AS. Seasonal density, sporozoite rates, and entomological inoculation rates of *Anopheles gambiae* and *Anopheles funestus* in high-altitude sugarcane growing zone in Western Kenya. Tropical Medicine & International Health. 1998;**3**:706-710

[36] Breman JG. The ears of the hippopotamus: manifestations, determinants, and estimates of the malaria burden. The American Journal of Tropical Medicine and Hygiene. 2001;**64**(1, 2S):1-11

[37] Kent RJ, Thuma PE, Mharakurwa S, Norris DE. Seasonality, blood feeding behavior, and transmission of *Plasmodium falciparum* by *Anopheles arabiensis* after an extended drought in southern Zambia. The American Journal of Tropical Medicine and Hygiene. 2007;**76**:267-274

[38] Himeidan Y, Elzaki MM, Kuleka EJ, Ibrahim M, Elhasson IM. Pattern of malaria transmission along the Rahad River basin, eastern Sudan. Parasites & Vectors. 2011;**4**:109

[39] Shililu JI, Ghebremeskel T, Mengistu S, Fekadu H, Zerom M, Mbogo C, et al. High seasonal variations in entomologic inoculation rate in Eritrea, a semi-arid region of unstable malaria in Africa. American Journal of Tropical Medicine and Hygiene. 2003;**69**:607-613

[40] Shaukat A, Breman JG, McKenzie FE. Using entomological inoculation rate to assess the impact of vector control on malaria parasite transmission and elimination. Malaria Journal. 2010;**9**:122

[41] Krafsur E. The bionomics and relative prevalence of *Anopheles* species with respect to the transmission of Plasmodium to man in western Ethiopia. Journal of Medical Entomology. 1977;**25**:180-194

[42] Fornadel CM, Norris LC, Glass GE, Norris DE. Analysis of *Anopheles arabiensis* blood feeding behavior in southern Zambia during the two years after introduction of insecticide-treated bed nets. The American Journal of Tropical Medicine and Hygiene. 2010;**83**:848-853

[43] Fornadel C, Norris LC, Norris DE. Centers for disease control light traps

for monitoring *Anopheles arabiensis* human biting rates in an area with low vector density and high insecticide treated bed net use. The American Journal of Tropical Medicine and Hygiene. 2010;**83**:838-842

[44] Ndiath M, Mazenot C, Gaye A, Konate L, Bouganali C, Faye O, et al. Methods to collect Anopheles mosquitoes and evaluate malaria transmission: A comparative study in two villages in Senegal. Malaria Journal. 2011;**10**(270):28

[45] Overgaard H, Sæbø S, Reddy MR, Reddy VP, Abaga S, Matias A, et al. Light traps fail to estimate reliable malaria mosquito biting rates on Bioko Island, Equatorial Guinea. Malaria Journal. 2012;**11**:56

[46] Petrarca V, Beier JC, Onyango F, Koros J, Asiago C, Koech DK, et al. Species composition of the *Anopheles gambiae* complex (Diptera: Culicidae) at two sites in western Kenya. Journal of Medical Entomology. 1991;**28**:307-313

[47] Mboera LE. Sampling techniques for adult Afrotropical malaria vectors and their reliability in the estimation of entomological inoculation rate. Tanzania Health Research Bulletin. 2005;**7**:117-124

[48] Massebo F, Balkew M, Gebermichael T, Lindtjørn B. Zoophagic behaviour of anopheline mosquitoes in southwest Ethiopia: Opportunity for malaria vector control. Parasites & Vectors. 2015;**8**:845. DOI: 10.1186/s13071-015-1264-9