

## indoor residual spraying in regions bordering Lake Victoria, Tanzania

Charles Kakilla<sup>1\*</sup>, Alphaxard Manjurano<sup>1\*</sup>, Karen Nelwin<sup>1</sup>, Jackline Martin<sup>1</sup>, Fabian Mashauri<sup>1</sup>, Safari M. Kinung'hi<sup>1</sup>, Eric Lyimo<sup>1</sup>, Doris Mangalu<sup>1</sup>, Lucy Bernard<sup>1</sup>, Nduka Iwuchukwu<sup>2</sup>, Dismasi Mwalimu<sup>3</sup>, Naomi Serbantez<sup>4</sup>, George Greer<sup>4</sup>, Kristen George<sup>5</sup>, Richard M Oxborough<sup>6</sup>, and Stephen M. Magesa<sup>2</sup>

1. National Institute for Medical Research, Mwanza, Tanzania.
2. PMI-AIRS Tanzania Project, Abt Associates, Mwanza, Tanzania.
3. National Malaria Control Program, Ministry of Health, Community Development, Gender, Elderly and Children, Dodoma, Tanzania
4. U.S. President's Malaria Initiative, Dar es Salaam, Tanzania
5. U.S. President's Malaria Initiative, U.S. Agency for International Development, Washington, DC, USA
6. PMI AIRS/VectorLink Project, Abt Associates, 6130 Executive Blvd, Rockville, MD 20852, USA

\*Correspondence: [kakillacharles@gmail.com](mailto:kakillacharles@gmail.com); [amanjurano@yahoo.co.uk](mailto:amanjurano@yahoo.co.uk)

## Abstract

### Background

Vector control through long lasting insecticidal nets and focal indoor residual spraying (IRS) is a major component of the Tanzania national malaria control strategy. In mainland Tanzania, IRS has been conducted annually around Lake Victoria basin since 2007. Due to pyrethroid resistance in malaria vectors, use of pyrethroids for IRS was phased out and from 2014 to 2017 pirimiphos-methyl (Actellic 300CS) was sprayed in regions of Kagera, Geita, Mwanza and Mara.

### Methods

WHO Cone bioassays were conducted monthly on interior house walls to determine residual efficacy of pirimiphos-methyl CS. Indoor CDC light traps with or without bottle rotator were hung next to protected sleepers indoors and also set outdoors (un-baited) as a proxy measure for indoor and outdoor biting rate and time of biting. A sub-sample of *Anopheles* were tested by PCR to determine species identity and ELISA for sporozoite rate.

### Results

Annual IRS with Actellic® CS between 2015 and 2017 was effective on sprayed walls for a mean of 7 months in cone bioassay. PCR of 2016 and 2017 samples showed vector populations were predominantly *An. arabiensis* (58.1%, n=4,403 IRS sites, 58%, n=2,441 unsprayed sites). There was a greater proportion of *An. funestus* s.s. in unsprayed sites (20.4%, n=858) than sprayed sites (7.9%, n=595) and fewer *An. parensis* (2%, n=85 unsprayed, 7.8%, n=591 sprayed). Biting peaks of *An. gambiae* s.l. followed periods of rainfall occurring between October and April, but were generally lower in sprayed sites than unsprayed. In most sprayed sites, *An. gambiae* s.l. indoor densities increased between January and February, i.e. 10-12 months after IRS. Based on these data and malaria case data, the timing of IRS was changed to November in Kagera and Geita Regions in 2018. The predominant species *An. arabiensis* had a sporozoite rate in 2017 of 2.0% (95% CI: 1.4-2.9) in unsprayed sites compared to 0.8% (95% CI: 0.5-1.3) in sprayed sites (p=0.003). Sporozoite rates also appeared to be lower for *An. funestus* collected in sprayed sites.

### Conclusion

IRS appeared to have substantial impact on malaria transmission, with sporozoite rate in *An. arabiensis* being 59% lower in sprayed sites than in unsprayed sites in 2017.

### Keywords

Malaria vectors, indoor residual spraying, pirimiphos-methyl, species composition, *Anopheles gambiae*, *Anopheles arabiensis*, seasonality, Tanzania.

## Background

In sub-Saharan Africa, recent gains in malaria control have been mostly accomplished through a substantial boost in vector control using long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). These tools have significantly contributed to a 50% reduction of *Plasmodium falciparum* infection prevalence in endemic countries between 2000 and 2015(1). IRS has been reported to successfully reduce malaria prevalence and incidence in several African countries in the past decade(2–4). In mainland Tanzania, IRS implementation funded by the United States (U.S.) President’s Malaria Initiative (PMI) was launched in 2007 in Muleba and Karagwe districts, located in Kagera Region. The initial locations were supported in response to a malaria epidemic in 2006(5). Thereafter, IRS activities with pyrethroid insecticides were progressively expanded to other districts in the Lake Victoria basin including the remaining five districts of Kagera Region in 2009; and in 2010 and 2011 to all 18 districts of Kagera, Mwanza, and Mara, covering 1.1 million structures and protecting nearly 6.3 million people(6).

Previous entomological monitoring in the Lake Victoria basin in 2015 indicated that(7) the malaria vector species composition varied by district with the predominant species being *An. arabiensis* in Mara Region, Muleba and Ngara districts and *An. gambiae* s.s in Magu and Geita districts. Pyrethroid resistance was documented by Kisinza *et al* (2017) in all districts that were tested in 2015 near Lake Victoria, including Musoma Rural, Magu, and Muleba. Due to the detection of pyrethroid resistance in malaria vectors, the use of pyrethroids for IRS was gradually phased out in accordance with WHO guidance that pyrethroids should be preserved for LLINs(8). The carbamate insecticide, bendiocarb (Ficam®, 80% WP) was used alongside the pyrethroid deltamethrin K-Othrine® (WG 250) from 2011 to 2013(5). From 2014 to 2017, a long-acting organophosphate formulation of pirimiphos-methyl (Actellic® 300CS) was sprayed annually in all targeted areas of the Lake Victoria basin in the regions of Kagera, Geita, Mwanza and Mara.

Despite widespread pyrethroid resistance being detected in malaria vectors throughout Tanzania(9,10), IRS in combination with pyrethroid LLINs has proven effective in mainland Tanzania(5,11) and also in Zanzibar(12). Partly due to vector control, reported malaria deaths in mainland Tanzania reduced by ~32%, from 15,819 in 2010 to 5,045 in 2016(13).

We report results of entomological surveillance covering ten sprayed sites and four unsprayed control sites in the Lake Victoria Basin. The main objective was to evaluate the entomological impact of IRS with pirimiphos-methyl CS against malaria vectors. Specifically, entomological data was collected to assess the persistence of residual efficacy of pirimiphos-methyl CS on sprayed walls, determine vector species composition, seasonality, feeding behaviour and malaria infectivity.

## Methods

### Study area and duration

Entomological surveillance was conducted in regions around Lake Victoria, north-western Tanzania. For three years between 2015 and 2017, between eight and ten districts of the Lake Victoria basin were sprayed annually with pirimiphos-methyl CS and entomological monitoring was conducted in sprayed and unsprayed sites. A list of districts and annual spray status is presented in Table 1 and Figure 1.

**Table 1: Annual spray status of districts around the Lake Victoria basin between 2015 and 2017.**

Region	District	2015	2016	2017
Kagera	Ngara	Sprayed 37,240 (98.7%)	Sprayed 52,885 (97.6%)	Sprayed 61,422 (97.3%)
	Biharamulo*	Sprayed 42,767 (93.3%)	Not sprayed	Not sprayed
	Muleba	Sprayed 81,294 (98.6%)	Not sprayed	Not sprayed
	Chato	Sprayed 53,899 (92.5%)	Sprayed 73,249 (95.8%)	Sprayed 83,163 (90.7%)
	Missenyi	Not sprayed	Sprayed 44,111 (97.3%)	Sprayed 49,494 (97.3%)
	Bukoba Rural	Not sprayed	Sprayed 63,346 (99.4%)	Sprayed 69,083 (98.5%)
Mwanza	Magu	Sprayed 58,234 (91.8%)	Not sprayed	Not sprayed
	Misungwi	Sprayed 47,638 (92.4%)	Not sprayed	Not sprayed
	Sengerema	Not sprayed	Sprayed 97,012 (92.3%)	Sprayed 122,476 (94.6%)
	Kwimba	Not sprayed	Sprayed 71,733 (90.3%)	Sprayed 90,634 (95.9%)
Simiyu	Busega**	Not sprayed	Not sprayed	Not sprayed
Mara	Rorya	Sprayed	Not sprayed	Not sprayed

		77,228 (91.6%)		
	Musoma Rural	Not sprayed	Sprayed 35,151 (95.8%)	Sprayed 40,981 (93.4%)
	Butiama	Not sprayed	Sprayed 50,066 (94.9%)	Sprayed 58,386 (94.3%)
	Tarime*	Not sprayed	Not sprayed	Not sprayed
Geita	Geita Town Council	Sprayed 21,363 (96.6%)	Sprayed (approx. 20,000 by Geita Gold Mine)	Sprayed (approx. 20,000 by Geita Gold Mine)
	Nyang'hwale	Not sprayed	Not sprayed	Sprayed 50,099 (95.5%)
	Bukombe**	Not sprayed	Not sprayed	Not sprayed
Mainland Total		419,753(94.2%)	487,553(94.9%)	625,738(95.1%)

\* Control for 2017 entomological survey

\*\* Control for 2016, 2017 entomological survey

### Residual efficacy of Actellic 300CS (pirimiphos-methyl)

Cone bioassays were conducted on interior wall surfaces according to WHO protocols to determine the quality of spray within 14 days of application and the duration of residual efficacy, which was monitored monthly until mortality was lower than 80% for two consecutive months(14). Batches of two to five days-old non-blood-fed female *Anopheles gambiae* s.s. (Kisumu strain) were tested by exposing them to sprayed surfaces under WHO plastic cones for 30 minutes, after which they were transferred to clean paper cups and kept in a field insectary for recording delayed mortality. *An. gambiae* Kisumu strain were known to be fully susceptible to pirimiphos-methyl and were reared in the NIMR Mwanza insectary at  $27 \pm 1^\circ\text{C}$ , and 60-80% relative humidity before being transported to the field in cool boxes for the assays. Knock-down and mortality were recorded 60 minutes post-exposure and after 24-hours holding. Portable untreated surfaces (approximately 30cm by 30cm) were constructed of cement, mud, burnt brick, whitewash and painted substrates and used as negative controls. Cone tests on untreated portable surfaces were conducted outdoors (to avoid the airborne effect of Actellic 300CS indoors) in a shaded area in parallel for each sprayed house. When mortality in negative controls was between 5% and 20%, the results were corrected using Abbot's formula, and those above 20% were discarded and the tests repeated(15). A summary of cone bioassay tests conducted is shown in Table 2 below.

**Table 2: Overview of monthly cone bioassay in sprayed houses to determine residual efficacy.** (a) Five surface types of wall tested were mud, cement, painted, white wash and burnt brick. (b) There were at least 2 houses per surface type. (c) In 2015, 3 cones were placed on treated wall surfaces (1.5m 1m, 0.5m); while in 2016 and 2017 only 2 cones were placed at 2m and 1m height from the floor respectively.

Year	No. of districts	No. of wall types tested <sup>(a)</sup>	No. of houses tested p/month <sup>(b)</sup>	Cone replicates p/month <sup>(c)</sup>	No. of <i>An. gambiae</i> Kisumu tested p/month
2015	7	5	70	210	2,100
2016	8	5	80	160	1,600
2017	10	5	100	200	2,000

### Mosquito sampling and rainfall data

We used three entomological sampling methods, indoor CDC light traps(16), indoor and outdoor CDC light trap fitted with bottle rotator (CBR)(17) and indoor Prokopack aspirators(18). The number of sites, houses used for trapping, duration of sampling and outcomes are presented in Table 3. Rainfall data during the period of monthly entomological monitoring were accessed from an online database system(19).

**Table 3: Mosquito sampling methods, number of sites sampled, frequency of trapping and outcomes.**

Method	Sites	Number of houses/traps	Frequency & year	Outcomes
CDC light trap	10 IRS sites + 4 control sites	2 houses per site per night; 1 light trap per house per night	28 nights per month 2016-17	<ul style="list-style-type: none"> <li>Species composition and indoor vector abundance</li> </ul>
CDC Light trap fitted with bottle rotator (CBR)	4 IRS sites + 4 control sites	10 houses per site per month; 2 CBRs per house per night (one indoors and one outdoors)	10 nights per month 2017	<ul style="list-style-type: none"> <li>Species composition</li> <li>Biting pattern / activity</li> <li>Blood meal analysis</li> <li>Parity rate</li> </ul>
Prokopack aspirator	4 IRS sites + 4 controls	10 houses per site per month.	20 days per month. 2017	<ul style="list-style-type: none"> <li>Species composition</li> <li>Indoor resting density</li> </ul>

### Indoor CDC light traps (2016-17)

In 2016 and 2017, two houses per night were selected for CDC light traps on 28 consecutive nights each month (for a total of 56 trap nights per month per site). The same houses were

used for sampling per site every month. House selection was based on a random pick of houses near the residence of community mosquito collectors. In selected houses, CDC light traps were installed indoors, *circa* 1.5m above the floor next to the head of the sleeping person(20). The person(s) was requested to sleep under an intact untreated mosquito net(s) provided by the project. CDC light traps were set to operate from 18:00 to 06:00. In the morning, captured mosquitoes were transferred into labeled paper cups and taken for preliminary morphological identification in the field office. All mosquitoes from traps were killed before conducting morphological identification and recording results according to species, sex and abdominal status.

### **CDC light trap with collection bottle rotator (CBR) (2017)**

One CDC light trap with automatic collection bottle rotator (CBR – John Hock model 1512) was set indoors and one outdoors at ten randomly selected houses per site for 10 nights per month. CBR traps were set from March to December (10 months) in 2017 and sampling was scheduled on nights near a new moon to minimize the effect of moonlight on the outdoor light-trap collection, and to reduce bias when comparing species distribution across seasons. An estimate of the presence and period of moonlight was calculated using an online lunar calendar(21). Indoor CBRs were set up in sleeping areas of houses, while outdoor CBRs were set up within a 10-meter radius of the house. Ethical concerns restrict use of human landing collection (HLC) for mosquito collection. Therefore, the CBR trapping was considered a proxy for human landing collection targeting host seeking vector mosquitoes. Indoor and outdoor human-biting rate of *Anopheles* and time of biting were determined in the selected sentinel sites. All mosquitoes from traps were killed before conducting morphological identification and recording results according to species, sex and abdominal status.

### **Indoor Prokopack aspirator (2017)**

The improved Prokopack aspirator (John Hock model 1419) was used for sampling indoor resting mosquitoes from 10 houses per day over 20 days within each selected sentinel site per month in 2017(18,22). Aspiration was carried out in the morning between 06:00 and 08:00am and was conducted in all rooms in the house, moving the aspirator across walls, ceiling and near furniture. To standardize the collection, the sampling was conducted for a total of 30 minutes per house, by two assistants working simultaneously in the same house for fifteen minutes each.

## **Laboratory analysis**

All collected samples were identified to species morphologically using the systematic key of Gillies and Coetzee (1987)(23). Female Anopheline mosquitoes collected in 2016 and 2017 were subsequently analyzed for presence of *P. falciparum* sporozoites by enzyme-linked immunosorbent assay (ELISA) technique according to the protocol of Burkot *et al.* (1984)(24) and slightly modified by Wirtz *et al.* (1987)(25). Polymerase chain reaction (PCR) was conducted to identify members of the *An. gambiae* s.l. complex and *An. funestus* s.l. group according to the protocols of Scott *et al.* (1993) and Wilkins *et al.* (2006), respectively(26,27). *Anopheles* collected from Prokopack aspirators, CDC light traps and CBR were tested for blood meal source, using the ELISA protocol described by Beier *et al.* (1988)(28).

## **Data analysis**

Indoor vector resting density was calculated as the total number of female *Anopheles* collected (by species), divided by the total number of rooms surveyed by Prokopack aspirator. The human biting rate was calculated as the total number of mosquitoes collected by CDC light trap, divided by the number of trap nights. Sporozoite rate was estimated as the proportion of female *Anopheles* found positive for the presence of circum-sporozoite proteins. Sporozoite rates in unsprayed and sprayed sites were compared by *t*-test. All statistical tests and 95% confidence intervals were calculated using R version 3.4.4.

## **Results**

### **Residual efficacy of Actellic 300CS (pirimiphos-methyl), 2015-17**

Overall results shortly after spraying showed that the quality of spraying in 2015, 2016 and 2017 was satisfactory and relatively homogeneous. All tests conducted <2 weeks after spray application resulted in mortality of 100%, with the exception of a lowest mortality recorded at 90.8% from one house in 2016. Monthly cone bioassay indicated a mean residual duration of seven months post-spraying (mortality >80% WHO defined mortality threshold), with a decrease in mortality to approximately 50-70% recorded 9 months post-spraying (Figure 2). Trends were similar for all wall substrates.



## Vector seasonality

### Indoor density of *An. gambiae* s.l. and *An. funestus* s.l. by CDC light trap (2016-17)

Figure 3 presents the mean nightly indoor catch of *An. gambiae* s.l. and *An. funestus* s.l. from indoor CDC light trap collections conducted monthly for 2 years from January 2016 to December 2017. *An. gambiae* s.l. was the predominant vector species in all sites throughout the sampling period over the two years period 2016 – 2017. *An. funestus* s.l. indoor densities were very low in most sites, with relatively high indoor densities only recorded in Chato (June-October) and Butiama (May-June). Density peaks were generally observed following periods of significant rainfall occurring between October and April (Figure 3).

Following IRS in February/March, indoor densities were generally low in sprayed sites, at <3 *An. gambiae* s.l. per trap/night between March and December (1-10 months after spraying). In the sprayed sites of Ngara (Kagera Region), Geita (Geita Region) and Kwimba (Mwanza Region), densities were particularly low year-round, never exceeding 1 per trap/night. However, in Missenyi (Kagera Region) indoor *An. gambiae* s.l. densities were particularly high between April and August at 4-8 per trap/night, despite IRS in February. While in Butiama a smaller indoor peak of *An. funestus* s.l. was reached in June at 3 per trap/night, 3 months after IRS.

In many sprayed sentinel sites, including Chato (Kagera Region), Sengerema (Mwanza Region), Musoma Rural and Butiama (Mara Region) relatively high *An. gambiae* s.l. indoor densities were recorded between January and February, which is 10-12 months after the previous IRS cycle, by which time insecticide efficacy had decreased substantially.

### **Biting rate for *Anopheles gambiae* s.l. using CDC light trap fitted with bottle rotator (CBR)**

In 2017, CBR traps were set from March to December. Data was combined for 4 sprayed sites (Sengerema, Musoma Rural, Chato and Bukoba Rural), and 4 unsprayed sites (Busega, Bukombe, Tarime and Biharamulo) to compare the mean biting rate indoors and outdoors. The total catch size per site using CBR (indoors and outdoors) over 200 trap nights per site indoors and outdoors (10 trap nights per month both indoors and outdoors for 10 months) was 4,616 *An. gambiae* s.l. from sprayed sites and 5,260 from unsprayed sites. The total *An. gambiae* s.l. collected per sprayed site was 333 from Sengerema, 290 from Musoma Rural, 3,809 from Chato and 184 from Bukoba Rural. While for unsprayed sites the total was 83 from Tarime, 2,795 from Bukombe, 2,303 from Biharamulo, and 79 from Busega.

In sprayed sites, the *An. gambiae* s.l. biting rate was greater outdoors than indoors at all times of night. This provides some evidence that *An. gambiae* s.l. (mostly *An. arabiensis*) may be modifying their behavior to avoid contact with insecticide sprayed walls. In unsprayed sites there also appeared to be more outdoor biting, but only late at night between 10pm and 3am. However, it should be noted that the majority of *An. gambiae* s.l. in all sites were collected later in the evening when the majority of people are likely to be indoors and protected by LLINs. Nevertheless, we observed a greater degree of outdoor biting risk early in the evening in sprayed sites compared to unsprayed sites (Figure 4).

### **Indoor resting densities of *Anopheles gambiae* s.l. in 2017 using Prokopack aspirators**

The mean number of *An. gambiae* s.l. collected by Prokopack aspirator resting indoors, was greater in the 4 unsprayed sites of Biharamulo, Bukombe, Busega and Tarime than in the 4 sprayed sites of Bukoba Rural, Chato, Sengerema and Musoma Rural. In general, the highest peak in resting density was observed between May and August after the long rain season, with Chato and Busega also having a smaller peak in March (Figure 6). There were no *An. gambiae* s.l. collected throughout the 2017 collection period in the sprayed site of Musoma Rural (Figure 5).

### **Species composition**

A total of 8,957 female *Anopheles* collected from 2016 to 2017 were analyzed by PCR for species identification. Results confirmed vector populations in sprayed districts to be

predominantly *An. arabiensis* (71%, n=3,768) with smaller proportions of *An. parensis* (11.1%, n=589), *An. funestus* s.s. (11%, n=585), *An. gambiae* s.s. (6.8%, n=361), and *An. rivulorum* (0.1%, n=3).

The predominant vector species in unsprayed districts was also *An. arabiensis* (66.9%, n=2,441), however there was a higher proportion of *An. funestus* s.s. (23.5%, n=858) and fewer *An. parensis* (2.3%, n=85), and similar proportion of *An. gambiae* s.s. (7.3%, n=267) as in sprayed sites.

### Sporozoite rate

Between 2016 and 2017 the overall *Plasmodium falciparum* sporozoite rate across all sites (sprayed and unsprayed) for all *Anopheles* (*funestus*, *arabiensis*, *gambiae*, and *parensis*) combined was estimated as 1.72% (286/16,670). The overall sporozoite infection rate was higher in unsprayed sites, estimated as 2.02% (115/5,686) than in sprayed sites at 1.56% (171/10,984). Mean sporozoite rates were generally less than 2% for all sprayed sites (from 2016 to 2017), with the highest rates scored at 4.5% (Ngara, 2017) and 3.9% (Biharamulo, 2016) in areas where significant proportions of *An. funestus* and *An. gambiae* were present. See additional file 1, table S1 for 2016 and 2017 sporozoite rates presented by site.

Results from 2017 were disaggregated by species (from PCR results) and spray status (Table 4). This could not be done with data from 2016. Results by species showed that *An. funestus* s.s. carried the highest sporozoite rate estimated at 4.07% (30/738) across unsprayed and sprayed sites combined. The mean *An. funestus* s.s. sporozoite rate estimated as 4.3% (27/630) in unsprayed sites and 2.8% (3/108) in sprayed sites, although the difference was not statistically significant ( $p=0.48$ ) (Table 4), possibly due to the small sample size in sprayed sites. The predominant species, *An. arabiensis* exhibited a relatively lower overall sporozoite rate in 2017 estimated as 1.34% (45/3,366), with a higher sporozoite rate in unsprayed sites (2.0%; with 95% CI: 1.4-2.9) compared to sprayed sites (0.8% with 95% CI: 0.5-1.3) ( $p=0.003$ ). Although not commonly considered as an important malaria vector, *An. parensis* had an overall sporozoite rate of 1.1% (5/435).

**Table 4: Sporozoite rates disaggregated by vector species and spray status from 2017 sampling.**

Mosquito species	Spray status	No. of samples	Number sporozoite	Sporozoite rate % (95% CI)	P-value
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		<b>analyzed</b>	<b>positive</b>		
<i>An. gambiae</i>	Sprayed	118	3	2.5 (0.5-7.2)	0.876
<b>s.s.</b>	Unsprayed	177	5	2.8 (0.9-6.5)	
<i>An. arabiensis</i>	Sprayed	1,924	16	0.8 (0.5-1.3)	<b>0.003</b>
	Unsprayed	1,442	29	2.0 (1.3-2.9)	
<i>An. funestus</i>	Sprayed	108	3	2.8 (0.6-7.9)	0.480
<b>s.s.</b>	Unsprayed	630	27	4.3 (2.8-6.2)	
<i>An. parensis</i>	Sprayed	362	4	1.1 (0.3-2.8)	0.840
	Unsprayed	73	1	1.4 (0.03-7.4)	

### Blood meal analysis

A total of 194 *Anopheles arabiensis* (identified by PCR) that were collected from January to September 2017 by indoor resting collections were tested for vertebrate host blood source (human, bovine, goat and dog) with 109 from sprayed sites (Sengerema, Kwimba, Bukoba rural and Missenyi) and 85 from unsprayed sites (Bukombe and Busega). Overall, the proportion of *An. arabiensis* that fed on humans (including mixed blood meals on both human and animal) was 59.3% (115/194), with cattle blood being the most common non-human source. An estimated 32.5% (63/194) of *An. arabiensis* fed on both human and animals, demonstrating opportunistic feeding behavior, while only 26.8% fed only on humans (Additional file 1, Table S2).

### Discussion

Cone bioassay results following indoor residual spraying (IRS) with Actellic CS show that it provided control of malaria vectors in north-western Tanzania for a mean of 7 months. Seven months residual duration lies in the higher end of performance for this insecticide formulation, considering a range of 2-9 months that was observed in 9 other PMI supported countries(29). Although *An. arabiensis* in the Lake zone of north-western Tanzania are resistant to pyrethroids, with high intensity resistance present observed in some sites, they were susceptible to pirimiphos-methyl during the study(7).

IRS campaigns were usually conducted in February and March, meaning that protection was provided through the year up to October/November. However, rainfall in north-western Tanzania is bi-modal, with a second peak of *An. gambiae* s.l. occurring in January and February, which is 10-12 months after the previous IRS cycle, by which time insecticide

efficacy had decreased substantially. In response to entomology data from this study and District Health Information System 2 (DHIS2)-derived reports on peak malaria cases, the timing of IRS has since been changed to November in Kagera and Geita Regions in 2018(30). Spraying towards the end of the year should provide better protection during the two major malaria peaks of December/January and June/July.

Consistent with results from other studies in neighboring western Kenya(31), we observed the peak biting rates of *Anopheles gambiae* s.l. occurring in unsprayed sites late at night, although was higher outdoors than indoors. Biting time and location (indoors/outdoors) can change depending on host availability(32) and selection for outdoor biting due to indoor insecticide exposure. Our results suggest that *An. gambiae* s.l. (mostly *An. arabiensis*) may have shifted to bite more often outdoors in sites where IRS has been conducted for several years(33). *An. arabiensis* were shown to be opportunistic in feeding behavior, with many having fed on both human and animal hosts (mostly cattle). This may partially explain why *An. arabiensis* was the predominant malaria vector species collected in sprayed sites, with *An. gambiae* s.s. and *An. funestus* s.s. more readily controlled due to their anthropophilic and endophilic nature (31,34). However, *An. arabiensis* was also the predominant species in unsprayed sites, indicating that other factors including climatic conditions and other control measures (particularly LLINs) have contributed to *An. arabiensis* dominating in this region.

*Anopheles funestus* had the highest sporozoite rate among all species of *Anopheles* collected in 2017, but only constituted 8% of *Anopheles* collected in sprayed sites and 20% in unsprayed sites. Our results suggest that there was a higher sporozoite carriage by *An. funestus* in unsprayed sites in comparison to sprayed sites, while there was also evidence for a species shift in sprayed sites within the *An. funestus* group. In some sprayed sites in Kagera it appeared that *An. parensis* (member of the *An. funestus* group) might have replaced *An. funestus* s.s., as was reported in coastal Kenya following IRS with DDT in the 1960s(35). IRS has been extremely successful in controlling *An. funestus* s.s. in several countries, with the species being highly anthropophilic and preferring to rest indoors. In the Pare/Taveta area of East Africa, where dieldrin was sprayed between 1954-1959 *An. funestus* complex was not found for 3 years after the end of spraying (Smith, 1962) but the more zoophilic species *An. rivulorum* (of the *An. funestus* group) became common thereafter. The finding of *An. parensis* with sporozoites indicates that this species is probably becoming an important secondary vector(36) in Tanzania, as has been demonstrated in South Africa (37).

An obvious limitation of this study is that there was no baseline monitoring of vector densities and sporozoite rates before IRS was first conducted in each site. There were also few unsprayed sites, which were relatively far from sprayed sites. These two factors make it difficult to directly determine the impact that IRS had on vector populations. Nevertheless, in the majority of sites, IRS with pirimiphos-methyl CS was successful in keeping vector densities relatively low for approximately 9 months after spraying. There was an exception in the sprayed site of Missenyi, where a particularly high density of *An. gambiae* s.l. was collected just a few months after spraying. Missenyi district is known to receive a relatively high amount of rainfall in March-May and most arable land is used for sugar cane cultivation that results in prolonged availability of larval habitats for Anophelines.

## **Conclusion**

The most important finding was that IRS appeared to have a substantial impact on malaria transmission, with the sporozoite rate in the predominant malaria vector species, *An. arabiensis*, being 59% lower in sprayed sites than in unsprayed sites in 2017. This is in keeping with a study in Kagera Region which showed that a combination of non-pyrethroid IRS together with pyrethroid LLINs resulted in fewer cases of malaria than villages with LLINs only(38).

## **Abbreviations**

CBR: collection bottle rotators

CDC: Centers for Disease Control

ICON 10 CS: lambda-cyhalothrin capsule suspension

ITNs: insecticide treated bed nets

IRS: indoor residual spraying; DDT: 4,4'-(2,2,2trichloroethane-1,1-diyl) bis (chlorobenzene)

KDT: knock down time

LLIN: long-lasting insecticide-treated net

MoHCDEC: Ministry of Health, Community Development, Gender, Elderly and Children

PMI: President's Malaria Initiative

WHO: World Health Organization

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**Competing interests**

Authors have no competing interests.

**Ethics approval and consent to participate**

The study pertained to the continuous national malaria programme around Lake Victoria intending to control malaria through IRS. Lake Zone Institutional Review Board (LZIRB) of the National Institute for Medical Research (NIMR), Tanzania granted ethical approval for this study. Furthermore, at district and village level, permission to carry out IRS monitoring was attained from District Medical Officers (DMOs) and village leaders. We verbally acquired informed consents from head of households to use their houses for cone wall bioassays and mosquitoes collection. We used of mosquito collection traps instead of human land catch (HLC).

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**Author contributions**

CK, AM, KN collected data from the field. EL, DM, and LB performed molecular lab assays. DM consulted on study. AM, RO and SM supervised the study. CK wrote first draft and all authors contributed to the review of final manuscript.

**Availability of data and materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Consent for publication**

Not applicable.

## References

1. Bhatt S, Weiss DJ, Cameron E, Bisanzio D, Mappin B, Dalrymple U, et al. The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. *Nature* [Internet]. 2015 Feb 28 [cited 2018 Aug 11];526(7572):207–11. Available from: <http://www.nature.com/nature/journal/v526/n7572/abs/nature15535.html>
2. Hamusse SD, Balcha TT, Belachew T. The impact of indoor residual spraying on malaria incidence in East Shoa Zone, Ethiopia. *Glob Health Action* [Internet]. 2012 Jan 8;5(1):11619. Available from: <https://www.tandfonline.com/doi/full/10.3402/gha.v5i0.11619>
3. Gimnig JE, Otieno P, Were V, Marwanga D, Abong'o D, Wiegand R, et al. The effect of indoor residual spraying on the prevalence of malaria parasite infection, clinical malaria and anemia in an area of perennial transmission and moderate coverage of insecticide treated nets in western Kenya. Snounou G, editor. *PLoS One* [Internet]. 2016 Mar 8;11(1):e0145282. Available from: <http://dx.plos.org/10.1371/journal.pone.0145282>
4. Kanyangarara M, Mamini E, Mharakurwa S, Munyati S, Gwanzura L, Kobayashi T, et al. Reduction in Malaria Incidence following Indoor Residual Spraying with Actellic 300 CS in a Setting with Pyrethroid Resistance: Mutasa District, Zimbabwe. Brooke B, editor. *PLoS One* [Internet]. 2016 Mar 8;11(3):e0151971. Available from: <http://dx.plos.org/10.1371/journal.pone.0151971>
5. Mashauri FM, Kinung'Hi SM, Kaatano GM, Magesa SM, Kishamawe C, Mwanga JR, et al. Impact of indoor residual spraying of lambda-cyhalothrin on malaria prevalence and anemia in an epidemic-prone District of Muleba, North-western Tanzania. *Am J Trop Med Hyg* [Internet]. 2013 Feb 18;88(5):841–9. Available from: <http://www.ajtmh.org/cgi/doi/10.4269/ajtmh.12-0412>
6. USAID. PRESIDENT ' S MALARIA INITIATIVE Malaria Operational Plan ( MOP ) Tanzania FY 2012. 2012.
7. Kisinza WN, Nkya TE, Kabula B, Overgaard HJ, Massue DJ, Mageni Z, et al. Multiple insecticide resistance in *Anopheles gambiae* from Tanzania: A major concern for malaria vector control. *Malar J*. 2017;16(1):439.
8. Organization WH. WHO guidance for countries on combining indoor residual spraying and long-lasting insecticidal nets. 2014;
9. Nkya TE, Akhouayri I, Poupardin R, Batengana B, Mosha F, Magesa S, et al.



- Insecticide resistance mechanisms associated with different environments in the malaria vector *Anopheles gambiae*: a case study in Tanzania. *Malar J* [Internet]. 2014 Feb 18;13(1):28. Available from:  
<http://malariajournal.biomedcentral.com/articles/10.1186/1475-2875-13-28>
10. Hemingway J, Ranson H, Magill A, Kolaczinski J, Fornadel C, Gimnig J, et al. Averting a malaria disaster: will insecticide resistance derail malaria control? *Lancet* [Internet]. 2016 Feb 19;387(10029):1785–8. Available from:  
<http://www.sciencedirect.com/science/article/pii/S0140673615004171>
  11. West PA, Protopopoff N, Wright A, Kivaju Z, Tagererwa R, Mosha FW, et al. Indoor Residual Spraying in Combination with Insecticide-Treated Nets Compared to Insecticide-Treated Nets Alone for Protection against Malaria: A Cluster Randomised Trial in Tanzania. Gething PW, editor. *PLoS Med* [Internet]. 2014 Mar 8;11(4):e1001630. Available from: <http://dx.plos.org/10.1371/journal.pmed.1001630>
  12. Haji KA, Thawer NG, Khatib BO, Mcha JH, Rashid A, Ali AS, et al. Efficacy, persistence and vector susceptibility to pirimiphos-methyl (Actellic®; 300CS) insecticide for indoor residual spraying in Zanzibar. *Parasites and Vectors* [Internet]. 2015 Mar 8;8(1). Available from: <http://www.parasitesandvectors.com/content/8/1/628>
  13. WHO. World Malaria Report [Internet]. 2017. Available from:  
<http://www.who.int/malaria/publications/world-malaria-report-2017/report/en/>
  14. WHO. Report of the WHO Informal Consultation: Tests procedures for insecticide resistance monitoring in malaria vectors, bio-efficacy and persistence of insecticides on treated surfaces. Geneva: World Health Organization: Parasitic Diseases and Vector Control. 1998.
  15. Abbott WS. A Method of Computing the Effectiveness of an Insecticide. *J Econ Entomol* [Internet]. 2015 Mar 25;18(2):265–7. Available from:  
<https://academic.oup.com/jee/article-lookup/doi/10.1093/jee/18.2.265a>
  16. Shiff CJ, Minjas JN, Hall T, Hunt RH, Lyimo S, Davis JR. Malaria infection potential of anopheline mosquitoes sampled by light trapping indoors in coastal Tanzanian villages. *Med Vet Entomol* [Internet]. 1995 Mar 24;9(3):256–62. Available from:  
<http://doi.wiley.com/10.1111/j.1365-2915.1995.tb00131.x>
  17. Barnard DR, Xue R-D. Laboratory evaluation of mosquito repellents against *Aedes albopictus*, *Culex nigripalpus*, and *Ochlerotatus triseriatus* (Diptera: Culicidae). *J Med Entomol* [Internet]. 2004 Mar 24;41(4):726–30. Available from:  
<https://www.bioone.org/doi/full/10.1603/0022-2585-41.4.726>

18. Maia MF, Robinson A, John A, Mgando J, Simfukwe E, Moore SJ. Comparison of the CDC Backpack aspirator and the Prokopack aspirator for sampling indoor-and outdoor-resting mosquitoes in southern Tanzania. *Parasit Vectors* [Internet]. 2011 Feb 18;4(1):124. Available from:  
<https://parasitesandvectors.biomedcentral.com/articles/10.1186/1756-3305-4-124>
19. Dekadal (10-day) Precipitation [Internet]. 2017 [cited 2017 May 23]. Available from:  
<http://iridl.ldeo.columbia.edu/maproom/Health/Regional/Africa/Malaria/MEWS/index.html>
20. Lines JD, Curtis CF, Wilkes TJ, Njunwa KJ. Monitoring human-biting mosquitoes (Diptera: Culicidae) in Tanzania with light-traps hung beside mosquito nets. *Bull Entomol Res* [Internet]. 1991 Mar 24;81(01):77. Available from:  
[http://www.journals.cambridge.org/abstract\\_S0007485300053268](http://www.journals.cambridge.org/abstract_S0007485300053268)
21. Moon Phases 2016 – Lunar Calendar [Internet]. 2017. Available from:  
<https://www.timeanddate.com/moon/phases/?year=2016>
22. Vazquez-Prokopec GM, Galvin WA, Kelly R, Kitron U. A new, cost-effective, battery-powered aspirator for adult mosquito collections. *J Med Entomol* [Internet]. 2009 Feb 18;46(6):1256–9. Available from:  
<http://www.bioone.org/doi/abs/10.1603/033.046.0602>
23. Gillies MT, Coetzee M. A supplement to the Anophelinae of Africa south of the Sahara (Afrotropical region). *Publ South African Inst Med Res* [Internet]. 1987;55(55):1–143. Available from:  
[http://ihi.eprints.org/1274/1/Gillies\\_%26\\_Coetzee\\_1984\\_Supplement\\_to\\_African\\_anophelines\\_1.pdf](http://ihi.eprints.org/1274/1/Gillies_%26_Coetzee_1984_Supplement_to_African_anophelines_1.pdf)
24. Burkot TR, Williams JL, Schneider I. Identification of *Plasmodium falciparum*-infected mosquitoes by a double antibody enzyme-linked immunosorbent assay. *Am J Trop Med Hyg.* 1984;33(5):783–8.
25. Wirtz RA, Zavala F, Charoenvit Y, Campbell GH, Burkot TR, Schneider I, et al. Comparative testing of monoclonal antibodies against *Plasmodium falciparum* sporozoites for ELISA development. *Bull World Health Organ* [Internet]. 1987 Mar 26;65(1):39. Available from:  
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2490858/>
26. Scott, J. A.; Brogdon, W. G.; Collins FH. Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. *Am J Trop Med Hyg.* 1993;49(4):520–9.

27. Wilkins EE, Howell PI, Benedict MQ. IMP PCR primers detect single nucleotide polymorphisms for *Anopheles gambiae* species identification, Mopti and Savanna rDNA types, and resistance to dieldrin in *Anopheles arabiensis*. *Malar J*. 2006;5.
28. Beier JC, Perkins P V., Wirtz RA, Koros J, Diggs D, Gargan TP, et al. Bloodmeal identification by direct enzyme-linked immunosorbent assay (ELISA), tested on *Anopheles* (Diptera: Culicidae) in Kenya. *J Med Entomol* [Internet]. 1988;25(1):9–16. Available from: <https://academic.oup.com/jme/article-abstract/25/1/9/847378>
29. Dengela D, Seyoum A, Lucas B, Johns B, George K, Belemvire A, et al. Multi-country assessment of residual bio-efficacy of insecticides used for indoor residual spraying in malaria control on different surface types: Results from program monitoring in 17 PMI/USAID-supported IRS countries. *Parasites and Vectors*. 2018;11(1).
30. MD B, Abt Associates. PMI Africa IRS (AIRS) Project Indoor Residual Spraying (IRS 2) Task Order Six, 2018 Tanzania End of Spray Report. 2018.
31. Bayoh MN, Walker ED, Kosgei J, Ombok M, Olang GB, Githeko AK, et al. Persistently high estimates of late night, indoor exposure to malaria vectors despite high coverage of insecticide treated nets. *Parasit Vectors* [Internet]. 2014 Mar 26;7(1):380. Available from: <http://parasitesandvectors.biomedcentral.com/articles/10.1186/1756-3305-7-380>
32. Hawkes F, Gibson G. Seeing is believing: the nocturnal malarial mosquito *Anopheles coluzzii* responds to visual host-cues when odour indicates a host is nearby. *Parasit Vectors* [Internet]. 2016 Feb 18;9(1):320. Available from: <https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-016-1609-z>
33. Reddy MR, Overgaard HJ, Abaga S, Reddy VP, Caccone A, Kiszewski AE, et al. Outdoor host seeking behaviour of *Anopheles gambiae* mosquitoes following initiation of malaria vector control on Bioko Island, Equatorial Guinea. *Malar J* [Internet]. 2011 Feb 18;10(1):184. Available from: <http://malariajournal.biomedcentral.com/articles/10.1186/1475-2875-10-184>
34. Githeko AK, Adungo NI, Karanja DM, Hawley WA, Vulule JM, Seroney IK, et al. Some Observations on the Biting Behavior of *Anopheles gambiae* s.s, *Anopheles arabiensis*, and *Anopheles funestus* and Their Implications for Malaria Control. *Exp Parasitol* [Internet]. 1996 Mar 26;82(3):306–15. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0014489496900387>
35. Gillies MT, Furlong M. An investigation into the behaviour of *anopheles parensis* gillies at Malindi on the Kenya coast. *Bull Entomol Res*. 1964;55(1):1–16.

36. Mulamba C, Irving H, Riveron JM, Mukwaya LG, Birungi J, Wondji CS. Contrasting Plasmodium infection rates and insecticide susceptibility profiles between the sympatric sibling species *Anopheles parensis* and *Anopheles funestus* s.s: A potential challenge for malaria vector control in Uganda. *Parasites and Vectors*. 2014;7(1).
37. Burke A, Moss YD, Duncan F, Qwabe B, Coetzee M, Koekemoer L, et al. *Anopheles parensis* contributes to residual malaria transmission in South Africa. *Malar J*. 2019;1–7.
38. Protopopoff N, Wright A, West PA, Tigererwa R, Mosha FW, Kisinza W, et al. Combination of insecticide treated nets and indoor residual spraying in northern Tanzania provides additional reduction in vector population density and malaria transmission rates compared to insecticide treated nets alone: A randomised control trial. *PLoS One*. 2015;10(11).

## Figure legends

### Figure 1: Study sites.

Map of entomological surveillance sites in districts surrounding Lake Victoria, NW Tanzania. Showing all sites from entomological monitoring survey conducted between 2015 and 2017.

### Figure 2: Mean monthly percentage mortality (24h) of *An. gambiae* (Kisumu).

Mean represents 24 hours mortality after 30 mins cone bioassay on mud, cement, painted, white wash and burnt brick walls that were sprayed with Actellic 300CS in 2015, 2016 and 2017. The red dotted line shows the WHO standard cut-off (80% mortality).

### Figure 3: Mean nightly indoor catch of *An. gambiae* s.l. and *An. funestus* s.l.

Indoor density of *An. gambiae* s.l. and *An. funestus* s.l. collected from CDC light traps for sampling period 2016 – 2017 (except for Nyang’hwale (sprayed, 2017) and Tarime (unsprayed, 2017) where data was only collected in 2017). (A) Kagera region with Biharamulo as control site. (B) Mwanza region with Busega (a close by site, in Simiyu region, as a control site. (C) Mara region with Tarime as control site. (D) Geita region with Bukombe as control sites. Arrows indicate time when IRS was conducted.

### Figure 4: Biting rate for *Anopheles gambiae* s.l.

Mean biting time of *An. gambiae* s.l. from CBR conducted indoors and outdoors. Mean hourly biting rate is indicated from 18:00 to 06:00 in sprayed and unsprayed areas.

**Figure 5: Indoor resting density for *Anopheles gambiae* s.l.**

(A) Kagera region with Biharamulo as control. (B) Geita, and Mwanza region with Bukombe and Busega as controls, respectively. (C) Mara region with Tarime as control. Arrows indicate time when IRS was conducted. Analysis of variance indicate significantly higher ( $p < 0.05$ ) indoor resting densities across regions (i.e. Kagera, Geita, Mwanza, and Mara).

**Figure 6: Mosquitoes collection and species composition.**

Species composition expressed as proportion of *Anopheles* species tested by PCR in respective years (A) 2016 (B) 2017. In 2016, 8 of 10 sites were sprayed with Actellic CS; in 2017 9 of 13 sites were sprayed with Actellic CS.

## Additional files

Additional file 1: Table S1. Sporozoite rate (all *Anopheles* tested) in all 14 districts for 2016 and 2017; \*denotes unsprayed control sites.

<b>Sporozoite rate (%), (total positive/total tested)</b>				
<b>Region</b>	<b>District</b>	<b>2016</b>	<b>2017</b>	<b>Overall 2016-17</b>
<b>Kagera</b>	Ngara	4.5% (10/220)	0.0% (0/50)	3.7% (10/270)
	Chato	1.2% (22/1,810)	1.1% (12/1,091)	1.2% (34/2,901)
	Missenyi	1.6% (22/1,357)	0.8% (9/1,066)	1.3% (31/2,423)
	Bukoba rural	1.3% (4/310)	1.1% (3/279)	1.2% (7/589)
	Biharamulo*	N/A	3.9% (22/560)	3.9% (22/560)
<b>Mwanza</b>	Sengerema	0.9%(13/1,422)	1.9% (3/158)	1.0% (16/1,580)
	Kwimba	2.3% (9/386)	0.0% (0/52)	2.1% (9/438)
<b>Simiyu</b>	Busega*	1.4% (8/586)	1.1% (9/806)	1.2% (17/1,392)
<b>Mara</b>	Musoma rural	2.2% (31/1,416)	0.0% (0/5)	2.2% (31/1,421)
	Butiama	2.9% (26/888)	1.8% (5/276)	2.7% (31/1,164)
	Tarime*	N/A	2.2% (17/772)	2.2% (17/772)
<b>Geita</b>	Geita TC	N/A	1.9% (1/52)	1.9% (1/52)
	Nyang'hwale	N/A	1.4% (2/146)	1.4% (2/146)
	Bukombe*	1.7% (38/2,250)	2.9% (21/712)	2.0% (59/2,962)

Additional file 2: Table S2. Results of ELISA to determine blood meal source of *Anopheles* collected by Prokopack aspirator and CDC light trap.

<b>District</b>	<b>No. tested</b>	<b>Human</b>	<b>Cow</b>	<b>Goat or dog</b>	<b>Mixed (human + animal)</b>	<b>Mixed (animal + animal)</b>
Sengerema	13	3	1	0	3	6
Missenyi	78	21	18	0	30	9
Bukoba Rural	17	4	0	4	6	3
Kwimba	1	1	0	0	0	0
Total sprayed sites	109	26.6% (29)	17.4% (19)	3.7% (4)	35.8% (39)	16.5% (18)
Bukombe	37	6	11	1	2	17
Busega	48	17	4	0	22	5
Total unsprayed sites	85	27.1% (23)	17.6% (15)	1.2% (1)	28.2% (24)	25.9% (22)