ISSN: 2573-9611

Research Article

Journal of Pediatrics & Neonatal Biology

Bio-Efficacy of Insecticides Used for Vector Control in Busia and Tororo Districts, Eastern Uganda

Faith Chemutai[1*], Joseph Kisakye[2], Agapitus Kato[3], Fredrick Kabbale[4] and Anthony Egeru[5]

[1]Department of Department of Zoology, Entomology and Fisheries Sciences, School of Biological Sciences, Makerere University P.O. Box 7062, Kampala, Uganda

[2,4] Department of Zoology, Entomology and Fisheries Sciences, School of Biological Sciences, Makerere University P.O. Box 7062, Kampala, Uganda

[3]Department of Entomology, Uganda Virus Research Institute

[5]Department of Environmental Management, School of Forestry, Environment and Geographical Sciences, Makerere University P.O. Box 7062, Kampala, Uganda

[*]Corresponding Author:
Faith Chemutai, Department of Department of Zoology, Entomology and Fisheries Sciences, School of Biological Sciences, Makerere University P.O. Box 7062, Kampala, Uganda.

Submitted: 24 Feb 2023; **Accepted:**06 Mar 2023; **Published:**16 Mar 2023

Citations: Chemutai, F., Kisakye, J., Kato, A., Kabbale, F., Egeru, A. (2023). Bio-Efficacy of Insecticides Used For Vector Control in Busia and Tororo Districts, Eastern Uganda. *J Pediatr Neonatal Biol*, *8*(1), 156-167

Abstract

Reductions in malaria burden worldwide coincides with the massive scale-up of malaria treatment and prevention measures, of which vector control is the major component, particularly in SSA. The key vector control interventions in Africa including Uganda, rely heavily on utilisation of long-lasting insecticidal nets (LLINs) and insecticide-based indoor residual spraying (IRS). This study assessed the bio-efficacy of insecticides used for vector control in LLINs and IRS in Busia and Tororo. Samples of Anopheles mosquito larvae were collected from various breeding grounds before rearing them in an insectary. Emerged adults were observed under a dissecting microscope and identified using standardized morphological keys after respective bioassays on used nets and sprayed walls. Independent two sample T-test was used to test for significant differences in the mean malaria vectors diversity, mean variations, mortalities by net and wall type. Results showed that mean mortalities of Anopheles mosquitoes to used brands of nets from Busia (9.86±11.35) and Tororo (9.64 ±11.12) varied insignificantly (t=0.119, p=0.906). A disaggregated analysis for each net used revealed that, the DAWA plus 2.0 registered a highest mean mortality of mosquitos, followed by PermaNet 3.0+PBO, Olyset and PermaNet 2.0 respectively. Fludora fusion revealed highest mortalities on plastered painted wall, followed by Brick plain then mud/wattle walls at all times. The trend is similar for Actellic, but unlike Fludora fusion, Actellic exhibited mortalities lower than the 80% threshold for all the wall types and T-test (T-test P values<0.001) indicated Actellic efficacy to be significantly lower than that of Fludora fusion on all the three wall substrates. This study has shown that vector behaviour, biology and physiology need consistent monitoring and surveillance for further entomological characterisation.

Keywords: Bimodal, Bio-Efficacy, Sentinel Areas, Oscillation, *Plasmodium* Vectors

Background

High malaria transmission rates in Sub-Saharan Africa (SSA) are attributed to the continuous presence of effective and competent *Plasmodium* vectors, *Anopheles gambiae* complex and the Anopheles funestus group which play a key role in transmitting the most dangerous malaria parasite species *Plasmodium falciparum* The core essentials that make these species highly effective *Plasmodium* vectors are their preference for humans as a source of blood combined with indoor resting habits and exploitation of breeding habitats created by human activities. Information of these vector innate feeding preferences and resting habits when combined with data on host accessibility, precisely forecasts the intensity of *Plasmodium* transmission *Plasmodium* transmission in Uganda is perennial with two peaks in March to May and September to December consistent to the rainfall seasons, favoring mosquitoes to breed and also during which the vector biting density increases [1-8].

Reductions in malaria burden worldwide coincides with the massive scale-up of malaria treatment and prevention measures, of which vector control is the major component, particularly in SSA [9]. The core *Plasmodium* vector control interventions in Africa including Uganda, rely heavily on utilisation of long-lasting insecticide nets (LLINs) and indoor residual spraying (IRS) which are insecticide-based relying on four chemical classes: organochlo-

rines, pyrethroids, carbamates and organophosphates. Whereas 14 formulations belonging to these classes are approved by the World Health Organization (WHO) for use in IRS, only pyrethroids are approved for use in LLINs because of their low human toxicity, repellent properties and rapid knock down and killing effect thus the community is protected from malaria (Helinski et al., 2015). Busia district uses only LLINs while Tororo district uses both LLINs and IRS in battling against *Plasmodium* vectors. Although LLINs and IRS have contributed significantly to reduced clinical malaria incidences due to their efficiency in some sceneries, there is paucity of evidence regarding their effectiveness following their deployment in a given region [9-13].

However, the advances made in ascertaining their efficiency are fragile due to the decreased effectiveness of the interventions partially as a result of vectors' lowered responsiveness towards the insecticides used in the control. Vector species have not only evaded exposure, but also changing of feeding from late to early biting, shifting from endophagic to exophagic, and avoiding resting on LLINs or the walls sprayed with insecticides [9, 14, 15]. Also, these vector control approaches have been noted to be ineffective against exophagic vectors and increased resistance to pyrethroids [3, 16-18]. This is more pertinent given the fact that elsewhere mosquitoes have become difficult to be controlled due to their change in biology, physiology and behavior, leading to decreased efficiency of vector-control interventions [3, 14, 17].

Despite the deployed vector control approaches, malaria status of Busia and Tororo districts is particularly high as the area is characterised by numerous and recurrent bushes, persistent stagnant water around homesteads, long rain seasons, low altitude and high temperatures. Busia and Tororo also accommodates two important boarder points of Busia and Malaba along the famous Trans-Africa highway, characterised by heavy traffic of people and merchandise from, through and to many other countries. All these factors favours the proliferation of Anopheles mosquitoes and reproduction of the parasites within them [2]. Additionally, limited surveillance and monitoring of mosquitoes for behavioral adaptations and changes in vector species' composition is the common challenge [19]. Together with the fact that there is also oscillation of mosquito vectors and the human-plasmodium carriers within the area, it could explain why amidst the intensified vector control measures, the regions still experience active *Plasmodium* transmission, especially during the peak of malaria vector breeding season that spans the summer months [20]. Therefore, there was a need to assess the bio-efficacy of insecticides used for vector control in the two methods, so as to ascertain the effectiveness of the different control frameworks in Busia and Tororo in order to implicate the role of variabilities in the two districts.

Materials and Methods

Description of Study Area

The study was conducted in two purposively selected districts of Busia and Tororo in Eastern Uganda as depicted in Figure 1, regarded as among the sentinel regions sharing eco-epidemiological features and characterised strata of high malaria transmission with the presence of mosquito species and higher insecticide pressure [18, 21].

Figure 1: Location of *Anopheles* Mosquito Larvae Sampling Sites in the two Districts

Busia and Tororo districts have a stable perennial malaria transmission with malaria prevalence rates ranging from 39 to 68% (Okia et al., 2018a). Busia district is located to the southeast and lies between 0o 46'N, 34o 0'E of Uganda near Kenya boarder and bordering Tororo district to the north [22]. Tororo town is approximately 10 km west of the town of Malaba at the border between Uganda and Kenya, located 205 km northeast of Kampala and lies between 0° 45′N, 34° 5′E (Latitude: 0.692780; Longitude: 34.181655) in Eastern Uganda and lies at an average elevation of 1,278 m above sea level.

Climate

The rainfall patterns of Busia and Tororo is bimodal, with the first rainy season (short rains) extending from March to May and a longer rainy season extending from August to November, with annual rainfall ranging from 1520 to 1800 mm. The mean annual temperature ranges from 16.20C to 28.70C. Average annual precipitation is 1,494 millimeters and relative humidity ranging from 52% to 89% [23].

Vegetation

Riverine zones and lowlands of these districts grow rice, altitude forests, savannah mosaic, swamp, wooded savannah and grass savannah [23]. During rainy months of the year, rice gardens flood and hold water for long periods, providing potential breeding sites for Anopheles mosquitoes [2].

Drainage

Busia District accommodates wetlands and rivers covering a total area of 57.173sq. km, while open water, Lake Victoria, cover 36.88sq. km. The most significant permanent swamp systems are along River Lumboka to the west, forming part of the boundary with Bugiri District, and River Malaba to the north bordering Tororo District. Tororo District accommodates river Malaba, moist Combretum savanna, wetlands and swamps [23].

Research Design

This study took a mixed methods research approach including efficacy testing of Anopheles mosquitoes to insecticides used and retrospective data analysis for malaria cases in the study area for the past nine years (from 2012 to 2020).

Sampling Design, Sampling Points and Sample Collection

Cluster randomized selection of sites as by was used to collect larvae in Busitema, Sikuda, Masafu Dabani, Masaba and Buhehe for Busia, and Nagongera, Mulanda, Petta, TC Kisoko, Merikit and Molo for Tororo. Sampling from stagnant water around homesteads, nearby swamps and rice paddies following a method outlined by [10, 24]. A sample size of 500 mosquito larvae was collected from each and every sub county. In the retrospective data used, semi-structured questionnaires were administered in the form of interview to the household representative while obtaining information about the type, frequency of vector control application and nature of insecticides used. The sample size obtained from the representative of each homestead was determined basing on the approach by as shown below:

$$n = \left(\frac{Z^2 PQ}{\alpha^2} \right)$$

Where: n = the sample number; Z = 1.96 (at 95% confidence level); P = the expected proportion of community households with information needed (15%); Q = 1-P; α is the margin of sampling error (5%).

$$\text{Implying, } n = \frac{1.96^2 * 0.15(1-0.15)}{0.05^2}$$

n = 195.9216 households which approximates to about 200 households

Thus, the estimated sample size for this study was at least 196 households [25]. Therefore, 200 households were sampled.

Mosquito Collection

Anopheles mosquito larvae were collected from seasons of February to June 2021 using scoopers from various breeding grounds then reared in an insectary at 25 0C and 80 % humidity. Water in the larval container was refreshed every 2–3 days. Pupae were harvested in a plastic cup and placed within a cage (bottom 27 cm × 27 cm, top 25 cm × 25 cm, height 27 cm), in which a cotton wool soaked in 10% glucose solution was placed in a 50 ml glass flask. The cage was kept on a table in a well-ventilated insectary room. The glucose solution was changed every 2-3 days according to the mosquito rearing protocol [24].

Malaria Vectors Diversity and Abundance Detection

Emerged adults from the reared larvae were morphologically identified using simplified standard morphological keys adopted from Gillies & Coetzee (1987) to deduce the species present (Kabbale *et al*., 2016). Whilst to check and to improve on the precision of the morphological identification, emerged adult mosquitoes were observed under a dissecting microscope and identified using standardized morphological keys as by Coetzee (2020).

Efficacy Testing on Used LLINs (PermaNet 2.0, DAWA Plus 2.0, Olyset and PermaNet 3.0)

Using an aspirator, the reared 4-6 days non-blood fed 25 *Anopheles gambiae* s.l mosquitoes in replicates of four, were fed into used nets fitted with bioassay cones in a designated test laboratory [26]. Four bioassay cones lined with self-adhesive tape were fixed on the net lined on a paperboard to mimic the placement of the net on bed for the assay. The mosquitoes were left in the exposure cones for 3 minutes, after which the number of knocked down (KD) mosquitoes were scored. All the exposed mosquitoes were then gently transferred to holding paper cups for 60 minutes, mortality was recorded and provided with 10% sugar solution soaked on cotton wool pads placed on top of the paper cups covered with an untreated net. Final mortality was recorded at 24 hour based on the status of mosquitoes as no longer standing, immobile or gliding along the curvature of the paper cups [26].

Efficacy of IRS Insecticides

In Tororo district, cone bio-assays were conducted on 3 different

sprayed wall types (plastered painted, Plain brick and mud/wattle) [27]. For each wall type, four replicates of 10 non-blood fed 4-6 day old *Anopheles* mosquitoes were exposed. Cones lined with self-adhesive tape were fixed on the sprayed walls for the assay, after which an aspirator was used to introduce the mosquitoes into the cones. The cones were placed at heights of 0.5 m, 1.0 m, and 1.5 m above the floor. The mosquitoes were left in the exposure cones for 30 minutes, after which the number of knocked down (KD) mosquitoes were scored. All the exposed mosquitoes were then gently transferred to holding paper cups for 30 minutes, mortality was recorded and provided with 10% sugar solution soaked on cotton wool pads placed on top of the paper cups covered with an untreated net. Final mortality was recorded at intervals of 24 hours after exposure based on the status of mosquitoes as no longer standing, immobile or gliding along the curvature of the paper cups (Hakizimana *et al*., 2016). Non-insecticides' impregnated walls were used as the control.

Malaria Times Series Data Collection

Time series data on malaria cases (2012–2020) from Health Centre level II, III, IV, private and government health facilities occurring across two districts of the study were obtained from the district health information management system (DHMIS). Particularly, routine weekly and monthly malaria surveillance data reported passively through public and high volume private health facilities for nine (9) years (2012-2020) were accessed from the DHMIS.

Data Analysis

Malaria Vectors Diversity and Abundance

To assess the malaria vectors diversity and abundances in Busia and Tororo districts, mosquito composition data was analysed in SPSS version 2.5.0 data analysis software (Fricker, 2001). To identify whether malaria vectors diversity varied significantly across the districts, the data were log transformed where an independent sample T-test was run.

Bio-Efficacy Cone Assays

To determine the bio-efficacy of the insecticides in LLINs and IRS as malaria vector control approaches used in Busia and Tororo, cone assay data (KD and mortalities) recorded in Excel sheets were exported to SPSS version 2.5.0 where an independent sample T-test was performed to test for the mean differences in mosquito mortality across the two districts. This test was repeated by net type in both districts, and wall spray type in Tororo district.

One-Way Analysis of Variance was used to test for significant differences in mean variations mortalities by type of net at a 95% confidence interval. The analysis was repeated using the disaggregation-by-cases command for different levels of exposure to nets in both districts. For wall spray in Tororo, the data were first disaggregated by 2-cases that is type of insecticide and wall type before a One-Way ANOVA was run.

Results

Malaria Vectors Diversity and Abundance

Table 1 show malaria mosquito diversity and abundance results. A total of 5,180 individuals of *Anopheles* mosquitoes emerged from the reared larvae, of which 3,675 (71%) samples were *Anopheles gambiae* s.l collected from both districts and 1,505 (29%) were *Anopheles funestus.*

Table 1: Malaria Vectors Diversity and Abundance in Busia and Tororo between Feb and June, 2021

Species	February		March		April		May		June	
	Busia	Tororo	Busia	Tororo	Busia	Tororo	Busia	Tororo	Busia	Tororo
A.gambiae S.l	199	254	313	447	428	222	519	308	566	419
A.funestus	189	0	227	0	323	0	361	0	405	0

According to Table 2, there was no significant difference between Busia and Tororo An. Gambiae abundances. The mean of An.Funestus of Busia and Tororo varied significantly ($t=13.081$, $p<0.005$).

Table 2: Variation in Malaria Vectors Diversity and sample means between Districts

Malaria Vectors	District	Sample Means	t-test	Sig.
A. gambiae s.l	Busia	58.33	2.32	0.159
	Tororo	98.33		
A. funestus	Busia	32.83	13.08	0.003
	Tororo	00.00		

Interestingly, all the An. Funestus collected in this study came from Busia district shown in Figure 2. Of the *An. gambiae* samples collected from Busia district, 7.5% came from Masafu, 7.7% from Sikuda,……..etc., and of those collected from Tororo, 8% came from Nagongera, 7.7% came from Petta. Of the *An. funestus* samples collected from Busia, Buhehe had the highest percentage (23.4%), followed by Sikuda (20%), Masaba (19%), Masafu (17.7%), Dabani (12%) and Busitema (7.8%) respectively.

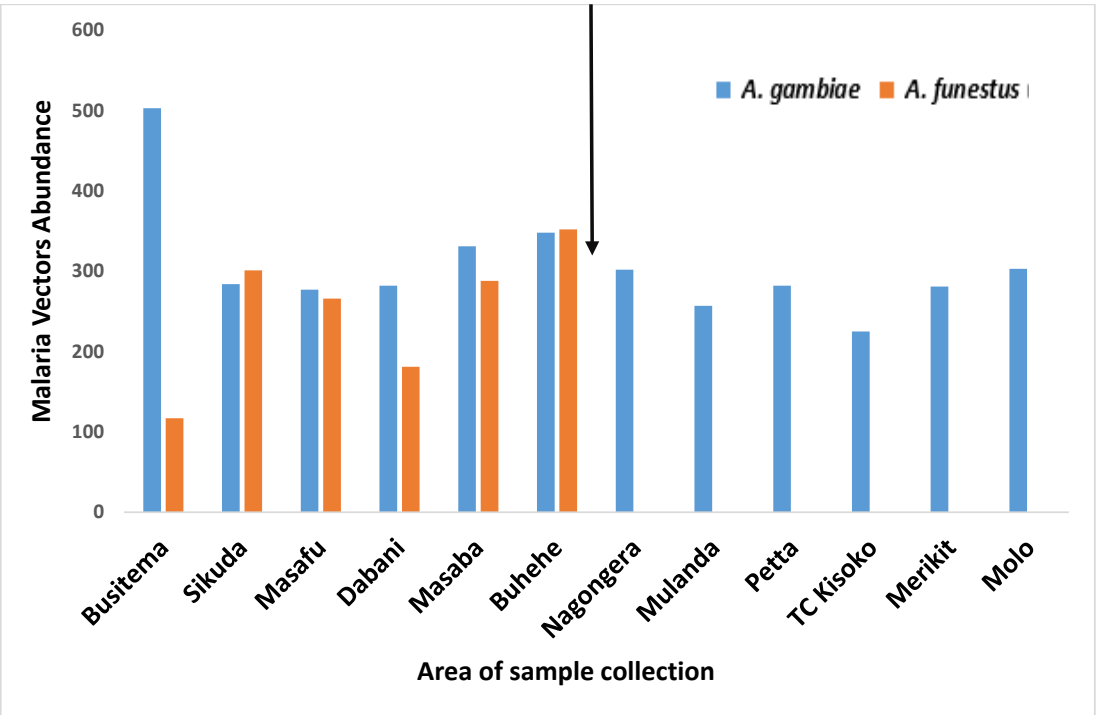

Figure 2: Malaria Vectors Diversity and Abundance across Data Collection Sites in Busia and Tororo Districts

Mosquito Mortalities in LLINs Cone Assays

According to Table 3 and Fig.3, out of four brands of LLINs tested for insecticides efficacy, 99% mortality was recorded for the deltamethrin 80 mg/m^2 impregnated DAWA plus 2.0 LLINs in Busia and 98% in Tororo. PermaNet 3.0 +PBO (84 mg/m^2) and Olyset (525 mg/m^2) permethrin respectively recorded mortalities of 94% and 88% in Busia, and mortalities of 92% and 84% in Tororo. PermaNet 2.0 recorded 68% mortality at 55 mg/m^2 permethrin in Busia, and mortalities of 62% in Tororo. According to Table 3, DAWA plus 2.0 (t=0.027; p >0.05), PermaNet 3.0 +PBO (t=0.100), Olyset (t=0.069) and PermaNet 2.0 (t=0.055).

Table 3: Mean Variations of the most Effective Net in the two Districts

Type of net District		Chemical type	Chemical conc. (mg/m^2)	Mean mortality (%)	Std. Deviation	t-value	p-value
DAWA Plus 2.0	Busia	Deltamethrin	80	99	0.6	0.027	0.979
	Tororo	Deltamethrin	80	98	1.1		
Olyset	Busia	Permethrin	525	88	2	0.069	0.945
	Tororo	Permethrin	525	84	3		
PermaNet 2.0	Busia	Deltamethrin	55	94	1.7	0.100	0.921
	Tororo	Deltamethrin	55	93	1.4		
PermaNet 3.0+PBO	Busia	Permethrin	84	68	3	0.055	0.956
	Tororo	Permethrin	84	62	2		

As can be observed in Figure 3, the DAWA plus 2.0 registered a highest mean mortality of mosquitoes in both districts, followed by PermaNet 3.0 +PBO, Olyset and PermaNet 2.0 in that order; and there was no significant difference in the efficacy of the LLINs between study districts

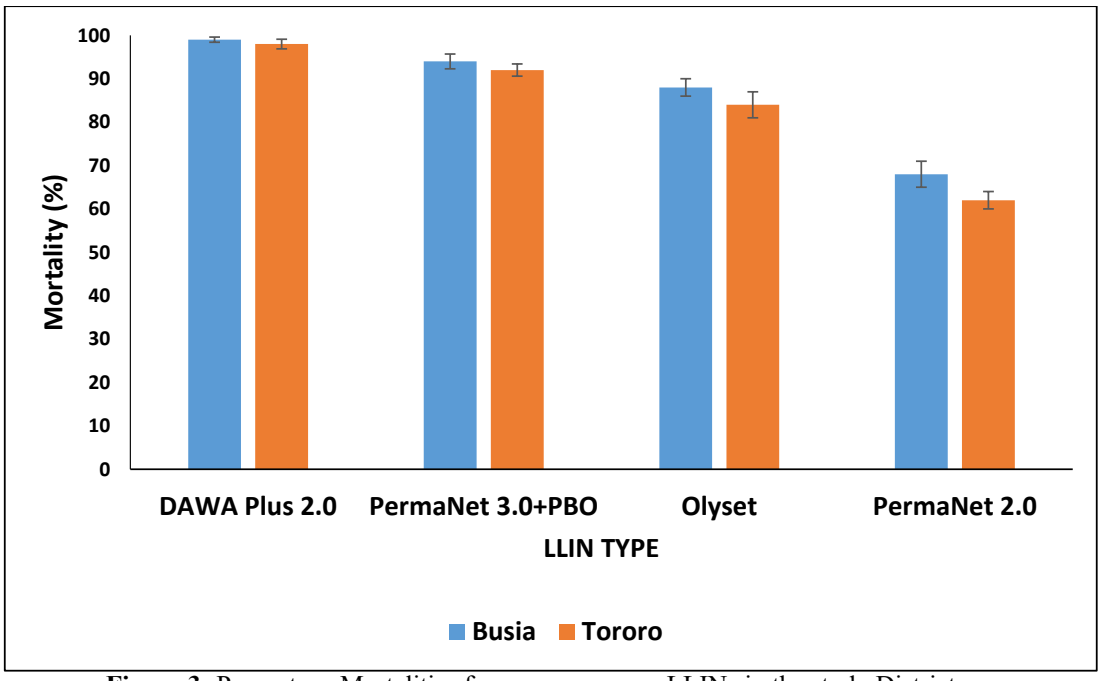

Figure 3: Percentage Mortalities for cone assays on LLINs in the study Districts

On disaggregating the data by type of net and time, the results revealed none of the mosquitoes died in all LLINs at the exposure time of 3 minutes. At holding time (knockdown) of 60 minutes, higher mortalities were registered by DAWA plus 2.0, followed by PermaNet 3.0, Olyset and PermaNet 2.0 respectively. However, the difference in mortalities caused by the LLINs were not significant (F=0.752, p=0.537). Under recovery time (24 hours), higher mosquito mortalities were further registered by DAWA plus 2.0, followed by PermaNet 3.0+PBO, Olyset and PermaNet 2.0, respectively. However, the difference in mortalities caused by the LLINs were still not significant (F=0.628, P=0.601) as shown in Table 4.

Table 4: Variation in mosquito mortalities post exposure to LLINs

Time	Net Type	Mean Mortalities	Std. Deviation	Std. Error	Minimum	Maximum	F	p-value
Exposure time (3mins)	DAWA Plus 2.0	0.0	0.0	0.0	0.0	0.0		
	Olyset	0.0	0.0	0.0	0.0	0.0		
	PermaNet 2.0	0.0	0.0	0.0	0.0	0.0		
	PermaNet 3.0+PBO	0.0	0.0	0.0	0.0	0.0		
	Total	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**		
Holding time (60mins)	DAWA Plus 2.0	16.3	12.0	3.5	0.0	25.0	0.752	0.537
	Olyset	15.6	11.5	3.3	0.0	24.0		
	PermaNet 2.0	10.4	7.9	2.3	0.0	18.0		
	PermaNet 3.0+PBO	15.7	11.6	3.3	0.0	25.0		
	Total	**14.5**	**10.8**	**1.6**	**0.0**	**25.0**		
Recovery time (24 hrs.)	DAWA Plus 2.0	16.4	12.1	3.5	0.0	25.0	0.628	0.601
	Olyset	15.8	11.7	3.4	0.0	25.0		
	PermaNet 2.0	11.0	8.2	2.4	0.0	18.0		

	PermaNet 3.0+PBO	15.8	11.7	3.4	0.0	24.0		
	Total	**14.8**	**10.9**	**1.6**	**0.0**	**25.0**		

Mosquito Mortalities Post Exposure to Treated Walls in Tororo District

Figure 4 and 5 show mortalities for Actellic® 300 CS and Fludora fusion on three wall types respectively. Fludora fusion revealed highest mortalities on plastered painted wall, followed by Brick plain then mud/wattle walls at all times.

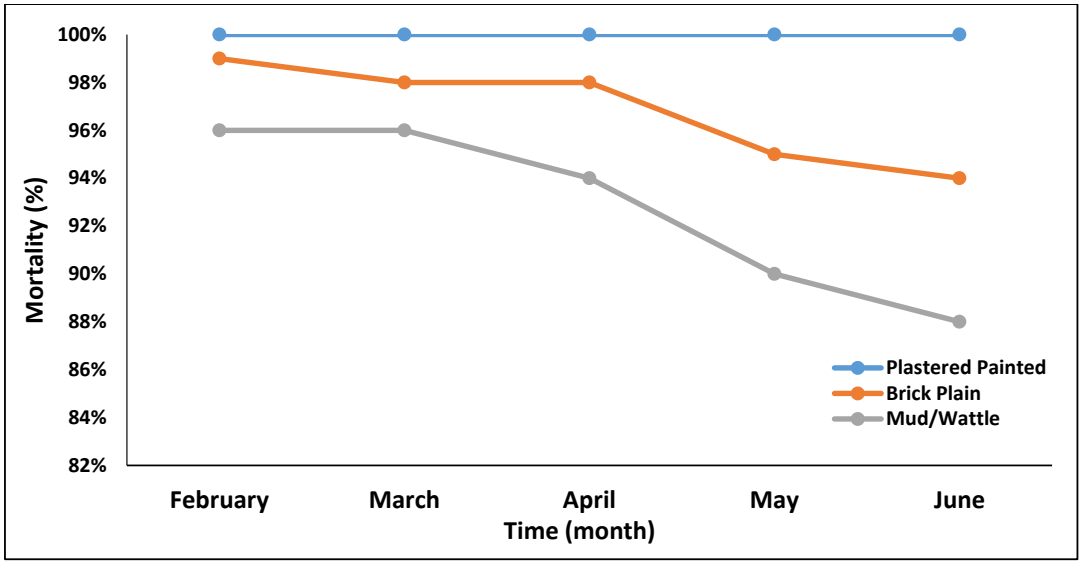

Figure 4: Efficacy of IRS Treated Fludora Fusion Walls Substrates during the Study Period

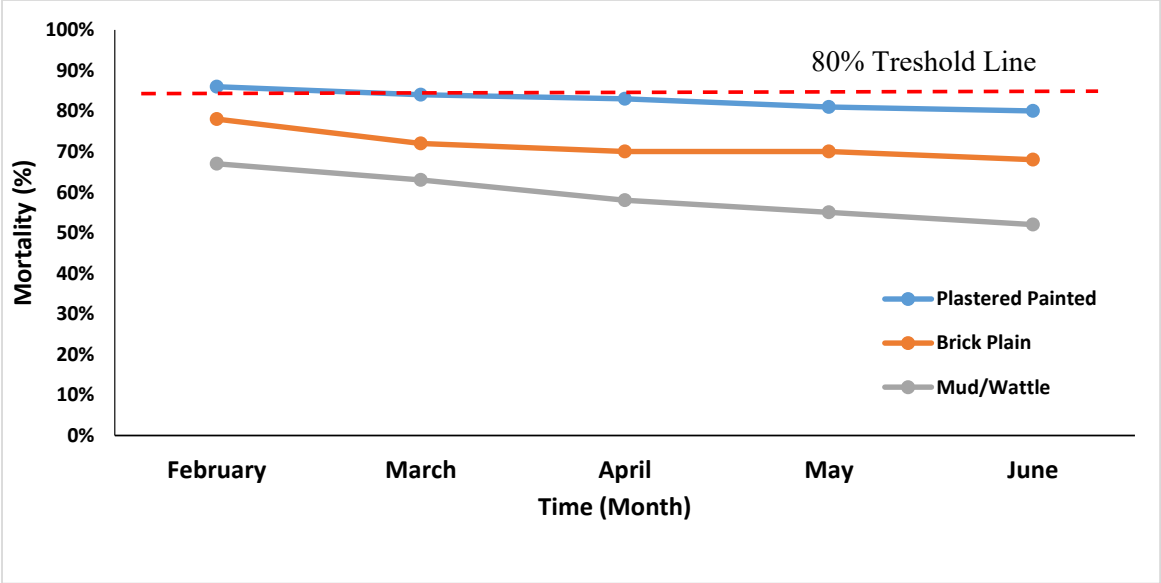

Figure 5: Efficacy of IRS Treated Actellic® 300 CS Walls Substrates during the Study Period

The trend is similar for Actellic, but unlike Fludora fusion, Actellic exhibited mortalities lower than the 80% threshold for all the wall types and T-test (T-test P values<0.001; Table 5) indicated its efficacy to be significantly lower than that of Fludora fusion on all the three wall substrates

Table 5: Variation in Efficacy of IRS Actellic® 300CS and Fludora Fusion treated walls

Wall type	Mean mortality		t. test		
	IRS Fludora Fusion	IRS Actellic® 300CS	T	Df	P
Plastered Painted	1±0	0.828±0.024	-10.72	8	<0.001
Brick Plain	0.968±0.022	0.716±0.038	-12.76	8	<0.001
Mud/Wattle	0.928±0.036	0.59±0.060	-16.11	8	<0.001

Discussion

Malaria Vectors Diversity and Abundance

The predominance of Anopheles gambiae s s.l as shown in Table 1& Fig. 2, could be attributed to the receptive lentic aquatic habitats including swamps and rice paddies, which flood and hold water for long periods during rainy months. These habitats are crucial for mosquito dynamics where many of their important life cycle processes take place [6].

The absence of Anopheles funestus in Tororo district depicted in Fig. 2 could be explained by the complementary effectiveness of the vector control interventions of LLINs & IRS under use in this region. The synergistic control strategy of LLINs plus IRS could have led to a shift in mosquito vector dynamics, whereby the most susceptible to a specific vector-control measure becomes less common [3]. Similar studies by conducted in Migori Kenya reflects the same whereby, LLINs and IRS reduced indoor Anopheles densities, shifts in vector species composition, changes in the time and location of mosquito biting, and changes in host selection, and increases in early exophily. However, this could not rule out the fact that there could be other prevailing ecological or human factors in Busia district responsible for their proliferation such as increased permanent breeding sites. The A. funestus that dominated in Busia district is known to breed all year round and prefer permanent, stagnant water bodies, while Anopheles gambiae sensu lato breed in temporary human created water bodies including rice paddies, pools, puddles, construction sites and hoof prints which were more prevalent in Tororo than Busia district [2, 5, 28].

In addition, the LLINS and IRS have also been associated with changes in sympatric Anopheles species composition. In Uganda, Kenya and elsewhere, sustained vector control has not only resulted in reductions in transmission intensity, but also changes in Anopheles species composition, their behaviour, biology and density [29].

Bio-Efficacy Testing on LLINs

The bio-efficacy of LLINs is comparatively similar, with DAWA plus having the highest mortalities. The higher mortalities observed for DAWA plus 2.0 net compared to other brands of nets used in the two districts in deterring Anopheles mosquitoes could be credited to the insecticides' active ingredients residual effect and bio-efficacy in killing the mosquitoes over time [30-32]. The relative superior performance of deltamethrin active ingredient than permethrin explains the improved bioefficacy of DAWA plus 2.0 compared to PermaNet 3.0 respectively. Similar explanation is consistent to DAWA plus 2.0 versus Olyset .The differential concentration of deltamethrin in DAWA plus 2.0 (80 mg/m2) is responsible for its relatively better performance compared to PermaNet 2.0 (55 mg/m2) by [30]. Similar studies by on comparisons between synergised LLIN and non-synergised LLIN reflects the same phenomena to this study whereby PermaNet 3.0 performed significantly better than PermaNet 2.0 [12].

The efficiency of LLINs relies on: the biology and behaviour of mosquito vectors based on their biting and resting behaviour as well as their susceptibility status to insecticides the insecticides selected as per active ingredient, bio-efficacy over time and durability [13, 31, 32]. Though the greater the overlap in activity time between mosquitoes biting and resting indoors and people being indoors, but not under bed nets could suggest why high malaria status in the region is still recorded (Ekoko et al., 2019).

Comparative effectiveness evaluations using local vector populations such as presented in this study provide valuable data to inform selection of appropriate interventions, this is pertinent to the consistent optimal bio-efficacy of DAWA plus 2.0 indicating that this net represents a viable option for areas with pyrethroid-resistant Anopheles species [7, 30].

Bio-efficacy of Actellic® 300CS and Fludora Fusion IRS

The bio-efficacy of plastered painted walls performing best regardless of insecticide type when compared to brick plain and mud/wattle walls has been observed in other studies and could be attributed to the oil emulsions combined with paint in the plastered wall enabling it to retain the chemical for a longer period of time hence higher residual activity [27, 31]. The effectiveness of IRS relies on: the biology and behavior of mosquito vectors based on their biting and resting behavior as well as their susceptibility status to insecticides the insecticides selected based on residual bio-efficacy over time [27, 32]. There is substantial variation in the duration of action: induced mortality, inhibited blood-feeding between same studies conducted in Ethiopia by with the same product. Some of these differences are attributed to procedural differences such as wall-type though others will reflect true differences in the behaviors and susceptibility of local mosquito populations [10, 33, 34].

The recent consistent change of insecticide type from bendiocarb to Actellic® 300CS and to Fludora fusion in Tororo district, means that for the first-time multiple non-parathyroid IRS products are available with different modes of action this is pertinent to their long lasting residual activity that achieve broadly equivalent reductions in malaria burden across Africa [28, 35, 36]. Similar studies in Zanzibar by demonstrated bendiocarb to have a shorter residual life span on sprayed surfaces of two to three months when compared to Actellic® 300CS and Fludora fusion, which have a

longer residual life efficiency of up to one year when used in mosaics [37].

Conclusions

Anopheles gambiae sensu lato is still predominant in both districts despite of employment of two different control strategies, and in addition Anopheles funestus was caught in different areas of Busia district. This suggests that the control strategies in place are not sufficient. More strategies are required to combat the problem. Both LLINs and IRS proved to have effect on Anopheles mosquitoes, but catching high numbers of endophilic mosquitoes suggest that the impact of LLINs and IRS on the primary malaria vectors (Anopheles gambiae s.l and Anopheles funestus) may be affected by change of behaviour of these mosquito populations. This leads to the conclusion that the current vector control interventions are effective against Plasmodium vectors, but will not lead to elimination of the disease unless additional tools are employed.

Recommendations

Molecular study should be carried out in order to distinguish the specific species in the Anopheles gambiae complex and Anopheles funestus group responsible for the Plasmodium mediation in the study area. This baseline characterization will provide a background of insecticide resistance mechanisms in mosquito populations in the different clusters, to enable effective management of insecticide resistance and at the same time facilitate continued vector control efforts.

Further studies are needed to quantify the individual contribution of each method when LLINs and IRS are deployed in combination. Additionally, to characterize the impact of long-lasting insecticidal nets and indoor residual sprays on vector density, behavior and species composition for accurate epidemiological modelling.

Acknowledgements

I gratefully acknowledge the role played by Infectious Disease Research Collaboration (IDRC) and its staff in Tororo (where I undertook the laboratory experiments). Additional support and guidance provided by Dr. Agapitus Kato, Dr. Joseph Kisakye, Dr. Anthony Egeru, Dr. Fredrick Kabbale and Dr. Eric Sande is greatly acknowledged. I acknowledge and appreciate the assistance given to me by the entire staff of the Department of Zoology, Entomology and Fisheries Sciences, Makerere University. I also greatly appreciate the assistance rendered to me by my friends Alex Musiime, Gordon Yofesi, Daniel Oyoo, Joseph Okoth and Ismail Onyige.

Authors Contribution Statement

F.C conceived the study, collected data for research, analysed the data and manuscript writing

J.K and A.E supported in designing the study, data analysis and manuscript writing

A.K supported with manuscript write up

F.K supported with the manuscript write up

All the authors reviewed the article

Ethical considerations

Clearance for this research was sought from the Ugandan National Council for Science and Technology (UNCST; ref. NS 253S), and the Makerere University School of Medicine Research & Ethics Committee (SOMREC; 2021–133).

Funding

This research was self, family and partially funded by the KFW German Bank under the supervision of Inter-University Council of East Africa (IUCEA) and East Africa Community (EAC).

Competing Interests

The Authors declares that they have no competing interests in terms of financial or non-financial status.

Data Availability

The Mosquito Larvae Collection

The larvae were collected from the study area and reared in the laboratory. Respective bioassays were undertaken on used sleeping nets and indoor residual sprays. After bio-efficiency tests they were stereoscopically dissected and morphologically identified under dissecting microscope.

I would like to ask you some questions regarding plasmodium vector control in your area. I am specifically asking about the type of control intervention you practice. The information collected from this interview will assist the decision makers with proper planning. Participation in this interview is voluntary. You may answer questions that you only desire, stop at any time or decline the interview. If you go on with the interview, the results captured in this interview will be treated confidentially. We will not disclose your name or details at any one moment and expect the interview to last 20-35 minutes. Thank you for sparing your valuable time to answer this questionnaire.

References

1. Collins, E., Vaselli, N. M., Sylla, M., Beavogui, A. H., Orsborne, J., Lawrence, G., Wiegand, R. E., Irish, S. R., Walker, T., & Messenger, L. A. (2019). The relationship between insecticide resistance , mosquito age and malaria prevalence in Anopheles gambiae s . l . from Guinea. Scientific Reports, November 2018, 1–12.
2. Oguttu, D. W., Matovu, J. K. B., Okumu, D. C., Ario, A. R., Okullo, A. E., Opigo, J., & Nankabirwa, V. (2017). Rapid reduction of malaria following introduction of vector control interventions in Tororo District , Uganda : a descriptive study. Malaria Journal, 1–8.
3. Musiime, A. K., Smith, D. L., Kilama, M., Rek, J., Arinaitwe, E., Nankabirwa, J. I., Kamya, M. R., Conrad, M. D., Dorsey, G., Akol, A. M., Staedke, S. G., Lindsay, S. W., & Egonyu, J. P. (2019). Impact of vector control interventions on malaria transmission intensity , outdoor vector biting rates and Anopheles mosquito species composition in Tororo , Uganda. Malaria Journal, 1–9.
4. Sherrard-smith, E., Skarp, J. E., Beale, A. D., Fornadel, C., Norris, L. C., & Moore, S. J. (2019). Mosquito feeding behav-

ior and how it influences residual malaria transmission across Africa. 116(30).
5. Kabbale, G. F., Anne, M. A., John, B. K., Enock, M., Anne, K., Anges, Y., & Ambrose, W. O. (2016). Molecular identification of Anopheles gambiae sensu stricto Giles (formerly Anopheles gambiae Savannah Form) in Kamuli District, Uganda. African Journal of Biotechnology, 15(39), 2124–2131.
6. Gunathilaka, N., Abeyewickreme, W., Hapugoda, M., & Wickremasinghe, R. (2015). Species Composition and Diversity of Malaria Vector Breeding Habitats in Trincomalee District of Sri Lanka. BioMed Research International, 2015.
7. Keven, J. B. (2015). The host-feeding ecology of mosquito vectors in the Anopheles punctulatus (Diptera : Culicidae) species complex in a malaria endemic province of Papua New Guinea. May.
8. Raouf, S., Mpimbaza, A., Kigozi, R., Sserwanga, A., Rubahika, D., Katamba, H., Lindsay, S. W., Kapella, B. K., Belay, K. A., Kamya, M. R., Staedke, S. G., & Dorsey, G. (2017). Resurgence of Malaria Following Discontinuation of Indoor Residual Spraying of Insecticide in an Area of Uganda With Previously High-Transmission Intensity. 65, 453–460.
9. Kleinschmidt, I., Mnzava, A. P., Kafy, H. T., Mbogo, C., Bashir, A. I., Bigoga, J., Adechoubou, A., Raghavendra, K., Knox, T. B., Malik, E. M., Nkuni, Z. J., Bayoh, N., Ochomo, E., Fondjo, E., Kouambeng, C., Awono-ambene, H. P., Etang, J., Akogbeto, M., Bhatt, R., … Donnelly, M. J. (2015). Design of a study to determine the impact of insecticide resistance on malaria vector control : a multi - country investigation. Malaria Journal, July.
10. Deressa, W., Loha, E., Balkew, M., Hailu, A., Gari, T., Kenea, O., Overgaard, H. J., Gebremichael, T., Robberstad, B., & Lindtjørn, B. (2016). Combining long-lasting insecticidal nets and indoor residual spraying for malaria prevention in Ethiopia : study protocol for a cluster randomized controlled trial. Trials, 1–16.
11. Antonio-nkondjio, C., Sandjo, N. N., Awono-ambene, P., & Wondji, C. S. (2018). Implementing a larviciding efficacy or effectiveness control intervention against malaria vectors : key parameters for success. 1–12.
12. Rek, J., Musiime, A., Zedi, M., Otto, G., Kyagamba, P., Rwatooro, J. A., Arinaitwe, E., Nankabirwa, J., Staedke, G., Drakeley, C., Rosenthal, P. J., Kamya, M., Dorsey, G., & Id, J. K. (2020). Non-adherence to long-lasting insecticide treated bednet use following successful malaria control in Tororo , Uganda. 1–14.
13. Kenea, O., Balkew, M., Tekie, H., Gebre-michael, T., Deressa, W., Loha, E., Lindtjørn, B., & Overgaard, H. J. (2016). Human-biting activities of Anopheles species in south-central Ethiopia. Parasites & Vectors, 1–12.
14. Ojuka, P., Boum, Y., Denoeud-Ndam, L., Nabasumba, C., Muller, Y., Okia, M., Mwanga-Amumpaire, J., De Beaudrap, P., Protopopoff, N., & Etard, J. F. (2015). Early biting and insecticide resistance in the malaria vector Anopheles might compromise the effectiveness of vector control intervention in Southwestern Uganda. Malaria Journal, 14(1), 1–8.
15. Nankabirwa, J. I., Briggs, J., Rek, J., Arinaitwe, E., Nayebare, P., Katrak, S., Staedke, S. G., Rosenthal, P. J., Rodriguez-barraquer, I., Kamya, M. R., Dorsey, G., & Greenhouse, B. (2019). Persistent Parasitemia Despite Dramatic Reduction in Malaria Incidence After 3 Rounds of Indoor Residual Spraying in Tororo , Uganda. 219.
16. Yadouleton, A. W., Padonou, G., Asidi, A., Moiroux, N., Bio-Banganna, S., Corbel, V., N'guessan, R., Gbenou, D., Yacoubou, I., Gazard, K., & Akogbeto, M. C. (2010). Insecticide resistance status in Anopheles gambiae in southern Benin.
17. Mnzava, A. P., Knox, T. B., Temu, E. A., Trett, A., Fornadel, C., Hemingway, J., & Renshaw, M. (2015). Implementation of the global plan for insecticide resistance management in malaria vectors : progress , challenges and the way forward. ???, 1–9.
18. Hakizimana, E., Karema, C., Munyakanage, D., Iranzi, G., Githure, J., Tongren, J. E., Takken, W., Binagwaho, A., & Koenraadt, C. J. M. (2016). Susceptibility of Anopheles gambiae to insecticides used for malaria vector control in Rwanda. Malaria Journal, 1–11.
19. Katureebe, A., Zinszer, K., Arinaitwe, E., Rek, J., Kakande, E., Charland, K., Kigozi, R., Kilama, M., Nankabirwa, J., Yeka, A., Mawejje, H., Mpimbaza, A., Katamba, H., Donnelly, M. J., Rosenthal, J., Drakeley, C., Lindsay, S. W., Staedke, S. G., Smith, L., … Dorsey, G. (2016). Measures of Malaria Burden after Long- Lasting Insecticidal Net Distribution and Indoor Residual Spraying at Three Sites in Uganda : A Prospective Observational Study. December 2014, 1–22.
20. Ssempiira, J., Nambuusi, B., Kissa, J., Agaba, B., Makumbi, F., Kasasa, S., & Vounatsou, P. (2017). The contribution of malaria control interventions on spatio-temporal changes of parasitaemia risk in Uganda during 2009-2014. Parasites and Vectors, 10(1), 450.
21. Tchouakui, M., Mugenzi, L. M., D. Menze, B., Khaukha, J. N., Tchapga, W., Tchoupo, M., ... & Wondji, C. S. (2021). Pyrethroid resistance aggravation in Ugandan malaria vectors is reducing bednet efficacy. Pathogens, 10(4), 415.
22. Githinji, E. K., Irungu, L. W., Ndegwa, P. N., Machani, M. G., Amito, R. O., Kemei, B. J., Murima, P. N., Ombui, G. M., Wanjoya, A. K., Mbogo, C. M., & Mathenge, E. M. (2020). Species Composition , Phenotypic and Genotypic Resistance Levels in Major Malaria Vectors in Teso North and Teso South Subcounties in Busia County , Western Kenya. 2020.
23. Profi, V. (2016). Tororo District.
24. Das, S., Garver, L., & Dimopoulos, G. (2007). Protocol for Mosquito Rearing (A. gambiae).
25. Easterby-Smith, M., Thrope, R., Jackson, P., Thorpe, R., & Jackson, P. (2013). Management research. In Sage.
26. Staedke, S. G., Kamya, M. R., Dorsey, G., Maiteki-Sebuguzi, C., Gonahasa, S., Yeka, A., ... & Donnelly, M. J. (2019). LLIN Evaluation in Uganda Project (LLINEUP)–Impact of long-lasting insecticidal nets with, and without, piperonyl butoxide on malaria indicators in Uganda: study protocol for a cluster-randomised trial. Trials, 20(1), 1-13.
27. Pmi, T. H. E., & Project, V. (2019). THE PMI VECTORLINK PROJECT ANNUAL ENTOMOLOGY REPORT.

28. Abong'o, B., Gimnig, J. E., Torr, S. J., Longman, B., Omoke, D., Muchoki, M., ter Kuile, F., Ochomo, E., Munga, S., Samuels, A. M., Njagi, K., Maas, J., Perry, R. T., Fornadel, C., Donnelly, M. J., & Oxborough, R. M. (2020b). Impact of indoor residual spraying with pirimiphos-methyl (Actellic 300CS) on entomological indicators of transmission and malaria case burden in Migori County, western Kenya. Scientific Reports, 10(1).
29. Mawejje, H. D., Kilama, M., Kigozi, S. P., Musiime, A. K., Kamya, M., Lines, J., Lindsay, S. W., Smith, D., Dorsey, G., Donnelly, M. J., & Staedke, S. G. (2021). Impact of seasonality and malaria control interventions on Anopheles density and species composition from three areas of Uganda with differing malaria endemicity. Malaria Journal, 20(1).
30. Okia, M., Ndyomugyenyi, R., Kirunda, J., Byaruhanga, A., Adibaku, S., Lwamafa, D. K., & Kironde, F. (2013). Bioefficacy of long-lasting insecticidal nets against pyrethroid-resistant populations of Anopheles gambiae s.s. from different malaria transmission zones in Uganda. Parasites and Vectors, 6(1), 1–10.
31. Opiyo, M. A., & Paaijmans, K. P. (2020). "We spray and walk away": Wall modifications decrease the impact of indoor residual spray campaigns through reductions in post-spray coverage. Malaria Journal, 19(1).
32. Tangena, J. A. A., Hendriks, C. M. J., Devine, M., Tammaro, M., Trett, A. E., Williams, I., Depina, A. J., Sisay, A., Herizo, R., Kafy, H. T., Chizema, E., Were, A., Rozier, J., Coleman, M., & Moyes, C. L. (2020). Indoor residual spraying for malaria control in sub-Saharan Africa 1997 to 2017: An adjusted retrospective analysis. In Malaria Journal (Vol. 19, Issue 1). BioMed Central Ltd.
33. Russell, T. L., Beebe, N. W., Cooper, R. D., Lobo, N. F., & Burkot, T. R. (2013). Successful malaria elimination strategies require interventions that target changing vector behaviours. 1–5.
34. Sherrard-smith, E., Grif, J. T., Winskill, P., Corbel, V., Pennetier, C., Djénontin, A., Moore, S., Richardson, J. H., Müller, P., Edi, C., Protopopoff, N., Oxborough, R., Agossa, F., Guessan, R. N., Rowland, M., & Churcher, T. S. (2018). Systematic review of indoor residual spray ef fi cacy and effectiveness against Plasmodium falciparum in Africa.
35. Abong'o, B., Gimnig, J. E., Torr, S. J., Longman, B., Omoke, D., Muchoki, M., ter Kuile, F., Ochomo, E., Munga, S., Samuels, A. M., Njagi, K., Maas, J., Perry, R. T., Fornadel, ---with pirimiphos-methyl (Actellic 300CS) on entomological indicators of transmission and malaria case burden in Migori County, western Kenya. Scientific Reports, 10(1), 1–14.
36. Kleinschmidt, I., Bradley, J., Knox, T. B., Mnzava, A. P., Kafy, H. T., Mbogo, C., Ismail, B. A., Lines, J., & Donnelly, M. J. (2018). Implications of insecticide resistance for malaria vector control with long-lasting insecticidal nets : a WHO-coordinated , prospective , international , observational cohort study. 18(June).
37. Haji, K. A., Thawer, N. G., Khatib, B. O., Mcha, J. H., Rashid, A., Ali, A. S., Jones, C., Bagi, J., Magesa, S. M., Ramsan, M. M., Garimo, I., Greer, G., Reithinger, R., & Ngondi, J. M. (2015). Efficacy , persistence and vector susceptibility to pirimiphos-methyl (Actellic ® 300CS) insecticide for indoor residual spraying in Zanzibar. Parasites & Vectors, 1–7.
38. Back, R. (2010). 1 . The burden of malaria Malaria kills children in. 17–23.
39. Coetzee, M. (2020). Key to the females of Afrotropical Anopheles mosquitoes (Diptera : Culicidae). Malaria Journal, 1–20.
40. Control, M., Swiss, E., & Winter, T. P. H. (2016). Vector Control Strategies and Successes … and challenges.
41. Ekoko, W. E., Ambene, P. A., Bigoga, J., Mandeng, S., Piameu, M., Nvondo, N., Toto, J. C., Nwane, P., Patchoke, S., Mbakop, L. R., Binyang, J. A., Donelly, M., Kleinschmidt, I., Knox, T., & Mbida, A. M. (2019). Patterns of anopheline feeding / resting behaviour and Plasmodium infections in North Cameroon , 2011 – 2014 : implications for malaria control. Parasites & Vectors, 1–12.
42. Fullman, N., Burstein, R., Lim, S. S., Medlin, C., & Gakidou, E. (2013). Nets, spray or both? The effectiveness of insecticide-treated nets and indoor residual spraying in reducing malaria morbidity and child mortality in sub-Saharan Africa. Malaria journal, 12(1), 1-10.
43. Godfray, H. C. J. (2013). Mosquito ecology and control of malaria. Journal of Animal Ecology, 82(1), 15–25.
44. Helinski, M. E. H., Nuwa, A., Protopopoff, N., Feldman, M., Ojuka, P., Oguttu, D. W., Abeku, T. A., & Meek, S. (2015). Entomological surveillance following a long-lasting insecticidal net universal coverage campaign in Midwestern Uganda. Parasites & Vectors, 1–11.
45. Indicator, M. (2009). Uganda Bureau of Statistics and ICF International Macro, 2010. Uganda Malaria Indicator Survey.
46. Kabbale, F. G., Akol, A. M., Kaddu, J. B., & Onapa, A. W. (2013). Biting patterns and seasonality of Anopheles gambiae sensu lato and Anopheles funestus mosquitoes in Kamuli District, Uganda. Parasites & vectors, 6, 1-9.
47. Kigozi, S. P., Kigozi, R. N., Sebuguzi, C. M., Cano, J., Rutazaana, D., Opigo, J., ... & Pullan, R. L. (2020). Spatial-temporal patterns of malaria incidence in Uganda using HMIS data from 2015 to 2019. BMC public health, 20(1), 1-14.
48. Ochomo, E., Bayoh, N. M., Kamau, L., Atieli, F., Vulule, J., Ouma, C., Ombok, M., Njagi, K., Soti, D., Mathenge, E., Muthami, L., Kinyari, T., Subramaniam, K., Kleinschmidt, I., Donnelly, M. J., & Mbogo, C. (2014). Pyrethroid susceptibility of malaria vectors in four Districts of western Kenya. Parasites and Vectors, 7(1), 1–9.
49. Okia, M., Hoel, D. F., Kirunda, J., Rwakimari, J. B., Mpeka, B., Ambayo, D., Price, A., Oguttu, D. W., Okui, A. P., & Govere, J. (2018a). Insecticide resistance status of the malaria mosquitoes : Anopheles gambiae and Anopheles funestus in eastern and northern Uganda. Malaria Journal, 1–12.
50. Okia, M., Hoel, D. F., Kirunda, J., Rwakimari, J. B., Mpeka, B., Ambayo, D., Price, A., Oguttu, D. W., Okui, A. P., & Govere, J. (2018b). Insecticide resistance status of the malaria mosquitoes: Anopheles gambiae and Anopheles funestus in eastern and northern Uganda. Malaria Journal, 17(1), 157.
51. Perugini, E., Guelbeogo, W. M., Calzetta, M., Manzi, S., Virgillito, C., Caputo, B., Pichler, V., Ranson, H., Sagnon, N.

F., Torre, A., & Pombi, M. (2020). Behavioural plasticity of Anopheles coluzzii and Anopheles arabiensis undermines LLIN community protective effect in a Sudanese - savannah village in Burkina Faso. Parasites & Vectors, 1–10.

52. Tukei, B. B., Beke, A., & Figueroa, H. L. (2017). Assessing the effect of indoor residual spraying (IRS) on malaria morbidity in Northern Uganda : a before and after study. Malaria Journal, 1–9.
53. Were, V., Buff, A. M., Desai, M., Kariuki, S., Samuels, A., Ter Kuile, F. O., Phillips-Howard, P. A., Patrick Kachur, S., & Niessen, L. (2018). Socioeconomic health inequality in malaria indicators in rural western Kenya: Evidence from a household malaria survey on burden and care-seeking behaviour. Malaria Journal, 17(1).
54. West, P. A., Protopopoff, N., Wright, A., Kivaju, Z., Tigererwa, R., Mosha, F. W., Kisinza, W., Rowland, M., & Kleinschmidt, I. (2014). 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. PLoS Medicine, 11(4).