Comparison of Different Trapping Methods to Collect Malaria Vectors Indoors and Outdoors in Western Kenya

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Abstract

Background

Enhanced vector surveillance, is one of the 4 pillars of the WHO's global vector control response (2017–2030). Human landing catches are the gold standard for entomological surveys but are difficult to implement and expose collectors to mosquito bites and potentially to malaria infection. Other surveillance tools such as light traps, pyrethrum spray catches and aspiration are less expensive and do not expose collectors to potentially infectious mosquitoes, but they are difficult to implement outdoors and/or to assess duration of collection/standardize collection effort. This study evaluated four mosquito trapping methods that may be cheaper, easier, and less risky to implement compared to human landing catch.

Methods

Three mosquito sampling methods (UV light traps, CDC light traps and Prokopack aspiration) were evaluated against human landing catches in two villages of Rarieda sub-county, in Siaya County, western Kenya. UV light traps, CDC light traps and human landing catches were conducted in three locations: inside houses, 10 meters from the house and 10 meters from the compound boundary. These were done every hour from 17:00 until 07:00. Prokopack aspiration was done indoors and outdoors of houses adjacent to the light trap and HLC houses from 07:00 until 11:00. Analyses of mosquito densities, species abundance and sporozoite infection prevalence were performed across all sampling methods. Species within the *An. gambiae* and *An. funestus* species complexes were identified using PCR. ELISAs were used to determine mosquito sporozoite infection prevalence. Data analysis was done in R statistical software.

Results

A total of 5,370 male and female *Anopheles* mosquitoes were sampled from 608 trapping efforts. *An. funestus* constituted 70.3% (n = 3,877) of the sampled *Anopheles* mosquitoes while *An. coustani* was 19.7% and *An. gambiae* s.l. was much lower at 7.2%. 93.8% of *An. funestus* s.l. samples processed through PCR were *An. funestus* s.s. and 97.8% of *An. gambiae* s.l. were confirmed to be *An. arabiensis*. Only *An. funestus* samples were positive for sporozoites, with a species specific sporozoite infection prevalence of 3.1%. Indoor aspiration captured the highest number of *An. funestus* (mean = 6.74; RR = 7.49 compared to indoor HLC, 95% CI 3.95–14.22, $P < 0.001$) followed by indoor UV-LT, (mean = 3.7; RR = 3.6, 95% CI 2.02–6.42, $P < 0.001$) and indoor CDC-LT (mean = 1.74; RR = 1.85, 95% CI 1.02–3.33, $P = 0.042$). In pairwise comparisons, significantly different numbers of *An. funestus* were collected by all indoor methods with the most collected by aspiration and the fewest by HLC. For *An. arabiensis*, indoor UV-LT and indoor CDC-LT each captured an average of 0.18 per trap-night which were significantly higher than HLC indoors. Outdoors, UV-LT collected significantly higher numbers of *Anopheles* mosquitoes across all species analyzed (*An. funestus*: mean = 1.69, RR = 4.27 compared to outdoor HLC, 95% CI

2.20–8.31, *P* < 0.001; *An. arabiensis*: mean = 0.22, RR = 15.64, 95% CI 1.97-124.36, *P* = 0.009; *An. coustani*: mean = 3.74, RR = 10.48, 95% CI 4.37–25.14, *P* < 0.001) when compared to outdoor HLC. Hourly biting in UV-LT and CDC-LT indicated different peaks compared to HLC for *An. funestus* collected indoors.

Conclusions

Anopheles funestus remains the predominant malaria vector in the region and was primarily caught indoors. *Anopheles arabiensis* were trapped in similar both indoors and outdoors while and *An. coustani* were mostly collected outdoors with UV-LTs. UV-LT and CDC-LT collected higher numbers of the primary *Anopheles* mosquitoes indoors and outdoors except for *An. funestus* indoors where aspiration was the most efficient method. The UV-LT generally collected more mosquitoes than the CDC-LT indicating UV-LTs may be an efficient tool for monitoring populations of *Anopheles* mosquitoes. Differences in hourly biting by different collection methods indicate the need to further investigate the behaviour of *An. funestus*.

Introduction

The Global Vector Control Response 2017–2030 (GVCR) provides a framework to enhance vector control through improved capacity and surveillance, and through better coordination and integrated action across sectors and diseases. One of the four pillars of this strategy is enhanced vector surveillance [1]. Robust vector surveillance is critical for monitoring currently recommended vector control tools as well as to evaluate novel control strategies [2]. WHO's objectives for vector surveillance include: characterizing receptivity (to evaluate vector presence and density to enable selection and stratification of interventions), tracking of malaria vector densities (for selection and timing of vector control deployment by biting time or seasonality of transmission), monitoring of insecticide resistance (IR) for selecting insecticides to be used by programmes, and identifying other threats to vector control efficacy including detecting gaps in intervention coverage [3, 4]. However, the range of entomological surveillance methods currently available may lack the sensitivity to detect subtle changes in vector behaviours or may not be adequate to evaluate the performance of novel vector control tools that may target a greater diversity of adult mosquito behaviours [5, 6].

The most common vector control tools—long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS)—target indoor biting mosquitoes that are most active when people are in bed (LLINs) or that spend time resting on walls inside the house (IRS). LLINs and IRS have a direct impact on vector bionomics [7] and historically have been monitored using human landing catches (HLC), CDC light traps, pyrethrum spray catches, and aspiration techniques.

HLC is considered the gold standard for sampling host-seeking mosquitoes [8] and for the estimation of entomological exposure rates [9, 10], for the evaluation of vector control interventions, and for the study of mosquito behaviour [9, 11, 12]. However, HLC is labor-intensive, exposes human collectors to potentially infectious mosquito bites, and is subjected to collector bias [13]. Other surveillance tools like light traps, pyrethrum spray catches and aspiration are less expensive and can be implemented more

widely, but the information they provide is generally limited to indoor abundance. These methods either cannot be implemented outdoors at all or are thought to be inefficient in capturing mosquitoes outdoors. Furthermore, they generally do not provide information on mosquito behaviour, particularly the time and location of mosquito biting. Additionally, in the context of evaluating novel vector control tools, it is prudent assess surveillance tools that can holistically provide information on subtle changes in mosquito behaviour, hence the inclusion of two outdoor locations in this study.

This study evaluated CDC light traps (CDC-LT), UV light traps (UV-LT) against HLC and Prokopack® aspiration (hereafter referred to as aspiration) conducted either inside or outside houses.

Methods

Study area

The study was conducted in Memba (-0.16118, 34.36639) and Mabinju (-0.17966, 34.37003) villages in Rarieda sub-county, Siaya county, western Kenya. Residents of the area live in scattered compounds which consist of an average of 3 houses occupied by closely related family members and interspersed with farmlands. The area immediately around the house structures is usually delineated from the surrounding farmland by a fence or hedges. The area experiences intense, year-round malaria transmission [14] with *Plasmodium falciparum* as the predominant malaria species and *An. funestus, An. arabiensis* and *An. gambiae* sensu stricto (s.s.) the main vectors [15, 16]. Historically, malaria transmission in western Kenya was very high with an estimated 300 infectious bites per person per year in the late 1980s and early 1990s [17]. Transmission has declined substantially since then, largely due to the scale up of insecticide treated nets through mass distributions targeting universal coverage (1 net for every 2 people) supplemented with routine distribution to pregnant women and children < 1 year [18]. However, the burden of malaria remains high with parasite prevalence at 19% in children aged 6 months to 14 years in the region [19]. Additionally, the deployment of malaria vector control tools such as ITNs has been accompanied by shifts in vector populations in this region beginning with the near complete disappearance of *An. funestus* [20], followed by a decline in *An. gambiae* s.s. [16] and a return of *An. funestus* [15]. These shifts in mosquito populations necessitate frequent evaluation of trapping tools.

Mosquito trapping methods

Light traps: CDC light traps (model 512) and UV light traps (model 912) (John W. Hock Company, Gainesville, Florida, U. S. A), without artificial attractants, were used. The CDC light trap uses an incandescent light, while the UV light traps are similar to CDC-LTs in design but with an ultraviolet light bulb. The traps were installed by hanging them approximately 1.5 m above the ground either indoors or outdoors. The indoor traps were placed next to a person sleeping under a bed net whereas the outdoor traps were placed either 10 meters from the structure (referred to hereafter as outdoors close) or 10m outside the compound (referred to hereafter as outdoors far) where indoor sampling was conducted. The outdoor traps were not baited. All light traps were powered by a rechargeable 12-volt battery and were switched on at 17:00. Collection cups for the traps were replaced every hour of the night by field staff in

all instances of outdoor traps and a subset of indoor light traps. Twelve traps were not collected hourly indoors as the compound owners refused entry after they had gone to bed. In those houses, light traps were collected at 07:00 the next morning. Data analysis from these houses included hourly mosquito activity up to the last time entry was granted; the rest of the collections till morning were excluded in the analysis of hourly biting but aggregate numbers of mosquitoes collected throughout the night were included in comparisons of trap efficiency.

Human landing catches: To reduce the risk of transmission of Covid-19, collectors were recruited from the compounds in which they lived. Collectors were males above the age of 18 years, organized into teams comprised of 6 volunteers. The team of six volunteers per compound were split into one indoor and two outdoor locations and they worked in two shifts. The first shift ran from 17:00 until 00:00 when the next team took over until 07:00. The volunteers were trained in HLC and provided with a flashlight, a mouth aspirator, mosquito collection cups and hurricane lamp. The collectors sat on a chair with their legs exposed from foot to knee and captured mosquitoes as soon as they landed on an exposed leg [21]. Collections were conducted over 45 minutes within each hour with a 15-minute break to allow collectors to rest and to change collection cups. Each hour's collection was kept separately in labelled paper cups. Supervisors were assigned to coordinate the collection activities and ensure volunteers were consistently engaged in mosquito collections throughout each collection night. HLC data was collected using tablets, with the forms programmed in CommCare® (Dimagi, Inc, Massachusetts, USA).

Resting collections (Aspiration): Prokopack aspirators (Model 1419, John W Hock Company, Gainesville, Florida, USA) were used to collect mosquitoes resting indoors and outdoors from 07:00 to 11:00. A total of ten sleeping structures from different compounds nearest to the light trap and HLC houses were conveniently selected for aspiration. Sampling was done by moving the aspirator along the walls and the roof, in dark corners, and underneath furniture in the house to collect indoor resting mosquitoes for 10 minutes in each structure. Outdoor sampling was performed by aspirating from clay pots and other water collection containers that were located within 5 meters of each sampled house. After every collection, the samples were released into an adult mosquito cage for sorting. The sampled mosquitoes from each collection were transferred to labeled paper cups per collection separating the outdoor and indoor catches.

Study design

Compounds from which mosquitoes were to be collected each night were randomly selected from the database of houses that had been identified in the study area and different households were randomized every night. The HLC, CDC-LT and UV-LT were rotated nightly among 10 compounds in each village. Each compound had only one trap type placed in three different positions: indoors, outdoors close and outdoors far. These locations were selected to enable a comprehensive assessment of where sugar fed mosquitoes were likely to be captured, as has been described in detail in a separate article [22]. Indoor light traps were placed next to a person under a bed net at a height of 1.5m. Outdoor light traps were placed at a height of 1.5m but were not baited with either a human or an animal. Aspiration collections were conducted every morning preceding the light trap and HLC collections at the indoor and outdoor

close locations but no aspirations were conducted at the outdoor far locations. The mosquito collections were done for four days every week over the two months' study duration.

Mosquito processing

Each paper cup was labeled with a unique collection and house code generated from a tablet for every sampled compound and then sent to the field laboratory in Lwak, Asembo for morphological assessment. Entomology field supervisors and a driver collected the paper cups with mosquitoes from the HLC collectors and light trap collection cups every hour of the night and placed them in cooler boxes containing ice packs for transport to the field laboratory. Upon reception at the field laboratory, the samples were immobilized with chloroform in a killing jar or by freezing at -20^{0}C. The mosquitoes were separated by species, sex, and the abdominal status for females and numbers collected per trap recorded. The mosquitoes were identified morphologically using taxonomic keys [23] to differentiate between *An. funestus* s.l. and *An. gambiae* s.l. and other secondary malaria vectors.

Molecular assays

Polymerase chain reaction (PCR) was used to differentiate between mosquitoes of the *An. gambiae* s.l. complex following the protocols described by Scott et al. [24] and between sibling species of the *An. funestus* complex using the protocols described by Koekemoer et al. [25]. Sporozoite infection rates were determined by enzyme linked immunosorbent assay (ELISA) using the protocol adapted from Wirtz et al. as described in the MR4 Methods in *Anopheles* Research [26, 27].

Data analysis

Vector abundance was assessed using descriptive statistics (means, SD, proportions, and 95% CI). The human biting rate (HBR) per person per hour was derived as an average of total number of anophelines captured divided by the total collection effort. For HLCs, no adjustments were made for the fact that collectors were operating for only 45 minutes within each hour. Generalized linear mixed models (GLMM) using Template Model Builder (glmmTMB) were fitted using negative binomial distribution for analysis of mosquito numbers by various collection methods. Models were adjusted for repeated measures using the structure ID as a random effect. Analyses of trap comparisons were conducted on the 3 most predominant species complexes *An. funestus* s.l., *An. gambiae* s.l. and *An. coustani* s.l. individually. For assessment of hourly trap catches, data were only including structures that had at least 12–14 collections during the night; structures/nights that did not achieve this threshold were excluded from these analyses of hourly collections. All data analyses were performed using R statistical software version 4.1.2 and the significance level was set at $\alpha = 0.05$.

Results

Abundance of Anopheles mosquitoes

A total of 5,370 male and female *Anopheles* mosquitoes were sampled during the study period from a total of 608 trapping efforts: CDC-LT (165), UV-LT (152), aspiration (158) and HLC (133). *An. funestus* constituted more than half (n = 3,777; 70.3%) of the sampled *Anopheles* mosquitoes with the rest being *An. gambiae* s.l. (n = 385; 7.2%), *An. coustani* (n = 1,061; 19.7%) and other *Anopheles* species (n = 147; 2.7%) including *An. pharoensis* (n = 120), *An. rufipes* (n = 16), *An. gibbinsi* (n = 5), *An. parensis* (n = 3), *An. maculipalpis* (n = 1), *An. chrysti* (n = 1), and *An. tenebrosus* (n = 1). Only *An. rufipes* (n = 3), *An. pharoensis* (n = 1) and *An. parensis* (n = 1) of the secondary Anophelines, other than *An. coustani* were collected indoors; the rest were trapped outdoors.

A proportion of the sampled mosquitoes (51% of *An. funestus* and 53% of *An. gambiae*) were processed for species identification by PCR and sporozoite detection using ELISA. Out of the 1,760 *An. funestus* s.l. samples analyzed by PCR, 1,650 (93.8%) were confirmed to be *An. funestus* s.s. and 45 (2.6%) *An. leesoni*, while 65 (3.7%) did not amplify. A total of 214 *An. gambiae* s.l. were processed through PCR out of which 209 (97.8%) were confirmed to be *An. arabiensis* and the remaining 5 (2.3%) samples did not amplify. A sample of the three predominant species *An. funestus* s.l. (n = 862), *An. arabiensis* (n = 168) and *An. coustani* (n = 358) were analyzed for *P. falciparum* sporozoite infection. Only *An. funestus* samples were positive, with a species specific sporozoite infection prevalence of 3.1% (27/862).

Comparison of mean numbers of Anopheles mosquitoes caught per trapping method each night/day

The average number of mosquitoes collected indoors each night by HLC was 0.97 for *An. funestus*, 0.03 for *An. arabiensis*, and 0.08 for *An. coustani*. When compared to indoor HLC, indoor aspiration method captured the highest number of *An. funestus* with a mean of 6.74, (RR = 7.49, 95% CI 3.95–14.22, $p < 0.001$) followed by indoor UV-LT with a mean of 3.70, (RR = 3.60, 95% CI 2.02–6.42, $p < 0.001$) then indoor CDC-LT with a mean of 1.74 (RR = 1.85, 95% CI 1.02–3.33, $p = 0.04$). Significantly more *An. arabiensis* were collected indoors by UV-LT and CD-LT with a mean of 0.18 each, followed by aspiration and HLC. For *An. coustani*, the CDC-LT collected the highest number of mosquitoes indoors mean of 0.29 although this difference was not statistically significant ($p = 0.104$). The indoor UV-LT collected a mean of 0.08 *An. coustani* per trap-night while no *An. coustani* were collected by indoor aspiration.

Outdoors, when data was aggregated to night of collection or day of collection (for aspiration), there were no differences in the means for the two outdoor locations (outdoor far and outdoor close) for either the CDC-LT, the UV-LT or HLCs for any of the species. The data sets for the two outdoor locations were therefore combined as outdoors in the daily mean analysis. Outdoor UV-LT collected significantly higher numbers of *Anopheles* mosquitoes across all species analyzed (*An. funestus* mean = 1.69, RR = 4.27, 95% CI 2.20–8.31, $p < 0.001$; *An. arabiensis* mean = 0.22, RR = 15.64, 95% CI 1.97-124.36, $p = 0.009$; *An. coustani* mean = 3.74, RR = 10.48, 95% CI 4.37–25.14, $p < 0.001$) when compared to outdoor HLC. Outdoor CDC-LT also collected significantly higher mosquitoes compared to outdoor HLC for all three species (Table 1). For outdoor aspiration, significantly fewer *An. funestus* were collected per sampling effort compared to HLC ($p = 0.003$).

Table 1
Comparison of mean numbers of *Anopheles* mosquitoes caught by UV-LT, CDC-LT and aspiration to HLC

Species	Collection position	Collection Method*	Mean daily trap catch	RR (95% CI)	*P*-Value
An. funestus	Indoors	CDC_LT[a]	1.74 (1.02–2.45)	1.85 (1.02–3.33)	0.042
		UV_LT[d]	3.70 (2.59–4.82)	3.60 (2.02–6.42)	< 0.001
		Aspiration[c]	6.74 (4.69–8.78)	7.49 (3.95–14.22)	< 0.001
		HLC[b] (Ref)	0.97 (0.61–1.39)	Ref	Ref
	Outdoors	CDC_LT[a]	1.00 (0.74–1.40)	2.75 (1.42–5.32)	0.003
		UV_LT[a]	1.69 (1.06–2.32)	4.27 (2.20–8.31)	< 0.001
		Aspiration[c]	0.06 (0.01–0.12)	0.17 (0.05–0.55)	0.003
		HLC[b] (Ref)	0.37 (0.15–0.60)	Ref	Ref
An. arabiensis	Indoors	CDC_LT[a]	0.18 (0.06–0.29)	5.61 (1.17–26.82)	0.031
		UV_LT[a]	0.18 (0.07–0.30)	5.87 (1.21–28.43)	0.028
		Aspiration[ab]	0.10 (0.03–0.17)	3.30 (0.63–17.30)	0.158
		HLC[b] (Ref)	0.03 (0–0.08)	Ref	Ref
	Outdoors	CDC_LT[ab]	0.15 (0.05–0.26)	10.81 (1.34–87.35)	0.026
		UV_LT[b]	0.22 (0.11–0.33)	15.64 (1.97–124.36)	0.009
		Aspiration[ac]	0.05 (0–0.11)	3.59 (0.38–34.28)	0.267
		HLC[c] (Ref)	0.01 (0–0.04)	Ref	Ref
An. coustani	Indoors	CDC_LT[a]	0.29 (0–0.64)	3.44 (0.78–15.22)	0.104
		UV_LT[a]	0.08 (0.02–0.15)	1.32 (0.24–7.32)	0.749
		Aspiration[a]	0	-	-

*Post hoc comparison of the trapping methods. Methods bearing the same letter do not differ significantly at 5% level

Species	Collection position	Collection Method*	Mean daily trap catch	RR (95% CI)	*P*-Value
		HLC[a] (Ref)	0.08 (0–0.18)	Ref	Ref
	Outdoors	CDC_LT[a]	2.14 (0.46–3.82)	10.74 (4.48–25.77)	< 0.001
		UV_LT[a]	3.74 (1.28–6.20)	10.48 (4.37–25.14)	< 0.001
		Aspiration[b]	0.23 (0.05–0.41)	0.70 (0.16–3.01)	0.637
		HLC[b] (Ref)	0.29 (0–0.58)	Ref	Ref

*Post hoc comparison of the trapping methods. Methods bearing the same letter do not differ significantly at 5% level

When a post hoc analysis was done to compare the performance in mean mosquito collection between traps, aspiration collected statistically more *An. funestus* indoors than UV-LT which in turn collected significantly more *An. funestus* compared to indoor CDC-LT. Outdoors, UV-LT and CDC-LT collected significantly more *An. funestus* compared to HLC or outdoor aspiration but there was no statistical difference between outdoor UV-LT and outdoor CDC-LT. UV-LT collected significantly higher number of *An. arabiensis* compared to HLC and aspiration outdoors while CDC-LT collected significantly more *An. arabiensis* compared to HLC only. Outdoor UV-LT and CDC-LT collected more *An. coustani* in relation to HLC and aspiration collections but there were no differences in mean numbers of *An. coustani* by the different trapping methods indoors.

Comparison of hourly mosquito collections by trapping method

The mean number of mosquitoes captured by hour and by location using the three different collection methods are presented in Fig. 1 and the hourly biting patterns are shown in Fig. 2. By HLC, biting for *An. funestus* increased around midnight reaching a plateau that remained consistent throughout the remainder of the night. In contrast, a peak of activity for *An. funestus* was observed by both CDC-LT and UV-LT between 7pm and 8pm which diminished rapidly but activity was still observed throughout the night by both methods. Outdoors, *An. funestus* showed similar patterns although they were less distinct given the lower numbers collected. For *An. coustani* outdoors, a peak in activity was observed by CDC-LT and UV-LT between 8pm and 9pm which declined rapidly though some activity was still observed throughout the night. Numbers of *An. coustani* collected by HLC outdoors or by any collection method indoors were too low to discern a pattern. Similarly, the numbers of *An. arabiensis* collected by the three methods both indoors and outdoors were too low to detect a clear pattern of activity throughout the night.

Discussion

This study compared the efficacy of different trapping methods, placed at different locations around the peridomestic space to identify the most suitable method or set of methods to use as potential alternatives to HLCs. *An. funestus* was predominantly caught resting indoors with aspiration being the most effective method of collection. Based on the mean numbers collected, UV-LT outperformed the CDC-LT in trapping *An. funestus* indoors. For all other comparisons, UV-LT collected more mosquitoes compared to the CDC-LT except for sampling *An. coustani* indoors; however, other than for *An. funestus* indoors, none of these observed differences were statistically significant. The UV-LT and CDC-LT trapped more mosquitoes than HLC both indoors and outdoors. Hourly biting rates in UV-LT and CDC-LT indicated different peaks in biting from that of HLC which raises questions about the physiological state and behavior of mosquitoes captured by the different collection methods.

The observation of *An. funestus* as the primary vector collected during the evaluation of these trapping methods coupled with the fact that these mosquitoes were mostly captured indoors demonstrates the resilience in this vector species after years of high coverage of ITNs. *An. funestus* reemerged [15] after almost being eliminated in the study area following the distribution of ITNs in 2000s [28]. Multiple research groups have reported resurgences of *An. funestus* despite sustained control efforts in multiple countries [15, 29]. The reemergence of *An. funestus* is likely associated with high levels of pyrethroid resistance that developed in this species [30, 31]. The fact that only indoor collected *An. funestus* were positive for sporozoites indicates that the bulk of malaria transmission in this area is likely propagated indoors by this species and complementary indoor vector control tools are needed to achieve malaria elimination.

All the *An. gambiae* s.l. caught by the different trapping methods were *An. arabiensis*. The dominance of *An. arabiensis* compared to *An. gambiae* s.s. following the scale up of ITNs was previously reported by Bayoh et al 2010 [16] indicating that *An gambiae* s.s. has not responded in the same way as *An. funestus* despite the presence of phenotypic and genotypic resistance in *An. gambiae* s.s [32]. *An. arabiensis* were mostly collected outdoors by light traps and aspiration from clay pots, consistent with the species' exophilic and exophagic behaviour previously reported in in the region [33–35]. This likely has enabled them to avoid indoor deployed interventions such as LLINs and IRS [11, 36, 37]. Despite not being detected in the current study, sporozoite positive *An. arabiensis* have been reported previously albeit at lower rates compared to *An. funestus* [15]. Given their tendency to feed and rest outdoors, *An. arabiensis* may contribute to residual transmission of malaria [38]. The presence of *An. coustani* in the peri-domestic space has been observed previously [39] but their importance for malaria transmission remains to be elucidated.

Comparison of different mosquito trapping methods indicates that mechanical aspirations indoors and UV-LT outdoors captured high numbers *An. funestus* mosquitoes. UV-LT performed well outdoors and indoors, second only to aspiration in the number of *An. funestus* mosquitoes collected indoors. UV-LTs generally collected more mosquitoes than CDC-LTs, possibly because the efficacy of incandescent light in CDC-LTs may be affected by other light sources in the night including moonlight [40]. Also, mosquitoes have diverse response to different light spectra as previously reported where mosquito response to

artificial light indicated that blue and green light is often more attractive than that in the yellow-orange and red regions of the visible spectrum [41, 42]. UV-LT is a largely unexplored trapping technique that could be useful for both indoor and outdoor trapping of mosquitoes especially when evaluating outdoor deployed vector control methods such as ATSBs as was recently done in Mali [43].

Fewer mosquitoes were collected by HLCs compared to both UV-LT and CDC-LT both indoors and outdoors. Similar results have been recorded when HLCs were compared to the Furvela tent trap, the host decoy trap, mosquito electrocuting traps and outdoor CDC light traps [40]. While HLCs are considered the gold standard for monitoring entomological measures of malaria transmission, the low numbers collected suggest they may underestimate entomological inoculation rates. The differences may be due to inefficiency of the mosquito collectors who are expected to remain awake throughout the night and capture mosquitoes in limited light conditions. However, they may also reflect the fact that light traps may capture more than just host-seeking mosquitoes [44–46].

Human landing catches remains the gold standard method for monitoring the abundance and host seeking behaviour of mosquitoes because it has been very difficult to find an alternative, easy to standardize method, that can be conducted in rural settings with limited access to electricity [47–50]. CDC-LT, and less frequently UV-LT, are routinely used in monitoring *Anopheles* abundance during entomological surveillance. These traps are usually set up in the evening and left to run uninterrupted the whole night and therefore are unable to account for the specific hours at which mosquitoes were trapped as an indicator of host seeking behaviour [9, 44, 51, 52]. Rotator light traps have been used to assess diel mosquito activity in studies of *Aedes* mosquitoes [53–55] and less frequently to monitor *Anopheles* activity [47, 56, 57]. In this study, CDC-LT and UV-LT bags were collected every hour through the night. Despite being labor intensive and intrusive, this method allowed a direct comparison of the mosquito host seeking behaviour patterns to those usually depicted by HLC. In western Kenya, previous HLC collections demonstrated a single peak in biting by *An. funestus* that extended from midnight until around 6am [58] similar to what was observed in this study. CDC-LT and UV-LT identified high mosquito activity early in the evening when people are unlikely to be under the protection of their bed nets and early in the morning indoors when people are getting out of bed and embarking on their daily activities. This differed from the HLC collections which is consistent with previous observations where biting was observed primarily when people were in bed and under their bed nets. Similar observations have been reported in the highlands of western Kenya [59] where it was suggested that transmission could occur at times when people were not under the protection of nets. However, the differences in collection times by the different methods raises questions about mosquito behaviour in the peridomestic space including those unrelated to host seeking. *An. arabiensis* densities were too low to derive any meaningful inferences on their behaviour indoors but like *An. coustani*, were observed to peak early in the evening outdoors.

Conclusion

This study indicates that aspiration, CDC-LTs and UV-LTs are efficient methods for trapping *Anopheles* mosquitoes indoors and outdoors but are not a true proxy of HLC collections. The heterogeneity present in trapping outcomes results from variations in traps, their location and mosquito species. The different trends observed between mosquitoes trapped by HLCs and light trap at different times of the night suggest that these methods collect mosquitoes with varied activities during the night and care must be taken when interpreting the results.

Abbreviations

CDC	Centers for disease control
ELISA	Enzyme linked immunosorbent assay
GVCR	Global Vector Control Response
HLC	Human landing catch
ID	Identification
IRS	Indoor residual spray
LLINs	Long lasting insecticidal nets
KEMRI	Kenya Medical Research Institute
PCR	Polymerase chain reaction
CDC-LTs	Center for disease control light traps
UV-LTs	Ultra violet light traps
WHO	World Health Organization

Declarations

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Author contribution

J. K., S.O., participated in study design, coordinated sample collection and processing and drafted the manuscript, V.M., B.A. performed statistical analysis and interpretation and manuscript reviews, D.P.M., M.D., C.O., participated in data interpretation, manuscript reviews and revisions, J.E.G., and E.O. conceptualized the study, supervised its implementation and analysis and offered technical support. All authors read and approved the final manuscript.

Disclaimer

The findings and conclusions in this manuscript are those of the authors and do not necessarily represent the official position of the Kenya Medical Research Institute or the US Centers for Disease Control and Prevention.

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Ethics approvals and consent to participate

The study was approved by the scientific and ethics review unit (SERU) of the Kenya Medial Research Institute (protocol number KEMRI/SERU/CGHR/123/3613) and by Institutional Review Board of the US Centers for Disease Control and Prevention (IRB# 7112). Written informed consent was obtained from all HLC collectors. The HLC collectors were given mefloquine malaria prophylaxis (Cheplapharm Arzneimittel GmbH, Ziegelhof 24, 17489 Greifswald, Germany) and regularly tested for malaria. The prophylaxis treatment started 1 week before collections began and were given repeat doses once every week through the collection period, until 4 weeks after collections ended. Verbal consent was sought from the compound head to use light traps and Prokopack aspiration in their compounds.

Competing interests

Not applicable

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Figures

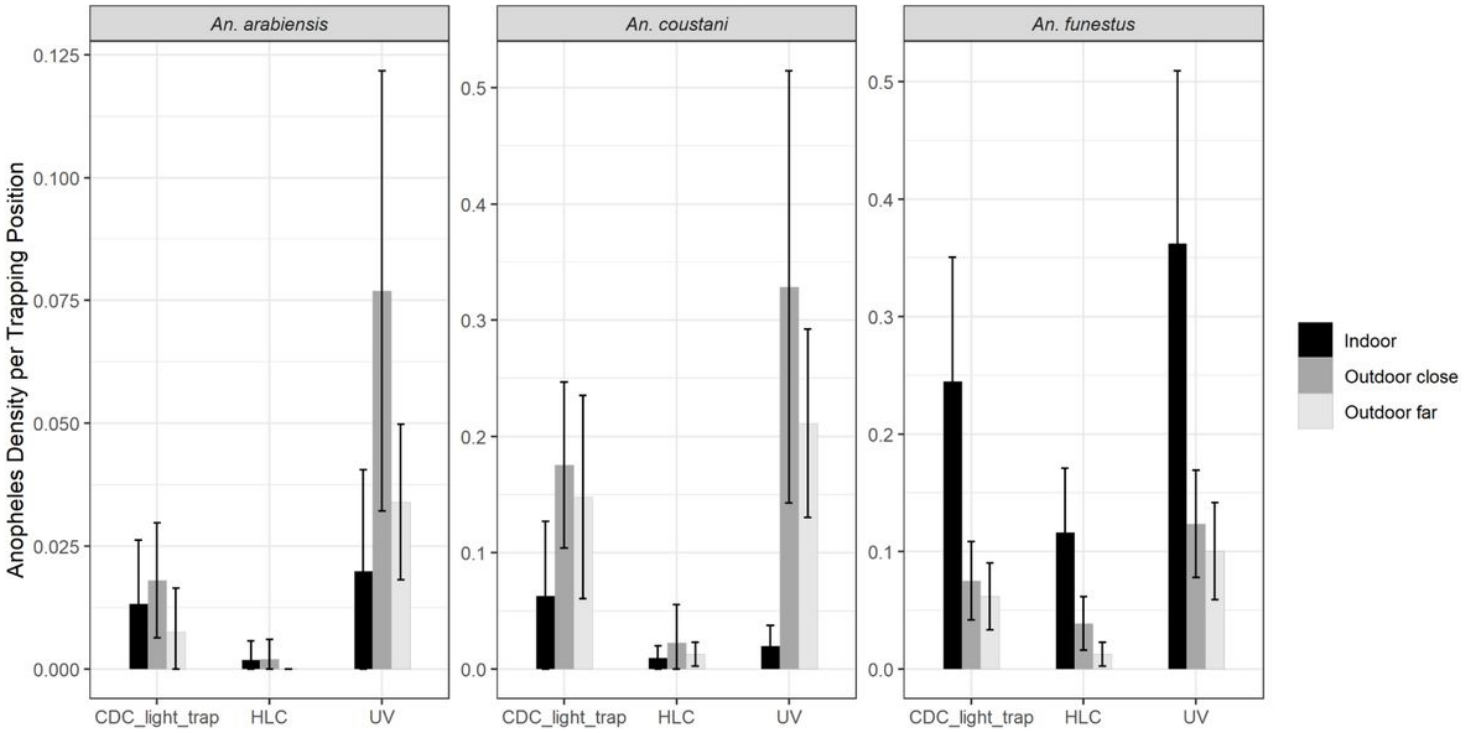

Figure 1

Comparison of UV-LT, CDC-LT and HLC at three different locations

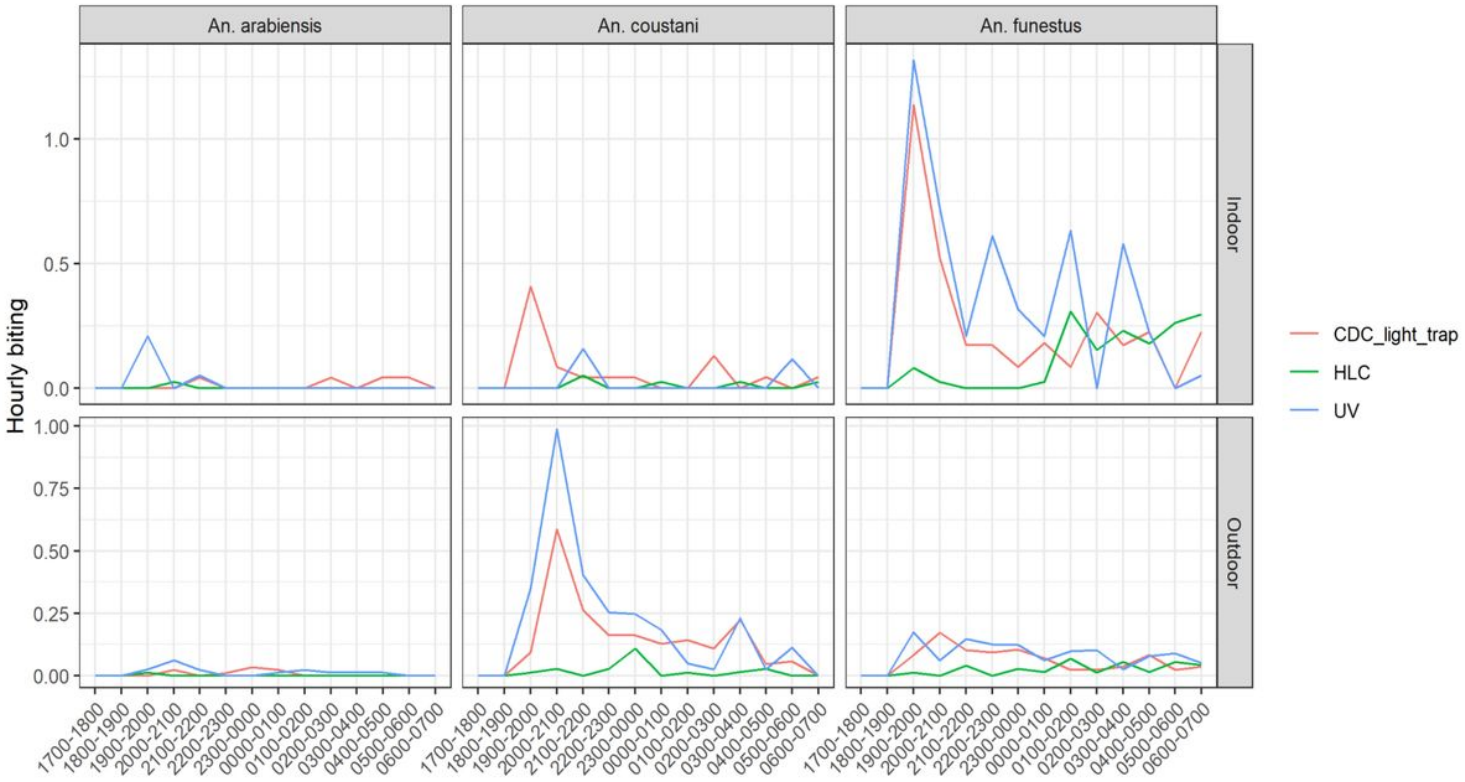

Figure 2

Comparison of hourly trap catches from indoor and outdoor locations