Assessment of the Effectiveness of Various Adult Mosquito Sampling Methods in a Thickly Populated Urban Slum Settlement - A study from Besant Nagar, Chennai, India

Sangamithra Ravishankaran  
National Institute of Malaria Research

Aswin Asokan  
National Institute of Malaria Research

Johnson Amala Justin N A  
Ministry of Health and Family Welfare

Shalu Thomas  
Madras Christian College

Vasna Joshua  
National Institute of Epidemiology

Manu Thomas Mathai  
Madras Christian College

Alex Eapen (✉ alexeapen@yahoo.com)  
National Institute of Malaria Research

Research

Keywords: CDC-Light trap, Urban slum, Anopheles stephensi, Resting collection, Vector incrimination

DOI: https://doi.org/10.21203/rs.3.rs-30372/v1

License: ☇ ☀ This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

Background

In an urban scenario, it is an arduous task to collect adult *Anopheles stephensi*, unlike the immature forms due to various reasons such as the complex spatial heterogeneity, intricacies in feeding, and resting preferences. Thus it is necessary to have more specific and accurate assessments of adult vector density by performing various collection methods and timeframes to achieve appropriate and sustainable vector control strategies.

Methods

The study was undertaken in two phases, (i) resting dawn collections in cattle sheds from Jan 2015 to Dec 2016 to assess the possibility of maximum collection of *An. stephensi* compared to dusk collections done previously and to find out the best appropriate collection strategy for vector control and (ii) light trap collections from Jan 2016 to Apr 2017 to determine the efficiency and reliability of CDC light trap in sampling the anophelines including the urban malaria vector, *Anopheles stephensi* in human dwellings besides, other mosquito species. The man-hour density of *An. stephensi* in cattle sheds were calculated and its infection rate was analyzed by circum-sporozoite ELISA (CS-ELISA). Binary logistic regression analysis was done to ascertain the variables associated with the collection of *Anopheles stephensi* using a light trap.

Results

The resting collections in cattle sheds indicated that dawn collections yielded more *Anopheles stephensi* than at dusk. The resting stage female (fully fed, semi-gravid, and gravid) mosquitoes were more in dawn collections than in dusk collections. The CDC-light trap collections revealed that *An. stephensi* collected indoors were more than outdoor in human dwellings. Further, vector incrimination results observed that 0.56% of the female *An. stephensi* from cattle sheds were infected in 2014 (dusk), 0.15%, and 0.09% in 2015 and 2016 respectively in dawn collections. Nevertheless, 2.3% of *An. stephensi* collected by CDC light trap in human dwellings were positive for *Plasmodium vivax* (*Pv210*) infection. Binary logistic regression analysis proved that the presence of *An. stephensi* in human dwellings was significantly influenced by seasons, the number of rooms in the house, number of household members, and also the use of repellents.

Conclusions

The different collection (resting and light trap) approaches were assessed to find out the appropriate collection method and time which could yield the maximum number of *An. stephensi* with the existing resources. The study revealed that dawn collections during the early hours as the most suitable time to collect wild *An. stephensi* in an urban setting. The present study thus would help in chalkling out an operationally feasible vector control strategy with the most appropriate methodology, timeframe useful for effective control of vectors.
**Background**

Malaria remains a life-threatening disease in many tropical and sub-tropical areas, caused by the protozoan parasite *Plasmodium*, transmitted by female *Anopheles* mosquitoes, which bite mainly between dusk and dawn. In 2018, India contributed 3.4% of an estimated 228 million cases and 2% of 4.05 lakh deaths globally. India shared the highest (47%) among the total *Plasmodium vivax* cases worldwide [1]. Tamil Nadu state recorded 3758 cases in 2018, 75% of which was contributed by Chennai [2]. Nonetheless, urban malaria is at low risk compared to rural malaria, accounting for 6–28% of the estimated global annual malaria disease incidence, and also warrants special attention in the perspective of global malaria elimination efforts [3]. Regardless of effective control measures by the Urban malaria scheme (UMS) of the local health department in Chennai, malaria still remains to be a threat due to its persistent nature. In Chennai, malaria is transmitted by the Asiatic urban malaria vector, *Anopheles stephensi*, which predominantly breeds in overhead tanks, besides, wells and other water storage habitats [4]. The adult vectors predominantly are found resting in cattle sheds and thatched structures [5].

In a real urban scenario, it is a strenuous task to find and collect adult *An. stephensi* unlike the immature forms due to the complex spatial heterogeneity, the resultant changes, besides, the intricacies in feeding, and resting preferences. The adult mosquitoes rest in places/areas, less disturbed, dark, and damp place with conducive microclimatic conditions [5, 6]. Urbanization and better economy have forced the urban dwellers to have modernized, long-lasting roofing structures such as concrete instead of the older thatched and tiled roof which provided ample places for the vector mosquitoes to hide and rest [7]. Further, the increased use of mosquito repellents has driven the less susceptible *An. stephensi* out and therefore are forced to find out suitable resting places in the vicinity of human and cattle populations [5, 8, 9]. Hence, identification of suitable collection methods, potential target sites, and appropriate time of collection is crucial in yielding better estimates of adult vector populations in urban environments.

The present study was done following the resting collections in cattle sheds (dusk) and pyrethrum spray sheet collection in human dwellings (dawn) [5]. The aim of the study was to check the possibility to have more accurate assessments for adult vector density by performing different collection methods (resting in cattle sheds and CDC light trap in human dwellings all night) and timeframes other than the existing routine methods.

**Methods**

**Study site**

The study was conducted at Besant Nagar (13.0002˚N, 80.2668˚E), a residential area with slums adjacent to the seashore in the south-eastern part of Chennai, characterized by its meso-endemic perennial transmission of malaria, predominantly *Plasmodium vivax*, by *Anopheles stephensi* [4, 5, 10]. The study was conducted in two phases, (i) resting dawn collections were undertaken in cattle sheds from Jan 2015 to Dec 2016 to find out the possibility of collecting the maximum number of *An. stephensi* compared to
dusk collections done previously and to ascertain the most appropriate collection strategy [5] and (ii) light trap collections from Jan 2016 to Apr 2017 to assess the efficiency and reliability of CDC light trap in sampling the anophelines including the local malaria vector *Anopheles stephensi*, in human dwellings besides, other mosquito species, *Culex* and *Aedes* species.

**Resting Collections (dawn) In Cattle Sheds**

Cattle sheds were surveyed in the dawn (04:30 – 06:00 am) using a flashlight and an oral/mouth aspirator. The sheds were selected based on the previous longitudinal surveys [5]. In each cattle shed, 15–30 minutes were spent depending on the size/area and presence or absence of mosquitoes at the time of collection. The collected mosquitoes were brought to the laboratory in a cold chain condition and identified to the species level following standard identification keys. The females were enumerated and graded based on their abdominal conditions. The late-stage fed, gravid, and/or semi-gravid appearance of the abdomen was considered as resting stages, while the unfed guts, and/or freshly fed as feeding stages [11]. The man-hour density (MHD) of *An. stephensi* was calculated by dividing the total number of female mosquitoes collected by total time spent for 1 hour period i.e., (Total female *An. stephensi* collected/total time spent) × 60 [5]. The mosquitoes were then processed for circumsporozoite sandwich enzyme-linked immunosorbent assay (ELISA) following the Malaria Research and Reference Reagent Resource Center (MR4) protocol [12].

**Light Trap Collections In Human Dwellings**

A total of 203 trap collections were carried out in 113 houses out of which, 107 (52.7%) were indoor and 96 (47.3%) outdoor. In respect of the light traps placed in 113 houses, 90 houses had both indoor and outdoor placement of traps. However, in the remaining 23 houses, the traps were either placed indoor (17) or outdoor (6) which was mainly based on the permission of the house owner/ head of the family and availability of appropriate locations/places to fix them. Among the 203 collection sites, *An. stephensi* was trapped in 98 collections which include 79 out of 167 collections (47.3%) in thatched houses, 4 out of 11 (36.4%) in concrete houses, 15 out of 21 (71.4%) in asbestos houses, and none in 4 tiled houses. A total of 224 female *An. stephensi* were collected, 180 in thatched houses, 9 in concrete, and 35 in asbestos roofed houses (Table 2).
### Table 2

*Anopheles stephensi* collections from various structure types in human dwellings (indoor and outdoor)

| Total houses | Placement types | Roof types | No. of collections | Total female An. stephensi collected n (%) | Abdominal condition | Male | No. of female An. stephensi tested for ELISA (%) |
|--------------|-----------------|------------|--------------------|-------------------------------------------|---------------------|------|-----------------------------------------------|
|              | Indoor          | Thatched   | 89                 | 44 (49.4)                                 | UF                  | 107  | 124                                          |
|              |                 |            |                    |                                           | HF                  | 46   | 5    |
|              |                 |            |                    |                                           | FF                  | 5    | 0    |
|              |                 |            |                    |                                           | SG                  | 35   | 18   |
|              |                 |            |                    |                                           | G                   | 20   | 8    |
|              |                 |            |                    |                                           | Total               | 107  | 124  |
|              | Outdoor         | Thatched   | 78                 | 35 (44.9)                                 | UF                  | 96   | 100  |
|              |                 |            |                    |                                           | HF                  | 52   | 52   |
|              |                 |            |                    |                                           | FF                  | 14   | 14   |
|              |                 |            |                    |                                           | SG                  | 15   | 15   |
|              |                 |            |                    |                                           | G                   | 9    | 9    |
|              |                 |            |                    |                                           | Total               | 96   | 100  |

UF-Unfed; HF-Half fed; FF-Fully fed; SG-Semi gravid; G-Gravid
It was noticed that *An. stephensi* trapped indoor were 24% more than outdoor. But the percentage distribution of *An. stephensi* among different structure types was almost similar in both indoor and outdoor. The abdominal condition of the trapped female *An. stephensi* indicated that unfed females were trapped more (43.8%) compared to other stages. The proportion of resting stage females was more indoor (59%) while the proportion of feeding stage females was more outdoor (66%) (Table 2). Among 224 female *An. stephensi* collected, 214 samples were tested for the presence of *Plasmodium* infection by CS-ELISA, out of which 5 (2.3%) were positive for *Plasmodium vivax* (Pv210) infection. Importantly, four positives were from thatched structures (1-indoor, 3-outdoor) and one from an outdoor asbestos structure.

*Anopheles stephensi* density peaked from February 2016 to March 2016 as a result of heavy rainfall due to the cyclonic effect in November and December 2015. The density also peaked during October 2016 and continued till December 2016 owing to heavy rains during September 2016 followed by a decline during the subsequent months (Fig. 2, 4). During the study period, the average temperature ranged from 26.05°C to 28°C in winter, although it was 29.65°C to 32.75°C and 27.65°C to 31.95°C in summer and monsoon seasons. The average relative humidity ranged from 68.5 to 75% in winter, 68 to 75.5% in summer, and 62 to 72.5% in the monsoon season.

Composition of other mosquito species collected along with *An. stephensi* in human dwellings in both indoor and outdoor is illustrated in Fig. 3b. Interestingly, more species were attracted/trapped indoor than outdoor. *Culex quinquefasciatus* was the predominant species both indoor and outdoor followed by *An. stephensi*.

Binary logistic regression analysis revealed that presence of *An. stephensi* was significantly influenced by summer season (Coefficient = 2.094, AOR = 8.117, *P* = 0.003, CI = 2.057–32.033 ) and pre monsoon season (Coefficient = 1.812, AOR = 6.120, *P* = 0.022, CI = 1.303–26.749) comparatively than the monsoon season after controlling other factors; the number of rooms in the house (Coefficient = 0.727, AOR = 2.069, *P* = 0.002, CI = 1.315–3.253); number of household members (Coefficient= -0.337, AOR = 0.714, *P* = 0.017, CI = 0.541–0.943) and use of repellents (Coefficient= -2.113, AOR = 0.121, *P*< 0.001, CI = 0.038–0.382).

### Variables And Data Analysis

The data was analyzed using SPSS version 21. The different parameters obtained and selected for analysis were seasonal variations, roof type of the houses, indoor or outdoor, the number of rooms in the house, number of household members, use of repellents, monthly data of mean temperature, mean relative humidity, total rainfall, and mean wind speed as they were likely to influence the availability of
vectors during the collection period [18–20]. Binary logistic regression analysis was performed to find out the parameters that affect the collection of *Anopheles stephensi* using a light trap.

**Results**

**Resting collections in cattle sheds**

Resting collections undertaken in cattle sheds from 2014 to 2016 showed that collections made at dawn (2015 and 2016) yielded more *Anopheles stephensi* than at dusk (2014) in the same collection settings (Table 1). A total of 214 dawn collections were made in 2015 and 206 collections in 2016 which yielded 2386 (MHD − 45.7) and 5759 (MHD − 115.7) female *An. stephensi* respectively. The resting stage female mosquitoes (fully fed, semi-gravid, and gravid) were more than the feeding stage females in both the dawn and dusk collections. Vector incrimination of female *An. stephensi* revealed that 0.56% were observed to be infected in dusk collections undertaken in 2014. However, it was 0.15% and 0.09% in dawn collections carried out in 2015 and 2016 respectively (Table 1). The reduction in vector incrimination in spite of the high vector density was also reflected in the declining malaria prevalence observed in the study site compared to the previous years. The adult vector density peaked during November 2015 and was maximum in July 2016 (Fig. 2). MHD was observed to be very high (115.7) in the 2016 dawn collection due to heavy rainfall (cyclones) during the end of 2015 and mid of 2016 (Fig. 2). The composition of other mosquito species collected along with *An. stephensi* in cattle sheds (2016) has been depicted in Fig. 3a. *Culex gelidus* was perceived to be more abundant (44.63%), followed by *An. stephensi* (38.14%) and *Culex quinquefasciatus* (16.23%). Other species such as *Armigeres subalbatus*, *Aedes aegypti*, *Anopheles subpictus*, *An. vagus*, *An. barbirostris*, *An. annularis* and *Mansonella annulifera* collected were in negligible numbers (1%).
Table 1
Dawn and dusk resting collections of *Anopheles stephensi* in cattle sheds and the infectivity status

| Year | Dawn No. of collected males (M) | No. of collected females (F) | Total No. collected | Abdominal condition n (%) | Proportion of infectivity |
|------|--------------------------------|-----------------------------|---------------------|---------------------------|--------------------------|
|      | Unfed | Half-fed | Fully-fed | Semi-gravid | Gravid | Total | Positive n (%) |
| Dusk 2014 | 18        | 20        | 74        | 21        | 42      | 45      | 56      | 67      | 23      | 72      | 4      | 3      | 0      | 1      |
|        | (5.7)  | (6.1)    | (7.6)    | (6.2)    | (6.9)   | (7.0)   | (6.1)   | (7.1)   | (3.1)   | (7.3)   | (0.56) | (0.3) | (0.4) | (0.1) |
| Dusk 2015 | 21        | 45        | 23        | 31        | 5       | 68      | 22      | 78      | 10      | 19      | 3      | 3      | 0      | 0      |
|        | (0.2)  | (2.8)    | (0.9)    | (3.3)    | (0.5)   | (2.8)   | (0.9)   | (3.3)   | (0.4)   | (3.3)   | (0.15) | (0.3) | (0.4) | (0.15) |
| Dusk 2016 | 20        | 11        | 57        | 29        | 2       | 15      | 54      | 33      | 7       | 44      | 4      | 4      | 0      | 0      |
|        | (0.0)  | (0.3)    | (0.9)    | (0.3)    | (0.5)   | (0.3)   | (0.9)   | (0.3)   | (0.1)   | (0.4)   | (0.09) | (0.3) | (0.4) | (0.09) |

*(Thomas et al., 2017)*

**Discussion**

The present study analyzed the efficiency in terms of the yield of adult vector collections during dawn and evaluated the CDC-light traps placed inside or outside of residential dwellings in the urban slum settings of Chennai. Thatched houses were surveyed more since a number of vector mosquitoes were collected in these structures besides, the results of the vector incrimination in earlier studies [5, 21]. While the aspirator collections in the cattle sheds and pyrethrum spray sheet collections in human dwellings were used as the main collection tools to get the adult vector density, collections with CDC light traps...
were used as a means to encompass the whole night period to collect the mosquito fauna foraging the different quarters of the night.

**Dawn Collection (resting) Of Mosquitoes**

The dawn resting collections in 2015 yielded 3 times the number of adult mosquitoes compared to the dusk collections in 2014. Surprisingly, there was an increase in vector density and it was even twice more in 2016 compared to the dawn collections in 2015. Although there was an escalation in the vector density, malaria incidence was declining due to the intensified vector surveillance activities targeting two vector-borne diseases (malaria and dengue) and hence the focussed intervention measures. In contrast, *Anopheles* mosquito surveillance in Madagascar revealed that the mosquito density was least during the early morning collections whilst they peaked at 00:00 hour [22]. Elsewhere in Uganda, the vector densities peaked up during early evenings and mornings [23]. The increased number of mosquitoes collected could be attributed to the collection time, as the early morning time corresponds to minimal disturbance and hence the mosquitoes could rest for a longer period. Furthermore, it may also be due to the possible chance of availability of all the mosquitoes, which had otherwise flown in seeking appropriate, cryptic resting places during the later hours of the night besides, fully fed mosquitoes resting and unable to fly after a full, heavy blood meal. Studies from Benin have reported a shift towards the early morning biting behaviour of malaria vectors [24]. This is analogous to the present findings observed in the highly populated urban slum settings. Such possibilities need to be examined further over the years for the successful implementation of effective control measures targeting the vectors.

**Cdc Light Trap Collection**

Overall, there was a 24% detected increase in the number of *An. stephensi* trapped indoors compared to outdoors. This is consistent with the other studies that the CDC light traps are good at trapping indoor mosquitoes, particularly the stage of host-seeking [25]. Further, unfed female *An. stephensi* were more compared to other stages in both indoor and outdoor. This could be due to the presence of the light trap, which partially diverts the mosquitoes from seeking potential hosts [26]. Unfed and half-fed were also more in outdoor collections compared to indoors. Whereas fully fed, the semi gravid and gravid stage was observed to be twice more indoor.

The presence of more fully fed, semi gravid, and gravid stage vector mosquitoes indoors reflects the resting preference in thatched structures and is accordant with the previous studies undertaken at the same site [5]. Moreover, when the proportion of vectors incriminated with malaria parasites was checked, it was found that the percentage of positivity was considerably higher (2.3%) than the previous study (0.65%). This finding affirms the importance of thatched structures as preferred resting sites of the malaria vectors in an endemic urban setting. Further, it points out that, in a busy urban scenario, identification of such a potential resting preference is really worth as it can help to locate the resting hotspots and can prioritize the vector surveillance efforts. This coupled with better allocations of supplies
in terms of resources, manpower can significantly aid in scaling down the disease prevalence. Nevertheless, the presence of more infected vectors in the outdoor underlines the need for evidencing outdoor biting and implementing effective control strategies.

Stratification by season revealed that the presence of *An. stephensi* was significantly influenced by the seasons. *An. stephensi* count varied throughout the study period, unlike in previous years. Over the years, it has been noticed that the climate of Chennai is unpredictable, similar to the scenario detected elsewhere in the world. Precipitation/rainfall varies (Fig. 2) every year which affects the mosquito density. Generally, the most abundant species captured in traps were *Culex quinquefasciatus*, probably because of its preference for human beings [27]. Hence, it is assumed that CDC light traps are beneficial in multiple ways, in terms of mosquito diversity assessments, species prevalence, vector control, and protection from the bites of other mosquito species while targeting *Anopheles* species. These findings are in tune with previous studies, which have shown that CDC light traps were attractive to various genera of mosquitoes [15, 28]. Overall, the number of *Aedes* species collected in this study was low and most of the captured ones were *Aedes aegypti*. Previous studies have also indicated the inefficiency of CDC light trap in capturing *Aedes* species [15, 29]. This could be ascribed to their diurnal biting nature and restricted ability to fly high [30]. The collected *Anopheles* mosquitoes represented all the grades of abdominal conditions. However, with a higher number of feeding stages, it is assumed that feeding status did seem to impact capture efficiency while comparing indoor and outdoor trap locations. This stands in support of a previous study that indicated a preferential capture of fully fed/unfed by CDC light traps in indoor locations in Zambia [31].

The number of female *An. stephensi* was significantly influenced by various factors. For instance, the use of repellents was having an adverse impact on the presence of the vectors. This may be due to the deterrent effects of the chemicals that forced the mosquitoes to drive away from the houses and its close proximity [8]. Also, the number of rooms was found to have a positive correlation with the number of *An. stephensi*. An increased number of rooms, though not necessarily, may indicate the presence of more inhabitants and thereby providing an escalation in the olfactory cues. Also, it will offer the vectors plenty of hideouts, which prevents them from being caught. Surprisingly, the number of inhabitants was negatively correlated with the number of *An. stephensi* collected by the light traps. It was assumed that, as the olfactory cues got stronger, the mosquitoes were preferably attracted to humans than to the traps. However, the number of female *An. stephensi* was not appeared to be influenced by climatic factors like local mean temperature, relative humidity, rainfall or wind speed, or the location/placement of traps, indoor or outdoor.

It is well known that adult collections of *Anopheles stephensi* in an urban setting are an onerous task unlike locating the immature breeding habitats. The situation is more acute and complicated when malaria is on a declining trend. This practical difficulty may be attributed to the successful and more organized vector control efforts targeting the potential breeding habitats thereby reducing/eliminating them. Further, the intensive vector-control efforts by the health department over the years against dengue has really resulted in a collateral benefit to malaria vector control as few of the clear breeding habitats are
mutually shared by both malaria and dengue vectors. In addition, the attitude and exercise of the community to switch over from the age-old cemented overhead tanks to easily available and economically cheaper fibre tanks with mosquito-proof lids provide ample occasions to prevent vector breeding and therefore low adult abundance. Interestingly wells which are also another potential breeding habitat of *Anopheles stephensi* have been drastically reduced and most of them are closed and converted to tube wells. This custom may be due to repeated monsoon failure over the years resulting in the low water table, increase in household consumption, all of which led to a compounding effect of wells being dried up which were otherwise perennial in nature. Wells due to its damp, dim conditions act as an ideal resting habitat for many mosquito species including malaria vectors. In the absence of such habitats, the vectors are forced to rest elsewhere with less disturbed, appropriate microclimate conditions and hence cannot be collected easily unlike the earlier period. The progressive and healthy urban economy over the years has also resulted in the execution of changing the roof structures of houses that were once thatched or tiled, which belonged to economically weaker, low middle-income group people to asbestos and concrete roof structures which do not serve as an ideal resting habitat for malaria vectors. Furthermore, the intense and regular indoor repellent usage over the years owing to the mosquito nuisance and also with the rising economy has driven the susceptible *An. stephensi* irrespective of the density to take shelter or rest outdoors, regardless of any ideal place it could find.

One of the limitations of the study was that parallel dusk collection (aspirator) data could not be generated due to the lack of manpower for undertaking collections simultaneously and refusal from the community in disturbing the limited privacy in urban settings especially a busy metropolitan city like Chennai. The trap placements were sparse, irregular and the number of traps differed considerably between months and house structures as these contingencies are sometimes bound to happen in field settings. The study period had to be extended for another 4 months to confirm the unusual peak density of mosquitoes following the devastating floods during Nov-Dec 2015 (rainfall − 752 mm). So in order to understand the unprecedented peak vector density and to find out the actual scenario, the study was extended to 4 more months (Jan- April 2017) of the following year, the period of which corresponded to the preceding year with normal precipitation (rainfall − 151.1 mm). It is unclear whether the 34% of vector mosquitoes were human blood-fed or bovine blood-fed. The reason for omitting the blood meal analyses was mainly because of a previous study [5] from the same site indicating 95% of the analyzed *An. stephensi* positive for bovine blood. Also, the unfed mosquitoes were not ascertained if they have newly emerged (nulliparous) or parous females that have not yet taken a blood meal. Further, we couldn’t confirm or exclude the possibility that concurrent usage of nets or repellents increased the number of outdoor collections and so decreased indoor biting. Nevertheless, the study construes the various findings that are intervention driven points translational to the vector control programme.

**Conclusion**

The different collection (resting and light trap) strategies were assessed to find out the appropriate collection method and time which could yield the maximum collection of *An. stephensi* with the available resources. In this regard, the present study indicated dawn collections during the early hours to be the
most appropriate time to collect wild *An. stephensi* in an urban setting. The study also highlighted the need for an in-depth adult mosquito/vector collection/trapping strategies (resting or light trap) in an urban setting to determine the capture/collection efficiency. Besides, an appropriate time interval targeting the precise location/site (indoor or outdoor) based on the mosquito/vector behavioural changes is a requisite to assess vector abundance for effective control measures. Elaborate and more extensive collection strategies in future depending on the resources may be useful to determine the capture efficiency in an endemic area.

**Abbreviations**

CDC
Centers for Disease Control and Prevention; CS-ELISA:Circumsporozoite ELISA; MR4:Malaria Research and Reference Reagent Resource Center; MHD:Man Hour Density; GPS:Global Positioning System;

**Declarations**

**Ethics approval and consent to participate**

The manuscript does not involve the use of any animal data or tissue. However, institutional ethical clearance of the project was obtained from the National Institute of Malaria Research of Indian Council of Medical Research, New Delhi (ECR/NIMR/EC/2010/100).

**Consent for publication**

Not applicable.

**Availability of data and materials**

The dataset generated during and/or analyzed during the current study shall be available on request mentioning the purpose by contacting the corresponding author.

**Competing interests**

The authors declare that they have no competing interests.

**Funding**

The work was supported by the National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH) Grant U19AI089676. The content of this manuscript is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

**Authors’ contributions**
AE designed the experiment with input from MTM and VJ. AE, JAJ, AA, and SR participated in study design. SR, ST, and AE wrote the manuscript. JAJ and AA conducted the experiment at the study site. VJ, SR, AA, and ST contributed to data analysis. AE, SR, VJ, and MTM edited the manuscript. All authors read and approved the final manuscript.

Acknowledgments

We thank the National Institute of Malaria Research and Indian Council of Medical Research for providing the necessary facilities and support. We gratefully acknowledge Dr. Amit Prakash Sharma (Director, ICMR-National Institute of Malaria Research), Dr. Neena Valecha (Former Director, ICMR-National Institute of Malaria Research), Dr. Jane Carlton from New York University, USA and Dr. Matthew B Thomas from Penn State University for their valuable suggestions; The staff of the NIMR field unit, Chennai; technical staff of Regional office of Health and Family Welfare (Govt. of India) at Besant Nagar, Chennai; the communities of Adyar, Besant Nagar, Thiruvanmiyur for permitting us to carry out the survey in their premises. This work was supported by the National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH) Grant U19AI089676. The content of this manuscript is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

References

1. World Health Organization. World malaria report 2019.
2. Health and Family Welfare Department, Government of Tamil Nadu. https://www.tnhealth.tn.gov.in/tngovin/dph/dphdbmal.php. Accessed 01 May 2020.
3. Mathanga DP, Tembo AK, Mzilahowa T, Bauleni A, Mtimaukanena K, Taylor TE, Valim C, Walker ED, Wilson ML. Patterns and determinants of malaria risk in urban and peri-urban areas of Blantyre, Malawi. Malar J. 2016;15(1):590.
4. Thomas S, Ravishankaran S, Justin JA, Asokan A, Mathai MT, Valecha N, Thomas MB, Eapen A. Overhead tank is the potential breeding habitat of *Anopheles stephensi* in an urban transmission setting of Chennai, India. Malar J. 2016;15(1):274.
5. Thomas S, Ravishankaran S, Justin NJ, Asokan A, Mathai MT, Valecha N, Montgomery J, Thomas MB, Eapen A. Resting and feeding preferences of *Anopheles stephensi* in an urban setting, perennial for malaria. Malar J. 2017;16(1):111.
6. Somah SL. Historical settlement of Liberia and its environmental impact. University Press of America; 1995. P. 108.
7. Wilson ML, Krogstad DJ, Arinaitwe E, Arevalo-Herrera M, Chery L, Ferreira MU, Ndiaye D, Mathanga DP, Eapen A. Urban malaria: understanding its epidemiology, ecology, and transmission across seven diverse ICEMR network sites. Am J Trop Med Hyg. 2015;93(3_Suppl):110–23.
8. Van Eijk AM, Ramanathapuram L, Sutton PL, Peddy N, Choubey S, Mohanty S, Asokan A, Ravishankaran S, Priya GS, Johnson JA, Velayutham S. The use of mosquito repellents at three sites
in India with declining malaria transmission: surveys in the community and clinic. Parasit Vectors. 2016;9(1):418.

9. Nagpal BN, Srivastava A, Dash AP. Resting behaviour of Anopheles stephensi type form to assess its amenability to control malaria through indoor residual spray. J Vector Borne Dis. 2012;49(3):175.

10. Thomas S, Ravishankaran S, Justin NJ, Asokan A, Kalsingh TM, Mathai MT, Valecha N, Montgomery J, Thomas MB, Eapen A. Microclimate variables of the ambient environment deliver the actual estimates of the extrinsic incubation period of Plasmodium vivax and Plasmodium falciparum: a study from a malaria-endemic urban setting, Chennai in India. Malar J. 2018;17(1):201.

11. WHO. Manual on Practical Entomology in Malaria: Methods and techniques. Geneva: World Health Organization. Division of Malaria Other Parasitic Diseases, Part 2;; 1975.

12. Wirtz R, Avery M, Benedict M. Specific Anopheles techniques 3.3 Plasmodium Sporozoite ELISA. Malaria Research and Reference Reagent Resource Center, MR4 2007, p. 11.

13. González M, Alarcón-Elbal PM, Valle-Mora J, Goldarazena A. Comparison of different light sources for trapping Culicoides biting midges, mosquitoes and other dipterans. Vet Parasitol. 2016;226:44–9.

14. Mwanga EP, Ngowo HS, Mapua SA, Mmbando AS, Kaindoa EW, Kifungo K, Okumu FO. Evaluation of an ultraviolet LED trap for catching Anopheles and Culex mosquitoes in south-eastern Tanzania. Parasit Vectors. 2019;12(1):418.

15. Sriwichai P, Karl S, Samsung Y, Sumruayphol S, Kiattibutr K, Payakkapol A, Mueller I, Yan G, Cui L, Sattabongkot J. Evaluation of CDC light traps for mosquito surveillance in a malaria endemic area on the Thai-Myanmar border. Parasit Vectors. 2015;8(1):636.

16. Nagpal BN, Srivastava A, Saxena R, Ansari MA, Dash AP, Das SC. Pictorial identification key for Indian anophelines. Delhi: Malaria Research Centre (ICMR); 2005. p. 40.

17. Nagpal BN, Sharma VP. Indian anophelines. New Delhi: Oxford and IBH Publishing Co. Pvt Ltd; 1995.

18. Karki S, Hamer GL, Anderson TK, Goldberg TL, Kitron UD, Krebs BL, Walker ED, Ruiz MO. Effect of trapping methods, weather, and landscape on estimates of the Culex vector mosquito abundance. Environ Health Insights. 2016:EHI-S33384.

19. Roiz D, Ruiz S, Soriguier R, Figuerola J. Climatic effects on mosquito abundance in Mediterranean wetlands. Parasit Vectors. 2014;7(1):333.

20. Thomas S, Ravishankaran S, Asokan A, Justin NJ, Kalsingh TM, Mathai MT, Valecha N, Eapen A. Socio-demographic and household attributes may not necessarily influence malaria: evidence from a cross sectional study of households in an urban slum setting of Chennai, India. Malar J. 2018;17(1):4.

21. Ondiba IM, Oyieke FA, Ong’amo GO, Olumula MM, Nyamongo IK, Estambale BB. Malaria vector abundance is associated with house structures in Baringo County, Kenya. PLoS ONE. 2018;13(6).

22. Tedrow RE, Rakotomanga T, Nepomichene T, Howes RE, Ratovonjato J, Ratsimbasaoa AC, Svenson GJ, Zimmerman PA. Anopheles mosquito surveillance in Madagascar reveals multiple blood feeding behavior and Plasmodium infection. PLoS Negl Trop Dis. 2019;13(7):e0007176.
23. Ojuka P, Boum Y, Denoeud-Ndam L, Nabasumba C, Muller Y, Okia M, Mwanga-Amumpaire J, De Beaudrap P, Protopopoff N, Etard JF. Early biting and insecticide resistance in the malaria vector *Anopheles* might compromise the effectiveness of vector control intervention in Southwestern Uganda. Malar J. 2015;14(1):148.

24. Moiroux N, Damien GB, Egrot M, Djenontin A, Chandre F, Corbel V, Killeen GF, Pennetier C. Human exposure to early morning *Anopheles funestus* biting behavior and personal protection provided by long-lasting insecticidal nets. PLoS One. 2014;9(8).

25. Mwanga EP, Ngowo HS, Mapua SA, Mmbando AS, Kaindoa EW, Kifungo K, Okumu FO. Evaluation of an ultraviolet LED trap for catching *Anopheles* and *Culex* mosquitoes in south-eastern Tanzania. Parasit Vectors. 2019;12(1):418.

26. Service MW. A battery-operated light-trap for sampling mosquito populations. Bull World Health Organ. 1970;43(4):635–41.

27. Samuel PP, Arunachalam N, Hiriyan J, Thenmozhi V, Gajanana A, Satyanarayana K. Host-feeding pattern of *Culex quinquefasciatus* Say and *Mansonia annulifera* (Theobald)(Diptera: Culicidae), the major vectors of filariasis in a rural area of south India. J Med Entomol. 2004;41(3):442–6.

28. Zaim M, Ershadi MR, Manouchehri AV, Hamdi MR. The use of CDC light traps and other procedures for sampling malaria vectors in southern Iran. J Am Mosq Control Assoc. 1986;2(4):511–15.

29. Li Y, Su X, Zhou G, Zhang H, Puthiyakunnon S, Shuai S, Cai S, Gu J, Zhou X, Yan G, Chen XG. Comparative evaluation of the efficiency of the BG-Sentinel trap, CDC light trap and Mosquito-oviposition trap for the surveillance of vector mosquitoes. Parasit Vectors. 2016;9(1):446.

30. McClelland. 1960. McClelland GA. Observations on the Mosquito, *Aëdes (Stegomyia) aegypti* (L.), in East Africa. II.—The Biting Cycle in a Domestic Population on the Kenya Coast. Bull Entomol Res. 1960;50(4):687 – 96.

31. Sikaala CH, Killeen GF, Chanda J, Chinula D, Miller JM, Russell TL, Seyoum A. Evaluation of alternative mosquito sampling methods for malaria vectors in Lowland South-East Zambia. Parasit Vectors. 2013;6(1):91.

**Figures**
Figure 1

Map of the study site indicating the locations of CDC-light trap placements
Figure 2

Monthly man hour density in cattle sheds with corresponding malaria incidence and rainfall
Figure 3
Mosquito species composition in cattle sheds and human dwellings (2016)
Figure 4

Anopheles stephensi collections in cattle sheds and human dwellings

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- GraphicalAbstractfinal.jpg