Comparison of different sampling methods to catch lymphatic filariasis vectors in a Sudan savannah area of Mali

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Abstract

Background

There is a need for better tools to monitor the transmission of lymphatic filariasis and malaria in areas undergoing interventions to interrupt transmission. Therefore, mosquito collection methods other than human landing catch (HLC) are needed. This study aimed to compare the Ifakara tent trap type C (ITTC) and the Biogents sentinel trap (BGST) to the HLC in areas with different vector densities.

Mosquitoes were collected in two villages in Mali from July to December in 2011 and 2012. The three methods were implemented at each site with one ITTC, one BGST and one HLC unit that consisted of one room with two collectors - one indoor and the other outdoor. The *Anopheles* collected in 2011 were individually dissected while those from 2012 were screened in pools using RT-PCR to determine the maximum infection prevalence likelihood (MIPL) for *Wuchereria bancrofti* and *Plasmodium falciparum*. The dissection of the females also allowed to assess the parity rates, as well its results.

Over the 2 years, the HLC method collected 1,019 *Anopheles*, yields that were 34- and 1.5-fold higher than those with the BGST and ITTC, respectively. None of the dissected *Anopheles* were infected. The RT-PCR results showed comparable MIPL between HLC and ITTC for *Wuchereria bancrofti* with one infected pool from each trap's yield (respectively 0.03% [0.0009% – 0.2%] and 0.04% [0.001% – 0.2%]). For *Plasmodium falciparum*, no infected pool was recovered from BGST.

The ITTC is a good alternative to HLC for xenomonitoring of programme activities.

Keywords: Lymphatic filariasis, malaria, Vector collection methods, *Anopheles gambiae*, Sudan savannah area, Mali.

Background

Lymphatic filariasis (LF) is an important public health problem in tropical and subtropical areas worldwide due to its chronic manifestations, elephantiasis and hydrocele[1]. LF is transmitted in West Africa by mosquitoes of the genus *Anopheles* [2]. Since 2000, the dual goals of the World Health Organization (WHO) and Global Programme to Eliminate Lymphatic Filariasis (GPELF) have been to eliminate LF as a public health problem in endemic areas by stopping transmission primarily using mass drug administration (MDA) and to alleviate the suffering of people already affected by the disease's chronic manifestations [3]. One of the main challenges of the GPELF has been the monitoring of transmission intensity during and after MDA. Although vector control and the use of xenomonitoring as a monitoring tool hold promise as important components of post MDA surveillance in the LF elimination process, xenomonitoring requires a safe and effective way of collecting mosquitoes at the community level that is representative of the vector fauna[4].

The efficiency of human-baited tent traps in comparison to human landing catch (HLC) is well established for *Anopheles gambiae sensu lato* (*An. gambiae s.l)* [5]. LF is unique because it is transmitted by anopheline and culicine mosquitoes including the genera *Anopheles*, *Culex*, *Aedes* and *Mansonia*. *Anopheles* mosquitoes are the principal vectors in rural areas in Africa but the genus *Culex* (*Culex* spp.) play an important role in LF transmission in urban communities in East Africa [6]. *Mansonia* has also been incriminated as a vector of LF in Guinea and Ghana. To date, HLC is the most frequently used method for *Anopheles* collections in many endemic areas of West Africa due in large part to the fact that it mimics the natural situation of mosquitoes trying to bite humans. However, HLC raises ethical concerns, including

the possibility that the collectors can be bitten by infected mosquitoes [7,8].

Additionally, HLC is labor intensive, and the mosquito yield is dependent on the collector's attractiveness to mosquitoes, ability and experience [7–9]. Thus, despite the fact that most of the existing mosquito data were generated using this method, its use is controversial and many ethics committees are reluctant to approve HLC for sampling mosquitoes.

To overcome these issues, alternative trapping methods have been explored with regard to ease of use, operator independence, cost of implementation and safety. Human-baited tent traps, like the ITTC (Ifakara tent trap type C), represent an alternative collection method that, like HLC, allow fresh specimen collection for live dissections and adequate storage for PCR or RT-PCR processing. ITTC has been reported to have yields more similar to those of the HLC as compared to several other methods [8,10–12].

Ideally, examination of vector abundance, distribution, species composition and infection rate should be assessed prior to initiation and at the end of MDA. Several LF endemic countries have stopped or are about to stop MDA in many implementation units. Given the increasing evidence for the importance of the association between vector competence and outcome of interventions against LF, effective vector sampling is becoming increasingly important [13]. Most studies conducted on ITTC have evaluated the performance of the traps in sampling malaria transmitting mosquito populations such as *An. gambiae s.l* [14–17]. None of the previous studies have compared the collected *Anopheles* infection rates for *Wuchereria bancrofti (W. bancrofti)* and *Plasmodium falciparum (P. falciparum)*, two co-endemic parasites transmitted by the same vectors in some settings. To ascertain the good reported correlation between ITTC yields and that of HLC in West African

settings, the current study was initiated to evaluate the ITTC and BGST as alternative mosquito sampling methods to replace the HLC in two villages in Mali that have different mosquito densities.

Methods

Study site identification and characteristics

Kolondieba district has an estimated population of 216,260 inhabitants distributed over 205 villages. The study was conducted in the villages of Bougoula (1,906 inhabitants) (longitude 11.045155758 and latitude -6.982963281) and Boundioba (3,201 inhabitants) (longitude 11.04218429 and latitude -6.984337661) that are located ~ 15 km apart, 276 km at the south of Bamako in the district of Kolondieba, region of Sikasso. This area has the highest annual rainfall in the country, ranging from 1,200 to 1,500 mm, with a rainy season that extends from July to December. Subsistence agriculture is the main occupation followed by panning for gold and wood harvesting from the forests. The district had already received five consecutive annual MDA rounds with ~80% annual epidemiological coverage rate when the present study was initiated in 2011. The endemicity levels of the study villages before MDA were unknown, although the neighboring sentinel site representing both villages was highly endemic before MDA initiation with a *W. bancrofti* antigen prevalence rate of 60% in 2000[18]. The two study villages share several important characteristics (climate, vegetation and housing style, ethnic group composition, and socio-cultural and health care seeking behaviors), despite the existence of a permanent backwater in Boundioba (but not Bougoula) that is an important potential larval habitat for mosquitoes.

Study Design

A longitudinal study with monthly mosquito collections was conducted from July to December in 2011 and 2012 in the two study villages. Mosquito collections were conducted three times a month in each study village in 2011 and six times a month in 2012 (in order to increase the number of collected mosquitoes). A total of 3 traps of each kind were used simultaneously per collection round.

Vector collection methods

Local teams were trained to set up the traps and collect the mosquitoes. The three collection tools were:

(i) The all-night HLC method - Mosquitoes attempting to feed were captured by adults seated on benches, with their feet and legs bared to the knee, using mechanical mouth aspirators type *colluzzi* and handheld battery-operated lamps. One collector operated indoors and the other outdoors at each collection point. The two collectors operated from 6 p.m to midnight before being replaced by two others who operated from midnight to 6 a.m.

(ii) The BGST- This is a simple suction trap constructed to use upward-directed air currents as well as visual cues to attract mosquitoes. The trap was used with a dispenser system (BG-Lure) that releases artificial human skin outdoors and needs no CO_2 [19]. Biogents sentinel traps (BGST) have been included in this study because of their efficiency in sampling culicine mosquitoes. The BGST is essentially a collapsible, white fabric container with an opening covered by white gauze, a diameter of 36 cm (14 inches), a height of 40 cm (1.3 feet), and a fan that sucks air into the trap through a black catch pipe. The airflow draws approaching mosquitoes into a catch bag.

(iii) The ITTC – This trap does not require electricity or moving parts and has been found to be able to collect well correlated numbers of *Anopheles* with the HLC

yields in rural and urban settings in Tanzania (5,8). An attractant, a villager, slept under each ITTC and was responsible for collecting the trapped mosquitoes using a mechanical mouth aspirator every two hours.

Logistics

Vectors were collected during the last two weeks of each collection month (from July to December). To control for random effects, the three trapping methods were implemented simultaneously at each of the three collection Zones, first in one village for three consecutive days and then in the other village for another three days in 2011 and every other day in each village in 2012. All of the collections occurred between 6:00 pm and 6:00 am at each of the three collection sites in the two villages. These sites were selected according to the village environmental characteristics and separated from each other by ≥200 m. Overall, the three areas we named 'Zones' were at the Northern side (Zone A), at the middle (Zone B) and at the Southern side of the village (Zone C). Zone A was close to the main breeding site in the village, Zone B was close to the original settlement area corresponding to the middle of the village, and Zone C was located close to the recently occupied area of the village. In each area, the locations of the three sampling methods were separated by approximately 100 m due to the relatively small size of the villages. Collections were set monthly (12 days collection in total) from July to December. During the same month, mosquito collection methods and location were fixed. The following month, the traps and methods were rotated. The collectors worked in two teams (a first team working from 6:00 p.m. to midnight and a second team from midnight to 6:00 a.m.) for both the HLC and the ITTC. Only *An. gambiae s.l* were further processed because the other species were of little epidemiological importance (do not transmit disease or were present in very low numbers). Collected mosquitoes were stored in

labelled screw top tubes containing a solution of 70% ethanol in 2011 or RNALater® solution in 2012 after sorting according to morphologically identifiable species, collection site and method. Whereas the specimens from 2012 were freshly stored and frozen the next day, those from 2011 were freshly dissected for parity rate and *W. bancrofti* infection status in the field before preservation of the carcass in alcohol and storage at room temperature thereafter.

Processing of specimens

Infection status and species identity were determined for the 2011 specimens stored in alcohol using the hemalum staining technique [20]. They were later dissected to look for *W. bancrofti* larval stages. In 2012, the mosquitoes were sorted and directly stored in RNA Later® solution for subsequent screening using PCR in the laboratory [21].

Given the fact that no infective mosquito was recovered in 2011 using the dissection, the 2012 collected mosquitoes tested using PCR provided an opportunity to test the same mosquito pools for the co-endemic malaria parasite *P. falciparum*. This not only provided an independent measure of the quality of the DNA extraction but allowed comparison of the yields of the three collection methods as related to the infection rate for one or both of these co-endemic parasites.

Parity rates and survival estimation

Mosquitoes to be dissected were kept fresh (about 100 per day per collection method) or preserved in 70% ethanol for future staining for *W. bancrofti* larval stage identification using Mayer's acid hemalum technique before individually dissection under a dissecting microscope [20]. Female *Anopheles* were individually placed on a slide into a drop of saline and dissected using a dissecting needle to remove the ovaries from the abdomen. A stereomicroscope (X40) was used to observe the

tracheole structure. Parity was determined by checking tracheole structure according to the method described by Detinova and Gillies[22].

Daily survival rates were calculated by Davidson's method based on the parity at the power of one divided by the duration of the gonotrophic cycle in days and were equal to the cubic root of the parity rate because the gonotrophic cycle occupies three days[23]. We used the gonotrophic cycle duration of three days observed in our insectary at the Faculty of Medicine of Bamako for *Anopheles* females collected in the study villages and reared for other experimental purposes (unpublished data).

Fresh specimen and dissection techniques

Hemalum staining is a standardized mosquito staining procedure that involves a series of 30 min immersions of the mosquitoes in 70%, 55% and 25% alcohol solutions[24]. Tubes containing approximately 20 mosquitoes are then stained in hemalum (Mayer's) stain (VWR, West Chester, PA) following a modification of Nelson (1958) for seven days before immersion in distilled water for three days[24]. The stained mosquitoes were then stored in glycerol before dissection to identify larvae of *W. bancrofti*. The dissection was done using a dissecting microscope by macerating the head, thorax and abdomen of the individual mosquito on a slide and covering it with a coverslip for observation under a stereomicroscope [25].

For *W. bancrofti* larval stage recovery from the female *Anopheles* specimens collected in 2011, the head, thorax and abdomen were examined separately in three drops of saline water using a stereomicroscope at X 200. The larval stages were identified according to the criteria of Nelson [26]. The mosquitoes collected in 2012 were stored in pools of one to 20 females in RNA later® solution [21] before processing using PCR for parasite DNA identification as previously described by

Rao et al in 2014 [27]. The pooling was done per village, month, collection method and mosquito morphology (considering *An. gambiae s.l* and *An. funestus* species).

Ethics statement

A collective village-wide oral consent was obtained from village elders, and all mosquito collectors signed an individual written consent. The study protocol and consent forms were approved by both the IRB of the Liverpool School of Tropical Medicine (LSTM) (reference#10.88RS) and that of the Malian National Institute of Research in Public Health, Bamako, Mali (reference #9/11/CE-INRSP).

Data management and analysis

In the field, mosquito identification and dissection results were noted on specific data recording sheets. The recorded data were later entered into Microsoft Access and analysed using SPSS version 14 (SPSS Inc., Chicago, IL) and GraphPad Prism software version 5 (GraphPad Software, La Jolla, CA). The collection methods were compared in terms of correlation between collection methods' mosquito yields using Spearman correlation test and the number of mosquitoes collected per night per trap over the study period. The parity rates and overall proportions of *An. gambiae* complex members were compared using their 95% confidence intervals.

A generalized linear mixed model, also called the random effects model [28,29], was used to assess the relative collection rates of the different collection methods as compared to the HLC [30,31]. Village and trap type were included as fixed effects in the model, and collection date was included as a random effect. A negative binomial model was fitted as there was evidence of overdispersion in the data. The confidence level was set at 95% for all statistical tests. For the vector infection level assessment, the PoolScreen software version 2 was used to determine the maximum infection prevalence likelihood (MIPL) and its 95% confidence interval [32].

Results

Characteristics of the collected mosquitoes

Based on the yields of individual collection rounds in 2011, *Culex* spp. had a significantly higher vector density, expressed in mean number of mosquitoes per person per night, (13 with 95% CI [5.24-20.85]) than *An. gambiae s.l* (2 with 95% CI [0.82-2.99]) in Boundioba. In Bougoula, a different scenario was observed with comparable mean densities for the two species with 8 [5.05-10.6] versus 11 [5.89-16.73], respectively for *Culex* spp. and *An. gambiae s.l.*

The percentage of *An. gambiae s.l.*in the total collected mosquitoes varied significantly by capture method. In 2011, *An. gambiae* complex mosquitoes represented 58.3% [55.92-60.55] of the total collected by HLC followed by 40% [37.75-42.35] by ITTC and only 1.7% [1.18-2.41] by BGST. The same trend was observed in 2012 with 54.5% [53.40-56.61], 44.9% [43.82-46.03] and 0.6% [0.42-0.] of the *Anopheles* captured by the HLC, the ITTC and the BGST, respectively. Overall, for the two villages combined, the BGST collected more *Culex* spp. each year than the two other methods, while HLC collected more *An. gambiae s.l.* than ITTC and BGST each year (Table 1).

Comparison of the Mosquito Collection Traps' yields

There was a strong and significant positive correlation between the HLC and ITTC yields of *An. gambiae s.l.* in both villages and over the two collection years. The correlation coefficients ranged from 0.66 to 0.84 and all p values were less than 0.007 (Table 2). The BGST yields were never significantly correlated with those of the HLC in the two villages over the two collection years with all correlation coefficients less than or equal to 0.28 (Table 2). The entire collected *Anopheles* using

298 the three collection methods in 2011 were dissected, and none was found infected
299 (data not shown).
300 In 2011, *Anopheles* parity and daily survival rates were comparable between
301 mosquitoes collected by HLC and those collected using the other methods in
302 Bougoula and Boundioba, as evidenced by overlapping 95% confidence intervals.
303 The entire collected mosquitoes were characterized by high parity (from 79.4 % to
304 94.4 %) and survival rates (from 92% to 98%) (Table 3).
305 A significant difference was observed in the collection rates for *An. gambiae s.l*
306 between villages (60% less for the village of Boundioba) and between the collection
307 methods (29% and 98% less for the ITTC and BGST, respectively, as compared to
308 the HLC) (Table 4).
309 **Mosquito collection traps' yields infection rates**
310 The *Anopheles* pools collected using BGST were not found to be infected. No *W.*
311 *bancrofti* infected pool was recovered in the village of Boundioba among the 49, 47
312 and 5 pools tested from the HLC, ITTC and BGST, respectively (data not shown).
313 As shown in table 5, *P. falciparum* was found in several pools from each study
314 village in 2012 with comparable overall MIPL of 2% [95%CI (1.6% – 2.4%)] and
315 1.3% [95%CI (0.7% – 2.1%)] respectively in Bougoula and Boundioba. In
316 Bougoula, a significantly higher MIPL was observed for the HLC collected
317 *Anopheles* 3% [95%CI (2.3% – 3.8%)] as compared to that for ITTC, which was 1%
318 [95%CI (0.9%-1.4%)]. In Boundioba, the HLC reported the highest MIPL but the
319 three methods showed comparable 95% confidence intervals for *P. falciparum* MIPL
320 (Table 5).
321
322 **Discussion**

323 Vector species composition varied between the two villages. *An. gambiae s.l* were
324 more frequent in the village of Bougoula in both collection years (Table 1), at each
325 assessment point and using all three collections methods as previously shown [33–35]
326 (data not shown). Such a dramatic difference in mosquito density between two
327 villages separated by only 17 km in the same region could be due to several factors,
328 including differences in the villages' ecological conditions, breeding site dispersal
329 and features, housing characteristics, and the frequency and abundance of rain [36,37].
330 The level of education, behaviors and occupations (type of crops and agricultural
331 methods used) of the population can also impact vector density, although these
332 characteristics are very likely to be similar between the populations of the two study
333 villages. Regardless of the reason for the observed differences in vector density, this
334 type of variability requires further study as it may impact both the success of MDA
335 and the implementation of post-MDA surveillance strategies in villages that are part
336 of the same LF evaluation unit.
337 Over the two years of the study, BGST yields were composed of *Culex* spp. more
338 frequently than those of the other two collection methods. Given the fact that *Culex*
339 spp. are not a vector of LF in West Africa, they are unimportant in the assessment of
340 LF transmission. Nonetheless, given the high number of *Culex* spp. collected, even if
341 they do not transmit LF, they may constitute a useful source for monitoring vector-
342 human contact, especially in areas where few *Anopheles* species exist (urban areas of
343 most endemic African countries) and where several rounds of MDA have lowered
344 both the LF infection and microfilaraemia rates. Finding *Culex* spp. infected with
345 any stage of *W. bancrofti* DNA may presage an increase or re-emergence of LF
346 transmission in areas where MDA has already reduced or stopped LF transmission [38].
347 **Collection methods' comparison**

348 The ability to follow the impact of entomological interventions or the re-emergence
349 of an infection previously interrupted or dramatically reduced requires repeated
350 assessments over a period of time. However, since vector density has important
351 implications with respect to the determination of most transmission parameters, the
352 use of different mosquito collection methods can make such comparisons difficult.
353 This especially applies to collection methods that do not collect mosquitoes trying to
354 bite humans. Of the two trapping methods tested, the ITTC showed better correlation
355 with the HLC than the BGST with respect to total yields of *An. gambiae s.l.* over the
356 transmission season. In fact, the BGST collected predominantly *Culex* spp., which do
357 not transmit LF or malaria in the study region.
358 Both the HLC and ITTC collected relatively old mosquitoes, which are more likely
359 to have participated in disease transmission, with a survival rate >92% and a parity
360 rate at least 79.4%. The high parity and survival rates of mosquitoes captured with
361 these two methods indicate the suitability of the collected fauna for transmission
362 assessment [39,40].
363 In terms of infected mosquito identification, HLC showed a higher MIPL for *P.*
364 *falciparum* in Bougoula as compared to the ITTC. For *W. bancrofti* and in the village
365 of Boundioba, the collection methods were still comparable with respect to the MIPL
366 overlapping 95% CI. With the pool screening, there seems to be an underestimation
367 of *P. falciparum* when infection prevalence as well as vector densities are high. Such
368 a scenario is likely to be more common for malaria than LF due to the high impact of
369 the MDA on LF endemicity levels in the study areas.
370 Overall, in each village, the three methods had comparable MIPL except in
371 Bougoula where the HLC had significantly higher MIPL than ITTC. This may be

372 due to the sample sizes that certainly may need to be higher to achieve statistical
373 significance for the observed phenomenon especially in the village of Boundioba.
374 In most endemic areas, LF elimination programs have been ongoing for several years
375 and there is an increased need for surveillance prior to, during and after stopping
376 MDA. This assessment is important in *Anopheles* mosquitoe transmission areas
377 where MDA impact seems low. Although the ideal package for surveillance has not
378 yet been determined, it will likely be a combination of blood and vector surveillance
379 on a regular basis with sustained community participation and ideally embedded into
380 the routine health care activities. The identification of the most cost-effective, safe
381 and reliable vector surveillance method is, therefore, of high importance. Whereas
382 the yield of *Anopheles* using HLC was twice that of the ITTC over the two years of
383 the study, the ITTC uses one collector per collection point as compared to two for
384 the HLC– one indoor and the other outdoor. Additionally, the cost of operation is
385 higher for the HLC because of the need for training and expertise, especially in the
386 setting of a community monitoring system that would be part of an integrated vector
387 management system in endemic areas [41]. Despite the initial cost of the tents, which
388 can pose a challenge, the ease of implementation, the possibility of using another
389 type of bait in the tent (natural or artificial) (12,32), the lack of operator impact on
390 the efficiency of the method, the capacity to collect both *Culex* spp. and *An. gambiae*
391 *s.l.* for xenomonitoring purposes, and the absence of ethical issues, are also
392 important factors in favor of the ITTC as compared to the HLC [41].
393 Despite these advantages, ITTC has some limitations as an entomological and
394 epidemiological surveillance tool because of its limited sensitivity, particularly in
395 high mosquito density settings. This problem is exacerbated when rain can enter the
396 trap when it is set up during the rainy season. In addition, the bulky nature of the trap

makes it impractical for indoor use and thus unsuitable for studying indoor biting mosquitoes. The bulkiness of the trap also poses particular problems in densely populated informal settlements in urban areas. The materials making up the trap make it too heavy and difficult to move between sampling sites. Lighter materials can be used to overcome this problem [14,44].

Conclusion

Our data suggest that ITTCappears to be a good alternative to HLC. Further studies in different endemicity settings are needed. Collection of *An. gambiae s.l.* using the ITTC provides numbers of specimens that are well correlated with those from the HLC, independent of the vector density. Similarly, the infection rates, as observed for malaria parasites, were comparable for the yields of these two mosquito collection methods. Consequently, ITTC provides an ethically acceptable alternative to HLC for use in monitoring mosquito vectors as part of entomological surveillance during and following interventions targeting LF or malaria elimination such as MDA and seasonal malaria chemoprevention. The bulkiness of the ITTC remains an issue that could be addressed by using different materials and comparing the new design to the HLC.

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