Am. J. Trop. Med. Hyg., 106(4), 2022, pp. 1247–1253
doi:10.4269/ajtmh.21-0667

Comparison of Different Sampling Methods to Catch Lymphatic Filariasis Vectors in a Sudan Savannah Area of Mali

Yaya Ibrahim Coulibaly,[1,2,3*] Moussa Sangare,[1,4] Housseini Dolo,[1] Salif Seriba Doumbia,[1] Siaka Yamoussa Coulibaly,[1] Ilo Dicko,[1] Abdoul Fatao Diabate,[1] Michel Emmanuel Coulibaly,[1] Lamine Soumaoro,[1] Abdallah Amadou Diallo,[1] Massitan Dembele,[5] Sekou Fantamady Traore,[1] Michelle Stanton,[2] Benjamin Guibehi Koudou,[6] Amy D. Klion,[7] Thomas B. Nutman,[8,9] Louise Kelly-Hope,[2] and Moses John Bockarie[2,10]

[1]*Filariasis Research and Training Unit, International Center of Excellence in Research (ICER-Mali), Bamako, Mali;* [2]*Center for Neglected Tropical Diseases, Liverpool School of Tropical Medicine, University of Liverpool, Liverpool, United Kingdom;* [3]*Dermatology Hospital of Bamako, Bamako, Mali;* [4]*Faculty of Health Sciences, Interdisciplinary School of Health Sciences, University of Ottawa, Ottawa, Ontario, Canada;* [5]*National Lymphatic Filariasis Elimination Program, Bamako, Mali;* [6]*Swiss Center for Scientific Research in Côte d'Ivoire (CSRS), Abidjan, Côte d'Ivoire;* [7]*Laboratory of Parasitic Diseases, Eosinophil Pathology Section, National Institutes of Health, Bethesda, Maryland;* [8]*Laboratory of Parasitic Diseases, Helminth Immunology Section, National Institutes of Health, Bethesda, Maryland;* [9]*World Health Organization, Vectors, Environment, and Society Research, Geneva, Switzerland;* [10]*School of Community Health Sciences, Njala University, Bo, Sierra Leone*

Abstract. 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, whereas those from 2012 were screened in pools using reverse transcription-polymerase chain reaction (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 *W. 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 *P. falciparum*, no infected pool was recovered from BGST. The ITTC is a good alternative to HLC for xenomonitoring of program activities.

BACKGROUND

Lymphatic filariasis (LF) is an important public health problem in tropical and subtropical areas worldwide because of its chronic manifestations, elephantiasis, and hydrocele.[1] Lymphatic filariasis 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 Program 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] Lymphatic filariasis 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 Ifakara tent trap type C (ITTC), represent an alternative collection method that, like HLC, allows fresh specimen collection for live dissections and adequate storage for polymerase chain reaction (PCR) or reverse transcription-polymerase chain reaction (RT-PCR) processing. Ifakara tent trap type C has been reported to have yields more similar to those of the HLC as compared with 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

*Address correspondence to Yaya Ibrahim Coulibaly, Filariasis Research and Training Unit, International Center of Excellence in Research (ICER-Mali), Faculty of Medicine and Odonto-Stomatology, BP: 1805, Point G, Bamako, Mali. E-mail: yicoulibaly@icermali.org

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* and *Plasmodium 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, this 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.

MATERIALS AND 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) (long. 11.045155758 and lat. −6.982963281) and Boundioba (3,201 inhabitants) (long. 11.04218429 and lat. −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 this 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 sociocultural and healthcare-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 (to increase the number of collected mosquitoes). A total of three 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 as follows:

1. 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 PM to midnight before being replaced by two others who operated from midnight to 6 AM.
2. The biogents sentinel trap (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 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.
3. 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 2 hours.

Logistics. Vectors were collected during the last 2 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 3 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 because of 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 PM to midnight and a second team from midnight to 6:00 AM) 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 labeled screw top tubes containing a solution of 70% ethanol in 2011 or RNAater® solution in 2012 after sorting according to morphologically identifiable species, collection site, and method. Although 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 RNAlater® solution (distributed by Thermo Fisher Scientific, Carlsbad, CA) 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 3 days.[23] We used the gonotrophic cycle duration of 3 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 minutes 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 7 days before immersion in distilled water for 3 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 ×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 RNAlater® 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 analyzed 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 (CIs).

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 with 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% CI.[32]

TABLE 1
Collected mosquitoes distribution per collection method in the two villages of the Kolondieba district in 2011 and 2012

Species collected		Total collected (%)	HLC (%)	[95% CI]	BGST (%)	[95% CI]	ITTC (%)	[95% CI]
Culex 2011	Bougoula	1,033 (37.50)	27 (2.6)	[1.76–3.72]	917 (88.8)	[86.73–90.59]	89 (8.6)	[7.02–10.44]
	Boundioba	1,722 (62.50)	22 (1.3)	[0.82–1.90]	1,664 (96.6)	[95.70–97.41]	36 (2.1)	[1.49–2.85]
	The two villages	2,755 (100)	49 (1.8)	[1.33–2.32]	2,581 (93.7)	[92.73–94.55]	125 (4.5)	[3.81–5.36]
An. gambiae 2011	Bougoula	1,494 (85.57)	844 (56.6)	[53.97–58.99]	18 (1.2)	[0.74–1.86]	631 (42.2)	[39.75–44.75]
	Boundioba	252 (14.43)	172 (68.3)	[62.31–73.78]	12 (4.8)	[2.61–7.95]	68 (27)	[21.78–32.72]
	The two villages	1,746 (100)	1,017 (58.3)	[55.92–60.55]	30 (1.7)	[1.18–2.41]	699 (40)	[37.75–42.35]
Culex 2012	Bougoula	2,464 (52.57)	463 (18.8)	[17.28–20.37]	1,761 (71.5)	[69.66–73.23]	240 (9.7)	[8.62–10.96]
	Boundioba	2,223 (47.43)	114 (5.1)	[4.27–6.11]	2,055 (92.5)	[91.29–93.49]	54 (2.4)	[1.85–3.13]
	The two villages	4,687 (100)	577 (12.3)	[11.39–13.27]	3,816 (81.4)	[80.28–82.51]	294 (6.3)	[5.61–6.99]
An. gambiae 2012	Bougoula	6,368 (81.81)	3,474 (54.6)	[53.33–55.77]	35 (0.5)	[0.39–0.76]	2,859 (44.9)	[43.68–46.12]
	Boundioba	1,416 (18.19)	769 (54.3)	[51.71–56.89]	9 (0.6)	[0.31–1.16]	638 (45.1)	[42.48–47.66]
	The two villages	7,784 (100)	4,243 (54.5)	[53.40–56.61]	44 (0.6)	[0.42–0.75]	3,497 (44.9)	[43.82–46.03]

An. gambiae = *Anopheles gambiae* complex; BGST = Biogents sentinel trap; *Culex* = *Culex* spp.; HLC = human landing catch; ITTC = Ifakara tent trap type C.

TABLE 2
Variations in the correlation level between the yields of the three mosquito collection methods by village and collection year

Village and collection year	Tests Spearman correlation test	Correlation between the yields from the collection methods HLC-ITTC	HLC-BGST
Bougoula in 2011	*r*	0.74	0.28
	P	< 0.001	0.16
Bougoula in 2012	*r*	0.74	0.08
	P	< 0.001	0.68
Bougoula over the 2 collection years	*r*	0.84	0.12
	P	< 0.001	0.35
Boundioba 2011	*r*	0.66	0.23
	P	0.007	0.40
Boundioba 2012	*r*	0.74	0.10
	P	< 0.001	0.58
Boundioba over the 2 collection years	*r*	0.77	0.07
	P	< 0.001	0.65

BGST = biogents sentinel trap; HLC = human landing catch; ITTC = Ifakara tent trap type C.

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* s.l. 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% (0.42–0.75) 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, whereas 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 the three collection methods in 2011 were dissected, and none was found infected (data not shown).

In 2011, *Anopheles* parity and daily survival rates were comparable between mosquitoes collected by HLC and those collected using the other methods in Bougoula and Boundioba, as evidenced by overlapping 95% CIs. The entire collected mosquitoes were characterized by high parity (from 79.4% to 94.4%) and survival rates (from 92% to 98%) (Table 3).

A significant difference was observed in the collection rates for *An. gambiae* s.l. between villages (60% less for the village of Boundioba) and between the collection methods (29% and 98% less for the ITTC and BGST, respectively, as compared with the HLC) (Table 4).

Mosquito collection traps' yields infection rates. The *Anopheles* pools collected using BGST were not found to be infected. No *W. bancrofti* infected pool was recovered in the village of Boundioba among the 49, 47, and 5 pools tested from the HLC, ITTC, and BGST, respectively (data not shown).

As shown in Table 5, *P. falciparum* was found in several pools from each study village in 2012 with comparable overall MIPL of 2% [95% CI (1.6–2.4%)] and 1.3% [95% CI (0.7–2.1%)], respectively, in Bougoula and Boundioba. In Bougoula, a significantly higher MIPL was observed for the HLC collected *Anopheles* 3% [95% CI (2.3–3.8%)] as compared with that for ITTC, which was 1% [95% CI (0.9–1.4%)]. In Boundioba, the HLC reported the highest MIPL but the three methods showed comparable 95% CIs for *P. falciparum* MIPL (Table 5).

DISCUSSION

Vector species composition varied between the two villages. *An. gambiae* s.l. were more frequent in the village of Bougoula in both collection years (Table 1), at each assessment point and using all three collections methods as previously shown[33–35] (data not shown). Such a dramatic difference in

TABLE 3
Variations of the *Anopheles gambiae* complex members' parity and survival rates per village in 2011

Collection methods	Villages	Parity rate, *n/N* (%) [95% CI]	Survival rate (%) [95% CI]
BGST	Bougoula	17/18 (94.4) [72.71–99.86]	(98) [90–100]
	Boundioba	11/12 (91.7) [61.52–99.79]	(97) [85–100]
HLC	Bougoula	706/844 (83.6) [80.98–86.08]	(92) [93–95]
	Boundioba	153/172 (89) [83.29–93.22]	(95) [94–98]
ITTC	Bougoula	533/631(84.5) [81.4–87.21]	(93) [93–96]
	Boundioba	54/68 (79.4) [67.88–88.26]	(96) [88–97]

BGST = biogents sentinel trap; HLC = human landing catch; ITTC = Ifakara tent trap type C; *n/N* = number parous divided by the total number dissected.

TABLE 4
Variation of the relative catch of the different collection methods yields according to the trap type and the village

Fixed effect	Relative catch	95% CI	*P* value
Trap type			
HLC (reference type)	1		
BGST	0.017	[0.012, 0.023]	$P < 0.0001$
ITTC	0.712	[0.593, 0.8551]	$P = 0.0003$
Village			
Bougoula	1		
Boundioba	0.404	[0.1753, 0.9322]	$P = 0.0336$

BGST = biogents sentinel trap; HLC = human landing catch; ITTC = Ifakara tent trap type C.

mosquito density between two villages separated by only 17 km in the same region could be because of several factors, including differences in the villages' ecological conditions, breeding site dispersal and features, housing characteristics, and the frequency and abundance of rain.[36,37] The level of education, behaviors, and occupations (type of crops and agricultural methods used) of the population can also impact vector density, although these characteristics are very likely to be similar between the populations of the two study villages. Regardless of the reason for the observed differences in vector density, this type of variability requires further study as it may impact both the success of MDA and the implementation of post-MDA surveillance strategies in villages that are part of the same LF evaluation unit.

Over the 2 years of the study, BGST yields were composed of *Culex* spp. more frequently than those of the other two collection methods. Given the fact that *Culex* spp. are not a vector of LF in West Africa, they are unimportant in the assessment of LF transmission. Nonetheless, given the high number of *Culex* spp. collected, even if they do not transmit LF, they may constitute a useful source for monitoring vector–human contact, especially in areas where few *Anopheles* species exist (urban areas of most endemic African countries) and where several rounds of MDA have lowered both the LF infection and microfilaraemia rates. Finding *Culex* spp. infected with any stage of *W. bancrofti* DNA may presage an increase or re-emergence of LF transmission in areas where MDA has already reduced or stopped LF transmission.[38]

Collection methods' comparison. The ability to follow the impact of entomological interventions or the re-emergence of an infection previously interrupted or dramatically reduced requires repeated assessments over a period of time. However, as vector density has important implications with respect to the determination of most transmission parameters, the use of different mosquito collection methods can make such comparisons difficult. This especially applies to collection methods that do not collect mosquitoes trying to bite humans. Of the two trapping methods tested, the ITTC showed better correlation with the HLC than the BGST with respect to total yields of *An. gambiae* s.l. over the transmission season. In fact, the BGST collected predominantly *Culex* spp., which do not transmit LF or malaria in the study region.

Both the HLC and ITTC collected relatively old mosquitoes, which are more likely to have participated in disease transmission, with a survival rate $> 92\%$ and a parity rate at least 79.4%. The high parity and survival rates of mosquitoes captured with these two methods indicate the suitability of the collected fauna for transmission assessment.[39,40]

In terms of infected mosquito identification, HLC showed a higher MIPL for *P. falciparum* in Bougoula as compared with the ITTC. For *W. bancrofti* and in the village of Boundioba, the collection methods were still comparable with respect to the MIPL overlapping 95% CI. With the pool screening, there seems to be an underestimation of *P. falciparum* when infection prevalence as well as vector densities are high. Such a scenario is likely to be more common for malaria than LF because of the high impact of the MDA on LF endemicity levels in the study areas.

Overall, in each village, the three methods had comparable MIPL except in Bougoula where the HLC had significantly higher MIPL than ITTC. This may be because of the sample sizes that certainly may need to be higher to achieve statistical significance for the observed phenomenon especially in the village of Boundioba.

In most endemic areas, LF elimination programs have been ongoing for several years and there is an increased need for surveillance before, during, and after stopping MDA. This assessment is important in *Anopheles* mosquitoe transmission areas where MDA impact seems low. Although the ideal package for surveillance has not yet been determined, it will likely be a combination of blood and vector surveillance on a regular basis with sustained community participation and ideally embedded into the routine healthcare activities. The identification of the most cost-effective, safe, and reliable vector surveillance method is, therefore, of

TABLE 5
Variation in the likelihood of *Anopheles gambiae* complex members' infection prevalence likelihood with *Plasmodium falciparum* in 2012 per collection method and per village

Bougoula 2012					
Collection method	# Tested	# Pools	Pools size range	# Positive pools	Pf infection prevalence likelihood* [95% CI]
HLC	3,460	185	[1–20]	79	3% [2.3–3.8%]
ITTC	2,836	157	[1–20]	25	1% [0.9–1.4%]
BGST	33	10	[1–7]	1	3% [0.09–14.7%]
Total	6,329	352	[1–20]	105	2% [1.6–2.4%]
Boundioba 2012					
Collection method	# Tested	# Pools	Pools size range	# Positive pools	Pf infection prevalence likelihood* [95% CI]
HLC	718	49	[1–20]	11	2% [0.8–3.1%]
ITTC	637	47	[1–20]	5	1% [0.3–1.9%]
BGST	9	5	[1–3]	0	0% [0–19.2%]
Total	1,355	101	[1–20]	16	1.3% [0.7–2.1%]

BGST = biogents sentinel trap; CI = confidence interval; HLC = human landing catch; ITTC = Ifakara tent trap type C; Pf = *P. falciparum;* # = number.

high importance. Although the yield of *Anopheles* using HLC was twice that of the ITTC over the 2 years of the study, the ITTC uses one collector per collection point as compared with two for the HLC—one indoor and the other outdoor. Additionally, the cost of operation is higher for the HLC because of the need for training and expertise, especially in the setting of a community monitoring system that would be part of an integrated vector management system in endemic areas.[41] Despite the initial cost of the tents, which can pose a challenge, the ease of implementation, the possibility of using another type of bait in the tent (natural or artificial),[12,32] the lack of operator impact on the efficiency of the method, the capacity to collect both *Culex* spp. and *An. gambiae* s.l. for xenomonitoring purposes, and the absence of ethical issues, are also important factors in favor of the ITTC as compared with the HLC.[41]

Despite these advantages, ITTC has some limitations as an entomological and epidemiological surveillance tool because of its limited sensitivity, particularly in high mosquito-density settings. This problem is exacerbated when rain can enter the 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 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.[14,42]

CONCLUSION

Our data suggest that ITTC appears 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.

Received June 11, 2021. Accepted for publication December 4, 2021.

Published online February 28, 2022.

Authors' addresses: Yaya Ibrahim Coulibaly, Filariasis Research and Training Unit, International Center of Excellence in Research (ICER-Mali), Bamako, Mali, Center for Neglected Tropical Diseases, Liverpool School of Tropical Medicine, University of Liverpool, Liverpool, United Kingdom, and Dermatology Hospital of Bamako, Bamako, Mali, E-mail: yicoulibaly@icermali.org. Moussa Sangare, Filariasis Research and Training Unit, International Center of Excellence in Research (ICER-Mali), Bamako, Mali, and Faculty of Health Sciences, Interdisciplinary School of Health Sciences, University of Ottawa, Ottawa, Ontario, Canada, E-mail: mbsangare@icermali.org. Housseini Dolo, Salif Seriba Doumbia, Siaka Yamoussa Coulibaly, Ilo Dicko, Abdoul Fatao Diabate, Michel Emmanuel Coulibaly, Lamine Soumaoro, and Abdallah Amadou Diallo, E-mails: hdolo@icermali.org, salifdoumbia@icermali.org, yamoussa@icermali.org, ilo@icermali.org, afatao@icermali.org, michou@icermali.org, soumla@icermali.org, and abdallahamadoudiallo@icermali.org. Massitan Dembele, National Lymphatic Filariasis Elimination Program, Bamako, Mali, E-mail: masdembele@yahoo.fr. Sekou Fantamady Traore, Filariasis Research and Training Unit, International Center of Excellence in Research (ICER-Mali), Bamako, Mali, E-mail: cheick@icermali.org. Michelle Stanton, Center for Neglected Tropical Diseases, Liverpool School of Tropical Medicine, University of Liverpool, Liverpool, United Kingdom, E-mail: michelle.stanton@lstmed.ac.uk. Benjamin Guibehi Koudou, Swiss Center for Scientific Research in Côte d'Ivoire (CSRS), Abidjan, Côte d'Ivoire, E-mail: guibehi.koudou@csrs.ci. Amy D. Klion, Laboratory of Parasitic Diseases, Eosinophil Pathology Section, National Institutes of Health, Bethesda, MD, E-mail: aklion@niaid.nih.gov. Thomas B. Nutman, Laboratory of Parasitic Diseases, Helminth immunology Section, National Institutes of Health, Bethesda, MD, and World Health Organization, Vectors, Environment and Society Research, Geneva, Switzerland, E-mail: tnuman@niaid.nih.gov. Louise Kelly-Hope, Center for Neglected Tropical Diseases, Liverpool School of Tropical Medicine, University of Liverpool, Liverpool, United Kingdom, E-mail: lkhope@liverpool.ac.uk. Moses John Bockarie, Center for Neglected Tropical Diseases, Liverpool School of Tropical Medicine, University of Liverpool, Liverpool, United Kingdom, and School of Community Health Sciences, Njala University, Bo, Sierra Leone, E-mail: bockarie@edctp.org.

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