

Comparisons of Human-Landing Catches and Odor-Baited Entry Traps for Sampling Malaria Vectors in Senegal

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ABSTRACT A comparative study of human-landing catches (HLCs) and odor-baited entry traps (OBETs) for sampling malaria vectors was conducted in two different bioclimatic areas of Senegal, the Sahelian and Sudano-Guinean phytogeographic zones, from September to December 2002. Mosquitoes were collected by the two methods both indoors and outdoors. The reliability of OBET samples was tested by comparing the two methods. Overall, HLC was more effective indoors and for surveying the anopheline fauna. Both methods were effective in sampling the four known malaria vectors in Senegal [*Anopheles gambiae* s.s., *An. arabiensis* Patton, *An. funestus* Giles, and *An. nili* (Theobald)], and mosquito age structures and infectivity rates did not differ between methods.

KEY WORDS human-landing catch, odor-baited entry trap, sampling method, malaria vectors, Senegal

THE ABUNDANCE OF HUMAN malaria vectors is usually estimated by human-landing (biting) catches (HLCs). This is the most direct, reliable and favored method, because it measures the frequency of human-mosquito contact (Service 1993). This contact between human and host-seeking *Anopheles* mosquitoes is a component of the entomological inoculation rate, which quantifies the dynamics of malaria transmission (Macdonald 1957). Drawbacks of this method include its dependence on the skills and experience of a mosquito collector and the natural human variation in attractiveness to mosquitoes. In addition, this method puts the collector at risk of infective mosquito bites and contracting malaria, which is ethically unsatisfactory. Because of these drawbacks, considerable effort has been made to find alternative methods that are at least as sensitive, specific, and reproducible and that are ethically acceptable. One of these methods is the odor-baited entry trap (OBET) developed by Costantini et al. (1993), which is evaluated in this work. The first use of this trap was to test for odor preferences of mosquitoes in response to air currents passed over alternative hosts (Costantini et al. 1998a, Costantini

and Diallo 2001, Duchemin et al. 2001) or to study the field response of mosquitoes to host kairomones (Costantini et al. 1996, 2001). These studies have shown that this trap can be successfully used to collect Afrotropical mosquito species, including important vectors of malaria such as *Anopheles gambiae* Giles s.l. and *An. funestus* Giles (Costantini et al. 1993, Duchemin et al. 2001). This article reports on the results of field trials in two different settings in Senegal to compare the OBET with the HLC method.

Materials and Methods

Study Sites. Fieldwork was carried out in two Senegalese villages that were selected on the basis of their malaria vectors and contrasting ecoclimatic settings: Barkédji in the Sahelian zone and Ngari situated in the Sudano-Guinean zone (Fig. 1). The village of Barkédji (15° 17' N, 14° 53' W) is located in the north central part of the country, in the Sahelian belt ≈350 km from Dakar, the capital city of Senegal. Its climate is characterized by a long dry season of 8–9 mo. It is situated between isohyets 200 and 400 mm with a hydrographic network of temporary ground pools filled with water at the onset of the rainy season remaining as the unique sources of water during the dry season from July to February. The dominant vegetation is herbaceous and shrubby savannah. *Anopheles gambiae* s.s. and *An. arabiensis* Patton are the main malaria vectors in this village with *An. arabiensis* predominating (Lemasson et al. 1997).

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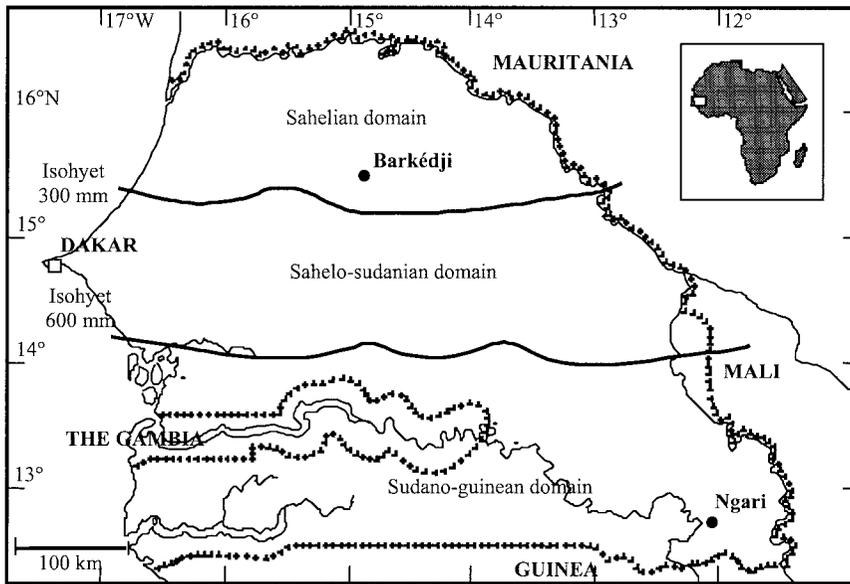


Fig. 1. Location of the two study villages in Senegal. Dotted lines are country borders.

The village of Ngari ($12^{\circ} 38' N$, $12^{\circ} 14' W$) is located in the wet, extreme southeastern part of Senegal. This area is characterized by Sudano-Guinean savanna vegetation and one rainy season from June to November, with annual rainfall totaling 1,200–1,300 mm. Natural vegetation is principally dense forest, although, as a consequence of human pressure, most of the forest has been replaced by rice and groundnut cultivations. Human malaria is transmitted by four species: *An. gambiae*, *An. arabiensis*, *An. funestus*, and *An. nili* (Theobald) (Dia et al. 2003).

Experimental Protocol. In each village, sampling of malaria vectors was carried out both indoors and outdoors by human landing catches (HLCs) and odor-baited entry traps (OBETs). The aim of the study was to compare the relative performance of these four sampling methods (i.e., indoor HLC, indoor OBET, outdoor HLC, and outdoor OBET), which were treated as separate treatments (see below, “Data Analysis”), and to assess possible biases of the OBET catches with respect to HLCs. To account both for temporal fluctuations of mosquito densities during the experiment as well as spatial heterogeneities in mosquito abundance between houses within the village, four houses were chosen in each village, and in each of them a sampling method was implemented according to the following plan: on the first night, an indoor HLC was carried out in the first house, an indoor OBET in the second house, an outdoor HLC in the third house, and an outdoor OBET in the fourth house. For the next three nights, the four collection methods were rotated among houses, such that each house was sampled once by each of the four collection methods. These four consecutive nights represent one replicate. Eight and five replicates were conducted in

Barkédji (from 13 September to 14 November 2002) and Ngari (from 17 October to 1 December 2002), respectively.

Sampling Procedures. The OBET was used as described by Costantini et al. (1993). The trap is composed of a tent with a sleeper whose odors are drawn to a cage trap by a fan via inflatable polythene tubing. For HLCs, at each collection site, two collectors/sleepers worked from 7:00 p.m. to 7:00 a.m.. The collectors stayed the same across the experiment, each sleeping every other night in the OBET tent to recover, collecting mosquitoes on the next night. In this way, individuals who collected mosquitoes by HLCs were the same used in the OBETs.

Field Processing of Mosquitoes. The mosquitoes captured in OBETs were retrieved from the trap with an aspirator the morning after collection. Anopheline mosquitoes were sorted and identified morphologically to species according to Gillies and De Meillon (1968). For each collection method, ovaries from a sample of anopheline mosquitoes were dissected to determine parity, by observing the degree of coiling of ovarian tracheoles (Detinova 1962). All mosquitoes were kept in tubes containing silica gel and preserved at $-20^{\circ}C$ in the laboratory until processing.

Laboratory Processing of Mosquitoes. Members of the *An. gambiae* complex were identified to species and molecular form by the procedures of Scott et al. (1993) and Favia et al. (2001). Malaria infections were determined on the crushed head and thoraces of anopheline mosquitoes by reactions with monoclonal antibodies of *Plasmodium falciparum*, *P. malariae*, and *P. ovale* circumsporozoite protein (CSP) in an enzyme-linked immunosorbent assay (ELISA)

according to Burkot et al. (1984) and Wirtz et al. (1987).

Data Analyses. The mean treatment catch across nights was calculated within each replicate to minimize pseudoreplication, and means were $\log(1 + x)$ -transformed before analysis to ensure homoscedasticity and normality of the data. To control for differences across replicates in mean numbers of vectors collected, a mixed model two-way analysis of variance (ANOVA) was performed, with replicates treated as random blocks and the four sampling methods as fixed treatments. ANOVAs were conducted with Generalized Linear Modeling software (Payne 1987), by specifying an identity link function and normal errors (Crawley 1993). Because interest was in comparing the performance of the OBET with respect to the HLC performed under similar conditions (i.e., alternatively either indoors or outdoors), after having verified the existence of statistically significant differences between treatments by an F-test, we subsequently limited individual treatments comparisons to the two planned contrasts: indoor HLC versus indoor OBET, and outdoor HLC versus outdoor OBET. Planned comparisons among treatments were performed by calculating least significant differences (LSDs). The analysis of residuals confirmed that the generalized linear model approach chosen conformed satisfactorily to the structure of the data. Analyses were performed separately for Barkédji and Ngari.

The parous and circumsporozoite protein rates were, respectively, calculated as the proportion of parous females and the proportion of CS-positive mosquitoes out of the total tested. For the analysis of differences in the parous and infectivity rates between sampling methods both indoors and outdoors, a logistic binomial model allowing for overdispersion was conducted in GLIM. To account for spatial, temporal, and specific difference in parity and infectivity rates, the data were stratified according to village, mosquito, and month of collection before analysis.

Results

Mosquito Collections. Overall, 2,695 females *Anopheles* mosquitoes comprising 10 species were collected: 1,239 from Barkédji and 1,456 from Ngari (Table 1). In both villages, all the species collected in the OBET also were obtained by landing catches. In Barkédji, a few *An. pharoensis* Theobald and *An. rufipes* (Gough) that were collected by HLC were not represented in the OBET samples. The same was observed in Ngari where *An. hancocki* Edwards, *An. squamosus* Theobald, and *An. ziemanni* Grünberg were only collected by HLC. *An. gambiae* s.l. was the commonest anopheline collected in both villages, accounting for 99% of totals in Barkédji and 44% in Ngari. In the latter village, *An. gambiae* s.l. was followed in abundance by *An. funestus* (34%) and *An. nili* (18%).

The molecular identification of specimens from the *An. gambiae* complex revealed that *An. gambiae* s.s. and *An. arabiensis* were present in both villages. The frequencies of the two species were statistically different between the HLC and OBET outdoors in Barkédji but not indoors (Table 2). In Ngari, no significant difference was observed between the frequencies of the two species outdoors or indoors for the OBET and HLC (Table 2). The only molecular form of *An. gambiae* s.s. found in Barkédji was the M form ($n = 16$), whereas only the S form ($n = 44$) was observed in Ngari village.

Sampling Method Comparison. As a more robust way of testing for statistical differences in the mean number of vectors collected by each of the four treatments, first we performed F-tests for treatment differences in the mixed model ANOVA before proceeding to the planned contrasts by LSD. In all cases, the individual treatment comparisons were warranted by statistically significant differences among the four treatments ($F_{3,12}$ and $F_{3,21} \geq 12.9$; $P < 0.001$).

In both villages, the numbers of mosquitoes collected in OBETs indoors were always lower than those obtained by HLC, for the four species considered

Table 1. Numbers of anophelines collected in Barkédji and Ngari villages by collection method and location

Mosquito species	Barkédji				Ngari			
	Indoor		Outdoor		Indoor		Outdoor	
	HLC	OBET	HLC	OBET	HLC	OBET	HLC	OBET
<i>An. coustani</i>					1	0	22	3
<i>An. flavicosta</i>					1	0	1	1
<i>An. funestus</i>					108	15	120	253
<i>An. gambiae</i> s.l.	367	38	431	392	91	1	180	372
<i>An. hancocki</i>					0	0	1	0
<i>An. nili</i>					51	3	113	100
<i>An. pharoensis</i>	4	0	1	0	1	0	14	0
<i>An. rufipes</i>	2	0	4	0	0	0	2	0
<i>An. squamosus</i>					0	0	1	0
<i>An. ziemanni</i>		-			0	0	1	0
Total	373	38	436	392	253	19	455	729

Table 2. Percentage (and number of individuals) of the *An. gambiae* complex collected by sampling method type and location

Village/mosquito species	Indoor			Outdoor		
	OBET	HLC	χ^2/P	OBET	HLC	χ^2/P
Barkédji						
<i>An. gambiae</i>	5.4 (2)	11.5 (6)	0.4 ^a /0.53	0 (0)	12.1 (8)	5.3 ^a /0.02
<i>An. arabiensis</i>	94.6 (35)	88.5 (46)		100 (55)	87.9 (58)	
Ngari						
<i>An. gambiae</i>	0 (0)	38.5 (20)		29.6 (16)	16.3 (8)	2.5/0.11
<i>An. arabiensis</i>	0 (0)	61.5 (32)		70.4 (38)	83.7 (41)	

Values in parenthesis indicate number of specimens identified.
^a Yates' correction applied to χ^2 .

(Table 3). Indoors, the OBET collected significantly fewer *An. gambiae* s.l. than HLC in Barkédji and the same pattern applied to the three other species in Ngari (Table 3). In outdoor collections, mean numbers collected by OBET and HLC were similar for *An. gambiae* s.l. in Barkedji and *An. nili* in Ngari. More *An. funestus* and *An. gambiae* s.l. were collected outdoors by OBETs than by HLCs in Ngari. However differences between the two collection methods were in no case statistically significant (Table 3).

Parous Rates. Samples of 874 females collected in Barkédji and 942 in Ngari were dissected for parity determinations (Table 4). Despite highly significant differences among species in individual parous rates ($F_{7,21} = 5.81$; $P < 0.001$), average parous rates among the four sampling methods across species/villages ranged 0.56–0.64, and these differences were not statistically significant ($F_{3,21} = 0.34$; $P = 0.80$).

Circumsporozoite Protein Rates. In total, 1,217 anopheline specimens collected in Barkédji and 1,365 collected in Ngari were tested by ELISA for CSP antigen (Table 5). Only *P. falciparum* and *P. malariae* were observed. The latter was found only in one specimen collected outdoors with the OBET in Barkédji. No specimens collected in the OBET indoors were CS positive in either village, but the sample size was in this case small. As for of parity, despite significant differences between species in individual CSP rates ($F_{7,21} = 3.55$; $P = 0.01$), average CSP rates among the four sampling methods across species/villages ranged 0.0–2.5%, and these differences were not statistically significant ($F_{3,21} = 0.72$; $P = 0.55$).

Discussion

The simultaneous field trials of HLC and OBET in the two different settings enabled the direct comparisons of collection methods and associated mosquito parity and infectivity rates. The main advantage of the OBET is that it samples host-seeking mosquitoes but avoids any risk of being bitten. Its reliability for sampling *Anopheles* malaria vectors has been already demonstrated during field choice tests of host preferences (Costantini et al. 1993, Costantini and Diallo 2001, Duchemin et al. 2001).

OBETs caught fewer anopheline species than HLCs, as did the Mbita trap (an entry, no-return device) in comparison with HLCs in the highlands of Madagascar (Laganier et al. 2003). Thus, the OBET may not be appropriate for the study of anopheline fauna. In contrast, CDC light traps captured anopheline fauna comparable with that of HLC (Le Goff et al. 1993, Faye et al. 1992). The fewer number of anopheline species other than malaria vectors in the OBET might be a result of their exophilic/zoophilic behavior (Gillies and De Meillon 1968); with the OBET, it is thought that mosquito entry is promoted by odors from the human host in the tent. Other behavioral traits could affect trap catches; for example, it is well known that ranging *An. pharoensis* (Snow 1975) and those entering huts (Snow 1987) fly at ground level, whereas in our study, the human odor plumes left the OBET at 1.5 m above the ground, in this way perhaps lowering the chances for this species to find the trap entrance.

Table 3. Mean number (\pm SE) of malaria vectors collected per human per night, and per trap per night in Barkédji and Ngari villages according to sampling method

Locality/mosquito species	Indoor	Indoor	P	Outdoor	Outdoor	P
	OBET	HLC		OBET	HLC	
Barkédji						
<i>An. gambiae</i> s.l.	1.2 \pm 0.3	11.5 \pm 3.0	***	12.3 \pm 2.9	13.5 \pm 3.2	ns
Ngari						
<i>An. funestus</i>	0.8 \pm 0.4	5.4 \pm 1.5	**	12.7 \pm 2.0	6.0 \pm 0.9	ns
<i>An. gambiae</i> s.l.	0.1 \pm 0.05	4.6 \pm 1.5	**	18.6 \pm 2.5	9.1 \pm 1.6	ns
<i>An. nili</i>	0.4 \pm 0.3	6.4 \pm 2.3	**	12.5 \pm 2.8	14.1 \pm 4.6	ns

Asterisks denote P values of LSD tests for individual comparisons between indoor treatments and between outdoor treatments: ns, not significant; $P > 0.05$, * $P \leq 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 4. Parous rates (%) of malaria vectors (\pm SE) caught by the four sampling methods in Ngari and Barkédji villages

Locality/mosquito species	Indoor		Outdoor	
	OBET	HLC	OBET	HLC
Barkédji				
<i>An. gambiae</i> s.l.	75.0 \pm 6.8 (38)	64.7 \pm 3.0 (258)	59.2 \pm 3.0 (277)	61.8 \pm 2.8 (301)
Ngari				
<i>An. funestus</i>	28.6 \pm 12.1 (14)	55.7 \pm 5.6 (79)	47.6 \pm 3.7 (185)	45.0 \pm 4.8 (109)
<i>An. gambiae</i> s.l.	100 (1)	58.3 \pm 7.1 (58)	43.4 \pm 3.4 (212)	44.4 \pm 4.5 (124)
<i>An. nili</i>	100 (1)	89.7 \pm 4.9 (39)	78.6 \pm 5.5 (56)	85.9 \pm 4.3 (64)

Values in parentheses indicate number of specimens dissected.

Numbers of mosquitoes collected in the indoor OBET were always significantly lower than those of the indoor HLC. One explanation for this result is that it reflects the fundamental difference between the OBET and HLC methods. In the case of HLC, both visual and physical stimuli are present. Host-seeking mosquitoes are first attracted by the olfactory stimulus and then will move toward the host via additional host stimuli such as visual cues, temperature, and humidity (Klowden 1996). Thus, the mosquito in response to such stimuli can target the appropriate site for taking its blood meal. By contrast, in the OBET, there are no visual host cues. The olfactory stimulus is channeled to the cage trap by a convective airstream current. When using the OBET indoors, because of the confined space and the absence of wind, the odor is dispersed all around the room. Mosquitoes sensing the odor when within a room have no more directional cues and are unable to locate the cage entry. Conversely, outdoors the OBET remains highly efficient, because the human odor is properly channeled by the airstream current and wind, so that odor-mediated anemotaxis can provide an efficient strategy to locate the trap entrance.

This is probably why in both villages; the mean numbers of vectors collected in the OBET outdoors were comparable with those of outdoor HLCs for all malaria vectors. This result also could be explained by the exophagic tendency of these species in our study area. Indeed, respectively, 52, 65.2, and 62.2% of *An. gambiae* s.l., *An. funestus*, and *An. nili* were found to be exophagic in a recent study (Dia et al. 2003). It also could be the result of a good performance of the OBET outdoors, because in other automatic collection equipment such as light traps, there is no intrinsic

variability of mechanical sampling techniques and the ability of the catcher is not involved so that all aggressive mosquitoes can move without barrier toward the entry cage (Costantini et al. 1993).

Despite the discrepancies observed in the mean numbers of vectors collected indoors, the age population structure and infectivity of the malaria vectors were overall similar when comparing between pairwise collection methods indoors and outdoors in both villages. The two methods therefore provided qualitatively comparable samples in collecting human-seeking malaria vectors populations as found previously for HLC and light traps (Lines et al. 1991, Faye et al. 1992, Le Goff et al. 1993, Costantini et al. 1998b) and more recently between HLC and Mbita traps (Laganier et al. 2003, Mathenge et al. 2004).

In view of the striking increase of antimalarial drug resistance (Trape 2001) and the necessity of studies to quantify disease exposure (for example, in vaccine and clinical trials), the OBET offers a valid alternative to landing catches on humans, because it does not expose individuals to infectious mosquito bites, and sampling is made passively all night without the need of skilled personnel. On the basis of our results, the OBET could prove useful as a method of estimation of malaria transmission and exposure outdoors, where CDC light traps have failed to give satisfactory results (Costantini et al. 1998b), although further studies are presumably needed before general (e.g., continent-wide) conclusions may be drawn. It is, however, encouraging that our results were consistent for several malaria vector species and in different ecological contexts.

Table 5. Circumsporozoite protein rates of *P. falciparum* (% \pm SE) for malaria vectors caught by the two collection methods in Ngari and Barkédji villages

Locality/mosquito species	Indoor		Outdoor	
	OBET	HLC	OBET	HLC
Barkédji				
<i>An. gambiae</i> s.l.	0 (38)	0.5 \pm 0.4 (366)	0.5 \pm 0.4 (389)	2.0 \pm 0.7 (424)
Ngari				
<i>An. funestus</i>	0 (15)	5.9 \pm 2.3 (102)	5.4 \pm 1.5 (240)	4.3 \pm 1.9 (115)
<i>An. gambiae</i> s.l.	0 (1)	2.2 \pm 1.6 (89)	2.2 \pm 0.8 (364)	0 (180)
<i>An. nili</i>	0 (3)	0 (51)	4.2 \pm 2.0 (96)	3.7 \pm 1.8 (109)

Values in parenthesis indicate number of specimens tested.

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