Attraction of *Anopheles* (Diptera: Culicidae) to Volatile Chemicals in Western Kenya

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ARTICLE

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ABSTRACT *Anopheles gambiae* s.l. and *Anopheles funestus* Giles are the primary vectors of malaria in East Africa. Identification of host-location olfactory cues may increase trap sensitivity for vector control and surveillance programs. Solid-state army miniature light traps were operated near sleeping humans in huts at night without lights and augmented with the potential attractants: L-lactic acid, Limburger cheese volatiles, hexanoic acid, and carbon dioxide. Mosquito response varied between species and gender. Female *An. funestus* exhibited a greater response to traps baited with L-lactic acid in combination with carbon dioxide than carbon dioxide alone in two different experiments.

KEY WORDS *Anopheles gambia*, *Anopheles funestus*, mosquito, attractants

HOST ODORS PROVIDE the olfactory cues by which mosquitoes locate blood meals (Takken 1991). Houses containing humans attract significantly more *Anopheles gambiae* (Giles) and *Anopheles funestus* (Giles) than do empty houses (Haddow 1942). Carbon dioxide, as found in human breath, attracts mosquitoes (Gillies 1980, Costantini et al. 1996, Gibson et al. 1997). Volatiles in human breath other than CO_2 reportedly do not play an important role in host-seeking by *An. gambiae* s.s. (De Jong and Knols 1995a), indicating that body odors may provide important host-location cues by which African malaria vectors identify humans as a specific mammalian host species.

Our field study tested components of human body odor in combination with CO_2 as potential attractants for the two primary African malaria vectors, *An. gambiae* and *An. funestus.* If attractive, these compounds may be used to increase trap effectiveness in malaria control programs.

Materials and Methods

Research Site. Our study was conducted in Kosogo village in the Kisumu District of western Kenya. Kosogo (33° 20′ E, 0° 30′ S) lies in the Kano Plains to the west of the Nandi Hills and north of the Kisii highlands. The region receives an average rainfall of 155 cm/yr, with the long rainy season lasting from April to June (Githeko 1992). The vegetation type was woody savanna, consisting of scattered *Acacia* and *Balanites* trees. The majority of the area surrounding Kosogo was covered with sugarcane. There were several drainage channels that run east and west from the hills toward the plains, providing suitable larval habitat for mosquitoes. A large, permanent, central sunlit pond surrounded by marshy vegetation and a variety of other temporary pools served as larval habitat for *An. gambiae* and *An. funestus.* All references to *An. gambiae* s.l. refer to *An. gambiae* and *An. arabiensis*, which were indistinguishable morphologically and known to occur in the study area.

Huts were constructed from dried mud, wooden poles, and thatch, with an occasional roof fortified with aluminum sheeting. Cattle and goats were housed in an enclosure 30 m from the huts.

Attractants. Two kilograms of dry ice in the form of small spheres (2 cm diameter) were placed in a padded envelope (21.59 by 27.94 cm, CareMail #2, Manco, Avon, OH) to ensure slow release throughout the night. Carbon dioxide alone was the control treatment. Hexanoic, L-lactic acid, and synthetic cheese volatiles were tested in combination with CO_2.

Attractants were pipetted onto a No. 2 (100% cotton) 3.8-cm long dental wick (Dental Supply, Des Moines, IA) to slow the release of the attractant and to ensure that it remained present for the entire night. For hexanoic acid (Sigma, Milwaukee, WI), 0.094 ml was combined with 1.06 μl of paraffin oil (100 mg of hexanoic acid), whereas for L-lactic acid (+) 98% (Sigma) 0.121 ml was combined with 79.3 μl of paraffin oil (100 mg of L-lactic acid). Analysis of Limburger cheese by gas chromatography-mass spectrometry showed that most volatiles were aliphatic fatty acids (Knols 1996). Although the composition may change, the primary chemical components of Limburger cheese were butanoic, propanoic, pentanoic, and hexanoic acids. These volatiles combined in proportions comparable to cheese and consisted of 45 μl of butanoic (0.35 g), 30 μl of propanoic (0.3 g), 20 μl of hexanoic (0.2 g), and 5 μl of valeric acid (0.05 g). To simulate Limburger cheese, 100 μg of the volatiles

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were combined with 100 μl of paraffin oil. Hanes cushion crew socks (86% cotton, 10% polyester, 2% nylon, 2% natural latex rubber, Hanes Socks, High Point, NC) were tested in an attempt to account for human foot odor chemicals. Socks were worn by the same human subject for 10 h and then one sock each was suspended 4 cm from the trap intake. Carbon dioxide was not used in combination with this treatment. A nonbaited trap was suspended within 1 m of an adult sleeping human.

Traps. Solid-state army miniature light traps (SSAM), powered by a 6-V battery, were suspended from the roof 0.3 m beneath the eves of the hut. Traps assigned attractants had the baited cotton wick attached by a wire 3 cm from the trap intake. If the treatment included CO_2, the release bag was located 15 cm from the trap intake. Light bulbs in all traps were removed to prevent visual cues from interfering with the testing of olfactory cues. Traps were baited each evening before 1830 hours and collected each morning after 0630 hours. Mosquitoes were transported to the Walter Reed Research Institute facilities in Kisumu for specific identification and compilation of data. If the fan in the trap was not operational during morning collection, the data from that trap was not included in the analysis.

Field Trials

Experiment 1. Each hut was assigned one trap per night positioned indoors with the rain shield on to prevent interference from falling debris. Six treatments (CO_2 control, L-lactic, hexanoic acid, synthetic cheese volatiles, socks, and humans) were assigned randomly to different huts each night to minimize confounding by unknown hut-specific factors. Depending on resource availability, traps were operated for up to 19 nights.

Experiment 2. Because of the attractive response to L-lactic acid in experiment 1, a direct comparison was conducted between traps baited with L-lactic acid in combination with CO_2 versus traps baited with CO_2 alone. Two traps were placed in each hut $\approx$4 m apart and were baited randomly with either L-lactic acid + CO_2 or with CO_2 alone. Traps were operated in 10 replicate huts for 14 nights.

Analysis of Data. PROC GLM (SAS Institute 1988, pp. 551–640) was used to analyze attractant response in experiment 1. A paired *t*-test analyzed the comparison in experiment 2 (PROC Univariate, 0.05 level of significance) (SAS Institute 1988).

Results

Experiment 1. More *An. funestus* were captured than *An. gambiae* in all treatments (Table 1). The average number of females captured per trap per night differed significantly among treatments for both *An. funestus* and *An. gambiae* (Table 1). The catch of female *An. gambiae* and *An. funestus* in traps baited with L-lactic acid + CO_2 was significantly greater than in traps baited only with CO_2 or other attractants. Only collections in traps baited with L-lactic acid + CO_2 were significantly greater than traps with CO_2 alone.

Experiment 2. Although not significantly different, a total of 60 more female *An. gambiae* were collected in traps baited with L-lactic acid + CO_2 compared with traps baited only with CO_2 (Table 2). In contrast, a total of >1,300 female *An. funestus* was collected in traps with L-lactic acid + CO_2 compared with >700 females with CO_2 alone, and the mean catch per trap per night also was significantly greater when lactic acid was included (Table 2).

Table 1. Average catch of female *Anopheles* species collected in traps baited with the attractants carbon dioxide (dry ice), hexanoic acid, L-lactic acid, synthetic Limburger cheese volatiles, sweaty socks, and human presence

Treatment	*n*	Means ± SE
	Anopheles gambiae	
Carbon dioxide	19	1.80 ± 0.152a
Hexanoic acid	18	1.36 ± 0.273ab
Human	19	0.97 ± 0.126b
L-lactic acid	19	2.91 ± 0.230c
Cheese volatiles	13	1.60 ± 0.292ab
Socks	8	1.13 ± 0.410ab
	Anopheles funestus	
Carbon dioxide	19	9.59 ± 0.588a
Hexanoic acid	18	7.70 ± 1.32a
Human	19	11.77 ± 0.851a
L-lactic acid	19	17.91 ± 1.44b
Cheese volatiles	13	8.63 ± 1.28a
Socks	8	5.02 ± 1.78a

Means in a column for a species followed by the same letter are not significantly different (least squares analysis, $P < 0.05$). *n*, trap nights

Discussion

Nonbaited traps set near humans allowed us to compare the efficiency of traps without any artificial enhancement to those with several attractants derived or related to human emanations. Cork (1996) found a strong electroantennogram response by *An. gambiae* to hexanoic acid, indicating its potential usefulness as an attractant. However, in our study fewer female *An. gambiae* and *An. funestus* responded to hexanoic acid baited traps than to either CO_2 or L-lactic acid. *An. funestus* also had a poorer response to hexanoic acid baited traps than to traps near sleeping humans, indicating that a more complex combination of chemicals are needed.

Table 2. *Anopheles* collected by traps baited with L-lactic acid in combination with carbon dioxide versus traps in the same hut baited with carbon dioxide only

Treatment	*n*	Means	Pr > *T*
	Anopheles gambiae		
Carbon dioxide	134	2.1	0.4399
L-lactic acid	134	1.6	
	Anopheles funestus		
Carbon dioxide	134	10.4	0.0001
L-lactic acid	134	5.8	

n, total trap nights.

Knols (1996) found that traps baited with Limburger cheese caught significantly greater numbers of mosquitoes than control traps. It was thought that the females of highly anthropophagic mosquito species were attracted by Limburger cheese odor because it resembled foot odor (Noble 1982). Our data indicated that female *An. gambiae* did not prefer the Limburger cheese volatiles, in the combination and concentration that were selected for testing, over the other attractants.

Ankles are the biting sites of choice for some *Anopheles* mosquitoes (De Jong and Knols 1995b). In an attempt to use olfactory emanations of total foot odor, including microorganisms, cotton socks were used for bait after being worn for a 10-h period. The response to this sock treatment was extremely low, perhaps due to the dissipation of the odor over time. This type of treatment also may depend on variations among the attractiveness of the humans wearing the socks. Without the presence of CO_2 and the moisture and heat production of a human foot, olfactory cues related to feet may not be attractive to these mosquitoes.

L-lactic acid in combination with CO_2 was the only treatment that significantly attracted more *Anopheles* mosquitoes than the CO_2 controls. Takken (1991) found L-lactic acid to have attractive properties for some species of mosquitoes in the laboratory.

Trap sensitivity may be increased with the use of L-lactic acid plus CO_2 as bait. More representative estimates of *Anopheles* populations in the environment may increase early warning capability (Collins and Paskewitz 1995) to identify vector population increases and to initiate intervention before mosquito population densities exceed pathogen transmission thresholds.

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