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BLOOD-MEAL ANALYSIS FOR ANOPHELINE MOSQUITOES SAMPLED ALONG THE KENYAN COAST

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ABSTRACT. A total of 1,480 *Anopheles gambiae* sensu lato and 439 *An. funestus,* collected from 30 sites along the Kenyan coast, were tested by direct enzyme-linked immunosorbent assay (ELISA) for blood-meal identification. Overall, the enzyme-linked immunosorbent assay (ELISA) identified 92 and 87% of the samples tested in *An. gambiae* s.l. and *An. funestus,* respectively. Of these, human IgG was detected in 98.97% (n = 1,347) of *An. gambiae* s.l. and 99.48% (n = 379) of *An. funestus.* Only 14 (1.03%) of the *An. gambiae* s.l. had fed on other vertebrate hosts tested, which were bovines, chickens, and goats. Additionally, only 2 *An. funestus* had fed on goats. In all the 28 sites that had bloodfed mosquitoes, *An. gambiae* s.l. had a human blood index greater than 0.9. Twenty-five of these sites had a human blood index greater than 0.9 for *An. funestus,* while the other 3 sites had no bloodfed mosquitoes. The *An. gambiae* s.l. were tested by polymerase chain reaction (PCR) for species identification. A total of 338 were *An. gambiae* s.s., 79 were *An. arabiensis,* and 12 were *An. merus.* The human blood index was 0.96, 0.91, and 1.0 for *An. gambiae* s.s., *An. arabiensis,* and *An. merus,* respectively. The *Plasmodium falciparum* sporozoite infection rates were 6.2% for species in the *An. gambiae* complex and 3.7% for *An. funestus.* These results emphasize that *An. funestus* and members of the *An. gambiae* complex on the Kenyan coast are highly anthropophilic, with nearly all specimens feeding on humans during every blood meal. The results further demonstrated active transmission of *P. falciparum* sporozoites by the primary vector species. This study suggests that the use of insecticide-treated nets will be effective for controlling biting mosquitoes inside houses along the coast of Kenya.

KEY WORDS ELISA, human blood index, human IgG, human–vector contact, PCR

INTRODUCTION

Blood-meal analysis in mosquitoes is of paramount importance in malaria epidemiological studies (Service et al. 1986, Service 1989). The feeding preference of anopheline mosquitos is an important parameter in the transmission of malaria parasites. The correct identification of the preferred host for malaria vectors is important because it determines the frequency at which a vector population feeds on humans. This is a measure of human–vector contact (Garret-Jones et al. 1980) and is useful in the estimation of vectorial capacity.

Extensive studies on feeding behavior of anopheline mosquitoes have been done in western Kenya (Joshi et al. 1975, Service et al. 1978, Highton et al. 1979, Beier et al. 1988, Petrarca and Beier 1992, Githeko et al. 1994). However, little information is available on the Kenyan coast (Mosha and Petrarca 1983, Mutero et al. 1984, Mbogo et al. 1993a), and therefore, there was a need to do extensive research on a district-wide scale.

Although the vector population on the Kenyan coast is thought to be highly anthropophilic, there was no information regarding the feeding behavior of the sibling species of the *Anopheles gambiae* complex. The main objective of this study was to determine the identification of blood-meal hosts from *Anopheles funestus* and members of the *An. gambiae* complex: *An. gambiae* s.s., *Anopheles arabiensis,* and *Anopheles merus* along the Kenyan coast. This study extends that of Mbogo et al. (1993a) both geographically and by furnishing species-specific data obtained through polymerase chain reaction (PCR) analysis. In addition, information also is provided on *P. falciparum* sporozoite infection rates for field-collected mosquitoes.

MATERIALS AND METHODS

Study sites

Thirty study sites (Fig. 1), which were distributed 2–60 km from the coastline, were selected for malaria vector studies in Malindi, Kilifi, and Kwale districts (Mbogo et al. 2003). In each district, 10 collection sites were selected and, at each collection site, 10 houses were randomly identified for mosquito sampling from June 1997 to May 1998. At the 30 sites, the houses were mainly constructed of mud walls and the roofs made of coconut thatch. In most houses, the windows were unscreened and walls had holes. This facilitated easy movement of mosquitoes in and out of the houses. In these sites, the primary agricultural activities included cash crops, such as cashew nuts, coconuts, mangoes, bananas, pawpaws, and oranges. The subsistence farming included growing of maize, cassava, beans, and peas. In these collection sites, the vertebrate hosts were mainly goats, chickens, and cattle. The

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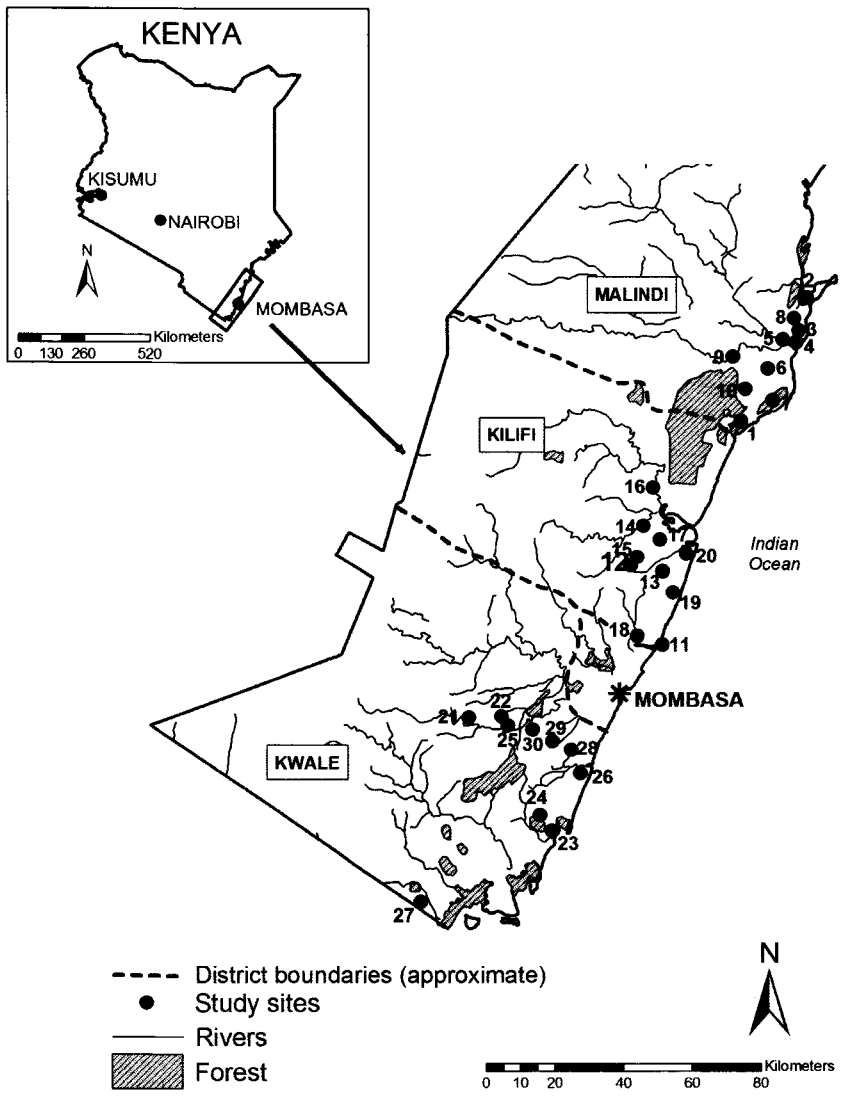

Fig. 1. A map of the 30 collection sites in the study area.

majority of the homesteads kept goats and chickens as the main domestic animals, whereas few households kept cattle.

Mosquito collection

An entomological survey was conducted once every 2 months in each collection site from June 1997 to May 1998. Ten houses were selected randomly at each collection site and sampled for indoor resting mosquitoes by pyrethrum spray sheet collection (PSC) (WHO 1975). The mosquitoes were placed in vials containing Carnoy's solution (3:1 ethanol:glacial acetic acid) for preservation, labeled, and then transported to the laboratory for analysis. The samples were kept frozen at −20°C until testing.

Laboratory processing

The anopheline mosquitoes were classified according to bloodfeeding stages (WHO 1975), and only the fully bloodfed females were used for enzyme-linked immunosorbent assay (ELISA) testing. In the laboratory, the anopheline mosquitoes were identified morphologically (Gillies and Coetzee 1987) and cut transversely between the thorax and the abdomen. The posterior part containing the blood meal was placed in a vial and then labeled by a unique number for the individual mosquito. The head–thorax was placed in a different vial labeled by mosquito number.

The *An. gambiae* s.l. were further identified to sibling species by the PCR method (Scott et al. 1993). Briefly, DNA was extracted from single

Table 1. Summary of all samples tested and identified by ELISA for *Anopheles gambiae* s.l. and *Anopheles funestus* collected in the districts of Malindi, Kilifi, and Kwale along the coast of Kenya from June 1997 to May 1998.

District	Species	No. tested	Bloodmeal sources				
			Human	Bovine	Chicken	Goat	Unidentified
Malindi	*An. gambiae* s.l.	896	850	2	0	5	39
	An. funestus	62	54	0	0	1	7
Kilifi	*An. gambiae* s.l.	272	259	0	2	1	10
	An. funestus	133	129	0	0	0	4
Kwale	*An. gambiae* s.l.	312	238	0	1	3	70
	An. funestus	244	196	0	0	1	47
Total		1,919	1,726	2	3	11	177

mosquito triturates using the method described by Collins et al. (1987). Eight microliters of the sample DNA were then used as the template for PCR amplification. Each amplified sample was run in 1.8% horizontal agarose-Tris-boric acid-EDTA gels and visualized by an ultraviolet transilluminator.

Blood-meal identification

Each mosquito abdomen was ground in 50 μl of phosphate-buffered saline (PBS), then 950 μl of PBS were added after grinding. Blood meals were identified by direct ELISA using antihost (IgG) conjugate against human and cow proteins in a single-step assay (Beier et al. 1988). The nonreacting samples were then tested subsequently using chicken and goat IgG. ELISA results were read visually (Beier and Koros 1991).

P. falciparum sporozoite ELISA

The head and thorax of each individual mosquito was ground using 50 μl of boiled casein-blocking buffer (BB) with Nonidet P-40 (NP-40; 5 μl NP40/1 ml BB); 200 μl of BB were then added, bringing the final volume to 250 μl. Fifty-microliter aliquots were tested by ELISA using monoclonal antibodies to detect circumsporozoite (CS) proteins of *Plasmodium falciparum* (Wirtz et al. 1987). The results were read visually (Beier and Koros 1991).

RESULTS

Blood meal identification

A total of 1,919 bloodfed anopheline mosquitoes collected from the 30 sites along the Kenya coast were tested by ELISA. Of these, 1,480 (77.1%) were *An. gambiae* s.l. and 439 (22.9%) were *An. funestus* Giles (Table 1). Overall, the ELISA identified 92 and 87% of the samples tested in *An. gambiae* s.l. and *An. funestus,* respectively. Of these, human IgG was detected in 98.97% (n = 1,347) of *An. gambiae* s.l. and 99.48% (n = 379) of *An. funestus.* Only 14 (1.03%) of the *An. gambiae* s.l. had fed on other vertebrate hosts tested, which were bovines, chickens, and goats. Additionally, only 2 *An. funestus* had fed on goats. None of the mosquitoes tested were found to have multiple blood meals.

The blood-meal analysis results according to each district showed that the highest proportion of the anopheline mosquitoes fed on humans (Table 2). However, at 2 sites, Dabaso and Chasimba, no bloodfed anopheline mosquitoes were collected. In all the 28 sites, *An. gambiae* s.l. had a human blood index greater than 0.9. Twenty-five of these sites had a human blood index greater than 0.9 for *An. funestus,* and the other 3 sites had no bloodfed mosquitoes.

PCR analysis, the blood-meal sources, and *P. falciparum* infection rates

Table 3 shows the PCR results for the *An. gambiae* complex, the blood-meal sources for each species, and the sporozoite infection rates. Of the *An. gambiae* s.l. tested, 429 were further analyzed by PCR for their sibling species. *An. gambiae* s.s., *An. arabiensis,* and *An. merus* showed a high degree of human bloodfeeding. There was no significant difference between the human blood index for *An. gambiae* s.s. and *An. arabiensis* (χ^2 = 0.009; df = 1; P = 0.556). The degree of feeding on the other vertebrate hosts was very low. Only 0.9% of the *An. gambiae* s.s. fed on other vertebrate hosts (chickens, bovine, and goats), while 1.3% of the *An. arabiensis* fed on goats.

The *P. falciparum* sporozoite infection rates for the *An. gambiae* complex and *An. funestus* were 6.2 and 3.7%, respectively. There was no significant difference between the infection rates of *An. gambiae* complex and *An. funestus* (χ^2 = 2.73, df = 1, P = 0.093). The *P. falciparum* infection rates were 1.9% (n = 338) and 1.4% (n = 79) for *An. gambiae* s.s. and *An. arabiensis,* respectively. There was no significant difference between the infection rates for *An. gambiae* s.s. and *An. arabiensis* (χ^2 0.05, P = 0.829). No sporozoite infections were found for the 12 *An. merus* tested.

Table 2. The proportion of *Anopheles funestus* and *Anopheles gambiae* s.l. that fed on human hosts from mosquito collections made from 10 sites in each of 3 districts along the Kenyan coast.

District	Site	*An. gambiae* s.l.		*An. funestus*	
		No. identified	% human	No. identified	% human
Malindi	Dabaso	0	0.0	0	0.0
	Garithe	163	98.2	1	100.0
	Kagombani	40	100.0	1	100.0
	Majenjeni	121	100.0	1	100.0
	Masheheni	74	98.6	1	100.0
	Maziwani	74	98.6	25	100.0
	Mbaraka Chembe	10	90.0	1	100.0
	Mjanaheri	77	98.7	0	0.0
	Mijomboni	156	99.4	10	90.0
	Paziani	131	98.5	15	100.0
Kilifi	Barani	34	100.0	66	100.0
	Chasimba	0	0.0	0	0.0
	Dindiri	52	98.1	23	100.0
	Jaribuni	67	98.5	10	100.0
	Kitsoeni	14	100.0	5	100.0
	Kitengwani	6	100.0	8	100.0
	Majajani	68	98.5	8	100.0
	Mtepeni	11	100.0	6	100.0
	Shariani	8	100.0	0	0.0
	Takaungu	2	100.0	3	100.0
Kwale	Amani	102	100.0	29	100.0
	Dumbule	2	100.0	2	100.0
	Gazi	16	93.8	20	100.0
	Magaoni	15	100.0	36	100.0
	Moyeni	11	90.9	5	100.0
	Mwaroni	2	100.0	2	0.0
	Tsuini	60	100.0	55	98.2
	Vinuni	23	100.0	41	100.0
	Vuga	1	100.0	7	100.0
	Ziwani	10	100.0	2	100.0

DISCUSSION

Our results show that *An. funestus* and members of the *An. gambiae* complex; *An. gambiae* s.s., *An. arabiensis* Patton, and *An. merus* Donitz, are highly anthropophilic. The high degree of human feeding may be a primary factor contributing to the efficiency of *P. falciparum* transmission on the Kenyan coast (Mbogo et al. 1993b). These results are similar to those reported by Mbogo and colleagues (1993a) in that they also found that a high proportion of *An. gambiae* s.l. and *An. funestus* had fed predominantly on humans irrespective of the availability of cattle and other domestic animals. No multiple blood meals were detected. All 3 members of the *An. gambiae* complex: *Anopheles gambiae* s.s., *An. arabiensis,* and *An. merus,* had fed predominantly on humans. The human blood index found in this study appears to be one of the highest on record for malarial transmission in Africa. It is surprising that *An. arabiensis* feed predominantly on humans at the coastal Kenya. This is unlike the situation in western Kenya (Beier et al. 1988, Petrarca et al. 1991, Petrarca and Beier 1992, Githeko et al. 1994) and in the Mwea irrigation scheme

Table 3. Blood-meal sources for the *Anopheles gambiae* s.l. and *Anopheles funestus* and the proportion that fed on each vertebrate host shown in parentheses.

Species[1]	No. tested	Bloodmeal sources				
		Human	Bovine	Chicken	Goat	Unidentified
An. gambiae complex	1,480	1,347 (91.01)	2 (0.15)	3 (0.22)	9 (0.61)	119 (8.04)
An. gambiae s.s.	338	323 (95.56)	1 (0.29)	1 (0.29)	1 (0.29)	12 (3.55)
Anopheles arabiensis	79	72 (91.14)	0 (0.00)	0 (0.00)	1 (1.27)	6 (7.59)
Anopheles merus	12	12 (100.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Anopheles funestus	439	379 (86.33)	0 (0.00)	0 (0.00)	2 (0.46)	58 (13.21)

[1] *An. gambiae* s.s., *An. arabiensis,* and *An. merus* of the *An. gambiae* complex were identified by polymerase chain reaction.

(Ijumba et al. 1990), where the availability of cows is a determining factor for bloodfeeding.

The limitations of our study include small sample sizes from some sites, lack of outdoor samples, some blood meals were not identified though all the mosquitoes were bloodfed and the study did not address potential differences in availability of hosts as in Mbogo et al. (1993a). However, further efforts are necessary to identify blood meals from outdoor resting mosquito populations. In conclusion, there is evidence that *An. funestus* and members of the *An. gambiae* complex; *An. gambiae* s.s., *An. arabiensis,* and *An. merus* along the Kenyan coast feed predominantly on humans. From a public-health point of view, it shows that the management and control of malaria vectors along the coast of Kenya may be easily targeted using the currently available tools for reducing human–mosquito contact.

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REFERENCES CITED

Beier JC, Koros J. 1991. Visual assessment of sporozoite and bloodmeal ELISA samples in malaria field studies. *J Med Entomol* 28:805–808.

Beier JC, Perkins PV, Wirtz RA, Koros J, Diggs D, Gargan II TP, Koech DK. 1988. Bloodmeal identification by direct enzyme-linked immunosorbent assay (ELISA), tested on *Anopheles* (Diptera: Culicidae) in Kenya. *J Med Entomol* 25:9–16.

Collins F, Mendez MA, Rasmussen M, Mehaffey PC, Besansky NJ, Finnerty V. 1987. A ribosomal RNA gene probe differentiates members of the *Anopheles gambiae* complex. *Am J Trop Med Hyg* 37:37–41.

Garret-Jones C, Boreman PFL, Bidwell DE. 1980. Feeding habits of anopheline (Diptera: Culicidae) in 1971–78, with reference to the human blood index: a review. *Bull Ent Res* 70:165–185.

Gillies M, Coetzee M. 1987. A supplement to Anophelinae of Africa south of Sahara (Afro-tropical region). Publication of the South Africa Institute of Medical Research 55:1–143.

Githeko A, Service MW, Mbogo CM, Atieli FK, Juma O. 1994. Origin of bloodmeals in in-door and out-door resting malaria vectors in Western Kenya. *Acta Trop* 58:307–316.

Highton R, Bryan H, Boreham PFL, Chandler JA. 1979. Studies on the sibling species *Anopheles gambiae* Giles and *An. arabiensis* Patton (Diptera: Culicidae) in the Kisumu area, Kenya. *Bull Ent Res* 69:43–53.

Ijumba JN, Mwangi RW, Beier JC. 1990. Malaria transmission potential of *Anopheles* mosquitoes in the Mwea-Tebere irrigation scheme, Kenya. *Med Vet Entomol* 4:425–432.

Joshi G, Service MW, Pradhan GD. 1975. A survey of species A and B of the *Anopheles gambiae* Giles complex in the Kisumu area of Kenya prior to insecticidal spraying with OMS-43 (fenitrothion). *Ann Trop Med Parasitol* 69:91–104.

Mbogo CM, Kabiru EW, Muiruri SK, Nzovu JG, Ouma JH, Githure JI, Beier JC. 1993a. Blood feeding behavior of *Anopheles gambiae* s.l. and *Anopheles funestus* in Kilifi District, Kenya. *J Am Mosq Control Assoc* 9:225–227.

Mbogo CM, Mwangangi JM, Nzovu J, Gu W, Yan G, Gunter J, Swalm C, Keating J, Regens JL, Shililu JI, Githure JI, Beier JC. 2003. Spatial and temporal heterogeneity of *Anopheles* mosquitoes and *Plasmodium falciparum* transmission along the Kenyan coast *Am J Trop Med Hyg* 68:734–742.

Mbogo CM, Snow RW, Kabiru EW, Ouma JH, Githure JI, Marsh K, Beier JC. 1993b. Low-level *Plasmodium falciparum* transmission and the incidence of severe malaria infection on the Kenyan Coast. *Am J Trop Med Hyg* 49:245–253.

Mosha F, Petrarca V. 1983. Ecological studies on *Anopheles gambiae* complex on the Kenya coast. *Trans R Soc Trop Med Hyg* 77:344–345.

Mutero C, Mosha FW, Subra R. 1984. Biting activity and resting behaviour of *Anopheles merus* Donitz (Diptera: Culicidae) on the Kenyan Coast. *Ann Trop Med Parasitol* 78:43–47.

Petrarca V, Beier JC. 1992. Intra-specific chromosomal polymorphism in the *Anopheles gambiae* complex as a factor affecting malaria transmission in Kisumu area of Kenya. *Am J Trop Med Hyg* 46:229–237.

Petrarca V, Beier JC, Onyango F, Koros J, Asiago C, Koech DK, Roberts CR. 1991. Species composition of *Anopheles gambiae* complex (Diptera: Culicidae) at two sites in Western Kenya. *J Med Entomol* 28:307–313.

Scott J, Brodgon WG, Collins FH. 1993. Identification of single specimens of *Anopheles gambiae* complex by polymerase chain reaction. *Am J Trop Med Hyg* 49: 520–529.

Service M. 1989. The importance of ecological studies on malaria vectors. *Bull Soc Vector Ecol* 14:26–38.

Service M, Joshi GP, Pradhan GO. 1978. A survey of *Anopheles gambiae* (species A) and *An. arabiensis* (species B) of the *An. gambiae* Giles complex in the Kisumu area of Kenya following insecticidal spraying with OMS-43 (fenitrothion). *Ann Trop Med Parasitol* 72:377–386.

Service M, Voller A, Bidwell DE. 1986. The enzyme-linked immunosorbent assay (ELISA) test for the identification of bloodmeals of haematophagous insects. *Bull Entomol Res* 76:321–330.

WHO [World Health Organization]. 1975. *Manual on practical entomology in Malaria.* Part II. *Methods and techniques* Geneva, Switzerland: World Health Organization Offset Publication.

Wirtz R, Zavala F, Charoenvit Y, Campbell GH, Burkot TR, Schneider I, Esser KM, Beaudoin RL, Andre RG. 1987. Comparative testing of *Plasmodium falciparum* circumsporozoite antibody. *Bull Wld Hlth Org* 65:39–45.