Contribution of *Anopheles funestus*, *An. gambiae* and *An. nili* (Diptera: Culicidae) to the perennial malaria transmission in the southern and western forest areas of Côte d'Ivoire

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The involvement of members of the *Anopheles gambiae* complex Giles and *An. funestus* Giles and *An. nili* Theobald groups in the transmission of *Plasmodium falciparum* was recently investigated in the villages of Gbatta and Kpéhiri, which lie, respectively, in forest areas in the west and south of Côte d'Ivoire.

Adult female mosquitoes were collected, using human landing catches, inside and outside dwellings. After identification and dissection, the heads and thoraces of all the anopheline mosquitoes were tested, in an ELISA, for circumsporozoite protein (CSP). All the female anopheline mosquitoes collected and identified to species using PCR were found to be *An. gambiae* s.s., *An. nili* s.s. or *An. funestus* s.s., with *An. gambiae* s.s. and *An. funestus* s.s. predominant in Gbatta but *An. nili* s.s. the most common species in Kpéhiri. In Gbatta, 3.1% of the female *An. gambiae* collected, 5.0% of the female *An. funestus* and 1.8% of the female *An. nili* were found CSP-positive. The corresponding values in Kpéhiri were even higher, at 5.9%, 6.2% and 2.4%, respectively. The estimated entomological inoculation rates (EIR) were very high: 302 infected bites (139 from *An. gambiae*, 146 from *An. funestus* and 17 from *An. nili*)/person-year in Gbatta and 484 infected bites (204 from *An. gambiae*, 70 from *An. funestus* and 210 from *An. nili*)/person-year in Kpéhiri.

In Gbatta, *An. gambiae* s.s. was responsible for most of the rainy-season transmission while *An. funestus* became the main malaria vector in the dry seasons. In Kpéhiri, however, *An. nili* appeared to be the main vector throughout the year, with *An. gambiae* of secondary importance and *An. funestus* only becoming a significant vector during the rainy season. Although, in both study sites, intense transmission was therefore occurring and the same three species of anopheline mosquito were present, the relative importance of each mosquito species in the epidemiology of the human malaria at each site differed markedly.

In most of the endemic countries in sub-Saharan Africa, human malaria remains one

of the leading causes of morbidity and mortality, particularly among young children, pregnant women and non-immune individuals (Bremner *et al.*, 2001; Guïyedi *et al.*, 2007). Although malaria can be

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severely limited, if not eliminated, by effective vector control, the implementation of any successful vector-control measures requires knowledge of the biology of the anopheline species present in the area to be targeted.

In tropical Africa, the parasites causing human malaria are mostly transmitted by primary vector species such as members of the *Anopheles gambiae* Giles complex (*An. gambiae* s.s., *An. arabiensis*, *An. quadriannulatus* A, *An. quadriannulatus* B, *An. bwambae*, *An. melas*, *An. merus* and, possibly, *An. comorensis*) and members of the *An. funestus* Giles and *An. nili* Theobald groups (Fontenille and Lochouarn, 1999; Becker *et al.*, 2010; see below).

In Côte d'Ivoire, over the last couple of decades, the role of *An. gambiae* s.l. in the transmission of malarial parasites in the savannah zone has been investigated several times (Dossou-Yovo *et al.*, 1994; Adja *et al.*, 2006; Diakité *et al.*, 2010; Koudou *et al.*, 2010); there have been relatively few studies on malaria transmission in the southern, forest zone. *Anopheles gambiae* s.s., which remains the most widespread malaria vector in tropical Africa, has two molecular forms, known as M and S, and it is the S form that predominates (9:1) in the north of Côte d'Ivoire (Toure, 2001).

In some areas of Africa, *An. funestus*, which is both endophilic and anthropophilic, is as important in transmitting malarial parasites to humans as any of the other vectors, and it can sometimes be the main vector (Fontenille *et al.*, 1997). This species, which can be observed throughout the year in forest zones in the Democratic Republic of Congo (Coene, 1993) and on rivers near to forest in Kenya (Githeko *et al.*, 1996), belongs to a group of 10 species (*An. funestus*, *An. vaneedeni*, *An. parensis*, *An. aruni*, *An. fuscivenosus*, *An. lesoni*, *An. confusus*, *An. rivulorum*, 'An. rivulorum-like' and *An. brucei*) that are morphologically similar, at least as adults (Gillies and Coetzee, 1987; Cohuet *et al.*, 2003; Coetzee and Fontenille, 2004). Together, these 10 species are usually known as the

'*An. funestus*' group. Two chromosomal forms of *An. funestus*, known as Kiribina and Folonzo, have recently been described (Michel *et al.*, 2006). Very little is known about mosquitoes of the *An. funestus* group in Côte d'Ivoire.

Anopheles nili s.l. has a wide geographical distribution across most of tropical Africa (Hamon and Mouchet, 1961) and is regarded as one of the five major malaria vectors in Africa (Fontenille and Simard, 2004). Four morphologically similar species (*An. nili* s.s., *An. somalicus*, *An. carnevalei* and *An. ovengensis*) are known as the '*An. nili* group' or *An. nili* s.l. (Brunhes *et al.*, 1999; Awono-Ambene *et al.*, 2004; Fontenille and Simard, 2004). Carnevale *et al.* (1992) described *An. nili* (s.l.) as a very efficient vector of *Plasmodium falciparum* in some villages in southern Cameroon. As with several other known vectors, there is little information available on *An. nili* s.l. in Côte d'Ivoire, although there would appear to be many habitats that could support the breeding of these mosquitoes, especially in the southern forest zone (the larvae of *An. nili* s.l. are typically found in vegetation or in dense shade along the edges of streams and large rivers).

The main aims of the present study were to identify the mosquito species (within the *An. gambiae* complex and *An. nili* and *An. funestus* groups) to be found in the forest areas of western and southern Côte d'Ivoire, and to evaluate the relative importance of each species in the transmission of *P. falciparum* to the local human populations.

MATERIALS AND METHODS

Study Area

The study was carried out in the villages of Gbatta (6°56'N, 8°13'W) and Kpéhiri (5°49'N, 6°37'W), which are located in the forest areas of western and southern Côte d'Ivoire, respectively (Fig. 1).

In Gbatta, the basic economic activity is agriculture (with rice and coffee the main crops), the population at the time of the

FIG. 1. Map of Cote d'Ivoire, showing the locations of the two study sites.

present study was about 820 (mostly of the Yacouba ethnic group) and most of the houses are built in the traditional style, with mud walls and thatched rooves. The perennial River Vi flows near the village, the single rainy season per year runs from April to October, the mean annual rainfall is 1600 mm, and the mean temperature is 24.8°C.

Kpéhiri, which lies approximately 800 m from the mighty Sassandra River and closer to the perennial River Nawa, had a population at the time of the present study of about 2200, who were mostly of the Bakoué ethnic group and cocoa or coffee farmers. The village's houses are of a more modern style than those of Gbatta, with walls of cement and/or mud and rooves of corrugated iron. The village experiences two rainy seasons each year, one running from mid-September to mid-November and the other from March to mid-July, and has a mean annual rainfall of 1400 mm and a mean temperature of 25.7°C.

Mosquito Collections

Adult mosquitoes were sampled, using overnight (i.e. 18.00 to 06.00 hours) human landing collections (HLC) inside and outside human dwellings, every 2 months, from May 2001 to May 2002 in Gbatta and from February 2002 to August 2002 in Kpéhiri.

In Gbatta, each bimonthly collection consisted of three consecutive nights of HLC, with one collector indoors and one outdoors at each of three sentinel collection points. In Kpéhiri, however, each bimonthly collection consisted of two consecutive nights of HLC, with one collector indoors and one outdoors at each of four sentinel collection points.

FIELD PROCESSING OF MOSQUITOES

After collection, mosquitoes were morphologically identified using the identification keys of Gillies and Coetzee (1987). The parity of each female anopheline mosquito was assessed by dissecting out the ovaries

and observing the degree of coiling of the ovarian tracheoles (Detinova, 1962). Each female anopheline mosquito collected was stored, individually, in a labelled microcentrifuge tube, with desiccant, for laboratory processing.

LABORATORY PROCESSING OF MOSQUITOES

A sub-sample of 465 mosquitoes — 234 (60 *An. gambiae* s.l., 98 *An. funestus* group and 76 *An. nili* group) from Gbatta and 231 (60 *An. gambiae* s.l., 78 *An. funestus* group and 93 *An. nili* group) from Kpéhiri — was used, in PCR-based assays, to identify the species of each complex/group present at the two sites. Separate assays were used for species within the *An. gambiae* complex (Scott *et al.*, 1993), *An. funestus* group (Koekemoer *et al.*, 2002; Cohuet *et al.*, 2003) and *An. nili* group (Kengne *et al.*, 2003).

The heads and thoraces of all 2898 female anopheline mosquitoes collected were tested, in an ELISA, for the circumsporozoite protein (CSP) of *P. falciparum*, using the method of Burkot *et al.* (1984) as modified by Wirtz *et al.* (1987).

Ethics

The study protocol was approved by the Zouan-Houin regional office of the Ministry of Public Health. All the mosquito collectors were adult volunteers (aged 18–35 years) from the study villages. Each of these volunteers gave his or her informed consent, was protected with appropriate antimalarial prophylaxis, and was immunized against yellow fever.

Data Analysis

The human biting rates (bites/person-night), sporozoite 'rates' (% of tested mosquitoes found ELISA-positive for CSP) and entomological inoculation rates (EIR; infective bites per person-night and person-year) were calculated for the different populations (Fontenille *et al.*, 1997).

All data were analysed using version 9.04 of the STATA software package (Stata

Corporation, College Station, TX). An exact binomial distribution was assumed in the calculation of 95% confidence intervals (CI) for the sporozoite 'rates' and human biting rates. The overall results for the two study villages were compared in χ^2 tests (sporozoite 'rates') or Kruskal–Wallis tests (human biting rates and EIR). A *P*-value of ≤ 0.05 was considered indicative of a statistically significant difference.

RESULTS

Composition and Abundance of Mosquito Fauna

In Gbatta, 4334 mosquitoes were collected in the landing catches, most (71.8%) of these were anopheline, and most (93.2%) of the anopheline mosquitoes collected were members of the *An. gambiae* complex (50.1%), *An. funestus* group (32.5%) or *An. nili* group (10.6%) (see Table 1).

In Kpéhiri, 3301 mosquitoes were collected in landing catches and, as in Gbatta, most of these (75.6%) were anopheline, and most (99.5%) of the anopheline mosquitoes collected were members of the *An. gambiae*

complex (25.8%), *An. funestus* group (8.4%) or *An. nili* group (65.3%) (see Table 1).

Among all the anopheline mosquitoes caught, from both study villages combined, members of the *An. nili* group (65.3%) predominated over *An. gambiae* s.l. (25.8%) and members of the *An. funestus* group (8.4%).

SPECIES IN THE *Anopheles gambiae* COMPLEX AND *An. funestus* AND *An. nili* GROUPS IDENTIFIED IN THE STUDY VILLAGES

All of the 120 female *An. gambiae* s.l. identified to species level in a PCR-based assay were found to be *An. gambiae* s.s. and most (73.2% of the 60 tested specimens from Gbatta and 60.9% of the 60 from Kpéhiri) were of the M molecular form.

Similarly, all of the females of the *An. funestus* group investigated by PCR were identified as *An. funestus* s.s., and all of the females of the *An. nili* group investigated by PCR were identified as *An. nili* s.s..

Given the results of the PCR, all of the members of the *An. gambiae* complex and *An. funestus* and *An. nili* groups in the two study villages were assumed to be *An.*

TABLE 1. The numbers of adult female mosquitoes collected on human bait in Gbatta between May 2001 and May 2002 and in Kpéhiri between the February and August of 2002

Species	No. of adult females collected in:		
	Gbatta	Kpéhiri	Both villages
<i>Anopheles gambiae</i> complex	1559	643	2202
<i>An. funestus</i> group	1012	210	1222
<i>An. nili</i> group	329	1630	1959
<i>An. coustani</i>	1	1	2
<i>An. paludis</i>	4	7	11
<i>An. pharoensis</i>	31	3	34
<i>An. ziemanni</i>	177	2	179
Any <i>Anopheles</i>	3113	2496	5609
<i>Mansonia</i> sp.	960	668	1628
<i>Culex</i> sp.	96	66	162
<i>Aedes</i> sp.	79	59	138
<i>Coquillettidia</i> sp.	13	0	13
<i>Eretmapodites</i> sp.	13	12	25
<i>Uranotaenia</i> sp.	60	0	60
Any species	4334	3301	7635

FIG. 2. Seasonal variation in the human-biting rates of *Anopheles funestus* (■), *An. nili* (□) and *An. gambiae* (■) in Gbatta village, from May 2001 to May 2002.

gambiae s.s., *An. funestus* s.s. and *An. nili* s.s., respectively.

Mosquito Population Dynamics

The overall biting rates in Gbatta and Kpéhiri, in bites/person-night, were estimated at 12.4 and 9.5 for *An. gambiae* s.s. ($P>0.05$), 8.0 and 3.1 for *An. funestus* s.s. ($P<0.001$) and 2.6 and 24 for *An. nili* s.s. ($P<0.001$), respectively.

In terms of human biting, *An. gambiae* s.s. appeared to be the predominant anopheline species in Gbatta, with a peak biting rate, of 43 bites/person-night, recorded in September 2001 (Fig. 2). The highest rates of human biting by *An. funestus* s.s. were recorded during dry seasons: in January 2002 in Gbatta (13.1 bites/person-night) and in August 2002 in Kpéhiri (7.8 bites/person-night). In Kpéhiri, the biting rates for *An. nili* s.s. remained relatively high (>20 bites/person-night) throughout the study period, which ran from the February to the August of 2002 (Fig. 3).

Overall, 57.8% and 60.5% of the *An. gambiae* s.s., 57.9% and 72.9% of the *An.*

funestus s.s. and 51.4% and 52.6% of the *An. nili* s.s. collected in Gbatta and Kpéhiri, respectively, were collected indoors, indicating that all three species were mainly endophagic.

Parity 'Rates' and Sporozoite Infections

Of the female anophelines collected, 1386 of those from Gbatta and 1512 of those from Kpéhiri were dissected and classified as parous or nulliparous. The estimated parity 'rates' and (95% confidence intervals) for the dissected specimens from Gbatta and Kpéhiri were, respectively, 59.8% (55.1%–64.4%) and 67.0% (62.0%–72.1%) for *An. gambiae* s.s. ($P=0.038$), 77.1% (74.1%–80.1%) and 55.2% (47.3%–63.6%) for *An. funestus* s.s. ($P<0.001$), and 62.4% (56.1%–69.0%) and 58.3% (55.3%–61.3%) for *An. nili* s.s. ($P=0.71$).

The overall sporozoites 'rates' and (95% confidence intervals) in Gbatta and Kpéhiri were, respectively, 3.1% (1.4%–4.7%) and 5.9% (3.4%–8.5%) for *An. gambiae* s.s., 5.0% (3.4%–6.6%) and 6.2% (2.2%–10.1%) for *An. funestus* s.s., and 1.8% (0.0%–3.6%)

FIG. 3. Seasonal variation in the human-biting rates of *Anopheles funestus* (■), *An. nili* (□) and *An. gambiae* (■) in Kpéhiri village, from February 2002 to August 2002.

and 2.4% (1.5%–3.4%) for *An. nili* s.s. (Table 2).

Entomological Inoculation Rates

The biting rates and sporozoite 'rates' recorded in the present study indicate that, in 2001–2002, *An. gambiae* s.s., *An. funestus* s.s. and *An. nili* s.s. were all vectors of *P. falciparum* in the two study villages, although their relative importance varied with the village and/or season.

During the 13-month survey in Gbatta, the EIR for *P. falciparum* was estimated at 24.9 infective bites/person-month, with *An. gambiae* s.s., *An. funestus* s.s. and *An. nili* s.s. responsible for 11.4, 12.0 and 1.5 of those infective bites, respectively (see Table 3). The monthly EIR recorded for *An. funestus* s.s. in Gbatta was thus eight times higher than the corresponding value for *An. nili* s.s.. Transmission of *P. falciparum* by *An. funestus* s.s. was observed at the end of the rainy season and during the dry season whereas *An. nili* s.s. only appeared to be significant vector of this pathogen in May (2001 and 2002) and September (2001).

During the 8-month survey in Kpéhiri, the monthly EIR was estimated at 39.9

infective bites/person-month, with *An. gambiae* s.s., *An. funestus* s.s. and *An. nili* s.s. responsible for 16.8, 5.7 and 17.4 of those infective bites, respectively (see Table 3). The EIR recorded for *An. funestus* s.s. in Kpéhiri was thus half the corresponding value in Gbatta but the EIR recorded for *An. nili* s.s. in Kpéhiri was 12 times higher than that observed in Gbatta. In Kpéhiri, *An. nili* s.s. appeared to be transmitting *P. falciparum* throughout the year (or, at least, in every month in which mosquitoes were collected in the village).

DISCUSSION

The results of the present study, implemented in Gbatta and Kpéhiri, demonstrated the sympatric presence of members of the *An. gambiae* complex and *An. funestus* and *An. nili* groups in the forest zone of Côte d'Ivoire. This confirmed old data collected by Doucet *et al.* (1960) and recent observations made during a systematic survey of the Culicine fauna of Côte d'Ivoire (Fofana *et al.*, 2010). All of the female specimens of the *An. gambiae* complex that were collected

TABLE 2. Sporozoite infection 'rates' (as indicated by ELISA positivity for Plasmodium falciparum circumsporozoite protein) in samples of the Anopheles gambiae s.s., An. funestus s.s. and An. nili s.s. caught in Gbattia or Kpéhiri

Village	Collection month	Anopheles gambiae s.s.				Anopheles funestus s.s.				Anopheles nili s.s.			
		Prevalence and (95% confidence interval)		No. of females:		Prevalence and (95% confidence interval)		No. of females:		Prevalence and (95% confidence interval)		No. of females:	
		Tested	Positive	(%)		Tested	Positive	(%)		Tested	Positive	(%)	
Gbattia	May 2001	75	3	4.0 (0.5–8.5)		37	0	0		26	0	0	
	July 2001	80	1	1.3 (1.2–3.7)		47	0	0		28	0	0	
	September 2001	95	4	4.2 (0.1–8.3)		116	8	6.9 (2.2–11.6)		66	1	1.5 (0.0–4.5)	
	November 2001	42	0	0		148	4	2.7 (0.1–5.3)		37	0	0	
	January 2002	8	0	0		282	19	6.7 (3.8–9.7)		15	0	0	
	March 2002	50	3	6.0 (0.8–12.8)		54	1	1.9 (0.0–5.6)		18	0	0	
	May 2002	75	2	2.7 (1.1–6.4)		58	5	8.6 (1.2–11.6)		29	3	10.3 (0.0–22.1)	
Kpéhiri	All	425	13	3.1 (1.4–4.7)		742	37	5.0 (3.4–6.6)		219	4	1.8 (0.0–3.6)	
	February 2002	11	0	0		12	1	8.3 (0.0–26.7)		145	10	6.9 (2.7–11.1)	
	April 2002	116	9	7.8 (2.8–12.7)		0	0	0		403	6	1.5 (0.3–2.7)	
	June 2002	127	7	5.5 (1.5–9.5)		4	0	0		194	4	2.1 (0.0–4.1)	
	August 2002	83	4	4.8 (0.1–9.5)		130	8	6.2 (2.0–10.3)		287	5	1.7 (0.2–3.3)	
	All	337	20	5.9 (3.4–8.5)		146	9	6.2 (2.2–10.1)		1029	25	2.4 (1.5–3.4)	

TABLE 3. Numbers of mosquitoes tested and estimates of the entomological inoculation rates (EIR), in infective bites/person-night, for *Anopheles gambiae* s.s., *An. funestus* s.s. and *An. nili* s.s. in Gbatta or Kpéhi

Village	Sampling month	<i>Anopheles gambiae</i> s.s.		<i>Anopheles funestus</i> s.s.		<i>Anopheles nili</i> s.s.	
		No. tested	EIR	No. tested	EIR	No. tested	EIR
Gbatta	May 2001	75	0.40	37	0	26	0
	July 2001	80	0.26	47	0	28	0
	September 2001	95	1.81	116	0.49	66	0.06
	November 2001	42	0	148	0.03	37	0
	January 2002	8	0	282	0.88	15	0
	March 2002	50	0.26	54	0.07	18	0
	May 2002	75	0.29	58	0.33	29	0.27
	All seven*	425	0.38	742	0.40	219	0.05
Kpéhi	February 2002	11	0	12	0.11	145	1.82
	April 2002	116	0.98	0	—	403	0.42
	June 2002	127	0.91	4	0	194	0.39
	August 2002	83	0.31	130	0.48	287	0.40
	All four†	337	0.56	146	0.19	1029	0.58

*These overall values for Gbatta indicate equivalent mean monthly EIR of 11.4, 12.0 and 1.5 infective bites/person-month for *Anopheles gambiae* s.s., *An. funestus* s.s. and *An. nili* s.s., respectively.

†These overall values for Kpéhi indicate equivalent mean monthly EIR of 16.8, 5.7 and 17.4 infective bites/person-month for *Anopheles gambiae* s.s., *An. funestus* s.s. and *An. nili* s.s., respectively.

during the present study and identified to species level were found to be *An. gambiae* s.s. and were predominantly of the M molecular form. No *An. arabiensis* were identified at either of the two study sites. The use of molecular tools developed in the last decade (Koekemoer *et al.*, 2002; Cohuet *et al.*, 2003) allowed the members of the *An. funestus* group present at the study sites also to be identified to species level. *Anopheles funestus* s.s., the main *Plasmodium* vector in this group (Coetzee and Fontenille, 2004), was the only member of the group detected in the study villages. Although this species is usually the only member of the *An. funestus* group represented in entomological surveys conducted elsewhere in West Africa, such as in Senegal (Cohuet *et al.*, 2004), Burkina Faso (Dabiré *et al.*, 2008) and Ghana (Coetzee *et al.*, 2006), the sampling techniques used often preferentially target anthropophilic (human landing catches) and/or endophagic (indoor spray catches) species such as *An. funestus* s.s.. All of the members of the *An. nili* group identified to species level in the present study — and in the

village of Ngari, which is located near the forest gallery in southern Senegal (Dia *et al.*, 2003) — were found to be *An. nili* s.s..

In Gbatta, in south-western Côte d'Ivoire, *An. gambiae* s.s. and *An. funestus* s.s. were collected all year round, with *An. gambiae* s.s. the predominant species during the rainy season. The numbers of adult *An. gambiae* s.s. usually increase a few weeks after the beginning of the rainy season, as the rainfall creates temporary breeding sites (puddles) that are very productive (Dossou-Yovo *et al.*, 1995). During the dry season included in the present study, the population of *An. gambiae* s.s. in Gbatta decreased and *An. funestus* s.s. became predominant as the climatic conditions favoured the development of breeding sites suitable for the latter species (Gilles and de Meillon, 1968; Brengues *et al.*, 1979). Thus, although the overall EIR of *An. funestus* s.s. was not statistically different from that of *An. gambiae* s.s., *An. funestus* s.s. helped maintain a high level of malarial transmission throughout the year.

In Kpéhi, *An. nili* s.s. were collected throughout the study period and gave

annual biting rates that were significantly higher than those recorded, for the same species, in Gbatta. Kpéhiri lies close to two rivers, not just one, and the water levels in those rivers are kept high year-round by rainfall entering tributaries in the north of the country. This flow of water supports many permanent mosquito breeding sites and it is the permanence and abundance of such sites that probably allow large populations of *An. nili* s.s. to develop in Kpéhiri (Krafsur, 1970). The water level in the River Vi, which flows close to Gbatta, is much reduced during the dry season. In Kpéhiri it is *An. nili* s.s. that helps maintain a high level of malarial transmission during the dry season. The same species has been found to play a major role in malarial transmission in some villages in a forested area of Cameroon (Carnevale *et al.*, 1992). In areas where several major vectors species are present, variation in ecological settings, both spatial and temporal, directly affects the relative role of each species in transmission (Antonio Nkondjio *et al.*, 2002; Bigoga *et al.*, 2007).

The present results demonstrate very high levels of *P. falciparum* transmission in forested areas of Côte d'Ivoire. Although the relative abundance and biting rate of each vector species varied between the two study villages, it was the presence of three vector species, exploiting different ecological niches, that allowed transmission to occur year-round. In one of the study villages, the main vector changed from *An. gambiae* s.s. in the rainy season to *An. funestus* s.s. in the dry seasons. In the other study village, it was *An. nili* s.s. that ensured year-round transmission and could therefore be considered the main malaria vector.

As larval control in the two study villages would be difficult to implement, it is suggested that malarial control should be based on long-lasting insecticidal nets, used at high coverage, and/or indoor residual spraying with a non-pyrethroid, long-lasting, insecticidal formulation.

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