



Malaria vectors and urbanization in the equatorial forest region of south Cameroon

Christophe Antonio-Nkondjio^{a,*}, Frédéric Simard^a,
Parfait Awono-Ambene^{a,b}, Pierre Ngassam^c, Jean-Claude Toto^a,
Timoléon Tchuinkam^{a,d}, Didier Fontenille^e

^a Laboratoire IRD de Recherche sur le Paludisme, Organisation de Coordination pour la lutte Contre les Endémies en Afrique Centrale (OCEAC), P.O. Box 288, Yaoundé, Cameroon

^b Centre de Recherches Médicales, Institut de Recherches Médicales et d'étude des Plantes Médicinales (IMPM), P.O. Box 6163, Yaoundé, Cameroon

^c Laboratoire de Biologie Générale, Faculté des Sciences, Université de Yaoundé I, P.O. Box 337, Yaoundé, Cameroon

^d Faculté des Sciences, Université de Dschang, P.O. Box 67, Dschang, Cameroon

^e Laboratoire de Lutte Contre les Insectes Nuisibles (LIN-UR 016), Institut de Recherche pour le Développement (IRD), 911, avenue Agropolis, P.O. Box 64501, 34394 Montpellier cedex 5, France

Received 26 March 2004; received in revised form 28 June 2004; accepted 7 July 2004

KEYWORDS

Malaria;
Plasmodium spp.;
Anopheles spp.;
Transmission;
Urbanization;
Cameroon

Summary Entomological surveys were carried out in the town of Mbalmayo and in the nearby rural village of Olama, within the equatorial forest zone of Cameroon. Mosquitoes were captured when landing on human volunteers and by pyrethrum spray catches. Malaria vectors captured were *Anopheles gambiae* Giles (M and S forms) and *A. moucheti* Evans in both areas, together with *A. funestus* Giles in Mbalmayo. One *A. marshallii* (Theobald) specimen infected by *Plasmodium falciparum* was found in Olama. *Anopheles moucheti* was the most abundant anopheline species caught in Olama, while *A. gambiae* was the most abundant in Mbalmayo. All these vectors were highly anthropophilic as indicated by the fact that only 5 of 201 blood meals analysed had been taken from non-human hosts. *Plasmodium falciparum* was the only malaria parasite species found in Mbalmayo, while *P. malariae* was also found in Olama. The annual entomological inoculation rate was estimated at 129 infective bites/person/year in Mbalmayo and 322 in Olama. Comparison with data published in 1955 from Mbalmayo, before expansion of the town, showed the impact of urbanization on the composition of the vector system and malaria transmission dynamics. Such changes should be considered when implementing sustainable control measures.

© 2004 Royal Society of Tropical Medicine and Hygiene. Published by Elsevier Ltd. All rights reserved.

* Corresponding author. Tel.: +237 223 22 32; fax: +237 223 00 61.
E-mail address: antonio_nk@yahoo.fr (C. Antonio-Nkondjio).

1. Introduction

During the twentieth century, ecological upheavals resulting from rapid urban development in Africa led to extensive modification of malaria epidemiology. In most African cities malaria transmission and vector densities are considerably lower than in surrounding rural regions (Gazin et al., 1987; Mendis et al., 2000; Robert et al., 1986; Trape and Zoulani, 1987a, 1987b). Many reasons such as destruction of breeding sites through improved drainage, domestic pollution, habitat improvement and increased use of personal protection measures, based mainly on insecticides, explain the lower vector densities in densely populated areas (Hay et al., 2000; Robert et al., 2003). Large variations are usually observed among districts within a city, as a result of heterogeneity in the distribution of human dwellings and population densities (Trape and Zoulani, 1987c). Hence, knowledge gained from studies of malaria transmission dynamics in rural settings, to which most current literature refers, is of limited value for a clear understanding of the much more complex epidemiological situation occurring nowadays in Africa.

In rural areas of equatorial Africa, the wide range of available breeding sites generally favour vector diversity and high densities. Malaria is perennial and is often transmitted by local vectors such as *Anopheles moucheti* and *A. nili* (Theobald), which are responsible for human biting rates (HBR) of more than 100 bites/person/night and entomological inoculation rates (EIR) reaching 300 infective bites/person/year (Antonio-Nkondjio et al., 2002; Carnevale et al., 1992; Njan Nloga et al., 1993). *Anopheles gambiae* and *A. funestus*, the most efficient malaria vectors in the world and major human malaria vectors throughout sub-Saharan Africa, are usually rare in forests unmodified by humans (Adam, 1956; Gillies and De Meillon, 1968). However, recent environmental changes resulting from intensified human activities, including deforestation for agricultural purposes or human settlements, promotes the introduction and proliferation of these highly anthropophilic vector species with serious consequences on malaria epidemiology.

We report here the results of a longitudinal entomological follow-up on malaria transmission dynamics conducted in 2000–2001 in the town of Mbalmayo, located within the equatorial forest area of south Cameroon, Central Africa. Adam (1955) published an entomological survey of malaria vectors and transmission dynamics in this locality. In the past 45 years, Mbalmayo has expanded and become urbanized, and it is now a town with about 66 500 inhabitants. Sampling was also

conducted in the village of Olama, 15 km away, in a rural environment. This study design allowed us to assess the impact of urbanization and deforestation on local malaria vector populations and their effects on malaria transmission dynamics.

2. Materials and methods

2.1. Study area

Mbalmayo (3°30'N, 11°26'E) is an urban area situated along the river Nyong, 50 km south of Yaounde, the capital of Cameroon. Since the early 1960s, Mbalmayo has experienced population growth from 5500 inhabitants in 1960 to 12 700 in 1964, 22 100 in 1976 and about 37 000 in 1987 (Santoir, 1995). Its population in 2004 is estimated to be about 66 500 (<http://www.world-gazetteer.com/c/c.cm.htm>). The village of Olama (3°24'N, 11°18'E) is situated 15 km south of Mbalmayo, downstream on the river Nyong, and currently has about 200 inhabitants.

Mbalmayo and Olama belong to the Congo–Guinean phytogeographic zone. They experience a typical equatorial climate, characterized by two rainy seasons extending from March to June and from September to November. Average annual rainfall during our study period was 1600 mm. The average minimum and maximum monthly temperature recorded by the national meteorological services ranged from 18–25 °C in July to 20–29 °C in March.

2.2. Mosquito collections

Adult mosquitoes were collected in alternate months from February 2000 to June 2001. Two sampling methods were used. First, human landing catches were conducted from 19:00 to 06:00 hours to assess the HBR. In Mbalmayo collections were made indoors and/or outdoors at seven collection places distributed along a transect from 20 to 500 m from the river; in Olama collections were made in three places both outdoors and indoors. Secondly, pyrethrum spray catches of resting females were carried out in the afternoon in 2–3 rooms, different from those used for night collections.

2.3. Field processing of anophelines

Anophelines were visually separated from other Culicidae and identified to species using morphological characteristics according to the identification keys of Gillies and De Meillon (1968) and Gillies and

Coetzee (1987). Every anopheline specimen was stored individually in a numbered tube containing dessicant, archived and kept at -20°C until processed in the laboratory in Yaounde.

2.4. Laboratory processing of anophelines

Blood meal sources of a sample of females captured by pyrethrum spray were identified by ELISA (Beier et al., 1988). The technique identified human, bovine, ovine (sheep and goat), equine (horse and donkey), pig or chicken hosts.

The head and thorax of female anophelines were tested for the presence of circumsporozoite protein (CSP) of *Plasmodium falciparum*, *P. malariae*, and *P. ovale* by ELISA, as described by Fontenille et al. (2001). *Plasmodium vivax* is not present in this region of Africa. The CSP rate and 95% confidence intervals were calculated. The EIR was calculated by multiplying the HBR from the landing catches by the CSP rate for each sampling period. Females belonging to the *A. gambiae* complex were identified to species using the PCR technique described by Scott et al. (1993). Specimens identified as *A. gambiae* were then tested for the M and S molecular forms using the diagnostic PCR-based assay of Favia et al. (2001). Specimens from the *A. nili* group were also identified to species using a recently described PCR technique (Kengne et al., 2003).

3. Results

3.1. Species diversity

In Mbalmayo, 1090 anopheline females were collected from April 2000 to June 2001. In Olama, 4653

mosquitoes were collected from February 2000 to June 2001. The anopheline species caught were: *Anopheles gambiae*, *A. funestus*, *A. moucheti*, *A. nili*, *A. paludis* Theobald, *A. ziemanni* Grünberg, and *A. marshallii*. *Anopheles marshallii* and *A. nili* were caught only in Olama (Table 1). *Anopheles moucheti* was predominant in collections from Olama, while *A. gambiae* was the most abundant species in Mbalmayo.

Of the 759 *A. gambiae* s.l. females captured, 120 of 721 in Mbalmayo and 29 of 38 in Olama were identified as *A. gambiae* s.s. by PCR; 97.5% of the *A. gambiae* s.s. collected in Mbalmayo and 65.5% of those collected in Olama were of the M molecular form, the remainder were of the S molecular form. Only *A. nili* s.s. (typical form) was found in Olama.

3.2. Mosquito behaviour

In Mbalmayo, *A. gambiae*, *A. funestus* and *A. moucheti* were caught throughout the study period (Figure 1A). The HBR was 11.3 bites/person/night for *A. gambiae*, 5.1 for *A. moucheti* and 0.8 for *A. funestus* and varied with the season. The maximum HBR for *A. gambiae* (27.8 bites/person/night) occurred in June 2000, in October 2000 for *A. moucheti* (8.4 bites/person/night), and in February 2001 for *A. funestus* (2.3 bites/person/night). In places where mosquitoes were collected both indoors and outdoors, 43% of *A. moucheti*, 52% of *A. gambiae* and 50% of *A. funestus* were collected indoors. Over 90% of *A. moucheti*, 85% of *A. gambiae* and 70% of *A. funestus* were collected in houses situated less than 200 m from the river, and densities of the two most abundant species, *A. moucheti* and *A. gambiae*, gradually decreased with distance from the river (Figure 2).

In Olama, *A. moucheti* was the most abundant malaria vector, accounting for more than 95% of

Table 1 Number of anophelines captured in Mbalmayo from April 2000 to June 2001 and in Olama from February 2000 to June 2001

Species	Mbalmayo		Olama		Mbalmayo in 1955 ^a
	Human landing	Indoor spray	Human landing	Indoor spray	
<i>A. funestus</i>	48	2	0	1	12
<i>A. gambiae</i>	686	35	26	12	230
<i>A. moucheti</i>	306	5	4118	432	1342
<i>A. paludis</i>	1	0	41	1	0
<i>A. marshallii</i>	0	0	9	0	0
<i>A. nili</i>	0	0	8	0	2
<i>A. ziemanni</i>	7	0	5	0	0
Total	1048	42	4207	446	1586

^a Results obtained by Adam (1955) in Mbalmayo are mentioned for comparison.

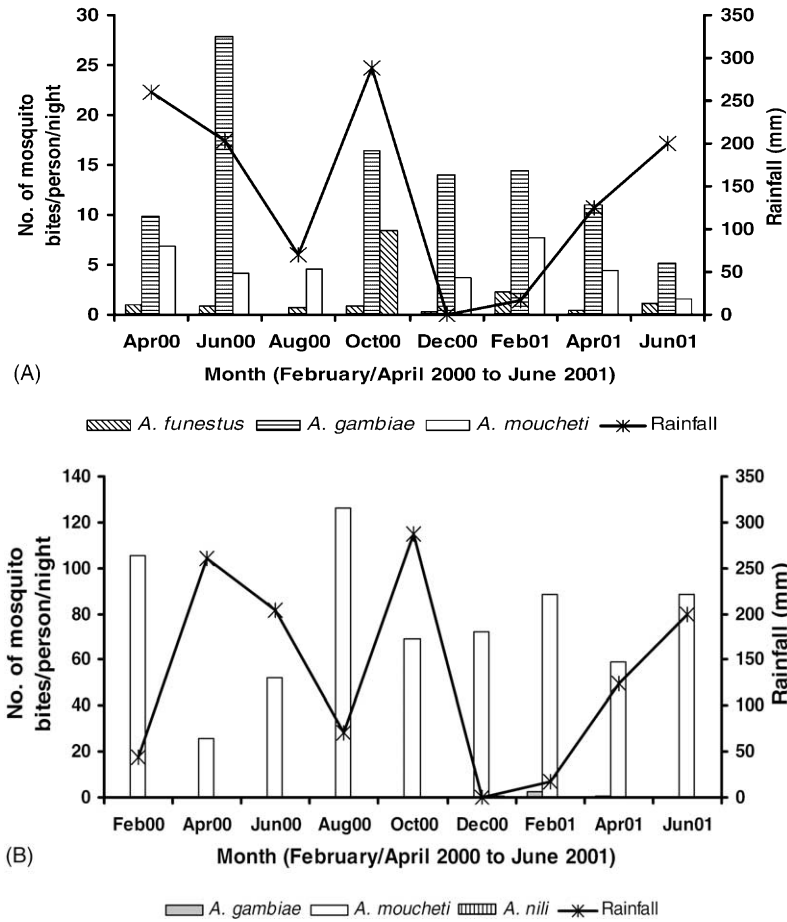


Figure 1 Rainfall and human biting rate for malaria vectors in (A) Mbalmayo from April 2000 to June 2001 and (B) Olama from February 2000 to June 2001.

the anophelines caught. This vector was present throughout the study period with an average HBR of 76.3 bites/person/night. *Anopheles gambiae* was collected in February and April 2001 (HBR=2.33 and 0.5 bites/person/night respectively), and

A. nili was collected only in December 2000 (HBR=0.83 bites/person/night). The *A. moucheti* HBR was always more than 20 bites/person/night with a maximum of 126 bites/person/night recorded in August 2000 (Figure 1B). Comparing both indoor and outdoor captures, more than 66% of *A. moucheti* were caught indoors.

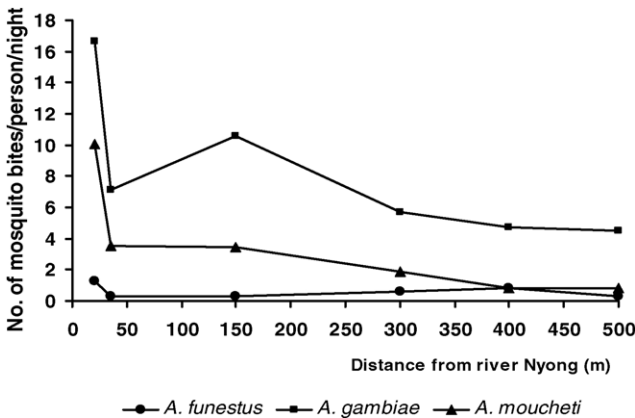


Figure 2 Human biting rate according to distance from river Nyong in Mbalmayo, Cameroon.

A total of 201 blood meals from resting females collected in both areas were tested by ELISA to determine their origin ($n=14$ in Mbalmayo; $n=187$ in Olama). All the blood meals tested for *A. gambiae* ($n=10$), *A. funestus* ($n=1$) and 185/190 *A. moucheti* had been taken from humans only. In Olama, *A. moucheti* was found with ovine ($n=3$), human + ovine ($n=1$) and human + bovine ($n=1$) blood.

3.3. Circumsporozoite protein rate

The results of the test for CSP are shown in Table 2. The CSP rate varied from 1.3 to 12.5%.

Table 2 Infective rate for *Plasmodium falciparum* calculated by circumsporozoite protein ELISA from the head and thoraxes of mosquitoes captured in Mbalmayo and Olama, Cameroon

Location	Species	No. tested	Positive	CSP rate (95% CI)
Mbalmayo	<i>A. moucheti</i>	299	4	1.3% (0.02–2.6)
	<i>A. gambiae</i>	714	32	4.5% (3–6)
	<i>A. funestus</i>	44	2	4.5% (0.56–15.5)
Olama	<i>A. moucheti</i>	4084	85 ^a	2.1% (1.6–2.6)
	<i>A. gambiae</i>	37	4	10.8% (1–26)
	<i>A. marshallii</i>	8	1	12.5% (0.3–52.7)

^a Two were *Plasmodium malariae*, all the rest were *P. falciparum*.

3.4. Entomological inoculation rate

Malaria transmission was perennial throughout the study period in both Mbalmayo and Olama. In Mbalmayo, the annual EIR was 129 infective bites/person/year. *Anopheles gambiae*, *A. moucheti* and *A. funestus* were responsible for 84, 11 and 5% of *P. falciparum* transmission respectively. Malaria transmission by *A. gambiae* was observed almost throughout the study period. *Anopheles moucheti* took part in the transmission from April to December 2000. Involvement of *A. funestus* was observed only in February 2001.

In Olama, the annual EIR was estimated at 322 infective bites/person/year. *Anopheles moucheti* was responsible for 94% of malaria transmission.

4. Discussion

Greater anopheline diversity was observed in the rural village of Olama, with seven species collected, than in the town of Mbalmayo, where five species were observed. Overall anopheline densities were also significantly higher in the rural than in the semi-urban environment, as commonly reported (Robert et al., 2003). All four known malaria vectors in Africa's equatorial forest region were collected: *A. gambiae*, *A. funestus*, *A. nili* and *A. moucheti*. *Anopheles gambiae* s.s., represented by its two molecular forms M and S, was the only member of the *A. gambiae* complex present in Olama and Mbalmayo. The scarcity of *A. nili* along the river Nyong (only eight specimens out of 5743 anophelines caught) is probably due to the slow river flow in this area, which is not favourable for *A. nili* breeding. Very high densities of *A. moucheti* were observed in Olama, whereas in Mbalmayo, these were much lower and *A. gambiae* was the most abundant species caught. By contrast, in 1955, *A. moucheti* was the most abundant species

caught in this location, its densities being around six times those of *A. gambiae* (Adam, 1955), as currently observed in the rural locality of Olama. *Anopheles moucheti* is a forest mosquito known to breed in slow-moving rivers, among vegetation islands made of *Pistia stratiotes* and *Paspallum* species (Gillies and De Meillon, 1968; Mouchet and Gariou, 1966). Their destruction, due to the clearance of vegetation along the river banks in Mbalmayo, may have caused the decrease in the relative proportions of *A. moucheti* in our collections. Moreover, *A. moucheti* is a highly endophilic mosquito that may have suffered from human habitat improvement and increased use of personal protection measures (such as insecticide sprays, coils or insecticide-treated nets) during the urbanization of Mbalmayo. This is indicated by the fact that only 43% of *A. moucheti* fed indoors in Mbalmayo, while in Olama, 66% of the females took their blood meals inside human dwellings. Development of the river banks for market gardening and deforestation for human settlement also created suitable breeding opportunities for the peridomestic *A. gambiae*. Species of the *A. gambiae* complex, especially *A. gambiae* s.s. and *A. arabiensis*, have been reported to take advantage of the colonization of swamps for housing and/or agricultural purposes in most sub-Saharan Africa periurban areas (Fondjo et al., 1992; Manga et al., 1992; Robert et al., 1986; Trape and Zoulani, 1987b). In Mbalmayo, vector densities were very high in the vicinity of the river Nyong, and rapidly decreased with distance from the river. This non-random distribution suggests low dispersion of the vectors in this environment, as formerly observed for *A. arabiensis* in the densely populated suburbs of Dakar, Senegal (Trape et al., 1992) and Khartoum, Sudan (El Sayed et al., 2000), and for both *A. arabiensis* and *A. funestus* in Maputo, Mozambique (Mendis et al., 2000). Similar observations were also reported for *A. nili* and *A. moucheti* in Cameroon (Le Goff et al., 1997; Njan Nloga et al., 1993).

Thus, the river Nyong and its banks appear as the most important permanent breeding source for all malaria vector species observed in Mbalmayo. However, the HBR of *A. gambiae* remained high (>5 bites/person/night) even in the collection site most remote from the river. This reflects the existence of numerous additional breeding places favourable to this species such as the rainwater-filled tyre tracks and puddles, which are scattered throughout the town and are particularly numerous in the non built-up, open areas.

High malaria transmission intensity was observed throughout the year in both sites we surveyed. The EIR in Mbalmayo estimated at 129 infective bites/person/year was consistent with the semi-urban status of this town, which is still growing. Although transmission is usually low in most African city centres (Awolola et al., 2002; Lindsay et al., 1990; Robert et al., 1986), high malaria transmission intensity is commonly observed in suburban areas, as this environment usually provides numerous suitable breeding sites for anophelines. For example, in the periphery of Yaounde (50 km north of Mbalmayo), malaria transmission is estimated to be about 300 infective bites/person/year (Antonio-Nkondjio et al., 2002) but is only 3–33 within the city limits (Fondjo et al., 1992; Manga et al., 1992; Nimpaye et al., 2001). In Kinshasa (Democratic Republic of Congo), Coene (1993) observed an annual EIR of 30 in the city centre, whereas it reached 455 in a suburban village 15 km away. Moreover significant differences in the intensity of malaria transmission in different districts of the same town have been reported in Brazzaville (Congo), Bouake (Cote d'Ivoire) and Kumasi (Ghana), depending on the practice of market gardening or irrigated urban agriculture in swampy areas or along streams (Afrane et al., 2004; Dossou-Yovo et al., 1998; Trape and Zoulani, 1987a).

Overall, four species were found with *P. falciparum* CSP: *A. gambiae*, *A. funestus*, *A. moucheti* and *A. marshallii*. *Anopheles marshallii* has previously been reported carrying *P. falciparum* CSP in Tanzania (Curtis et al., 1999). Secondary vectors such as *A. hancocki*, *A. coustani* or *A. wellcomei* were also reported to be infected in the region (Adam, 1956; Fontenille et al., 2000; Wanji et al., 2003). This observation confirms the fact that, wherever they are present, genetically competent anopheline species, that are not usually recognized as malaria vectors, may contribute to the overall transmission intensity. However, direct observation of live sporozoites in the salivary glands of females is needed to prove the role of such species in malaria transmission. In most situations, it is highly improbable that

these species could maintain parasite transmission on their own, in the absence of major vector species.

In Olama, 94% of the transmission was due to *A. moucheti* whereas in Mbalmayo, *A. gambiae* was the major vector, responsible for 84% of the transmission, the remaining being due to *A. moucheti* and *A. funestus*. Despite very high densities, the CSP index of *A. moucheti* was less than 2%, as commonly reported for this species (Adam, 1956; Antonio-Nkondjio et al., 2002; Manga et al., 1995; Njan Nloga et al., 1993). On the other hand, *A. gambiae* and *A. funestus* are usually much more efficient vectors, with CSP indices of 5% and above (Antonio-Nkondjio et al., 2002; Fontenille and Lochouarn, 1999). Our estimate of 10.8% of infective *A. gambiae* females in Olama, however, was based on a small sample size ($n=37$). The presence in Mbalmayo of peridomestic *A. gambiae* and *A. funestus*, together with rural *A. moucheti* emphasizes the semi-urban status of this locality. The fact that *A. gambiae* and *A. funestus* tend to supplement transmission by *A. moucheti* may have dramatic consequences in terms of stability and intensity of transmission. Furthermore, high prevalence of resistance in local *P. falciparum* strains may further jeopardize efficient malaria control in this area (Soula et al., 2000). Precise knowledge of malaria transmission dynamics and of the different vector species involved is therefore required to devise and monitor effective means for malaria control. Our study sheds light on the effect of rapid urbanization on the entomological parameters of transmission and the composition of the vector system, and substantial differences were demonstrated that should be taken into account when implementing vector control measures.

Conflicts of interest statement

The authors have no conflicts of interest concerning the work reported in this paper.

Acknowledgements

This work was supported in part by grant no. A20727 from the UNDP/World Bank/WHO Special programme for Research and Training in Tropical Diseases (TDR) to C.A.N., the French Institut de Recherche pour le Développement (IRD) and by the French Ministry of Research through the Pal+ initiative. We are indebted to C. Wondji, J.-Y. Meunier, S. Zebaze-Kemleu and R. Nyambam for valuable help in the field and laboratory.

References

- Adam, J.P., 1955. Quelques anophèles nouveaux pour la faune Camerounaise. *Ann. Parasitol.* 300, 389–394.
- Adam, J.P., 1956. Note faunistique et biologique sur les anophèles de la région de Yaoundé et la transmission du paludisme en zone forestière du sud Cameroun. *Bull. Soc. Path. Exot.* 49, 210–220.
- Afrane, A.Y., Klinkenberg, E., Drechsel, P., Owusu-Daaku, K., Garms, R., Kruppa, T., 2004. Does irrigated urban agriculture influence the transmission of malaria in the city of Kumasi, Ghana? *Acta Trop.* 89, 125–134.
- Antonio-Nkondjio, C., Awono-Ambene, P., Toto, J.C., Meunier, J.Y., Zebaze-Kemleu, S., Nyambam, R., Wondji, C.S., Tchuinkam, T., Fontenille, D., 2002. High malaria transmission intensity in a village close to Yaounde, the capital city of Cameroon. *J. Med. Entomol.* 39, 350–355.
- Awolola, T.S., Okwa, O., Hunt, R.H., Ogunrinade, A.F., Coetzee, M., 2002. Dynamics of the malaria-vector populations in coastal Lagos, south-western Nigeria. *Ann. Trop. Med. Parasitol.* 96, 75–82.
- Beier, J.C., Perkins, P.V., Wirtz, R.A., Koros, J., Diggs, D., Gargam, T.P.I.I., Koech, D.K., 1988. Blood meal identification by direct enzyme-linked immunosorbent assay (ELISA) tested on *Anopheles* (Diptera: Culicidae) in Kenya. *J. Med. Entomol.* 25, 9–16.
- Carnevale, P., Le Goff, G., Toto, J.C., Robert, V., 1992. *Anopheles nili* as the main malaria vector of human malaria in villages of southern Cameroon. *Med. Vet. Entomol.* 6, 135–138.
- Coene, J., 1993. Malaria in urban and rural Kinshasa: the entomological input. *Med. Vet. Entomol.* 7, 127–137.
- Curtis, C.F., Pates, H.V., Takken, W., Maxwell, C.A., Myamba, J., Priestman, A., Akinpela, O., Yayo, A.M., Hu, J.T., 1999. Biological problems with the replacement of a vector population by *Plasmodium*-refractory mosquitoes. *Parassitologia* 41, 479–481.
- Dossou-Yovo, J., Doannio, J., Diarrassouba, S., Chauvancy, G., 1998. Impact d'aménagements de rizières sur la transmission du paludisme dans la ville de Bouaké, Côte d'Ivoire. *Bull. Soc. Pathol. Exot.* 91, 327–333.
- El Sayed, B.B., Arnot, D.E., Mukhtar, M.M., Baraka, O.Z., Dafalla, A.A., Elnaiem, D.E., Nugud, A.H., 2000. A study of the urban malaria transmission problem in Khartoum. *Acta Trop.* 25, 163–171.
- Favia, G., Lanfracotti, A., Spanos, L., Siden-Kiamos, I., Louis, C., 2001. Molecular characterization of ribosomal DNA polymorphisms discriminating among chromosomal forms of *Anopheles gambiae* s.s. *Insect Mol. Biol.* 10, 19–23.
- Fondjo, E., Robert, V., Le Goff, G., Toto, J.C., Carnevale, P., 1992. Le paludisme urbain à Yaoundé (Cameroun). 2 – Etude entomologique dans deux quartiers peu urbanisés. *Bull. Soc. Path. Exot.* 85, 57–63.
- Fontenille, D., Lochouart, L., 1999. The complexity of the malaria vectorial system in Africa. *Parassitologia* 41, 267–271.
- Fontenille, D., Wandji, S., Djouaka, R., Awono-Ambene, H.P., 2000. *Anopheles hancocki*, vecteur secondaire du paludisme au Cameroun. *Bull. Liais. Doc. OCEAC* 33, 23–26.
- Fontenille, D., Meunier, J.Y., Antonio-Nkondjio, C., Tchuinkam, T., 2001. Use of circumsporozoite protein enzyme-linked immunosorbent assay compared with microscopic examination of salivary glands for calculation of malaria infectivity rates in mosquitoes (Diptera: Culicidae) from Cameroon. *J. Med. Entomol.* 38, 451–454.
- Gazin, P., Robert, V., Ouedraogo, V., Carnevale, P., 1987. Le paludisme urbain à Bobo-Dioulasso (Burkina Faso). 2. Les indices paludologiques. *Cah. ORSTOM Ser. Ent. Med. Parasitol.* 24, 121–128.
- Gillies, M.T., De Meillon, B., 1968. The Anophelinae of Africa South of the Sahara, second ed. South African Institute of Medical Research, Johannesburg.
- Gillies, M.T., Coetzee, M., 1987. A Supplement to the Anophelinae of Africa South of the Sahara. South African Institute of Medical Research, Johannesburg.
- Hay, S.I., Rogers, D.J., Toomer, J.F., Snow, R.W., 2000. Annual *Plasmodium falciparum* entomological inoculation rates (EIR) across Africa: literature survey, internet access and review. *Trans. R. Soc. Trop. Med. Hyg.* 94, 113–127.
- Kengne, P., Awono-Ambene, P., Antonio-Nkondjio, C., Simard, F., Fontenille, D., 2003. Molecular identification of the *Anopheles nili* group of African malaria vectors. *Med. Vet. Entomol.* 17, 67–74.
- Le Goff, G., Carnevale, P., Robert, V., 1997. Low dispersion of anopheline malaria vectors in the African equatorial forest. *Parasite* 2, 187–189.
- Lindsay, S.W., Campbell, H., Adiamah, J.H., Greenwood, A.M., Bangali, J.E., Greenwood, B.M., 1990. Malaria in a peri-urban area of the Gambia. *Ann. Trop. Med. Parasitol.* 84, 553–562.
- Manga, L., Toto, J.C., Carnevale, P., 1995. Malaria vectors and transmission in an area deforested for a new international airport in southern Cameroon. *Ann. Soc. Belge Méd. Trop.* 75, 43–49.
- Manga, L., Robert, V., Messi, J., Desfontaine, M., Carnevale, P., 1992. Le paludisme urbain à Yaoundé. Cameroun. Une étude entomologique dans deux quartiers centraux. *Mém. Soc. R. Belge Ent.* 35, 155–162.
- Mendis, C., Jacobsen, J.L., Gamage-Mendis, A., Bule, E., Dgedge, M., Thompson, R., Cuamba, N., Barreto, J., Begtrup, K., Sinden, R.E., Hogg, B., 2000. *Anopheles arabiensis* and *A. funestus* are equally important vectors of malaria in Matola coastal suburb of Maputo, southern Mozambique. *Med. Vet. Entomol.* 14, 171–180.
- Mouchet, J., Gariou, J., 1966. *Anopheles moucheti* au Cameroun. *Cah. ORSTOM Sér. Ent. Méd. Parasitol.* 4, 71–81.
- Nimpaye, H., Van Der Kolk, M., Fontenille, D., Boudin, C., 2001. Le paludisme urbain à Yaoundé (Cameroun) en 2000. Etude entomologique dans le quartier central Dakar. *Bull. Liais. Doc. OCEAC* 34, 11–14.
- Njan Nloga, A., Robert, V., Toto, J.C., Carnevale, P., 1993. *Anopheles moucheti*, vecteur principal du paludisme au sud-Cameroun. *Bull. Liais. Doc. OCEAC* 26, 63–67.
- Robert, V., Gazin, P., Ouédraogo, V., Carnevale, P., 1986. Le paludisme urbain à Bobo-Dioulasso (Burkina Faso). Etude entomologique de la transmission. *Cah. ORSTOM Sér. Ent. Méd. Parasitol.* 24, 121–128.
- Robert, V., Macintyre, K., Keating, J., Trape, J.F., Duchemin, J.B., McWilson, W., Beier, J.C., 2003. Malaria transmission in urban sub-Saharan Africa. *Am. J. Trop. Med. Hyg.* 68, 169–176.
- Santoir, C., 1995. La population I: Evolution de 1967 à 1976. La population II: Densités rurales en 1976. In: Santoir, C., Bopda, A. (Eds.), Atlas régional du Sud Cameroun. ORSTOM-MINREST, Paris, pp. 19–27.
- Scott, J.A., Brogdon, W.G., Collins, F.H., 1993. Identification of single specimens of the *Anopheles gambiae* complex by polymerase chain reaction. *Am. J. Trop. Med. Hyg.* 49, 520–529.
- Soula, G., Ndounga, M., Foumane, V., Ollivier, G., Youmba, J.C., Basco, L., Boudin, C., Samé Ekobo, A., Ringwald, P., 2000. Bilan de la résistance de *Plasmodium falciparum* à la chloroquine au Cameroun et alternatives thérapeutiques. *Bull. Liais. Doc. OCEAC* 33, 15–27.
- Trape, J.F., Zoulani, A., 1987a. Malaria and urbanization in Central Africa: the example of Brazzaville. Part II: Results of en-

- tomological surveys and epidemiological analysis. *Trans. R. Soc. Trop. Med. Hyg.* 81 (Suppl. 2), 10–18.
- Trape, J.F., Zoulani, A., 1987b. Etudes sur le paludisme dans une zone de mosaïque Forêt-Savane d'Afrique Centrale, la région de Brazzaville. I-Résultats des enquêtes entomologiques. *Bull. Soc. Pathol. Exot.* 80, 84–99.
- Trape, J.F., Zoulani, A., 1987c. Malaria and urbanization in Central Africa: the example of Brazzaville. Part III: Relationships between urbanization and the intensity of malaria transmission. *Trans. R. Soc. Trop. Med. Hyg.* 81 (Suppl. 2), 19–25.
- Trape, J.F., Lefebvre-Zante, E., Legros, F., Ndiaye, G., Bouganali, H., Druilhe, P., Salem, G., 1992. Vector density gradients and the epidemiology of urban malaria in Dakar, Senegal. *Am. J. Trop. Med. Hyg.* 47, 181–189.
- Wanji, S., Tanke, T., Atanga, S.N., Ajonina, C., Tendongfor, N., Fontenille, D., 2003. Anopheles species of the mount Cameroon region: biting habits, feeding behaviour and entomological inoculation rates. *Trop. Med. Int. Health.* 8, 643–649.

Available online at www.sciencedirect.com

