Journal of Cell and Animal Biology Vol. 3 (3), pp.033-038, March, 2009
Available online at http://www.academicjournals.org/JCAB
ISSN 1996-0867 © 2009 Academic Journals

Full Length Research Paper

Seasonal variations of malaria transmission in Western Cameroon highlands: Entomological, parasitological and clinical investigations

Jean Atangana[1],[2]*, Etienne Fondjo[1], Abraham Fomena[3], Joseph Lebel Tamesse[4], Salomon Patchoké[1], Hamadou N. M. Ndjemaï[1,3] and Prosper A. Bessong Ndong[1]

[1]National Malaria Control Programme, Ministry of Public Health, Cameroon P.O. Box 14386, Yaoundé, Cameroon.
[2]Laboratory of Zoology, Faculty of Science, University of Yaoundé I P.O. Box 812, Yaoundé, Cameroon.
[3]Laboratory of Parasitology, Faculty of Science, University of Yaoundé I P.O. Box 812, Yaoundé, Cameroon.
[4]Laboratory of Zoology, Ecole Normale Supérieure, University of Yaoundé I, P.O. Box 614, Yaoundé, Cameroon.

Accepted 14 January, 2009

A cross-sectional study on malaria transmission was carried out in Mangoum, a village situated in the western highland region of Cameroon in October 2005 and May 2006, to assess malaria parasite prevalence in a cohort of children and vectors biting habits and entomological inoculation rate. Mosquitoes were collected by landing catches on volunteers. Members of the *Anopheles gambiae* complex and molecular forms were identified using Polymerase Chain Reaction method. Infection intensity was determined by counting the number of infected red blood cells against 200 leucocytes. A total of 1195 *Anopheles* were collected, 183 and 1012 respectively in the dry and rainy seasons. Two *Anopheles* species were identified: *A. gambiae* s.l. and *Anopheles funestus*. *A. gambiae* s.s. was the only member of the *A. gambiae* complex found and the main malaria vector in this region. The sporozoïte rate of *A. gambiae* was higher in the rainy season (9%). The average inoculation rate was 90 infective bites per man per year. A total of 699 children were examined. Two parasites species were identified: *Plasmodium falciparum* and *Plasmodium malariae*. The mean parasite rate was 41. 3%. In the age range of 2 - 9 years, the parasite rates were 49% in the dry season and 34.7% in the rainy season. The mean spleen rate of the age range 2 - 9 years varies from 26. 7% in rainy season to 22. 3% in the dry season, and was not significantly different (p = 0, 3). The Western highlands region can be considered as an area of high malaria transmission intensity.

Key words: Malaria, transmission, *Anopheles* vectors, biting habits, sporozoite rate, parasites species, western Cameroon highlands.

INTRODUCTION

Malaria still remains one of biggest public health problems of mankind. The world Organization estimates that malaria affects about 300 - 500 million people with annual mortality of about 1.5 - 2.5 million. More than 90% of these cases are registered in sub-Saharan Africa with more than 70% of inhabitants in some areas of this re-ion being chronically infected by *Plasmodium falciparum* (OMS, 1998a; Fontenille and Lochouarn, 1999). Despite the endemicity of the disease in the country large variations exist between ecological foci depending on agricultural practices, the presence of rivers or swamps, the urban status of the region and elevation. Although, many studies have been carried on malaria transmission across the country, these studies showed that: *A. gambiae*, *A. arabiensis*, *A. pharoensis*, *A. coustani* and *A. funestus* are the main vectors in the northern part. The first two species are sympatric throughout the country with a variable frequency according to the climatic conditions. *A. funestus* is found almost in all bio-climatic areas near Swamps or Rivers (Same-Ekobo et al., 2001, Antonio-Nkondjio et al. 2008). In the southern part, *A. gambiae*, *A. Moucheti*, *A. funestus A. nili* and *A. hancocki* are the main malaria vectors.

*Corresponding author. E-mail: j_atana@yahoo.fr. Tel: (237) 22 22 39 17. Fax: (237) 22 22 39 57.

Parasitological data also showed that *P. falciparum* is the predominant plasmodial species, followed by *P. malariae* found everywhere in the country but with a low percentage and *P. ovale* found only in the north and coastal parts of the country (Moyou, 1977; Fondjo, 1996, Couprié et al., 1985). Few have highlighted the impact of elevation on malaria transmission pattern. In highland areas it's though that malaria transmission rate is low due to the scarcity of vectors and their low survival rate (Wanji et al., 2003; Githeko et al., 2006). Malaria transmission is being recorded mainly during rainy season. In other to assess the malaria transmission pattern in the Cameroon highlands region, we conducted two cross-sectional studies in the area of Mangoum situated in the western highlands area of Cameroon during the dry and rainy seasons. These studies were both carried out on both human and vectors to determine the level of malaria prevalence in there two important groups.

MATERIALS AND METHODS

Study site

The study was carried out in October 2005 (beginning of dry season) and May 2006 (rainy season) in Mangoum village (5° 28' N, 10°33' E, 1100 m of altitude). The area is characterized by two seasons of unequal duration: a long rainy season running from March – September having an annual rainfall of about 2000 mm. The mean annual temperature is 22°C. Mangoum is essentially an agricultural region having many swamps favourable for the proliferation of mosquitoes. The principal crops grown are market garden produce (tomatoes, water melon, vegetables) and foods crops (maize, yams, irish potatoes, beans etc). Beside this, they also carry out small farming of bovine, caprine, ovine, porcine and poultry.

Adult mosquito collections and field processing

Mosquitoes were collected after landing on a human volunteer from 07: 00 pm to 06: 00 am. Collections were done in 4 places both outdoors and indoors.

Anophelines were identified using morphological characteristics according to Gillies and De Meillon (1968), and Gilles and Coetzee (1987) and the collected mosquitoes were conserved individually in tubes containing silicagel and kept at -20°C further analysis in the laboratory.

Laboratory processing of anophelines

Members of the *A. gambiae* complex were identified using molecular diagnostic tools (Favia et al., 2001; Scott et al., 1993). M and S molecular forms were also identify using molecular diagnostic tools (Favia et al., 2001). DNA extracted from the leg or the wing was used for this analysis. Infections were detected either by direct observation of *Plasmodium* sporozoites in the salivary glands.

The physiological age of adult female *Anopheles*, and hence parity ratio (proportion of female mosquitoes having laid eggs at least once), was determined through the dissection of ovaries and examination of tracheoles (Detinova, 1962). The identification of sporozoites obtained from specimens' dissection was carried out with a microscope with a resolution power of 40X. The sporozoite rate was calculated as a ratio of the total number of mosquitoes infected divided by the total number of mosquitoes dissected. The entomological inoculation rate (*EIR*), that is, the number of infective bites per person per night is calculated, by multiplying the number of mosquito bites per person per night by the mosquitoes' infective rate.

Parasite prevalence

During each entomological survey a cohort of children aged 0 – 15 years old living in the two different quarters in the village were identified for the presence of malaria parasites. The checking of the presence of malaria parasites in the sampled population was carried out using the finger-prick method to obtain thick and thin smears. Immediately after the finger pricked, any child complaining of fever, had his or her thick-blood smear stained in 10% Giemsa for 10 min in the field and examined for the presence of malaria parasites. If parasites are seen, the child is treated with paracetamol and coartem. The remaining other slides were stained in 3% Giemsa for 30 min and the presence of parasites were checked and infections intensity was determined by counting the number of infected red blood cells against 200 leucocytes.

The study was approved by the ethics review committee of Cameroon Medical research.

Statistical analysis

Data were analysed with version 6.0 of the Epi info software package. We calculated 95% confidence intervals (CI) for sporozoïte rates. Chi 2-test was use to compare overall parity ratio, sporozoïte, parasite and spleen rates between seasons and between age groups of children. All the tests were performed at the 5% significance level.

RESULTS

Anopheles species: Biting density, biting habits and endophagy

Between October 2005 and May 2006, a total of 1195 Anophelines were collected: 1012 (84.7%) in the rainy season (Table 1a) and 183 (15.3%) in the dry season (Table 1b). Two *Anopheles* species were identified: *A. gambiae* s.l. and *A. funestus*. *A. funestus* was rare and found only during the dry season. Polymerase chain reaction (PCR) analysis of *A. gambiae* complex revealed that all specimens tested were all *A. gambiae* s.s. from the S molecular form. The two major species of *Anopheles* showed heterogeneity of their biting rates at the rainy and dry seasons (Table 1a, b). *A. gambiae* was the most important species in both seasons with 33.7 bites per man per night (b/m/n) in the rainy season and 4.9 b/m/n in the dry season. The hourly variations of the human biting rate of the two species of *Anopheles* are shown in Figure 1. Both species display the same cycle during the night with major peak of activity between midnight and 2 am. Both *Anopheles* species varied in their feeding behaviour during the two seasons. *A. gambiae* was more endophagous during the rainy season (Figure 2).

Parity ratio of malaria vectors

Table 2 shows the numbers of *Anopheles* tested and

Table 1. Sporozoïte and infectious biting rate of malaria vectors in the Western mountain Cameroon region.

Anopheles species	Number Captured biting	Man Nights	Number dissected	Sporozoïte rate [95% CI]	Biting rate (b/m/n)	Infective biting rate (ib/m/year)
a) Rainy season						
Anopheles gambiae	1012	30	393	9% [6-12]	33.3	90
Anopheles funestus	0	0	0	0	0	0
b) **Dry season**						
Anopheles gambiae	147	30	128	6% [2-10]	4.9	9
Anopheles funestus	36	30	20	5% [-2-12. 1]	1.2	1.8

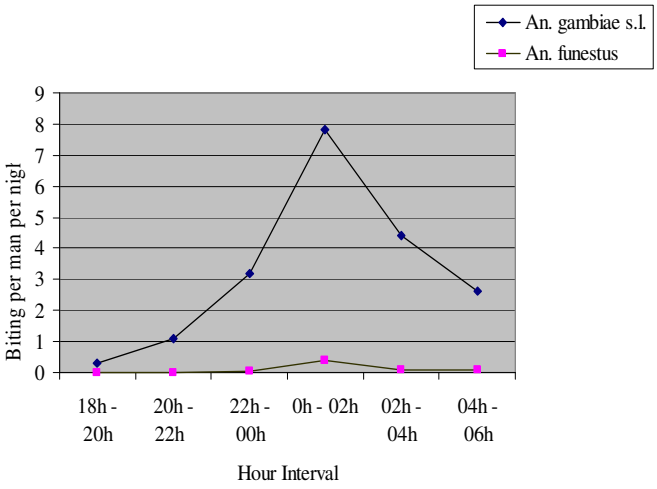

Figure 1. Night biting cycle of the malaria vectors in the Western mountain Cameroon region.

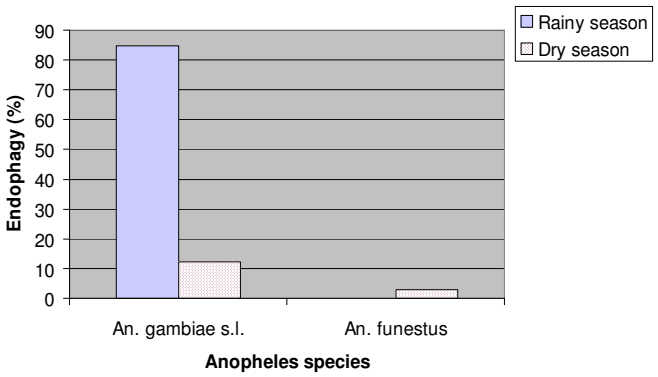

Figure 2. Proportions of the *Anopheles* biting indoors (endophagy) during the rainy and dry season

those having laid eggs at least once, hence the estimated parity ratios during the different surveys. In the rainy season, the parity ratio of *A. gambiae s.l.* was 81.4 and 64.4% in the dry season, with a very high significant difference (χ^2 = 13.69; ddl = 1; p < 0.001). Parity ratio of *A. funestus* was estimated only in the dry season and it was 80.6%. There was no significant difference between two malaria vectors in the dry season (χ^2 = 2.90; ddl = 1; p = 0.08).

Infection rate and entomological inoculation rate

The infection rate of *A. gambiae* s.s. was compared between the rainy season (9%) and the dry season (6%) and there was no significant different, (χ^2 = 1. 06; ddl = 1; p = 0. 3). *A. funestus* was found infected only during the dry season. The *EIR* was calculated based on *Anopheles* collected from landing catches (Table 1a, b). The highest infective biting rate was recorded with *A. gambiae* during the rainy season (7.5 infective bites per person per month).

Malaria prevalence

Malaria prevalence is meso-endemic in Mangoum village. The parasite prevalence of *P. falciparum* was 83.5% (264/316) while malaria prevalence of *P. malariae* was 16.5% (52/316). *P. ovale* was not found during the study.

In 2005, the mean parasite rate was 38. 8% in Mangoum village. The parasite rate in the different age groups: 2 – 4, 5 – 9 and 10 – 15 years varied significantly and was higher in the age group of 2 – 4 years (χ^2 = 37.7; d.d.l = 3; p < 0.001) . In May 2006, the mean parasite rate was 26.7%, the difference age groups was equally significant (χ^2 = 25.76; d.d.l = 3; p < 0.01). The difference in the parasite rates between the seasons showed a very high significant difference (χ^2 = 11.5; d.d.l = 1; p < 0.01) (Table 3).

During the dry season, the mean spleen rate was 22.3%. This rate showed no significant difference between the age groups (χ^2= 0, 79; d.d.l = 1; p = 0, 3). During the rainy season, the spleen rate was 26.7%. This rate showed no significant different between the age groups (χ^2 = 0.17; d.d.l = 1; p = 0.6), with respect to the two seasons, the spleen rate was compared (χ^2 = 1.04; d.d.l = 1; p = 0.3) (Table 4).

DISCUSSION

The main species of *Anopheles* in the Western highland region of Cameroon are *A. gambiae s.s.* and *A.* These results confirmed the confinement of the sibling species *A.*

Table 2. Number of mosquitoes tested, number of mosquitoes that laid eggs at least once, and variation of parity ratios (in percentage, including 95 % confidence interval) of *Anopheles gambiae s.l.* and *An. funestus* in Mangoum

	***Anopheles gambiae* s.l.**		***An. funestus***	
	Number tested (laid eggs)	**Parity ratio [95% CI]**	**Number tested (laid eggs)**	**Parity ratio[95 %CI]**
Rainy season	392 (319)	81.4 [76,6-86.1]	0 (0)	0
Dry season	104 (67)	64.4 [55.2-73.6]	31 (25)	80.6 [66.6-94.5]

Table 3. Seasonal distribution of parasite rates according to age groups and seasons.

	Parasite rates (%)		
Age classes	**Rainy season**	**Dry season**	**Total**
< 2 years	14.4 % (9/67)	32 % (16/50)	21.4 % (25/117)
2 - 4 Years	35.4 % (34/96)	55.7 % (49/89)	44.9 % (83/185)
5 – 9 years	33.8 % (41/121)	43.3 % (45/103)	38.4 % (86/224)
10 – 15 years	18 % (16/90)	19.3 % (16/83)	18.5 % (32/173)
Overall	26.7 % (100/374)	38.8 % (126/325)	33.3 % (226/699)

Table 4. Seasonal distribution of the spleen rate in the children of 2 to 9 years old.

	Spleen rates (%)		
Age classes	**Rainy season**	**Dry season**	**Total**
2 - 4 Years	28.1 % (27/96)	25.8 % (22/89)	26.5 % (49/185)
5 – 9 years	25.6 % (31/121	19.4 % (20/103)	22.8 % (51/224)
Overall	26.7 % (58/217)	22.3 % (42/188)	24.7 % (100/405)

funestus gambiae s.s. and *A. funestus* to the forested areas of Africa (Coetzee et al., 2000; Wanji et al., 2003). *A. gambiae* s.s. and *A. funestus* are the principal malaria vectors in this study area as they were found infected. These species are well known as efficient vectors in Cameroon and other areas of Africa (Shililu et al., 1998; Lindblade et al., 1999; Jambou et al., 2001; Koudou et al., 2005; Githelo et al., 2006; Antonio Nkondjio et al., 2006). The biting cycle of *Anopheles* species were according to the habitual diagram of the night biting cycle of *Anopheles (*Gillies and De Meillon, 1968) with the biting peak observed between midnight and 2 am. *A. gambiae s.s.* and *A. funestus* displayed variations in their biting behaviour according to the season of the year, being more endophagous during the rainy season. This change may be related to the weather conditions in the study area. During the wet season, the rainfall is very heavy and the temperature low. These environmental factors may probably influence these two anopheles species to be more endophagous than exophagous during the rainy season. This seasonal shift in the feeding behaviour of *A. gambiae s.s.* and *A. funestus* would have important implications for the vector control in the Western highlands region of Cameroon during the rainy season, as insecticide treated nets (ITS) or indoor residual spraying could be very effective in preventing these two *Anopheles* species from biting, or reducing their vectorial capacity.

Previous research conducted in Mount Cameroon region, closely to our study site showed high parity ratios of *A. gambiae s.l.* accompanied by high anthropophily of these mosquitoes (Wanji et al., 2003). Here, we confirmed high parity ratios of these malaria vectors in western highlands region of Cameroon.

In Mangoum, as in most of central Africa, sporozoite rates were higher in *A. gambiae s.l.* and *An. funestus* than other malaria vectors (Fontenille and Locchouarn, 1999). The total entomological inoculation rate was around 101 infected bites per human per year. *A. gambiae s.l.* compared with *A. funestus* was the main malaria vector in Mangoum. Previous studies conducted in nearby closely suburbs reported annual entomological inoculation rate of 161 infected bites per human per year, only to *A. gambiae* (Wanji et al., 2003).

This result finding also showed that the predominant plasmodial species in Mangoum was *P. falciparum* followed by *P. malariae*. The relatively high frequency of *P. falciparum* parasitism had already been signalled in Cameroon by other authors (Couprié et al., 1985; Touze and Charmot, 1993). Elsewhere, *P. ovale* was not found during our study. However, many investigations conducted in Cameroon revealed the presence of this plasmodial species (Moyou, 1977; Robert et al., 1992; Fondjo, 1996). Despite the very low numbers of malaria vectors

collected in the dry season and a subsequently low *EIR*, a large number of children (38.8%) were infected. This indicates a high efficiency of transmission by the vectors. Although the possibility that most of the children acquired their infections during the rainy season cannot the ruled out, this seems unlikely because the numbers of vectors and *EIR* were very high, particularly in the *A. gambiae* during the rainy season. Finally, our result confirmed that the spleen rates were lower than the parasite rates in the 2 – 9 years age group and remained less sensible to seasonal variations. Previous research carried out in the coastal and forest areas of the country came to the same conclusions (Merlin et al., 1985; Josse et al., 1987; Tang-Line-Foot, 1987).

This study shows that, Western Cameroon highlands region is an area of high malaria transmission intensity, inhabited by major vectors of malaria in Africa. Every night during the rainy season, and every ten nights during the dry season, an inhabitant of this region may receive three infective bites from *Anopheles*. This area can also be classified as a meso-endemic zone of malaria transmission. The parasite and spleen rates, the highly endophagous behaviour of *Anopheles* species, coupled with high EIRs, indicate the necessity of introducing vector control measures in the region as one of the strategies control malaria. This vector control intervention needs to focus on providing an effective personal protection for the most vulnerable groups against vector contact rather than aiming at reducing the potential for transmission at the regional level. The most appropriate vector control option in this area could be the use of insecticide treated nets (ITNs) as this tool is currently the most effective and practical vector control option in areas where malaria is highly transmitted (Diallo et al., 1999; Guillet, 2001).

ACKNOWLEDGEMENTS

This study received financial support from Fonds Mondial de Lutte contre le SIDA, la Tuberculose et le Paludisme, and from National malaria control programme, Ministry of Public Health, Cameroon. We are indebted to Dr Ateuchia Pascal, Dr Bouba and Mr Fossong Bathelemy for their technical assistance. We are also especially grateful to Dr Antonio-Nkondjio Christophe who revised the final manuscript and to the volunteers who participated to this study.

REFERENCES

Antonio- Nkondjio C, Hinzoumbe C, Simard F, Awono- Ambene P, Chouaibou M. ,Tchuinkam T and Fontenille D (2006). Complexity of the malaria vectorial system in Cameroon: Contribution of secondary vectors to malaria Transmission. Entomological Society of America. 43: 1215-1221.

Antonio- Nkondjio C, Atangana J, Ndo C, Awono-Ambene P, Fondjo E, Fontenille D and Simard Frederic (2008). Malaria transmission and rice cultivation in Lagdo, northern Cameroon. Transactions of the Royal Society of Tropical Medecine and Hygiene.102: 352-359.

Coetzee M, Craiy M, Sueur D (2000). Distribution of African mosquitoes belonging to the *Anopheles gambiae* complex. Parasitol. Today. 16: 74-77.

Couprié B, Claudot Y, Same Ekobo A, Issoufa H, Leger-Debruyne M, Tribouley J et Rippert C (1985). Etude épidémiologique du paludisme dans les regions rizicoles de Yagoua et de Maga (Nord Cameroun). Bull. Soc. Path. Ex. 78:191 – 204.

Detinova TS (1962). Méthodes à appliquer pour classer par groupe d'âges des Diptères présentant une importance médicale. Org. Mond. Santé. 47: 220.

Diallo DA, Habluetzel A, Cuzin-Ouattara N (1999). Widespread distribution of insecticide-impregnated curtains reduces child mortality, prevalence and intensity of malaria infection, and malaria transmission in rural Burkina Faso. Parassitologia. 41: 377-381.

Favia G, Lanfrancotti A, Spanos L, Siden-Kiamos I, Louis C (2001). Molecular Chacterization of ribosomal DNA polymorphisms discriminating among chromosomal forms of *Anopheles gambiae* s.s.. Insect. Mol. Biol. 10:19-23.

Fondjo E (1996). Etude du comportement du complexe *Anopheles gambiae* et la transmission du paludisme dans deux faciès écoclimatiquess au Mali et au Cameroun. Thèse Doctorat 3ème cycle. Min. Ens. Sec. Sup. Rech. Sc. ISFRA. Mali, p 92.

Fontenille D et Lochouarn l (1999). The complexity of the malaria vectorial system in Africa. Parassitologia. 41: 267-271.

Gillies MT, De Meillon B (1968). The Anopheline of Africa South of the Sahara (Ethiopian Zoogeographical region). Pub. Sth. Afr.Inst. Med. Res., Johannesburg. 54: 343.

Gillies MT, Coetzee Maureen (1987). A supplement to the Anophelinae of Africa South of the SAHARA (Afrotropical Region). Publications of the South African Institute for Medical Research. 55: 143.

Githeko KA, Ayisi MJ, Odada KP, Atieli KF, Ndenga AB, Githure IJ, Yan G (2006). Topography and malaria transmission heterogeneity in western Kenya highlands: Prospects for focal vector control. Malaria J. 5:107.

Guillet P (2001). Insecticide-treated nets in Africa: where do we stand? Africa Health. 23: S22-S24.

Jambou R, Ranaivo I, Rahadmalala et al. (2001). Malaria in the highlands of Madagascar after five years of indoor spraying of DDT. Transactions of the Royal Society of Tropical Medecine and Hygiene. 95: 14 -18.

Josse R, Merlen M, LE Hesran JY, Avennec F, Foumane Ngane V, Awono Zogo D, Eteki D, Combe A, Tribouley J et Rippert C (1987). Évaluation des indices épidémiologiques du paludisme en saison des pluies à Yaoundé (Républiques du Cameroun). Bull. Liais. OCEAC Yaoundé. 79:39-48.

Koudou BG, Yao T, Doumbia M, Nsanzabana C, Cisse G, Girardin O, Dao D, N'Goran EK, Vounatsou P, Bordmann G, Keiser J, Tanner M and Utzinger J (2005). Malaria transmission dynamics in central Côte d'Ivoire: the influence of changing patterns of irrigated rice agriculture. Med. Veter. Entomol. 19: 27 – 37.

Lindblade KA, Walker ED, Onapa AW, Katungu J, Wilson ML (1999). Highland malaria in Uganda: prospective analysis of an epidemic associated with EL Nino. Transactions of the Royal Society of Tropical Medecine and Hygiene. 91: 387-388.

Merlin M, LE Hesran JY, Josse R, Josseran R, Sicard JM, LE Mao G, Eteki D, Combe A, Tribouley J et Rippert C (1986). Évaluations des indices cliniques, parasitologiques et immunologiques du paludisme dans la région de la baie de Bonny en Afrique Centrale. Bull. Soc. Path. Exot. 79: 707-720.

Moyou SR (1977). Les aspects actuels du paludisme au Cameroun. Thèse Médecine, Bordeaux, Paris.OMS (1998a)- Évaluation de la santé, In Rapport sur la santé dans le monde. La vie au 21 ème siècle, une perspective pour tous. Organisation Mondiale de la Santé (ed) Genève, Suisse, Pp. 52-61.

Robert V, Van den Broek A, Stevens P, Slootweg R, Petrarca V, Coluzzi M, Le Goff G, Di Decco MA, Carnevale P (1992). Mosquitoes and malaria transmission in irrigated rice-fields in the Benoue valley of Northern Cameroon. Acta Trop. 52:201-204.

Same EA, Fondjo E, Eouzan JP (2001). Grands travaux et maladies à vecteurs au Cameroun: Impact des amenagements ruraux et urbains sur le paludisme et autres maladies à vecteurs. IRD, Paris, p. 221.

Scott JA, WG Brogdon and FH Collins (1993). Identification of single specimens of the *Anopheles gambiae* by polymerase chain reaction.

Am. J. Trop. Med. Hyg. 49: 520-529.
Shililu JI, Maier WA, Seitz HM and Orago AS (1998). Seasonal density, sporozoite rate and entomological inoculation rates of *Anopheles gambiae* and *A. funestus* in a high altitude sugarcane growing zone in western Kenya. Trop. Med. Int. Health. 3: 706-710.
Tang-Line-Foot (1987). Évolution récente des indices paludométriques au Cameroun. Thèse de Doctorat d'État, Université de Bordeaux II. p 76.
Touze JE, Charmot G (1993). Le paludisme à *Plasmodium falciparum* : situation actuelle et perspective. Cahiers Santé. 3: 217-229.
Wanji S, Tanke T, Atanga NS, 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