

Malaria Transmission in Far Northern Cameroon: Characterization of Anopheline Species and the Sporozoite Infection Rate

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ABSTRACT

As part of a longitudinal study to assess the transmission of malaria in far northern Cameroon, Anopheline species were characterized and their infection rates determined. Entomological studies were carried out in July and October 1998. Night captures of vectors using human landing catches were conducted indoors and outdoors from four different localities in Maga (Mahouda, Simatou, Altiniéré and Farahoulou). The species of female *Anopheles* mosquitoes were morphologically identified, and the presence of *Plasmodium* infection assessed by the enzyme-linked immunosorbent assay for circumsporozoite proteins. *Anopheles gambiae s.l.* (98%) and *An. funestus* (2%) were the malaria vectors found. Average man biting rate was higher in the irrigated rice cultivating areas than in areas situated far from irrigation. Variation in the average sporozoite antigen rates was not significant. The entomological inoculation rate was higher in the irrigated rice paddies of Mahouda (9.03%) and Simatou (10.06%) than in the non-irrigated areas of Altiniéré (2.07%) and Farahoulou (1.7%). Our data suggest that transmission varies during July and October, *An. gambiae s.l.* responsible for 99% of malaria transmission and *An. funestus* for 1%.

Key words: malaria, transmission, sporozoite rates *Plasmodium falciparum*, *Anopheles gambiae*, *Anopheles funestus*, irrigation, rice paddy, Cameroon.

RÉSUMÉ

Les espèces *Anophèles* et leur taux d'infection ont été déterminés dans le cadre d'une partie d'une étude longitudinale de la transmission du paludisme dans la province de l'extrême nord Cameroun. Les études entomologiques ont été menées de Juillet et Octobre 1998. Les captures nocturne des vecteurs sur appâts humains ont été faites à l'intérieur et à l'extérieur des maisons dans quatre localités de Maga (Mahouda, Simatou, Altiniéré et Farahoulou). Les espèces d'*Anophèles* femelles ont été morphologiquement identifiées et la présence d'infection Plasmodiale étudiée par la technique Immunoenzymatique (ELISA) pour la recherche de protéines circumsporozoite. *Anophèles gambiae s.l.* (98%) et *An. funestus* (2%) ont été les vecteurs de paludisme trouvés. La moyenne des piqûres par personne a été plus élevée dans les régions de culture irriguée de riz que dans les régions situées à distances des irrigations. La variation de la moyenne de la prévalence d'antigènes de sporozoites n'a pas été significative. Le taux d'inoculation entomologiques a été plus élevé dans les rizières de Mahouda (9.03%) et Simatou (10.06%) que dans les régions non irriguées d'Altiniéré (2.07%) et Farahoulou (1.7%). Nos données suggèrent que la transmission varie de Juillet et Octobre et que *An. gambiae* et *An. funestus* sont respectivement responsables de 99% et 1% de la transmission du paludisme.

Mots clés : malaria, transmission, taux de sporozoites, *Plasmodium falciparum*, *Anophèles gambiae*, *Anophèles funestus*, rizières, Cameroon

INTRODUCTION

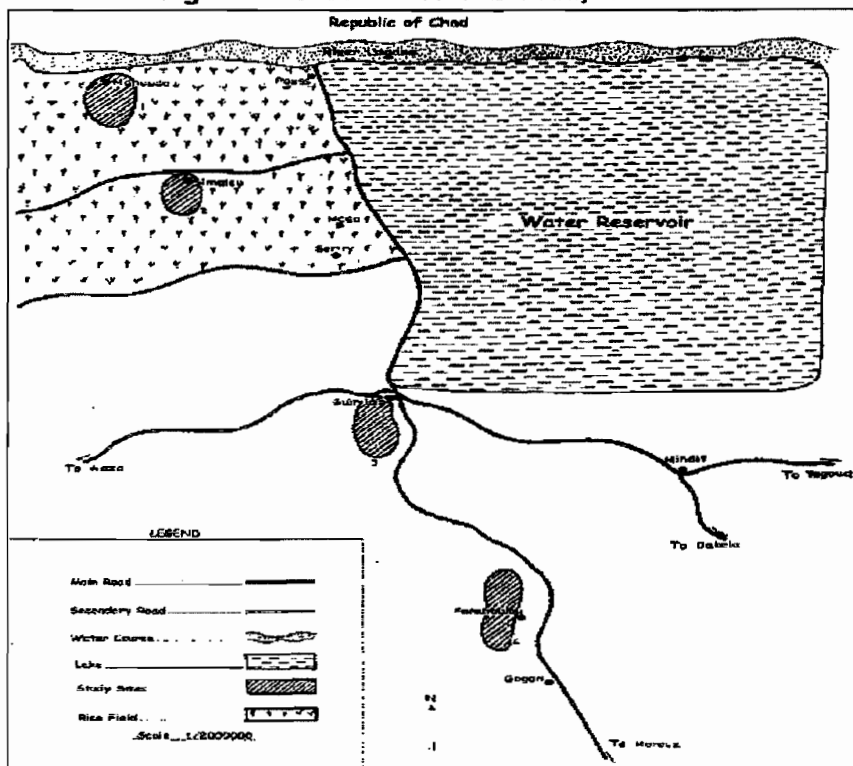
Exclusively the female *Anopheles* mosquito transmits malaria, an important tropical disease. The disease affects about 400 million people, causing at least 1.5 million deaths annually worldwide (WHO, 1996). Malaria is caused by protozoan parasites of the genus *Plasmodium* of which four (*P. falciparum*, *P. malariae*, *P. ovale* and *P. vivax*) affect humans. Of these, *P.falciparum* is the most virulent and prevalent in Africa, which harbours more than 90% of the global clinical and death cases of malaria (WHO, 1996). The high prevalence of malaria in Africa is attributed to the presence of human and natural factors that support breeding of the very efficient mosquito vectors of *P. falciparum* as well as indigence and political instability. In order to predict the course of malaria epidemic or its occurrence, sufficient information is required on the dynamics of its transmission. Transmission, known to vary in time and space occur either perennially or seasonally depending on the vector species involved (McKenzie *et al*, 1987; Collins *et al*, 1995). Five very efficient vectors of malaria in Africa include *An. gambiae*, *An. arabiensis*, *An. funestus*, *An. nili* and *An. moucheti* (Carlwood *et al*, 1998; Simard *et al*, 1999; Fontenille *et al*, 1997). In some parts including Cameroon, these vectors occur in sympatry and transmit either at the same time or during different seasons (Gillies and De Meilon, 1968; Gillies and Coetzee, 1987; Burkot *et al*, 1994; Temu *et al*, 1998). The malaria

vectors more especially *An. gambiae s.s.* and *An. arabiensis* of the *An. gambiae* complex have the widest distribution in Africa. They play a fundamental role in the transmission of malaria, but members of the complex as well as other potential vectors exhibit differences in behaviour, seasonal prevalence and levels of vectorial efficiency. All five species frequently occur in Cameroon (Fontenille *et al*, 1999). Unlike in the southern parts of Cameroon where studies have been conducted to evaluate malaria transmission (Manga *et al*, 1992; Manga *et al*, 1997; Fondjo *et al*, 1992; Le Goff *et al*, 1992, Mouchet *et al*, 1998), little is known about the transmission of malaria in the Maga area of far northern Cameroon where the ecosystem has been modified by the cultivation of rice. As part of a longitudinal study to assess the level of malaria transmission in the Maga area and evaluate the effect of rice cultivation on it, we characterized the Anopheline species and determined their entomological inoculation rates.

MATERIALS AND METHODS

The study was conducted in four different localities in far northern Cameroon (Figure 1): Mahouda and Simatou in the irrigated rice cultivating area of Maga, Altinière and Farahoulou, outside the irrigated zone. Maga is located 13°N; 14.05°W

Figure 1: Location of the Study Sites



Night captures of mosquitoes using human landing catches were conducted indoors and outdoors during the months of July in the rainy season and October in the dry season of 1998. Collection was done for three nights per month in each locality for six human nights. The species of female *Anopheles* mosquitoes was morphologically identified (Gillies and De Meillon, 1968; Gillies and Coetzee, 1987) and the heads and thoraces dissected and preserved on cotton wool over silica gel in separate tubes. These were then transported to the laboratory where the heads and thoraces were immediately homogenized in blocking buffer (0.5% Casein, 0.1N NaOH, 1xPBS) and stored at -20°C until assessed for infectivity by sporozoite ELISA according to the method of Wirtz et al, (1987a and 1987b) and Burkot et al, (1984). Positive controls and uninfected laboratory reared negative control mosquitoes were included in the assay. Where more than 30 mosquitoes of the *Anopheles gambiae* complex was captured, a random sample of at least 30 mosquitoes was used to identify the sibling species following the PCR method described by Collins et al. (1990), Scott et al. (1993) and as modified by Temu et al, (1998). One leg or wing from each mosquito was placed in a 0.2ml Eppendorf tube to which 12.5ml of PCR master mix containing species specific primers, deoxynucleotide triphosphates, PCR buffer and Taq polymerase was added. The PCR amplification procedure followed an initial denaturation step of 94°C for 2min followed by a 30-cycle denaturation at 94°C for 30sec, annealing at 50°C for 30sec, elongation at 72°C for 8min using a PTC-100 thermal cycler (MJ Research Inc., Waltham, MA). The amplified PCR product was electrophoresed on a 2.5% ethidium bromide treated agarose gel to estimate the sizes of the products and determine the species.

RESULTS

A total of 843 female mosquitoes were captured from the four localities comprising 826 (98%) *An. gambiae* s.l. [281(32%) *An. gambiae* s.s. and 545(66%) *An. arabiensis*] and 17 (2%) *An. funestus*. Of the 843 tested by ELISA 140 infections were detected, 138 in *An. gambiae* s.l. (sporozoite rate = 16.7%) and 2 in *An. funestus* (sporozoite rate of 11.8%). Other *Anopheles* species found were *An. Pharoensis* and *An. rufipes*. Details of catch per locality, man-biting rates (ma), sporozoite rates (SR) and the entomological inoculation rate (EIR = number of infective bites /man/night) are shown in tables 1, 2 and 3 respectively. The average man-biting rate of the two vector species was higher in Mahouda and Simatou situated in the irrigated rice fields compared to Altiniéré and Farahoulou situated far from irrigation. Variation in the sporozoite antigen rates per locality were not significant ($\chi^2 = 0.849$, df

= 39, p = 0.83). The entomological inoculation rate (number of infective bites/man/night) was observed to be higher in Mahouda and Simatou than in Altiniéré and Farahoulou.

DISCUSSION

Anopheles gambiae s.l. (*An. gambiae* s.s. and *An. arabiensis*) and *An. funestus* were the only malaria vectors in Maga in July and October 1998. The density of these two vectors was seen to vary considerably in between the localities. *Anopheles gambiae* s.s. and *Anopheles arabiensis*, both members of the *An. gambiae* complex comprise the major vector species in North Cameroon (Fontenille et al, 2000, Fondjo et al, 1996; Fondjo et al, 1999). Known to breed in shallow, sunlit, freshwater pool of high organic content, the presence of rice paddies in Mahouda and Simatou could possibly account for their higher density in these areas and may in part, better explain their implication in the transmission of malaria during the dry and wet seasons. Similar observations have been made in studies in The Gambia (Thomson et al, 1994; Lindsay et al, 1991). *An. funestus* populations tend to fluctuate with changes in the water table; however, akin to studies during different seasons in Tanzania (Temu et al, 1998) their generally low density both in the rainy season and in the rice paddies compared to *An. gambiae* s.l. suggest that it is not a major vector in the Maga area.

Overall, the higher biting rate in Mahouda and Simatou is possibly due to water in the irrigated rice paddies that supports breeding of the mosquito vectors. Both vectors tend to be more aggressive in July than in October, probably because of the rains in July providing water for breeding. According to Botha De Meillon (1951), the fact that climate plays a part in the diversity of behaviour of African anophelines in different localities is indisputable. However, differences in ecology of the population owing to human activities also have an influence. Thus, the increased biting rates in October in areas situated far from irrigation is paradoxical and suggests that human activity may also be responsible; This human activity may be amenable to intervention.

Variations in the sporozoite antigen index per locality for both vector species were not different in the irrigation and non-irrigation zones. The entomological inoculation rate is higher in Mahouda and Simatou because of their location in the irrigated rice fields and not due to differences in the sporozoite rates. Our data provide preliminary information on transmission of malaria in the Maga area of far northern Cameroon and how rice cultivation affects the epidemiology of malaria in this region.

Table 1: Distribution of *Anopheles gambiae* siblings in different localities of the Maga during the dry and wet seasons.

Study site	Collection period	<i>Anopheles gambiae</i> complex		
		# Tested by PCR	<i>An. Gambiae s.s</i>	<i>An. Arabiensis</i>
mahouda	JULY (rainy season)	30	11	19
	OCTOBER (Dry season)	30	13	17
Simatou	JULY (rainy season)	30	5	25
	OCTOBER (Dry season)	30	8	22
Altiniéré	JULY (rainy season)	30	4	26
	OCTOBER (Dry season)	30	10	20
Farahoulou	JULY (rainy season)	9	1	8
	OCTOBER (Dry season)	30	14	16
TOTAL	JULY (rainy season)	99	21(21%)	78 (79%)
	OCTOBER (Dry season)	120	45 (37.5%)	75(62.5%)
Combined Proportion (July & October)		Total = 219	66/219 (30%)	153/219 (70%)

Table 2: Numbers and biting rates for the different *Anopheles* species collected from different localities in Maga during the rainy and dry seasons.

STUDY SITE	<i>Anopheles gambiae s. l.</i>					<i>Anopheles funestus</i>					Combined average ma
	JULY (Rainy season)		OCTOBER (Dry season)		Average ma	JULY (Rainy season)		OCTOBER (Dry season)		Average ma	
	Total #	ma	Total #	ma		Total #	ma	total #	ma		
Mahouda	152	73.08	71	21.25	52.35	1	1.41	1	0.37	1	52.35
Simatou	183	83.33	167	52.62	71.05	10	1.41	4	0.62	1,1	72.15
Altiniéré	50	6.6	93	22.37	12.95	0	0	1	0.25	0.05	13
Farahoulou	9	1.1	100	21.75	9.4	0	0	0	0.37	0.15	9.55

Table 3: Combined average sporozoite rates and EIR (infective bites/man/night) for the two vectors from different localities of Maga during the two seasons.

Study site	<i>Anopheles gambiae s.l.</i>					<i>Anopheles funestus</i>					Average	
	Total collected	Tested for CSP	Positive for CSP	SR (%)	EIR (ma x SR)	Total collected	Tested for CSP	Positive for CSP	SR (%)	EIR (ma x SR)	SR (%)	EIR
Mahouda	351	351	59	16.8 ± 3	8,9	14	14	2	14.3(2/14)	0,14	16.7 ± 3	9,03
Simatou	223	223	34	15.2 ± 4	10,6	2	0	0	0	0	15.2 ± 4	10,6
Altiniere	109	109	18	16.5 ± 6	2,07	0	0	0	0	0	16.5 ± 6	2,07
Farahoulou	143	143	27	18.9 ± 6	1,7	0	0	0	0	0	18.9 ± 6	1,7

Variation in the sporozoite antigen rates per locality is not Significant ($\chi^2=0.849$, $df=39$, $p=0.83$), while ma

For the two vector species is much higher in mahouda and simatou in the rice fields.

SR: Sporozoite rate (proportion of mosquitoes positive by ELISA/total mosquitoes tested by ELISA)

ma: Man biting rate

EIR: entomological inoculation rate (SR x ma)

= Number

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