

Anopheles species of the mount Cameroon region: biting habits, feeding behaviour and entomological inoculation rates

Samuel Wanji^{1,2}, Theodore Tanke¹, Sali N. Atanga², Caroline Ajonina¹, Tendongfor Nicholas¹ and Didier Fontenille³

¹ University of Buea, Department of Life Sciences, Buea, Cameroon

² Research Foundation in Tropical Diseases and Environment, Buea, Cameroon

³ Laboratoire IRD-ORSTOM, OCEAC, Yaoundé, Cameroon

Summary

There is a lack of data on the *Anopheles* fauna, its biology and the roles played by different vector species in the transmission of malaria in the mount Cameroon region. The biting habits, feeding behaviour and entomological inoculation rates of different *Anopheles* species during the dry and rainy season were investigated. A total of 2165 *Anopheles* was collected, 805 in the rainy season and 1360 in the dry season. Five *Anopheles* species were identified: *Anopheles gambiae* s.l., *An. funestus*, *An. hancocki*, *An. moucheti* and *An. nili*. *An. gambiae*, *An. funestus* and *An. hancocki*, recorded during both seasons, were the main vectors of malaria in the region. *An. gambiae* s.s. was the only member of the *An. gambiae* (Giles) complex. These three species had their peak activity between 1 and 2 AM. A human blood index (HBI) of 98.29% was recorded for fed *Anopheles*. The sporozoite rate, for all vectors together, was significantly higher in the rainy season (9.4%) than in the dry season (4.2%) with all the species infected by *Plasmodium falciparum*. The average inoculation rate was 0.44 infective bites per man per night, which adds up to 161 infective bites per year in this study area. Analyses of relative abundance and infection rate of malaria vectors at different sites situated along a transect of 20 km during the dry season showed high heterogeneity in biting and sporozoite rates. No malaria vector was caught at 1200 m a.s.l. The mount Cameroon region should be considered an area of high malaria transmission intensity.

keywords malaria, *Anopheles* vectors, biting habits, infective rate, mount Cameroon region

Introduction

Although tremendous progress has been made in understanding and fighting malaria over the last century, the disease remains one of the biggest public health problems of mankind. The World Health Organization estimates that malaria affects some 300–500 million people, killing 1.5–2.5 million every year. More than 90% of these cases are registered in sub-Saharan Africa with >70% of inhabitants in certain areas of this region being chronically infected by *Plasmodium falciparum*. Fontenille and Lochouarn (1999) have discussed the complexity of malaria vectorial systems in Africa, with different species involved in transmission in different bio-geographical zones.

The principal vectors of malaria in Cameroon are *Anopheles gambiae* s.s. and *An. funestus*, which are widespread. *An. arabiensis* is restricted to the northern part of the country and *An. melas* to the coastal zones of the southern part. *An. hancocki*, *An. nili* and *An. moucheti* play

a secondary role in the transmission of malaria in the forest block, and their relative importance may vary from one bio-ecological zone to another (Carnevale *et al.* 1992; Njan *et al.* 1993; Fontenille *et al.* 2000; Nkondjio *et al.* 2002).

The mount Cameroon region can be considered as an area of hyperendemic malaria. Parasitological and clinical studies recently revealed a spleen rate of 62% (65 of 105) and a parasite rate of 71% (75 of 105) in children of 2–9 years of age (unpublished data). Despite this high incidence of malaria in the region, no research has been carried out on malaria transmission, and on the roles played by different vectors at different seasons of the year. Cameroon is characterized by several bio-geographical zones and the national programme for malaria control under the roll-back malaria initiative requires information on the malaria transmission from all the bio-ecological zones of the country. This paper reports on work carried out in the mount Cameroon region on the biting habits, feeding behaviours and entomological inoculation rates (EIR) of malaria vectors, during two seasons of the year.

Materials and methods

Study area

Mount Cameroon, the highest mountain in West Africa and an active volcano, rises from the Atlantic Ocean at the Gulf of Guinea and culminates at 4100 m above sea level in Buea. The mountain is formed of a continuous pile of terraces from the base to the summit; at an altitude of 50 m from the coast, is a sedimentary plain that extends from Limbe to Mutengene and Tiko. From Mutengene, the terrain gradually elevates to an altitude of 800–1200 m in Buea town. Hydrologically, some 20 streams are of prime importance and empty into the Atlantic Ocean. Two of these, Ombe and Onge, flow southeast and northwest, respectively.

In this forested area of southern Cameroon, the equatorial climate has been modified by the double influence of the ocean and the mountain. The temperatures are lower than in the other areas of the southern part of the country: the mean values of the minimum temperatures are 20 °C in December and 18 °C in August, the mean values of the maximum temperatures are 35 °C in August and 30 °C in March. Rainfall is also important. Debundsha, located at the western flanks of mount Cameroon registers up to 11 000 mm of rainfall, making this area one of the wettest in the world. The mount Cameroon area has a long rainy season that starts in March and ends in late October with maximum rainfall in August and September. The dry season starts in late October and ends in February.

More than 400 000 people live around mount Cameroon in the towns of Tiko, Limbe, Mutengene, Buea and Muyuka. The population is composed of indigenous Bakweri people, Creoles (from Liberia and Sierra Leone) and immigrants from other parts of Cameroon, especially the North West Province.

Mosquito collection

The study was conducted during the rainy months of June, July, August and September 1998 and the dry months of November and December 1999, January and February 2000. Four stations representing the major settlements along a gradient of altitude (transect) in the mount Cameroon region were selected for the study: Mutengene (4°05' N; 9°18' E, 100 m a.s.l.), Molyko (4°08' N; 9°17' E (600 m a.s.l.), Likoko (4°13' N; 9°14' E, 800 m a.s.l.) and Vasingi (4°17' N; 9°15' E, 1200 m a.s.l.). Mosquitoes were collected by the same workforce at all stations during the two seasons through standardized house-resting collections and human-biting collections.

Two human-landing collections were carried out at each month of the 8 months of collection in the four stations, using two teams each with four trained collectors. The

activities of the collectors were monitored throughout the night by a supervisor. The first team worked from 6 PM to 12 AM, and the second from 12 AM to 6 AM. Two homes were selected per station with one collector being indoors (generally in the bedroom) and the other outdoors. Collections took place over eight man-nights per station per month with different houses used for subsequent collections. Tubes were labelled hourly and mosquitoes that came to feed on collectors were captured using aspirators and then transferred into the tubes. This gave a total of 256 man-nights during the entire period of collection.

Pyrethrum spray collections as described by Service (1977) were carried out in all four stations every month of the 8 months of collection. Fifteen bedrooms were selected randomly from each locality, with at most three bedrooms from the same house; 480 rooms were sprayed during the entire period of the study. The mosquitoes collected in test tubes were kept in cold boxes and taken to the laboratory for processing.

Collections were made using the same team of collectors who expressed consent to a research protocol approved by the Ethical Committee of the University of Buea and the Research Foundation in Tropical Diseases and Environment. All volunteers were given prophylactic treatment for malaria.

Identification of mosquitoes

Two approaches were used for the identification of mosquitoes: the morphological identification of different species of *Anopheles* and the molecular identification of members of the *An. gambiae* s.l. All mosquitoes collected were identified using the morphological key of Gillies and De Meillon (1968) and Gillies and Coetze (1987) and 'The IRD Software for the Identification of *Anopheles* of the Afro-Tropical Region' by Hervy *et al.* (1998).

Anopheles gambiae s.l. mosquitoes collected in the dry season were further identified into the sibling species using polymerase chain reaction (PCR) techniques demonstrated by Paskewitz and Collins (1990), with minor modifications. Two methods were used: the direct method where a single leg or a wing of mosquito was placed directly into PCR reaction mixture and amplified; and in situations where this method gave negative results, probably because of significant DNA degradation, the extraction method was used.

Estimation of infective rate of *Anopheles*

Two methods were used for the estimation of sporozoite rates. Initially, microscopy was used for *Anopheles* collected in the rainy season and later on the enzyme-linked

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immunosorbent assay (CSP-ELISA) was used for dry season mosquitoes as soon as this assay system was established in the laboratory (Fontenille *et al.* 2001). The head and thorax of *Anopheles* caught in the dry season were separated from the abdomen and tested for the presence of *P. falciparum*, *P. ovale* and *P. malariae* CSP by the method described by Burkot *et al.* (1984) and modified by Wirtz *et al.* (1987).

Determination of source of blood meal of *Anopheles*

Fed *Anopheles* from the house-resting collection were dissected and blood smeared on Whatman filter paper. The technique used for the ELISA was that described by Beier *et al.* (1988). Antibodies used for the test were of human, goat, chicken and bovine origin. Only *Anopheles* collected during the dry season were tested using this method.

Estimation of physiological age

Physiological age was calculated using the method of Detinova (1962) where *Anopheles* mosquitoes collected during the rainy season were dissected and their ovaries observed for tracheolar appearance.

Expression of results

The following entomological indices of malaria transmission were determined: the sporozoite rate – the proportion of *Anopheles* with positive salivary gland after dissection or positive by CSP-ELISA; the biting rate – the number of *Anopheles* bites experienced by a collector during an entire night of activity (the biting cycle is the hourly variation of the biting rate and it is also referred to as the aggressiveness of the species); the infective biting rate – the

product of the biting rate and the sporozoite rate estimated from human-landing catches; the coefficient of endophagy – the ratio of the number of *Anopheles* caught biting indoors to that caught biting outdoors; the human blood index (HBI) – the proportion of *Anopheles* that fed on humans; and the parity rate – the proportion of *Anopheles* that were found parous.

Simple chi-square analysis was performed to compare different proportions and the chi-square analysis of trend was performed to find out if there is any relationship between the variation in the altitude and the *Anopheles* infectivity. All the tests were performed at the 5% significance level.

Results***Anopheles* species: biting densities, feeding behaviour and infection rates**

Between June 1998 and February 2000, a total of 2165 *Anopheles* were collected: 805 (37.2%) in the rainy season (Table 1a) and 1360 (62.8%) in the dry season (Table 1b). Five *Anopheles* species were identified: *An. gambiae*, *An. funestus*, *An. hancocki*, *An. nili* and *An. moucheti*. *An. nili* and *An. moucheti* were rare and found only during the dry season. PCR analysis of the sibling species of *An. gambiae* revealed that all the 125 specimens tested were *An. gambiae* s.s. The major species of *Anopheles* collected from the mount Cameroon region were therefore *An. gambiae* s.s., *An. funestus* and *An. hancocki*.

The three major species of *Anopheles* showed different biting rates at the rainy and dry seasons (Table 2a,b). *An. gambiae* was the most aggressive species at both seasons with three bites per man per night in the rainy season and 5.53 bites per man per night in the dry season.

Table 1 Species and sporozoite rates of malaria vectors found in the Mount Cameroon region

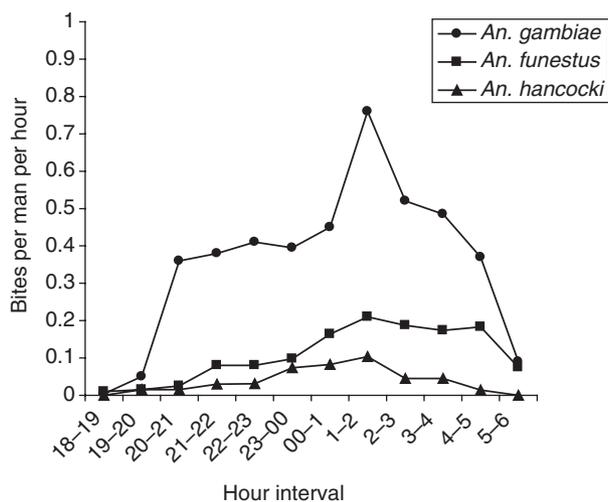
<i>Anopheles</i> species	Number captured	Number dissected	Number positive	Sporozoite rate (95% CI)
(a) Rainy season				
<i>An. gambiae</i>	457	157	22	14.01% (8.5–19.4)
<i>An. funestus</i>	310	127	8	6.29% (2.0–10.5)
<i>An. hancocki</i>	38	38	0	0
<i>Anopheles</i> species	Number captured	Number tested (ELISA)	Number positive	Sporozoite rate (95% CI)
(b) Dry season				
<i>An. gambiae</i>	956	551	25	4.53% (2.8–6.26)
<i>An. funestus</i>	314	245	8	3.26% (1.04–5.48)
<i>An. hancocki</i>	83	79	4	5.06% (0.23–9.89)
<i>An. moucheti</i>	5	5	0	0
<i>An. nili</i>	2	2	0	0

Table 2 Entomological inoculation rates of malaria vectors in the Mount Cameroon region (1998–2000)

<i>Anopheles</i> species	Number captured biting	Man-nights	Number dissected	Sporozoite rate (95% CI)	Biting rate (bites/man/night)	Infective biting rate (infective bites/man/night)
(a) Rainy season						
<i>An. gambiae</i>	384	128	105	15% (8.1–21.8)	3	0.45
<i>An. funestus</i>	238	128	64	6.0% (0.2–11.8)	1.86	0.11
<i>An. hancocki</i>	35	128	35	0	0.27	0
<i>Anopheles</i> species	Number captured biting	Man-nights	Number tested by ELISA	Sporozoite rate (95% CI)	Biting rate (bites/man/night)	Infective biting rate (infective bites/man/night)
(b) Dry season						
<i>An. gambiae</i>	706	128	367	2.17% (0.7–3.6)	5.53	0.12
<i>An. funestus</i>	96	128	95	4.2% (0.17–8.2)	0.75	0.03
<i>An. hancocki</i>	79	128	76	5.3% (0.27–10.37)	0.62	0.03

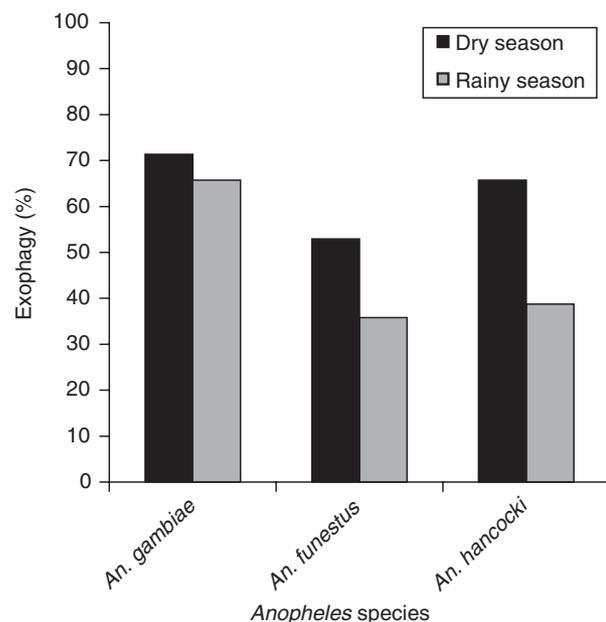
The hourly variations of the aggressiveness of the three major species of *Anopheles* are shown in Figure 1. All three species display the same biting cycle during the night with a minor peak of activity between 8 and 11 PM and a major peak of activity between 1 and 4 AM.

The three main species of *Anopheles* varied in their feeding behaviour during the two seasons (Figure 2); *An. funestus* and *An. hancocki* were more exophagous during the dry season while *An. gambiae* was both endo- and exophagous in equal measure during both seasons. The three main species also displayed heterogeneity in their sporozoite rates and EIR for the two seasons. *An. gambiae* was more infected during the rainy season (14%; 22 of 157) while *An. hancocki* was more infected during the dry season (5%; four of 79). *An. hancocki* was found infected only during the dry season. *An. funestus* maintains

**Figure 1** Night biting cycle of the malaria vectors in the Mount Cameroon region (1998–2000).

relatively stable infection rates in both seasons: 6.3% (eight of 127) during the rainy season and 3.26% (eight of 245) during the dry season. The EIR was estimated based on *Anopheles* collected from landing catches (Table 2a,b). The highest infective biting rate was recorded with *An. gambiae* during the rainy season (0.45 infective bite per man per night).

Overall infective biting rates of 0.61 and 0.2 were obtained for the three species of *Anopheles* for the rainy and dry seasons, respectively. Therefore, an individual may receive an infective bite every other night during the rainy season and once every five nights during the dry season.

**Figure 2** Proportions of *Anopheles* biting outdoors (exophagy) during the rainy and dry seasons.

Species infecting *Anopheles* sp. in the study area

Of 882 *Anopheles* collected during the dry season and tested for *P. falciparum*, *P. malariae*, and *P. ovale*, all 37 infected specimens had *P. falciparum*.

Source of blood meal of *Anopheles*

Of 235 fed *Anopheles* collected during the dry season and tested for sources of blood meal, 156 *An. gambiae*, 72 *An. funestus* and three *An. hancocki* had fed on humans. One *An. funestus* had fed both on humans and goats. Four specimens were negative, suggesting that they may have taken blood from sources different from those with available antibodies.

Parous proportions and longevity of *Anopheles*

The parous proportions for the three major species of *Anopheles* of the mount Cameroon region were as follows: *An. gambiae* 80.6 ± 5.14%; *An. funestus* 78.0 ± 6.32%; *An. hancocki* 80% ± 6.76%. Assuming an interval of 3 days between blood meals, the probability of surviving for 1 day was very high (0.92–0.93) for all three species of *Anopheles* and their life expectation was 12–14 days.

Relative abundance and circumsporozoite rates of malaria vectors at different points of a transect of altitude during the dry season

For vector density analyses, 888 *Anopheles* were used (all species considered) collected during the dry season (Table 3). There was great heterogeneity in the biting rates at different altitudes taken together ($\chi^2 = 16.89$, $df = 3$, $P < 10^{-3}$). The highest biting rate was recorded at Molyko (600 m a.s.l.), the lowest at Vasingi (1200 m a.s.l.), where no mosquito was captured during the 32 man-nights of collection.

As for CSP rates and infective biting rate, 545 *Anopheles* (all species taken together) collected from different points of the gradient of altitude and analysed for

circumsporozoite protein also displayed heterogeneity in their infection rates ($\chi^2 = 12.42$, $df = 3$, $P < 10^{-2}$). The highest infection rate was recorded at Mutengene (9.37%), and the lowest at Molyko (1.3%). There was no linear trend between sporozoite rates and altitudes. The EIR did not vary significantly between Mutengene, Molyko and Likoko ($\chi^2 = 3.2$, $df = 3$, $P > 0.10$).

Discussion

The main species of *Anopheles* in the mount Cameroon region are *An. gambiae* s.s., *An. funestus* and *An. hancocki*. These results confirm the confinement of the sibling species *An. gambiae* s.s. to the forested areas of Africa (Coetzee *et al.* 2000). *An. gambiae* s.s. and *An. funestus* are the major vectors of malaria in this study area as they were found infected during the dry and rainy seasons. These two species are well known as efficient vectors of malaria in other areas of Africa and Madagascar (Meyus *et al.* 1962; Manga *et al.* 1997; Shililu *et al.* 1998; Lindblade *et al.* 1999; Jambou *et al.* 2001). The presence of *An. hancocki* in the mount Cameroon region and its implication in malaria transmission is noteworthy; *An. hancocki* has only rarely been found infected with *Plasmodium* species (Vaucl & Campourcy 1943; Fontenille *et al.* 2000). In the mount Cameroon region it was found that *An. hancocki* had a relatively high sporozoite rate (5%) during the dry season, topping the malaria transmission; hence it should be considered an important malaria vector there.

Anopheles species of the mount Cameroon area were highly anthropophagous (almost 100% of specimens tested fed on human beings despite the presence of other blood sources such as goats, dogs, chickens and pigs that are commonly reared by the inhabitants of this area). These findings are in agreement with other reports from equatorial Africa regions (Nkondjio *et al.* 2002) and are in contrast with the anthropophagous rate of *Anopheles* in west Africa and savannah regions where the blood meal preference is determined by the availability of potential hosts (Lemasson *et al.* 1997; Killeen *et al.* 2001).

Table 3 Human biting rates and entomological inoculation rates of malaria vectors at different altitudes of the mount Cameroon region during the dry season (1999–2000)

Station (altitude)	Number collected	Biting rate	Number tested	Number positive	CSP rate (95% CI)	Infective biting rate
Mutengene (100 m)	69	2.15	64	6	9.3% (2.48–16.9)	0.20
Molyko (600 m)	645	20.15	309	4	1.3% (0.04–2.54)	0.26
Likoko (800 m)	174	5.43	172	6	3.4% (0.75–6.21)	0.19
Vasingi (1200 m)	0	–	–	–	–	–

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Anopheles funestus and *An. hancocki* displayed variations in their biting behaviour according to the time of year, being more endophagous during the rainy season and more exophagous during the dry 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. *An. gambiae* seems to be more adapted to the weather conditions of this area as its biting behaviour was similar in the two seasons. This seasonal shift in the feeding behaviour of *An. funestus* and *An. hancocki* would have important implications for the vector control in the mount Cameroon region during the rainy season, as insecticide treated nets (ITN) or residual spraying could be very effective in preventing these two *Anopheles* species from biting, or reducing their vectorial capacity.

By employing the same workforce at different points of a transect of altitude from 100–1200 m a.s.l., it was found that the malaria vectors were heterogeneously distributed along the transect. At Vasingi, a station at 1200 m a.s.l, no mosquito was collected during the research period, probably because of weather conditions: temperatures at this altitude are usually <15 °C. The absence of mosquitoes could also be due to the lack of appropriate breeding sites. The slope at this altitude is abrupt and the streams flow very fast.

The relative abundance of vectors at altitudes where they were found varied greatly, with fewer *Anopheles* collected at low-altitude Mutengene than high-altitude Molyko and Likoko. The relatively low abundance of malaria vectors at Mutengene is paradoxical as this station is characterized by high temperature (30–36 °C) and a high relative humidity throughout the year. These results could be explained by the fact that the locality is more urbanized than the other two stations and that relatively lower hygienic conditions lead to the contamination of breeding places with polluted materials, making them unsuitable for the development of *Anopheles* larvae (Bruce-Chwatt 1985). *Culex* spp. and *Mansonia* sp. (data not shown) were also collected at this station during the surveys. Mutengene nevertheless seems to be very suitable for malaria transmission as the highest infectivity rate was recorded there.

In contrast to Mutengene, Molyko displayed high malaria density. Molyko is a newly urbanized area, which is experiencing a rapid increase of population since the creation of the University of Buea in 1993. The increase in population at this station is associated to the environmental modifications for the infrastructure required for accommodating and supporting the new population and to the creation of more man-made breeding places for *Anopheles* (Trape & Zoulani 1987).

Plasmodium falciparum was the only parasite found in the vectors using ELISA-CSP, but this does not mean that it is the only species of malaria parasite in this area. In a recent study (unpublished data) conducted in 105 children in the area (71% prevalence), the specific prevalence of *P. malariae* was 4%. No *P. ovale* was found. Therefore it is more likely that *P. falciparum* is the dominant species in this hilly area.

This study shows that mount Cameroon region is an area of high malaria transmission intensity, inhabited by the major vectors of malaria in Africa. Every other night during the rainy season, and every five nights during the dry season, an inhabitant of this region may receive an infective bite from *Anopheles*. The abundance of *Anopheles* species, their highly anthropophagous behaviour and their longevity, coupled with high EIRs, indicate the necessity of introducing the vector control measures in the region as one of the strategies of fighting malaria. Such vector control needs to focus on providing an effective personal protection for the most susceptible age 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 ITN as these tools are currently the most effective and practical vector control option in areas where malaria is highly transmitted (Diallo *et al.* 1999; Guillet 2001).

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Authors

Caroline Ajonina, Tendongfor Nicholas and Theodore Tanke, University of Buea, Department of Life Sciences, P.O. Box 63, Buea, Cameroon. E-mail: ntendongfor@yahoo.com

Sali N. Atanga, Research Foundation in Tropical Diseases and Environment, P.O. Box 474, Buea, Cameroon. E-mail: atangandi@yahoo.com

Dr Didier Fontenille, Laboratoire IRD-ORSTOM, OCEAC, BP. 288, Yaoundé, Cameroon. E-mail: didier.fontenille@mpl.ird.fr

Dr. Samuel Wanji, Research Foundation in Tropical Diseases and Environment, P.O. Box 474, Buea-Cameroon. E-mail: swanji@yahoo.fr or refotde@yahoo.fr (corresponding author).