Contents lists available at ScienceDirect

Transactions of the Royal Society of Tropical Medicine and Hygiene

journal homepage: <http://www.elsevier.com/locate/trstmh>

Climate change and altitudinal structuring of malaria vectors in south-western Cameroon: their relation to malaria transmission

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ARTICLE INFO

Article history:

Received 28 July 2009

Received in revised form 11 February 2010

Accepted 11 February 2010

Available online xxx

Keywords:

Malaria vectors

Altitudinal structuring

Sporogonic cycle

Climate

South-western Cameroon

ABSTRACT

An entomological survey was conducted in Cameroon between October 2004 and September 2005, in nine localities targeted for malaria vector control based on adult productivity and variability. Mosquitoes were collected by human-landing catches (HLCs) and pyrethrum spray catches. A total of 12 500 anophelines were collected and dissected: *Anopheles gambiae* s.l. (56.86%), *An. funestus* s.l. (32.57%), *An. hancocki* (9.38%), and *An. nili* (1.18%). Applying PCR revealed that specimens of the *An. funestus* group were *An. funestus* s.s. and *An. gambiae* complex were mostly *An. melas* and *An. gambiae* s.s. of the M and S molecular forms with the M forms being the most predominant. The natural distribution patterns of *Anopheles* species were largely determined by altitude with some species having unique environmental tolerance limits. A human blood index (HBI) of 99.05% was recorded. Mean probability of daily survival of the malaria vectors was 0.92, with annual mean life expectancy of 21.9 days and the expectation of infective life was long with a mean of 7.4 days. The high survival rates suggest a high vector potential for the species. This information enhances the development of a more focused and informed vector control intervention.

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1. Introduction

Plantation agriculture is an important form of land-use in south-western, Cameroon. The area under plantation agriculture has expanded rapidly in past decades. They are often associated with the widespread modification or disturbance of the natural landscape through artificial practices such as the permanent removal of natural

vegetation, poor drainage systems, soil improvement and application of chemicals. It is worth noting that the presence and abundance of mosquito breeding sites associated with plantation agriculture often results in a corresponding increase of malaria vectors.¹ Ecological studies in many areas of Africa show that altitude and bioclimatic structure have an important impact on vector distribution.² Temperature, rainfall, relative humidity and altitude are the four major factors affecting the presence and abundance of anopheline mosquitoes in any given area.³

This stimulated interest at a time when the Malaria Eradication Programme was initiated by the World Health Organization. This paper therefore, describes a longitudinal entomological survey carried out with the objectives

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Figure 1. Map of the study area showing the main sites sampled and the main ecological barriers. The small rectangular boxes indicate the sampling localities. The dense continuous white spot indicates the primary forest around the Mountain. The small white plots of land indicate the remains of the primary forest after deforestation.

of establishing baseline entomological data for future control activities in the region and to evaluate the impact of altitude and crop plantation on malaria vector distribution.

2. Materials and methods

2.1. Study area

The study was conducted in nine localities as illustrated on the map (Figure 1). Each locality was visited twice every month throughout October 2004 to September 2005. The geographical coordinates, altitudes, demography and type of agricultural practises carried out in each locality is shown on Table 1. The area lies within the tropical rain forest region of Central Africa and harbours the largest agro-industrial scheme in the country and sub region, the Cameroon Development Corporation (CDC). These areas

vary greatly in socioeconomic status such that there are well constructed houses with piped water as well as poorly constructed houses with no piped water or occasional septic tank services especially in the camps. Plantation workers are housed in camps with few medical facilities at their disposal. More than 450 000 people live in these areas and the population is highly heterogeneous comprising people from almost every ethnic community in Cameroon, some parts of neighbouring Nigeria, Niger and Ghana. In all the study areas where the survey was done, the plantation workers stay inside their houses from approximately 21:00 h, and rarely use mosquito nets. Houses are concentrated in the same area and built of the same style, using planks or bricks for the walls and iron roofs with no ceilings and approximately two to five persons living per room. The homesteads are separated by large plantations which form the main agricultural activity of the people in these areas.

Table 1
Human population densities and the different type of agricultural practices carried out at different altitudes of the Mount Cameroon region.

Locality	Geographic coordinates	Altitude (m a.s.l.)	Population density	Agricultural practice and crop type
Bonakanda	09° 16' 21" E, 04° 12' 21" N	958	456	Intensive subsistence farming
Vasingi	09° 14' 12" E, 04° 09' 34" N	1100	1965	Intensive subsistence farming
Bokova	09° 27' 33" E, 04° 21' 14" N	903	314	Intensive subsistence farming
Tole	09° 14' 0" E, 04° 13' 0" N	800	28 179	Tea, Eucalyptus, Coffee, Plantain, Tobacco
Muyuka	09° 21' 20" E, 04° 12' 49" N	341	31 384	Rubber, Grapes, Orange, Coffee, Cocoa, Tobacco, Cassava, Sugar cane
Mutengene	09° 18' 29" E, 04° 05' 57" N	284	47 478	Oil palm, Oranges, Coffee, Maize, Plantain, Pineapples
Likomba	09° 18' 31" E, 04° 02' 43" N	118	27 595	Oil palm, Rubber, Mango, Cocoa, Coffee
Debundscha	08° 59' 0" E, 04° 07' 0" N	10	8 680	Oil palm, Mangrove, Fishing
Tiko	09° 21' 36" E, 04° 04' 30" N	0	55 914	Oil palm, Banana, Rubber, Coconut, Pepper, Mangrove, Fishing

Figure 2. Indoor and outdoor monthly variation in the density of anopheline vectors of human malaria (line) in relation to rainfall (bars) in the Mount Cameroon region.

2.2. Study design

Included in the survey were two types of entomological surveys: day collections and night captures. For the day collections, a team of three people estimated indoor resting density using the pyrethrum spray catch (PSC) method in 36 randomly chosen houses (three per visit) between 15:00 h and 17:00 h. This consisted of covering all exposed surfaces with white sheets, spraying the rooms and collecting all fallen specimens. Night captures were conducted at two houses in each month per locality, at least 40 m apart, between 18:00 h and 06:00 h with a personnel change at midnight. At each house, a collector was posted indoors and another outdoors with a flashlight and a mouth aspirator.⁴ Collection teams were rotated among blocks each month in an attempt to limit temporal and/or collector bias. Each month, prior to continuing surveys of non-sampled households, an attempt was made to inspect premises that were previously closed or where access had been refused. Access to these areas was attempted at least three times.

2.3. Estimation of physiological age

Anopheles mosquitoes captured on human volunteers were dissected for determination of their physiological age.^{5,6} The parous rate was determined by dividing the number of parous *Anopheles* by the total number dissected. The daily survival rate or the probability of a mosquito surviving one day was calculated.⁷ Based on the assumption that the anopheline population is relatively stable within

the area with an interval of two days between each blood meal, we calculated the life expectancy and the infective life expectancy of all the anopheline species.⁸

2.4. Laboratory processing of *Anopheles*

Mosquitoes collected were identified to species level using two main approaches: morphologically using the morphological key and the IRD software for the identification of *Anopheles* of the Afro-Tropical Region.^{9,10} Samples of *An. gambiae* complex and *An. funestus* group were stored individually in labelled tubes that contained desiccant and kept at -20 °C for further laboratory processing by PCR. Up to three PCR reactions were conducted for identification of one specimen. Sub-samples of *An. gambiae*¹¹ and *An. funestus*¹² from different sites were processed by the PCR technique, involving a combination of the protocols.

The origin of the blood meal was determined by enzyme-linked immunosorbent assay.¹³ The technique identified human, bovine, ovine, equine, pig, and chicken blood.

2.5. Statistical analysis

Variation in adult mosquito densities between villages and months was compared using one-way analysis of variance (ANOVA) using a general linear model (GLM) for all experiments.¹⁴ Where significant differences were observed in ANOVA, means were compared at the $P=0.05$ significance level using the Tukey's honestly significant difference (HSD) test. Cross correlation analysis was used to

Table 2
Distribution of malaria vectors and the molecular forms of *Anopheles gambiae* complex with respect to different altitudes.

Locality	Altitude (m a.s.l.)	Anopheles species						
		Anopheles gambiae s.l.						
		<i>An. funestus</i> s.s.	<i>An. hancocki</i>	<i>An. nili</i>	<i>An. gambiae</i> s.s.	<i>An. melas</i>	M form	S form
Vasingi	1100	-	-	-	-	-	-	-
Bonakanda	958	-	-	-	-	-	-	-
Bokova	903	-	-	-	2	0	-	2
Tole	800	1429	583	-	375	0	0	8
Muyuka	341	701	109	-	1302	0	5	18
Mutengene	284	1547	420	119	399	0	139	163
Likomba	118	256	54	8	747	0	41	0
Debundscha	10	-	-	-	2019	96	186	0
Tiko	0	138	7	21	1553	0	53	0
Total		4071	1173	148	6397	96	424	191

- Dashes indicate no data was collected.

assess the relationship between rainfall and Anopheline density at different altitudes. Statistical analyses was done using log transformed [$\log_{10}(n + 1)$] on anopheline counts to normalize the data.

3. Results

3.1. Species composition and abundance

A total of 15 109 mosquitoes belonging to four genera and eight species were collected over the one-year period. The genus *Anopheles* was more diverse with five species, followed by *Aedes* with two species, *Culex* and *Mansonia* with one species each. Among the anophelines, *An. gambiae* s.l. was the most predominant mosquito species (54.6%) both in the dry and rainy season in all samples collected followed by *An. funestus* (34.3%). Other anophelines included *An. hancocki* (9.9%), and *An. nili* (1.2%). The number and the relative proportion of each of the vector species strongly varied with rainfall both indoor and outdoor throughout the year (Figure 2). Species of malaria vectors differed significantly by locality ($\chi^2 = 152$, $df = 8$, $P < 0.001$). The number of anophelines captured in total from the palm plantations was 3.29-fold higher than in the banana, 2.7-fold higher than in the rubber and 2.4-fold higher than in the tea plantations. Significantly higher numbers of *An. gambiae* were observed in the palm plantations both indoors and outdoors than in either of the other agro-ecosystems (ANOVA, $F = 58.160$, $P < 0.001$). *An. gambiae* was proportionately more abundant throughout the survey than *An. funestus*, *An. hancocki* and *An. nili* from November to August and proportionately less in September and October. But it showed great variation and had a classic peak of abundance towards the rainy season (February, April and May). There was no significant difference in the abundance of *An. gambiae* and *An. funestus* in the months of June, July and August, which coincided with the peak rainy season. The differences in mosquito numbers were statistically significant for both *An. gambiae* ($t = 3.25$, $df = 8$, $P < 0.001$) and *An. funestus* ($t = 2.32$, $df = 8$, $P < 0.001$).

Comparing malaria vector abundance both in the tea and palm plantations, similar densities of *An. funestus* were

obtained, with higher densities observed in the month of August in the tea plantation and September in the palm, both coinciding with the peak rainy season. In contrast, *An. hancocki* abundance in the tea plantation was 83.29-fold higher than in the Tiko banana plantation. There was no significant site-to-site variation for *An. hancocki*. Other anophelines with site-to-site variation included *An. nili* mainly occurring at altitude lower than 284 m a.s.l., but with lower densities as the altitude decreased from the Likomba palm plantation at 118 m a.s.l. to the Tiko banana plantation.

There was a significant monthly variation in the densities of *An. funestus*, *An. gambiae*, *An. hancocki* and *An. nili* (ANOVA, $P < 0.001$). In Debundscha palm plantation, the density of *An. gambiae* was significantly correlated with the dry season when the temperatures were quite high with lower rainfall. The proportion of *An. funestus* and *An. nili* in any ecosystem where both species occur was strongly correlated with rainfall ($r^2 = 0.948$, $n = 5$, $P < 0.001$). A significant correlation was observed in the population densities of *An. funestus* and *An. gambiae* co-existing at different altitudes ($r^2 = -0.434$, $n = 7$, $P < 0.001$). Also, significant correlations were found with temperature and anopheline population densities ($P < 0.001$).

PCR identification within the *An. funestus* group revealed that all the specimens tested from all the localities ($n = 243$) were *An. funestus* s.s. Furthermore, 313 females of the *An. gambiae* complex identified by PCR-RLFP showed that *An. melas* was restricted only to the Debundscha palm plantations at an altitude of 10 m a.s.l. representing 3.5% of the total population of *An. gambiae* s.l. (Table 2). All other 302 specimens representing 96.5% were *An. gambiae* s.s. (100% of M molecular form). This also led to the identification of a mixture of the S and M molecular forms (54:46%) in Mutengene and Muyuka (78:22%) with transitional ecotypes, midway between the high and lowlands but the S molecular forms predominated in both areas. Despite the coexistence of the two molecular forms, no hybrid was recorded implying a completion of the speciation. At altitude above 800 m a.s.l. (Tole and Bokova) considered as the highlands of Mount Cameroon, *An. gambiae* s.s. (100% of S molecular forms) was the sole member of the *An. gambiae* complex.

Figure 3. Indoor and outdoor night biting cycle (number of bites per human per hour) for each vector species in the Mount Cameroon region.

3.2. Biting behaviour

There were considerable differences in biting behaviour among members of the anophelines. *An. gambiae* s.s., *An. funestus*, *An. hancocki* and *An. nili* were highly anthropophilic, feeding preferentially on humans. When capturing anophelines, both indoors and outdoors during the same night, it was observed that 60.6% of *An. gambiae*, 54.4% of *An. hancocki*, 67.6% of *An. nili* and 46.2% of *An. funestus* were captured outdoors. There was a significant difference between the indoor feeding behaviour of different anopheline vectors ($\chi^2 = 4.72$, $df = 3$, $P = 0.001$).

In general *An. gambiae* s.s. has, on account of its high anthropophily, developed a strong tendency for exophagy in all the localities except in the Tole tea plantation (60.5% endophagy). The more endophilic *An. funestus*, was the most frequent species among the endophilic vectors captured, but it tends to be more exophagic both in the Muyuka rubber and Tole tea plantations with 56.6% and 65.8% respectively. *An. hancocki* was more exophagic than *An. funestus* and *An. gambiae*. *An. nili* represented only 0.9% of the endophilic vectors captured. Overall, there was a significant variation in the biting behaviour at an altitude of 800 m and altitudes below 300 m ($P < 0.001$).

3.3. Feeding preference

A total of 210 blood meal spots were tested by ELISA for host identification. These were collected from both indoor and outdoor resting females, including 63 *An. funestus*, 112 *An. gambiae*, 31 *An. hancocki* and four *An. nili*. All specimens had fed on human hosts, except two *An. gambiae* females who had taken mixed blood meals (chicken blood). Both specimens were outdoor resting

females. The effect of species human blood-feeding rates for mosquitoes collected at different altitudes was significant ($df = 3$, $P < 0.001$), with the rate for *An. gambiae* s.l. significantly higher than for *An. funestus*.

3.4. Biting cycles

All the anopheline captured both indoors and outdoors were nocturnal feeders, with most of the blood-feeding occurring toward the second part of the night and did not vary during the year followed by a sharp decline from 03:00 h to 06:00 h (Figure 3). In total, 57.5% ($n = 2341$) of the bites of *An. funestus*, 54.2% ($n = 3520$) of *An. gambiae*, 39.5% ($n = 463$) of *An. hancocki*, and 37.8% ($n = 56$) of *An. nili* occurred after midnight. The peak indoor-biting activity of *An. gambiae* s.l. and *An. funestus* was between 01:00 h and 02:00 h. Contrarily, *An. hancocki* showed a peak indoor-biting behaviour during the first part of the night between 22:00 h and 23:00 h. For *An. nili* there was a consistent indoor and outdoor-biting behaviour with peaks between 21:00 h and 02:00 h.

3.5. Parity, daily survival rate, life expectancy and expectation of infective life

In total, 11 885 anophelines were classified either as parous or nulliparous. The parous rates were as follows: *An. gambiae* 76%, *An. funestus* 83.5%, *An. hancocki* 79.7% and *An. nili* 86.5%. Overall, more than 79% of the anophelines caught in the region were parous, however a significant difference was observed between the parous rates of anophelines caught in different agro-ecosystems ($\chi^2 = 258.9$, $df = 8$, $P = 0.001$). Also, the parous rate among the *Anopheles* species caught at lower altitudes was observed to be significantly

higher than those caught at higher altitudes ($X^2 = 32.27$, $P < 0.001$). The probability of the malaria vectors surviving one day was very high with *An. funestus*, *An. gambiae*, *An. hancocki* and *An. nili* recording 0.91, 0.87, 0.89 and 0.93 respectively. The probability for *An. gambiae* s.l., *An. funestus* s.s., *An. hancocki* and *An. nili* to survive long enough to constitute a potential infection source was estimated at 3.7%, 11.5%, 6.6% and 17.5% respectively. The expectation of infective life of *An. gambiae* s.l., *An. funestus* s.s., *An. hancocki* and *An. nili* was 9.5, 4.1, 6.2 and 15.3 days respectively. Overall 53.86%, 38.94%, 48.4% and 59.9% of *An. funestus*, *An. gambiae*, *An. hancocki* and *An. nili* respectively would be expected to live the 10 days necessary for the *Plasmodium falciparum* sporozoites to be formed. Those surviving the 10 days would have a further life expectancy of 24.4, 16.5, 19.8 and 31.7 days respectively.

3.6. Climatological data

In this forested area of southern Cameroon, monthly mean maximum temperatures were significantly associated with the different agro-ecosystems, but minimum temperature was not significantly associated with the localities. The climate suitability zone that described the range of *An. gambiae* s.s. lay with total annual precipitation of 230–9224 mm, maximum annual temperature range of 25–31 °C, minimum annual temperature range of 18–20 °C, mean maximum temperature during the wet season 25–30 °C and mean minimum temperature 18–24 °C. For *An. funestus* the climate suitability zone was defined as lying within areas with total annual precipitation of 230–2817 mm, maximum annual temperature of 25–29 °C, minimum annual temperature of 20–23 °C, mean maximum temperature during the wet season 21–28 °C and mean minimum temperature 18–22 °C. Maximum rainfall in this area was usually recorded in the months of August and September. An overall mean maximum annual relative humidity of 88% was recorded.

4. Discussion

There was a strong positive correlation between the abundance of the *Anopheles* species and the maximum, mean and minimum temperatures, whereas, for *An. hancocki*, the correlation was significant (positive) only for minimum temperature. These results suggest that temperature has a marked effect on mosquito abundance.¹⁵ The distribution of the *Anopheles* species is highly erratic within their distribution range, probably due to local environmental factors such as precipitation and temperature, and maybe physical factors such as altitudinal barriers and habitat availability, and biotic factors such as abundance of vertebrate hosts.

An. funestus s.s. is the only member of the *An. funestus* group identified in the Mount Cameroon region but they were not collected in Debundscha, the rainiest district in Cameroon and Africa in general. *An. funestus* was the most predominant species in the highlands and would play a major role in malaria transmission in this ecotype. Both recently described M and S molecular forms of *An.*

gambiae s.s. were represented in our sample. The results of molecular study shows that both M and S forms of *An. gambiae* s.s. are sympatric and colonize the ecological zones midway between the high and the lowlands of the Mount Cameroon region. The M forms largely restricted to the coastal sea lines while the S forms are largely restricted to the highlands. Difference in ecological distribution between molecular forms of *An. gambiae* s.s. was found, with Debundscha samples consisting exclusively of M forms. No 'hybrid' molecular patterns were detected. These findings add more information to the available data on the distribution and relative occurrence of each form, making available baseline information for a thorough assessment of the biological and epidemiological consequences of this genetic subdivision. *An. melas* and *An. gambiae* s.s. are sympatric in the coastal region, and when *An. melas* is predominant, endophagy, endophily and anthropophily are very marked. This allows for a better understanding of their contribution to malaria transmission in mangrove ecosystems.¹⁷

All the *Anopheles* species are highly anthropophilic, feeding preferentially on humans and on account of their high anthropophily, have developed a strong tendency for endophagy and exophagy as well as endophily and exophily. These biological variations indicate that they are more efficient malaria vectors with a capacity to sustain malaria transmission at lower vector population densities. The feeding behaviour of all the malaria vectors varied along their geographic distribution, partly because of the high degree of human population heterogeneity and land use. The significant degree of exophagic biting behaviour observed for the species may be partly as a result of the location of the villages along the different altitudes. Dwellings have incomplete walls and the habits of the human population facilitate human-vector contact and enhanced exophagy of mosquitoes. These species showed a marked night-time biting behaviour both indoors and outdoors, with intense activity taking place towards and after midnight and decreasing gradually until dawn when the population immediately disappear to their shelters. The readiness of *Anopheles* species to feed at a particular time of the night is probably due to environmental conditions, which might have influenced the temperature and high relative humidity. The resulting outdoor feeding behaviour of the *Anopheles* species increases their chance of becoming infected and re-infected with the malaria parasite¹⁸ and therefore the likelihood of having high infection rates.

The variability of the biting pattern of *Anopheles* species along their geographical distribution was very stable with species showing unimodal, bimodal or trimodal biting peaks at different altitudes which suggests that the biting pattern of the species represents their adaptation to anthropophagy. This has important implications for control as insecticide treated nets will reduce human–vector contact for *An. gambiae* s.l. and *An. funestus* s.s., whereas for *An. hancocki* and *An. nili* alternative methods of personal protection, such as the use of repellents, are recommended, although the latter measure might increase the risk of malaria by diverting mosquitoes to non-users.¹⁹

The mean parous rates for all the anopheline species are high, as none was less than 70%. This indicates that a

large proportion of female mosquitoes in the study sites had already practiced haematophagy.²⁰ Such high parous rates of anophelines has been associated with the non-application of control measures and the availability of potential breeding sites close to human residences.²¹ The probability of daily survival of the mosquitoes remained very high throughout the year, suggesting that the vectors are well adapted to the environmental conditions in the Mount Cameroon region. The results of this study indicate that anopheline mosquitoes are long-lived, with mean annual life expectancy ranging between 16.5 and 27.7 days. This observation is of considerable importance with respect to vector efficiency to transmit malaria parasites as the high longevity of adult female mosquitoes allows for increased opportunities to encounter an infected human host, the malaria parasites to develop, and to transmit the parasites in later blood meals to previously uninfected hosts.²⁰ The mean annual probability of anopheline mosquitoes surviving the sporogonic cycle was low, ranging from 0.25 to 0.48. This is probably because only a relatively small proportion of anopheline populations live long enough for the *Plasmodium* sporogonic cycle to be completed. Estimates of daily survivorship of the total *Anopheles* species in the Mount Cameroon region ranges from 0.87 to 0.93, which indicates that at the end of each day at least 87 to 93%, will have survived. Assuming this survivorship is constant throughout the adult life of the anopheline mosquitoes, over 60% of female anophelines would survive longer than a 14-day extrinsic incubation period. The ability of the vectors to bite indoors was observed to increase with increased altitude and vice versa. Transmission could occur at higher altitudes because the adult mosquitoes rest more indoors, where temperatures are 3–5 °C higher than outside.²² Therefore control measures that rely on indoor residual spraying may actually impact malaria transmission more through their effect on adult longevity than through their effect on the population of adult mosquitoes.

Currently, this study provides ample evidence to indicate that *Anopheles* species are able to adapt at different altitudes where the primary forests have been replaced completely by large plantations of banana, tea, coffee, sugar cane and rubber. Significant differences in host-feeding patterns do exist in sympatric *Anopheles* species and host preference have shown that these mosquitoes will feed on humans at different altitudes even when other hosts are available. Thus, our conclusion is that all the *Anopheles* species are the most likely human malaria vectors in the study areas based on their seasonal distribution, frequency, endophilic and anthropophilic behaviour. Altitude has a great influence on micro-climate parameters and consequent variation on malaria vector structure. Additional studies are needed to assess the importance of each vector species as a biting nuisance and their potential to transmit malaria because not all the species are involved equally in the transmission of malaria to humans at different altitudes. However, because they are simultaneously involved in malaria transmission this poses a high risk for all inhabitants, indigenous workers as well as non-immune workers. The occurrence of these most efficient vectors of malaria warrants the implementation of energetic and long-lasting malaria control measures.

Authors' contributions: MCT participated in the design of the study, conducted the field work, analysed and interpreted the data and drafted the manuscript. WIN, JM and NJ were involved in designing and implementing the study and writing of the manuscript. NT assisted in the data analysis and writing of the manuscript. FS helped with molecular analysis and interpretation of results. WS provided financial support and participated in the conception and design of the study and approval the final version of the manuscript to be published. MCT and SW are guarantors of the paper.

Acknowledgments: The authors thank the Department of Human Resources and Health of the Cameroon Development Corporation (CDC) for granting permission to carry out these studies in their estates. Special thanks to Dr John Githure for his critical comments on the manuscript. We thank the mosquito collection volunteers for their support in mosquito sampling and collection. We appreciate the constant participation of the residents of the nine areas.

Funding: None.

Conflicts of interest: SW is the Director of the Research Foundation of Tropical Diseases and Environment (REFOTDE), Cameroon. NT is employed as Assistant Director of the Research Foundation of Tropical Diseases and Environment. JM, WIN, NJ and MCT declare that they have no competing interests.

Ethical approval: Permission to carry out this study was obtained from the Human Resources and Health departments of the Cameroon Development Corporation (CDC), and ethical clearance was properly sought from the National Ethical Review Committee of Cameroon but was deemed unnecessary. A sensitization rally was organized with the population during which the purpose of the study was clearly explained. Participation in the study was voluntary.

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