Current knowledge of malaria transmission in Cameroon: The vectors and their efficiency

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Abstract

The malaria vectorial system in Cameroon is highly complex with two or more vectors involved in malaria transmission in almost all the bio-ecological zones. This paper focuses on the behavioural and genetic differences observed among populations within each malaria vector species. The entomological inoculation rate (EIR) profiles indicate that the risk of exposure to infected mosquito is highly heterogeneous and seasonal. This paper also emphasises the need to generate spatial and temporal data on transmission intensity on smaller scales to guide targeted control of malaria operations in the country. Furthermore, EIR estimates in the present review provide means of quantifying levels of exposures to infected mosquitoes in different regions of the country and could be important in evaluating the efficacy of vector control measures.

Introduction

In Cameroon, as in many other countries of the Central Africa region, malaria continues to be a major cause of morbidity and mortality despite enormous investment efforts. Fontenille and Louchouarn (1999) have stressed that the percularity of malaria problem in Africa South of the Sahara is attributable to the mosquito vectors of tropical Africa, which probably constitute the most powerful vectorial system anywhere available to malaria parasites. This system, which generally causes high inoculation rates, is also remarkably stable in a wide range of geographical and ecological conditions and it appears to be very flexible, both in exploiting new man-made environments and in its response to malaria control activities.

In most parts of Africa, several vectors transmit malaria in each locality, in some cases at the same time and in other cases during different seasons (Fontenille et al. 1997). In central Africa, it is not rare to capture more than four different vectors during the same night (*An. funestus, An. gambiae, An. nili, An. hancocki* and *An. moucheti.* Therefore, there is an enormous heterogeneity in malaria transmission intensity in affected areas of Africa, and implementation of targeted malaria control operations would require an understanding of the forces that drive transmission.

Within the *Anopheles* species are a group of closely related and morphologically indistinguishable species of which two or more co-exist in many areas (Coetzee *et al.* 2000). Because individual's species within the species complexes differ in host-biting preference, abundance and vector competence, identification of the mosquito vectors to species level and mapping

species distribution in heterogeneous environments are critical. The assessment of these indices would give a snapshot of the potential risk of infection and elucidate the patterns of disease transmission (Githeko *et al.* 1993).

Cameroon is characterized by several bio-geographical zones and several studies have compared transmission among different bio-ecological and even among villages within the same area, and have reported significant variations in transmission intensity among villages separated only by short distances and even between rural and urban settings within the same locality (Wanji *et al.*, 2003, Fontenille & Louchouarn, 1999). An overview of the magnitude of these spatial variations is critical to comprehend the transmission dynamics of the disease. In this paper we focus on *An. gambiae, An. funestus, An. moucheti* and the frequently overlooked *An. nili* and *An. hancocki*. These mosquitoes are abundant in some areas of high human population densities, and are responsible for malaria transmission to million of people in the country.

Anopheles gambiae

Three members of the *An. gambiae s.l.* complex are found in Cameroon: *An. melas* colonises mangrove swamps along the Atlantic coast, South West of the country, *An. arabiensis* extends from the dry savanna in the North (Southern border of Lake Chad) down to the evergreen forest edge and *An. gambiae s.s.* is widespread throughout the country (Simard *et al.*, 1999). these species, *An. gambiae* is virtually everywhere in Cameroon and transmits malaria to humans in humid, forested environment in the South as well as in dry savannas and rice fields in the north; in rural, semi-urban and urban settings and at low or high altitudes. This complex is replete with chromosomal inversion polymorphism within species and aspects of inversion polymorphism in the *An. gambiae* complex have been reviewed by very few investigations. Three of the five recognized chromosomal form of *An. gambiae* have been observed. All specimens collected South of the country and in the rain forest domain with < 1500 mm rainfall per year, belonged to the Forest chromosomal form, with standard (2R+, 2L+) chromosomes and/or very low frequency of 2Rb inversion (Simard *et al.*, 1999).

A rapid rDNA polymerase chain reaction (PCR) assay as described by Scott *et al.* (1993) has allowed for the accurate identification of members within the *An. gambiae* complex. The *An. gambiae* s.s., are further identified to the molecular forms M and S according to Favia *et al.* (2001). Most studies focus on *An. gambiae*, because it is a widely spread mosquito population, and relatively easy to colonize. A lot of attention has been given to the genetic studies of *An. gambiae* and a lot is still in progress. Cohuet *et al.* (2004) reports that all the specimens of *An. gambiae* complex from the Savannah-forest transition areas are mostly *An. gambiae* sensu stricto of the S molecular. Recently, our team in Buea, in collaboration with OCEAC have also reported similarly findings in the Mount Cameroon region. These results show that members of the *An. gambiae* complex were

An. gambiae s.s., *An. gambiae* S and M forms and *An. melas.* It is interesting to note that only the M form of *An. gambiae* is present in the coastal region of Debundscha (50m above sea level), whereas the S and M forms co-exist in Mutengene (204m a.s.l). The study of the association of these inversions and their frequencies confirms that *An. gambiae* s.s.,includes two populations showing partial reproductive isolation.

An. arabiensis is the most anthropophilic member of the *An. gambiae* complex and occurs in most parts of tropical Africa. *An. arabiensis* is restricted to the northern part of Cameroon where rice fields are cultivated twice every year. It is more adapted to rice growing fields. It is not only important; it is an efficient vector of malaria especially where transmission is unstable. Its implication in malaria transmission, suggests that its control would have a major impact on reducing the disease in the community. Studies show that *An. arabiensis* (sibling species of the *An. gambiae* complex) is present throughout the year with an average of 32 bites per person per night. The entomological inoculation rate is estimated at 0.82 infective bites per person per night from mid June to mid September (Robert *et al.,* 1992). Thus rice fields seem to promote the development of *An. arabiensis,* a major vector of malaria and therefore rice cultivation can be considered in this area as a 'source of life and death' (Laventure *et al.,* 1996).

On the other hand, Etang *et al.* (2003) carried out a large-scale survey of *An. gambiae* Giles susceptibility to insecticides and discovered that dieldrin, DDT, Permethrin and deltamelthrin resistance was present in some mosquito populations. Within the *An. gambiae* complex, resistant individuals belonged to *An. gambiae* s.s., and *An. arabiensis.* Both M and S molecular forms of *An. gambiae* s.s., are also resistant to insecticide treatment. None of the surviving mosquitoes was positive for the Kdr "Leu-Phe" mutation using a PCR diagnostic assay. These results suggest involvement of other resistant mechanism(s).

In the Mount Cameroon region, *An. gambiae s.s.,* is observed throughout the year and is the most aggressive species at both seasons with 3 bites per person per night in the rainy season and 5.53 bites per person in the dry season. The human blood index (HBI) was 98.2 % for fed *Anopheles. Anopheles gambiae* displayed heterogeneity in its sporozoites rates for the rainy and dry season. It is more infected during the rainy season (14 %). An individual of this region will receive an infective bite from *An. gambiae s.s.,* after every third night during the rainy season (i.e. 0.45 infective bite/person/night).

In the Savannah-forest region, *An. gambiaes* is the only anopheline species present with a daily biting rate of 2.4 bites per person per night. The sporozoitic index of *An. gambiae* is 9.4 %. The entomological inoculation rate is estimated at 0.225 infective bites per person per night (i.e. 82 infective bites per person per year). Malaria transmission is low during the year with a remarkable increase in September, when the rainfall is at its peak.

In the urban areas of Yaounde, the transmission level from *Anopheles* to man revealed a calculated entomological inoculation rate of 34 infective bites per person per year (Van der Kolk *et al.*, 2003).

In villages near Sanaga River, inhabitants of this area received about 67 infective bites per year from *An. gambiae* (Carnevale *et al.*, 1992).

Anopheles funestus

Anopheles funestus is present all over the country and it is very heterogeneous. It is known since the 1930s that this group is composed of several species closely related to each other, which can only be differentiated by minor characteristics of their larvae or adults. Koekemoer *et al.* (2002) have identified five members of the *An. funestus* group: *An. funestus, An. leesoni, An. parensi* (species of the subgroup *An. funestus*) and *An. vaneedeni*.The fifth species identified was named *An. rivulorum*-like. Cohuet *et al.* (2004), reports that all the specimens of *An. funestus* group from the Savannah-forest transition were *An. funestus* sensu stricto. Human *Plasmodia* has only been found in *An. funestus*, which is an excellent vector having a high vectorial capacity. Cohuet *et al.* (2005) described the population genetic structure of *An. funestus* in Cameroon. Microsatellite markers revealed no particular departure from panmixia within each local population. Cytogenetic studies demonstrated high levels of chromosomal heterogeneity both within and between populations. Strong deficiency of heterokaryotypes was found, indicating a subdivision of *An. funestus* in chromosomal forms.
In total more than 60 % of the bites of *An. funestus* occurred after midnight when capturing mosquitoes, both indoors and outdoors, during the same night. It is also important to know that more than 50 % of *An. funestus* were captured indoors, suggesting these anopheline populations are mainly endophagic.

In the Savannah-forest area, *An. funestus* is highly anthropophilic recording a human blood index of 99.3 %. It is responsible for 88 % of the total malaria transmission, with a *Plasmodium falciparum* circumsporozoite rate of 6.8 %.

Despite the great vectorial importance of this species, little attention has been devoted to *An. funestus* to clearly understand its dynamics. Complementary studies are absolutely necessary on this species. The clear characterization of members of the *An. funestus* group will be of great importance in the fight against malaria transmission. Other species of the *An. funestus* group after intensive studies have been found to be non-vectors or vectors of malaria to a much lesser extend: *An. parensis, An. rivolurum,* or *An. vaneedeni.* In some high agricultural activity areas, *An. funestus* can be, by far, the major malaria vector.

Anopheles nili

Kengne and others have recently developed a species-specific PCR assay, which enables the rapid identification of three members of the *An. nili* group based on the sequence analysis of portions of the rDNA: *An. nili*

typical form, *An. nili oveng* form and *An. carnevalei*. Awono-Ambene *et al.* (2004) later unravelled the fact that "Oveng form" is genetically distinct from the other members of the *An. nili* group and so named it, *An. ovengensis*, after its geographical origin. *An. nili* breeds along riversides (Carnevale *et al.* 1992). The observation of live *Plasmodium falciparum* sporozoites in *An. ovengensis* mosquitoes suggests its probable role in human malaria transmission, at least in villages located near rivers (Awono-Ambene *et al.* 2004). They are very efficient malaria vectors, in villages along the Sanaga River, South of Cameroon. *An. nili* was always present throughout the year and recorded an average number of 23.9 ± 9.1 bites per person per night with a *maximum* of 42 bites per person in September and a minimum of 11 bites per person in November. Thus the annual biting rate of *An. nili* was about 8754 bites per person. It was therefore responsible for perennial transmission of malaria in these villages along the river and the total inoculation rate due to this species reached 105 infective bites per person per night.

Anopheles moucheti

Morphological and behavioural differences among populations suggest that several species are present in the *An. moucheti* group: *An. moucheti moucheti* sensu stricto, *An. moucheti nigeriensis* and *An. moucheti bervoetsi* (Antonio-Nkondjio *et al.* 2002). Using allozyme markers, morphological and/or geographical populations of *An. moucheti* were genetically differentiated. Brunhes *et al.* (1998), in his report considered that *An. moucheti moucheti* and *An. moucheti nigeriensis* were synonymous and that *An. moucheti bervoetsi* is a sub-species (geographical population) of *An. moucheti*.

Members belonging to the *An. moucheti* group are equatorial forest mosquitoes. This mosquito has also been reported in Namibia (De Meillon, 1951). This mosquito is an efficient vector of *Plasmodium* and it is quite often the major vector of malaria in forest regions and sometimes the only vector. In areas where this species is alone an annual entomological rate of 322 infective per person per year can be recorded (Njan Nloga *et al.*, 1993). The population of *An. moucheti* in the forest zone are highly anthropophilic.

Anopheles hancocki

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 (Vancel & 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 malaria transmission. Hence, it should be considered as an important malaria vector. More research is required on the bionomic, behaviour and the taxonomic status of this group of *Anopheles* species.

Conclusions

In conclusion, we would emphasize that the malaria vectorial system in Cameroon is very complex and any vector control measure should take

into account the details of this heterogeneity, especially on the population genetic structure and the gene flow among and within the vector populations.

It is important to note that *An. gambiae* is not the only efficient vector of transmission, so special attention should also be given to other vectors especially with the emergence of new molecular biology techniques. Since knock down resistance (Kdr) is widespread at a high frequency, it would be interesting to use them as markers to investigate the degree of genetic isolation between different sibling species of *An. gambiae* s.s., which are sympatric in the areas. Research on *An. gambiae* s.l., resistance should be promoted in Cameroon, to improve malaria vector control programs and to implement resistance management strategies.

The level of transmission remains very high for villages located in the suburbs of cities. Transmission is maintained year round in urban areas despite the low vector densities, because high rate of human feeding facilitates direct contact with gametocyte carriers and efficient malaria parasite transmission by vectors population (Mbogo *et al.,* 1993). These comparisons show the stability of anopheline populations in villages where no vector control operations have been carried out. Such a high level of biting and inoculation justifies a vector control programme. In Cameroon, because plasmodia are transmitted at a high rate and continuously throughout the year by one or more vectors species, implementation of an efficient vector control strategy can not be easily attainable, despite the high anthropophilic and endophilic nature of the vectors.

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