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Blood feeding pattern and seasonal distribution of dengue mosquito, *Aedes aegypti* (Diptera: Culicidae) in Southern Benin

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Abstract

To assess the seasonal distribution of *Ae. aegypti* and its blood feeding pattern in Cotonou, southern Benin, a cross seasonal entomological study on adults of *Ae. aegypti* was carried out from May 2013 to April 2014 in southern Benin. *Ae. aegypti* were collected by Human Landing Catches (HLC), Indoor Pyrethrum Spray Catches (PSC), Biogents (BG) sentinel trap and Gravid traps (GT) in order to evaluate the seasonal distribution of *Ae. Aegypti* in southern Benin. Moreover, blood-meal sources were searched from mosquitoes collected using Polymerase Chain Reaction (PCR). During the year of study, a total number of 18,658 mosquitoes were collected. 15,204 were sampled by HLC; 303 with BG trap, 3,038 with PSC, 48 with the GT. From 13,834 females, 1,380 were blood-fed. Adult's collection was high during the two rainy seasons (June to July and October to November) but declined in the two dry seasons (December to March and August to September). The average of Human Biting Rates (HBR) obtained during the rainy seasons (79.6 bites/ person/ night) was significantly higher than those obtained during the dry seasons (58.62 bites/p/n) ($p < 0.05$). Identification of blood-meal sources indicated humans as the primary host. These findings showed the presence of *Ae. aegypti* year round at Cotonou in southern Benin. Data on blood feeding patterns of *Ae. aegypti* collected during this study showed that the most important host are humans. These results provide valuable information about potential dengue virus transmission cycles and will help to get a greater understanding about DENV ecology in Benin.

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Introduction

The incidence and distribution of dengue-related illness has grown dramatically in recent decades (Bhatt *et al.*, 2013).With nearly 400 million infections each year, dengue virus (DENV) is the world's most important mosquito-borne virus (mobovirus) (Bhatt *et al.,* 2013). DENV is mainly transmitted by the vector mosquito *Aedes aegypti* which belongs to the family *Culicidae* and order Diptera.

Ae. aegypti mosquitoes are the most important vector of urban DENV and yellow fever virus (Brown *et al.*, 2011). A sylvatic DENV is maintained in non-human primates and forest-dwelling mosquitoes and is known to occur in Africa (Hanley *et al.*, 2013). *Aedes* spp are widely distributed in Africa. When their distribution is combined with rapid population growth, unplanned urbanization, and increased international travel, extensive transmission of DENV is likely in Africa (Gubler, 1997). DENV infection is caused by one of four DENV serotypes (DENV 1, DENV 2, DENV 3 and DENV 4). All four DENV serotypes have been also isolated in Africa (Amarasinghe *et al.,* 2011).

The knowledge about epidemiology of DENV epidemiology and its impact on public health in Africa remains is poor. Because of low awareness by health care providers and lack of diagnostic testing and systematic surveillance, the impact of DENV for human health in Africa has not been defined.

Environmental factors including weather variables may play a significant role in the transmission of DENV (Bi *et al.*, 2007; Wongkoon *et al.*, 2013). Temperature, rainfall, and relative humidity are major parameters influencing the incidence of dengue fever (Thammapalo *et al.*, 2005).

Recently, outbreaks of dengue fever have been reported in many African countries. In fact, 696 suspected cases were recorded in Senegal with 196 confirmed in 2009 (Sylla *et al.*, 2009). In Cape Verde, an estimated 210,000 clinical cases were documented of which 174 fitted the WHO definition of severe dengue fever including 6 fatalities (Sylla *et al.*, 2009). The re-emergence of dengue fever has been observed also in Mauritius (Issack *et al.*, 2010), in Cameroon (Peyrefitte *et al.*, 2006), and in Senegal (Sylla *et al.*, 2009). However, in Benin, a country of West Africa, most available data on dengue fever and *Ae. aegypti* date back more than 30-40 years despite the presence of this mosquito throughout the year (Yadouleton *et al.*, 2014).

In order to implement effective and sustainable arbovirus vector control measures in southern Benin, particularly at Cotonou, the economic capital, there is an urgent need to determine the prevalence and seasonal distribution of *Ae. aegypti.*

Materials and methods

Study sites

The study was carried out in southern Benin at Cotonou, the economic capital, from Mai 2013 to April 2014. Southern Benin is characterized by two rainy seasons (April- July and October- November) and two dry seasons (December-March and August-September).

The choice of the study area is based on the weak level of urbanization (Figure 1). A lot of undeveloped land lies in between the properties and standing water gives good breeding sites for mosquito larvae. Additionally, a lot of second hand tires from Europe and Asia, which are good breeding sites for *Ae. aegypti,* are stored and sold in this area.

Mosquito sampling.

Collections of adult mosquitoes were carried out monthly from May 2013 to April 2014. Adults collection were organized in households where old tired from Asia and Europe are stored. These mosquitoes were collected using following sampling methods:

First, Indoor and outdoor Human Landing Catches (HLC) were performed by four collectors monthly over two consecutive days (11:00 AM to 6:00 PM), in 4 randomly selected sites in the Dandji area.

Fig. 1. Map of Benin and Cotonou showing the investigated area Dandji.

Second, Indoor Pyrethrum Spray Catches (PSC) in 4 other selected compounds; the same compounds in each sampling method being consistently used throughout the study.

Collectors gave prior informed consent and received anti-malaria prophylaxis and yellow fever immunization. They were organized in teams of two for each collection point and they rotated between locations within houses every two hours.

In addition to the population collected by HLC and PSC, two traps of Biogents (BG) sentinel trap and Gravid traps (GT) were used twice a week for adults of *Ae. aegypti* collection.

All mosquitoes were transported to the laboratory, and identified on chill tables according to species and sex using morphological characteristics (Edwards, 1941; Rueda, 2004)

Entomological parameters

Entomological indicators of DENV transmission intensity at the study area were evaluated:

First, the mosquito fauna composition, which is the number of mosquitoes collected during the cross seasonal entomological study from Mai 2013-April 2014.

Second, the human biting rate (HBR), which is the number of mosquitoes biting a person during a given time period (bites/p/t) (time being night, month or year) (rewrite; identical with the previous publication).

Identification of blood-meal sources

All mosquitoes with fresh or visible blood remnants were sorted out and 114 blood fed mosquitoes were chosen for blood meal identification according to the trapping month. DNA was extracted from the abdomens using the Invitek Pathogen Kit (Invitek, Berlin, Germany).

Isolated DNA serves as DNA templates in subsequent PCR reaction. PCR primers are based on vertebrate cytochrome B gene (Leisnham and Juliano, 2012). PCR reactions were performed with the HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA). Amplicons were sequenced by Sanger sequencing and sequences were analysed by several programs and compared with sequences from reference databases (GenBank etc.) to identify the blood meal source.

Ethical considerations

Ethical approval for this study was granted by the Ethical Committee of the Ministry of Health in Benin. Verbal consent was asked to the head of each household for the spray catches and consent of collectors was obtained prior to HLC. In case of refusal, permission was sought from the next household.

Results

Mosquito fauna composition and physiology status of samples mosquitoes

A total number of 18,593 mosquitoes were collected from Mai 2013 to April 2014. Among these populations of mosquitoes, 15,204 were collected by HLC; 303 from BG, 3,038 from PSC and 48 from the GT traps (Figure. 2). The mosquito populations collected by HLC was significantly higher than those collected from others methods of catching (P<0.05) (Figure. 2). Among the 18,593 of *Ae. aegypti* collected, female populations (13,834) was significantly higher than male populations (p<0.05). From the 13,593 *Ae. aegypti* collected, 1,334 females were blood fed.

Table 1. Composition of adult mosquito collection.

Methods of mosquitoes collection	nb. of *Ae. aepypti* (female/male)	nb of blood fed mosquitoes (%)	total nb
HLC	12739/2465	772	15204
PSC	819/2219	599	3038
BG	228/75	9	303
GT	48/0	0	48
Total	13834/ 4759	1380	18593

Seasonal abundance of Ae. aegypti population at Dandji

Adult's collection (Table 1) was high during the two rainy seasons (June to July and October to November,) but declined in the two dry seasons (December to March and August to September) (Figure. 2).

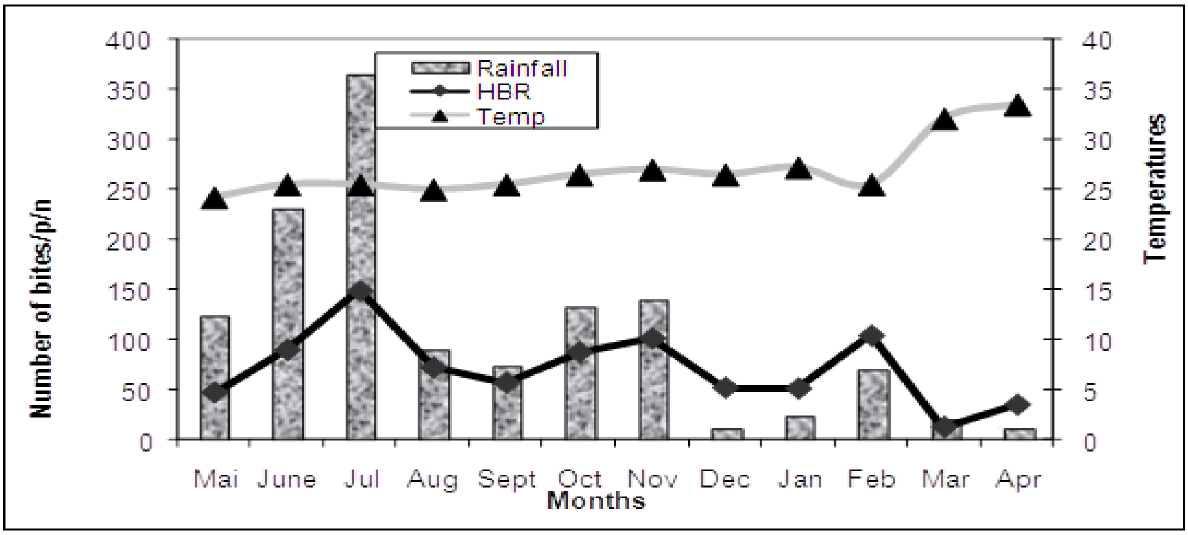

Fig. 2. Seasonal variation of the number of human bites/person/night, temperature and rainfall at Dandji from Mai 2013 to April 2014.

In fact, the highest Human Biting Rates (HBR) of *Ae. aegypti* was found during the rainy seasons in July (149.69 bites/p/n) and the lowest during the dry seasons in March (12.89 bites/p/n) (Figure. 3). At Dandji, from Mai 2013 to April 2014, the average HBR obtained during the rainy seasons (79.6 bites/ p/n) was significantly higher than those obtained during the dry seasons (58.62 bites/p/n) (p< 0.05).

Blood feeding pattern of Ae. aegypti

A total of 114 *Ae. aegypti* were analyzed for the source of the blood meal (Figure. 3). For *Ae. aegypti* four different hosts were identified: human (94.7%), sheep (1.8%), cattle (2.6%) and the Maxwell's duiker (0.9%) a small antelope.

Discussion

Adult's collection was high during the two rainy seasons (June to July and October to November) but declined in the two dry seasons (December to March and August to September). However, the increased number of *Ae. aegypti* captured in February 2014 (Figure. 2) can be explained by the exeptionell increase of rainfall during this period of dry season; certainly an effect of climate change (Leisnham and Juliano, 2012).

With HLC the highest numbers of mosquitoes could be caught followed by PSC. The sampling with BG sentinel and gravid traps were less effective due to problems with electricity, destruction and robbery. Several factors can explain the spatial distribution of *Ae. aegypti* in southern Benin. Among theses, anthropological factors play an important role. Results from our study showed that most *Ae. aegypti* were collected in areas were second hand tires from Europe and Asia are stored and sold. These tires were the most productive *Ae. aegypti* larvae breeding sites and thus, independently of season. This result confirmed what was found in the Central African Republic (Kamgang *et al.*, 2013) and in southern Cameroon (Fontenille *et al.*, 2001).

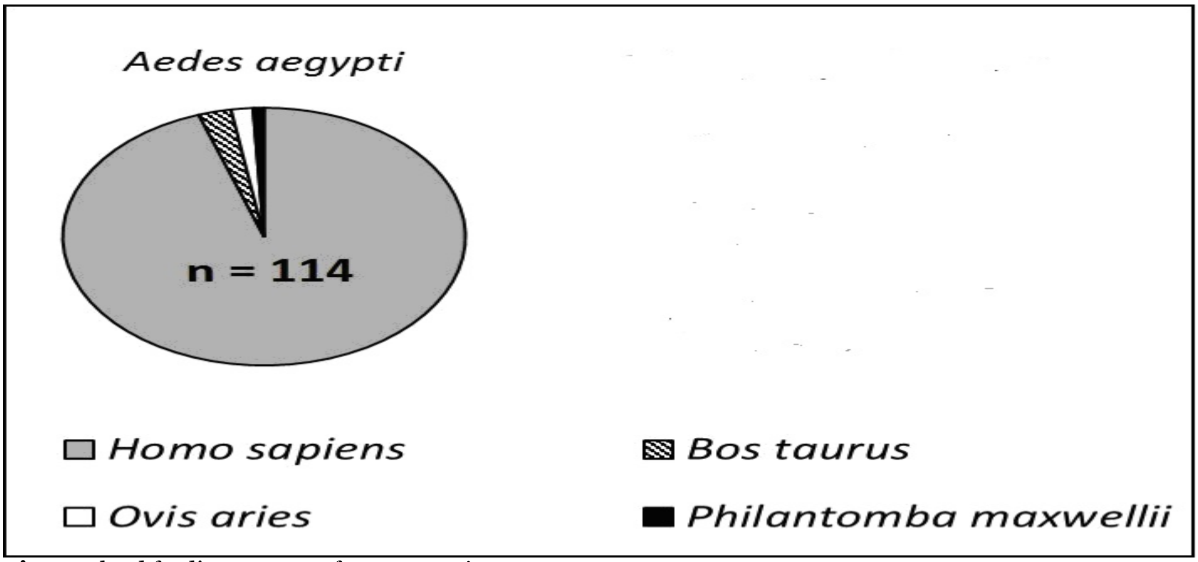

Fig. 3. Blood feeding pattern of *Ae. Aegypti.*

In fact, the advantages of this second hand tire trade of are considerable. They contribute to the improvement of living conditions by supplying income and employment to urban populations. However, the positive economic and social impact of this trade is limited by the fact that these tires are good breeding sites for *Ae. aegypti,* and therefore contribute to the development of large mosquito populations in these areas. Despite their massive presence in the tires, some larvae were found also in abandoned peri-domestic containers and discarded tanks. This finding is in agreement with observations made in Cameroon (Fontenille *et al.*, 2001; Simard *et al.*, 2005; Kamgang *et al.,* 2010) where peri-domestic containers represented the bulk of the containers infested by *Ae. aegypti* or *Ae. albopictus*.

Moreover, in many sub-Saharan towns, unplanned urbanization and lack of waste management lead to widespread water collection, thus favoring the proliferation of *Aedes* spp (Amarasinghe *et al.*, 2011). The study area Dandji, is located in a zone with a weak level of urbanization. This factor added to the presence of second hand tire trade can explain the high populations of *Ae. aegypti* in this area and can trigger the transmission of dengue and yellow fever.

The absence of *Aedes albopictus* in our study can be explained by the climate condition which certainly is not suitable for this mosquito development. However, since 2000, *Ae. albopictus* has invaded several central African countries, including Cameroon (Kamgang *et al.,* 2010), Gabon (Coffinet *et al.*, 2007), Equatorial Guinea (Toto *et al.*, 2003) and the Central African Republic (Diallo *et al.,* 2010) where it occurs in human-dominated environments previously colonized by *Ae. aegypti*. The coexistence of *Ae. aegypti* and *Ae. albopictus* has been documented in several regions in the world, where the larvae sometimes share common developmental sites (Kittayapong and Strickman, 1993). It's therefore crucial that more investigation need to be done to search for the presence of this mosquito in Benin. It could be shown that *Ae. aegypti* primarily feeds on humans, which is in line with other studies investigating the blood feeding pattern of this vector (Ponlawat and, Harrington., 2005). The high frequency of human meals in this undeveloped urban site suggests that the anthropophilic feeding behavior can have a big impact on DENV transmission. This anthropophilic feeding behavior can explain the highest population of *Ae. aegypti* collected by HLC compared to other method of collections.. But it remains important to consider the potential effects of collection method when analyzing and interpreting the results of mosquito blood-feeding behavior. In further experiments *Ae. aegypti* have to be captured in outdoor resting sites and results have to be compared.

In Benin, there is an increasingly attention being given to malaria vector control (Yadouleton *et al.*, 2011) while *Ae. aegypti* is an important nuisance that often condition community acceptance of vector control tools. Because of low awareness by health care providers and lack of diagnostic testing and systematic surveillance, DENV infections only have been reported from travelers returning to France and Japan (Moi *et al.*, 2010; Ujiie *et al.*, 2012). These findings are supposed to be a ringing for planning vector control programs on *Ae. aegypti* in Benin. Moreover, data on blood feeding patterns of *Ae. aegypti* collected, could provide valuable information about potential DENV hosts others than humans and will help to get a greater understanding about DENV ecology in Benin.

It is therefore crucial for health authorities of Benin to develop a program to fight against this mosquito and avoid an outbreak of DENV as it was the case in many sub-Saharan Africa countries cited above.

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