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Study of the Behavior and Entomological Parameters of Anopheles in Two Health Zones of The North-Ubangi Province, Democratic Republic of Congo

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ABSTRACT

An entomological study was conducted using two mosquito sampling technical, pyrethrum spray catch (PSC) and Human Landing Catch (HLC), inside and outside houses in two Health Zones in North Ubangi Province give the names of Health zones. This study was conducted from 31 March to 14 April 2021. This province is located in the north of the DRC, in a forested area. The objective of this work was to identify the behavior of *anopheles* and their entomological parameters. In total, 784 samples of *Anopheles* were captured, 575 by HLC (236 inside the houses and 339 outside), and 209 by (PSC). The *anopheles* captured in these two environments belong to the following 3 species: *Anopheles gambiae* s.l, (n= 769 i.e. 98%) *Anopheles funestus* grp (n=10 i.e. 1%), *Anopheles paludis* (n=5 i.e. 1%). The behavioral study reveals that *Anopheles gambiae* s.l is more exophagous than endophagous with a indoors peak startings a little later between 23:00 and 02:00 hours while the outdoors peak starts a little earlier at 19:00 hours. The density of *Anopheles gambiae* s.l. is 98% of all *Anopheles* mosquitoes collected. The determination of the sporozoite index by the ELISA method gave 58% of the infested *Anopheles*. *Anopheles gambiae* s.l is the major vector of malaria in this environment and is more abundant.

INTRODUCTION

Malaria is a parasitic disease caused by Plasmodium species transmitted by the mosquitoes of the genus *Anopheles* the Culicidae family (OMS 2004). According to the world Health Organization (WHO 2020), The considerable increase in the number of cases (14 million) and 69000 more people die from malaria in 2020 compared to 2019 (627000 against 558000), Nearly two-thirds (47000) additional.

The degree of human-vector contact is an important component in the transmission of this disease. For a mosquito to lay eggs, it must take at least one blood meal. It is during this meal that, the mosquito takes the pathogen from an infected human and injects it into a healthy human during another blood test (Mbogo *et al.*, 1993). Human-vector contact is therefore of paramount importance in developing and assessing the impact of vector-borne disease control strategies (Simon *et al.*, 2008). In Africa, the main vectors are *Anopheles gambiae* s.l, *An. arabiensis*, and *An. funestus* (Wat'senga *et al.*, 2018).

In the Democratic Republic of the Congo, a part from a few studies carried out by the President's Malaria Initiative (PMI) and Médecin Sans Frontier (MSF), very little data exists on the behaviour of *Anopheles* in the province of North Ubangi. The behavior of *Anopheles gambiae* s.l in urban and rural areas remains poorly known, yet *An. gambiae* s.l is the major vector of malaria in the DRC (Afolabi *et al.*, 2006). However, in, the prevalence of malaria was 62,472 cases and 39 deaths for the Gbado-Lite health zone and 90,332 cases with 69 deaths for the Karawa health zone (District Health Information 2020). Therefore, it is preferable to study the behavior and the entomological parameters of this vector in order to regenerate information to help the control programme in making decisions on complementary control interventions in the province of Nord-Ubangi in the DRC. The general objective of this study is to contribute to the understanding of *Anopheles* feeding and resting behavior, the species composition and to determine the *Anopheles* sporozoitic index in Gbado-Lite and Karawa health zones in the Nord-Ubangi province of the DRC.

MATERIALS AND METHODS

Study Area:

The present study was carried out in, Karawa Health Zone (N 3°21'33.978"

E20°18'20.19") and in Gbado-Lite Health Zone (N 4°16'53.12388" E 21°0'17.4617") in the province of North-Ubangi. The selection of both areas as study sites was justified in that they are an ecotone belonging to the Ubangi ecoregion. Two types of houses were identified in these health zones: traditional type of houses (Banco walls) and modern type of houses (brick walls). From a demographic point of view, the population of the Karawa health zone is 304,978, and that of the Gbado-Lite health zone 169, 847.

Study Period:

This was a descriptive study that was conducted from 31 March to 14 April 2021 during the rainy season.

Mosquito Collection:

Human Landing Catch (HLC):

Human Landing Catch (HLCs) were conducted to determine mosquito biting time, and biting locations and to identify species. Adult mosquitoes were captured on ten consecutive nights in 20 different houses on each randomly selected night, with one person inside and one outside each selected house. Mosquito collectors Capturers rotated inside and outside houses after six hours (Wat'senga *et al.*, 2009). All *Anopheles* mosquitoes collected by HBC were identified morphological features and then preserved in 1.5 ml Eppendorf tubes on silica gel. The collected *Anopheles* were preserved separately in the tubes according to the time interval set, i.e., after every one hour with a time bag. This was done in order to determine the time of activity of each species of mosquito.

Pyrethrum Spray Catch (PSC):

PSCs were conducted from 06:00 to 09:00 in the morning in the same areas as HLC but in different houses randomly selected to estimate resting mosquito density, digestive status, and sporozoite index.

Before the PSCs were carried out, all occupants were asked to leave the house. All rooms including the living room

were sprayed with a commercially available aerosol (Baygon 600 ml containing pyrethroid to kill mosquitoes resting inside the houses (endophilic). Fifteen minutes after spraying, all fallen mosquitoes were collected on the white sheets laid on the flat floor surfaces. Female *Anopheles* were classified according to the abdominal stage (fed or unfed). Blood fed mosquitoes were thereafter classified into 4 stages (Unfed, Blood-fed, Half-gravid and gravid) (Wat'senga *et al.*, 2009). Hundred (100) *Anopheles gambiae* s.l were then tested by ELISA to determine the proportion of the circumsporozoite index or sporozoite index (SI). In order to assess the vectorial competence of different *Anopheles* species towards *Plasmodium* (SI represents the proportion of infected mosquitoes out of the total number tested), we tested for the circumsporozoite antigen (Ag) of the sporozoite or oocyst membrane envelope in female *Anopheles* using a specific monoclonal antibody coupled to an enzyme, peroxidase, according to the technique described by Wirtz *et al.*, (2009). Each mosquito collected was correctly labeled, stored in a 1.5 ml Eppendorf tube with silica gel, and identified morphologically using a binocular light microscope at 40x magnification.

Entomological Parameters and Data Analysis:

Entomological data were collected on survey sheets. The following

entomological parameters were determined i) the anopheline density corresponding to the number of mosquitoes captured per house; ii) the species identification using the Identification Key for Female *Anopheles* Mosquitoes of the Afro-Tropical Region (Gillies and Meillon 1968; Gillies and Coetzee 1987); iii) the period of aggressiveness and iv) the infestation rate (IR) of the mosquitoes expressed by the proportion of mosquitoes carrying the *P. falciparum* circumsporozoite antigen (CSP) over the total number of mosquitoes tested. *falciparum* circumsporozoite antigen (CSP) out of the total number of mosquitoes tested. Data were entered into Microsoft Excel and analyzed to compare different proportions.

RESULTS

Specific Composition:

During the study period, 784 *Anopheles* were collected in the two selected sites. Three species of *Anopheles* (*An. gambiae* s.l, *An. funestus* grp, *An. paludis*) were identified, *An. gambiae* s.l. was the most abundant (n = 769 or 98,1%), followed by *An. funestus* s.l (n = 10 or 1,3%) and *An. paludis* (n = 5 or 0,6%). More *Anopheles* species was higher from HLC (73%: 575/784) than from PSC (27%: 209/784).

The species composition is presented by zone and by collection method in figures (1 & 2).

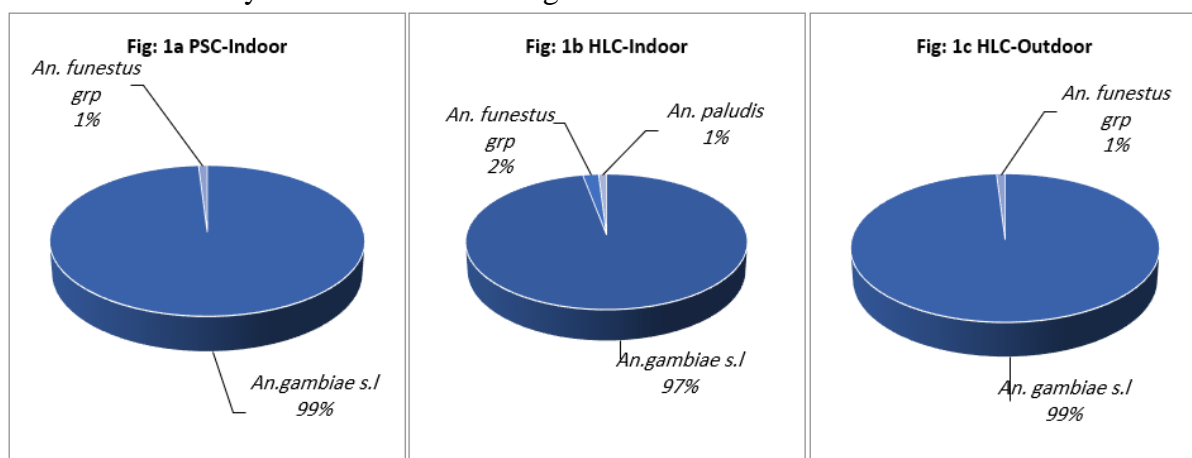


Fig. 1: Distribution of *anopheles* captured by PSC and HLC Indoors and outdoors in the Karawa Health Zone.

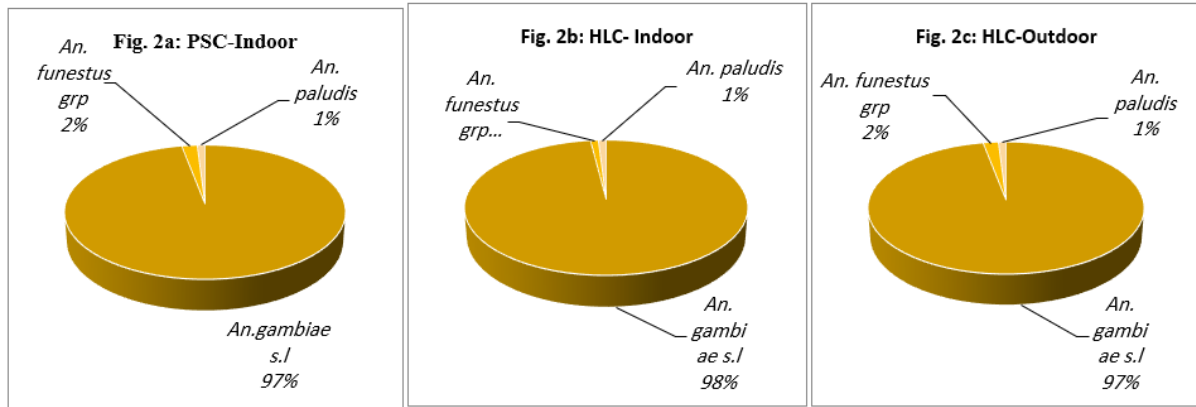


Fig. 2: Distribution of *anopheles* captured by PSC and HLC Indoors and outdoors in the Gbado-Lite Health Zone.

The condition of the abdomen of *Anopheles* mosquitoes collected using pyrethrum spraying inside the houses (PSC) is presented in Table 1 below. We noted that the majority of female *Anopheles* captured were blood fed in both sites, followed by the semi-gravid *Anopheles*. The species

Anopheles gambiae s.l was more abundant in both sites while *Anopheles funestus* was captured at extremely low numbers in both health zones and *Anopheles paludis* was only captured in the Gbado-Lite health zone.

Table 1: Abdominal status of malaria vectors collected resting indoors through PSC karawa and gbado-lite

KARAWA HEALTH AREA					
SPECIES	UNFID	BLOOD-FED	HALF-GRAVID	GRAVID	TOTAL
<i>An. gambiae</i> s.l	16(14%)	65(58%)	33(27%)	2(3%)	116(100%)
<i>An. funestus</i> s.l	0(0%)	1(100%)	0(0%)	0(0%)	1(100%)
Total <i>Anopheles</i>	16(14%)	66(56%)	33(28%)	2(2%)	117(100%)
GBADO-LITE HEALTH AREA					
<i>An. gambiae</i> s.l	10(11%)	46(52%)	33(37%)	0(0%)	89(100%)
<i>An. funestus</i> sl	0(0%)	1(50%)	1(50%)	0(0%)	2(100%)
<i>An. paludis</i>	1(100%)	0(0%)	0(0%)	0(0%)	1(100%)
Total anopheles	11(12%)	47(51%)	34(37%)	0(0%)	92(100%)

Period of Aggressiveness of Malaria Vectors Collected Indoors and Outdoors by HLC:

Generally speaking, the peak period of *Anopheles* bites or aggressiveness indoors was late in the evening, between

22:00 and 02:00 in both sites, whereas the peak was observed outdoors because the habitats remain outside for a long time, which means that the vectors are more active already in the early evening between 18:00 and 19:00.

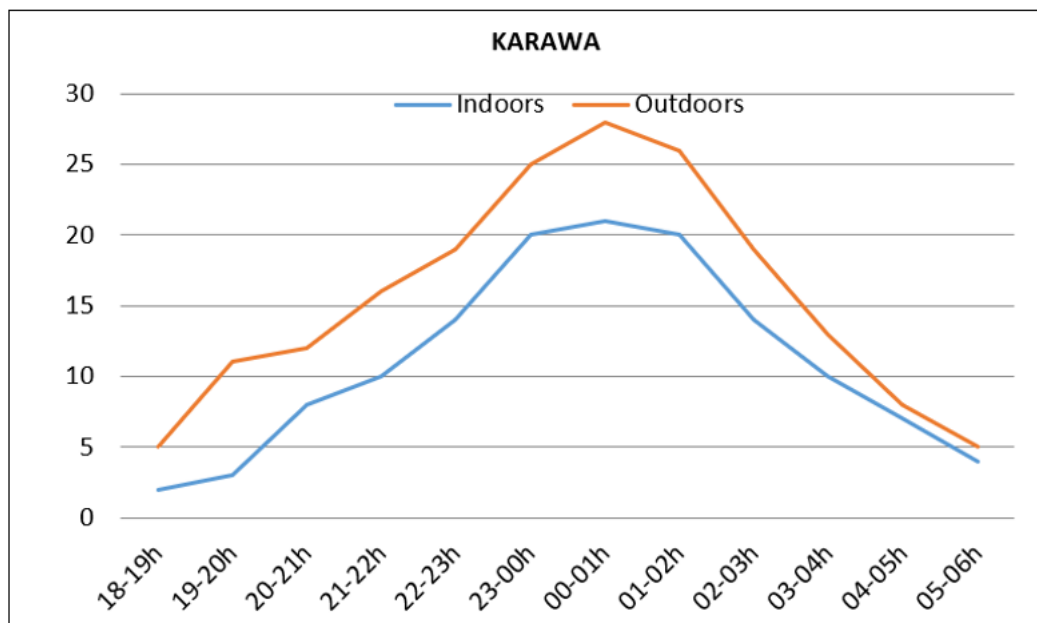


Fig. 3a: Time schedule of malaria vector bites collected indoors and outdoors by HLC

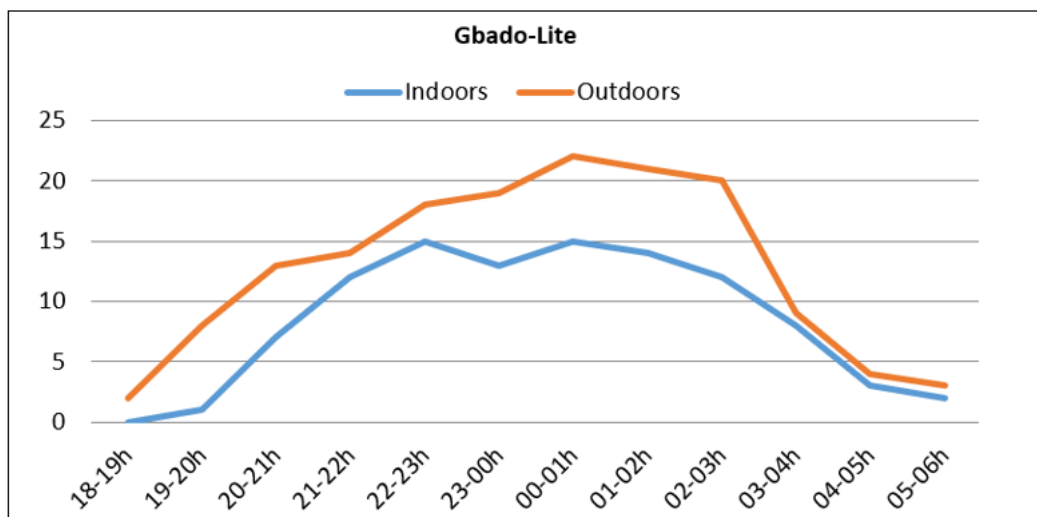


Fig. 3b: Time schedule of malaria vector bites collected indoors and outdoors by HLC

ELISA Test Results:

Table 2 shows the ELISA test results. The data in Table 2 shows that on the two sampling sites, there were 17 CS

positives mosquitoes at ZS Gbado-Lite and 12 CS positives mosquitoes at ZS Karawa. The number of negatives mosquitoes was 38 at ZS Karawa and 33 at ZS Gbado-Lite.

Table 2 ELISA *Anopheles gambiae* s.l

Sites	ELISA <i>Anopheles gambiae</i> s.l		Tested number	Percentage for site
	Positive (+)	Negative (-)		
ZS Gbado-lite	17	33	50	34
ZS Karawa	12	38	50	24
Total			100	58

DISCUSSION

Three species of *Anopheles*, namely *An. gambiae* s.l., *An. funestus* and *An. paludis* were captured in Karawa and Gbado-Lite HZs in North Ubangi Province RDC. After a series of night captures in 20 households for the HLC and another 20 houses for the PSC, it was found that *An. gambiae* s.l. is the main malaria vector in both sites. This species constituted 98% of the population of vectors collected and presented a sporozoite index of 58%; this reveals the high risk of malaria transmission in these two sites during the year 2021.

The study carried out on the feeding and resting behavior of *Anopheles* and the entomological parameters (such as population density and sporozoitic index) in the health zones allowed the incrimination of this species in disease transmission in both health zones. These results corroborate those recorded in previous studies carried out in several locations of the provincial city of Kinshasa (Wat'senga *et al.*, 2009). Out of a total of 784 *Anopheles*, 575 (73%) specimens were captured by Human Landing Catch "HLCs" and 209 (26.6%) specimens by Pyrethrum Spray Catch "PSC".

The sporozoitic index (SI) is one of the key parameters for assessing malaria transmission in sites. It provides information that varies from one setting to another. The sporozoite indices in both sites gave 58%. While corroborating previous observations (Karch *et al.*, 1992). This result shows the existence of the intensity of malaria transmission

Time and place of aggressivity are important parameters that tell us when and where mosquitoes are active. In general, the peak period for indoor biting of *Anopheles* was late at night, between 10 pm and 6 am, which mirrored the outdoor biting patterns at both sites. These results corroborate those reported on entomological activities implemented by the National Institute of Biomedical Research of the DRC.

The climate of the province is favorable for the proliferation of malaria vectors, with temperatures varying between 27 and 28°C throughout the year and a short dry season that begins in early November and ends in late February (Entomological Monitoring Report 2020).

The development of various agricultural products such as rice and groundnuts, as well as the development of fish ponds, introduces an ecological context that is very favorable for the development of *An. gambiae* s.l. Several studies have shown the impact of rice cultivation on the proliferation of *Anopheles*, especially *An. gambiae* s.l. (Koudou *et al.*, 2007).

Conclusion and Suggestions:

The aim of this study was to conduct an entomological survey on the behavior and entomological parameters of malaria vectors in the two health zones of North-Ubangi (Gbado-Lite and Karawa) in the Republic of the Congo.

This study revealed that:

Three species were identified namely *An. gambiae* s.l (98,1%), *An. funestus* s.l (1,3%), and *An. paludis* (0,6%). The species *gambiae* s.l is the most abundant. The anopheles captured showed more exophagous than endophagous behavior and that outdoor activity starts earlier at 19 h00' than indoor activity a little later at 23 h00'. Four abdominal stages were observed in the vectors, unfed, Blood-fed, Half-gravid and gravid respectively in Karawa: 14%, 56%, 28%, 2% and Gbado-Lite: 12%, 51%, 37%, and 0%. In both health zones, the majority were fed and Half-gravid. ELISA tests indicate that *Anopheles gambiae* presented 58% of, sporozoites, thus the major vector of malaria in both zones.

Because the *Anopheles gambiae* species feeds outside (more exophagous), it would be better to add complementary vector control methods, as the current method of control with LLINs is not

sufficient, especially as the vectors are starting to change their feeding behavior.

List of abbreviations

ELISA: Enzyme-linked Immunosorbent Assay

HLC : Human Landing Catch

PSC : Pyrethrum Spray Catch

NMCP: National Malaria Control Program

LLIN: Long-lasting insecticidal net

Zs : Health area

S.I: Circumsporozoïtique index or sporozoite index

PMI : President's Malaria Initiative

MSF : Médecin Sans Frontier

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