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Study of entomological parameters involved in the transmission of *Plasmodium* parasite in *Anopheles gambiae* in the city of Maroua, Far North Region Cameroon.

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Abstract
In the year 2011, an entomological study based on the evaluation of the anthropophilic fauna and especially on the determination of entomological parameters of the transmission of *Plasmodium* parasite was carried out in Maroua town Cameroon. Of a total of 1177 mosquitoes caught on human beings, female *Anopheles* represented only by *Anopheles gambiae* showed a fairly low number of (136/1177) that is 11.55% of the total number of Culicidae captured. Anthropophilic Culicinae, consisting solely of *Culex quinquefasciatus* alone accounts for 88.45% with a workforce of (1041/1177) of the total mosquitoes collected on human beings. The general aggressiveness rate is then Ar = 4.08 bites/man/night, is an annual aggressiveness 1491.68 bites/man/year in Maroua during the study period. The transmission study dealt only with the principal malaria vector, 136 female *Anopheles gambiae* caught on man gave an average aggression rate Ar = 0.47 bites/man/night, representing an annual rate of 171.55 bites/man/year aggressiveness. Out of 131 salivary glands of *Anopheles gambiae* dissected, only 13 females showed *Plasmodium* sporozoites in their salivary glands, a sporozoite index SI = 0.0 99. Infected female *Anopheles* bites in Maroua is estimated at Entomological Inoculation Rate (EIR) = 0.05infected bite/man/night, or 18.25 infected bite/man/year. This reflects a low level of the transmission of *Plasmodium* during the study period in Maroua. The average annual rate of parturity is estimated at PR = 51%.

Keywords: *Anopheles gambiae,* Entomological study, parameters, *Plasmodium*, transmission, Maroua, Cameroon.

1. Introduction

In the world, 220 million cases of malaria, with 660 000 deaths, were reported in 2010 [1]. Africa remains the most affected continent with 90% of global mortality. The six most affected countries are Nigeria, the Democratic Republic of Congo, Tanzania, Uganda, Mozambique and Côte d'Ivoire [1]. More than a million people were gratified in ten years through global action thanks to the distribution of impregnated nets. However, in Sub-Saharan countries, a slowdown in net distribution was observed. While 145 million nets were provided to the population in 2010, only 66 million were provided in 2012 [1]. Among the diseases known as transmissible to human being, emphasis should be laid on mosquito vectors such as *Culex, Aedes* and *Anopheles* [2]. Relentless efforts are observed against the scientific community who carry out the control of the diseases transmitted by mosquitoes, namely mosquito resistance to insecticides; hygiene in certain localities and economic problems related to high costs of synthetic insecticides [3]. In most major cities of the tropical zones, Maroua a Savannah city of the Northern Cameroon, is currently experiencing rapid growth not only to the natural increase of the population and/or the rural exodus, but also to the creation of the University of Maroua. This development is rapid and in a disorganized manner, so that the planning services and hygiene are unable to effectively clean the urban environment. This results in wastewater collection, the potential larvae breeding sites, particularly favorable to the development of immature stages of mosquitoes that bite especially inside our rooms. Faced with this situation, the World Health Organization recommends the development of new strategies to overcome this problem. One method that has proven its effectiveness is the fight against the adult vector populations directly involved in the transmission of parasitic diseases. So, to ensure the existence of the relationship between vector/Man/Parasite in a given area, it is very important to assess the degree of entomological parameters of the transmission of the parasite.

The overall objective of this paper is to evaluate the main entomological parameters directly involved in the transmission of the *Plasmodium* parasite, known as the *Anopheles gambiae*. The specific objectives are to:

- make an inventory of the anthropophilic Culicidae fauna of Maroua;
- dissect salivary glands of female *Anopheles* to search for sporozoites;
- dissect the abdomens to determine the physiological age of female *anopheles* and calculate entomological evidence form the dissected organs to assess the magnitude of its transmission in Maroua town

1.1 Materials and methods

1.1.1 Presentation of the study site

Maroua, Capital of the Far North Region Cameroon and the Diamare Division is located between 10 ° 31' -10 ° 39 'North latitude and 14 ° 13' -14 ° 24' East longitude, at an altitude of 400 m in the Savannah (4). Maroua has three Sub-Divisions. It stretches along the shores of Mayo- Kaliao River, longitudinally from West to East. Its climate is sudano - sahelian with an average temperature of 35 °C maximum in March and April to 45 °C and 25 °C minimum [5]. A very short rainy season alternates with a relatively long dry season depending on the years. The annual rainfall is about 800 to 900 mm distributed over 50 to 60 days of precipitation [6]. The vegetation consists of thorny sahelian steppes and is dominated by *Balanites aegyptiaca*, *Boswellia dalzielii*, *Anogeissus leiocarpus*, *Azadirachta indica*, *Khaya senegalensis* [7]. The population of the city of Maroua increased from 201,372 (RGPH 1987) inhabitants to 400 000 in 2013 according to the Maroua I Council.

1.1.2 Methodology

1.1.2.1 Capture of adult mosquitoes on human subjects

To capture mosquitoes, the conventional method of capture is used [9]. The captures were made throughout 2011. Three sub-Divisions were selected to perform nightly catch. These neighbourhoods were Founangué (Pont-Vert), Domayo and Lopéré. The choice of these neighbourhoods depended mainly on the presence of one or more permanent breeding sites. In each of the three neighbourhoods, 4 captured houses were selected; corresponding to 4 men-nights. This makes a total of 12 homes captured. The capture rate was 2 passages per month, or 24 outputs in total. What is 4 x 3 neighborhood houses (=12 catchers) x 12 months x 2 outputs = 288 men-nights. House captures remained fixed and unchangeable until the end of the work. The choice of selected houses obeys a number of criteria, namely the proximity of permanent breeding sites, the number of people living in the house, the populous neighbourhood character and willingness of household heads to accept catchers in their homes. Two night catches were made for a month with a two-week interval in every neighbourhood and every home capture. To avoid biasness in observations due to fatigue and drowsiness of catchers; the first team of four begins capturing from 7 pm to 12 am. The second team replaces the first one from 12 am to 6 am. However, preventive measures have been taken in advance to protect catchers' infections that result from mosquito bites by making them antimalarials before introducing them in the houses of capture.

1.1.2.2 Method of capture: All anthropophilic Culicidae fauna of the Maroua city have been captured. The catchers were equipped with a flashlight, test tubes, pens and small bags labeled in a one hour time slot. The capturer with breeches or trousers rolled up sits inside the house of capture. The capturer torches on his legs every three minutes to see if a mosquito set to feed itself with blood. If a mosquito is detected, it is captured using a glass test tube closed with a cotton wool once inside. The tubes containing the mosquitoes were stored in bags depending on the time slots of one hour each. Captured mosquitoes were identified, and then the salivary glands and abdomens of female *Anopheles* were dissected early in the morning.

1.1.2.3 Identification of *Anopheles*

Identification keys of Gillies and De Meillon [10] and Gillies and =Coetzee [11] were used to separate the female *Anopheles* mosquitoes from the others.

1.1.2.4 Dissection of female *Anopheles* [12]

1.1.2.5 Dissection of the salivary gland

The dissection was performed in the laboratory of the Institute of Agricultural Research for Development (IRAD) and following an established protocol (Figure 1). A drop of saline NaCl 9% was deposited on a glass slide mounted on an optical microscope, the wings and legs of the female *Anopheles* were pulled after the stunned shaking of the test tube. The female *Anopheles* was posed in a profile position with the near physiological solution on the blade head. With pliers sharp tips or mounted needles, the head was severed with a jerk and a little pressure was exercised on the chest to bring out the salivary glands. Once the salivary glands are out, the rest of the body was kept for the dissection of the abdomen. Salivary glands were placed in physiological saline and covered with a coverslip, and then a slight pressure was applied to the preparation using the 100X objective to pop the salivary glands to release any eventual sporozoites, and the whole was left for about 3 minutes to allow the full release sporozoites. Sporozoites are the infective forms of *Plasmodium* in female *Anopheles*. Preparation was then observed under an optical microscope at 10X and 40X.

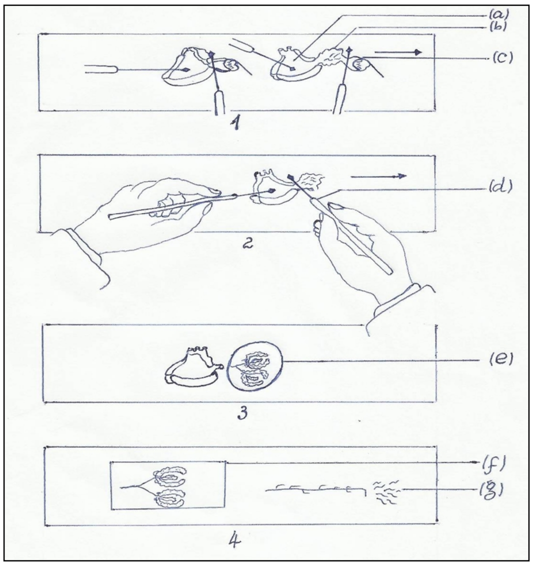

Fig 1: Various stages of dissection of the salivary glands of adult female *Anopheles* (Illustrated by Saotoing WHO, 2005).

Key: 1-4 = stages of dissection (a) = thorax of *Anopheles* (b) = salivary gland of *Anopheles* (c) = head of *Anopheles* (d) = needle attached (e) = saline + salivary glands (f) = salivary gland covered with leaf (g) = sporozoites.

1.1.2.6 Dissection of the ovaries

The abdomen of the female *Anopheles* was placed on a slide with the lap portion placed at the edge of a drop of distilled water, a section was made between segments 6 and 7; maintaining *Anopheles* on the trunk, the terminal part of the abdomen was made; released ovaries were placed on a microscope slide in a drop of distilled water and the whole was left to dry for at least 6 hours (**Fig. 2**). The ovaries, completely dried, easily distinguish ovarian structures nulliparous and parous. A nulliparous female presents tracheoles ovarian curled and those of female ovary barrier consist in scalp tracheoles distended network.

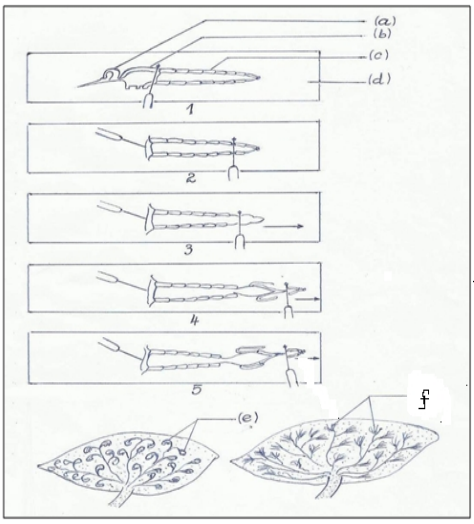

Fig 2: Different stages of ovarian dissection of adult female *Anopheles* (Illustrated by Saotoing, WHO, 2005)

Key: 1-5 = stages of dissection of the abdomen of a female *Anopheles*, (a) = head of *Anopheles*, (b) = thorax of *Anopheles*, (c) = abdomen of *Anopheles*, (d) = microscope slide, (e) = nulliparous ovary, (f) = parous ovary

1.1.2.7 Entomological indexes calculated

Indexes usually calculated in the entomological studies are:

- the biting rate (BR).This is the average number of mosquito bites per man per unit time. BR= number of mosquitoes caught in unit time / number of man-nights.
- the sporozoite rate (SR). The sporozoite index represents the percentage of female *Anopheles* with sporozoites of *Plasmodium* in their salivary glands.
- the entomological inoculation rate (EIR). This is the number of infected bites (IB) a man receives in a given period. The entomological inoculation rate is calculated using the formula of Ross (1911): EIR = SR.IB.
- the parturity rate (PR).This is the ratio of parous throughout parous and nulliparous.
- survival rate (P). The survival rate (P) is the probability of survival daily female *Anopheles*. It is obtained by the formula of Davidson (1954) and determined as follows: $P = \sqrt[l]{p/(p+Np)}$. l= duration of the gonotrophic cycle (3 days), p= number of parous and Np= number of nulliparous females.).
- the vectorial capacity (VC). Vectorial capacity (Jones and Garret- Schidrawi, 1969) represents the rate of potentially infectious contacts. $VC = (SR^2).p^n / -\log_e p$.
- life expectancy (LE) and the expectation of infective life (EIL). This is a parameter that can predict, within a given population, the proportion of individuals *(Anopheles*) who, after taking a blood meal, infecting live long enough to transmit the *Plasmodium*. These parameters are measured respectively in the formula of MacDonald (1957) and Moskovsky (in Detinova, 1963) as follows: $LE = \ell / -\log_e p$ and $EIL = p^n / -\log_e p$.
- the stability index (Si). This index is used to classify areas in "stable" (Ist > 2.5) or "unstable" (Ist < 0.5) or intermediate. $Si = a / -\log_e p$. a= number of people bitten / day).

1.1.2.8 Data analysis

To analyze the results, the T-tests (T), the Chi-Square ($\chi 2$) and the comparison test (Z) of 2 medium n_1 and n_2 populations were used to compare two averages between means [13]. The correlation (rho) test to establish any correlations between variables was also used.

1.2 Results and Discussion

1.2.1 Entomological data transmission of *Plasmodium* in Maroua

1.2.2 Anopheles biting rate (BR)

A total of 136 female *Anopheles gambiae* was captured on humans in 2011. The average rate of anopheline aggressiveness during the year 2011 was then estimated at BR = 0.47 bite/man/night in Maroua, an annual aggressiveness of BR=171.55 bites/man/year. However, we noticed a fairly low rate of daily aggression which was the highest in October (BR= 0.95 b/m/n) and lowest in January (BR = 0.08 b/m/n). A similar study was conducted in two rice growing villages in Côte d'Ivoire and a density of *Anopheles gambiae* aggressive BR = 30 bite/man/night was obtained, representing an annual rate of aggression BR = 10950 bites/man/year [14]. This rate was much higher than the one obtained in Maroua. This leads to the conclusion that the aggressive density depends largely on study areas. A similar study was conducted in ecological zones in Yaounde which revealed that the aggressive density was higher in the peripheral zone (Nkol Bisson) with BR = 1813 bites/man/year than in the center of the city (Nkol Bikok) with BR = 284 [15]. Other authors have carried out a study on malaria transmission in Cotonou, Benin, in three different representative aspects of the city and discovered aggressive densities of *Anopheles gambiae*, which vary according to the degree of urbanization in the neighbourhoods [16]. The average annual number of bites was found to be equal to 1179 in the center of the city, 3,666 in the outlying districts and 3,363 in intermediate zones. In the Senegal River basin, a similar study was performed and an average of daily injection of 0.28 p/m/n was found [17]. In most cases, the average proportion of biting rate rotated around some months and during heavy rainfall, from June to August. This observation corroborates that of others who discovered that Yagoua and Maga in the Far North of Cameroon, the mortality rate due to malaria by *Anopheles gambiae* increased dramatically during the rainy season [18]. Furthermore, a study conducted in the Northern part of Cameroon showed that *Anopheles gambiae* larvae mostly grow at the beginning and the end of the rainy season [19]. A positive correlation (r = 0.38), although small, was found between the rainfall and the number of captured *Anopheles*. The *Anopheles* biting rates appear to be low in Maroua. This is due not only to the scarcity of permanent breeding sites, but also the loss of female *Anopheles* in stray animals kept at home or in pets. A simple observation reveals that three households host at least one pet in Maroua. Dry and especially after the crop harvest season, most pets are free and the main streets of Maroua are invaded. Hence, this provides an alternative meal for mosquitoes, thus creating a biological shield and thereby increasing their life expectancy.

1.2.3 Sporozoite Index (SI)
Out of the 131 salivary glands of female *Anopheles gambiae* dissected, only 13 showed *Plasmodium* sporozoite, with a sporozoite Index SI = 0.99. The sporozoite rate did not depend on the abundance of rain as a low correlation (r = 0.04) was found between these two parameters. An entomological study of urban malaria in Cotonou, Benin has identified an average sporozoite rate of SI =1.7 % [16]. A similar study conducted in two central districts of the city of Yaounde revealed a sporozoite rate of 5.2% [20]. The disparity between these indexes depends on environmental factors and the vectorial capacity of the mosquito. If we keep the number of female *Anopheles* captured and dissected positively 13/134 in Maroua, 35/2054 in Cotonou and 3/58 in Yaoundé, we notice that female *Anopheles* in Maroua seem quite dynamic in the transmission of *Plasmodium* to humans. It should be noted that small mosquitoes exist throughout the year in Maroua. The existence of sporozoites in the salivary glands of mosquitoes in Maroua revealed that there is a contact between humans and *Anopheles* mosquitoes and the transmission of *Plasmodium* is real.

1.2.4 Entomological inoculation rate (EIR)
Infected female *Anopheles* bites in Maroua is EIR = 0.05 infected bite /man/night, or 18.25 infected bite/man/year. In Yaounde (Cameroon 13 infected bites per person and per year was obtained [20]. In the Sahel region of Burkina Faso, a number of 21 infected bites/man/year was found [21]. This number is not far from the annual infected bites received in the sahelian Region of Maroua (Cameroon) (18.25 infected bites/man/year). In Northern Senegal, there was less than 5 infective bites per man and per year [22]. In the coastal urban of Cotonou (Republic of Benin) a daily inoculation rate was EIR = 1.02 infected bite /man/night [20]. In all cases, the correlation seems to be very low between the number of female *Anopheles* caught and positive salivary glands in the explored regions. In fact, in Maroua, there is a fairly high relationship (r = 0.51) between the number of dissected *Anopheles* and the number of positive salivary glands.

1.2.5 Parturity rate (PR)
Out of the 132 ovaries of females *Anopheles* dissected, 67 showed ovarian pares with an average annual parturity rate of PR = 51%. The examination of the physiological age of the imaginative population of *Anopheles gambiae* in Maroua showed that this population varies slowly during the year. In the city of Maroua, the average parturity rates varies from 46% in dry season and 54% in the rainy season with an average of 50%. A non-significant difference ($\chi2 = 3.38$, df = 1, $\alpha = 0.05$) was observed between the parturity rate for two seasons. However, we noted a slight predominance of parturity rate during the rainy season. A low correlation (r = 0.08) was observed between the rate of parous and rainfall and the number of *Anopheles* caught and ovaries pares (r = 0.40). Half of the imaginative population of *Anopheles gambiae* being a barrier, it is necessary to classify the city of Maroua as a risk area or a zone known as epidemiologically dangerous. Parous rates are comparable to those obtained at Tingrela in Burkina Faso, where the variation in the percentage of parous females ranged from 32.6% to 93.3% with an average of 53.9% in 1966 and 1967 respectively [23]. In fact, a study was conducted on urban malaria in Burkina Faso and an average rate of 42% of parous throughout *Anopheles gambiae* captured was obtained [22]. Similarly, a fluctuation rate was observed in the forest zone of Southern Cameroon where the rates were ranged from 45.3% parous in the beginning of the short rainy season and 72.8 % at the end of the rainy seasons [24]. In Côte d'Ivoire, in the region of Man, it was showed that the variation in the percentage of parous females of *Anopheles gambiae* population ranged between 62% and 86% [25]. In the rainforest of Sassandra in Côte d'Ivoire, it was also found that the oscillations in the rate of parous females of *Anopheles gambiae* ranged between 61% to 79% [26]. Entomological studies in Senegal showed very high parturity rates of about 70% in *Anopheles gambiae* [27]. From these observations, it should be noted that the parous rates are high in wetland Savannah region. A similar study in Cotonou, Benin was carried out on the physiological age of the females of 2282 *Anopheles gambiae* led to obtaining an average rate of 49% parturity [20]. This parturity rate (49%) is in close relation with that obtained in Maroua (51%). The highest parturity rates were observed at the end of the rainy season. This fact is explained by the stability of larvae breeding sites and the absence of the leaching phenomenon at the beginning and at the end of the rainy seasons [19]. The rainfall is erratic, leaving time required for mosquito larvae to develop normally [24, 28]. The entomological parameters are shown in the table below.

Table 1: Entomological data of the transmission of *Plasmodium* in Maroua at 2011.

Bilan	Jan11	Feb	Mar	Apri	May	June	July	Aug	Sep	Oct	Nov	Dec	T
Rainfall	0	0	0	4	120	110	188	277	93	0	0	0	
Caught	2	4	4	7	10	13	18	14	19	23	16	6	136
Catchers	24	24	24	24	24	24	24	24	24	24	24	24	288
SG diss.	0	4	4	7	10	12	17	14	18	21	16	6	131
Salivary gld^{+}	0	0	0	0	1	2	2	1	2	3	2	0	13
BR	0.08	0.16	0.16	0.29	0.41	0.54	0.75	0.58	0.79	0.95	0.66	0.25	0.47
SI	0	0	0	0	0.10	0.16	0.11	0.07	0.11	0.14	0.18	0	0.07
EIR	0	0	0	0	0.05	0.12	0.08	0	0.16	0.12	0.08	0	0.05
Dissected ov	2	2	4	6	10	12	16	14	18	20	16	12	132
Parous ov.	1	1	1	3	6	7	8	7	11	11	8	6	70
PR	50	50	25	50	54	60	50	52	58	55	50	53	51%

Keys: S Gdiss: Salivary glands dissected; EIR: Entomological inoculation Rate; BR: Biting rate; SI: Sporozoite index; PR: Parturity rate; ov: ovary.

1.2.6 Survival rate (SR)
Daily survival rates of imaginative population of *Anopheles gambiae* in Maroua was calculated using the Davidson formula of (1954) are: $p_{ds} = 0.79$ and $p_{rs} = 0.82$ respectively in the dry and rainy seasons. A non-significant difference ($\chi2 = 3.39$, df = 1, $\alpha = 5\%$) was observed between daily survival rates of the two seasons. The average daily survival rates of *Anopheles gambiae* in the city of Maroua is p = 0.82. This rate, approaching the number 1 is high enough to maintain the *Anopheles* alive to meet the preferred host who is man. In fact, a similar study was conducted in Burkina Faso and a daily survival rate was p = 0.71 [29].

These same authors conducted a similar study in traditional rural areas and found a daily survival rate of 0.91 [29]. The average survival rate obtained in Maroua is slightly lower than that obtained by others authors where in the forest zone of Cameroon, they obtained daily survival rates of 0.87 and 0.85 respectively for *Anopheles nili* and *Anopheles gambiae*, and 0.85 and 0.84 for *Anopheles moucheti* in the villages of So- assi and Nlong-assi respectively [30, 24]. The difference between daily survival rates in the Savannah and forest areas is due to the fact that malaria transmission is continuous in forest regions, and it is seasonal in the Savannah zones [24]. It has been reported that the low temperature variations are the cause of limited modifications for the duration of ovarian maturation changes in the forest areas [24]. However, temperature varies considerably in the Savannah and is sometimes the cause of the differences in daily survival rates.

1.2.7 Life expectancy and infective life expectancy (LE and ILE)

The annual average life expectancy in *Anopheles gambiae* Maroua was 15.78 days during the period of study. Distributed according to the seasons, it is 12.76 days in the dry season and 15.80 days in the rainy season. A non-significant difference ($\chi2 = 1.58$, df = 1, $\alpha = 5\%$) was observed between the life expectancies of the two seasons. In the same vein, it has also discovered that life expectancy is slow in the South of Cameroon and high during the early rainy season (9.4 days) and low during the dry season (3.8 days) in *Anopheles moucheti* [24]. Female *Anopheles* still tend to live longer in the rainy season than in the dry season. This shows that *Anopheles gambiae* is persistent all the time in the city of Maroua. Annual infective life average calculated, revealed that it was Ei = 0.72 in the *Anopheles gambiae* of Maroua. Distributed according to the seasons, it is 0.50 in the dry season and 0.72 in the rainy season. It is comparable to that obtained in the South of Cameroon where *Anopheles moucheti* has an infective expectancy life of 0.63 and 0.70 in the hamlets of So-assi and Nlong –assi [24]. The variations are due to the difference in vegetation, climate, rainfall and involved vector agents. According to certain authors, , the more longevity *Anopheles* has, the higher is the probability of transmitting *Plasmodium* to a larger number of people increases [32].

1.2.8 Vectorial capacity (VC)

The vectorial capacity represents the rate of potential infectious contacts (or daily spread of malaria rate) between individuals through the *Anopheles* vector [33]. The vectorial capacity is also defined as "the daily rate of a future inoculations initiated by each infected case." In the city of Maroua, the daily vectorial capacity is 0.15. This means that *Anopheles gambiae* could theoretically be responsible for 0.15 new infection every day in case Maroua town is not protected. This parameter is much lower than that obtained, in the forest region of South Cameroon, an average value of vectorial capacity of 7 new infections per day are noticed [24]. The annual vectorial average capacity in the city of Maroua is estimated about VC = 55.67 new infections per year.

1.2.9 Stability index (Si)

The annual average stability index of malaria in Maroua was Si = 1.73 in 2011. This value is used to classify the city of Maroua town as intermediate transmission zone. This index is lower than that obtained in the forest, stabilized area index of 2.82 at So –assi [24]. In fact, the difference is that malaria transmission is almost continuous and permanent in forest area as opposed to the areas of the Sahel and Savannah where transmission depends much more on seasons. In general, the transmission is high during the rainy season and becomes low or zero during the dry season in the Sahel regions. The disappearance of houses suitable for larval development of the *Anopheles* larvae is responsible for this situation. In the same vein, that successive droughts cause the loss of usual lodgings, thereby causing the reduction or total disappearance of mosquitoes in a given environment [34].

2. Conclusion

The anthropophilic Culicidae fauna of the city of Maroua and the species night consisted mainly of *Culex. quinquefasciatus* and *Anopheles gambiae*. The average rate of Anopheline aggressiveness during the year 2011 was Ar = 0.47 bite/person/night, with an annual aggressiveness of 171.55 bite/person/year. The Pont-Vert neighbourhood, is the one that has provided more *Anopheles* than Lopere and Domayo neighbourhoods. More than half of infected *Anopheles* mosquitoes were also captured in Pont-Vert neighbourhood. In general, the infected bites of female *Anopheles* in Maroua was EIR = 0.05 infected bite /man/night, or 18.25 infected bite /man/year. *Anopheles gambiae* is theoretically responsible for 0.15 new infections each day in the case Maroua city lacks protection during the period of study. Most female *Anopheles* were captured at the beginning and at the end of the rainy season. The annual average index of the stability of malaria in Maroua was Si = 1.73, which classified the city of Maroua as an intermediate malaria transmission area in the year 2011. In days ahead, it should be good to conduct a parasitological study of malaria in Maroua to assess the proportions of malaria and healthy carriers of the *Plasmodium.*

3. Thanks

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