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RESEARCH ARTICLE

IMPACT OF THE USE OF MOSQUITO NETS ON MALARIA TRANSMISSION IN DJOUMOUNA (BRAZZAVILLE-CONGO).

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Abstract

Malaria transmission has been studied in Djoumouna, south of Brazzaville between October 2016 and May 2017. The objective of the study was to evaluate the transmission of malaria. The captures of the residual fauna were made according to three situations, the absence, the presence or the defectiveness of the mosquito nets. The mosquito nets observed were originally long-lasting insecticide-treated mosquito net (LLIN) acquired during the 2011-2012 mass distribution campaign. Efficacy tests conducted with these nets reveal very low mortality rates (ranging from 2.5% to 17.5%). This mortality indicates the almost non-existence of the insecticidal effect, therefore, these LLIN were considered as simple nets. *Anopheles gambiae s.l.* at 99.68% was predominant, followed by *Culex quinquefasciatus* at 0.32%. The highest anopheline density was observed in rooms without mosquito nets, followed by rooms with defective nets. On average, malaria transmission is infective per person every two weeks in rooms without mosquito nets; it is an average of one infective bite per month, in the rooms with defective mosquito net and infective sting every 6 months in rooms with mosquito net in good condition. The use of healthy LLIN in 2012 has been an important factor in reducing malaria transmission. In 2017, the deterioration of these LLIN or their absence has been an element in the rise of malaria transmission.

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Introduction:-

Malaria is a life-threatening parasitic disease. In 2016, WHO estimated the incidence of this serious disease at 216 million cases worldwide, including 445,000 deaths. All geographical areas in the southern hemisphere, including the intertropical zone (Africa, Southeast Asia, Latin America and the Pacific Islands) are strongly affected (OMS, 2017). The transmission of this infection throughout the world is unequally distributed, its intensity varies according to the geographical zones and the climatic conditions (Mouchet et al., 1993); it is also a function of the development conditions of the competent vectors, the plasmodial species in question (*Plasmodium falciparum*, *P. malariae*, *P. ovale*, *P. vivax* and *P. knowlesi*), anthropogenic factors and the receptivity of humans to the parasite. These conditions make the transmission of malaria vary considerably from one country to another, from one department to

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another (Carnevale et al., 2009). Sub-Saharan Africa is the region most affected by this scourge. In fact, a rate of 90% of cases and a mortality of 91% linked to malaria are recorded there. Pregnant women and children under five account for 92% of the cases in sub-Saharan Africa (OMS, 2017).

In Congo, malaria is stable and its transmission is permanent. It operates in a hyper or holoendemic mode throughout the territory. This infection is the main factor of morbidity and mortality. In 2016, 374,252 cases of malaria were recorded in public health facilities (PNLP, 2017).

To combat this endemic, the use of long-lasting insecticide-treated mosquito nets (LLINs) has become one of the major pillars of the malaria prevention policy. Indeed, this use has been adopted as the main control measure in the 2008-2012 strategic plan (PNLP, 2013)

However, as a result of this measure, few entomological studies have been conducted on the level of malaria transmission. We have therefore undertaken to evaluate, during this work, the transmission of malaria in Djoumouna, a locality in the city of Brazzaville, after the mass distribution campaign of LLINs in 2011 and 2012 (PNLP, 2013) and the appearance resistance to pyrethroids in *An. gambiae s.l.* in many localities of the country from the year 2002 (Bitsindou et al., 2006; PNLP, 2016). An inventory of anopheline fauna and an assessment of the intensity of malaria transmission during the rainy season were carried out and here we report the results obtained between October 2016 and May 2017.

Material and Methods:-

Field of study

The study took place at 25 km southeast of Brazzaville, in the peri-urban town of Djoumouna, geo-localized at 4.36 S for 15.15 E and an average altitude of 217 m (Getamap, 2018). The population is estimated at 635 inhabitants and this locality experienced many tumults related to civil unrest in 1997 and 1998-1999. These tumults provoked an important rural exodus of young people mainly towards Brazzaville. Very few people have returned to the locality and the present inhabitants practice subsistence farming and livestock farming. The grouped dwellings are constructed of cement bricks made of terracotta or cob or are made of sheet metal with a corrugated iron roof. These homes usually have gaps that can pass many arthropods including mosquitoes.

Located in the Department of Pool, Djoumouna is in a degraded secondary forest area. Its climate is humid tropical type with two distinct seasons: a rainy season from October to May and a dry season from June to September. The average annual rainfall is 1370 mm and the average temperature is 25.5°C. The locality adjoins four rivers: Iomba, Kinkoué, Loumbangala and Djoumouna. These rivers supply water to a series of ponds used for fish farming. These ponds are regularly emptied for the recovery and sale of fish or when the channels that irrigate them are obstructed. At these times, they present on their bed numerous puddles of water favorable to the proliferation of the vectors of malaria. In addition, many natural lodgings exist in the field; they are represented by pools of water, wells and public fountains installed in the locality. This situation is likely to generate anophelids all year long.

The vegetation is composed of species belonging to different families: Fabaceae, Rubiaceae like *Sherbourniazenkeri*, Euphorbiaceae, Poaceae, Apocynaceae and Malvaceae are the most diverse families in number of species (Miabangana et al., 2016).

Materials

The equipment used for the realization of this work was composed of flashlight, vacuum cleaner mouth (2), storage jar with a capacity of 130 cl, mosquito net tulle, cotton, elastic bracelets, cooler with a capacity of 6 liters for the transport of preserving jars, a sponge, a thermo-hygrometer brand BEURER and collection cards.

Methods

The surveys took place throughout the months of the rainy season. Mosquitoes were collected from the residual fauna in the bedrooms between 7 am and 11 am, with an average of 15 minutes for each room selected; 3 consecutive sessions per month were performed, giving 24 sessions of catches made in 8 months.

To do this, two investigators were recruited. Using a flashlight, the investigators searched for and collected mosquitoes from the endophile fauna in the rooms on the walls, ceiling, behind or under furniture, clothes hung under the bed, on and under the mosquito net. Each spotted mosquito was sucked up with a vacuum cleaner mouth and then introduced, for its conservation, in a jar with a capacity of 130 cl whose opening was closed with mosquito

net tulle maintained by an elastic strap. For each house visited, all the mosquitos collected in the bedrooms were introduced into the same jar bearing a number on the collection card, corresponding to the dwelling concerned.

On the collection sheet were the following information: the number of the dwelling, the geographical coordinates of the study site, the collection technique used, the number of interviewers, the date and time of capture, the temperature, the relative humidity, the number of rooms in each house inspected, the number of persons who slept in each room the day before the survey, the number of mosquito nets, whatever their quality, per house (the place and the date and their status), the number of mosquitoes collected by genus and species. For female anopheles, the physiological state (fasting, sip, semi-gravid, gravid), the size and rate for each type were determined.

When transported to the laboratory, all the jars were placed in a 6-liter cooler, the bottom of which was lined with a sponge soaked in water to maintain an average temperature of 24.5°C and relative humidity average of 75%. These temperature and humidity ranges help keep mosquitoes alive during the journey from Djoumouna to the insectarium. Under these conditions, mosquitoes can be kept for up to 5 hours after collection. Temperature and relative humidity were recorded in homes with a thermo-hygrometer brand BEURER.

Entomological indices

The mosquitoes were brought back to the laboratory for their identification using the key of GILLIES and DEMEILLON (Gillies et al., 1968). After identification, the entomological parameters of the transmission were calculated. Samples were enumerated per dwelling for the determination of aggressive densities (ai) per month and anopheline resting density per room (ADR). After binocular dissection of females of *An. gambiae s.l.*, the determination of physiological age was made after examination of the appearance of ovarian tracheoles^[11], and the degree of partitioning (TP) was calculated. Sporozoite (salivary gland) microscopic research was used to determine the sporozoite index (SI).

The entomological inoculation rate (TIE) was determined from the aggressive density (ai) and the infection rate (IS).

Evaluation of the effectiveness of LLINS

Efficacy tests in cones (WHO) were carried out on 3 LLINS distributed between 2011 and 2012 during the distribution campaign by the health system (PNLP / CSI). The physical characteristics of each LLINS allowed to classify them into 2 categories: LLINS in good condition (1 copy) and LLINS defective (2 copies).

The LLINS in good condition is made of polyethylene and was impregnated with Deltamethrin at a dose of 68mg / m². It was produced in 2010.

The information collected indicated that the two defective LLINSs were made of polyester and had been impregnated with Deltamethrin at a dose of 55mg / m².

Malaria case

Data on malaria cases were collected at the Integrated Health Center (CSI) of Djoumouna and cover the years 2012 to 2017, with the exception of 2014 for which no data was available. In addition, only the data for the last 5 months of the year (August to December) are complete for the years 2012 to 2017. Indeed, the missing data would be inherent to the many socio-political disturbances that occurred in the study area.

Statistical analysis of the data

The data from this study were captured with the Excel 2010 software and processed by the ANOVA statistical test. This statistical test allowed a multivariate analysis to explain the dependence of the quantitative variables on the qualitative variables (the absence, the presence and the state of the net) at the significance level of 5% using the Xlstat software. .

Results:-

A total of 24 residual wildlife collection sessions were conducted in 85 bedrooms. The cumulative number of people involved in the study who slept in these rooms the day before our visits was 729 people. In the 85 rooms inspected, 78 mosquito nets were observed, one net per room. Of the 78 mosquito nets observed: 25 (32%) were in good condition and 53 (or 68%) in a defective state. Of the 85 rooms visited, seven were without mosquito nets.

The mosquito nets observed in the rooms, were at the origin of the LLINs distributed during the 2011-2012 distribution campaign. However, it is recognized that the average duration of effectiveness of a LLINS is 3 years. Based on this observation, we considered that the mosquito nets observed in the field did not really have the specificities of the LLINs at the time of the study (October 2016 to May 2017).

Depending on the absence, presence and condition (good / defective) of the nets observed in the rooms, the results obtained are shown in Table 1 below:

Table 1:-Distribution of females of *An. gambiae s.l.* collected in Djoumouna according to their physiological state.

State of mosquito nets	Number of rooms visited	Number of persons in rooms visited	Average number of persons per room	Number of Anopheles females collected	Average Anopheline Density per Room	unfed	fed	Semi-gravid	gravid
Without mosquito net	7	35 (4, 8%)	5	84 (12,8%)	12	0	58 (69,1%)	22 (26,2%)	4 (4,7%)
good net	25	102 (14%)	4,1	40 (6,1%)	1,6	5 (12,5%)	15 (37,5%)	10 (25%)	10 (25%)
defective net	53	592 (81, 2%)	11,2	532 (81,1%)	10	11 (2%)	368 (69,2%)	134 (25,2%)	19 (3,6%)
Total	85	729	8,6	656	7,7	16 (2,4%)	441 (67,2%)	166 (25,3%)	33 (05,1%)

Table 1, above, shows the number of rooms visited, the number of persons who slept in rooms just prior to the enumerators' passage, the number of anopheline females collected, the average anopheline density per chamber, the distribution of mosquitoes according to the absence / presence and the state of the mosquito net, the number and the physiological state of the females of *Anopheles* collected according to the state of the mosquito nets in the rooms where the collections were made.

A total of 659 female mosquitoes (all species combined) were collected of which: 656 (or 99, 55%) *An. gambiae s.l.*; 1 (0, 15%) *An. funestus* and 2 (0, 30%) *Culex quinquefasciatus*. Of the *An. gambiae s.l.* females collected, 84 (12, 8%) came from the non-mosquito-controlled rooms, 40 (6, 1%) came from the rooms with mosquito nets in good condition and 532 (81, 1%) from the rooms with mosquito nets. defective. It appears that 12, 5% of *Anopheles* collected in rooms with intact mosquito nets were observed under fasting conditions.

The visit of the bedrooms correlated, the absence or presence of mosquito nets (good or bad condition) to the number of *Anopheles* collected

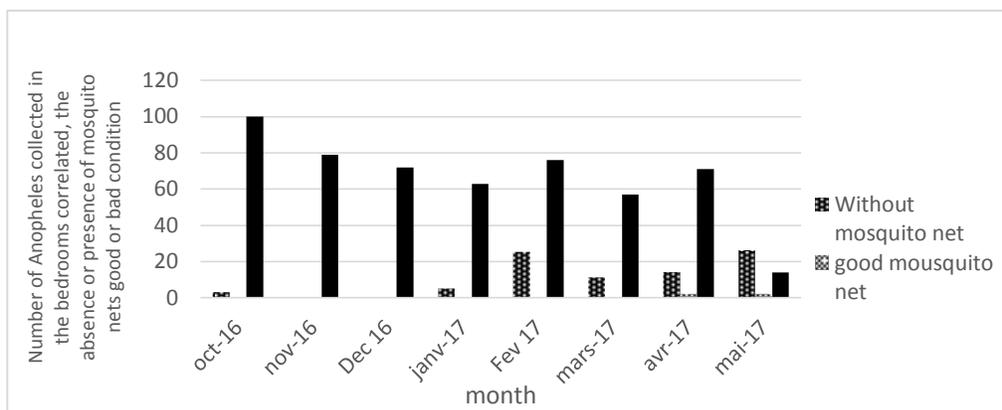


Figure 1:-Monthly distribution of anopheles collected

The use of the ANOVA test shows that there is a link between the number of *Anopheles* collected, the presence or absence of mosquito nets and the state of the mosquito net ($p < 0.0001$).

Table 2 below presents the different entomological indicators of malaria transmission.

Table 2:-Distribution of Entomological Indicators of Malaria Transmission

State of mosquito nets	Number of persons having slept in the rooms visited	Aggressive density (ma)	Number of mosquitoes dissected	Infected mosquitoes	Sporozoitic index (IS)	Number of pairs	Percentage/rate	Entomological inoculation rate (TIE)	Either infective sting every
Without mosquito net	35	2,3 p/h/n	84	2	2,40%	52	61,90%	0,06	17 days
good net	102	0,24 p/h/n	40	1	2,50%	23	57,50%	0,006	166,7 days or every 5.6 months
defective net	592	0,9 p/h/n	532	12	2,30%	313	58,80%	0,02	50 days or every 1,7months
Total	729	0,9 p/h/n	656	15	2,30%	388	59,10%	0,02	50 days or every 1,7 months

Regarding inoculation rates, the results indicate that the values of this indicator vary with the increase in values of aggressive anopheles densities (ai). Malaria transmission varies from an infective bite per person every 2 weeks (17 days), for people sleeping without a mosquito net, to an infective bite per person every 5 to 6 months, for people who use a mosquito net in good condition (Table 2). The transmission is infertile puncture per person every two months on average for people sleeping under a torn mosquito net.

The similarity of the results of the different rates (sip, semi-gravid and fraction), whatever the state of the nets, reinforced the determination of the team to carry out the efficacy tests of the insecticide used to impregnate the mosquito nets. Remember that these nets were acquired as LLINS during the mass distribution campaign in 2011-2012.

Table 3: Results of efficacy Tests on *Anopheles gambiae s.l.* (Djougouna Strain) 2018

Typical of LLINs	Number of female test of females tested	KD rate (60 min.)	% Mortality (24h)	Number of control females	Mortality of control females	Sensitivity level
good LLINs	80	2,5	2,5	20	0	Resistance
defective LLINs A	80	3,25	5%	20	0	Resistance
defective LLINs B	80	3,75	7%	20	0	Resistance

The tests were conducted with 300 females, of which 240 tested on mosquito nets that were originally LLINs and 60 control females tested on a normal (non-impregnated) net.

The results of mortality rates and KD rates obtained indicate that the effectiveness of LLINs is very low, 6 years after their acquisition. The KD rate at 60 min is 2,5% for the mosquito net in good condition and respectively 3,25% and 3,75% for the two defective mosquito nets. The mortality was 2,5% for the nets in good condition while the mortality was respectively 5% for the defective LLINs A and 7% for the defective LLINs B. The mortality rate of the control females was zero.

Climate data

Temperatures in the bedrooms ranged from 26, 4 ° C to 32, 2 ° C. The relative humidity was between 63% -89%.

Malaria case during 5 years in Djoumouna

The number of malaria cases recorded during 5 years (from 2012 to 2017, except 2014) in the locality of Djoumouna is presented in Table 2 and Figure 2.

Table 4:-Distribution of Malaria incidence in Djoumouna from 2012 to 2017 (SCI / Djoumouna.).

Year	2012	2013	2015	2016	2017
All pathologies recorded in SCI	231	490	331	290	1288
Malaria cases registered with SCI	85	237	188	198	933
% of malaria cases recorded in SCI	36,8	48,4	56,8	68,3	72,4

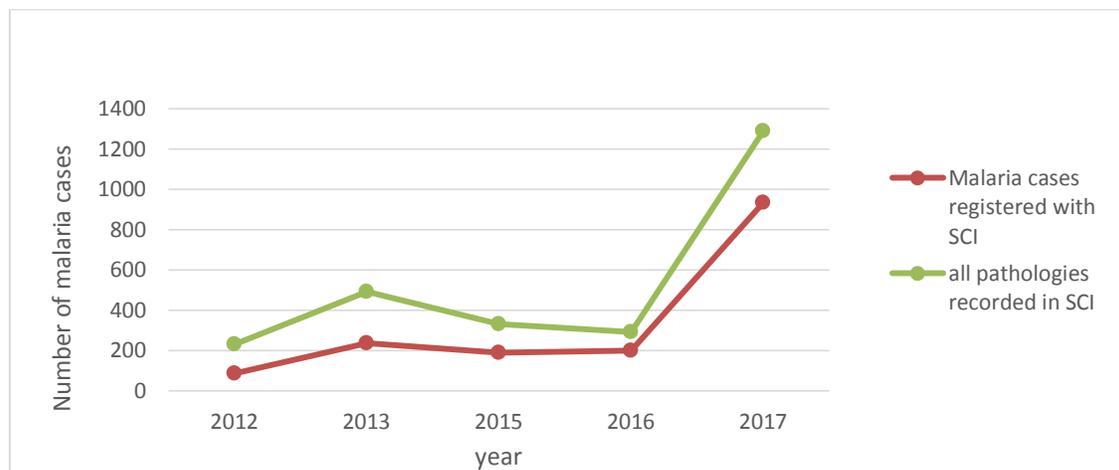


Figure 2:-Malaria incidence in Djoumouna from 2012 to 2017 (SCI / Djoumouna.).

Discussion:-

The locality of Djoumouna has abiotic characteristics which favor the perpetuity of the transmission of malaria. There are 4 streams crossing the locality to which we must add opportunistic breeding sites of *An. gambiae s.l.* as the many fish ponds (Carnevale et al., 1978, 1979a, 1979b).

As has been observed in many African countries, it is recognized that hydro-settlements (ponds, rice fields and dams, etc.) are suitable habitats for vector development (*An. gambiae s.l.*, *An. Funestus*) and thus favorable to the long-term transmission of malaria (Ravoahangimalala et al., 2003; Doannio et al., 2002).

Temperature and humidity are among the most important environmental criteria in the life cycle of culicidae. The temperatures and humidity recorded in the bedrooms are included in the temperature and humidity fringe that allow the life cycle of the Anopheles (MR4, 2016; OMS, 2014) to be realized.

These degrees of temperature have an impact on the number of egg-laying, the maturation of eggs, the frequency of blood meals, the population dynamics and the density of these. These humidity levels have an impact on the survival of adult stages.

In Djoumouna, the use of mosquito nets is an integral part of the population's behavior in the fight against malaria. However, the main obstacle to acquiring a new mosquito net is financial. Indeed, to ensure the protection of a family, it is generally necessary to have more than two mosquito nets, which is not always obvious for heads of families whose incomes are precarious.

Although other species were captured in the locality (*An. Moucheti*; *An. Paludis*; *An. Hancocki*; *An. Funestus* and *An. Nili*) in previous studies (Carnevale et al., 1979, 1985; Bitsindou, 1983) *An. gambiae s.l.* is the main anopheline species collected during our study. It provides the bulk of malaria transmission, as has been observed in several countries in sub-Saharan Africa (Bitsindou, 1983; Zoulani et al., 1994; Trape et al., 1987; Ndiath et al., 2012; Metelo et al., 2015; Julvez et al., 1998; Faye et al., 1998).

As with sow, semi-gravid and gravid females, the results show that partition rates, females, semipregnated, gravid and sporozoite rates are comparable regardless of presence, absence or the condition of the mosquito net (Tables 1 and 2). These data confirm that we are in the presence of the same anopheles population.

Relatively high rates of siphon females and semi-pregnant females reveal endophile behavior in the targeted anopheline population. This observation shows that at the time of the study, ie 5 years after the mass distribution, the remanence of the LLINS is no longer appropriate because the duration of this remanence is in average of 3 years (Darriet et al., 2000; OMS, 2002). This reduction in effectiveness or loss of remanence is often accentuated by maintenance (washing with detergent) and by the misuse of these LLINS which cause tearing of the latter (OMS, 2002). This assertion is corroborated by the low mortality rates (2, 5%, 5% and 7%) indicated by the results of efficacy tests performed.

According to the WHO, a net meets the criteria for conical bioassays when, after several washings, the net induces mortality $\geq 80\%$ after 24 hours at exposure and / or $\geq 95\%$ KD after 60 minutes of exposure (WHO, 2011). It is in this evolutionary context that we deliberately considered mosquito nets not as LLINS but as simple mosquito nets throughout our presentation.

However, despite the almost non-existent insecticidal effect of targeted LLINs, the use of mosquito nets in good condition or even torn or in poor condition significantly reduces the transmission of malaria at the community level in Djoumouna. Logically, it appears that people who sleep without mosquito net are those who are the most stung. The comparison results show that, even when torn apart, mosquito nets contribute a bit to the reduction in the number of bites received by people sleeping under this mosquito net. In fact, depending on the state of the net, it has been observed that malaria transmission is on average (i) an infective bite per person every 5,6 months in the case of the use of intact mosquito nets, (ii) an infective bite per person every 1, 7 months in houses with torn bed nets and an infective bite per person every two weeks in persons who do not sleep under a mosquito net. Regarding the use of mosquito nets in good condition, our results show that if they are used appropriately and wisely, the malaria transmission is less than one infective bite per person every 6 months, in average.

From the late '70s to the early' 90s, studies have shown that performance related to net use can be significantly improved with the use of LLINs distributed during mass distribution campaigns. The use of LLINS usually reduces the rate of entomological inoculation of malaria in the communities that use them. In Djoumouna and surrounding areas, the systematic use of LLINs by the population has resulted in a dramatic reduction in the rate of entomological inoculation, which has increased from an average of 1000 infective bites / person / year, in the 1970s, to 7 infective bites / person / year (Carnevale et al., 1978; Julvez et al., 1998; OMS, 2002), in the 1980s. In Burkina Faso, entomological inoculation rates increased from 55ib/p/yr to 3 ib/p/yr after using Deltamethrin-treated bed nets (Robert et al., 1991). These results are also in agreement with those obtained by other authors in Africa (Metelo et al., 2015; Faye et al., 1998), who observe decreases in aggressive density after use of impregnated mosquito nets. In general, the low number of sessions and the method of capture used could explain the low number of vectors collected. It is recognized that the numbers of residual fauna are still lower than the numbers obtained with landing catch.

The evolution of malaria cases from 2012 to 2017 (except 2014) shows a significant variation in malaria transmission in Djoumouna. Indeed, from 2012 to 2013, a rise in cases is observed. Then a steady decline is recorded from 2013 to 2016, then the figure shows a rise in cases with a peak in 2017. The rise in cases observed between 2016 and 2017 could be explained by the lack of protection of populations by the nets (loss of remanence) and could also reveal the need to implement a mass distribution of LLINS at the national level or at least at the local level.

The effect of the mass use of LLINs has been, for the community at large, a reduction in the level of malaria transmission that has been enhanced by ACTs (Artemisinin-based Combination Therapy) for Malaria access

treatment between 2013 and 2016. ACT treatment reduced parasite pools for the entire human community. These results are in line with those obtained throughout the national territory for the year 2012 during which 117,610 cases of malaria were recorded (PNPL, 2013). In Djoumouna, malaria data obtained at CSI from 2012 to 2017 (except 2014) showed a drastic reduction in cases of this pathology during the period when the effectiveness of the insecticide used for the impregnation of the LLINs was still in place. This decline was due to the massive use of LLINs distributed during the campaign in 2011-2012 which resulted in a significant reduction in malaria transmission. The coverage rate of the LLINs had been higher than 80% in the department of Pool (PNPL, 2012). Studies of the use of LLINs in other African countries have shown that a high coverage rate of LLIN use results in a significant reduction in parasite density (Yadouleton et al., 2017), and that synergy of ACT treatments and the use of LLINs had the epidemiologic effect of the collapse of the number of malaria cases in a holoendemic zone (SPE, 2013).

However, several social behaviors can be evoked to explain the rise of malaria cases in 2013: the diversion of the LLINs from their use by some beneficiaries to make fishing nets or barriers to protect crops against birds and other pests cultures; the refusal of some people, allergic to insecticides used, claus-trophobes or intolerant to the heat to continue to sleep under the LLINs; difficulty in sleeping under LLINs the negligence of unrolling the LLINs to sleep under it; the resale of the LLINs; loss of LLINs. The results obtained show that after the period of effectiveness of the remanence, which is on average 3 years after the mass distribution, the LLINs lose their role of chemical barrier. After this period, all that remains for them is the role of physical barrier which in the long run is damaged by the deterioration of its integrity. These observations (loss of persistence and deterioration of LLINs) result in a reduction in population coverage as a result of a dramatic increase in malaria cases that could spread nationally for the years 2018 and beyond, as long as no mass distribution is implemented.

Conclusion:-

The presence of *Plasmodium* and the existence of numerous fish ponds in Djoumouna favorable to the proliferation of *An. gambiae s.l.* make the locality of Djoumouna and its surroundings an area of intense and lasting transmission of malaria. Although simple mosquito nets protect against vector bites, LLINs are an effective public health tool for controlling malaria and other vector-borne diseases. However, for this to be the case, this effectiveness must be subject to the effectiveness and regularity of the implementation of a mass distribution of LLINs every 3 years and to an adequate awareness of the use of those at the level of the populations concerned. The respect of this regularity, the good sensitization of the populations on the use of the LLINs and the adequate management of the malaria attacks in the health centers are the sine qua non conditions to maintain at the lowest level the transmission of the malaria.

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