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Effect of indoor residual spraying on the entomological parameters of malaria transmission in Nassian, North-East in Côte d'Ivoire

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ABSTRACT: To reduce malaria-related morbidity and mortality in Côte d'Ivoire, the National Malaria Control Program selected Indoor Residual Spraying as a complementary strategy to long-lasting insecticide-treated nets widely distributed in the country. As such, the current study was conducted to collect baseline information before IRS from May 2019 to August 2020 and to evaluate its effect on entomological parameters of malaria transmission from September 2020 to August 2021 after the intervention in Nassian, a high malaria endemic district of Côte d'Ivoire. Vectors were identified both morphologically and molecularly. the parity rate and the presence of *Plasmodium falciparum* sporozoite was determined.

A total of 7,401 females of *Anopheles* were collected, of which 73.4 % before IRS and 26.6 % after IRS. *Anopheles gambiae s.s.* (85.2 %), *An. funestus s.l.* (14.2 %), *An. nili* (0.3%) and of *An. pharoensis* (0.3%) were collected. In baseline, the mean biting rate of *Anopheles gambiae s.s* was 17.5 bites/person/night. The mean parity rate was 81.5% with an average entomological inoculation rate of 1.2 infective bites/person/night. However, after IRS implementation, these parameters decreased respectivelly to 6.1 bites/person/night, the parity rate to 69.3 % and the EIR to 0.2 infective bites /person/night.

A significant decrease of all entomological parameters was observed after the implementation of the IRS showing the positive effect of IRS on the vectors. However, following monitoring should be required including an epidemiological assessment to support the impact that was observed. This will guide the National Malaria Control Programme for future decision making and advocacy.

KEYWORDS: Malaria, *Anopheles gambiae,* IRS, Nassian, Côte d'Ivoire.

1 INTRODUCTION

Malaria is a major global public health concern and a leading cause of morbidity and mortality in most tropical regions of the world [1]. Globally, the World Health Organization (WHO) estimated 249 million malaria cases which 94 % cases and 95 % of morbidity in the African region in 2022 [2].

Long-lasting insecticide treated-nets (LLINs) and indoor residual spraying (IRS) of insecticides ever since represent the two WHO recommended vector control strategies that have shown effective impact on the disease [3]. Many studies have shown the efficacy of IRS and LLINs in reducing malaria transmission and prevalence [4], [5], [6]. However, these methods, especially LLINs, rely on the use of pyrethroid insecticides for which mosquitoes have developed resistance.

In Côte d'Ivoire, malaria remains the deadliest endemic disease despite the efforts of the government and its partners. The disease is still the first reason for consultation, counting for 33%, and the majority of the population (81%) living in areas with an incidence ranging from 300 to over 500 per thousand [7]. The incidence of malaria has increased from 154.6 to 229 $^{0}/_{00}$ in the general population and from 286.9 to 596.4 $^{0}/_{00}$ in children under 5 years of age from 2016 to 2019 [8], [9], [10], [11]. Malaria transmission is stable throughout the year with peaks during the rainy season [12]. To reduce malaria-related morbidity and mortality in Côte d'Ivoire, the NMCP adopted mass and routine distributions of LLINs every three years while IRS was advocated as a complementary strategy during the national strategic plan (2016-2020). To this end, the health districts of Nassian and Sakassou were selected to receive the first IRS campaign using clothianidin-based insecticides.

Although IRS has proven to be a key strategy for malaria elimination in many countries in the world (Asia, Russia, Europe, Latin America) and used in some African countries (Rwanda, Sénégal, Madagascar, Mali, Burkina Faso) for vector control **[**13], [14], IRS in Côte d'Ivoire was just localized and had never been implemented widely. Conducting a community IRS using new insecticide requires impact monitoring and evaluation to guide the NMCP in the perspective of extending to other districts. This study was therefore conducted to assess entomological parameters of malaria vectors before and after IRS implementation in Nassian health district.

2 Material And Methods

2.1 Study Sites

The study was conducted in the Nassian health district (8° 27' N; 3° 28' W) in the Bounkani region at the North-Eastern of Cote d'Ivoire (Fig. 1). The climate is of Sudanian type, with four seasons, a dry season which lasts from 4 to 5 months and It extends from November to March in recent years and a rainy season lasts 7 à 8 months, from April to October with a short dry season (July - August) [15]. The annual average rainfall varies between 900 and 1200 mm. The monthly average temperature varies between 24,3 °C and 28°C [16], this district covers an area of over 164 km2 with a population estimated to 48,170 inhabitants in 2018 in [15], [16], [17]. Two types of houses were found in Nassian: traditional (mud walls) and modern (cement walls).

Two sites, one in urban and one in rural setting, were selected for mosquito collections. Nassian, an urban area (8° 27' 11" N latitude and 3° 32' 4.22"W longitude with 4,785 peoples in 2014), and Parhadi, a village (8° 27' 18" N latitude; 3° 28' 3" W longitude with 2,823 peoples in 2014). In the health district of Nassian, malaria transmission is permanent with an upsurge during the rainy season. Mosquito breeding sites are created particularly during the rainy seasons. The choice of this district for the implementation of the first phase of IRS in Côte d'Ivoire lied on its high mararia endemicity [18]. In 2016 and 2017, the Nassian health district recorded the highest incidence of malaria cases (377.4 ‰ and 369.7‰ respectively) in the general population of Côte d'Ivoire [10,11]. Nassian recorded the highest infection rate and reported the second highest malaria incidence of the country in 2018–2019 [18].

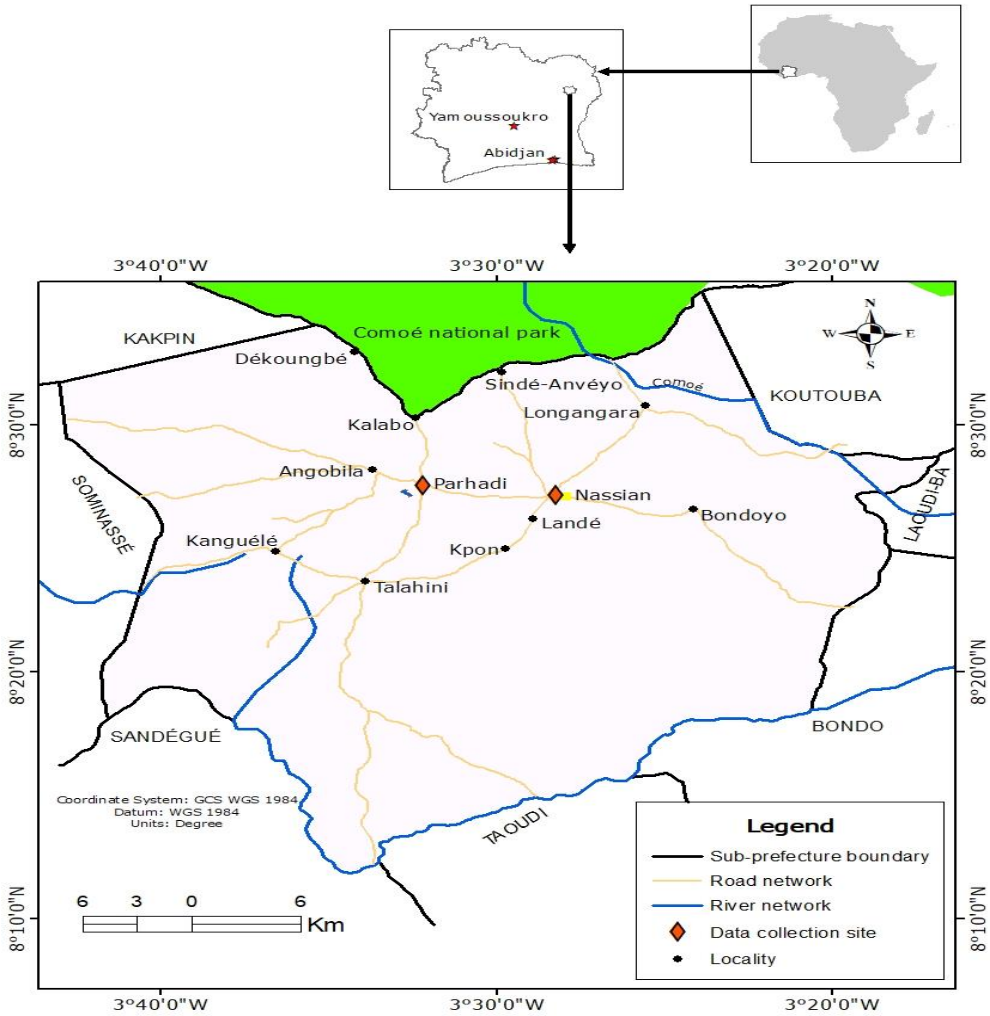

Fig. 1. Maps showing the study sites

2.2 Adult Mosquito Collection

Adult mosquitoes were collected using human landing catch (HLC), pyrethrum spray catch (PSC), and Centers for Disease Control and Prevention (CDC) light trap methods [19], [20]. The HLCs were conducted during two consecutive nights in four houses (two urban and two rural) from about 6: 00 p.m to 6: 00 a.m per month. The PSCs were conducted in 30 houses (15 in each setting) per month. They were carried out between 6: 00 a.m. and 8: 00 a.m. during two consecutive days per month. The CDC light trap collections were performed in four houses (two per setting) during two consecutive nights per month. CDC light traps were installed inside the bedrooms. The trap was suspended in a room at 1.5 meters from the floor and collection started from about 6: 00 p.m. to 6: 00 a.m. The same houses were maintained for HLC and CDC light trap collections, while randomly selected houses were used each month for PSC collections depending on the availability of households (Fig. 2). The entomological parameters assessed were vector composition, seasonality, biting behavior, sporozoite rate, parity rate, and entomological inoculation rate (EIR). To measure the impact of the intervention on these entomological parameters, we compared the values indicated from baseline and after the first IRS.

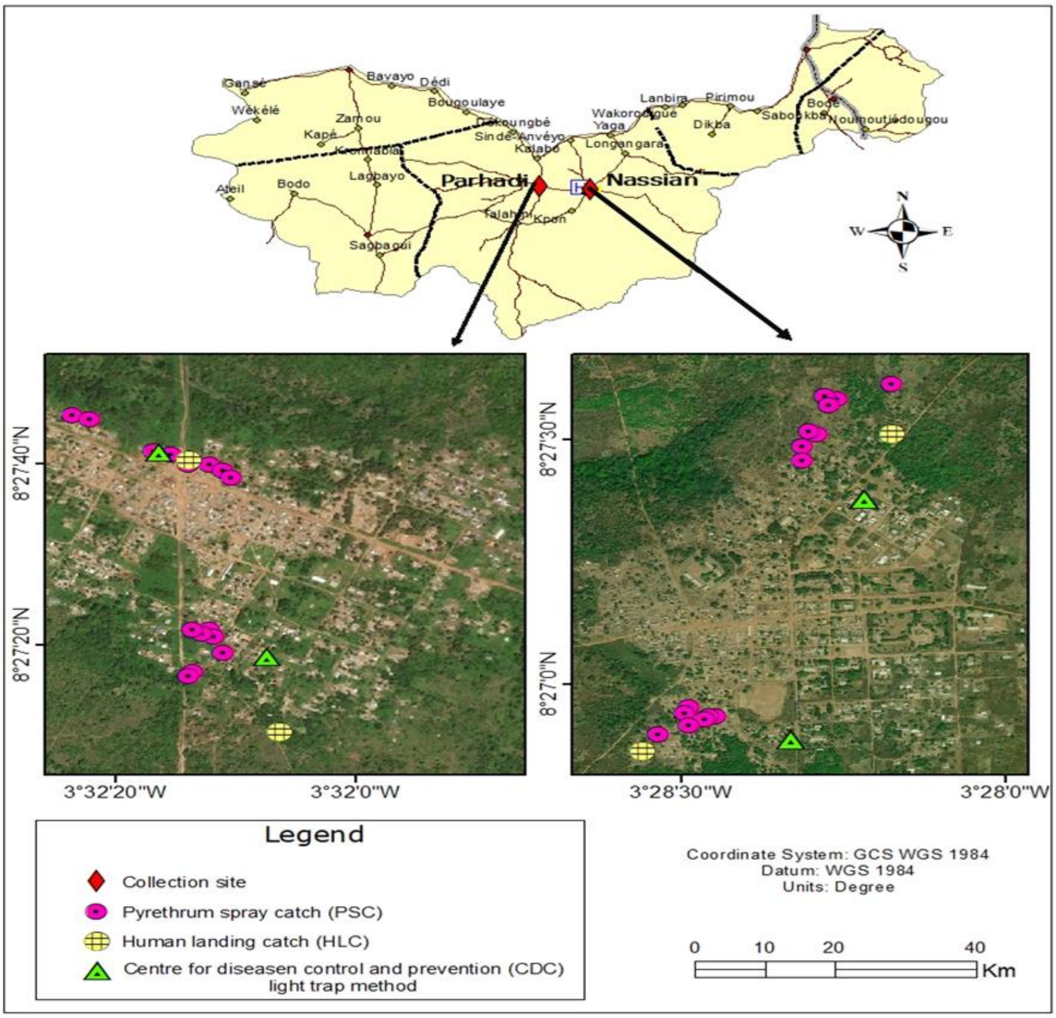

Fig. 2. Map of department of Nassian showing study area and mosquitoes collections sites in Nassian and Parhadi

2.3 Field Mosquito Processing

All mosquitoes collected, were identified and recorded. *Anopheles* mosquitoes were identified using identification keys to species level [21], [22]. Female of a subsample of *An. gambiae s.l.* from HLC were dissected to estimate parity rate, by observing the coiling degree of ovarian tracheoles [23]. All mosquitoes were stored individually in Eppendorf tubes contained silica gel, labelled according to the study site point and date of collection. These sample were subjected to subsequent molecular analysis in the laboratory at the Centre Suisse de Recherches Scientifiques en Côte d'Ivoire to identify the species of the *Anopheles complex,* infection status, using polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA), [24].

2.4 Laboratory Processing Of Mosquitoes

A subsample of *An. gambiae* s.l was preserved from the HLC, PSC, and CDC-light trap collections, respectively, to determine subspecies of *An. gambiae* s.l. Those subjects were used for molecular identification using SINE200 PCR according to the protocol of Santolamazza *et al* [24]. The procedure begins with mosquito DNA extraction, followed by amplification and electrophoretic migration, and ends with visualization. The DNA of each individual mosquito was extracted using the protocol designed by Collins *et al* [25]. *An. gambiae* complex species were identified as *An. gambiae* s.s, *An. Coluzzii,* or hybrids of the two species, following the SINE PCR protocol described by Santolamazza *et al [*24*].*

The sporozoite infection status was determined from head and thorax of a subsample of parous mosquitoes collected by HLC. The ELISA technique for the detection of *Plasmodium falciparum* circumsporozoite infection is the commonly used method for determining the infection rate of mosquitoes according to the WHO [26]. Only *Plasmodium falciparum* was detected, since it is the predominant causal agent of malaria in Côte d'Ivoire [13, 27].

2.5 Statistical Analysis

Data were entered using Epi info 7.0 software and transferred to Excel 2016. STATA version 19.10.1.559 (IBM, New York, USA) software used for statistical analysis. Pearson's Chi-square test was used to compare the proportion of parameters (density, human biting rate, parity rate, infection of antigen sporozoite rate and entomological inoculation rate) between the entomological data before and after intervention. A difference is considered as significant when the p-value is less than 0.05.

2.6 Ethical Considerations

The surveys were conducted in accordance with the study protocol approved by the National Ethics Committee for Life Sciences and Health (CNESVS) of the Ministry of Health and Public Hygiene. The consent of all volunteers was obtained before their participation in the study. No houses were selected for capture without the advice of the head the family or his representative.

3 Results

3.1 Mosquitos' Species Composition

From May 2019 to August 2021, A total of 8, 993 mosquitoes were collected in the two studies sites regardless the three capture method, 69.8 % (6, 277) in baseline and 30.2 % (2 716) after IRS ($\chi 2$ = 956.711, p = 0.001). *Anopheles* was predominant (82.3 %), while *Culicines* accounted for 17.7 % of the mosquitoes collected. *Anopheles gambiae s.l.* (85.2 %) was the most common mosquito followed by *Anopheles funestus s.l* (14.3 %) (Table 1). Other species of *Anopheles* were also collected but in very small proportions. Only 0.3 % (20) of *An. nili* and 0.2 % (18) of *An. pharoensis* were collected. We analyzed 608 samples of *An. gambiae s.l.* for their species and all were determined to be *An. gambiae* sensu stricto (s.s.), there were no *An. coluzzii* in baseline.

Table 1. Abundance of Anopheles mosquitoes collected between May 2019 and August 2021 in Nassian and Parhadi using three collection methods

	Density for May 2019 ‐ August 2020			Total Baseline	Density for septembre 2020 ‐ August 2021			Total after IRS	TOTAL
Species	**HLC**	**PSC**	**CDC-LT**		**HLC**	**PSC**	**CDC-LT**		
An. gambiae s.s	3 917	736	67	4 720 (86.9 %)	1 179	386	21	1 586 (80.5 %)	6 306 (85.2 %)
An. funestus s.l	470	207	24	701 (12.9 %)	262	83	9	354 (18 %)	1 055 (14.3 %)
An. nili	1	0	0	1 (0 %)	20	1	0	21 (1 %)	22 (0.3 %)
An. pharoensis	10	0	0	10 (0.2 %)	8	00	0	8 (0.4 %)	18 (0.2 %)
TOTAL	4 398 (84.8%)	943 (13.8%)	91 (1.4%)	5 432 (100 %)	1 469 (71.3 %)	470 (26.3 %)	30 (2.4%)	1 969 (100%)	7 401 (100%)

HLC: Human land catch; PSC : Pyrethrum spray catch ; CDC-LT : Center disease control ‐ Light trap

3.2 Dynamic Of Anopheles Gambiae S.S And Anopheles Funestus Collected By Human Land Catch Method In Baseline

Anopheles populations in urban (54 %) and rural (46 %) zones presented the same dynamics in baseline although it had a statistically significant difference in density between the two zones (*($\chi 2$ = 18.52,* p = 0.001). May to August was a period of low density with 3.9 % (n= 170) of Anopheles, and low transmission (0.09 infective bites/ person/night). However, from September to December, 79.8 % (n = 3508) of Anopheles mosquitoes were collected representing the period of high density and high transmission (1.95 ib/p/n). Finally, from January to August 2020, 16.3% (n= 709) Anopheles were collected (Fig. 3), with an entomological inoculation rate at 0.45 ib/p/n. Anopheles vectors had a endophagy rate in both the two studies zones. Anopheles ga*mbiae* s.s. biting activities were higher between 00: 00 and 05: 00 hours during the study period, both indoors and outdoors (Fig. 4). The lowest biting rates of An. gambiae s.s. were observed from May to August 2019 (2,6 b/p/n). However, from September onwards, there was an increase of biting rates until December (50,6 b/p/n) with a peak in October (100,8 b/p/n) after the rainfall peak in September (Fig. 5). As soon as An. Gambiae populations decreased, An. Funestus populations increased from November (12.6 b/p/n).

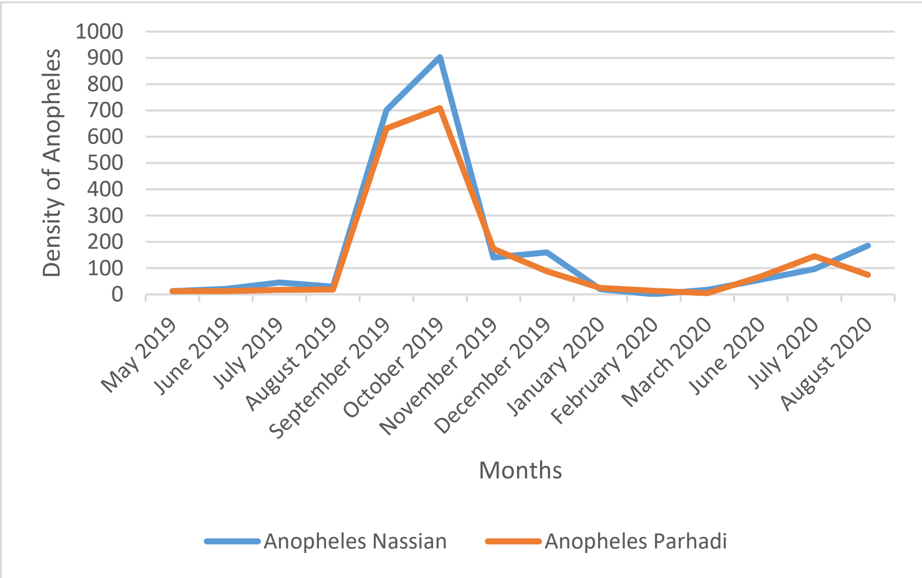

***Fig. 3.** Density of Anopheles vectors in Nassian and Parhadi*

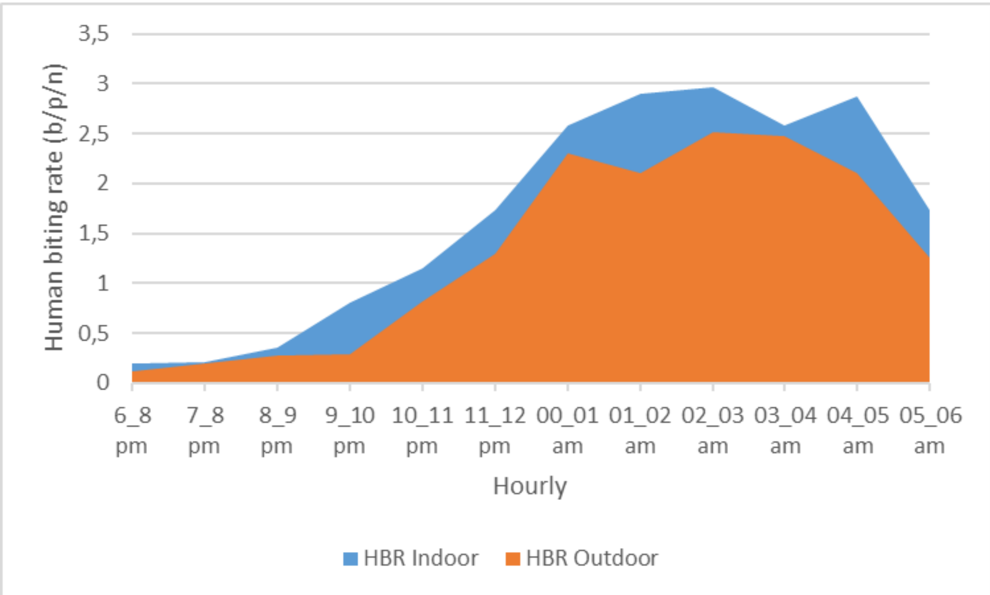

***Fig. 4.** Biting cycle of Anopheles vectors using HLC in baseline*

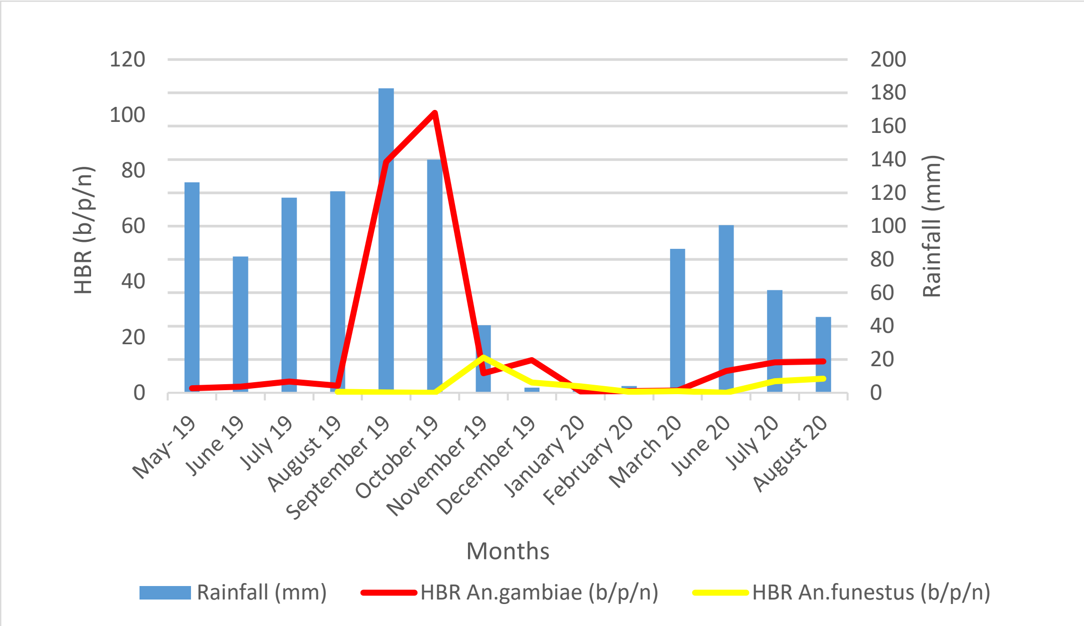

Fig. 5. ***Seasonal variation of human biting rate of An. gambiae s.s. and An. funestus s.l in relation to rainfall***

3.3 Parity Rate

Out of 1,608 dissected mosquitoes (*An. gambiae s.s* and *An. funestus s.l*), 1,311 were found parous before IRS. The average parity rate was as very high from May 2019 to August 2020 (81.5%). There is no significant difference of parity between periods of low and high biting rate.

3.4 Decision Making For The Appropriate Period To Achieve The Indoor Residual Spraying.

As a baseline to indoor spraying in Nassian, entomological monitoring of malaria vectors showed two malaria transmission periods: May to August which was a period of low density and low transmission with an average entomological inoculation rate EIR of 0.09 infective p/h/n. The second period, from September to August with an EIR increased to 0.2 infective b/p/n. Thus, IRS was conducted in August 2020 just prior to the peak of the transmission.

3.5 Effect Of IRS On Entomological Parameters On Malaria Transmission Human Biting Rate And Peak Biting Of Anopheles Species

3.5.1 Variation Of Entomological Parameters Of Anopheles Gambiae S.S

After IRS, the dynamics of vector species abundance showed two periods as before IRS: from September to December after the IRS us high density period and January to August, a low density period. There is a change in biting behavior and abundance of anopheles after IRS; *An. gambiae s.s*. tended to bite both outsides (Fig. 6). The low abundance observed during the dry season from January to March 2020 increased from June onwards. However, after IRS, the lowest densities were observed with only n= 1441 *Anopheles* vectors were collectedfrom September 2020 to August 2021 compare to Septembre 2019 to August 2020, before IRS (n = 4216) **(**$\chi 2$ = 940.63, *p* = 0.001). The peak of *An. gambiae s.s* caught by HLC in October decreased to 58.7 % after IRS, The average human biting rate for *An. gambiae s.s*. decreased from 17.5 to 6.1 p/h/n after IRS with 65 % of reduction, (Fig. 7).

Overall, out of a total of 1 078 head-thoraxes of *An. gambiae* s.s was assessed by ELISA, 72 were positive for the circumsporozoite antigen of *Plasmodium falciparum* before IRS, [Fig. 8]. The average proportion of *An. gambiae* s.s. mosquitoes infected by *P. falciparum* was 6.6 %. Two peaks were observed in the variation of the sporozoite index for *An. gambiae* s.s. A peak of 6.8% was recorded in July during low biting rate period and another for the high biting rate period with a maximum of 11.2% in November.

The entomological inoculation rate of *An. gambiae s.s.* decreased from 1. 2 infective bites/p/n in baseline to 0.2 infective bites/p/n after IRS with a maximum of 3.2 infective b/p/n in October 2019, [Fig. 9].

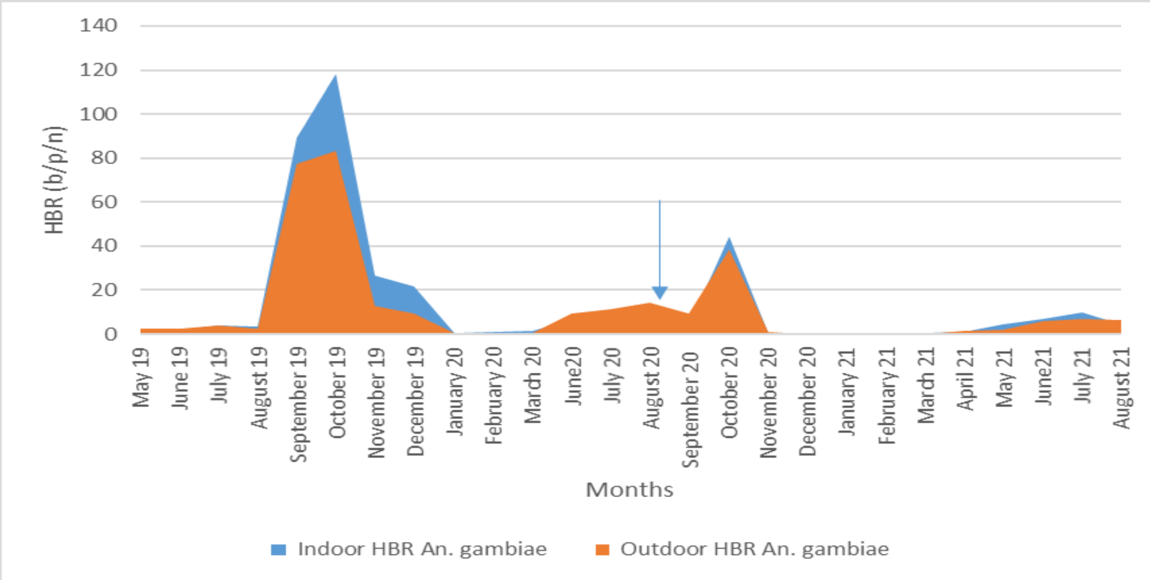

Fig. 6. Variation of Monthly human biting rate (b/p/n) of An. gambiae s.s

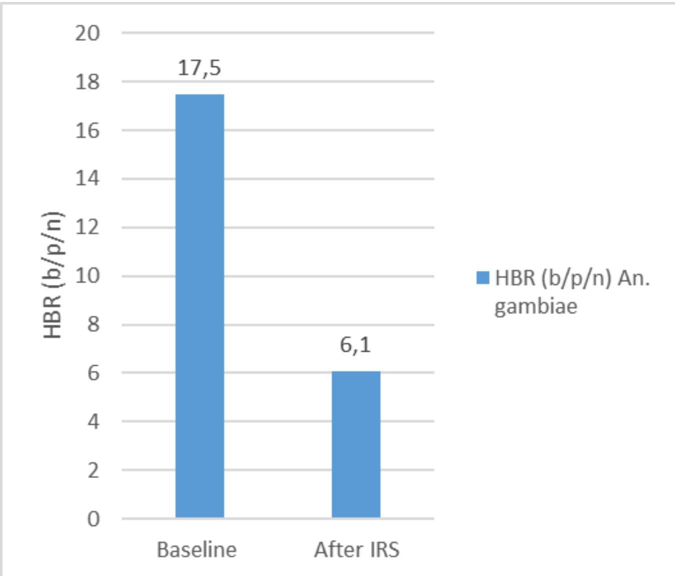

Fig. 7. Variation of Monthly human biting rate (b/p/n) of An. gambiae s.s

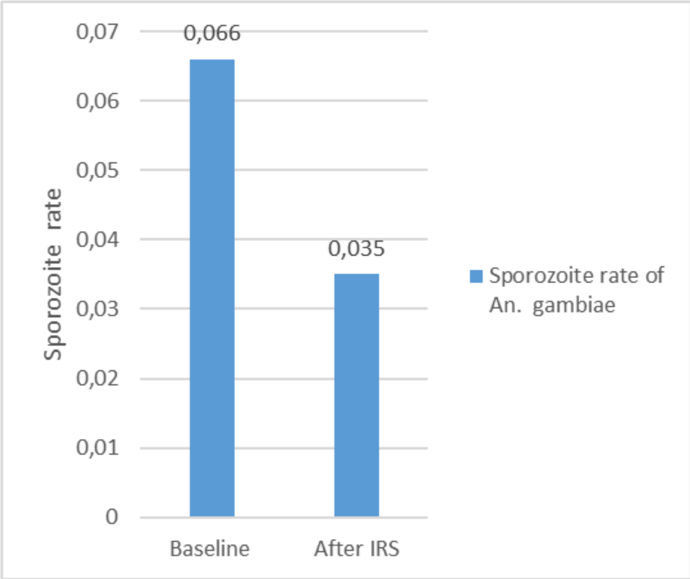

Fig. 8. Variation of sporozoite rate of Anopheles gambiae s.s before and after IRS in Nassian

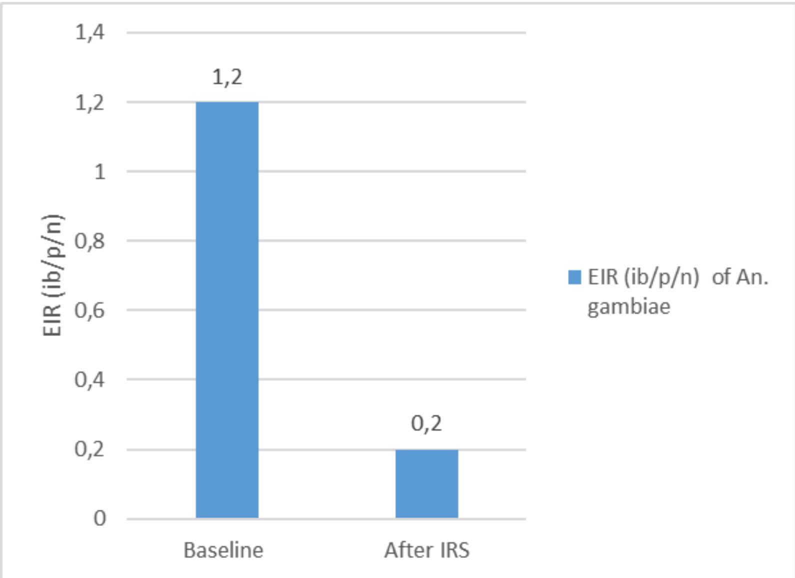

Fig. 9. Variation of entomological inoculation rate of Anopheles gambiae s.s before and after IRS in Nassian

3.6 Variation Of Entomological Parameters Of Anopheles Funestus S.L

After IRS, a reduction of *An. funestus s.l* entomological parameters was observed. The human biting rate decreased of 64 % despite being present every months of 2020 (Fig.10). The peak of *An. Funestus s.l* caught by HLC in November 2019 at the start of the dry season decreased after intervention. *Anopheles funestus* represented 22.5% of the infected mosquitoes before IRS. The *An. funestus s.l* average entomological inoculation rate, wich was to 0.23 ib/p/n for baseline decreased to 0.06 ib/p/n after IRS in 2020.

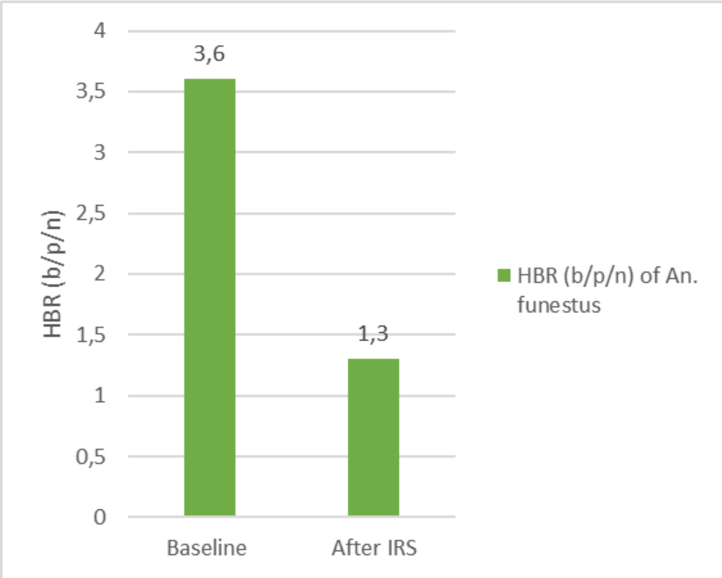

Fig. 10. Variation of Human biting rate of An. funestus before and after IRS in Nassian

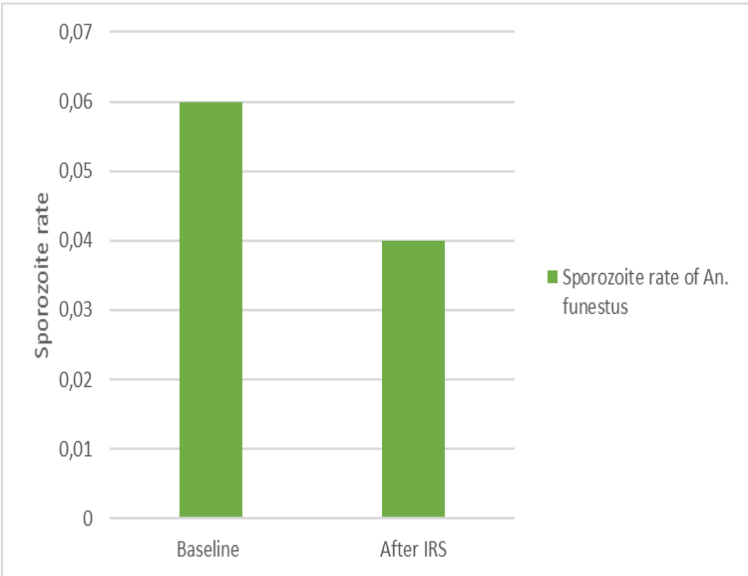

Fig. 11. Variation of spozoite index of An. funestus before and after IRS in Nassian

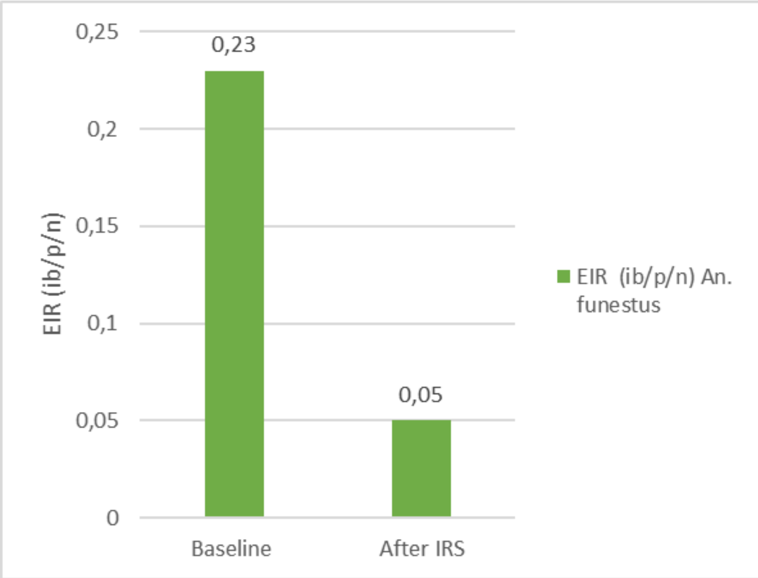

Fig. 12. Variation of entomological inoculation rate of An. funestus before and after IRS in Nassian

3.6.1 Variation Of Parity Rate

Out of 989 dissected mosquitoes (*An. gambiae s.s* and *An. funestus s.l*), 686 were found parous after IRS. The average parity rate decreased from September 2020 to August 2021 (69.3 %). There is a significant difference of parity between before and after IRS ($\chi 2$= 6.782, *p* = 0.009).

4 Discussion

Anopheles gambiae s.s was the main vector of malaria in Nassian, it was following by An. funestus in baseline and after IRS. Malaria transmission in Nassian was caused by two vectors: *An. gambiae* s.s and *An. funestus* s.l, the two main malaria vectors in West Africa [28], [29]. High *An. gambiae* s.l. densities in both urban and rural area would be due of the lower level of urbanization of Nassian [30]. The main malaria vector species identified by PCR was *An. gambiae* s.s recognized as the major vector of malaria present in humid savannah zone in Côte d'Ivoire [29]. The density peak was observed during the rainy season for *An. gambiae* s.s. It started in September and reached its maximum in October. This peak was consecutive to the rainfall peak. Indeed, in the absence of lowland rice fields and market gardening in the study area, *Anopheles gambiae* s.l breeding sites were temporary and sunny water collections related to rainfall. Those, were naturals puddles, small collection of water that no longer flow when the rains decreased in October. The results obtained in the present study were consistent with those generally obtained in the savannah region of west Africa where malaria transmission is related with fluviometric rhythm and the density of vector populations [31].

In baseline period, the study showed that the period of high density and high transmission was from September to December. Thus, indicating the appropriate period for carrying out IRS which would be August to cover the period of high transmission. This is because the required coverage must be achieved prior to the period of high transmission and must be maintained for the duration of the period [3].

As with the human landing catch, the densities of An. gambiae and An funestus, were relatively high from january to august 2020 compared to 2019. However, after IRS the peak of human biting rate in September-October of *An. gambiae* was reduced by 73.7% from 2019 to 2020. For *An. Funestus, it* decreased of 8.18% in 2019 to 3.5 % in 2020, although it was present all year with a pic in July before IRS. This significant difference could be due to the IRS conducted in August 2020. These results were consistent with those obtained in Mali which a significantly lower density was observed between the test and control village up to 6 months after IRS **[**33**].** Indeed, a spray is effective for 3 to 6 months, sometimes up to a year depending on the formulation of the insecticide used and the type of surface sprayed [1,38]. After IRS, *An. gambiae* s.s and *An. funestus* s.l tended to bite both outside. This change in behavior could be due to IRS as observed in Benin [34]. Hourly activity was always intense in the second half of the night between 00: 00 et 05: 00 am.

The parity rate and the EIR before and after were good indicators to assess the impact of an indoor residual spraying [14]. The parity rate is very high in baseline period. There was no significant difference between periods of low and high biting rate (p= 0.944). The probability of mosquitoes becoming infected and transmitting malaria remained high throughout the year. This justified the high infection rates that were observed for *An. gambiae* s.s and *An. funestus* s.l, as well as the EIR that reached its peak in October. These results validated the choice of Nassian for the pilot phase for IRS in Côte d'Ivoire and consistent with those obtained in urban areas of permanent and high malaria transmission with very hight parity rate [35]. This study showed that *An. funestus* s.l. maintained malaria transmission at the end of the rainy season and during the dry season when those of *An. gambiae* s.s. were very low. However, in 2020, from January to March, during the dry season, the parity rate decreased as well as the abundance of malaria mosquito vectors as authors have found in a study in Burkina Faso [36**]**. From the first rains, in June the parity rate increased with the densities of *An. gambiae* s.s but with a decrease compared to 2009. This behavior of *Anopheles* may be due to the availability of larval sites linked to rainfall**.** The average parity rate for *An. gambiae* s.s and *An. funestus* s.l decreased after IRS compared to baseline (92.6%) from September to December. The particularly high infection rates for *An. gambiae s.s* in baseline were confirmed by the results of the study conducted by Ndiath *et al* in which the S form (*An. gambiae s.s*) was more susceptible to *Plasmodium falciparum* infection than the M form (*Anopheles coluzzi)* [37]. The infection rate was significantly decreased after IRS for *An. gambiae*. s.s and *An. funestus* s.l. The reduction in entomological inoculation rate compared to baseline periods reaffirmed the impact of IRS on entomological parameters of malaria transmission in the study area.

5 CONCLUSION

This study showed that the highest abundance of local populations of *Anopheles* vectors and highest malaria transmission occurred from September to December in Nassian, Côte d'Ivoire. Therefore, the most appropriate time for IRS implementation for an effective control of local malaria vectors in the Nassian health district should be before September to December. IRS reduced the occurrence of high densities for the *An. gambiae* peak in October and the *An. funestus* peak in November and the longevity of species vectors. A reduction in the entomological inoculation rate after IRS was also observed. A significant decrease of all entomological parameters was observed after the implementation of the IRS showing the positive effect of IRS on the vectors. However, following monitoring should be required including an epidemiological assessment to support the impact that was observed. This will guide the National Malaria Control Programme for future decision making and advocacy.

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