Working paper (20-30 pages)

1) Introduction

The scale-up of control strategies targeting malaria vectors (*Anopheles* mosquitoes) such as indoor residual spraying (IRS), insecticide-treated bed nets (ITNs) and the destruction of breeding sites has led to huge progress in the control of malaria in Sub-Saharan African countries [1, 2, 3]. For example, in Senegal, the proportion of mortality attributed to malaria fell from 18.2% to 3.6% between 2006 and 2014 [4, 5]. However, in recent years, the widespread use of insecticides in agriculture [6] and bed net treatment [7, 8] contributed to the evolution of resistant mosquito strains. Resistance to pyrethroids is a particular threat for malaria control, since pyrethroids are currently the only recommended and approved insecticides for treating bed nets, primarily because of their low toxicity to humans compared to other pesticides [9]. Therefore, there is great concern that rapidly spreading pyrethroid resistance may annul recent successes in the fight against malaria with ITNs [10, 11, 12].

There are two main molecular mechanisms involved in insecticide resistance in general, and pyrethroid resistance in particular: target site resistance and metabolic resistance. Target site resistance results from a mutation at a site targeted by the insecticide (in the mosquito), that lowers the impact of the insecticide. Two well-known molecular markers of target site resistance are the *kdr* and the *Ace1* loci. At the *kdr* locus, resistance is often associated with a replacement of the 1014L 'sensitive' allele (hereafter referred to as *kdr-S*, for sensitive) by either the 1014S or the 1014F (hereafter referred to as *kdr-R*), both 'resistant' alleles. The 1014F allele was originally widespread in West Africa while the 1014S was originally widespread in East Africa, so these two resistant alleles are also sometime referred to as *kdr-w* and *kdr-e*, respectively. But these alleles have now widened their geographical distributions [13]. In this project, *Ace1* alleles associated with susceptibility (wild type allele) and resistance (G119S allele) are referred to as *Ace1-S* and *Ace1-R*, respectively. Metabolic resistance entails changes in the metabolism of the insecticide before it reaches its target. Currently there are no markers for metabolic resistance, though leads are being investigated [14].

Two laboratory-controlled studies indicate that *kdr-R* and *Ace1-R* alleles lead to increased *Anopheles'* susceptibility to *Plasmodium* [15, 16]. This may in turn lead to increased *Plasmodium* infection rates in mosquitoes, and therefore, in some situations, to an increased entomological inoculation rate (EIR) compared to the situation before insecticides were widely used. Thus, if the frequency of resistant alleles is high and if *Plasmodium* infection is increased in resistant mosquitoes compared to sensitive mosquitoes [17], there is a risk that malaria transmission may become even higher than it was before the scale-up of ITNs.

In Dielmo, a demographic surveillance site (DSS) 280 km south from Dakar, Senegal, a rebound in malaria prevalence was indeed recently recorded both in *kdr-R* mosquitoes and in humans [10, 18]. In this situation, the impact of ITNs was strong enough to suppress malaria prevalence in villages to a low but persisting level despite the frequency of insecticide-resistant mosquitoes approaching 10% in 2010. However, we do not know how further increases in the frequency of resistance alleles or human behavioural changes would impact EIR, and therefore malaria prevalence in this location. Furthermore, in Dielmo [19] and in Benin [20], there is a suspicion that some mosquito species are changing their behaviour, allowing them to bite in day time, when people are not protected by mosquito nets. It is possible that the mosquitoes biting in day time are sensitive to pyrethroids because they avoid contact with ITNs. Changes in the indoor/outdoor biting ratio also have been recorded after the introduction of ITNs in Tanzania [21]. But, there is controversy that *kdr-R* alleles might impact mosquito longevity (data show an impact under some conditions but not all) [22], and that infection of *kdr-R* mosquitoes might impact their level of resistance [23], revealing potentially complex interplays between these important factors of vectorial capacity.

The vectorial capacity of *Anopheles* to transmit malaria depends not only on these entomological factors, but, amongst others, also on human behaviour regarding the use of bed nets and insecticides [24, 25], and on current and future frequencies of resistance alleles in anopheline populations. Therefore, the vectorial capacity and its consequences on malaria transmission are expected to vary from one place to another and over time.

This project aimed **to assess how rates of malaria transmission depend on the alleles associated with pyrethroid resistance, and concomitant behavioural changes in mosquitoes and humans.**

An international approach was needed to assess the extent and the geographical variability of the risk of insecticide-resistant *Anopheles* increasing susceptibility to *Plasmodium* infection, and its impact on malaria epidemiology and therefore on malaria control. The complexity of genetic, entomological and human behavioural factors also justifies a trans-disciplinary approach. Entomological field and lab work are both needed to measure *Plasmodium* susceptibility of *kdr-R* and *Acel-R* mosquitoes in order to assess how widespread the phenomenon is likely to be, and whether it is specific to some mosquito populations. We also need to measure various entomological parameters like mosquito biting behaviour, because variations in these parameters will also impact malaria epidemiology. Evolutionary biology is

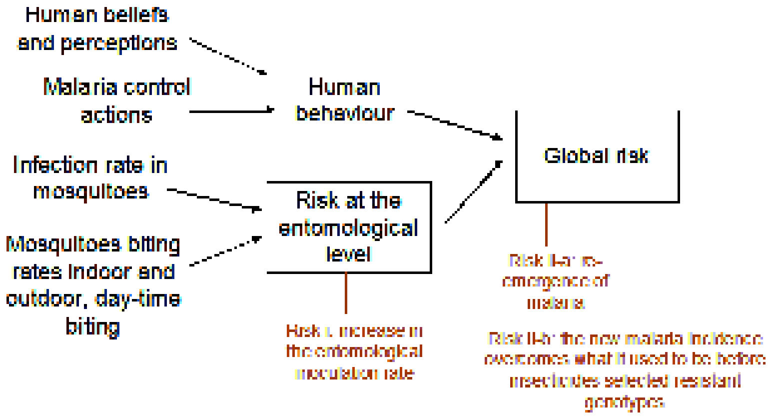

Figure 1. Factors assessed for the risk analysis

needed to understand the projected frequencies of insecticide-resistant mosquitoes in each location. Modelling is needed to integrate these effects and assess how the variations in entomological parameters might affect the risk of malaria transmission at the entomological level (hereafter referred to as risk i – Figure 1). Sociological data from different locations is necessary to assess how human behaviour moderates the entomological risk. Whatever the allele frequency in mosquitoes, and whatever the probability of being infected in the absence of protection, the overall malaria risk might be negligible if humans protect themselves effectively from mosquitoes. This actual risk, resulting from the combination of entomological and human factors, will hereafter be referred to as the ‘global risk’ (risk ii), and will be assessed using the risk analysis tools from epidemiology.

The suspected increasing susceptibility of *kdr-R* and *Ace1-R Anopheles* to *Plasmodium* opens new important questions:

(i) Are *kdr-R* and *Ace1-R Anopheles* with increased susceptibility to *Plasmodium* infection (compared to *kdr-S* and *Ace1-S Anopheles*, respectively) widespread?

(ii) The two known *kdr-R* alleles are suspected to exhibit different levels of pyrethroid resistance. Is there also a difference in the level of susceptibility to *Plasmodium* exhibited by *Anopheles* carrying these two different *kdr-R* resistance alleles?

(iii) In a mosquito population where insecticide-resistant *Anopheles* are more susceptible to *Plasmodium* than insecticide-susceptible *Anopheles*, how does *Plasmodium* prevalence in mosquitoes increase with the frequency of resistance alleles?

(iv) If *Anopheles* susceptibility to *Plasmodium* does not depend on mosquito genotype at insecticide-resistance loci, the only risk associated with the spread of resistance alleles coding for pyrethroid resistance would be a return to a situation comparable to the situation before pyrethroid-based malaria control strategies were implemented. However, if *Anopheles* susceptibility to *Plasmodium* is increased in insecticide-resistant mosquitoes, is there a risk (at the entomological level) that malaria transmission might increase to a level higher than what it used to be before the implementation of insecticide-based control strategies in some locations?

(v) Are changes in mosquito biting behaviour from indoor nocturnal biting towards outdoor or day-time biting inversely linked to genes coding for resistance? Are outdoor biting mosquitoes less susceptible to *Plasmodium*?

(vi) Malaria risk perception is likely to decrease in the population with increasing success of malaria-control strategies. Do people with a lower risk perception of malaria protect themselves less against mosquitoes?

(vii) In locations where insecticide-based control methods have been implemented for a long period, insecticide resistance alleles in mosquitoes are likely to be common, not only locally, but also in neighbouring areas. Is there an increased risk of exposure to infectious bites for populations living in these neighbouring areas that might not directly be benefiting from control strategies?

(viii) Given the risk at the entomological level (risk i) based on *Plasmodium* susceptibility (question iv), and mosquito behaviour (question v) and given the human behaviour across time and space (questions vi and vii), how large is the resulting (global) risk that an increased susceptibility of *kdr-R* or *Ace1-R Anopheles* to *Plasmodium* could threaten malaria control strategies and lead locally or internationally to situations where malaria incidence would increase again (risk ii-a) or, further, be worse than before the implementation of insecticide-based control strategies (risk ii-b)?

2) Methods

In order to answer these questions, we had the following objectives:

(i) To measure *Plasmodium* infection rates in insecticide resistant and susceptible *Anopheles* genotypes and other entomological parameters of interest (biting rates and behaviour, frequencies of resistance alleles, mosquito susceptibility to insecticides at different stages of the life cycle) in three different localities. These measurements will help answer questions i, ii, iii and v.

(ii) To evaluate human perception of the risk associated to malaria in different localities, and assessing how human behaviour regarding malaria control methods changes and is expected to vary across space and time (in particular in the future), in order to assess how it impacts the risk of malaria transmission locally and globally (questions vi and vii).

(iii) To draw realistic scenarios from the evaluation of human behaviour (regarding how they protect themselves from mosquitoes), and using these scenarios to evaluate how the theoretical risk assessed at the entomological level (risk i) could translate into a global risk, at local and international scales, to find situations where malaria incidence would increase while control strategies had shown some successes (risk ii).

a) Acquisition and analysis of entomological data

We sampled mosquito populations (larvae and adults) in three locations across West Africa: Agboville in Côte d'Ivoire, Tori-Bossito in Benin, and Dielmo in Senegal.

In Senegal, the level of resistance to pyrethroid is known to be lower than in Benin and Côte d'Ivoire, with a frequency of *kdr-R* alleles around 10% in Dielmo (IRD-URMITE, personal communication). Dielmo HDSS was chosen because of the availability of a 10 year longitudinal sampling of mosquitoes. In Dielmo, a high level of malaria control is implemented. The epidemiology of malaria for humans in Dielmo has been studied intensively for two decades, providing a unique resource for analysing the efficiency of insecticide-based control methods there.

In Côte d'Ivoire, Agboville research station has a very high level of resistance to all the insecticides used for public health [26]. The frequency of *kdr* resistant alleles is more than 80%. In order to get enough *kdr-S* mosquitoes to be compared to *kdr-R* mosquitoes, the sampled mosquitoes where crossed with an Ivorian sensitive mosquito strain from the lab, which is the only solution as the situation (more mosquitoes are resistant) is the same everywhere in Côte d'Ivoire.

In Benin, Tori-Bossito was chosen because this location has a lower frequency of resistance alleles (77%) compared to the other study sites investigated by Ossé and colleagues [27]. Indeed, the frequency of resistance alleles is very high in Benin [28] and we needed to increase our chance to collect enough susceptible mosquitoes to be able to compare them with resistant mosquitoes when testing for susceptibility to Plasmodium infection.

This data collection in each locality made it possible to tackle objectives i and provide answers to questions i, ii, iii and v. In particular:

- *Data collection 1* (human biting rate, wild adult mosquito infection rates): The human biting rate is commonly measured by human landing catch, and were measured in conditions where (a) individuals are protected by ITNs (indoors) and (b) individuals are not protected by mosquito nets (outdoors). These biting densities were recorded until recently in Dielmo [19]. We recorded them in our three sites with the same study design as [19]. Mosquitoes were collected with human-landing catches from 19:00 to 11:00 for three consecutive nights per month during one season (six months). Note that the time period of catches is usually from 19:00 to 07:00 but mosquitoes were collected until 11:00 to assess potential changes toward day-time biting. These mosquitoes were identified to species (morphological identification + subspecies identification of the *An. gambiae* complex by the diagnostic PCR assay [29]), their infectious status was determined by ELISA-CSP; and sub-samples were genotyped for the *kdr* and *Ace1* loci by PCR.

- *Data collection 2* (Experimental assessment of mosquitoes sensitivity to *Plasmodium*): Mosquito larvae were collected and raised and then infected using direct membrane feeding with *P. falciparum* gametocyte-containing blood donated by volunteer patients. In Tori Bossito and Agboville the presence of oocysts in the stomach was determined by dissection under light microscopy after 6-8 days, while in Dielmo the presence of sporozoites in the salivary glands was determined by ELISA-CSP after 14 days. Mosquito species, molecular forms and genotype at *kdr* and *Ace1* loci were identified by PCR (same methodology as [16] to be able to compare results). These measures of infection are complementary as oocysts may or may not lead to sporozoites depending on the success of the parasite in infecting the mosquito. Analysis of sporozoites directly measures which mosquitoes can infect humans, while oocysts can be used to measure both density and occurrence of infection.

- *Data collection 3* (Permethrin susceptibility tests): In order to link the genotypes of resistance (*kdr* alleles) with phenotypes of resistance, the resistance of adult females to permethrin, and when possible, to a set of other insecticides (deltamethrin, malathion, and DDT), was assessed with the WHO tube/cylinder susceptibility test protocol.

b) Acquisition and analysis of sociological data

The Knowledge, Attitudes, Practises and Beliefs (KAPB) of populations were measured in the three sites. Several quantitative and qualitative methods were used for data collection:

- *Household surveys*: In order to capture behaviour of people regarding the use of ITNs and other repellents for mosquito and/or malaria control a household questionnaire were administered in selected households of each site in the three countries. The questionnaire was built around knowledge and practises of populations regarding malaria control strategies, acceptability of tools proposed by malaria control programs and alternative methods.
- *Participative research:* This primarily used the photovoice method (participatory photography). Photovoice is a process by which people can identify, represent, and enhance their community through a specific photographic technique. This consists of entrusting cameras to local residents to enable them to act as recorders, and potential catalysts of social action and change, in their own communities. The technique consisted of giving disposable cameras to three groups of people (from rural, suburban and urban setting) in each location with the request to take photos of what represents the best malaria control strategy in their surroundings. The selection of participants was based on the criteria of age, sex, socio-economic and environmental conditions. The goal was to: (i) enable people to record and reflect on their community's strengths and concerns regarding malaria and control strategies; (ii) promote critical dialogue and knowledge about personal and community issues concerning malaria control strategies and future options; (iii) provide policy makers with clear recommendations from populations. Selected photos gathered during the photovoice method were used as basis for focus group discussions (FGDs) with members of various communities on their attitudes and perceptions. In each site, 12 FGDs were conducted in three selected locations with socio-economic and environmental differences.
- *Semi-structured interviews*: Additionally, face-to-face interviews were conducted with selected resource persons from the communities as well as with health personnel, traditional, municipal and administrative authorities. Interviews goal was to contribute to document control strategies from local communities to regional and national agencies.

Findings from the sociological studies are critically important to assess risks accurately and inform policies for vector or malaria control at the national level in the respective countries.

c) Transversal integration of research outputs: risk analysis

The last step of our methodology was to integrate all the results in an analysis of the risk of a setback in malaria control due to the evolution of resistance and increased *Anopheles* sensitivity to Plasmodium. This analysis considered three components:

- the mosquito infection status and its determinants (in particular, the genotype at loci involved in resistance evolution: kdr, Ace1)
- the mosquito behaviour (propensities to bite indoor and outdoor, time of biting) and its evolution;
- human behaviour (propensity to be indoors and outdoors during the night and mosquito avoiding behaviour) and its evolution.

Integrating all these components is critical for a proper assessment of the risk because it is expected to be the highest in the particular situation where both entomological factors (increased susceptibility to *Plasmodium* of resistant *Anopheles* that became frequent) and human behaviour (decreased vigilance) play against the effectiveness of malaria control.

From the three components (mosquito biology, mosquito behaviour, and human behaviour), a number of scenarios can be drawn. For example, one scenario could be that most *Anopheles* carry resistance alleles that increase their probability of being infected, that they have a high propensity to bite outdoors, and that despite the preventive actions from authorities most humans stay outdoors at night and do not protect themselves from mosquitoes. A risk is a probability of occurrence of a scenario and its size of impact [30], and was estimated qualitatively (i.e. we estimated whether the risk is low/ medium or high) for various scenarios in each location, to identify the main local threats to malaria control, and the control options.

The above example scenario would probably have serious adverse consequences for malaria incidence. If it has a high probability, the development of malaria control strategies that do not involve changing population behaviour or insecticide would be strongly recommended (e.g. strategies that can be implemented at the level of the village). In a similar scenario but with lower coverage of effective preventive interventions, and greater awareness of the risk of malaria transmission, strategies with greater focus on increasing coverage of prevention could be recommended. A set of alternative actions could be recommended in some scenarios, in which case their respective costs would determine the priorities.

3) Results

a) Entomological data

(i) Senegal

- **Human landing catches**

In Dielmo, Senegal, longitudinal data has been collected from 2006 to 2017 regarding the propensity of mosquitoes to bite (biting rates) and their species and genotype at the kdr locus and their status of infection regarding *Plasmodium falciparum*. Biting rates until 2016 have been published in Sougoufara et al. [31].

In Dielmo there is a wide diversity of mosquito species, including non-transmitting mosquitoes in particular from the *Culex* and *Aedes* genera. From 2006 to 2016, the proportion of *Anopheles* decreased from half to about a quarter of the fauna captured. There was a huge decrease in the Dielmo *Anopheles* population from 2006 to 2016, putatively linked to the introduction of mosquito nets.

Year	Person-night	Anopheles	Total	% Anopheles	Total / PN
2006	72	2656	5107	52.01	70.93
2007	96	2049	3371	60.78	35.11
2008	104	1786	5206	34.31	50.06

2009	140	2671	8003	33.37	57.16
2010	144	2041	4163	49.03	28.91
2011	144	1254	4751	26.39	32.99
2012	144	557	4057	13.73	28.17
2013	144	1097	2265	48.43	15.73
2014	144	747	1769	42.23	12.28
2015	144	589	2558	23.03	17.76
2016	48	89	387	23.00	8.06

Table 1. Number of mosquitoes collected between July 2006 and April 2016 in Dielmo. Value in the right show the relative proportions of Anopheles and the number of mosquitoes of all species captured by person per night. PN (number of person-nights per year)

The proportion of Anopheles mosquitoes biting early in the morning rather that during the night has not changed significantly across time. The proportion of mosquitoes biting rather indoor or outdoor changed toward a higher proportion of bites occurring outdoor, but because the number of bites indoor dropped faster than the number of bites outdoor, not necessarily because mosquitoes that were biting indoor switch to bite outdoor (Figure 2).

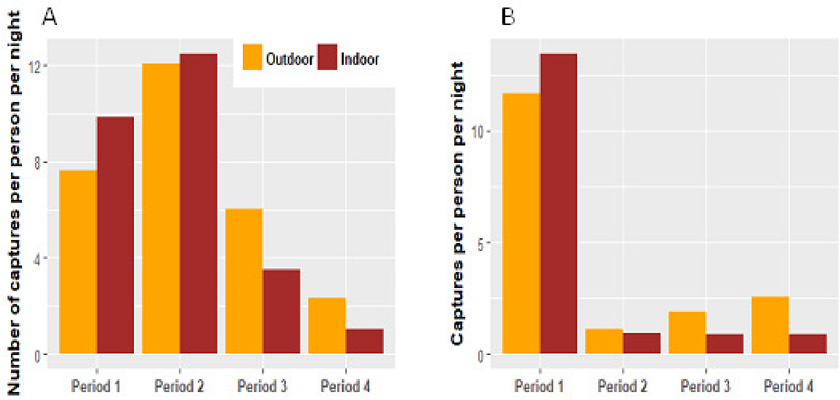

Figure 2. Variations in the indoor and outdoor biting rates of An. gambiae s.l [A] and An. funestus [B] per period according to the model: Period 1 (July 2005 - June 2008), Period 2 (July 2008 - June 2011), Period 3 (July 2011-June 2014), Period 4 (August 2014 - April 2016)

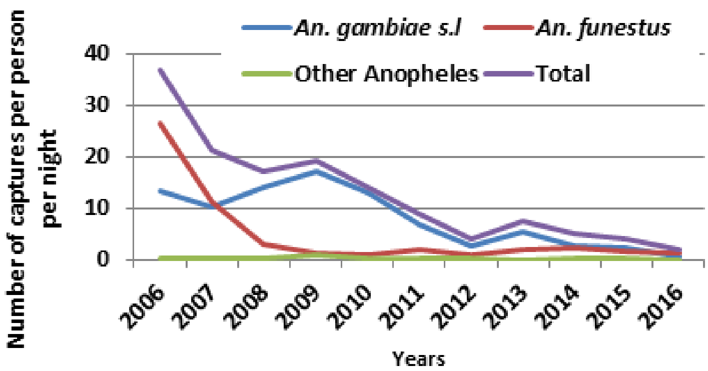

Figure 3: Yearly dynamic in the number of mosquitoes captured per person-night in Dielmo

The two malaria vectors in Dielmo are *An. gambiae* and *An. funestus*. Rates of infection were calculated for 4 periods: Period 1 (July 2006 - June 2008) before the implementation of mosquito nets, Period 2 (July 2008 - June 2011), Period 3 (July 2011-June 2014), Period 4 (August 2014 - April 2016) after the introduction and renewal of mosquito nets. For *An. gambiae* the rates increased and then decreased, while for *An. funestus* they decreased and then increased (Table 2). There is thus a trend toward an increased proportion of the resistant alleles. However the change is much smaller than expected based on previous data, making it unlikely that any change in the epidemiology of malaria can be related to such changes in frequency.

Species	Period	Tested	Positive	Infection rate
An. gambiae	Period 1	1044	11	1.05%
	Period 2	4820	58	1.20%
	Period 3	2077	46	2.21%
	Period 4	448	2	0.45%
An. funestus	Period 1	2075	33	1.59%
	Period 2	506	3	0.59%
	Period 3	645	1	0.16%
	Period 4	507	4	0.79%

Table 2. Infection rates of Anopheles captured in Dielmo by period

In parallel, the proportion of resistant alleles at the kdr locus increased (Table 3):

	Year				
Species	**2011**	**2012**	**2013**	**2014**	**2016**
An. arabiensis	8.20%	4.05%	8.82%	11.25%	12.20%
An. coluzzii	0	0	0	0	0
An. gambiae s.s	NA	NA	NA	0	0

Table 3. Proportion of the kdr-west resistant mutation across years in Dielmo.

- **Experimental infection**

Regarding the experimental infection, there is a significant association between the rate of infection and the mosquito phenotype of resistance to pyrethroids.

	Dead (sensitive phenotype)	Alive (resistant phenotype)
Not infected	19	66
Infected	8	71
% of infection	29%	52%

Table 4. Relationship between rate of infection and phenotype of resistance

According the analysis, the difference in infection rates corresponds to an odds ratio of 2.72 (95% confidence interval=1.13-7.44), resistance being a risk factor for the infection by Plasmodium.

- **Study of resistance**

WHO susceptibility tests carried by Cailleau et al. at IPD (unpublished) showed that Dielmo mosquitoes were partially resistant to pyrethroids. Mortality rates were 78% for permethrin (n=81) and 92% for deltamethrin (n=131) after 1h of exposure.

CDC bottle bioassays carried by Thiaw et al. [32] showed that Dielmo mosquitoes were fully susceptible to lambdacyhalothrin, bendiocarb and fenitrothion. Overall, mortality rates of 97, 94.6, 93.5, 92.1, and 90.1% were, respectively, observed for permethrin, deltamethrin, pirimiphos-methyl, etofenprox and alphacypermethrin after 30 minutes of exposure. Resistance to DDT was observed, with a mortality rate of 62%. The use of EA significantly improved the susceptibility of *An. gambiae* s.l. to DDT by inhibiting GSTs ($p = 0.03$). PCR revealed that *An. arabiensis* was the predominant species (91.3%; IC 95 86.6–94%) within *An. gambiae* complex from Dielmo, followed by *Anopheles coluzzii* (5.4%; IC 95 2.7–8.1%) and *Anopheles gambiae s.s.* (3.3%; IC 95 0.6–5.9%). Both 1014F and 1014S alleles were found in *An. arabiensis* population with frequencies of 0.08 and 0.36, respectively, and 0.23 and 0.13in *An. coluzzii*. In An. gambiae s.s. population, only kdr L1014F mutation was detected, with a frequency of 0.17. It was observed that some individual mosquitoes carried both alleles, with 19 specimens recorded for *An. arabiensis* and 2 for *An. coluzzii*. The presence of L1014F and L1014S alleles were not associated with resistance to pyrethroids and DDT in *An. arabiensis*.

(ii) Benin

- **Human landing catches**

As found by Yadouleton et al. at CREC [33], during the year of study, a total of 45,351 mosquitoes were collected from HLC and 3,858 from PSC. Of the 45,351 females caught by HLC, 25% (11,320/745,351) were *Anopheles*. The main malaria parasite, *P. falciparum* was transmitted by *An. coluzzii* in this area with two rainy seasons (April- July and October- November) and two dry seasons (December-March and August-September).

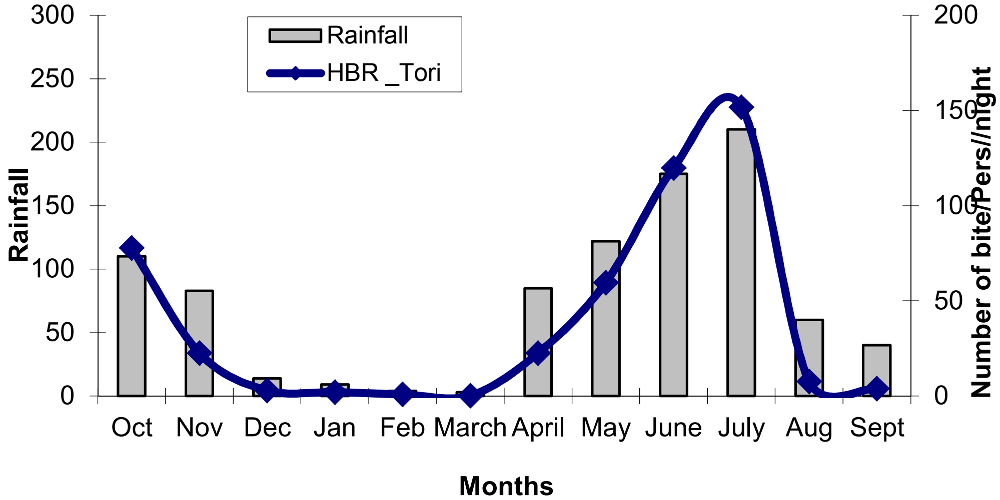

Figure 4: Number of Human bites/person/night and rainfall at Tori-Bossito from October 2016 to September 2017.

The Human Biting Rate (HBR) was estimated from the Human Landing Catches (HLC). The highest biting rates of *An. gambiae s.l.* was found in July during the rainy season (151.79 bites/p/n) and the lowest in March (0.20 bites/p/n) during the dry season (Fig 3).

The average of bites/person/night during the two rainy season (April-July and from October - November) was 60.9 bites/p/n and approximatively 20 times significantly higher than those obtained during the two dry season (March, August-September) (2.96 bites/p/n) ($P < 0.05$).

The global proportion of infected mosquitoes was 0.06% (39/692). The entomological infection rate (EIR) was estimated from the overall collection. Transmission, as measured by HBR , catches and EIR was high during the two rainy seasons (April to July and October to November) but declined in the two dry seasons (December to March and August to September).

Of the large number of mosquitoes collected by HLC (11,326), a random sample of 692 were tested for the L1014F (kdr West) genotype and for infection status. The L1014F frequency was 0.86 in *An. coluzzii* and only 16 were SS regarding the kdr-locus. The ace-1^R mutation also occurred but at frequency < 0.1. Only 39 of this subsample were infected, hence, the sample size was inadequate to power a test of the link between the resistance genotype and infection (Table 5) (this is feasible because a larger sample can be tested).

	Natural infections		**Experimental Infection**	
Genotype	**Not infected**	**Infected**	**Not infected**	**Infected**
RR	616	38	175	25

RS	21	1	16	1
SS	16	0	11	0

Table 5. Number of oocyst-infected wild-exposed mosquitoes for the three kdr genotypes.

From this data however we can estimate the proportion of the R allele in the population to be around 96% [CI 95.8% - 96.4%] and the frequency of oocyst infection in wild conditions to be 5.6% [CI 5.2% – 6.0%].

- **Experimental infection**

A total of 228 mosquitoes were experimentally exposed to gametocyte-infected blood donors. Unfortunately, too few sensistive mosquitoes and a too small rate of infection do not, again, provide enough power to test the relationship between the genotype of resistance and the susceptibility of mosquitoes to Plasmodium (Table 5). From this data we can estimate the proportion of the R allele in the lab-raised population to 90% [CI 90.4% - 92.5%] and the frequency of oocyst infection in this population as 11.4% [CI 10.1% - 12.7%].

- **Study of resistance**

WHO susceptibility tests showed that Torri-Bossito mosquitoes where resistant to DDT and pyrethroids, but susceptible to bendiocarb. Mortality rates where 4% (2016) and 3% for DDT, 32% (2016 and 2017) for permethrin, 65% (2016) and 62% (2017) for deltamethrin, and 100% (2016 and 2017) for bendiocarb (n=100 for each year and each molecule).

(iii) Côte d'Ivoire

- **Human landing catches**

The seasonal pattern of biting in Agboville is given in Figure 5.

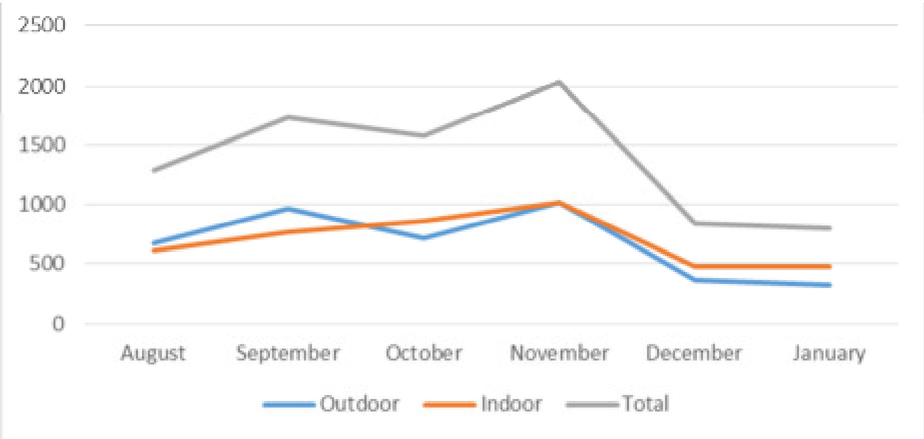

Figure 5. Time series of indoor and outdoor biting rates by An. gambiae across the rainy season in Agboville

During the rainy season, from August to January 2017, 9,247 female mosquitoes were caught by HLC. 90,1% (8,331/9,247) belonged to the *Anopheles* gambiae complex and less than 0.13% (12/9,247) to other *Anopheles* species. Half (49%) of *An. gambiae* females were caught outdoor, 51% were caught indoor. September and November are the months with the highest biting densities. The nocturnal activity pattern is given in Figure 6

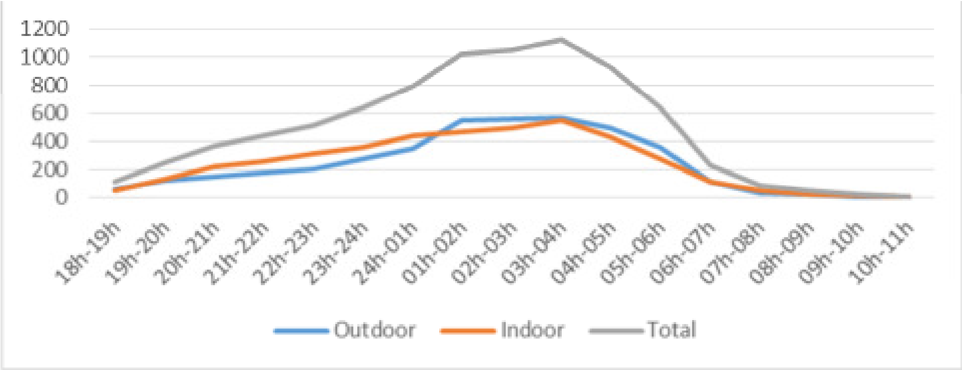

Figure 6. Biting rhythm of An. gambiae in Agboville.

The number of bites per person per night varied from 44 to 113 over the period. However the mosquitoes were caught in three different districts and biting densities were quite heterogeneous:

	Hopital	**Sogefia**	**Sokora**	**Total**	**Bites/Person/night**
August	229	147	914	1290	71.67
September	213	227	1289	1729	96.06
October	154	105	1312	1571	87.28
November	142	180	1715	2037	113.17
December	130	57	658	845	46.94
January	85	52	662	799	44.39

Table 6. Total mosquitoes caught and biting densities by district in Agboville.

Of the more than 8,000 *An. gambiae* females, 2551 were dissected for oocysts, revealing an infection rate approximately constant across seasons of about 3%. Similarly, 1606 females were processed for their status of sporozoite infection, with an infection rate varying between 2 and 5% with an average of 3% (Table 7).

	Oocysts			**Sporozoites**		
	Infected	**Total tested**	**Infection rate**	**Infected**	**Total tested**	**Infection rate**
August	0	361	0.000	6	279	0.022
September	28	433	0.065	11	337	0.033
October	12	429	0.028	12	299	0.040
November	15	438	0.034	1	247	0.004
December	11	444	0.025	11	222	0.050
January	15	446	0.034	10	222	0.045
Total	81	2551	0.032	51	1606	0.032

Table 7. Results of oocyst dissections and sporozoite testing by month (Agboville).

Those infected by oocysts were genotyped for their *kdr* locus and the number of oocysts was counted, providing data on the relationship between the genotype at the kdr locus and the intensity of infection, and revealing a trend toward a higher intensity of infection in homozygous RR genotypes, however, this was not significant (Chi-square = 3.814; P=0.148).

	Natural infections			**Experimental Infection**		
Genotype	**N**	**Mean number of oocysts**	**Median number of oocysts**	**N**	**Mean number of oocysts**	**Median number of oocysts**

RR	36	11.2	6	12	12.6	6.5
RS	34	6.1	3	16	8.8	5
SS	11	4.5	2	14	6.6	4.5
Global	81	8.1	3.5	42	9.1	5

Table 8. Oocyst counts by resistance genotype (Agboville).

- **Experimental infection**

A total of 3125 mosquitoes were experimentally exposed to sporozoite-infected blood donors 6 days post emergence. Of these, 2032 mosquitoes reached 14 days and were dissected to look for oocysts. Of these, 42 (0.02%) were infected and the intensity of infection was higher for RR genotypes compared to RS genotypes, and higher for RS genotypes compared to SS genotypes (Table 8).

- **Study of resistance**

Mosquitoes from Agboville were found to be resistant to the four families of insecticides used in public health as resistance to these compounds were all less than 90%. The resistance ration varied from 2 for malathion to more than 14 for bendiocarb. We were not able to assess this ratio for DDT since that none of mosquitoes were knocked down by this chemical.

Mosquitoes	Insecticides	Mortality(%)	TKD50(min)	Resistance Ratio	Resistance status
Kisumu	Deltamethrin	100	22.99	1	S
	Bendiocarb	100	28.6	1	S
	Malathion	100	30.9	1	S
	DDT	100	99.32	1	S
wild	Deltamethrin	6.38	99.32	4.59	R
	Bendiocarb	8.89	419.30	14.66	R
	Malathion	66.05	61.88	2	R
	DDT	2.17	/	/	R

Table 9. Mortality rate, KD50 (time necessary for 50% of mosquitoes to be knocked down) and resistance ratio.

b) Socio-anthropological data

(i) Comparative analysis of quantitative data

A number of households were investigated in each location (N=299 in Tori Bossito, Benin, N=701 in Agboville, Côte d'Ivoire, N=187 in Dielmo, Senegal and N=112 in Dakar, Senegal) to gather information regarding how populations understand malaria and how they use mosquito nets and other vector control tools. The results are presented here are for the most relevant questions. All figures are percentages (of respondents answering a given question). The complete set of percentages is available in tabular form from the investigators.

- **What causes malaria?**

Only answers chosen by at least 1% of respondents in at least one given location are presented. About half of total respondents attribute malaria to mosquitoes (choice 1_moustique) only, but this figure goes from as low as 37% in Torri Bossito in Benin, where a variety of alternative causes were also provided, to 67% in Agboville in Côte d'Ivoire. We can see that the sun (choice 2_soleil) is cited as a complementary cause (in addition to mosquitoes) very often (>20 % of the total number of respondents). Food (5_alimentation) and being tired (4_fatigue) come next as potential explanations for malaria (>10% and >3% of respondents, respectively). Rain (3_Pluie) was rarely cited.

Other causes cited were most often poor sanitation, dirt, dirty water, stagnant water, and lack of hygiene. A few respondents attributed the disease to trash, tse-tse flies, domestic animals, kitchen fires, dirty blood, climate change, or god.

- **When is one more exposed to malaria ?**

For most respondents in most localities, exposure to malaria occurs on the evening (choice 4_soir). To the exception of Dielmo where respondent estimate that exposition to malaria occurs at any time (5_attmoment) for one third of respondents, or rather on the morning (1_matin) for another one third. What is generally advised to avoid malaria?

Most respondents answered "sleep under mosquito nets" (1_dormirMoustiquaire) as the one behaviour advised to avoid malaria. However, cleaning surroundings (2_assainirEntourage) was also ranked very high as prominent advice. Other behaviours including go to the hospital (3_allerHopital), avoid some food (6_eviterCertainsAlimts), wear protecting clothes (4_vetementsProtect), or avoid hard work (5_eviterTravauxPenibles) are only cited as a complement of the two other propositions, or marginally alone. How do you evaluate the prevalence rate of malaria in your locality?

In Tori Bossito and Dakar, most respondents (>90%) estimate the rate of malaria to be decreasing (1_enBaisse). In Agboville this is also the most general appreciation although more respondents do not know (4_nsp). In Dielmo answers are much more diverse, with still a bigger number of ~39% estimating the rate to be decreasing, but one third estimating this rate to be increasing (2_augmente) and another third estimating this rate to be constant (3_constant). This is very interesting to note as the epidemiological data show a huge decrease of malaria rates in Dielmo : an actual decrease does not necessarily lead to a perception of such a decrease.

- **Do you think people are still as afraid of malaria today as they used to be?**

In Torri Bossito and Agboville, a majority indicate they are no more afraid of malaria than they used to be. In Dakar about half of respondents answer they are still afraid, but no more so than before, and in Dielmo a majority of respondents answer they are still afraid. In all the locations, a substantial number of individuals indicate they are less afraid, which will be very important to take into account in a risk analysis as this might make them less vigilant in protecting themselves.

However, to assess this risk it is important to understand why they can be less afraid or not. Among those who answered yes, a majority think that malaria still kills as much as it used to or more (1_tueAutantOuPlus), except in Dakar where other reasons are given, in particular the fact that independently of its rate, malaria is a dangerous disease that kills (some mention their experience of losing someone). The lack of treatment (2_pasTraitement) can still be cited in Agboville or Dielmo (which is very surprising in Dielmo as the HDSS provide the treatment systematically and for free), as well as the increase in the number of mosquitoes (3_plusMoustiques) in particular in Agboville.

Among those who answered no, many cite the efficiency of modern drugs (2_medicamentsPlusEfficaces) and of the fight against malaria vectors (3_lutteVecteursPlusEfficace) as reassuring. Other reasons (other) are provided in particular in Dakar as the lower rate of malaria (quite a frequent answer actually), the availability of treatment, the fact they know the disease better, or more marginally, the fact districts/houses are cleaner. The existence of diseases that are worse (1_maladiesPlusGraves) can also be cited but is less often.

- **Are actions against malaria taken in your area?**

Most respondents are aware of actions taken against malaria in their locality, except in Agboville where a majority there is no such action or are not aware of it.

- **At what time of the day do you notice the more mosquitoes in your district/village?**

Respondents notice more mosquitoes on the evening (3_soiree) rather than in morning (1_matinee) or afternoons (2_apresmidi). However in Dielmo, a significant number of respondents answer they observe mosquitoes at any time of the day (4_aTTmoment , 16%). This can be correlated to the very high number of diurnal (non-malaria transmitting) mosquitoes in this locality, around 75% in the last years, compared to e.g. Côte d'Ivoire where non *Anopheles* mosquitoes represent 10% of catches (see entomological data). However, in Benin as well non-*Anopheles* mosquitoes represent about 75 of the fauna captured, and still mosquitoes are rather perceived in the evening. The difference could be explained by a different composition of this non-*Anopheles* fauna.

- **At what time of the day do mosquitoes bite the most in your district/village ?**

The answers to this question are very similar to the question on when mosquitoes are noticed, indicating people notice the presence of mosquitoes when they are been bitten. At what time of the year do you notice most mosquitoes in your district/village?

Mosquitoes are noticed the most during the rainy season in most localities, except in Dielmo where they are also perceived a lot during the dry season. Again, this can be due to the high number and species composition of non-*Anopheles* (non malaria-transmitting) mosquitoes in this locality, which can persist throughout the year.

- **In recent years, do you notice more or less mosquitoes in your district/village?**

In Tori Bossito and Dakar, respondents clearly notice fewer mosquitos in their district or village than they used to. However, in Agboville and Dielmo, the perception is not as clear. The longitudinal entomological data in Dielmo allows these figures to be compared with actual trends and show that despite a real decrease in the number of mosquitoes captured, at least at night, the people do not necessarily perceive it.

- **What are the issues generated by mosquitoes?**

Disease (3_maladie) was cited by most respondents as one of the issues generated by mosquitoes in all localities, although a bit less in Agboville (78% versus more than 95% in other localities). Bites (1_piqure) were actually coming as the first issue in Agboville (83% of respondents cited noise as an issue). Noise (2_bruit) was cited by 8% of respondents in Dakar up to 33% of respondents in Dielmo, insomnia (4_insomnie) was cited by only 1,77% in Tori Bossito but 29% in Dielmo, and buttons were cited by only 2.65% in Tori Bossito but 57% in Dielmo. Other causes were cited (as financial issues, death) but were marginal.

Mosquitoes are perceived as causing issues beyond the fact they cause a disease by 87% of individuals in Agboville, but only 23% in Tori Bossito. Regarding issues motivating for using a mosquito net, as bites and buttons, 8% of people from Tori Bossito cite these issues only, but much higher proportions of inhabitants from the other locations (from 30% in Dakar to 84% in Agboville, 60% in Dielmo).

What kind of diseases can be transmitted by mosquitoes?

Malaria (2_paludisme) is cited most often as a disease that can be transmitted by mosquitoes. It is cited alone by 80% and 82% of respondents in Tori Bossito and Dakar, 71% of respondents in Agboville and 47% of respondents in Dielmo. However, it is also often cited with other diseases. Typhoid fewer (3_fievreTyphoide) is cited by 9% of respondents in Agboville and 4% in Dielmo. However it is not known at all in Torri Bossito and Dakar. Dengue (1_dengue) is almost completely unknown. AIDS (4_sida) is very high in Dielmo and quite high as well in Tori Bossito, with 9% and 2.5% of respondants citing it. Other diseases that were cited were Elephantiasis (2) , Onchocercosis, Ebola, Diarrhoea (3), Diabetes, Cholera, Insomnia, Headache, Tuberculosis, Anaemia (6), Yellow fever, and 7 respondents replied 'a lot of diseases'.

- **Before going to sleep at night, what is your preferred place to rest?**

In most places, outdoor in fresh air (4_courEnPleinAir) is the preferred place to rest, while outdoor in front of the court (5_dehorsDvtCour) is also frequently cited. The bedroom (1_chambre) is most cited alone only in Dielmo (32%) while the living room (2_salon) come second in this place. Answers representing less than 0.5% of the total number of respondents are not provided.

For an analysis of risks (of being bitten), it is useful to analyse these answers in terms of resting indoor versus outdoor. Therefore we counted all answers with only bedroom (1_chambre) or living room (2_salon) as "indoor", all answers with other choices as "outdoor" and all answers with a mix of 1 or 2 and other choices as "indoor or outdoor". We find out that in Dakar, more than 90% of respondents rest outdoor, while 83% do in Agboville and 74% in Tori-Bossito. It is only in Dielmo that half of respondents rest rather indoor. Up to 12-14% of respondents can rest indoor or outdoor depending on days in Agboville and Dielmo, and 4-5% in Tori Bossito and Dakar.

- **Is malaria necessarily caused by the mosquito?**

For a majority of respondents in all locations, mosquitoes are a necessary cause of malaria. However, this majority is short in Dielmo (actually closer to half of respondents). It is around 70% in other locations.

- **Amongst these vector-control tools, which do you use in your household?**

In the following table, evidence-based methods are counted alone or in combination with other methods, while other methods are counted only when used alone or in combination together. Data is not available for Senegal. Most households use impregnated mosquito nets (82% and 85%), only few non impregnated nets. A quarter of respondents use insecticide spray in Agboville and 43% in Dielmo (only a few cite AID). Alternative methods are used alone in only 10% of households in Agboville and 2.5% in Dielmo.

- **Does your household own at least one mosquito net?**

A huge majority of households 93-98% own mosquito nets in all localities, although a small bit less in Agboville (88%). By comparing these figures with how many households use these mosquito nets, we can estimate the proportion of households who have a net but do not use it to be around 5% in Agboville and 9% in Dielmo.

- **Do you use this net?**

In agreement with previous imprecise estimates (5% and 9%), 3.5% of respondents in Agboville and 12% in Dielmo declaring they own a net also declared that they do not use it.

- **Who is sleeping under the mosquito net?**

In most households using a mosquito net (70-80%), all inhabitants are sleeping under the net (5_toutMonde). In a dozen of households (10-13%) only children (1_enfants) sleep under the net. In Agboville, only elders (4_viellesPersonnes) sleep under the net. Pregnant women and children only (1_enfants 2_enceintes) sleep under the net in 2-3% of households. Choices represented by less than 0.5% of households are not presented in the table (but are combination of several choices and therefore corresponds to most inhabitants of the household).

- **Do you think mosquitoes are now more resistant to insecticides than in the past?**

A non-negligible proportion of respondents (from 35% in Dakar to 75% in Agboville) are aware of issues of mosquito's resistance.

- **How would you qualify the advice given during sensitization campaigns?**

Most respondents (>80% in all locations) consider the information given during sensitization campaigns as important (2_Important) or rather important (3_AssezImportant), showing concern and openness to information from respondents.

(ii) Senegal

- **Most common diseases in the locality**

The diseases perceived as being the most frequent in the research areas, especially in urban areas (Medina Gounass and Ndiarème Limamoulaye), are headaches, coughs, asthma, hypertension, diabetes and diarrhoea among children. Malaria was rarely mentioned first, it is often cited last and sometimes it is the presentation of researchers and their object of study that help them make the connection. The fact that malaria is rarely mentioned is due to a reported decrease in malaria but not to the rarity of the mosquito in the area.

In Ndiop and Dielmo, areas that can be considered as rural, malaria is cited among the most common diseases of the locality but specifically in this area borreliosis comes back in the discourse, because of the recrudescence of this disease. However, many means of communication and care are set up in these villages (posters in the concessions, information on the disease ...). Malaria care is fully supported by a health centre (and health and disease surveillance system) managed by IRD and Institut Pasteur. This has contributed to its decline in these areas to the point that it is no longer considered a "problem" since the establishment of the centre in 1992. Indeed, this care is both preventive and curative (distribution of mosquito nets, RDTs, supply of drugs and monitoring of treatment).

- **Malaria is no longer considered as a major issue in these areas**

In both urban and rural areas, malaria is not considered a major health problem. The disease has decreased over the years. However, the insalubrity and the problems related to sanitation that prevail in certain urban areas are concerns (Medina Gounass, Ndiarème Limamoulaye, Ndiop).

«R1 : nous avons aussi le problème de vidange de nos toilettes parce qu'on fait recours au vidange manuel en creusant dans la rue et ça ne nous arrange pas. Les enfants n'ont nulle part ou aller et les rues sont étroites en plus nos toilettes sont remplies. Des fois même pour faire appel aux voitures de vidange, on est obligé d'épargner 50000 à 60000fcfa pour le faire mais avec la famille ça devient difficile. Si vous nous aidez à propos de cela ça serait mieux aussi nous avons un problème d'espace. »

➔ *"R1: we also have the problem of emptying our toilets because we use manual emptying digging in the street and it does not suit us. The children have nowhere to go and the streets are narrow and our toilets are full. Sometimes even to use the cars of emptying, one is obliged to save 50,000 to 60,000 CFA francs to do it but with the family it becomes difficult. If you help us about that it would be better. Also we have a space problem." FGD_Guédiawaye_Ndiarème Limamoulaye_Femme*

« R4 : ça serait mieux si vous nous avez touché des moustiquaires. Ensuite nos enfants nos pas d'espace ou jouer. Mais le vrai problème c'est le manque de rigole parce que nos enfants jouent dans la rue et nous déversons nos eaux de ménage aussi dans la rue raison pour laquelle nos enfants ne peuvent pas surpasser ces maladies telles que le palu, la diarrhée et les toux et des fois même ça touche aux adultes parce qu'on a un problème d'espace. Bref c'est ça qui nous pose problème sincèrement. »

➔ *R4: It would be better if you touched us with mosquito nets. Then our children have no space where to play. But the real problem is the lack of flow ditches because our children play on the street and we also pour our household water into the street, which is why our children cannot surpass these diseases such as malaria, diarrhoea and coughing and sometimes that even affects adults because we have a space problem. In short this is what poses us problem sincerely. FGD_Guediawaye_Ndiareme Limamoulaye_Femme*

However, there are means of struggle that are implemented, including regarding the Medina Gounass canal which has reduced malaria in the area and the activities of Set-Settal, which are still rare.

- **Perceived causes of malaria**

Malaria perceived causes are multiple and depend on many factors. Women associate the causes of malaria with housework during pregnancy, divine will, fatigue, climate change and influenza. They also mention poverty as a cause, the fact that children do not eat enough. The sun, the green mangos, the winter period with the rain are also associated as causes.

Another perceived cause of malaria is the mosquito for some. The latter transmits the disease through the bite. The type of mosquito is specified as well as the puncture and transmission time of the disease (community stakeholder). Community actors specify that it is the female *Anopheles* that transmits the disease at specific times of the night (21h to 6h in the morning).

« R3 : il y a deux façons de moustique le male et la femelle. C'est la femelle c'est-à-dire l'anophèle femelle qui donne le palu à cause de sa piqure. Mais le male crie et dérange seulement. »

➔ *R3: There are two kinds of mosquitoes the male and the female. It is the female, that is to say, the female Anopheles who gives malaria because of its bite. But the male screams and disturbs only. FGD_Guediawaye_Ndiareme Limamoulaye_Femme*

Others (head of household) specify a time when the female *Anopheles* bites and transmits malaria (3am), even going to specify the type of mosquito (big black mosquito). The most likely place for mosquito bites is the "outside", these places are unavoidable according to the populations surveyed, the whole family meets in the courtyard to share the evening meal and discuss before going to sleep in the rooms where the mosquito nets hang. These moments of "ngonal" are seen as moments of transmission of malaria, but many say they do not spend these moments in the room and under the mosquito net.

In addition, the bite of the mosquito is associated with other "aggravating" factors, including the sun, the rain season, insalubrity and stagnant water. In Medina Gounas and Ndiarème Limamoulaye the existence of retention basin promotes the presence of mosquitoes. There are "unavoidable" unhygienic spaces in some areas, such as Ndiop and Dielmo, where because of the lack of sanitation, people are forced to create their own system, which is to let the sewage run behind the houses. This practice is seen as promoting the presence and reproduction of mosquitoes.

Dielmo is bordered by a small permanent river, the Nema, whose marshy banks is seen as offering year-round larval breeding to *Anopheles*. The Nema is not present in N'Diop.

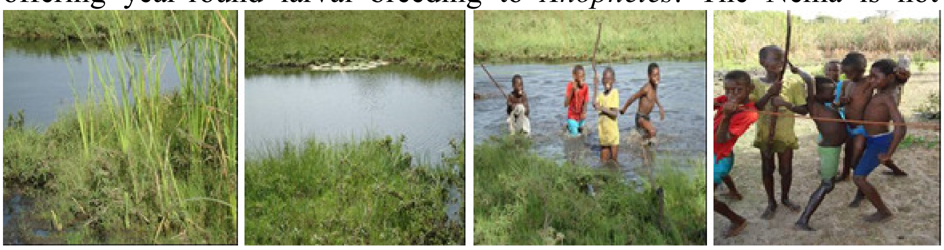

Figure 7. River Nema and children in Dielmo

Figure 8. Environment for market gardening in Dielmo

This river has become a favourite playground for children, who usually meet there in the early afternoon, when their parents are busy in the market gardening of the "naako". The "nema" is a place of sociability quite important in the village for the children. It should be noted that in Dielmo, the playgrounds and sociability are not as numerous for children: the village is not very large and is structured in two neighbourhoods and a hamlet: Kaolack and Ndangane, Santhie Mouride. Apart from this function, the "nema" allows women to carry out market gardening activities during the dry season. If during the rainy season the predominant activity is agriculture, the "naako" usually occupy women during the dry season. They are located near the "Nema" which allows them to benefit from the water to water the plants. There is also considerable mosquito breeding in the area.

We noted a strong presence of cattle and sheep in and around the village.

In Medina Gounass, the poor sanitationty and the presence of the retention basin are perceived as causes of malaria. The presence of wastewater in the streets (Medina Gounass and Ndiareme Limamoulaye) and behind the houses (Ndiop and Dielmo), is perceived as having health consequences, including malaria but also respiratory diseases (though from the entomologists perspective the mosquitoes that this induces are probably Culex, not Anopheles). The perceived consequences of malaria include suspension of the professional activities of people with the disease and of their studies.

From a qualitative point of view, the mosquito-related cause is often associated with others causes relating to the hygiene and sanitation conditions of the living environment (stagnant water, uncovered food, etc.), to the consumption of some foods (cow's milk, roe fruit, green mango), to some natural causes (sun and rain) or even supernatural causes. There is a multi-causal explanation in which mediators like stagnant water, dirt, sewage, take an important place.

- **Risks associated with malaria in urban and rural areas**

Populations identify a set of practices at risk of leading to malaria disease: Do not sleep under mosquito nets, « ngonal », walk under the sun, pour sewage into the nature, the practice of market gardening activity among women of Dielmo, flowing toilets (due to lack of septic tanks), no maintenance of bathrooms, luggage in the rooms.

The most at-risk perceived categories are children under 5, pregnant women and the elderly. In children under 5 years of age the risks associated with malaria are death, disability (deafness, mobility), seizures, paralysis, diarrhoea, constipation. In pregnant women, the perceived risks are abortion, anaemia or giving birth to a premature baby. In the elderly, the risk is also associated with death. In addition, some do not respond in terms of vulnerable categories but those who do not sleep under mosquito nets. They are considered the most exposed people.

In general, the means of prevention mentioned and used are practically related to the causes associated with malaria. The main means of prevention is the mosquito net, impregnated or not, according to all the targets. The use of drugs as a preventive measure is observed among household heads who, because of an incorporated memory of quinine, use quinine and Fansidar (Guédiawaye) to protect their families from malaria in addition to mosquito nets. In young people, it is seen as better to give drugs as a means of prevention because they do not sleep under mosquito nets. Other means are evoked as the spray, spirals, the fan and the incense with strong smell, the use of the bleach and the soap for the dishes and the washing of the hands, but also to avoid the sun. The care of the children, so that they are clean as well as the hygiene of the food are also evoked.

In terms of community dynamics to manage and prevent malaria, they are more important in Guédiawaye than in Ndiop Dielmo. To avoid illness, the youth association in Guédiawaye performs set-setal in the neighbourhood, frequent house cleaning, and cutting trees to reduce the risk of contracting malaria. This practice is more important in urban areas of Guédiawaye than in Ndiop Dielmo where sanitation activities are less important and less initiated.

- **Modes of protection against the vector and perceptions of their effectiveness**

If the mosquitoes are evoked as the cause of malaria, one will evoke the mosquito net, as well as other local or popular ways to hunt mosquitoes or reduce their nuisance: Santang, pump, traditional leaf, incense (morning and before sleep) . Individual and collective hygiene measures are mentioned when the perceived causes refer to the environment, to the living environment: weeding during the rainy season. Moreover, the preventive means are conceived and used here in a syncretic way. For example, for the mothers who are babysitting, the known and appreciated preventive means are mosquito nets, plants (used to chase the mosquito), Yotox, spiral, fan. There is also the use of medicines (paracetamol and doliprane) to give to the child in case of hot body and a mixture of honey plant, lemon and water (to give each beginning of the rain season). For pregnant women, the net is cited as a good preventive measure, as well as the respect of the CPN, the drugs prescribed during the pregnancy, as well as the measures of individual and collective hygiene, the use of Yotox and Santang (decoction of plants used in the form of fumigation). The Santang is lit right after twilight to chase away evil spirits. The use of these different products is current and compete with mosquito nets.

Regarding to the effectiveness of preventive measures such as mosquito nets and pumps, people have become more dubious, because they think that these tools are no longer enough to repel mosquitoes or kill them:

« R1 : Pour une dernière intervention, je ne suis pas d’accord sur le fait qu’on dit que les moustiquaires sont imprégnées pour une longue durée parce qu’il suffit que les moustiquaires durent trois mois et tu vois les moustiques sur la moustiquaire. Je pense qu’on devrait imprégner les moustiquaires chaque six mois »

➔ *"R1: For a last intervention, I do not agree with the fact that it is said that mosquito nets are impregnated for a long time because it is enough that the mosquito nets last three months and you see mosquitoes on the net . I think mosquito nets should be impregnated every six months. " FGD_PV_Dielmo*

If the mosquito net is perceived to be more effective than other means of fight (allows sleep without any mosquito bites), people think that those distributed free of charge have a product that is not strong, short-lived and cannot keep mosquitoes away. In the opinion of the interviewees, the length of the nets is not often adapted when covering the sleeping spaces on the floor. This point of view is linked to an imaginary of free: since it is given, it is thought that designers do not put enough fabric to fully cover the beds (especially those placed on the ground). It also suggests that the quality of the tissue is poor and that there are few insecticides in impregnated nets, thus reinforcing the discomfort that these nets cause.

- **Views on Vector Resistance and Resilience Strategies**

The communities recognize that mosquitoes have become resistant to malaria control interventions. They perceive various different explanations for this, both relating the characteristics of interventions and their own daily experience. They perceive a lack of efficacy in insecticides, and consequently adopt a variety of alternative ways of combatting malaria.

For some, the mosquitoes are thought to have familiarized themselves with the product (the insecticide) and adapted to it. For others, mosquitoes are "protected" because they live only on grass and blood, in addition to the fact that the products no longer have sufficiently high dose. Because of this perceived resistance, people think that mosquitoes have changed their habits and bite more during the day than at night.

« E : Vous avez évoqué pas mal de choses, mais dans ce que vous venez de nous montrer qu'est-ce qui participe aux résistances des moustiques ? R1 : Selon moi depuis qu'on a commencé à imprégner les moustiquaires, les moustiques deviennent de plus en plus résistants. Auparavant les moustiques ne piquaient que le soir mais maintenant ils peuvent nous piquer à longueur de journée. »

➔ *"E: You mentioned a lot of things, but in what you just showed us what is involved in the resistance of mosquitoes? R1: In my opinion since we started to impregnate mosquito nets, mosquitoes have become more and more resistant. Previously mosquitoes were only biting in the evening but now they can bite us all day long. "*

« R4 : Regardes, il s'agit d'un jardin, je m'active sur ça. Tu vois un enfant dans les herbes en train de laver les aubergines qu'il a cueilli avec de l'eau et que dans cet endroit on trouve beaucoup de moustiques ce qui est dangereux pour lui. »

➔ *"R4: Look, this is a garden, I'm focusing on it. You see a child in the grass washing with water the eggplants that he has picked and in this place there are many mosquitoes which is dangerous for him."*

This perception that vector habits has changed has also led people to think that it is no longer useful to sleep under mosquito nets every night, because according to their perceptions it is especially during the day that mosquitoes have become dangerous.

The factors responsible for this growing resistance are mainly related to the everyday behaviour of the population. The activities or practices of the populations tend to favour perception of resistance and especially the presence of mosquitoes. In Dielmo, the practice of market gardening is seen as a favourable factor for the reproduction and resistance of mosquitoes. The water used for this activity is not frequently renewed. In Ndiop and Dielmo, the water of the toilets is not drained, it stagnates behind the houses and this is the decoration of the whole village.

In Guédiawaye, Ndiop and Dielmo, the mosquito resistance and changes in the attitudes of the population are systematically linked to a low perceived effectiveness of the preventive means that are proposed: the use of mosquito nets, now more and more contested and people are trying to turn to popular understanding or sanitation approaches to move away from nets. At the same time spraying is desired, but there are disagreements about this with some considerations (e.g. regarding the need to move items to enable spraying, stains on the walls) disfavouring it. Because of resistance, paradoxically, at the same time, people think that malaria is no longer as problematic as it used to be, and that the increased virulence of mosquitoes suggests that the disease is something other than malaria. Strategies have evolved in time, elders report there was formally the use of quinine, and insecticide dusting. However, mosquito nets have been more efficient, favouring the reduction of malaria, and medical management also improved over time with the availability of drugs, tests and treatments. Today, virulent mosquito bites at unusual times and places suggest some other disease.

To improve the situation and better manage vector resistance, the local people propose the following measures:

- Sanitation ;
- Dusting with insecticide and spraying ;
- Drug prevention through seasonal chemo prevention of malaria (CPS) ;
- Increase the insecticide dose of the net and other insecticides ;
- Involvement of local authorities in health problems, especially malaria.

(iii) Benin

The data from this research were collected and processed using the prospective approach and the content analysis method. Individual interviews were conducted with households and the photovoice technique and led to the organization of three focus groups in the villages of: Agouako, Gbegoudo and Akadjame with women aged 35 and over (six per village). These women were chosen because of their responsibility in the household, in particular because they are responsible for children and are therefore the privileged witnesses of the effects of malaria. These villages were selected because of their peri-urban characteristics on the one hand and their lowland environments on the other hand. These reasons make them crossover environments of several categories of experiments on malaria disease but also large-scale breeding sites of mosquito vectors of the disease.

A variety of problems related to the fight against malaria were identified in the municipality of Tori.

- **Economic issues**

As a result of low income and subsistence difficulties, some people resell impregnated mosquito nets to buy food or enrol their children in school. Thus, mosquito nets are no longer used to prevent malaria that is growing in the region. Also, the low level of income does not allow them to buy new mosquito nets once the one obtained for free is used. In addition, on the agricultural level, the commune is subject to a glaring lack of means for the processing of agricultural products; This leads the cooperatives to substitute the net for the work of processing cassava, for example to gari, as witnessed by the words of the following informant:

« Le principal problème à la base de tout ici c'est la pauvreté. Nous ne mangeons pas à notre faim à cause de la pauvreté. Aucune activité ne marche sérieusement parce que le revenu est très faible. Alors nous utilisons les moustiquaires pour en faire ce qu'on peut ».

➔ *"The main problem underlying everything here is poverty. We do not eat enough because of poverty. No activity works seriously because the incomes are very low. So we use mosquito nets for all kinds of purpose. Woman, 33, shopkeeper, Tori-Bossito, Acadjamè village.*

- **Social issues**

At the social level, the problems relate to population growth, low education and illiteracy. These constitute impediments to the implementation of the malaria prevention policy:

« Le problème qui se pose ici c'est d'abord le faible niveau d'instruction. Cela ne nous permet pas de prendre des précautions pour limiter les naissances et on a beaucoup d'enfants dont la prise en charge est difficile. Si on peut nous apprendre les méthodes modernes de planification, ça nous aidera beaucoup ; car lorsqu'on fait beaucoup d'enfants, le nombre de moustiquaire est insuffisant »

➔ *"The problem here is first and foremost the low level of education. That does not allow us to take precautions to limit births and we have many children, whose care is difficult. If someone could teach us methods of modern family planning, it would help us a lot; because when we have many* *children, the number of mosquito nets is insufficient " Female, 28, without a fixed occupation, Tori-Bossito, Agouako village.*

- **Cultural issues**

Knowledge of some traditional measures reduces the enthusiasm of populations to adopt recommended interventions. The reasons given here are related, on the one hand, to the cost of modern measures and, on the other, to mistrust of everything that comes from elsewhere and which concerns health. This is what the following informant states:

« Les mesures dites modernes ont un coût alors que sans rien les gens peuvent aller chercher leur feuille à côté pour en faire une tisane ou autre chose. C'est donc un contexte difficile pour l'application des mesures modernes. »

➔ *"The so-called modern measures have a cost. Even with no resources can go and collect herbs nearby to herbal tea or something else. It is therefore a difficult context for the application of modern measures." Man, 44, teacher, Tori-Bossito, village Agouako.*

- **Political issues**

Populations are not involved in the development of health policies. This means that often their real aspirations are not taken into account by projects:

« Il ne faut plus que les gens restent en haut la bas pour prendre des décisions en notre nom. Ce n'est pas toujours ce que nous voulons qu'ils décident. La preuve regardez cette moustiquaire qu'on distribue partout mais que les gens n'utilisent pas. Nous voulons qu'on nous consulte avant de décider des choses pour nous ce qui n'est pas actuellement le cas. »

➔ *"The people at the top should not make decisions on our behalf. It's not always what we want them to decide. For instance, look at mosquito nets that are distributed everywhere but that people do not use. We want to be consulted before things are decided for us, which is not currently the case." Man, Gbégoudo, 35, logger, Tori-Bossito, Gbégoudo village.*

In addition, impregnated mosquito-net distributors reduce access by patronage and exerting pressure. Also, preventive medications like chloroquine that were used by all, now exist only for pregnant women (intermittent preventive treatment) and children under 5 years (intermittent preventive infant treatment).

- **Environmental issues**

A number of problems might be related to the poor planning of buildings and the heat caused by the destruction of the environment. Climate change could causes excessive heat that causes people to sleep on the yard or spend more time at night without protecting themselves from mosquitoes. The following words illustrate it:

« Nous avons très chaud surtout en saison sèche alors que la moustiquaire est source de chaleur. C'est pourquoi il est difficile de l'utiliser pendant cette période. »

➔ *"We are very hot especially in the dry season and the net is a source of heat. This is why it is difficult to use during this period. » Man, 39 years old, Mason, Agouako*

- **Technological issues**

The quality of the mosquito nets does not encourage their use. The mosquito net of rectangular shape is not always adaptable to the forms of the buildings in the municipality of Tori-Bossito. People also do not approve of the vast majority of Olyset LLINs and prefer soft LLINs.

(iv) Côte d'Ivoire

- **Quantitative data on mosquito net use and its determinants**

In Cote d'Ivoire, the study was conducted in the department of Agboville. Agboville is a town in south-eastern Cote d'Ivoire, situated at 80 km north to Abidjan, the Economic capital city of the country. The selection of Agboville as the study site for this research was motivated by the fact that the region is considered at potential high risk of malaria by the Malaria National control program. Additionally, it has high rates of insecticide resistance in mosquitoes. Available background data, included LLIN coverage from household surveys (Table 10).

	May	**June**	**July**	**Aug.**	**Sept.**	**Oct.**	**total**
Total number of individuals leaving in investigated households.	3335	11582	2053	4780	9237	9267	40254
Number of households investigated	169	465	252	603	1131	1185	3805
Number of households with LLINs	467	1 803	246	5	1 063	1 157	4741
Number of households with LLINs hanging	467	1 803	246	579	1 063	1 157	4736
Number of bed units in the households	NA	NA	1 004	2 146	3 828	4 315	NA
Number of individuals who slept under an LLINs the night before the investigation	2215	9048	1808	3647	7567	7915	32200
% of individuals who slept under an LLIN the night before the investigation	66.42	78.12	88.07	76.30	81.92	85.41	79.99

Table 10. Coverage of mosquito nets in the city of Agboville (source PNLP Côte d'Ivoire).

Several social science techniques were used to measure Knowledge, Attitudes, Practices and Beliefs (KAPB) of the populations in selected sites in the Department of Agboville. This involved both qualitative and quantitative techniques. For quantitative data collection, a household survey was carried (n=700). This study used a questionnaire to assess knowledge and practices of the populations with regard to the strategies of fight against the malaria, the acceptability of the tools proposed by the programs of fight against malaria and the alternative methods. Qualitative methods involved semi-structured interviews (n=30) with a selection of community resource persons as well as with the health personnel, and traditional, municipal and administrative authorities. Also participatory methods such as photovoice (n=12) and focus group discussions (n=24) were used. The aim of that research strategy was to: (i) enable people to record and reflect on the strengths and concerns of their community with regard to malaria control strategies; (ii) promote critical dialogue and knowledge on personal and community issues regarding malaria control strategies and future options; (iii) provide decision-makers with clear recommendations from the people.

In this research, three study sites were selected based on socio-economic and ecological criteria: 1 rural site (Offoumpo), sub-urban area (Sokoura) and urban site (Residentiel). In those sites, the selection of participants to the study was based on criteria of age, gender and socio-economic status. The data collection took place from December 2016 to April 2017.

- **Perception of malaria by populations**

Malaria was cited by 97.4% of informants as one of the most common diseases in the region. Malaria is generally characterized by populations in terms of symptoms and causality. If headache and fever are identified as the main symptoms of malaria, it is not clear if what they claim as malaria is exactly malaria as fever and headache can be the symptoms of other diseases. A diversity of causes are assigned to malaria. Most of the people associate the disease with mosquitos' bites. But this knowledge varies according to the level of education and exposure to awareness campaigns. Misconceptions persist among populations that still link the cause of malaria to natural elements such as the sun or feeding habits.

Based on their daily experience, populations elaborate their knowledge of malaria on elements of their surrounding environment or practices. Daily activities such as farming, trading and long-distance walks characterized by long exposure to sun are believed to expose people to malaria. Malaria is also correlated with excessive consumption of certain foods especially oily ones. As far as the level of education is concerned, the less people are educated, the more they tend to associate the causes of malaria with diet and the sun.

- **Perception of the vector of malaria**

The mosquito is considered by the population as causing at the same time diseases and nuisances. The survey revealed that populations experience a high level of mosquito nuisance, regardless of the study environment. The presence of mosquitoes is often related to social explanations such as the transgression of traditions.

Populations identified several natural and cultural drivers to the presence of mosquitoes. With regard to periods of intense mosquito aggression, respondents recognized that mosquitoes are more present during the rainy season when there are several pools of stagnant water around the village or neighbourhood. It is also the harvesting time for some fruits and vegetables (mangoes, maize, etc.) that attract mosquitoes. However, most people recognized that mosquitoes are present, regardless the season or the period of the year. As long as environmental issues are not taken into account, mosquitoes will always be present. The town of Agboville is surrounded by lowlands and swamps, often used for rice cultivation, that constitute breeding sites for mosquitoes. Additional to that, the sanitation system is poorly developed in rural and sub-urban areas, contributing to the breeding of mosquitoes. Lack of hygiene in the household and its surrounding is also identified as attracting mosquitoes. This comprises accumulation of dirty clothes, garbage dumps, scrubs, etc. Human activities such as food production (e.g. cassava processing for the production of a local dish called attiéké), drainage system of waste water from households, urban agricultural activities (gardening) or livestock keeping are also favouring factors to the spread of mosquitoes.

Whether one is outside or inside, one feels the mosquito bites. However, the difference between the outside and the inside in terms of mosquito bites could be observed the most in the evening. Most of informants do not directly link mosquito bites to malaria. They are more worried about nuisances caused by mosquito bites

- **Social perceptions of malaria vector control tools**

Several vector control tools are used by populations. These are fumigating coils (locally called moustico), insecticide sprays, and bednets. Tools like fan have also been mentioned by few people. Each of the most cited tools has advantages and disadvantages. The coils are affordable by all the households even in remote rural areas, but their efficiency is seriously questioned. The exposition of some people to the fumigating coils could cause rheum and allergies. Insecticides sprays are efficient in killing mosquitoes, but their long-lasting effect increasingly challenged especially in a context of increased resistance of mosquitoes to insecticides. Additionally, the sprays are not affordable by poor households. Long-lasting, insecticide-treated mosquito nets (LLIN) are also used. This tool is increasingly accepted as the results of mass distribution campaigns at the national levels. However, despite the awareness campaigns, the use of this tool still encounters resistance. Many people reported not using betnets received from the government. They prefer to use them for other purposes such as sponges for bath and dishes, garden fences (to fight against pests), dryers, goal nets, etc. This shows that the mass distribution of LLIN could contribute to fight malaria but has limits.

- **Perception of mosquito resistance to insecticide**

According to data collected in the field, the respondents admit that the mosquitoes have changed their behaviour in the sense that most of the repellents used nowadays hardly kill mosquitoes. Many people believe that the role assigned to the insecticide of a control tool is nothing more than a pretext that hides the very intention of the manufacturers. For them, more than fighting intentions, the design and sale of insecticides serve other purposes. This explains the tendency of mosquitoes to adapt to products. As a result, the mosquitoes reappear with the disintegration of the repulsion device. As if to say that the repellent effect for which the populations use the tools is not observed. In addition, with the combination of mosquito control tools, the repellent effect of some is annihilated by that of others and vice versa.

Some informants recommended that strategies for the vector control should focus more on hygiene and sanitation and not on the use of chemical repellents. Thus, for them, the resurgence of mosquitoes despite the use of insecticides is linked to the deficiency in the sanitation of the living environment.

- **Control strategies from health authorities**

To the question on the knowledge of strategies from the authorities for vector control, informants mention: (i) mosquito net distribution (53.54%); (ii) environmental sanitation (29.00%) and, (iii) awareness raising around preventive control tools (28.28). %). Populations say they are not directly involved in the fight against malaria in the locality. However, they state that community health workers organize home-based discussions and discussions on community behaviour change topics. The strategies put in place by the institutional actors are appreciated, but these strategies must be adapted to the environment and to the concern of the populations. Indeed, according to the majority of informants, prevention means proposed before were more effective than those proposed today. For them the integration of general spraying at the expense of the distribution of impregnated mosquito nets would be beneficial for mosquito control. This should be accompanied with sanitation campaigns that are more effective for malaria vector control than any other means.

c) Modelling

In Dielmo, we were able to estimate entomological infection rates for bed net users and non-users by weighting biting rates outdoor and indoor (obtained from a statistical model) by inhabitants hourly habits to rest rather indoor versus indoor.

Specy	Periods	Exposure		Infection rates			EIR/year Protected	EIR/year Unprotected	Relative hazard	True protective efficacy of an ITN (P^*)
		Protected (B_p)	Unprotected (B_u)	Tested	Positive	s= Positive/Tested	$EIRp=B_p*365*s$	$EIRu=B_u*365*s$	λ_p. B_p / B_u EIR_p / EIR_u	$P^* = 1-\lambda_p$
An. gambiae	Period 1	3,32	9,28	1044	11	1,05%	12,76	35,68	36,65%	64,24%
	Period 2	4,46	11,71	4820	58	1,20%	19,61	51,43	38,37%	61,87%
	Period 3	1,92	4.12	2077	46	2,21%	15,55	33,30	47,44%	53,30%
	Period 4	0,69	1,33	448	2	0,45%	1,12	2,16	54,31%	48,02%
An. funestus	Period 1	4,76	12,77	2075	33	1,59%	27,65	74,14	37,29%	62,71%
	Period 2	0,40	0,97	506	3	0,59%	0,87	2,10	41,37%	58,63%
	Period 3	0,58	1,17	645	1	0,16%	0,33	0,66	50,06%	49,94%
	Period 4	0,72	1.26	507	4	0,79%	2,06	3,64	56,75%	43,25%
		$B_{p\ (gambiae)} + B_{p\ (funestus)}$	$B_{u\ (gambiae)} + B_{u\ (funestus)}$	Tested	Positive	s	$EIR_{p\ (gambiae)} + EIR_{p\ (funestus)}$	$EIR_{u\ (gambiae)} + EIR_{u\ (funestus)}$	EIR_p / EIR_u	$P^{**} = (1 - EIR_p / EIR_u)$
Total	Period 1	8,08	22,05	3119	44	1,41%	40,41	109,82	36,79%	63,21%
	Period 2	4,87	12,68	5326	61	1,15%	20,48	53,53	38,25%	61,75%
	Period 3	2,51	5.29	2722	47	1,73%	15,88	33,96	46,77%	53,23%
	Period 4	1,41	2,59	955	6	0,63%	3,19	5,80	54,97%	45,03%

Table 11. Mean number of bites across nights expected on bed net users (10:00 to 05:00) and non-users according to the following periods: Period 1 (July 2006 - June 2008) before the implementation of mosquito nets, Period 2 (July 2008 - June 2011), Period 3 (July 2011-June 2014), Period 4 (August 2014 - April 2016) after the introduction and renewals of mosquito nets.

We could see that the EIR decreased a lot, both for users and non-users, mainly because of the dramatic decrease in the number of bites (the mosquito population felt down dramatically). The rate of infection, although not steadily decreasing, finally plays a minor role in the EIR values.

However, all inhabitants, users or not are still bitten several time a year (3-5) by an infected mosquito, putting them at a persistent risk of malaria infection.

We also combined an existing mathematical model for mosquito population dynamics that included the evolution of resistance, with a model for malaria in mosquitoes. This new model allows us to determine the theoretical impact of varying the sensitivity of Anopheles mosquitoes to Plasmodium infection as a function of resistance status on malaria transmission. We have parameterised this model to the entomological data collected in Dielmo, Senegal and Agboville, Côte d'Ivoire and are currently running simulations to determine the impact on transmission.

4) Integrated risk-analysis

In order to assess the risk of seeing a setback in malaria control due to the evolution of resistance and the increased Anopheles sensitivity to Plasmodium, we aimed to take into account the three components of the mosquito infection status and its determinants (in particular, the genotype at loci involved in resistance evolution: *kdr*, *Ace1*), the mosquito behaviour (propensity to bite indoor and outdoor, time of biting) and its change over time and the human behaviour (propensity to be indoors or outdoors during the night, and mosquito avoidance behaviour) and its change over time.

Regarding the biology of infection of mosquitoes, we found that in Dielmo, Senegal, and Agboville, Côte d'Ivoire, resistant mosquitoes (bearing the L1014F allele) tend to have higher infection rates than sensitive mosquitoes, both in wild populations or via experimental infection, but in Benin, there were not enough sensitive mosquitoes were available for a comparison with resistant mosquitoes. These results support results obtained by previous studies on lab strains [15, 23, 34]. In Dielmo, we observed that the trend in infection rates was not steady: the infection rates of *An. gambiae* first increases after the implementation of impregnated mosquito nets, from 2006 to 2014, but then decreased to a rate never seen before during the longitudinal survey from 2014 to 2016. Conversely in *An. funestus*, the rate of infection decreased from 2006 to 2014, and then increased again. These changes in rates of infections can be linked to various factors. The decreases can be linked to the prophylaxis given to humans to treat malaria, and to the dramatic drop in the number of bites (linked to a likely drop in the population size of mosquitoes). The increases could be due to a lowered vigilance in malaria control (e.g. populations are using less the nets) or also potentially to the increase in the frequency of the *kdr-R* mosquitoes in the population (as seen, from ~8 to ~12% in e.g. *An. arabiensis*). The latter explanation would account more easily for the interspecific differences in trends of infection (resistant alleles being rarer in *An. funestus*), but the change of the frequency was very slow (only a 4 points increase). It would be of great concern if this is the only explanation (as future evolution of resistance alleles would then have a very high impact), but hopefully, the global drop in the number of bites will largely compensate for any increase in the rate of infection of mosquitoes in Dielmo (Table 11). In other locations (Côte d'Ivoire and Benin), the proportion of resistant mosquitoes already being very high, however, the situation regarding their frequency and therefore the rate of infection is unlikely to worsen, unless there is also a link with the frequency of *Ace 1*, which remains to be investigated (its frequency being too low for now to get enough *Ace1-S* mosquitoes to be compared with those who are *Ace1-R* regarding their susceptibility to infection by *Plasmodium*).

Mosquitoes still bite mostly in night-time in all our three sites, not early in the evening or after people wake in the morning. This pattern is quite reassuring. There is a switch to an higher proportion of outdoor bites in Dielmo, but this is because the number of outdoor bites has not decreased as fast as the rate of indoor biting, not because mosquitoes that were biting indoors are now biting outdoors. This is also reassuring. In other sites the proportion of bites occurring indoor rather than indoor is similar to that before the implementation of nets in Dielmo, suggesting that the same pattern regarding this behaviour might hold.

Regarding human behaviour, a variety of issues have been identified that still prevent a number of individuals to use mosquitoes nets properly, and a few factors have been identified that might led, in the future, both to an increased coverage or to a lowered coverage, depending on which factors will predominate. Factors can be classed into economic (poverty), social (education, etc.), cultural (beliefs and traditions), political (frustration regarding how the malaria issue is handled), environmental (heat) and technological (nets quality).

Economic factors play a role that could evolve positively is poverty is reduced in the future: populations use nets for other purpose than what they are supposed to be used for, e.g. agriculture (to protect crops from pests), cassava transformation, or wedding dresses. This allows population to earn a few coins which can be very helpful in case of dramatic poverty. Populations also indicate that it is difficult for them to renew nets when they are broken, in particular in Benin, which could be solved by distributing new nets more often. On the other hand the proportion of families owning and using at least one net is still very high in all three locations, despite these issues.

Social factors could play a major role as well. A lot of beliefs persist regarding how malaria is understood, and how it is transmitted. Not everyone makes the link with mosquito bites, while this is very important for them to use nets. Hence, there is room for progress regarding the coverage with education. We can be optimist in this regard as populations consider information campaigns as important, indicating they are rather receptive. However, when informing populations, it is very important to help them to distinguish mosquitoes that transmit malaria from those that don't, and to understand they are different kind of mosquitoes with different behaviours, as mosquitoes biting in daytime (non-transmitting species) convinced some people that mosquitoes have adapted to nets by changing their behaviour and therefore that nets are not useful anymore. Given the inconvenience of using nets (heat, discomfort due to the texture), this is likely to be a factor that might lead to a lowered coverage. The fact that the entomological data do not indicate that changes in mosquito behaviour have occurred that would make nets inefficient (resistance to the insecticide lowers the efficiency but the mechanical barrier of the net remains), makes it easier to identify the appropriate message: net use continues to be highly desirable. It may also be important to take into account the perceptions of malaria as either dangerous or benign. It is not always considered the most problematic disease in the study sites, and it might be important to underline that the decline in malaria is tightly tied to control measures and that vigilance has to be maintained for the control efficiency to persist.

Cultural aspects might also play a role, and are linked to education as well. Populations have "alternative" methods they used to implement traditionally. The use of these alternative methods is linked to economic aspects (they are less expensive) and political aspects (state implemented methods are perceived as a way to serve obscure goals). Regarding political aspects, answering populations most emergent needs (as sanitation) could help, as populations would see their ideas are taken into account (the implementation of control tools would be less perceived as a top-down approach).

Regarding environmental issue, it will be difficult for the concerned countries to really weight in the balance to fight climate change, although the effect on net use could be put on the list of arguments. Locally however, there could be policies and support to populations for constructions to be fresher, so that populations are more motivated to use nets. This would also help populations to adhere to state policies as this would show state entitlement to improve their comfort.

Eventually, regarding technological issues, it should be possible to take into account inhabitant's preferences regarding the shape and texture of nets, in order to improve coverage. It would also be useful to find a mechanism to deliver nets for free but letting populations know their actual price (e.g. by a label on the package), so nets are not perceived as low quality nets because of their low (or no) cost.

To sum up, we are not in the worst scenario possible either regarding entomological aspects or sociological aspects, but the risks are not null. The risk is therefore medium, and efforts have to be maintained to improve net coverage and make sure no drawback is observed in malaria control.

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