International Journal of Entomology Research
ISSN: 2455-4758; Impact Factor: RJIF 5.24
Received: 01-01-2020; Accepted: 02-02-2020; Published: 01-03-2020
www.entomologyjournals.com
Volume 5; Issue 2; 2019; Page No. 01-08

Feeding behavior and insecticide resistance status of the *Anopheles gambiae* complex in prelude of indoors residual spraying in Baroueli, Mali

Amadou Guindo[1], Ladji Camara[2], Abdourhamane Dicko[3], Sekou F Traore[4], Mamadou Coulibaly[5], Dr. Mamadou B Coulibaly[6]

[1, 2, 4, 5] Malaria Research and Training Center, Faculty of Medicine and Dentistry, University of Sciences, techniques and Technologies of Bamako, Mali

[3] Programme National de Lutte contre le Paludisme, Mali

Abstract

In prelude of implementing IRS in the Baroueli district through PMI/USAID's support, entomologic baseline studies were conducted. Mosquitoes were collected monthly using pyrethrum spray catches and human landing catches from June to October 2010. Bioassays were conducted to assess insecticide resistance in vectors using WHO kits. The collections comprised 81.2% of *Anopheles spp*, 18% of *Culex spp* and 0.8% of *Aedes spp* (n=11583). *Anopheles gambiae s.l.* represented 80% of the Anophelines (n=9402). Within *An. gambiae s.l., An. coluzzii* represented 83.2% of the total collection (N=1403) against 10.8% for *An. arabiensis* and 6.0% for *An. gambiae*. EIRs by PCS were 0, 2.3, 7.8, 5.8 and 1.4 infective bites per human per month from June to October, respectively. By HLCs EIRs were 0, 5.3, 28.5, 19.1 and 0. The numbers of mosquitoes collected indoors (98.7, SD=109.9) and outdoor (85.8, SD=106.2) were comparable (independent T-test, p=0.856). Bioassays showed resistance to pyrethroids and DDT while bendiocarb showed high efficiency (100% mortality) which led the National Malaria Control Programme to shifting from pyrethroids to bendiocarb for IRS. These results indicate that malaria transmission might be considerable outdoor as well as indoors and that the major malaria vector is resistant to pyrethroids but susceptible to carbamates.

Keywords: carbamates, outdoor transmission, pyrethroids, susceptible, vector-borne disease

1. Introduction

In 2017 WHO estimated the number of malaria cases to be 219 million around the world with about 435, 000 deaths (WHO, 2018). Although the number of cases decreased between 2000 and 2015, malaria still remains a major public health problem in Sub-Saharan Africa. Even if malaria has been eradicated or eliminated from some areas of the world, countries in the global equatorial zone remain malaria endemic (Feachem *et al*. 2009) [8].

In 2017 Malian National Statistics reported more than two million malaria cases with 1,050 deaths making malaria the deadliest parasitic disease in the country. Malaria accounts for 42% of the health-seeking motives in health facilities in the general population (EDSM-V 2012-2013). The Ministry of Health through the National Malaria Control Program (NMCP) has developed a malaria control policy that is based on four major interventions: 1] rapid diagnosis and treatment, 2] intermittent preventive treatment in pregnant women (IPT), 3] seasonal malaria chemoprevention and 4] vector control.

In Mali Anopheles gambiae s.l. and Anopheles funestus s.l. are the two major malaria vectors. The first is a complex of at least eight sibling species (An. gambiae, An. coluzzii, An. arabiensis, An. merus, An. melas, An. quadriannulatus A, An. quadriannulatus B and An. bwambae) (Coluzzi *et al*. 1979, Hunt *et al*. 1998, Coetzee *et al*. 2013) but only An coluzzii, An. gambiae and An. arabiensis have so far been reported from Mali (Toure *et al*. 1998) [18]. Anopheles funestus is a complex of at least eight sibling species (An. funestus s.s., An. rivulorum, An. parensis, An. vaneedeni, An. aruni, *An. confuses*, *An. brucei* and *An. fuscivenosus)*. Only *An. funestus s.s.* has been reported from Mali. There are a very few studies on *An. funestus*; however, field-based studies have implicated it as the principle vector during the cool, dry season when the densities of *An. gambiae* are extremely low (Gillies and De Meillon, 1968, Diuk-Wasser *et al*, 2005).

Vector control is a key component of the WHO global malaria control strategy (WHO 1992). That strategy has been mainly based on large-scale distribution of insecticide-treated bed nets (Choi *et al*. 1995). This strategy has been adopted by the National Malaria Control Programs in Africa. The current large scale vector control interventions in use in Mali are the mass distribution of long lasting insecticidal bed nets (LLINs) and indoor residual spraying (IRS). LLINs are offered through routine distribution (i.e. pregnant women at prenatal visits and children under 5 years of age). Mali has adopted universal coverage however the Baroueli district is not yet covered. Recently there has been a revival of indoor residual spraying (IRS) which was used successfully in eradicating malaria in certain areas of the world. The WHO is encouraging malaria control programs to consider including IRS in their strategy. IRS is most efficient where the target vector is endophilic and susceptible to the insecticide to be sprayed (WHO, 2006). Evidences have shown that in a neighboring country of Mali, Senegal, *An. gambiae* is strongly endophilic (Faye *et al*. 1997) [7] though it might be important to revisit this question to make sure this still applies after several years of interventions targeting indoor resting/feeding mosquitoes.

Both methods (ITNs and IRS) require the use of insecticides for a long period of time therefore may lead to the selection of resistance in the vector to the insecticide that is used. Pyrethroids are, currently, the preferred insecticides for ITNs because of their low mammalian toxicity and their biodegradation. Various types of insecticides including pyrethroid, organophosphate and carbamate families are used in IRS. It is inevitable that the implementation of one or both these methods over a long period of time will result into the development of insecticide resistance in target insects.

In 2005 Mali was accepted in a group of fifteen African countries that are beneficiaries of the US President' Malaria Initiative (PMI) which has a vector control component based on IRS and large-scale distribution of LLINs. IRS started in 2008 with two districts (Bla and Koulikoro). In 2011 it was extended to Baroueli. The successful implementation of such interventions requires knowledge on the biology of local vectors prior to the interventions. It is important to know what vectors species are present, the different sub-populations of the species, their implication in malaria transmission, their feeding/resting behavior and their insecticide susceptibility status. These baseline data are crucial for a realistic impact assessment of the entomological interventions and for evidence-based decision making. Here we present the results of an entomologic baseline study that we conducted, in 2010, in the Baroueli district located in the Segou region in Mali prior to IRS. A lack of or poor baseline data would compromise the validity of an impact assessment, jeopardize the success of the whole program and lead to a squandering of resources without accomplishing the goals of the interventions.

2. Materials and methods

2.1 Study sites

Baroueli is a district of about 200 000 inhabitants located in the Segou region Northeast of Bamako the capital city of Mali. At 150 km from Bamako the district of Baroueli is easy to access and relatively close to the capital where the laboratories for analysis are located. Its geographical coordinates are 13.04N and 6.50W. The transmission of malaria is seasonal with the bulk occurring during the rainy season (June to October) following the general pattern in Mali (Coulibaly *et al*. 2013) [3, 5]. According to national statistics, in Segou region, the incidences were 120.7%o and 40.3% o for uncomplicated and complicated malaria respectively (SLIS 2010). Vector control is based on two main strategies: LLINs distribution and IRS. Studies have shown that 87.4% of children under five years old and 71.0% of pregnant women were using LLINs in 2010 (Traore *et al*. 2010) [20].

Data collections were, simultaneously, made in three villages namely Boidie, Kamba and Soungola. Data were combined for the analyses.

2.2 Mosquito collection methods

Mosquitoes were collected on the field using pyrethrum spray catches (PSCs) and human landing catches (HLCs) following the protocol described elsewhere (Diuk-Wasser *et al*, 2005). Collections were conducted once a month from June to October 2010. The great majority of malaria transmission happens during this period with the peak in October. This period is also the rainy season where mosquito densities are high. During the dry season (November-May) mosquito densities are so low that the proposed analysis is extremely difficult. PSCs were conducted in the morning from 08H00 to 10H00 in thirty rooms randomly selected in each of the three villages every month. The same rooms were sprayed every month. HLCs were organized in two randomly selected rooms from 18H00 to 06H00 the next morning in each of the three villages. Two volunteers (one indoor and one outdoor) collected mosquitoes with an hour switch (indoor and outdoor) from 18H00 to 00H00 and two others collected from 00H00 to 06H00. The same two rooms were used every month for the whole survey.

2.3. Determined entomological parameters

2.3.1 Vector species identification.

Individual mosquitoes were identified to species using molecular typing described elsewhere (Fanello *et al* 2002). Only specimens that were morphologically identified to be *Anopheles gambiae s.l.* were kept for molecular analysis.

2.3.2. Vector densities and human blood index (HBI).

These parameters were determined using mosquitoes collected by Pyrethrinum spray catches (PSC) for convenience. PSC targeted only indoors resting mosquitoes. That allows estimating the density per room. PSC collected mosquitoes include half gravid and full blood fed specimens that were used to determine the human blood index as mosquitoes that are collected through HLCs are all unfed. Vector densities refer to the average number of females mosquitoes per room. The human biting rates refer to the average number of bites per person per time unit (here the time unit is the month).

The human blood index or the anthropophily refers to the proportion of the specimens fed on human blood. The blood sources were determined using ELISA following the protocol described in Beier *et al*, 2002 [1] (Beier 2002) [1]. Table 1 shows the formulas used to calculate these two parameters.

2.3.3. Human biting rates (HBR), parasite infection rates and the entomological inoculation rates (EIR).

These three parameters were determined using both methods. Our goal here is to present estimates from both methods rather than comparing parameters between methods. The human biting rates refer to the number of mosquito bites per person per month

The parasite infection rates refer to the proportion of mosquitoes found to be harboring *Plasmodium falciparum*. Enzyme linked immune-sorbent assays (ELISA) for detecting circumsporozoit (CSP) were used to determine the infection status by *Plasmodium falciparum* following the protocol described in Beier *et al* 2002. Subsamples were randomly selected for the ELISA testing [1].

The entomological inoculation rates refer to the average number of infective bites per person per time unit (we used month). EIRs measure the level of malaria transmission. Table 1 shows the formulas used to calculate each of the three parameters.

Table 1: Formulas used to calculate the entomological parameters

Entomological parameters	Formula
Densities	Total number of collected mosquitoes/Total number of rooms
Human blood index	(Total number of positive in ELISA for human blood/Total number of tested mosquitoes)x100
Human biting rates (HLC)	Total number of collected mosquitoes/(Total number of volunteers x number of rounds)
Human biting rates (PSC)	Total number of mosquitoes/number of residents that have slept in the rooms the previous night
Parasite infection rates in mosquitoes	(Total positive in ELISA/Total tested)x100
Entomological inoculation rates (HLC)	HBR x (Total positive in ELISA/Total tested)

2.3.4 Proportion of mosquitoes biting indoor vs outdoor and determination of the parous rates.

After collection, mosquitoes are tallied to determine the proportions caught indoor and outdoor. Then subsamples (indoor and outdoor) were dissected to determine the parous rate. Mosquitoes were recorded as nulliparous when the ovaries contained tightly coiled ovarioles. They were recorded as parous when the ovaries contained relaxed ovaries. This follows the methods described by Detinova (Detinova, 1962).

2.3.5 Insecticide susceptibility testing.

The WHO bio-assay kits were used to test for the susceptibility/resistance (WHO 1998) [22] of vectors in the district. The insecticides used were: deltamethrin 0.05%, lambda-cyhalothrin 0.05%, bendiocarb 0.1%, permethrin 0.75% and DDT 4%. The assays were conducted only once in September when mosquitoes densities were still high. Unfed 2-5 days old female mosquitoes from the first generation progeny of wild caught individuals were used. Wild females were caught using room search collection. The number of exposed mosquitoes was one hundred (100) for each village. This represents four (4) replicates of 25 specimens. The control tube also contained 25 specimens.

2.3.6 Data analysis

Proportions of the components of *An. gambiae* were compared using the Pearson chi-squared with a cut off of 0.05. The means numbers of mosquitoes collected indoors and outdoors were compared using independent T-test.

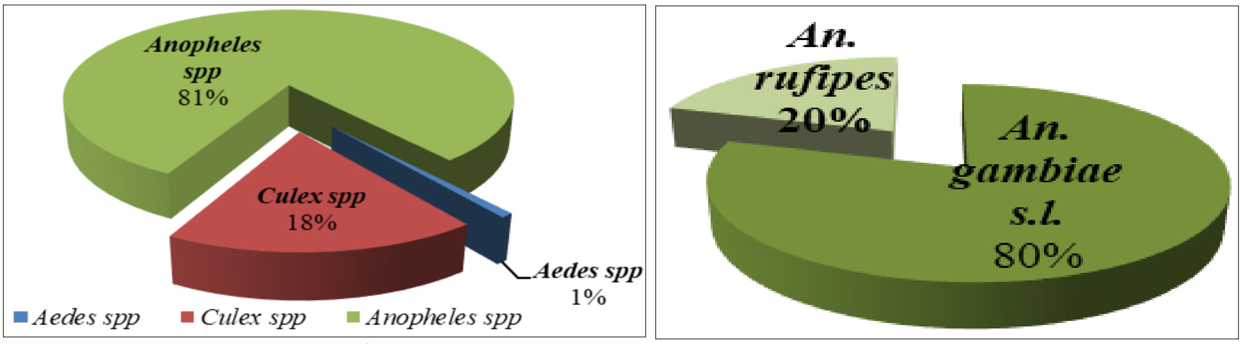
2.3.7 Ethical considerations

This work was conducted under a cooperative agreement approved by the Malian Government and verbal consents were obtained from house owners and mosquito collectors after the protocols were explained to them in the local language. The protocol was reviewed retrospectively by the Malian IRB (Nº2012-84/CE/FMPOS).

3. Results

3.1. Mosquito species identification

Overall three species of mosquitoes were morphologically identified from the collected specimens from June to October 2010 in three villages in Baroueli. The identified species were *Anopheles spp* (81.2%), *Culex spp* (18%) and *Aedes spp* (0.8%) on a total of 11583 specimens. Among the anophelines there were 80% of *Anopheles gambiae s.l.* and 20% of *Anopheles rufipes* (N = 9402) (See fig 1). *Anopheles gambiae* represented 64.6% of the total collection. These results stem from PSC collection.



A B

Fig 1: Different species of mosquitoes morphologically identified in Baroueli (A) and the proportions of anophelines (B) from June to October 2010

Molecular typing was conducted in the laboratory on randomly selected *Anopheles gambiae s.l.* specimens to identify sibling species. *An. coluzzi* represented 83.2% of the total collection (N =1403) against 10.8% for *An. arabiensis* and 6.0% for *An. gambiae*. When compared by pair the frequencies of *An. coluzzii* were significantly higher than those of *An. gambiae* (N = 1251, Chi2 = 36.2, df = 4, $P<10^{-3}$) and those of *An. arabiensis* (N = 1319, Chi2 = 74.9, df = 4, $P<10^{-3}$) respectively. Also the difference between the proportions of *An. arabiensis* and *An. gambiae* was significant (N = 236, Chi2=236, df = 4, $P<10^{-3}$). In all months *An. coluzzi* predominated with a frequency higher than that of *An. gambiae* (Fig 2). No *An. gambiae* was recorded in June.

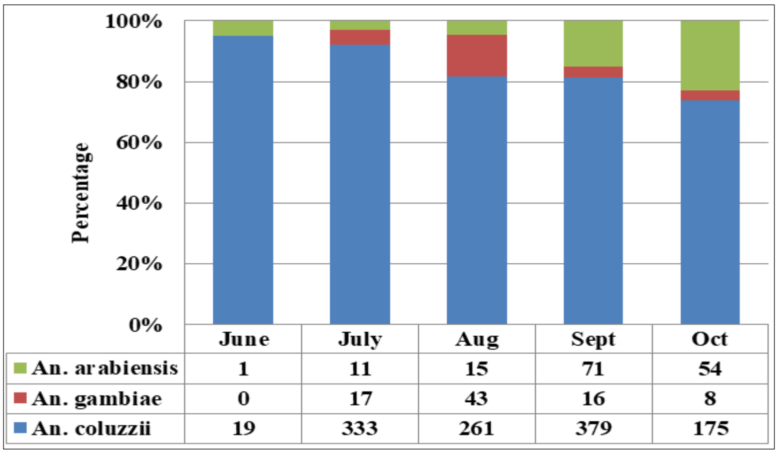


Fig 2: Malaria vector species composition in Baroueli from June to October 2010

3.2. Density and human blood index (HBI)

Overall, the anopheline densities were low in June (0.3 female anopheles per room). Densities increased progressively through July (4.7), August (28.3) and September (46.8) then decreased in October (3.0). This pattern follows the abundance pattern of the rainfalls (Fig 3). The highest densities were recorded in August and September.

Overall the human blood index was low. It was 87.8 % (n = 16), 41.1% (n = 331), 40% (n = 402), 38.7% (n = 483) and 52.6% (n = 137) in June, July, August, September and October respectively.

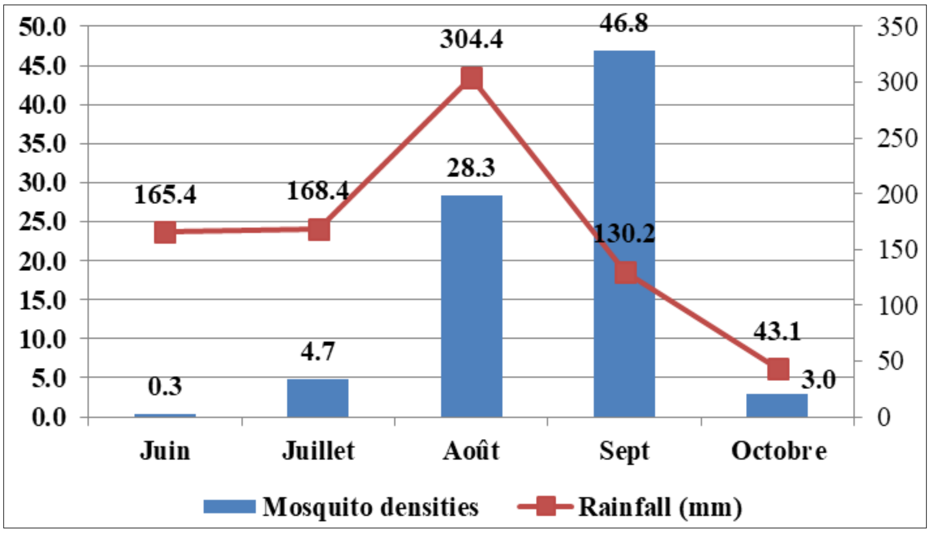


Fig 3: Densities of An. gambiae s.l. per room per month and the rainfall per month

3.3. Indoors/outdoors biting and Parous rates

Different species were caught during the human landing catches. These included *An. gambiae*, *An. rufipes*, *An. pharoensis*. However only *An. gambiae s.l.* specimens were included in the analysis. Table 2 shows the counts and the human biting rates of *An. gambiae s.l* per month indoors and outdoors. Subsamples if not all collected mosquitoes were dissected and their ovaries pulled and checked under a light microscope for parity.

Statistical analysis show no significant differences between the mean counts indoors (98.7, SD=109.9) and that obtained outdoors (85.8, SD = 106.2) (independent T-test: t = 0.187, df = 8, p = 0.856). Over all the months the proportions of mosquitoes collected indoors and outdoors were 53.5% and 46.5% (N = 922) respectively.

Table 2: Counts of female and HBR *Anopheles gambiae s.l.* caught by HLC (indoors and outdoors) in Baroueli from June to October 2010

Month	Indoors		Outdoors		Total
	Total count	HBRHLC	Total count	HBRHLC	
June	8	2.0	0	0	8
July	47	11.8	39	9.75	86
August	146	36.5	119	29.8	265
September	270	67.5	257	64.25	527
October	22	5.5	14	3.5	36
Mean(HBRHLC)		24.7(SD=27.5)		21.5 (SD=26.6)	
Mean (Count)	98.7		85.8		922

Table 2 shows the human biting rates indoors and outdoors (24.7 ± 27.5 and 21.5 ± 26.6 respectively) from the human landing collections. Statistical analysis showed that the HBRs are not significantly different (t = 0.187, df = 8, P = 0.856). Analyses of the *Plasmodium falciparum* CSP (Circumsporozoite protein) showed that 2.6% (n = 152) of *An. coluzzi* collected indoor were infected while 4.6% (n = 152) were infected outdoor. For *An. gambiae* 8.9% (n = 45) were infected indoor while 3.1% (n = 32) were infected outdoor (Table 3)

Table 3: P. falciparum infection (CSP) rates per species within the An. gambiae complex and per collection site (indoor vs outdoor)

	An coluzzii		An gambiae	
	Indoor	outdoor	Indoor	outdoor
Infection rate	2.6 % (152)	4.6% (152)	8.9% (45)	3.1% (n=32)
P-value (Chi2)	P=0.560		P=0.643	

Subsamples of *Anopheles gambiae s.l.* caught by human landing catches were dissected to pull their ovaries. The latter were observed under a microscope to determine the parous rates. These were 75% (n = 8), 97.7% (n = 84), 91.3% (n = 242), 88.6% (n = 467) and 97.2% (n = 35) in June, July, August, September and October respectively.

3.4 Human biting rates, *Plasmodium falciparum* infection rates and EIR for the *An. gambiae* complex

Table 4 shows the HBR, the infection rates and the EIRs using both PSC and HLC for *the An. gambiae* complex. The purpose here is not comparing the two methods but instead showing the tendencies using both mosquito collections. In both methods the three parameters showed a similar trend except the infection rates using PCS. The HBR, the infection rates and the EIRs were low at the beginning of the rainy season, high towards the middle and low towards the end of the season. However, from PSC data, the infection rates were low at the beginning of the rainy season, got high in July-August, decreased in September then increased in October to the highest level (10.7%, n = 271).

Table 4: Human biting rates, infection rates and EIRs per month using PSC and HLC in Baroueli from June to October 2010 for the *An. gambiae* complex

Month	Human biting rates		Infection rates (%)		EIR	
Year	PSC	HLC	PSC (n)	HLC (n)	PSC	HLC
June-10	1.4	10	0.0 (27)	0.0(7)	0.0	0.0
July-10	30.8	107.5	7.5 (427)	4.9(41)	2.3	5.3
August-10	112.1	331.3	7.0 (757)	8.6(93)	7.8	28.5
September-10	247	658.8	2.4 (808)	2.9(234)	5.9	19.1
October-10	13.3	45	10.7 (271)	0.0(25)	1.4	0.0

Though the HBRs and the EIRs showed similar trends from both HLCs and PSCs the estimates of these parameters were higher in HLC than in PSC (fig 4).

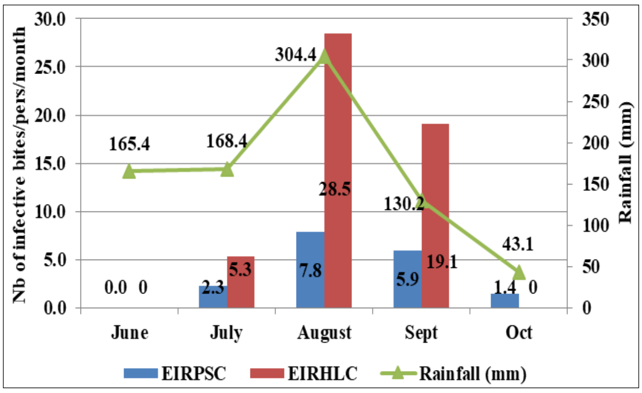


Fig 4: Entomologic inoculation rates from PSC data (blue bars) and from HLC data (red bars) of An. gambiaes.l. and rainfalls (in mm on the right vertical axis) per month in Baroueli from June to October 2010

3.5 Insecticide susceptibility assays

Figure 5 shows the mean mortality and knockdown rates in each of the three villages and in Baroueli using the above insecticides respectively. The insecticide susceptibility assays showed low mean mortality rates (<80%) for all tested pyrethroids and DDT. However bendiocarb, a carbamate, showed 100 mortality rates.

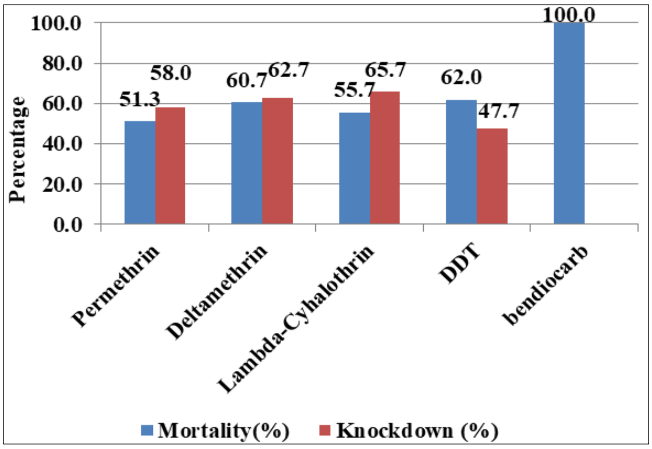


Fig 5: Results from WHO tube bio-assays using permethrin 0.75%, deltamethrin 0.05%, lambda-cyhalothrin 0.05%, DDT 4% and bendiocarb 0.1% in Baroueli.

4. Discussion

Vector borne-diseases constitute major health problems around the world. According to WHO they are responsible for about 17% of the global burden of parasitic and infectious diseases (WHO, 2008). The burden is heavier in low resources countries.

Vector control plays an important role in the control of such diseases. Evidences are available from the malaria eradication campaign from the 1950s to today. Vector control has relied heavily on the use of insecticides through

Intervention strategies such as indoors residual spraying and insecticide-treated bed nets. Recently the World Health Organization and its partners have developed an integrated approach for vector control. This is an intersectoral approach that also has the potential to cover multiple diseases simultaneously. As the importance of vector control is obvious, it is crucial to understand the biology of the vectors before any intervention is implemented. Entomologic baseline surveys are important and provide information at the interventions sites on local vector species composition and seasonal variations, their resting and biting behavior and their role in disease transmission. The resting and biting behaviors of insect vectors of human diseases have influences on the choice of the control strategies. Recently, in Burkina Faso, a new cryptic subgroup within *Anopheles gambiae s.s.*, the major malaria vector in Sub-Saharan Africa, was described based on the resting behavior. This subgroup has been presumed to be exophilic and they were found to be more susceptible to *Plasmodium falciparum* infection (Riehle *et al*. 2011) [14]. This could have serious implications on current large scale vector interventions such as IRS and ITNs as these methods target indoors resting/biting mosquitoes. Through baseline survey the response of local vectors to insecticides could be determined. This allows decision makers to avoid wastes of resources. Finally Baseline survey will also serve as bases for monitoring and evaluation of interventions. Collected data during and/or after interventions could be compared to the baselines to measure the impacts. The lack or the poor implementation of such baselines surveys may compromise the effectiveness of rather efficient interventions. Our study provides information, in Baroueli in prelude of IRS, on the malaria vector species composition, its biting behavior and its response to five insecticides used in public health for disease control. This will help decision makers select the most suitable insecticide. Among the three mosquito species recorded in Baroueli the *An. gambiae complex* showed the highest frequencies compared to *Aedes spp* and *Culex spp*. It is well established in Mali that the *An. gambiae complex* is the major malaria vector and transmits lymphatic filariasis too along with *An. funestus* ((Toure *et al*. 1998) [18]; (Touré 1979) [19] in (Gyapong *et al*. 2008) [9]; (Toure *et al*. 1986)) [17]. Results from the molecular typing within the *An. gambiae complex* were expected. In Baroueli we found *An. gambiae s.l.* to be composed of *An. coluzzi*, *An. gambiae* and *An. arabiensis*. The first predominated over the last two throughout the study period within the transmission season (June to October). Another species of *Anopheles* was encountered: *An. rufipes*. This species has been reported as zoophagic. Therefore it was not included in our analysis however it is worth mentioning it as there could be a possibility for it to be a potential vector to take the relay, one day, if interventions could eliminate or drastically reduce the populations of the *An. gambiae* complex. *Aedes spp* represented about 1% of the PSC collected mosquitoes. In Mali *Aedes spp* are thought to bite and rest outdoors (personal observations). That might be an explanation for the low densities of this mosquito in our PSC collections as the collection was intended for indoors resting mosquitoes. This species, responsible for the transmission of yellow fever, dengue, zika fever and other arbovirus diseases, needs more investigations in Mali. National reports and independent evidences (Phoutrides *et al*. 2011) [13] refer to the circulation of dengue and there are statistics about yellow fever in Mali. That makes this vector a major one in public health. In Mali, so far, the *Culex spp* has not yet been reported to be vectors for any major diseases. It is instead considered as a nuisance mosquito. It looks more abundant in cities than rural areas (personal observations). It is noteworthy because it is very abundant though less than the *An. gambiae* complex in our study. It bites indoors and outdoors and seems to be more resistant to insecticides than *Anopheles*. Therefore residents from IRS covered areas might still complain about mosquito bites even if the malaria burden was reduced. Analysis of the abundance (Densities per room) *Anopheles gambiae s.s.* showed temporal variations. The densities were low at the start and at the end of the transmission season and high at the middle. They followed a similar trend as the rain abundance. This was true for the species distribution too. These were expected as similar patterns have been described elsewhere in Mali (Sogoba *et al*. 2007) [16]. In contrast the human blood index was unexpectedly low throughout the transmission season even though it has shown the same tendency as the human biting rates and the entomological inoculation rates. Other studies in Mali showed similar results (unpublished). Outside Mali, in Senegal a study showed relatively higher rates (Ndiath *et al*. 2008) [12]. The human blood index was higher in June than in July but this is due to the denominator effect as in June the total number of tested mosquitoes was 16 whereas in July it was 331. Therefore this should be taken into account in interpreting such results.

The mean numbers of mosquitoes collected indoors and outdoors were comparable whereas it was believed that higher proportions of mosquitoes fed indoors. Results indicate comparable proportions of the *An. gambiae complex* blood feeding indoors and outdoors. This was observed in Tanzania after large deployment of ITNs (Russell *et al*. 2011) [15]. This has important implications for vector controls strategies targeting indoors resting and orbiting mosquitoes (e.g. IRS and LLINs). If a similar proportion of mosquitoes is biting and or resting outside it will be necessary to not only develop more efficient tools to collect and characterize those vectors but also to develop control tools (as complements to existing tools) that target them outside. In addition the human behavior might play in favor of vectors biting outside in Mali. This country being in the tropical zone and because of the high temperatures, many people prefer staying outside until after midnight before they go under bed nets (field observations). Knowing that the peak biting period for *Anopheles gambiae* (complex) is between 8-10 pm to 4-6 am (Mourou *et al*, 2010) people might already get infective bites outside. This is supported by the results that showed comparable CSP infection rates in mosquitoes collected indoor and outdoor for both *An. coluzzi* and *Anopheles gambiae*. Even though the numbers seem low for *An. gambiae* these trigger the alarms that there could be as much malaria transmission occurring outdoor as indoor. Hence the necessity to revisit the current strategies and develop new strategies of malaria control with more attention to outdoor transmission of the disease.

Already in June-July, though collected numbers are relatively low, the parous rates were high. This indicates that a considerable proportion of *Anopheles gambiae* (complex) females had chance to blood feed at least once indicating a possible active malaria transmission. This is supported by the infection rate (with *Plasmodium falciparum*) which, while null in June, was 7.5% in July.

The human biting rates (HBRs), the mosquito infection rates and the EIRs were analyzed using both PSC and HLC data with no intention for comparison. The general tendency for all three parameters shows low estimates at the beginning and at the end of the rainy season and higher estimates at the middle. This matches that of the mosquito densities. Though the intention is not to compare these parameters between the two collection methods it is noticeable that the estimates are globally higher using HLC data. Because in HLCs collected mosquitoes are active (looking for blood) and the number of volunteers collecting is relatively low (1 or 2 per room per time unit) the results might be subject to overestimation. In contrast in PSCs collected mosquitoes are not active, the biting rate is reported on the number of people having slept in a particular room the night before the collection and only the blood fed and the half-gravid are used for determining the biting rates. This could lead to underestimating the HBRs. For these reasons it is advisable, whenever possible, to conduct both methods in establishing the HBRs. This calls also for the needs for harmonization of the way these parameters are estimated across laboratories as shown by Kelly hope *et al* in 2009 (Kelly-Hope and McKenzie 2009) [11].

The insecticide susceptibility assays showed less efficacy for the pyrethroids (deltamethrin, permethrin, lambda-cyhalothrin) and DDT whereas they showed a very high efficacy of bendiocarb which is a carbamate. These results are similar in all three villages in the Baroueli district. This has an important implication for malaria vector control as pyrethroids are the mostly used insecticides in public health for their low toxicity for mammals. These results were used by the National Malaria Control Programme for shifting to bendiocarb for IRS. This highlights the necessity of developing an insecticide resistance management plan for the vector control strategies deployed in malaria endemic countries in order to assure successful vector control interventions based on the use of insecticides.

5. Conclusions

An. coluzzi, *An. gambiae* and *An. arabiensis* were the three members of the An. gambiae complex that were encountered in this study with the first being the most predominant in abundance. This study shows that the proportions of mosquitoes biting outdoor and indoor are comparable. Data covering larger geographical and ecological areas might be needed to generalize this finding but this definitely questions the traditional belief of having the great majority of biting occurring indoor. The insecticide resistance testing on the *An. gambiae s.l.* showed resistance to pyrethroids and DDT but susceptibility to carbamates.

6. Acknowledgements

We thank Richard Sakai, Adama Dao and Moussa Keita for reviewing the manuscript; USAID/PMI for the financial support.

7. Disclaimer

This study is made possible by the generous support of the American people through (USAID). The contents are the responsibility of the MRTC/University of Sciences, Techniques and Technologies of Bamako, and do not necessarily reflect the views of USAID or the United States Government.

8. References

1. Beier J. Vector Incrimination and Entomological Inoculation Rates. Methods in Molecular Medicine. 2002; 72: 3-11.
2. Choi HW, Breman JG, Teutsch SM, Liu S, Hightower AW, Sexton JD, *et al*. The effectiveness of insecticide-impregnated bed nets in reducing cases of malaria infection: a meta-analysis of published results. Am J Trop Med Hyg. 1995; 52:377-82.
3. Coetzee M, Hunt RH, Wilkerson R, Della Torre A, Coulibaly MB, Besansky NJ, *et al*. Anopheles coluzzii and Anopheles amharicus, new members of the Anopheles gambiae complex. Zootaxa. 2013; 3619:246-74.
4. Coluzzi M, Sabatini A, Petrarca V, DiDeco MA. Chromosomal differentiation and adpatation to human environments in the *Anopheles gambiae* complex. *Royal Society of Tropical Medicine and Hygiene*. 1979; 73:483-497.
5. Coulibaly DS, Rebaudet M, Travassos Y, Tolo M, Laurens AK. Kone K. Spatio-temporal analysis of malaria within a transmission season in Bandiagara, Mali. Malar J. 2013; 12:82.
6. EDSM-V. Enquête Démographique et de santé. CPS/SSDSPF-INSTAT-INFOSTAT-ICF International Bamako, Mali, 2012.
7. Faye O, Konate J, Mouchet D, Fontenille N, Sy G, Hebrard, L. Indoor resting by outdoor biting females of Anopheles gambiae complex (Diptera:Culicidae) in the Sahel of northern Senegal. J Med Entomol. 1997; 34:285-9.
8. Feachem RA, Phillips A, Targett GA. Shrinking the Malaria Map; A Prospectus on Malaria Elimination. First edition, 2009. ISBN-13:978-0-615-27387-7.
9. Gyapong JO, Boakye DA, Traore SF. Impact of Mass Drug Administration on Anopheles-Transmitted Filariasis In Africa: Emerging Data From Ghana And Mali, 2008. WHO/TDR (http://www.tropika.net/svc/review/050510-Lymphatic_Filariasis_MDA_Ghana_and_Mali#R13 R20)
10. Hunt RH, Coetzee M, Fettene M. The *Anopheles gambiae* complex: a new species from Ethiopia. *Trans. R. Soc. Trop. Med. Hyg. 1998;* 92:231-235.
11. Kelly-Hope LA, McKenzie FE. The multiplicity of malaria transmission: a review of entomological inoculation rate measurements and methods across sub-Saharan Africa. Malar J. 2009; 8: 19.
12. Ndiath MO, Brengues C, Konate L, Sokhna C, Boudin C, Trape JF, *et al*. Dynamics of transmission of Plasmodium falciparum by Anopheles arabiensis and the molecular forms M and S of Anopheles gambiae in Dielmo, Senegal. Malar J. 2008; 7:136.
13. Phoutrides EK, Coulibaly MB, George CM, Sacko A, Traore S, Bessoff K, *et al*. Dengue Virus Seroprevalence Among Febrile Patients in Bamako, Mali: Results of a 2006 Surveillance Study. Vector Borne Zoonotic Dis, 2011.
14. Riehle MM, Guelbeogo WM, Gneme A, Eiglmeier K, Holm I, Bischoff ET, *et al*. A cryptic subgroup of Anopheles gambiae is highly susceptible to human malaria parasites. Science. 2011; 331:596-8.

15. Russell TL, Govella NJ, Azizi S, Drakeley CJ, Kachur SP, Killeen GF, *et al*. Increased proportions of outdoor feeding among residual malaria vector populations following increased use of insecticide-treated nets in rural Tanzania. Malar J. 2011; 10:80.
16. Sogoba N, Doumbia S, Vounatsou P, Baber I, Keita M, Maiga M, *et al*. Monitoring of larval habitats and mosquito densities in the Sudan savanna of Mali: implications for malaria vector control. Am J Trop Med Hyg. 2007; 77:82-8.
17. Toure YT, Petrarca V, Coluzzi M. [Comparative estimate of the rates of infection with sporozoites and filaria in various forms of the Anopheles gambiae complex in a village in Mali]. Ann Ist Super Sanita. 1986; 22: 215-7.
18. Toure YTV, Petrarca SF, Traore A, Coulibaly HM, Maiga O, Sankare M, *et al*. The distribution and inversion polymorphism of chromosomally recognized taxa of the *Anopheles gambiae* complex in Mali, West Africa. *Parassitologia*. 1998; 40:477-511.
19. Touré YT. Bio-écologie des anophèles (Diptera, culcidea) dans une zone rurale de savane soudanienne au Mali (village de Banambani). Incidence sur la transmission du paludisme et de la filariose de bancroft au Mali. Thèse de 3ème cycle, Centre Pédagogique Supérieur, 1979.
20. Traore KS Mariko, Doumbia B, Berthé S. Enquête sur la prevalence de l'anémie palustre chez les enfants (EA&P) au Mali. Ministère de la Santé, Programme National de Lutte contre le Paludisme (PNLP), INFO-STAT, Bamako, Mali ICF Macro, Calverton, Maryland, USA, 2010.
21. WHO. Grandes lignes du plan d’action de l’OMS pour la lutte contre le paludisme 1993-2000. Conférence ministérielle sur le paudisme, Amsterdam, 1992.
22. WHO. Test procedures for insecticide resistance monitoring in malaria vectors, bio-efficacy and persistance of insecticides on treated surfaces. WHO, Geneva, Switzerland, 1998. WHO/CDS/CPC/MAL/ 98.12.