PRESIDENT'S MALARIA INITIATIVE

The PMI AIRS Project
Indoor Residual Spraying Task Order Six

THE DEMOCRATIC REPUBLIC OF THE CONGO

STRENGTHENING VECTOR CONTROL & ENHANCED ENTOMOLOGICAL MONITORING

FINAL REPORT

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ACRONYMS

AIRS	Africa Indoor Residual Spraying Project
CDC	Centers for Disease Control and Prevention
DRC	Democratic Republic of Congo
EIR	Entomological Inoculation Rate
ELISA	Enzyme-linked Immunosorbent Assay
HBR	Human Biting Rate
HLCs	Human Landing Catches
IRS	Indoor Residual Spraying
Kdr	knockdown resistance
LLIN	Long Lasting Insecticide-treated nets
NIBR	National Institute of Bio-medical Research/Institut National de Recherche Bio-médicale
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Collection
USAID	United States Agency for International Development
WHO	World Health Organization
WHOPES	World Health Organization Pesticide Evaluation Schemes

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1. Executive Summary

In 2015, *An. gambiae* s.l. was the predominant malaria vector caught through human landing catch (HLC) and pyrethrum spray catch (PSC) in most sentinel sites. Molecular species identification showed that all *An. gambiae* s.l. from 2014 were either *An. gambiae* s.s. or *An. coluzzii*, and further analysis is ongoing to determine proportions of each species. The exceptions were Kapolowe (Katanga) and Lodja (Kasaï Orientale) where the most common species was *An. paludis*. *An. funestus* s.l. was also collected in relatively large numbers in Mikalayi (Kasaï Occidental). *An. paludis* in Lodja were highly anthrophilic with an unusual early biting peak and predominantly were biting outdoors; however, no sporozoites were detected from 1,366 samples collected in 2015.

In general, indoor biting by *An. gambiae* s.l. was primarily late at night between 22:00 – 05:00. In Lodja, there was significant outdoor biting but this was largely after 22:00. Early outdoor *An. paludis* biting in Lodja was intense and peaked between 19:00-20:00. Climatic conditions and mosquito seasonality, measured by abundance, varied at sentinel sites across this large country. The peak in *Anopheles* mosquito abundance was July-September in Kabondo and Mikalayi, compared with April-June in Kapolowe and Kingasani. In other sentinel sites, no distinct peaks were observed. Even in sites where peaks were observed, there continued to be relatively high *Anopheles* densities year round. The human biting rate was highest in Lodja (Kasaï Orientale) and Kabondo (Province Orientale). In Lodja, the indoor human biting rate (HBR) of *An. gambiae* s.l. was >35 bites person/night year round. There was also significant outdoor biting potential with >43 bites person/night year round. In Kabondo, the indoor biting rate of *An. gambiae* s.l. was more seasonal, with a peak of 96 bites person/night between July-September. In Katana, Kalemie, and Kapolowe the *An. gambiae* s.l. biting rate was low at ≤3 bites person/night year round. The 2015 *An. gambiae* s.l. sporozoite rate varied between 2-10% by sentinel site.

PSC collected relatively large proportions of *An. gambiae* s.l. across most sites, indicating overnight indoor resting habits. HLC indicated that the majority of *An. gambiae* s.l. biting took place indoors. The exception was in Lodja where PSC caught few *An. gambiae* s.l. and similar proportions were caught by HLC both indoors and outdoors. The vast majority of *An. gambiae* s.l. captured by PSC were blood-fed at all sentinel sites, with few half-gravid, gravid, or unfed. This indicates that *An. gambiae* s.l. probably entered houses to blood-feed, before resting on house walls until morning, but exited before they become half-gravid or gravid. PSC did not collect *An. paludis* in Kapolowe and Lodja, confirming the exophilic tendencies of this species. *An. funestus* s.l. was also captured with PSC in large numbers in Kapolowe and Mikalayi.

An. gambiae s.l. were fully susceptible to bendiocarb and pirimiphos-methyl at all seven sites in 2015. DDT resistance was widespread, with low mortality recorded at all sites. Permethrin resistance was recorded at five of seven sites, with emerging resistance at two sites. Conversely, *An. gambiae* s.l. were fully susceptible to deltamethrin at five of seven sites, with emerging resistance at two. Results of samples collected in 2014 showed that the knockdown resistance (kdr*)* L1014F allele was present at high frequency in Kabondo, Kingasani, and Tshikaji, while the frequency was low in Lodja and Kapolowe. Pre-exposure to piperonyl-butoxide (PBO) followed by permethrin fully restored susceptibility in several sites and indicates that metabolic resistance through mixed-function oxidases is an important component of resistance mechanisms.

As the majority of malaria vectors at sentinel sites were biting indoors and late at night, use of long-lasting insecticide-treated nets (LLINs) should provide some protection in most locations. There was a significant amount of outdoor biting, but this was mostly late at night and the level of importance will depend on local cultural nighttime practices. Future LLIN distribution campaigns should take into consideration the finding of widespread permethrin resistance and may consider purchasing LLINs containing different pyrethroids such as deltamethrin, to which *An. gambiae* s.l. were largely susceptible. PSC collections indicated that *An. gambiae* s.l. and *An. funestus* s.l. rested indoors overnight and that control through bendiocarb or pirimiphos-methyl IRS could be a feasible option in the future. It is vitally important for the National Malaria Control Program of DR Congo to develop a plan for combating insecticide resistance using the World Health Organization (WHO) Global Plan for Insecticide Resistance Management (GPIRM) for guidance.

Other activities, such as the evaluation of the Suna trap (not yet started), susceptibility testing using Center for Disease Control and Prevention (CDC) bottles (in progress), 2015 kdr-gene frequency, blood-meal analysis and species identification, will be reported on later.

2. INTRODUCTION

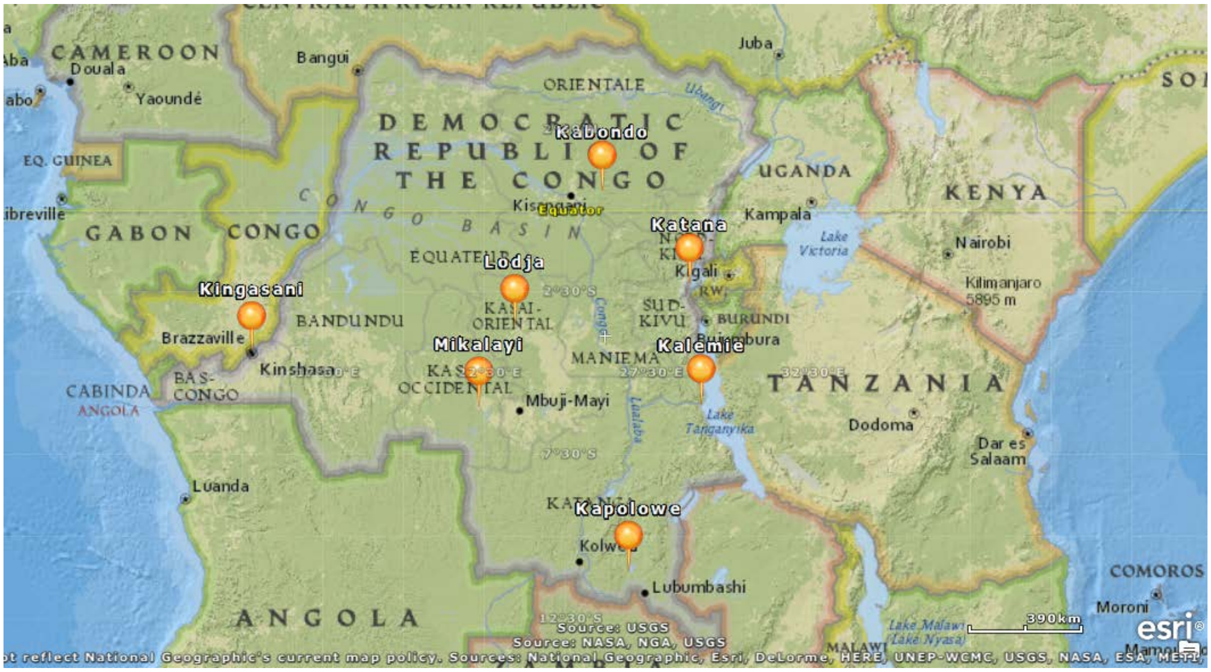
Abt Associates conducts entomological monitoring and surveillance through the President's Malaria Initiative Africa Indoor Residual Spraying (PMI AIRS) Project in the Democratic Republic of the Congo (DRC). This report covers the entomological activities undertaken in seven sites (Kingasani, Kalemie, Katana, Mikalayi, Lodja, Kapolowe and Kabondo) in the DRC from November 2014 – January 31, 2016. The results reflect the objectives in the Work Plan 2015, which are listed as follows:

- Identify malaria vector species in the seven sentinel sites;
- Determine the susceptibility level of the main vector of malaria, *Anopheles gambiae* s.l., to all four classes of insecticides approved by the World Health Organization Pesticide Evaluation Scheme (WHOPES, 2013) for public health in seven sentinel sites;
- Compare vector density and behavior;
- Determine the sporozoite rate and the entomological inoculation rate.
- Evaluation of Suna trap for sampling host-seeking malaria vectors in selected areas in the DRC; and
- Surveillance of *An. paludis* in Lodja.

3. METHODOLOGY

To obtain mosquitoes from the field the following methods were used: human landing catches, pyrethrum spray catch and collection of larvae. These methods were used in all seven sentinel sites (Kabondo, Lodja, Katana, Kalemie, Kapolowe, Mikalayi and Kingasani) during three collection periods (January to March 2015, April to June 2015, and July to September 2015).

FIGURE 1 : 2015 SENTINEL SITES FOR ENTOMOLOGICAL ACTIVITY



3.1 HUMAN LANDING CATCHES

Human landing catches (HLCs) were conducted indoors and outdoors in one selected health area per site (health zone). In each targeted health area and collection period, human landing catches were conducted in eight randomly selected houses. The human biting rates were calculated based on eight person-nights both indoor and outdoor during each collection period. Each night mosquitoes were sampled by two people indoors and two outdoors in two houses in each of the sentinel sites. The two collectors in each location were assigned in two shifts: one person from 6 p.m. to 12 a.m. and another from 12 a.m. to 6 a.m. Collections were done for four nights (each night with two different houses).

All *Anopheles* mosquitoes caught during the night were identified by species morphologically using the identification key of Gillies and De Meillon (1968) [1]. A sub-sample of sibling species was preserved in 1.5 ml eppendorf tubes on silica gel for further molecular analysis.

The heads and thoraxes of the vector species were properly labeled and analyzed using enzyme-linked immunosorbent assay (ELISA) for circumsporozoite protein antigen identification by the National Institute of Biomedical Research (NIBR). The legs and wings of female vector species were saved for Polymerase Chain Reaction (PCR) analyses in NIBR to identify sibling species and molecular forms of *Anopheles gambiae* s.s., while the abdomens were saved for blood-meal analysis.

3.2 PYRETHRUM SPRAY CATCHES

Pyrethrum spray catches (PSCs) was used to estimate the indoor resting density of mosquito species. In the selected health area, 10 houses were used for indoor PSC during each monitoring period. The PSC was carried out between 6:00 a.m. and 10:00 a.m. Before the PSC was performed, all occupants were asked to move water, food, and anything that could not be sprayed with insecticide out of the house. Information on the number of people, including the number of children under 5 and the number of pregnant women and animals who slept in the house the previous night, type of house, wall types, the number of treated nets present in the house, and usage of LLINs was collected. White sheets were placed on the floor from wall to wall and on surfaces to collect knocked down mosquitoes. The rooms were sprayed with a commercially available insecticide spray to knock down/kill mosquitoes resting inside the houses. Twenty minutes after spraying, all fallen mosquitoes were collected from the white sheet. Female *Anopheles* mosquitoes were classified according to abdomen status as unfed, fed, gravid, or half-gravid. Each mosquito collected was properly labeled for processing by NIBR for sibling species identification using PCR and other lab-based analysis following standard procedures.

Data were recorded for every house sampled by PSC or HLC showing the proportion of houses with at least one mosquito net in use in the house. Data are presented in the same section as PSC results as the presence or absence of ITNs may influence the duration of indoor resting. Presence of at least one ITN was confirmed by visual inspection.

3.3 Insecticide Susceptibility Testing

Insecticide resistance tests were conducted in seven sentinel sites. Adult mosquitoes reared from field-collected larvae and pupae were used for the test. The objective was to determine frequency of phenotypic resistance per sentinel site. Two- to five-day-old, non-blood-fed female *An. gambiae* s.l. were tested according to the standard WHO protocols using diagnostic dosages of insecticide impregnated papers. At least one insecticide from each class of insecticide was tested, including bendiocarb (0.1%), deltamethrin (0.05%), permethrin (0.75%), pirimiphos-methyl (0.1%), and DDT (4%).

During the test, female adult mosquitoes were exposed to discriminating dosages of insecticide in four replicates for one hour. The test tubes were kept in a vertical position. Exposure tests were accompanied by two control tests where mosquitoes were exposed to filter papers impregnated with oil. The numbers of mosquitoes knocked down were recorded following the WHO protocol of 2013. After one hour of exposure, mosquitoes were transferred to holding tubes internally lined with insecticide-free papers. After they were transferred to the holding tube, both the test and control mosquitoes were supplied with sugar solution (10%) with cotton pads and kept in a box to maintain optimum temperature and humidity. Mortality was recorded after the 24-hour recovery period. Mosquitoes were preserved for molecular analysis at NIBR to identify mechanism of resistance.

3.4 Use of the Synergist PBO in Susceptibility Tests

Susceptibility tests of *Anopheles gambiae* s.l. to permethrin (0.75%) following pre-exposure to PBO were conducted in all seven sites using the WHO tube test. The method used to perform this activity was the same as the susceptibility test of *Anopheles gambiae* s.l. but in two steps. The first step was to expose *Anopheles gambiae* s.l. for 60 minutes to papers treated with PBO at 5%. Mosquitoes were removed from the tube and introduced (second step) in another tube with papers impregnated with permethrin (0.75%) for an exposure time of 60 minutes. Mosquitoes were then removed and transferred to paper cups with access to sugar solution for recording of mortality 24h after exposure.

3.5 Surveillance of *An. paludis* at Lodja Sentinel Site

Alongside longitudinal vector monitoring conducted three times per year in all seven sentinel sites, a more detailed monthly surveillance study was established in the Lodja site in Sankuru Province to determine whether *Anopheles paludis* is an important malaria vector in the area.

This study was developed due to the high abundance of the human biting *An. paludis* in Lodja; second only to *Anopheles gambiae* s.l., the main vector of malaria in DRC. *Anopheles paludis* is a member of the

An. coustani group and is found across equatorial Africa, preferring forests or wooded savanna and breeding sites consisting of large clear water bodies such as ponds, pools and rivers, often shaded by forest and with some aquatic vegetation present. *Anopheles paludis* is considered to be an important malaria vector species in DRC. Vector incrimination of this species was first done in 1945 by Wolfs in Mbandaka, Northern DRC [2]. Subsequently human biting of *An. paludis* has been reported in several places in DRC, and in 1991 a sporozoite rate of 6.2% (6/97) was recorded in Kenge, Bandundu Province (South West DRC) [3]. However, the importance of this species across the country may vary as no sporozoites were found in a study done in Lubumbashi, Katanga Province, South East DRC in 1946 [4].

Considering the high sporozoite rate found in Bandundu in 1991, our hypothesis was that *An. paludis* was an important malaria vector species in Lodja, based on the high degree of anthropophily. To determine whether this hypothesis was true it was important to conduct detailed surveillance throughout the year. From January to December 2015, mosquito collections were undertaken monthly by indoor PSC and by indoor and outdoor HLC. The main objective was to determine the importance of *Anopheles paludis* in malaria transmission in Lodja.

The specific objectives were:

1) To determine the timing of peak vector-human biting of *An. paludis* throughout the year using indoor and outdoor HLC.
2) To determine whether *An. paludis* rest indoors by conducting indoor PSC.
3) To determine the relative abundance of human host-seeking *Anopheles paludis* in Lodja by season.
4) To conduct direct circumsporozoite ELISA index analysis to detect the presence of *Plasmodium falciparum*, the main malaria parasite in DRC, in the salivary glands.
5) To calculate the entomological inoculation rate of *An. paludis* in Lodja.

3.6 Evaluation of the Suna Trap

Evaluation of the Suna Trap will be conducted at the Kingasani sentinel site. Currently, human landing catches are used indoors and outdoors in DRC to monitor human biting rates, but this method is labor intensive and results in a small sample size. Therefore, a comparison will be made between HLC, CDC light traps and the Suna Trap both indoors and outdoors using a Latin square rotation design. The trapping materials have been received from CDC/Atlanta and results will be included in the next report in 2016.

4. SPECIES COMPOSITION FOR HLC AND PSC COLLECTIONS

4.1 PROVINCE ORIENTALE, KABONDO SENTINEL SITE

TABLE 1: DISTRIBUTION OF MOSQUITOES COLLECTED IN KABONDO BY GENUS, SPECIES, AND METHOD

Site	KABONDO				
Period	FIRST SESSION: JANUARY – MARCH 2015				
Method	HLC			PSC	
Species	Indoors	Outdoors	Total		Total
An. gambiae s.l.	104	23	127	92	219
Culex spp.	1955	3286	5241	247	5488
Period	SECOND SESSION: APRIL – JUNE 2015				
An. gambiae s.l.	66	39	105	114	219
Other *Anopheles*	6	4	10	0	10
Period	THIRD SESSION : JULY – SEPTEMBER 2015				
An. gambiae s.l.	771	545	1316	654	1970
Period	OVERALL ANOPEHELES : JANUARY – SEPTEMBER 2015				
An. gambiae s.l.	941	607	1548	860	2408
Other *Anopheles*	6	4	10	0	10

Over the three trapping periods the main malaria vector captured was *An. gambiae* s.l. Of 1548 *An. gambiae* s.l. captured by HLC, 61% (941/1548) were captured biting indoors. Species identification using PCR is ongoing. The high number of *An. gambiae* s.l. indoors showed the vector to be endophagic in the area. *An. swahilicus* was captured (indoors and outdoors) for the first time during the trip in session two. PSC caught 36% (860/2408) of the total *An. gambiae* s.l. caught. Peak *An. gambiae* s.l. biting rates were in July-September, with 85% (1316/1548) caught by HLC in this period.

4.2 KATANGA PROVINCE, KALEMIE SENTINEL SITE

TABLE 2: DISTRIBUTION OF MOSQUITOES COLLECTED IN KALEMIE BY GENUS, SPECIES, AND METHOD

Site	KALEMIE				
Period	FISRT SESSION: JANUARY – MARCH 2015				
Method	HLC			PSC	
Species	Indoors	Outdoors	Total		Total
An. gambiae s.l.	3	10	13	28	41
Total 1: Anophelinae	3	10	13	28	41
Culex spp.	68	76	144	4	148
Mansonia spp.	12	11	23	3	26
Aedes spp.	1	0	1	0	1
Total 2: Culicinae	81	87	168	7	175
Period	SECOND SESSION: APRIL – JUNE 2015				
An. gambiae s.l.	13	14	27	33	60
An. funestus s.l.	2	2	4	19	23
An. nili	1	0	1	0	1
An. salbaii	0	0	0	1	1
An. tenebrosus	3	2	5	0	5
An. christyi	0	1	1	0	1
Total 1: Anophelinae	19	19	38	53	91
Culex spp.	242	196	438	24	462
Mansonia spp.	150	66	216	1	217
Total 2: Culicinae	392	262	654	25	679
Period	THIRD SESSION: JULY – SEPTEMBER 2015				
An. gambiae s.l.	23	19	42	40	82
An. funestus s.l.	1	0	1	16	17
An. salbaï	1	7	8	0	8
An. tenebrosus	1	1	2	0	2
Total	26	27	53	56	109
Period	OVERALL ANOPEHELES : JANUARY – SEPTEMBER 2015				
An. gambiae s.l.	39	43	82	101	183
An. funestus s.l.	3	2	5	35	40
Other *Anopheles*	6	11	17	1	18

The overall number of *Anopheles* captured through HLC was relatively low in all trapping sites. *An. gambiae* s.l. was the main malaria vector collected, with similar proportions captured biting indoors and outdoors, while PSC collected 55% of *An. gambiae* s.l.

4.3 KATANGA PROVINCE, KAPOLOWE SENTINEL SITE

TABLE 3: DISTRIBUTION OF MOSQUITOES COLLECTED IN KAPOLOWE BY GENUS, SPECIES, AND METHOD

Site	KAPOLOWE				
Period	FIRST SESSION: JANUARY-MARCH 2015				
Method	HLC			PSC	
Species	Indoors	Outdoors	Total		Total
An. gambiae s.l.	9	4	13	61	74
An. paludis	67	49	116	0	116
Total 1: Anophelinae	76	53	129	61	190
Culex spp.	1188	1179	2367	0	2367
Mansonia spp.	137	301	438	0	438
Total 2: Culicinae	1325	1480	2805	0	2805
Period	SECOND SESSION: APRIL - JUNE 2015				
An. gambiae s.l.	0	0	0	4	4
An. funestus s.l.	18	3	21	96	117
An. paludis	231	298	529	0	529
Total 1: Anophelinae	249	301	550	100	650
Culex spp.	669	1621	2290	16	2306
Mansonia spp.	38	92	130	7	137
Total 2: Culicinae	707	1713	2420	23	2443
Period	THIRD SESSION: JULY - SEPTEMBER 2015				
An. gambiae s.l.	0	1	1	14	15
An. funestus s.l.	0	0	0	8	8
An. caliginosus	88	117	205	0	205
An. nili	0	0	0	1	1
An. tenebrosus	0	1	1	0	1
An. ziemanni	0	1	1	0	1
Total 3: Anophelinae	88	120	208	23	231
Period	OVERALL ANOPEHELES : JANUARY – SEPTEMBER 2015				
An. gambiae s.l.	9	5	14	79	93
An. paludis	298	347	645	0	645
An. caliginosus	88	117	205	0	205
Other *Anopheles*	0	2	2	1	3

Between January and September most *An. gambiae* s.l. were captured by PSC (79/93; 85%) rather than through HLC. *An. paludis* were captured in large numbers between January and June but all were captured by HLC, with slightly more (347/645; 54%) caught outdoors. No *An. paludis* were captured by PSC. *An. caliginosus* was the most abundant species captured at Kapolowe in the period between July and September 2015 by HLC method (205 specimens). It was slightly more abundant outdoors (117/205; 57%) than indoors (88/205; 43%) but none were captured by PSC.

4.4 SUD KIVU PROVINCE, KATANA SENTINEL SITE

TABLE 4: DISTRIBUTION OF MOSQUITOES COLLECTED IN KATANA BY GENUS, SPECIES, AND METHOD

Site	KATANA				
Period	FIRST SESSION: JANUARY – MARCH 2015				
Method	HLC				
Species	Indoors	Outdoors	Total	PSC	Total
An. gambiae s.l.	2	3	5	101	106
Total: Anophelinae	2	3	5	101	106
Culex spp.	146	174	320	98	418
Total: Culicinae	146	174	320	98	418
Period	SECOND SESSION: APRIL – JUNE 2015				
An. gambiae s.l.	7	11	18	113	131
An. paludis	2	3	5	0	5
Total 1: Anophelinae	9	14	23	113	136
Culex ssp.	207	164	371	98	469
Total 2: Culicinae	207	164	371	98	469
Period	THIRD SESSION: JULY – SEPTEMBER 2015				
An. gambiae s.l.	8	9	17	67	84
An. funestus s.l.	7	3	10	14	24
Total	15	12	27	81	108
Period	OVERALL ANOPEHELES : JANUARY – SEPTEMBER 2015				
An. gambiae s.l.	17	23	40	281	321
An. funestus s.l.	7	3	10	14	24
An. paludis	2	3	5	0	5

The main vector captured in Katana was *An. gambiae* s.l., although the majority were caught by PSC with only 12% (40/321) by HLC. *An. gambiae* s.l. were caught all year round with small numbers of *An. funestus* s.l. captured from July-September only.

4.5 KINSHASA PROVINCE, KINGASANI SENTINEL SITE

TABLE 5: DISTRIBUTION OF MOSQUITOES COLLECTED IN KINGASANI BY GENUS, SPECIES, AND METHOD

Site	KINGASANI				
Period	FIRST SESSION: JANUARY-MARCH 2015				
Method	HLC			PSC	Total
Species	Indoors	Outdoors	Total		
An. gambiae s.l.	29	60	89	7	96
Culex spp.	137	220	357	30	387
Mansonia spp.	60	190	250	63	313
Total Culicinae	197	410	607	93	700
Period	SECOND SESSION: APRIL – JUNE 2015				
An. gambiae s.l.	86	58	144	200	344
Culex spp.	158	135	293	20	313
Mansonia spp.	1	2	3	0	3
Total Culicinae	159	137	296	20	316
Period	THIRD SESSION: JULY – SEPT 2015				
An. gambiae s.l.	17	41	58	18	76
An. funestus s.l.	0	1	1	0	1
Total Anophelinae	17	42	59	18	77
Period	OVERALL ANOPEHELES : JANUARY – SEPTEMBER 2015				
An. gambiae s.l.	132	159	291	225	516
An. funestus s.l.	0	1	1	0	1

The primary vector species captured in Kingasani was *An. gambiae* s.l. with 56% (291/516) collected through HLC during this collection period. Out of 291 samples collected by HLC 55% were captured outdoors. The largest densities of *An. gambiae* s.l. were captured between April-June, mostly through PSC.

4.6 KASAÏ ORIENTAL PROVINCE, LODJA SENTINEL SITE

TABLE 6 : DISTRIBUTION OF MOSQUITOES COLLECTED IN LODJA*
BY GENUS, SPECIES, AND METHOD

Site	**LODJA**				
Period	FIRST SESSION: JANUARY – MARCH 2015				
Method	HLC				
Species	Indoor	Outdoor	Total	PSC	Total
An. gambiae s.l.	278	675	953	102	1,055
An. funestus s.l.	90	120	210	0	210
An. paludis	105	1,139	1,244	4	1,248
Total 1: Anophelinae	473	1,934	2,407	106	2,513
Culex spp.	263	286	549	0	549
Mansonia	235	179	414	0	414
Total 2: Culicinae	498	465	963	0	963
Period	SECOND SESSION: APRIL – JUNE 2015				
An. gambiae s.l.	624	595	1,219	19	1,238
An. funestus s.l.	29	17	46	0	46
An. paludis	189	474	663	0	663
Total 1 : Anophelinae	842	1,086	1,928	19	1,947
Culex spp.	534	415	949	0	949
Mansonia spp.	468	173	641	0	641
Total 2 : Culicinae	1,002	588	1,590	0	1,590
Period	THIRD SESSION: JULY – SEPTEMBER 2015				
An. gambiae s.l.	346	252	598	46	644
An. funestus s.l.	30	14	44	1	45
An. paludis	241	847	1,088	5	1,093
An. nili	5	0	5	0	5
Total 3: Anophelinae	622	1,113	1,735	52	1,787

Period	OVERALL ANOPEHELES : JANUARY – SEPTEMBER 2015				
An. gambiae s.l.	1,248	1,522	2,770	167	2,937
An. funestus s.l.	149	151	300	1	301
An. paludis	535	2,460	2,995	9	3,004
Other *Anopheles*	5	0	5	0	5

*Collections were conducted monthly from January 2015 to December 2015

Compared to the other sites, more collections were done in Lodja due to the *An. paludis* study that was conducted monthly from January 2015 to December 2015. This is reported in detail in section 13.

The most abundant species was *An. gambiae* s.l. with the vast majority captured through HLC and only 6% (167/2,937) through PSC. *An. gambiae* s.l. were captured in large numbers throughout the year. *An. paludis* were also caught year-round, with the majority captured biting outdoors (2,460/2,995; 82%). *An. funestus* s.l. were captured in relatively low numbers, with the peak population between January and March.

4.7 Kasaï Occidental Province, Mikalayi Sentinel Site

TABLE 7 : DISTRIBUTION OF MOSQUITOES COLLECTED IN MIKALAYI BY GENUS, SPECIES, AND METHOD

Site	MIKALAYI				
Period	FIRST SESSION: JANUARY – MARCH 2015				
Method	HLC			PSC	Total
Species	Indoors	Outdoors	Total		
An. gambiae s.l.	95	77	172	124	296
An. paludis	1	0	1	0	1
Total 1: Anophelinae	96	77	173	124	297
Culex ssp.	57	58	115	0	115
Mansonia ssp.	25	41	66	0	66
Total 2: Culicinae	82	99	181	124	305
Period	SECOND SESSION: APRIL – JUNE 2015				
An. gambiae s.l.	43	62	105	117	222
An. funestus s.l.	15	13	28	6	34
An. paludis	4	3	7	0	7
An. implexus	0	0	0	8	8
An. nili	0	0	0	1	1
An. rufipes	1	0	1	0	1

An. christyi	2	0	2	0	2
Total: Anophelinae	65	78	143	132	275
Culex spp.	80	89	169	0	169
Mansonia spp.	14	14	28	0	28
Coqueltidia spp.	14	20	34	0	34
Total : Culicinae	108	123	231	0	231
Period	THIRD SESSION: JULY – SEPTEMBER 2015				
An. gambiae s.l.	103	129	232	111	343
An. funestus s.l.	156	216	372	160	532
An. nili	1	0	1	0	1
An. paludis	0	2	2	0	2
An. implexus	0	0	0	1	1
Period	OVERALL ANOPEHELES : JANUARY – SEPTEMBER 2015				
An. gambiae s.l.	241	268	509	352	861
An. funestus s.l.	171	229	400	166	566
Other *Anopheles*	9	5	14	10	24

An. gambiae s.l. were captured in similar numbers during each trapping period, while *An. funestus* s.l. showed a concentrated peak in population size between July and September. Both species were captured biting indoors and outdoors at similar frequencies, with large numbers collected resting indoors with PSC.

5. BLOOD DIGESTION STAGE OF MALARIA VECTORS COLLECTED USING PSC

Abdominal status results for the seven sentinel sites are presented in Tables 8-14. At all sentinel sites, the majority of *An. gambiae* s.l. and *An. funestus* s.l. collected through PSC were blood-fed. There were very few gravid females, indicating that blood-fed mosquitoes exited before becoming fully gravid, presumably to seek suitable oviposition sites. There were few half-gravid *An. gambiae* s.l. and *An. funestus* s.l. in Kalemie (Table 9), Kapolowe (Table 10), Katana (Table 11), and Mikalayi (Table 14). This appears to indicate that mosquitoes were blood-feeding, resting inside on walls but exiting after a relatively short time before most became half or fully gravid. In Kabondo (Table 8), Kingasani (Table 12) and Lodja (Table 13) the proportion of half-gravid *An. gambiae* s.l. and *An. funestus* s.l.was slightly higher and may be an indication of slightly longer resting times. Early exiting in some sentinel sites may be due to excito-repellency of pyrethroid LLINs. However, there are no clear trends indicating that the proportion of semi-gravid *Anopheles* found resting indoors was correlated with net coverage. Particularly, low net coverage was recorded in Kalemie with only 24% of sentinel houses having at least 1 net during 2015 (Table 15), yet there were few semi-gravid *Anopheles* collected.

5.1 PROVINCE ORIENTALE, KABONDO SENTINEL SITE

TABLE 8: BLOOD DIGESTION STAGE OF MALARIA VECTORS COLLECTED IN KABONDO USING PSC

Site	KABONDO				
Period	FIRST SESSION: JANUARY – MARCH 2015				
Species	Fed	Unfed	Half-gravid	Gravid	Total
An. gambiae s.l.	38 (41%)	24 (26%)	13 (14%)	17 (19%)	92
Period	SECOND SESSION: APRIL – JUNE 2015				
An. gambiae s.l.	56 (49%)	18 (16%)	7 (6%)	33 (29%)	114
Period	THIRD SESSION: JULY – SEPT 2015				
An. gambiae s.l.	414 (63%)	153 (23%)	71 (11%)	16 (2%)	654

5.2 KATANGA PROVINCE, KALEMIE SENTINEL SITE

TABLE 9: BLOOD DIGESTION STAGE OF MALARIA VECTORS COLLECTED IN KALEMIE USING PSC

Site	KALEMIE				
Period	FIRST SESSION : JANUARY – MARCH 2015				
Species	Fed	Unfed	Half-gravid	Gravid	Total
An. gambiae s.l.	16 (57%)	10 (36%)	1 (4%)	1 (4%)	28
Period	SECOND SESSION : APRIL – JUNE 2015				
An. gambiae s.l.	26 (79%)	1 (3%)	6 (18%)	0	33
An. funestus s.l.	13 (68%)	0	6 (32%)	0	19
An. salbaii	1 (100%)	0	0	0	1
Total	40 (77%)	0	12 (23%)	0	52
Period	THIRD SESSION : JULY - SEPTEMBER 2015				
An. funestus s.l.	16 (100%)	0	0	0	16
An. gambiae s.l.	40 (100%)	0	0	0	40
Total	56 (100%)	0	0	0	56

5.3 KATANGA PROVINCE, KAPOLOWE SENTINEL SITE

TABLE 10: BLOOD DIGESTION STAGE OF MALARIA VECTORS COLLECTED IN KAPOLOWE USING PSC

Site	KAPOLOWE				
Period	FIRST SESSION: JANUARY – MARCH 2015				
Species	Fed	Unfed	Half-gravid	Gravid	Total
An. gambiae s.l.	34 (56%)	27 (44%)	0	0	61
Period	SECOND SESSION: APRIL – JUNE 2015				
An. gambiae s.l.	96 (100%)	0	0	0	96
An. funestus s.l.	4 (100%)	0	0	0	4
Total	100 (100%)	0	0	0	100
Period	THIRD SESSION: JULY – SEPTEMBER 2015				
An. funestus s.l.	0	3 (38%)	0	5	8
An. gambiae s.l.	0	1 (7%)	2 (14%)	11 (79%)	14
An. nili	0	0	0	1 (100%)	1
Total	0	4 (17%)	2 (9%)	17 (74%)	23

5.4 SUD KIVU PROVINCE, KATANA SENTINEL SITE

TABLE 11: BLOOD DIGESTION STAGE OF MALARIA VECTORS COLLECTED IN KATANA USING PSC

Site	KATANA				
Period	FIRST SESSION : JANUARY – MARCH 2015				
Species	Fed	Unfed	Half-gravid	Gravid	Total
An. gambiae s.l.	85 (84%)	6 (6%)	9 (9%)	1 (1%)	101
Period	SECOND SESSION : APRIL – JUNE 2015				
An. gambiae s.l.	113 (100%)	0	0	0	113

Period	THIRD SESSION : JULY – SEPTEMBER 2015				
An. funestus s.l.	14 (100%)	0	0	0	14
An. gambiae s.l.	67 (100%)	0	0	0	67
Total	81 (100%)	0	0	0	81

5.5 KINSHASA PROVINCE, KINGASANI SENTINEL SITE

TABLE 12: BLOOD DIGESTION STAGE OF MALARIA VECTORS COLLECTED IN KINGASANI USING PSC

Site	KINGASANI				
Period	FIRST SESSION: JANUARY – MARCH 2015				
Species	Fed	Unfed	Half-gravid	Gravid	Total
An. gambiae s.l.	3 (43%)	3 (43%)	1 (14%)	0	7
Period	SECOND SESSION : APRIL – JUNE 2015				
An. gambiae s.l.	134 (67%)	20 (10%)	40 (20%)	6 (3%)	200
Period	THIRD SESSION : JULY – SEPTEMBER 2015				
An. gambiae s.l.	6 (33%)	0	6 (33%)	6 (33%)	18

5.6 KASAI ORIENTAL PROVINCE, LODJA SENTINEL SITE

TABLE 13: BLOOD DIGESTION STAGE OF MALARIA VECTORS COLLECTED IN LODJA USING PSC

Site	LODJA				
Period	FIRST SESSION: JANUARY – MARCH 2015				
Species	Fed	Unfed	Half-gravid	Gravid	Total
An. gambiae s.l.	68 (67%)	15 (15%)	19 (19%)	0	102
An. paludis	3 (75%)	1 (25%)	0	0	4
Total	71 (67%)	16 (15%)	19 (18%)	0	106
Period	SECOND SESSION: APRIL – JUNE 2015				
An gambiae s.l.	13 (68%)	4 (21%)	0	2 (11%)	19
Period	THIRD SESSION: APRIL – JUNE 2015				
An. funestus s.l.	0	1 (100%)	0	0	1
An. gambiae s.l.	15 (33%)	25 (54%)	6 (13%)	0	46
An. paludis	1 (20%)	4 (80%)	0	0	5
Total	16 (31%)	30 (58%)	6 (12%)	0	52

5.7 KASAI OCCIDENTAL PROVINCE, MIKALAYI SENTINEL SITE

TABLE 14: BLOOD DIGESTION STAGE OF MALARIA VECTORS COLLECTED IN MIKALAYI USING PSC

Site	MIKALAYI				
Period	FIRST SESSION: JANUARY – MARCH 2015				
Species	Fed	Unfed	Half-gravid	Gravid	Total
An gambiae s.l	118 (95%)	6 (5%)	0	0	124
Period	SECOND SESSION: APRIL – JUNE 2015				

An. gambiae s.l.	103 (88%)	14 (12%)	0	0	117
An. funestus s.l.	5 (83%)	1 (17%)	0	0	6
An. nili	1 (100%)	0	0	0	1
An. implexus	4 (50%)	4 (50%)	0	0	8
Total 2: Anophelinae	**113 (86%)**	**19 (14%)**	**0**	**0**	**132**
Period	THIRD SESSION: JULY – SEPTEMBER 2015				
An. funestus s.l.	158 (99%)	2 (1%)	0	0	160
An. gambiae s.l.	109 (98%)	2 (2%)	0	0	111
An. implexus	1 (1%)	0	0	0	1
Total 3 : Anophelinae	**267 (98%)**	**5 (2%)**	**0**	**0**	**272**

5.8 PRESENCE OF MOSQUITO NETS IN HOUSES USED FOR VECTOR SAMPLING

TABLE 15: NUMBER OF HOUSES USED FOR HLC AND PSC WHICH HAD AT LEAST ONE MOSQUITO NET PRESENT IN THE HOUSE.

Periods / Sites	January - March 2015				April – June 2015				July – September 2015				General Total	
	HLC	PSC	TOTAL	Proportion	HLC	PSC	TOTAL	Proportion	HLC	PSC	TOTAL	Proportion	Number	Proportion
1. Kabondo	4	8	12	0.67	6	10	16	0.89	6	10	16	0.89	44	0.81
2. Kalemie	1	9	10	0.56	0	1	1	0.06	0	2	2	0.11	13	0.24
3. Kapolowe	7	2	9	0.50	7	1	8	0.44	7	4	11	0.61	28	0.52
4. Katana	4	8	12	0.67	3	4	7	0.39	2	1	3	0.17	22	0.41
5. Kingasani	8	4	12	0.67	6	4	10	0.56	4	4	8	0.44	30	0.56
6. Mikalayi	6	6	12	0.67	8	5	13	0.72	8	8	16	0.89	41	0.76
7. Lodja	7	8	15	0.83	0	1	1	0.06	4	5	9	0.50	25	0.46
Total	37	45	82	**0.65**	30	26	56	**0.44**	31	34	65	**0.52**	203	**0.54**

#The number of houses sampled per period was 8 for HLC and 10 for PSC.

The data in Table 15 represents houses which had at least one mosquito net present. The average net coverage varied between time periods and sites. Annually, the mean number of sentinel houses with nets was high in Kabondo at 81% and lowest in Kalemie at 24%.

6. Human Biting Rate of Malaria Vectors Indoors and Outdoors

6.1 Province Orientale, Kabondo Sentinel Site

TABLE 16: HUMAN BITING RATE OF *AN. GAMBIAE* S.L. IN KABONDO SENTINEL SITE, EASTERN PROVINCE (JANUARY-MARCH, APRIL-JUNE, AND JULY-SEPTEMBER 2015)

Sites	Species	Area	Variable	Jan/Mar	April/June	July/Sept

The human biting rate indoors and outdoors was variable during 2015. The highest human biting rate was recorded during July/September indoors (96 bites/person/night) and 68 bites/person/night outdoor. During all periods the human biting rate was higher indoors than outdoors.

6.2 Katanga Province, Kalemie Sentinel Site

TABLE 17: HUMAN BITING RATES IN KALEMIE SENTINEL SITE, KATANGA PROVINCE (JANUARY-MARCH, APRIL-JUNE AND JULY-SEPTEMBER 2015)

Sites	Species	Area	Variable	Jan/March	Apr/June	July/Sept
	An. gambiae s.l.	Indoor	Total mosquitoes	3	13	23
			Person-nights	8	8	8
			HBR/night	0.4	3	3
		Outdoor	Total mosquitoes	10	14	19
			Person-nights	8	8	8
			HBR/night	1	2	2
	An. funestus	Indoor	Total mosquitoes	0	2	1
			Person-nights	8	8	8

Sites	Species	Area	Variable	Jan/March	Apr/June	July/Sept
KALEMIE	s.l.		HBR/night	0	0.3	0.1
		Outdoor	Total mosquitoes	0	2	0
			Person-nights	8	8	8
			HBR/night	0	0.3	0
	An. nili	Indoor	Total mosquitoes	0	1	0
			Person-nights	8	8	8
			HBR/night	0	0.1	0
		Outdoor	Total mosquitoes	0	0	0
			Person-nights	8	8	8
			HBR/night	0	0	0
	An. tenebrosus	Indoor	Total mosquitoes	0	3	1
			Person-nights	8	8	8
			HBR/night	0	0.4	0.1
		Outdoor	Total mosquitoes	1	2	1
			Person-nights	8	8	8
			HBR/night	0.1	0.3	0.1
	An. salbaii	Indoor	Total mosquitoes	0	0	0
			Person-nights	8	8	8
			HBR/night	0	0	0
	An. salbaii	Outdoor	Total mosquitoes	0	1	7
			Person-nights	8	8	8
			HBR/night	0	0.1	0.9

According to Table 17 all the Anophelinae captured in Kalemie were active indoors and/or outdoors with the biting rates varying greatly. Biting rates for *An. gambiae* s.l. varied from 0.4 to 3 bites per person per night indoors and between 1 and 2 bites per person per night outdoors.

6.3 KATANGA PROVINCE, KAPOLOWE SENTINEL SITE

TABLE 18: HUMAN BITING RATE OF MALARIA VECTORS IN KAPOLOWE SENTINEL SITE, KATANGA PROVINCE (JANUARY-MARCH, APRIL-JUNE, JULY-SEPTEMBER 2015)

Sites	Species	Area	Variable	Jan/Mar	Apr/June	July/Sept
			Total mosquitoes	9	0	0

Sites	Species	Area	Variable	Jan/Mar	Apr/June	July/Sept
KAPOLOWE	*An. gambiae* s.l.	Indoor	Person-nights	8	8	8
			HBR/night	1.1	0	0
		Outdoor	Total mosquitoes	4	0	1
			Person-nights	8	8	8
			HBR/night	0.5	0	0.1
	An. paludis	Indoor	Total mosquitoes	67	231	0
			Person-nights	8	8	8
			HBR/night	8	29	0
		Outdoor	Total mosquitoes	49	298	0
			Person-nights	8	8	8
			HBR/night	6	37	0
	An. funestus s.l.	Indoor	Total mosquitoes	0	18	0
			Person-nights	8	8	8
			HBR/night	0	2	0
		Outdoor	Total mosquitoes	0	3	0
			Person-nights	8	8	8
			HBR/night	0	0.4	0

The Table above shows that the human biting rate in Kapolowe was, in general, low for all species of Anophelinae except *An. paludis*, with peak biting during the Apr/June timeframe.

6.4 SUD KIVU PROVINCE, KATANA SENTINEL SITE

TABLE 19: HUMAN BITING RATE OF MALARIA VECTORS IN KATANA SENTINEL SITE, SUD KIVU PROVINCE (JANUARY-MARCH, APRIL-JUNE, JULY-SEPT 2015)

Sites	Species	Area	Variable	Jan/Mar	Apr/June	July/Sept
KATANA	*An. gambiae* s.l.	Indoor	Total mosquitoes	2	7	8
			Person-nights	8	8	8
			HBR/night	0.3	1	1
		Outdoor	Total mosquitoes	3	11	9
			Person-nights	8	8	8
			HBR/night	0.4	1	1
	An. paludis	Indoor	Total mosquitoes	0	2	0
			Person-nights	8	8	8
			HBR/night	0	0.3	0
		Outdoor	Total mosquitoes	0	3	0
			Person-nights	8	8	8
			HBR/night	0	0.4	0

	An. funestus s.l.	Indoor	Total mosquitoes	0	0	7
			Person-nights	8	8	8
			HBR/night	0	0	1
		Outdoor	Total mosquitoes	0	0	3
			Person-nights	8	8	8
			HBR/night	0	0	0.38

All biting rates were relatively low in Katana. *An. gambiae* s.l. were caught during each period, *An. paludis* biting was only during April-June 2015, and *An. funestus* s.l. biting was only during July-Sept 2015.

6.5 KINSHASA PROVINCE, KINGASANI SENTINEL SITE

TABLE 20: HUMAN BITING RATE OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. IN KINGASANI SENTINEL SITE, KINSHASA PROVINCE JANUARY-MARCH, APRIL-MAY, JULY-SEPT 2015.

Sites	Species	Area	Variable	Jan/Mar	Apr/June	July/Sept
KINGASANI	*An. gambiae* s.l.	Indoor	Total mosquitoes	29	59	17
			Person-nights	8	8	8
			HBR/night	4	7	2
		Outdoor	Total mosquitoes	60	31	41
			Person-nights	8	8	8
			HBR/night	8	4	5
	An funestus s.l.	Indoor	Total mosquitoes	0	0	0
			Person-nights	6	8	8
			HBR/night	0	0	0
		Outdoor	Total mosquitoes	0	0	1
			Person-nights	8	8	8
			HBR/night	0	0	0

An. gambiae s.l. biting occurred throughout the year (Table 20). The biting rate varied from 2 to 7 bites/human/night indoors and 7.5 to 5 bites/human/night outdoors.

6.6 KASAI ORIENTAL PROVINCE, LODJA SENTINEL SITE

TABLE 21: HUMAN BITING RATE OF MALARIA VECTORS IN LODJA SENTINEL SITE, EASTERN KASAÏ PROVINCE (JANUARY-MARCH, APRIL-MAY, JULY-SEPTEMBER 2015).

Sites	**Species**	**Area**	**Variable**	Jan/Mar	Apr/June	July/Sept
	An. gambiae s.l.	Indoor	Total mosquitoes	278	624	346
			Person-nights	24*	24	24
			HBR/night	12	26	14
		Outdoor	Total mosquitoes	675	595	252
			Person-nights	24	24	24

LODJA			HBR/night	28	25	11
	An. paludis	Indoor	Total mosquitoes	105	189	241
			Person-nights	24	24	24
			HBR/night	4	8	10
		Outdoor	Total mosquitoes	1139	474	847
			Person-nights	24	24	24
			HBR/night	47	20	35
	An. funestus s.l.	Indoor	Total mosquitoes	90	29	20
			Person-nights	24	24	24
			HBR/night	4	1	1
		Outdoor	Total mosquitoes	120	17	9
			Person-nights	24	24	24
			HBR/night	5	1	1

*In Lodja collections were done monthly, therefore 24 houses were sampled for the 3-month period.

All three species (*An. gambiae* s.l., *An. paludis* and *An. funestus* s.l.) had high biting rates in Lodja at all times from February to September 2015. The biting rates were high both indoors and outdoors.

6.7 KASAI OCCIDENTAL PROVINCE, MIKALAYI SENTINEL SITE

TABLE 22: HUMAN BITING RATE OF MALARIA VECTORS IN MIKALAYI SENTINEL SITE, KASAI OCCIDENTAL PROVINCE

Province	Species	Area	Variable	Jan/Mar	April/June	July/Sept
	An. gambiae s.l.	Indoor	Total mosquitoes	95	43	103
			Person-nights	8	8	8
			HBR/night	12	5	13
		Outdoor	Total mosquitoes	77	62	129
			Person-nights	8	8	8
			HBR/night	10	8	16
	An. paludis	Indoor	Total mosquitoes	1	4	0
			Person-nights	8	8	8
			HBR/night	0.1	0.5	0
		Outdoor	Total mosquitoes	0	3	2
			Person-nights	8	8	8
			HBR/night	0.0	0.4	0.3
		Indoor	Total mosquitoes	0	15	156
			Person-nights		8	8
			HBR/night	-	2	20

Province	Species	Area	Variable	Jan/Mar	April/June	July/Sept
MIKALAYI	*An. funestus* s.l.	Outdoor	Total mosquitoes	0	13	216
			Person-nights		8	8
			HBR/night	-	2	27

An. gambiae s.l. was captured roughly equally across the year, while *An. funestus* biting peaked during the July/Sept timeframe (Table 22).

6.8 SUMMARY OF HUMAN BITING RATES ACROSS ALL SENTINEL SITES

TABLE 23 : SUMMARY OF HUMAN BITING RATE (BITES/PERSON/NIGHT) OF *AN. GAMBIAE* S.L. BY SENTINEL SITE

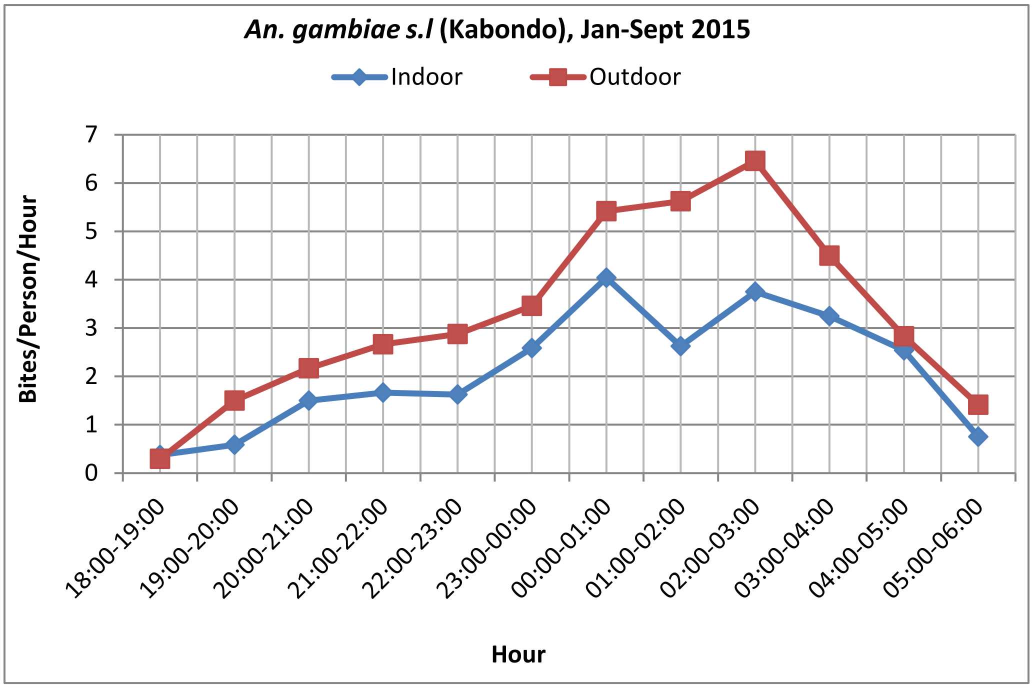
Periods	Location	Kabondo	Kalemie	Kapolowe	Katana	Kingasani	Lodja	Mikalayi
January/ Mach	Indoor	13	0.4	1.1	0.3	4	12	12
	Outdoor	3	1	0.5	0.4	8	24	10
April/June	Indoor	9	2	0	1	7	26	5
	Outdoor	5	2	0	1	3	25	8
July/Sept	Indoor	96	3	0	1	2	14	33
	Outdoor	68	2	0.1	1	5	11	43

Generally, *An. gambiae* s.l. had a higher biting rate than others vectors (Tables 16-22). The human biting rate for *An. gambiae* s.l. (Table 23) was variable from one site to another and also showed seasonal variations within the sites. The highest biting rates were recorded in Kabondo, Lodja and Mikalayi.

7. Biting Times of Malaria Vectors Indoors and Outdoors

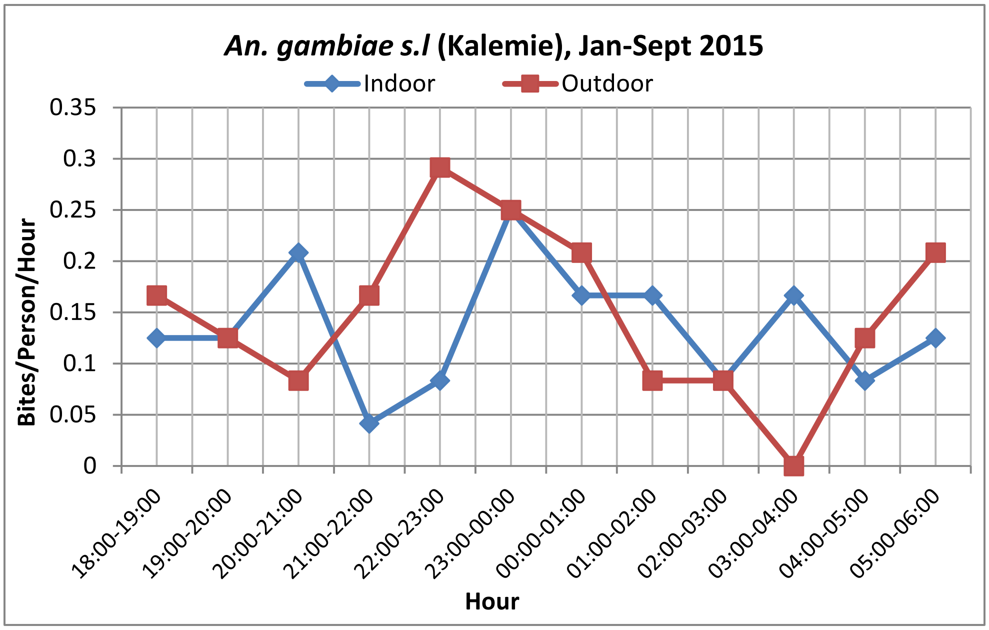
7.1 Province Orientale, Kabondo sentinel site

FIGURE 2: BITING ACTIVITY OF *AN. GAMBIAE* S.L. AT KABONDO (JANUARY-SEPTEMBER 2015)



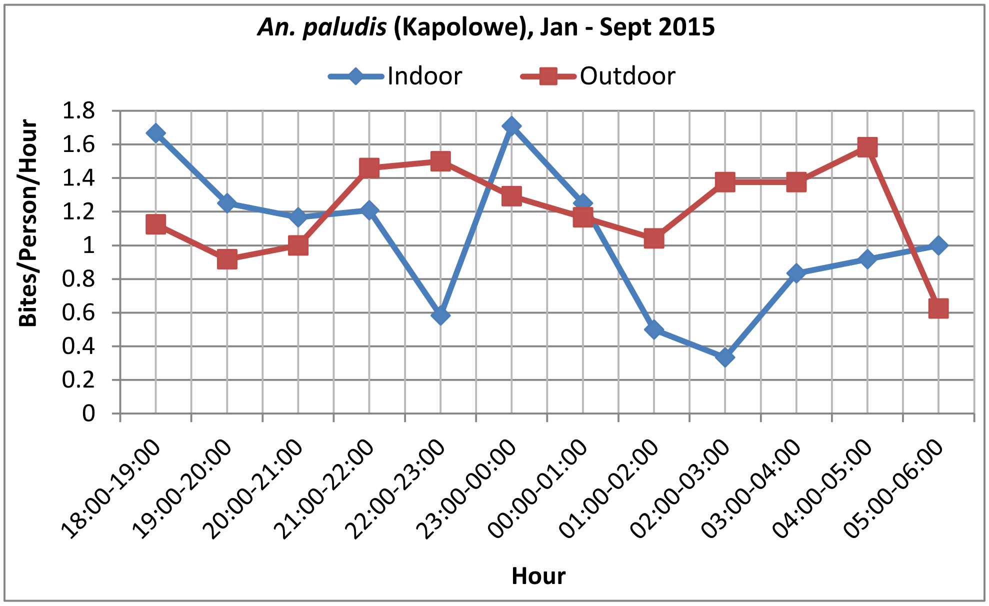
7.2 KATANGA PROVINCE, KALEMIE SENTINEL SITE

FIGURE 3: BITING ACTIVITY OF *AN. GAMBIAE* S.L. AT KALEMIE (JANUARY-SEPTEMBER 2015)



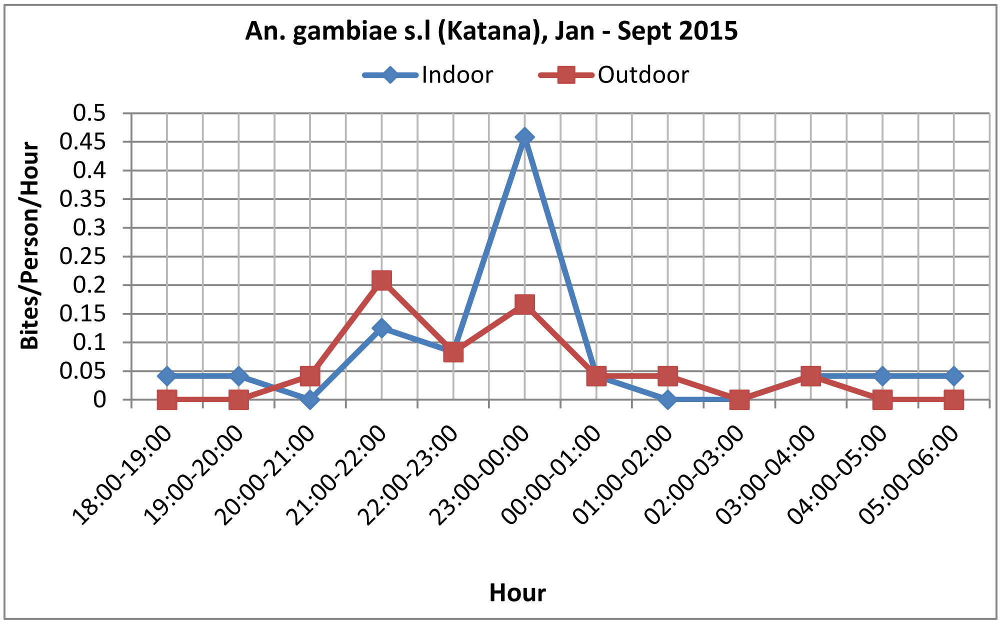
7.3 KATANGA PROVINCE, KAPOLOWE SENTINEL SITE

FIGURE 4: BITING ACTIVITY OF *AN. PALUDIS* AT KAPOLOWE SENTINEL SITE (JANUARY-SEPTEMBER 2015)



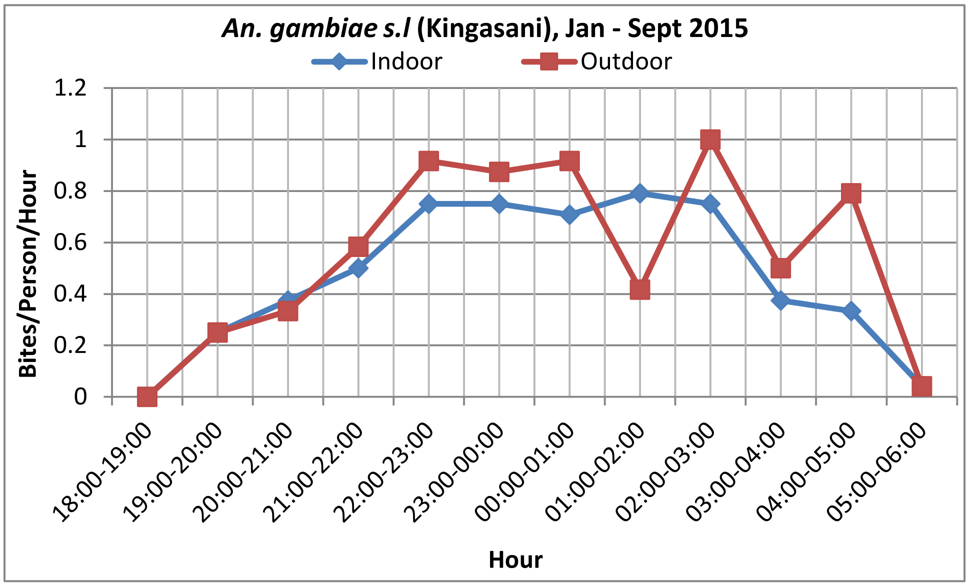
7.4 SUD KIVU PROVINCE, KATANA SENTINEL SITE

FIGURE 5: BITING ACTIVITY OF *AN. GAMBIAE* S.L. AT KATANA (JANUARY-SEPTEMBER 2015)



7.5 KINSHASA PROVINCE, KINGASANI SENTINEL SITE

FIGURE 6: BITING ACTIVITY OF *AN. GAMBIAE* S.L. AT KINGASANI (JANUARY-SEPTEMBER 2015)



7.6 Kasai Oriental Province, Lodja sentinel site

FIGURE 7A: BITING ACTIVITY OF *AN. GAMBIAE* S.L. AT LODJA (JANUARY-SEPTEMBER 2015)

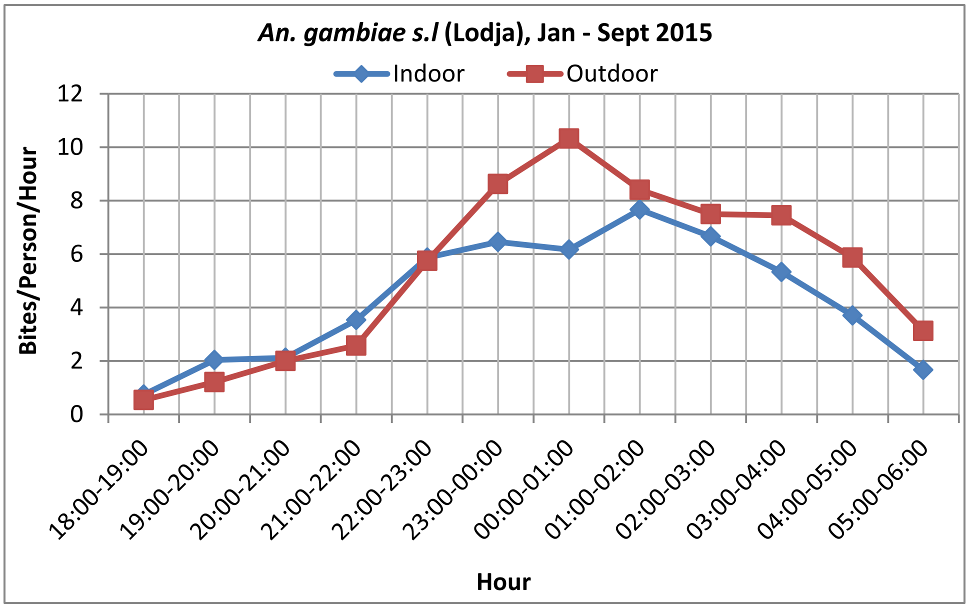


FIGURE 7B: BITING ACTIVITY OF *AN. FUNESTUS* S.L. AT LODJA (JANUARY-SEPTEMBER 2015)

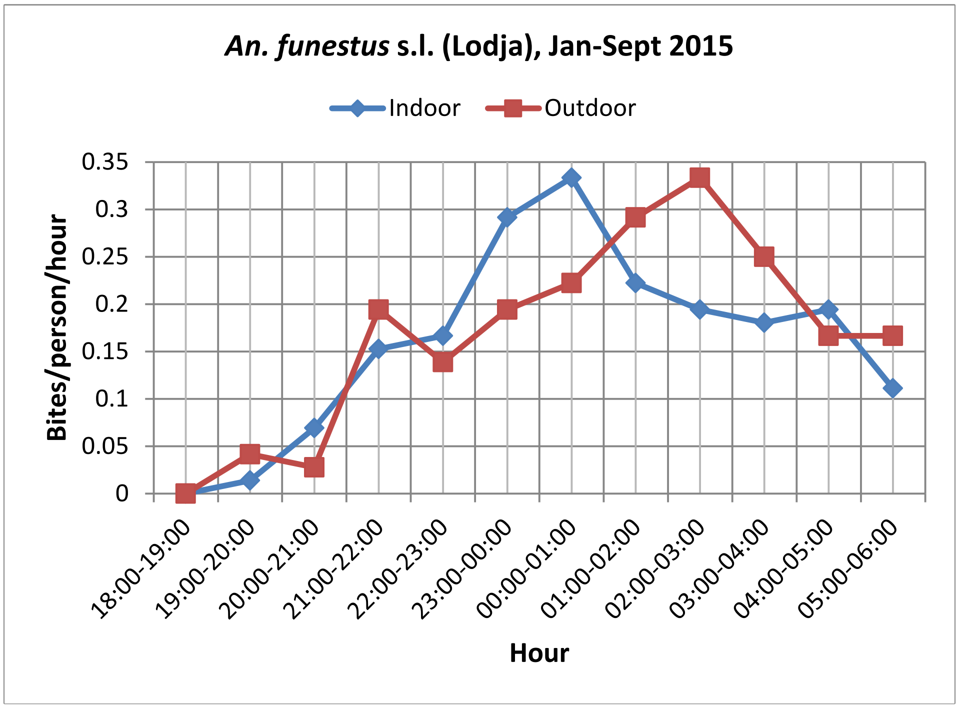
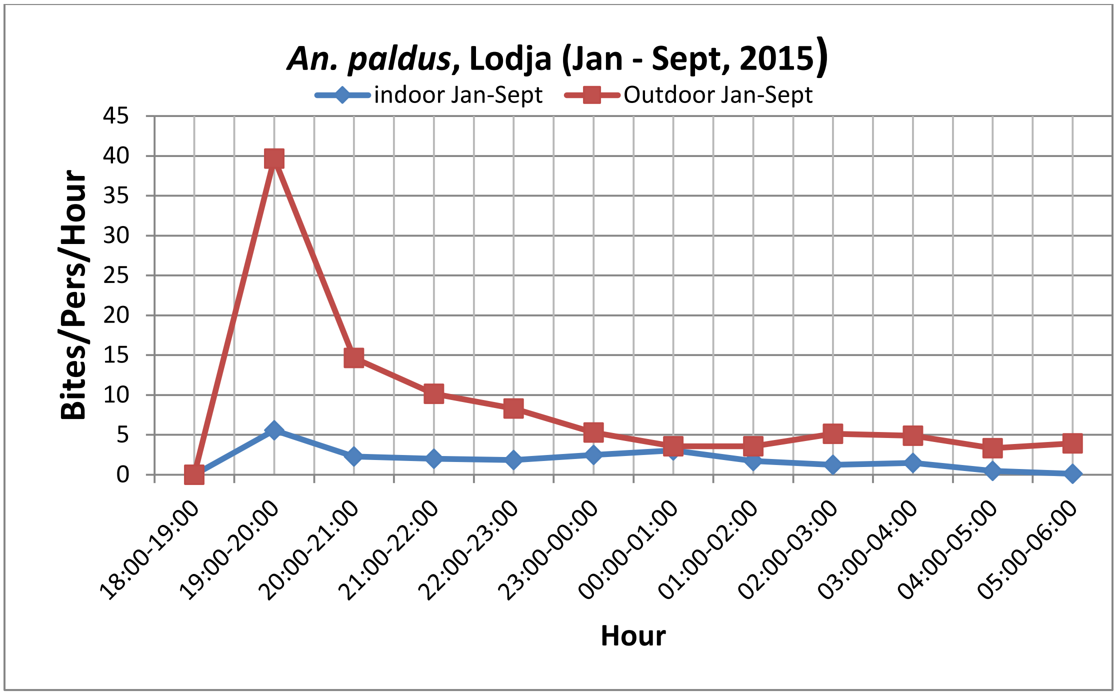


FIGURE 7C: BITING ACTIVITY OF *AN. PALUDIS* AT LODJA (JANUARY-SEPTEMBER 2015)



7.7 KASAI OCCIDENTAL PROVINCE, MIKALAYI SENTINEL SITE

FIGURE 8A: BITING ACTIVITY OF *AN. FUNESTUS* S.L. AT MIKALAYI (JANUARY-SEPTEMBER 2015)

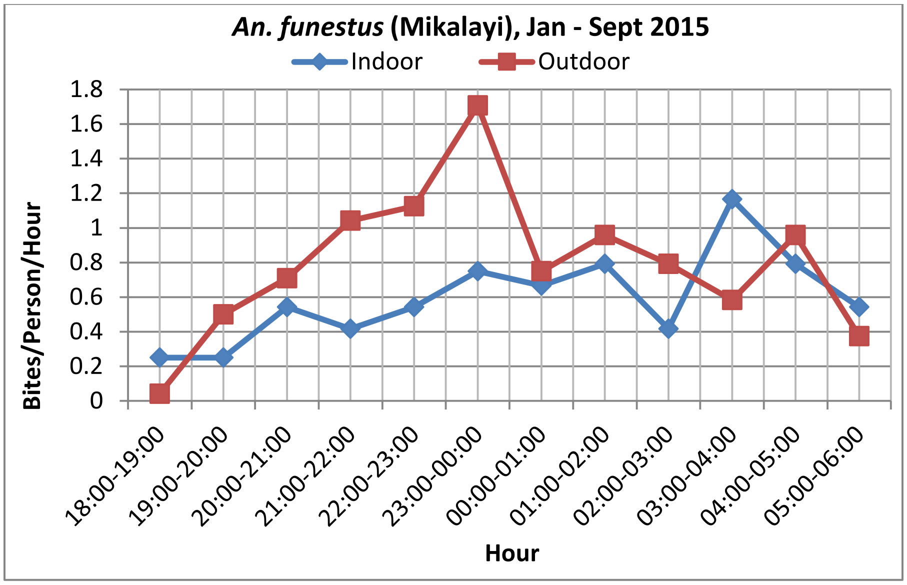
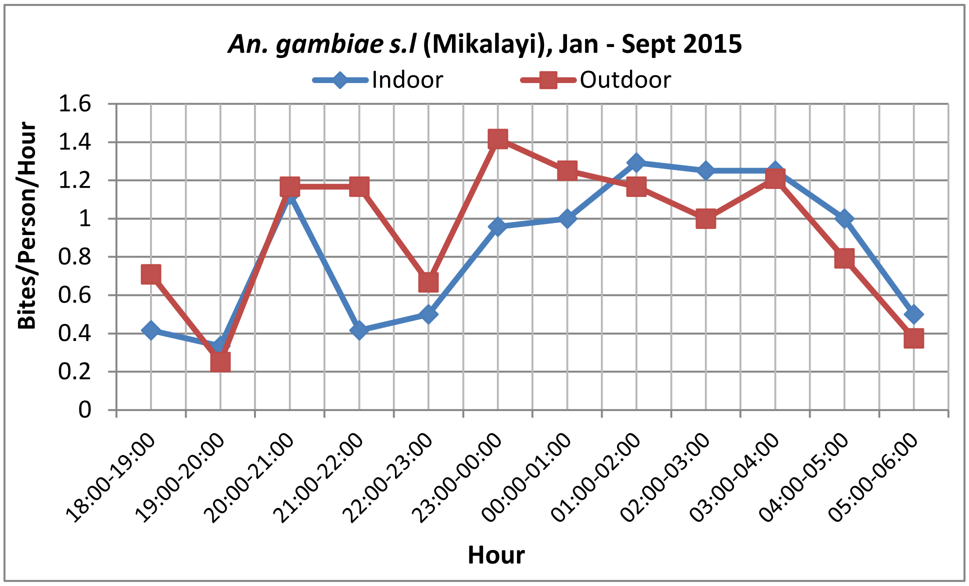


FIGURE 8B: BITING ACTIVITY OF *AN. GAMBIAE* S.L. AT MIKALAYI (JANUARY-SEPTEMBER 2015)



An. gambiae s.l. were captured biting in peak numbers late at night between 23:00 and 3am indoors in Kabondo (Fig 2), Kingasani (Fig 6) and Lodja (Fig 7A). In Lodja there was a similar trend outdoors for *An. gambiae* s.l. and most biting was after 22:00 when many people may be indoors. In Kabondo the outdoor and indoor biting rates and times were also very similar. In Mikalayi (Fig 8B) there was some evidence of early evening and late night biting by *An. gambiae* s.l. both indoors and outdoors. Due to the relatively low numbers of mosquitoes collected, biting time trends could not be clearly established for *An. gambiae* s.l. in Kalemie (Fig 3), Katana (Fig 5) and Kapolowe (not shown). *An. funestus* s.l. were captured in Mikalayi, Katana and Lodja; with the majority of biting between 22:00 and 5am in Lodja (Fig 7B) both indoors and outdoors. In Mikalayi (Fig 8A) and Katana (not shown) the biting densities were lower and no clear trends were observed. In Lodja *An. paludis* had a very early evening biting peak outdoors between 19:00 – 20:00, but biting rates remained relatively high throughout the night outdoors and low indoors (see section section 13 for more details). *An. paludis* in Kapolowe had a different biting pattern, there was no clear peak during the early evening and biting trends were similar both indoors and outdoors. More work is needed to determine whether *An. paludis* is a species complex (see section 13).

8. SUSCEPTIBILITY TESTS OF *AN. GAMBIAE* S.L. TO FOUR CLASSES OF INSECTICIDE

TABLE 24: RESULTS OF SUSCEPTIBILITY TESTS OF *ANOPHELES GAMBIAE* S.L. TO FOUR DIFFERENT INSECTICIDES IN 2015

Sentinel sites	Insecticides	Control exposed	Control died (%)	Number exposed (test)	Observed 24 hrs Mortality	Status
KABONDO	Pirimiphos-methyl 0.1%	20	0	100	100%	S
	Bendiocarb 0.1%	20	0	100	100%	S
	Deltamethrin 0.05	20	0	100	85%	R
	Permetrhin (0.75%)	20	0	100	52%	R
	DDT 4%	20	0	100	37%	R
KAPOLOWE	Pirimiphos-methyl 0.1%	20	0	80	100%	S
	Bendiocarb 0.1%	20	0	80	100%	S
	Deltamethrin 0.05	20	0	80	100%	S
	Permetrhin (0.75%)	20	0	80	53%	R
	DDT 4%	20	0	80	37%	R
KINGASANI	Pirimiphos-methyl (0.1%)	20	0	80	100	S
	Bendiocarb (0.1%)	20	0	80	100	S
	Deltamethrin (0.05%)	20	5	80	97	PR
	Permetrhin (0.75%)	20	0	80	91	PR
	DDT 4%	20	20	80	8	R
KATANA	Pirimiphos-methyl (0.1%)	25	0	100	100	S
	Bendiocarb (0.1%)	25	0	100	100	S

Sentinel sites	Insecticides	Control exposed	Control died (%)	Number exposed (test)	Observed 24 hrs Mortality	Status
	Deltamethrin (0.05%)	25	0	100	98	S
	Permetrhin (0.75%)	25	0	100	92	PR
	DDT 4%	ND	ND	ND	ND	ND
KALEMIE	Pirimiphos-methyl (0.1%)	25	0	100	100	S
	Bendiocarb (0.1%)	25	0	100	100	S
	Deltamethrin (0.05%)	25	0	100	100	S
	Permethrin (0.75%)	25	0	100	55	R
	DDT 4%	25	0	100	33	R
LODJA	Pirimiphos-methyl 0.1%	25	0	100	100	S
	Bendiocarb 0.1%	25	0	100	100	S
	Deltamethrin 0.05%	25	0	100	100	S
	Permetrhin (0.75%)	25	0	100	68	R
	DDT 4%	25	0	100	8	R
MIKALAYI	Pirimiphos-methyl 0.1%	20	0	100	100	S
	Bendiocarb 0.1%	25	0	100	100	S
	Deltamethrin 0.05%	25	0	100	100	S
	Permetrhin (0.75%)	25	0	100	30	R

The results from the WHO tube tests showed that *An. gambiae* s.l. was susceptible to pirimiphos-methyl and bendiocarb in all the sites. The vector was resistant to DDT in all sites where the tests were conducted. *Anopheles gambiae* s.l. was susceptible to deltamethrin at Lodja, Kapolowe, Katana, Kalemie, and Mikalayi sentinel sites, and resistant at Kabondo sentinel site. Possible resistance to deltamethrin was observed at the Kingasani sentinel site. *An. gambiae* s.l. was resistant to permethrin at Kapolowe, Kalemie, Mikalayi, Kabondoand Lodja sites. Possible resistance to permethrin was indicated for Kingasani and Katana sites.

9. SUSCEPTIBILITY TESTS OF *ANOPHELES GAMBIAE* S.L. TO PERMETHRIN AND THE SYNERGIST PBO

TABLE 25 : SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. TO PERMETHRIN USING A SYNERGIST PBO

Sentinel sites	Insecticides	Control exposed	Control died (%)	Number exposed (test)	Observed 24 hrs Mortality (%)	Effect of PBO exposure on susceptibility to permethrin
KABONDO	Permethrin (0.75%)	50	0	100	R (62)	Susceptibility increased
	Permethrin (0.75%) + PBO	50	0	100	R (86)	
KALEMIE	Permethrin (0.75%)	50	0	100	R (94)	Susceptibility increased
	Permethrin (0.75%) + PBO	50	0	100	R (96)	
KAPOLOWE	Permethrin (0.75%)	50	0	100	R (53)	Susceptibility increased
	Permethrin (0.75%) + PBO	50	0	100	S (100)	
LODJA	Permethrin (0.75%)	50	0	100	R79	Susceptibility increased
	Permethrin (0.75%) + PBO	50	0	100	S100	
MIKALAYI	Permethrin (0.75%)	50	0	100	R (30)	Susceptibility increased
	Permethrin (0.75%) + PBO	50	0	100	S (100)	
KINGASANI	Permethrin (0.75%)	50	0	100	R (92)	Susceptibility increased
	Permethrin (7.5% + PBO	50	0	100	S (99)	
KATANA	Permethrin (0.75%)	50	0	100	S (99)	Susceptibility increased
	Permethrin (7.5%) + PBO	50	0	100	S (100)	

All test results showed that the use of PBO increased the susceptibility of *Anopheles gambiae* s.l. exposed to permethrin. Resistance had been either completely or partially reversed for most of the sites, indicating the presence of metabolic resistance mediated by increased or modified activities of mono-oxygenase enzymes. This may be the only resistance mechanism involved in sites where resistance has been totally abolished (100%).

10. Sporozoite rates of *Anopheles gambiae* s.l. in 2014 and 2015

TABLE 26 : SPOROZOITE INDEX OF *AN. GAMBIAE* S.L. FROM HLC IN 2014

Sentinel Sites	*Anopheles gambiae* s.l. analyzed	*Anopheles gambiae* positive	Sporozoite rate (%)
Kabondo	176	12	7
Tshikaji	179	7	4
Kapolowe	241	6	3
Lodja	342	13	4
Kingasani	142	14	10
Fungurume	0	0	0
Mikalayi	54	8	15

The highest sporozoite index during 2014 was recorded at Mikalayi (15% of *Anopheles gambiae* s.l.) and the lowest was observed at Kapolowe sentinel site (3% of *Anopheles gambiae* s.l.).

TABLE 27: SPOROZOITE RATE OF *AN. GAMBIAE* S.L. IN 2015

Sentinel Sites	*Anopheles gambiae* s.l. analyzed	*Anopheles gambiae* s.l. positive	Sporozoite Index (%)
Kabondo	77	3	4
Kalemie	58	6	10
Kapolowe	65	1	2
Lodja	274	7	3
Kingasani	223	11	5
Katana	214	3	2
Mikalayi	246	13	5

The highest sporozoite rate for 2015 was observed at Kalemie sentinel site (10% of *Anopheles gambiae* s.l.) and the lowest rate was recorded at Kapolowe sentinel site and at Katana sentinel site (1.5% of *Anopheles gambiae* s.l.).

11. GENETIC IDENTIFICATION OF *ANOPHELES GAMBIAE* S.L. FROM 2014 SAMPLES

All Anophelinae captured in sentinel sites were identified morphologically to species complex level using the key of Gilles et al., 1968. In order to identify to species level, conventional PCR was performed.

The molecular biology unit conducted analysis of 819 samples of *Anopheles gambiae* s.l. from all the sites. Out of those, DNA was extracted from 212 legs and 607 whole mosquitoes. All DNA extraction was done using the procedure described in the Promega Technical Manual (Wizard Genomic DNA, Purification kit, Promega, USA) , using the following reagents:

- RNase solution
- Nuclei Lysis solution
- Cell Lysis solution
- DNA Rehydration Solution (10 mM Tris, 1mMEDTA)

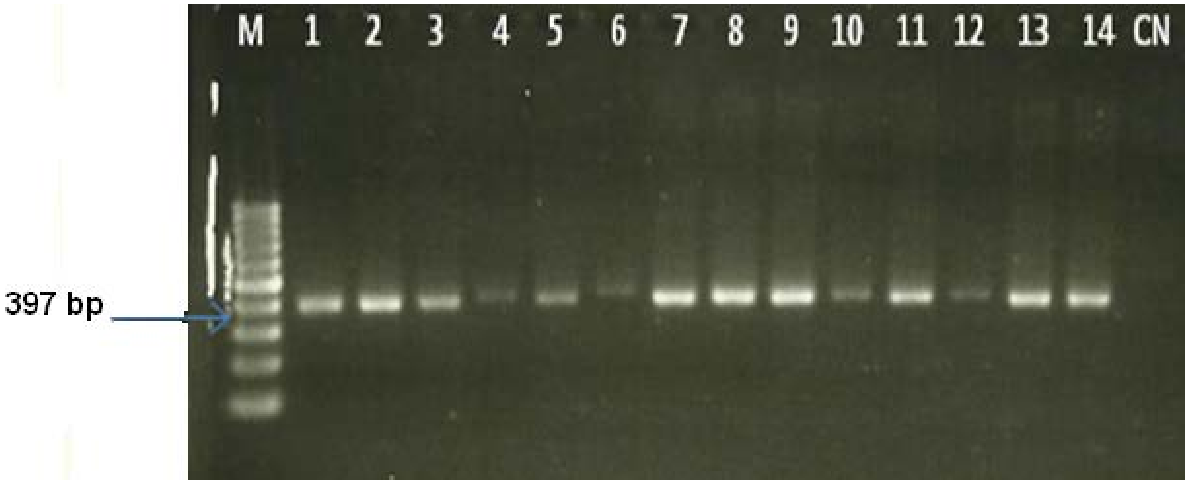
Amplification was conducted according to Favia et al., 2001 using the primers listed below:

- UN-Universal primer (GTGTGCCCCTTCCTCGATGT)
- AR-*Anopheles arabiensis* (AAGTGTCCTTCTCCATCCTA)
- GA-*Anopheles gambiae* s.s./*An. coluzzii* (CTGGTTTGGTCGGCACGTTT)
- ME-*Anopheles merus/melas* (TGACCAACCCACTCCCTTG

All DNA analyzed according the PCR technique (Favia et al. 2001) were *Anopheles gambiae* s.s./An. coluzzii and corresponded to 397bp (Fig. 9).

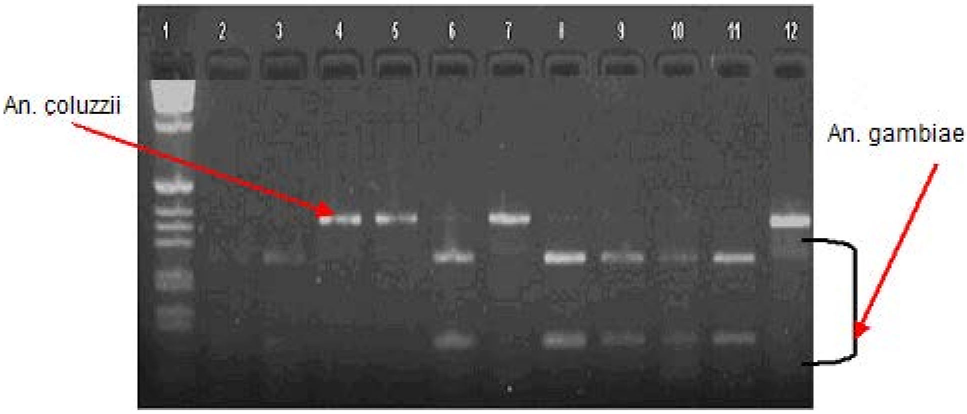
FIGURE 9: THE AGAROSE GEL (2%) SHOWING THE BANDS OF *ANOPHELES GAMBIAE* S.S./*AN. COLUZZII* AFTER PCR

M = marker; CN = negative control;
Anopheles gambiae s.s./*An. coluzzii* =1-14 columns



The DNA showing *Anopheles gambiae* s.s./*An. coluzzii* were submitted to enzymatic restriction using 1 µl of *Haemophilus hemolyticus*, *serotype I* (Hha I), 2 µl of Multi Core Buffer, 2 µl of BSA, 1.5 ml of distilled and 24 µl of DNA. With this enzyme, the DNA was restricted to one band of 367 for *An. coluzzii* (formerly *An. gambiae* M Form) or 2 bands (257 bp and 110 bp) which characterize *An. coluzzii* (formerly the S Form (Fig. 10)).

FIGURE 10: BANDS OF DNA OF *ANOPHELES GAMBIAE* S.S. AND *AN. COLUZZII* AFTER MIGRATION ON 2% AGAROSE GEL



Marker: column 1 (1 Kbp);

An. coluzzi: columns 4, 5, 7 & 12 (367 bp)

An. gambiae s.s.: columns 3, 6, 8, 9, 10 (257pb and 110 bp)

Out of 605 *Anopheles gambiae* s.l. collected in 2014 in different sites and analyzed for *Anopheles* species, 347 were *Anopheles gambiae* s.s/*An. coluzzii,* while the remaining 258 failed to produce bands (Table 27). The failure of a large proportion to produce bands may be due to morphological misidentification of mosquitoes, meaning that there were species other than *An. gambiae* s.l. A more likely explanation is that during this period there were numerous power outages (there is no backup generator), which may have affected the quality of some of the reagents and also resulted in DNA degradation of samples as some were stored without silica gel. Sample storage is currently being addressed.

TABLE 28: IDENTIFICATION OF AN. GAMBIAE S.L. COMPLEX TO SPECIES LEVEL AND DISTRIBUTION BY SENTINEL SITES IN 2014

Species	KABONDO	KINGASANI	KAPOLOWE	LODJA	MIKALAYI	TSHIKAJI	FUNGURUME	Total
An. gambiae s.s./*An. coluzzii* (GA)	132	43	18	55	60	39	0	347
An. arabiensis (AR)	0	0	0	0	0	0	0	0
An. melas/merus (ME)	0	0	0	0	0	0	0	0
Total	132	43	18	55	60	39	0	347

According to Table 27, all *Anopheles gambiae* s.l. from all the sites were *Anopheles gambiae* s.s./*An. coluzzii*. No other member of the *Anopheles gambiae* complex was identified in the sites.

TABLE 29: DISTRIBUTION OF ANOPHELES GAMBIAE AND COLUZZII IN THE SENTINEL SITES IN DRC (2014)

Molecular forms	Sentinel sites in DRC						Total
	Kabondo	Kingasani	Kapolowe	Lodja	Mikalayi	Tshikaji	
An. gambiae ss	0	13	12	23	12	9	69
An. coluzzii	50	17	17	7	18	23	132
Total	50	30	29	30	30	32	201

Two species of the *An. gambiae* complex were identified in the sentinel sites through the DRC. The first one was *An. gambiae* s.s., which represented 34.3 % (69/201) of all samples analyzed and An. coluzzii, which represented 65.7 % (132/201). Note that only 201 species out of 347 were analyzed due to a lack of primers. The DNA not analyzed is still available and can be analyzed.

12. Genetic identification of *Anopheles gambiae* s.l. from 2015 samples

For the 2015 samples identified morphologically as *Anopheles gambiae* s.l. the method of Wilkins et al. (2006) was utilized for species identification.

The mix for species identification consisted of the following:

- PCR H2O : 8,3µl
- Buffer 5X Go Taq : 5µl
- dNTP : 2,5µl
- MgCl2 : 1µl
- IMP-UN (GCTGCGAGTTGTAGAGATGCG) : 1µl
- AR-3T (GTGTTAAGTGTCCTTCTCCgTC) : 1µl
- GA-3T (GCTTACTGGTTTGGTCGGCAtGT) : 1µl
- ME-3T (CAACCACTCCCTTGACGaTG) : 1µl
- IMP-S1 (CCAGACCAAGATGGTTCGcTG) : 1µl
- IMP-M1 (TAGCCAGCTCTTGTCCACTAGTtTT) : 1µl
- Taq Pol. : 0,2µl
- DNA : 2µl

The amplification conditions were:

- Denaturation 1: 95°C for 5 minutes X 1 cycle
- (Denaturation : 95°C/30sec, hybridisation : 58°C/30sec, elongation : 72°C/30sec) X30 cycles
- Final elongation: 72°C for 5 minutes X 1 cycle

279 DNA extractions were conducted with approximately 40 mosquitoes tested per sentinel site. Of these 12 (4%) did not amplify and are presumed to be other *Anopheles* species. 101 (36%) specimens were identified as *An. gambiae* s.s., 5 (2%) An. coluzzii and 160 (57%) specimens were identified as hybrids of *An. gambiae* s.s. / *An. coluzzii*.

TABLE 30: SPECIES COMPOSITION BY SENTINEL SITE IN 2015

Species	Kabondo	Kapolowe	Katana	Kingasani	Kalemie	Lodja	Mikalayi
An. gambiae s.s.	36	23	5	8	25	3	1
An. coluzzii	0	0	0	2	2	1	0
Hybrid *An. gambiae s.s.* / *coluzzii*	1	16	35	30	4	35	39
An. arabiensis	0	0	0	0	0	1	0
Other *Anopheles*	3	1	0	0	8	0	0
Subtotal	40	40	40	40	39	40	40

The high proportion of *An. gambiae* s.s. / *An. coluzzii* hybrids reported is unusual, as typical hybrid rates in neighboring countries are <1%, with the highest rates recorded at around 20% in Equatorial Guinea. After conducting an STTA at INRB together with Dr Neil Lobo of University of Notre Dame, it was concluded that the high reported rate of hybrids is most likely due to contamination. The PCR assay depends on a base pair mismatch, which would work if the conditions were perfect. However, if they were not, it is easy to get non-specific amplification, especially if most of the primer binds. The presence of a range of band intensities on gel images observed at INRB appears to be an indication of contamination or non-specific binding of primers. In general the conditions and laboratory procedures at INRB were observed to be very good. However, contamination could have easily occurred. For example, a needle was used to pierce the PCR tube before denaturing; if the needle punctures of the tubes introduced a small amount of DNA, a faint band may occur that looks like a hybrid.

Way forward

Two hundred archived specimens from 2015 collections identified morphologically as *An. gambiae* s.l. will be dissected so that 3 legs and 1 wing are sent to University of Notre Dame, USA and 3 legs and 1 wing kept at INRB. University of Notre Dame will conduct species identification using the methods of Wilkins (2006) and also Santolamazza (2008). In parallel INRB will conduct analysis of the same specimens using the method of Wilkins (2006). Results will be compared between laboratories and methodologies. If the results are different between laboratories for the Wilkins method, troubleshooting will be conducted to determine steps that can be done to improve the quality of results. The method of Santolamazza is likely to be more robust than that of Wilkins as it is based on a single copy and irreversible SINE200 insertion and, thus, is not subjected to peculiar evolutionary patterns affecting rDNA markers (Santolamazza, 2008). Therefore, this method may be preferred for future analysis. More detailed laboratory quality assurance training has been included in the work plan for 2017 along with routine sending of samples to University of Notre Dame for quality assurance double testing of specimens. A supplementary annex will be added to this report with the revised molecular analysis following the conclusion of the troubleshooting process.

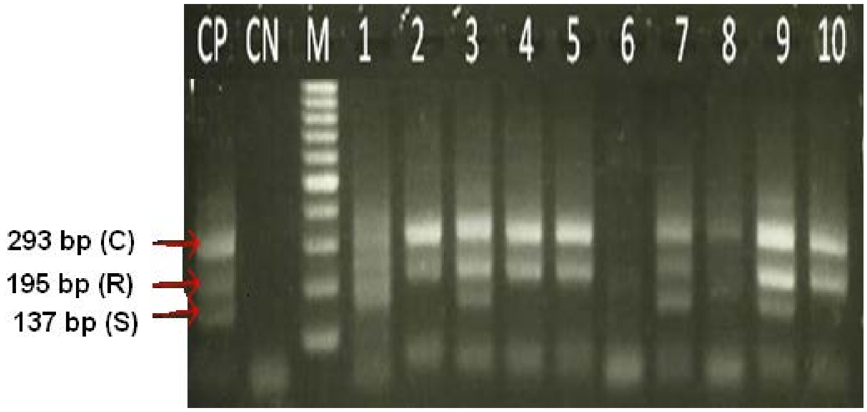
13. FREQUENCY OF KDR RESISTANCE MARKERS FROM 2014 SAMPLES

The DNA of all *Anopheles gambiae* s.s./*An. coluzzii* were analyzed for presence of kdr-L1014F alleles according to Martinez-Torres et al. (1998) [5] and Basilua et al. (2013) [6]. The following primers were used to identify the kdr-L1014F:

- AgD1 (ATAGATTCCCCGACCATG)
- AgD2 (AGACAAGGATGATGAACC)
- AgD3 (AATTTGCATTACTTACGACA)
- AGD4 (CTGTAGTGATAGGAAATTTA).

After the PCR, we obtained images, such as Figure 11.

FIGURE 11: BANDS OF DNA SHOWING KDR GENOTYPIC STATUS OF *ANOPHELES GAMBIAE* S.S./*AN. COLUZZII*



CP = positive control; CN = negative control; C = internal control; M = marker (ladder); R = Resistance (195 bp); S = Susceptibility (137 bp); Heterozygote specimen RS: 1, 3, 7 and 9; Homozygote specimen RR: 2, 4, 5, 8 and 10; Negative specimen: 6.

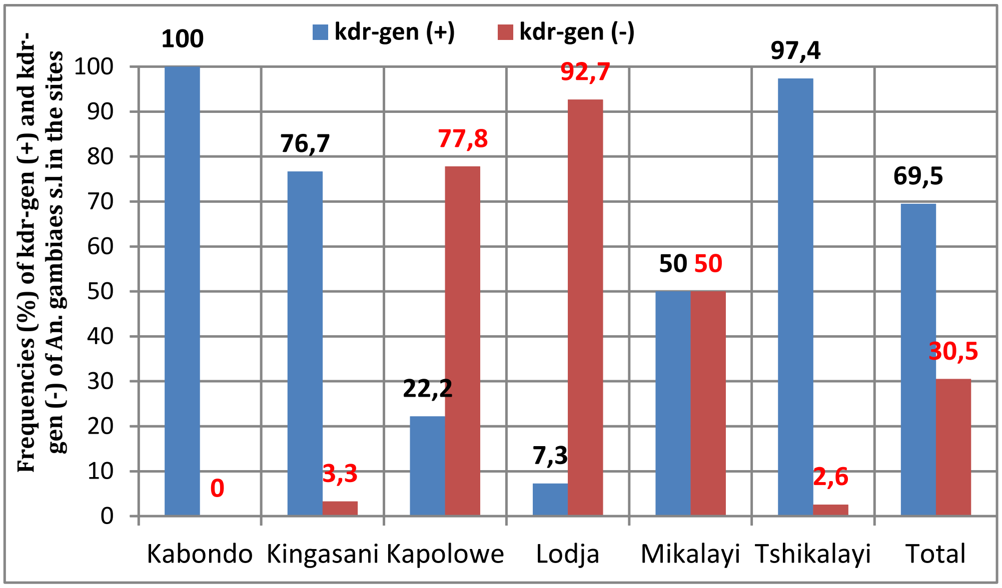
TABLE 31: DISTRIBUTION OF *ANOPHELES GAMBIAE* S.S./*AN. COLUZZII* BY SENTINEL SITE ACCORDING TO THEIR KDR L1014F GENOTYPIC STATUS IN 2014

STATUS	KABONDO	KINGASANI	KAPOLOWE	LODJA	MIKALAYI	TSHIKAJI	Total
Resistant (RR)	127	30	4	3	22	25	211

Susceptible (SS)	0	10	14	51	30	1	106
Heterozygous (RS)	5	3	0	1	8	13	30
Total	132	43	18	55	60	39	347

Table 31 shows that the kdr status of *Anopheles gambiae* s.s varied from one site to another. Out of 347 *Anopheles* analyzed, 241 (211+30) specimens were positive for the L1014F allele (70 %; 241/347). Within the positive, 211specimens were homozygote resistant (88 %; 211/241) and 30 were heterozygote resistant (12 %; 30/241). The highest proportion of specimens homozygote resistant were recorded at Kabondo (96 %; 127/132), followed by Kingasani (70%; 30/43). The number of homozygote resistant samples was low in Kapolowe (22%; 4/18) and Lodja (5%; 3/55). For the heterozygote resistant specimens, the highest value was recorded in Tshikaji (33%; 13/39). For each site, we have compared the kdr L1014F positive and negative allele frequencies as shown in Fig.12.

FIGURE 12: CUMULATIVE FREQUENCIES OF *ANOPHELES GAMBIAE* S.S/ AN. *COLUZZII* WITH KDR L1014F (WEST) ALLELES PRESENT WHERE KDR-GEN (+) REFERS TO MOSQUITOES THAT WERE KDR-HETEROZYGOUS OR KDR-HOMOZYGOUS IN 2014



The kdr status of *Anopheles gambiae* s.s./*An. coluzzii* varied from one site to another. The highest frequencies of kdr L1014F were recorded in Kabondo (100 %) followed respectively by Tshikaji (97 %), Kingasani (77 %) and Mikalayi 50%.

14. SURVEILLANCE OF *AN. PALUDIS* AT LODJA SENTINEL SITE

Monthly abundance of host-seeking and resting *An. paludis* Jan-Dec 2015

The abundance of host-seeking *An. paludis* was determined by HLC indoors and outdoors while indoor resting abundance was determined by PSC. Results are presented by month in Figures 13-14 and Table 30.

FIGURE 13: MONTHLY TOTAL ABUNDANCE OF HOST-SEEKING *AN. PALUDIS* BY HLC (INDOOR + OUTDOOR)

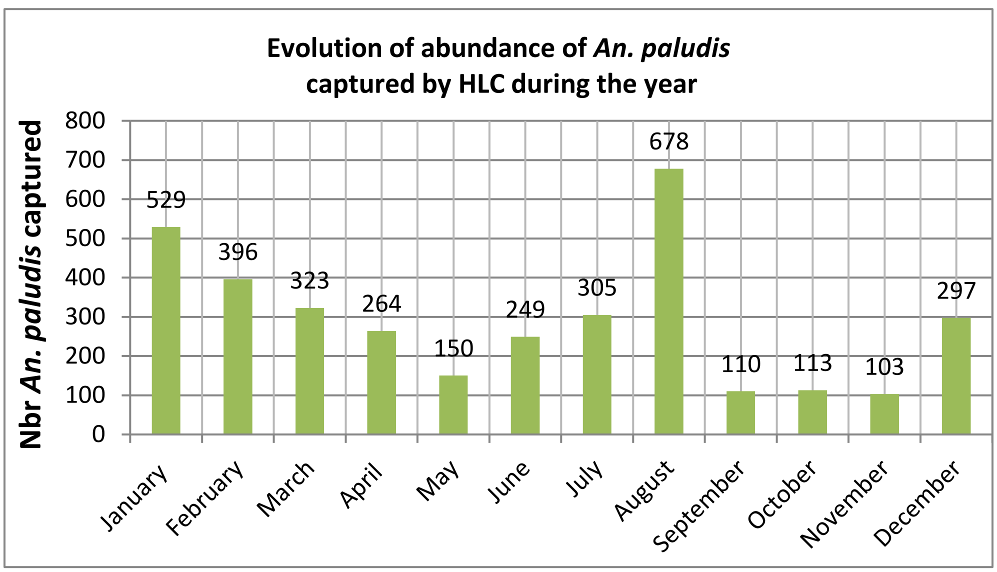


FIGURE. 14: 2015 MONTHLY INDOOR AND OUTDOOR HOST-SEEKING *AN. PALUDIS* BY HLC.

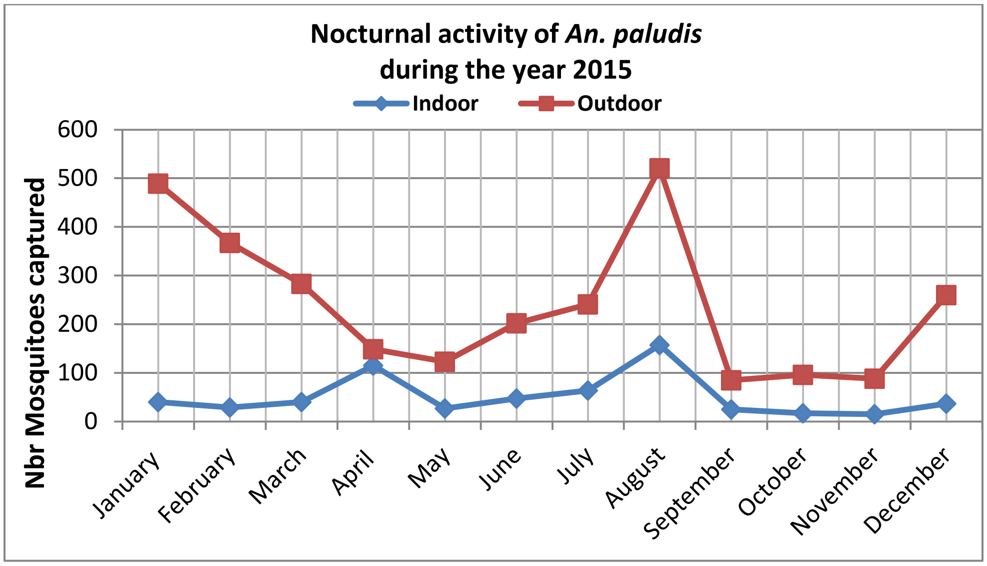


Figure 14 shows that *An. paludis* was captured every month by HLC in Lodja, with considerable seasonal variation. The highest number of *An. paludis* collected by HLC was in January at 527, with 20 bites per person/night outdoors (489/24) and 2 indoors (38/24). The number collected by HLC decreased gradually between January and April, followed by a stabilization of numbers between April and July at 150-305 per month. The lowest numbers of *An. paludis* were observed in September and October 2015. The proportion of host-seeking *An. paludis* captured by HLC outdoors was significantly greater than the proportion caught indoors, at 83% (2,904/3,503) and 17% (599/3,503), respectively (χ^2=1251, P<0.001) (Figure 14). Indoors, the highest numbers of *An. paludis* caught by HLC were recorded in April and August 2015, and low numbers were recorded the rest of the year. The number of indoor resting *An. paludis* captured by month using PSC was far lower than those host-seeking caught by HLC (Table 32). There were seven months (of twelve) where PSC failed to catch a single *An. paludis* and the maximum caught was six.

TABLE 32: 2015 MONTHLY ABUNDANCE OF *AN. PALUDIS* IN LODJA BY COLLECTION METHOD AND LOCATION (INDOORS OR OUTDOORS)

	HLC		Total	PSC	Total
Month	**Indoor**	**Outdoor**		**Indoor**	
January	38	489	527	2	529
February	29	367	396	0	396
March	38	283	321	2	323
Total Jan-March	**105**	**1,139**	**1,244**	**4**	**1,248**
April	115	149	264	0	264
May	27	123	150	0	150
June	47	202	249	0	249
Total April- June	**189**	**474**	**663**	**0**	**663**
July	64	241	305	0	305
August	152	521	673	5	678
September	25	85	110	0	110
Total July- Sept	**241**	**847**	**1088**	**5**	**1093**

October	12	96	108	5	113
November	15	88	103	0	103
December	37	260	297	0	297
Total Oct-Dec	**64**	**444**	**508**	**5**	**513**
Overall Total Jan - Dec	**599**	**2,904**	**3,503**	**14**	**3,517**

Transmission of malaria

In total, 3,517 *An. paludis* were captured in Lodja. Out of these 3,503 were collected by HLC and 14 by PSC (Table 30). 1,366 of 3,517 (39%) *An. paludis* collected in Lodja were analyzed for circumsporozoite index (CS) by NIBR in DRC. Out of all specimens of *An. paludis* analyzed none were positive for the presence of sporozoites.

TABLE 33: NUMBERS OF *AN. PALUDIS* FROM HLC ANALYSED FOR PRESENCE OF CIRCUMSPOROZOITES

	An. paludis analyzed	% of total collected	*An. paludis* Captured
NIBR, DRC	1,366	39%	3,517

Molecular Identification of Morphologically Identified *Anopheles paludis* samples collected from sentinel sites (DRC)

Anopheles paludis (n=311) from Lodja and *An. caliginosus* (n=30) from Kapolowe were sent to the group of Dr. Neil Lobo in order to accurately determine species composition through sequencing of ITS2 and CO1 regions at the Genomics Core Facility, University of Notre Dame. This preliminary molecular analysis is the first of its kind in the DRC for this species and will contribute to better understanding of basic biology and improve intervention strategies.

An *in silico* comparison was used to determine species groups. Raw ITS2 and CO1 sequences were manually cleaned and then aligned using the Seqman pro assembler (Lasergene v10.1.1, DNASTAR Inc., Madison, WI) followed by database comparisons using published methodologies towards identifying sequence groups indicative of species. High sequence identity (99% or greater) to voucher specimen sequences in the database was primarily used for final species confirmation [7].

Both ITS2 and CO1 sequencing and analysis revealed the presence of more than one species within the morphologically identified group (*An. paludis* or *An. caliginosus*) (Table 33). Of the *An. paludis* samples, sequences were obtained from 298 (11 did not amplify). Specimens identified as *An. paludis* consisted of 2 ITS2 groups (presumably 2 cryptic species) and 3 CO1 groups (pointing to possible introgression between the species much like the *An. gambiae* complex). Specimens identified as *An. caliginosus* consisted of 2 ITS2 groups (presumably 2 cryptic species) and 2 CO1 sequences (which matched the ITS2 samples) pointing to 2 distinct groups/species. Since these sequences generated are novel and not present in the database (90% identity cutoff), accurate species determination is not possible. Group Coustani (Reid & Knight 1961), is known to consist of 9 distinct species *An. caliginosus* De Meillon 1943, *An. coustani* Laveran 1900, *An. crypticus* Coetzee 1994, *An. fuscicolor* Van Someren 1947, *An. namibiensis* Coetzee 1984, *An. paludis* Theobald 1900, *An. symesi* Edwards 1928, *An. tenebrosus* Donitz 1902, *An. ziemanni* Grunberg 1902), which may also have cryptic or sibling species within each them. At this point, we are unable to assign exact species but plan to associate specific morphological attributes and hence species to molecular determinants. It is important to note that both species identified within the *An. caliginosus* group were also identified as 'unknown *An. coustani*-related species' in the Kenya highlands [8, 9]. Preliminary tests to look for sporozoite DNA in the head and thoraxes of the mosquitoes have demonstrated that the *An. caliginosus* were all negative, while samples in both ITS2 groups for *An. paludis* were found to contain *Plasmodium falciparum* and *P. ovale* DNA. The unusually high rate of positives (17 of 298; 5%) mandated a retesting of all positive samples to rule out contamination. Results were replicated. The high number of *Plasmodium* DNA positive *An. paludis* specimens may be due to the sensitivity of the PCR used (0.2parasites/ul; 250x more than microscopy) and also, does not indicate infectivity.

TABLE 34: MOLECULAR ID USING ITS2 AND CO1 SEQUENCES

Morphological species	**Number of**	**ITS2 Group (number of samples)**	**CO1 Group (number of samples)**	**Plasmodium DNA species**	**Notes**

	specimens			(number of samples)	
An. paludis	311	*An. cf paludis* 1 (18)	CO1 Type A (7)	Negative	
			CO1 Type B (11)	Po (1); Pf (1)	
		An. cf paludis 2 (286)	CO1 Type C (100)	Po (3)	
			CO1 Type B (172)	Po (4); Pf (3), ?, (2)	The 2 unknown Plasmodium amplifications require re-sequencing
An. caliginosus	30	Contig 752 (25)	CO1 Type D (26)	Negative	Identified in Kenya
		Contig 754 (4)	CO1 Type E (4)	Negative	Identified in Kenya

Summary

An. paludis is known to be a major malaria vector in parts of DRC, particularly in Bandundu Province [3]. Initial trapping indicated that large numbers of *An. paludis* were collected in Lodja site (Sankuru Province), in addition to *An. gambiae* s.l. and *An. funestus* s.l. Therefore, it was important to determine whether *An. paludis* was an important malaria vector in this area and to determine the behavioral characteristics of this species.

Monthly surveillance done in Lodja during 2015 has provided the following information:

1) *An. paludis* was very abundant in Lodja and highly anthropophilic with large numbers collected every month by HLC.

2) The number of *An. paludis* captured by month was variable, apparently, with climate (relative humidity and temperature) playing an important role. The population was more abundant in the rainy season.

3) *An. paludis* was found to be predominantly exophagic with far more host-seeking caught outdoors by HLC.

4) The number of *An. paludis* captured resting indoors by PSC was very low and indicates the species to be largely exophilic. It appears that those which blood-fed indoors exited before dawn.

5) Circumsporozoite (CS) ELISA was conducted on 41% of *An. paludis* total catch by NIBR. The results suggest that *An. paludis* is unlikely to be a malaria vector of primary importance in Lodja. However, PCR testing at Notre Dame has indicated presence of *P. falciparum* and *P.ovale* sporozoites. The PCR technique used (Lobo, unpublished data) is approximately 250x more sensitive than ELISA.

6) Molecular species identification using ITS2 and CO1 sequencing has revealed that there are likely to be 2 cryptic species each within samples morphologically identified as *An. paludis* and *An. caliginosus*.

These results raise further important questions with the primary questions of interest being:

- Why was there considerable malaria transmission by *An. paludis* from Bandundu Province (South-west) and yet none detected by ELISA from *An. paludis* from Lodja site?
- What is the risk of malaria transmission from *An. paludis* at other sentinel sites where large numbers have been collected?

It has been shown that *An. paludis* in Lodja is a highly anthrophilic species with an unusual early biting peak and which predominantly bites outdoors. These early, outdoor biting tendencies mean that LLINs are unlikely to offer much if any protection from being bitten. While it was found in this study that no sporozoites were present by ELISA, the finding of Karch et al in 1992 that high sporozoite rates were found in Bandundu is a warning sign that this species group may be of great significance elsewhere in DRC. We think it is important to continue to determine the importance of *An. paludis* group in areas where it is a major human-biting species, such as Kapolowe (Katanga Province) where it is caught in greater numbers than *An. gambiae* s.l.

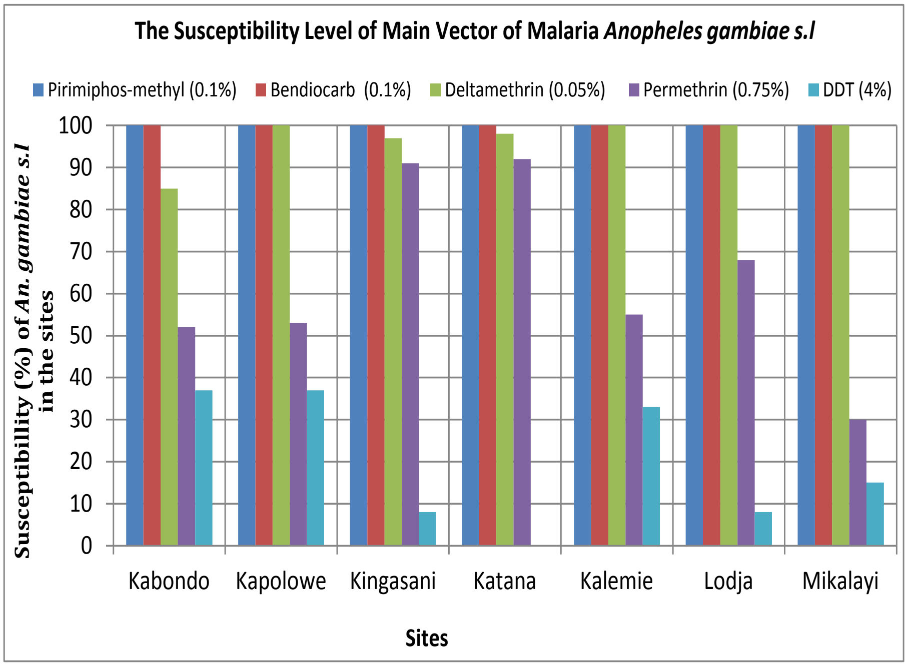
15. SUMMARY

TABLE 35: *ANOPHELES* MOSQUITO SPECIES IN THE SEVEN SENTINEL SITES

Anopheles captured	Kabondo	Kapolowe	Kingasani	Katana	Kalemie	Lodja	Mikalayi
An. gambiae s.l.	X	x	x	x	x	x	x
An. funestus s.l.	X	x	x	x	x	x	x
An. paludis		x		x		x	x
An. implexus							x
An. nili	X	x					x
An. rufipes							x
An. christyi					x		x
An salbaii					x		x
An. ziemanni		x					
An. tenebrosus		x			x		
An. caliginosus		x					
An. swahilicus	X						

As shown in Table 35, 12 species of *Anopheles* were caught in the seven sentinel sites. They were distributed differently in the sentinel sites. Out of these species, four are recognized as being malaria vectors in DRC: *An. gambiae* s.l., *An. funestus* s.l., *An. paludis* and *An. nili.* The eight other species of *Anopheles,* which are not recognized as malaria vectors in DRC, are: *An. implexus, An. rufipes, An. christyi, An. salbaii, An. ziemanni, An. tenebrosus, An. caliginosus and An. swahilicus.* Eight species of *Anopheles* were identified in Mikalayi. *An. gambiae* s.l. and *An. funestus* s.l. were caught from all sentinel sites. *An. paludis* was identified in Kapolowe, Katana and Lodja sites. *An. salbaii* was caught in two sentinel sites (Kalemie and Mikalayi). *An. swahilicus* was caught only in Kabondo.

FIGURE 15: THE SUSCEPTIBILITY LEVEL (% MORTALITY) OF THE MAIN VECTOR OF MALARIA, *Anopheles Gambiae* s.l.



Anopheles gambiae s.l. were tested to five insecticides belonging to four classes of insecticides recommended by WHOPES for public health in seven sentinel sites: DDT 4% (organochlorines), pirimiphos-methyl 0.1% (organophosphates), bendiocarb 0.1% (carbamates), deltamethrin 0.05% (pyrethroid), and permethrin 0.75% (pyrethroid). Pirimiphos-methyl 0.1% and bendiocarb 0.1% killed all *Anopheles gambiae* s.l. it came into contact with. *An. gambiae* s.l. was susceptible to deltamethrin 0.05% everywhere except at Kingasani and Katana. Mosquitoes were resistant to permethrin 0.75% and DDT 4% everywhere.

COMPARISON OF VECTOR DENSITIES AND BEHAVIOR

Densities varied for all species of *Anopheles* from one site to another, from period to period. In a sentinel site, the situation varied with the environmental conditions (wind, temperature, humidity) and the house conditions where mosquitoes were captured.

ENTOMOLOGICAL INOCULATION RATE

Sporozoite rates were variable from one site to another, from one period to another; even in the same sentinel site, the sporozoite rate changed from one season to another. (Tables 36 and 37)

To obtain the data included in Table 36 as the entomological inoculation rate indoor/outdoor, the mean sporozoite index for *An. gambiae* s.l. in a site was multiplied by the human bites rate (HBR) and the result divided by 100. To determine the daily entomological inoculation rate, the analysis for *An. gambiae* s.l. for sporozoite index (SI) was first done, after which the agressivity (HBR= number of bites per human per night) of *An. gambiae* s.l. (indoor and outdoor) was calculated as follows: total number of mosquitoes collected by HLC over all periods/by the total number of person nights across all periods for each species of malaria vector incrimined. The daily Entomological Inoculation Rates (EIRs) were calculated indoors and outdoors as follows:

The EIR = SI (general for the house)*HBR; for example:

1) Indoor for Kabondo: the EIR = (SI/100)*HBR indoor = (3.9)/100*39.2 = 1.5288 = 1.53

2) Outdoor for Kabondo: the EIR = (SI/100)*HBR outdoor = (3.9)/100*25.29 = 0.98631= 0. 98 (or 0.99)

TABLE 36: ENTOMOLOGICAL INNOCULATION RATE INDOOR/OUTDOOR FOR *AN. GAMBIAE* S.L. IN SENTINEL SITES, 2015

Sentinel Sites	Sporozoite index (SI)	Human bites rates (HBR)		Daily Entomological Inoculation Rates (EIR)	
		Indoor	Outdoor	Indoor	Outdoor
Kabondo	3.9	39.2	25.29	1.53	0.98
Kalemie	10.4	1.63	1.79	0.17	0.18
Kapolowe	1.5	0.38	0.21	0.01	0.0
Lodja	2.6	17.3	21.1	0.45	0.55
Kingasani	4.9	4.38	5.50	0.21	0.27
Katana	1.5	0.71	0.96	0.01	0.01
Mikalayi	5.3	4.50	4.13	0.24	0.22

According to Table 36, the risk for humans sleeping indoors to contract malaria was greatest in Lodja (1.4 infective bites /person/night) and lowest in Kapolowe (0.01 infective bites/person/night). Outdoors, the greatest EIR (1.6 infective bites/person/night) was observed in Lodja and the lowest EIR (0.0 infective bites/person/night) in Kapolowe. In general, humans were at risk in all sites of contracting malaria indoors and outdoors.

TABLE 37: ENTOMOLOGICAL INOCULATION RATE INDOOR/OUTDOOR FOR *AN. GAMBIAE* S.L. IN SENTINEL SITES, 2014

Sentinel Sites	Sporozoite index (SI)	Human bites rates (HBR)		Daily Entomology Inoculation Rates (EIR)	
		Indoor	Outdoor	Indoor	Outdoor
Kabondo	6.8	22.7	12.1	1.54	0.82
Tshikaji	3.9	4.2	5.2	0.16	0.20
Kapolowe	2.5	24.5	20.6	0.61	0.52
Lodja	3.8	8.3	13.4	0.32	0.51
Kingasani	9.9	2.7	9.2	0.27	0.91
Fungurume	0	-	-	-	-
Mikalayi	14.8	1.4	0.6	0.21	0.09

Table 37 shows that the EIR varied from one site to another, indoors and outdoors. The highest value indoor was recorded at Kabondo sentinel site (1.54 infective bites/person/night) and lowest at Tshikaji (0.16 infective bites/person/night). Outdoors, the lowest EIR was observed at Mikalayi with 0.09 infective bites/person/night. The highest was 0.91 infective bites/person/night in Kingasani. The lack of data for Fungurume sentinel site, where IRS was conducted, is because no *An. gambiae* s.l. were captured during the period when the captures were made.

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