PMI VECTORLINK NIGER ANNUAL ENTOMOLOGICAL REPORT
APRIL 2018-MARCH 2019

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Acronyms

CDC	Centers for Disease Control and Prevention
CERMES	Centre de Recherches Medicales et Scientifiques
CSP	Circumsporozoite
DDT	Dichloro-Diphenyl-Trichloroethane
ELISA	Enzyme-Linked Immuno-Sorbent Assay
GPS	Global Positioning System
HBR	Human Biting Rate
HLC	Human Landing Catch
IRD	Indoor Resting Densities
IRM	Insecticide Resistance Monitoring
IRS	Indoor Residual Spraying
KDR	Knock Down Resistance
LLIN	Long Lasting Insecticidal Net
NMCP	National Malaria Control Program
MOP	Malaria Operational Plan
PBO	Piperonyl butoxide
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
RFLP	Restriction Fragment Length Polymorphism
SINE	Short Interspersed Element
USAID	United States Agency for International Development
WHO	World Health Organization

Executive Summary

Entomological monitoring of malaria vectors was conducted in selected sites of Niger, from August 2018 to March 2019 to generate data to support the National Malaria Control Program (NMCP) in making strategic vector control decisions.

Longitudinal vector monitoring using human landing catch (HLC) and pyrethrum spray catch (PSC) was conducted in six districts (Agadez, Gaya, Ingal, Niamey V, Tessaoua, Zindarou) selected by the NMCP. The VectorLink Niger project assessed vector composition, distribution, behavior, sporozoite infection, parity, and entomological inoculation rate (EIR) of malaria vectors. The project also tested the susceptibility of *Anopheles gambiae* s.l. mosquitoes against pyrethroid insecticides, pirimiphos-methyl, bendiocarb and chlorfenapyr using World Health Organization (WHO) susceptibility test kits, and Centers for Disease Control and Prevention (CDC) bottle assays for chlorfenapyr, in nine sites including the six longitudinal vector surveillance sites. When resistance was observed, resistance intensity and synergist effect of piperonyl butoxide (PBO) were also evaluated in the sites where enough larvae were collected.

The results of the vector surveillance undertaken either monthly (Niamey V) or bimonthly (all other sites except Balleyara) showed that *An. gambiae* s.l. was the main and predominant malaria vector species in the country. *An. gambiae* s.l. represented more than 96% (11,113 of 11,520) of the vectors collected throughout the collection period and in all sites. PSCs yielded more vectors than HLCs with 7,849 *An. gambiae* s.l. (across all sites) as compared with than 3,265, respectively.

The biting behavior of *An. gambiae* s.l. was variable across sites with endophilic tendency in Gaya and Tessaoua while more biting occurred outdoors in the other sites. The average peak biting occurred mostly between 11:00 pm and 3:00 am.

Resistance to the three pyrethroids tested (deltamethrin, permethrin and alpha-cypermethrin) was observed in all nine sites. Chlorfenapyr susceptibility was recorded at the dose of 200 µg/bottle in all six sites where the test was completed. The vector also showed resistance to pirimiphos-methyl and bendiocarb in most sites, except in Zindarou and Keita for pirimiphos-methyl and Zindarou for bendiocarb.

At the time of approval of this report, the molecular results were not yet available. The results will be attached to this report as an addendum as soon as they are available.

The data collected within the VectorLink Niger 2018 Work Plan period of performance will support NMCP in strategically deploying vector control interventions in the country.

1. INTRODUCTION

In September 2017, Abt Associates was awarded a five-year Task Order, the U.S. President's Malaria Initiative (PMI) VectorLink Project, to support PMI, as well as the United States Agency for International Development (USAID) Missions, Country Offices, and Bureaus with malaria programs, in planning and implementing vector control programs with the overall goal of reducing the burden of malaria in Africa.

Malaria is endemic in Niger and is the leading cause of death and morbidity combined, disproportionately affecting children under five years of age[1]. According to Niger's Annual Health Statistic Report (2017), there were over 3,021,595 malaria cases, and 3,021 malaria deaths in 2017, putting it among the countries with the highest per capita rate of malaria fatalities globally.

There are three malaria endemicity zones in Niger: hypo-endemic, meso-endemic and hyper-endemic. According to the PMI FY2018 Niger Malaria Operational Plan (MOP), the vast majority of Niger's population (94%) lives in the two southernmost (meso-endemic and hyper-endemic) zones where malaria is most prevalent. The rainy season in Niger lasts about three to four months, from June to September, with peak malaria transmission during the second half (August-September).

In 2018, PMI VectorLink project conducted entomological monitoring activities to support the country to establish baseline data in anticipation of future expanded insecticide-based vector control activities. Comprehensive vector bionomics and resistance monitoring, paired with health facility-based malaria incidence data and population density will help generate a robust foundation of data for decision making as part of the integrated vector control strategy in future years.

[1] PMI Niger Malaria Operational Plan FY 2017

2. METHODOLOGY

2.1 ENTOMOLOGICAL MONITORING SITES

Five longitudinal monitoring sites (Niamey V, Gaya, Tessaoua, Agadez and Balleyara) spread across the three endemicity zones, were proposed in the 2018 Work Plan for bimonthly bionomic data collection. In October 2018, after the first round of collections had been conducted in the five original sites, Balleyera was replaced with Ingal and Zindarou, per NMCP, Centre de Recherches Medicales et Scientifiques (CERMES), and PMI's recommendation (Figure 1).

An additional three sites (Keita, Tchintabaraden, and Zinder) were selected for annual insecticide resistance monitoring only (Figure 1).

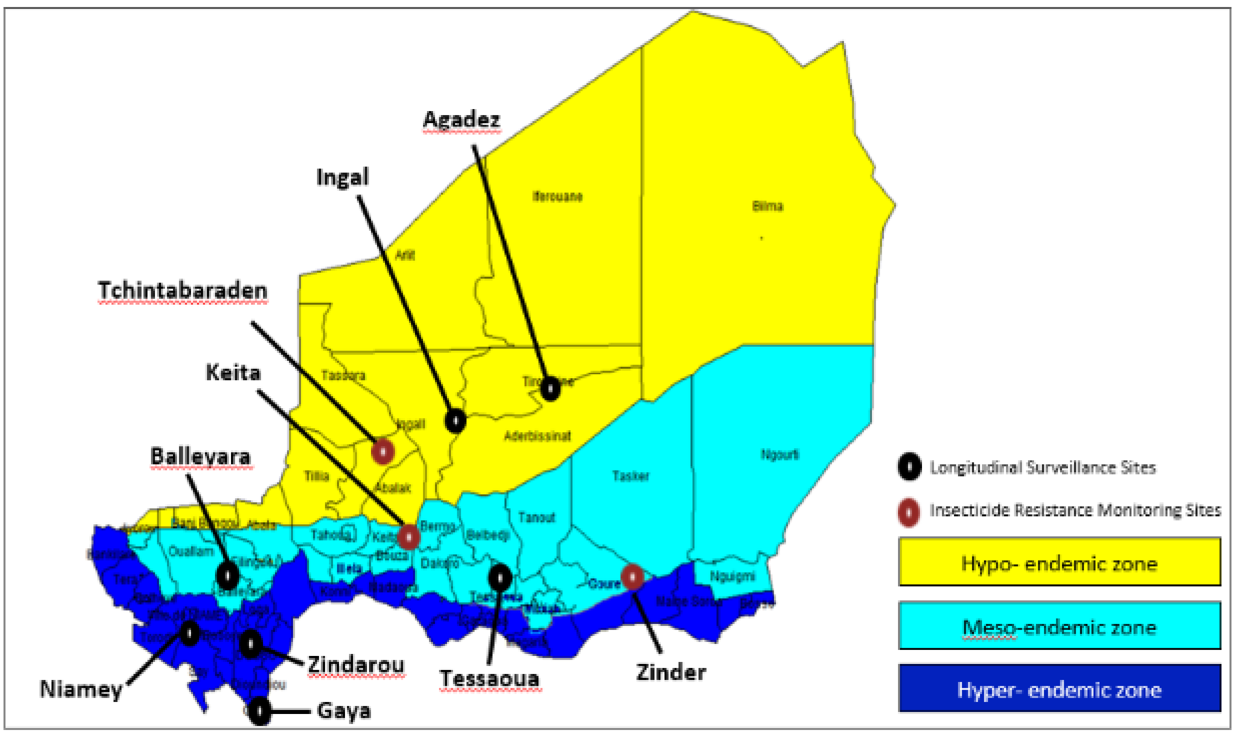


Figure 1: Map of PMI VectorLink entomological monitoring sites in Niger

2.2 VECTOR BIONOMICS MONITORING

Adult mosquito collections were done using human landing catches (HLCs) and pyrethrum spray catches (PSCs). HLCs were done in two houses for two nights per site per collection period (monthly or bimonthly) in the same houses. HLCs were performed indoors and outdoors to collect adult mosquitoes landing on human baits. With legs exposed up to the knees to attract host-seeking mosquitoes, one pair of mosquito collectors (human bait) were seated indoors and outdoors from

6:00 pm to 00:00 am and a second pair from 00:00 am to 6:00 am. The collectors switched between indoors and outdoors on an hourly basis. The collectors used flashlights and hemolysis tubes to collect mosquitoes that landed on their legs before the mosquitoes could bite. The tubes were covered with cotton after individual collection of mosquitoes. The teams transferred the mosquitoes hourly to custom-made bags for a total of 12 hours.

The PSCs were conducted in ten houses per collection period. The same houses were sampled during each collection period. The PSCs were carried out during morning hours, between 6:00 am and 8:00 am. White cloth/sheets were placed on the floor from wall to wall in sampled rooms. The rooms were sprayed with the commercial pyrethroid + PBO insecticide after closing windows and doors and covering or removing any drinking water and food items. For houses with open eaves, collectors sprayed from outside through the eaves before entering and spraying indoors. Ten minutes after spraying, all mosquitoes knocked down by the chemical were collected from the white sheets. The mosquitoes were kept in petri dishes and then sorted by species using an identification key. The abdominal status of all female anophelines was determined, and individuals were sorted into four categories: unfed, blood-fed, half-gravid, and gravid. The collection methods and times are shown in Table 1.

Table 1: Longitudinal monitoring collection methods

Collection method	Time	Frequency	Sample
HLC	6:00 pm to 6:00 am	Two nights per site	Two houses per site
PSC	6:00 am to 8:00 am	One day per site	Ten houses per site

All mosquitoes collected with both methods were morphologically identified by genus and species or species complex using a binocular microscope and identification keys (Gillies, M.T. & Coetzee, M. 1987). The identification was done by a team of trained technicians from CERMES and NMCP. A subsample of *An. gambiae* s.l. from each site was dissected for parity rate estimation. All mosquitoes were preserved on silica gel in Eppendorf tubes for further laboratory processing to identify sibling species, resistance mechanisms, infection status, and blood meal source using Polymerase Chain Reaction (PCR) and Enzyme-Linked Immunosorbent Assay (ELISA).

The indicators listed in Table 2 were calculated based on the number of mosquitoes collected through each collection method.

Table 2: Vector surveillance indicators by collection method

Collection Method	Indicator	Unit of Measure
HLC	Human Biting Rate	# bites / person / night
	Peak biting time	Hour of highest bites
	Parity Rate	Percentage of parous mosquitoes (out of total dissected)
	Exophagic Rate	Percentage of mosquitoes biting outside
	Endophagic Rate	Percentage of mosquitoes biting inside
PSC	Indoor Resting Density	# mosquitoes / house / day
	% of fed females	Percentage of fed mosquitoes (out of total collected by PSC)

2.3 INSECTICIDE RESISTANCE MONITORING

The field teams visited each site for larval collections and insecticide susceptibility tests. *An. gambiae* s.l. larvae and pupae were collected from different larval habitats across each site using the dipping method. Collected larvae and pupae were pooled and reared to adults in the field laboratory. The adult mosquitoes were kept under controlled conditions (25 ºC ± 2 ºC and 70% ± 10% humidity) and fed with 10% sugar solution soaked in cotton. The global positioning system (GPS) coordinates of the larval collection sites were recorded for the geo mapping of the larval breeding sites and distribution in each locality.

Insecticide susceptibility testing was conducted using World Health Organization (WHO) tube tests on 2-5-day old adult female *An. gambiae* s.l. reared from larvae collections. The mosquitoes were exposed to the diagnostic doses of each insecticide for one hour and the percent mortality was recorded after 24 hours post-exposure. Key findings include:

The diagnostic concentrations of deltamethrin (0.05%), permethrin (0.75%), alpha-cypermethrin (0.05%), bendiocarb (0.1%) and pirimiphos-methyl (0.25%) were tested on site. The resistance status was determined following WHO criteria with < 90% as confirmed resistance, 90% - 97% as possible resistance, and ≥98% as susceptible (WHO, 2016).

Synergist assays with piperonyl butoxide (PBO) were conducted for deltamethrin, permethrin and alpha-cypermethrin according to the WHO susceptibility test protocol to determine the involvement of P450s in any pyrethroid resistance detected in a site, and to assess whether PBO long lasting insecticidal nets (LLINs) would be an effective vector control option in the country. One-hour pre-exposure of the mosquitoes to PBO 4% was done before exposure to each pyrethroid insecticide and mortality was recorded 24 hours post-exposure. A high increment of the mortality after pre-exposure to PBO represents an involvement of enzyme activities such as P450s in the insecticide resistance of the population tested.

Resistance intensity at 5x and 10x the diagnostic concentration of deltamethrin, permethrin, alpha-cypermethrin and pirimiphos-methyl were also tested and the intensity of the resistance was defined following the WHO criteria of high, moderate or low intensity (WHO, 2016).

CDC bottle assays were used to test resistance to chlorfenapyr at the doses of 100µg/bottle and 200µg/bottle. Testing was done following the protocol of Brogdon et al. (2010) but exposure was completed for one hour and mortality recorded up to 72 hours.

2.4 MOLECULAR CHARACTERIZATION

Insecticide resistance in mosquitoes can be related to target site mutations. Among them, resistance to pyrethroids and dichloro-diphenyl-trichloroethane (DDT) is described as a substitution of the amino acid leucine to either phenylalanine (L1014F, referred as *kdr* -West) or serine (L1014S, referred as *kdr*-East) at the position 1014 in the sodium channel gate. For organophosphate and carbamate insecticide, target site mechanism, known as *ace-1* is a substitution of an amino acid glycine with serine at position 119.

About 50 randomly selected *An. gambiae* s.l. mosquitoes among the dead per site and the surviving mosquitoes from the WHO susceptibility tests (all insecticides) were further analyzed to identify species and assess molecular markers of insecticide resistance. The DNA of each individual mosquito was extracted using the protocol designed by Collins et al. (1987). The presence of *kdr*-West and East was characterized using the conventional Polymerase Chain Reaction (PCR) restriction fragment length polymorphism (RFLP) method described by Matinez-Torres et al. (1999).

A subsample of *An. gambiae* s.l. collected by HLC and PSC from all the vector surveillance sites were also identified to the species level. The *An. gambiae*, *An. coluzzii*, and hybrids of both species were analyzed following the Short Interspersed Element (SINE) protocol described by Santolamazza et al. (2008).

The sporozoite infection of mosquitoes collected using HLC was determined using PCR and blood meal source of mosquitoes collected through PSC was analyzed using Enzyme-Linked Immunosorbent Assay (ELISA).

3. RESULTS

3.1 VECTOR BIONOMICS

The original work plan approved by PMI in June 2018 included five longitudinal monitoring sites (Agadez, Tessaoua, Balleyara, Niamey V, and Gaya). Each site had a specific collection schedule to ensure sufficient data could be collected to monitor trends in each endemicity zone. In September 2018, the NMCP requested that Balleyara be replaced by Ingal and Zindarou. Therefore, the PMI VectorLink Niger project completed , one round of monitoring in Balleyara, two in Zindarou and Ingal, three in Gaya, four in Agadez and Tessaoua, and six in Niamey V (Table 3).

Table 3: Adult mosquito collections completed in the PMI VectorLink Niger 2018 Work Plan period of performance (April 2018 – March 2019)

	2018					2019		
	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
Agadez	X		X		X		X	
Ingal*			X		X		X	
Tessaoua	X		X		X		X	
Balleyara*	X							
Zindarou*			X		X			
Niamey V	X		X		X	X	X	X
Gaya	X		X		X			

* Collections were completed in Balleyara in August 2018 per the approved work plan prior to modifying the sites (dropping Balleyara and adding Ingal and Zindarou) in October 2018 per NMCP request.

3.1.1 SPECIES COMPOSITION

A total of 11,520 *Anopheles* were collected across seven sites. *An. gambiae* s.l. was most abundant (96.5%; n=11,113) followed by *An. funestus* (1.7%; n= 200) and, *An. rufipes* (1.5%; n=175), *An. pharoousis An. nili,* and *An. squamosus*, all combined made up less than 0.5% of the total collection.

Of the 11,113 *An. gambiae* s.l. collected, 3,265 (29.4%) were collected by HLC across all sites from three rounds of monitoring in Gaya, one in Balleyara, two round in Zindarou and Ingal, four round in Agadez and Tessaoua, and six round in Niamey V. *An. gambiae* s.l was the only *Anopheles* mosquito collected in Agadez (n=16), Tessaoua (n=184), Balleyara (n=370), and Ingal, (n=5). The overall highest densities of *An. gambiae* s.l. using HLC method were recorded in Gaya (n=1,526) and Niamey V (n=892) (Figure 2).

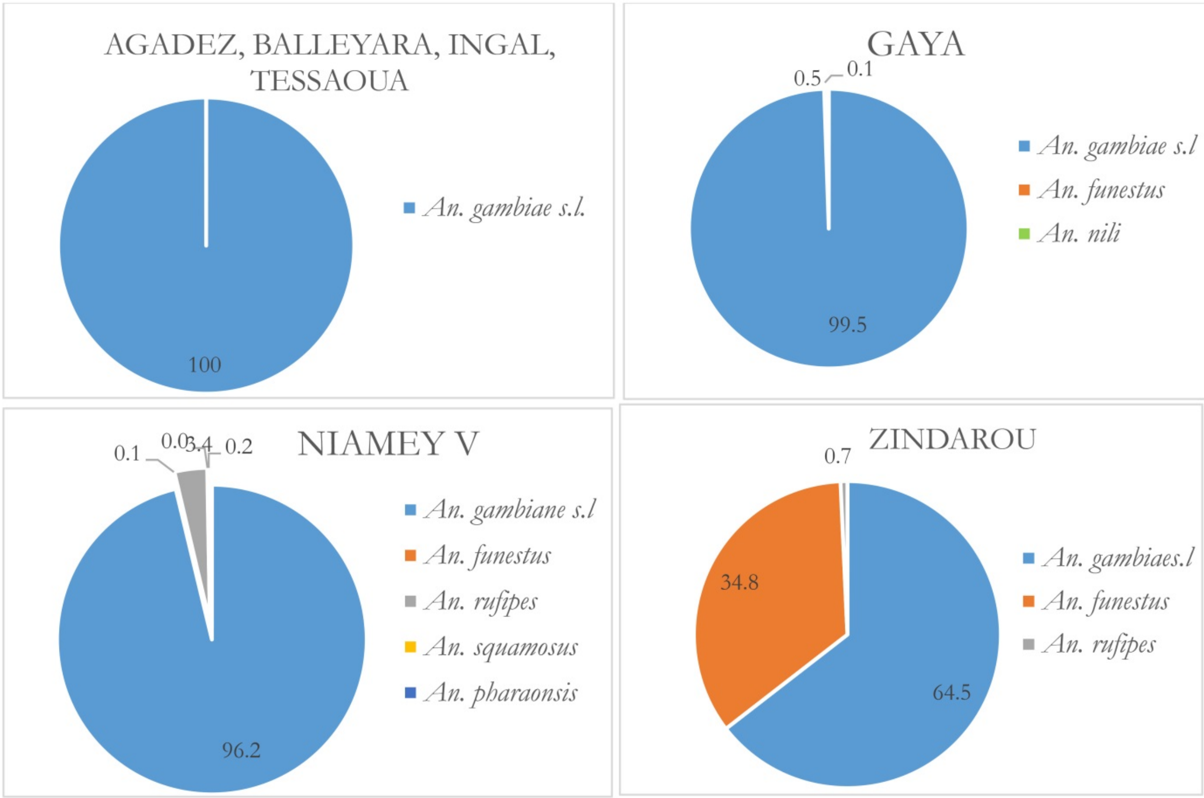
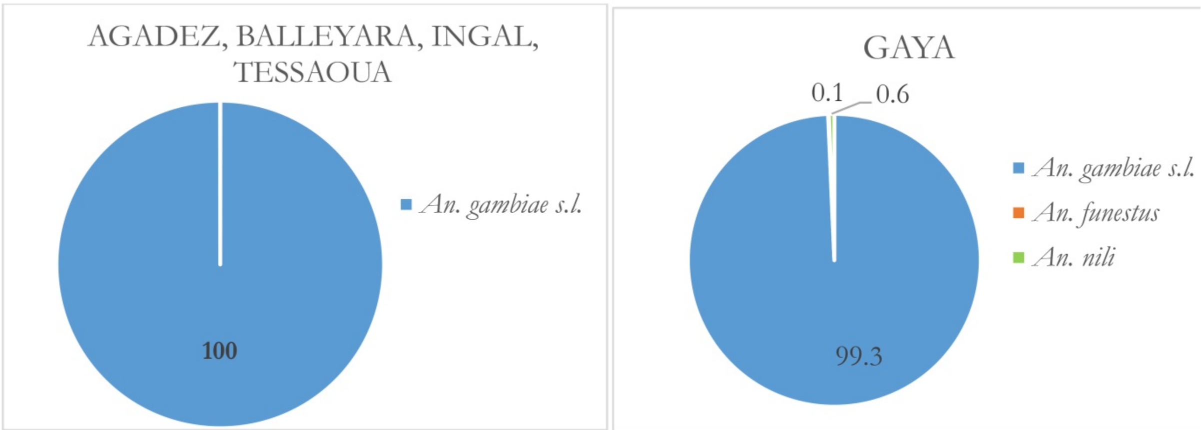


Figure 2: Species composition of mosquitoes collected by HLC

Of the 7849 *An. gambiae* s.l. collected by PSC, the majority were from Niamey V (49.9 %; n= 3918), followed by Gaya (33.8%, n=2654) then Balleyara (7.6 %; n= 596) where only a single collection was done and Tessaoua (6, 1%; n=478). In the two other sites the *An. gambiae* s.l. collected represented less than 2% (Figure 3).

Also, all anopheles collected from Agadez, balleyara, Ingal and Tessaoua were *An. gambiae* s.l., while 99.3% and 95.6% of the anopheles of Gaya and Niamey V respectively were *An. gambiae* s.l. Zindarou recorded the lowest percentage with 45.3% of *An. gambiae* s.l. and 52.6% of *An. funestus*.



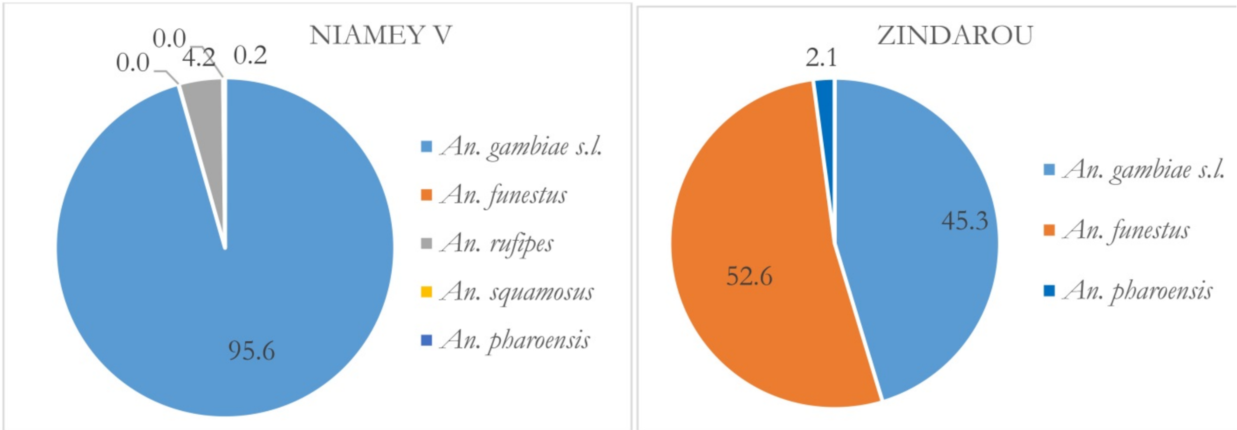
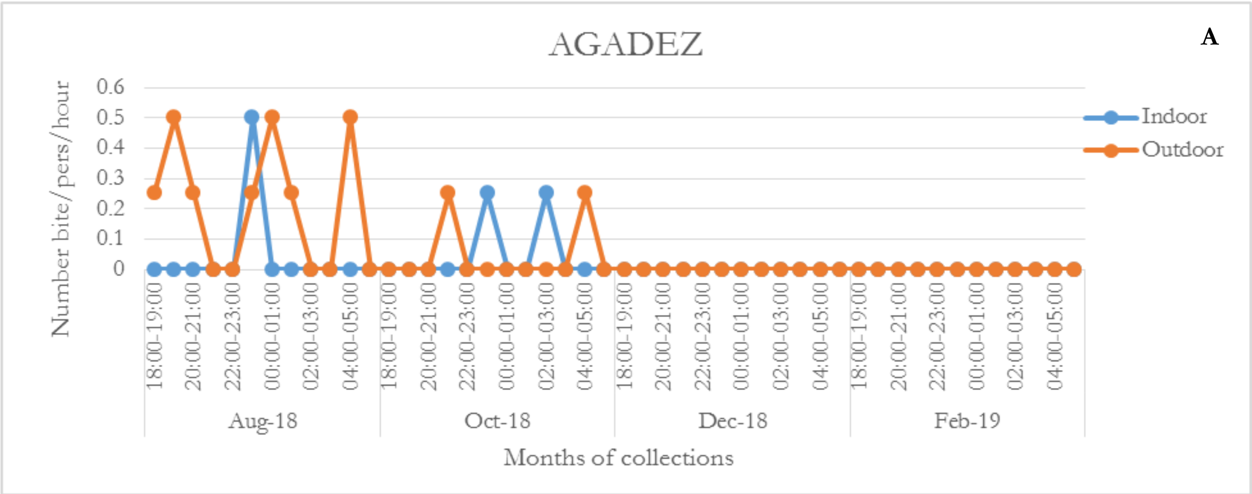


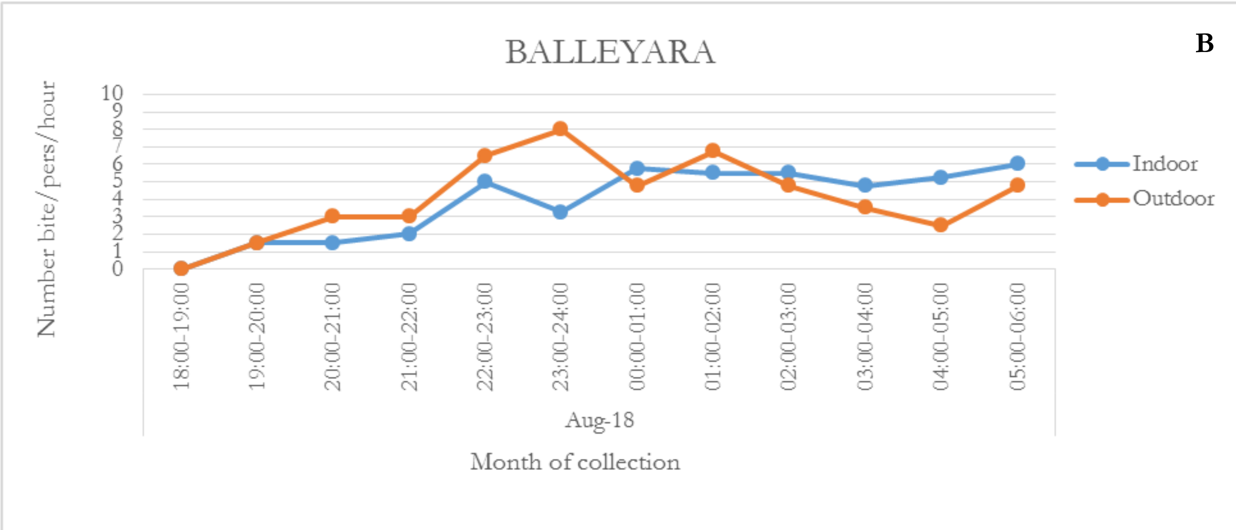
Figure 3: Species composition of mosquitoes collected by PSC

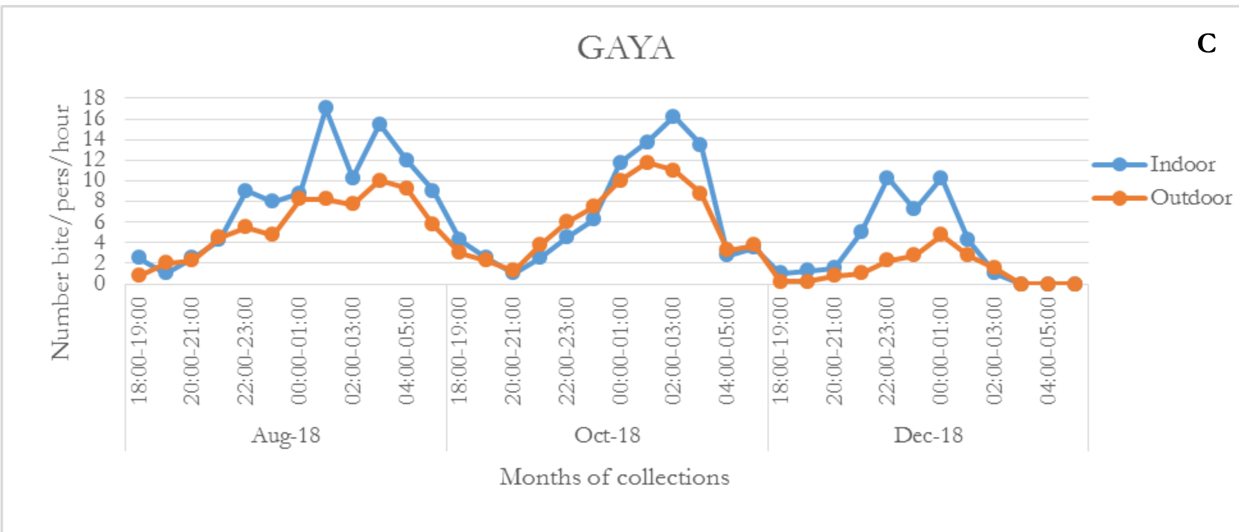
3.1.2 BITING BEHAVIOR OF *AN. GAMBIAE S.L.*

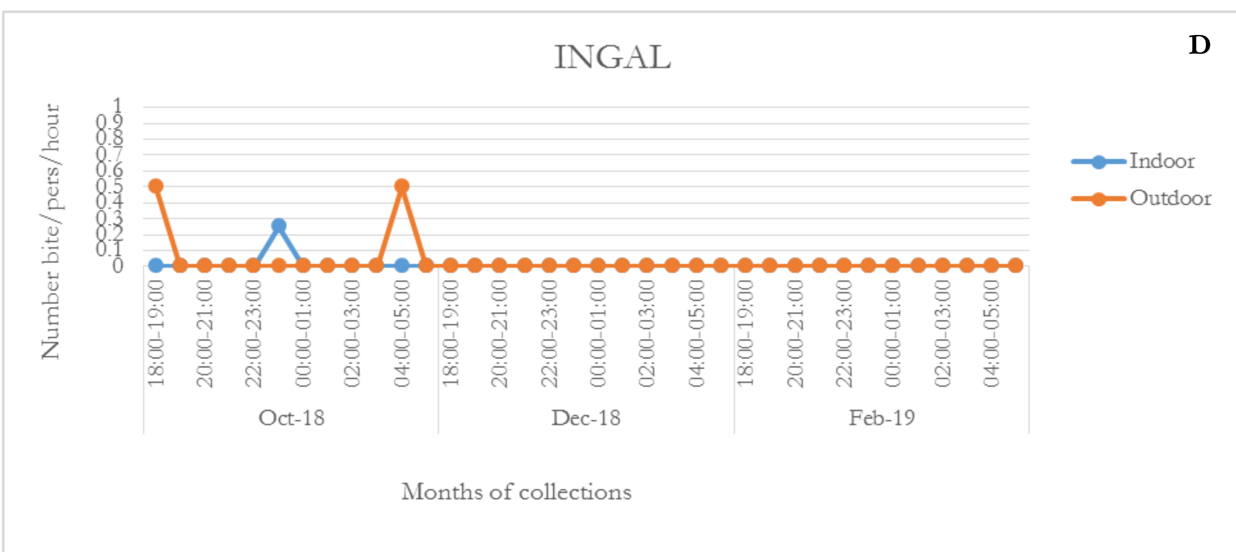
An. gambiae s.l. showed variable biting behavior across the seven districts. The densities of *An. gambiae* s.l. were overall higher outdoors in Ingal (80.0%), Agadez (75.0%), Niamey V (52.7%) Zindarou (52.7%) and Balleyara (52.0%) as compared to indoors. On the other hand, *An. gambiae* s.l. was generally endophilic in Gaya (58.7%) and Tessaoua (55.7%) (Figures 4a-g).

Overall, *An. gambiae* s.l. females biting activity was highest between 10:00 pm and 03:00 am, indoors and outdoors across all sites. In Agadez and Balleyara, the biting rates peaked between 10:00 pm and 12:00 am indoors, and 12:00 am and 2:00 am outdoors. In Gaya and Niamey V, peak biting times were between 11:00 pm and 3:00 am for both indoors and outdoors. Ingal recorded a peak biting time around 4:00 am outdoors and 11:00 pm indoors.




BALLEYARA
B
Number bite/pers/hour
10
9
8
7
6
5
4
3
2
1
0
18:00-19:00
19:00-20:00
20:00-21:00
21:00-22:00
22:00-23:00
23:00-24:00
00:00-01:00
01:00-02:00
02:00-03:00
03:00-04:00
04:00-05:00
05:00-06:00
Aug-18
Month of collection
Indoor
Outdoor


GAYA
C
Number bite/pers/hour
18
16
14
12
10
8
6
4
2
0
18:00-19:00
20:00-21:00
22:00-23:00
00:00-01:00
02:00-03:00
04:00-05:00
Aug-18
Oct-18
Dec-18
Months of collections
Indoor
Outdoor


INGAL
D
Number bite/pers/hour
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
18:00-19:00
20:00-21:00
22:00-23:00
00:00-01:00
02:00-03:00
04:00-05:00
Oct-18
Dec-18
Feb-19
Months of collections
Indoor
Outdoor

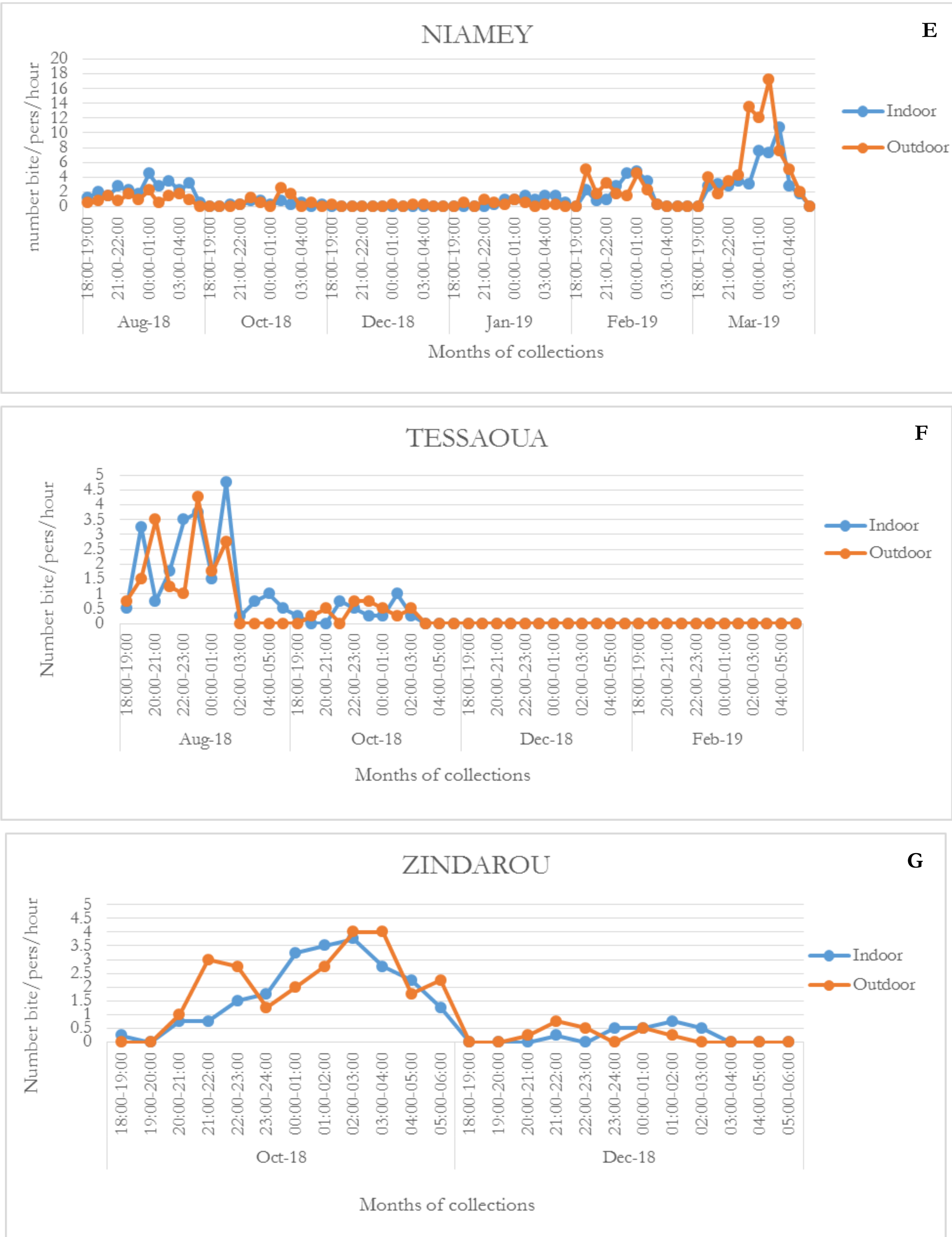


Figure 4: Biting rate of *An. gambiae* s.l. collected using HLC in seven sites (A: Agadez; B: Balleyara; C: Gaya; D: Ingall; E: Niamey V; F: Tessaoua; G: Zindarou)

3.1.3 Human Biting Rate of *An. gambiae s.l.* over HLC collection

During the high transmission season, from August to December, Gaya recorded the highest indoor human biting rate (HBR) with an overall rate of 99.3; 87.3 and 41.8 bites per person per night (b/p/n) in August, October and December 2018, respectively, followed by Balleyara (99 b/p/n in August) , and Tessaoua (59.8 b/p/n in August). Agadez (0.5 b/p/n) and Ingall (0.25 b/p/n) recorded the lowest HBR during the same period.

The outdoor biting rate was the highest in Balleyara in August (87.3 b/p/n), followed by Gaya (71.8 b/p/n) and Tessaoua (61 b/p/n) in the same month (Figure 5).

The overall HBR of *An. gambiae* s.l. recorded from December 2018 to March 2019 showed that only Niamey V yielded a higher rate in March 2019 indoor (45 b/p/n) and outdoor (71 b/ p/n). No *An. gambiae* s.l. wwere collected in any other sites (Agadez, Ingal and Tessaoua) where vector surveillance was conducted from December 2018 to March 2019.

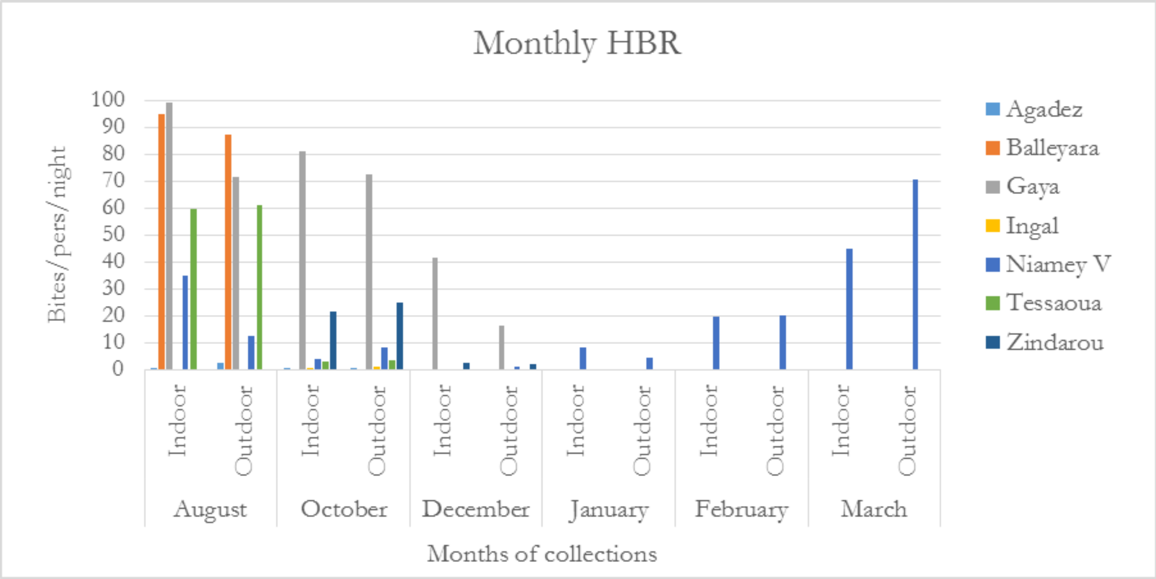


Figure 5: *An. gambiae* s.l. indoor and outdoor biting rates by site (August 2018 to March 2019)

3.1.4 Parity Rates

Parity rates were high in all the sites across collection period, though the numbers were relatively low in most of the sites except Gaya and Niamey V. All the mean parity rates were above 50% in all sites. (Table 4).

Table 4: Parity rate of An. gambiae s.l. by site (August 2018 – March 2019)

		Aug-18	Oct-18	Dec-18	Jan-19	Feb-19	Mar-19
AGADEZ	Total Collected	12	4	0		0	
	Total dissected	12	4	0		0	
	# Parous	10	4	0		0	
	% Parity	**83.3**	**100.0**	**0.0**		**0.0**	
BALLEYARA	Total Collected	370					
	Total dissected	101					
	# Parous	71					
	% Parity	**70.3**					
GAYA	Total Collected	675	619	232			
	Total dissected	184	292	144			
	# Parous	88	215	90			
	% Parity	**47.8**	**73.6**	**62.5**			
INGAL	Total Collected		5	0		0	
	Total dissected		5	0		0	
	# Parous		4	0		0	
	% Parity		**80.0**	**0.0**		**0.0**	
NIAMEY	Total Collected	166	59	4	50	160	463
	Total dissected	160	47	4	43	67	203
	# Parous	84	17	3	25	49	134
	% Parity	**52.5**	**36.2**	**75.0**	**58.1**	**73.1**	**66.0**
TESSAOUA	Total Collected	156	49	0		0	
	Total dissected	116	44	0		0	
	# Parous	74	30	0		0	
	% Parity	**63.8**	**68.2**	**0.0**		**0.0**	
ZINDAROU	Total Collected		177	19			
	Total dissected		99	19			
	# Parous		46	15			
	% Parity		**46.5**	**78.9**			

3.1.5 Indoor Resting Density

The overall mean indoor resting density of the vector was calculated using the number of *An. gambiae* s.l. collected by PSC in 10 houses per site throughout the collection period. The mean monthly density was highest in Niamey V in August, February, and March with an average of 96; 120 and 158 *An. gambiae* s.l. per house respectively, compared to the six other sites. Highest densities were recorded in August in all sites but, exceptionally, Niamey V recorded peak densities in February and March as well. Low or no density was found in Agadez, Tessaoua and Ingall between October and February (Figure 6).

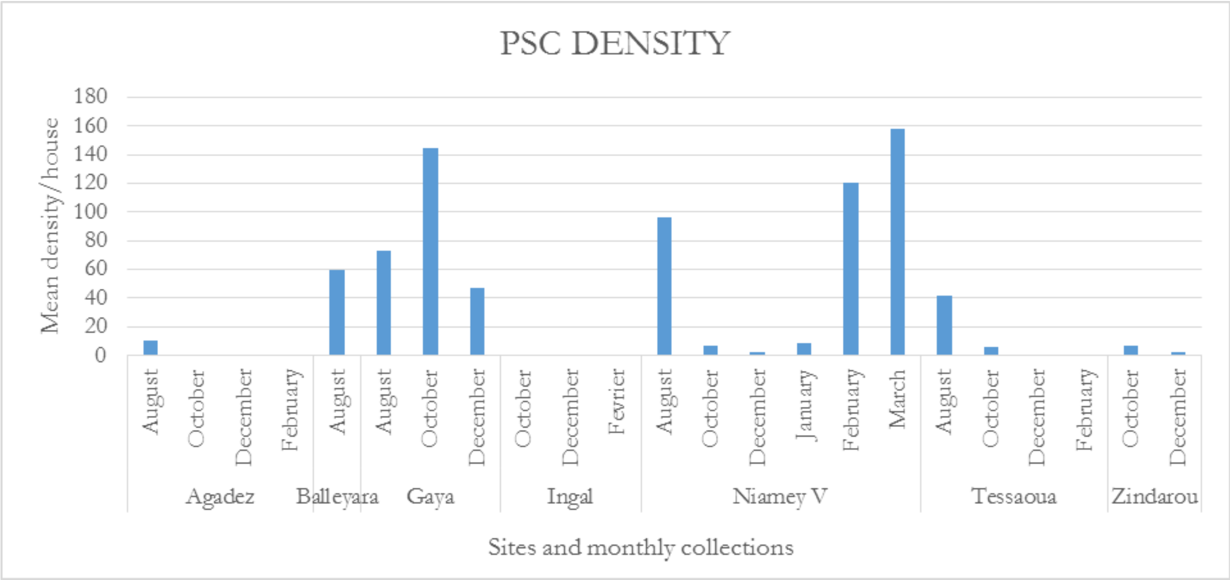


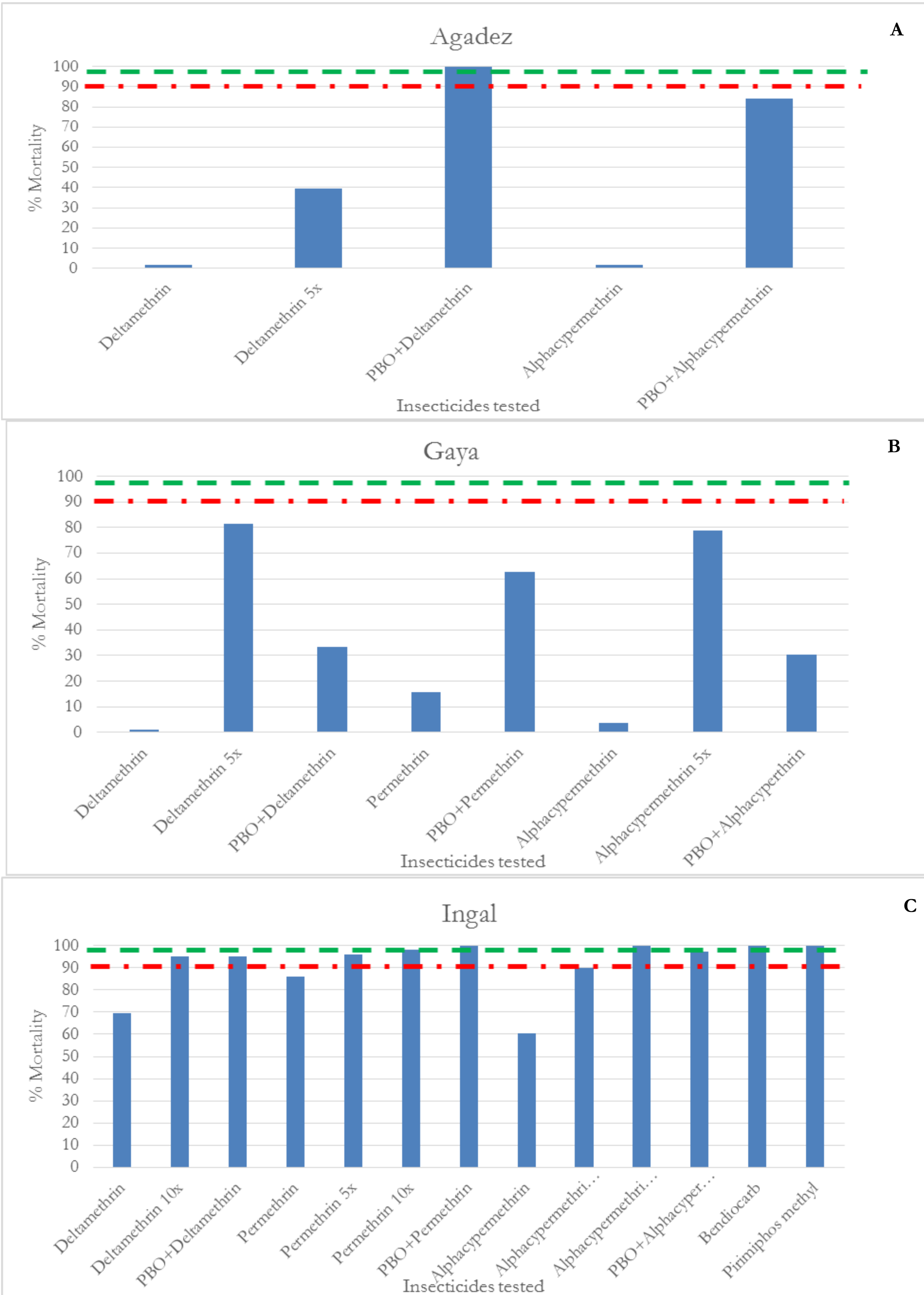
Figure 6: Mean density of *An. gambiae* s.l. per house per site using PSC (August 2018 – March 2019)

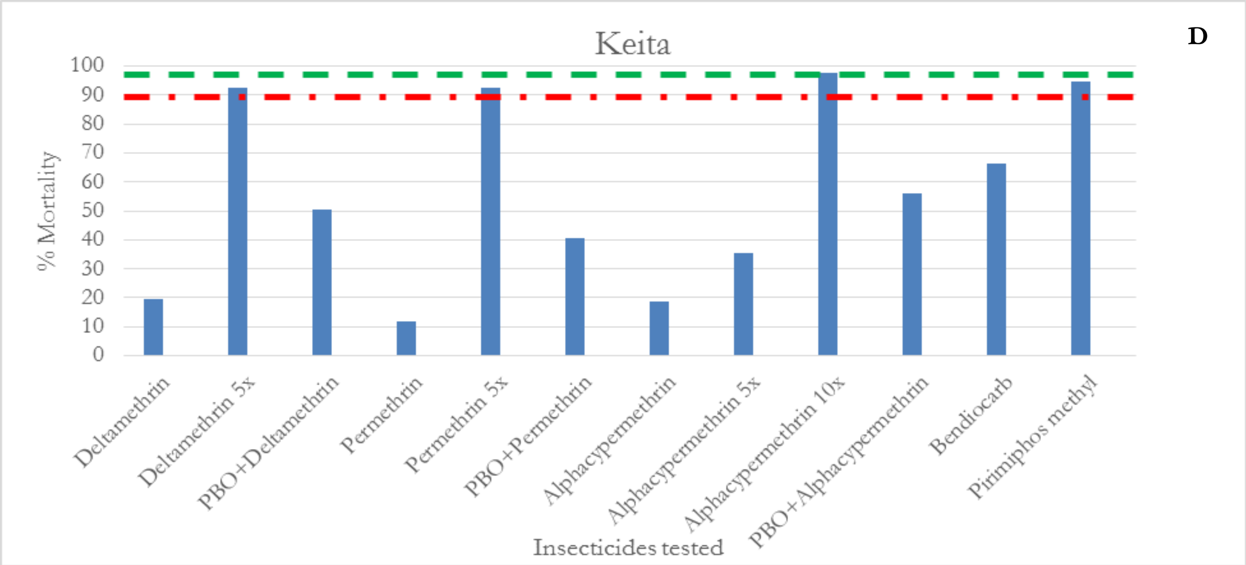
3.2 Insecticide Resistance

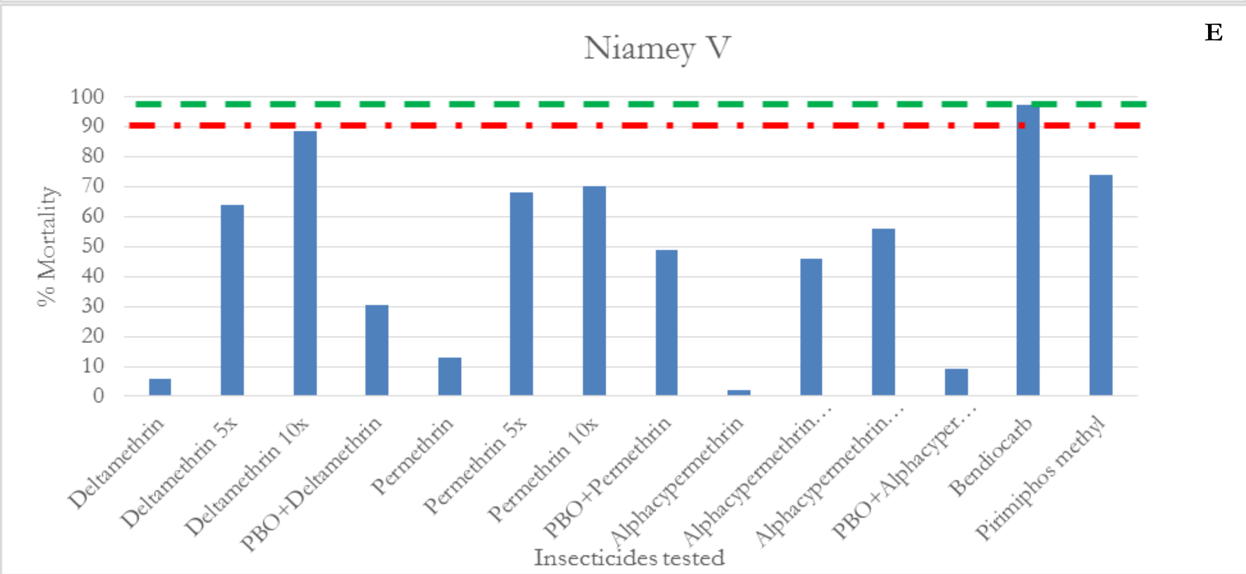
Figures 7a-i illustrate the resistance profile to the different insecticides against which *An. gambiae* s.l. collected from eight different sites were tested. For all the figures, the horizontal dashed red line represents the 90% threshold for resistance and the green line represents the 98% threshold for susceptibility.

All insecticides were tested in Niamey V, Tchintabaraden and Ingal, but not all insecticides could be tested in the remaining six sites due to the limited number of collected mosquito larvae. Detailed results are presented in Table A-1 of the Annex. Key findings include:

- Resistance was observed to the diagnostic dose of all pyrethroids in all sites. Pirimiphos-methyl and bendiocarb showed susceptibility in Ingal and Zindarou, but resistance to both insecticides was observed in Niamey V and Tchintabaraden. The remaining sites did not yield sufficient larvae to test pirimiphos-methyl and bendiocarb.
- Further testing revealed a high intensity of resistance to deltamethrin, permethrin and alpha-cypermethrin in the sites where the full set of tests was completed. Only *An. gambiae* s.l. from Keita showed low resistance intensity to deltamethrin and permethrin (100% mortality at 5x the diagnostic dose).
- The pre-exposure of mosquitoes to PBO before deltamethrin, permethrin and alpha-cypermethrin yielded significant increased mortality in most of the sites surveyed ($p>0.05$).


Agadez
A
% Mortality
100
90
80
70
60
50
40
30
20
10
0
Deltamethrin
Deltamethrin 5x
PBO+Deltamethrin
Alphacypermethrin
PBO+Alphacypermethrin
Insecticides tested
Gaya
B
% Mortality
100
90
80
70
60
50
40
30
20
10
0
Deltamethrin
Deltamethrin 5x
PBO+Deltamethrin
Permethrin
PBO+Permethrin
Alphacypermethrin
Alphacypermethrin 5x
PBO+Alphacyperthrin
Insecticides tested
Ingal
C
% Mortality
100
90
80
70
60
50
40
30
20
10
0
Deltamethrin
Deltamethrin 10x
PBO+Deltamethrin
Permethrin
Permethrin 5x
Permethrin 10x
PBO+Permethrin
Alphacypermethrin
Alphacypermethri…
Alphacypermethri…
PBO+Alphacyper…
Bendiocarb
Pirimiphos methyl
Insecticides tested


Keita
D
% Mortality
100
90
80
70
60
50
40
30
20
10
0
Deltamethrin
Deltamethrin 5x
PBO+Deltamethrin
Permethrin
Permethrin 5x
PBO+Permethrin
Alphacypermethrin
Alphacypermethrin 5x
Alphacypermethrin 10x
PBO+Alphacypermethrin
Bendiocarb
Pirimiphos methyl
Insecticides tested


Niamey V
E
% Mortality
100
90
80
70
60
50
40
30
20
10
0
Deltamethrin
Deltamethrin 5x
Deltamethrin 10x
PBO+Deltamethrin
Permethrin
Permethrin 5x
Permethrin 10x
PBO+Permethrin
Alphacypermethrin
Alphacypermethrin…
Alphacypermethrin…
PBO+Alphacyper…
Bendiocarb
Pirimiphos methyl
Insecticides tested

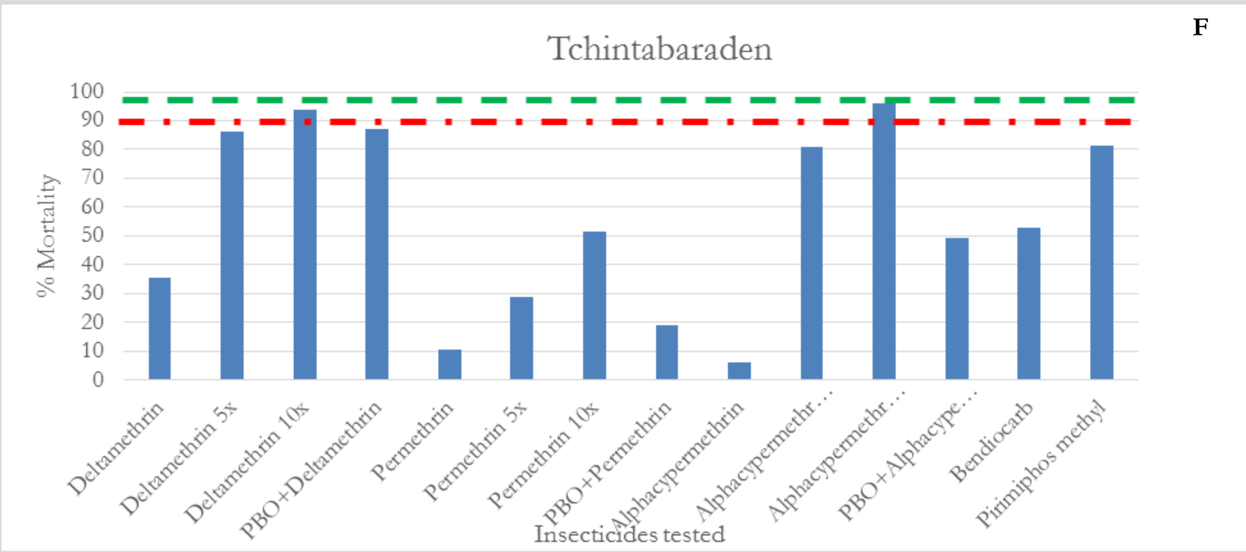

Tchintabaraden
F
% Mortality
100
90
80
70
60
50
40
30
20
10
0
Deltamethrin
Deltamethrin 5x
Deltamethrin 10x
PBO+Deltamethrin
Permethrin
Permethrin 5x
Permethrin 10x
PBO+Permethrin
Alphacypermethrin
Alphacypermethr…
Alphacypermethr…
PBO+Alphacype…
Bendiocarb
Pirimiphos methyl
Insecticides tested

Figure 7: Insecticide resistance status of *An. gambiae* s.l. from sentinel sites (A: Agadez; B: Gaya; C: Ingall; D: Keita; E: Niamey V; F: Tchintabaraden; G: Tessaoua; H: Zindarou/Boboye; I: Zinder)

The results of the CDC bottle assays using chlorfenapyr are shown in the figures 8 and 9 and Table A-2 of the Annex. Six sites out of nine were tested and susceptibility was recorded in all sites after 72 hours post-exposure at the dose of 200ug/bottle. For all the figures, the horizontal dashed red line represents the 90% threshold for resistance and the green line represents the 98% threshold for susceptibility. The Abbott formula was used to correct the observed mortality when the control mortality was > 5% and < 20%.

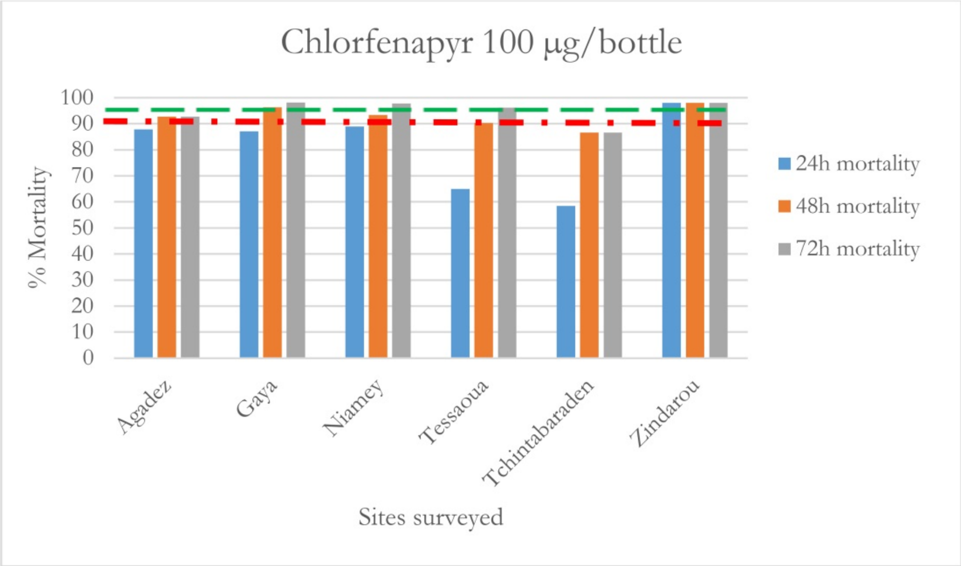


Figure 8: Susceptibility of *An. gambiae* s.l. to chlorfenapyr 100µg/bottle

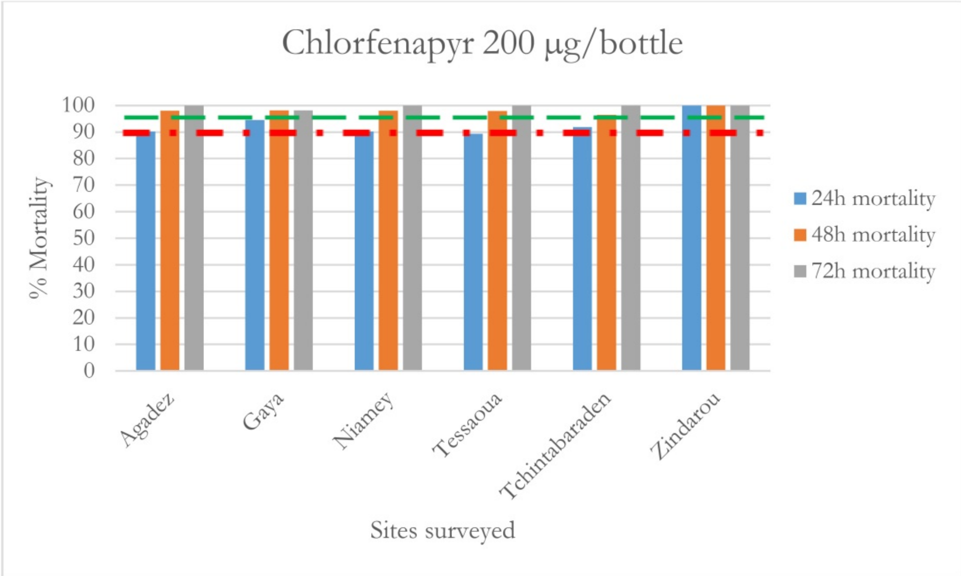


Figure 9: Susceptibility of *An. gambiae* s.l. to chlorfenapyr 200µg/bottle

3.3 MOLECULAR CHARACTERIZATION

At the time of submission of this report, the molecular analyses are underway at CERMES. They will be inserted into this report as soon as they are available.

4. Conclusion

Bionomics data collected during the first work plan year of the PMI VectorLink Niger project showed that *An. gambiae* s.l. was the predominant malaria vector in all seven sites using both HLC and PSC methods.

Out of the 11,520 anopheles collected, PSC produced the highest numbers (n=7849) and particularly, Niamey V recorded the highest density among the sites followed by Gaya. Both sites are irrigated rice field areas and the availability of favorable and permanent larvae habitats has contributed to the large number of vectors collected. The geographical position of Niamey V has also contributed to the monthly collection undertaken specifically for this site compared to the others. However, the absence of vectors observed in all the others sites after December should be considered when planning of the collection periods for each site in future years. Niger has a long dry season covering the period of November to June and very short rainy season of a maximum of four months from July to October during which the highest malaria incidence occurs. Therefore, vector surveillance should be planned in these specific sites mostly during the rainy season for obtaining enough vectors for further analysis and decision making.

The results of HLCs also showed that the *An. gambiae* s.l. biting rate was higher in Gaya during the rainy season both outdoors and indoors followed by Tessaoua and Balleyara. Exceptionally, Niamey V recorded higher biting rates in the dry season (February and March). The rainfall in Niamey V and the presence of rivers has likely contributed to the perennially higher densities and also the presence of several other vectors such as *An. rufipes* and *An. funestus*. Niamey V experienced flooding in January of 2019, which may also have contributed to the presence and increased density of malaria vectors in February and March. Such a phenomenon could cause malaria epidemic in the area. Therefore, the current data could help NMCP to better prepare for unseasonable outbreaks of malaria and/or others mosquito borne diseases.

Anopheles gambiae s.l. was resistant to all three pyrethroids tested (deltamethrin, permethrin and alpha-cypermethrin) in all sites and high resistance was recorded in almost all the sites where the tests were conducted, except Keita. Though pre-exposure to PBO could not restore full susceptibility in all sites, except with permethrin and alpha-cypermethrin in Zinder, significant increment of mortality was recorded. These data collected for the first time in the country will inform NMCP's approach for insecticide-treated net procurement and distribution. The benefit of the pre-exposure to PBO, which was observed, could support PBO net distribution and stratification in the country, especially in the areas of high malaria incidence. Furthermore, susceptibility observed to chlorfenapyr could support the contribution to control resistant vectors by using second generation nets.

The susceptibility testing of pirimiphos-methyl was completed in five of the nine sentinel sites and showed resistance of *An. gambiae* s.l. in three of the sites and suspected resistance in one site. Only two sites recorded full susceptibility. This is a cause for concern as the country develops a vector control strategy including indoor residual spraying (IRS). Additional data is need to be collected to confirm the trend that was observed in the situation where IRS could be an option for controlling malaria vectors during the short rainy season with high malaria incidence.

5. References

Brogdon W, Chan A (2010). Guidelines for Evaluating Insecticide Resistance in Vectors using the CDC Bottle Bioassay/ Methods in anopheles research. Second edition. CDC Atlanta USA: CDC technical report. P 343.

Gillies, M.T. and Coetzee, M. (1987) A Supplement to the Anophelinae of Africa South of the Sahara. South African Institute for Medical Research, 55, 1-143.

Martinez-Torres D, Chevillon C, Brun-Barale A, Berge J, Pasteur Na, Pauron D: Voltage dependent Na^+ channel in pyrethroid resistant *Culex pipens* L mosquitoes. *Pesticide Science* 1999, 55(10):1012-1020.

Santolamazza F, Mancini E, Simard F, Qi Y, Tu Z, della Torre A. Insertion polymorphisms of SINE200 retrotransposons within speciation islands of *Anopheles gambiae* molecular forms. Malar J. 2008; 7:163

WHO (2016): Test procedures for insecticide resistance monitoring in malaria vector mosquitoes – 2nd ed ISBN 978 92 4 151157 5

6. ANNEX

Table A-1: WHO susceptibility test results, expressed as percent mortality 24 hours after 60 min exposure to impregnated papers (60 min pre-exposure to PBO was done for all synergist assays)

	% Mortality (n)													
	Deltamethrin 0.05%				**Permethrin 0.75%**				**Alpha-cypermethrin 0.05%**					
Site	1X (N)	5X (N)	10X (N)	PBO + deltamethrin	1X (N)	5X (N)	10X (N)	PBO + Permethrin	1X (N)	5X (N)	10X (N)	PBO + alphacypermethrin	Bendiocarb 0.1% (N)	Pirimiphos methyl 0.25% (N)
Agadez	1.6 (62)	39.4 (33)		100 (83)					1.6 (63)			84.1 (63)		
Gaya	1.2 (84)	81.6 (87)		33.3 (69)	15.6 (96)			62.5 (88)	3.8 (80)	78.7 (75)		30.2 (86)		
Ingal	69.7 (109)		95 (101)	95.1 (103)	86 (100)	96.1 (102)	98.1 (104)	100 (103)	60.4 (106)	89.7 (78)	100 (102)	97.2 (100)	100 (100)	100 (100)
Keita	19.5 (77)	92.4 (79)		52.9 (87)	11.8 (85)	92.4 (79)		40.3 (86)	18.5 (81)	35.3 (85)	97.5 (80)	55.8 (77)	66.3 (89)	94.7 (76)
Niamey V	5.8 (86)	64 (100)	88.4 (75)	30.7 (88)	13 (100)	68.1 (75)	70 (100)	49 (100)	2 (100)	46 (100)	55.9 (68)	9.2 (98)	97.3 (75)	74.1 (85)
Tchintabaraden	35.3 (93)	86.2 (109)	93.6 (100)	87.2 (94)	10.6 (96)	28.7 (103)	51.6 (81)	19.2 (101)	6 (100)	80.8 (105)	96.2 (84)	49.5 (91)	52.9 (98)	81.3 (84)
Tessaoua	33 (100)			72 (100)	22 (100)			45 (100)	22.8 (92)		53.8 (91)			
Zindarou	13.8 (94)	64.3 (42)	83 (47)	60.7 (84)	29.8 (84)	86.7 (45)	87 (77)	46.6 (88)	9 (78)			53.4 (88)	92.4 (105)	100 (43)
Zinder	77 (100)	100 (100)		77 (100)	77.9 (100)	100 (100)		100 (100)	93 (100)			100 (100)		

Table A-2: CDC bottle assay results expressed as percent mortality 24, 48, and 72 hours after 60 minute exposure to chlorfenapyr

	Mortality (%)								
	Agadez			Gaya			Niamey V		
Dose	24h	48h	72h	24h	48h	72h	24h	48h	72h
100ug/bottle	87.8	92.7	92.7	87.0	96.3	98.1	88.9	93.3	97.8
200ug/bottle	90.2	98.0	100.0	94.5	98.2	98.2	93.9	100.0	100.0
Control Acetone	0.0	0.0	0.0	0.0	0.0	0.0	4.0	4.0	4.0

	Tessaoua			Zindarou			Tchintabaraden		
Dose	24h	48h	72h	24h	48h	72h	24h	48h	72h
100ug/bottle	65.0	90.2	96.1	98.0	98.0	98.0	58.4	86.5	86.5
200ug/bottle	89.3	97.9	100.0	100.0	100.0	100.0	91.9	96.5	100.0
Control Acetone	6.7	6.7	6.7	0.0	0.0	0.0	0.0	0.0	0.0

Resistant confirmed *Suspected resistance* *Susceptible*