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PMI VECTORLINK NIGER ANNUAL ENTOMOLOGY REPORT APRIL 2021–MARCH 2022

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ACRONYMS

Ace	Acetylcholinesterase
b/p/n	bites per person per night
CDC	Centers for Disease Control and Prevention
CDC LT	Centers for Disease Control and Prevention Light Trap
CERMES	Centre de Recherches Médicale et Sanitaire
EIR	Entomological Inoculation Rate
ELISA	Enzyme-Linked Immunosorbent Assay
f/r	females/room
f/r/d	Females per Room per Day
HBR	Human Biting Rate
HLC	Human Landing Catch
ib/p/m	Infective Bites per Person per Month
ib/p/n	Infective Bites per Person per Night
ITN	Insecticide-treated Net
kdr	Knock Down Resistance
NMCP	National Malaria Control Program
P.	*Plasmodium*
PBO	Piperonyl Butoxide
PCR	Polymerase Chain Reaction
PMI	U.S. President's Malaria Initiative
PSC	Pyrethrum Spray Catch
SOP	Standard Operating Procedure
WHO	World Health Organization

EXECUTIVE SUMMARY

The U.S. President's Malaria Initiative (PMI) VectorLink Niger project conducted entomological monitoring of malaria vectors in Niger from June to December 2021, representing the highest malaria vector density and transmission periods including the rainy season across the selected sites, to support the National Malaria Control Program (NMCP) in making strategic vector control decisions. Longitudinal vector surveillance was done using human landing catch (HLC), pyrethrum spray catch (PSC), and Centers for Disease Control Light Trap (CDC LT) collection methods in six sentinel sites selected by the NMCP and the Centre de Recherches Medicale et Sanitaire. The six longitudinal monitoring sites included Agadez (very low transmission zone), Keita (low transmission zone), and Balleyara, Gaya, Guidimouni, and Niamey V (moderate transmission zone). The data collection period and frequency were planned on a site-specific timeline. PMI VectorLink Niger assessed vector composition, distribution, behavior, sporozoite infection, parity, and entomological inoculation rate (EIR) of the malaria vectors collected. The project also tested the susceptibility status of *Anopheles gambiae* s.l. mosquitoes in 15 sentinel sites, which included the six routine surveillance sites and nine additional sites (Boboye, Madaoua, Madarounfa, Matamey, Sabon Kafi, Say, Tchintabaraden, Tessaoua, and Tillabery). The World Health Organization insecticide susceptibility test kits and the Centers for Disease Control and Prevention bottle bioassays were used to test the susceptibility of *An. gambiae* s.l. against diagnostic concentrations of pyrethroid (alpha-cypermethrin, deltamethrin, and permethrin), organophosphate (pirimiphos-methyl), neonicotinoid (clothianidin), and pyrrole (chlorfenapyr) insecticides. Pyrethroid resistance intensity and synergist effect of piperonyl butoxide (PBO) were also evaluated in all sites when resistance was confirmed. *Anopheles gambiae* s.l. and *An. funestus* species were determined from randomly selected samples from the longitudinal surveillance samples and insecticide susceptibility tested mosquitoes from all sites using polymerase chain reaction (PCR). For insecticides susceptibility tested samples, knock down resistance (*kdr*) west and east and acetylcholinesterase (*ace*-1) mutations were characterized using subsamples of dead and/or live mosquitoes.

Six *Anopheles* species were recorded throughout the collection period including *An. gambiae* s.l., *An. funestus* s.l., *An. rufipes*, *An. pharoensis*, *An. nili,* and *An. ziemanni*. *An. gambiae* s.l. represented more than 82.4% (16,223 of 19,698) of the vectors collected during the collection period in all sites. PSCs yielded higher numbers of *An. gambiae* s.l. (64.5%; 10,289), across all sites, than HLCs (33.3%; 5,396) and CDC LTs (3.3%; 540). *An. funestus* s.l. was recorded in all sites except in Agadez and represented 16.3% (n=3,231) of mosquitoes collected. The biting behavior of *An. gambiae* s.l. and *An. funestus* s.l. varied across sites with an endophagic tendency in all sites except in Balleyara and Niamey V in August, Gaya in September, and Guidimouni in October, where the mosquitoes bit more outdoors. The mean peak biting of *An. gambiae* s.l. occurred mostly between 10:00 pm and 3:00 am, whereas average peak biting of *An. funestus* s.l. occurred mostly between 9:00 pm and 4:00 am as in 2019 and 2020. Vector species composition and behavior recorded in 2021 were similar to those of 2020, except that *An. funestus* s.l. was found in more sites.

The PCR analysis revealed the presence of three species of *An. gambiae* s.l. (*An. coluzzii, An. arabiensis,* and *An. gambiae*) and two species of *An. funestus* s.l. (*An. funestus* s.s. and *An. leesoni*). *An. coluzzii* was the most common species of the *An. gambiae* complex in all sites, followed by *An. arabiensis,* while *An. funestus* s.s. was the major vector of the *An. funestus* group. Unlike 2019 and 2020 where one *An. parensis* was recorded per year, no *An. parensis* was identified in 2021.

A total of, 2,078 *An. gambiae* s.l. collected by HLC were analyzed using Enzyme-Linked Immunosorbent Assay (ELISA) to determine the *Plasmodium falciparum* infection of the mosquitoes tested. Twenty-four mosquitoes (1.2%), all *An. coluzzii,* were found positive for *Plasmodium falciparum* parasite. In 2021, no *An. arabiensis* and *An. gambiae* s.s. were found infected. The entomological inoculation rate (EIR) calculated for each site per month, was highest in Gaya (23.15 infective bites per person per month o), followed by Guidimouni (4.82 ib/p/m) and Niamey V (4.41 ib/p/m). No infective bite was recorded in Agadez over four months of collection. In Guidimouni, 2% of *An. funestus* s.l. were found to be infected and the mean EIR was 17.85 ib/p/m.

Resistance to the three pyrethroids tested (deltamethrin, permethrin, and alpha-cypermethrin) was observed in all 15 sites. Pre-exposure of *An. gambiae* s.l. to PBO before pyrethroids did not completely reverse the resistance status of the mosquito populations in any of the sites surveyed except in Niamey V with alpha-cypermethrin and in Balleyara, Guidimouni, Keita, Madarounfa, and Niamey V with deltamethrin. Nonetheless, a substantial increase in mortality was observed for pyrethroids overall when preceded by PBO exposure, and particularly for deltamethrin. High resistance intensity to alpha-cypermethrin, deltamethrin, and permethrin was observed in all 15 sites except in Guidimouni, Keita, and Niamey V, where the resistance intensity to deltamethrin was moderate. *An. gambiae* s.l. also showed full susceptibility to pirimiphos-methyl in seven of the 15 sites (compared to the previous year where only 5 sites recorded susceptibility) while resistance in the other 8 sites was still moderate resistance intensity.

Susceptibility of *An. gambiae* s.l. to chlorfenapyr at the dose of 100 µg/bottle was recorded in six of the 15 sites (Balleyara, Gaya, Guidimouni, Keita, Tessaoua, and Tillabery) and susceptibility at 200ug/bottle was recorded in the nine other sites. Susceptibility to both clothianidin 2% (WHO tube) and clothianidin 4µg/bottle (CDC bottle) was recorded at all 15 sites.

The *kdr-w* and *kdr-e* mutations were recorded across the 15 sites at frequencies between 0.44 and 0.78 and between 0.11 and 0.68, respectively, and the *ace*-1 was between 0.07 and 0.16. In Guidimouni, where insecticide susceptibility tests were conducted against *An. funestus* s.l., resistance to all pyrethroids was observed (22% mortality for alpha-cypermethrin, 55% for deltamethrin, and 85% for permethrin). However, PBO synergism restored full susceptibility to all pyrethroids, and the resistance intensity observed for all three pyrethroids was low. Also, susceptibility to pirimiphos-methyl and chlorfenapyr was recorded against *An. funestus* s.l.

Similar to 2019 and 2020, the highest peak vector density and transmission were recorded between August and October in all the sites, with *An. coluzzii* being the main vector. This supports the NMCP ongoing sensitization on ITNs use for malaria prevention especially during the peak density and transmission period. Additionally, the vector populations were resistant to all pyrethroids with the involvement of both the *kdr* west and east along with the *ace-1* mutations. This implies the involvement of multiple pyrethroid resistance mechanisms in Niger and upports the need for strategic deployment of vector control interventions by considering the insecticide resistance status of the vectors across the country.

1. INTRODUCTION

Malaria is endemic in Niger and is the leading cause of death and disability combined, disproportionately affecting children under five years of age. According to Niger's Annual Health Statistic Report (2020), there were over 4,332,909 malaria cases, and 6,098 malaria deaths in 2020, putting it among the countries with the highest per capita rate of malaria fatalities globally.

Since 2020, the country has been stratified into four endemicity strata, very low transmission, low transmission, moderate transmission, and very high transmission, to help the National Malaria Control Program (NMCP) determine district-level priorities. Most of the population (94%) resides in the two southernmost zones (moderate and high transmission), where malaria is most prevalent (PMI Malaria Operational Plan 2020). The rainy season in Niger lasts three to four months, from June through September, with peak malaria transmission during the second half (August–September).

Understanding why and where transmission is persisting, ensuring effective vector control, and monitoring trends are critical to accelerating progress toward malaria elimination. To achieve these, the role of entomological surveillance is very important as it provides information on vector species, including their spatial and temporal distribution, density, ecology, biting, feeding, and resting behavior, as well as the rate of infectivity, transmission, and susceptibility to the insecticides used in vector control interventions.

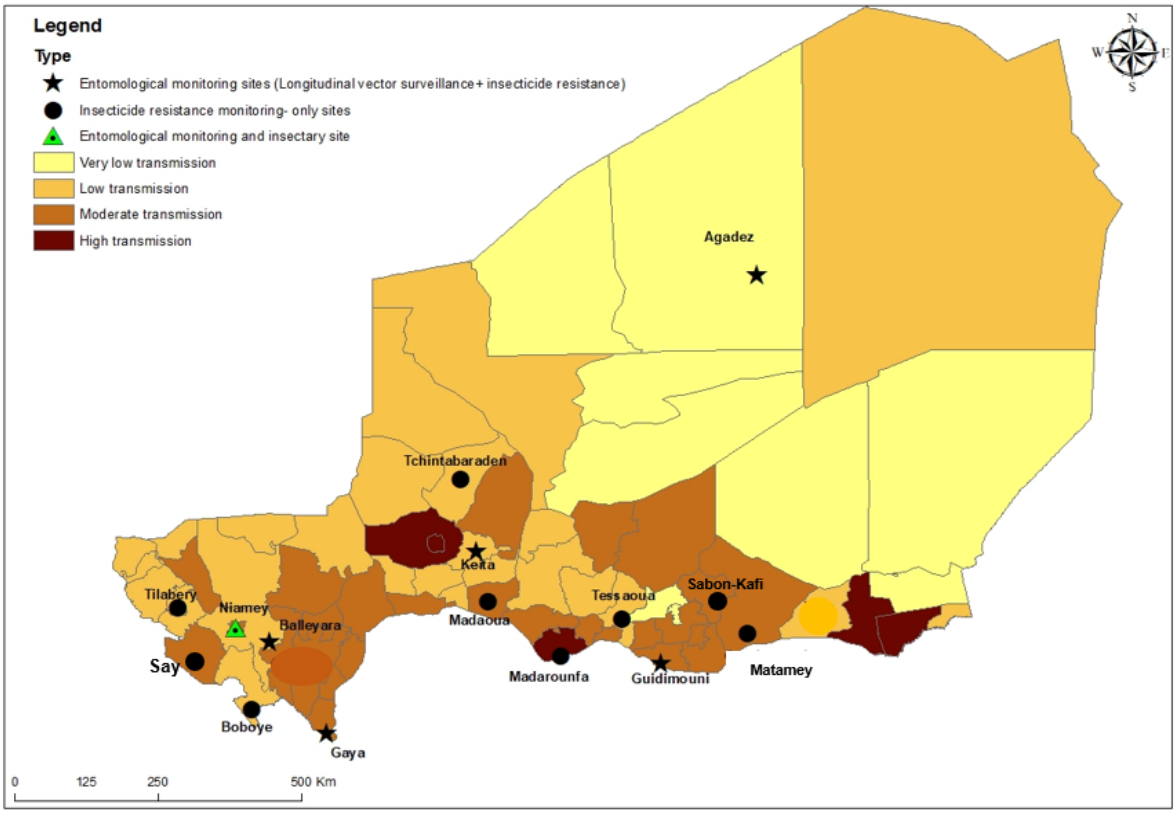
In 2021, the U.S. President's Malaria Initiative (PMI) VectorLink Niger Project conducted longitudinal vector surveillance in six sentinel sites and insecticides resistance in 15 sites, including the six vector surveillance sites. The data collected will provide the NMCP with trends on entomological indicators of malaria transmission and insecticide resistance status of the vectors. Paired with health facility-based malaria incidence data and population density, these results will support the NMCP on evidence-based decision making for vector control interventions, such as a stratified distribution of insecticide-treated nets (ITNs) across the country.

2. METHODS

2.1 ENTOMOLOGICAL MONITORING SITES

PMI VectorLink Niger conducted longitudinal entomological vector surveillance in six sentinel sites, selected by the NMCP and the Centre de Recherche Medicale et Sanitaire (CERMES) from June to December 2021, coinciding with the highest density, transmission period and rainy season of the selected sites. The selected sites are located in three of the four country's malaria endemicity zones: Agadez is in the very low transmission zone, Keita in the low-transmission zone, and Balleyara, Gaya, Guidimouni, and Niamey V in the moderate transmission zones (Figure 1). Insecticide resistance monitoring was also conducted in 15 sites selected across the country, including the six sites for vector surveillance and nine additional sites (Boboye, Madaoua, Madarounfa, Matamey, Sabon Kafi, Say, Tchintabaraden, Tessaoua, and Tillabery), covering all the endemicity zones (Figure 1).

FIGURE 1: ENTOMOLOGICAL MONITORING SITES SUPPORTED IN 2021 BY PMI VECTORLINK NIGER



2.2 LONGITUDINAL MONITORING

PMI VectorLink Niger collected adult mosquitoes using human landing catches (HLCs), pyrethrum spray catches (PSCs), and Centers for Disease Control light traps (CDC LTs) monthly in all six longitudinal monitoring sites. Due to the different ecological and geographical location of the sites, the vector surveillance data collections were planned on a site-specific timeline. In the very low transmission zone site (Agadez), four consecutive monthly collections were conducted during the rainy season, which coincided with the highest transmission season (July to October). In the sites of Balleyara and Keita, five consecutive monthly collections

were planned during the highest transmission season (July to November), and in the sites of Gaya, Guidimouni, and Niamey V, seven consecutive monthly collections were planned during the highest transmission season (June to December) (Table 1). The collection timeline was selected based on previous years' reports of no mosquitoes having been collected in the other months of the year for each of the sites.

TABLE 1: COLLECTION TIMELINE IN THE LONGITUDINAL MONITORING SITES

	Jun-2021	Jul-2021	Aug-2021	Sep-2021	Oct-2021	Nov-2021	Dec-2021
Agadez		X	X	X	X		
Balleyara		X	X	X	X	X	
Gaya	X	X	X	X	X	X	X
Guidimouni	X	X	X	X	X	X	X
Niamey V	X	X	X	X	X	X	X
Keita		X	X	X	X	X	

All collections were conducted as described in Table 2 and following PMI VectorLink standard operating procedures (SOPs). (Complete SOPs can be found at https://pmivectorlink.org/resources/tools-and-innovations/.) HLCs were conducted during two nights per site per month. The same two houses were used at every month's collection, indoors and outdoors, and from approximately 6:00 pm to 6:00 am. PSCs were carried out during morning hours, between 7:00 am and 10:00 am in the same 10 houses during every month's collection. CDC LTs were installed indoors in the same two houses in each site each month. The CDC LT collection houses were different from those used for HLCs and PSCs.

TABLE 2: LONGITUDINAL ADULT MOSQUITO COLLECTION METHODS

Collection Method	Time	Frequency	Collection Area	Sample
HLC	6:00 pm to 6:00 am	Two consecutive nights per site per month	Indoor and outdoor	Two houses per site (same houses every time)
PSC	7:00 am to 10:00 am	Once per site per month	Indoor	Ten houses per site (same houses every time)
CDC LT	6:00 pm to 6:00 am	Two consecutive nights per site per month	Indoor	Two houses per site (same houses every time)

All mosquitoes were morphologically identified using identification keys (Coetzee 2020). A sub-sample of *Anopheles* species from each site collected by HLC was dissected to determine the parity and to estimate the longevity of the vector population of each site. The targeted number to be dissected was around 100 mosquitoes per site per collection period. Therefore, all the vectors were dissected for parity when the number collected was below 100. Above that, a percentage of the total collected was considered to estimate the number of mosquitoes around 100 to be dissected per site. All *Anopheles* vector species were labeled and preserved on silica gel in Eppendorf tubes for further laboratory analysis to identify sibling species, resistance mechanisms using Polymerase Chain Reaction (PCR), the sporozoite infection rates (Circumsporozoite (CSP)) and blood meal source using Enzyme-Linked Immunosorbent Assay (ELISA). The indicators listed in Table 3 were calculated based on the mosquitoes collected through each collection method.

TABLE 3: VECTOR SURVEILLANCE INDICATORS BY COLLECTION METHOD

Collection Method	Indicator	Unit of Measure
HLC	Species composition of mosquitoes collected using HLC method	Percentage of each mosquito species collected out of all mosquitoes collected by HLC
	Human biting rate	Mean number of bites/person/nights
	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	Species composition of mosquitoes collected using PSC method	Percentage of each mosquito species collected out of all mosquitoes collected by PSC
	Indoor resting density	Mean number of mosquitoes/house/days
	% of fed females	Percentage of fed mosquitoes out of total collected by PSC
CDC LT	Species composition of mosquitoes collected using CDC LT method	Percentage of each mosquito species collected out of total collected by CDC LT

2.3 MOLECULAR CHARACTERIZATION

Molecular analysis of sub-samples was conducted, including species identification of the adult mosquitoes that had been collected through longitudinal vector surveillance and those reared in the field, and used for insecticides susceptibility testing.

2.3.1 SPECIES IDENTIFICATION

A sample of 2,406 *An. gambiae* s.l. collected using the three methods (1,450 by HLC, 593 by PSC, and 363 by CDC LT) from all six sites were identified to the species level. DNA from each mosquito was extracted using the protocol designed by Collins *et al.* (1987). *An. gambiae* s.s., *An. coluzzii*, and *An. arabiensis* were identified by PCR following the Short-Interspersed Element (SINE) protocol described by Santolamazza *et al.* (2008). The species of *An. funestus* group were also identified within a sub-sample of 189 mosquitoes using the multiplex protocol designed by Koekemoer *et al.* (2002). Also, 1787 *An. gambiae* s.l. mosquitoes were randomly selected from the dead and surviving mosquitoes used for insecticide susceptibility tests and controls (alpha-cypermethrin n=533 mosquitoes, deltamethrin n=536, permethrin n=520, pirimiphos methyl n=126, and control n=74), from all sites, were further analyzed for species identification.

2.3.2 CIRCUMSPOROZOITE AND BLOOD MEAL ANALYSIS

A total of 2,078 mosquitos collected using HLC at all sites were analyzed for *Plasmodium* (*P.) falciparum* infection using ELISA methods following the protocol (Wirtz *et al.* 1987). The sporozoite rate was calculated as the ratio of the number of circumsporozoite positive mosquitoes over the total number of mosquitoes analyzed by site. The monthly entomological inoculation rate (EIR) represents the product of the sporozoite rate and the human biting rate (HBR) per month. As the sites do not have the same number of months of monitoring, the monthly mean EIR was estimated using the number of collection months per site.

The blood meal source of 600 mosquitoes collected from all sites using PSC (480) and CDC LTs (120) was also determined using PCR following the methods of Kent and Norris (2005). The human blood index was calculated using the number of mosquitoes found with human blood out of the total number of mosquitoes tested.

2.4 INSECTICIDE RESISTANCE MONITORING

From August through December 2021, PMI VectorLink Niger completed insecticide resistance monitoring in 15 sites across the country including the six longitudinal monitoring sites (Figure 1, above) following the PMI

VectorLink SOPs. *An. gambiae* s.l. larvae and pupae were collected in each site from several larval habitats, pooled, and reared to adulthood in the field laboratory. Insecticide susceptibility tests were conducted on 2–5-day-old *An. gambiae* s.l. adult females using World Health Organization (WHO) tube tests (SOP 06/01) for all insecticides except chlorfenapyr and clothianidin 4ug/bottle, which were tested using Centers for Disease Control and Prevention (CDC) bottle assays (SOP 04/01).

For each insecticide, 80–100 female *An. gambiae* s.l. were tested in four tubes of 20–25 mosquitoes, and an additional 40–50 were tested in two control tubes (20–25 each). The diagnostic concentrations of permethrin (0.75%), deltamethrin (0.05%), alpha-cypermethrin (0.05%), and pirimiphos-methyl (0.25%) were tested in all sites.

When resistance was confirmed to the diagnostic concentrations, resistance intensity was also tested using 5 and 10 times the diagnostic concentration. Synergist assays with piperonyl butoxide (PBO) were conducted for alpha-cypermethrin, deltamethrin, and permethrin according to the WHO tube test protocol to determine the involvement of cytochrome P450s in pyrethroid resistance.

Clothianidin 2% papers were treated locally using a protocol designed by PMI VectorLink. The susceptibility testing was conducted as described above, and the mortality was recorded every 24 hours for up to seven days post-exposure. CDC bottle assays were also conducted using clothianidin 4 µg /bottle with one-hour exposure, and mortality recorded at 24 hours.

CDC bottle assays were conducted using chlorfenapyr at the doses of 100µg/bottle and 200µg/bottle with one-hour exposure, and mortality was recorded every 24 hours for up to three days (72 hours).

Both clothianidin 2% and 4 µg /bottle as well as both doses of 100 and 200 µg /bottle of chlorfenapyr were tested due to the lack of approved diagnostic doses and testing procedures for those insecticides.

For all tests, resistance and intensity were defined following the WHO criteria (WHO 2016):

Resistance status at diagnostic doses:

- 98% or greater mortality indicates susceptibility.
- Between 90 and 97% mortality indicates possible resistance, requiring confirmation.
- Less than 90% mortality indicates confirmed resistance.

Resistance intensity with mortality at 5x and/or mortality at 10x:

- 98–100% at 5x: low resistance intensity
- <98% at 5x and 98–100% at 10x: moderate resistance intensity
- <98% at 10x: high resistance intensity

TABLE 4: INSECTICIDES TESTED

Testing Procedures	Insecticides	Diagnostic Concentration (DC)	Intensity Assay		Diagnostic Exposure Time	Delayed Mortality Post-exposure
			5X DC	10X DC		
WHO tube test	Deltamethrin	0.05%	0.25%	0.50%	60 mins	24 hours
	Permethrin	0.75%	3.75%	7.50%	60 mins	24 hours
	Alpha-cypermethrin	0.05%	0.25%	0.50%	60 mins	24 hours
	Pirimiphos-methyl	0.25%	1.25%	2.5%	60 mins	24 hours
	Clothianidin	2.00%	n/a		60 mins	Up to 7 days
	Piperonyl butoxide	4.00%	n/a		60 mins synergist and 60 mins pyrethroid	24 hours
CDC bottle assay	Chlorfenapyr	100 and 200 µg/bottles	n/a		60 mins	Up to 72 hours
	Clothianidin	4 µg/bottles	n/a		60 mins	24 hours

2.4.1 CHARACTERIZATION OF INSECTICIDE RESISTANCE MARKERS

Markers of insecticide resistance were determined in 120 mosquitoes per site after susceptibility testing to determine the frequency of the mutations among the population tested. The presence of knock down resistance *(kdr)* west and east was characterized using the conventional PCR restriction fragment length polymorphism (RFLP) method as described by Martinez-Torres *et al.* (1999). The protocol described by Weill *et al.* (2004) was used to determine the acetylcholinesterase (*ace)-1* mutation at all sites within the mosquito population tested.

2.5 DATA PRESENTATION AND STATISTICAL ANALYSIS

The District Health Information Software (DHIS-2)-based VectorLink Collect database has been used for entomological data management in Niger since 2020. All entomological data collected in Niger in 2021 were analyzed in VectorLink Collect. The platform includes comprehensive dashboards to synthesize and describe vector bionomics and insecticide resistance summary results. Currently, the NMCP, CERMES, and PMI Niger have been trained to have ongoing access to these results dashboards to support timely decision making.

3. RESULTS

3.1 LONGITUDINAL MONITORING

Four monthly collections were completed in Agadez, five in Balleyara and Keita, and seven in Gaya, Guidimouni, and Niamey V, using the three collection methods (HLCs, PSCs, and CDC LTs).

3.1.1 SPECIES COMPOSITION

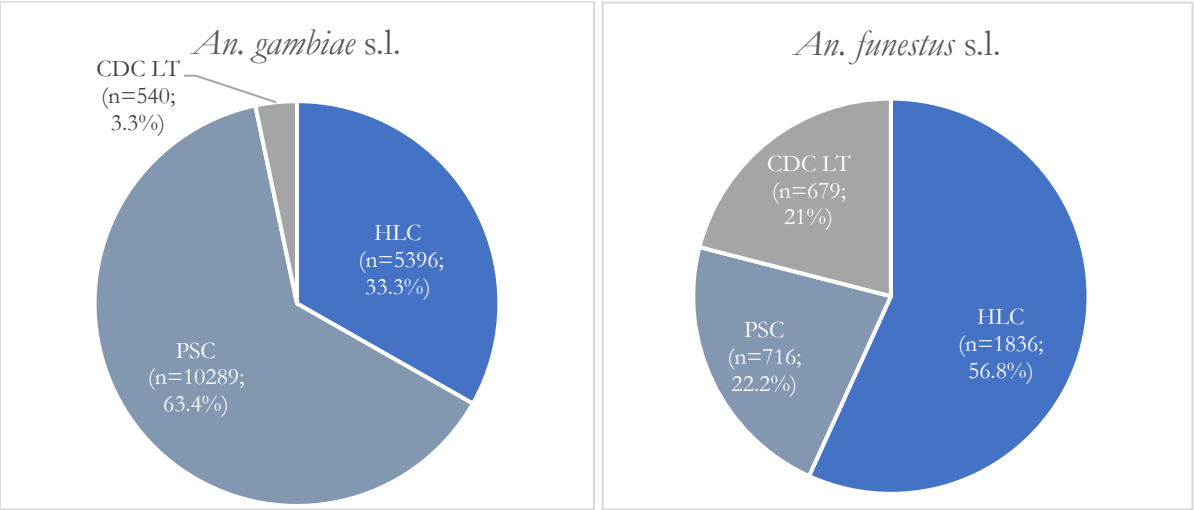
A total of 19,698 *Anopheles* mosquitoes were collected across the six longitudinal monitoring sites using all three collection methods. *An. gambiae* s.l. was the most abundant (82.4%; n=16,225), followed by *An. funestus* s.l. (16.4%; n=3,231), *An. rufipes* (0.7%; n=140), *An. pharoensis* (0.5%; n=89), *An. nili* (0.04%; n=7), and *An. ziemanni* (0.03%; n=5) while a single *An. coustani* (0.01%; n=1) was recorded (Table 5).

TABLE 5: SPECIES COMPOSITION AND NUMBER OF ANOPHELES MOSQUITOES COLLECTED USING ALL THREE COLLECTION METHODS AT ALL SITES

	Agadez	Balleyara	Gaya	Guidimouni	Keita	Niamey V	Total	% of All Species
An. gambiae s.l.	37	662	11,432	1,476	1,166	1,452	16,225	82.4
An. funestus s.l.	0	9	296	2,912	5	9	3,231	16.4
An. rufipes	0	9	103	6	4	18	140	0.7
An. pharoensis	0	0	56	12	0	21	89	0.5
An. nili	0	0	7	0	0	0	7	0.04
An. ziemanni	0	0	3	2	0	0	5	0.03
An. coustani	0	0	0	0	0	1	1	0.01
Total	**37**	**680**	**11,897**	**4,408**	**1,175**	**1,501**	19,698	

Of the 16,225 *An. gambiae* s.l. collected (across all sites), 5,396 (33.3%) were collected by HLC, 10,289 (63.4%) by PSC, and 540 (3.3%) by CDC LT. Of the 3,231 *An. funestus* s.l. collected, 1,836 (56.8%) were collected using HLC, 716 (22.2%) using PSC, and 679 (21.0%) using CDC LT (Figure 2).

FIGURE 2: NUMBER AND PERCENTAGE OF AN. GAMBIAE S.L. AND AN. FUNESTUS S.L. COLLECTED USING ALL THREE COLLECTION METHODS AT ALL SITES*

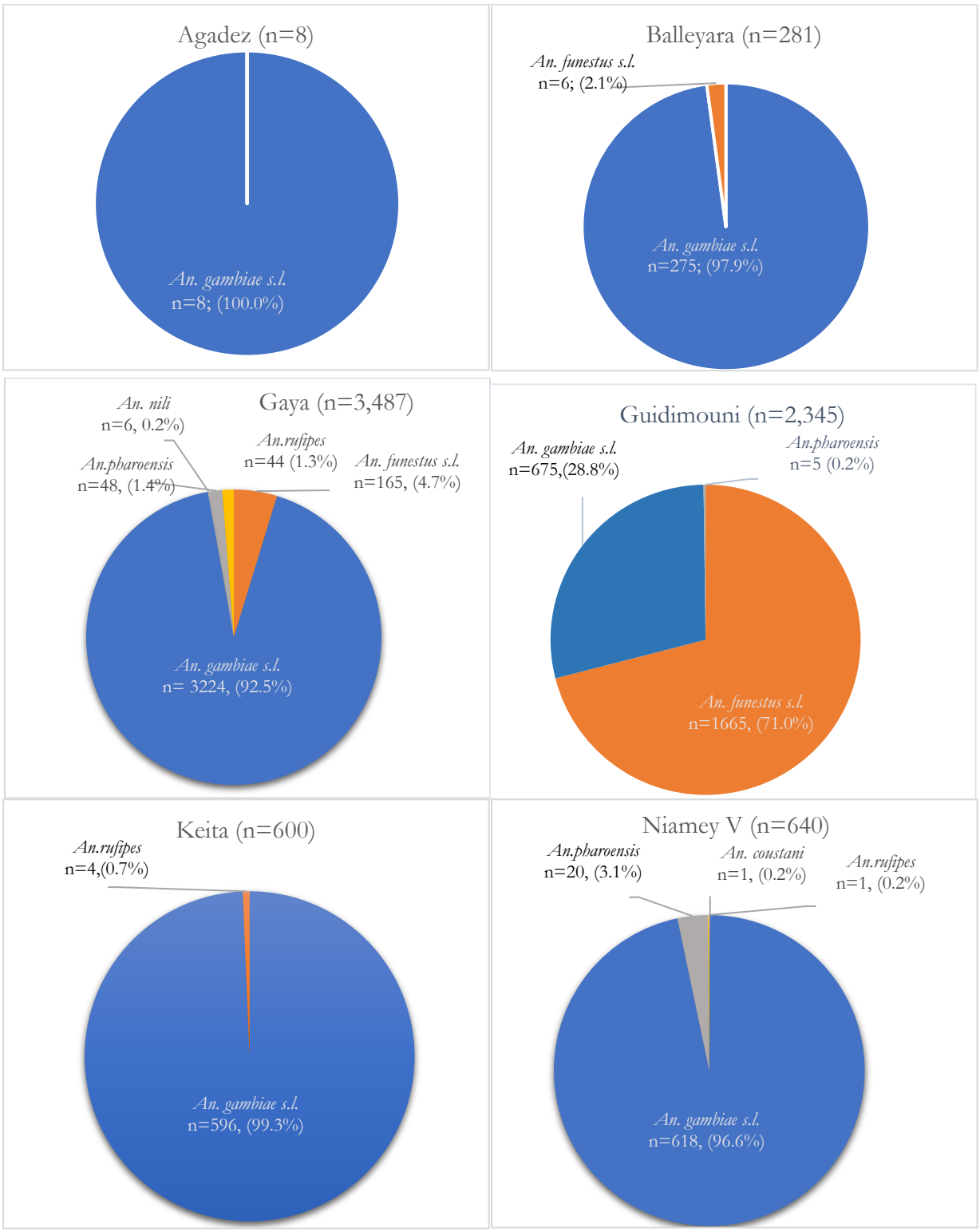


* *For each collection methods, n= number of vectors collected followed by the percentage in parenthesis*

3.1.2 SPECIES COMPOSITION OF *ANOPHELES* COLLECTED USING HLC

Of the 7,361 *Anopheles* mosquitoes collected by HLC, *An. gambiae* s.l. was the dominant malaria vector (73.3%, n=5,396) across five of the six sites: Agadez 100%; n=8), Balleyara (97.9%; n=275/281), Gaya (92.85%; n=3,224/3,487), Keita (99.3%; n=596/600), and Niamey V (96.6%; n=618/640) while. Guidimouni recorded the lowest proportion of *An. gambiae* s.l. (28.8%; n=675/2,345). There, *An. funestus* s.l. was predominant (71%; n=1,665/2,345), which is consistent with the past years' results, The other *Anopheles* vectors collected by HLC were *An. funestus* s.l. (25%, n=1,836), *An. pharoensis* (1%, n=74), *An. rufipes* (0.7%, n=49), *An. nili* (0.1%, n=6), and a single *An. coustani* (0.01%, n=1.(Figure 3; Annex Table A1).

FIGURE 3: SPECIES COMPOSITION OF ANOPHELES COLLECTED USING HLC, BY SITE (N=7,361)



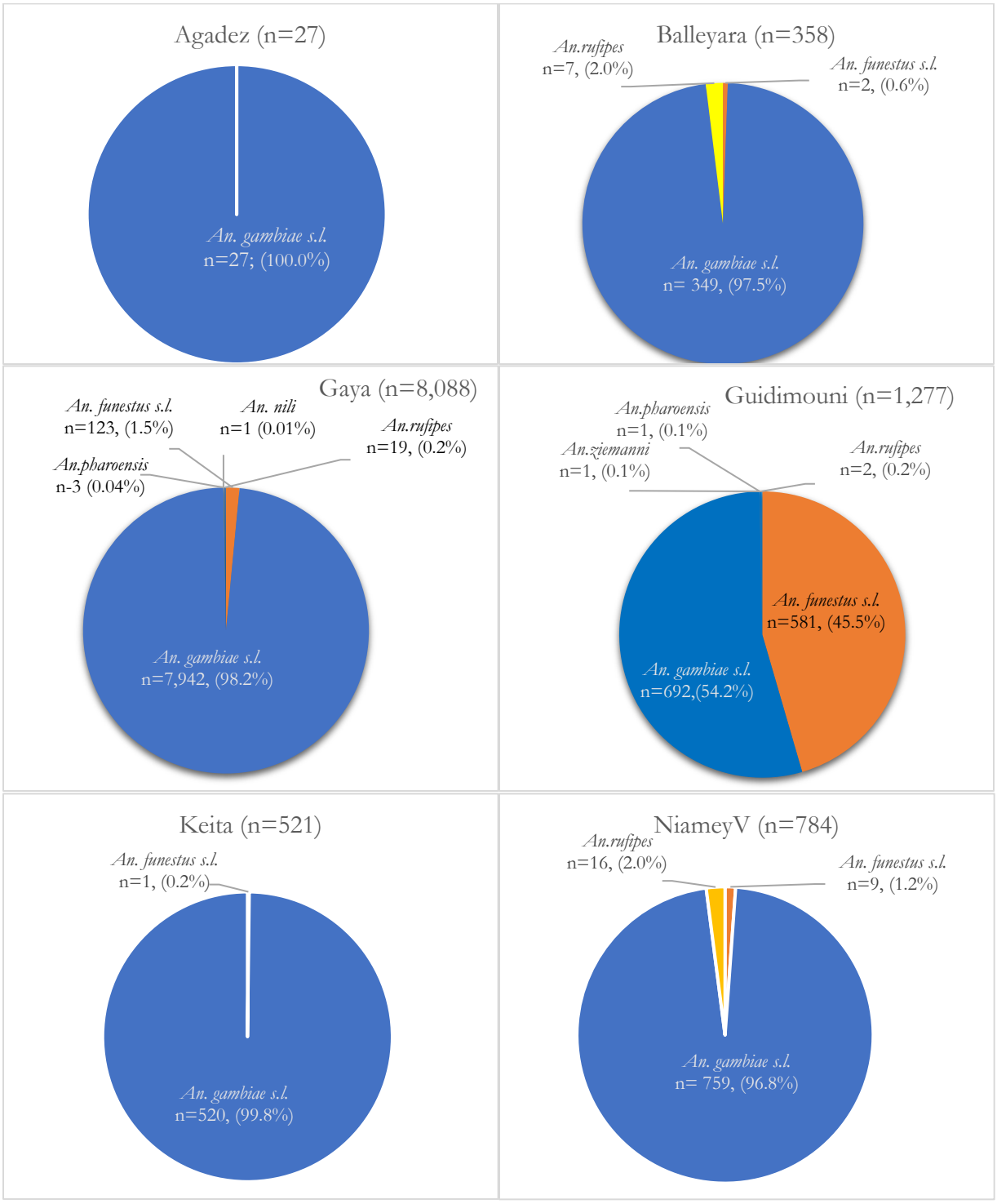
**For each species, n=number collected followed by the percentage in parenthesis.*

3.1.3 SPECIES COMPOSITION OF *ANOPHELES* COLLECTED USING PSC

Among the 11,055 *Anopheles* mosquitoes collected using the PSC method, *An. gambiae* s.l. was the predominant vector, representing 93.1% (n=10,289) of the total *Anopheles* species and the main species recorded in all the sites: Agadez (100%; n=27), Balleyara (97.5%; n=349), Gaya (98.2%; n=7,942), Keita (99.8%; n=526), Niamey

V (96.8%; n=759), and Guidimouni (54.2%, n=692). *An. funestus* s.l. was recorded in five of the six sites: Balleyara (0.6%; n=2), Gaya (1.5%; n=123), Guidimouni (45.5%; n=581), Keita (0.2%; 1), and Niamey (1.2%; 9). Forty-four *An. rufipes* (0.4%), four *An. pharoensis* (0.01%) and only one *An. ziemanni* were collected using the PSC method (Figure 4, Annex Table A2).

FIGURE 4: SPECIES COMPOSITION OF ANOPHELES COLLECTED USING PSC, BY SITE (N=11,055)



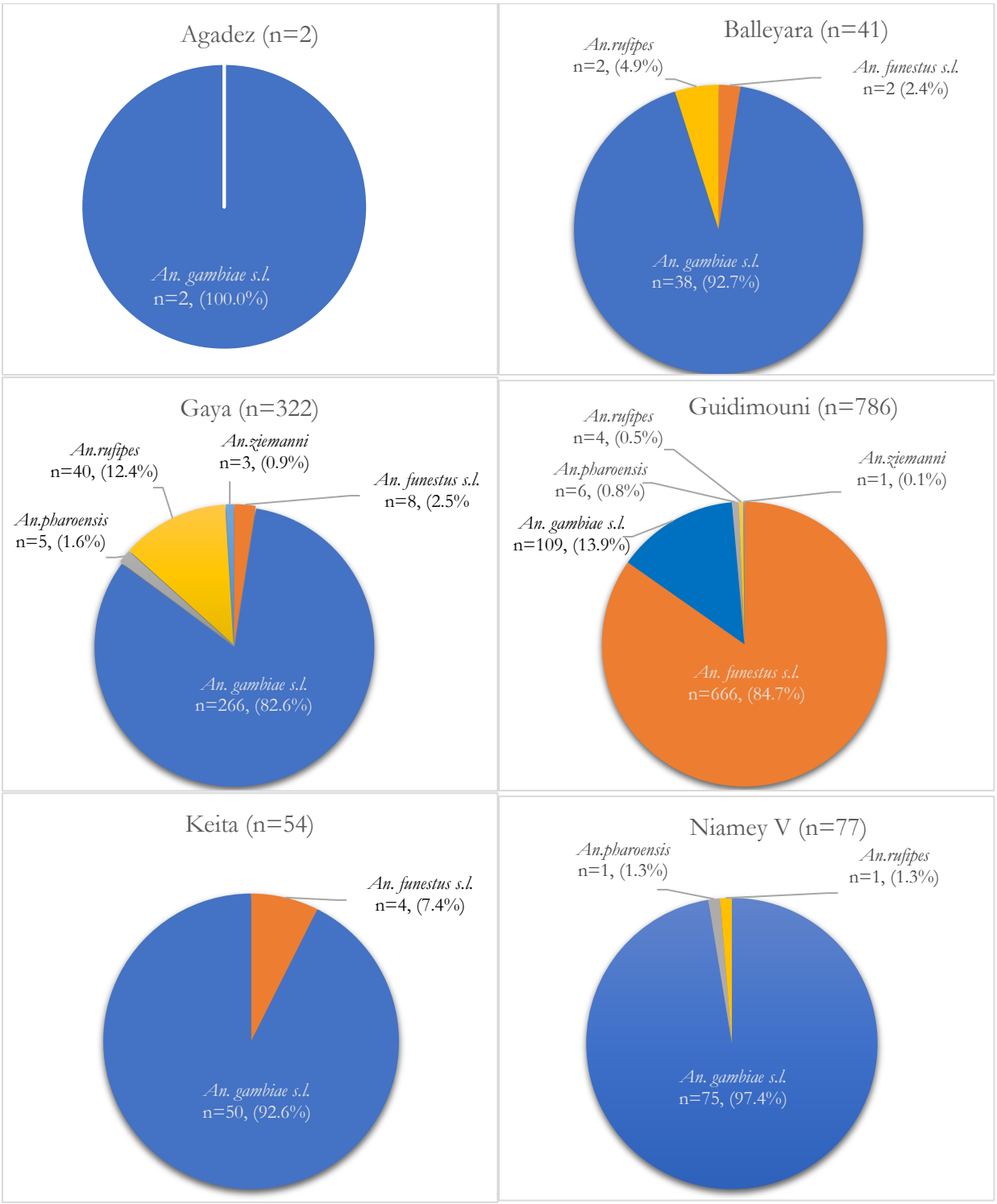
** For each species, n=number collected followed by the percentage in parenthesis.*

3.1.4 SPECIES COMPOSITION OF ANOPHELES COLLECTED BY CDC LIGHT TRAPS

The CDC LTs were less productive than the HLCs and PSCs in the different sites. A total of 1,282 *Anopheles* were caught at the six sites (Figure 5). *An. gambiae* s.l. was the predominant vector collected in all sites except

Guidimouni, but *An. funestus* s.l. was the most collected and represented 53.6% (n=679) of the total *Anopheles* caught. *An. gambiae* s.l. represented 82.6% in Gaya (n=266) and more than 90.0% of the *Anopheles* species collected by CDC LT at four sites: Agadez (100%; n=2), Balleyara (92.7%; n=38), Keita (92.6%; n=50), and Niamey V (97.4%; n=75). In Guidimouni, *An. funestus* s.l. was predominant (84.7%, n=666) (Annex Table A3).

FIGURE 5: SPECIES COMPOSITION OF ANOPHELES COLLECTED USING CDC LTS, BY SITE (N=1,282)



** For each species, n=number collected followed by the percentage in parenthesis.*

3.1.5 HUMAN BITING RATE OF *ANOPHELES* VECTORS COLLECTED USING HLC

3.1.6 HBRS OF *AN. GAMBIAE* S.L.

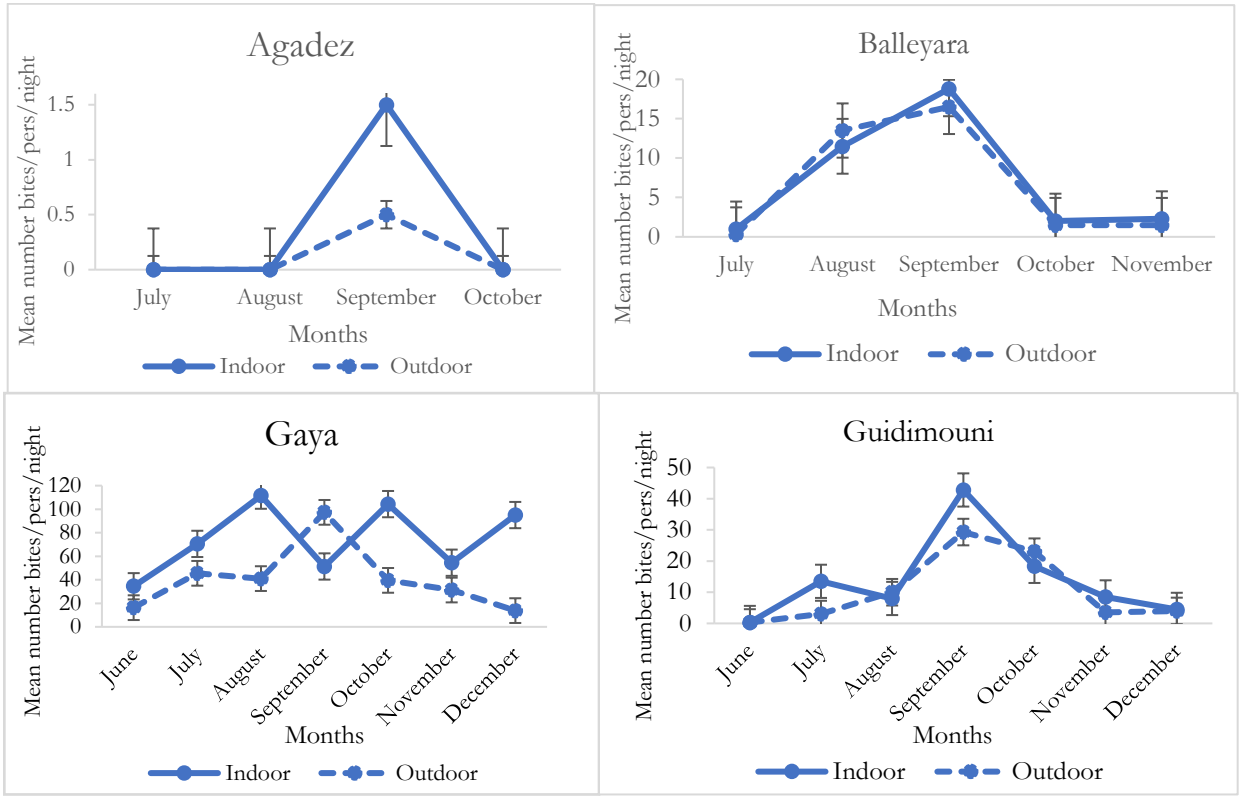
Agadez, with four monthly collections, recorded the lowest mean HBR of 0.25 bites/person/night (b/p/n), which was observed only in September both indoors (1.5 b/p/n) and outdoors (0.5 b/p/n) (Figure 6, Annex Table B1).

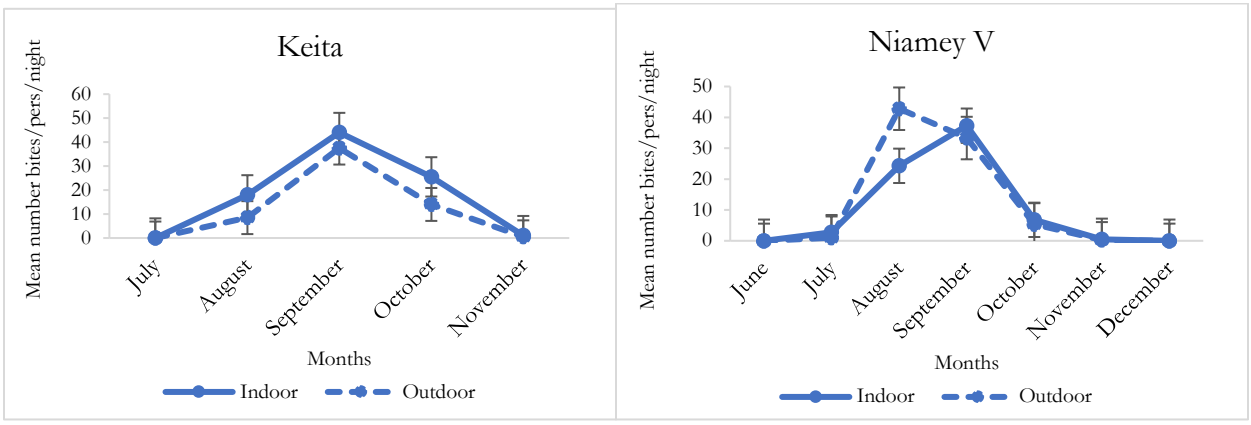
In the two sites where five monthly collections were conducted, Keita recorded the highest mean HBR (14.9 b/p/n), followed by Balleyara (6.9 b/p/n). The peak HBR was observed in September in both sites, with 18.8 b/p/n indoors and 16.5 b/p/n outdoors in Balleyara; and 44.0 b/p/n indoors and 37.5 b/p/n outdoors in Keita.

In the three sites where seven monthly collections were completed, Gaya recorded the highest *An. gambiae* s.l. mean HBR (57.6 b/p/n), followed by Guidimouni (12.1 b/p/n) and Niamey V (11.0 b/p/n). The peak HBR in these three sites was recorded in Gaya in August (111.5 b/p/n indoors) and September (97.3 b/p/n outdoors), followed by Guidimouni in September (42.8 b/p/n indoors, 29.3 b/p/n outdoors) and Niamey V with 37.3 b/p/n in August outdoors and 42.8 b/p/n in September indoors (Figure 6).

Overall, the *An. gambiae* s.l. mean HBR was higher indoors than outdoors during most of the collection months in all sites except in Balleyara and Niamey V in August; Gaya in September; Guidimouni in October when the outdoor HBR was slightly higher.

FIGURE 6: MEAN INDOOR AND OUTDOOR HBRS OF AN. GAMBIAE S.L., BY MONTH AND BY SITE



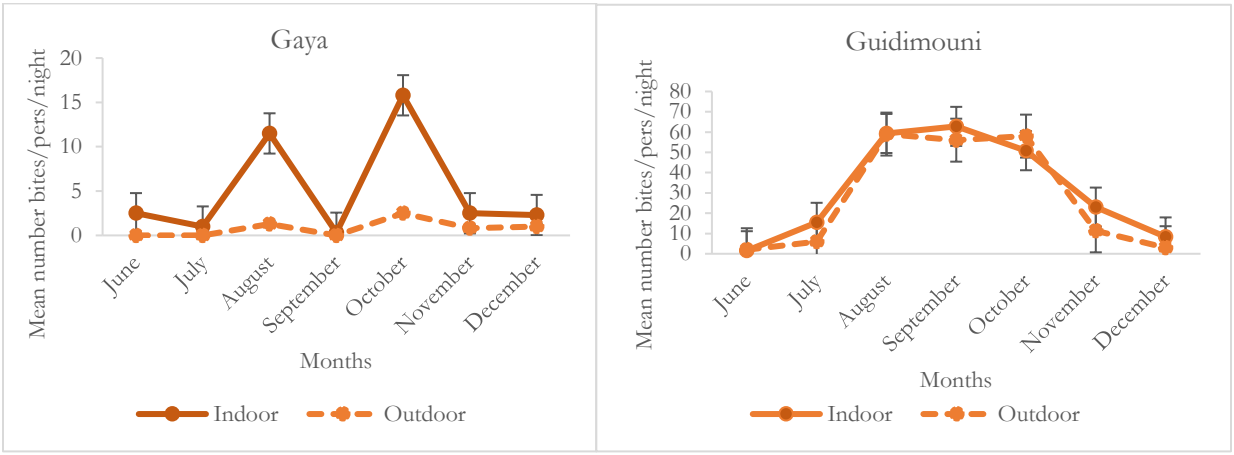


Error bars in the figures represent the standard errors.

3.1.7 HBRs OF *AN. FUNESTUS* S.L.

In the two sites where large numbers of *An. funestus* s.l. were collected, Guidimouni recorded the highest mean HBR (29.8 b/p/n), followed by Gaya (3 b/p/n) (Figure 7). In Gaya, the peak HBR of *An. funestus* s.l. was recorded in October (15.8 b/p/n indoor and 2.5 b/p/n outdoor); in Guidimouni, the peak indoor HBR (62.8 b/p/n) was recorded in August and the peak outdoor HBR (59 b/p/n) was recorded in September.

FIGURE 7: MEAN INDOOR AND OUTDOOR HBRS OF AN. FUNESTUS S.L. FROM GAYA AND GUIDIMOUNI, BY MONTH

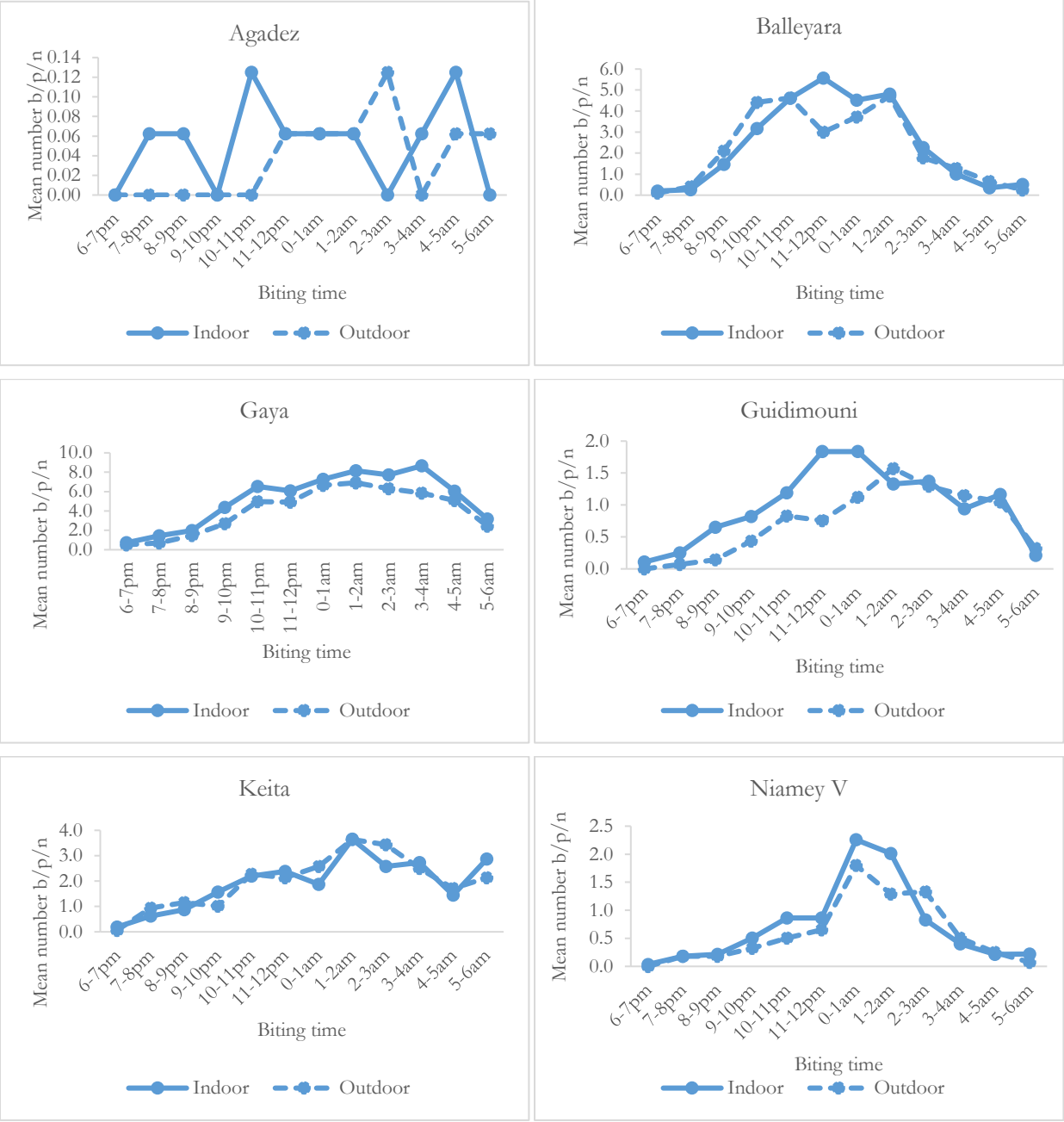


Error bars in the figure represent the standard errors.

3.1.8 BITING CYCLE OF *AN. GAMBIAE* S.L.

Overall, the biting activity of *An. gambiae* s.l. was highest between 11:00 pm and 3:00 am during the collection period, both indoors and outdoors, in all sites. In Agadez, the peak biting was recorded between 11:00 pm and 4:00 am indoors and between 2:00 am and 3:00 am outdoors. It was between 11:00 pm and 1:00 am in Balleyara both indoors and outdoors, and between 11:00 pm and 3:00 am in Gaya, Guidimouni, Keita, and Niamey, both indoors and outdoors (Figure 8, Annex Table B2).

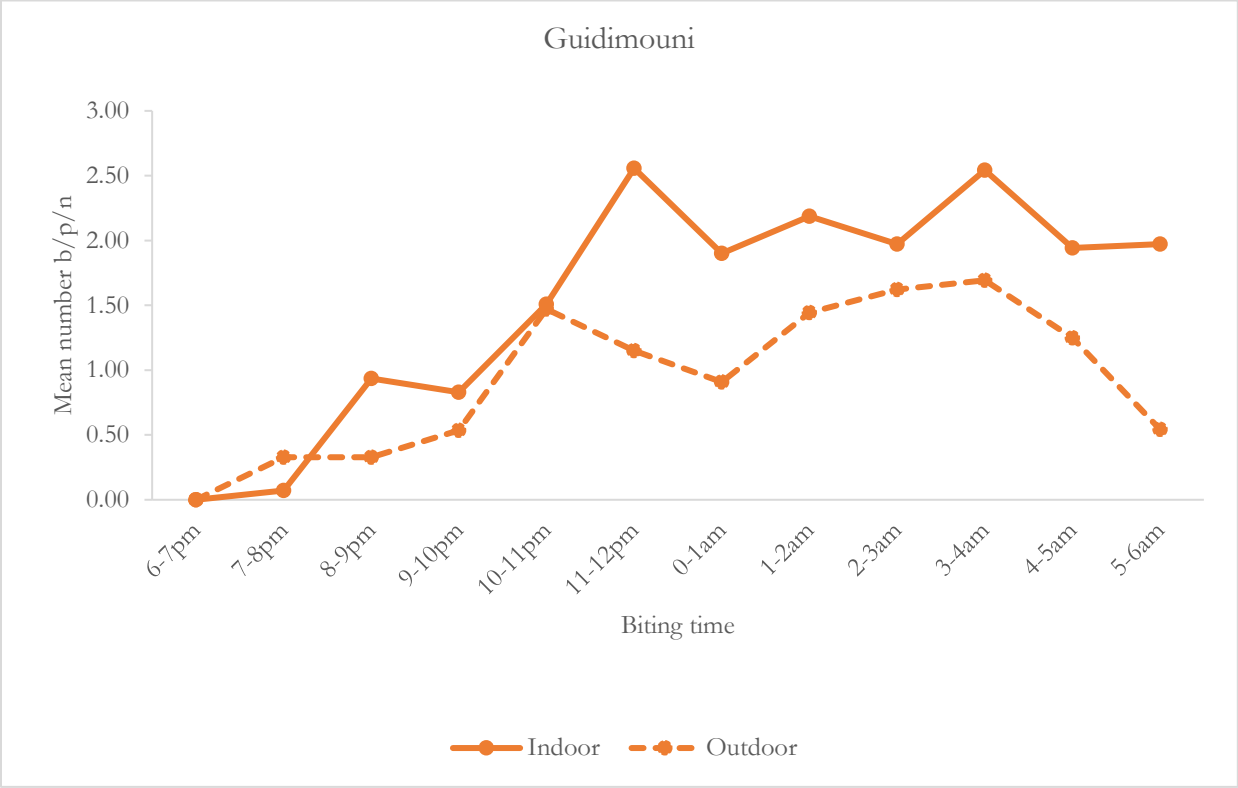
FIGURE 8: BITING CYCLE OF AN. GAMBIAE S.L., BY SITE



3.1.9 BITING CYCLE OF *AN. FUNESTUS* S.L.

An. funestus s.l. peaks biting occurred between 11:00 pm and 12:00 am indoors and between 11:00pm and 3:00 to 4:00 am outdoors (Figure 9, Annex Table B2).

FIGURE 9: BITING CYCLE OF AN. FUNESTUS S.L. IN GUIDIMOUNI

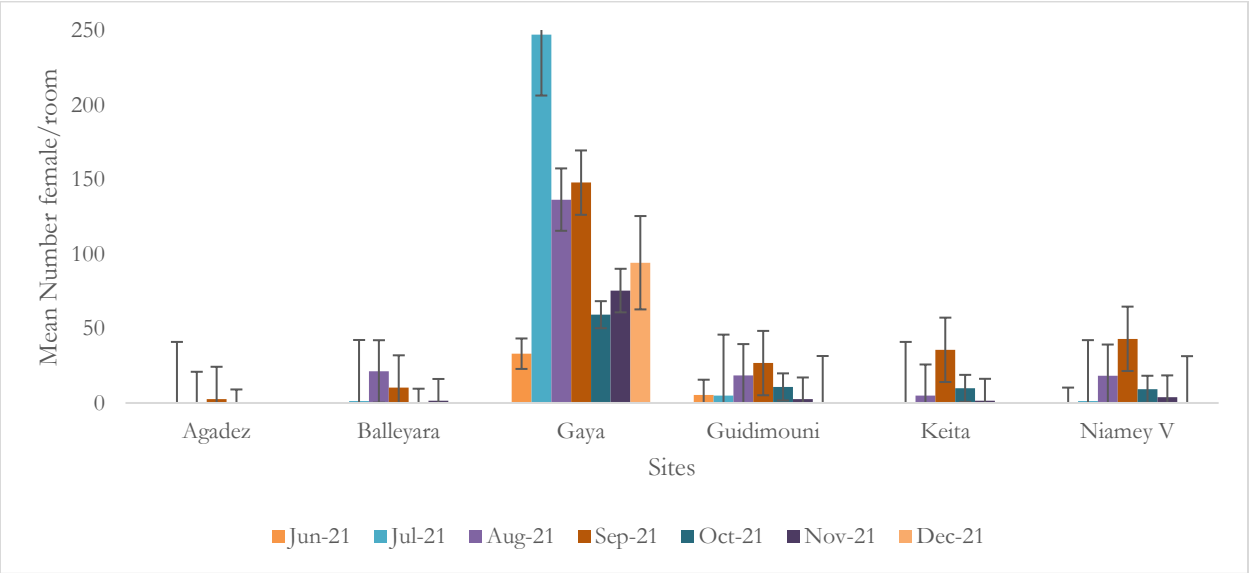


3.1.10 INDOOR RESTING DENSITY

3.1.11 MONTHLY INDOOR RESTING DENSITY OF *AN. GAMBIAE* S.L. COLLECTED USING PSC

The mean indoor resting density of each site was calculated using the density of *An. gambiae* s.l. collected through PSCs from the 10 houses surveyed every month. Gaya recorded the highest monthly mean indoor resting density (126.9 females/room (f/r)), which is consistent with past year's collections, followed by Niamey V (12.6 f/r) and Guidimouni (10.6 f/r). The peak *An. gambiae* s.l. indoor resting densities were recorded in September in four of the six sites: Agadez (2.7 f/r), Guidimouni (26.8 f/r), Keita (35.7 f/r), and Niamey V (43.1 f/r), while Gaya recorded the peak density in July (247.5 f/r) and Balleyara did in August (21.2 f/r) (Figure 10, Annex Table B3).

FIGURE 10: MONTHLY MEAN INDOOR RESTING DENSITY OF AN. GAMBIAE S.L. PER HOUSE, BY SITE

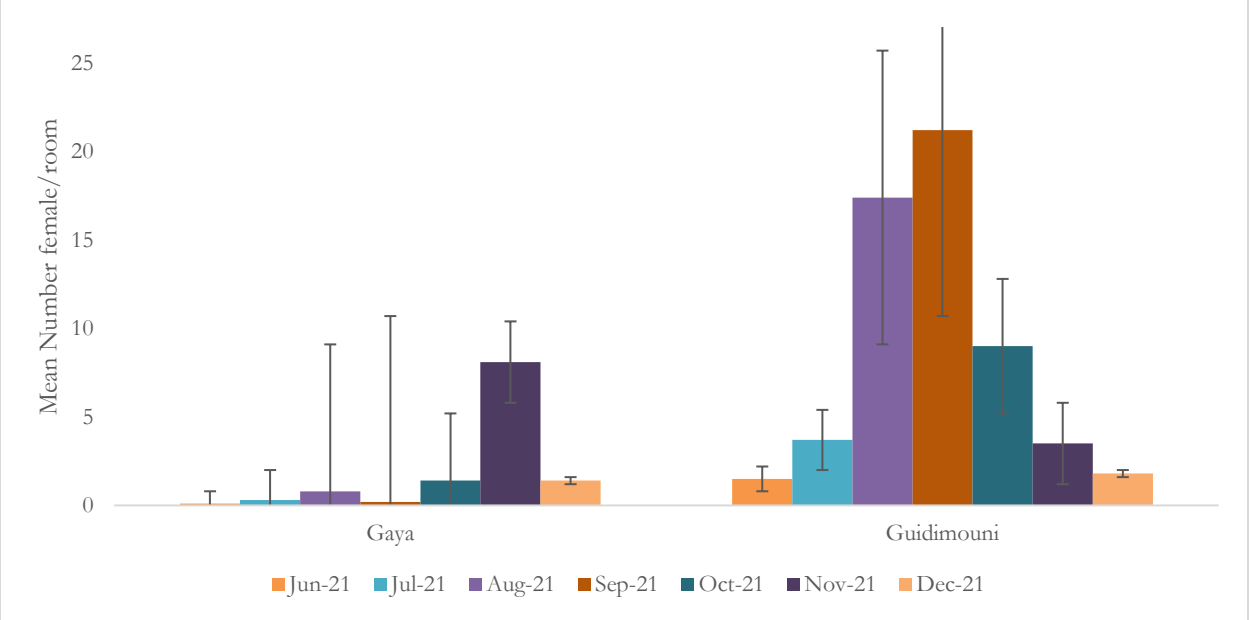


**Error bars represent the standard errors.*

3.1.12 MONTHLY INDOOR RESTING DENSITY OF *AN. FUNESTUS* S.L. COLLECTED USING PSC

The greatest numbers of *An. funestus* s.l. were collected in Gaya and Guidimouni by PSC, at a monthly average of 1.8 and 8.3 f/r, respectively, from June to December (Figure 11). In Balleyara and Niamey V, *An. funestus* s.l. were collected by PSC only in November, and a single specimen of *An. funestus* s.l. was collected in Keita, also in November. Guidimouni recorded the highest indoor resting density in all months.

FIGURE 11: MONTHLY MEAN DENSITY OF AN. FUNESTUS S.L. PER HOUSE, BY SITE



**Error bars represent the standard errors.*

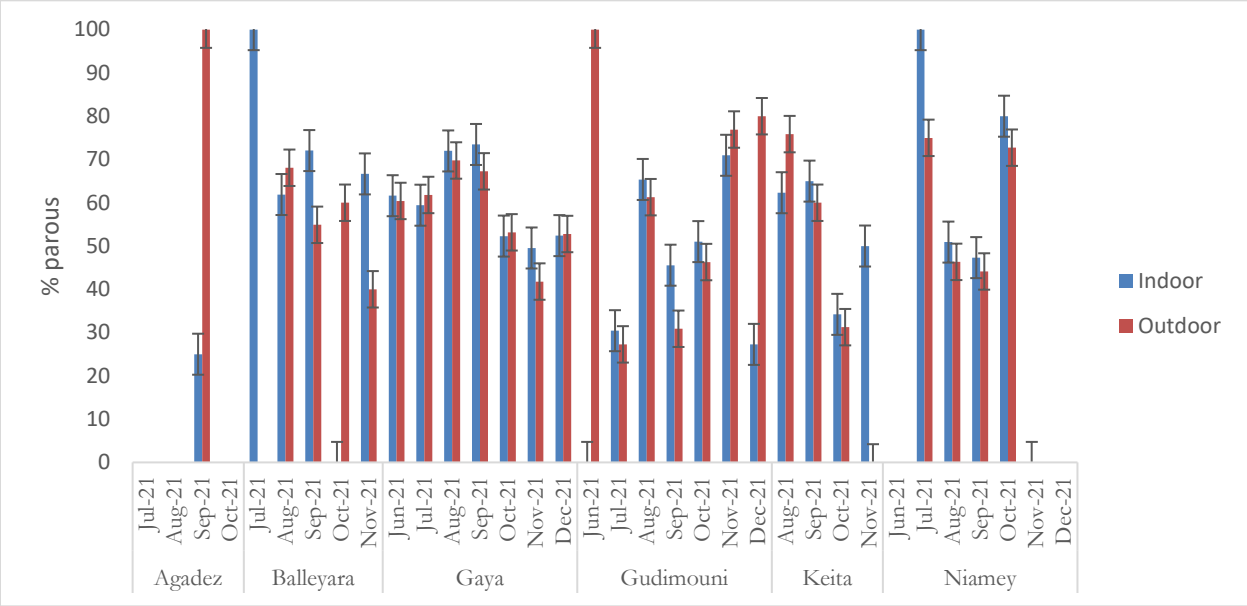
3.1.13 PARITY RATE

3.1.14 PARITY RATE OF *AN. GAMBIAE* S.L.

A total of 2,797 (1,676 indoor, 1,121 outdoor*) An. gambiae* s.l. collected from Agadez (5), Balleyara (230), Gaya (1,437), Guidimouni (426), Keita (303), and Niamey V (396) were dissected to determine parity.

Of the dissected mosquitoes, 2 (40.0%) from Agadez were parous, as were 148 (64.3%) from Balleyara, 854 (59.4%) from Gaya, 200 (46.9%) from Guidimouni, 176 (58.1%) from Keita, and 304 (76.8%) from Niamey V. (Figure 12, Annex Table B4).

FIGURE 12: MONTHLY INDOOR AND OUTDOOR PARITY RATE OF AN. GAMBIAE S.L., BY SITE

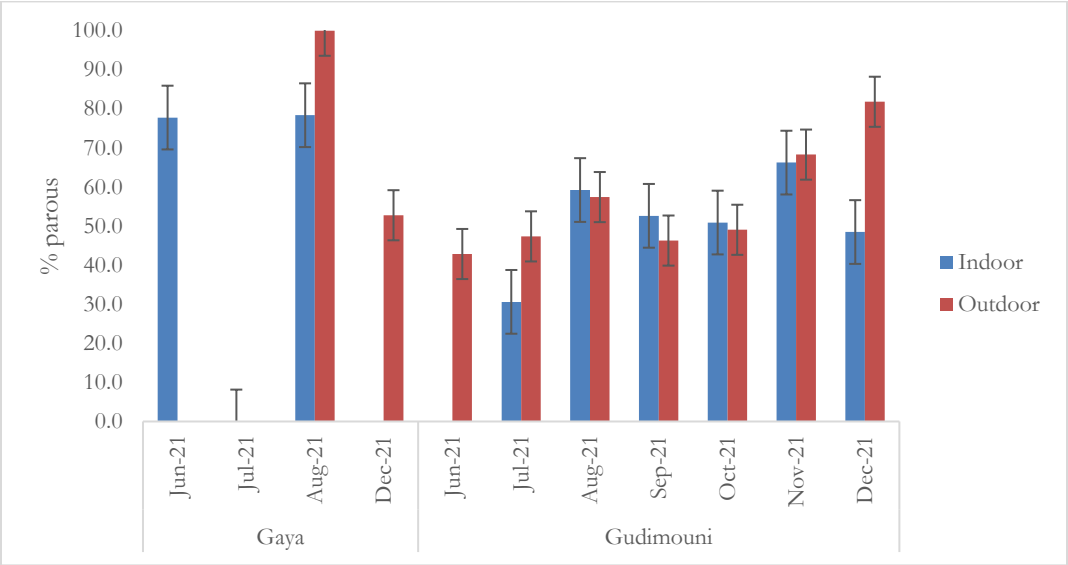


**Error bars represent the standard errors.*

3.1.15 PARITY RATE OF *AN. FUNESTUS* S.L.

A total of 788 (494 indoors, 294 outdoors*) An. funestus* s.l. from Gaya (66) and Guidimouni (714) were dissected for parity assessment. Of the dissected mosquitoes, 46 (69.7%) in Gaya and 387 (54.2%) in Guidimouni were parous. The mean parity rates in both sites were above 50% (Figure 13, Annex Table B4).

FIGURE 13: MONTHLY INDOOR AND OUTDOOR PARITY RATE OF AN. FUNESTUS S.L. IN GAYA AND IN GUIDIMOUNI



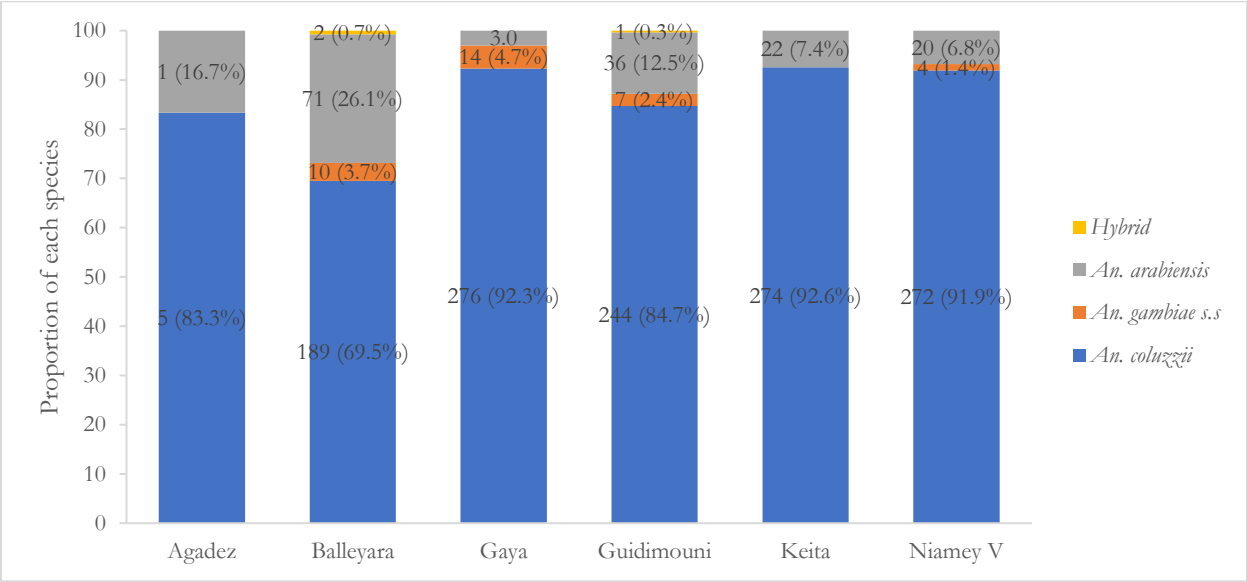
**Error bars represent the standard errors.*

3.2 MOLECULAR CHARACTERIZATION

3.2.1 SPECIES COMPOSITION OF *AN. GAMBIAE* S.L. COLLECTED BY HLC

A total of 1,471 *An. gambiae* s.l. collected by HLC were tested for species composition across all sites and 1457 were successfully amplified. *An. coluzzii* was the predominant species collected (86.5%; n=1,260), followed by *An. arabiensis* (10.9%; n=159), *An. gambiae* (2.4%; n=35). There were 3 hybrid samples of *An. gambiae* s.s./*An. coluzzii*. *An. gambiae* s.s. was collected in four of the six sites: Balleyara (3.7%; n=10), Gaya (4.7%; n=14), Guidimouni (2.4%; n=7), and Niamey V (1.4%; n=4). (Figure 14, Annex Table C1).

FIGURE 14: SPECIES COMPOSITION OF AN. GAMBIAE S.L. COLLECTED BY HLC, BY SITE (N=1,457)



**Note: Numbers in the bars represent the proportion and number per species of the complex.*

3.2.2 SPECIES COMPOSITION OF *AN. GAMBIAE* S.L. COLLECTED BY PSC

Of the 600 *An. gambiae* s.l. analyzed, 593 were successfully amplified. *An. coluzzii* was the predominant species in all sites (81.5%; n=483), followed by *An. arabiensis* (14.2%; n=84) and *An. gambiae* s.s. (4.4%; n=26). *An. gambiae* s.s. was not collected in Guidimouni (Figure 15, Annex Table C1).

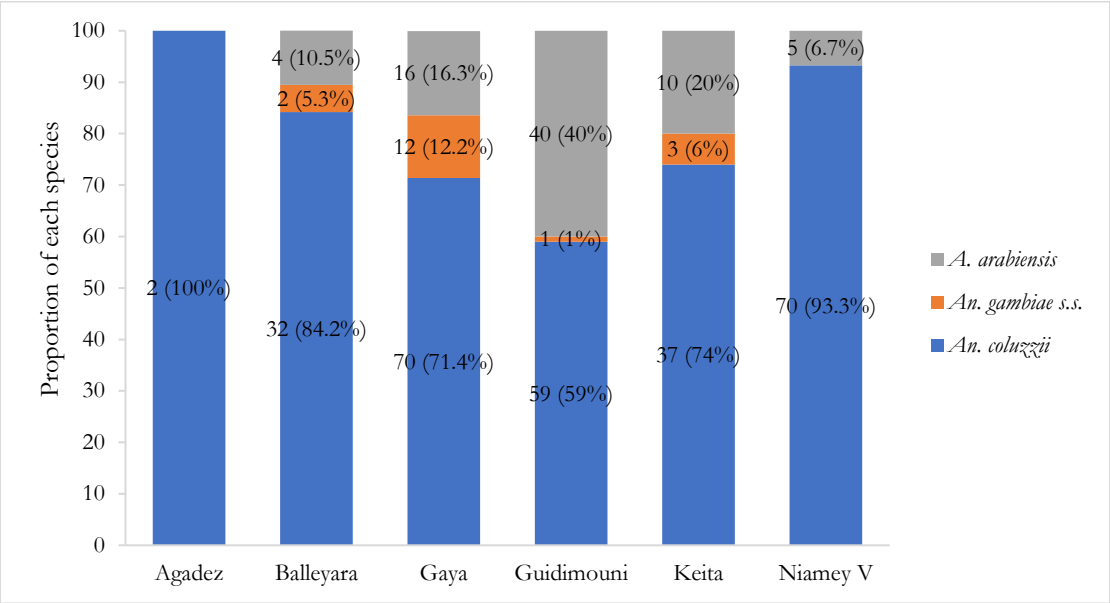
FIGURE 15: SPECIES COMPOSITION OF AN. GAMBIAE S.L. COLLECTED BY PSC, BY SITE (N=593)

Note: Numbers in the bars represent the proportion and number per species of the complex.

3.2.3 SPECIES COMPOSITION OF *AN. GAMBIAE* S.L. COLLECTED BY CDC LT (N=363)

Of the 365 *An. gambiae* s.l. collected by CDC LT and tested across all sites, 363 were successfully amplified. *An. coluzzii* was the predominant species in all sites (74.5%; n=270), followed by *An. arabiensis* (20.7%; n=75) and *An. gambiae* s.s. (4.96%; n=18). No hybrid was found within the CDC LT collected samples (Figure 16, Annex Table C1).

FIGURE 16: SPECIES COMPOSITION OF AN. GAMBIAE S.L. COLLECTED BY CDC LT, BY SITE (N=363)

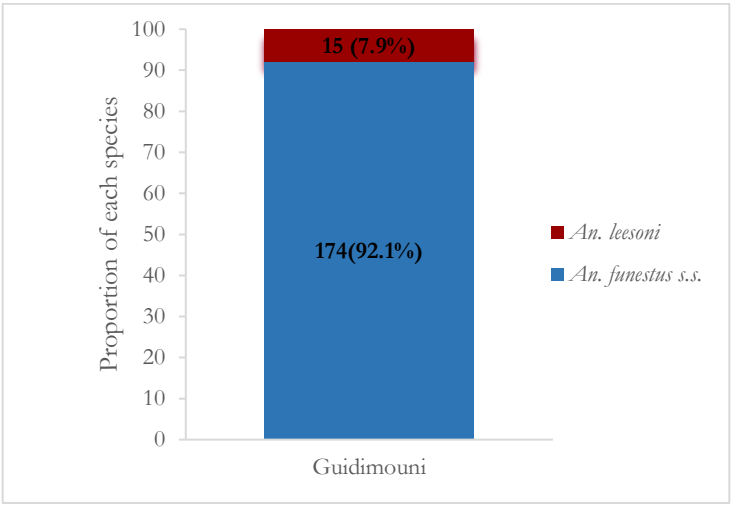


**Note: Numbers in the bars represent the proportion and number per species of the complex.*

3.2.4 SPECIES COMPOSITION OF *AN. FUNESTUS* S.L. COLLECTED BY HLC (N=189)

Of the 666 *An. funestus* s.l. collected by HLC, 200 from Guidimouni were molecularly analyzed for species identification and 189 successfully amplified. *An. funestus* s.s. (92.1%; n=174) was the predominant species over *An. leesoni* (7.9%; n=15) (Figure 17). .

FIGURE 17: SPECIES COMPOSITION OF AN. FUNESTUS GROUP COLLECTED IN GUIDIMOUNI (N=189)



**Note: Numbers in the bars represent the proportion and number per species of the complex.*

3.2.5 *PLASMODIUM* SPOROZOITE INFECTION RATES OF *AN. GAMBIAE* S.L.

Of the 2,078 *An. gambiae* s.l. collected by HLC and tested by PCR, 24 were positive for the *P. falciparum* parasite. Sporozoite infection rates ranged from 0.00% in Agadez to 1.46% in Balleyara, with an average of 1.15% across the six sites (Table 6). The P. falciparum infection rate in *An. gambiae* s.l. indoor (mean of 1.15%) and outdoor

(mean 1.16%) was similar at all sites. Niamey V recorded the highest indoor infection rate (2.15%) followed by Balleyara (1.40%) and Gaya (1.11%), while Guidimouni recorded the highest outdoor infection rate (1.81%), followed by Gaya (1.69%) and Balleyara (1.53%). No circumsporozoite infection was recorded in Agadez in *An. gambiae* s.l. collected by HLC.

TABLE 6: PLASMODIUM SPOROZOITE INFECTION RATES OF AN. GAMBIAE S.L. COLLECTED BY HLC (N=2078)

Localities	Number Tested Indoor	Indoor Positive	Indoor Sporozoite Rate (%)	Number Tested Outdoor	Outdoor Positive	Outdoor Sporozoite Rate (%)	Total Tested	Total Positive	Mean Total Sporozoite Rate (%)
Agadez	3	0	**0.00**	3	0	**0.00**	6	0	**0.00**
Balleyara	143	2	**1.40**	131	2	**1.53**	274	4	**1.46**
Gaya	271	3	**1.11**	177	3	**1.69**	448	6	**1.34**
Guidimouni	229	2	**0.87**	221	4	**1.81**	450	6	**1.33**
Keita	212	1	**0.47**	238	1	**0.42**	450	2	**0.44**
Niamey V	186	4	**2.15**	264	2	**0.76**	450	6	**1.33**
Total	**1,044**	**12**	**1.15**	**1034**	**12**	**1.16**	**2078**	**24**	**1.15**

3.2.6 ENTOMOLOGICAL INOCULATION RATE OF *AN. GAMBIAE* S.L.

High EIR was recorded in the three sites with seven months data collection. Gaya recorded the highest mean EIR with 23.15 infected bites per person per month (ib/p/m), followed by Niamey V (4.41 ib/p/m) and Guidimouni (4.82 ib/p/m). In the two sites with five months collections, Balleyara recorded the highest mean EIR with 3.02 ib/p/m followed by Keita with 1.97 ib/p/m (Table 7, Annex Table C2).

TABLE 7: ENTOMOLOGICAL INOCULATION RATE OF AN. GAMBIAE S.L. COLLECTED BY HLC

Locality	Months of collection	Sporozoite Rate	Mean HBR (b/p/n)	Mean EIR (=ib/p/n)	Mean monthly EIR (ib/p/m)
Agadez	4	0.000	0.25	0.00	0.00
Balleyara	5	**0.015**	6.89	0.10	3.02
Gaya	7	**0.013**	57.59	0.77	23.15
Guidimouni	7	**0.013**	12.07	0.16	4.82
Keita	5	**0.004**	14.90	0.07	1.97
Niamey V	7	**0.013**	11.06	0.15	4.41

3.2.7 *PLASMODIUM* SPOROZOITE INFECTION RATES AND ENTOMOLOGICAL INOCULATION RATES OF *AN. FUNESTUS* S.L.

Of the 200 *An. funestus* s.l. tested by PCR, all from Guidimouni, 4 (2%) were positive for *P. falciparum* parasite (Table 8). Only *An. funestus* s.s. was found to be infected.

The mean monthly EIR of *An. funestus* s.l. in Guidimouni over the five-month collection period was estimated to 17.85 ib/p/m (Table 8).

TABLE 8: ENTOMOLOGICAL INOCULATION RATE OF AN. FUNESTUS S.L. COLLECTED USING HLC IN GUIDIMOUNI

Locality	Total Number Tested	Total Positive	Sporozoite Rate	Mean HBR (b/p/n)	Mean EIR (ib/p/n)	Mean EIR* (ib/p/m)
Guidimouni	200	4	0.02	29.75	0.60	17.85

* *The collection period was seven months.*

3.2.8 HOST PREFERENCE

A total of 565 *An. gambiae* s.l collected using PSC method, from five sites (without Agadez, where no blood-fed specimens were collected) were analyzed by PCR (35 out of 600 failed to amplify) and 64.60% (n=365) were positive showed evidence for human blood meal (Table 9). The highest human blood index was observed in Guidimouni (74.77%; n=83), followed by Balleyara (71.05%; n=81) and Niamey V (61.21%, n=71). Overall 5.84%, 6.02% and 23.54% samples tested were positive for sheep, goat and cow blood meals respectively with the highest (31.90%) animal (cow) blood index recorded in Niamey V.

TABLE 9: BLOOD MEAL SOURCES AND HUMAN BLOOD INDEX OF AN. GAMBIAE S.L. COLLECTED USING PSC, BY SITE

Sites	Total Tested	Human		Goat		Cow		Sheep	
		N	%	N	%	N	%	N	%
Balleyara	114	81	71.05	9	7.89	20	17.54	4	3.51
Gaya	112	70	62.50	4	3.57	27	24.11	11	9.82
Guidimouni	111	83	74.77	4	3.60	18	16.22	6	5.41
Keita	112	60	53.57	13	11.61	31	27.68	8	7.14
Niamey V	116	71	61.21	4	3.45	37	31.90	4	3.45
Total	565	365	64.60	34	6.02	133	23.54	33	5.84

3.3 INSECTICIDE RESISTANCE MONITORING

3.3.1 INSECTICIDE SUSCEPTIBILITY AGAINST *AN. GAMBIAE* S.L.

Between August and December 2021, PMI VectorLink Niger completed insecticide resistance testing on the *An. gambiae* s.l. collected in the 15 sites (Figures 18–23, Annex Table D1), and on the *An. funestus* s.l. in Guidimouni. (Figure 24).

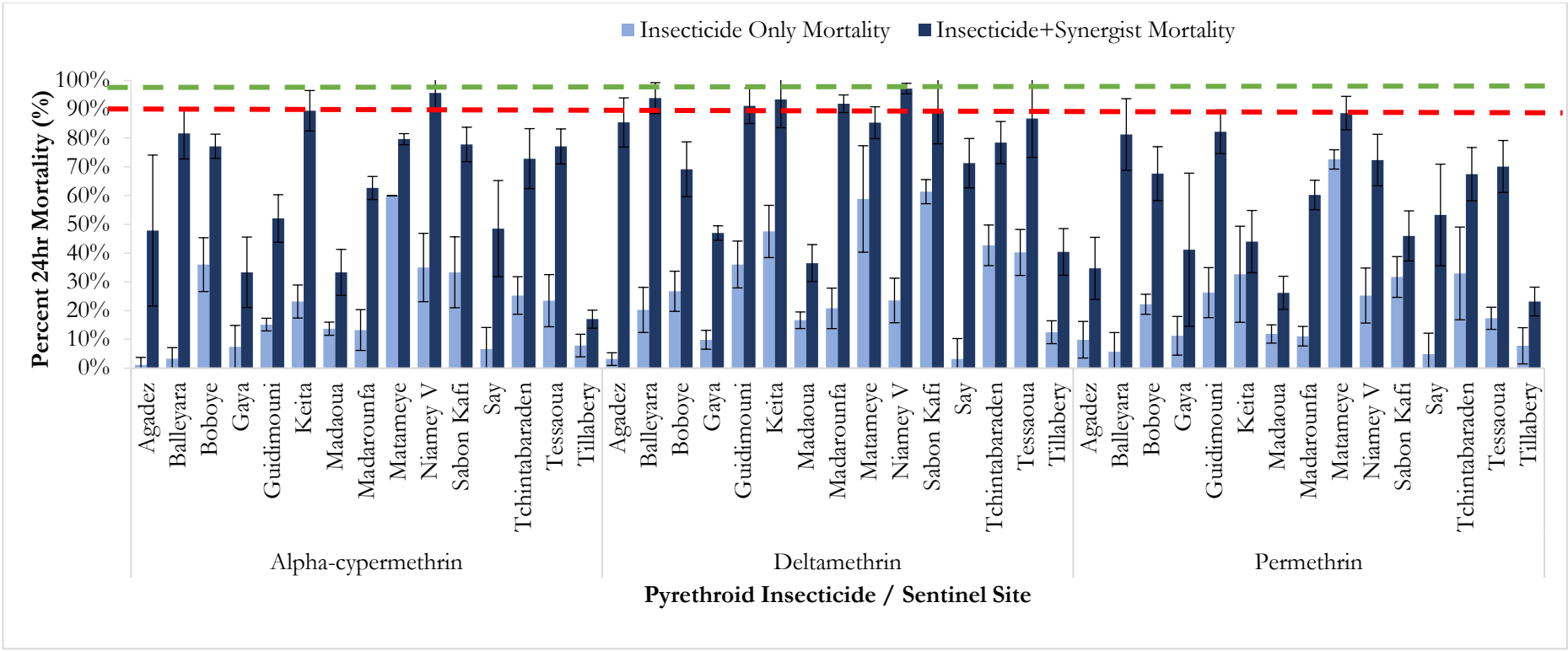
As found in 2020, resistance to the three pyrethroids tested (deltamethrin, permethrin, and alpha-cypermethrin) was observed in all 15 sites. Exposure of *An. gambiae* s.l. to PBO before exposure to the pyrethroids did not completely reverse the resistance status of the mosquito populations except with alpha-cypermethrin in Niamey V and with deltamethrin in Balleyara, Guidimouni, Keita, Madarounfa, and Niamey V. Nonetheless, a substantial increase in mortality was observed for pyrethroids overall and particularly for deltamethrin. High resistance intensity to alpha-cypermethrin, deltamethrin, and permethrin was observed at all sites, except for deltamethrin in Guidimouni, Keita, and Niamey V, where the resistance was moderate.

An. gambiae s.l. was susceptible to pirimiphos-methyl in seven of the 15 sites and resistant in the other eight sites at a moderate resistance intensity. Susceptibility to chlorfenapyr at the dose of 100 µg/bottle was recorded in six of the 15 sites (Balleyara, Gaya, Guidimouni, Keita, Tessaoua, and Tillabery,), while the nine remaining

sites recorded susceptibility at 200ug/bottle. Susceptibility to clothianidin 2% and 4µg/bottle was recorded at 14 of the 15 sites tested. The test could not be carried out in Madaoua due to a limited number of larvae.

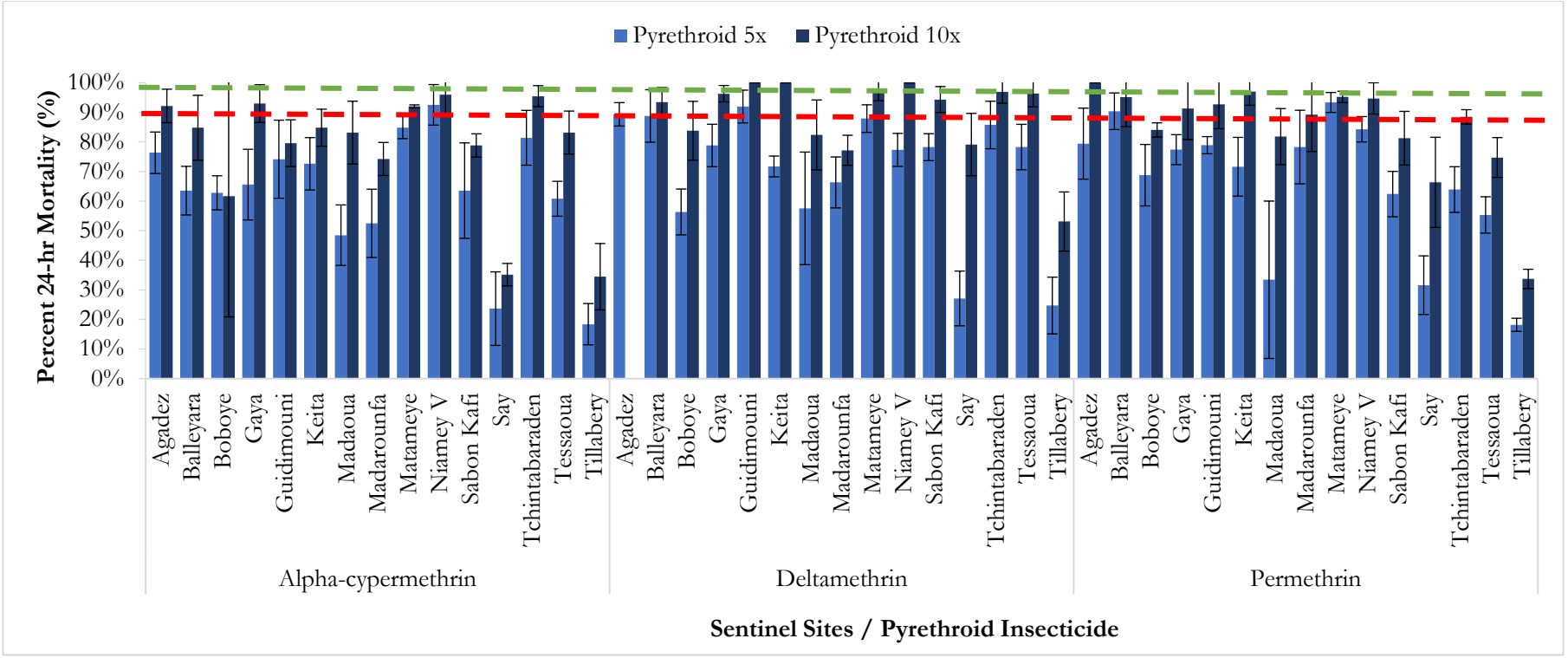
Note: The horizontal dashed red line in Figures 18–24 represents the 90% threshold for resistance and the green line represents the 98% threshold for susceptibility.

FIGURE 18: 24-HOUR MORTALITY OF AN. GAMBIAE S.L. AFTER EXPOSURE TO PYRETHROIDS (ALPHA-CYPERMETHRIN 0.05%, DELTAMETHRIN 0.05%, AND PERMETHRIN 0.75%), WITH AND WITHOUT PRE-EXPOSURE TO PBO 4%, BY SITE



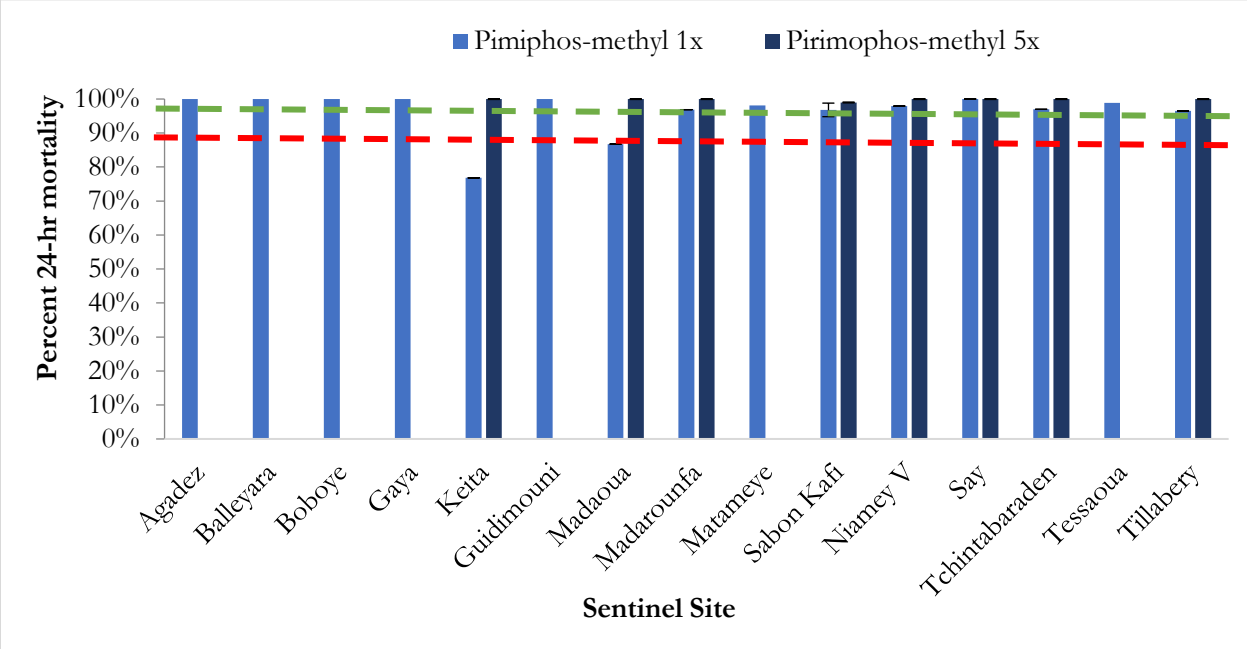
**Error bars represent the standard deviation.*

FIGURE 19: INTENSITY ASSAY OF PYRETHROIDS (ALPHA-CYPERMETHRIN (0.25% AND 0.5%), DELTAMETHRIN (0.25% AND 0.5%), AND PERMETHRIN (3.5% AND 7.5%) AGAINST AN. GAMBIAE S.L., BY SITE



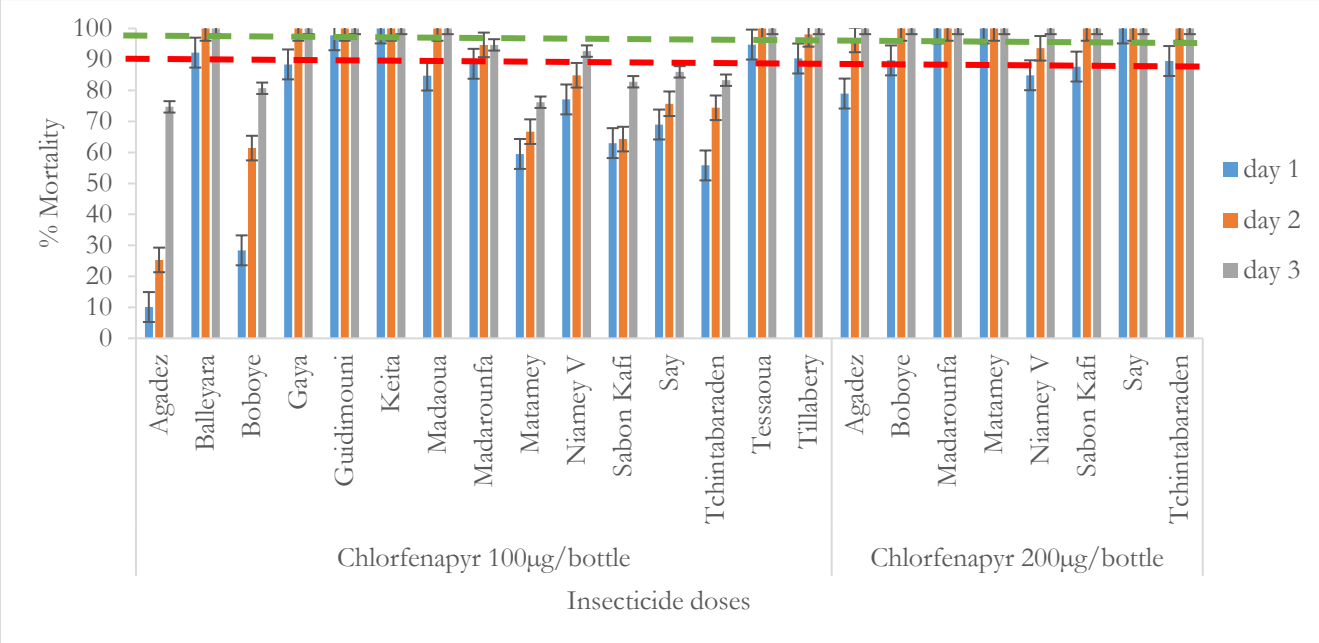
**Error bars represent the standard deviation.*

FIGURE 20: 24-HOUR MORTALITY OF AN. GAMBIAE S.I. AFTER EXPOSURE TO PIRIMIPHOS-METHYL 0.25%, BY SITE



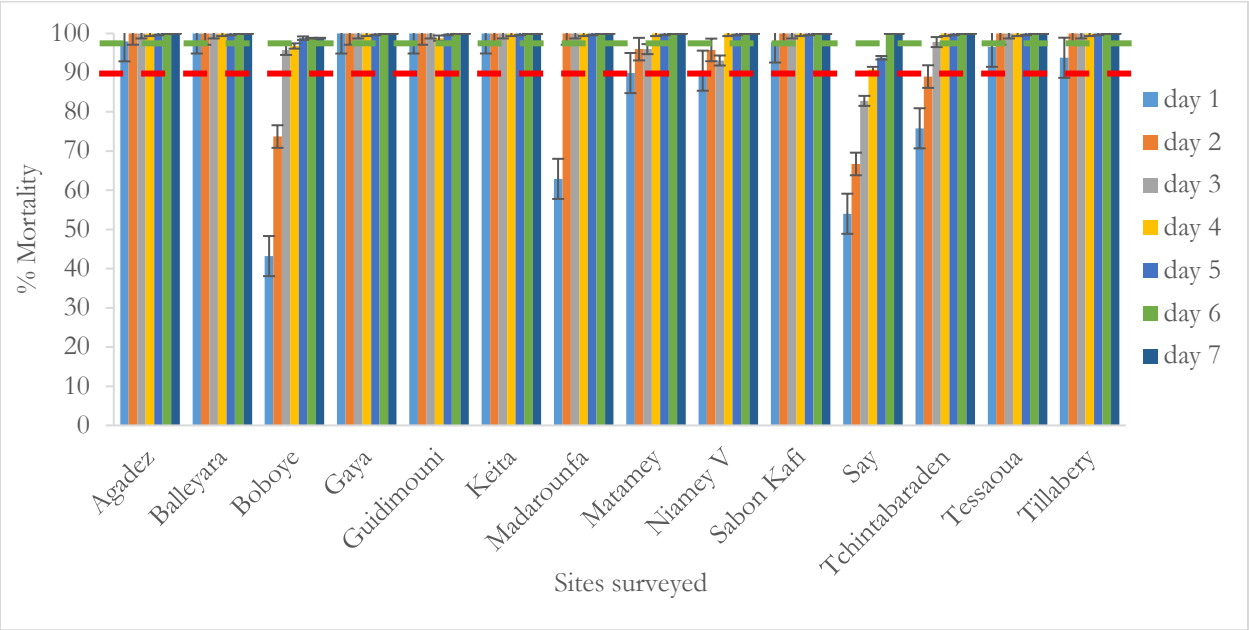
**Error bars represent the standard deviation.*

FIGURE 21: SUSCEPTIBILITY OF AN. GAMBIAE S.L. TO CHLORFENAPYR 100 µG/BOTTLE AND 200 µG/BOTTLE, BY SITE



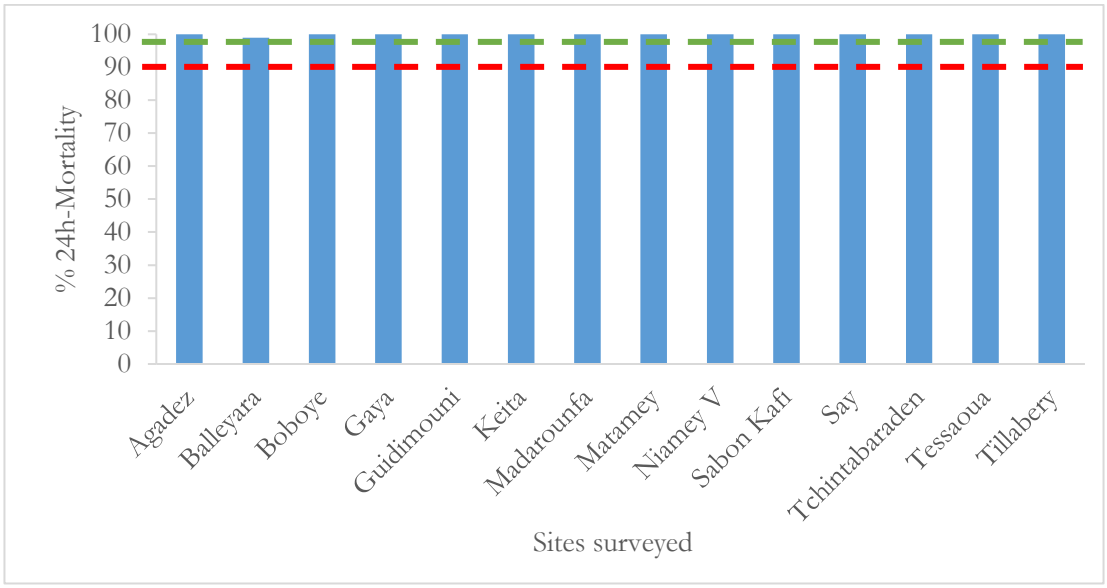
**Error bars represent the standard error.*

FIGURE 22: SUSCEPTIBILITY OF AN. GAMBIAE S.L. TO CLOTHIANIDIN 2%, BY SITE



**Error bars represent the standard error.*

FIGURE 23: SUSCEPTIBILITY OF AN. GAMBIAE S.L. TO CLOTHIANIDIN 4µG/BOTTLE BY SITE TO CLOTHIANIDIN 4 µG /BOTTLE AT GUIDIMOUNI

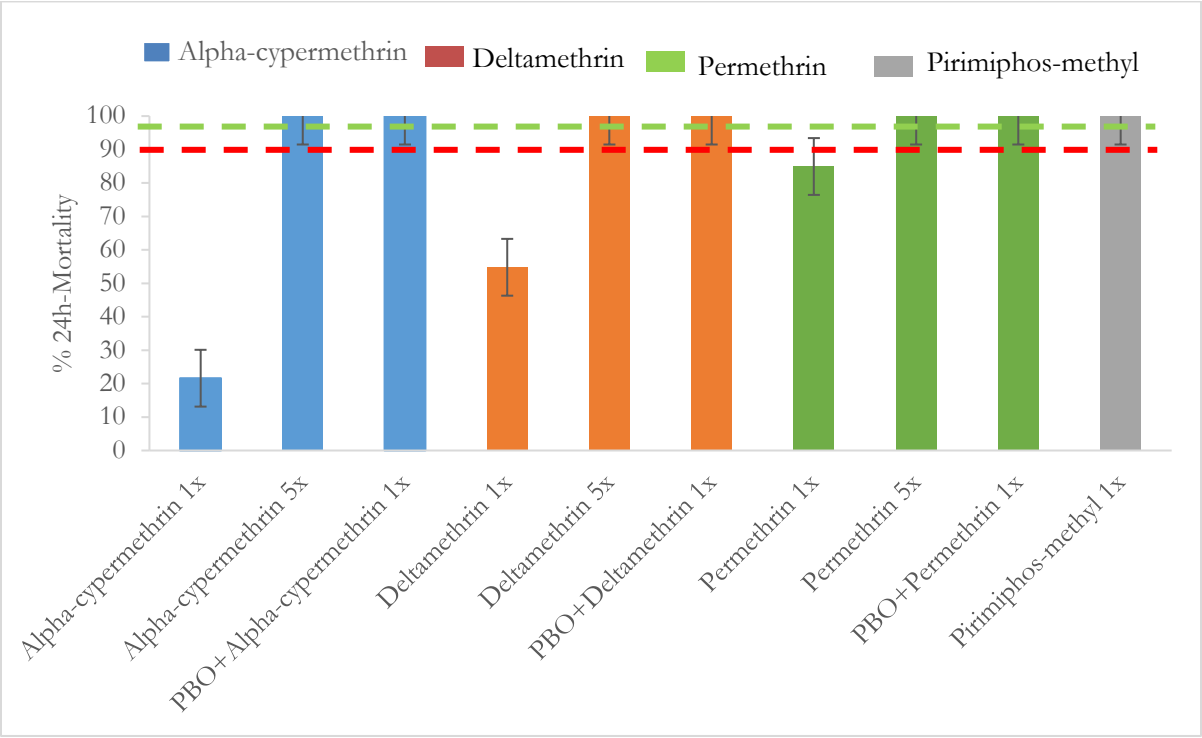


3.3.2 INSECTICIDE SUSCEPTIBILITY AGAINST *AN. FUNESTUS* S.L.

In 2021, insecticide resistance tests were conducted against *An. funestus* s.l. at Guidimouni, where the species was found in large numbers during adult mosquito collection. *An. funestus* s.l. was resistant to all three pyrethroids (22% mortality for alpha-cypermethrin, 55% for deltamethrin, and 85% for permethrin) tested. Synergism using PBO restored full susceptibility and the resistance intensity was found to be low for all three pyrethroids (Figure 24). Furthermore, full susceptibility (100%) of *An. funestus* s.l. was recorded against

diagnostic concentration of pirimiphos-methyl (Figure 24). Chlorfenapyr (100 µg/bottle) tested using the CDC bottle assay and clothianidin at the dose of 2%, also yielded 100% mortality (susceptibility per 3 or 7 days mortality recording) but not shown in the figure).

FIGURE 24: SUSCEPTIBILITY, PBO SYNERGISM AND RESISTANCE INTENSITY OF AN. FUNESTUS S.L. TO PYRETHROIDS (ALPHA-CYPERMETHRIN 0.05% DELTAMETHRIN 0.05%, PERMETHRIN 0.75%) AND SUSCEPTIBILITY TO PIRIMIPHOS-METHYL 0.25% AT GUIDIMOUNI

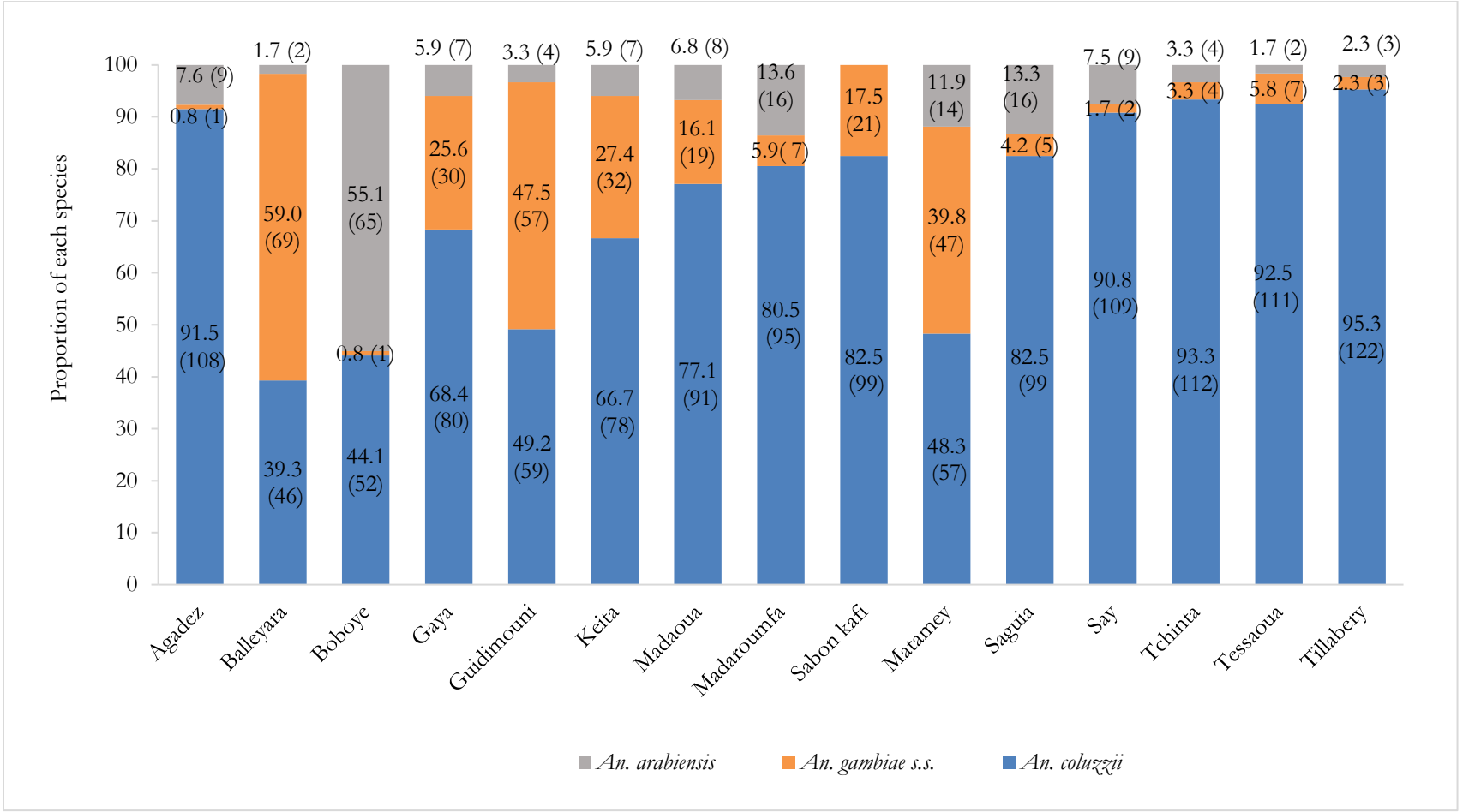


3.4 SPECIES COMPOSITION OF *AN. GAMBIAE* S.L. TESTED FOR INSECTICIDE SUSCEPTIBILITY

A total of 1,810 randomly selected mosquitoes from among the *An. gambiae* s.l. mosquitoes tested for insecticide susceptibility across all 15 sites were analyzed for species identification and for target site mutation mechanisms such as the *kdr* markers: *kdr-w*, *kdr-e,* and *Ace-1*.

Out of the 1,810 analyzed for species identification, 1,789 were successfully amplified (21 failed) for the detection of *An. gambiae* s.l. species complex. Of the number amplified, 1,318 were *An. coluzzii* (73.7%), followed by *An. gambiae* s.s. (17.1%; n=305). The proportion of *An. coluzzii* was below 50% in only four of the 15 sites (Balleyara (39.3%, n=46), Boboye (44.1%, n=52), Guidimouni (49.2%, n=59), and Matamey (48.3%, n=57)). *An. arabiensis* (166; 9.3%) was found in all 15 sites except Sabon Kafi (Figure 25, Annex Table D3).

FIGURE 25: SPECIES COMPOSITION OF AN. GAMBIAE S.L. OF MOSQUITOES TESTED FOR INSECTICIDE SUSCEPTIBILITY MONITORING, BY SITE (N=1,789), FOR EACH BAR, THE % OF EACH SPECIES IS FOLLOWED BY THE NUMBER TESTED IN BRACKET



Note: Numbers in the bars represent the proportion and number per species of the complex.

3.5 INSECTICIDE RESISTANCE MARKERS

Out of 1,787 mosquitos tested for resistance mechanism across the 15 sites (23 of the 1,810 failed to amplify), the *kdr-w* frequencies varied from 44% to 78% with a mean of 66% across all sites. The highest *kdr-w* frequency was recorded in Gaya and Madaoua, each with 78%, followed by Tillabery (76%), while the lowest was in Boboye (44%). The highest *ace-1* allele frequency was found in Balleyara (18%), followed by Madaoua (16%). The PCR results showed the presence of *kdr-e* with frequencies varying from 11% in Boboye to 68% in Gaya (Table 10, Annex Table D2), which is consistent with the 2020 results.

TABLE 10: TARGET SITE INSECTICIDE RESISTANCE MECHANISM ACROSS SITES (N=1,787)

Localities	N tested	Mutation *kdr -west*				Mutation *kdr*-east				Mutation *ace*-1			
		RR	RS	SS	freq	RR	RS	SS	freq	RR	RS	SS	freq
Agadez	118	72	17	29	0.68	50	14	54	0.48	10	6	102	0.11
Balleyara	119	54	38	27	0.61	23	22	74	0.29	18	6	95	0.18
Boboye	118	45	14	59	0.44	12	1	105	0.11	14	2	102	0.13
Gaya	118	83	18	17	0.78	67	27	24	0.68	7	3	108	0.07
Guidimouni	117	66	28	23	0.68	48	21	48	0.50	9	4	104	0.09
Keita	118	45	37	36	0.54	46	13	59	0.44	12	7	99	0.13
Madaoua	119	79	27	13	0.78	68	17	34	0.64	18	3	98	0.16
Madarounfa	118	65	23	30	0.65	59	17	42	0.57	13	3	102	0.12
Matamey	117	50	18	49	0.50	56	35	26	0.63	8	5	104	0.09
Niamey V	120	76	15	29	0.70	60	17	43	0.57	13	8	99	0.14
Sabon Kafi	117	71	19	27	0.69	15	0	102	0.13	12	4	101	0.12
Say	119	74	21	24	0.71	66	2	51	0.56	9	5	105	0.10
Tchintabaraden	120	52	36	32	0.58	49	16	55	0.48	9	3	108	0.09
Tessaoua	120	78	22	20	0.74	46	34	40	0.53	11	7	102	0.12
Tillabery	129	80	35	14	0.76	69	29	31	0.65	11	4	114	0.10
Total	**1787**	**990**	**368**	**429**	**0.66**	**734**	**265**	**788**	**0.48**	**174**	**70**	**1543**	**0.12**

Note: freq=frequency; RR=homozygous resistant; RS=heterozygous resistant; SS=homozygous susceptible

4. DISCUSSION AND CONCLUSION

PMI VectorLink Niger conducted longitudinal vector surveillance data collection monthly from June to December 2021 in six selected sentinel sites, on a site-specific collection timeline that considered the geographical location of each site. A total of 35 collection efforts were done across all six sites.

Bionomic data collected using HLC, PSC, and CDC LT methods showed that *An. gambiae* s.l. was the predominant malaria vector (82.4% of the total *Anopheles* collected at all sites). However, the percentage *of An. gambiae* s.l. collected was 7.9% less than that of 2020 in the same six sites and timelines. *An. funestus* s.l. represented the second major *Anopheles* vector (16.4% of all *Anopheles* collected at all sites) and recorded an increased percentage of 10.1% compared to 2020. More *An. gambiae* s.l. were collected through PSC than through HLC or CDC LTs indoor , suggesting that vectors prefer to rest inside dwellings. This trend supports the continued focus on universal ITN coverage, the main vector control intervention implemented in Niger to prevent malaria transmission. Of the 19,698 *Anopheles* collected, PSC produced about 11,055 , and Gaya recorded the highest density among the six sites, followed by Niamey V. Both sites are irrigated rice field areas, which are favorable and permanent larval habitats. With the higher density of *An. gambiae* s.l. recorded, particularly in Gaya and Niamey V, and considering their agricultural status, it may be appropriate to implement additional vector control interventions such as larval source management to target larval stages during rice cultivation and reduce the number of emerging mosquitoes.

The results of the HLCs also showed that the peak biting time was between 10:00 pm and 3:00 am on average in all sites, both indoors and outdoors. The *An. gambiae* s.l. indoor and outdoor HBRs were higher in Gaya for all months surveyed compared to the other sites, except in September in Keita both indoors and outdoors. The peak biting time observed was similar to those recorded in previous years (2019, 2020) in all the sites where the collection was conducted.

Furthermore, in 2021, *An. funestus* s.l. was found in five of the six sites compared to two sites in 2020. with the highest density recorded in Guidimouni through all three collection methods. The predominance of *An. funestus* s.l. in Guidimouni was similar to the trends recorded during the previous years' surveys with peak densities recorded in August and September. Though *An. funestus* s.l. was found in five sites, Gaya recorded the second highest number after Guidimouni, while few specimens were recorded in the other three sites.

The molecular analysis of samples collected showed that *An. coluzzii* was the predominant malaria vector species in the 15 sites surveyed for insecticide susceptibility and also in the six bionomics monitoring sites regardless of the collection method. However, a variation in *An. gambiae* s.s. and *An. arabiensis* species proportions was observed in all the sites with *An. gambiae* s.s. found in all sites, but at a lower proportion than in 2020. *An. arabiensis* was recorded through all collection methods in all sites except by CDC LT in Agadez. No specimen was collected through PSC in Agadez. Unlike in 2020, where hybrids *An. gambiae./An. coluzzii* were found in 10 sites in in 2021, hybrid was found in only three sites (Balleyara, Guidimouni, and Niamey V).

For the *An. funestus* group in Guidimouni, two sub-species were identified in 2021 including *An. funestus* s.s. as the predominant species, and *An. leesoni*. The data recorded each year within *An. funestus* group species showed that several species could be found in Guidimouni as *An. rivulorum-like* was detected in 2019 and *An. parensis* in 2019 and 2020. This species composition will need further investigation by sampling a larger number of specimens for laboratory analysis, accounting for seasonality.

The overall parity rate was high across all sites, exceeding 50.0% except in Agadez and Gaya, showing a high probability for vectors to carry the malaria parasite. The 2021 rate was similar to that recorded in 2019 and 2020 with an average of 55.2% parous vectors. Niamey V recorded the highest parity rate in 2021 with an average of 76.8%. The data also showed that *An. gambiae* s.l. fed on both humans and animals with a high rate of human-vector contact. Hence, the need to increase awareness of the correct use of ITNs and support the implementation of complementary vector control interventions to reduce the *Anopheles* mosquito longevity in Niger.

For three consecutive years (2019, 2021, 2021), Gaya recorded the highest EIR, followed by Balleyara and Niamey V. Furthermore, Gaya, Guidimouni, Niamey V, and Balleyara recorded the highest sporozoite infection rates among the *An. gambiae* s.l. collected by HLC, thereby increasing the risk of populations to malaria during the peak transmission period between August and October. No infected mosquito was recorded in Agadez as observed in previous years, consistent with its very low endemicity status as described by the epidemiological map of the country. Therefore, an integrated vector control and case management strategy using additional measures such as a combination of interventions (ITN mass campaign distribution, Seasonal Malaria Chemoprevention, indoor residual spraying, larviciding, etc.) could be put in place to accelerate malaria elimination in the very low transmission zones.

Similar to 2020, the mean EIR of *An. funestus* s.l. was 17.85 ib/p/m in Guidimouni higher than that of *An. gambiae* s.l. (4.82 ib/p/m) at this site. Transmission is persistent in both the meso- and hyper-endemic areas located in the southern and wettest part of the country, where the highest EIRs were recorded. Despite the ITN distribution campaigns for universal coverage in all districts of these areas, the continuous malaria transmission observed could be attributed to the limited utilization of distributed ITNs among the entire population, which is around 60% for children under five years old and 80% among pregnant women according to the 2021 Niger Malaria Indicator Survey. The transmission level in the different parts of the country may warrant reinforcement of malaria prevention communication to encourage the population to practice prevention. Furthermore, it was observed that the mosquitoes were equally infected indoors and outdoors in the sites surveyed. This is another cause of concern as the current vector control intervention implemented by the NMCP only target indoor biting and resting vectors.

An. gambiae s.l. was resistant to all three pyrethroids tested (alpha-cypermethrin, deltamethrin, and permethrin) in all 15 sites, consistent with trends reported for the past three years. High pyrethroid resistance was observed in all sites except in Guidimouni, Keita, and Niamey V, with moderate resistance for deltamethrin, and in Agadez for permethrin. Exposure to PBO before exposure to the three pyrethroids considerably increased the mortality of the mosquitoes but did not restore full susceptibility in any site. This suggests that enzymes such as P450s may be involved in the insecticide resistance of the vectors in some sites. Also, chlorfenapyr at the dose of 100µg/bottle yielded susceptibility in six of the 15 sites tested. In the nine other sites, susceptibility was recorded at the dose of 200 µg/bottle. These data, collected by the project for four consecutive years, have informed and guided the NMCP's malaria vector control strategy decisions, particularly for ITN procurement and the distribution approach for the future ITN mass distribution campaign. The NMCP and its partners have taken the results of the resistance to pyrethroids into consideration to begin the distribution of the new generation ITNs (PBO ITNs and Interceptor G2) for the 2022 ITN mass distribution campaign and for all future mass and routine distribution. However, the NMCP should prioritize efforts to enhance the use of ITNs within communities especially intensifying messages on mosquito peak biting times for better prevention against vector bites and reduced malaria transmission.

Susceptibility of *An. gambiae* s.l. to pirimiphos-methyl was observed in seven of the 15 sites while moderate resistance intensity was observed in the remaining eight sites. Nevertheless, caution needs to be taken with the use of organophosphate-based pesticides that are commonly used in agriculture in several countries, including

Niger. On the other hand, *An. gambiae* s.l. was susceptible to clothianidin at the dose of 2% in all sites surveyed as observed in previous years. The test of clothianidin at the dose of 4 ug/bottle conducted in 2021 showed full susceptibility of *An. gambiae* s.l. at all sites. These results may inform the choice of insecticide should the NMCP ever consider implementing indoor residual spraying in Niger.

As recommended in 2020, PMI VectorLink Niger conducted insecticide resistance monitoring to determine the insecticide susceptibility of *An. funestus* s.l. in Guidimouni and found that the vector was resistant to all pyrethroids (with a low resistance intensity. Pre-exposure to PBO restored full susceptibility to all pyrethroids. Full susceptibility of *An. funestus* s.l. to pirimiphos-methyl, clothianidin, and chlorfenapyr was recorded.

Both *kdr*-w and *kdr*-e, and *ace*-1 mutations were present in *An. gambiae* s.l. population in all 15 sites and thus were involved in the high resistance observed within the vector populations to pyrethroids and pirimiphos methyl in most of the sites. In 2021, the *kdr*-e frequencies were not as high as in 2020 compared to the *kdr* -w but they were still above 50% except in Boboye, Sabon Kafi, Balleyara, Agadez, and Tchintabaraden. High *kdr*-w frequencies were also recorded in all sites except Matamey. Gaya and Madaoua recorded the highest frequency of both *kdr*-w (78%) followed by Tillabery (76%) and Tessaoua (74%). For *kdr*-e, Gaya also recorded the highest frequency (68%) followed by Tillabery (65%) and Matamey (63%). All these sites are agricultural areas with intensive use of pesticides in addition to having received pyrethroid-only ITNs during universal coverage from the most recent mass distribution campaign. The *ace*-1 frequencies were still low in the country, with Balleyara recording the highest frequencies followed by Madaoua and Niamey V. However, *ace*-1 frequencies have increased since 2018, warranting a close follow-up of the use of carbamate and organophosphate-based insecticides in agriculture across the country.

Per the recent review and validation of the country's integrated vector control and insecticide resistance management plan, the NMCP may need to continuously consider the data collected annually to adapt vector control strategies and resistance management considering the available data in other to propose appropriate vector control tools that could be effective in the different target districts of the country. The analysis of the data generated during the past four years has supported a strategic intervention plan with the introduction of PBO-ITNs in the country in 2022. Though, the data provided by VectorLink over the past four years guided the NMCP on procuring PBO-ITNs and interceptor G2 nets to be distributed during the 2022 mass distribution campaign the NMCP should continue aiming for larger coverage of the endemic areas with new types of ITNs (interceptor G2 and PBO Nets).. Whereas current NMCP policy places ITNs at the forefront of vector control strategies, the present vector bionomic data (density and EIR) indicate high malaria transmission and high human/vector contact within the most populated part of the country. This calls for additional vector control interventions such as indoor residual spraying or larval source management in area of rice cultivation, to complement the distribution and use of ITNs.

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ANNEX A: SPECIES COMPOSITION

TABLE A1: SPECIES COMPOSITION PER SITE OF MOSQUITOES COLLECTED USING HLC

Sentinel Sites	Species	Jun-21		Jul-21		Aug-21		Sep-21		Oct-21		Nov-21		De-21c		Total		Total
		In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	
Agadez	*An. funestus s.l.*			0	0	0	0	0	0	0	0					0	0	0
	An. gambiae s.l.			0	0	0	0	6	2	0	0					6	2	8
Balleyara	*An. funestus s.l.*			0	0	0	0	0	5	0	0	1	0			1	5	6
	An. gambiae s.l.			4	1	46	54	75	66	8	6	9	6			142	133	275
Gaya	*An. funestus s.l.*	10	0	4	0	46	5	1	0	63	10	10	3	9	4	143	22	165
	An. gambiae s.l.	138	65	282	182	446	164	205	389	417	158	218	125	380	55	2086	1138	3224
	An. nili	0	0	1	0	1	0	1	0	0	0	0	3	0	0	3	3	6
	An. pharoensis	4	4	1	2	1	4	0	6	1	2	3	3	11	6	21	27	48
	An. rufipes	0	0	2	2	0	0	15	18	0	0	6	1	0	0	23	21	44
Guidimouni	*An. funestus s.l.*	6	8	62	24	237	236	251	224	203	232	92	45	33	12	884	781	1665
	An. gambiae s.l.	1	1	54	12	32	40	171	117	73	92	34	14	18	16	383	292	675
	An. pharoensis	0	0	0	0	1	1	0	0	2	0	0	1	0	0	3	2	5
Keita	*An. funestus s.l.*			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	An. gambiae s.l.			0	0	72	34	176	150	102	56	4	2			354	242	596
	An. rufipes			0	0	0	0	0	0	4	0	0	0	0	0	4	0	4
Niamey V	*An. coustani*	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1
	An. funestus s.l.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	An. gambiae s.l.	0	0	11	4	97	171	149	133	27	21	4	1	0	0	288	330	618
	An. pharoensis	0	0	0	0	0	0	6	10	2	2	0	0	0	0	8	12	20
	An. rufipes	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1
	Total	159	78	421	227	979	709	1056	1121	903	579	381	204	451	93	4350	3011	7361

***Agadez has 4 months of collection (July-October); Balleyara and Keita 5 months (July-November); Gaya, Guidimouni, and Niamey V 6 months (June-December)**

TABLE A2: SPECIES COMPOSITION PER SITE OF MOSQUITOES COLLECTED USING PSC

Sentinel Sites	Species	Jun-21	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21	Total
Agadez	*An. funestus s.l.*		0	0	0	0			0
	An. gambiae s.l.		0	0	27	0			27
Balleyara	*An. funestus s.l.*		0	0	0	0	2		2
	An. gambiae s.l.		13	212	104	5	15		349
	An. rufipes		0	1	5	1	0		7
Gaya	*An. funestus s.l.*	1	3	8	2	14	81	14	123
	An. gambiae s.l.	331	2,475	1,366	1,480	593	755	942	7942
	An. nili	0	0	0	0	0	1	0	1
	An. pharoensis	0	0	1	0	0	1	1	3
	An. rufipes	10	0	1	0	5	3	0	19
Guidimouni	*An. funestus s.l.*	15	37	174	212	90	35	18	581
	An. gambiae s.l.	54	49	186	268	108	25	2	692
	An. pharoensis	1	0	0	0	0	0	0	1
	An. rufipes	0	1	0	1	0	0	0	2
	An. ziemanni	0	0	1	0	0	0	0	1
Keita	*An. funestus s.l.*		0	0	1	0	0		1
	An. gambiae s.l.		0	49	357	98	16		520
Niamey V	*An. funestus s.l.*	0	0	0	0	0	9	0	9
	An. gambiae s.l.	1	12	183	431	92	39	1	759
	An. nili	0	0	0	0	0	0	0	
	An. pharoensis	0	0	0	0	0	0	0	
	An. rufipes	0	0	0	16	0	0	0	16
	Total	413	2,591	2,181	2,904	1,006	982	978	11,055

***Agadez has 4 months of collection (July-October); Balleyara and Keita 5 months (July-November); Gaya, Guidimouni, and Niamey V 6 months (June-December).**

TABLE A3: SPECIES COMPOSITION PER SITE OF MOSQUITOES COLLECTED USING CDC LT

Sentinel Sites	Species	Jun-21	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21	Total
Agadez	*An. funestus s.l.*		0	0	0	0			0
	An. gambiae s.l.		0	0	2	0			2
Balleyara	*An. funestus s.l.*		0	0	0	1	0		1
	An. gambiae s.l.		5	14	19	0	0		38
	An. rufipes		0	0	1	1	0		2
Gaya	*An. funestus s.l.*	1	0	0	0	0	0	7	8
	An. gambiae s.l.	24	25	2	10	2	5	198	266
	An. pharoensis	0	0	0	0	0	0	5	5
	An. rufipes	38	1	0	0	0	1	0	40
	An. ziemanni	0	0	0	0	3	0	0	3
Guidimouni	*An. funestus s.l.*	2	0	42	449	145	4	24	666
	An. gambiae s.l.	1	0	3	42	40	20	3	109
	An. pharoensis	0	0	0	0	6	0	0	6
	An. rufipes	0	0	0	0	3	0	1	4
	An. ziemanni	0	0	0	0	1	0	0	1
Keita	*An. funestus s.l.*		0	3	1	0	0		4
	An. gambiae s.l.		0	8	21	21	0		50
Niamey V	*An. funestus s.l.*	0	0	0	0	0	0	0	0
	An. gambiae s.l.	0	0	65	2	8	0	0	75
	An. pharoensis	0	0	0	0	1	0	0	1
	An. rufipes	0	0	0	0	1	0	0	1
	Total	66	31	137	547	233	30	238	1 282

***Agadez has 4 months of collection (July-October); Balleyara and Keita 5 months (July-November); Gaya, Guidimouni, and Niamey V have 6 months (June-December).**

ANNEX B: HUMAN BITING RATES INDOOR RESTING DENSITY AND PARITY

TABLE B1: MONTHLY HUMAN BITING RATE RESULTS FROM HLCS OF AN. GAMBIAE S.L. AND AN. FUNESTUS S.L

Sentinel Site	Species	Indoor/ Outdoor Biting Rate	Jun-21	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21
Agadez	*An. gambiae* s.l.	Indoor		0	0	1.5	0		
		Outdoor		0	0	0.5	0		
Balleyara	*An. gambiae* s.l.	Indoor		1	11.5	18.8	2	2.3	
		Outdoor		0.3	13.5	16.5	1.5	1.5	
	An. funestus s.l.	Indoor		0	0	0	0	0.3	
		Outdoor		0	0	1.3	0	0	
Gaya	*An. gambiae* s.l.	Indoor	34.5	70.5	111.5	51.3	104.3	54.5	95
		Outdoor	16.3	45.5	41	97.3	39.5	31.3	13.8
	An. funestus s.l.	Indoor	2.5	1	11.5	0.3	15.8	2.5	2.3
		Outdoor	0	0	1.3	0	2.5	0.8	1
Guidimouni	*An. gambiae* s.l.	Indoor	0.3	13.5	8	42.8	18.3	8.5	4.5
		Outdoor	0.3	3	10	29.3	23	3.5	4
	An. funestus s.l.	Indoor	1.5	15.5	59.3	62.8	50.8	23	8.3
		Outdoor	2	6	59	56	58	11.3	3
Keita	*An. gambiae* s.l.	Indoor		0	18	44	25.5	1	
		Outdoor		0	8.5	37.5	14	0.5	
Niamey	*An. gambiae* s.l.	Indoor	0	2.8	24.3	37.3	6.8	1	0
		Outdoor	0	1	42.8	33.3	5.3	0.3	0

***Agadez has 4 months of collection (July-October); Balleyara and Keita 5 months (July-November); Gaya, Guidimouni, and Niamey V 6 months (June-December).**

TABLE B2: HOURLY HUMAN BITING CYCLE OF AN. GAMBIAE S.L. BY SITE

	Agadez		Balleyara		Gaya		Guidimouni		Keita		Niamey V	
	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor
6-7pm	0.00	0.00	0.2	0.1	0.72	0.51	0.11	0.00	0.19	0.06	0.04	0.00
7-8pm	0.06	0.00	0.26	0.4	1.46	0.71	0.25	0.07	0.63	0.94	0.18	0.18
8-9pm	0.06	0.00	1.47	2.11	1.97	1.47	0.65	0.14	0.88	1.15	0.21	0.18
9-10pm	0.00	0.00	3.17	4.42	4.37	2.70	0.82	0.44	1.56	1.01	0.51	0.32
10-11pm	0.13	0.00	4.61	4.62	6.53	4.96	1.19	0.83	2.20	2.28	0.86	0.51
11-12pm	0.06	0.06	5.57	3	6.09	4.91	1.84	0.76	2.39	2.14	0.86	0.65
0-1am	0.06	0.06	4.52	3.72	7.29	6.66	1.84	1.12	1.88	2.58	2.26	1.80
1-2am	0.06	0.06	4.81	4.72	8.16	6.91	1.33	1.57	3.65	3.64	2.01	1.29
2-3am	0.00	0.13	2.27	1.78	7.71	6.31	1.37	1.29	2.58	3.44	0.83	1.33
3-4am	0.06	0.00	1.01	1.27	8.66	5.84	0.94	1.15	2.73	2.50	0.40	0.51
4-5am	0.13	0.06	0.36	0.66	6.06	5.08	1.16	1.05	1.45	1.71	0.21	0.25
5-6am	0.00	0.06	0.51	0.25	3.17	2.44	0.21	0.32	2.88	2.14	0.22	0.07

***Agadez has 4 months of collection (July-October); Balleyara and Keita 5 months (July-November); Gaya, Guidimouni, and Niamey V 6 months (June-December).**

TABLE B3: MONTHLY INDOOR RESTING DENSITY OF AN. GAMBIAE S.L. AND AN. FUNESTUS S.L. COLLECTED BY PSC, BY SITE

		Jun-21	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21
Agadez	*An. gambiae s.l.*		0	0	2.7	0		
Balleyara	*An. gambiae s.l.*		1.3	21.2	10.4	0.5	1.5	
	An. funestus s.l.		0	0	0	0	0.2	
Gaya	*An. gambiae s.l.*	33.1	247.5	136.6	148	59.3	75.5	94.2
	An. funestus s.l.	0.1	0.3	0.8	0.2	1.4	8.1	1.4
Guidimouni	*An. gambiae s.l.*	5.4	4.9	18.6	26.8	10.8	2.5	0.2
	An. funestus s.l.	1.5	3.7	17.4	21.2	9	3.5	1.8
Keita	*An. gambiae s.l.*		0	4.9	35.7	9.8	1.6	
	An. funestus s.l.		0	0	0.1	0	0	
Niamey V	*An. gambiae s.l.*	0.1	1.2	18.3	43.1	9.2	3.9	0.1
	An. funestus s.l.	0	0	0	0	0	0.9	0

***Agadez has 4 months of collection (July-October); Balleyara and Keita 5 months (July-November); Gaya, Guidimouni, and Niamey V 6 months (June-December).**

TABLE B4: PARITY RESULTS OF AN. GAMBIAE S.L. AND AN. FUNESTUS S.L. COLLECTED INDOORS AND OUTDOORS

Sentinel Site	Period of Time	*An. gambiae* s.l.				*An. funestus* s.l.			
		% parous Indoor	# Examined Indoor	% parous Outdoor	# Examined Outdoor	% parous Indoor	# Examined Indoor	% parous Outdoor	# Examined Outdoor
Agadez	Jun-21								
	Jul-21		0	0	0				
	Aug-21		0	0	0				
	Sep-21	25.0	4	100	1				
	Oct-21		0	0	0				
Balleyara	Jul-21	100.0	4		0				
	Aug-21	61.9	42	68.1	47				
	Sep-21	72.1	68	54.9	51			25.0	4
	Oct-21	0.0	2	60.0	5				
	Nov-21	66.7	6	40.0	5				
Gaya	Jun-21	61.6	86	60.4	48	77.8	9		0
	Jul-21	59.4	106	61.8	89	0.0	4		0
	Aug-21	72.0	157	69.8	86	78.4	37	100	5
	Sep-21	73.5	98	67.3	113				
	Oct-21	52.3	174	53.2	79				
	Nov-21	49.5	111	41.8	67	50.0	8	33.3	3
	Dec-21	52.4	187	52.8	36				
Guidimouni	Jun-21	0.0	1	100.0	1		5	42.9	7
	Jul-21	30.4	46	27.3	11	30.6	49	47.4	19
	Aug-21	65.4	26	61.3	31	59.2	130	57.4	101
	Sep-21	45.6	79	30.9	68	52.6	76	46.3	54
	Oct-21	51.0	49	46.3	54	50.9	55	49.1	53
	Nov-21	71.0	31	76.9	13	66.3	80	68.3	41
	Dec-21	27.3	11	80.0	5	48.5	33	81.8	11

Sentinel Site	Period of Time	*An. gambiae* s.l.				*An. funestus* s.l.			
		% parous Indoor	# Examined Indoor	% parous Outdoor	# Examined Outdoor	% parous Indoor	# Examined Indoor	% parous Outdoor	# Examined Outdoor
Keita	Jul-21								
	Aug-21	62.3	69	75.9	29				
	Sep-21	65.0	80	60.0	65				
	Oct-21	34.2	38	31.3	16				
	Nov-21	50.0	4	0.0	2				
Niamey V	Jun-21								
	Jul-21	100.0	8	75.0	4				
	Aug-21	50.9	55	46.3	82				
	Sep-21	47.3	112	44.1	102				
	Oct-21	80.0	20	72.7	11				
	Nov-21	0.0	3						
	Dec-21								

***Agadez has 4 months of collection (July-October); Balleyara and Keita 5 months (July-November); Gaya, Guidimouni, and Niamey V 6 months (June-December).**

ANNEX C: MOLECULAR SPECIES IDENTIFICATION

TABLE C1: SPECIES COMPOSITION OF THE AN. GAMBIAE S.L. COMPLEX COLLECTED USING PSC (N=593), HLC (N=1,457), AND CDC LT (N=363)

Sites	PSC							HLC									CDC LT						
	Total No. tested	*An. arabiensis*		*An. coluzzii*		*An. gambiae s.s.*		Total No. tested	*An. arabiensis*		*An. coluzzii*		*An. gambiae s.s.*		*Hybrid*		Total No. tested	*An. arabiensis*		*An. coluzzii*		*An. gambiae s.s.*	
		No.	%	No.	%	No.	%		No.	%	No.	%	No	%	No.	%		No	%	No.	%	No.	%
Agadez	**0**	0	0.0	0	0.0	0	0.0	6	1	16.7	5	83.3	0	0.0	0	0.0	2	0	0.0	2	100	0	0.0
Balleyara	**118**	26	22.0	80	67.8	12	10.2	272	71	26.1	189	69.5	10	3.7	2	0.7	38	4	10.5	32	84.2	2	5.3
Gaya	**119**	6	5.0	108	90.8	5	4.2	299	9	3.0	276	92.3	14	4.7	0	0.0	98	16	16.3	70	71.4	12	12.2
Guidimouni	**118**	19	16.1	99	83.9	0	0.0	288	36	12.5	244	84.7	7	2.4	1	0.3	100	40	40	59	59	1	1
Keita	**119**	18	15.1	98	82.4	3	2.5	296	22	7.4	274	92.6	0	0.0	0	0.0	50	10	20	37	74	3	6
Niamey V	**119**	15	12.6	98	82.4	6	5.0	296	20	6.8	272	91.9	4	1.4	0	0.0	75	5	6.7	70	93.3	0	0.0
Total	**593**	**84**	**14.2**	**483**	**81.5**	**26**	**4.4**	**1457**	**159**	**10.9**	**1260**	**86.5**	**35**	**2.4**	**3**	**0.2**	**363**	**75**	**20.7**	**270**	**74.4**	**18**	**4.96**

TABLE C2: INDOOR AND OUTDOOR ENTOMOLOGICAL INOCULATION RATES OF AN. GAMBIAE S.L., BY SITE

Localities	Sporozoite Rate		Mean HBR (b/p/n)		Mean EIR (=ib/p/n)		Mean EIR (=ib/p/m)		Mean total EIR (=ib/p/m) *
	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor	Indoor/ m	outdoor/ m	
Agadez	0	0	0.38	0.13	0.00	0.00	0.0	0.0	0.0
Balleyara	0.014	0.015	7.12	6.66	0.10	0.10	3.0	3.0	3.0
Gaya	0.011	0.017	74.51	40.67	0.82	0.69	24.6	20.7	22.7
Guidimouni	0.009	0.018	13.7	10.44	0.12	0.19	3.7	5.6	4.7
Keita	0.005	0.004	17.7	12.1	0.09	0.05	2.7	1.5	2.1
Niamey V	0.022	0.008	10.31	11.81	0.23	0.09	6.8	2.8	4.8
Total Mean	0.012	0.012	20.62	13.64	0.25	0.16	7.4	4.9	6.2

* *The mean collection period EIR was calculated over each site collection months.*

ANNEX D: INSECTICIDE SUSCEPTIBILITY TEST RESULTS AND RESISTANCE MARKERS OF THE FIFTEEN SITES MONITORED

TABLE D1: RESULTS OF AN. GAMBIAE S.L. SUSCEPTIBILITY TEST AGAINST THE DIFFERENT INSECTICIDES

		Agadez	Balleyara	Birni N' Gaoure	Gaya	Guidimouni	Keita	Madaoua	Madarounfa
Insecticide Tested	Concentration	Percent Mortality (Number Tested)							
Alpha-cypermethrin	0.05%	1.1% (90)	3.3% (92)	36% (100)	7.4% (81)	15.2% (99)	16.8% (82)	13.9% (101)	13.3% (98)
PBO+Alpha-cypermethrin		47.8% (92)	81.6% (98)	77.1% (105)	33.3% (80)	52% (101)	88.6% (95)	33.3% (103)	62.6% (99)
Alpha-cypermethrin	0.25%	76.3% (93)	63.5% (85)	62.8% (86)	65.6% (90)	74.2% (89)	72.6% (95)	48.5% (99)	52.5% (101)
Alpha-cypermethrin	0.50%	94% (102)	84.8% (92)	61.6% (86)	92.9% (85)	79.6% (93)	84.8% (100)	83.1% (89)	74.2% (97)
Deltamethrin	0.05%	3.2% (95)	20.3% (79)	26.7% (101)	9.9% (81)	36% (86)	42.4% (101)	16.7% (96)	20.8% (101)
PBO+Deltamethrin		85.4% (96)	93.9% (98)	69.1% (94)	47% (93)	91.2% (91)	92.8% (92)	36.5% (104)	91.9% (99)
Deltamethrin	0.25%	92.2% (103)	88.8% (89)	56.3% (87)	78.8% (99)	92% (87)	71.7% (93)	57.5% (73)	66.3% (92)
Deltamethrin	0.50%	NC	93.4% (76)	83.8% (80)	96.3% (81)	100% (89)	100% (103)	82.4% (102)	77.2% (92)
Permethrin	0.75%	9.9% (91)	5.7% (88)	22.2% (90)	11.3% (80)	26.3% (99)	28.9% (98)	11.9% (101)	11.1% (90)
PBO+Permethrin		34.7% (98)	81.3% (96)	67.6% (105)	41.2% (85)	82.2% (98)	40.9% (100)	26.2% (103)	60.2% (93)
Permethrin	3.75%	79.4% (102)	90.4% (83)	68.8% (80)	77.4% (84)	78.9% (90)	71.6% (93)	33.4% (99)	78.3% (92)
Permethrin	7.50%	100% (99)	95.1% (82)	84% (94)	91.3% (80)	92.7% (96)	97% (70)	81.8% (89)	89.2% (93)
Pirimiphos-methyl	0.25%	100% (102)	100% (90)	100% (80)	100% (77)	100% (90)	76.8% (84)	86.7% (98)	96.9% (96)
Pirimiphos-methyl	1.25%	NA	NA	NA	NA	NA	100% (97)	100% (95)	100% (95)
Clothianidin	2%	100% (100)	100% (87)	100% (95)	100% (95)	100% (91)	100% (100)	100% (94)	100% (97)
Clothianidin	4 µg/bottle	100% (95)	100% (99)	100% (89)	100% (101)	100% (98)	100% (78)	NA	100% (86)
Chlorfenapyr	100 µg/bottle	100% (79)	100% (77)	80.7% (88)	100% (64)	100% (89)	100% (89)	100% (92)	94.7% (79)
Chlorfenapyr	200 µg/bottle	100% (81)	NA	100% (72)	NA	NA	NA	100% (97)	100% (85)

		Matameye	Niamey V	Sabon Kafi	Say	Tchintabaraden	Tessaoua	Tillabery
Insecticide Tested	Concentration	Mortality*	Mortality*	Mortality*	Mortality*	Mortality*	Mortality*	Mortality*
Alpha-cypermethrin	0.05%	60% (90)	35% (100)	33.3% (88)	6.7% (105)	25.3% (91)	23.5% (98)	7.9% (89)
PBO + Alpha-cypermethrin	0.05%	79.6% (103)	95.7% (92)	77.8% (84)	48.5% (101)	72.8% (81)	77.1% (87)	17% (88)
Alpha-cypermethrin	0.25%	84.8% (99)	92.5% (107)	63.5% (96)	23.7% (93)	81.4% (86)	60.8% (102)	18.4% (87)
Alpha-cypermethrin	0.50%	92% (100)	96% (99)	78.8% (99)	35.1% (111)	95.5% (88)	83.2% (95)	34.4% (90)
Deltamethrin	0.05%	58.8% (102)	15.7% (102)	61.4% (87)	3.2% (95)	42.7% (96)	40.2% (87)	12.5% (104)
PBO + Deltamethrin	0.05%	85.3% (109)	96.9% (107)	89.3% (90)	71.3% (87)	78.4% (88)	86.8% (91)	40.4% (99)
Deltamethrin	0.25%	87.9% (99)	77.3% (92)	78.2% (101)	27.1% (93)	85.7% (98)	78.3% (92)	24.7% (85)
Deltamethrin	0.50%	97.3% (111)	100% (94)	94.3% (88)	79.1% (101)	96.9% (98)	96.4% (83)	53.1% (81)
Permethrin	0.75%	72.6% (113)	25.3% (99)	31.7% (82)	4.9% (102)	32.9% (85)	17.3% (98)	7.8% (90)
PBO + Permethrin	0.75%	88.7% (106)	72.3% (94)	46% (87)	53.3% (92)	67.4% (86)	70.1% (96)	23.2% (82)
Permethrin	3.75%	93.3% (105)	84.3% (89)	62.4% (93)	31.6% (95)	63.9% (108)	55.3% (94)	18.2% (88)
Permethrin	7.50%	95.2% (104)	94.7% (102)	81.3% (96)	66.3% (98)	88.5% (104)	74.7% (95)	33.7% (95)
Pirimiphos-methyl	0.25%	98.1% (108)	97.9% (96)	96.8% (94)	100% (105)	97% (99)	98.9% (88)	96.5% (86)
Pirimiphos-methyl	1.25%	NA	100 %(106)	99% (99)	100% (83)	100% (96)	NA	100% (85)
Clothianidin	**2%**	**100% (95)**	**100% (102)**	**100% (80)**	**100% (87)**	**100% (91)**	**100% (89)**	**100% (80)**
Clothianidin	**4 µg/bottle**	**100% (95)**	**100% (100)**	**100% (88)**	**100% (87)**	**100% (87)**	**100% (85)**	**100% (95)**
Chlorfenapyr	**100 µg/bottle**	**76.2% (84)**	**97.9% (104)**	**82.8% (80)**	**86% (87)**	**83.3% (86)**	**100% (81)**	**100% (10)**
Chlorfenapyr	**200 µg/bottle**	**100% (75)**	**100% (101)**	**100% (86)**	**100% (77)**	**100% (86)**	NA	**100% (80)**

Resistance confirmed *Possible resistance* *Susceptible; NC = Not completed due to lack of mosquitoes; NA = Not applicable*

TABLE D2: FREQUENCY OF RESISTANCE ALLELES PER DEAD AND ALIVE STATUS OF MOSQUITOES

Sites		Resistance Alleles														
		Kdr-w					*Kdr-e*					*Ace-1*				
		Total	RR	RS	SS	*freq*	Total	RR	RS	SS	*freq*	Total	RR	RS	SS	*freq*
Agadez	dead	49	31	5	13	*0.68*	49	22	5	22	*0.50*	49	5	3	41	*0.13*
	alive	69	41	12	16	*0.68*	69	28	9	32	*0.47*	69	5	3	61	*0.09*
Boboye	dead	62	26	8	28	*0.48*	64	7	0	57	*0.11*	62	7	0	55	*0.11*
	alive	56	19	6	31	*0.39*	54	5	1	48	*0.10*	56	7	2	47	*0.14*
Balleyara	dead	56	25	17	14	*0.60*	56	8	11	37	*0.24*	56	10	0	46	*0.18*
	alive	63	29	21	13	*0.63*	63	15	11	37	*0.33*	63	8	6	49	*0.17*
Gaya	dead	58	32	14	12	*0.67*	58	31	19	8	*0.70*	58	3	0	55	*0.05*
	alive	60	51	4	5	*0.88*	60	36	8	16	*0.67*	60	4	3	53	*0.09*
Guidimouni	dead	67	39	15	13	*0.69*	67	20	14	33	*0.40*	67	7	1	59	*0.11*
	alive	50	27	13	10	*0.67*	50	28	7	15	*0.63*	50	2	3	45	*0.07*
Keita	dead	60	19	20	21	*0.48*	60	21	6	33	*0.40*	60	4	2	54	*0.08*
	alive	58	26	17	15	*0.59*	58	25	7	26	*0.49*	58	8	5	45	*0.18*
Madaoua	dead	56	40	13	3	*0.83*	57	29	10	18	*0.60*	57	7	2	48	*0.14*
	alive	63	39	14	10	*0.73*	62	39	7	16	*0.69*	62	11	1	50	*0.19*
Madarounfa	dead	59	36	11	12	*0.70*	59	26	7	26	*0.50*	59	9	2	48	*0.17*
	alive	59	29	12	18	*0.59*	59	33	10	16	*0.64*	59	4	1	54	*0.08*
Matamey	dead	60	34	12	14	*0.67*	60	27	19	14	*0.61*	60	1	2	57	*0.03*
	alive	57	37	7	13	*0.71*	57	29	16	12	*0.65*	57	11	2	44	*0.21*
Niamey V	dead	85	55	10	20	*0.71*	85	39	12	34	*0.26*	85	6	6	73	*0.11*
	alive	35	21	5	9	*0.67*	35	21	5	9	*0.67*	35	7	2	26	*0.23*
Sabon Kafi	dead	59	23	8	28	*0.46*	59	6	0	53	*0.10*	59	4	2	53	*0.08*
	alive	58	27	10	21	*0.55*	58	9	0	49	*0.16*	58	4	3	51	*0.09*
Say	dead	50	33	10	7	*0.76*	50	32	1	17	*0.65*	50	3	2	45	*0.08*
	alive	69	41	11	17	*0.67*	69	34	1	34	*0.50*	69	6	3	60	*0.11*
Tchintabaraden	dead	65	28	17	20	*0.56*	65	25	11	29	*0.47*	65	3	0	**62**	*0.05*
	alive	55	24	19	12	*0.61*	55	24	5	26	*0.48*	55	6	3	**46**	*0.14*
Tessaoua	dead	61	41	12	8	*0.77*	61	26	20	15	*0.59*	61	6	5	50	*0.14*
	alive	59	37	10	12	*0.71*	59	20	14	25	*0.46*	59	5	2	52	*0.10*
Tillabery	dead	62	40	19	3	*0.80*	62	36	11	15	*0.67*	62	5	2	55	*0.10*
	alive	67	40	16	11	*0.72*	67	33	18	16	*0.63*	67	6	2	59	*0.10*

TABLE D3: SPECIES COMPOSITION OF THE AN. GAMBIAE S.L. OF INSECTICIDE RESISTANCE (N=1,789)

Sites	*Total number tested*	*An. arabiensis*		*An. coluzzii*		*An. gambiae* s.s.	
		Number	%	Number	%	Number	%
Agadez	118	9	7.63	108	91.53	1	0.85
Balleyara	117	2	1.71	46	39.32	69	58.97
Boboye	118	65	55.08	52	44.07	1	0.85
Gaya	117	7	5.98	80	68.38	30	25.64
Guidimouni	120	4	3.33	59	49.17	57	47.50
Keita	117	7	5.98	78	66.67	32	27.35
Madaoua	118	8	6.78	91	77.12	19	16.10
Madarounfa	118	16	13.56	95	80.51	7	5.93
Matamey	118	14	11.86	57	48.31	47	39.83
Niamey V	120	16	13.33	99	82.50	5	4.17
Sabon Kafi	120	0	0.00	99	82.50	21	17.50
Say	120	9	7.50	109	90.83	2	1.67
Tchintabaraden	120	4	3.33	112	93.33	4	3.33
Tessaoua	120	2	1.67	111	92.50	7	5.83
Tillabery	128	3	2.34	122	95.31	3	2.34
Total	**1789**	**166**	**9.28**	**1318**	**73.67**	**305**	**17.05**