U.S. PRESIDENT'S MALARIA INITIATIVE

PMI VECTORLINK NIGER ANNUAL ENTOMOLOGY REPORT APRIL 2019-MARCH 2020

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ACRONYMS

Ace	acetylcholinesterase
CDC	Centers for Disease Control and Prevention
EIR	Entomological Inoculation Rate
ELISA	Enzyme-linked Immunosorbent Assay
f/r/d	Females/Room/Day
HBR	Human Biting Rate
HLC	Human Landing Catch
ib/p/m	Infective bites per Person per Month
IRS	Indoor Residual Spray
ITN	Insecticide-treated Net
kdr	Knock Down Resistance
NMCP	National Malaria Control Program
P.	*Plasmodium*
PBO	Piperonyl Butoxide
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
WHO	World Health Organization

EXECUTIVE SUMMARY

The VectorLink Niger project conducted entomological monitoring of malaria vectors in Niger from April 2019 to March 2020 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) methods was conducted in 10 sentinel sites (Agadez and Ingall in hypo- endemic areas; Balleyara, Fararrat and Tessaoua in meso-endemic areas; Gaya, Guidimouni, , Niamey V, Say and Zindarou in hyper endemic areas) selected by the NMCP. Routine surveillance were undertaken every two months in nine sites, and monthly in Niamey. 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 of *An. gambiae* s.l. mosquitoes against diagnostic concentrations of pyrethroid (alpha-cypermethrin, deltamethrin, and permethrin), organophosphate (pirimiphos-methyl), carbamate (bendiocarb), neonicotinoid (clothianidin), and pyrrole (chlorfenapyr) insecticides. World Health Organization (WHO) susceptibility test kits and Centers for Disease Control (CDC) bottle assays for chlorfenapyr were used in nine of the 10 sentinel sites. The resistance intensity and synergist effect of piperonyl butoxide were also evaluated in the nine sites tested and when resistance was observed. The results of the routine surveillance showed that *An. gambiae* s.l. was the predominant malaria vector species in the country. *An. gambiae* s.l. represented more than 95% (15,469 of 17,091) of the vectors collected during the period in all sites. PSCs yielded more vectors than HLCs with 9,821 *An. gambiae* s.l. (across all sites) as compared with 5,648, respectively. *An. funestus* s.l. was found in three sites and represented 7.7% (n=1,317) of mosquitoes collected. The biting behavior of *An. gambiae* s.l. and *An. funestus* s.l. vary across sites with endophilic tendency in all sites except Zindarou, where it was mostly exophilic. The average peak biting of *An. gambiae* s.l. occurred mostly between 10:00 pm and 3:00 am while *An. funestus* s.l. average peak biting occurred mostly between 10:00 pm and 4:00 am.

The molecular characterization revealed the presence of three species of *An. gambiae* s.l. (*An. coluzzii, An. arabiensis,* and *An. gambiae* and three species of *An. funestus* s.l. (*An. rivulorum-like, An. rivulorum,* and *An. parensis*). *An. coluzzii* was the major vector of the *An. gambiae* complex in all sites followed by *An. arabiensis,* while *An. rivulorum-like* was the major vector of the *An. funestus* complex followed by *An. rivulorum.* For both methods collection 2,770 *An. gambiae* s.l. were analyzed by PCR and 32 were positive for *Plasmodium falciparum* parasite (HLC 23/1871 and PSC 9/899). *An. coluzzii* representing the predominant vector, was the most infected in all sites. *An. arabiensis* was found infected only in Balleyara with HLC method collection and in Say and Tessaoua with PSC method collection. No *An. gambiae* was infected. The Entomological Inoculation Rate (EIR) was higher in hyper-endemic areas including Gaya (180.72 infective bites per person per month (ib/p/m)), Zindarou (168.12 ib/p/m), Niamey V (75.90 ib/p/m) than in the meso-endemic sites: Fararrat 0 ib/p/m, Tessaoua 15.72 ib/p/m) and no infective bites were recorded in hypo-endemic sites (Agadez and Ingall). For *An. funestus* s.l., the EIR was similar in sites where larger numbers of the vector collected were tested: Guidimouni (6.05, ib/p/m) and Zindarou (5.13 ib/p/m).

Larval collections for insecticide resistance testing were productive in nine of the 10 sites (no larvae were collected in Ingall). Resistance to the three pyrethroids tested (deltamethrin, permethrin, and alpha-cypermethrin) was observed in all nine sites. High deltamethrin resistance was observed in all nine sites while permethrin and alpha-cypermethrin showed moderate resistance in Gaya and Tessaoua. Pre-exposure of mosquitoes to PBO before exposure to the pyrethroids did not completely reverse the resistance status of the *An. gambiae* s.l. populations in any of the sites surveyed except in Agadez Chlorfenapyr susceptibility was recorded at the dose of 200 µg/bottle in all nine sites, while clothianidin susceptibility was recorded in seven

of the nine sites. The vector also showed full susceptibility to pirimiphos-methyl in six of the nine sites and moderate resistance in the other three sites. *An. gambiae* s.l. showed susceptibility to bendiocarb in Balleyara only.

The *kdr-w* and *Ace*-1 mutations were recorded at frequencies between 0.22 and 0.38 and between 0.05 and 0.09, respectively, across the nine sites.

These results showed that the vector density and transmission in Niger are concentrated within a short period of the year and particularly in the southern and endemic part of the country. This suggests that the NMCP should continue intensifying the ongoing national seasonal malaria chemoprevention (SMC) campaigns during the peak density and transmission period. Additionally, as the level of *kdr* mutation is still low, the traditional (pyrethroid only) ITNs can continue to be distributed in Niger but the distribution should be stratified to enable the most appropriate deployment of ITNs in areas where a certain insecticide is more effective against the local mosquito population.

I. INTRODUCTION

Malaria is endemic in Niger and is the leading cause of death and disability combined, disproportionately affecting children under five years of age.[1] According to the Annual Health Statistics Report (2018), there were over 3,621,972 malaria cases and 3,761 malaria deaths in Niger in 2018, putting it among the countries with the highest per capita rate of malaria fatalities globally. The country is divided into three malaria endemicity zones from south to north: hyper-endemic, meso-endemic, and hypo-endemic. According to the President's Malaria Initiative (PMI) FY2019 Niger Malaria Operational Plan,[2] the vast majority of Niger's population (94%) lives in the two southernmost zones (meso-endemic and hyper-endemic), where malaria is most prevalent. The rainy season in Niger lasts about three to four months, from June through September, with peak malaria transmission during the second half (August-September).

In 2019, the PMI VectorLink Project conducted entomological monitoring activities in 10 sentinel sites to collect data to support the country's National Malaria Control Programme (NMCP) in its insecticide-based vector control activities, such as routine and mass campaign distribution of insecticide-treated nets (ITN) to achieve universal coverage in Niger. The data collected will provide the NMCP with trends on entomological parameters of malaria transmission (vector species, density, distribution, infection rates, behavior, etc.) and insecticide resistance. Paired with health facility-based malaria incidence data and population density, these results will generate a robust foundation for evidence-based decision making, especially for better choice of ITNs and implementation of an integrated vector control strategy in future years.

[1] PMI Niger Malaria Operational Plan FY 2017, https://www.pmi.gov/docs/default-source/default-document-library/malaria-operational-plans/fy17/fy-2017-niger-malaria-operational-plan.pdf

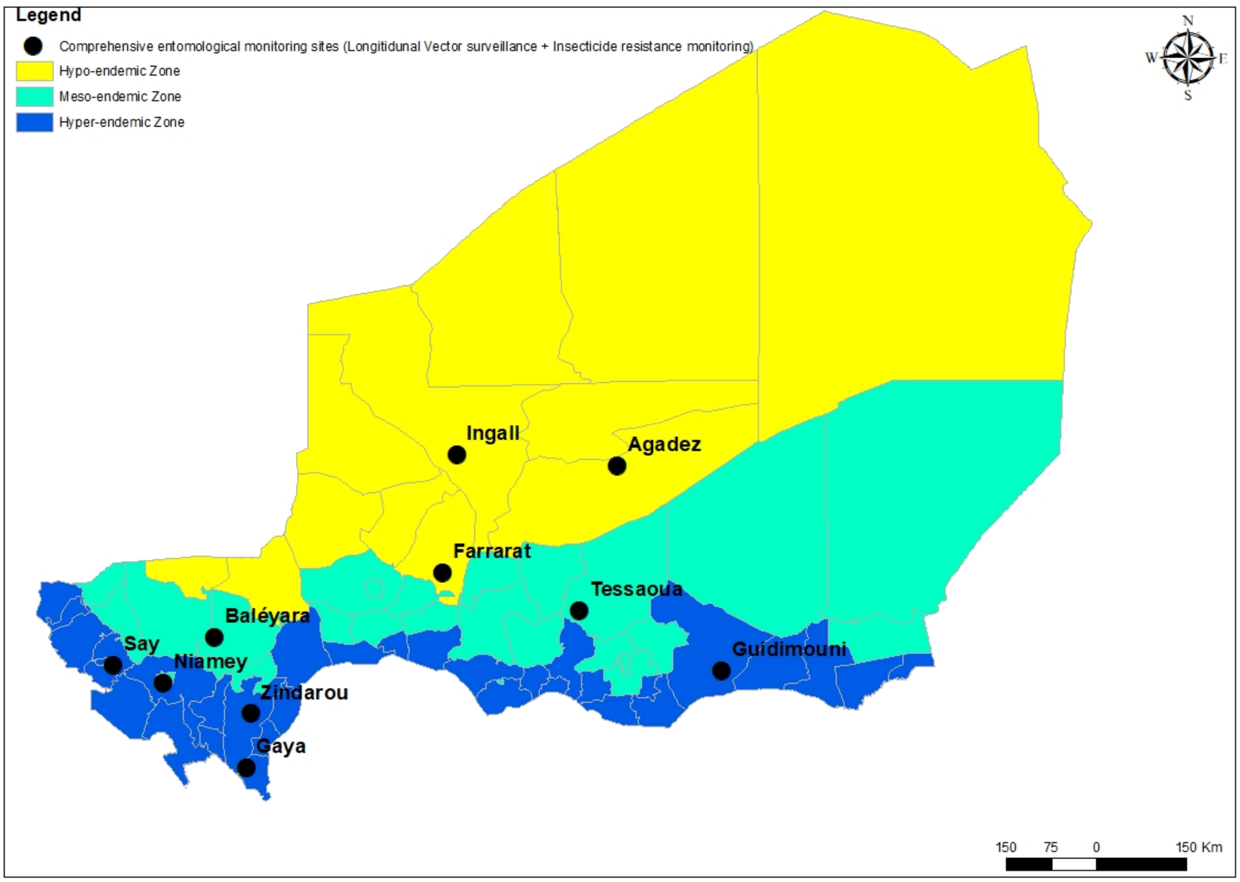
[2] https://www.pmi.gov/docs/default-source/default-document-library/malaria-operational-plans/fy19/fy-2019-niger-malaria-operational-plan.pdf?sfvrsn=5

2. Methods

2.1 Entomological Monitoring Sites

From April 2019 to March 2020, VectorLink Niger conducted longitudinal entomological surveillance (vector surveillance and insecticide resistance monitoring) in 10 sentinel sites, which were selected by the NMCP and Centre de Recherches Medicale et Sanitaire (CERMES). The selected sites, shown in Figure 1, are located across the country's three malaria endemicity zones: Agadez and Ingall in the hypo-endemic zone, Balleyara, Fararrat, and Tessaoua in the meso-endemic zone, and Niamey V, Gaya, Guidimouni, Say, and Zindarou in the hyper-endemic zone.

Figure 1. Entomological Monitoring Sites Supported By PMI VectorLink Niger



2.2 Longitudinal Monitoring

Longitudinal monitoring was conducted every two months in nine sites; in Niamey, collections were done on a monthly basis. Adult mosquito collections were done using human landing catches (HLCs) and pyrethrum spray catches (PSCs) and following the VectorLink Standard Operating Procedures (SOPs).[3]

[3] https://pmivectorlink.org/resources/tools-and-innovations/

HLCs were conducted during two consecutive nights per site per month, in the same two houses, both indoors and outdoors from 6:00 pm to 6:00 am. PSCs were carried out between 7:00 am and 10:00 am in the same 10 randomly selected houses each collection period. The samples were identified morphologically and preserved in 1.5 ml Eppendorf tubes containing silica gel. 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	7:00 am to 10:00 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 identification keys (Gillies and Coetzee 1987). A sub-sample of *Anopheles gambiae* s.l. collected by HLC method from each site was dissected to determine the parity and estimate the longevity of the vector population of each site. All *Anopheles* species were labelled and preserved on silica gel in Eppendorf tubes for further laboratory analysis 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 mosquitoes collected through each collection method.

TABLE 2: VECTOR SURVEILLANCE INDICATORS BY COLLECTION METHOD

Collection Method	Indicator	Unit of Measure
HLC	Human biting rate	Mean number of 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	Mean number of mosquitoes / house / day
	% of fed females	Percentage of fed mosquitoes (out of total collected by PSC)

2.3 INSECTICIDE RESISTANCE MONITORING

Larvae and pupae of *An. gambiae* s.l. were collected from different larval habitats in each site, pooled, and reared to adulthood in the field. Insecticide susceptibility tests were conducted on 2–5-day-old adult females using the WHO tube tests and CDC bottle assays in accordance with the PMI VectorLink SOPs.

The diagnostic concentrations of permethrin (0.75%), deltamethrin (0.05%), alpha-cypermethrin (0.05%), bendiocarb (0.1%), and pirimiphos-methyl (0.25%) were tested in all sites in which larvae were harvested and also lambda-cyhalothrin 0.05% in six of those sites where larger number of larvae were collected as lambda-cyhalothrin paper was available. Resistance was defined following the WHO criteria, with less than 90% mortality as confirmed resistance, 90–97% mortality as possible resistance, and 98% or greater as susceptible. When insecticide resistance was confirmed, resistance intensity (high, moderate, and low) was also tested at five and 10 times the diagnostic concentration of permethrin, deltamethrin, alpha-cypermethrin, and pirimiphos-methyl.

Synergist assays with piperonyl butoxide (PBO) were conducted for deltamethrin, permethrin, and alpha-cypermethrin using WHO tube tests to determine the involvement of P450s in pyrethroid resistance.

CDC bottle assays were conducted using chlorfenapyr at the doses of 100µg/bottle and 200µg/bottle. Paper was treated with clothianidin (13mg/paper) following PMI VectorLink SOPs, and the susceptibility testing was conducted using WHO tube tests.

2.4 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 susceptibility testing, and determination of *Plasmodium* (*P.*)-infected mosquitoes, blood meal sources, and target site resistance markers.

2.4.1 SPECIES IDENTIFICATION

The 1,710 *An. gambiae* s.l. collected by HLC (820) and PSC (890) from all the vector surveillance 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 *An. funestus* group species were also identified within a sub-sample of 386 mosquitoes using the protocol designed by Koekemoer et al. (2002). Also, averagely 150 *An. gambiae* s.l. mosquitoes were randomly selected from the dead and surviving mosquitoes from the WHO susceptibility tests all insecticides (alpha-cypermethrin n=154 mosquitoes, deltamethrin n= 162, permethrin n= 191, lambda-cyhalothrin n=137, bendiocarb n=150, pirimiphos methyl n=160 and control n=121) in each site and were further analyzed to identify species.

2.4.2 CIRCUMSPOROZOITE ANALYSIS AND BLOOD MEAL SOURCES

The *P. falciparum* infection rates of 2770 mosquitoes collected using HLC (1871) and PSC, (899) was determined using PCR methods following the protocol of Echeverry et al. (2017). 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 daily entomological inoculation rate (EIR) represents the product of the sporozoite rate and the human biting rate (HBR) per night.

The blood meal source of a total of 1344 mosquitoes collected through PSC at all sites 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 observed out of the total number of mosquitoes tested.

2.4.3 CHARACTERIZATION OF INSECTICIDE RESISTANCE MARKERS

The insecticide resistance markers of 100 mosquitoes identified per site after susceptibility testing were assessed to determine the frequency of the mutations among the population tested. The presence of *knock down resistant (Kdr)-West* 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 the sites where carbamate resistance was observed.

3. RESULTS

3.1 LONGITUDINAL MONITORING

Six bimonthly collections were completed in nine sites and 12 monthly collections were completed in Niamey V, using the two collection methods (HLCs and PSCs). Table 2 shows the collections completed in each of the 10 sites in this reporting period.

TABLE 3: MOSQUITO COLLECTION PERIOD FROM AT EACH SENTINEL SITE FROM APRIL 2019 TO MARCH 2020

	Apr 2019	May 2019	Jun 2019	Jul 2019	Aug 2019	Sep 2019	Oct 2019	Nov 2019	Dec 2019	Jan 2020	Feb 2020	Mar 2020
Agadez	X		X		X		X		X		X	
Balleyara		X		X		X		X		X		X
Fararrat	X		X		X		X		X		X	
Gaya		X		X		X		X		X		X
Guidimouni	X		X		X		X		X		X	
Ingall	X		X		X		X		X		X	
Niamey V	X	X	X	X	X	X	X	X	X	X	X	X
Say		X		X		X		X		X		X
Tessaoua	X		X		X		X		X		X	
Zindarou		X		X		X		X		X		X

3.1.1 SPECIES COMPOSITION

A total of 17,091 *Anopheles* were collected across the 10 sites. *An. gambiae* s.l. was the most abundant (90.5%; n=15,469), followed by *An. funestus* s.l. (7.7%; n=1317), *An. rufipes* (1%; n=168), *An. pharoensis* (0.7%; n=125), and *An. nili*, (0.11%; n=12) (Figure 2).

Of the 15,469 *An. gambiae* s.l. collected, 5648 (36.5%) were collected by HLC and 9821 (63.5%) by PSC (Figure 3).

FIGURE 2: TOTAL *ANOPHELES* COLLECTED (N= 17,091) AT ALL SITES USING HLC (40.1%, N=6850) AND PSC (59.9%, N=10,241)

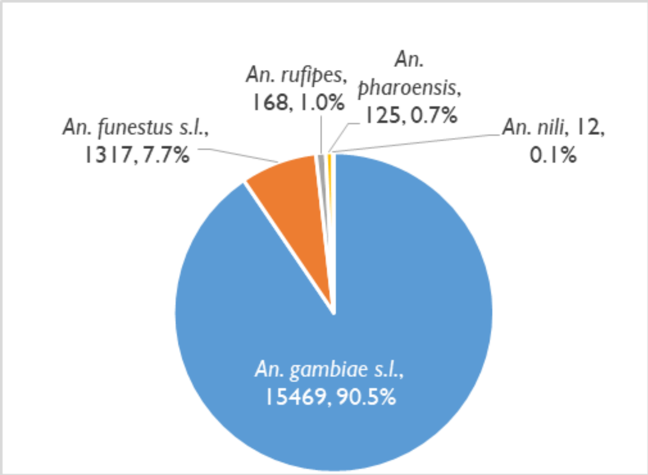
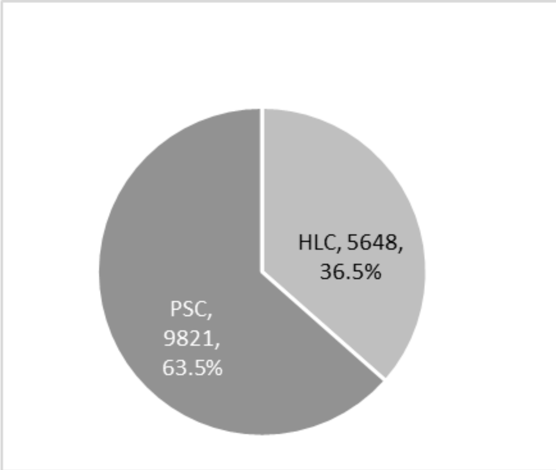


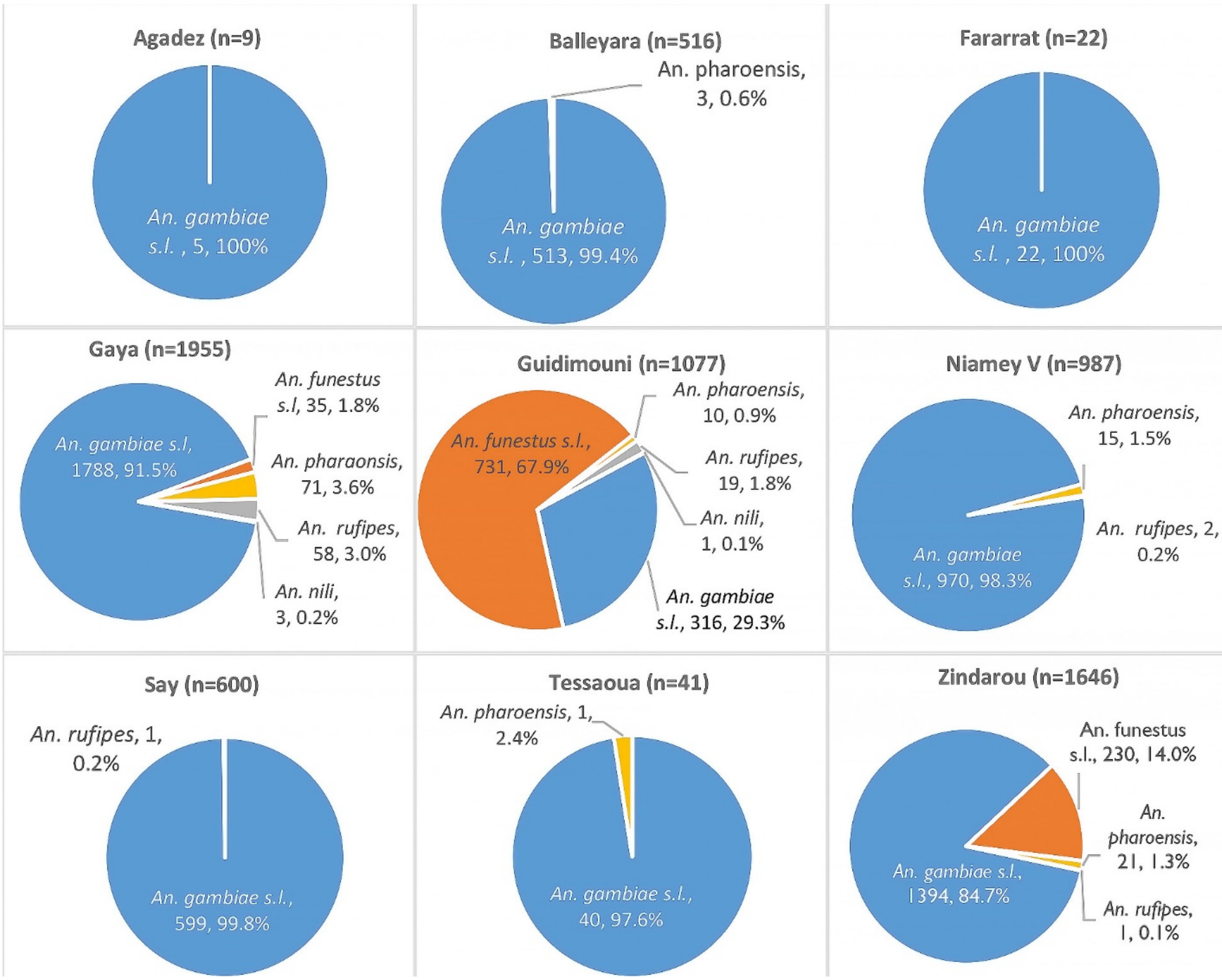
FIGURE 3: TOTAL *AN. GAMBIAE* S.L. COLLECTED (N=15,469) AT ALL SITES USING HLC(36.5%, N=5,648) AND PSC (63.5%, N=9,821)



SPECIES COMPOSITION OF MOSQUITOES COLLECTED USING HLC

Figure 4 illustrates the species composition of mosquitoes collected by HLC in each site. A total of 6850 *Anopheles* mosquitoes were collected using HLC across all 10 sites. *An. gambiae* s.l. was the only *Anopheles* species collected in three sites, Agadez (n=5), Fararrat (n=22), and Ingall (n=1), and it was the main species recorded in six sites: Balleyara (99.4% n=513), Gaya (91.3%; n=1788), Niamey V (98.3% n=970), Say (99.8%, n=599), Tessaoua (97.6%, n=40), and Zindarou (84.7%, n=1394). The other *Anopheles* vectors collected by HLC were *An. funestus* s.l., *An. nili*, *An. pharoensis,* and *An. rufipes*. Guidimouni recorded the lowest proportion of *An. gambiae* s.l. (29.3%, n=316/1077); *An. funestus* s.l. represented the predominant *Anopheles* vector there, at 67.9% (n=731/1077) of the total collected.

FIGURE 4: SPECIES COMPOSITION OF THE ANOPHELES COLLECTED USING HLC, BY SITE*

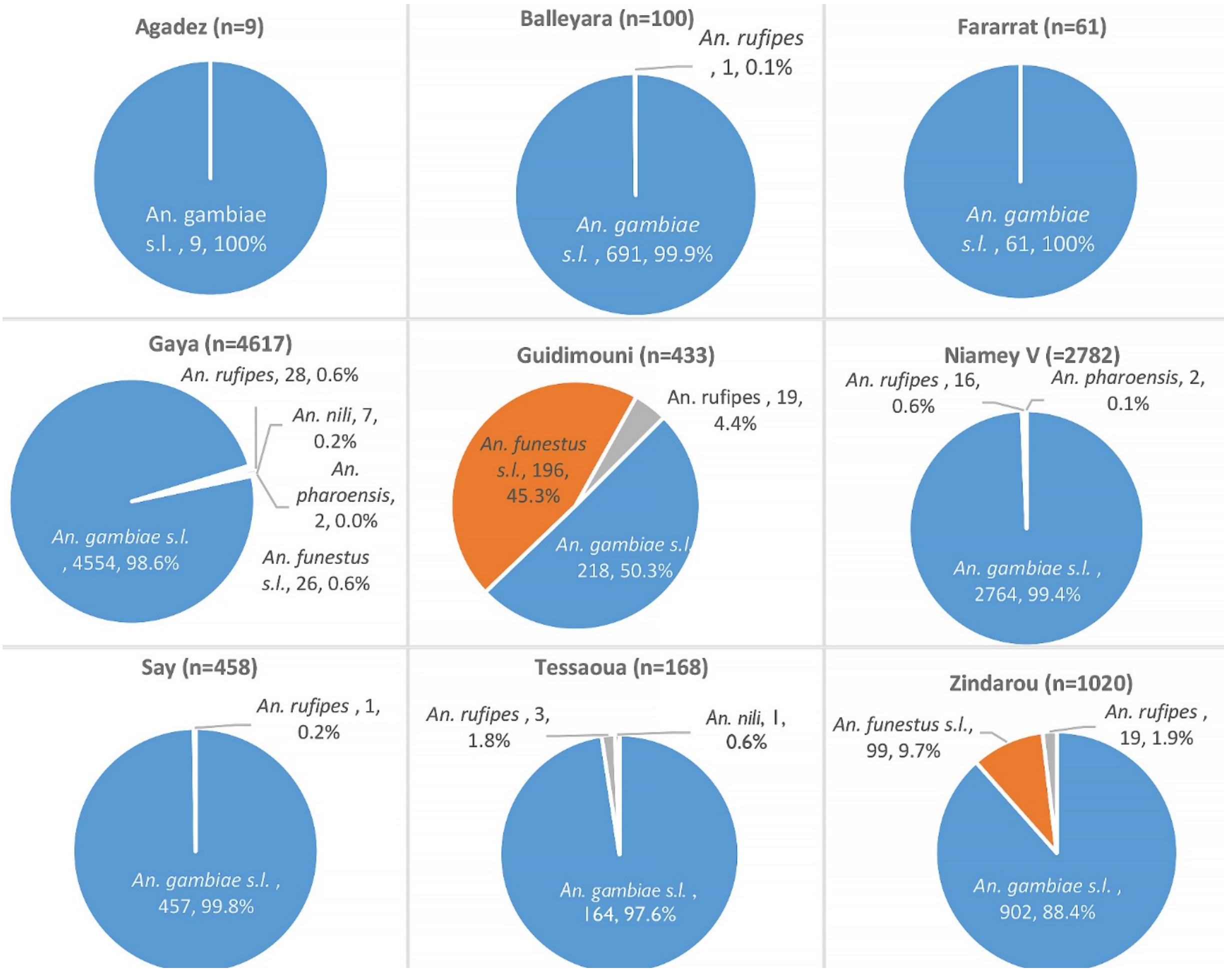


**Ingall yielded only a single An. gambiae* s.l. *as Anopheles mosquito*

SPECIES COMPOSITION OF MOSQUITOES COLLECTED USING PSC

Figure 5 describes the vector composition of *Anopheles* mosquitoes collected using PSC in each site. A total of 10,241 *Anopheles* were caught in all sites. *An. gambiae* s.l. was the predominant vector collected, representing 96% (n=9821) of the total *Anopheles* species. *An. gambiae* s.l. was the only *Anopheles* collected (100%) using PSC in Agadez (n=9), Fararrat (n=61), and in Ingall (n=1). *An. gambiae* s.l. represented more than 98% of the *Anopheles* species collected by PSC in all the other sites—Balleyara (99.96% n=691), Gaya (98.78%, n=4554), Niamey (99.35%, n=2764), Say (99.78%, n=457), Tessaoua (98%, n=164)—except Zindarou (88%, n=902) and Guidimouni (50.3%, n=218), where a higher number of *An. funestus* s.l was recorded than in any other site (10%, n=99) and (45.3%, n=196) respectively.

FIGURE 5: SPECIES COMPOSITION OF THE *ANOPHELES* COLLECTED USING PSC, BY SITE*



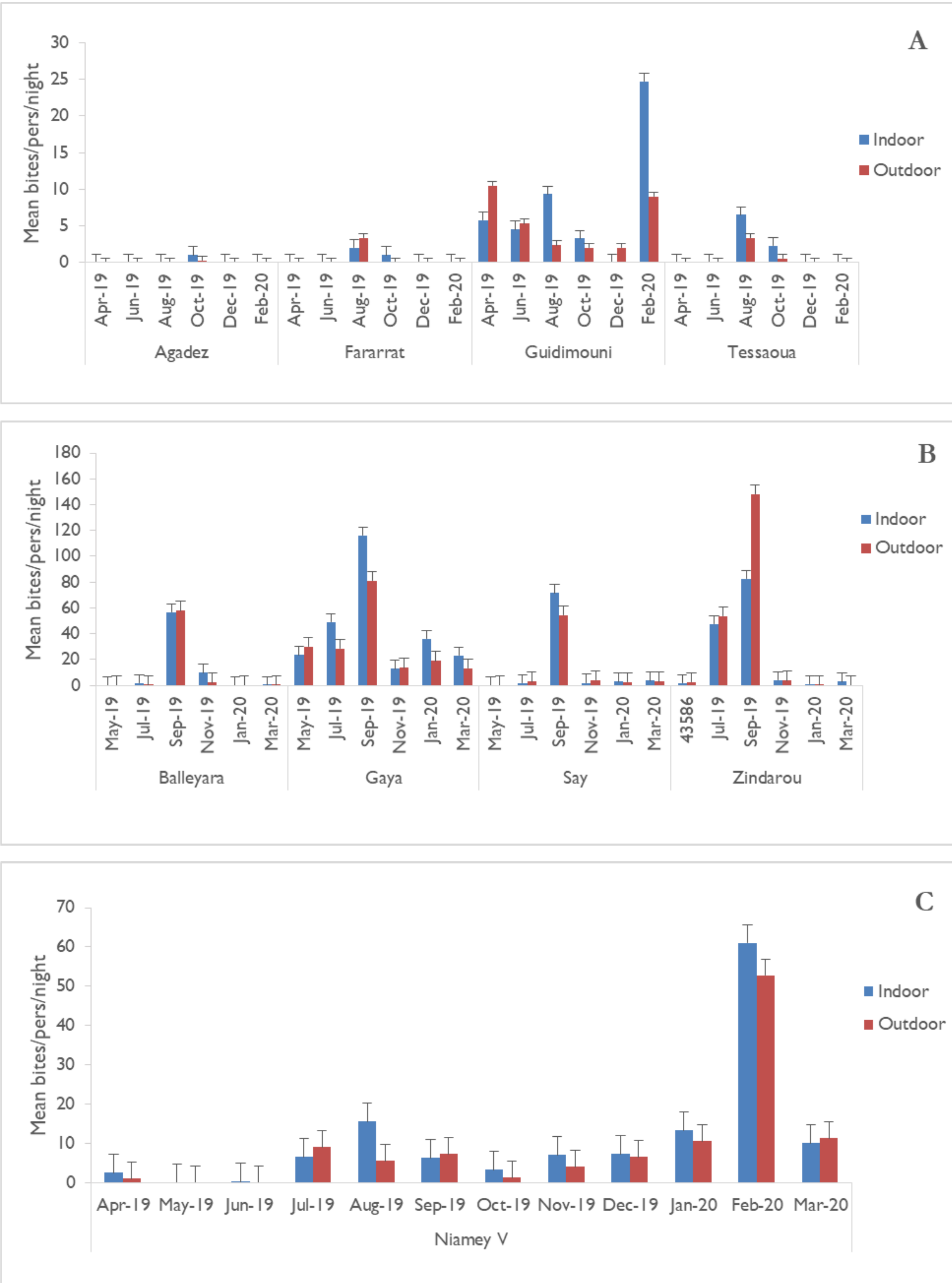
***Ingall yielded only a single An. gambiae s.l.**

3.1.2 HUMAN BITING RATE (HBR) OF *AN. GAMBIAE* S.L. COLLECTED

The sites of Agadez, Fararrat, Guidimouni, and Tessaoua, where monitored bimonthly starting in April 2019, through February 2020, and recorded the lowest HBRs with less than 10 b/p/n each (Figure 6A). Gaya and Zindarou recorded the highest average HBRs of *An. gambiae* s.l., 42.33 and 35.38 bites/person/night (b/p/n), respectively, among the four sites (Ballayara, Gaya, Say, and Zindarou) that were monitored bimonthly starting in May 2019, through March 2020 (Figure 6B). The HBRs in Say and Balleyara were 13.71 b/p/n and 11.29 b/p/n, respectively. In all four sites, the peak biting during the reporting period was observed in September (Figure 6B). The *An. gambiae* s.l. HBR was higher indoors in three of the four sites; in Zindarou, the highest HBR was recorded outdoors, also in September. During its 12 months of monitoring, Niamey V recorded an average of 11.84 b/p/n (Figure 6C). The overall HBR of *An. gambiae* s.l. recorded from January to March 2020 showed that only Niamey V yielded a higher rate in February indoor (61.0 b/p/n) and outdoor (52.75 b/ p/n) (Figure 6 A, B, C; Annex Table A-1).

FIGURE 6: HUMAN BITING RATES OF *AN. GAMBIAE* S.L., BY SITE, BY MONTH

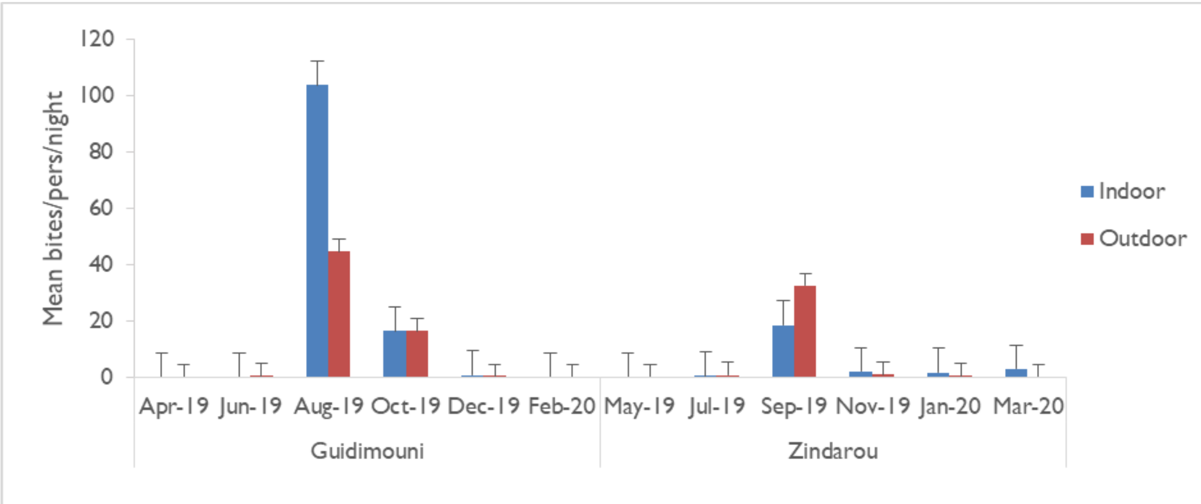
"Error bars of each figure represent the standard errors"



Human Biting Rate of An. funestus s.l.

Both Guidimouni and Zindarou recorded HBRs of *An. funestus* s.l. with an average of 15.29 and 5.03 b/p/n respectively over the period. Guidimouni recorded the highest HBR in August (103.75 b/p/n indoor and 44.75 b/p/n outdoor), and Zindarou has the highest in September (18.5 b/p/n indoor and 32.5 b/p/n outdoor) (Figure 7)

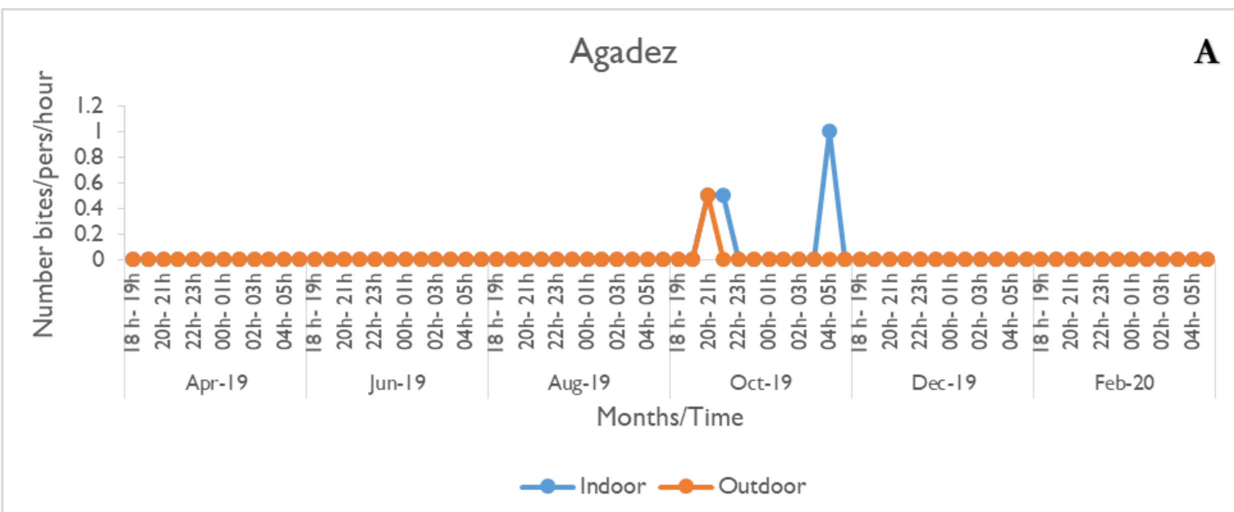
Figure 7: Human Biting Rates of *An. funestus* s.l. from Guidimouni and Zindarou by Month "Error bars of each figure represent the standard errors"

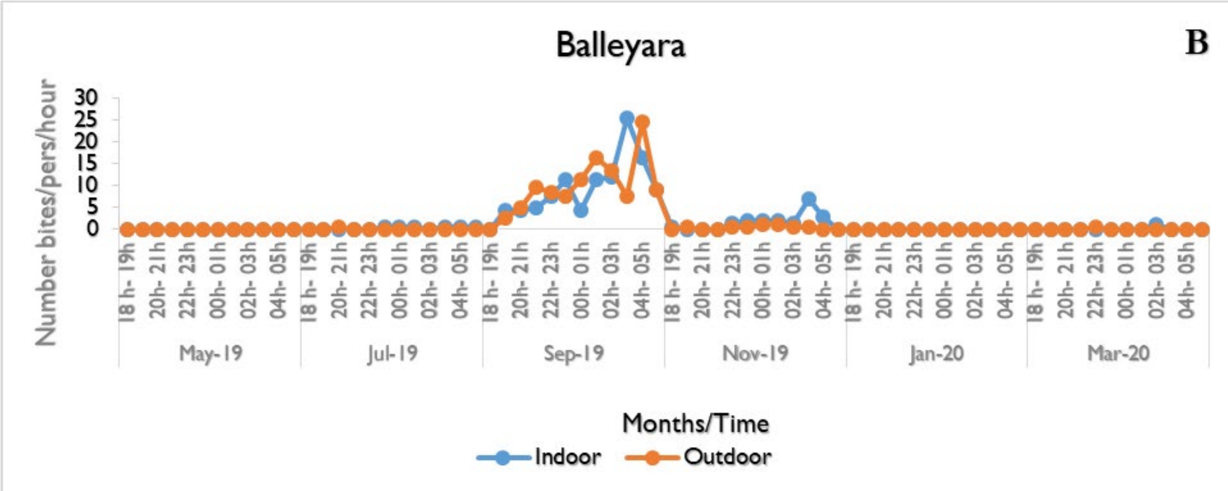


3.1.3 Biting Cycle of *An. gambiae* s.l.

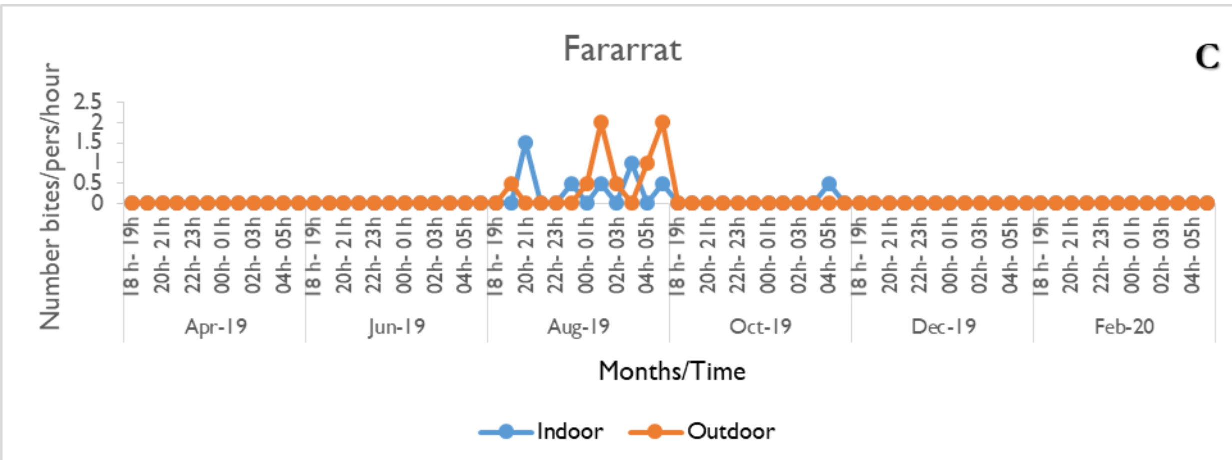
Overall, the biting activity of *An. gambiae* s.l. was highest between 10:00 pm and 03:00 am during the collection period, both indoors and outdoors, in all the sites (Figures 8 (A-I)).

Figure 8: Biting Rate and Cycle of *An. gambiae* s.l. in All Nine Sites

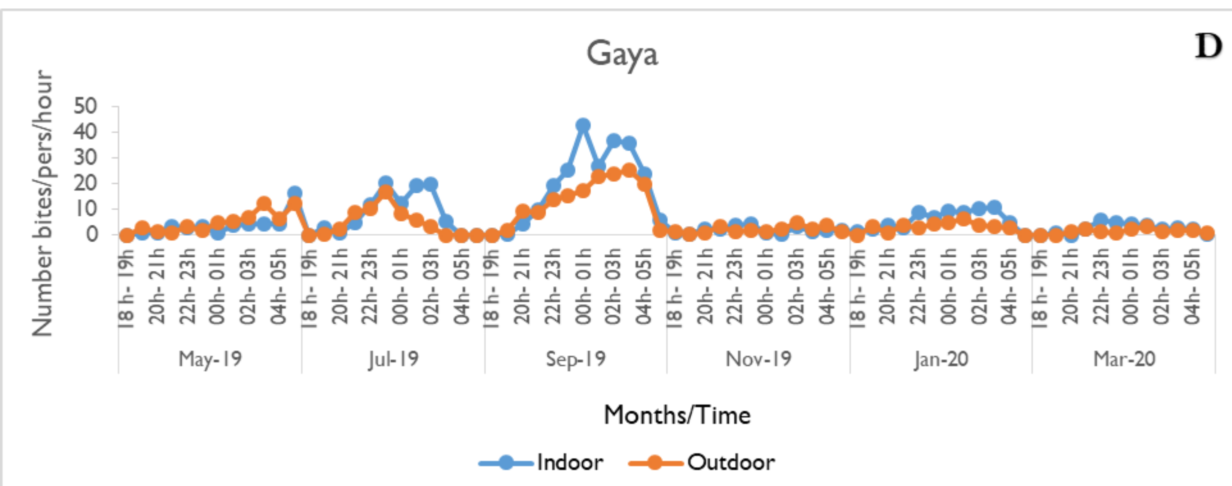




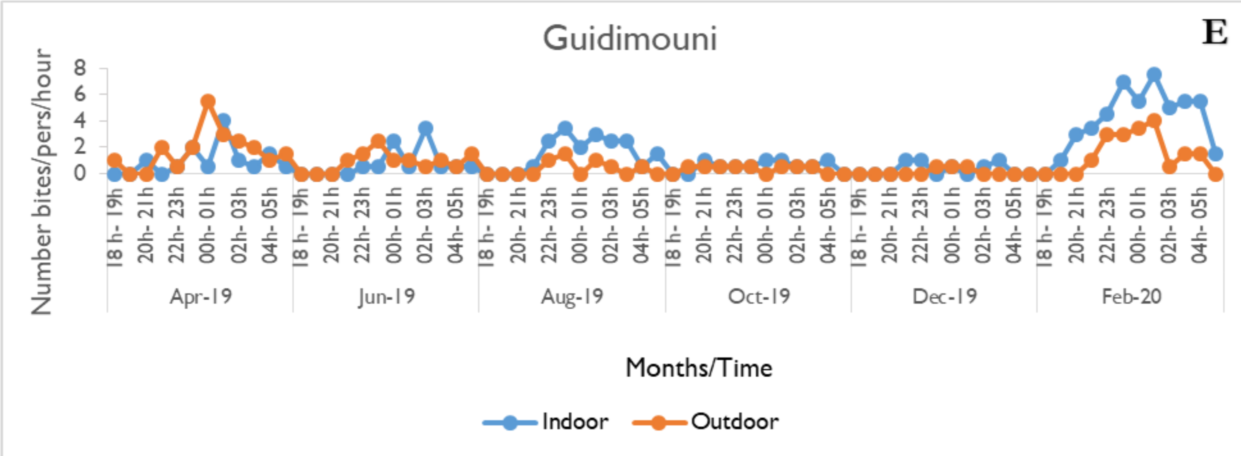
Balleyara
B
Number bites/pers/hour
Months/Time
Indoor
Outdoor

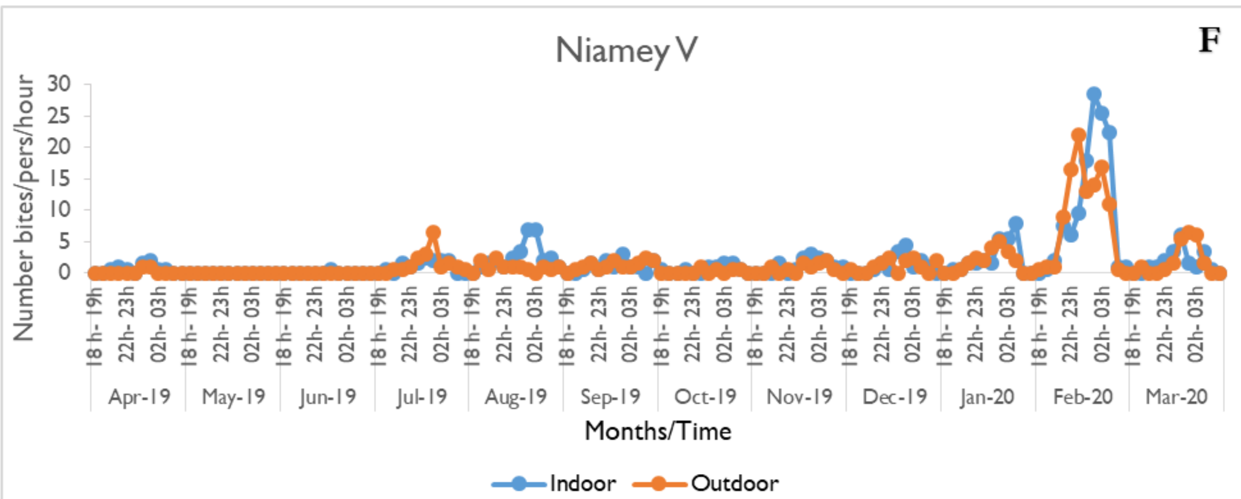


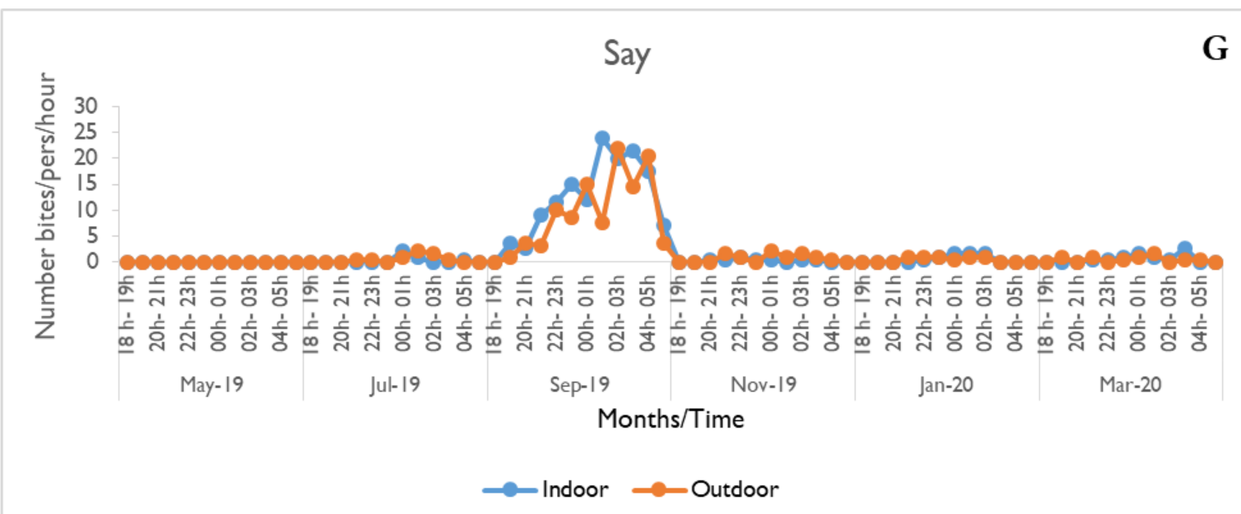
Fararrat
C
Number bites/pers/hour
Months/Time
Indoor
Outdoor

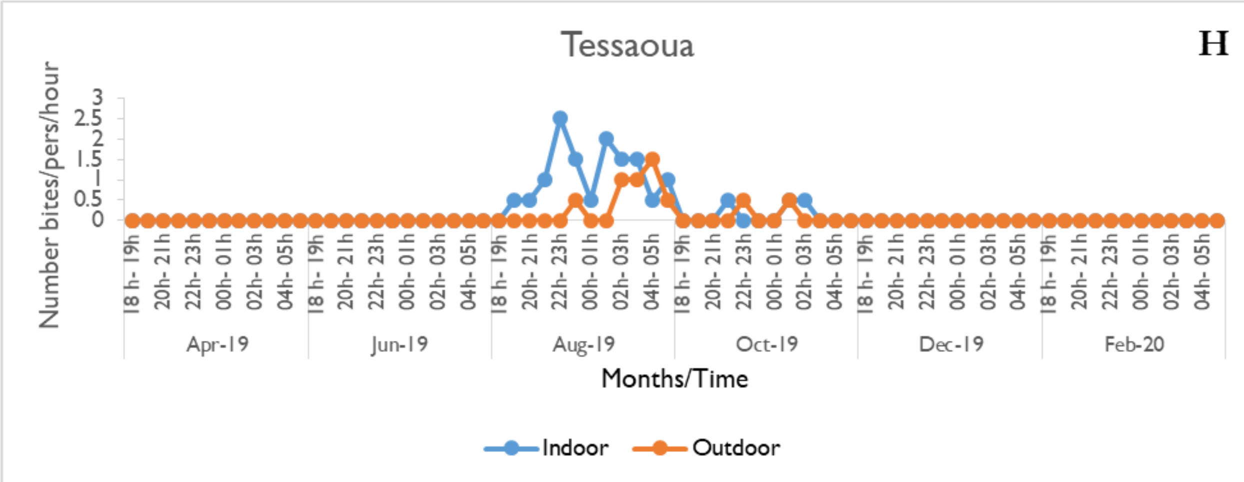


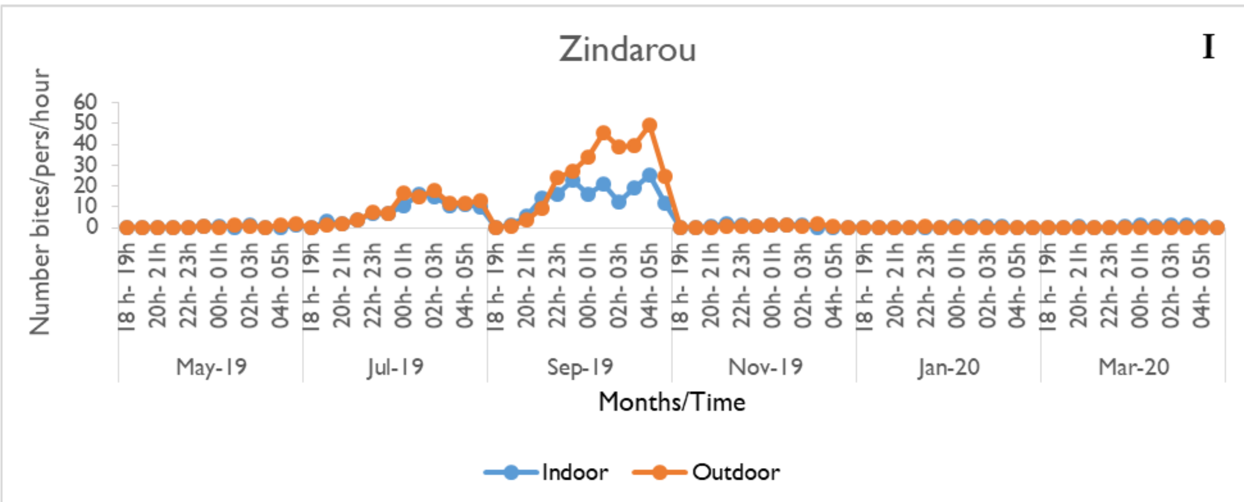
Gaya
D
Number bites/pers/hour
Months/Time
Indoor
Outdoor


Guidimouni
E
Number bites/pers/hour
Apr-19
Jun-19
Aug-19
Oct-19
Dec-19
Feb-20
Months/Time
Indoor
Outdoor


Niamey V
F
Number bites/pers/hour
Apr-19
May-19
Jun-19
Jul-19
Aug-19
Sep-19
Oct-19
Nov-19
Dec-19
Jan-20
Feb-20
Mar-20
Months/Time
Indoor
Outdoor


Say
G
Number bites/pers/hour
May-19
Jul-19
Sep-19
Nov-19
Jan-20
Mar-20
Months/Time
Indoor
Outdoor

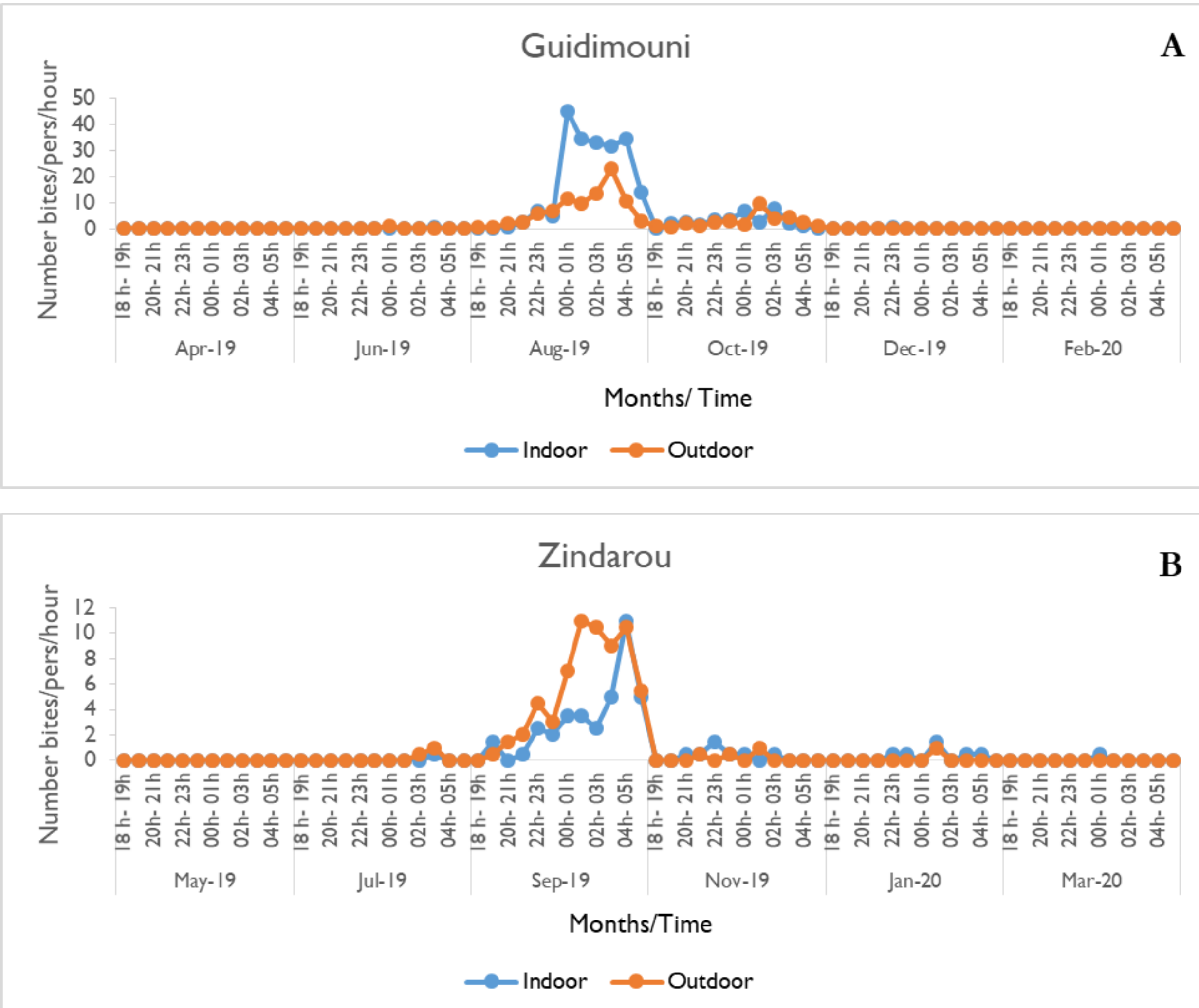

Tessaoua
H
Number bites/pers/hour
Months/Time
Apr-19
Jun-19
Aug-19
Oct-19
Dec-19
Feb-20
Indoor
Outdoor


Zindarou
I
Number bites/pers/hour
Months/Time
May-19
Jul-19
Sep-19
Nov-19
Jan-20
Mar-20
Indoor
Outdoor

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

Guidimouni recorded a high number of *An. funestus* s.l., followed by Zindarou. Figures 14 and 15 show the biting cycle of *An. funestus* s.l., with the peak biting occurring between 10:00 pm to 4:00 am, both indoors and outdoors (Figures 9A & B).

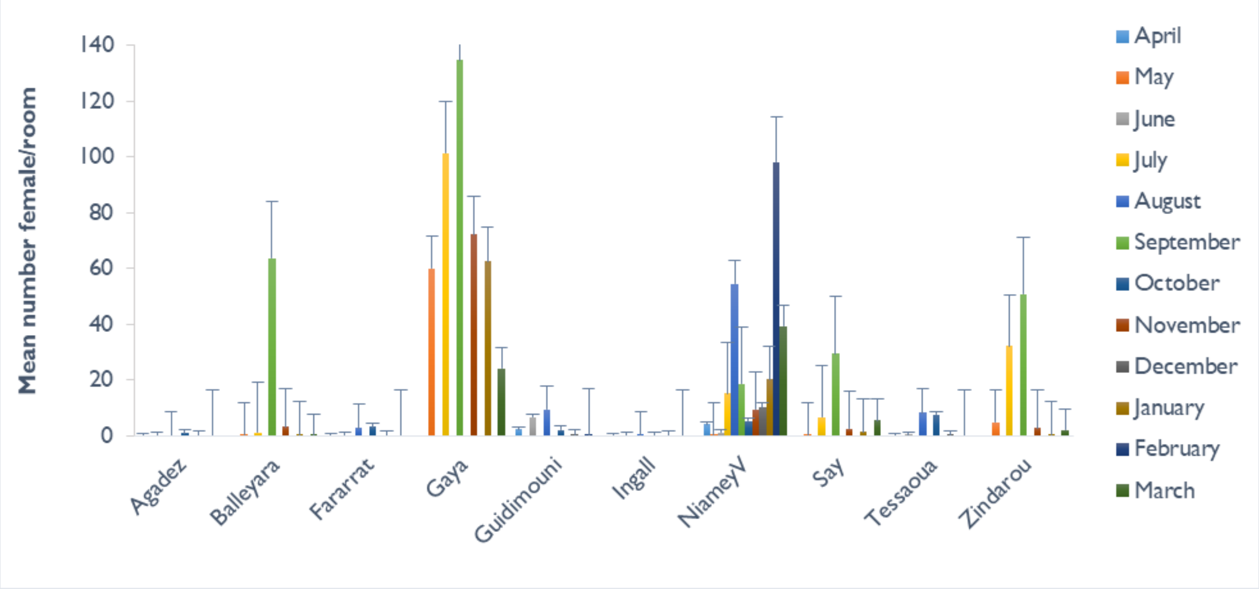
FIGURE 9: BITING RATE AND CYCLE OF *AN. FUNESTUS* S.L. IN GUIDIMOUNI AND ZINDAROU



3.1.5 INDOOR RESTING DENSITY

The average 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. Over the 12-month period, Gaya recorded the highest mean indoor resting density (75.9 females/room/day (f/r/d)), followed by Niamey V (23.0 f/r/d) and Zindarou (15.4 f/r/d). During the high transmission season, *An. gambiae* s.l. indoor resting densities were high in September in Gaya (more than100 f/r/d), followed by Balleyara (68.8 f/r/d) and Zindarou (48.1 f/r/d)); Niamey V recorded its highest indoor resting density (98.0 f/r/d) in February and also than any other sites in February (Figure 10).

FIGURE 10: MONTHLY MEAN DENSITY OF *AN. GAMBIAE* S.L. PER HOUSE "ERROR BARS OF EACH FIGURE REPRESENT THE STANDARD ERRORS"



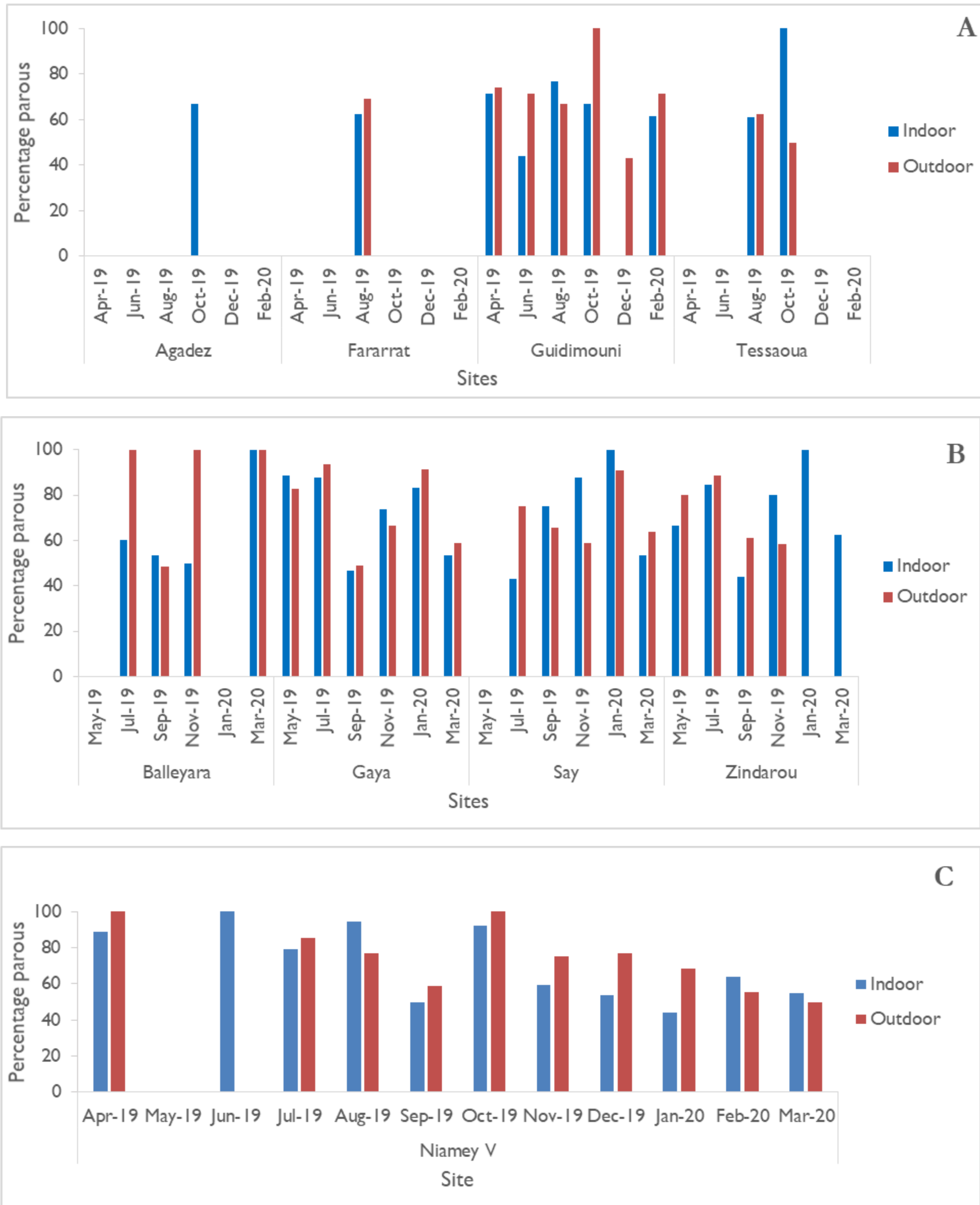
3.1.6 PARITY RATE OF *AN. GAMBIAE* S.L.

A total of 3,176 (1,710 indoor, 1,466 outdoor*) An. gambiae* s.l. collected over 12 months in Niamey V and six months in the other eight productive sites were dissected to determine parity: Agadez (3), Balleyara (342), Fararrat (21), Gaya (795), Guidimouni (119), Niamey V (147), Say (139), Tessaoua (31), and Zindarou (471). The single mosquito collected at Ingall was not dissected.

Of the dissected mosquitoes, 2 (66.7%) were parous in Agadez, 167 (51.5%) in Balleyara, 14 (66.7%) in Fararrat, 573 (72.1%) in Gaya, 83 (69.7%) in Guidimouni, 117 (79.6%) in Niamey V, 97 (69.8%) in Say, 19 (61.3%) in Tessaoua, and 385 (81.7%) in Zindarou. All the mean parity rates were above 50% in all sites (Figure 11 A, B, C; Annex Table A-2).

FIGURE 11: MONTHLY INDOOR AND OUTDOOR PARITY RATE OF AN. *GAMBIAE* S.L., BY SITE

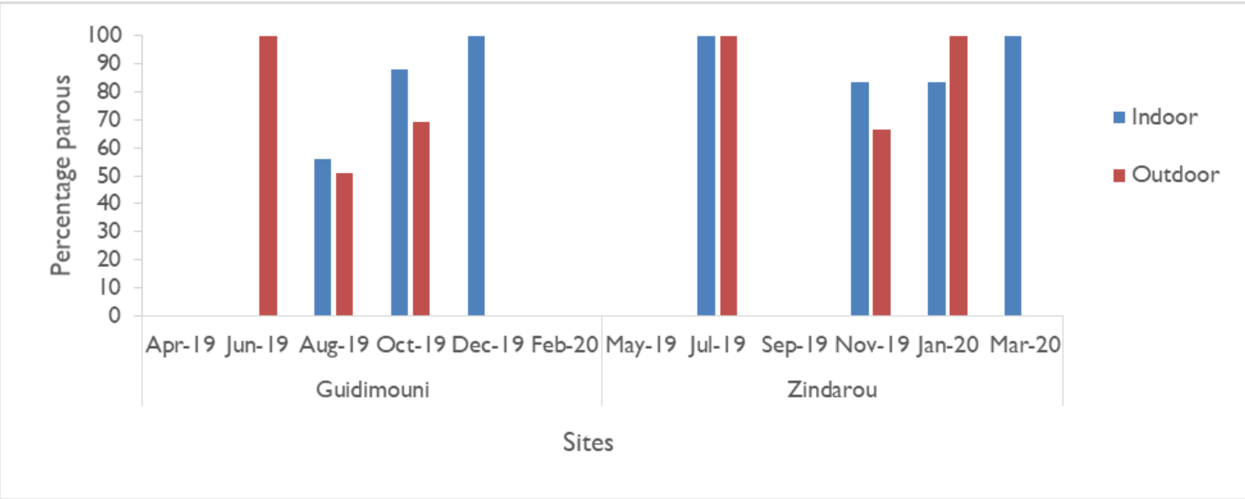
(A: Agadez, Farrarat, Guidimouni, and Tessaoua, Monitored In April, June, August, October, December 2019 And February 2020; B: Balleyara, Gaya, Say, and Zindarou, Monitored In May, July, September, November 2019 And January- March 2020 And C: Niamey V, Monitored for 12 consecutive months)



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

A total of 346 (248 indoor, 98 outdoor) *An. funestus* s.l. were dissected for parity assessment in Guidimouni (234 indoor, 91 outdoor) and Zindarou (14 indoor, 7 outdoor). Of the dissected mosquitoes, 192 (58.35%) in Guidimouni were parous, as were 18 (85.71%) in Zindarou (Figure 12, Annex Table A-3).

FIGURE 12: MONTHLY INDOOR AND OUTDOOR PARITY RATE OF *AN. FUNESTUS* S.L.



3.2 INSECTICIDE RESISTANCE MONITORING

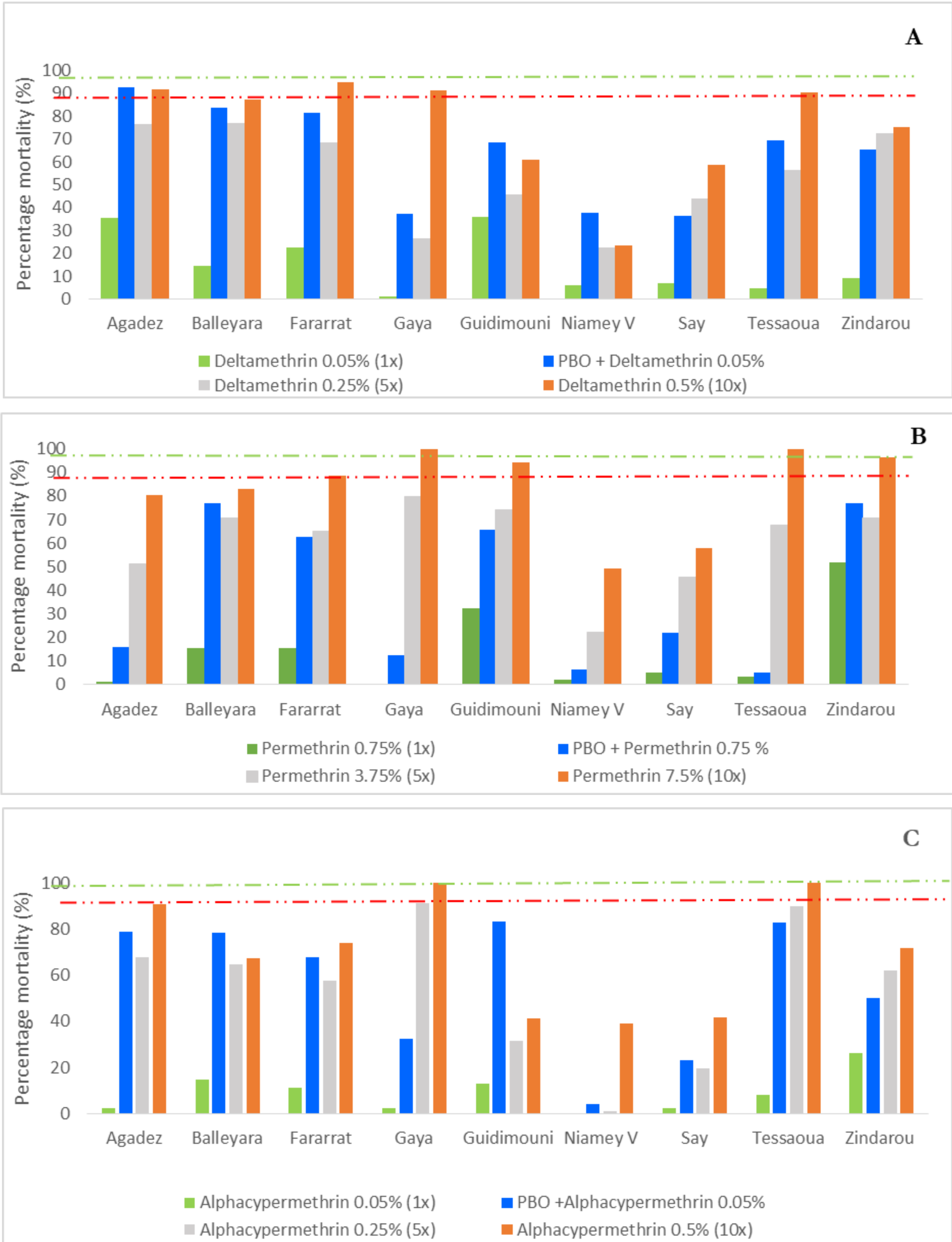
Between August and December 2019, PMI VectorLink Niger completed insecticide resistance testing on the *An. gambiae* s.l. collected in nine sites. (No larvae were found in Ingall after three attempted collections.) Figure 13 (A, B, C, D) illustrates the mosquitoes' resistance profile to the different insecticides. (Detailed results are shown in Annex Table A-4.)

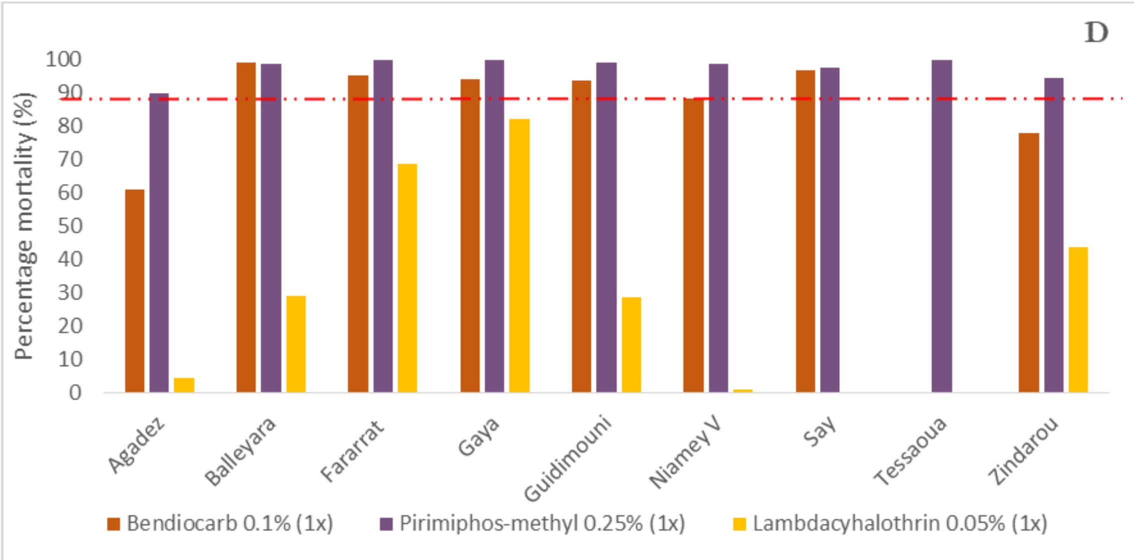
High pyrethroid resistance was observed for all the pyrethroids (deltamethrin, permethrin, and alpha-cypermethrin) tested in six of the sites (Agadez, Balleyara, Guidimouni, Niamey V, Say, and Zindarou). Moderate resistance was noted for deltamethrin in Agadez and Fararrat and for alpha-cypermethrin and permethrin in Gaya and Tessaoua. Susceptibility to pirimiphos-methyl was recorded in seven sites (all but Agadez and Zindarou). Resistance to lambda-cyhalothrin was observed at the six sites where tested and similar to all other pyrethroids tested.

Pre-exposure of mosquitoes to PBO before exposure to the pyrethroids did not completely reverse the resistance status of the *An. gambiae* s.l. populations in any of the sites surveyed except in Agadez, where moderate resistance was noted with PBO and deltamethrin. Nonetheless, a substantial increment in mortality was observed for all pyrethroids and particularly deltamethrin.

Note: The horizontal dashed red line in Figures 13 (A, B, C, D) represents the 90% threshold for resistance and the green line represents the 98% threshold for susceptibility.

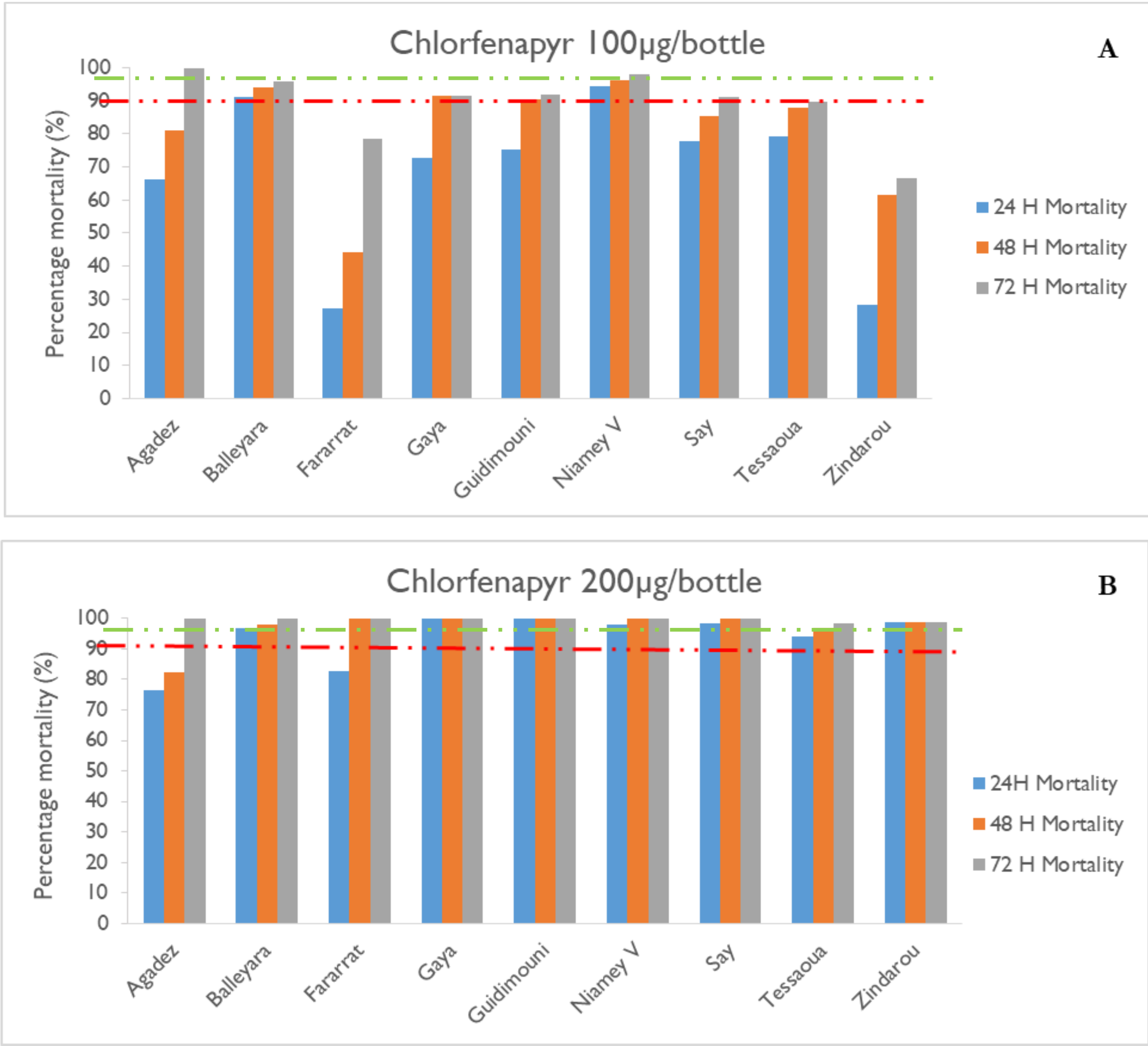
FIGURE 13: *AN. GAMBIAE* S.L. SUSCEPTIBILITY TO INSECTICIDES TESTED (A FOR DELTAMETHRIN, B FOR PERMETHRIN, C FOR ALPHA-CYPERMETHRIN AND D FOR BENDIOCARB, PIRIMIPHOS METHYL AND LAMBDA-CYHALOTRHIN)





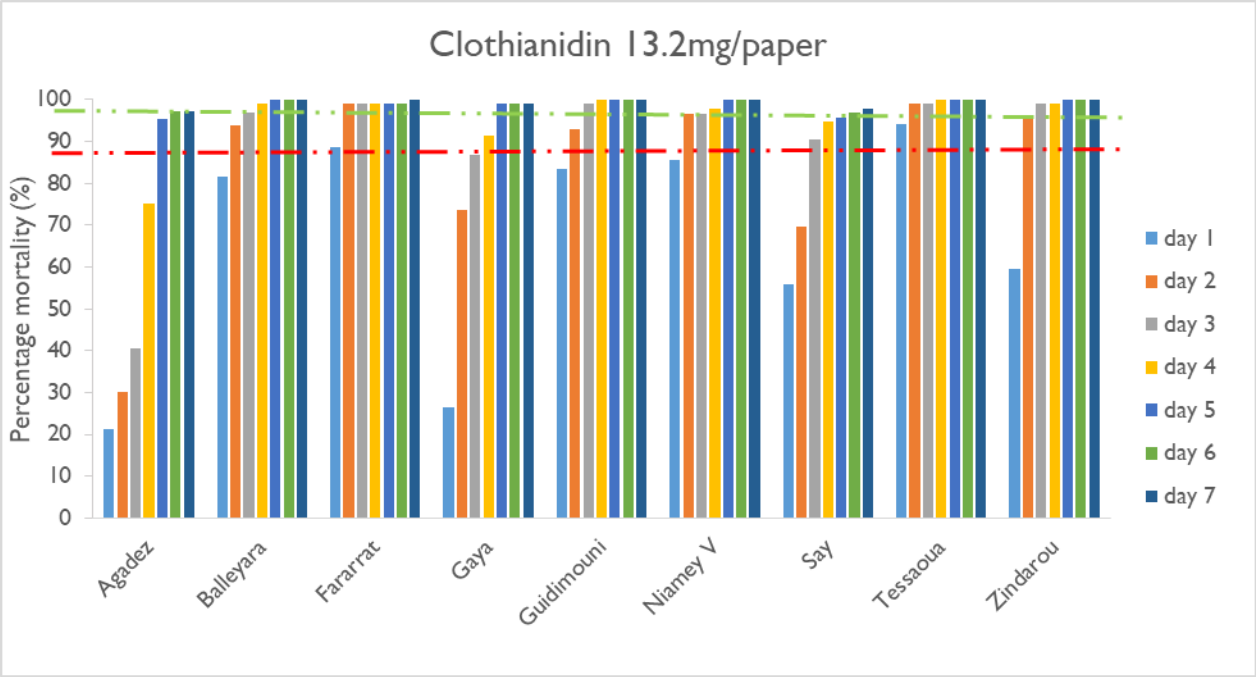
The results of the CDC bottle assays using chlorfenapyr are shown in Figures 14 A & B. Susceptibility to chlorfenapyr at 100µg/bottle was observed only in Agadez and Niamey V, after 72 hours. The lowest mortality rate at 100 µg/bottle was recorded in Zindarou (Figure 14A). Susceptibility to chlorfenapyr 200µg/bottle was recorded in all nine sites (Figure 14B).

FIGURE 14: SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. TO CHLORFENAPYR 100µG/BOTTLE AND 200µG/BOTTLE BY SITE



Full susceptibility to clothianidin was observed in seven of nine sites after seven days of delayed mortality recording. Possible resistance was observed in Agadez and Say (Figure 15).

FIGURE 15: SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. TO CLOTHIANIDIN, BY SITE

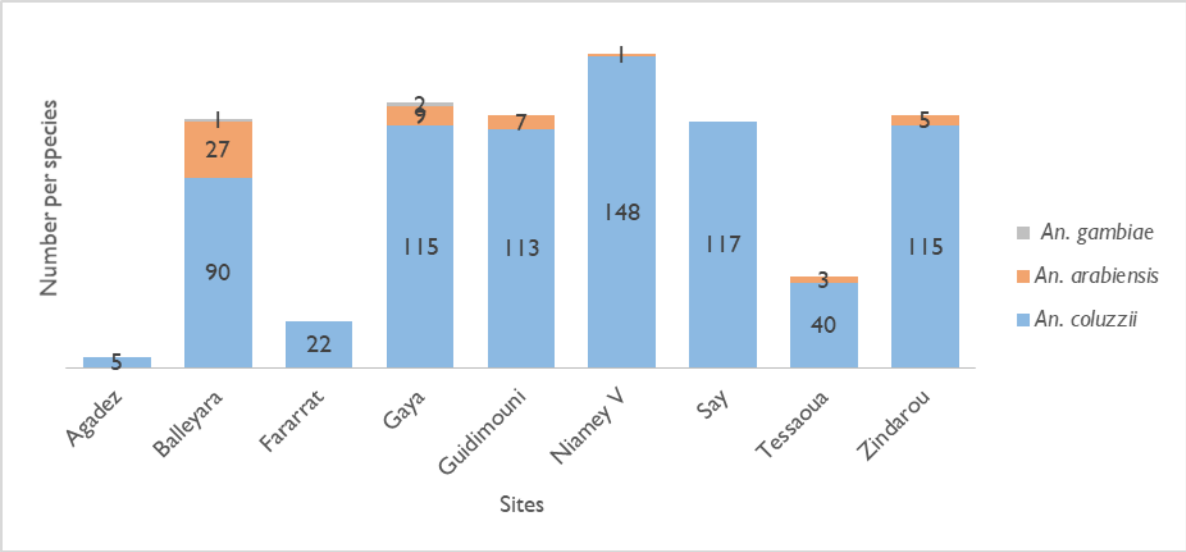


3.3 MOLECULAR CHARACTERIZATION OF *AN. GAMBIAE* S.L.

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

Of the 821 *An. gambiae* s.l. collected by HLC, *An. coluzzii* was the predominant species collected across all 10 sites (93.3%, n=766) and was the only species collected in Agadez, Fararrat, Ingall, and Say. *An. arabiensis* (6.3%, n=52) was collected in six of the sites: Balleyara (22.9%, n=27), Gaya (7.1%, n=9), Guidimouni (5.8%, n=9), NiameyV (0.7%, n=1), Tessaoua (7%, n=3), and Zindarou (4.2%, n=5). The highest number was collected in Balleyara, followed by Gaya and Tessaoua (Figure 16, Annex Table A-5). *An. gambiae* (0.4%, n=3) was collected only in Balleyara and Gaya.

FIGURE 16: SPECIES COMPOSITION OF AN. GAMBIAE COMPLEX COLLECTED BY HLC (N=821)*

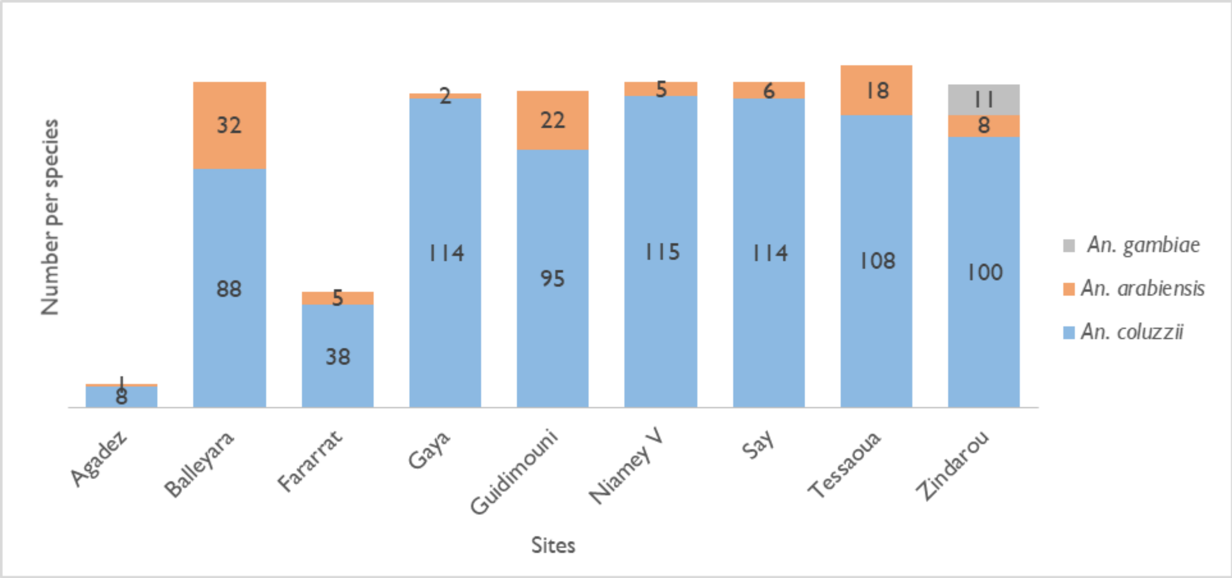


*A single An. coluzzii was collected at Ingall

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

Of the 891 *An. gambiae* s.l. collected by PSC and tested across all sites, *An. coluzzii* was the predominant species in all sites (87.6% n=781) followed by *An. arabiensis* (11.1%, n=99). *An. gambiae* (1.2%, n=11) was collected only in Zindarou, and *An. coluzzii* was the only species found in Ingall (n=1) (Figure 17, Annex A-5).

FIGURE 17: SPECIES COMPOSITION OF *AN. GAMBIAE* S.L. COMPLEX COLLECTED BY PSC (N=891)*

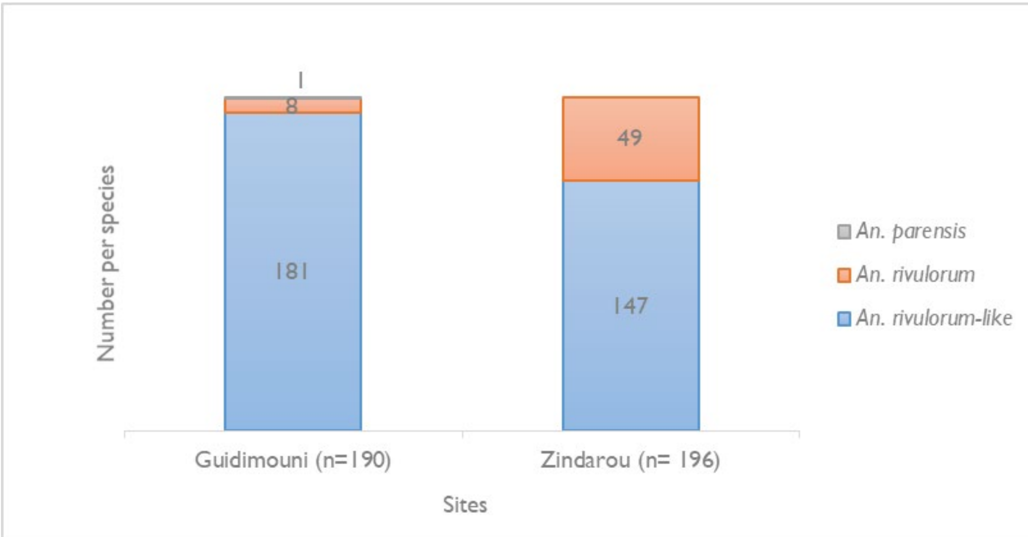


*A Single An. Coluzzii Was Collected At Ingall

3.3.3 Species Composition of *An. funestus* s.l. Complex Collected by HLC (n=386)

Of the 1077 *An. funestus* s.l. collected by HLC, a sample of 386 was tested of molecular characterization. *An. rivulorum-like*, at 95.3%, n=190 in Guidimouni and 75%, n=199 in Zindarou, was the predominant species, followed by *An. rivulorum* at 4.2.1%, n=190 and 25%, n=196 in the two sites, while *An. parensis* was identified only in Guidimouni (0.5%, n=1) (Figure 18).

Figure 18: Species Composition of *An. funestus* Complex Collected by HLC (n=386)



3.3.4 Plasmodium Sporozoite Infection Rates of *An. gambiae* s.l.

Of the 2,770 *An. gambiae* s.l. tested by PCR, 32 (HLC 23/1871 and PSC 9/899) were positive for *P. falciparum* parasite. For HLC collection, sporozoite infection rates were low, ranging from 0 to 0.0233, with an average of 0.0123 across sites. (Table 4). The infection rate of *An. gambiae* s.l. was higher among those collected indoors (mean of 1.64% all sites included) than outdoors. Say recorded the highest indoor infection rate (2.53 %) followed by Gaya (2.31%) while Zindarou recorded the highest outdoor infection rate (1.23%) followed by Niamey V (1.18%). Additionally, of the 899 *An. gambiae* s.l. mosquitoes collected using PSC in all nine sites, nine were infected. The sporozoite infection rates ranged from 0 to 0.0168. The highest sporozoite rates were recorded in Gaya, Say and Zindarou (mean of 1.67%) (Table5).

Table 4: *Plasmodium* Sporozoite Infection Rates of *An. gambiae s.l.* Collected by HLC

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	4	0	0.00	1	0	0.00	5	0	0.00
Balleyara	115	2	1.74	185	2	1.08	300	4	1.33
Fararrat	10	0	0.00	12	0	0.00	22	0	0.00
Gaya	173	4	2.31	127	0	0.00	300	4	1.33
Guidimouni	189	2	1.06	111	0	0.00	300	2	0.67
Niamey V	215	2	0.93	85	1	1.18	300	3	1.00
Ingall	0	0	0.00	1	0	0.00	1	0	0.00
Say	158	4	2.53	142	0	0.00	300	4	1.33
Tessaoua	32	0	0.00	11	1	9.09	43	1	2.33
Zindarou	138	3	2.17	162	2	1.23	300	5	1.67
Total	**1034**	**17**	1.64	**837**	**6**	0.72	**1871**	**23**	**1.23**

TABLE 5: *PLASMODIUM* SPOROZOITE INFECTION RATES OF *AN. GAMBIAE S.L.* COLLECTED BY PSC

Localities	Number tested	Positive	Sporozoite Rate (%)
Agadez	9	0	0.00
Balleyara	120	1	0.83
Fararrat	43	0	0.00
Gaya	120	2	1.67
Guidimouni	120	0	0.00
Niamey V	120	1	0.83
Say	120	2	1.67
Tessaoua	128	1	0.78
Zindarou	119	2	1.68
Total	899	**9**	1.00

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

The highest EIR was observed in Gaya with 30.12 infected bites per person per month (ib/p/m) followed by Zindarou with 28.02 ib/p/m. Balleyara and Say both recorded 10.41 ib/p/m. Niamey, Tessaoua, and Guidimouni recorded 6.31, 2.65, and 2.52 ib/p/m respectively (Table 6).

TABLE 6: ENTOMOLOGICAL INOCULATION RATE OF *AN. GAMBIAE* S.L. COLLECTED BY HLC

Localities	Sporozoite Rate		*HBR (bpm)*		*EIR (=ib/p/m)*			Period EIR (ib per collection period)*
	Indoor *IR*	Outdoor IR	Indoor HBR	Outdoor HBR	Indoor EIR	Outdoor EIR	Total EIR	
Agadez	0	0	4.80	0.90	0	0	0	0
Balleyara	0.0174	0.0108	410.40	303.00	7.14	3.27	10.41	62.46
Fararrat	0	0	15.00	16.50	0.00	0	0	0
Gaya	0.0231	0	1303.80	927.00	30.12	0	30.12	180.72
Guidimouni	0.0106	0	237.60	155.40	2.52	0	2.52	15.12
Niamey V	0.0093	0.0118	332.10	272.70	3.09	3.22	6.31	75.72
Say	0.0253	0	411.30	337.50	10.41	0	10.41	62.46
Tessaoua	0	0.0909	43.80	29.10	0.00	2.65	2.65	15.90
Zindarou	0.0217	0.0123	700.20	1042.50	15.19	12.82	28.02	168.12
Total Mean	**0.0119**	**0.014**	**384.30**	**342.60**	**4.57**	**4.80**	**9.37**	**NA**

* The EIR period was calculated over 6 months for all sites except Niamey 12 months

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

Of the 400 *An. funestus* s.l. tested by PCR, four were positive for *P. falciparum* parasite, one indoors in Guidimouni and three outdoors in Zindarou (Table 7). *An. rivulorum* was the only species found infected in the two sites.

The highest sporozoite infection rate of *An. funestus* s.l. was found in Zindarou (1.5%) while Guidimouni recorded the highest EIR (6.05 ib/p/m) (Table 8). Infection rate and EIR of *An. funestus* s.l. were higher indoors than outdoors in both sites.

TABLE 7: *PLASMODIUM* SPOROZOITE INFECTION RATES OF *AN. FUNESTUS* S.L. COLLECTED USING HLC

Localities	Indoor Number Tested	*Indoor Positive*	*Sporozoite Rate (%)*	Outdoor Number Tested	*Outdoor Positive*	Sporozoite Rate (%)	Total Number Tested	Total Positive	Mean Total Sporozoite Rate (%)
Guidimouni	100	1	1.00	100	**0**	0.00	200	1	0.5
Zindarou	67	2	2.99	133	**1**	0.75	200	3	1.5
Total	**167**	**3**	**1.80**	**233**	**1**	**0.43**	**400**	**4**	**1**

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

Localities	**Sporozoite Rate**		*HBR (bites per person per month)*		*EIR (=ib/p/m)*			*Period EIR (ib per collection period)**
	Indoor *SR*	**Outdoor SR**	**Indoor HBR**	**Outdoor HBR**	**Indoor EIR**	**Outdoor EIR**	**Total EIR**	
Guidimouni	0.01	0	605.1	309.90	6.05	0.00	6.05	36.31
Zindarou	0.0299	0.0075	127.8	174	3.82	1.31	5.13	30.76
Total Mean	**0.0200**	**0.0038**	**366.45**	**241.95**	**4.94**	**0.65**	**5.59**	**33.53**

* The EIR period was calculated over 6 months for both sites.

3.3.7 HOST PREFERENCE

A total of 1,344 mosquitoes were successfully analyzed by PCR from all nine sites (72 out of 1416 failed to amplify) and 70.5% showed evidence of having had a human blood meal (n=947). The highest human blood index was observed in Fararrat (87.5%, n=40) followed by Tessaoua (77.6%, n=116), and Balleyara (73.7%, n=194) (Table 9). The results also showed other blood meal sources, including bovine and ovine (also Table 9).

TABLE 9: BLOOD MEAL SOURCES AND HUMAN BLOOD INDEX OF *AN. GAMBIAE* S.L. COLLECTED USING PSC ACROSS ALL SITES

Sites	Total Tested	Human		Bovine		Ovine		Human/ Bovine		Human/Ovine		Total Human Blood Detected	Human Blood Index
		N	%	N	%	N	%	N	%	N	%	N	%
Agadez	7	5	71.4	2	28.6	0	0.0	0	0.0	0	0.0	5	71.4
Balleyara	194	141	72.7	31	16.0	18	9.3	2	1.0	0	0.0	143	73.7
Fararrat	40	35	87.5	0	0.0	5	12.5	0	0.0	0	0.0	35	87.5
Gaya	207	129	62.3	53	25.6	25	12.1	0	0.0	0	0.0	129	62.3
Guidimouni	196	140	71.4	46	23.5	10	5.1	0	0.0	0	0.0	140	71.4
Niamey V	196	126	64.3	54	27.6	7	3.6	8	4.1	1	0.5	135	68.9
Say	194	132	68.0	45	23.2	14	7.2	**3**	1.5	0	0.0	135	69.6
Tessaoua	116	85	73.3	12	10.3	14	12.1	4	3.4	1	0.9	90	77.6
Zindarou	194	135	69.6	43	22.2	16	8.2	0	0.0	0	0.0	135	69.6
Total	**1344**	**928**	**69.0**	**286**	**21.3**	**109**	**8.1**	**17**	**1.3**	**2**	**0.1**	**947**	**70.5**

3.4 INSECTICIDE RESISTANCE MARKERS

A total of 1,056 out of the 14,189 *An. gambiae* s.l. mosquitoes from insecticide susceptibility tests across all nine productive sites were analyzed for k*dr*-w and *Ace-1* mutations, out of which 844 were *An. coluzzii* (97.6%). *Kdr*-west frequencies varies from 0.22% to 038% with a mean of 0.31%. The highest *kdr*-w frequency was recorded in Guidimouni (0.38%) followed by Balleyara (0.36%), while the highest Ace-1 frequency was found in Balleyara and Niamey V (0.09) (Table 10 and Annex Table A-6).

TABLE 10: THE MOLECULAR SPECIES AND INSECTICIDE RESISTANCE MECHANISM ACROSS SITES

Localities	Number Tested	Species			*kdr-w* Mutation				*Ace-1* Mutation			
		An. gambiae s.s.	*An. arabiensis*	*An. coluzzii*	RR	RS	SS	freq	RR	RS	SS	Freq
Agadez	**117**	0	0	117	21	10	86	***0.22***	4	7	106	***0.06***
Balleyara	**118**	2	32	84	39	8	71	***0.36***	10	2	106	***0.09***
Fararrat	**118**	3	10	105	31	8	79	***0.30***	3	8	107	***0.06***
Gaya	**117**	3	15	99	35	13	69	***0.35***	6	6	105	***0.08***
Guidimouni	**117**	0	88	29	40	10	67	***0.38***	4	7	106	***0.06***
Niamey V	**113**	0	3	110	22	9	92	***0.23***	6	8	99	***0.09***
Say	**118**	0	8	110	24	8	86	***0.24***	7	2	109	***0.07***
Tessaoua	**119**	0	23	96	30	23	66	***0.35***	4	10	105	***0.08***
Zindarou	**119**	14	11	94	33	9	77	***0.32***	2	9	108	***0.05***
Total	**1056**	22	190	**844**	275	98	693	***0.31***	46	59	951	***0.07***

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

4. DISCUSSION AND CONCLUSION

Bionomic data collected using both HLC and PSC methods showed that *An. gambiae* s.l. was the predominant malaria vector (90.5%) at the 10 sentinel sites in which VectorLink Niger carried out activities under its 2019 work plan. *An. funestus* s.l. was found in only three sites: the highest proportion was in Guidimouni (67.9%), followed by Zindarou and Gaya. This larger number of *An. funestus* s.l. recorded in Guidimouni and Zindarou will need further investigation, such as insecticide resistance monitoring, to understand the impact of the vector control tools deployed in those areas on the vector population.

Of the 15,469 *Anopheles* collected, PSC produced the highest number (n=9821). Gaya recorded the highest density of all the sites followed by Niamey V. 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 proximity of the site of Niamey V to the laboratory enabled collections to be done monthly at little extra cost.

Niger has a long (eight-month) dry season from November to June and a very short (four-month) rainy season from July to October, during which malaria incidence is highest. To maximize resources, the low densities or absence of vectors observed in most sites after December should be considered when planning the collection periods for those sites in future years—vector surveillance should be planned for these sites mostly during the rainy season.

The results of the HLCs also showed that the peak biting time was recorded between 10:00 pm and 4:00 am in all sites both indoors and outdoors. The *An. gambiae* s.l. indoor HBR was highest in Gaya during the rainy season while the outdoor biting rate was highest in Zindarou, also during the rainy season. Niamey V was the only site to record its highest HBR in the dry season (February). The overall parity rate was high across nine sites, showing a high probability for vectors to carry the malaria parasite.

As in 2018, the molecular analysis of entomological samples that VectorLink Niger collected from April 2019 to March 2020 showed that *An. coluzzii* was the predominant malaria vector in nine sites for vectors susceptibility and in 10 sites for bionomic monitoring using both HLC and PSC collection methods. *An. arabiensis* was found in all the susceptibility monitoring sites except Agadez, in nine of the 10 sites using the PSC method and in six of the 10 using HLC. *An. gambiae* was present and collected only in Zindarou using PSC method and in two of the 10 sites (Balleyara and Gaya) using HLC. In Balleyara, Fararrat, Gaya, and Zindarou, the larvae collected for susceptibility monitoring included all three species, *An. gambiae*, *An. coluzzii,* and *An. arabiensis*.

Molecular characterization also revealed the presence of three species within the *An. funestus* group in the two sites where a larger number of *An. funestus* s.l. was collected. In both sites, *An. rivulorum-like* was the predominant species followed by *An. rivulorum,* while *An. parensis* was present only in Guidimouni.

In 2019, Gaya recorded the highest EIR, followed by Zindarou. Furthermore, Gaya, Say and Zindarou recorded higher sporozoite infection rates within the *An. gambiae* s.l. collected by PSCs, therefore increasing the risk of populations to contract malaria through the bite of infected vectors. All three sites recorded their highest biting rates during the transmission period covering August to September and are also located in the hyper endemic zone of the country. Similar to 2018, no infected mosquito was recorded in the hypo-endemic zones of Agadez or Ingall. Nor was any infected mosquito recorded in Fararrat, although it is in the meso-endemic area. Additionally, *An. funestus* s.l. was found infected with *P. falciparum* in Guidimouni and Zindarou with a higher EIR in Guidimouni than that of *An. gambiae* s.l. in this site. The data confirm the low transmission observed in Agadez and Ingall and contribute to the hypo endemicity of both areas as described by the epidemiological map of the country. Further strategies could be put in place for malaria elimination in those areas. Transmission is persistent in meso and hyper-endemic areas despite nationwide pyrethroid treated only ITN coverage in all the districts of these area which could be attributed to the utilization rate of the ITNs by the population and the geo-localization of the areas (Southern and wettest part of the country). Per the latest data recorded on ITN utilization in the country in 2012, 23.5% of the children under 5 years and 19.9% of the pregnant women were using the ITNs received (NMCP 2013), though these rates may have gradually increased. But, there is a need to continuously intensify communication for the correct use of ITNs when sleeping in both inside and outside houses.

An. gambiae s.l. fed on both humans and animals and showed that human and vector contact is still occurring, hence the need to increase awareness of the correct use of ITNs.

Both *kdr* and *Ace*-1 mutations were present in all of the nine productive sites. The *kdr-w* was present in the nine sites, thus contributed to the high resistance to pyrethroids observed within the susceptibility tests of the site vectors. Guidimouni recorded the highest frequency of *kdr-w* followed by Balleyara, both of which are market gardening areas with intensive use of pesticides in addition to universal ITN distribution. The *Ace*-1 frequencies are still low in the country with Balleyara and Niamey V recording the highest frequencies.

In Niamey V, rainfall and the presence of the Niger River contributes to perennially high vector densities. Niamey V experienced flooding in January 2020, which may have contributed to the presence and increased density of malaria vectors in February, bringing the risk of a malaria epidemic to the area. Therefore, the current data on mosquito density in Niamey could help the NMCP to better prepare for unseasonable outbreaks of malaria and/or other mosquito-borne diseases in Niamey (or elsewhere) at an unexpected time of the year.

An. gambiae s.l. was resistant to all three pyrethroids tested (deltamethrin, permethrin, and alpha-cypermethrin) in all nine sites and high resistance was recorded in six of the sites. Moderate mosquito resistance to deltamethrin was observed in Agadez and Fararrat and to alpha-cypermethrin and permethrin in Gaya and Tessaoua. Pre-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 except for deltamethrin in Agadez. This suggests that enzymes such as P450s may be involved in the insecticide resistance of the vectors in some sites. These data collected for the second consecutive year of PMI/VectorLink project in the country will inform the NMCP's approach to ITN procurement and distribution. The benefit of the observed pre-exposure to PBO could support PBO net distribution and stratification in the country, especially in the areas of high malaria incidence.

Chlorfenapyr at the dose of 200µg/bottle yielded full susceptibility at all the sites tested suggesting it usefulness in vector control as demonstrated in the new generation of ITNs such as interceptor G2. Susceptibility to pirimiphos-methyl, observed in seven of the nine sites, suggests that it may be a good candidate for Indoor Residual Spray in the future. Clothianidin at the dose of 13.2mg yielded full susceptibility in seven sites and moderate resistance in Agadez and Say suggests that it also may be a good candidate for IRS in the future in the country .

The NMCP previously developed an insecticide resistance monitoring and management plan that is scheduled to be revised in 2020. To better adapt vector control strategies and resistance management, the NMCP should use the current data collected and trends observed in this report when revising the plan. Whereas current NMCP policy places ITNs at the forefront of vector control strategies, the data indicate that other tools and other vector control strategies, such as new generation ITNs and/or IRS, may be more effective at reducing the densities and longevity of mosquitoes to reduce malaria transmission in areas of high malaria incidence.

5. References

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6. ANNEX: DATA TABLES

TABLE A-1: BITING RATE RESULTS HLC OF *AN. GAMBIAE* S.L.

Sites	Month	Indoor Human Biting Rate (b/p/n)	Outdoor Human Biting Rate (b/p/n)	Average Human Biting Rate (b/p/n)
Agadez	Apr-19	0	0	0
	Jun-19	0	0	0
	Aug-19	0	0	0
	Oct-19	1	0.25	0.63
	Dec-19	0	0	0.00
	Feb-20	0	0	0.00
Balleyara	May-19	0	0	0.00
	Jul-19	1.8	0.3	1.05
	Sep-19	56.6	57.8	57.20
	Nov-19	9.75	2.25	6.00
	Jan-20	0	0	0.00
	Mar-20	0.25	0.25	0.25
Fararrat	Apr-19	0	0	0.00
	Jun-19	0	0	0.00
	Aug-19	2	3.3	2.65
	Oct-19	1	0	0.50
	Dec-19	0	0	0.00
	Feb-20	0	0	0.00
Gaya	May-19	23.5	30	26.75
	Jul-19	49.2	28.7	38.95
	Sep-19	116.1	81	98.55
	Nov-19	12.75	13.5	13.13
	Jan-20	36	19	27.50
	Mar-20	23.25	13.25	18.25
Guidimouni	Apr-19	5.75	10.5	8.13
	Jun-19	4.5	5.3	4.90
	Aug-19	9.3	2.3	5.80
	Oct-19	3.25	2	2.63
	Dec-19	0	2	1.00
	Feb-20	24.75	9	16.88
Niamey V	Apr-19	2.5	1	1.75
	May-19	0	0	0.00
	Jun-19	0.3	0	0.15
	Jul-19	6.5	9	7.75
	Aug-19	15.5	5.5	10.50
	Sep-19	6.3	7.3	6.80
	Oct-19	3.25	1.25	2.25
	Nov-19	7	4	5.50
	Dec-19	7.25	6.5	6.88
	Jan-20	13.25	10.5	11.88
	Feb-20	61	52.75	56.88

Sites	Month	Indoor Human Biting Rate (b/p/n)	Outdoor Human Biting Rate (b/p/n)	Average Human Biting Rate (b/p/n)
	Mar-20	10	11.25	10.63
Say	May-19	0	0	0.00
	Jul-19	1.8	3	2.40
	Sep-19	71.75	54.5	63.13
	Nov-19	2	4.25	3.13
	Jan-20	3	2.75	2.88
	Mar-20	3.75	3	3.38
Tessaoua	Apr-19	0	0	0
	Jun-19	0	0	0
	Aug-19	6.5	3.3	4.4
	Oct-19	2.25	0.5	1.38
	Dec-19	0	0	0.00
	Feb-20	0	0	0.00
Zindarou	May-19	1.8	2.75	2.3
	Jul-19	47.5	53.5	50.5
	Sep-19	82.5	148.3	115.4
	Nov-19	4.25	3.75	4.00
	Jan-20	1	0.25	0.63
	Mar-20	3	0	1.50

TABLE A-2: PARITY RESULTS OF *AN. GAMBIAE* S.L. COLLECTED INDOORS AND OUTDOORS

Sites	Month	Indoor			Outdoor		
		Dissected	Parous	Parity rate	Dissected	Parous	Parity rate
Agadez	Apr-19	0	0	0	0	0	0
	Jun-19	0	0	0	0	0	0
	Aug-19	0	0	0	0	0	0
	Oct-19	3	2	66.67	0	0	0
	Dec-19	0	0	0	0	0	0
	Feb-20	0	0	0	0	0	0
Balleyara	May-19	0	0	0	0	0	0
	Jul-19	5	3	60	1	1	100
	Sep-19	181	97	53.6	155	75	48.39
	Nov-19	16	8	50	3	3	100
	Jan-20	0	0	0	0	0	0
	Mar-20	1	1	100	1	1	100
Fararrat	Apr-19	0	0	0	0	0	0
	Jun-19	0	0	0	0	0	0
	Aug-19	8	5	62.5	13	9	69.23
	Oct-19	0	0	0	0	0	0
	Dec-19	0	0	0	0	0	0
	Feb-20	0	0	0	0	0	0
Gaya	May-19	79	70	88.6	92	76	82.61
	Jul-19	198	174	87.9	109	102	93.58
	Sep-19	186	87	46.8	131	64	48.85
	Nov-19	23	17	73.9	18	12	66.7
	Jan-20	96	80	83.3	68	62	91.2
	Mar-20	69	37	53.6	46	27	58.7
Guidimouni	Apr-19	14	10	71.4	35	26	74.29
	Jun-19	16	7	43.8	21	15	71.43
	Aug-19	30	23	76.7	3	2	66.67
	Oct-19	9	6	66.7	3	3	100
	Dec-19	0	0	0	7	3	42.9
	Feb-20	26	16	61.5	7	5	71.4

Sites	Month	Indoor			Outdoor		
		Dissected	Parous	Parity rate	Dissected	Parous	Parity rate
Niamey V	Apr-19	9	8	88.9	3	3	100
	May-19	0	0	0	0	0	0
	Jun-19	1	1	100	0	0	0
	Jul-19	19	15	78.9	34	29	85.29
	Aug-19	35	33	94.3	13	10	76.92
	Sep-19	16	8	50	17	10	58.82
	Oct-19	13	2	92.31	5	5	100
	Nov-19	27	16	59.26	16	12	75
	Dec-19	28	15	53.57	26	20	76.92
	Jan-20	52	23	44.23	35	24	68.57
	Feb-20	149	95	63.76	125	69	55.2
	Mar-20	33	18	54.55	36	18	50
Say	May-19	0	0	0	0	0	0
	Jul-19	7	3	42.9	68	51	75
	Sep-19	68	51	75	52	34	65.38
	Nov-19	8	7	87.5	17	10	58.82
	Jan-20	12	12	100	11	10	90.91
	Mar-20	15	8	53.3	11	7	63.64
Tessaoua	Apr-19	0	0	0	0	0	0
	Jun-19	0	0	0	0	0	0
	Aug-19	23	14	60.9	8	5	62.5
	Oct-19	3	3	100	2	1	50
	Dec-19	0	0	0	0	0	0
	Feb-20	0	0	0	0	0	0
Zindarou	May-19	6	4	66.7	10	8	80
	Jul-19	179	151	84.4	210	186	88.57
	Sep-19	25	11	44	41	25	60.98
	Nov-19	10	8	80	12	7	58.3
	Jan-20	4	4	100	1	0	0
	Mar-20	8	5	62.5	0	0	0

TABLE A-3: PARITY RESULTS OF *AN. FUNESTUS* S.L. COLLECTED INDOORS AND OUTDOORS

Sites	Month	Indoor			Outdoor		
		Dissected	Parous	Parity rate	Dissected	Parous	Parity rate
Guidimouni	Apr-19	0	0	0	0	0	0
	Jun-19	0	0	0	2	2	100
	Aug-19	207	116	56.04	63	32	50.79
	Oct-19	25	22	88	26	18	69.23
	Dec-19	2	2	100	0	0	0
	Feb-20	0	0	0	0	0	0
Zindarou	May-19	0	0	0	0	0	0
	Jul-19	1	1	100	2	2	100
	Sep-19	0	0	0	0	0	0
	Nov-19	6	5	83.3	3	2	66.7
	Jan-20	6	5	83.3	2	2	100
	Mar-20	1	1	100	0	0	0

TABLE A-4: *AN. GAMBIAE* S.L. SUSCEPTIBILITY TO INSECTICIDES TESTED

Insecticide Tested	Agadez	Balleyara	Fararrat (Keita)	Gaya	Guidimouni	Niamey V	Say	Tessaoua	Zindarou (Boboye)
	Number tested (% mortality)	**Number tested (% mortality)**	**Number tested (% mortality)**	**Number tested (% mortality)**	**Number tested (% mortality)**	**Number tested (% mortality)**	**Number tested (% mortality)**	**Number tested (% mortality)**	**Number tested (% mortality)**
Deltamethrin (0.05%)	76 (35.5)	96 (14.6)	84 (22.6)	91 (1.1)	95 (35.8)	98 (6.1)	102 (6.9)	83(4.8)	99 (9.1)
PBO + Deltamethrin (0.05)	88 (92.8)	99 (83.8)	98 (81.6)	99 (37.4)	98 (68.4)	82 (37.8)	94 (36.2)	95 (69.5)	96 (65.6)
Deltamethrin (0.25%)	90 (76.7)	101 (77.2)	89 (68.5)	91 (26.4)	105 (45.7)	98 (22.4)	98 (43.9)	87 (56.3)	95 (72.6)
Deltamethrin (0.5%)	109 (91.7)	88 (87.5)	97 (94.8)	101 (91.1)	100 (61)	68(23.5)	102 (58.8)	51 (90.2)	100 (75)
Permethrin (0.75%)	82 (1.2)	103 (15.5)	121 (15.7)	100 (0)	96 (32.3)	91(2.2)	99 (5.1)	85 (3.5)	79 (51.9)
PBO + Permethrin (0.75%)	81 (16)	99 (76.8)	102 (62.7)	96 (12.5)	105 (65.7)	80 (6.3)	105 (21.9)	80 (5)	78 (76.9)
Permethrin (3.75%)	102 (51)	102 (70.7)	94 (64.9)	93 (79.6)	108 (74.1)	90 (22.2)	94 (45.7)	96 (67.7)	89 (70.8)
Permethrin (7.5%)	101 (80.2)	94 (83)	95 (88.4)	92 (100)	106 (94.3)	89 (49.4)	93 (58.1))	47 (100)	109 (96.3)
Alpha-cypermethrin (0.05)	82 (2.4)	95 (14.7)	100 (11)	90 (2.2)	86 (12.8)	91(0)	84 (2.4)	86 (8.1)	87 (26.4)
PBO + Alpha-cypermethrin (0.05)	90 (78.9)	102 (78.4)	93 (67.7)	92 (32.6)	84 (83.3)	93 (4.3)	86 (23.3)	81 (82.7)	74 (50)
Alpha-cypermethrin (0.25)	97(68)	97(64.9)	104(57.7)	102 (91.2)	100 (31.5)	90 (1.1)	92 (196)	50 (90)	100 (62)
Alpha-cypermethrin (0.5)	96 (90.6)	98 (67.3)	88 (73.8)	102 (100)	107 (41.1)	74 (39.2)	84 (41.7)	51 (100)	95 (71.6)
Lambda-cyhalothrin (0.05%)	88 (4.5)	72(29.2)	102(68.6)	106(82.1)	104 (28.8)	105 (1)	97 (0)	x	105 (43.8)
Bendiocarb (0.1%)	96 (61.1)	98 (99)	85 (95.3)	101 (94.1)	97 (93.8)	77 (88.3)	90 (96.7)	x	99 (78)
Pirimiphos-methyl (0.25%)	81 (90.1)	95 (98.9)	92 (100)	109 (100)	101 (99)	88 (98.9)	90(97.8)	55 (100)	93 (94.6)

Resistant confirmed *Possible resistance* *Susceptible;* x=test not completed

TABLE A-5: SPECIES COMPOSITION OF THE *AN. GAMBIAE* S.L. COMPLEX COLLECTED USING PSC AND HLC (N=1712)

Sites	PSC							HLC						
		An. coluzzii		*An. arabiensis*		*An. gambiae* s.s.			*An. coluzzii*		*An. arabiensis*		*An. gambiae* s.s.	
	Total number	*number*	*%*	*number*	*%*	*number*	*%*	*Total number*	*number*	*%*	*number*	*%*	*number*	*%*
Agadez	**9**	8	88.9	1	11.1	0	0	**5**	5	100.0	0	0.0	0	0.0
Balleyara	**120**	88	73.3	32	26.7	0	0	**118**	90	76.3	27	22.9	1	0.8
Fararrat	**43**	38	88.4	5	11.6	0	0	**22**	22	100.0	0	0.0	0	0.0
Gaya	**116**	114	98.3	2	1.7	0	0	**126**	115	91.3	9	7.1	2	1.6
Guidimouni	**117**	95	81.2	22	18.8	0	0	**120**	113	94.2	7	5.8	0	0.0
Ingall	**1**	1	100	0	0	0	0	**1**	1	100	0	0	0	0.0
Niamey V	**120**	115	95.8	5	4.2	0	0	**149**	148	99.3	1	0.7	0	0.0
Say	**120**	114	95.0	6	5.0	0	0	**117**	117	100.0	0	0.0	0	0.0
Tessaoua	**126**	108	85.7	18	14.3	0	0	**43**	40	93.0	3	7.0	0	0.0
Zindarou	**119**	100	84.0	8	6.7	11	9.2	**120**	115	95.8	5	4.2	0	0.0
Total	**891**	**781**	**87.6**	**99**	**11.1**	**11**	**1.2**	**821**	**766**	**93.3**	**52**	**6.3**	**3**	**0.4**

TABLE A-6: FREQUENCY OF RESISTANCE ALLELES PER DEAD AND ALIVE STATUS OF MOSQUITOES

		Resistance Alleles								
		kdr					*Ace-1*			
sites		**Total**	**RR**	**RS**	**SS**	***freq***	**RR**	**RS**	**SS**	***freq***
Agadez	dead	28	4	8	22	***0.21***	1	3	24	***0.09***
	alive	89	17	2	64	***0.11***	3	4	82	***0.06***
Balleyara	dead	66	22	5	39	***0.20***	8	0	58	***0.12***
	alive	52	17	3	32	***0.19***	2	2	48	***0.06***
Fararrat	dead	74	18	6	50	***0.16***	1	3	70	***0.03***
	alive	44	13	2	29	***0.17***	2	5	37	***0.10***
Gaya	dead	47	17	4	26	***0.40***	1	3	43	***0.05***
	alive	70	18	9	43	***0.32***	5	3	62	***0.09***
Guidimouni	dead	71	21	8	42	***0.35***	3	3	65	***0.06***
	alive	47	19	2	26	***0.43***	1	4	42	***0.06***
Niamey V	dead	28	6	3	19	***0.27***	0	0	28	***0***
	alive	85	16	6	63	***0.22***	6	8	71	***0.12***
Say	dead	38	7	4	27	***0.24***	1	0	37	***0.03***
	alive	80	17	4	69	***0.24***	6	2	72	***0.09***
Tessaoua	dead	26	9	3	15	***0.40***	0	1	25	***0.02***
	alive	93	21	20	52	***0.33***	4	9	80	***0.09***
Zindarou	dead	52	16	5	31	***0.36***	1	5	46	***0.07***
	alive	67	17	4	46	***0.28***	1	4	62	***0.04***