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PMI VECTORLINK MALI

ANNUAL ENTOMOLOGICAL MONITORING REPORT

JANUARY – DECEMBER 2018

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ACRONYMS

CS	Capsule suspension
CSP	Circumsporozoite protein
EIR	Entomological inoculation rate
ELISA	Enzyme-linked immunosorbent assay
HLC	Human landing catch
IRS	Indoor residual spraying
LBMA	Laboratoire de Biologie Moléculaire Appliquée, Molecular Biology Laboratory
LLIN	Long-lasting insecticidal net
N-GEN IRS	Next generation indoor residual spraying
NMCP	National Malaria Control Program
PBO	Piperonyl butoxide
PMI	U.S. President's Malaria Initiative
PSC	Pyrethrum spray catch
WG	Wettable granules

EXECUTIVE SUMMARY

In 2018, Actellic 300CS (capsule suspension) was sprayed in Bandiagara and Bankass (Mopti Region) for the second consecutive year and SumiShield 50WG was sprayed for the first time in Mopti and Djenné (also Mopti Region). The 2018 Mali indoor residual spraying (IRS) campaign was originally scheduled to begin on July 9th. Due to the increasing frequency of security incidents within the IRS target area leading up to and following the July 20th presidential election, VectorLink Mali suspended all activities, and the IRS campaign was implemented from August 30 to October 2, 2018. To monitor the impact of spraying on entomological indicators, mosquitoes were collected in four sprayed sites using pyrethrum spray catch (PSC) and human landing catch (HLC). An unsprayed control site, Tominian in neighboring Segou Region, and two sites in Koulikoro Region—Koulikoro (former IRS site) and Kati (unsprayed site)—were also monitored to determine the impact of IRS relocation.

IRS with Actellic 300CS in Bandiagara and Bankass had good residual efficacy of approximately four to six months on mud and cement walls. Cone bioassay demonstrated that SumiShield 50WG lasted a minimum of six months and still yielded 100% mortality (with 72h holding) six months after spraying. As the high transmission season is short in Mopti Region, either Actellic 300CS or SumiShield 50WG can be used to provide control during the whole season (assuming IRS is conducted in July).

Among the six different *Anopheles* species encountered (*An. gambiae* s.l., *An. funestus* s.l., *An. pharoensis*, *An. nili*, *An. ziemanni* and *An. rufipes*), *An. gambiae* s.l. was by far the most frequently sampled and accounted for 97.5% of *Anopheles* collected (14,033/14,390). Preliminary molecular species analysis performed on *An. gambiae* s.l. sampled from June to August in Koulikoro and Kati showed that *An. coluzzii* was the major species (>90%) in both sites.

Indoor resting densities were particularly high in the unsprayed control site of Tominian in October 2018, reaching a mean peak of 49.3 *An. gambiae* s.l. per house. Over the three-month period after IRS implementation (October to December) the mean indoor resting density of *An. gambiae* s.l. was 21.4 for Tominian (unsprayed), 8.2 for Mopti (SumiShield 50WG), 7.6 for Djenné (SumiShield 50WG), 2.0 for Bandiagara (Actellic 300CS) and 0.4 for Bankass (Actellic 300CS). All sprayed sites had lower indoor resting densities than Tominian (unsprayed control site). Indoor resting densities were higher in the sites sprayed with SumiShield 50WG than those sprayed with Actellic 300CS. The increase in Djenné from a mean of 0.4 *An. gambiae* s.l. per house post IRS with Actellic 300CS in 2017 (September to December) to 7.6 per house in 2018 (October to December) after spraying SumiShield 50WG is concerning. This may be due to the more slow-acting nature of SumiShield WG, meaning that mosquitoes may survive long-enough to be captured by morning pyrethrum spray catch.

Overall, peak *An. gambiae* s.l. indoor and outdoor biting rates were in September and October in the Mopti and Segou Regions. By district, post-IRS (October to December) mean *An. gambiae* s.l. human biting rates were: 14.5 indoors, 11.5 outdoors in Tominian (unsprayed); 5.0 indoors, 8.4 outdoors in Mopti (SumiShield 50WG); 3.9 indoors, 2.5 outdoors in Djenné (SumiShield 50WG); 0.2 indoors, 0.1 outdoors in Bandiagara (Actellic 300CS) and 0.3 indoors, 0.3 outdoors in Bankass (Actellic 300CS). All sprayed sites had lower *An. gambiae* s.l. biting rates than Tominian (unsprayed control site). While biting rates were approximately 15-20 fold higher in Mopti and Djenné (sprayed with SumiShield 50WG) than Bandiagara and Bankass (sprayed with Actellic 300CS), this appears to be unrelated to the insecticide sprayed, as biting rates in Mopti and Djenné were also relatively high in 2017 (5-15 fold higher than Bandiagara and Bankass) when sprayed with Actellic 300CS. This indicates mosquitoes are harder to control in these two locations (possibly due to numerous larval sites from river flood plains) regardless of the insecticide used.

An. gambiae s.l. biting rates in the former IRS district of Koulikoro were extremely high and reached a mean of around 100 bites per person per night during the peak in August. Despite these high biting rates, indoor

resting densities in Koulikoro were relatively low even though walls were unsprayed. As the area was sprayed for several years, there may have been some *An. gambiae* s.l. behavioral modification resulting in fewer vectors resting indoors until morning. Preliminary sporozoite data for Koulikoro and Kati showed relatively low sporozoite rates of 1.8% and 0.3%, respectively. While this is a fairly low infection rate, the high human biting rates during the peak transmission period will likely result in a high chance of people in Koulkoro and Kati becoming infected with malaria unless protected. Full entomological inoculation rate calculations will be reported after completion of molecular analysis by Laboratoire de Biologie Moléculaire Appliquée, expected in August 2019.

I. INTRODUCTION

Malaria vector control in Mali chiefly depends on nationwide use of pyrethroid long-lasting insecticidal nets (LLINs) and targeted application of indoor residual spraying (IRS) in high transmission areas.

PMI supports the National Malaria Control Program's (NMCP) strategy to reduce malaria transmission through targeted IRS in select high-risk areas. Starting in 2008, PMI supported three IRS campaigns in the districts of Bla and Koulikoro, adding a third district (Baraoueli) in 2011. In 2015, PMI reduced the IRS sites to two districts because of the added cost of spraying a new insecticide formulation, Actellic 300CS, to help mitigate pyrethroid resistance in *An gambiae* s.l.. In 2016, Mali benefited from the UNITAID-funded Next Generation Indoor Residual Spraying (NGenIRS) project, which included a short-term co-payment on long-lasting IRS insecticides. This allowed Mali to expand geographic coverage of IRS back to three districts, targeting 242,684 structures and protecting 778,884 people in total. In 2017, PMI shifted IRS operations from the districts of Koulikoro, Baroueli, and Fana in the south of Mali to Mopti Region in central Mali. This was based on data from a 2015 study showing a 60 percent malaria prevalence rate in this region compared to 30 percent nationally. In addition to its higher malaria prevalence, Mopti region does not benefit from the same level of malaria control resources as other areas.

The objective of VectorLink Mali in 2018 was to reduce malaria-associated morbidity and mortality in four districts of Mopti region – Mopti, Bandiagara, Bankass, and Djenné – by spraying 215,558 structures (67,417, 95,604, 26,998, and 25,539, respectively). Due to security issues, the IRS target was adjusted to 205,612 structures (60,992, 95,344, 24,948, and 24,328, respectively). The 2018 Mali IRS campaign was originally scheduled to begin on July 9. Due to the increasing frequency of security incidents within the IRS target area leading up to and following the July 20 presidential election, VectorLink Mali suspended all activities temporarily, and the IRS campaign was implemented from August 30 to October 2, 2018. In the end, IRS was conducted in a total of 46 out of 98 (47%) health areas across the four districts. The Mali NMCP vector control policy requires the rotation of insecticides with different modes of action for resistance management. SumiShield 50WG is a neonicotinoid insecticide that was used for the first time in Mali in the districts of Djenné and Mopti, with Actellic 300CS sprayed in Bandiagara and Bankass.

Entomological monitoring was conducted between June and December 2018 in the four IRS districts previously cited as well as in Tominian (unsprayed control site located around 50 km from the Mopti region). Koulikoro (former IRS site, last sprayed in 2016) and Kati (unsprayed control) in Southern Mali were also monitored as part of determining the impact of IRS relocation to Mopti Region.

The objectives of 2018 entomological monitoring were to monitor malaria vector biting rates, indoor resting densities, behavior and entomological inoculation rates in Mopti Region before and after IRS in comparison to the control site of Tominian; to determine residual efficacy of SumiShield 50WG and Actellic 300CS on walls following IRS; and to evaluate the impact of discontinuing IRS in Koulikoro on entomological inoculation rates.

2. METHODOLOGY

2.1 STUDY AREA, ENTOMOLOGICAL SURVEILLANCE SITES

In 2018, VectorLink Mali collected data on key entomological indicators from four surveillance sites in the Mopti Region (all sprayed with IRS) as well as one unsprayed site in neighboring Segou region and two sites (one former IRS, one control) in Koulikoro Region, as shown in Figure 1 and described in Table 1. All four sites in Mopti are located in IRS-targeted districts. The unsprayed control site in the neighboring Segou region is within 50km of Mopti region. Entomological surveillance was performed in Tienfala (Koulikoro) where IRS was conducted annually between 2013 and 2016 to determine the entomological inoculation rate over a two-year period after IRS withdrawal.

Figure 1: Map of Mali Showing IRS Entomological Surveillance Sites and Regional Boundaries

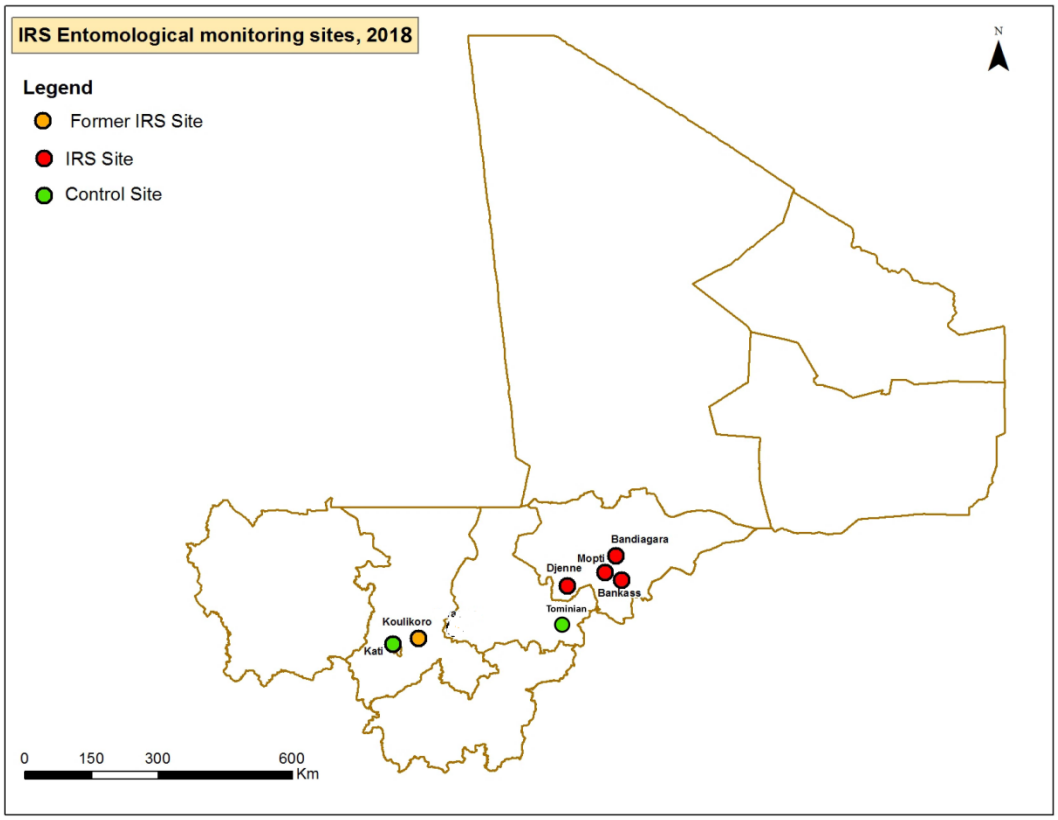


Table 1: IRS Entomological Surveillance Sites for 2018

Region	District	Health Area	Site (village)	Spray Status	Geographic Zone	IRS History
Mopti	Mopti	Tongorongo	Tongorongo (June to July) Serema (Sept to Dec)	Sprayed	Sahelian	OP 2017 – SS 2018
	Bandiagara	Bandiagara central	Dandoly	Sprayed		OP 2017-2018
	Bankass	Bankass central	Bankass	Sprayed		OP 2017-2018
	Djenné	Madiama	Madiama	Sprayed	Sahelian Flooded	OP 2017-SS 2018
Segou	Tominian	Ouan	Ouena	Unsprayed	Sahelian	Unsprayed 2017-18
Koulikoro	Kati	N'gabakoro-droit	Sala	Unsprayed	Northern Sudanese	Unsprayed 2013-18
	Koulikoro	Tienfala	Tienfala	Sprayed		Carbamate 2013-14 OP 2015-16 Unsprayed 2017-18

2.2 HUMAN LANDING CATCH

Human landing catches (HLCs) were carried out in each site for two consecutive nights each month from 06:00 pm to 06:00 am in four randomly selected houses (in the same houses every month) to determine the human biting rates for *An. gambiae* s.l. During each night of HLC, two collectors, each equipped with a mouth aspirator and a flashlight (Figure 3), sat in each house: one indoors (living room) and the second outdoors (within 2m of the house). The following morning, mosquito identification was performed using the Gillies & de Meillon (1968) key.

2.3 PYRETHRUM SPRAY CATCH

ZZ PAFF aerosol containing permethrin 0.25%, tetramethrin 0.20%, D-Phenothrin 0.01% and PBO 0.34% was used to perform pyrethrum spray catch (PSC) in 20 houses per site to sample indoor resting mosquitoes. Ten houses per day were inspected from 7:00 a.m. to 12:00 a.m. during two consecutive days. Ten to fifteen minutes after the spraying of houses, the knocked-down mosquitoes were collected from the white canvas previously laid down on the floor of the houses. Mosquitoes were put in labeled petri dishes and were then morphologically identified to genus (*Anopheles* or Culicines) then separated by sex. All female *Anopheles* were investigated for their abdominal status (unfed, fed, half gravid and gravid). Female *Anopheles gambiae* s.l. were stored in 1.5 ml labeled Eppendorf tubes containing 70% ethanol for further molecular lab analyzes.

2.4 WALL CONE BIOASSAY AND FUMIGANT TESTS

In total, cone bioassays were conducted in 24 structures (14 in Djenne and Mopti sprayed with SumiShied 50WG and 10 in Bandiagara and Bankass sprayed with Actellic 300CS). Quality assurance of the spraying and monthly bioassays of the insecticide were assessed in all targeted districts one to two days after IRS and subsequently on a monthly basis, using cone bioassays on sprayed walls. Those bioassays were carried out with an insectary colony of pyrethroid susceptible *An. coluzzii* exposed on a sprayed wall at varying heights (0.5 m, 1.0 m, and 1.5 m from the floor) following WHO protocols. Negative controls were performed in parallel with mosquitoes exposed to untreated blocks that were tested in an unsprayed area.

The contribution of airborne effects to overall mortality in cone bioassays was also assessed using fumigant bioassays. Thus, 10-12 females *An. coluzzii* susceptible strain were introduced into a cage set on a chair, approximately 10 cm away from the sprayed wall and about 1m above the floor.

For all testing, exposure time was 30 mins, and subsequent mortality was recorded 24h later for Actellic 300CS and every 24h for seven consecutive days for SumiShield 50WG.

2.5 Molecular Analysis of *An. gambiae* s.l. (PCR For Species Identification And ELISA to Detect Sporozoites)

Abdomens, legs and wings of adult mosquitoes caught in the field in 2018 by VectorLink Mali were analyzed by polymerase chain reaction (PCR) for species identification according to the protocol described by Santolamazza *et al.*, 2008. This method allows identification of *Anopheles gambiae*, *coluzzii* and *arabiensis*. Heads and thoraces for the same specimens collected through routine monthly HLC were used for circumsporozoite protein (CSP) enzyme-linked immunosorbent assay (ELISA) according to the protocol of Beier *et al.* (2002).

Data analysis by method:

- **PSC:** Mean density = Total number of collected *An. gambiae* s.l. / Total number of houses surveyed.
- **HLC**: Human biting rate = Total number of collected *An. gambiae* s.l. / Total number of human nights
- **Indoor/Outdoor biting ratio**: A Chi-square test was used to compare the proportion of indoor and the outdoor biting rates.
- **Sporozoite index** = (Total number of infected *An. gambiae* s.l. / Total number of *An. gambiae* s.l. tested with ELISA) x100.

In addition, Z-test for difference in proportions was used to compare sporozoite index between two populations.

3. RESULTS

3.1 MOSQUITO VECTOR SPECIES COMPOSITION

Over the study period of June to December 2018, a total of 21,367 mosquitoes were collected from seven sites through HLC, of which 42.2% were *An. gambiae* s.l. and 59.1% were Culicine mosquitoes (Figure 2). During the same period 7,709 were collected by PSC, of which 70.7% were *An. gambiae* s.l. After combining HLC and PSC data, *Anopheles* mosquitoes accounted for 49.5% (14,390/29,076) of the total catch compared with 50.5% Culicine mosquitoes. Among the six different *Anopheles* species (*An. gambiae* s.l., *An. funestus* s.l., *An. pharoensis*, *An. nili*, *An. ziemanni* and *An. rufipes*) encountered, *An. gambiae* s.l. was by far the most frequently sampled and accounted for 97.5% of *Anopheles* collected (14,033/14,390). Molecular analysis was suspended from November 2018 to March 2019 while contractual documentation was updated. Preliminary molecular species analysis performed on *An. gambiae* s.l. sampled from June to August in Koulikoro and Kati, showed that *An. coluzzii* was the major species (>90%) in both sites (Figure 3). The remaining samples (10% in Kati and 4.4% in Koulikoro) did not amplify by PCR, with the most likely explanation being either low quality DNA or morphological misidentification (with samples not belonging to the *An. gambiae* species complex).

Figure 2: Mosquito species identification for all mosquitoes collected by human landing catch and pyrethrum spray catch in all seven sites.

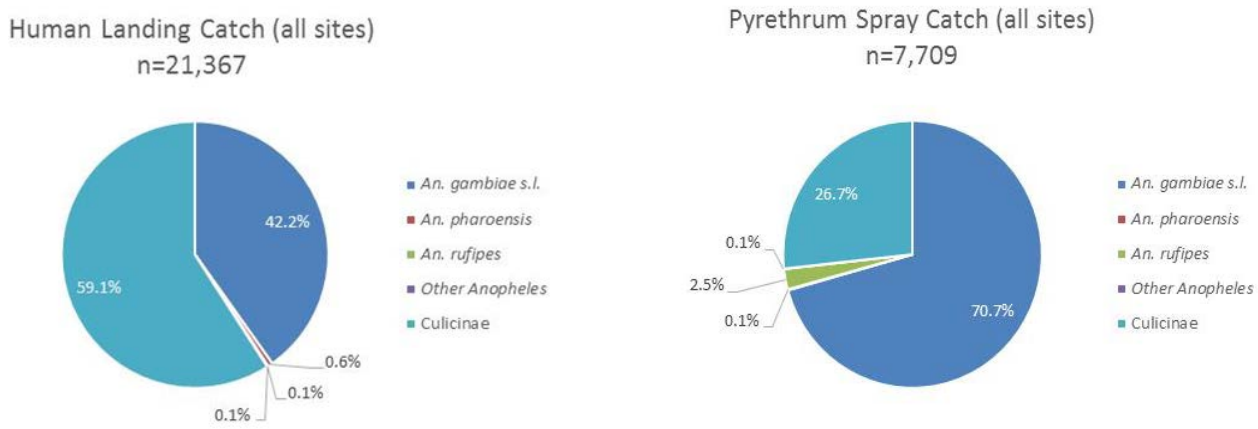


Figure 3: *An. gambiae* s.l. molecular species identification for Koulikoro and Kati (June-August).

3.2 INDOOR VECTOR RESTING DENSITY

Figure 4 presents monthly PSC data for four sprayed sites in Mopti Region and one unsprayed site. The indoor vector density was very low with a mean of ≤3 *An. gambiae* s.l. per house per day in all sites in Mopti Region during the pre-spray period of June and July. Sampling could not be conducted in August due to security concerns. Indoor resting densities were particularly high in Tominian (control site) and Mopti District in September during IRS implementation.

Indoor resting densities increased in Sept-October in all sites, as expected following the peak of the rainy season. In the unsprayed site of Tominian, resting densities reached a mean peak of 49.3 *An. gambiae* s.l. per house. Despite IRS with SumiShield 50WG in Mopti and Djenné, indoor resting densities were high in October, one month after spraying, with mean densities of 21.35 *An. gambiae* s.l. per house in Mopti and 15.4 per house in Djenné. Over the three month period after IRS implementation (October to December), the mean indoor resting density of *An. gambiae* s.l. was 21.4 for Tominian (unsprayed), 20.2 for Mopti (SumiShield 50WG), 7.6 for Djenné (SumiShield 50WG), 2.0 for Bandiagara (Actellic 300CS) and 0.4 for Bankass (Actellic 300CS). While densities were clearly higher in the sites sprayed with SumiShield 50WG than those sprayed with Actellic 300CS, it is important to compare these findings with 2017 data to account for site variation. Table 2 presents a summary of mean indoor resting densities in 2017 compared with 2018 and the relative density ratio between the two years. Of greatest concern was the nineteen–fold increase in indoor resting density in Djenné in 2018 when SumiShield 50WG was sprayed, compared with 2017 when Actellic 300CS was sprayed. There was also a nearly two-fold increase in density in Mopti in 2018 following the switch to SumiShield 50WG. However, in Bandiagara, where Actellic 300CS was sprayed in both years, there was also a 1.4-fold increase, indicating that the change to SumiShield 50WG may not be the only factor that resulted in increased resting densities in Djenné and Mopti.

Figure 4: Box and whisker plot showing monthly *An. gambiae* s.l. collected by PSC for four sites in Mopti Region that were sprayed and one unsprayed site in Segou Region.

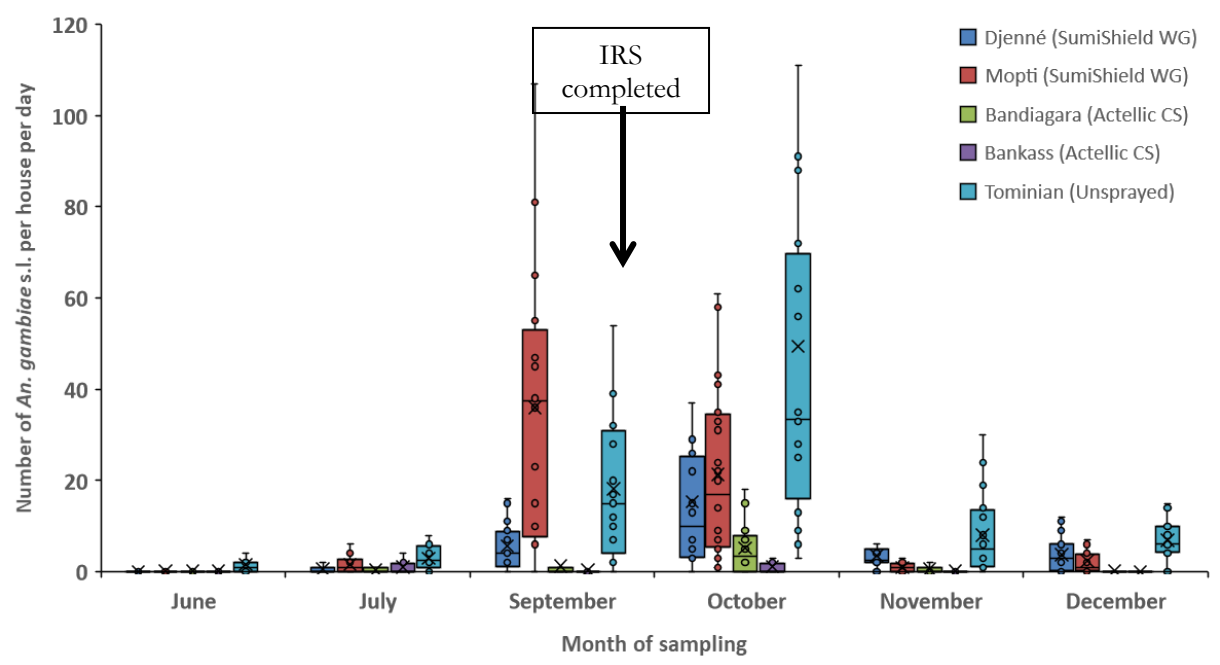
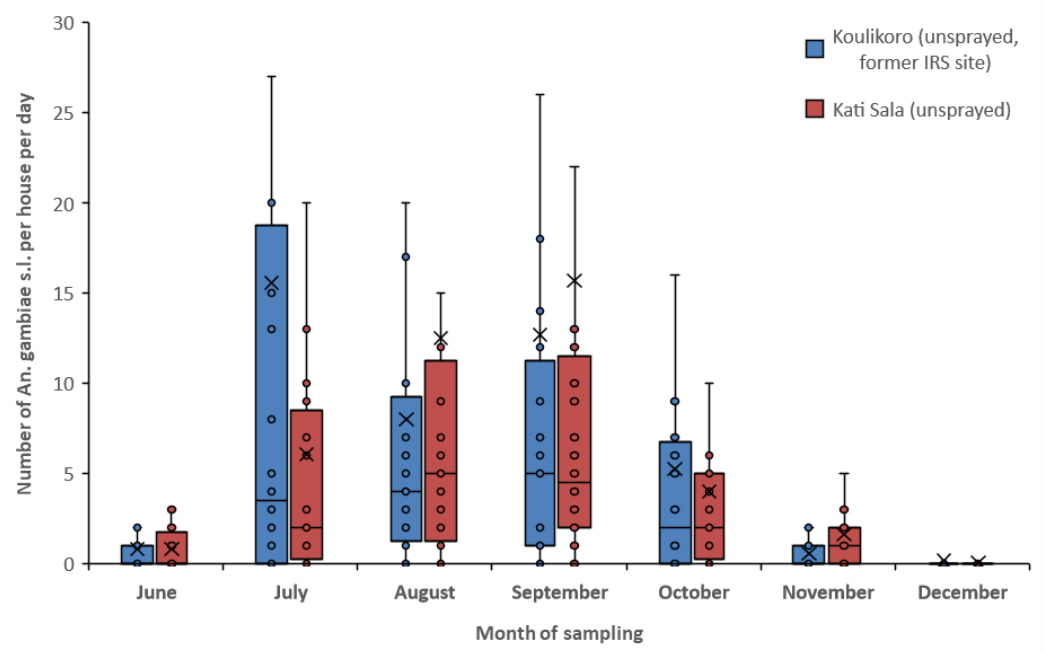


Table 2: Comparison of mean *An. gambiae* s.l. post-IRS indoor resting densities during September to December 2017 and October to December 2018.

Site	IRS Status	Mean indoor resting density post-IRS (PSC)	Ratio
Tominian	2017: No data collected 2018: Unsprayed	NA 21.4	NA
Djenné	2017: Actellic 300CS 2018: SumiShield 50WG	0.4 7.6	19.0
Mopti	2017: Actellic 300CS 2018: SumiShield 50WG	4.5 8.2	1.8
Bandiagara	2017: Actellic 300CS 2018: Actellic 300CS	1.4 2.0	1.4
Bankass	2017: Actellic 300CS 2018: Actellic 300CS	1.1 0.4	0.4
Koulikoro	2017: Unsprayed 2018: Unsprayed	6.0 2.0	0.3
Kati Sala	2017: Unsprayed 2018: Unsprayed	5.4 1.9	0.4

Figure 5 presents monthly PSC data for two unsprayed sites in Southern Mali including Koulikoro, a former IRS site that was last sprayed in 2016. Indoor resting densities were similar in both sites, with a mean indoor resting density of 6.1 *An. gambiae* s.l. per house in Koulikoro and 5.8 per house in Kati over the period June to December.

Figure 5: Box and whisker plot showing monthly *An. gambiae* s.l. collected by PSC for two sites that were unsprayed in 2018 (one former IRS site, last sprayed in 2016).



3.3 *AN. GAMBIAE* S.L. HUMAN BITING RATES

Overall, the peak in *An. gambiae* s.l. indoor and outdoor biting rates were in September and October in the Mopti and Segou regions (although there was no monitoring in August) (Figures 6 and 7), while it was in August and September in the Koulikoro region (Figures 8 and 9).

By district, post-IRS (October to December) mean *An. gambiae* s.l. human biting rates were: 14.5 indoors, 11.5 outdoors in Tominian (unsprayed); 5.0 indoors, 8.4 outdoors in Mopti (SumiShield 50WG); 3.9 indoors, 2.5 outdoors in Djenné (SumiShield 50WG); 0.2 indoors, 0.1 outdoors in Bandiagara (Actellic 300CS) and 0.3 indoors, 0.3 outdoors in Bankass (Actellic 300CS). The highest biting rates were in the unsprayed site of Tominian, followed by Mopti and Djenné, which were both sprayed with SumiShield 50WG. According to the biting rates it may appear that SumiShield WG is not working as well as Actellic CS. However, it should be noted that in 2017 Mopti also had relatively high biting rates, compared to other sprayed sites, when sprayed with Actellic 300CS (Table 3).

The mean *An. gambiae* s.l. human biting rates in Koulikoro (former IRS site) were particularly high in August and September, with more than 80 bites per person per night both indoors and outdoors. The trends were very similar in Kati, the neighboring unsprayed control site (Figures 8 and 9). Figure 10 examines overall mean biting rates (indoor and outdoor combined) for Koulikoro and Kati between August and December in 2016, 2017 and 2018. Overall, following withdrawal of IRS from Koulikoro (last sprayed in 2016), *An. gambiae* s.l. biting rates have become extremely high in 2017 and 2018, although the increase in biting rates in Kati, which has never been sprayed, indicates other factors may have contributed to the increased biting rates. Nevertheless, the biting risk is extremely high in Koulikoro and Kati and even a low sporozoite rate (e.g. <1%) would result in a high risk of malaria infection.

Figure 6: Box and whisker plot showing monthly indoor *An. gambiae* s.l. biting rate by HLC for four sites in Mopti Region that were sprayed and one unsprayed site in Segou Region.

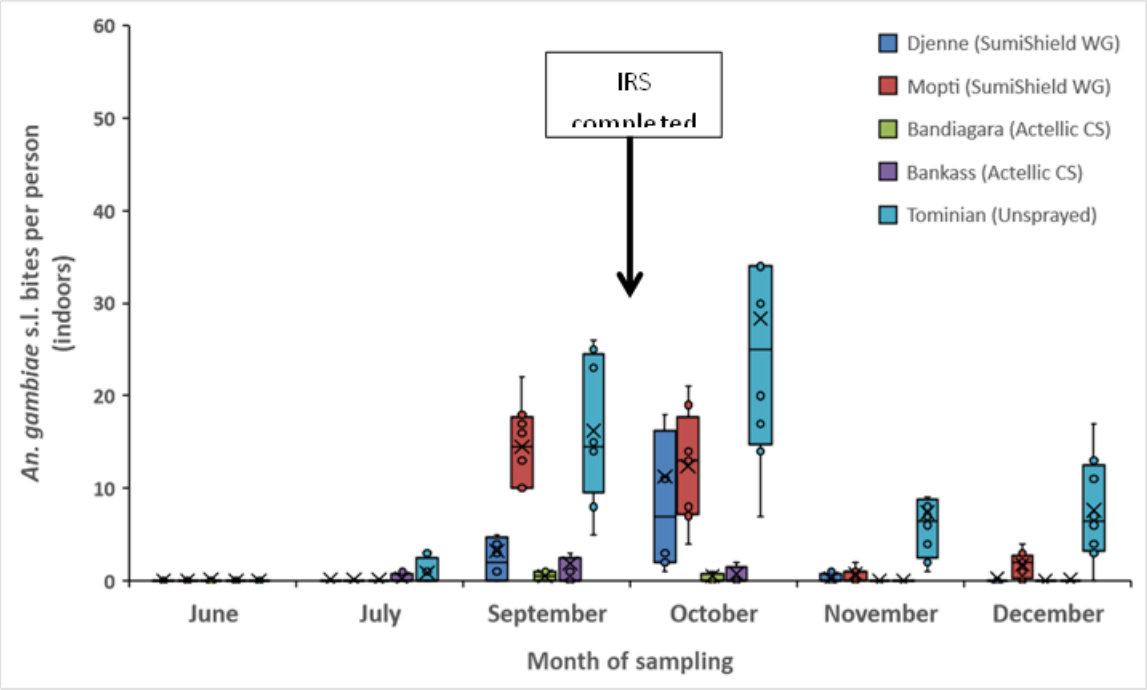


Figure 7: Box and whisker plot showing monthly outdoor *An. gambiae* s.l. biting rate by HLC for four sites in Mopti Region that were sprayed and one unsprayed site in Segou Region.

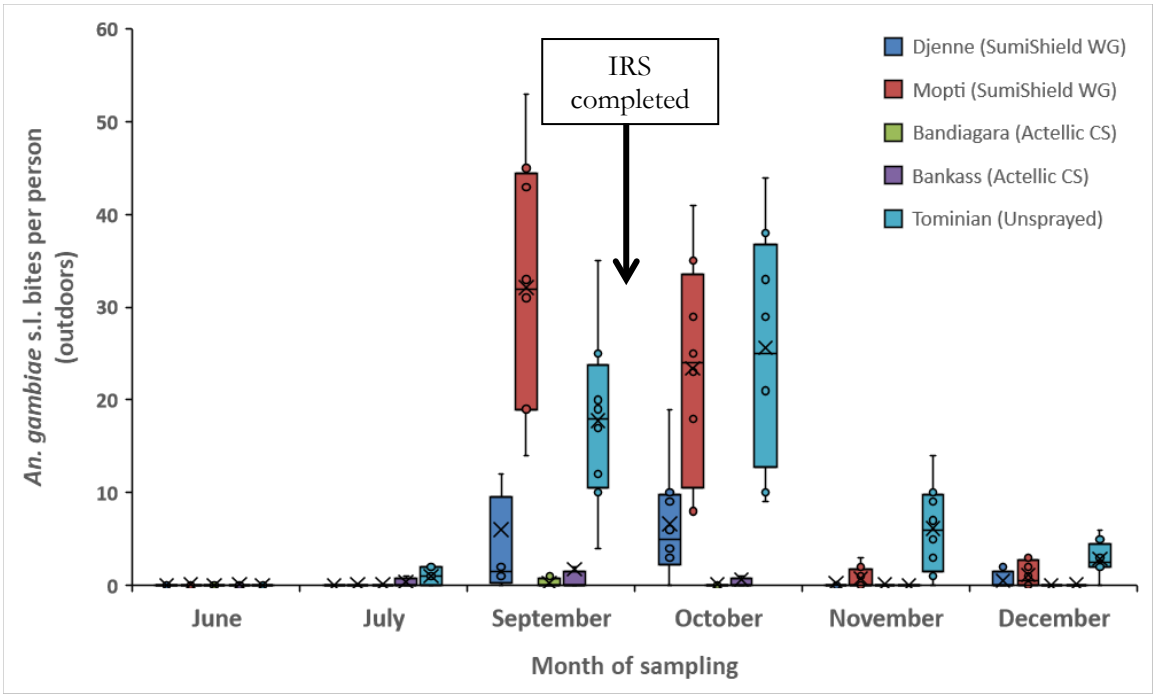


Figure 8: Box and whisker plot showing monthly indoor *An. gambiae s.l.* biting rate by HLC for two sites that were unsprayed (one former IRS site, one control).

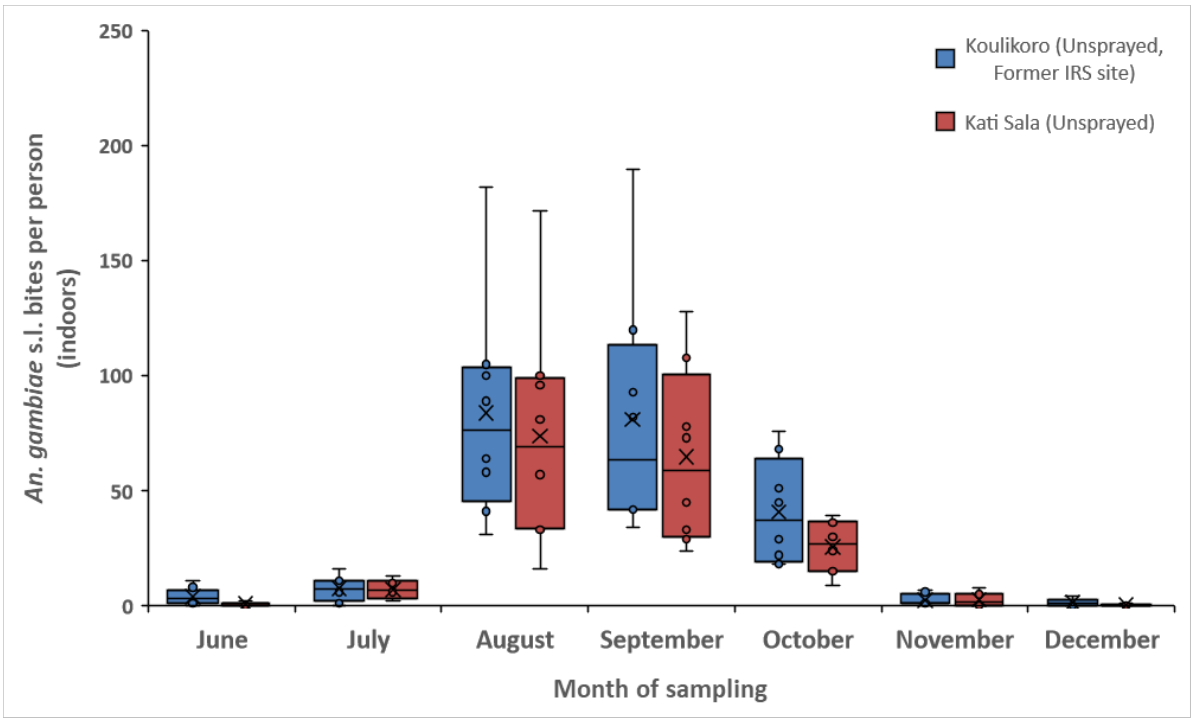


Figure 9: Box and whisker plot showing monthly outdoor *An. gambiae* s.l. biting rate by HLC for two sites that were unsprayed (one former IRS site, one control).

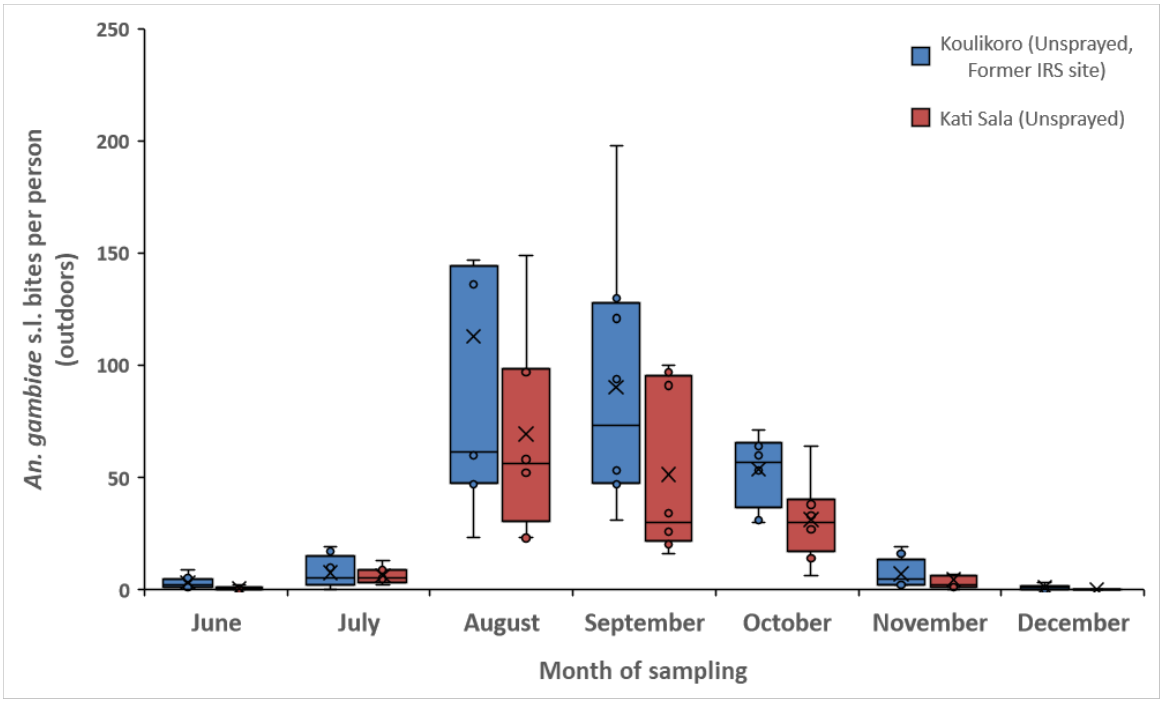
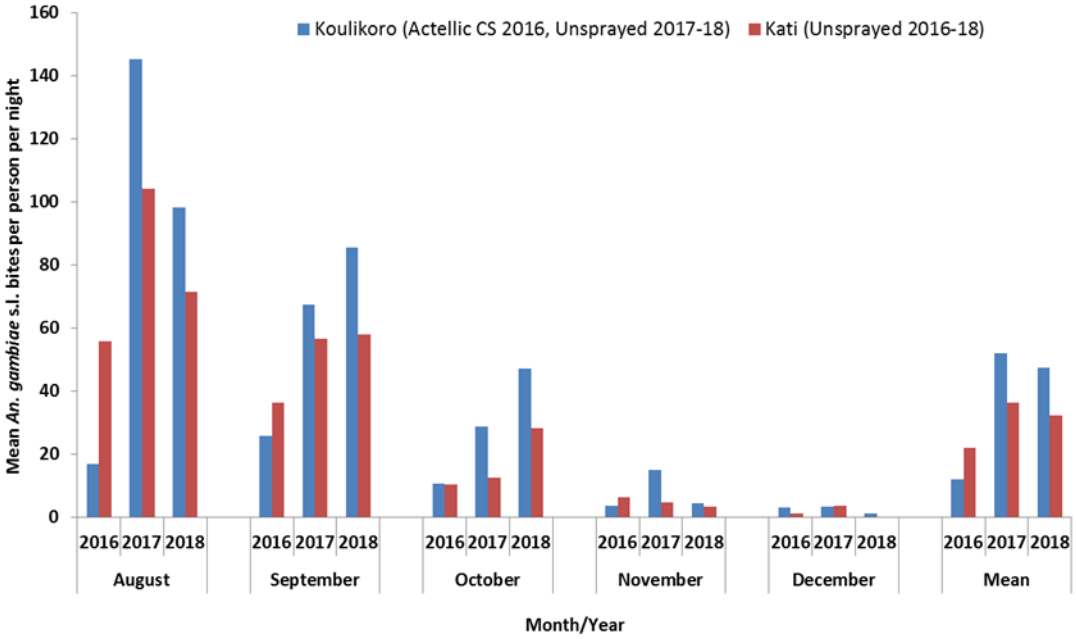


Table 3: Comparison of mean *An. gambiae* s.l. post-IRS indoor and outdoor biting rates during September to December 2017 and October to December 2018.

Site	IRS Status	Mean indoor biting rates post-IRS (HLC)	Ratio	Mean outdoor biting rates post-IRS (HLC)	Ratio
Tominian	2017: No data collected 2018: Unsprayed	NA 14.5	NA	NA 11.5	NA
Djenné	2017: Actellic 300CS 2018: SumiShield 50WG	2.6 3.9	1.5	1.0 2.5	2.5
Mopti	2017: Actellic 300CS 2018: SumiShield 50WG	5.9 5.0	0.8	9.2 8.4	0.9
Bandiagara	2017: Actellic 300CS 2018: Actellic 300CS	0.8 0.2	0.3	0.7 0.1	0.1
Bankass	2017: Actellic 300CS 2018: Actellic 300CS	0.4 0.3	0.8	0.6 0.3	0.5
Koulikoro	2017: Unsprayed 2018: Unsprayed	55.0 41.9	0.8	49.1 53.0	1.1
Kati Sala	2017: Unsprayed 2018: Unsprayed	34.4 33.4	1.0	38.6 31.3	0.8

Figure 10: Variation of mean biting rates (overall for indoor and outdoor) in *An. gambiae* s.l. in Koulikoro (former IRS site) & Kati (control site) between 2016 (IRS period) & 2017-2018 (IRS withdrawal period).



3.4 BITING TIMES OF *AN. GAMBIAE* S.L.

Figures 11 to 15 show the biting times of *An. gambiae* s.l. as recorded by HLC indoors and outdoors between 6:00 pm and 6:00 am. Data is not presented for Bandiagara and Bankass (both sites sprayed with Actellic 300CS) as less than 50 *An. gambiae* s.l. were collected by HLC over the study period. Biting trends were similar at all sites, with biting rates gradually increasing over the first half of the night and reaching a peak during the second half of the night between midnight and 6:00 am. Biting rates were still high during the last hour of collection between 5:00 am and 6:00 am. It will be beneficial to extend HLC in 2019 until 8:00 am to see if biting continues during the first hours after dawn, when people are less likely to be protected by mosquito nets.

Figure 11: Mean *An. gambiae* s.l. hourly biting rates in Djenné (sprayed with SumiShield 50WG) (n=121 indoors, n=117 outdoors), June to December 2018.

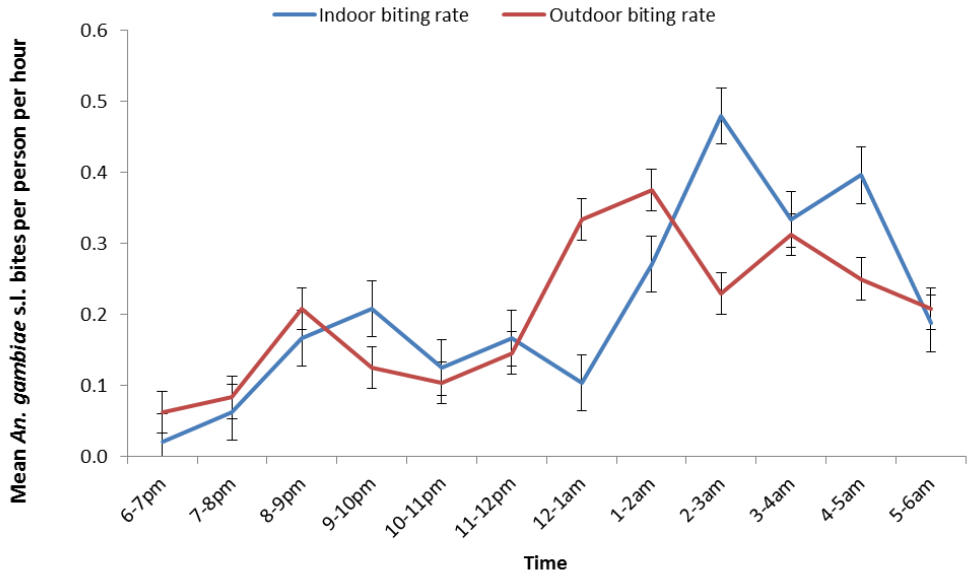


Figure 12: Mean *An. gambiae* s.l. hourly biting rates in Mopti (sprayed with SumiShield 50WG), June to December 2018 (n=253 indoors, n=444 outdoors).

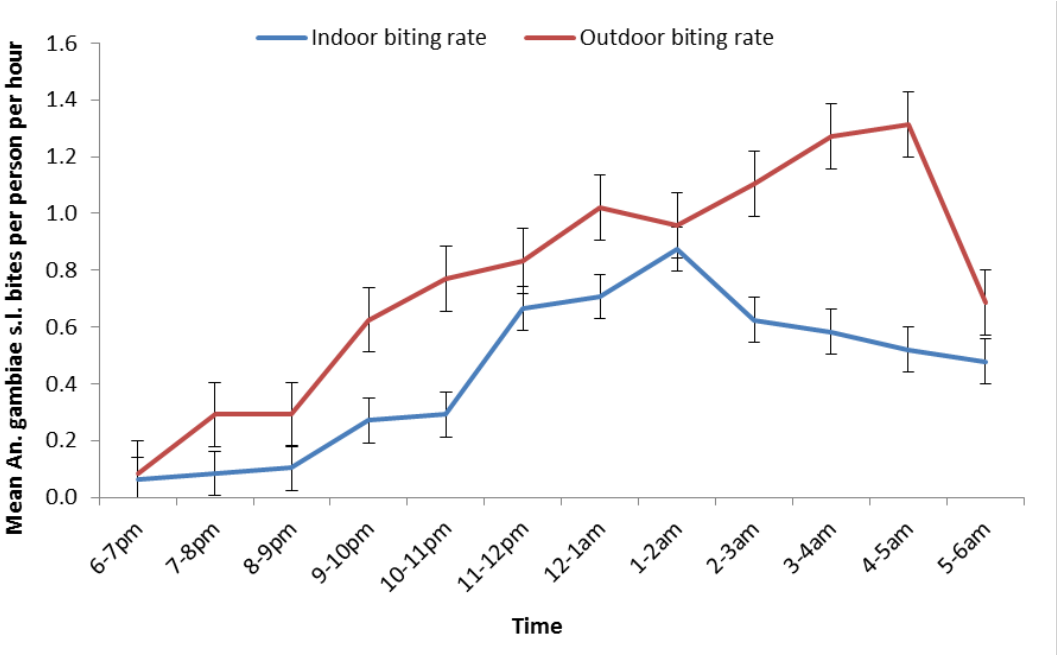


Figure 13: Mean *An. gambiae* s.l. hourly biting rates in Tominian (unsprayed), June to December 2018 (n=484 indoors, n=427 outdoors).

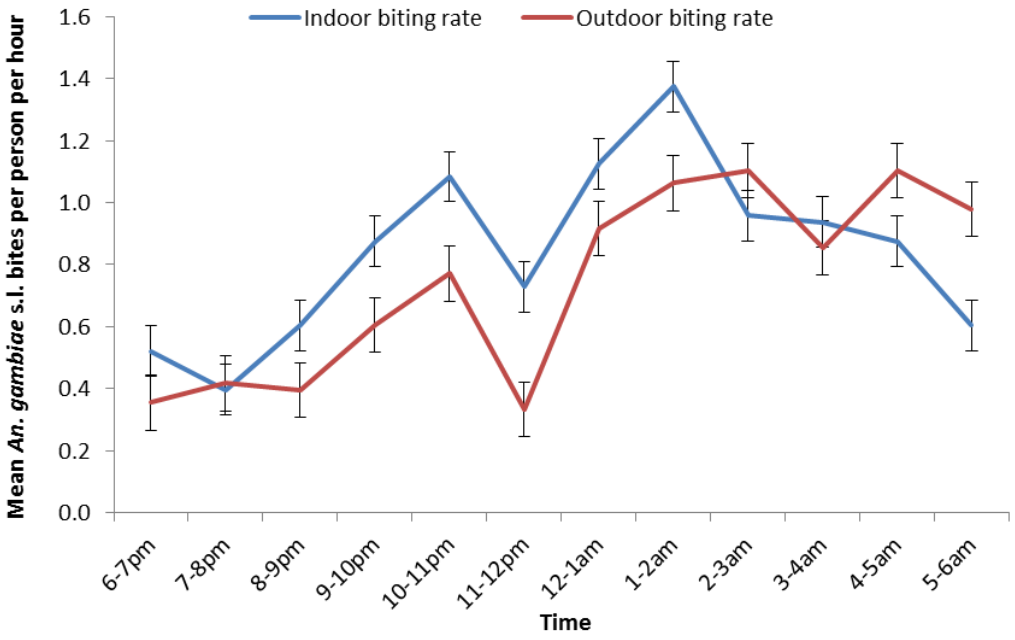


Figure 14: Mean *An. gambiae* s.l. hourly biting rates in Koulikoro (former IRS site), June-December 2018 (n=1,766 indoors, n=2,205 outdoors).

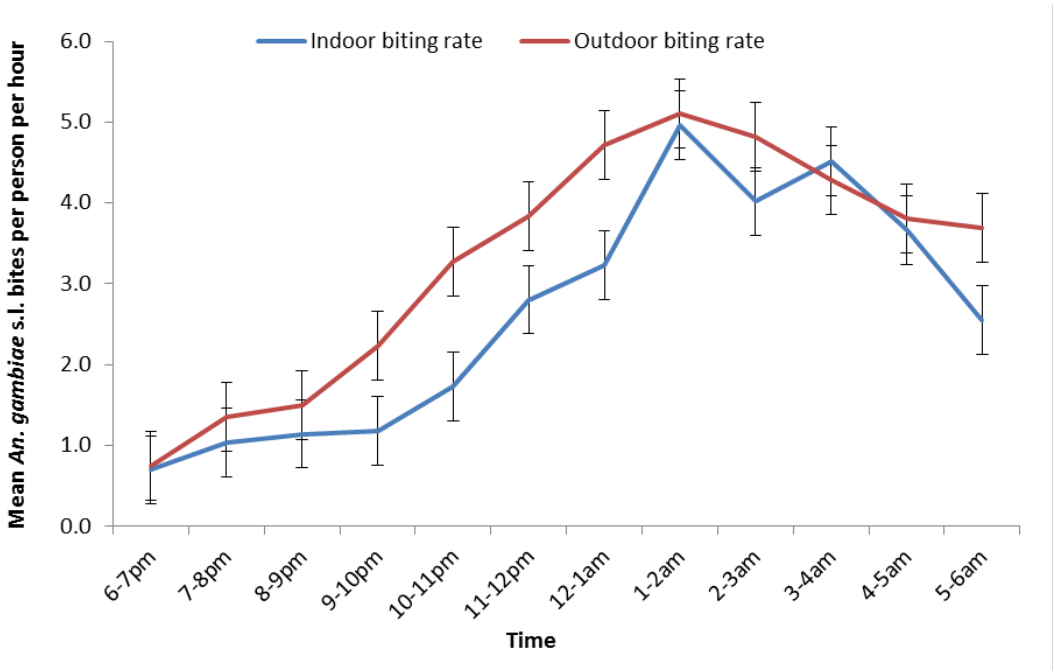
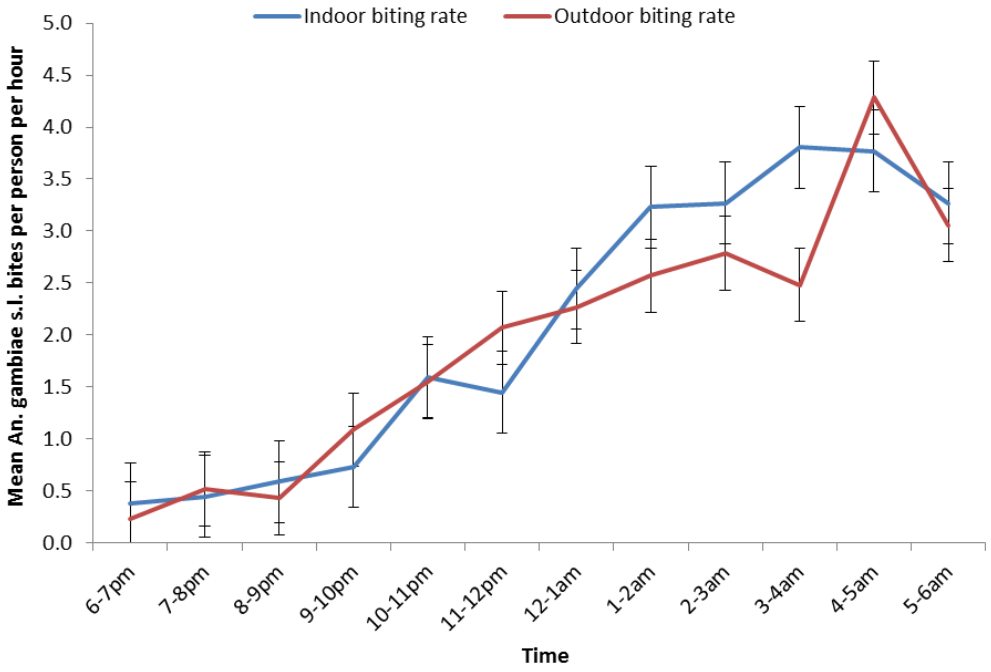


Figure 15: Mean *An. gambiae* s.l. hourly biting rates in Kati (unsprayed), June-December 2018 (n=1,398 indoors, n=1,307 outdoors).



3.5 BITING LOCATION OF *AN. GAMBIAE* S.L.

The mean indoor and outdoor *An. gambiae* s.l. biting rates for each site are displayed in Table 4. Pre-IRS, *An. gambiae* s.l. biting rates were generally low and there were similar biting rates indoors and outdoors at all sites. Post-IRS, the trend was the same for all sites except Mopti (SumiShield 50WG) and Koulikoro (former IRS site) where there was significantly more biting outdoors (Table 4).

Table 4: *An. gambiae* s.l. mean human biting rate per night, indoors and outdoors, June-December 2018.

Period/site	Indoor	Outdoor	In:Out Ratio
Pre IRS			
Djénné (SumiShield 50WG)	0.06[a]	0.00[a]	01:00
Mopti (SumiShield 50WG)	0.06[a]	0.13[a]	0.32 : 0.68
Combined SumiShield sites	0.06[a]	0.07[a]	0.48 : 0.52
Bandiagara (Actellic 300CS)	0.06[a]	0.13[a]	0.32 : 0.68
Bankass (Actellic 300CS)	0.13[a]	0.25[a]	0.34 : 0.66
Combined Actellic sites	0.01[a]	0.19[a]	0.33 : 0.67
Tominian (Unsprayed)	0.44[a]	0.50[a]	0.47 : 0.53
Koulikoro (Former IRS site)	5.69[a]	5.38[a]	0.51 : 0.49
Kati (Unsprayed)	3.88[a]	3.38[a]	0.53 : 0.47
Post IRS			
Djénné (SumiShield 50WG)	3.75[a]	3.66[a]	0.51 : 0.49
Mopti (SumiShield 50WG)	**7.88[a]**	**13.81[b]**	0.36 : 0.64
Combined SumiShield sites	**5.82[a]**	**8.74[b]**	0.4 : 0.6
Bandiagara (Actellic 300CS)	0.25[a]	0.13[a]	0.66 : 0.34
Bankass (Actellic 300CS)	0.69[a]	0.63[a]	0.52 : 0.48
Combined Actellic sites	0.47[a]	0.38[a]	0.55 : 0.45
Tominian (Unsprayed)	14.91[a]	13.09[a]	0.53 : 0.47
Koulikoro (Former IRS site)	**41.88[a]**	**53.00[b]**	0.44 : 0.56
Kati (Unsprayed)	33.40[a]	31.33[a]	0.52 : 0.48

3.6 *PLASMODIUM* SPOROZOITE RATE

The interim data collected before laboratory work was suspended shows a sporozoite rate of 1.8% [95% CI: 0.8-2.8%] in Koulikoro and 0.3% [95% CI 0-0.7%] in Kati for *An. gambiae* s.l. sampled from June to September 2018 (Table 5). The ELISA tests are about to be re-started following the issuing of a new contract to LBMA.

Table 5: Sporozoite rate in Koulikoro (former IRS site) and Kati (unsprayed site).

Sites	Parameters	Jun	Jul	Aug	Sep	Total	95% CI
Koulikoro (former IRS site)	Number tested	62	200	200	200	**662**	
	Number positive	1	6	4	1	**12**	
	Sporozoite rate %	1.6	3	2	0.5	**1.8**	0.8-2.8
Kati (unsprayed)	Number tested	25	200	197	300	**722**	
	Number positive	0	0	1	1	**2**	
	Sporozoite rate %	0	0	0.5	0.3	**0.3**	0.1-0.7

3.7 ENTOMOLOGICAL INOCULATION RATE (EIR)

The EIR will be estimated after molecular analyses are complete. Laboratory analysis is expected to resume in April and will be reported in a separate supplementary report in August 2019.

3.8 RESIDUAL DURATION OF INSECTICIDE FORMULATIONS

Mosquito mortality was 100% three months after spraying Actellic 300CS in Bandiagara and decreased to approximately 80% four, five and six months after spraying (Figure 16). In Bankass mortality was 100% four months after IRS with Actellic 300CS, followed by a decrease on all substrates to approximately 80% up to six months after IRS (Figure 17).

Figure 16: Percentage mortality (24h) from cone bioassay with insectary reared *An. coluzzii* tested on walls sprayed with Actellic 300CS in Bandiagara (Mopti Region).

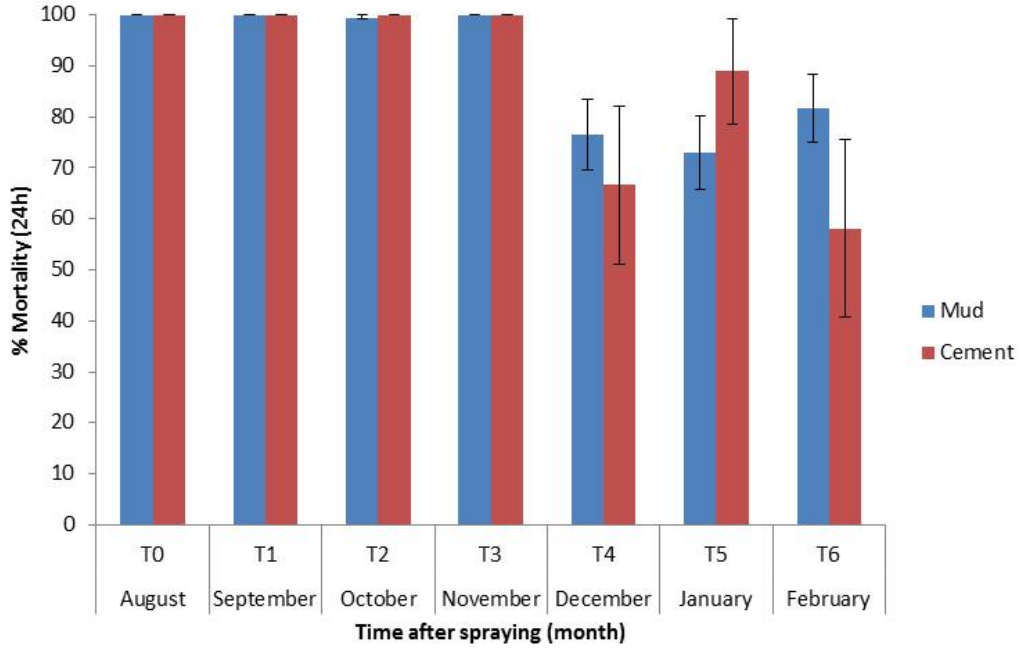
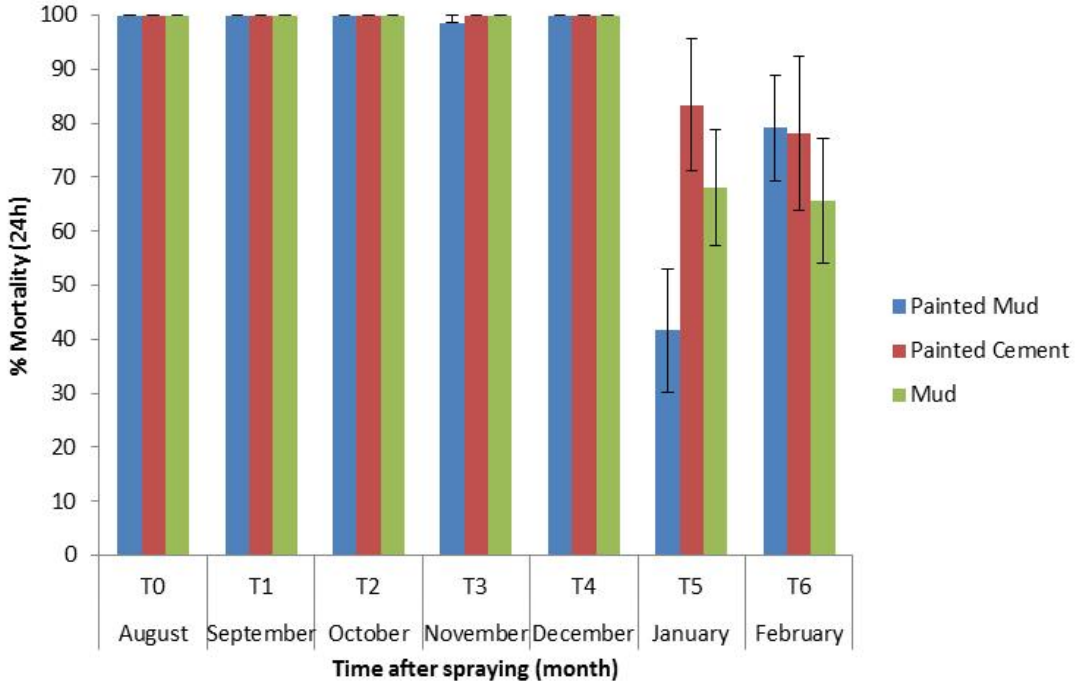


Figure 17: Percentage mortality (24h) from cone bioassay with insectary reared *An. coluzzii* tested on walls sprayed with Actellic 300CS in Bankass (Mopti Region).



In Djenné, mortality 24h after exposure was >90% on all substrates four months after spraying of SumiShield 50WG (Figure 18). There was a large decrease in mortality on painted cement after five months and painted mud after six months. Extended holding of mosquitoes for 72h resulted in 100% mortality six months after spray application (Figure 19).

Figure 18: Percentage mortality (24h) from cone bioassay with insectary reared *An. coluzzii* tested on walls sprayed with SumiShield 50WG in Djenné (Mopti Region).

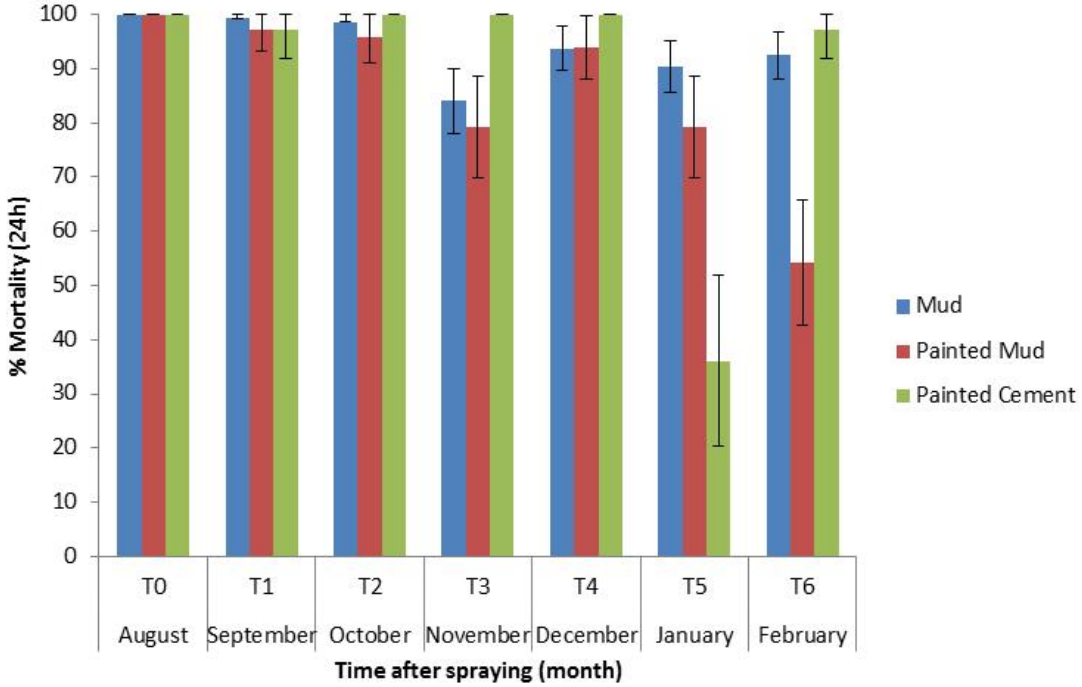
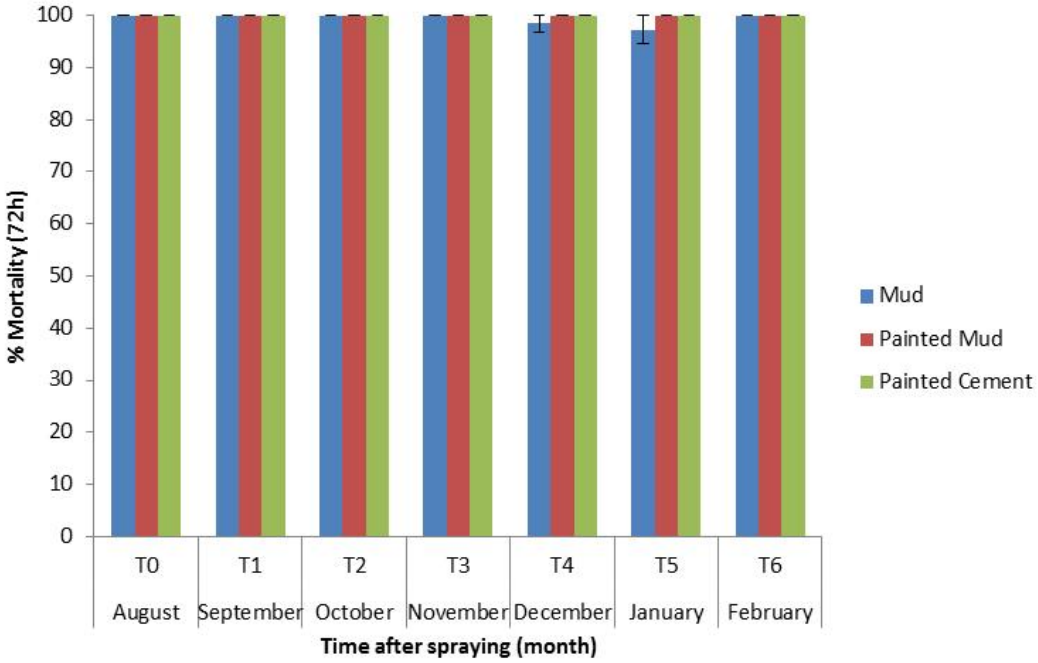


Figure 19: Percentage mortality (72h) from cone bioassay with insectary reared *An. coluzzii* tested on walls sprayed with SumiShield 50WG in Bankass (Mopti Region).



In Mopti Town, mortality 24h after exposure was >80% on all substrates five months after application of SumiShield 50WG, with a small decrease in mortality on cement after six months (Figure 20). As in Bankass, extended holding of 72h resulted in 100% mortality on all substrates after six months (Figure 21).

Figure 20: Percentage mortality (24h) from cone bioassay with insectary reared *An. coluzzii* tested on walls sprayed with SumiShield 50WG in Mopti Town (Mopti Region).

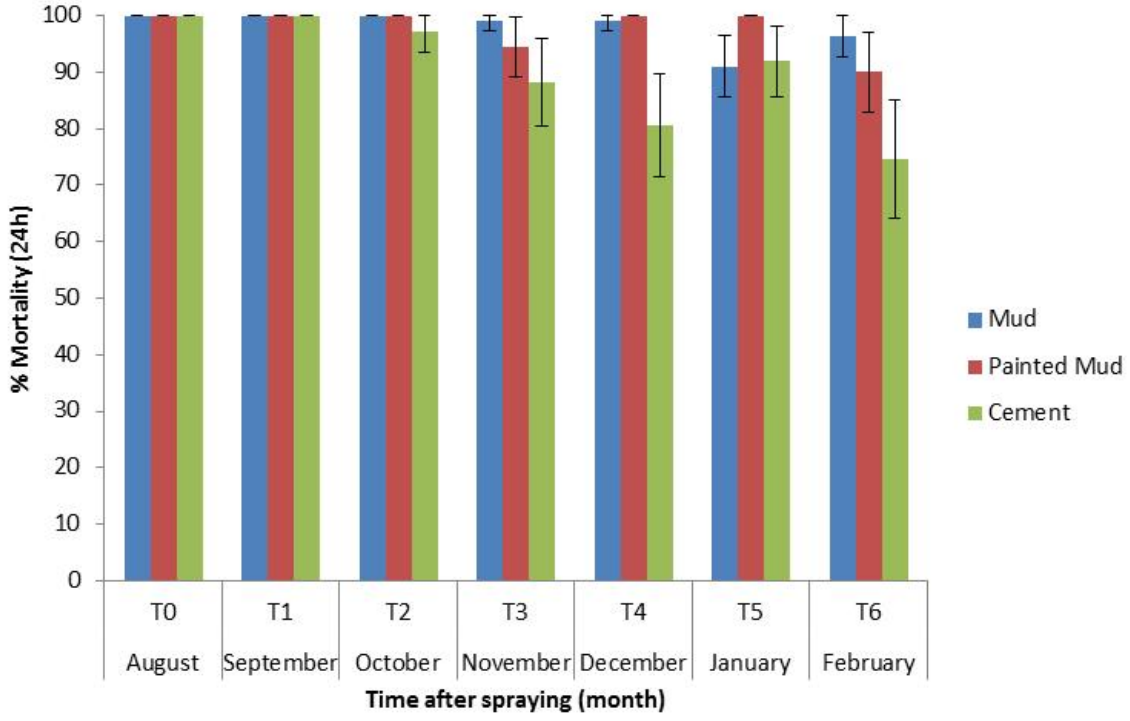
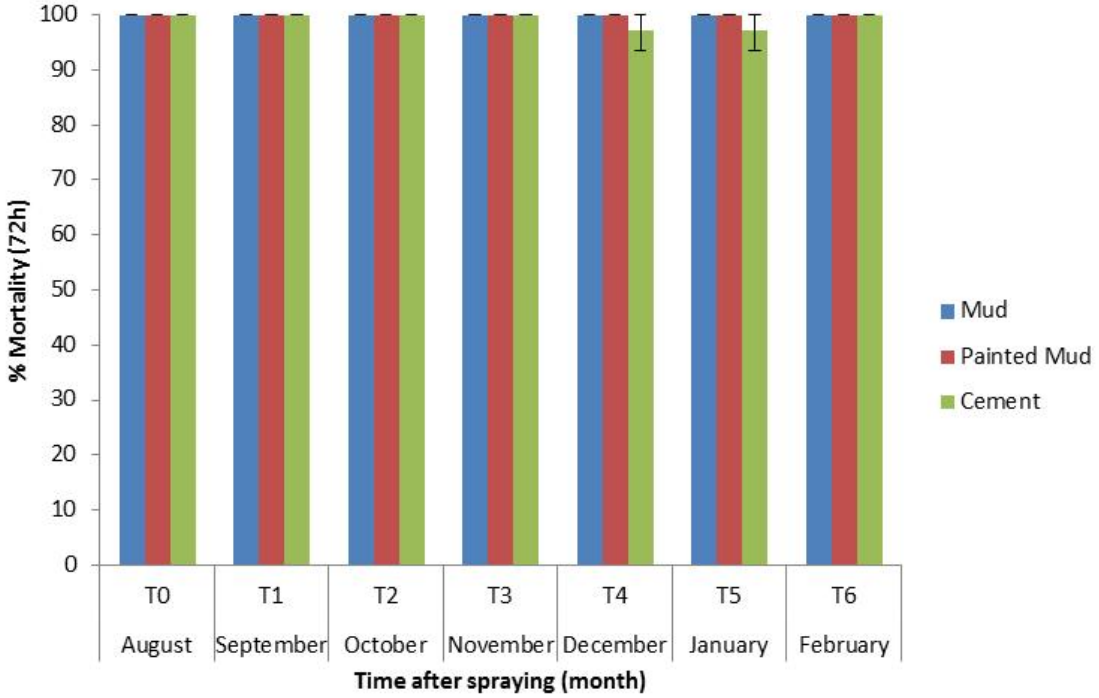


Figure 21: Percentage mortality (24h) from cone bioassay with insectary reared *An. coluzzii* tested on walls sprayed with Actellic 300CS in Bankass (Mopti Region).



4. Discussion

IRS with Actellic 300CS in Bandiagara and Bankass had good residual efficacy of approximately six months on mud and cement walls. Cone bioassay demonstrated that SumiShield 50WG lasted a minimum of six months, and still provided 100% mortality (with 72h holding) six months after spraying. As the high transmission season is short in Mopti Region, either Actellic 300CS or SumiShield 50WG can be used to provide control during the whole season (assuming IRS is conducted in July). *An. gambiae* s.l. were susceptible to both pirimiphos-methyl and clothianidin in all IRS districts (see 2018 insecticide resistance report). IRS was conducted late this year due to security concerns and had sub-optimal impact on vector densities. In 2019 the spray campaign is slated to start on July 1st, which should have a greater impact on *An. gambiae* s.l. populations and provide greater protection.

Due to security limitations it was difficult to quantify the impact of the IRS as there was only one control site in neighboring Segou Region. A major limitation is that we don't know how comparable the sites are, as we had no opportunity to collect sufficient baseline data before implementation of IRS. Nevertheless, all sprayed sites had lower indoor resting densities and biting rates than the unsprayed control site of Tominian. Indoor resting densities and biting rates were particularly low in the two districts sprayed with Actellic 300CS (Bandiagara and Bankass). Indoor resting densities were higher in districts sprayed with SumiShield 50WG, even one month after spraying when IRS should be particularly efficacious. Comparison with 2017 results showed that Mopti district had relatively high indoor resting and biting rates even when sprayed with Actellic 300CS. As Mopti District had higher *An. gambiae* s.l. abundance in both years we are not certain whether SumiShield WG is less effective than Actellic CS. *An. gambiae* s.l. are probably harder to control in Mopti District due to the annual flooding of the Niger and Bani rivers, resulting in a multitude of larval sites.

The nineteen fold increase of *An. gambiae* s.l. indoor resting densities post-IRS in Djenné in 2018 when sprayed with SumiShield 50WG is concerning. This may potentially be due to the more slow-acting nature of SumiShield WG, meaning that mosquitoes can survive long-enough to be captured by morning pyrethrum spray catch. The increase in biting rates, however, was to a much lesser degree than with resting densities. As biting rates were still high during the last hour of collection between 5:00 am and 6:00 am we will extend HLC in 2019 until 8:00 am to see if biting continues during the first hours after dawn, when people are less likely to be protected by mosquito nets.

Biting rates in the former IRS district of Koulikoro were extremely high and reached a mean of around 100 bites per person per night during the peak in August. Despite the high biting rates in Koulikoro, indoor resting densities were relatively low, even though walls were unsprayed. As the area was sprayed for several years, there may have been some *An. gambiae* s.l. behavioral modification resulting in fewer vectors resting indoors until morning. Preliminary sporozoite data for Koulikoro and Kati showed relatively low sporozoite rates of 1.8% and 0.3%, respectively. While this is a fairly low infection rate, the high human biting rates during the peak transmission period will likely result in a high chance of people in Koulkoro and Kati becoming infected with malaria, unless protected. Full EIR calculations will be reported after completion of molecular analysis by LBMA.

Some of the improvements to be made for the 2019 entomological campaign are to increase the number of paired control sites for longitudinal monitoring (security permitting) so that there are equal numbers of control and sprayed sites and to extend HLC to 8am to capture morning biting.

The entomological results suggest that Actellic CS and SumiShield WG should be used in rotation in Mali for insecticide resistance management, as both insecticides lasted long enough to cover the peak transmission season (according to cone bioassay results). The high *An. gambiae* s.l. biting rates in Koulikoro indicates that IRS relocation has had negative consequences, resulting in increased malaria vector biting rates and most likely increased malaria transmission.