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# THE PMI VECTORLINK PROJECT MOZAMBIQUE

## ADDENDUM TO JULY 2017-JUNE 2018 ANNUAL REPORT

## (INCORPORATING MOLECULAR ASSAY DATA)

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# ACRONYMS

| | |
|---|---|
| **AIRS** | Africa Indoor Residual Spraying |
| ***Ace-1*** | Acetylcholinesterase-1 gene |
| **CDC** | Centers for Disease Control and Prevention |
| **DNA** | Deoxyribonudeic Acid |
| **EIR** | Entomological Inoculation Rate |
| **HLC** | Human Landing Catch |
| **ib/p/n** | Infective Bites per Night |
| **IRS** | Indoor Residual Spray |
| **ITNs** | Insecticide-treated Nets |
| ***kdr*** | Knockdown Resistance |
| **NMCP** | National Malaria Control Program |
| ***P.*** | *Plasmodium* |
| **PCR** | Polymerase Chain Reaction |
| **PMI** | President's Malaria Initiative |
| **PSC** | Pyrethrum Spray Catch |
| **RFLP** | Restriction Fragment Length Polymorphism |
| **USAID** | United States Agency for International Development |
| **WHO** | World Health Organization |
| **WRBU** | Walter Reed Biosystematics Unit |

# EXECUTIVE SUMMARY

In Mozambique, Abt Associates implemented the President's Malaria Initiative (PMI) Africa Indoor Residual Spraying (AIRS) project from July 2017 to February 28, 2018; since March 1, 2018, Abt has been implementing the follow-on PMI VectorLink project, in close collaboration with Mozambique's National Malaria Control Program, the Provincial Directorate of Health in Zambézia Province, the District Services for Health, Women and Social Welfare, as well as the Ministry of Agriculture and Food Security and the Ministry of Land, Environment and Rural Development at the provincial and district levels.

During the 2017 spray campaign, PMI AIRS Mozambique conducted IRS with pirimiphos-methyl in seven target districts (Derre, Maganja da Costa, Milange, Mocuba, Molumbo, Mopeia, and Morrumbala). To achieve proper targeting of IRS, monthly entomological monitoring was performed using Centers for Disease Control and Prevention (CDC) light traps, human landing catches (HLCs), pyrethrum spray collections, and cone wall bioassays (used only in sprayed areas). Seasonal insecticide susceptibility tests were carried out in the seven sprayed districts.

In Mopeia district, a cluster-randomized trial was conducted to investigate the impact and cost effectiveness of combining IRS with a non-pyrethroid, next-generation IRS product and standard ITNs in an area with high malaria transmission. Key methodological considerations related to the study were comparison of vector density, biting rate, and sporozoite rates between treatment and control arms. CDC light trap collections and HLCs were used for sampling mosquitoes in the study areas.

VectorLink Mozambique submitted the comprehensive Annual Entomological Monitoring Report in December 2018, before molecular assay results were available. This addendum supplements that report by presenting molecular assay results associated with samples collected in 2017/18.

Advanced molecular assays were performed on a proportion of samples collected from July 2017 to June 2018 in Mopeia IRS intervention and control areas as well as the other intervention districts of Maganja da Costa, Mocuba, Morrumbala, Milange, Derre, and Molumbo and the control district of Molevala. Vector identification by polymerase chain reaction revealed a great diversity of anopheline species, up to 9 siblings of the major vectors *Anopheles gambiae* s.l. and *An. funestus* s.l. as well as several minor vector species. A test for accuracy in field morphological identification skills of the field team on samples collected by CDC light traps revealed high levels of accuracy (96.7%–100%) on all species except the rare *An. pretoriensis* (66.6%) and *An. tenebrosus,* (85.7%). Similar observations were made on samples collected using HLC.

Assays to detect malaria parasite sporozoites were performed against *Plasmodium* (*P.*) *falciparum* and *P. vivax* infections only. Vectors found to harbor these infections included *An. funestus* s.l., *An. funestus* s.s., *An. coustani*, *An. rivulorum*, *An. namibiensis*, *An. arabiensis* and *An. gambiae* s.s. In areas of Mopeia and Molevala district, which served as non-IRS controls, the overall crude *P. falciparum* sporozoite rates were found to be higher than in intervention areas. Similarly, the entomological inoculation rate estimates recorded higher values in the Mopeia control areas and Molevala district. This is a strong indicator of reduced malaria transmission in intervention areas as a result of IRS implementation.

Assays performed for detection and identification of mutations on genetic resistance markers, knockdown resistance (*kdr*) and Acetylcholinesterase-1 (*Ace-1)* genes revealed existence of a fully susceptible genotype in all vectors in all districts. Only 2.3% of *An. arabiensis* were found to carry the *Ace-1* marker for resistance. On the other hand, the *kdr-E* marker demonstrated variable combinations of all the three genotypes screened. Overall, the results demonstrate reduced transmission in intervention areas, ostensibly due to the IRS implementation.

# 1. INTRODUCTION

Indoor residual spraying (IRS) and insecticide-treated nets (ITNs) remain the primary mosquito vector control interventions in many parts of world, including sub-Saharan Africa, where the disease continues to be a public health concern.

Through the support of the President's Malaria Initiative (PMI), the Africa Indoor Residual Spraying (AIRS) project implemented five rounds of indoor residual spraying (IRS) in Mozambique's Zambézia province. During the 2017 IRS campaign, AIRS Mozambique sprayed in seven target districts: Derre, Maganja da Costa, Milange, Mocuba, Molumbo, Mopeia, and Morrumbala.

Both PMI AIRS and the follow-on PMI VectorLink Mozambique program also conducted entomological monitoring activities in Zambezia, and supported the National Malaria Control Program's (NMCP's) entomological activities in Nampula to enhance capacity for entomological monitoring.

Entomological monitoring was conducted in five of the IRS intervention districts (Maganja da Costa, Milange, Mocuba, Mopeia, and Morrumbala) as well as unsprayed Molevala district, which was used as the control. During April 2018, mosquito sampling and other entomology activities were suspended at all sites while the project team dealt with logistical issues associated with the transition from PMI AIRS to PMI VectorLink.

In Mopeia, entomological monitoring data were collected using Centers for Disease Control and Prevention (CDC) light traps and human landing catches (HLCs). In Maganja da Costa, Milange, Mocuba, Morrumbala, and Molevala, entomological monitoring data were collected using pyrethrum spray catch (PSC) as well as CDC light trap and HLC methodologies. For susceptibility tests in all districts, Prokopack aspirators were used to collect adult *An. funestus* s.l. and larval *An. gambiae* s.l.

The main objective of the entomology surveillance activities is to provide entomological data to inform decision making on IRS targeting and on choice of insecticide, and to measure impact. The information generated from the surveillance includes identifying vectors; estimating transmission levels, vector abundance and seasonality, vector biting patterns and location, and vector behavior; measuring the quality of spraying; and monitoring the decay rate of insecticides sprayed on different types of wall surface. The project was also tasked with building NMCP capacity to do insectary management and associated field vector surveillance.

This addendum report presents molecular data associated with the results presented earlier in the Entomological Monitoring Annual Report for 2017/18, which VectorLink Mozambique submitted in December 2018. It covers the period July 2017 to June 2018. From July 1, 2017, to February 28, 2018, activities took place under PMI AIRS; from March 1 to June 30, 2018, they were under PMI VectorLink.

# 2. METHODOLOGY

This section describes briefly each mosquito collection method used. The various sampling methodologies were detailed in the Entomological Monitoring Annual Report submitted in December 2018 and the same houses were sampled each month for all collection methods.

## 2.1 PYRETHRUM SPRAY CATCH

In each selected sentinel district (Maganja da Costa, Milange, Mocuba, and Morrumbala, as well as Molevala), two villages were selected for pyrethrum spray catch (PSC). Ten houses per village were chosen in each district, for a total of 20 houses per district. The houses were selected randomly at different distances to cover the area selected in each village. The samples were identified morphologically and preserved in 1.5 ml Eppendorf tubes containing silica gel. A proportion of the samples collected were sent for polymerase chain reaction (PCR) analysis to the Walter Reed Biosystematics Unit (WRBU) laboratory.

## 2.2 HUMAN LANDING CATCHES

HLCs were conducted in the intervention districts of Maganja da Costa, Milange, Mocuba, Mopeia, and Morrumbala as well as the control district of Molevala. With the exception of Mopeia, two houses were sampled in each district in a selected village on three consecutive nights to obtain six person-nights of collection per district per month (2 houses x 3 collection nights = 6 person-nights). In Mopeia, one house in each village in the intervention and control areas was selected for a total of eight houses (four in intervention and four in control districts). Collected mosquitoes were killed using cotton soaked in chloroform; identified; counted by species, location, and hour of collection; and preserved in 1.5 ml Eppendorf tubes with silica gel. A proportion of the samples collected by HLC were sent for PCR analysis at the WRBU laboratory.

## 2.3 CDC LIGHT TRAPS

CDC light traps were installed close to occupied nets in four houses in each of the intervention districts of Maganja da Costa, Milange, Mocuba, Mopeia, and Morrumbala as well as the control district of Molevala. Additionally, monthly CDC light trap collections were conducted in Quelimane city to monitor trends following no IRS in Quelimane city in 2017. In the six districts and Quelimane city, data were collected for three consecutive nights, resulting in 12 trap-nights per month per house for each district.

In Mopeia, 10 villages were selected (five in the intervention and five in the control areas) as sentinel sites for CDC light trapping using the same method above. Eight houses were selected in each village. Data were collected from 6 pm to 6 am for three consecutive nights. This necessitated setting up a total of 240 traps nights per month, equally split between Mopeia's intervention and control areas.

A proportion of the samples collected by this method were sent for analysis by PCR to the WRBU laboratory

## 2.4 VECTOR SUSCEPTIBILITY TESTING

Immature malaria vectors were collected from different larval habitats in Mocuba, Morrumbala, Maganja and Mopeia districts from January to March 2018. In September and October 2017, adult *An. funestus* mosquitoes were collected using Prokopacks and immediately used for susceptibility testing.

Field-collected *An. gambiae* s.l. larvae were reared to adult stage in the insectary. Batches of 25 females, sugar-fed and 3–5 days old, were subsequently subjected to World Health Organization (WHO) tube tests following the standard WHO 2016 protocol. These females were exposed to pirimiphos-methyl 0.25%, alpha-

cypermethrin 0.05%, permethrin 0.75%, DDT 4%, bendiocarb 0.1%, and deltamethrin 0.05% on WHO impregnated filter papers for 60 minutes.

Intensity assays were conducted by exposing wild-caught vector mosquitoes to insecticide dosages of 5× and 10× the diagnostic concentrations of permethrin and alpha-cypermethrin, according to the standard WHO bioassay method. All exposures were for one hour, and final mortality was scored after a 24-hour holding period during which a 10% sugar solution was made available to surviving mosquitoes.

Piperonyl butoxide (PBO) synergist assays were conducted using 3–5-day-old *An. gambiae* s.l. mosquitoes reared from field-collected larvae.

For clothianidin susceptibility tests, freshly treated filter papers[1] were inserted into plastic cylinders and tested according to standard WHO susceptibility test protocols. The exposure time was 60 minutes. Methodologies used for the above synergist assays, susceptibility tests, and strength of resistance were detailed in the Entomological Monitoring Annual Report of December 2018. Similar to other collections, a portion of samples from these tests were subjected to PCR assays at the WRBU laboratory to identify sibling species and detect presence of knockdown (*kdr)* and Acetylcolinesterase-1 (*Ace-1*) genes.

## 2.5 Selection of Samples for Molecular Assay

About 20% of annual samples to be assayed for each collection method in each district was pre-determined for molecular species identification during the planning phase of the activity. The number was estimated based on past mosquito abundance and available budget. The number was pro-rated to get the number of mosquitoes to be collected each month by each collection method. For each month's collection method, each mosquito sample was allocated a random number. The random numbers were run through the Excel random selection tool to pick up to the monthly target for each district's collection method. A total of 3,465 anopheline samples from the Mopeia study (1,655 from intervention areas and 1,810 from control areas) and 4,929 samples from the remaining districts were selected and submitted to WRBU for molecular analysis.

## 2.6 Advanced Molecular Assays

The advanced molecular assays performed by WRBU laboratory included the vector species identification, detection of malaria parasites *Plasmodium* (*P.*) *falciparum* and *P. vivax,* and detection of insecticide resistance genetic markers *kdr* and *Ace-1*.

## 2.7 Vector Species Identification

Following morphological identification of individual samples in the field, a selected proportion were amplified by PCR and directly sequenced using deoxyribonucleic acid (DNA) barcoding (mtDNA COI) and ITS2 (nDNA) primers and protocols for species confirmation. These initial screens provided verified positive controls for the PCR-based species diagnostic assays for downstream identifications. Where species diagnostic primers revealed unexpected band sizes, ITS2 or COI sequences were performed to verify species identity (Koekemoer et al. 2002). The MR4 *An. gambiae* s.l. assay for species diagnosis (Scott et al., 1993) was found to be highly reliable. *An. arabiensis* samples were identified using this assay. Specimens amplified as *"An. gambiae"* using this assay were further speciated into *An. gambiae* s.s. or *An. coluzzii* using the Fanello et al. (2002) restriction fragment length polymorphism (RFLP) PCR assays.

## 2.8 Detection and Identification of Malaria Parasite Species

The multiplex *falciparum-vivax* species diagnostic assay proposed by MR4 proved highly unreliable. Instead, the primers were used individually to detect *P. falciparum* and *P. vivax* in two separate reaction sets. These were

[1] Treated according to the AIRS protocol.

followed by sequencing to verify the accuracy of the primers in speciating. All controls and empty tube wells tested negative, as expected.

## 2.9 DETECTION OF INSECTICIDE RESISTANCE MARKERS

To determine the prevailing resistance mechanisms, molecular assays were used to detect presence of *kdr* and *Ace-1* genes in mosquito samples that had undergone WHO susceptibility test assays in the field.

# 3. RESULTS

## 3.1 MOLECULAR ASSAY RESULTS

A total of 8,394 samples were delivered for analysis at WRBU. Out of the total samples sent, 1036 specimens were found to be decomposed and covered with fungus, which degraded their DNA and made them unsuitable for either morphological or molecular identification. The remainder were identified by either morphology (to complex only) or by DNA methods. A total of 5382 samples were identified by DNA methods. A few failed to amplify or gave strange amplicon sequences for the specific assays.

### 3.1.1 IDENTIFICATION OF VECTOR SPECIES

Molecular assays performed on the samples identified the following anopheline species, mainly belonging to the two subgenera *Anopheles* and *Cellia*. Those belonging to *Anopheles* were *An. coustani*, *An.* cf. *coustani*, *An.* cf. *coustani* 2 NFL-2015, and *An. squamosus*. Those belonging to Cellia were *An. arabiensis*, *An. coluzzii*, *An. gambiae* s.s., *An. funestus* s.s., *An. parensis*, *An. rufipes*, *An. rivulorum*, *An. rivulorum*-like, *An. vaneedeni, An. leesoni, An. pharoensis, An. namibiensis, An. longipalpis,* and *An. pretoriensis*. In addition, *An.* MBI-12 and one potential *An. gambiae s.s.* × *An. coluzzii* hybrid cross was also identified through sequencing. For those samples that could not amplify, morphological identification by the WRBU team identified them as *An. gambiae* s.l. and *An. funestus* s.l.

Table1 shows details of species identification of 2,557 samples collected using different methods in Mopeia intervention and control areas. A higher diversity, collected mainly by HLCs performed indoors and outdoors, was observed in the control areas, with 17 siblings or species groups, in contrast to the IRS intervention areas, which yielded only 12 species. Even the common anopheline species were collected in significantly greater numbers in control areas compared with IRS intervention areas. This indicates potentially higher mosquito densities in the control areas as a result of mosquito density suppression by IRS.

**TABLE 1. NUMBERS AND SPECIES OF ANOPHELINE MOSQUITOES IDENTIFIED IN IRS INTERVENTION AND CONTROL AREAS IN MOPEIA DISTRICT**

| Final Species ID by WRBU | | | | $X^2$ | P |
|---|---|---|---|---|---|
| Intervention area | | Control area | | | |
| Species ID | n | Species ID | n | | |
| *An. funestus* s.l. | 192 | *An. funestus* s.l. | 397 | 71.35 | $2.99*10^{-17}$ |
| *An. funestus* s.s. | 308 | *An. funestus* s.s. | 1132 | 471.51 | $1.5*10^{-104}$ |
| *An. gambiae* s.l. | 16 | *An. gambiae* s.l. | 24 | 1.6 | 0.205 |
| *An. gambiae* s.s. | 13 | *An. gambiae* s.s. | 25 | 3.79 | 0.05 |
| *An. arabiensis* | 108 | *An. arabiensis* | 134 | 2.79 | 0.0946 |
| Unidentified | 48 | Unidentified | 12 | 21.6 | $3.36*10^{-6}$ |
| *An. cf. coustani* 2 NFL-2015 | 4 | *An. cf. coustani* 2 NFL-2015 | 5 | 0.11 | 0.738 |
| *An. coluzzii* | 2 | *An. coluzzii* | 4 | 0.67 | 0.414 |
| *An. coustani* | 32 | *An. coustani* | 15 | 6.15 | 0.0131 |
| *An. longipalpis* | 1 | | | 1 | 0.317 |
| *An. namibiensis* | 2 | *An. namibiensis* | 5 | 1.29 | 0.2568 |
| *An. rivulorum* | 19 | *An. rivulorum* | 45 | 10.56 | 0.001154 |

| Final Species ID by WRBU | | | | $X^2$ | P |
|---|---|---|---|---|---|
| Intervention area | | Control area | | | |
| Species ID | n | Species ID | n | | |
| *An. squamosus* | 2 | *An. squamosus* | 2 | 0.0 | 1 |
| | | *An. leesoni* | 1 | 1.0 | 0.317 |
| | | *An. parensis* | 1 | 1.0 | 0.317 |
| | | *An. pretoriensis* | 3 | 3 | 0.083 |
| | | *An. rivulorum*-like | 1 | 1.0 | 0.317 |
| | | *An. rufipes* | 1 | 1.0 | 0.317 |
| | | *An. spp* unknown | 1 | 1.0 | 0.317 |
| | | *An.* MBI-12 | 1 | 1.0 | 0.317 |
| Total | 748 | | 1809 | | |

### *FIELD TEAM ACCURACY IN VECTOR SPECIES IDENTIFICATION*

Table 2 compares 1,982 vector species identified by the team in the field and by molecular assays performed at the WRBU laboratory. The samples were collected by CDC light traps in Mopeia district.

**TABLE 2. RESULTS OF VECTOR SPECIES IDENTIFICATION FOR ANOPHELINES COLLECTED IN MOPEIA USING CDC LIGHT TRAPS AS IDENTIFIED BY THE MOZAMBIQUE FIELD TEAM IN COMPARISON WITH WRBU IDENTIFICATION USING MOLECULAR ASSAYS AND MORPHOLOGICAL KEYS FOR SOME OF THE UNAMPLIFIED SAMPLES**

| Area | Field team morphological ID | # identified by field team | Final ID at WRBU by PCR and morphology | # identified by WRBU | Total ID | Number discordant | % accuracy |
|---|---|---|---|---|---|---|---|
| Control | *An. funestus* s.l. | 1297 | *An. funestus* s.l. | 267 | 1297 | 0 | 100 |
| | | | *An. funestus* s.s. | 1001 | | | |
| | | | *An. leesoni* | 1 | | | |
| | | | *An. parensis* | 1 | | | |
| | | | *An. rivulorum* | 26 | | | |
| | | | *An. spp* unknown | 1 | | | |
| | *An. gambiae* s.l. | 122 | *An. arabiensis* | 88 | 122 | 1 | 99.18 |
| | | | *An. gambiae* s.l. | 12 | | | |
| | | | *An. gambiae* s.s. | 20 | | | |
| | | | *An. funestus* s.s. | 1 | | | |
| | | | *An. coluzzii* | 1 | | | |
| | *An. tenebrosus* | 7 | *An.* cf. *coustani* 2 NFL-2015 | 1 | 7 | 0 | 100 |
| | | | *An. coustani* | 3 | | | |
| | | | *An. namibiensis* | 3 | | | |
| | *An. pretoriensis* | 3 | *An. pretoriensis* | 2 | 3 | 1 | 66.66 |
| | | | *An. rufipes* | 1 | | | |
| | *An. coustani* | 4 | *An. coustani* | 4 | 4 | 0 | 100 |

| Area | Field team morphological ID | # identified by field team | Final ID at WRBU by PCR and morphology | # identified by WRBU | Total ID | Number discordant | % accuracy |
|---|---|---|---|---|---|---|---|
| | *An. ziemanni* | 1 | *An. namibiensis* | 1 | 1 | 0 | 100 |
| | *An. squamosus* | 1 | *An. coustani* | 1 | 1 | 1 | 0 |
| Intervention | *An. funestus* s.l. | 461 | *An. funestus* s.l. | 152 | 461 | 15 | 96.70 |
| | | | *An. funestus* s.s. | 281 | | | |
| | | | *An. rivulorum* | 12 | | | |
| | | | *An. gambiae* s.s. | 9 | | | |
| | | | *An. arabiensis* | 5 | | | |
| | | | *An. longipalpis* | 1 | | | |
| | *An. gambiae* s.l. | 76 | *An. arabiensis* | 68 | 76 | 1 | 98.68 |
| | | | *An. coluzzii* | 2 | | | |
| | | | *An. gambiae* s.l. | 5 | | | |
| | | | *An. funestus* s.l. | 1 | | | |
| | *An. tenebrosus* | 7 | *An.* cf. *coustani* 2 NFL-2015 | 4 | 7 | 1 | 85.71 |
| | | | *An. coustani* | 1 | | | |
| | | | Unidentified | 1 | | | |
| | | | *An. namibiensis* | 1 | | | |
| | *An. ziemanni* | 2 | Unidentified | 1 | 2 | 0 | 100 |
| | | | *An. coustani* | 1 | | | |
| | *An. coustani* | 1 | *An. funestus* s.s. | 1 | 1 | 1 | 0 |

Field team identification was limited to species and species group level, whereas the molecular assays could identify down to the sibling species level. Overall, this analysis demonstrates the appreciable skills of the field team in *Anopheles* species morphological identification: accuracy was high, estimated at 96.7% to 100% for most species, in particular abundant and major vector species. Poor accuracy in identification was limited to two minor species, namely, *An. pretoriensis* (66.60%) and *An. tenebrosus* (85.71%). Similar observations have been made on a similar dataset on anophelines collected using the HLC method (see Annex A for details).

### 3.1.2 Detection and Identification of Malaria Parasites *P. falciparum* and *P. vivax*

Detection and identification of malaria parasites was restricted to *P. falciparum* and *P. vivax*. The multiplex *falciparum-vivax* species diagnostic assay proved highly unreliable, resulting in spurious binding and unexpected product sizes. Following sustained failures to get reliable results in multiplex, the primers were instead used individually to detect *P. falciparum* and *P. vivax* in two separate reaction sets. Tables 3, 3.1, 4 and 4.1 show results for the *P. falciparum* and *P. vivax* assays, respectively stratified for intervention and control areas of Mopeia district, established from mosquitoes collected using CDC light trap and human landing catch collections. Results of PCR assay are given for each vector species with the respective sporozoite rate carriage calculated based on those results.

## TABLE 3. NUMBERS OF MOSQUITO VECTOR SPECIES POSITIVE FOR *P. FALCIPARUM* SPOROZOITES AS DETECTED BY PCR ASSAYS FROM CDC-LT COLLECTIONS

| Mopeia district | Final species ID as confirmed by PCR or morphology (for unamplified samples) by WRBU | *P. falciparum* detection assay results | | |
|---|---|---|---|---|
| | | # PCR positive | # PCR negative | (%) sporozoite rate* |
| Intervention | *An. funestus* s.l. | 2 | 151 | 1.31 |
| | *An. funestus* s.s. | 5 | 277 | 1.77 |
| | *An. rivulorum* | 0 | 12 | 0 |
| | *An. gambiae* s.s. | 2 | 7 | 22.2 |
| | *An. arabiensis* | 0 | 73 | 0 |
| | *An. longipalpis* | 0 | 1 | 0 |
| | *An. coluzzii* | 0 | 2 | 0 |
| | *An. gambiae* s.l. | 0 | 5 | 0 |
| | *An.* cf. *coustani* 2 NFL-2015 | 0 | 4 | 0 |
| | *An. namibiensis* | 0 | 1 | 0 |
| | Unidentified | 0 | 2 | 0 |
| | *An. coustani* | 0 | 2 | 0 |
| ***Total intervention area - All species*** | | ***9*** | ***537*** | ***1.65*** |
| Control area | *An. arabiensis* | 0 | 88 | 0 |
| | *An.* cf. *coustani* 2 NFL-2015 | 0 | 1 | 0 |
| | *An. coustani* | 1 | 7 | 12.5 |
| | *An. funestus* s.l. | 4 | 262 | 1.5 |
| | *An. funestus* s.s. | 36 | 966 | 3.59 |
| | *An. gambiae* s.l. | 0 | 12 | 0 |
| | *An. gambiae* s.s. | 0 | 20 | 0 |
| | *An. gambiae/coluzzii* | 0 | 1 | 0 |
| | *An. leesoni* | 0 | 1 | 0 |
| | *An. namibiensis* | 1 | 3 | 25 |
| | *An. parensis* | 0 | 1 | 0 |
| | *An. pretoriensis* | 0 | 2 | 0 |
| | *An. rivulorum* | 2 | 24 | 7.69 |
| | *An. rufipes* | 0 | 1 | 0 |
| | *Anopheles* spp. Unknown | 0 | 1 | 0 |
| ***Total control area - All species*** | | ***44*** | ***1390*** | ***3.16*** |

* Based on sporozoite detection by PCR.

In the intervention area, *P. falciparum* sporozoites were detected in *An. funestus* s.l. (1.31%), *An. funestus* s.s. (1.77%) and *An. gambiae* s.s. (22.2%) only, with an overall sporozoite rate estimated at 1.65%. Higher infections were detected in the control area, with a mean sporozoite estimated at 3.16%. Vector species found to carry *P. falciparum* sporozoites in the control area included *An. coustani* (12.5%), *An. funestus* s.l.. (1.5%), *An. funestus* s.s. (3.59%), *An. namibiensis* (25%), and *An. rivulorum* (7.69%). Surprisingly, none of the *An. gambiae* s.s. or *An. arabiensis* examined—from the control area—was found to carry *P. falciparum* sporozoites.

*An. funestus* s.s. and *An. coustani* were found positive for *P. falciparum* sporozoites in HLC samples from the control and intervention areas respectively. The sporozoite rate for *An. funestus* s.s. was 1.54%, while the *An coustani* sporozoite rate was 3.23%. The overall mean sporozoite rate estimated for all *Anopheles* species was 0.53% in the control and 0.52% in the intervention areas (Table 3.1).

## TABLE 3.1. NUMBERS OF MOSQUITO VECTOR SPECIES CARRYING *P. FALCIPARUM* SPOROZOITES AS DETECTED BY PCR ASSAYS OF HLC COLLECTIONS

| Mopeia District | Final species ID as confirmed by PCR or morphology (for unamplified samples) by WRBU | *P. falciparum* detection assay results | | |
|---|---|---|---|---|
| | | #PCR Positive | #PCR Negative | (%) sporozoite rate |
| Control area | *An. funestus* s.l. | 0 | 130 | 0 |
| | *An. funestus* s.s. | 2 | 128 | 1.54 |
| | *An. rivulorum* | 0 | 19 | 0 |
| | *An. rivulorum*-like | 0 | 1 | 0 |
| | *An. arabiensis* | 0 | 47 | 0 |
| | *An. gambiae* s.l. | 0 | 12 | 0 |
| | *An. coluzzii* | 0 | 3 | 0 |
| | *An. gambiae* s.s. | 0 | 5 | 0 |
| | *An. pretoriensis* | 0 | 1 | 0 |
| | *Anopheles rufipes* | 0 | 1 | 0 |
| | *An. coustani* | 0 | 8 | 0 |
| | *An. squamosus* | 0 | 3 | 0 |
| | *An cf. coustani* 2 NFL-2015 | 0 | 7 | 0 |
| | *An. squamosus* by morphology | 0 | 2 | 0 |
| | *Anopheles* sp unknown MOZ-01 | 0 | 1 | 0 |
| | *An. namibiensis* | 0 | 5 | 0 |
| ***Total control area - All species*** | | 2 | 373 | 0.53 |
| Intervention | *An. funestus* s.l. | 0 | 39 | 0 |
| | *An. funestus* s.s. | 0 | 41 | 0 |
| | *An. arabiensis* | 0 | 35 | 0 |
| | *An. gambiae* s.l. | 0 | 11 | 0 |
| | *An. gambiae* s.s. | 0 | 4 | 0 |
| | *An cf. coustani* 2 NFL-2015 | 0 | 4 | 0 |
| | *An. coustani* | *1* | 30 | 3.23 |
| | *An. squamosus* | 0 | 6 | 0 |
| | *An. namibiensis* | 0 | 1 | 0 |
| | *An. squamosus* by morphology | 0 | 2 | 0 |
| | *Anopheles coustani* by morphology | 0 | 8 | 0 |
| | *An. lesson* | 0 | 1 | 0 |
| | *An. parensis* | 0 | 1 | 0 |
| | *An. rivulorum* | 0 | 9 | 0 |
| ***Total Intervention area - All species*** | | 1 | 192 | 0.52 |

In the intervention area, *P. vivax* sporozoites were detected in *An. funestus* s.l. (0.65%) and *An. gambiae* s.s. (11.10%) from CDC light trap collections, with an overall sporozoite rate estimated at 0.36%. Fewer infections were detected in the control area with a mean sporozoite rate estimated at 0.28%. The only vector species found to carry *P. vivax* sporozoites in the control was *An. funestus* s.s. (0.39%). (Table 4)

## TABLE 4. NUMBERS OF MOSQUITO VECTOR SPECIES POSITIVE FOR *P. VIVAX* SPOROZOITES AS DETECTED BY PCR ASSAYS OF CDC-LT COLLECTIONS

| Mopeia district | Final species ID as confirmed by PCR or morphology (for unamplified samples) by WRBU | *P. vivax* detection assay results | | |
|---|---|---|---|---|
| | | # PCR positive | # PCR negative | (%) sporozoite rate* |
| Intervention | *An. funestus* s.l. | 1 | 152 | 0.65 |
| | *An. funestus* s.s. | 0 | 282 | 0 |
| | *An. rivulorum* | 0 | 12 | 0 |
| | *An. gambiae* s.s. | 1 | 8 | 11.1 |
| | *An. arabiensis* | 0 | 73 | 0 |
| | *An. longipalpis* | 0 | 1 | 0 |
| | *An. coluzzii* | 0 | 2 | 0 |
| | *An. gambiae* s.l. | 0 | 5 | 0 |
| | *An. cf. coustani* 2 NFL-2015 | 0 | 4 | 0 |
| | *An. namibiensis* | 0 | 1 | 0 |
| | Unidentified | 0 | 2 | 0 |
| | *An. coustani* | 0 | 2 | 0 |
| ***Total intervention area - All species*** | | ***2*** | ***544*** | ***0.36*** |
| Control area | *An arabiensis* | 0 | 88 | 0 |
| | *An. cf. coustani 2 NFL-2015* | 0 | 1 | 0 |
| | *An. coustani* | 0 | 8 | 0 |
| | *An. funestus* s.l. | 0 | 266 | 0 |
| | *An. funestus* s.s. | 4 | 998 | 0.39 |
| | *An. gambiae* s.l. | 0 | 12 | 0 |
| | *An. gambiae* s.s. | 0 | 20 | 0 |
| | *An. gambiae/coluzzii* | 0 | 1 | 0 |
| | *An. leeson* | 0 | 1 | 0 |
| | *An. namibiensis* | 0 | 4 | 0 |
| | *An. parensis* | 0 | 1 | 0 |
| | *An. pretoriensis* | 0 | 1 | 0 |
| | *An. rivulorum* | 0 | 26 | 0 |
| | *An. rufipes* | 0 | 1 | 0 |
| | *Anopheles spp. Unknown* | 0 | 1 | 0 |
| ***Total control area - All species*** | | ***4*** | ***1419*** | ***0.28*** |

* Based on sporozoite detection by PCR.

*Plasmodium vivax* sporozoites (20%) were found only in *An. gambiae* s.s. collected by HLC in the control area, and none on samples from the intervention area in Mopeia. This is in contrast to samples collected from the CDC-LT collections, where none of the members of the *An. gambiae* complex were positive for *P. vivax* infection in the control area in Mopeia. (Table 4.1)

## TABLE 4. 1. NUMBERS OF MOSQUITO VECTOR SPECIES POSITIVE FOR *P. VIVAX* SPOROZOITES AS DETECTED BY PCR ASSAYS OF HLC COLLECTIONS

| Mopeia District | Final species ID as confirmed by PCR or morphology (for unamplified samples) by WRBU | *P. vivax* detection assay results | | |
|---|---|---|---|---|
| | | #PCR positive | #PCR Negative | (%) sporozoite rate |
| Control area | *An. funestus* s.l. | 0 | 130 | 0 |
| | *An. funestus* s.s. | 0 | 130 | 0 |
| | *An. rivulorum* | 0 | 19 | 0 |
| | *An. rivulorum*-like | 0 | 1 | 0 |
| | *An. arabiensis* | 0 | 47 | 0 |
| | *An. gambiae* s.l. | 0 | 12 | 0 |
| | *An. coluzzii* | 0 | 3 | 0 |
| | *An. gambiae* s.s. | 1 | 4 | 20 |
| | *An. pretoriensis* | 0 | 1 | 0 |
| | *Anopheles rufipes* | 0 | 1 | 0 |
| | *An. coustani* | 0 | 8 | 0 |
| | *An. squamosus* | 0 | 3 | 0 |
| | *An cf. coustani* 2 NFL-2015 | 0 | 7 | 0 |
| | *An. squamosus* by morphology | 0 | 2 | 0 |
| | *Anopheles* sp unknown MOZ-01 | 0 | 1 | 0 |
| | *An. namibiensis* | 0 | 5 | 0 |
| ***Total control area - All species*** | | **1** | **374** | 0.27 |
| Intervention | *An. funestus* s.l. | 0 | 39 | 0 |
| | *An. funestus* s.s. | 0 | 41 | 0 |
| | *An. arabiensis* | 0 | 35 | 0 |
| | *An. gambiae* s.l. | 0 | 11 | 0 |
| | *An. gambiae* s.s. | 0 | 4 | 0 |
| | *An cf. coustani* 2 NFL-2015 | 0 | 4 | 0 |
| | *An. coustani* | 0 | 31 | 0 |
| | *An. squamosus* | 0 | 6 | 0 |
| | *An. namibiensis* | 0 | 1 | 0 |
| | *An. squamosus* by morphology | 0 | 2 | 0 |
| | *Anopheles coustani* by morphology | 0 | 8 | 0 |
| | *An. lesson* | 0 | 1 | 0 |
| | *Anopheles parensis* | 0 | 1 | 0 |
| | *Anopheles rivulorum* | 0 | 9 | 0 |
| ***Total Intervention area - All species*** | | **0** | **193** | **0** |

### 3.1.3 Entomological Inoculation Rate (EIR) Estimates

Table 5 and 5.1 shows the transmission risk of *P. falciparum* and *P. vivax* infections, respectively, in intervention and control areas in Mopeia through bites by the different vector species as estimated from the CDC light trap collections. The EIR is a measure of exposure, and expressed as infective bites an unprotected person is likely to experience in the area in one night. This was estimated as infective bites per trap per night (ib/t/n) from the CDC light trap collections or infective bites per person per night (ib/p/n) from the human landing catches.

**Table 5. *P. falciparum* Entomological Inoculation Rate as Estimated from Sporozoite Rates and Number of Mosquitoes Collected Per Trap Night (b/t/n) by the CDC Light Trap Collections in Mopeia Control and Intervention Areas ( July 2017 to June 2018)**

| Intervention area | | | | | Control area | | | |
|---|---|---|---|---|---|---|---|---|
| Species collected | Mean monthly numbers collected | *b/t/n | #Sporozoite rate | EIR | Mean monthly numbers collected | *b/t/n | #Sporozoite rate | EIR |
| *An. funestus* s.l. | 155.45 | 1.30 | 0.0161 | 0.0208 | 482.83 | 4.024 | 0.0315 | 0.1269 |
| *An. gambiae* s.l. | 6.91 | 0.06 | 0.2222 | 0.0128 | 29.00 | 0.242 | 0.0000 | 0.0000 |
| *An. coustani* | 0.09 | 0.00 | 0.00 | 0.0000 | 0.42 | 0.003 | 0.1250 | 0.0004 |
| *An. maculipalpis* | 0.00 | 0.00 | 0.00 | 0.0000 | 0.00 | 0.000 | 0.0000 | 0.0000 |
| *An. natalensis* | 0.00 | 0.00 | 0.00 | 0.0000 | 0.00 | 0.000 | 0.0000 | 0.0000 |
| *An. pharoensis* | 0.00 | 0.00 | 0.00 | 0.0000 | 0.00 | 0.000 | 0.0000 | 0.0000 |
| *An. pretoriensis* | 0.00 | 0.00 | 0.00 | 0.0000 | 0.25 | 0.002 | 0.0000 | 0.0000 |
| *An. squamosus* | 0.00 | 0.00 | 0.00 | 0.0000 | 0.08 | 0.001 | 0.0000 | 0.0000 |
| *An. tenebrosus* | 0.64 | 0.01 | 0.00 | 0.0000 | 0.92 | 0.008 | 0.0000 | 0.0000 |
| *An. ziemanni* | 0.18 | 0.00 | 0.00 | 0.0000 | 0.08 | 0.001 | 0.0000 | 0.0000 |
| *An. rufipes* | 0.09 | 0.00 | 0.00 | 0.0000 | 0.00 | 0.000 | 0.0000 | 0.0000 |
| *An. salbaii* | 0.00 | 0.00 | 0.00 | 0.0000 | 0.00 | 0.000 | 0.0000 | 0.0000 |

*Estimated from pooled indoor collections by eight (CDC LT) installed over three consecutive nights per month, in five villages for a total of 120 traps nights per month.

#Sporozoite rate determined from CDC light trap data, expressed in decimal.

+Value combines both *An. funestus* s.s. and *An. funestus* s.l. rates, as well as *An. gambiae* s.s. and *An. gambiae* s.l..

Based on the data from CDC light trap collections, the main vectors transmitting *P. falciparum* were identified as members of the *An. funestus* s.l, and *An. gambiae* s.l. at the intervention sites in Mopeia. However, only *An. gambiae* s.l and *An. coustani* were found to transmit *P. falciparum* in the control area. A higher risk, estimated at over six folds, was shown to exist in the control areas (0.1269 ib/t/n), inflicted by *An. funestus* s.l., compared with the risk experienced in the intervention areas (0.0208 ib/t/n), inflicted by *An. funestus* s.l. For *P. vivax,* the EIR determined from CDC light trap collections for *An. funestus* s.l. also showed a higher risk (about five folds) in the control area with 0.0138 ib/t/n compared to 0.0030 ib/t/n in the intervention area. EIR values of 0.0128 and 0.0041 ib/t/n were recorded for *P. falciparum* and *P. vivax*, respectively by *An. gambiae* s.l. at the intervention sites. However, no infective bites were detected by this species at the control sites.

## TABLE 5.1. *P. VIVAX* ENTOMOLOGICAL INOCULATION RATE AS ESTIMATED FROM SPOROZOITE RATES AND NUMBER OF MOSQUITOES COLLECTED PER TRAP PER NIGHT (B/T/N) BY THE CDC LIGHT TRAP COLLECTIONS IN MOPEIA CONTROL AND INTERVENTION AREAS ( JULY 2017 TO JUNE 2018)

| | Intervention area | | | | Control area | | | |
|---|---|---|---|---|---|---|---|---|
| Species collected | Mean monthly numbers collected | *b/t/n | #Sporozoite rate | EIR | Mean monthly numbers collected | *b/t/n | #Sporozoite rate | EIR |
| *An. funestus* s.l. | 155.45 | 1.30 | 0.0023 | 0.0030 | 526.73 | 4.389 | 0.0032 | 0.0138 |
| *An. gambiae* s.l. | 6.91 | 0.06 | 0.0714 | 0.0041 | 31.64 | 0.264 | 0.0000 | 0.0000 |
| *An. coustani* | 0.09 | 0.00 | 0.00 | 0.0000 | 0.45 | 0.004 | 0.0000 | 0.0000 |
| *An. maculipalpis* | 0.00 | 0.00 | 0.00 | 0.0000 | 0.00 | 0.000 | 0.0000 | 0.0000 |
| *An. natalensis* | 0.00 | 0.00 | 0.00 | 0.0000 | 0.00 | 0.000 | 0.0000 | 0.0000 |
| *An. pharoensis* | 0.00 | 0.00 | 0.00 | 0.0000 | 0.00 | 0.000 | 0.0000 | 0.0000 |
| *An. pretoriensis* | 0.00 | 0.00 | 0.00 | 0.0000 | 0.27 | 0.002 | 0.0000 | 0.0000 |
| *An. squamosus* | 0.00 | 0.00 | 0.00 | 0.0000 | 0.09 | 0.001 | 0.0000 | 0.0000 |
| *An. tenebrosus* | 0.64 | 0.01 | 0.00 | 0.0000 | 1.00 | 0.008 | 0.0000 | 0.0000 |
| *An. ziemanni* | 0.18 | 0.00 | 0.00 | 0.0000 | 0.09 | 0.001 | 0.0000 | 0.0000 |
| *An. rufipes* | 0.09 | 0.00 | 0.00 | 0.0000 | 0.00 | 0.000 | 0.0000 | 0.0000 |
| *An. salbaii* | 0.00 | 0.00 | 0.00 | 0.0000 | 0.00 | 0.000 | 0.0000 | 0.0000 |

The results from the HLCs ( Table 5.2 and 5.3) showed that in the intervention area, the only species found carrying *P. falciparum* was *An. coustani* and none of other anopheline mosquitoes identified were carrying either *P. falciparum* or *P. vivax*. The EIR for *An. coustani* was estimated as 0.0031 infective bites per person per night in the intervention areas. In the control area two anopheline species were found carrying malaria parasite namely *An. funestus* s.l. and *An. gambiae* s.l.. *An. funestus* s.l. was carrying *P. falciparum* parasite while *An. gambiae* s.l. was carrying *P. vivax*. The EIR was estimated as 0.02 and 0.01 infective bites per person per night respectively.

## TABLE 5.2. *P. FALCIPARUM* ENTOMOLOGICAL INOCULATION RATE AS ESTIMATED FROM SPOROZOITE RATES AND NUMBER OF MOSQUITOES COLLECTED BY HLC PER PERSON PER NIGHT (B/P/N) IN MOPEIA CONTROL AND INTERVENTION AREAS ( JULY 2017 TO JUNE 2018)

| | Intervention area | | | | Control area | | | |
|---|---|---|---|---|---|---|---|---|
| Species collected | Mean monthly numbers collected | *b/p/n | #Sporozoite rate | EIR | Mean monthly numbers collected | *b/p/n | #Sporozoite rate | EIR |
| *An. funestus* s.l. | 8.55 | 0.356 | 0 | 0 | 51.45 | 2.144 | 0.0071 | 0.02 |
| *An. gambiae* s.l. | 4.45 | 0.186 | 0 | 0 | 13.27 | 0.553 | 0 | 0 |
| *An. coustani* | 2.64 | 0.110 | 0.0286 | 0.0031 | 0.00 | 0.000 | 0 | 0 |
| *An. maculipalpis* | 0.09 | 0.004 | 0 | 0 | 0.00 | 0.000 | 0 | 0 |
| *An. natalensis* | 0.27 | 0.011 | 0 | 0 | 0.09 | 0.004 | 0 | 0 |
| *An. pharoensis* | 0.09 | 0.004 | 0 | 0 | 0.09 | 0.004 | 0 | 0 |
| *An. pretoriensis* | 0.09 | 0.004 | 0 | 0 | 0.27 | 0.011 | 0 | 0 |
| *An. squamosus* | 0.55 | 0.023 | 0 | 0 | 0.73 | 0.030 | 0 | 0 |
| *An. tenebrosus* | 1.18 | 0.049 | 0 | 0 | 1.45 | 0.061 | 0 | 0 |
| *An. ziemanni* | 0.73 | 0.030 | 0 | 0 | 0.18 | 0.008 | 0 | 0 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *An. rufipes* | 0.00 | 0.000 | 0 | 0 | 0.09 | 0.004 | 0 | 0 |
| *An. salbaii* | 0.00 | 0.000 | 0 | 0 | 0.09 | 0.004 | 0 | 0 |

## TABLE 5.3. *P. VIVAX* ENTOMOLOGICAL INOCULATION RATE AS ESTIMATED FROM SPOROZOITE RATES AND NUMBER OF MOSQUITOES COLLECTED BY HLC PER PERSON PER NIGHT (B/P/N) IN MOPEIA CONTROL AND INTERVENTION AREAS ( JULY 2017 TO JUNE 2018)

| | Intervention area | | | | Control area | | | |
|---|---|---|---|---|---|---|---|---|
| **Species collected** | **Mean monthly numbers collected** | ***b/p/n** | **#Sporozoite rate** | **EIR** | **Mean monthly numbers collected** | ***b/p/n** | **#Sporozoite rate** | **EIR** |
| *An. funestus* s.l. | 8.55 | 0.36 | 0 | 0 | 47.17 | 1.97 | 0.00 | 0.00 |
| *An. gambiae* s.l. | 4.45 | 0.19 | 0 | 0 | 12.17 | 0.51 | 0.0149 | 0.01 |
| *An. coustani* | 2.64 | 0.11 | 0 | 0 | 0.00 | 0.00 | 0 | 0 |
| *An. maculipalpis* | 0.09 | 0.00 | 0 | 0 | 0.00 | 0.00 | 0 | 0 |
| *An. natalensis* | 0.27 | 0.01 | 0 | 0 | 0.08 | 0.00 | 0 | 0 |
| *An. pharoensis* | 0.09 | 0.00 | 0 | 0 | 0.08 | 0.00 | 0 | 0 |
| *An. pretoriensis* | 0.09 | 0.00 | 0 | 0 | 0.25 | 0.01 | 0 | 0 |
| *An. squamosus* | 0.55 | 0.02 | 0 | 0 | 0.67 | 0.03 | 0 | 0 |
| *An. tenebrosus* | 1.18 | 0.05 | 0 | 0 | 1.33 | 0.06 | 0 | 0 |
| *An. ziemanni* | 0.73 | 0.03 | 0 | 0 | 0.17 | 0.01 | 0 | 0 |
| *An. rufipes* | 0.00 | 0.00 | 0 | 0 | 0.08 | 0.00 | 0 | 0 |
| *An. salbaii* | 0.00 | 0.00 | 0 | 0 | 0.08 | 0.00 | 0 | 0 |

A total of 1,135 *Anopheles* mosquitoes collected by CDC light traps in Molevala, Maganja da Costa, Milange, Morrumbala, Mocuba, and Quelimane city districts were screened for *P. falciparum* sporozoites by PCR. Table 6 shows the numbers and proportions of vector species found to carry *P falciparum* infections from each district. The last column provides the mean of all vectors screened. Annex B provides detailed data on the assays. Molevala, the control district, was found to have the highest overall sporozoite rates, estimated at 3.56%, followed by Maganja da Costa at 0.93% and Quelimane at 1.0%. No *P. falciparum* sporozoite infection was detected in vectors from the other districts (Milange, Morrumbala, and Mocuba).

## TABLE 6. PERCENTAGE AND NUMBER OF MOSQUITO VECTOR SPECIES FOUND TO BE CARRYING *P. FALCIPARUM* SPOROZOITES AS DETECTED BY PCR ON SAMPLES FROM CDC LIGHT TRAPS IN MOLEVALA, MAGANJA DA COSTA, MILANGE, MORRUMBALA, MOCUBA AND QUELIMANE DISTRICTS.

| District | Percentages of vector species carrying *P. falciparum* sporozoites (numbers in parenthesis represents numbers tested) | | | | | | All vectors combined* |
|---|---|---|---|---|---|---|---|
| | *An. rivulorum* | *An. funestus* s.s. | *An. gambiae* s.l. | *An. arabiensis* | *An. gambiae/coluzzii* | *An. pretoriensis* | |
| Molevala (control) | 6.25(16) | 4.04(198) | 6.45(31) | 3.03(99) | 0(8) | 0(0) | 3.56(401) |
| Maganja da Costa | 0(3) | 2.1(95) | 0(25) | 0(35) | 0(21) | 0(0) | 0.93(217) |
| Milange | 0(0) | 0(111) | 0(10) | 0(28) | 0(4) | 0(0) | 0(189) |
| Morrumbala | 0(0) | 0(8) | 0(1) | 0(103) | 0(0) | 100(1) | 0(130) |
| Mocuba | 0(0) | 0(25) | 0(9) | 0(15) | 0(1) | 0(0) | 0(97) |
| Quelimane | 0(0) | 0(69) | 0(10) | 0(0) | 33.3(3) | 0(0) | 1(101) |

*See Annex B for details on additional vectors screened.

Much higher *P. falciparum* sporozoite rates were estimated by PCR screening of *Anopheles* vectors collected by HLCs compared with CDC light trap collections. Table 7 shows a comparison of the estimates for all districts. Detailed HLC data screening results are in Annex C.

## TABLE 7. VARIABILITY IN *P. FALCIPARUM* SPOROZOITE RATES ESTIMATED BY PARASITE DETECTION ASSAY RESULTS FROM CDC LIGHT TRAP AND HLC COLLECTION IN THE MOLEVALA, MAGANJA DA COSTA, MILANGE, MORRUMBALA AND MOCUBA

| District | Mean overall *P. falciparum* sporozoite rate estimates | |
|---|---|---|
| | CDC light trap | HLC |
| Molevala (control) | 3.56 | 7.28 |
| Maganja da Costa | 0.93 | 7.43 |
| Milange | 0.00 | 1.42 |
| Morrumbala | 0.00 | 0.00 |
| Mocuba | 0.00 | 0.00 |

The EIR estimates established using the two datasets yielded variable results. Table 8 shows EIRs estimated using sporozoite rates established from PCR parasite screening of vectors collected by CDC light traps and HLCs with respective mean biting rates in all IRS districts (Maganja da Costa, Milange, Morrumbala, Mocuba) as well as the control district (Molevala). The highest mean sporozoite rate (3.56%) was found in CDC light trap data from Molevala and consequently the highest EIR (0.22 ib/t/n) was recorded there. The same was not true with HLC data, which show Maganja da Costa as having the highest sporozoite rate (7.43%) and consequently the EIR estimate (0.31 ib/p/n), due to low value on the bites per person per night observed in Maganja da Costa, compared with Molevala the highest EIR was observed in Molevala despite of lower sporozoite rate (7.28%).

**TABLE 8. *P. FALCIPARUM* EIRS ESTIMATED USING SPOROZOITE RATES ESTABLISHED FROM PCR PARASITE SCREENING OF VECTORS COLLECTED BY CDC LIGHT TRAPS OR HLCS WITH RESPECTIVE MEAN BITING RATES (ESTIMATED AS B/P/N) IN IRS AND CONTROL DISTRICTS, JULY 2017 TO JUNE 2018**

| District | Mean monthly numbers collected (HLC) | Mean monthly numbers collected (CDC LT) | *b/p/n | *b/t/n | Estimated from CDC light trap collection | | Estimated from HLC collection | |
|---|---|---|---|---|---|---|---|---|
| | | | | | #Sporozoite rate (%) | EIR | #Sporozoite rate (%) | EIR |
| Molevala (control) | 63.55 | 74.92 | 5.30 | 6.24 | 3.56 | 0.22 | 7.28 | 0.39 |
| Maganja da Costa | 50.55 | 53.67 | 4.21 | 4.47 | 0.93 | 0.04 | 7.43 | 0.31 |
| Milange | 35.09 | 27.75 | 2.92 | 2.31 | 0 | 0.00 | 1.42 | 0.04 |
| Morrumbala | 68.78 | 55.78 | 5.73 | 4.65 | 0 | 0.00 | 0 | 0.00 |
| Mocuba | 6.11 | 11.22 | 0.51 | 0.94 | 0 | 0.00 | 0 | 0.00 |

* Estimated from pooled indoor and outdoor HLCs by four collectors over three consecutive nights per month for a total of 12 person nights per month and four CDC LT over three consecutive nights per month for a total of 12 traps per night per month.

#pooled sporozoite rate combining all vector species screened for *P. falciparum*.

Results of PCR assays on CDC light trap collections show that infections with *P. vivax* were detected in vectors collected from Morrumbala and Quelimane districts only, with the overall crude sporozoite rates estimated as 0.76% (131) and 3.96% (101), respectively. All *P. vivax* infections were detected in *An. funestus* s.s. and *An. funestus* s.l. (see Annex D for details). Results from HLCs revealed infections in samples from Molevala (1.4%), Maganja da Costa (0.82%), and Morrumbala (0.97%). All infections were detected in *An. funestus* s.s. and *An. gambiae* s.s. (see Annex E for details).

### 3.1.4 DETECTION OF RESISTANCE MARKERS KDR AND ACE-1

Assays for detection of *kdr* and *Ace-1* genes were performed on 1,034 samples selected from among individual mosquitoes previously exposed to insecticide test papers (pirimiphos-methyl and alpha-cypermethrin) through WHO susceptibility tests in the field. These were collected from seven districts of Derre, Maganja da Costa, Milange, Mocuba, Morrumbala and Mopeia. Table 9 shows results both assays in terms of number tested, number failing to amplify for each assay, and number positive for the three genotypes (heterozygous, resistant, and susceptible) broken down by district and species. A proportion of samples that failed to amplify for each assay is indicated, with 241 failures for *kdr*-E and 51 failures for *Ace-1* assays. Failures might have been caused by deterioration of DNA material due to suboptimal storage conditions. Assays for *Ace-1* marker revealed the presence of 100% susceptible genotype for all species tested in all districts. Neither resistant nor heterozygote genotype could be detected for the *Ace-1* marker.

## TABLE 9. RESULTS OF PCR ASSAYS FOR RESISTANCE MARKERS *KDR* AND *ACE-1*, PERFORMED ON *AN. GAMBIAE* S.L. SAMPLES COLLECTED FROM STUDY DISTRICTS AND EXPOSED TO FIELD INSECTICIDE SUSCEPTIBILITY TESTS, SHOWING STATUS FOR EACH VECTOR SPECIES TESTED IN EACH DISTRICT

| District | Morpho-logical species ID by field team | Final species ID as confirmed by PCR or morphology for unamplified samples by WRBU | | *kdr-E* | | | | *Ace-1* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Samples ID | # identified | # failed to amplify | # heterozygous | # resistant | # susceptible | # failed to amplify | # heterozygous | # resistant | # susceptible |
| Morrumbala | *An. gambiae* s.l. | Unidentified | 38 | 29 | 1 | 5 | 5 | 1 | 0 | 0 | 37 |
| | | *An. arabiensis* | 192 | 62 | 22 | 6 | 102 | 14 | 0 | 0 | 178 |
| | | *An. gambiae* s.s. | 18 | 10 | 0 | 6 | 2 | 1 | 0 | 0 | 17 |
| | | *An. gambiae/coluzzii* | 4 | 2 | 0 | 1 | 1 | 0 | 0 | 0 | 4 |
| Mopeia | *An. gambiae* s.l. | *An. arabiensis* | 37 | 22 | 0 | 1 | 14 | 1 | 0 | 0 | 36 |
| | | *An. gambiae* s.s. | 20 | 2 | 3 | 4 | 11 | 0 | 0 | 0 | 20 |
| | | *An. merus* | 2 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 |
| Milange | *An. gambiae* s.l. | Unidentified | 5 | 4 | 0 | 0 | 1 | 1 | 0 | 0 | 4 |
| | | *An. arabiensis* | 256 | 15 | 1 | 0 | 240 | 10 | 0 | 0 | 246 |
| | | *An. gambiae s.l.* | 4 | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 4 |
| | | *An. gambiae* s.s./*coluzzii* | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| | | *An. gambiae* s.s. | 32 | 0 | 7 | 7 | 18 | 1 | 0 | 0 | 31 |
| | | *An. gambiae/coluzzii* | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 2 |
| Derre | *An. gambiae* s.l. | *An. arabiensis* | 6 | 1 | 0 | 1 | 4 | 0 | 0 | 0 | 6 |
| | | *An. gambiae* s.s. | 42 | 6 | 11 | 11 | 14 | 1 | 0 | 0 | 41 |
| | | *An. gambiae/coluzzii* | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| | | *An. merus* | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Molumbo | | Unidentified | 6 | 1 | 2 | 1 | 2 | 1 | 0 | 0 | 5 |

| District | Morpho-logical species ID by field team | Final species ID as confirmed by PCR or morphology for unamplified samples by WRBU | | *kdr-E* | | | | *Ace-1* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Samples ID | # identified | # failed to amplify | # heterozygous | # resistant | # susceptible | # failed to amplify | # heterozygous | # resistant | # susceptible |
| | *An. gambiae* s.l. | *An. arabiensis* | 39 | 12 | 7 | 2 | 18 | 1 | 0 | 0 | 38 |
| | | *An. gambiae* s.s. | 64 | 11 | 6 | 28 | 19 | 1 | 0 | 0 | 63 |
| | | *An. merus* | 16 | 6 | 2 | 5 | 3 | 0 | 0 | 0 | 16 |
| Maganja | *An. gambiae* s.l. | Unidentified | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | | *An. arabiensis* | 62 | 20 | 1 | 29 | 12 | 4 | 0 | 0 | 58 |
| | | *An. gambiae s.l.* | 2 | 2 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| | | *An. gambiae* s.s. | 51 | 16 | 0 | 0 | 9 | 2 | 0 | 0 | 49 |
| | | *An. gambiae/coluzzii* | 5 | 1 | 0 | 0 | 4 | 1 | 0 | 0 | 4 |
| | | *An. merus* | 3 | 1 | 0 | 2 | 0 | 2 | 0 | 0 | 1 |
| | | *An. quadriannulatus* | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Mocuba | *An. gambiae* s.l. | Unidentified | 6 | 2 | 0 | 0 | 4 | 0 | 0 | 0 | 6 |
| | | *An. arabiensis* | 81 | 7 | 11 | 3 | 60 | 5 | 0 | 0 | 76 |
| | | *An. gambiae* s.s. | 36 | 3 | 8 | 15 | 10 | 1 | 0 | 0 | 35 |

Table 10 shows further details of PCR assay results for resistance marker *kdr-E* performed on *An. gambiae* s.l. samples collected from the study districts to show frequencies of each genotype for each species identified from each district. A frequency of 100% susceptible genotype was recorded for *An. gambiae/coluzzii* in Milange and *An. merus* in Maganja da Costa, and 99% was recorded for *An. arabiensis* in Mopeia. A frequency of 100% heterozygote genotype was recorded for *An. gambiae* s.l. in Milange. The highest frequency for the resistance genotype was recorded for *An. gambiae* s.s. (75%) in Morrumbala and for *An. arabiensis* (69%) in Maganja da Costa.

## TABLE 10. DETAILS OF PCR ASSAY RESULTS S FOR RESISTANCE MARKERS *KDR-E* PERFORMED ON WILD *AN. GAMBIAE* S.L. SAMPLES COLLECTED FROM STUDY DISTRICTS TO SHOW FREQUENCIES OF EACH GENOTYPE FOR EACH SPECIES IDENTIFIED FROM EACH DISTRICT

| District | Field morphological species ID | WRBU final species ID as confirmed by PCR or morphology for unamplified samples | Kdr-E | | |
|---|---|---|---|---|---|
| | | *Sample ID* | #Heterozygous (%) | #Resistant (%) | #Susceptible (%) |
| Morrumbala | *An. gambiae s.l.* | *An. arabiensis* | 22(17%) | 6(5%) | 102(78%) |
| | | *An. gambiae* s.s. | 0(0%) | 6(75%) | 2(25%) |
| | | *An. gambiae/coluzzii* | 0(0%) | 1(50%) | 1(50%) |
| Mopeia | *An. gambiae* s.l. | *An. arabiensis* | 0(0%) | 1(7%) | 14(93%) |
| | | *An. gambiae* s.s. | 3(17%) | 4(22) | 11(63%) |
| | | *An. merus* | 0(0%) | 1(50%) | 1(50%) |
| Milange | *An. gambiae* s.l. | *An. arabiensis* | 1(0.5) | 0(0%) | 240(99.5%) |
| | | *An. gambiae s.l.* | 3(100%) | 0(0%) | 0(0%) |
| | | *An. gambiae* s.s./*coluzzii* | 0(0%) | 0(0%) | 1(100%) |
| | | *An. gambiae* s.s. | 7(22) | 7(22%) | 18(56%) |
| | | *An. gambiae/coluzzii* | 1(100%) | 0(0%) | 0(0%) |
| Derre | *An. gambiae* s.l. | *An. arabiensis* | 0(0%) | 1(20%) | 4(80%) |
| | | *An. gambiae* s.s. | 11(31%) | 11(31%) | 14(38%) |
| Molumbo | *An. gambiae* s.l. | *An. arabiensis* | 7(26%) | 2(7%) | 18(67%) |
| | | *An. gambiae* s.s. | 6(11%) | 28(53%) | 19(36%) |
| | | *An. merus* | 2(20%) | 5(50%) | 3(30%) |
| Maganja | *An. gambiae* s.l. | *An. arabiensis* | 1(2%) | 29(69%) | 12(29%) |
| | | *An. gambiae* s.s. | 0(0%) | 0(0%) | 9(100%) |
| | | *An. gambiae/coluzzii* | 0(0%) | 0(0%) | 4(100%) |
| | | *An. merus* | 0(0%) | 2(100%) | 0(0%) |
| Mocuba | *An. gambiae* s.l. | *An. arabiensis* | 11(15%) | 3(14%) | 60(81%) |
| | | *An. gambiae* s.s. | 8(24%) | 15(46%) | 10(30%) |

# 4. DISCUSSION

The species identification performed at WRBU using molecular assays and morphology (for the samples failing to amplify) revealed a high level of *Anopheles* vector species diversity: 18 species and species groups. The identified anophelines belong mainly to the two subgenera, *Anopheles* and *Cellia*. Those belonging to *Anopheles* are *An. coustani*, *An.* cf. *coustani* 2 NFL-2015, and *An. squamosus*. Those belonging to *Cellia* are *An. arabiensis*, *An. coluzzii*, *An. gambiae* s.l., *An. gambiae* s.s., *An. funestus* s.l., *An. funestus* s.s, *An. parensis*, *An. rufipes*, *An. rivulorum*, *An. rivulorum*-like, *An. vaneedeni, An. pharoensis, An. namibiensis, An. longipalpis*, and *An. pretoriensis.* In addition, *An.* MBI-12 and one potential *An. gambiae s.s.* × *An. coluzzii* hybrid cross was also identified through sequencing.

By far, *An. funestus* s.s. was the most abundant species in both IRS intervention and control areas. Higher vector diversity was observed in the unsprayed control areas. The assays revealed the presence of two members of the *An. coustani* group in samples from Mopeia, Mocuba, and Quelimane. These included *An. coustani*, and *An.* cf. *coustani* 2 and *An. namibiensis* .

Comparison of species identification performed by our field team and confirmed by the WRBU laboratory using morphological and molecular tools demonstrates a high level of reliability in the field team's identification skills. Their accuracy was estimated at between 96% and 100% for common anopheline species (*An. gambiae*, *An. funestus, An. coustani, An. ziemanni*); accuracy was below that range for only two rare species collected, *An. tenebrosus*, at 66.6%, and *An. pretoriensis,* at 85.7%.

Parasite detection in vectors was limited to two parasites only, namely, *P. falciparum* and *P. vivax*. PCR assays were performed to detect and identify the two infections. In the Mopeia intervention area, assays on mosquitoes collected by CDC light traps show that *P. falciparum* sporozoites were detected in *An. funestus* s.l., *An. funestus* s.s. and *An. gambiae* s.s. , with an overall sporozoite rate estimated at 1.65%. Higher infections were detected in the control area with a mean sporozoite estimated at 3.16%. Vector species found to be carrying *P. falciparum* sporozoites in the control area included *An. coustani*, *An. funestus* s.l., *An. funestus* s.s., *An. namibiensis,* and *An. rivulorum*. Surprisingly, none of the *An. gambiae* s.s. or *An. arabiensis* from the control areas was found to be carrying *P. falciparum* sporozoites. The higher sporozoite rate observed in control areas could be attributed to the effect of IRS on vector longevity, leading to reduced infection rates in intervention areas.

Malaria vector species found with *P. falciparum* or *P. vivax* were not always the same for CDC light trap collections and human landing catches. Unlike the CDC light trap collections, only *An. coustani* was found with *P. falciparum* sporozoites at the intervention sites in Mopeia from samples collected by human landing catches.

EIR estimates were also found to be different for the two collection methods. EIR estimates for Mopeia, based on sporozoite rates in *An. funestus* s.l. from CDC light trap collections show that there is higher exposure to *P. falciparum* in control areas (0.1269 ib/t/n) than in intervention (0.028 ib/t/n) areas. This shows about six fold higher risk to *P. falciparum* infection for unprotected people in control areas compared with intervention areas. Based on HLC samples, *P. falciparum* infection was found in *An. funestus* s.l. only at the control site with an EIR estimate of 0.02 ib/p/n.

In the rest of the districts, assays of samples collected by CDC light traps show that *P. falciparum* infections were detected in vectors from two districts only, one being the control district of Molevala (3.56%) and the other one being intervention districts of Maganja da Costa 0.93%). Results from assays of samples collected by HLC yielded much higher sporozoite rate estimates: 7.28% in Molevala, 7.43% in Maganja da Costa, and 1.42% in Milange . These observations on differences of infection rates by the two collection methods can be explained by the fact that the two collection methods might target mosquitoes at different physiological stages.

Estimates of EIR in the rest of the districts show *P. falciparum* transmission to be active in Molevala control district and Maganja da Costa. With infection results from CDC light trap collections, lower EIR estimates were scored for Moleva (0.22 ib/p/n) and Maganja da Costa (0.04 ib/p/n). However, using HLC collection data,

higher estimates were scored, 0.39 ib/p/n for Molevala and 0.31 ib/p/n in Maganja da Costa. The observed discrepancy results from different sporozoite scores from the two collection methods for the reasons described above.

Assays for detection of mutations in resistance markers *kdr-E* and *Ace-1* show that there are no mutations on the *Ace-1* marker among all vector populations screened from all seven districts surveyed. The *kdr-E* marker demonstrated variable combinations of all the three genotypes screened. The data are now available for further genetic analysis to establish the status of the *kdr-E* genetic marker and its contribution to insecticide resistance in Mozambique.

The July 2017 to June 2018 period posed challenges to preserving mosquito samples, which led to a sizeable proportion of the samples being infected with fungus to the extent that their DNA lost quality, resulting in non-amplification of these samples.

The higher sporozoite rates and corresponding EIRs in the control areas of Mopeia and Molevala districts is strong evidence of the positive contribution of IRS as well as ITNs, in suppressing malaria transmission in Mozambique.

# 5. REFERENCES


Fanello, C, Santollamazza, F, della Torre A. 2002. Simultaneous identification of species and molecular forms of the *Anopheles gambiae* complex by PCR-RFLP. *Medical and Veterinary Entomology* 16: 461-464.

Gillies MT, Coetzee M. 1987. A supplement to the Anophelinae of Africa south of the Sahara (Afrotropical region). *South African Inst Med Res* [Internet] (55): 1-143. Available from: http://ihi.eprints.org/1274/1/Gillies_%26_Coetzee_1984_Supplement_to_African_anophelines_1.pdf

Koekemoer, LL, Kamau, L, Hunt, R, Coetzee, M. 2002. A cocktail polymerase chain reaction assay to identify members of the *Anopheles funestus* (Diptera, Culicidae) group. *American Journal of Tropical Medcine & Hygiene* 66: 804-811.

Neil F. Lobo, Brandyce St. Laurent, Chadwick H. Seakale, Busiku Hamainza, Javan Chand, Dingani Chinula, Sindhu M. Krishnankutty, Jonathan D. Mueller, Nicholas A. Deason, Quynh T. Hoang, Heather L. Boldt, Julie Thumloup, Jennifer Stevenson, Aklilu Seyoum, Frank H. Collins 2015. Unexpected diversity of *Anopheles* species in Eastern Zambia: implications for evaluating vector behavior and interventions. *Scientific Report*: 5, 17952.

Scott JA, Brogdon W, Collins F. 1993. Identification of Single Specimens of the *Anopheles gambiae* complex by the Polymerase Chain Reaction. *Am J Trop Med Hyg* [Internet] 4(49): 520-9. Available from: http://files/105/Scott (1993)+PCR for anopheles.pdf

World Health Organization (WHO). 2016. *Test Procedures for Insecticide Resistance Monitoring in Malaria Vector Mosquitoes*. 2nd Edition. Geneva.

# ANNEXES

## ANNEX A. HLC RESULTS FROM MOPEIA INTERVENTION AND CONTROL AREAS TO SHOW LEVEL OF ACCURACY OF FIELD MORPHOLOGICAL IDENTIFICATION VERSUS WRBU MOLECULAR AND MORPHOLOGICAL IDENTIFICATION.

| Area | Field team morphological ID | # identified by field team | Final ID at WRBU by PCR and morphological | # identified by WRBU | Total ID | # discordant | % accuracy |
|---|---|---|---|---|---|---|---|
| Control | *An. funestus* s.l. | 280 | *An. funestus* s.l. | 130 | 280 | 0 | 100 |
| | | | *An. funestus* s.s. | 130 | | | |
| | | | *An. rivulorum* | 19 | | | |
| | | | *An. rivulorum*-like | 1 | | | |
| | *An. gambiae* s.l. | 64 | *An. arabiensis* | 46 | 64 | 0 | 100 |
| | | | *An. gambiae* s.l. | 12 | | | |
| | | | *An. coluzzii* | 3 | | | |
| | | | *An. gambiae* s.s. | 3 | | | |
| | *An. natalensis* | 1 | Unidentified | 1 | 0 | 0 | 0 |
| | *An. pharoensis* | 1 | Unidentified | 1 | 0 | 0 | 0 |
| | *An. pretoriensis* | 3 | Unidentified | 1 | 3 | 1 | 66.66 |
| | | | *An. gambiae* s.s. | 1 | | | |
| | | | *An. pretoriensis* | 1 | | | |
| | *An. rufipes* | 1 | *Anopheles* MBI-12 | 1 | 1 | 0 | 0 |
| | *An. salbaii* | 1 | *An. coustani* | 1 | 1 | 0 | 0 |
| | *An. squamosus* | 8 | Unidentified | 4 | 8 | 2 | 75 |
| | | | *An. gambiae* s.s. | 1 | | | |
| | | | An. squamosus | 2 | | | |
| | | | *Culex* MBI-27 | 1 | | | |
| | *An. tenebrosus* | 14 | Unidentified | 3 | 14 | 1 | 92.85 |
| | | | *An. cf. coustani* 2 NFL-2015 | 4 | | | |
| | | | *An. coustani* | 6 | | | |
| | | | *An. namibiensis* | 1 | | | |
| | *An. ziemanni* | 2 | Unidentified | 2 | 2 | 0 | 0 |
| Intervention | *An. coustani* | 29 | Unidentified | 10 | 29 | | |
| | | | *An. cf. coustani* 2 NFL-2015 | 1 | | | |
| | | | *An. coustani* | 18 | | | |
| | *An. funestus* s.l. | 94 | Unidentified | 22 | 94 | 0 | 100 |

| Area | Field team morphological ID | # identified by field team | Final ID at WRBU by PCR and morphological | # identified by WRBU | Total ID | # discordant | % accuracy |
|---|---|---|---|---|---|---|---|
| | | | *An. funestus* s.l. | 39 | | | |
| | | | *An. funestus* s.s. | 26 | | | |
| | | | *An. rivulorum* | 7 | | | |
| | *An. gambiae* s.l. | 49 | *An. arabiensis* | 35 | 49 | 0 | 100 |
| | | | *An. gambiae* s.l. | 11 | | | |
| | | | *An. gambiae* s.s. | 3 | | | |
| | *An. maculipalpis* | 1 | *An. gambiae* s.s. | 1 | 1 | | |
| | *An. natalensis* | 3 | Unidentified | 1 | 3 | 2 | 33.3 |
| | | | *An. cf. coustani* 2 NFL-2015 | 1 | | | |
| | | | *An. coustani* | 1 | | | |
| | *An. pharoensis* | 1 | *An. squamosus* | 1 | 1 | 1 | 0 |
| | *An. pretoriensis* | 1 | *An. coustani* | 1 | 1 | 1 | 0 |
| | *An. squamosus* | 6 | Unidentified | 6 | 6 | 0 | Unknown |
| | *An. tenebrosus* | 13 | Unidentified | 4 | 13 | 2 | 84.61 |
| | | | *An. cf. coustani* 2 NFL-2015 | 2 | | | |
| | | | *An. coustani* | 5 | | | |
| | | | *An. namibiensis* | 1 | | | |
| | | | *An. squamosus* | 1 | | | |
| | *An. ziemanni* | 8 | Unidentified | 3 | 8 | 0 | 100 |
| | | | *An. coustani* | 5 | | | |

## ANNEX B. NUMBERS OF MOSQUITO VECTOR SPECIES CARRYING *P. FALCIPARUM* SPOROZOITES AS DETECTED BY PCR ON SAMPLES FROM CDC LIGHT TRAP COLLECTIONS IN DISTRICTS OF MOLEVALA, MAGANJA, MILANGE, MORRUMBALA, MOCUBA AND QUELIMANE CITY.

| Districts | Field team morphological ID | | Final species ID as confirmed by PCR or morphology (for unamplified samples) by WRBU | | *P. falciparum* detection assay results | | Sporozoite rate (%) |
|---|---|---|---|---|---|---|---|
| | | | | | PCR Negative | PCR Positive | (PCR) |
| Molevala | *An. funestus* s.l. | 231 | *An. vaneedeni* | 1 | 1 | 0 | 0 |
| | | | *An. rivulorum* | 15 | 15 | 1 | 6.25 |
| | | | *An. funestus* s.s. | 198 | 190 | 8 | 4.04 |
| | | | *An. funestus* s.l. | 16 | 16 | 0 | 0 |
| | | | Unidentified | 1 | 1 | 0 | 0 |
| | *An. gambiae* s.l. | 175 | *An. merus* | 1 | 1 | 0 | 0 |
| | | | *An. gambiae* s.s./coluzzii | 4 | 4 | 0 | 0 |
| | | | *An. gambiae* s.s. | 34 | 34 | 0 | 0 |
| | | | *An. gambiae/coluzzii* | 4 | 4 | 0 | 0 |
| | | | *An. gambiae* s.l. | 31 | 29 | 2 | 6.45 |
| | | | *An. funestus* s.s. | 1 | 1 | 0 | 0 |
| | | | *An. arabiensis* | 99 | 96 | 3 | 3.03 |
| | | | Unidentified | 1 | 1 | 0 | 0 |
| Total Molevala | | | | | 393 | 14 | 3.56 |
| Maganja | *An. coustani* | 1 | *An. namibiensis* | 1 | 1 | 0 | 0 |
| | *An. funestus s.l.* | 114 | *An. funestus* s.l. | 16 | 16 | 0 | 0 |
| | | | *An. funestus* s.s. | 95 | 93 | 2 | 2.1 |
| | | | *An. rivulorum* | 3 | 3 | 0 | 0 |
| | *An. gambiae* s.l. | 102 | *An. arabiensis* | 35 | 35 | 0 | 0 |
| | | | *An. gambiae* s.l. | 25 | 25 | 0 | 0 |
| | | | *An. gambiae* s.s./coluzzii | 2 | 2 | 0 | 0 |
| | | | *An. gambiae* s.s. | 21 | 21 | 0 | 0 |
| | | | *An. gambiae/coluzzii* | 19 | 19 | 0 | 0 |
| Total Maganja da Costa | | | | | 215 | 2 | 0.93 |
| Milange | *An. coustani* | 5 | *An. coustani* | 4 | 4 | 0 | 0 |
| | | | *An. coustani* by morp. | 1 | 1 | 0 | 0 |
| | *An. funestus* s.l. | 138 | *An. funestus* s.l. | 27 | 27 | 0 | 0 |
| | | | *An. funestus* s.s. | 111 | 111 | 0 | 0 |
| | *An. gambiae* s.l. | 46 | *An. arabiensis* | 28 | 28 | 0 | 0 |
| | | | *An. gambiae* s.l. | 10 | 10 | 0 | 0 |

| Districts | Field team morphological ID | | Final species ID as confirmed by PCR or morphology (for unamplified samples) by WRBU | | *P. falciparum* detection assay results | | Sporozoite rate (%) |
|---|---|---|---|---|---|---|---|
| | | | | | PCR Negative | PCR Positive | (PCR) |
| | | | *An. gambiae* s.s. | 4 | 4 | 0 | 0 |
| | | | *An. gambiae/coluzzii* | 4 | 4 | 0 | 0 |
| Total Milange | | | | | 189 | 0 | 0 |
| Morrumbala | *An. coustani* | 1 | No ID | 0 | 0 | 0 | 0 |
| | *An. funestus* s.l. | 15 | *An. funestus* s.l. | 6 | 6 | 0 | 0 |
| | | | *An. funestus* s.s. | 8 | 8 | 0 | 0 |
| | | | No ID | 1 | 0 | 0 | 0 |
| | *An. gambiae* s.l. | 104 | *An. arabiensis* | 103 | 103 | 0 | 0 |
| | | | *An. gambiae* s.l. | 1 | 1 | 0 | 0 |
| | *An. natalensis* | 5 | Unidentified | 1 | 1 | 0 | 0 |
| | | | *An. maculipalpis* | 4 | 4 | 0 | 0 |
| | *An. pretoriensis* | 1 | *An. coustani* | 1 | 0 | 1 | 100 |
| | *An. rufipes* | 7 | *An. rufipes* | 7 | 7 | 0 | 0 |
| *Total Morrumbala* | | | | | 130 | 0 | 0 |
| Mocuba | *An. funestus* s.l. | 40 | *An. funestus* s.l. | 15 | 15 | 0 | 0 |
| | | | *An. funestus* s.s. | 25 | 25 | 0 | 0 |
| | *An. gambiae* s.l. | 60 | *An. arabiensis* | 15 | 15 | 0 | 0 |
| | | | *An. gambiae* s.l. | 9 | 9 | 0 | 0 |
| | | | *An. gambiae* s.s. | 32 | 32 | 0 | 0 |
| | | | *An. gambiae/coluzzii* | 1 | 1 | 0 | 0 |
| | | | NO ID | 3 | 0 | 0 | 0 |
| Total Mocuba | | | | | 97 | 0 | 0 |
| Quelimane | *An. funestus* s.l. | 74 | *An. funestus* s.l. | 5 | 5 | 0 | 0 |
| | | | *An. funestus* s.s. | 69 | 69 | 0 | 0 |
| | *An. gambiae* s.l. | 24 | *An. gambiae* s.l. | 10 | 10 | 0 | 0 |
| | | | *An. gambiae* s.s. | 11 | 11 | 0 | 0 |
| | | | *An. gambiae/coluzzii* | 3 | 2 | 1 | 33.33 |
| | *An. tenebrosus* | 3 | *An. cf. coustani* 2 NFL-2015 | 2 | 2 | 0 | 0 |
| | | | *An. namibiensis* | 1 | 1 | 0 | 0 |
| Total Quelimane | | | | | 100 | 1 | 1 |

## ANNEX C. NUMBERS OF MOSQUITO VECTOR SPECIES CARRYING *P. FALCIPARUM* SPOROZOITES AS DETECTED BY PCR, SAMPLES FROM HLC COLLECTIONS IN DISTRICTS OF MOLEVALA, MAGANJA, MILANGE, MORRUMBALA, AND MOCUBA.

| Districts | Field team morphological ID | Final ID at WRBU by PCR and morphological | *P. falciparum* detection assay results | | | % sporozoite rate |
|---|---|---|---|---|---|---|
| | | | PCR negative | PCR positive | Pending | |
| Molevala | *An. coustani* | Unidentified | 0 | 0 | 2 | 0 |
| | | An. coustani | 0 | 0 | 6 | 0 |
| | | *An. funestus* s.s. | 0 | 0 | 2 | 0 |
| | | *An. maculipalpis* | 0 | 0 | 1 | 0 |
| | *An. funestus* s.l. | *An. funestus* s.l. | 30 | 2 | 0 | 6.25 |
| | | *An. funestus* s.s. | 172 | 20 | 0 | 10.41 |
| | *An. gambiae* s.l. | *An. arabiensis* | 1 | 0 | 0 | 0 |
| | | *An. funestus* s.s. | 1 | 0 | 0 | 0 |
| | | *An. gambiae* s.l. | 4 | 0 | 0 | 0 |
| | | *An. gambiae* s.s. | 122 | 4 | 0 | 3.17 |
| | | *An. gambiae/ coluzzii* | 1 | 0 | 0 | 0 |
| | *An. pretoriensis* | Unidentified | 0 | 0 | 2 | 0 |
| | | *An. coustani* | 0 | 0 | 2 | 0 |
| | | *An. rufipes* | 0 | 0 | 4 | 0 |
| | *An. rufipes* | Unidentified | 0 | 0 | 2 | 0 |
| | | *An. rufipes* | 0 | 0 | 9 | 0 |
| | *An. tenebrosus* | *An. coustani* | 0 | 0 | 2 | 0 |
| | | *An. rufipes* | 0 | 0 | 2 | 0 |
| **Total Molevala** | | | **331** | **26** | | **7.28** |
| Maganja da Costa | *An. coustani* | *An. coustani* | 0 | 0 | 3 | 0 |
| | *An. funestus* s.l. | *An. funestus* s.l. | 27 | 4 | 0 | 12.50 |
| | | *An. funestus* s.s. | 141 | 18 | 0 | 11.3 |
| | | *An. rivulorum* | 9 | 2 | 0 | 18.18 |
| | *An. gambiae* s.l. | *An. arabiensis* | 78 | 2 | 0 | 2.56 |
| | | *An. coluzzii* | 1 | 0 | 0 | 0.00 |
| | | *An. gambiae* s.l. | 2 | 0 | 0 | 0.00 |
| | | *An. gambiae* s.s. | 73 | 1 | 0 | 1.35 |
| | | *An. gambiae/ coluzzii* | 5 | 0 | 0 | 0.00 |
| | *An. pharoensis* | Unidentified | 0 | 0 | 2 | 0.00 |
| | *An. tenebrosus* | Unidentified | 0 | 0 | 1 | 0.00 |
| | | *An. coustani* | 0 | 0 | 3 | 0.00 |
| **Total Maganja da Costa** | | | **336** | **27** | | **7.43** |
| Milange | *An. coustani* | Unidentified | 0 | 0 | 13 | 0.00 |
| | | *An. coustani* | 0 | 0 | 13 | 0.00 |
| | | *An. maculipalpis* | 0 | 0 | 3 | 0.00 |

| Districts | Field team morphological ID | Final ID at WRBU by PCR and morphological | *P. falciparum* detection assay results | | | % sporozoite rate |
|---|---|---|---|---|---|---|
| | | | PCR negative | PCR positive | Pending | |
| | *An. funestus* s.l. | Unidentified | 0 | 0 | 1 | 0.00 |
| | | *An. funestus* s.l. | 62 | 0 | 0 | 0.00 |
| | | *An. funestus* s.s. | 125 | 3 | 0 | 2.34 |
| | *An. gambiae* s.l. | *An. arabiensis* | 76 | 1 | 0 | 1.30 |
| | | *An. gambiae* s.l. | 7 | 0 | 0 | 0 |
| | | *An. gambiae* s.s. | 4 | 0 | 0 | 0 |
| | | *An. funestus* s.l. | 1 | 0 | 0 | 0 |
| | *An. pretoriensis* | *An. maculipalpis* | 0 | 0 | 2 | 0 |
| | *An. tenebrous* | Unidentified | 1 | 0 | 0 | 0 |
| | | *An. rufipes* | 0 | 0 | 1 | 0 |
| | *An. ziemanni* | *An. coustani* | 0 | 0 | 1 | 0 |
| **Total Milange** | | | **276** | **4** | | **1.42** |
| Morrumbala | *An. funestus* s.l. | *An. funestus* s.l. | 2 | 0 | 0 | 0 |
| | | *An. funestus* s.s. | 2 | 0 | 0 | 0 |
| | *An. gambiae* s.l. | *An. arabiensis* | 91 | 0 | 0 | 0 |
| | | *An. gambiae* s.s. | 3 | 0 | 0 | 0 |
| | *An. natalensis* | Unidentified | 0 | 0 | 1 | 0 |
| | *An. pretoriensis* | *An. rufipes* | 0 | 0 | 3 | 0 |
| **Total Morrumbala** | | | **98** | **0** | **0** | **0** |
| Mocuba | *An. funestus* s.l. | *An. funestus* s.l. | 3 | 0 | 0 | 0 |
| | | *An. funestus* s.s. | 8 | 0 | 0 | 0 |
| | | *An. rivulorum* | 20 | 0 | 0 | 0 |
| | *An. gambiae* s.l. | *An. arabiensis* | 10 | 0 | 0 | 0 |
| | | *An. gambiae* s.s. | 14 | 0 | 0 | 0 |
| **Total Mocuba** | | | **55** | **0** | | **0.00** |

## ANNEX D. NUMBERS OF MOSQUITO VECTOR SPECIES CARRYING *P. VIVAX* SPOROZOITES AS DETECTED BY PCR, SAMPLES FROM CDC LIGHT TRAPS IN DISTRICTS OF MOLEVALA, MAGANJA, MILANGE, MORRUMBALA, MOCUBA, AND QUELIMANE CITY

| Districts | Field team morphological ID | | Final species ID as confirmed by PCR or morphology (for unamplified samples) by WRBU | | *P. vivax* detection assay results | | Sporozoite rate (%) |
|---|---|---|---|---|---|---|---|
| | | | | | PCR Negative | PCR Positive | (PCR) |
| Molevala | *An. funestus* s.l. | 231 | *An. vaneedeni* | 1 | 1 | 0 | 0 |
| | | | *An. rivulorum* | 15 | 15 | 0 | 0 |
| | | | *An. funestus* s.s. | 198 | 198 | 0 | 0 |
| | | | *An. funestus* s.l. | 16 | 16 | 0 | 0 |
| | | | Unidentified | 1 | 1 | 0 | 0 |
| | *An. gambiae* s.l. | 175 | *An. merus* | 1 | 1 | 0 | 0 |
| | | | *An. gambiae* s.s./coluzzii | 4 | 4 | 0 | 0 |
| | | | *An. gambiae* s.s. | 34 | 34 | 0 | 0 |
| | | | *An. gambiae/ coluzzii* | 4 | 4 | 0 | 0 |
| | | | *An. gambiae* s.l. | 31 | 31 | 0 | 0 |
| | | | *An. funestus* s.s. | 1 | 1 | 0 | 0 |
| | | | *An. arabiensis* | 99 | 99 | 0 | 0 |
| | | | Unidentified | 1 | 1 | 0 | 0 |
| **Total Molevala** | | | | | **406** | **0** | **0** |
| Maganja | *An. coustani* | 1 | *An. namibiensis* | 1 | 1 | 0 | 0 |
| | *An. funestus s.l.* | 114 | *An. funestus* s.l. | 16 | 16 | 0 | 0 |
| | | | *An. funestus* s.s. | 95 | 95 | 0 | 0 |
| | | | *An. rivulorum* | 3 | 3 | 0 | 0 |
| | *An. gambiae* s.l. | 102 | *An. arabiensis* | 35 | 35 | 0 | 0 |
| | | | *An. gambiae* s.l. | 25 | 25 | 0 | 0 |
| | | | *An. gambiae* s.s./*coluzzii* | 2 | 2 | 0 | 0 |
| | | | *An. gambiae* s.s. | 21 | 21 | 0 | 0 |
| | | | *An. gambiae/ coluzzii* | 19 | 19 | 0 | 0 |
| **Total Maganja da Costa** | | | | | **217** | **0** | **0** |
| Milange | *An. coustani* | 5 | *An. coustani* | 4 | 4 | 0 | 0 |
| | | | *An. coustani* by morp. | 1 | 1 | 0 | 0 |
| | *An. funestus* s.l. | 138 | *An. funestus* s.l. | 27 | 27 | 0 | 0 |
| | | | *An. funestus* s.s. | 111 | 111 | 0 | 0 |
| | *An. gambiae* s.l. | 46 | *An. arabiensis* | 28 | 28 | 0 | 0 |
| | | | *An. gambiae* s.l. | 10 | 10 | 0 | 0 |
| | | | *An. gambiae* s.s. | 4 | 4 | 0 | 0 |
| | | | *An. gambiae/ coluzzii* | 4 | 4 | 0 | 0 |
| **Total Milange** | | | | | **189** | **0** | **0** |
| Morrumbala | *An. coustani* | 1 | No ID | 0 | 0 | 0 | 0 |

| Districts | Field team morphological ID | | Final species ID as confirmed by PCR or morphology (for unamplified samples) by WRBU | | *P. vivax* detection assay results | | Sporozoite rate (%) |
|---|---|---|---|---|---|---|---|
| | | | | | PCR Negative | PCR Positive | (PCR) |
| | *An. funestus* s.l. | 15 | *An. funestus* s.l. | 6 | 6 | 0 | 0 |
| | | | *An. funestus* s.s. | 8 | 7 | 1 | 12.5 |
| | | | No ID | 1 | 0 | 0 | 0 |
| | *An. gambiae* s.l. | 104 | *An. arabiensis* | 103 | 103 | 0 | 0 |
| | | | *An. gambiae* s.l. | 1 | 1 | 0 | 0 |
| | *An. natalensis* | 5 | Unidentified | 1 | 1 | 0 | 0 |
| | | | *An. maculipalpis* | 4 | 4 | 0 | 0 |
| | *An. pretoriensis* | 1 | *An. coustani* | 1 | 1 | 0 | 0 |
| | *An. rufipes* | 7 | *An. rufipes* | 7 | 7 | 0 | 0 |
| **Total Morrumbala** | | | | | **130** | **1** | **0.76** |
| Mocuba | *An. funestus* s.l. | 40 | *An. funestus* s.l. | 15 | 15 | 0 | 0 |
| | | | *An. funestus* s.s. | 25 | 25 | 0 | 0 |
| | *An. gambiae* s.l. | 60 | *An. arabiensis* | 15 | 15 | 0 | 0 |
| | | | *An. gambiae* s.l. | 9 | 9 | 0 | 0 |
| | | | *An. gambiae* s.s. | 32 | 32 | 0 | 0 |
| | | | *An. gambiae/coluzzii* | 1 | 1 | 0 | 0 |
| | | | NO ID | 3 | 3 | 0 | 0 |
| **Total Mocuba** | | | | | **100** | **0** | **0** |
| Quelimane city | *An. funestus* s.l. | 74 | *An. funestus* s.l. | 5 | 4 | 1 | 20 |
| | | | *An. funestus* s.s. | 69 | 66 | 3 | 4.34 |
| | *An. gambiae* s.l. | 24 | *An. gambiae* s.l. | 10 | 10 | 0 | 0 |
| | | | *An. gambiae* s.s. | 11 | 11 | 0 | 0 |
| | | | *An. gambiae/coluzzii* | 3 | 3 | 0 | 0 |
| | *An. tenebrosus* | 3 | *An. cf. coustani* 2 NFL-2015 | 2 | 2 | 0 | 0 |
| | | | *An. namibiensis* | 1 | 1 | 0 | 0 |
| **Total Quelimane city** | | | | | **97** | **4** | **3.96** |

## ANNEX E. NUMBERS OF MOSQUITO VECTOR SPECIES CARRYING *PLASMODIUM VIVAX* SPOROZOITES AS DETECTED BY PCR IN SAMPLES FROM HLC IN DISTRICTS OF MOLEVALA, MAGANJA, MILANGE, MORRUMBALA, AND MOCUBA.

| Districts | Field team morphological ID | Final ID at WRBU by PCR and morphological | *P. vivax* detection assay results | | | % sporozoite rate |
|---|---|---|---|---|---|---|
| | | | PCR negative | PCR positive | Pending | |
| Molevala | *An. coustani* | Unidentified | 0 | 0 | 2 | 0 |
| | | An. coustani | 0 | 0 | 6 | 0 |
| | | *An. funestus* s.s. | 0 | 0 | 2 | 0 |
| | | *An. maculipalpis* | 0 | 0 | 1 | 0 |
| | *An. funestus* s.l. | *An. funestus* s.l. | 32 | 0 | 0 | 0 |
| | | *An. funestus* s.s. | 188 | 4 | 0 | 10.00 |
| | *An. gambiae* s.l. | *An. arabiensis* | 1 | 0 | 0 | 0 |
| | | *An. funestus* s.s. | 1 | 0 | 0 | 0 |
| | | *An. gambiae* s.l. | 4 | 0 | 0 | 0 |
| | | *An. gambiae* s.s. | 125 | 1 | 0 | 0.79 |
| | | *An. gambiae/coluzzii* | 1 | 0 | 0 | 0 |
| | *An. pretoriensis* | Unidentified | 0 | 0 | 2 | 0 |
| | | *An. coustani* | 0 | 0 | 2 | 0 |
| | | *An. rufipes* | 0 | 0 | 4 | 0 |
| | *An. rufipes* | Unidentified | 0 | 0 | 2 | 0 |
| | | *An. rufipes* | 0 | 0 | 9 | 0 |
| | *An. tenebrosus* | *An. coustani* | 0 | 0 | 2 | 0 |
| | | *An. rufipes* | 0 | 0 | 2 | 0 |
| **Total Molevala** | | | **352** | **5** | | **1.40** |
| Maganja da Costa | *An. coustani* | *An. coustani* | 0 | 0 | 3 | 0 |
| | *An. funestus* s.l. | *An. funestus* s.l. | 29 | 2 | 0 | 6.25 |
| | | *An. funestus* s.s. | 158 | 1 | 0 | 3.13 |
| | | *An. rivulorum* | 11 | 0 | 0 | 0.00 |
| | *An. gambiae* s.l. | *An. arabiensis* | 80 | 0 | 0 | 0.00 |
| | | *An. coluzzii* | 1 | 0 | 0 | 0.00 |
| | | *An. gambiae* s.l. | 2 | 0 | 0 | 0.00 |
| | | *An. gambiae* s.s. | 74 | 0 | 0 | 0.00 |
| | | *An. gambiae/coluzzii* | 5 | 0 | 0 | 0.00 |
| | *An. pharoensis* | Unidentified | 0 | 0 | 2 | 0.00 |
| | *An. tenebrosus* | Unidentified | 0 | 0 | 1 | 0.00 |
| | | *An. coustani* | 0 | 0 | 3 | 0.00 |
| **Total Maganja da Costa** | | | **360** | **3** | | **0.82** |
| Milange | *An. coustani* | Unidentified | 0 | 0 | 13 | 0.00 |
| | | *An. coustani* | 0 | 0 | 13 | 0.00 |

| Districts | Field team morphological ID | Final ID at WRBU by PCR and morphological | *P. vivax* detection assay results | | | % sporozoite rate |
|---|---|---|---|---|---|---|
| | | | PCR negative | PCR positive | Pending | |
| | | *An. maculipalpis* | 0 | 0 | 3 | 0.00 |
| | *An. funestus* s.l. | Unidentified | 1 | 0 | 0 | 0.00 |
| | | *An. funestus* s.l. | 62 | 0 | 0 | 0.00 |
| | | *An. funestus* s.s. | 128 | 0 | 0 | 0.00 |
| | *An. gambiae* s.l. | *An. arabiensis* | 77 | 0 | 0 | 0.00 |
| | | *An. gambiae* s.l. | 7 | 0 | 0 | 0 |
| | | *An. gambiae* s.s. | 4 | 0 | 0 | 0 |
| | | *An. funestus* s.l. | 1 | 0 | 0 | 0 |
| | *An. pretoriensis* | *An. maculipalpis* | 0 | 0 | 2 | 0 |
| | *An. tenebrous* | Unidentified | 0 | 0 | 1 | 0 |
| | | *An. rufipes* | 0 | 0 | 1 | 0 |
| | *An. ziemanni* | *An. coustani* | 0 | 0 | 1 | 0 |
| **Total Milange** | | | **280** | **0** | | **0.00** |
| Morrumbala | *An. funestus* s.l. | *An. funestus* s.l. | 2 | 0 | 0 | 0 |
| | | *An. funestus* s.s. | 2 | 0 | 0 | 0 |
| | *An. gambiae* s.l. | *An. arabiensis* | 91 | 1 | 1 | 1.98 |
| | | *An. gambiae* s.s. | 3 | 0 | 0 | 0 |
| | *An. natalensis* | Unidentified | 1 | 0 | 0 | 0 |
| | *An. pretoriensis* | *An. rufipes* | 3 | 0 | 0 | 0 |
| **Total Morrumbala** | | | **102** | **1** | | **0.97** |
| Mocuba | *An. funestus* s.l. | *An. funestus* s.l. | 3 | 0 | 0 | 0 |
| | | *An. funestus* s.s. | 8 | 0 | 0 | 0 |
| | | *An. rivulorum* | 20 | 0 | 0 | 0 |
| | *An. gambiae* s.l. | *An. arabiensis* | 10 | 0 | 0 | 0 |
| | | *An. gambiae* s.s. | 14 | 0 | 0 | 0 |
| **Total Mocuba** | | | **55** | **0** | | **0.00** |