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THE PMI VECTORLINK MADAGASCAR ADDENDUM TO THE 2019-2020 FINAL ENTOMOLOGICAL MONITORING REPORT

JULY 2019 - AUGUST 2020

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Acronyms

EIR	Entomological Inoculation Rate
ELISA	Enzyme-Linked Immunosorbent Assay
HBR	Human Biting Rate
HLC	Human Landing Catch
IPM	Institut Pasteur of Madagascar
KD	Knockdown
NMCP	National Malaria Control Program
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
RFLP	Restriction Fragment Length Polymorphism
WHO	World Health Organization

1. INTRODUCTION

This addendum report presents results from the laboratory analysis of entomological samples, which were not included in the approved PMI VectorLink Madagascar 2019-2020 Final Entomological Monitoring Report. The molecular analysis work, initially scheduled to be conducted by Institut Pasteur of Madagascar (IPM), was later transferred to the National Malaria Control Program (NMCP) laboratory for completion as IPM switched their priorities to focus more on the COVID-19 pandemic at the time. The report, which covers the period of July 2019 to August 2020, includes the following data:

- Molecular species identification of *An. gambiae* s.l. and *An. funestus* s.l. mosquitoes collected during monthly adult collections and those used in insecticide susceptibility tests.
- *Plasmodium* falciparum sporozoite rates and entomological inoculation rates (EIRs).
- Allelic frequencies of genetic markers of insecticide resistance.

2. METHODOLOGY

2.1. VECTOR SPECIES COMPOSITION

A total of 2,417 *Anopheles gambiae* s.l. collected through Human Landing Catch (HLC) indoors (766) and outdoors (1,651), and 119 collected using Prokopack indoors (49) and outdoors (70) from all the vector surveillance sites were identified to the species level. The DNA of each individual *An. gambiae* s.l. mosquito was extracted using the protocol designed by Collins et al. (1987) and analyzed using Polymerase Chain Reaction (PCR) following the Short-Interspersed Element (SINE) protocol described by Santolamazza et al. (2008). A total of 453 *An. funestus* s.l. samples collected from nine sites surveyed were further identified at the subspecies level using the protocol of Koekemoer et al. (2002) from DNA extracted using the protocol of Collins et al. (1987). The other three sites either did not yield *An. funestus* s.l. (Betaindambo and Bezaha) or produced a single specimen (Manakaravavy). Of those, 406 were collected through Human Landing Catch (HLC) indoors (155) and outdoors (251) and, 47 collected using Prokopack indoor (20) and outdoor (27). In addition, 483 randomly selected *An. gambiae* s.l. mosquitoes among the dead per site and the surviving mosquitoes from the WHO susceptibility tests (all insecticides) conducted in Vavatenina (174) and Mahambo (309) were further analyzed for species identification.

2.2. ENTOMOLOGICAL INOCULATION RATES

Plasmodium falciparum infection rates of *An. gambiae* s.l. collected using HLC were determined using enzyme-linked immunosorbent assay (ELISA) method described by Burkot et al and modified by Wirtz et al., for sporozoite detection in the head and thorax of mosquitoes. This method uses a monoclonal antibody that recognizes a repetitive epitope on the circumsporozoite protein *of P. falciparum*. *Plasmodium falciparum* sporozoite ELISA Reagent Kits (MRA-890) were obtained from BIE Resources (NIAID, NIH, USA). Diluted *P. falciparum* sporozoite proteins supplied by the Center for Disease Control (CDC, Atlanta, USA) were used as positive controls, while ground male mosquitoes were used as negative controls. Determination of positive samples were done after reading optical densities (OD) at 405 nm on an ELISA plate reader. Positive samples were determined by OD readings 2-fold greater than the negative controls. The sporozoite rate was calculated as the ratio of the number of circumsporozoite-positive mosquitoes over the total number of mosquitoes analyzed by site. The monthly entomological inoculation rate (EIR) is calculated as the product of the sporozoite rate and the human biting rate per month (HBR). The EIR measures exposure to infectious bites and represents the intensity of malaria transmission. The mean EIR was calculated per month of collection using the HBR and the sporozoite rate recorded after analysis. The mean EIR per sentinel site was later calculated by multiplying the EIR recorded per month by the number of collection months.

2.3. CHARACTERIZATION OF INSECTICIDE RESISTANCE MARKERS

The insecticide resistance markers of 483 mosquitoes identified after insecticide susceptibility tests were assessed to determine the frequency of the mutations associated with resistance among the population tested from Vavatenina and Mahambo. The presence of Knock down resistance (*Kdr)*-West and East was characterized using the conventional Polymerase Chain Reaction (PCR) restriction fragment length polymorphism (RFLP) method as described by Matinez-Torres et al. (1999). The protocol described by Weill et al. (2004) was used for the determination of the (acetylcholinesterase) *ace-1* mutation within the population of each site.

3. RESULTS

3.1. SPECIES COMPOSITION OF *AN. GAMBIAE* COMPLEX

A total of 2,751 *An. gambiae* s.l. were sampled from the 12 sites surveyed. *Anopheles gambiae ss* and *An. arabiensis* were the two species found in the different sites with *An. arabiensis* being the predominant species in eight of the sites surveyed. The proportion of *An. arabiensis* varied from 96.4 percent in the locality of Manakaravavy to 100 percent in Manakaravavy, Betaindambo, Irina and Ankilivalo. The lowest proportion was recorded in Vavatenina (0.6%).

Table 1: Distribution of *An. gambiae* s.l. Species

Regions	Districts	Localities	# Tested	*An. gambiae s.s.*	*An. arabiensis*
Analanjirofo	Fenerive Est	Mahambo	619	601 (97.1%)	18 (2.9%)
Analanjirofo	Vavatenina	Vavatenina	480	477 (99.4%)	3 (0.6%)
Atsimo Andrefana	Ampanihy	Manakaravavy	110	4 (3.6%)	106 (96.4%)
Atsimo Andrefana	Betioky	Bezaha	129	0 (0.0%)	129 (100.0%)
Atsimo Andrefana	Toliara I	Betaindambo	52	0 (0.0%)	52 (100.0%)
Atsimo Andrefana	Toliara II	Tsaragiso	232	1 (0.4%)	231 (99.6%)
Diana	Antsiranana I	Anamakia	97	0 (0.0%)	97 (100.0%)
Haute Matsiatra	Ambalavao	Mahasoa	224	1 (0.4%)	223 (99.6%)
Ihorombe	Ihosy	Irina	185	0 (0.0%)	185 (100.0%)
Menabe	Mahabo	Ankilivalo	383	0 (0.0%)	383 (100.0%)
Vatovavy Fitovinany	Manakara	Ampasimpotsy	160	158 (98.8%)	2 (1.2%)
Vatovavy Fitovinany	Manakara	Marofarihy	80	61 (76.3%)	19 (23.7%)
Total			**2,751**	**1,303**	**1,448**

3.2. SPECIES COMPOSITION OF *AN. FUNESTUS* GROUP

A total of 453 *An. funestus* group was identified to sibling species in nine sites. *An. funestus* and *An. leesoni* were the two species found in the different sites. *An. leesoni* was found in two of the nine sites with the proportion of 6.0% in Ampasimpotsy and 6.3% in Marofarihy.

Table 2: Distribution of *An. funestus* Group Species

Regions	Districts	Localities	# Tested	*An. funestus* s.s.	*An. leesoni*
Analanjirofo	Fenerive Est	Mahambo	92	92 (100%)	0 (0.0%)
Analanjirofo	Vavatenina	Vavatenina	109	109 (100%)	0 (0.0%)
Atsimo Andrefana	Toliara II	Tsaragiso	7	7 (100%)	0 (0.0%)
Diana	Antsiranana I	Anamakia	6	6 (100%)	0 (0.0%)
Haute Matsiatra	Ambalavao	Mahasoa	70	70 (100%)	0 (0.0%)
Ihorombe	Ihosy	Irina	2	2 (100%)	0 (0.0%)
Menabe	Mahabo	Ankilivalo	3	3 (100%)	0 (0.0%)

Regions	Districts	Localities	# Tested	*An. funestus* s.s.	*An. leesoni*
Vatovavy Fitovinany	Manakara	Ampasimpotsy	84	79 (94.0%)	5 (6.0%)
Vatovavy Fitovinany	Manakara	Marofarihy	80	75 (93.7%)	5 (6.3%)
Total			**453**	**443**	**10**

3.3. ELISA CIRCUMSPOROZOITE DETECTION

ELISA CSP tests were carried out on 3,560 *Anopheles*, including 1,303 *An. gambiae* ss, 1,448 *An. arabiensis*, 443 *An. funestus*, 10 *An. leesoni*, 148 *An. mascarensis* and 208 *An. coustani* collected from July 2019 to August 2020. Four *An. gambiae* and five *An. arabiensis* were positive for *Plasmodium falciparum*. *Anopheles gambiae* s.l. of seven sites (Ampasimpotsy, Anamakia, Betaindambo, Bezaha, Mahasoa, Manakaravavy and Marofarihy) out of the 12 surveyed did not record any mosquito carrying the sporozoite infections. The sporozoite rates recorded in the five remaining sites varied from 0.3 percent in Mahambo for *An. gambiae* s.s. to 1.1 percent in Irina for *An. arabiensis*. Furthermore, the other potential vectors analyzed (443 *An. funestus* s.s., 10 *Anopheles leesoni*, 148 *An. mascarensis* and 208 *An. coustani*) were negative for sporozoite infection in all the 12 investigation sites.

3.4. ENTOMOLOGICAL INOCULATION RATE (EIR)

The mean EIR representing the product of the HBR and the sporozoite rate represents 0.24 infectious bites/person/month (ib/p/m) in Ankilivalo, 0.18 in Tsaragiso, 0.48 ib/p/m in Irina and 0.60 ib/p/m in Vavatenina and Mahambo (Table 3).

Table 3: EIR of Malaria Vectors Collected Using HLC

	An. arabiensis				*An. gambiae s.s.*			
Sites	**Mean HBR**	**Sporozoite Rate**	**EIR (ib/p/n)**	**EIR (ib/p/m)**	**Mean HBR**	**Sporozoite Rate**	**EIR (ib/p/n)**	**EIR (ib/p/m)**
Ankilivalo	1.5	0.005	0.008	0.24	0	0	0	0
Irina	1.5	0.011	0.016	0.48	0	0	0	0
Mahambo	0	0	0	0	6.6	0.003	0.020	0.60
Tsaragiso	1.6	0.004	0.006	0.18	0	0	0	0
Vavatenina	0	0	0	0	5.1	0.004	0.020	0.60

3.5. MOLECULAR DETECTION OF RESISTANCE MARKERS

Molecular markers of resistance were determined among samples of *An. gambiae* ss and *Anopheles arabiensis* resistant to pyrethroids (deltamethrin, permethrin, alpha-cypermethrin and lambda-cyhalothrin), from WHO tube test in two sites (Mahambo and Vavatenina).

468 *An. gambiae* ss and seven *An. arabiensis*, analyzed for *kdr*-west and 461 *An. gambiae* ss and seven *An. arabiensis* for *kdr*-east were homozygous susceptible (SS) in the two localities, showing that the mutation does not exist in the area. The *Ace*-1R mutation was tested in Vavatenina, and all the samples tested were also homozygous susceptible. The distribution of the species of *An. gambiae* and *An. arabiensis*, as well as the allelic frequencies of the *kdr*-west, *kdr*-east and *Ace*-1 mutations by locality are presented in Table 4.

Table 4: Frequency of *Kdr* (West and East) and *Ace-1*

Localities	# Tested	Species (%)	# Tested	*Kdr-West*					# Tested	*Kdr-East*					# Tested	*Ace-1*			
				RR	RS	SS	Freq	Not amplified		RR	RS	SS	Freq	Not amplified		RR	RS	SS	Freq
Mahambo	304	*An. gambiae* s.s. (98.4%)	304	0	0	298	0	6	304	0	0	294	0	10	0	NA	NA	NA	NA
	5	*An. arabiensis* (1.6%)	5	0	0	5	0	0	5	0	0	5	0	0	0	NA	NA	NA	NA
Vavatenina	172	*An. gambiae* s.s. (98.9%)	172	0	0	170	0	2	172	0	0	167	0	5	75	0	0	75	0
	2	*An. arabiensis* (1.2%)	2	0	0	2	0	0	2	0	0	2	0	0	0	NA	NA	NA	0
TOTAL	**483**		**483**	**0**	**0**	**475**	**0**	**8**	**483**	**0**	**0**	**468**	**0**	**15**	**75**	**0**	**0**	**75**	**0**

RR: Homozygous Resistant
RS: Heterozygous Resistant
SS: Homozygous Susceptible.
Freq: Frequency

4. Conclusions

Molecular analysis of entomological samples collected from July 2019 to August 2020 under the PMI VectorLink Madagascar project, including bionomic and susceptibility monitoring in the sentinel sites, showed that *An. gambiae* s.s. was the predominant malaria vector in only four of the twelve sites surveyed while *An. arabiensis* represented the main vector in all the other eight remaining sites and was the only species recorded in five sites.

Two species of the *An. funestus* group were also found, including *An. funestus* and *An. leesoni* with predominance of *An. funestus* in the two sites (Ampasimpotsy and Marofarihy) where both are present. Only *Anopheles arabiensis and Anopheles gambiae* s.s. were positive for sporozoite infection in five sites (Ankilivalo, Irina, Tsaragiso, Mahambo and Vavatenina), while no infection was recorded in the other sites. Additionally, all other *Anopheles* species tested did not yield any sporozoite infection, showing that only *An. gambiae* s.l. represents the main vector species found in the surveyed sites. The *An. gambiae* s.s. and *An. arabiensis* entomological inoculation rate was low in all sites where *Plasmodium* infection was recorded and ranged between 0.006 ibpn in Tsaragiso and 0.020 ibpn in Mahambo and Vavatenina. This trend may contrast with malaria endemicity recorded in Madagascar where malaria is considered to be an endemic disease affecting most of the population. Also, the data calls for additional mosquitoes to be analyzed for sporozoite infection detection to increase the likelihood of finding more infections if probable. Furthermore, additional parasite such as *P. vivax* will need to be investigated as already reported in the country.

The *kdr*-east, *kdr*-west and the *Ace*-1R mutations were absent within the vector populations of all localities tested and correlate with the phenotypic status of the mosquitoes recorded during the WHO susceptibility test. However, caution will need to be taken and close follow-up to be considered as few sites are now showing possible resistance to deltamethrin and confirmed resistance to either alpha-cypermethrin or permethrin, as described in the annual report. However, the outcomes could still support the deployment of any recommended vector control interventions using any of the tested insecticides.

5. REFERENCES

Burkot TR, Williams JL, Schneider I: Identification of *Plasmodium falciparum*-infected mosquitoes by a double antibody enzyme-linked immunosorbent assay. Am J Trop Med Hyg. 1984 Sep;33(5):783-8. doi: 10.4269/ajtmh.1984.33.783.

Collins, F. H., Mendez, M. A., Rasmussen, M. O., Mehaffey, P. C., Besansky, N. J., Finnerty, V. (1987). A ribosomal RNA gene probe differentiates member species of the *Anopheles gambiae* complex. Am J Trop Med Hyg, Vol. 37, 37-41.

Echeverry, D., Deason, N., Makuru, V., Davidson, J., Xiao, H., Niedbalski, J., Lobo, N. (2017). Fast and robust single PCR for Plasmodium sporozoite detection in mosquitoes using the cytochrome oxidase I gene. Malaria Journal, Vol.16.

Koekemoer, L. L., Kamau, L., Hunt, R. H., Coetzee, M. (2002). A cocktail polymerase chain reaction assay to identify members of the *Anopheles funestus* (Diptera: Culicidae) group. Am J Trop Med Hyg, Vol. 66, 804-11.

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

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

Weill M, Malcom C, Chandre F, Mogensen K, Berthomieu K, Marquine M, Raymond M. The unique mutation in Ace-1 giving high insecticide resistance is easily detectable in mosquito vectors. Insect Mol Biol. 2004; 13:1–7.

Wirtz, RA, Zavala, F, Charoenvit, Y, Campbell, GH, Burkot, TR et al. (1987). Comparative testing of monoclonal antibodies against *Plasmodium falciparum* sporozoites for ELISA development. Bulletin of the World Health Organization, 65 (1), 39 - 45. https://apps.who.int/iris/handle/10665/264328.