ZAP | The Zika AIRS Project

PMI Africa Indoor Residual Spraying (PMI AIRS) Task Order Six

ZAP HAITI ENTOMOLOGICAL MONITORING

MID-TERM REPORT

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CONTENT

Content…… i

Acronyms…. ii

1. Introduction and Objectives 1

2. Entomological monitoring sites and methods 3

2.1 Entomological monitoring sites 3
2.2 Entomological monitoring methods 3
2.2.1 Mosquito egg density by Ovitraps 3
2.2.2 Larval surveys 4
2.2.3 Demographic pupae surveys 4
2.3 Results…….. 5
2.3.1 Egg density… 5
2.3.2 Larval indexes 8
2.3.3 Pupae surveys 10
2.3.4 Adult mosquito identification from collected pupae 11

3. Discussion and Conclusion 12

ACRONYMS

AIRS	Africa Indoor Residual Spraying
Bti	*Bacillus thuringiensis israelensis*
CDC	Centers for Disease Control and Prevention
CZS	Congenital Zika Syndrome
GAT	Gravid *Aedes* Traps
GBS	Guillain-Barré Syndrome
LAC	Latin American and Carribbean
MSPP	Ministry of Public Health and Population / *Ministere de la Sante Publique et de la Population*
USAID	United States Agency for International Development
VC	Vector Control
ZAP	Zika AIRS Project
ZIKV	Zika Virus

1. INTRODUCTION AND OBJECTIVES

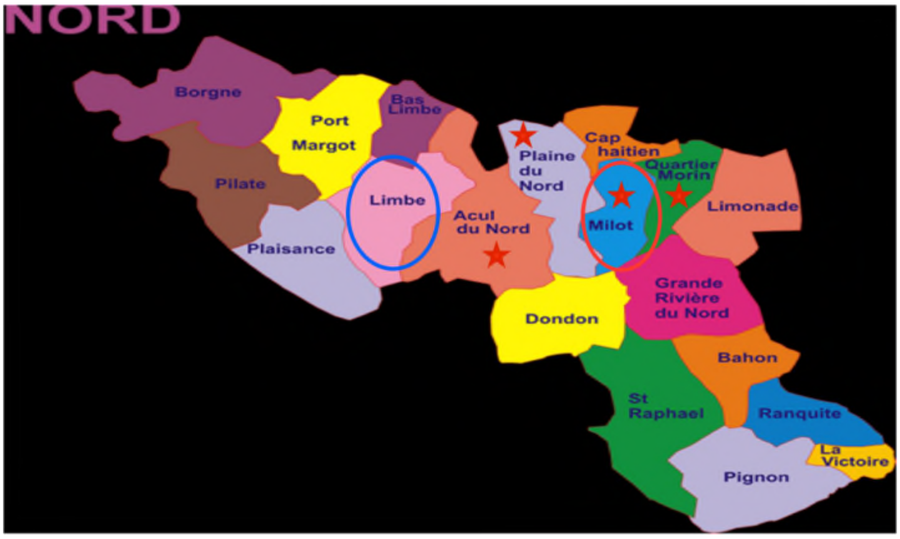
Chikungunya and Zika have recently emerged as important diseases in the Latin American and Caribbean (LAC) region, alongside dengue, all of which are transmitted by the *Aedes* mosquito. Zika virus (ZIKV) was first reported in early 2016 in Haiti, although virus isolation has since identified ZIKV in blood samples from December 2014 in Gressier, West Department (Lednicky et al. 2016).[1] Data available through the Pan American Health Organization show that ZIKV reporting has not been updated since the middle of 2016 for Haiti, reflecting the lack of data available on suspected and confirmed cases, cases of congenital Zika syndrome (CZS), and Guillain-Barré syndrome (GBS). Informal data obtained by the Zika AIRS Project (ZAP) Haiti shows that the *Ministere de la Sante Publique et de la Population* (MSPP) has found at least 10 cases of microcephaly in the Northern Department. Precise entomological data on the distribution of *Aedes aegypti* and *Aedes albopictus* are also significantly lacking. Predominate species include *Culex quinquefasciatus*, *Ae. albopictus*, and *Ae. aegypti.* A study from 2013 in the Northern Department, where ZAP Haiti activities are ongoing, found species composition between these species to be 3 percent, 27 percent, and 20 percent respectively.[2] The majority of the larvae in the study were collected in man-made habitats. The study also showed, through ecological modeling, that all three species will likely increase in the future due to rapid urbanization and lack of vector control (VC) activities

Since there is no vaccine available for Zika and other arboviruses, VC remains the most important tool for control and prevention. In Haiti, MSPP uses truck-mounted and handheld thermal fogging with malathion, and *Bacillus thuringiensis israelensis* (Bti) using backpack sprayers and hand application. However, applications are sporadic and inconsistent, and VC teams are under-staffed and under-capacity. In September 2016, Abt Associates received additional funds from the United States Agency for International Development (USAID) to expand the African Indoor Residual Spraying (AIRS) program to the LAC region for the purpose of strengthening the capacity of the MSPP to respond to Zika. ZAP Haiti, in close collaboration with MSPP, is currently implementing a VC program operating in Milot, Plaine-du-Nord, Acul du Nord, and Quartier Morin. There are ongoing discussions with MSPP and USAID for scale-up to other areas depending on the funding level (Figure 1). To measure the effect of the interventions, ZAP Haiti is implementing different strategies of entomological monitoring including ovitraps, larval surveys, demographic pupae surveys, and surveillance of adult mosquitoes. ZAP Haiti selected Milot and Limbe as the two communes for entomological monitoring. The latter was selected as a control site where ZAP is not operating at the VC level (Figure 1).

[1] Lednicky, J., De Rochars, V. M. B., El Badry, M., Loeb, J., Telisma, T., Chavannes, S., et al. Okech, B. (2016). Zika virus outbreak in Haiti in 2014: molecular and clinical data. *PLoS neglected tropical diseases*, *10*(4), e0004687.

[2] Samson, D. M., Archer, R. S., Alimi, T. O., Arheart, K. L., Impoinvil, D. E., Oscar, R., et al. Qualls, W. A. (2015). New baseline environmental assessment of mosquito ecology in northern Haiti during increased urbanization. *Journal of Vector Ecology*, *40*(1), 46-58.

Figure 1. VC and entomological monitoring sites selected by ZAP Haiti and MSPP in the Northern Department.



Note: VC intervention communes are indicated above with a "red star." The entomological monitoring sites, Milot and Limbe communes, are shown in red and blue circles.

In accordance to the work plan and in collaboration with the MSPP, ZAP Haiti has taken charge of, and implemented, the following tasks discussed in this report:

- Develop and review a complete entomological monitoring program for the selected sentinel sites
- Recruit and train staff for the technical entomological activities
- Coordinate with the MSPP to avoid redundancy of activities in the selected sentinel sites
- Identify and procure the entomological equipment and supplies necessary for the monitoring program
- Monitor the density of eggs on a weekly basis, with and without attractant infusion
- Perform larval surveys at least three times per month in the selected sentinel houses
- Perform demographic pupal surveys at least three times per month in the selected sentinel houses.

ZAP Haiti is now in the final stages of preparing to implement the following activities:

- Insecticide susceptibility assays with the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) methodologies using the same sites selected for entomological monitoring. The results of this particular activity will be presented in a separate report by August 2018.
- Monitoring the density of adult mosquitoes by three different catching methods: BG-Sentinel 2 traps, Gravid *Aedes* Traps (GAT) and Prokopack aspirators.

ZAP Haiti VC implementation began in the Milot Commune (intervention site selected for entomological monitoring) during the week of October 9, 2017; hence entomological data in this report does not yet overlap with Bti application in Milot commune and all data can be considered part of the ZAP Haiti baseline assessment. Hurricane Irma and the hiring process of seasonal workers are contributed to the delayed VC implementation in Milot commune.

2. ENTOMOLOGICAL MONITORING SITES AND METHODS

2.1 ENTOMOLOGICAL MONITORING SITES

The sites for entomological monitoring were selected in collaboration with the MSPP and the Haiti USAID mission, and the main selection criteria were: epidemiological relevance, social (population size) and geographical (vegetation, roads, town structure) similarities, and overall accessibility from Cap-Haitien. Limbe was selected as the control site, where ZAP Haiti is not performing any VC activities but the MSPP continues with their regular vector control program. This site is located 66 meters above sea level with a population of 70,000 (2003 census). Geographically, Limbe is composed of Limbe town and a few small urban centers divided between farmland. Average temperatures range from 27°C (August) to 23°C (January), with an average rainfall of 1,849 mm per year.

Milot commune was selected for monitoring the activities of VC. Milot is 105 meters above sea level with a population of 32,000 people (2003 census). Geographically, Milot is composed of Milot town and a few small urban centers divided between farmland. Average temperatures range from 26°C (August) to 22°C (January), with an average rainfall of 1,753 mm per year.

MSPP does not currently have an *Ae. aegypti* VC strategy in the Nord Department. VC teams are funded by the Global Fund and are largely concerned with malaria. Their work is irregular and no specific activities were reported to ZAP Haiti for Limbe or Milot during this report period of June 1, 2017 to September 30, 2017.

2.2 ENTOMOLOGICAL MONITORING METHODS

2.2.1 MOSQUITO EGG DENSITY BY OVITRAPS

Ovitraps are an efficient and cost-effective method for monitoring the dynamics of *Aedes spp*. mosquito density. Although there are several publications with variations on the details of the ovitraps, general guidelines do not exist.[3] The design of ZAP Haiti's ovitrap model consists of placing two black plastic containers, roughly 600 ml capacity, covered in the inner face by a paper oviposition surface and filled with 450 ml of either clean water or attractant solution. The rationale for using the attractant solution is that it can provide information even in areas with low mosquito density and can deliver more realistic data. Ovitraps were located in the intra or peri-domiciliary area of each house, according to the permission of the householder and the characteristics of the house.

[3] See: Velo, E., et al. (2016) Enhancement of Aedes albopictus collections by ovitrap and sticky adult trap. *Parasit Vectors* 9: 223; Carrieri, M., et al. (2017) Quality control and data validation procedure in large-scale quantitative monitoring of mosquito density: the case of Aedes albopictus in Emilia-Romagna region, Italy. *Pathog Glob Health* 111(2): 83-90; Manrique-Saide, P., et al. (2014) Multi-scale analysis of the associations among egg, larval and pupal surveys and the presence and abundance of adult female Aedes aegypti (Stegomyia aegypti) in the city of Merida, Mexico. *Med Vet Entomol* 28(3): 264-272.

The attractant infusion is prepared using one pound (454 grams) of acacia leaf in five gallons (18.9 L) of water, which is fermented for two days. This is equivalent to 10.8 grams of diluted acacia leaf per ovitrap (450 ml). The oviposition papers from the ovitraps are collected every five days after the ovitrap installation, and they are taken to the entomological laboratory where the eggs are counted by using dissection microscopes.

These activities are being performed in 100 houses in the commune of Limbe and 100 in Milot. Each house has two ovitraps; one with water and the other with attractant infusion. Ovitraps are set with a target distance of 200 m between them to avoid potential contamination and bias in the information: the fly range of *Ae. aegypti* mosquitoes is of typically no more than200 m,[4] so the possibility of the same mosquito laying eggs in two different houses with ovitraps must be minimized. The location and deployment dates of the ovitraps are detailed in Table 1.

Table 1. Location and installation date of ovitraps in the entomological monitoring sites of ZAP Haiti.

Sentinel site	Number of ovitraps located outside the house	Number of ovitraps located inside the house	Total number of ovitraps	Starting date
Limbe (Control)	22	77	99	June 12, 2017
Milot (Intervention)	41	59	100	May 31, 3017

2.2.2 LARVAL SURVEYS

Larval surveys are the gold standard for monitoring *Ae. aegypti* mosquitoes. These surveys provide information on the infestation indexes of the mosquitoes in particular populations. The strategy is based on the discrimination of the different types of habitats based on its productivity, guiding the health authorities to focus the larval control in particular types of breeding sites. It is important to note that the productivity measured with this surveys is based in presence/absence, and not in density.

The method consists of the thorough search of mosquito larvae within or around the houses, recording the presence of mosquito larvae in each of them. The information to be obtained in these surveys, better known as infestation indexes, can be summarized in three indexes: House Index (HI), Container Index (CI) and Breteau Index (BI).

In each of the entomological monitoring sites, 200 houses were selected for larval surveys, half of them also monitored with ovitraps, and half of them with only surveys and distributed in a paired fashion (a spatial distribution map using GPS coordinates is currently planned). The frequency of the surveys is three times per month, each lasting 10 days.

2.2.3 DEMOGRAPHIC PUPAE SURVEYS

Demographic pupae surveys measure the pupae productivity of containers in quantitative terms. Thus, the productivity index is much more informative only regarding presence/absence. Also, because larvae are susceptible to factors such as competence, food availability, predators, and xenobiotics, their life expectancy is lower; pupae do not feed and have a relatively short life stage (two to three days), so their presence can be considered as an indicator of adult mosquito production. Additionally, ingestible larvicides such as Bti can act slowly on larvae as they dilute, so larval presence/absence is a poor

[4] Russell, R. C., et al. (2005) Mark-release-recapture study to measure dispersal of the mosquito Aedes aegypti in Cairns, Queensland, Australia. *Med Vet Entomol* 19(4): 451-457.

indicator of Bti performance, while pupae presence is a good one. Although governments in the region understand these advantages, this activity is not performed regularly in national monitoring programs due to challenges at logistical and technical levels.

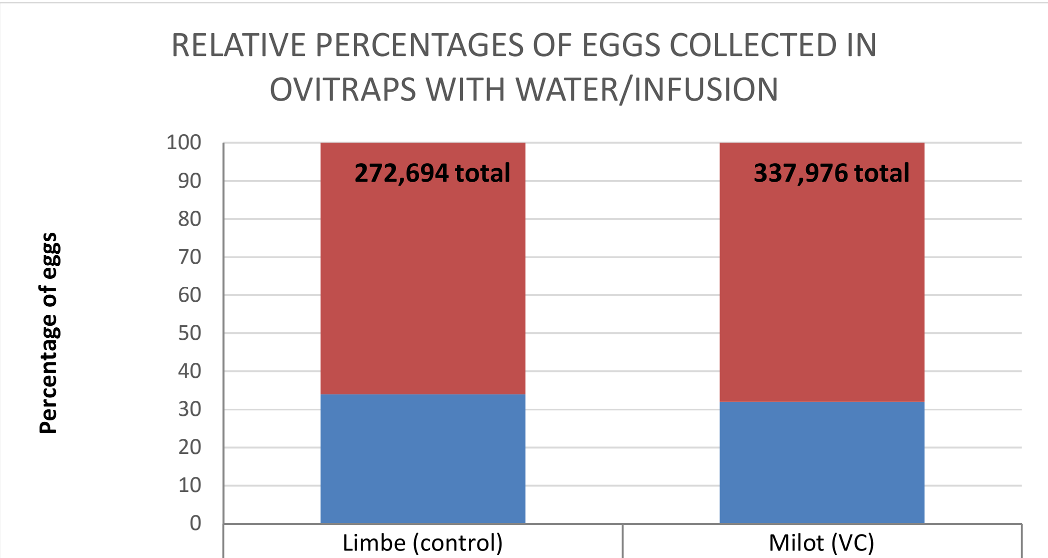
Demographic pupae surveys are conducted in a similar manner as larval surveys; they include counting or estimating the individual pupae count. As such, water deposits are searched for pupae, counted (based on total pupal count) and transferred to the entomological laboratory to be raised to the adult stage, and classified taxonomically. Demographic pupal surveys are being performed in the same frequency and location as the larval surveys (a spatial distribution map using GPS coordinates is currently planned).

2.3 Results

2.3.1 Egg density

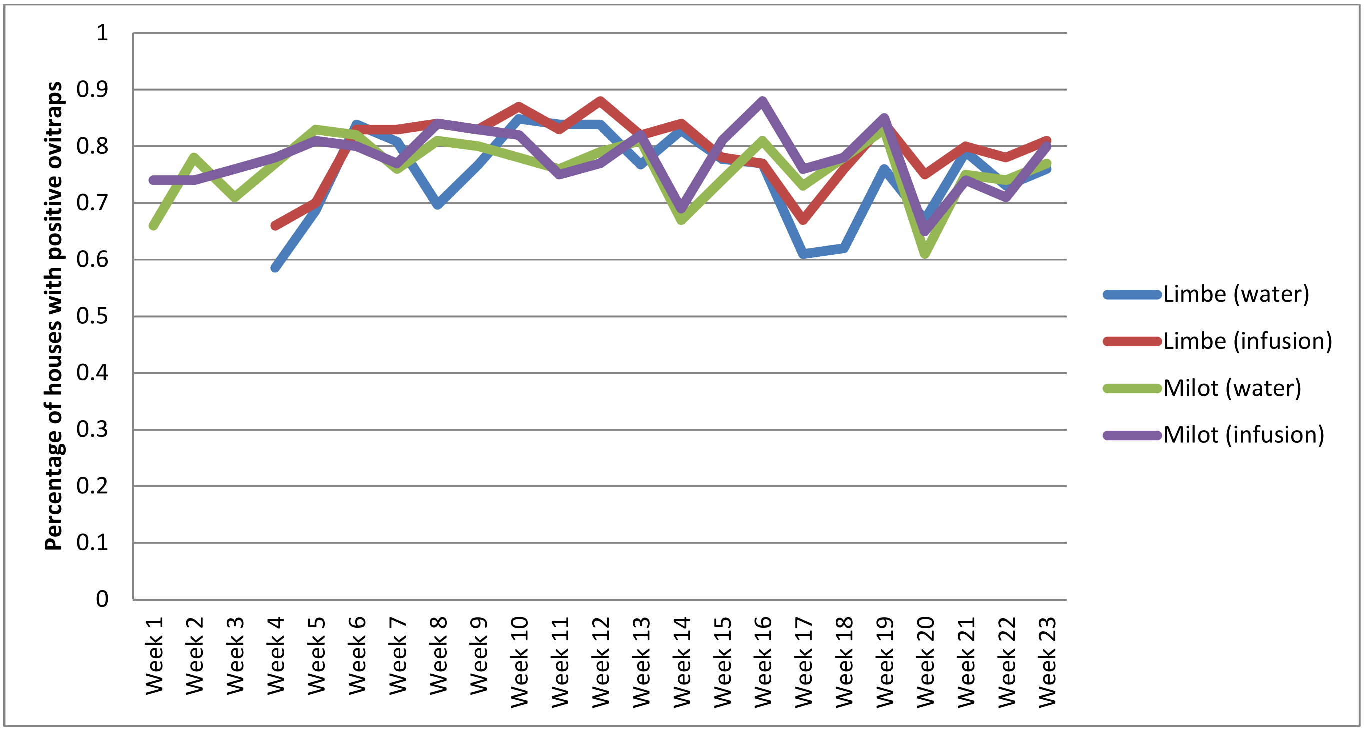
The results presented in this report were obtained between May 31 and September 30, 2017. A total number of 610,670 eggs from *Ae. aegypti* and *Ae. albopictus* were collected and counted, and from these 33 percent of eggs were collected in ovitraps with clean water and 67 percent in ovitraps with attractant infusion. The entomology program began collecting ovitrap data in Milot three weeks prior to beginning activities in Limbe; hence data for Milot represents 23 weeks of collections, whereas we have data from Limbe for 20 weeks of collections. This explains the difference in eggs collected, shown in Figure 2 separated by water-only and infusion ovitraps. As shown, the ovitraps with attractant infusion were more efficient at collecting eggs than the ovitraps with only water. Ovitrap indexes, calculated as the percentage of positive ovitraps, are shown in Figure 3 below.

Figure 2. Relative percentages of eggs collected in ovitraps with and without attractant infusion in the two current entomological monitoring communes in Haiti. The numbers over each bar represent the total sample size for each site.



	Limbe (control)	Milot (VC)
Total infusión	66.0	68.0
Total water	34.0	32.0

Figure 3. Ovitrap indexes per week, discriminating between ovitraps with and without attractant infusion, for the two sites of entomological monitoring in Haiti.



For the sake of our analysis, the added number of ovitraps with and without attractant infusion will be considered as the total eggs/ovitrap/house. Please note that Bti application had not yet begun in Milot commune during this period (VC activities started the week of October 9, 2017). The average number of eggs per house and per house per day, as well as the Ovitrap index, are shown in Figures 4, 5, and 6 respectively.

Figure 4. Egg density in the entomological monitoring sites per week (each 5 days), between May 31 and September 30, 2017. Average eggs per house adds the eggs counted in the two ovitraps in the house, with and without infusion. Bti application did *not* yet start in the intervention site (Milot commune) at this time.

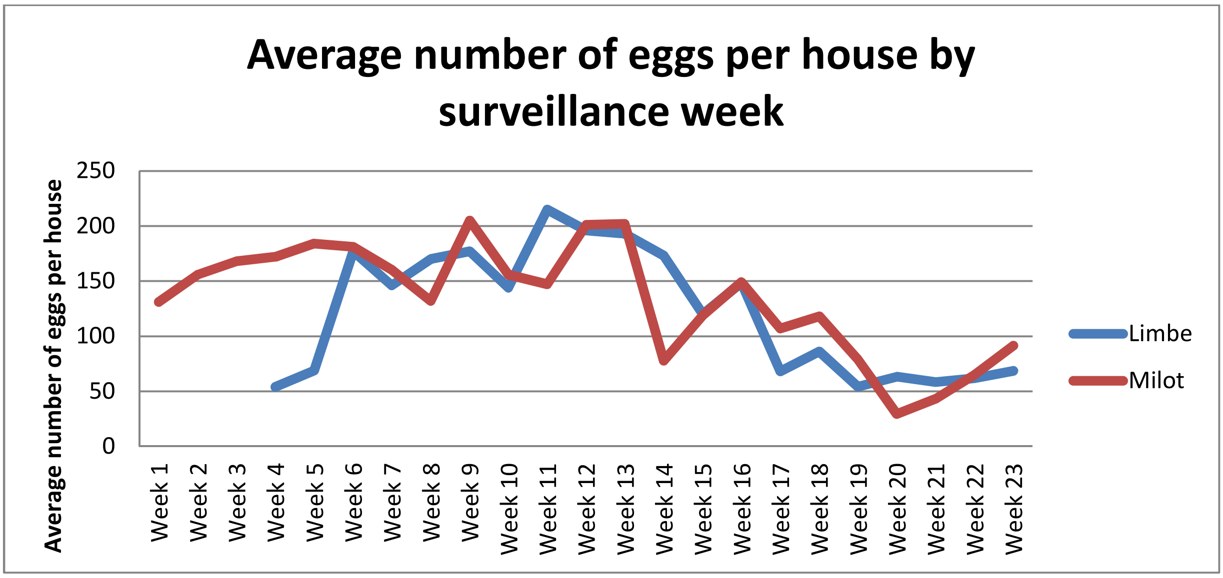


Figure 5. Average number of eggs per house per day in the entomological monitoring sites between May 31 and September 30, 2017.

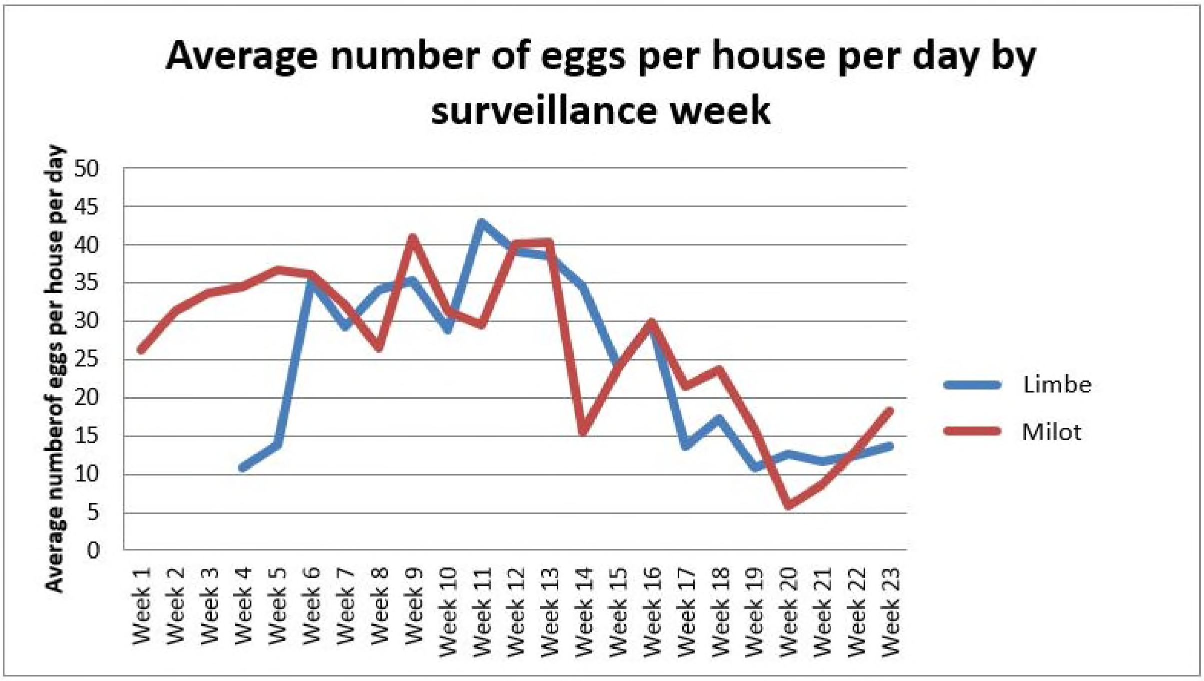
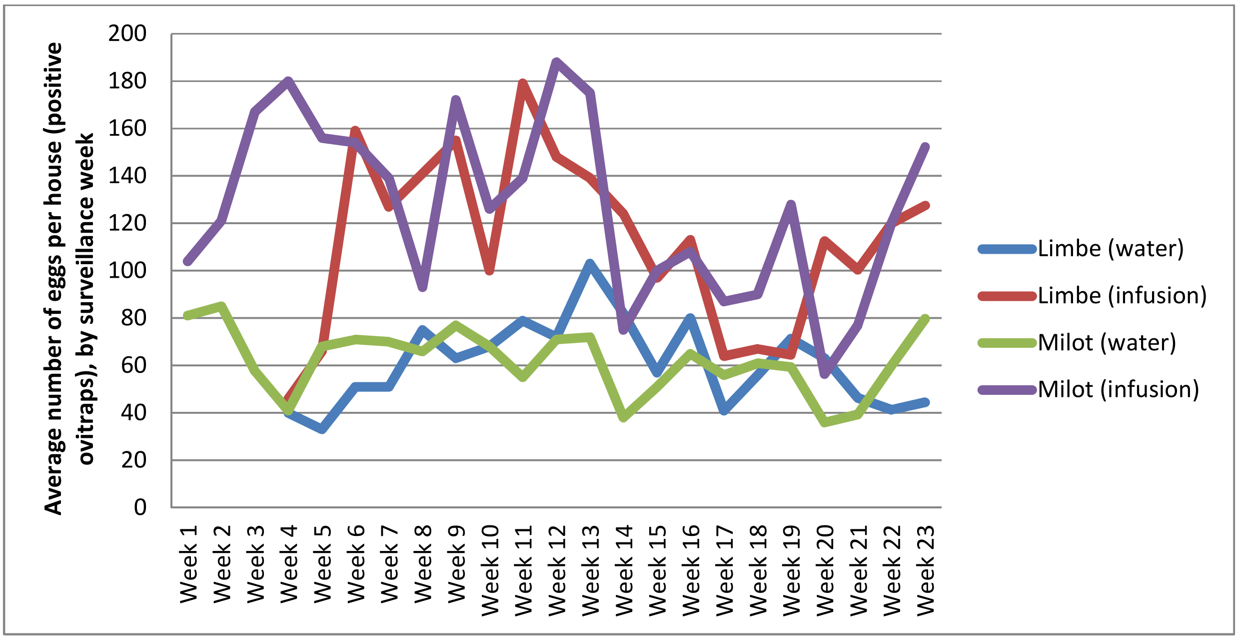


Figure 6. The Ovitrap index: number of eggs per house with only positive ovitraps, by surveillance week (May 31 to September 30, 2017) in Milot (control) and Limbe (intervention) communes.



2.3.2 LARVAL INDEXES

The results presented in this report were collected between June 1 and September 30, 2017. The total number of potential habitats searched was 14,332 from which 1,355 (9.5%) were found positive for larvae. In general, the most common positive type of habitat were ZAP Haiti ovitraps (37%) and small/medium sized plastic containers (27% of all positive habitats) (Figure 7). The Aedic indexes for each sentinel site are shown in Table 2.

Figure 7. Relative percentages of larval presence in different types of water containers in Milot and Limbe communes, ZAP Haiti's two current entomological monitoring sites

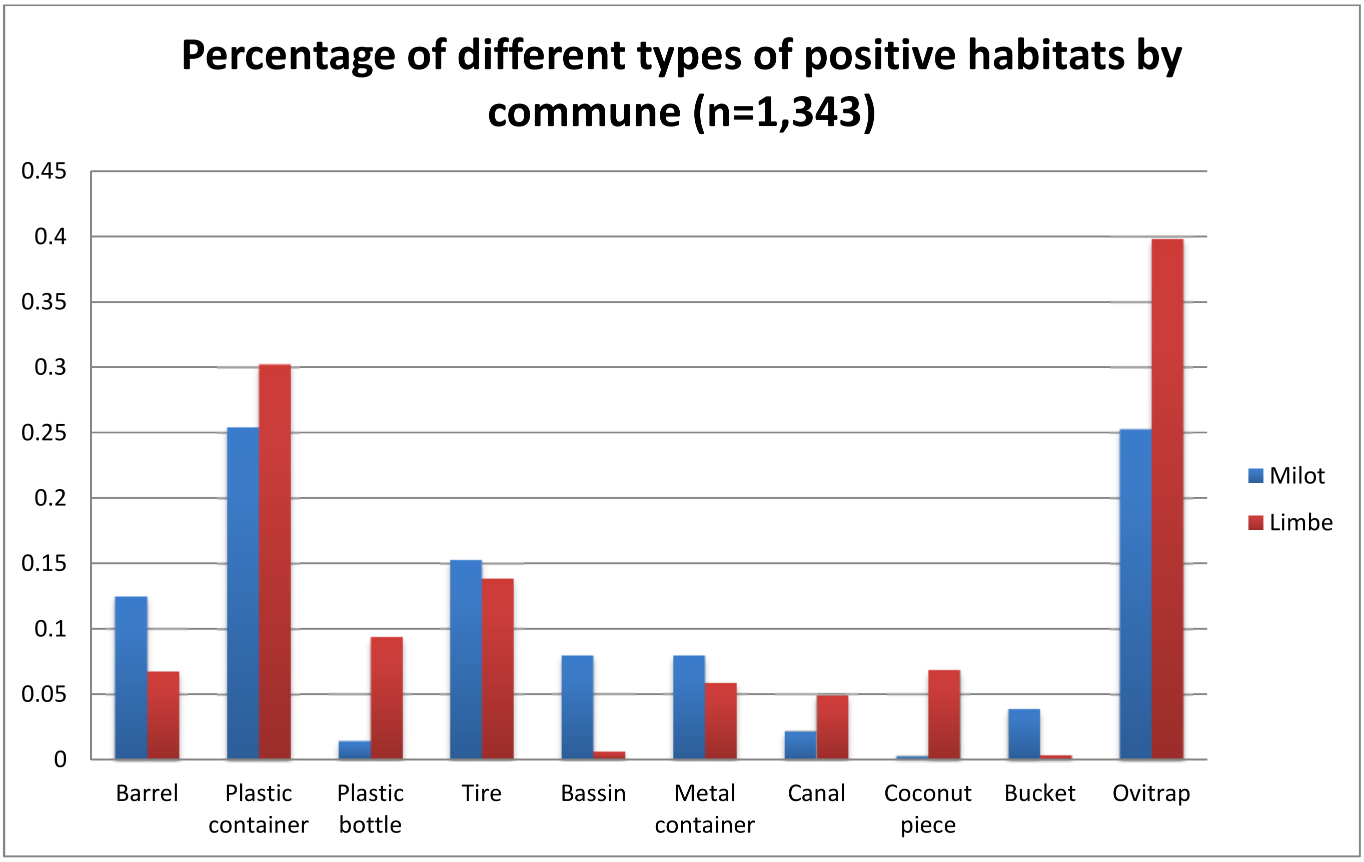


Table 2. Aedic indexes in the two entomological monitoring sites between June 1 and August 31, 2017. HI: House Index; CI: container Index; BI: Breteau Index.

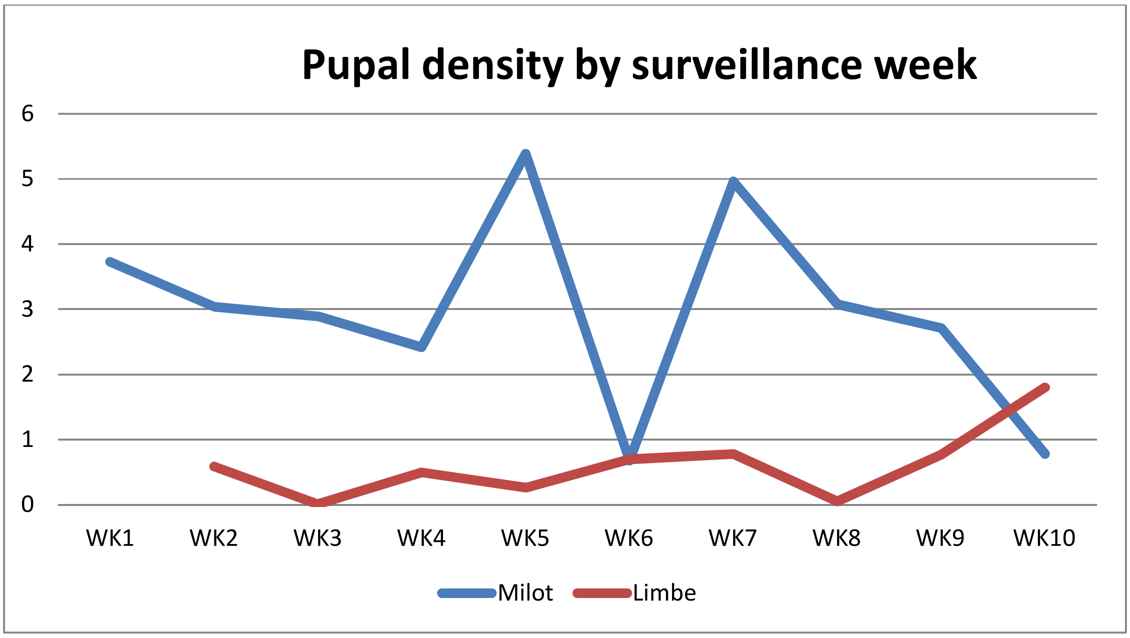
Site	Index	Week 1	Week 2	Week 3	Week 4	Week 5
Limbe (Control site)	HI	-	17%	12%	15%	2.5%
	CI	-	12%	9.5%	18%	2%
	BI	-	34	25	26	3.5
Milot (Intervention site)	HI	18%	14%	14%	12%	12%
	CI	13%	9%	12%	9%	11%
	BI	33.5	23	29	21.5	29

Site	Index	Week 6	Week 7	Week 8	Week 9	Week 10
Limbe (Control site)	HI	22%	43%	24%	14%	34%
	CI	21%	13%	9%	4%	9%
	BI	41.5	82.5	43.5	19.5	53
Milot (Intervention site)	HI	10%	46%	19%	22%	32%
	CI	6%	13%	7%	6%	8%
	BI	13	72	30	40	53

2.3.3 PUPAE SURVEYS

The demographic pupae surveys aim to correlate the density of pupae with the number of searched houses. In the Limbe site, the highest pupae density was found in Week 7 (18-19 August, 2017), and the lowest in Week 3 (27-28 June, 2017). The indexes describe these apparent trends and dynamics in both Milot and Limbe (Figure 8).

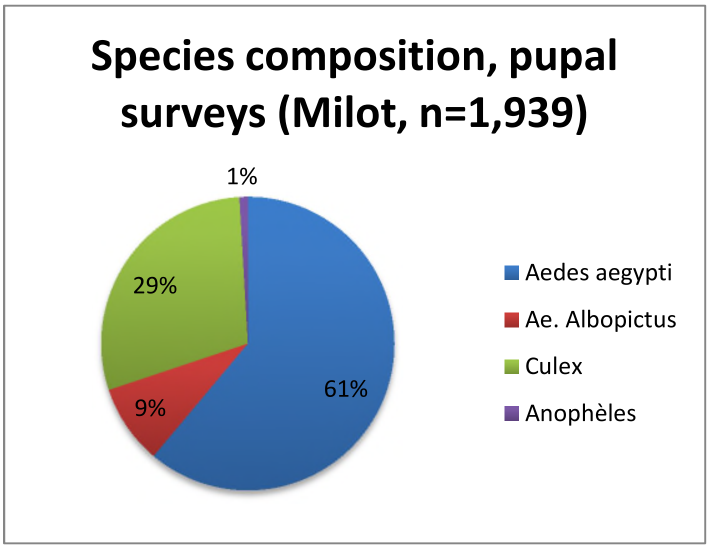
Figure 8. Number of pupae per house per week in the entomological monitoring sites in Haiti.

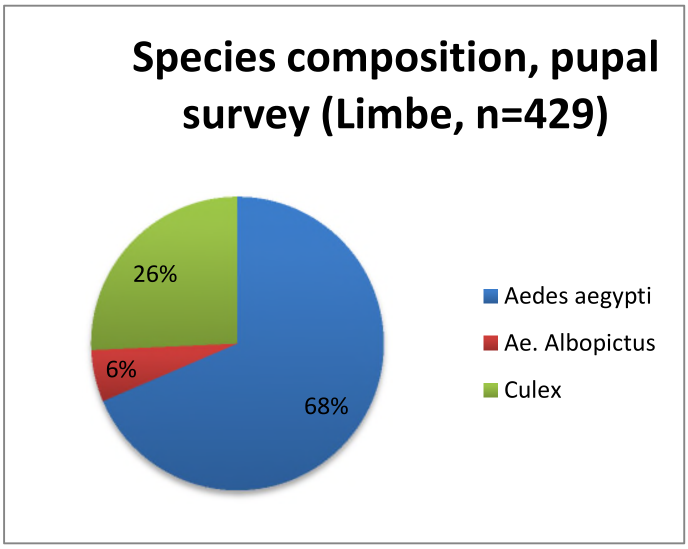


2.3.4 ADULT MOSQUITO IDENTIFICATION FROM COLLECTED PUPAE

The ZAP Haiti team has not yet begun raising adult mosquitoes from eggs collected in oviposition papers; rather, mosquitoes have been raised from pupae collected in the field. As shown in Figure 9, the predominate mosquito species identified included *Aedes aegypti*, followed by *Culex* mosquitoes and *Aedes albopictus*.

Figure 9. Species composition of the eggs collected with the ovitraps in Limbe (control) and Milot (intervention) communes between June 1 and September 30, 2017. Sample size for Milot is n=1,939 (82%); sample size for Limbe is n= 429 (18%).





3. Discussion and Conclusion

As shown above, the larval and pupae survey data show important differences between Limbe and Milot communes. These are most likely due to differences in access to drinking water and water storage practices. Limbe has greater access to water infrastructure (i.e., boreholes), and households tend to not store rainwater and refill storage containers frequently. In contrast, Milot has many hand-dug open wells, and households tend to store rainwater and refill storage containers less frequently. ZAP Haiti is planning further investigations to confirm these observations.

Lastly, the entomology team is in the final stages of planning the implementation of BG-sentinel traps and prokopack aspirators in 20 households in Milot and Limbe. We are also in the final stages of establishing a temporary insectarium, ready by the end of October, to raise adult mosquitoes for insecticide resistance testing.