Short-Term Scientific Mission Grant – REPORT

Action number: CA17108.

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A novel tool for the reduction of the dengue vector *Aedes aegypti* based on intelligent monitoring

The objective of the STSM was to understand and follow the entire process of the project "M.I. Aedes", a project that has been tested in several regions of Brazil with great success to reduce the burden of *Aedes*-vector borne diseases. I assisted all the phases that make up the "M.I. *Aedes*" project in the city of Araraquara (Sao Paulo, Brazil). In the following pages I have prepared a summary diagram of the action plans with a brief explanation of the details. I also provide in the last page a personal opinion of the project and compare and discuss it with the current scenario in Europe.

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City of Araraquara (SP)
240.000 inhabitants

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Recruitment of ca. 920 houses grouped in 7 districts

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Team of 7 operators visit the 920 houses (ca. 130 house/person/week)

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Operators visit the households using electric bikes

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Surveillance of mosquito *Aedes* traps

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Collection of females (*Aedes albopictus* and *Aedes aegypti*) from sticky traps

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The information is typed in the M.I. *Aedes* application

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Vials with *Aedes* females are revised

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Vials are sent by post to the Molecular Laboratory Analysis Center

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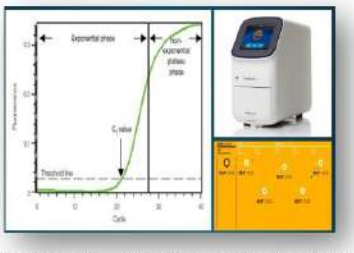

Aedes pools are processed within 48 h and analysed by RT-PCR to Dengue, Zika, Yellow fever, Chiqungunya and Mayaro viruses

11

Positive arbovirus detection in mosquitoes is rapidly notified

Suspected or positive cases of dengue (mainly) in humans are also rapidly notified

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Health authorities define the specific areas to implement CONTROL ACTIONS

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Several control actions are executed by local sanitary teams in households

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A service for removal useless stuff is also provided by public local authorities

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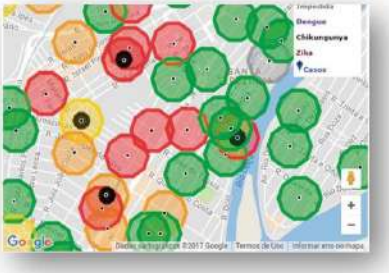


Informative ans risk maps are done in order to facilitate the execution of the tasks and interpretation of the process

In pararel, the public health authorities also perform an activity of awarennes during the entire year.

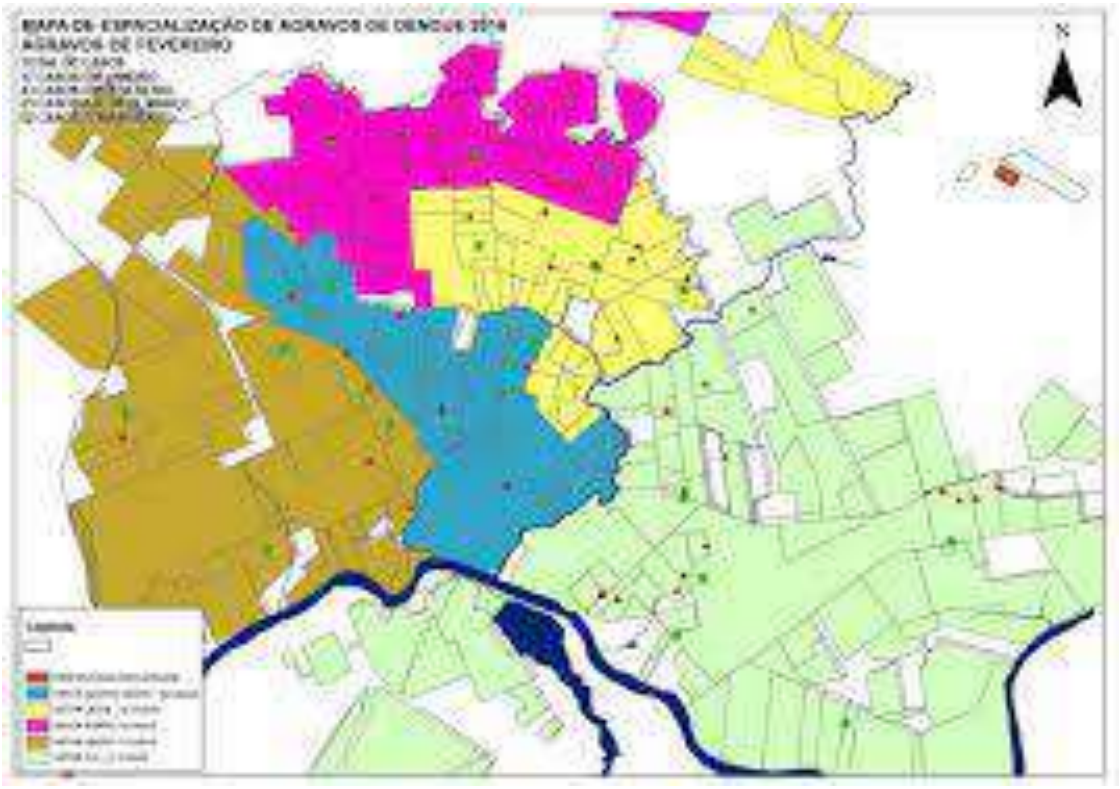
PHASE 1: SELECTION OF THE STUDY AREA

Araraquara is a medium city size located in southeast Brazil in the middle of Sao Paulo State. The population is 238,339 (2020 est.) in an area of 1,004 km^2 (388 sq mi) and 664 meters above sea level. It is also known as "the abode of the sun," because of its impressive sunset and because of its hot atmosphere, especially in summer. The city to carried out the project was selected on the basis of the reasonable size and high number of cases of dengue. The ECOVEC-company (Rentokil group) was assigned to carried out this project-entomological campaign.

Image of the city of Araraquara, SP-Brazil.

In red is denoted the mesoregion of Araraquara, SP-Brazil.

PHASE 2: DISTRICTS AND RECRUITMENT OF PARTICIPANTS



The city is divided in seven areas grouped in similar number of neighborhoods (households). A random door to door recruitment strategy was select to recruit suitable householders by local health authorities to participate in the project. A household is selected every ca. 200 m^2 given a total of ca. 131 households by every district, which result in the recruitment of a total of 920 households. Some particular criteria was followed to the inclusion of the participants in the project. Geographical data, full name, address, and telephone number of the householders along with other information was noted and incorporated to the databases.

PHASE 3 & 4: SURVEILLANCE OPERATORS AND WORK PLANNING

The city of Araraquara is visited on a weekly basis (from week 1 to week 52) by a team composed of 7 surveillance/monitoring operators of the company ECOVEC (Rentokil group). Each operator equipped with yellow uniforms and badges, an electric bike and a mobile phone (using google maps) visits the recruited houses (approximately 30 houses every day/operator). In case, nobody is available to allow the entrance in the selected house, the operator should try at least three attempts. In the last instance, the operator should call the householder. The house will remain unvisited only if the previous actions resulted unsuccess.

Each operator follows a daily route and use the goggle maps application to find the houses easily.

Detail of some members of the Ecovec team prior to start the daily work

Selected houses are noted in google maps and operators follow the better route to complete the visit of the households.

PHASE 5.1: COLLECTION OF *AEDES* MOSQUITOES


Aedes-mosquito traps installed in the back of the households

After getting the permission of the householders, each operator spots the mosquito trap and open it to check the following variables:

I. Check the identification number of the house (barcode).
II. Note the water level: empty, medium, full
III. Presence of larvae in the water (YES/NO)
IV. Attractant is ok? (YES/NO)
V. Sticky trap is in good condition? (YES/NO)
VI. Are there water-holding containers for breeding? (YES/NO)
VII. Presence of eggs? (YES/NO)
VIII. Presence of mosquitoes (YES(NO): *Culex* = number of male and female, *Aedes albopictus* = number of male and females, *Aedes aegypti* = number of male and females

The trap is cleaned internally and externally, the water is replaced, glued or individual eggs and larvae/pupae are also removed by a brush. Please note that dirty water should not emptied in sinks or scuppers. Water should be emptied on the floor or ground or grass.

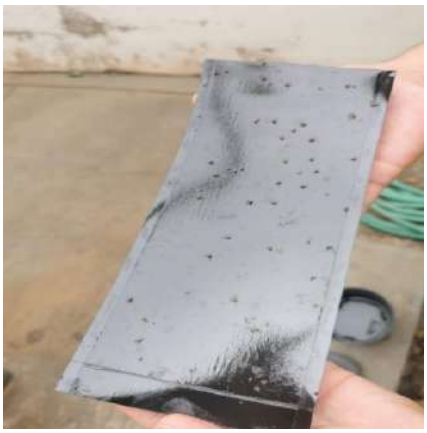



Image of a brush cleaning the inner walls of the trap and a sticky card with glued insects

PHASE 5.2: TRAP OF MOSQUITOES

Detail of the different parts of the *Aedes*-mosquito trap

MosquiTRAP consists of a matte black container (33 cm high by 15 cm wide), divided into two parts: a lower base, filled with approximately 300 ml of tap water, and an upper part with a funnel shaped opening, facilitating the mosquito's entry and hindering its exit. An odorless sticky card (black colour) that holds the captured mosquitoes is attached from the water line in the lower base to the upper part of the trap.

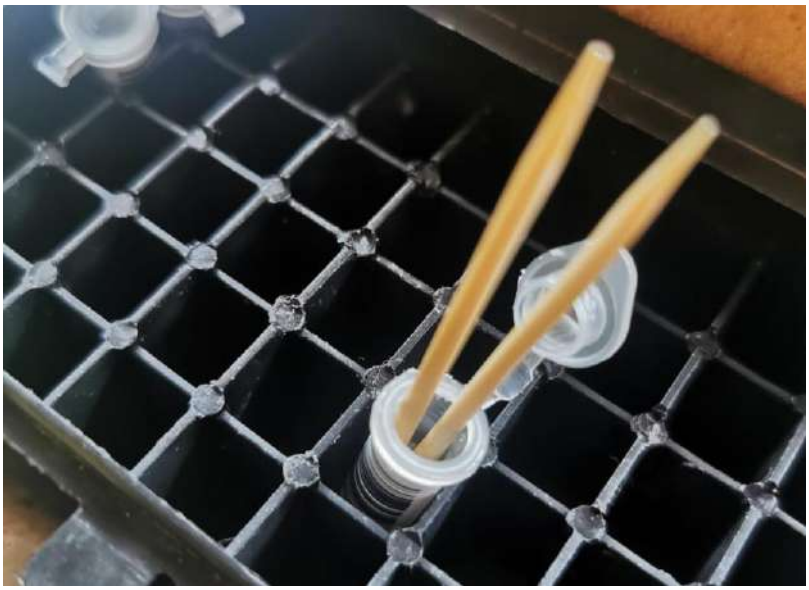
The device that releases the synthetic oviposition attractant (AtrAedes) was attached to the sticky card.

The traps were attached in a visible place, at a maximum height of 1.5m above ground, sheltered from sun and rain, out of reach of domestic animals and children.

PHASE 6: COLLECTION OF *AEDES* MOSQUITOES

Glued *Aedes* mosquitoes were carefully removed from the sticky cards and individually introduced into small vials (labelled with barcodes) with a preserving liquid. Disposable wooden sticks were used to remove the female mosquitoes. Other insects were discarded. In case the sticky card was very dirty, it was replaced by a new one. Overall, sticky cards are replaced every three months.

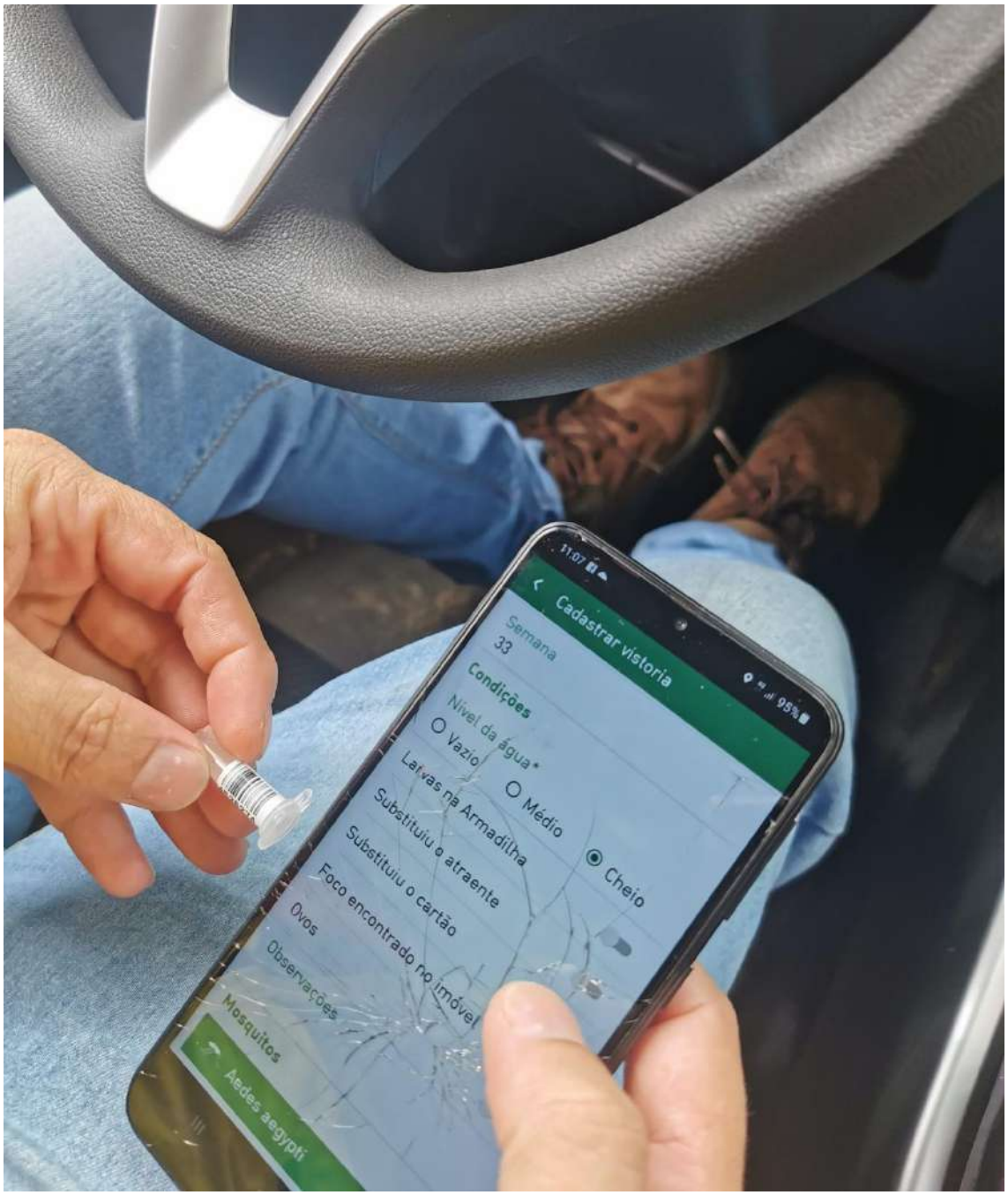
Removal of *Aedes* females with cleaned forceps or with wooden sticks



Insertion of *Aedes* mosquitoes in vials with preserving liquid

PHASE 7: TYPING DATA IN THE PHONE APPLICATION (MI-DENGUE)

The operator types the information gathered in a specific phone application called “M.I. Aedes”. The data are sent to a web server for further analysis.



PHASE 8: DOUBLE-CHECK THE CONTENT OF VIALS WITH MOSQUITOES

Every Sunday, all the vials collected by the operators along the week are gathered and stored. Barcode, number of mosquitoes and species identification is double-checked prior to shipping in order to avoid mistakes.

Image of vials containing mosquito females

Image of the shipping cages

PHASE 8: SHIPPING THE VIALS FOR MOLECULAR ANALYSIS

Every Monday, vials of the previous week are sent by post to the Laboratory of Molecular Analysis located in Minas Gerais State.

PHASE 10: MOLECULAR ANALYSIS

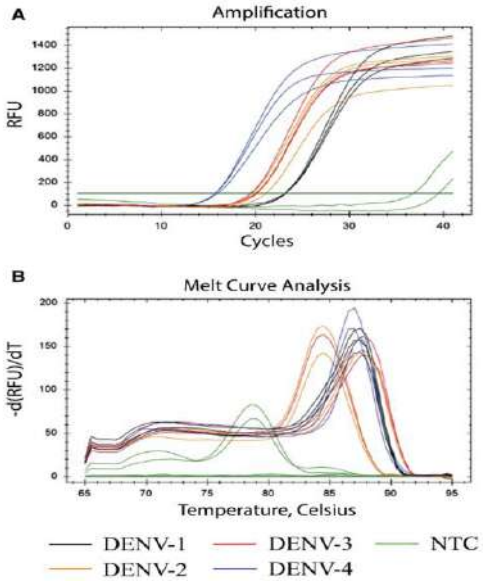
The trapped *Ae. aegypti* and *Ae. albopictus* female mosquitoes are placed in Eppendorf tubes with guanidine and analyzed by reverse transcriptase RT-PCR. In Brazil, the MI-Virus platform was tested in hundreds of municipalities to detect and map not only *Ae. aegypti* abundance but also the presence of mosquito populations infected with different arboviruses such as DENV, CHIKV, and ZIKV.

PHASE 11: POSITIVE CASES IN MOSQUITOES AND HUMANS

This is one of the most critical and important stages of the whole process. Hospitals and outpatient clinics are obliged to report dengue cases (chikungunya cases are rare, since the predominant disease is caused by two dengue serotypes) to the health authorities.

As a general rule, the accredited doctors will diagnose confirmed cases of dengue by serology and clinical symptoms. In case of a dengue epidemic, in order to reduce costs, diagnosis will be made only by serology. Both suspected and confirmed cases of dengue are reported.

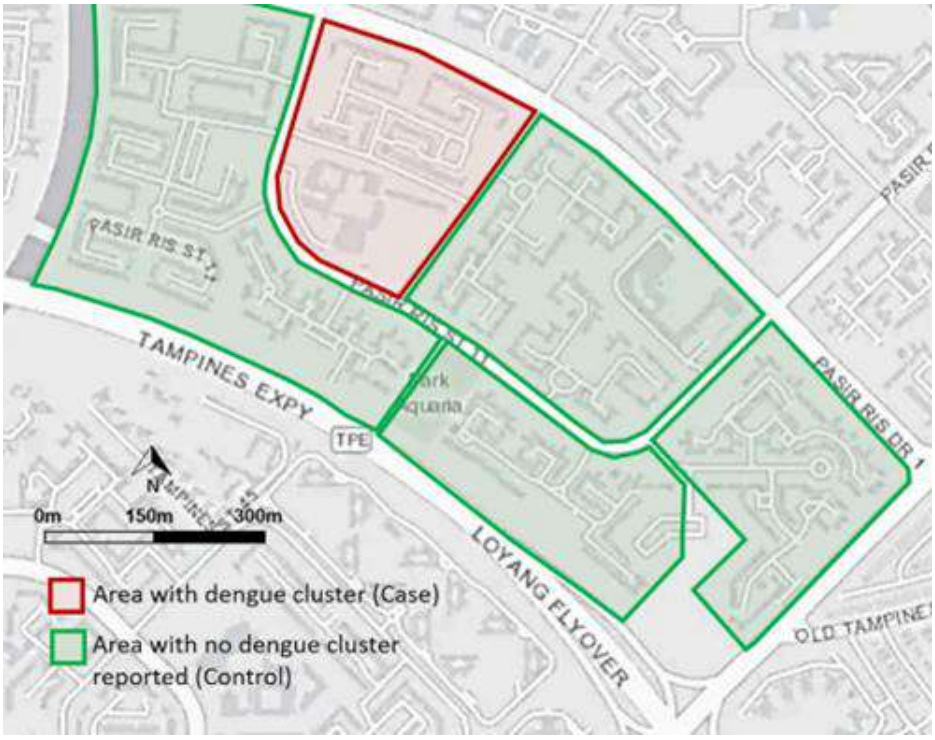
At the same time, within the MI *Aedes* project, *Aedes aegypti* pools positive for dengue and other arboviruses are reported. The Molecular Biology laboratory in Minas Gerais notifies the different health authorities the results by e-mail on a weekly basis.



PHASE 12: DEFINING CONTROL ACTIONS

With the information received from the cases of dengue in humans and positive *Aedes* females, the health authorities of the entomological surveillance of the municipality of Araraquara will be in charge of defining and organizing the points where the control tasks will be carried out. The health agent will organize a team to visit to the positive points.

The area to implement control actions will be established, depending on multiple reasons, as a general rule about 250 meters around, or in other words, encompassing a total of 8 quartierons.



PHASE 13: CONTROL ACTIONS

Once the area is defined, the control operators have the challenge to try to enter in all the households within the defined area (encompassing approximately 300 metros around the positive case or encompassing 8 houses around). They consider a success if they are able to carried out control actions in at east 85% of the households. Unfortunately, many times the householder recused to allow control operators to enter or simply deny the entrance. This point is one of the challenges to be improved as the extension of the applications measured do not reach the enough level to consider an action successful. The reasons to recuse the entrance are variable (old people, illness people, ignorance, fear of being robber etc.). The operators should achieve this aim before 7 days.

Control actions include the following actions:

- Removal or cleaning of larval sites
- Removal of artificial containers and rubbish that accumulate rainwater
- Awareness/sensibilization of the householders (all family members)
- Application of the adulticide called Cielo-Ultra-Low-Volume formulation composed of imidacloprid (30 g/kg; 3% p/p) + praletrin (7,5 g/kg; 0,75% p/p) + 96,25%. This chemical is provided by the council team. The application is focused primarily on the entrance of the house and in the backyards. Householders are encouraging to wait at least 30 min before visited the treated areas.
- In case of extreme emergency (epidemic peak of dengue) vehicle-mounted applications are carried out along the public streets.

An operator applying termo-fogging in the back of a house

PHASE 14: RUBBISH ELIMINATION

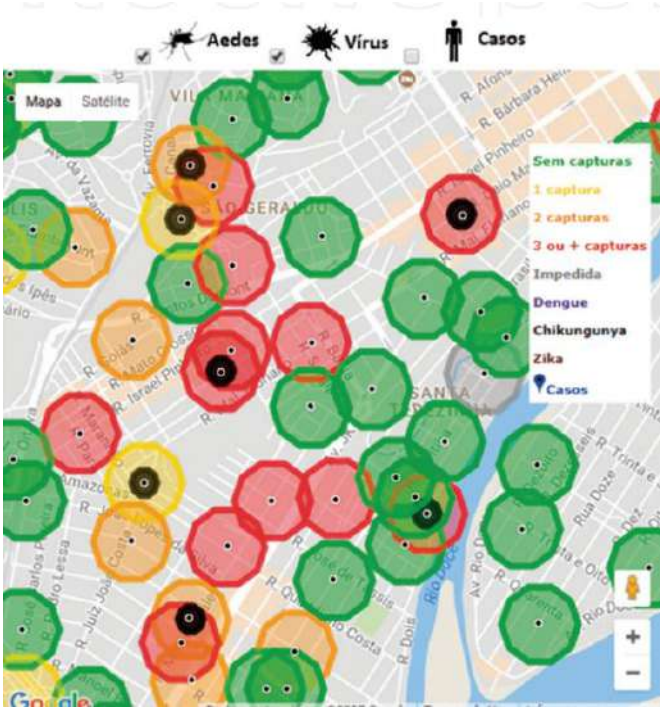
The public health authorities provide a new service to the citizenships. Since last year, householders have the opportunity to remove the useless stuff. This enterprise idea aimed to reduce the resting and breeding sites of *Aedes* mosquitoes. This service operates every two or three months.

Operators removing rubbish, useless stuff, and rubble from houses

PHASE 15: LONG TERM FOLLOW-UP AND MAPPING

By georeferencing the sampling sites, data obtained during surveillance is used to generate maps that show the areas of high and low infestation. This information provides real-time data and allows spatial analyses to determine vector control actions and to evaluate their impact on mosquito. The continuous surveillance of Aedes population allied with mathematical modeling strategies allows reliable predictions of infestation.

The georeferenced maps presented weekly by the MI-Aedes platform allowed health managers to identify the infestation status of city blocks by the colors green, yellow, orange, and red, according to the number of adult *Ae. aegypti* females captured. The weekly data evaluating vector infestation levels became an important information for dengue control programs because it helped public health managers to optimize *Ae. aegypti* control activities with improved precision of the target activities to the infested blocks. A weekly vector control indicator established by the entomological "mean female Aedes index "(MFAI) was used to compare among the different areas. Further research showed how the health authorities used the platform to evaluate the performance of the control measures employed by them within the area covered by the MI-Aedes.



Example of the map data obtained by the MI-Aedes platform and MI-Virus Dark dots at colored circles mean MosquiTRAP (GIS) position. Colored circles mean infestation status of mosquito abundance. Black circles mean infected *Aedes aegypti* by dengue virus captured by MosquiTRAP. Source: Ecovec company.

ALONG THE YEAR: AWARENESS AND PREVENTIVE PRACTICES

The health authorities provide a public service along the year in the entire region of Araraquara in order to provide knowledge, awareness and preventive practices for *Aedes*-arboviruses transmission. The team composed of at 45 health agents visit all the houses of Araraquara at least 3-4 times per year in order to aware the householders about good practices, evaluate the state of the members of the family, removal of larval sites, use of repellents, etc.

Agents of health authorities doing “door to door” practices

Agents of health authorities removing potential larval sites of *Aedes* mosquitoes

FINAL CONSIDERATIONS

It has been a great pleasure to have the opportunity to follow the operation of the *MI. Aedes* work-project in the city of Araraquara, Sao Paulo, Brazil. I have observed some facts that have caught my attention and I want to highlight them because they are very different when compared to my experiences in Europe. The following statements are my final considerations:

1. It is evident that, the tropical climate of Brazil allows *Aedes* mosquitoes to remain active all year round, maintaining continuous breeding cycles, while in Europe, their **activity is very limited during winter season** or even non-existent in the coldest parts of the continent. It is also true that global warming is changing this scenario in Europe, but we still cannot consider southern Europe as anything but subtropical, being clearly different from the situation in Brazil.

2. *Aedes albopictus* control in Europe focuses mainly on the larval control, by the application of biological larvicides (bti) on scuppers in **public urban areas.** It is believed that most of the larval sites of the Asian mosquito tiger are located in public urban areas and not in private areas. This contrasts with the situation in Brazil, where *Aedes aegypti* larval sites mainly occur inside households. For this reason, **it is much easier to control *Aedes albopictus* in Europe as we do not need to enter in houses** and is only in very rare occasions it is necessary to perform control actions in adult stages.

3. It is also evident that **cultural practices differ markedly between the two countries**. In Brazil, a moderate percentage of householders tend to accumulate disposable/useless stuff in their properties (entrance or back of the houses) which is infrequent in Europe (with exceptions). Obviously, this situation increases the available of resting and larval sites for *Aedes* mosquitoes.

4. The Brazilian health system should **seek new ways to reduce the time response to conduct control actions** (response time up to 7 days) in contrast to Europe, where in case of dengue cases they are able to provide a control action response in less time (24-48 h). I am perfectly aware that this issue can only be improved with better policies and more personnel, which depends on the available budget. I don't think Brazil is doing a bad job in this area **but my personal experience in Spain and Brazil is that specialized private pest control companies are better prepared to provide a response in case of outbreaks/arbovirus peaks/emergencies so the privatizing of this task might me a solution.**

5. The current epidemiological status of *Aedes*-arbovirus transmission between Europe and Brazil is completely different, as in Europe dengue/chikungunya/zika **outbreaks solely occurs occasionally during summer season** which makes much easier and cheaper to carry out any control and program control actions.

   I have many reasons to think that the situation and scenarios in Europe and Latin America are completely different and thus, different responses and control actions are required.