Pan American Health Organization (PAHO)

Department of Communicable Diseases & Health Analysis
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Technical Note on Transgenic Mosquitoes Engineered for *Aedes aegypti* Control. August 2014.

Background

The most effective measure currently in use to curb the transmission of dengue is suppressing the population of its main vector, *Aedes aegypti*, which to date is the only vector of the disease in the Americas. Nevertheless, the dengue virus transmission mechanism is complex and involves other factors, such as human and vector population density, the immunological profile of the human population, the circulating dengue serotypes, climate and environmental conditions, and the presence or absence of other potential vectors such as *Aedes albopictus*. It is therefore essential that strategies to control this disease address all of these factors.[1]

Thus, the Pan American Health Organization/World Health Organization (PAHO/WHO) and its Member States have been making a concerted effort since 2003 to implement the Integrated Management Strategy for Dengue Prevention and Control (IMS-Dengue) by producing a technical report, one of whose chapters deals with integrated vector management. The purpose of this component of the strategy is to create controlled *Ae. aegypti* breeding sites and reduce[2] vector population density.

In light of the above, the need to develop new tools for controlling the dengue vector has become a key element in current regional strategies. For some years, Oxford Insect Technology (Oxitec) has been engineering a genetically modified Rockefeller strain of *Ae. aegypti*, OX513A, as one more potential tool for combatting the vector. This strain has a transgene that promotes *Ae. aegypti* mortality in the larval stage, where the promoters responsible for the transgene's expression are found. The strain has a genetic marker expressed by a fluorescent protein and a conditional lethality system. This fluorescence makes it possible to identify the larvae and adult males in the laboratory. The conditional lethality system is mediated by the presence or absence of tetracycline. In the absence of this antibiotic, a protein lethal to these insects is produced, so that when males with that gene are released and fertilize wild females, the *Ae. aegypti* population declines, since their progeny die in the larval[3] stage.

Current Situation

This technology is still in the experimental stage, and laboratory tests have been conducted comparing the wild and transgenic strains under various biological parameters. Working with Oxitec, scientists have conducted field studies on the release of genetically engineered mosquitoes in the Cayman Islands (2009), Malaysia (2010), and Brazil (2011).[4,5,6]

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1 World Health Organization (WHO). (2012). Global strategy for dengue prevention and control (WHO/HTM/NTD/VEM/2012.5)

2 San Martin JL & Brathwaite-Dick O. (2007) La Estrategia de Gestión Integrada para la Prevención y el Control del Dengue en la Región de las Américas. Rev Panam Salud Publica, vol.21, n.1: 55-63

3 Phuc HK, Andreasen MH, Burton RS, Vass C, Epton MJ, et al. (2007) Late acting dominant lethal genetic systems and mosquito control. BMC Biology 5:11

4 Harris, A.F., et al. 2011. Field performance of engineered male mosquitoes. Nat Biotechnol. 29:1034-1037.

5 Harris, A.F., et al. 2012. Successful suppression of a field mosquito population by sustained release of engineered male mosquitoes. Nat Biotechnol. 30:828-830.

6 Lacroix R, et al.(2012) Open Field Release of Genetically Engineered Sterile Male *Aedes aegypti* in Malaysia. PLoS ONE 7(8): e42771. doi:10.1371/journal.pone.0042771

Based on the findings from the Cayman Islands studies, two publications were issued. The first describes and analyzes the techniques used in the field work, one of whose principal results was evidence that genetically engineered males could be paired with and fertilize wild females. The second publication focuses on suppression of the wild population, with the authors concluding that there was an 80% reduction in the wild *Ae. aegypti* population in comparison with the experiment's control area. The Malaysia study concluded that the behavior of the genetically engineered strain is similar to that of the wild strain under field conditions, making it a promising tool for dengue vector control.[7,8,9]

The studies in Brazil are as yet unpublished, but from the information garnered from meetings on the experiments and Brazil's Ministry of Health, the following should be noted:

- This study project is part of a collaboration agreement between two companies, MOSCAMED and Oxitec, with technical assistance from the University of São Paulo, to test use of the transgenic mosquito technology in Brazil.
- The strain used in the tests was OX513A, which is patented by Oxitec and registered in Brazil. Prior to the mosquitoes' release, authorization was obtained from the National Technical Commission on Biosafety (CTNBio) in December 2010. CTNBio is the Brazilian government's regulatory agency for this type of product.
- The Bahia State Health Secretariat Health (SESAB) is a major source of project funding.
- The studies were conducted in areas of two municipalities: Juazeiro and Jacobina, Bahia.
- Dengue prevention and control activities were not suspended in these areas during the study.
- At the Regional Meeting of the Integrated Management Strategy for Dengue Prevention and Control entitled "Why can't we control *Aedes aegypti*? Current situation and next steps," held in Panama from 19 to 22 November 2013, Oxitec presented data similar to that of the Brazil study, showing an 85% reduction in *Ae. aegypti* population density and an 81% reduction in the relative ovitrap index compared with an untreated area (Itabera/Juazeiro Fig.1). At three more study sites--Mandacaru (treated), Canaiba and Maniçoba (controls)--the data show as much as a 95% reduction in the relative ovitrap index (Fig.2).

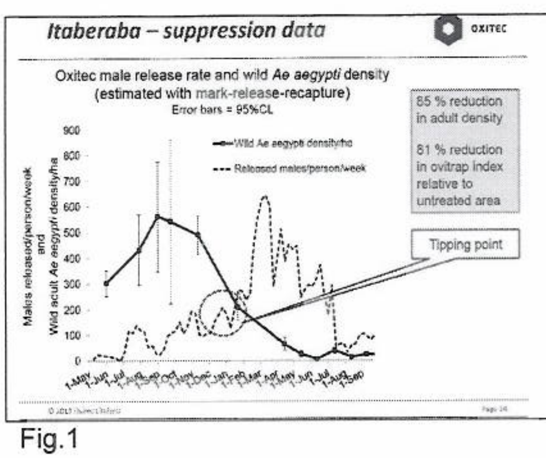

Fig.1

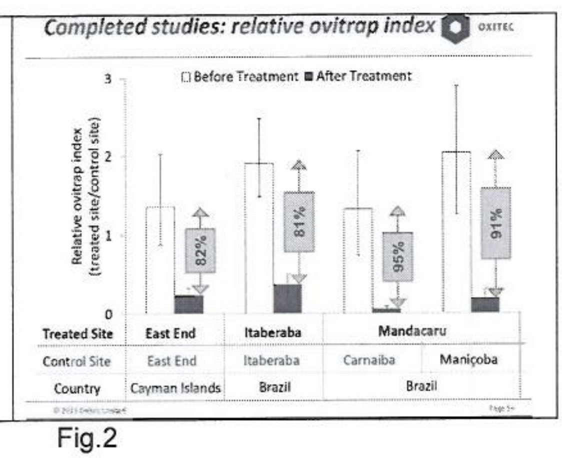

Fig.2

[7] Harris, A.F., et al. 2011. Field performance of engineered male mosquitoes. Nat Biotechnol. 29:1034-1037.
[8] Harris, A.F., et al. 2012. Successful suppression of a field mosquito population by sustained release of engineered male mosquitoes. Nat Biotechnol. 30:828-830.
[9] Lacroix R, et al. 2012. Open Field Release of Genetically Engineered Sterile Male *Aedes aegypti* in Malaysia. PLoS ONE 7(8): e42771. doi:10.1371/journal.pone.004277

It should be noted that Brazil's National Dengue Control Program does not employ this strategy for dengue vector control, *because, to date, there is no technical consensus on the use of this tool in public health.*

An important issue regarding the use of genetically engineered mosquitoes that must be addressed is regulation. While each country has the sovereign right to regulate any activity within its territory, the countries generally follow international guidelines. Use of genetically engineered organisms is governed by the Cartagena Protocol on Biosafety, and countries that are signatories to the protocol must adhere to its rules and principles. "*The Protocol thus creates an enabling environment for the environmentally sound application of biotechnology, making it possible to derive maximum benefit from the potential that biotechnology has to offer, while minimizing the possible risks to the environment and to human health.*" It is important to point out, however, that the World Health Organization, through TDR, recently published the report "Genetically Modified Insects: Science, Use, Status and Regulation," to assist the countries and bring them up to date on this issue.[10,11] In addition all countries are encouraged to read and follow the recently published *Guidance Framework for Testing of Genetically Modified Mosquitoes* developed and published by WHO/TDR after extensive consultation. The document can be downloaded on the website:

http://www.who.int/tdr/publications/year/2014/guide-fmrk-gm-mosquit/en/

The WHO also has a Vector Control Advisory Group (VCAG) on new tools, created in 2013, to serve as an advisory body on new forms of vector control for malaria and other vector-borne diseases. The VCAG has been charged with:

- Reviewing and evaluating the value to public health, "proof of principle " (epidemiological impact), of new tools, approaches, and technologies; and
- Issuing recommendations on the use of these new tools for vector control in the context of integrated vector management in multidisease scenarios.

The VCAG is currently reviewing the submission of Oxitec and the final decision will be made by the expert committee at its meeting in November 2014.

Studies conducted and funding

The Member States have the freedom and autonomy to decide how to conduct or fund studies of this nature. In the case of Brazil, the Government of Bahia made the decision to financially support the project. It should be noted that external funding of the tests can help ensure that there are no conflicts of interest.

Knowledge gaps

Significant scientific advances have clearly been achieved. However, certain issues remain that must be resolved to support decision makers, namely:

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[10] UN, 2000. Cartagena Protocol on Biosafety to the Convention on Biological Diversity. https://www.cbd.int/doc/legal/cartagena-protocol-en.pdf

[11] Beech J. C. et al. Genetically Modified Insects: Science, Use, Status and Regulation. Collection of Biosafety Reviews Vol. 6 (2012): 66-124. http://www.icgeb.org/biosafety/publications/collections.html

- To date, there are no studies demonstrating that the use of this technology affects the prevalence of the disease or how the dengue virus transmission dynamic in endemic areas might be affected. The available data indicate that the *Ae. aegypti* population can be suppressed;

- Exactly what is the minimum number of transgenic mosquitoes necessary for suppressing the wild population in an area? How much time is needed to achieve this?

- How should operations to control outbreaks or epidemics be handled in areas where genetically engineered mosquitoes have been released, since insecticides cannot be used (UBV) to control the adult vector?;

- Since the number of mosquitoes necessary to suppress the wild population is related to the size of the area to be treated, what would the potential model be for producing and releasing enough mosquitoes to achieve *Ae. aegypti* population suppression in major urban areas, where transmission of the dengue virus (epidemics and outbreaks) is more intense?;

- How to guarantee that the transgenic mosquitoes will not contribute to dengue transmission, since the methodology of separating males and females is manual and up to 0.5% of female transgenics with the potential to transmit the virus and larvae can be released and up to 15% of the larvae can survive in the presence of tetracycline in food or the environment (tetracycline is an antibiotic widely used in agriculture and veterinary medicine[12]);

- What is the effect on total reduction of *Ae. aegypti* in an area treated with transgenic mosquitoes if the country cannot control the reintroduction of wild strains of the same species through passive transport by migrant populations or geographical changes in neighboring areas that do not have alternative control systems (vectors have no borders)?

- Is there a possibility that the niche left vacant by *Ae. aegypti* will be filled by other mosquitoes such as *Ae. albopictus*, which is a major dengue vector in Asia?;

- Can this transgenic strain of mosquitoes develop resistance to the lethal effects of the gene, resulting in an increase in surviving transgenic mosquitoes?;

- In the absence of fumigation during the release of the genetically engineered strain, can other insect populations in the area, some of them potential vectors of dengue or other infections, increase? The area cannot be fumigated as long as transgenic mosquitoes are being released;

- Can the wild vector rebound in areas bordering the location where the genetically engineered vector is released, increasing the density of the wild vector in neighboring areas as a result of its displacement, and could this increase the number of expected cases and cause outbreaks or epidemics?

Sustainability

Another issue raised by this technology is its sustainability, since its results are dependent on continuous release of the mosquitoes. This makes it necessary to compare its

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[12] Wallace H. Genetically Modified Mosquitoes: Ongoing Concerns. Third World Network. Penang, Malaysia, 2013.

cost-effectiveness with that of other measures already in use or programmed and its medium- and long-term financial feasibility.

Final considerations

1. From 27 to 28 May 2014, PAHO/WHO, through its Dengue Regional Program, held the Meeting on the State of the Art in Prevention and Control of Dengue in the Americas. The event brought together scientists, collaborating centers, national program directors, and representatives of industry (including Oxitec). Some of the meeting's objectives were to: *a) Review the currently available knowledge and experience for surveillance, detection, diagnosis, management, treatment, and prevention of dengue; b) Derive conclusions and recommendations that serve the Dengue Regional Program to modify and update its strategies and technical cooperation plans, as well as its role in the fight to prevent and control this disease in the Americas;*

2. During the meeting, the "*Update and development of new technologies in dengue control and prevention*" roundtable was held, at which time the transgenic mosquito technology was presented as one of the tools currently in development;

3. Based on the discussions, one of the conclusions reached was that the existing tools for vector control are effective in eliminating *Ae. aegypti*, although questions about their sustainability and adequacy for community-based use remain. One of the recommendations was that PAHO/WHO assist the countries of the Region with decision-making on the use of new dengue prevention and control technologies, following protocols for their introduction that meet the needs of national dengue programs (information pending publication);

4. Several countries with highly developed scientific and technical capabilities in vector control have research projects under way in this area. It is very important for the results of those studies to be published and for the scientific community to evaluate and assess them. Since this is an innovation in vector control and PAHO/WHO is the WHO Regional Office for the Americas, it is equally important for the VCAG to evaluate, assess, and issue recommendations on the use of these new tools for vector control within the context of integrated vector management. The VCAG is currently reviewing the submission of Oxitec and the final decision will be made by the expert committee at its meeting in November 2014.

5. PAHO will continue following the developments related to the development of genetically modified mosquitoes and will be in a position to issue policy guidance regarding the mass use of this technology following the assessment of all available evidence and the recommendations of the VCAG.