Evaluation of Innovative Strategies for *Aedes aegypti* Control:

Challenges for their Introduction and Impact Assessment

Pan American Health Organization

World Health Organization
REGIONAL OFFICE FOR THE Americas

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Executive Summary

Vector control has become a complex task, given the number of alternatives available for the control of the various stages of mosquito populations, the diversity of available tools, and the range of intended objectives of the various strategies. Although all these strategies contribute to vector control, it has not been particularly easy to assess their impact on the burden of the disease. Experience has shown that there is no "magic bullet" or panacea that will provide a solution that is effective, lasting, economical, easy to implement, and sustainable over time.

When deciding whether to adopt new tools for the control of *Aedes*-transmitted infections (including vaccines), it is essential to have a guide for assessing the capacity of local programs for their introduction, implementation, scale-up, monitoring, and impact assessment. This guide reviews the broad range of available interventions, provides the scientific and technical elements needed to understand the potential, the advantages, and the limitations of the various technologies, as well as their possible overall effects. It also explains the relevant operational requirements for introducing any new technologies in vector control programs in the Region.

Several technological innovations have demonstrated success in controlling agricultural pests, and there are good (theoretical) prospects for their successful application in the public health field. They may also offer new opportunities for improving the performance of control programs. However, they also pose technical and operational challenges that must be considered before incorporating them in the inventory of control tools.[1]

Of particular note in the array of new technologies is the use of genetically modified mosquitoes (GMM) and mosquitoes biologically modified with *Wolbachia* (BMW) to control populations of mosquito disease vectors, whether by: (1) suppressing wild populations

with lethal genes that limit reproduction or reduce the survival of the mosquitoes, or (2) replacing populations with mosquitoes resistant to viral infections (low vector competence).

In general, innovations that involve vector modification are based on two strategies that can be categorized according to the outcome obtained (population elimination or replacement) or implantation dynamics (self-sustaining or self-limiting). Implicit in each are numerous conditions for ensuring coverage, dispersal, mosquito volume and release frequency, monitoring needs, costs, etc. (Table 1).

The objective is to sustainably eliminate mass reproduction of mosquitoes or to reduce the mosquitoes' potential to transmit infections of public health importance. The effects of technological innovations on reproductive capacity, survival of the infected vector, and interference in vector competence or vectorial capacity have demonstrated that these tools hold promise in the field of public health. Nevertheless, it would be premature to suggest that they have the potential to eliminate dengue and other arboviral diseases transmitted by *Aedes aegypti*, as there is no practical evidence of the feasibility of their scale-up and their efficacy at the operating levels at which it is wished to apply them.[2]

Table 1. Innovations in genetic modification of mosquitoes and biological modification with *Wolbachia*

Result of the intervention	Implantation dynamic	
	Self-limiting	Self-sustaining
Population suppression	Sterilization (SIT) Self-limiting genes (RISL) Female-killing mosquitoes	Lethal genes *Wolbachia* (cytoplasmic incompatibility)
Population replacement or substitution (interference with infection)	RNA transgenes Transposons (mobile genetic elements)	*Wolbachia* (interference with infection) Reduction of vector competence

Because practical use of such innovations is still limited, they would have to be phased in gradually to allow sufficient time to prepare the personnel responsible and the target communities, monitor production and introduction processes, assess their possible repercussions, and identify any problems for implementation and scale-up. The

evaluation of innovations should include parameters of efficacy and impact that have not been included or evaluated correctly in the assessments of other types of interventions. In addition, vector control programs that incorporate these new technologies will need to make significant adjustments in their structure, organization, focus, and approach to be able to deploy them effectively.

Considerations for the introduction of technological innovations (biological and genetic) for Aedes aegypti *control*

The innovations described here are being applied in the field and, although evidence of epidemiological impact does not exist for all of them, steps should be taken to present, as soon as possible, any findings with regard to their impact on the burden of disease.

When these innovations are introduced, they should not be seen as innovations that will replace existing interventions, but rather as a complement to regional control programs, as there are strengths and weaknesses that will need to be addressed for adequate large-scale application.

Tables 2 and 3 list the basic requirements that local control programs need to meet in order to incorporate these innovations into their control interventions.

The introduction of modified mosquitoes entails a linear process of preparation, release, dissemination of populations of genetically or biologically modified mosquitoes, and the replacement of wild populations with infected or modified populations. However, in each of these stages there are situations that, even when well-controlled, should be rigorously monitored and evaluated to ensure the continuity of the stages and the ultimate success of the interventions (Table 4).

Table 2. Requirements for the adoption of technological innovations

Infrastructure and programmatic requirements	Suppression strategies (reduce vector)	Replacement strategies (block transmission)
History of the use of similar technologies for control of agricultural pests (insect sterilization and others)	Desirable	Optional
Regulatory and legislative framework for the use of biotechnologies in the health field: • Environmental • Biosafety • Bioethics	Essential Essential Essential	Essential Essential Essential
Protocols for mass production of modified mosquitoes	Essential	Essential
Portfolio (dossier) of evidence on safety, quality, and efficacy of the product	Essential	Essential
PAHO recommendation through the Regional Program for Public Health Entomology and Vector Control Vector Control Advisory Group (VCAG)	Optional	Optional
Collaboration agreements with ministries of health (national or federal, provincial or state, and municipal), as appropriate to the country	Essential (national)	Essential (national)
Implementation plan • Guaranteed sources of funding • Long-term funding plan • Input logistics (production, distribution, release, monitoring, and evaluation)	Essential Desirable Essential	Essential Desirable Essential
Physical infrastructure for production of GMM/BMW • Insectarium • Laboratory (entomological) • Material resources for entomological monitoring • Trained technical personnel associated with the vector control program	Desirable Essential Optional Essential Essential	Desirable Desirable Essential Essential Essential
Multidisciplinary scientific support group for vector control personnel (action research)	Desirable	Desirable
Entomological surveillance system (ability to monitor spatial, temporal, and impact changes)	Essential	Desirable
Epidemiological surveillance system (ability to monitor spatial, temporal, and impact changes, including diagnostic capacity: serology, PCR, isolation)	Desirable	Essential
Baseline situation assessment (entomological and epidemiological) in the area where the innovations will be implemented	Desirable	Essential
Structured awareness-raising and communication campaign (expected impact messages): • Decision-makers • Technical personnel • NGOs (environmental, civil society groups) • Communications media • Communities (formal and informal community groups)	Essential Yes Yes Yes Desirable Yes	Essential Yes Yes Desirable Desirable Yes
Community participation agreements with communities involved (informed consent), as appropriate to the country	Essential	Essential

Table 3. Requirements for the implementation of the innovations

Implementation	Suppression strategies	Replacement strategies
Community engagement and participation in the design, organization, and monitoring of the innovations (local groups)	Desirable	Desirable
Definition of criteria for selection of the areas of intervention (entomological and epidemiological)	Essential	Essential
Integration with local vector control programs	Essential	Essential
Criteria established for coverage, frequency, and volume of mosquitoes released	Essential	Essential
Entomological surveillance (frequency, coverage, level of detail)	Essential	Optional
Case surveillance (confirmed, hospitalized)	Desirable	Essential
Serological/virological surveillance, PCR, and serotypes	Desirable	Essential
Virological and entomogical surveillance (PCR in females), molecular biology (genetic and biological fingerprint)	Optional	Essential
Traditional entomological surveillance: • larval surveys • oviposition • pupae • adults found in homes	Essential Optional Optional Desirable	Essential Optional Optional Desirable
Specialized entomological monitoring (range of flight, fecundity, fertility, parity, survival, vector competence), in line with local capacities	Optional	Desirable
Monitoring of performance and competitiveness (mosquito fitness)	Essential	Essential
Measurement of entomological impact	Essential	Desirable
Measurement of epidemiological impact	Essential	Essential
Monitoring of spread of the innovation (establishment and maintenance)	Essential	Essential
Communication of results to decision-makers, personnel, and communities	Essential	Essential
Measurement of community acceptance and satisfaction	Desirable	Desirable

Table 4. GMM and BMW introduction and evaluation processes

Stages	Pre-release	Release of GMM or BMW		Replacement and maintenance	Evaluation
Processes and target population	Experimental and preparation	Introduction	Dissemination and establishment	Suppression or replacement of wild populations	Impact on population and transmission
Wild populations	Population monitoring: typology of breeding sites, densities, seasonality			Invasion of species, migration	Resistance to replacement
Infected / modified populations	Mass production/ product monitoring	Release frequency and volume	Coverage and sustainability	Maintenance of vector competence (fertility, survival, dispersal)	Reduction of vector competence and vectorial capacity

The use of all these tools involves the mass release of biologically or genetically modified mosquitoes. Their effect on the vector population may be transitory (disappearing when the release of transgenic insects ceases) or permanent (if the released mosquitoes replace the target population). Mass production, which has been one of the most important constraints, requires specialized facilities to ensure adequate production monitoring and control in terms of the vector competence of the modified species.

These innovations cannot be regarded as panaceas, and their inclusion in control programs must be assessed in the light of local capacities and the integrated use of other control tools. In fact, like all available tools, GMM and BMW techniques should be used as part of an integrated scheme of tools (synergy), with specific targets (eggs, larvae, and adults) and stages to enhance their efficiency and maximize the individual and combined effect of the various control interventions.

These technologies should not be viewed, either, as a means of protecting specific individuals, houses, or neighborhoods, but rather as tools for protecting large areas such as cities or high-risk areas. The scale of application and the rate of scale-up required to cover such extensive areas is an open question, not only because of the magnitude of the task but also because of the resources required to carry it out.

A central aspect of the incorporation of such technologies is that the countries affected by dengue and other arboviral diseases do not currently have the necessary infrastructure, trained personnel, or political (financial) support needed to implement a genetic or biological control program, especially in the large urban areas that account for the greatest burden of dengue, Zika, and chikungunya. The countries of the Region need to invest in strengthening their vector control programs and human resources in order to be better prepared to implement and evaluate the available technological innovations in the short, medium, and long terms.

Objectives and Content

This guide for the assessment of new control tools—such as genetically modified mosquitoes (GMM) and mosquitoes modified biologically with *Wolbachia* (BMW)—describes the state of the art of these technological innovations, the organizational mechanisms necessary for introducing them, and the essential indicators for measuring their entomological and epidemiological effect in the short, medium, and long terms.

In order to introduce new technologies for the control of *Aedes aegypti*, the basic infrastructure to implement them in the field must be in place and there must be a plan for measuring the strengths and weaknesses of local control programs for their introduction, monitoring, impact assessment, scale-up, and sustainability.

The evaluation must include parameters of efficacy and impact that either have not been included in the evaluation of other interventions or else have not been satisfactorily evaluated. Furthermore, vector control programs that incorporate these new technologies will have to make significant adjustments in their structure, organization, and approach to be able to implement and evaluate them effectively.

The content of this document is based on four premises that serve as guiding principles for the introduction of new vector control technologies. They presuppose that local vector control programs:

- have evidence for determining that tools are effective;
- intend to introduce technological innovations as complementary tools;
- possess the capacity to put the tools into practice and evaluate them;
- have the elements required to decide when and where to introduce or scale-up interventions.

The document is organized from a comprehensive perspective. In other words, it is not designed to measure, exclusively and independently, mechanisms for the introduction, implementation, and assessment of new technologies; rather, it makes use of prior experiences in the assessment of various available tools in order to:

(1) describe the technical and operational challenges associated with their implementation;

(2) generate the evidence needed to ensure their efficacy, effectiveness, and sustainability;

(3) propose mechanisms for avoiding mistakes made in the past in the implementation and evaluation of new tools;

(4) seek ways of strengthening the capacities of vector control programs to enable adequate follow-up and make any adjustments needed to ensure the effective application of control tools.

The document highlights the role that technological innovations may play in enhancing *A. aegypti* control programs in the Region of the Americas and outlines the training and strengthening needs of operational programs.

Introduction

The Region of the Americas has a long history of vector-borne disease control. The evidence reveals the success of various programs in the past. The control of yellow fever and malaria in Cuba and Panama under the direction of William Gorgas (1901–1910), the elimination of *Anopheles gambiae* in Brazil (1940), the elimination of *Aedes aegypti* between 1950 and 1960 led by Fred Soper under the auspices of PAHO, the elimination of transmission of Chagas disease by *Triatoma infestans* in Brazil and Uruguay, and the recent elimination of onchocerciasis from 11 of the 13 endemic foci in Colombia, Ecuador, Mexico, and Guatemala (2013–2016) are recent examples of interventions that have combined the use of insecticides, sanitary engineering, and effective vaccines or medicines, supported by community participation and other control methods.

Many of these achievements could be reversed in the near future as a result of lack of commitment on the part of governments, technical weaknesses of control programs, shortages of properly trained human resources, low coverage of control activities, insecticide resistance, and other determinants that are affecting the epidemiology of vector-borne diseases at present. These challenges point up the need to improve the use of current tools and to adopt technological innovations.

Infections transmitted by *A. aegypti* have become growing public health problem in developing countries and a latent danger for developed countries, whether as a result of imported cases or the risk of introduction as a result of the existence of potential vectors in their territory. In recent decades there has been a significant rise in reports of dengue,[3] chikungunya[4], and Zika[5] virus infections and an increase in the transmission of yellow fever and other arboviral diseases in urban areas.[6,7]

It is estimated that nearly half the world's current population lives in areas at risk for dengue. Transmission is occurring in more than 100 countries and between 300 and 500 million people are infected annually, 96 million of whom have clinical manifestations and 500,000 have severe cases, with around 25,000 deaths. The infection is endemic in the Americas and in the Southeast Asia, Western Pacific, Africa, and Eastern Mediterranean regions. In the last 50 years the incidence has increased thirtyfold, a trend that shows no sign of abating. The epidemiological scenario reveals that the number of cases is increasing, that outbreaks are larger and longer-lasting, and that the affected areas and populations are continually expanding.[8,9] Achieving the WHO goal of reducing mortality by 50% and morbidity by 25%[10] by 2020 will be a challenge, given the lack of good surveillance systems that can correctly quantify the burden of disease and the deficiencies of vector control programs in the endemic countries.

The geographic spread of dengue from 1970 to date reveals the limited capacity of control programs to effectively contain the disease's spread. The recent introduction of the chikungunya and Zika viruses and their rapid spread across the continent also shows the limited response capacity of control programs and the limited effectiveness of the strategies employed.

Among the problems identified are the following:

(1) lack of linkage between vector control and elimination programs and health services, especially in the areas of epidemiological surveillance and patient care;

(2) reliance on the exclusive and intensive use of insecticides;

(3) lack of participation by all sectors involved in vector control (community, schools, municipal authorities, public services, urban infrastructure, etc.);

(4) low coverage of at-risk areas, combined with low intensity or frequency of vector control measures;

(5) application of the same measures to different risk situations;

(6) brief duration of the impact of interventions (limited effectiveness);

(7) mobility of the personnel hired (turnover and insufficiency of staff) and lack of training of program technical staff;

(8) lack of financing to modernize equipment and sustain the program (sustainability) beyond critical risk situations;

(9) lack of political will on the part of governments that fail to appreciate the true magnitude of the problem.[11,12]

A. aegypti control originated as an ambitious hemispheric campaign aimed at eliminating the vector from the Region. It began as a "vertical" control program based on a specialized organization that was separate from health services. The program's technical approach relied on the exclusive use of larvicides and insecticides. After many decades of applying this approach, other approaches were gradually adopted that brought this rigid and vertical structure into closer alignment with the views of affected populations (social and community participation). These new approaches emphasized behavior change and the promotion of domestic practices for the control of breeding sites. The focus on shared social responsibility made it easier to develop a multisectoral, multidisciplinary, participatory, social, and environmentally responsible program.[13] However, the capacity of local levels to maintain such a strategy continuously is limited, and steps are generally taken to strengthen it only in epidemic situations.

Traditional control tools, social participation and communication—among others—have taken time, have been applied partially or insufficiently, and have not been implemented in a timely, continuous, or sustainable manner, nor have they been well evaluated. The fact that they have been deemed to be of limited effectiveness therefore has more to do with the way they have been implemented or evaluated than with their demonstrated effectiveness in certain contexts. New technologies, in addition to demonstrating their effectiveness, should be incorporated into an integrated vector management (IVM) approach[14] in order to improve strategies and help to solve the operational and organizational challenges common to all traditional interventions.[1]

Interventions for *Aedes aegypti* Control

The available interventions can be classified according to the vector stage they target (egg, larva, pupa, or adult), the type of control or options (physical-mechanical, environmental, biological, chemical, behavioral, genetic, etc.), the means of application (air, land, spatial, focal, or targeted), and the user (responsible party) or level of application (individual, family, household, neighborhood, community, municipality).

The availability of a broad range of water containers in urban environments has given rise to an extensive range of tools designed to prevent them from becoming mosquito breeding sites: from specific measures, such as physical manipulation (covering, turning over, or washing out containers or eliminating breeding sites), biological interventions (use of fish, copepods, bacteria, etc.), or application of chemical larvicides, to more comprehensive measures, such as educational strategies (promotion of good practices, behavioral change) and environmental modifications (clean-up campaigns, basic sanitary engineering).

Insecticides have been the tool of choice for interventions targeting adult mosquitoes, although the ability of field professionals to apply them in accordance with technical requirements has been limited. Nevertheless, there are other tools designed to interfere with oviposition (traps), prevent the emergence of adults (polystyrene, covers, or nets over containers), prevent mosquito contact with humans (repellents, nets, and curtains), and limit mosquito survival (insecticides). All have benefited from and been strengthened by community participation and social mobilization (Figure 1).

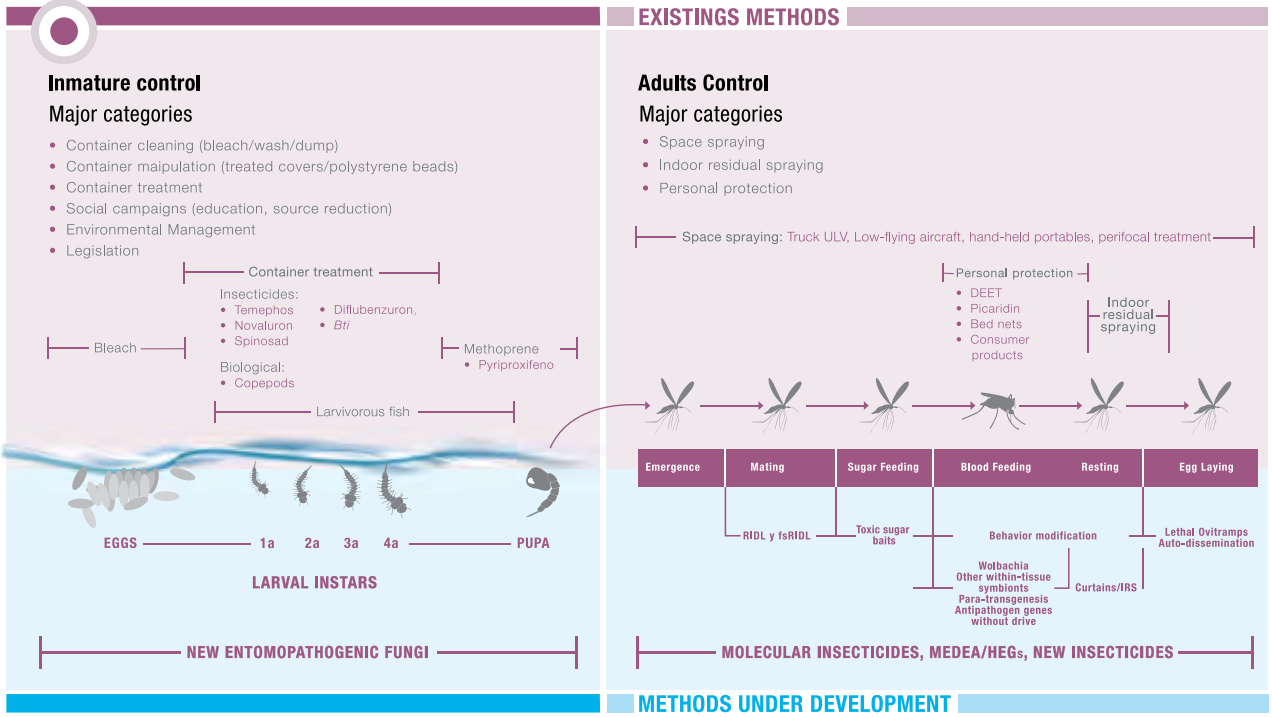
Innovations in biological and genetic modification of mosquito vectors

Genetic modification of insects has been well received in the agricultural sector owing to its impact on the control of pests that affect crops. In contrast with the intensive use of

insecticides, the lack of apparent ecological impact, specificity, and absence of resistance (unconfirmed) make genetically modified insects promising candidates for vector control. Nevertheless, their use in the field of human health has generated some concerns among the scientific community and raised doubts in the area of public health and in vector control programs. A very effective communication strategy will therefore be needed in order to publicize the evidence, benefits, and inherent risks and prevent rejection in target communities.

The objective is to sustainably eliminate the mass reproduction of mosquitoes or, at least, to curb their potential to transmit infections of public health importance, the aim being to limit the use of control interventions that are operationally complicated to apply extensively, frequently, and in a timely manner and that require excessive budgets, as they must be continually repeated (in other words, they are not sustainable). This does not, however, mean that technological innovations are more economical.

Figure 1. Interventions targeting different *Aedes aegypti* stages



From Achee NL, Gould F, Perkins TA, Reiner RC Jr, Morrison AC, Ritchie SA, et al. A critical assessment of vector control for dengue prevention. *PLoS Negl Trop Dis* 2015; 9 (5): e0003655. Reprinted with permission.

Biological and genetic vector control techniques have several features in common, which also distinguish them from traditional measures, including the following:

(1) dependence on vertical (maternal) transmission of heritable elements (resistance genes and *Wolbachia*);

(2) species specificity;

(3) environmental friendliness;

(4) active development in the treated population (through active mate-seeking by females);

(5) noninvasiveness of domestic spaces;

(6) large-scale applicability (essential).

A challenge common to both innovative control methods and traditional measures is to achieve the coverage needed to ensure that they are effective and sustainable.

In general, vector modification innovations are based on two strategies, which can be categorized according to the outcome obtained (population elimination or replacement) or the implantation dynamics (self-sustainable or self-limiting). Implicit in each are many conditions and risks for ensuring their effectiveness; for example, the requirements for a self-limiting strategy will be quite different from those for a self-sustaining (permanent) strategy in terms of coverage, dispersal, volume of mosquitoes and frequency of release, monitoring needs, costs, etc. (Table 5).

Table 5. Innovations in genetic modification of mosquitoes (GMM) and biological modification with *Wolbachia* (BMW)

Result of the intervention	Implantation dynamic	
	Self-limiting	Self-sustaining
Population suppression	Sterilization (SIT) Self-limiting genes (RISL)* Female-killing mosquitoes	Lethal genes *Wolbachia* (cytoplasmic incompatibility)
Population replacement or substitution (interference with infection)	RNA transgenes Transposons (mobile genetic elements)	*Wolbachia* (interference with infection) Reduction of vector competence

*Previously known as RIDL

Population suppression: The aim of population suppression strategies is to act on the demographics of the vector population in order to reduce it to a minimum and thus prevent the maintenance of transmission or to entirely eliminate the population from the environment in which the intervention is carried out. Such strategies reduce or eliminate populations by sterilizing males (using radiation); by inducing anomalies that reduce the viability of eggs, the survival of larvae and adults, or the reproductive capacity of populations, as a result of cytoplasmic incompatibility (CI) caused by *Wolbachia* bacteria; or by introducing dominant lethal genes that feminize males, reduce fertility, or kill females in early stages.

Population substitution or replacement: This strategy aims to replace vector populations with modified populations that are resistant to the viral infection. One of the most innovative mechanisms is *Wolbachia* transinfection. Other mechanisms involve the introduction of transgenes that indirectly reduce vector competence by altering vector survival, physiological functions (flight, feeding), or susceptibility to infection (interference).

Self-limiting strategies: These strategies call for the repeated release of large numbers of mosquitoes (inundative release) in order to maintain the gene flow in the treated population. They are reversible when releases cease.

Self-sustaining strategies: These strategies called for repeated release of modified mosquito populations in sufficient quantities to allow them to establish themselves as the dominant population (replacement). They are intended to persist in the population, which could give rise to unforeseen risks.

Control interventions for suppression of vector populations

The new technologies make use of recent progress with regard to the physiology and molecular biology of the insects in order to modify them genetically (GMM), which involves the insertion of genes in the mosquito genome or the transfer of *Wolbachia* infection, either in order to eliminate vector populations (which do not hatch, die prematurely, kill only females, or are rendered unsustainable) or in order to induce resistance to pathogen infection (by inhibiting viral replication and thereby shortening survival) in the *Wolbachia*-infected or genetically modified mosquitoes[15] (Table 6).

Sterile insect technique (SIT)

Sterile insect technique (SIT) encompasses all innovative methods that reduce the reproductive capacity of vectors.[16,17] Although the term suggests that there are no progeny (sterility by radiation), these insects are in fact capable of mating, but their offspring are not viable.

Radiation: Irradiation of male mosquitoes induces dominant gene mutations that are lethal for the offspring of the females with which they mate (the eggs die after being fertilized).[18] The limitations of radiation sterilization of males include potential negative effects on their mating performance (weakness), reduced flight capacity (dispersal) in comparison with wild mosquitoes, the operational need to separate females from males before releasing them, and the need to release enormous numbers of sterile males in order to compete with wild species (Table 6).[19,20]

Table 6. Innovations in genetic and biological control of vectors, by type, target population, and outcome

Type of modification	Target population		Expected outcome	
	Modified males	Wild females	Males	Females
Sterile mosquitoes				
Irradiation	Sterile males	(-)	Non-viable eggs	
Self-limiting gene (RISL) (previously known as "RIDL": release of insects carrying a dominant lethal gene)				
Female-specific promoter (FsRISL)	Dominant gene inserted (+)	(-)	Dominant gene carrier	Females unable to fly
Lethal gene Stage-specific	Early-acting lethality (+)	(-)	Larvae do not develop	
	Late-acting lethality (+)	(-)	Larvae do not become pupae	
Immunity genes				
RNAi genes	RNAi carrier	(-)	RNAi carrier	Resistant to infection (DENV2)
Wolbachia: Interventions not involving genetic modification				
Cytoplasmic incompatibility (CI)	*Wolbachia* carrier	(-)	Embryonic-stage death, absence of offspring (reduces egg hatch rate) or reduction of survival (vectorial capacity)	
	Wolbachia (+) and (-)	*Wolbachia* (+) of different strain		
	Wolbachia carrier	*Wolbachia* (+) of the same strain	Viable offspring	
Reduction of survival	(-)	*Wolbachia* carrier (eggs)	Reduced adult survival, diminished vectorial capacity	
Inhibition of viral replication (interference)	(-)	*Wolbachia* carrier (eggs)		Reduced vector competence

Adapted from: Elizabeth A. McGraw, Scott L. O'Neill. Beyond insecticides: new thinking on an ancient problem. *Nature Reviews, Microbiology* 2013; 11 (3): 181-193.

Genetic modification of vectors

DNA recombination

This type of reengineering introduces dominant lethal mutations in the genome of the mosquitoes that render their offspring non-viable. Other strategies consist in releasing insects carrying a dominant gene that is lethal for females—i.e., it produces conditional sterility or selective lethality. The released males transmit this gene to the offspring of wild females, killing the female offspring and thus reducing the population in a manner similar to SIT. This effect is sex-specific, as only females die; hence, the insects are referred to as "female killers."[21] Another technique is based on the insertion of endonuclease genes or homing endonuclease genes (HEG) which confer resistance to infection, fertility genes, or sex-determining genes, but these strategies are still under development[22] (Table 6).

The most advanced transgenetic strategy—now known as RISL *(release of insects carrying a self-limiting gene)* and previously known as RIDL *(release of insects carrying a dominant lethal gene*)—is the production of mosquitoes with a self-limiting gene in their genome that interrupts the development of vectors (early or late-stage lethality) and prevents them from reaching adulthood. These strategies make it possible to choose the stage of development to be affected.[23] The purpose of its application in control programs would be to destroy wild populations through the release of male mosquitoes carrying the lethal gene.[24,25]

In cohorts of RISL-transgenic *A. aegypti*, the products can be lethal only for females.[26] The expression of the gene specifically affects their wing muscles, rendering the offspring of the females incapable of flying (they do not survive), and therefore reduces the production of wild mosquitoes. If the release of female-specific RISL (FsRISL) males continues, it can succeed in eliminating the population within 10 to 20 weeks.[27,28,29] However, some field tests with FsRISL males have found that they exhibit less competitive mating behavior than wild males, which could lead to low population suppression rates (Table 6).

Studies to test the OX513A RIDL (self-limiting RISL gene) technique in *A. aegypti* have demonstrated that the modified males were less competitive than wild males.[30] Another experimental trial in Malaysia[31] found that the lifespan of modified mosquitoes was comparable to that of wild species, but their range of dispersal was smaller. The suppression of *A. aegypti* populations in the Cayman Islands reached 80% (2010), while in three locations in Brazil (2010) sustained release over a period of a year resulted in 95% suppression[32] (Table 7).

Population suppression strategies using Wolbachia-infected mosquitoes

The introduction of *Wolbachia pipientis* was described for the first time in the 1920s, and its potential to control insect pests has been known for 50 years. Bacteria of the *Wolbachia* genus have various properties that enable them to serve as a powerful biological dengue control intervention:

(1) they enhance the reproductive performance of infected females, which facilitates their spread;

(2) they are compatible with many hosts and can infect genetically distant species;

(3) they produce a wide range of effects (elimination of populations, reduction of survival, interference with virus transmission) that, if well managed, could improve the control of transmission and reduce the disease burden.[33]

Wolbachia bacteria are widely distributed in natural populations of insects and are capable of infecting between 40% and 76% of species; however, natural infection does not occur in the principal vector of dengue (*A. aegypti*), although it does occur in *A. albopictus*. It was not until 2005 that *A. aegypti* was successfully transinfected with *Wolbachia* from *A. albopictus*.

The *Wolbachia* genus comprises four clades (A, B, C, and D), but only two infect insect vectors (A and B). Infections with strains of clades A and B (*w*AlbA and *w*AlbB) have been found in A. albopictus, while the wMel strain is found in *Drosophila* populations worldwide. The transfer of *Wolbachia* infection from more distant species (*Drosophila*) to *A. aegypti* made it possible to reduce the vector's survival, limiting its ability to transmit dengue virus infection[34] (Table 7).

Wolbachia infection induces a set of reproductive disruptions in the host that are strengthened by maternal (vertical) transmission and are expressed in various ways: inducing early death of embryos or of eggs that fail to hatch; causing the feminization of males (male genotype with female phenotype);[35] modifying the sperm of the male to suppress fecundity and egg viability; causing the elimination or death of males (infected males die and females are infected);[36] inducing parthenogenesis in females (reproduction in females without sperm); and producing cytoplasmic incompatibility (CI) when infected males mate with uninfected females, resulting in non-viable eggs that fail to hatch and die.[37]

Table 7. Mosquito modification: Type of intervention and effects on *Aedes aegypti*

Type of genetic modification and effects on the vector	Lethal genes				Wolbachia			
	Irradiated mosquitoes (sterile)	RISL (previously RIDL)	Sex-specific	Mobile genes	*w*Mel	*w*MelPop *(popcorn)*	*w*MelPop-CLA	*w*Alb A and B
Eggs and larval development	Not produced	OX513A late lethality		Early or late mortality	Reduced growth period or death (90%)	Shorter egg development; reduced oviposition and viability	Increased metabolism	
Cytoplasmic incompatibility (CI)					Yes	Yes, less fixation of the infection	Yes	Yes
Resistance to or interference with infection (vector competence)				RNAi: Inhibits membrane proteins	Reduced susceptibility to dengue (70%-89%), chikungunya, yellow fever, and West Nile viruses; less interference, lower viral titers in saliva and body	Dengue, chikungunya, yellow fever, and West Nile virus: affects the intrinsic incubation period (IIP) by reducing survival; Reduced susceptibility, greater interference in saliva and body	Significant reduction of DENV	DENV: Reduced susceptibility and viral titers in saliva and body
Fertility	Sterile males	Sterility	Eliminates females (female killer)	Sterility		Reduction	Hatch rate reduced by 60%	Increase
Mating	Effective	FsRIDL (OX3604C) Does not fly, affects the thoracic muscles	Elimination	Effective	Effective	Similar to wild species	Reduction	Effective
Feeding						Reduced feeding; larger males	Reduced number and size of bloodmeals; “bendy” proboscis	
Dispersal		FsRIDL: Does not feed or fly				Movement affected		
Survival		FsRIDL: Reduction		Survival	Little effect: 10%	Adult survival reduced by 50%	50% reduction of adults and eggs	×
Population replacement					Infection present in 90% after 3 years (Australia)	×	Not as effective	×
Population extinction or suppression	×	×	×	×				

Wolbachia also has effects on oogenesis (generation of eggs), feeding, and the development (survival) of infected vectors, in addition to its most important effect, which is interference in infection by various pathogens.[38,39] The diversity of effects is due to the fact that *Wolbachia* infection can be established in various tissues, including those of the brain, thorax, salivary glands, muscles, abdomen, fatty tissue, reproductive system, and Malpighian tubules. The density of the infection depends on the strain of *Wolbachia*, and its strategic location in the tissues of the digestive system (salivary glands and midguts) can affect the vector competence of the infected species (Table 7).[40,41,42,43]

Cytoplasmic incompatibility

Cytoplasmic incompatibility (CI) causes incompatibility between eggs and sperm of the same species, which leads to the death of the progeny during the embryonic stage. The different strains and variants of *Wolbachia* have different capacities for inducing CI. There are two types of CI: on the one hand, incompatibility between sperm infected with *Wolbachia* and the egg of an uninfected female, which renders her eggs inviable. On the other hand, when infected sperm fertilizes an infected egg, the offspring are viable and the infection is transmitted transovarially, which results in greater reproductive success and faster spread. There is believed to be a bacterial infection threshold, but it may vary in different bacterium–host combinations: it may be complete (all offspring die), as is presumed to occur in mosquitoes, or partial, as in *Drosophila* (Tables 6 and 7).[44]

An essential condition for the success of *Wolbachia* infection is that the released male mosquitoes are competitive enough to prevail over wild males. Studies in *A. aegypti* have found that infection with *w*Mel and *w*MelPop strains of *Wolbachia* does not reduce the competitiveness of males bred in an insectarium, but negative effects could appear if the strain of mosquitoes released is better adapted to the breeding conditions in the insectarium than to those in the place of release (natural conditions).

Replacement interventions to promote resistance to viral infection

Exogenous gene expression and endogenous gene modification

Genetic techniques are designed to induce the expression of exogenous genes or modify endogenous genes (transgenesis) in order to increase the immune response of the vector or replace wild populations with mosquitoes carrying a gene that makes them resistant to pathogen infection (interference).[45]

The early strategies used mobile genetic elements (transposons) and the transforming genes were introduced into the genome of the mosquito.[46,47] An important limitation of

transgenesis strategies using viral genes is that effective control interventions must inhibit all serotypes. Furthermore, once introduced, the transferred gene must be maintained in the treated wild population.[48] For experimental purposes, infection-refractory mosquitoes have also been engineered by inserting into them a transgene that modifies the physiology of the infected tissues (salivary glands, hemolymph, digestive tract, thoracic musculature, or Malpighian tubules), thereby inhibiting or limiting reproduction of the virus in the vectors.[49,50]

Wolbachia-*mediated resistance to viral infection*

Wolbachia infection in *A. aegypti* can block the development of RNA viruses such as the dengue (DENV),[51] chikungunya (CHIKV),[38,39] Zika (ZIKV), yellow fever (YFV)[52], and West Nile (WNV)[53] viruses. There is disagreement regarding the mechanism of action of the bacterial infection, since the immunity genes induced by *Wolbachia* in the insect are not the same as those induced by the viral infection. The physiological mechanisms that interfere with pathogen infection in the presence of *Wolbachia* are unclear, although they have been shown to be related to activation of the immune system of the infected vector, with greater melanization of the hemolymph, which is responsible for encapsulating foreign bodies, and with cell competition for the resources necessary to perform various functions (fatty acids).[54]

The effect of *Wolbachia* on mosquito survival and viral interference is variable.[55] The wMelPop strain has a detrimental effect on larval development but a good antiviral effect. The *w*Mel strain, on the other hand, has less effect on mosquito fitness but also shows less viral interference. There is an apparent correlation between the density of the *Wolbachia* infection and resistance to viral infection, which means that, in order to block virus transmission, the *Wolbachia* infection must reach high densities in the population.[56] Experimental infections with *w*Mel and *w*MelPop differ in density and distribution in the infected tissues: *w*MelPoP shows higher densities and greater inhibition of DENV infection.[57] The *Wolbachia* infection density required to block viral infection can also have effects on the fitness of the insect, preventing it from spreading after being introduced into a population.[58] These possible limitations seem not to have occurred in the interventions carried out to date. In studies with *w*Mel in Australia, it was found that the reduction of experimental infection in the tissues of mosquitoes collected in the field was similar to that in mosquitoes observed in the laboratory and that it persisted for nearly two years in the infected populations.[59]

The induction of resistance to viral diseases as a result of infection with various strains of *Wolbachia* transferred from *Drosophila* to *Aedes* reduces survival or suppresses RNA virus infection.[60,61] *Wolbachia* have also been transferred from *A. albopictus* to *A. aegypti*, which

eliminates viral replication by other mechanisms.[62] To date, all the strains introduced in *A. aegypti* have shown complete or near-complete interference, and transinfection of *A. aegypti* has been shown to interfere more with the pathogen than natural infections in *A. albopictus*.[63,64]

The effects of *Wolbachia* on the competitiveness of released mosquitoes can reduce the effectiveness of interventions when the period of development is longer or adult size is smaller as a result of environmental changes such as temperature fluctuations.[65] The high densities required coupled with long periods of drought can increase costs and raise the threshold of infection needed to achieve the invasion of *w*MelPop in a wild population. The *w*MelPop strain spreads more readily under conditions of rapid larval development and at low densities. It is therefore important to assess breeding site characteristics and mosquito population dynamics[66] (Table 7).

Considerations for the introduction of biological and genetic innovations for *Aedes aegypti* control

Several technological innovations (GMM and BMW) have been proposed for the control of populations of mosquitoes that are disease vectors, such as turning them into carriers of genes that will:

(1) eliminate wild populations with lethal genes;

(2) reduce mosquito survival;

(3) replace populations with mosquitoes resistant to viral diseases.

The approach proposed for introducing these innovations appears to be a simple linear process of preparation, release, dispersal, and replacement of wild populations with modified populations. However, it is important to note that in each of these stages there are processes that, even when they are well managed, must be rigorously monitored and evaluated in order to ensure the continuity of the stages and the ultimate success of the intervention. Depending on the strategy (suppression or replacement), the monitoring and evaluation requirements will need to be tailored to the characteristics of the product (GMM or BMW) (Table 8).

Table 8. Stages of introduction and evaluation of GMM and BMW

Stages	Prerelease	Release of GMM or BMW		Replacement and maintenance	Evaluation
Processes and target population	Experimental and preparation	Introduction	Spread and establishment	Suppression or replacement of wild populations	Impacts on population and transmission
Wild populations	Population monitoring: typology of breeding sites, densities, seasonality			Species invasion, migration	Resistance to replacement
Infected / modified populations	Mass production/ product monitoring	Frequency and volume of release	Coverage and sustainability	Maintenance of vector competence (fecundity, survival, dispersal)	Reduction of vector competence and vectorial capacity

Adapted from: Ritchie S. Rear and release: a new paradigm for dengue control. *Austral Entomology* 2014; 53 (4): 363 367.

Stage 1: Prerelease: experimental and preparation

In general, this stage has been well documented and there is sufficient evidence to introduce these innovations in the field and test their effectiveness in mass release interventions.[67] Mechanisms for ensuring the generation of strain lines selected as potentially useful already exist, as do proven mechanisms for transinfection of mosquitoes and evidence on their invasive potential. It is essential to systematize all the evidence on the performance (fitness) of modified or infected vectors, such as periods of development, fecundity, mating, survival, dispersal, etc., as well as the evidence demonstrating pathogen interference in the populations of introduced species.[33,68,69]

Efficacy tests: Evidence for decision-making

It is critical for the research industries and institutions that promote such interventions to have compiled a dossier or portfolio detailing the methods and procedures for sustaining the innovations, including the results of laboratory studies of biosafety and efficacy under controlled conditions, descriptions of the potential effects and risks for both the environment and health, and a plan for production and application, with strategies for scale-up and evaluation. This plan should indicate the type of involvement and the level of collaboration (technical, academic, political) expected from national authorities, the necessary financing, and the agencies responsible for supporting the introduction of such innovations. The process should be carried out transparently and with a clear plan for accountability that seeks above all to provide evidence, identify benefits, point out limitations, and propose solutions[70] (Table 9).

Table 9. Prerelease stage: activities and indicators

Experimental and preparation. Processes and activities	Process indicators by type of activity		
	Production of GMM/BMW	Entomological surveillance	Epidemiological surveillance
	Control program	Health services	
Tests of efficacy, usefulness, and logistics Situation assessment	Capacity for mass production, input logistics Product quality control	Entomological assessment, monitoring of densities, seasonality, typology, and breeding site productivity	Endemic channel by location, probable cases, confirmed cases, reported cases, circulating serotypes and viruses
Safety tests: laboratory	Infrastructure and staff training, plan for risk analysis, detection, and management	Viral and entomological surveillance	Infrastructure and diagnostic capacity (NS1, IgM, IgG, PCR, isolation)
Tests of suitability, consent, and acceptance	Legislation, regulation, awareness-raising, and ethical, cultural, and social issues; stakeholder persuasion. Communication strategy	Perception of risk, acceptance, or resistance in the community	Perception of the risk of disease. Seroprevalence studies
Feasibility tests: receptivity conditions	Selection of control site and areas, design and duration of the study, isolation	Presence of the vector, conditions of the population, morbidity and mortality, coexistence with control programs, environmental conditions; mathematical models	

Logistics tests: Mass production capacity

The process for initiating the release of mosquitoes (infected with *Wolbachia* or genetically modified) begins with their large-scale production. To accomplish this, the receiving countries must be in a position to finance the technique, introduce it, and adapt it for mass production. In other words, they should have a manual of operating procedures, with the necessary infrastructure and technology (insectariums, laboratories, and "mosquito factories") and the necessary equipment and trained personnel to produce and release mosquitoes and to monitor and evaluate the impact of such interventions. Such innovations require a team made up of a variety of specialists, including entomologists, epidemiologists, social scientists, communications experts, molecular biologists, mathematical modelers, and technical field personnel. All should be linked to local vector control, epidemiological surveillance, and health care programs and to the receiving communities (Table 9).

Input logistics: It is crucial to establish the capacity to produce large numbers of mosquitoes and to have knowledge of the populations of wild species in order to estimate the ratio of males to be released in relation to the wild males against which they will be competing. This requires prior entomological work, including a situation assessment of candidate areas.

Usefulness tests: Quality control of the product

The dynamic nature of technological innovations means that there must be monitoring mechanisms for continually assessing the maintenance of biological or genetic traits in the modified species, as well as changes in populations of wild vectors. This will be increasingly important as the scale of application increases, when greater production will be required and release mechanisms will be diversified or intensify in frequency. The main concern at this point is that the interventions are intended to bring about the modification of a species at the population level, and it is therefore necessary to incorporate parameters and indicators that will ensure that demographic changes go in the desired direction (see "Evaluation: Measurement of Impact"). Nevertheless, it will be necessary to ensure that the innovation is being monitored throughout the process (Table 9).

Safety tests: Plan for risk analysis, detection, and management

Notwithstanding the potential merits of these innovations, it is necessary to design a plan for analysis and monitoring of risks and management of contingencies. The process of designing the plan should start in the laboratory and go on to cover field trials, including adverse environmental and ecological impacts. It should also take account of the position and perceptions of the participating communities. The plan should include detection and analysis of risks as perceived by users (control programs) and recipients (communities) and should provide for their prevention or their management in the event that they do occur; communication of the solutions adopted should also be included. In order to draw up a plan, it will be necessary to identify the circumstances that could trigger a negative effect, the level of exposure, the degree of uncertainty as to the potential effect, the actions that should be taken in order to remedy the situation, and the mechanisms for keeping risks within an acceptable level[71] (Table 9).

Suitability tests: Legislation and regulatory mechanisms

At the outset, it should be established that any new strategies are included within an integrated vector management approach and that they will act in synergy with traditional interventions aimed at reducing breeding sites, larval densities, and adult populations. However, while the regulatory processes for introduction, application logistics, evaluation designs, and safety aspects have already been established and standardized for traditional interventions, they will need to be adapted or redesigned for the innovations to be introduced.[72] Although the majority of innovations are already ready to be tested in the field (phase III), there are important considerations with regard to the regulation necessary[73] to obtain approval for the introduction of these and future innovations (Table 9).

Consent tests: Ethical, social, and cultural considerations

When this type of biotechnological innovation is introduced, there is a risk that it will be assumed that there are ethical requirements only in the area of research (biological product) and that social and cultural considerations are not so important, since human beings are not the direct target of the intervention. However, the investigators involved have ethical responsibilities with regard to both the process of application and evaluation and the residents of the areas in which the innovations will be introduced.

Obtaining the informed consent of the people who will take part, directly or indirectly, in the implementation of the innovations is a minimum requirement, that should be broadened as needed in order to address the needs of the communities concerned. It is also important to address certain social and cultural requirements of the populations with respect to their perceptions, expectations, and needs, not only for information but also for evidence and assurances that their health and that of their family members will not be affected by the application, monitoring, and evaluation of the technological innovations.[74]

Although these aspects are included as central elements of the preparation phase, many of these activities will have to be conducted throughout the trial, and they may become even more important as the trial advances and the results begin to be registered (Table 9).

Acceptance testing: Raising awareness among stakeholders

A central element in the introduction of technological innovations for *A. aegypti* control is to break with a long history of education and information campaigns and social participation schemes that have promoted changes (effective or not) in individual and community practices in order to eliminate, protect, or control the variety of vector breeding sites found in the domestic and surrounding environment.

Convincing members of the community that it is a good idea to release mosquitoes to do the "dirty work," when they have already internalized the opposite view, requires an extraordinary process of awareness-raising and communication to turn community members into participants and partners. The information and awareness campaign should explain the features of the innovations (strengths and weaknesses), the release procedures (areas, dates, etc.), the potential risks and, especially, the activities in which the community should intervene or participate (Table 9).

This awareness-raising campaign begins with informing and training the various actors at the various levels (national or federal, state or provincial, municipal and local) and the communicators responsible for reformulating the health promotion strategy and the portfolio of educational messages to include the benefits of the new vector control approach. This is a fundamental step, especially if the innovations are introduced as

strategies intended to complement the activities of traditional vector control programs (fumigation and elimination).

The population should also be given several opportunities to raise concerns and receive responses, a basic step in avoiding the spread of rumors and incorrect information.

Viability tests: Site selection

In order to move from the controlled conditions of the laboratory to a natural environment, where it is hoped to achieve a performance similar to that achieved during the development phase, it is necessary to identify the most suitable areas for assessing the benefits and impact of the technological innovations, as well as detecting any potential operational problems for release, monitoring, and the achievement of success.[72]

There are two general conditions for the introduction and evaluation of these innovations in the field:

(1) receptivity conditions that make it possible to introduce and evaluate the strategies;

(2) epidemiological conditions that make it possible to measure the effect or impact of the proposed interventions, be it suppression of vector populations (entomological) or interference in infection or transmission (epidemiological).

These conditions are not exclusive; both are necessary for successful implementation of the interventions and proper evaluation of their impact.

Receptivity conditions

- Presence of the target vector population (*A. aegypti*) with little or no competition from another vector species (*A. albopictus*). In the event that there is a secondary vector, the evaluation design should take into account its existence and its role in transmission;
- Existence of an official regulatory structure to support the introduction of new vector control tools;
- Political, financial, academic, and social commitment to carry out the field trials;
- Infrastructure: well-trained personnel, diagnostic laboratories, insectarium, inputs for mass production and distribution, release, and monitoring;
- Local research team with experience (entomologists, epidemiologists, sociologists, communications experts, etc.): a critical mass of personnel who are committed to collaborating in the evaluation;
- Social and institutional approval to avoid resistance to the field trials;
- Local safety conditions that will ensure that the research can be carried out.

Epidemiological and entomological conditions

Presence of the vector: An assessment that is as up-to-date and accurate as possible is needed to identify vector populations; oviposition sites; breeding site productivity; cryptic and most productive breeding sites; egg, larval, pupal, and adult densities; seasonal variations, etc. In short, it is necessary to have a surveillance and information system that provides entomological information in a continuous and well-organized manner and that makes it possible to monitor the most important entomological indices and variables.

The degree of detail necessary will depend on the type of intervention (suppression or replacement). However, in either case it should be ensured, after a certain time, that the species of interest is being identified and that wild species that have migrated from surrounding areas are not being included when population suppression is measured, nor are secondary vectors not affected by the intervention being considered when the impact of the intervention on transmission is assessed.

Population conditions: It is necessary to study the human populations that are vulnerable to the infection in terms of spatial distribution (urban or rural), population pyramid (age structure and sex), and other demographic variables (density, education, marginalization, mobility), and housing conditions (dwellings, sanitation, ecology, etc.). All these variables influence the conditions necessary for the spread of the vector, although they will not be modified by the intervention.

Presence of the disease: The ultimate aim of implementing the proposed technological innovations is to reduce or eliminate the burden of disease, which means that the interventions must be carried out in endemic and high-risk areas in order to be able to assess their real impact. To determine impact on transmission (replacement of populations), prior studies of seroprevalence will need to be conducted or a solid epidemiological surveillance system will have to be in place so that the occurrence of cases in different transmission situations (before, during, and after the period of transmission) can be detected promptly. Assessing impact will be more complicated if Zika and chikungunya coexist with dengue or may be introduced in the areas studied.

An essential condition for assessing impact on transmission is that the population in the trial site must be very large (in terms of area occupied or density) in order to measure the effect of the intervention on the disease. The relationship between population density and transmission levels will be crucial for determining the type of trial, the sample size, and the anticipated effect. The areas studied (intervention and control) must have high transmission levels in order to measure impact. These elements will determine the level

of coverage that the intervention should have and the number of modified mosquitoes required to cover the area, compete with wild populations, survive over time, and ultimately replace or eliminate the wild populations.

Duration of the intervention: Depending on the type of intervention (suppression or replacement) and the level of implementation (self-limited or self-sustaining), a timeframe can be established for the study. If the aim of the intervention is population suppression, the following should be taken into account: the size of the area to be treated, the time required for the modified species to be released and established, and the length of time needed to measure the elimination of the wild population from the niche that the modified species is intended to occupy. An important variable is the seasonality of wild populations, since the findings of the evaluation can change significantly if eggs are not taken into account—for example, eggs in diapause. In fact, this aspect is fundamental, since suppression interventions will have greater impact if they are applied when densities are low, whether for seasonal reasons or because of the effects of a control intervention.

In the case of an intervention intended to block the infection in the mosquito (replacement or interference), measurement of impact on transmission will require a design appropriate to the type of intervention to be measured. This means, first, having a control site that is similar (in terms of demographic, epidemiological, and ecological conditions) to the treated site and, second, prior knowledge of immunity (seroprevalence), intensity of transmission (seasonality), and the circulating serotypes that may be affected by the innovation, whether GMM or BMW. It should be anticipated that the evaluation of this type of introduction will span several transmission periods, not only to ensure sufficient cases, but also to correctly assess the sustained impact without the findings being confounded by transmission cycles associated with the natural immunity of the population.

Isolation: This geographical condition is desirable both for treated and control sites to limit conditions that might confound or contaminate the desired effects. With small-scale population suppression strategies, the impact of migration of wild species increases, while in larger-scale studies this effect will be less noticeable. In the case of replacement (interference) strategies, it is also necessary to take into account the mobility of the human population, which may be affected (exposed) outside the study site. It may be possible to protect the treated and control sites with buffer zones, where targeted vector control is carried out to limit migration.

Coexistence with local control programs: Given the endemic nature of the trial sites, it is to be expected that, in a situation of epidemiological alert, vector control measures may mask the effects of both suppression and replacement interventions, since they

may alter the survival of the biologically or genetically modified species or affect local transmission. Communities may already have been sensitized to control strategies, and control programs cannot remain indifferent in the face of an epidemiological alarm situation, a fact that should be clearly acknowledged before initiating the intervention and that will have to be borne in mind in both treated and control areas.

Adverse environmental conditions: To the extent possible, the presence of natural phenomena (hurricanes, floods, etc.) or migration processes that may alter the results of the study should be considered when selecting the trial site, as should conditions that may affect the safety of the research team (violence, kidnappings, etc.).

Mathematical modeling (tests of viability): In view of the paucity of available empirical information, it will be essential to utilize mathematical models that describe scenarios and predict the behavior of mosquito populations in response to population suppression or replacement strategies and their linkage with quantifiable entomological and epidemiological parameters.[75]

Stage 2: Release: Introduction, dispersal, and establishment

Introduction: Implementation and monitoring

The introduction of these innovations in the field will be a process defined by the site selected (in terms of geographical and population size), the design of the evaluation, and degree of isolation. The logical strategy for introduction is to employ a phased approach of increasing scale (areas to be covered) to calculate (monitor) operational efforts and input requirements, in addition to evaluating effects and requirements for greater scale-up (Table 10).

Vector control prior to the intervention: The effect of any technological innovation will be enhanced if it is introduced when mosquito population densities are lower, whether because of seasonal effects or as a result of intensive and extensive spraying. This operational advantage will also make it possible to release fewer modified or infected males. It is recommended that releases be scheduled before any seasonal effect has occurred or after a targeted control intervention.[15,76]

Table 10. Introduction, dispersal, and establishment: Activities and indicators

Stages	Process indicators by type of activity		
	Production of GMM or BMW	Entomological surveillance	Epidemiological surveillance
Introduction	Implementation	Monitoring	
Control intervention	Spraying and control prior to the intervention		Probable, confirmed, reported cases, outbreaks, circulating serotypes
Coverage (where)	Size and scale-up	Entomological surveys, tests of dispersal, mean oviposition rate, hatch rate, size, survival rate. Viral infection in adults	
Seasonality (when)	Timeliness		
Frequency (how many)	Mass release of mosquitoes		
Monitoring	Establishment and continuity of GMM or BMW		
Ethical, cultural, and social issues	Participation indicators	Perception of risk or protection with new species	Perception of risk of disease
Dispersal and establishment	Impact measurement		
Range of dispersal	Dispersal and longevity (survival) / densities	Entomological surveys, tests of dispersal, mean oviposition rate, hatch rate, size, survival rate. Viral infection in adults	Probable, confirmed, reported cases, serotypes
Mosquito competitiveness	Mating, fecundity, interference		Number of clusters, number of cases per cluster, serotypes, and circulating viruses
Reintroduction or invasion of wild species	Spread or continuous release. Establishment and continuity of the GMM or BMW		Seroconversion in cohorts, studies of mobility
Ethical, cultural, and social issues	Participation indicators	Perception of risk or protection with new species	Perception of risk of disease

Release process (how, where, when, and how many times)

Each of the strategies described will require a different number and frequency of release, depending on the potential they offer for penetration of wild populations and on whether they are self-limiting or self-sustaining. For example, strategies intended to change the proportion of females to males, produce sterility, or affect survival will require fewer releases than those that are designed to become established through reproduction of the population, such as *Wolbachia* infection and strategies that promote immunity, produce late lethality or affect some function (flight) or the metabolism of the insect.[1]

To determine the number of mosquitoes that need to be released, it is essential to estimate the size of the wild populations, a figure that cannot be derived from traditional entomological indicators. The ratio of modified male mosquitoes to wild males can be calculated on the basis of population density, area, dwellings, or number of wild males and will also depend on the mode of release (fixed or random points of release).[31,77] Nevertheless, the release of mosquitoes is a measure that may lead to resistance and lack of acceptance in the community, since the presence of mosquito pests (even if they do not bite) will be more noticeable.

Another important parameter is that the density of the *Wolbachia* infection or the gene driver in the mosquito population must be very high or be well established after repeated releases. To be effective the genetic modification or *Wolbachia* infection should ensure rapid reproduction to eliminate or replace wild populations. In addition, the proportion of females accidentally released should be low (under 2%) in order not to increase viral transmission.

A balance can be struck between the density of the released mosquito population, the time required for its establishment (fixation), and the timeframe for deeming the wild population to have been suppressed or replaced. All these conditioning factors call for knowledge of the population of wild species and biological parameters that are not available to control programs.

Stage 3: *Replacement and maintenance*

Continuous monitoring of vector populations will make it possible to assess whether the individuals introduced have become established and displaced the wild populations. In suppression strategies, the expected time should initially be envisaged in months and then prolonged to confirm that the wild populations have been displaced, that they have not been reintroduced through migration or invasion from surrounding areas, and that the dominant vector no longer exists. In replacement strategies, the situation is more complicated because the mosquito populations do not disappear, but rather are replaced by others that are not efficient vectors. The parameter for evaluation is not the presence or absence of the vector but the capacity of the existing mosquitoes to transmit dengue, chikungunya, and Zika.

This evaluation requires appropriate designs for measuring impact on transmission that also include entomological parameters such as vector dispersal, individual competitiveness in terms of mating, rates of *Wolbachia* infection or of genetic markers for resistance to infection, invasion of wild strains, and recovery of populations (Table 11).

The effects of interventions using GMM or BMW are not immediate, since displacement and, especially, replacement by populations of modified mosquitoes can be achieved only after several generations of wild mosquitoes (weeks or months). Maintaining the population requires the ongoing release of adequate numbers of modified mosquitoes. This calls for long-term financial commitments for the construction and maintenance of production facilities and inputs and for administrative and operational personnel.

The administrative, operational, and financial planning stage should therefore take account of the expected contribution of the intervention in relation to the resources needed to maintain the other interventions included in the integrated vector management program.

Table 11. Suppression or replacement stage: Activities and indicators

<table>
<tr><th rowspan="2">Stages</th><th colspan="3">Process indicators by type of activity</th></tr>
<tr><th>Production of GMM or BMW</th><th>Entomological surveillance</th><th>Epidemiological surveillance</th></tr>
<tr><th>Replacement or elimination</th><th></th><th colspan="2">Impact measurement</th></tr>
<tr><td>Maintenance</td><td>Evolution of the effectiveness</td><td rowspan="2">Entomological surveys, tests of dispersal, mean oviposition rate, hatch rate, size, survival rate. Viral infection in adults</td><td rowspan="2">Probable, confirmed, reported cases, number of clusters and cases per cluster, serotypes, seroconversion in cohorts, studies of mobility</td></tr>
<tr><td>Reintroduction</td><td>Migration or densities, establishment, continuity of GMM or BMW</td></tr>
<tr><td>Ethical, cultural, and social issues</td><td>Indicators of acceptance, messages transmitted, etc.</td><td>Perception of risk, protection with new species</td><td>Perception of risk of disease</td></tr>
<tr><th colspan="4">Suppression</th></tr>
<tr><td>Self-sustaining or self-limiting</td><td>Analysis of effectiveness, genetic variation of densities</td><td>Vector competence, infection or genetic marker in eggs and adults</td><td>Probable, confirmed, reported cases, number of localities affected, serotypes</td></tr>
<tr><td>Ethical, cultural, and social issues</td><td>Involvement indicators</td><td>Perception of risk, satisfaction</td><td>Perception of risk of disease</td></tr>
</table>

Decision-making for scale-up

A central element throughout the process of release and evaluation is continuous feedback from stakeholders and decision-makers at the local level. The results of the process of introduction, monitoring, and evaluation should be shared (communicated and endorsed) by program and community leaders. The possibility of scaling up the interventions to higher levels of application will hinge on the success of this communication strategy.

Evaluation: Impact Measurement

Interventions that are in phase III of development need to demonstrate that they have value for public health if they are to be recommended by WHO. It is therefore imperative to assess epidemiological effects.[78]

The innovations described here are being applied in the field and, although evidence of epidemiological impact does not exist for all of them, steps should be taken to present, as soon as possible, any findings regarding their impact on the burden of disease.

Even though there have been various systematic reviews on the effect or impact of a broad range of *A. aegypti* control interventions, the conclusions on their effectiveness are contradictory, mixed, or insufficient. This is due, among other factors, to the target of the intervention, the variety of study designs used, the type of intervention, its duration and coverage, the methodology used to evaluate it, and the indicators used and their limited ability to measure impact on the reduction of transmission or incidence of disease.

In many cases, the interventions evaluated combined different types of intervention with varying effects on vector densities; however, evidence of impact is seldom well supported or its quality is questionable.[79,80,81,82] Common problems include insufficient coverage (in space and time), imprecision of measurement processes, and the type of impact assessment, which points up the urgent need to improve processes for evaluating the introduction of new interventions.[79]

The most well-recognized epidemiological parameters are incidence of disease or infection, specific mortality, and prevalence of the infection in the population. In diseases such as dengue, which produces a significant proportion of asymptomatic cases and nonspecific febrile cases, seroconversion is considered a good proxy for past infection or disease.[83] It is essential to have standardized clinical case definitions and well-established

serovirological diagnostic procedures that make it possible to draw comparisons between studies and regions.[84]

Traditional entomological indicators do not predict risk or epidemiological impact well,[85] although they are useful for monitoring vector densities and are essential for evaluating population suppression strategies (SIT, irradiated mosquitoes, CI due to *Wolbachia*, lethal genes), and it should therefore be ensured that traditional entomological surveillance is well established in the trial sites. Since larval surveys do not predict adult populations well (not sensitive enough),[86] it will be necessary to expand the range of tools to include pupal surveys[87] and surveys of adult densities in housing, in order to fill the gaps in the information obtained through traditional methods.[88,89]

A substantive issue is that the effects of population-based (not individual) interventions should be evaluated at the population level. Unlike traditional interventions that act on different populations (individuals, families, dwellings, neighborhoods), innovations have another level of application and therefore another level of impact is expected.

Simply stated, the effect of the intervention should be measured at the level at which it is applied (direct effects on those exposed and not exposed to the intervention), whether the level is individuals, household, or neighborhood. Population-based interventions should measure the direct effects at the same level of application (dwellings protected and not protected, areas sprayed and not sprayed, etc.), although some may have indirect protective effects if the population coverage is very extensive (Table 12).

The studies proposed to evaluate effect on transmission are described below.

Cluster-randomized trial: Clusters trials are the best methodological option, but the populations studied have to be large, the geographical area has to be extensive, and the duration has to be significant in order to measure effect on transmission (two cycles of transmission). These studies are very expensive and require the participation of specialized teams. A stepped wedge design, involving the phased roll-out of the intervention in the control areas, can be used,[90] or observational studies can be conducted that complement one another and make it possible to obtain a more integrated evaluation of the effect of the proposed interventions.[91,92]

Table 12. Level of application of Aedes aegypti control interventions

Type of intervention		Level of application and impact			
		Individual	Household	Neighborhood or district	Locality
Physical	Clean-up of breeding sites		×	×	
	Basic sanitation		×	×	×
	Health promotion	×	×		
	Physical barriers: mosquito nets, curtains		×		
Chemical	Chemical larvicides		×		
	Repellents (individual or spatial)	×	×		
	Household spraying		×		
	Fogging		×	×	
Combined/ insecticides	Nets and curtains		×		
	Clothing	×			
Biological	Copepods		×	×	
	B. thuringiensis var. israelensis		×	×	
	Fish		×	×	
	Wolbachia				×
Modified mosquitoes	Genetically modified				×
	Irradiated mosquitoes				×

Guide for carrying out studies of the efficacy of control interventions

WHO has prepared a detailed guide for assessing the efficacy of control measures.[78] The principal steps for evaluating proposed technological innovations are set out (in an adapted form) below. Although this is not an exhaustive guide, it does include the steps that should be followed to evaluate innovations for suppression and replacement or interference with infection (Table 13).

Table 13. Evaluation of the efficacy of *Aedes aegypti* control innovations

Steps	Suppression	Replacement or interference with infection
Register the protocol and obtain ethical and administrative review	Essential	Essential
Select the control strategy	Type of intervention, population (treatment and control) with the entomological outcome measures	Type of intervention, population (operated and control) with the epidemiological and entomological measures
Units of analysis	Level of application (neighborhood, community)	Locality, geographical area
Study design	Pre-post, time series, cross-sectional, ecological	Randomized controlled trials (clusters), phased incorporation, cohort, time series
Sample size	According to the locality selected	Extensive areas of high transmission in order to measure impact on the disease or infection
Control of external variables	Invasion from surrounding areas, migration of species	Human mobility and migration of species
Implementation	Production, release, and maintenance of the modified species	Introduction, replacement, maintenance, and evaluation of effect on transmission
How to measure results	Entomological indicators, frequency, and number of mosquitoes released, monitoring	Entomological and epidemiological indicators, monitoring of effect over two cycles of transmission
Information system	Entomological surveillance system	Entomological and epidemiological surveillance system
Infrastructure	Insectariums, entomology and diagnostic laboratory (PCR in mosquitoes, molecular biology)	Diagnostic laboratory (serology, virus isolation, PCR and molecular biology)
Personnel	Well-trained entomologists and technical field staff	Well-trained epidemiologists, entomologists, and medical, paramedical, and technical field staff

Adapted from: Wilson A, Lindsay S, et al. *How to design vector control efficacy trials: Guidance on phase III vector control field trial design provided by the Vector Control Advisory Group*. Geneva: World Health Organization; 2017.

Entomological indicators

In the case of population suppression strategies, entomological parameters will be the main indicators used to measure the pace of elimination and its maintenance over time. The evaluation will need to be conducted at the population level (egg, larval, and adult density over time), but will also need to be stage-specific, depending on the type of biological or genetic modification: sterility, early or late lethality, dispersal (flight), feeding, etc.

If only the reduction of vector populations is achieved, the study should measure the vector density threshold below which transmission of DENV infection cannot occur or estimate the rates of reduction of the infection in the treated populations in comparison with those in control areas (Table 14).[93]

Table 14. Evaluation of strategies for suppression and replacement of *Aedes aegypti* populations (entomological indicators)

Biological-functional phase or stage Vector competence	Indicators	Strategy	
		Suppression / elimination	Replacement / interference with infection
Traditional entomology			
Egg	Oviposition	Desirable	Optional
Larva	CI, HI, BI, 3rd and 4th stage indices	Essential	Essential
Pupa	Pupal surveys by individual, house, area	Desirable	Optional
Adult	Adult index per house *(A. aegypti)* Females per house	Optional	Desirable
Proactive entomology			
Parity rate	Ratio nulliparous / multiparae	Desirable	Optional
Fecundity (production)	Mean number of eggs per female	Desirable	Optional
Fecundity (hatch)	Percentage of eggs that hatch per unit of time	Desirable	Optional
Survival	Mean age (days)	–	Desirable
Dispersal	Competitiveness (range of flight)	Desirable	Optional
Vector competence			
Viral infection rate Intrathoracic route or fed with infected blood	Percentage of female mosquitoes infected	-	Essential
Wolbachia infection density	Percentage of infected eggs or adults	Desirable	Essential
Genetic marker	Percentage of eggs or adults with genetic marker	Desirable	Essential

Assessment of interference with infection

To evaluate replacement strategies, both entomological and epidemiological indicators will be necessary. With regard to the former, it will not be sufficient to record the presence or absence of eggs or egg, larval, pupal, or adult density; it will also be necessary to investigate the progeny and the strains from which such populations come (wild or modified) and their spread in the population, as well as their performance in terms of oviposition, hatch rate, size of larvae and pupae, mating with wild species, fecundity, range of dispersal, age (survival), bite rate, vector competence (infection of adult females or eggs), etc.

If the aim is population replacement or interference with infection, entomological parameters will be needed to measure vector competence, which will also require a good laboratory that can carry out molecular biology tests that demonstrate the infection in adult females (Tables 14 and 15).

Table 15. Control interventions and epidemiological impact indicators by type of epidemiological surveillance

Type of surveillance		Passive		Active		Proactive	
Type of intervention		Probable cases	Positive cases	Secondary cases in families of positive cases	*Clusters* of positive cases	Sero-conversion (cohorts)	Virus in mosquitoes
Physical	Clean-up of breeding sites	×	×				
	Basic sanitation	×	×				
	Health promotion	×	×				
	Physical barriers: mosquito nets, curtains	×	×	×			
Chemical	Temephos	×	×	×			
	Repellents	×	× (individual)	× (spatial)			
	Household spraying	×	×	×	×		×
	Fogging	×	×	×	×		×
Combined/ insecticides	Nets and curtains	×	×	×			×
	Clothing	×	×				
Biological	Copepods	×	×	×			
	B. thuringiensis var. *israelensis*	×	×	×			
	Fish	×	×	×			
	Wolbachia	×	×	×	×	×	×
Modified mosquitoes	Genetically modified	×	×	×	×	×	×
	Irradiated mosquitoes	×	×	×	×	×	×

Estimating the Costs of Control Innovations

Estimating the costs of *Aedes aegypti* control interventions is a complex task, given the diversity of control options (physical, biological, chemical, educational), the technical and human resources involved, the geographical coverage, the need for repeated application, and variations in effectiveness due to the transitory effects of each intervention.

Moreover, the cost of control programs has increased owing to the increase in numbers of local health workers, program technical staff, inputs for vector control, and requirements for sustaining the entomological and epidemiological surveillance systems needed to monitor the desired impact.

In recent decades numerous studies on the costs associated with the dengue burden have been published at different levels (local, national, regional,[94] or global[95]). They use different metrics of harm (outpatient[96] or hospitalized dengue cases) and cost (direct and indirect). The estimates are variable owing to differences in calculation methods and the weight given to existing cases (infected or reported),[97] estimated underreporting, and the severity of the disease and the associated disability.[98,99]

In Latin America, cost estimate exercises have been carried out in Brazil,[100] Cuba,[101] Nicaragua,[102] Panama,[103] Puerto Rico,[104] and Mexico.[105] These studies focused on the estimation of direct and indirect costs of the disease, but few included the costs associated with prevention, surveillance, and, especially, control measures.

The estimates are limited to estimating the cost of epidemics in a locality or country, which may range from US$ 0.3 million (in Santiago de Cuba) to US$ 103 million (for the country as a whole) and from US$ 299 to US$ 594 per case.[106] In Nicaragua, the cost of an epidemic was estimated at US$ 2.7 million and the cost per case at US$ 44. In Puerto Rico, the cost of an epidemic was US$ 1.2 million and the cost per case was between US$ 23 and US$ 36. In Panama, the direct and indirect costs of an epidemic—

including surveillance, prevention, and control services—amounted to US$ 16.9 million, while the approximate cost per case was US$ 332 for outpatient cases and US$ 1,065 for hospitalized cases.

Experience has shown that these studies generally do not estimate the cost of operating vector control programs—a cost that is substantial. The evidence indicates that including surveillance, prevention, and vector control in the estimate can boost the costs by 39% in Thailand, 43% in Panama, and up to 49% in Puerto Rico.[107]

For technological innovations based on genetic modification or *Wolbachia* infection, new variables must be incorporated in the cost estimate: the construction of facilities to mass produce the modified insects, materials and resources for their repeated release, continuous monitoring of their penetration and replacement of the native population, and epidemiological impact assessment.

The estimated average cost of producing irradiated mosquitoes or mosquitoes with lethal genes, for example, is US$ 813 per million insects released, while the cost per case prevented is between US$ 20 and US$ 30 if the ratio of mosquitoes released per person is 10:1. These estimates assume that the lethality and competitiveness of the released mosquitoes are very high and are sustained throughout the period of intervention, and that any reductions in these parameters would necessitate more releases, which would obviously increase the costs. These estimates do not include the cost of the entomological surveillance needed to monitor impact. In contrast, a study by Suaya et al. (2009) found that the average cost of providing care for a case of dengue ranges from US$ 86 to US$ 190, and in the most severe cases the cost may rise to between US$ 357 and US$ 793.[25]

These data suggest that technological innovations may substantially reduce the costs of intervention. However, models need to be adjusted to the context of countries that lack the basic infrastructure to achieve the required levels of mass mosquito production and also lack a corps of specialized professionals who can monitor and evaluate such interventions.

Countries that decide to incorporate these technological innovations into their control programs will have to assume these additional costs (which will not be low) and continue to cover them alongside the costs of traditional vector control programs. Since these interventions are considered a complement to, not a substitute for, the control program, it will be very important to generate the epidemiological, entomological, and economic information needed to identify the scenarios in which they can be appropriately implemented, scaled up, monitored, and evaluated.

Meeting these information needs will also entail additional costs for countries, but they have not yet been included in any model for the application of these innovations.

Final Considerations

The innovations described here are associated with various benefits:

- They are environmentally friendly, since, unlike insecticides, they do not leave any toxic residue.
- Their specificity makes them more effective, since they affect a dominant vector in the transmission of an infection.
- They are relatively easy to produce on a large-scale.
- Their sustainability over time and the absence of resistance gives them an advantage over traditional control tools.
- They do not require access to housing (they are not invasive), unlike other interventions, which makes them even more attractive as a control tool.

An additional benefit of the release of genetically modified or infected adult males is that they do not bite or bother people, nor do they transmit infection.

The introduction of other technological innovations that promise greater coverage, impact, and sustainability is proposed to improve the effectiveness and lasting impact of interventions. These innovations also entail difficulties with regard to organizational and operating capacity which should be addressed before, during, and after their introduction as control measures.

An additional problem is the combination of strategies (not their integration) and differential evaluation of their impact, since an intervention can modify the physical availability of breeding sites (clean-up), but may not reduce vector densities or necessarily control the most stable and productive breeding sites.

Moreover, it has not been demonstrated that the reduction in egg or larval densities achieved with the available interventions is correlated with a decline in transmission. Nevertheless, the combined use of old strategies and the incorporation of new vector control tools creates several challenges:

(1) the use of indicators that specifically measure mosquito density at all stages of development (egg, larva, pupa, adult) in order to specifically evaluate available interventions;

(2) the definition of risk thresholds;

(3) the technical capacity of programs (human resources, equipment, financing) to carry out the intervention with the frequency and coverage required to undertake a proper assessment.[108,109]

The evidence suggests that technological innovations should be viewed as tools to complement vector control programs and that they should be introduced in a phased manner and in carefully selected locations until evidence of sustained impact has been collected and the potential risks associated with evolution of the modified species and the genetic or biological marker introduced have been addressed.

The role of each available strategy and technological innovation should be analyzed and defined, as should their level of use and the mechanisms for their introduction, monitoring, and evaluation. Bearing in mind the proposed guiding principles of this technical guide, it can be concluded that:

- There is sufficient evidence to indicate that they are effective tools; the evidence is not conclusive, but it is suggestive of their potential.
- Technological innovations should be complementary tools; the problem is determining how they are to be integrated into traditional control programs.
- Control programs should have the capacity to implement and evaluate them; there are significant training, organizational, and operational needs to be addressed in order to apply these innovations in specific situations.
- When and where they should be introduced and scaled-up: many conditions must be met to ensure the satisfactory application of technological innovations, from political commitment to community acceptance. Many technical and operational issues must also be addressed before contemplating their introduction.

References

1. McGraw EA, O'Neill SL. Beyond insecticides: new thinking on an ancient problem. *Nature Reviews, Microbiology* 2013; 11 (3): 181-193.
2. Scott TW, Takken W, Knols BG, Boëte C. The ecology of genetically modified mosquitoes. *Science* 2002; 298 (5591): 117-119.
3. Stanaway JD, Shepard DS, Undurraga EA, Halasa YA, Coffeng LE, et al. The global burden of dengue: an analysis from the Global Burden of Disease Study 2013. *Lancet Infect Dis* 2016; 16 (6): 712-723.
4. Weaver S, Forrester NL. Chikungunya: Evolutionary history and recent epidemic spread. *Antiviral Research* 2015; 120: 32-39.
5. Bogoch II, Brady OJ, Kraemer MUG, German M, Creatore MI, Kulkarni MA, et al. Anticipating the international spread of Zika virus from Brazil. Lancet 2016; 387 (10016): 335-336.
6. Vasconcelos PF, Calisher CH. Emergence of Human Arboviral Diseases in the Americas, 2000-2016. *Vector Borne Zoonotic Dis* 2016; 16 (5): 295-301.
7. Hotez PJ, Murray KO. Dengue, West Nile virus, chikungunya, Zika — and now Mayaro? *PLoS Negl Trop Dis* 2017; 11 (8): e0005462.
8. Slosek J. *Aedes aegypti* mosquitoes in the Americas: a review of their interactions with the human population. *Soc Sci Med* 1986; 23 (3): 249-257.
9. Messina JP, Brady OJ, Scott TW, Zou C, Pigott DM, Duda KA, et al. Global spread of dengue virus types: mapping the 70 year history. *Trends Microbiol* 2014; 22 (3): 138-146.
10. World Health Organization. *Sustaining the drive to overcome the global impact of neglected tropical diseases: Second WHO report on neglected diseases*. Geneva: WHO; 2013. http://www.who.int/iris/handle/10665/77950
11. Pan American Health Organization. The feasibility of eradicating *Aedes aegypti* in the Americas. *Rev Panam Salud Publica* 1997; 1 (1): 381-388.
12. Brathwaite Dick O, San Martín JL, Montoya RH, Del Diego J, Zambrano B, Dayan GH. Review: The history of dengue outbreaks in the Americas. *Am J Trop Med Hyg* 2012; 87 (4): 584-593.

13. Spiegel J, Bennett S, Hattersley L, Hayden M, Kittayapong P, Nalim S, et al. Barriers and bridges to prevention and control of dengue: the need for a social-ecological approach. *EcoHealth* 2005; 2 (4): 273-290.

14. World Health Organization. *Global Strategic Framework for Integrated Vector Management*. Geneva: WHO; 2004. http://whqlibdoc.who.int/hq/2004/WHO_CDS_CPE_PVC_2004_10.pdf

15. Wilke AB, Marrelli MT. Genetic control of mosquitoes: population suppression strategies. *Rev Inst Med Trop Sao Paulo* 2012; 54 (5): 287-292.

16. Knipling EF. Possibilities of insect control or eradication through the use of sexually sterile males. *Parasitology* 1955; 32 (3): 207-218.

17. Alphey L, Benedict M, Bellini R, Clark GG, Dame DA, Service MW, et al. Sterile-insect methods for control of mosquito-borne diseases: an analysis. *Vector Borne Zoonotic Dis* 2010; 10 (3): 295-311.

18. Bakri A, Mehta K, Lance DR. Sterilizing insects with ionizing radiation. En: Dyck VA, Hendrichs J, Robinson AS (eds.). *Sterile insect technique. Principles and practice in area-wide integrated pest management*. Países Bajos: Springer; 2005; pp. 233-68.

19. Andreasen MH, Curtis CF. Optimal life stage for radiation sterilization of Anopheles males and their fitness for release. *Med Vet Entomol* 2005; 19 (3): 238-244.

20. Helinski ME, Knols BG. Mating competitiveness of male *Anopheles arabiensis* mosquitoes irradiated with a partially or fully sterilizing dose in small and large laboratory cages. *J Med Entomol* 2008; 45 (4): 698-705.

21. Windbichler N, Papathanos PA, Crisanti A. Targeting the X chromosome during spermatogenesis induces Y chromosome transmission ratio distortion and early dominant embryo lethality in *Anopheles gambiae*. *PLoS Genet* 2008; 4 (12): e1000291.

22. Deredec A, Burt A, Godfray HC. Population genetics of using homing endonuclease genes in vector and pest management. *Genetics* 2008; 179 (4): 2013-2026.

23. Phuc HK, Andreasen MH, Burton RS, Vass C, Epton MJ, Pape G, et al. Late-acting dominant lethal genetic systems and mosquito control. *BMC Biol* 2007; 5: 11.

24. Thomas DD, Donnelly CA, Wood RJ, Alphey LS. Insect population control using a dominant, repressible, lethal genetic system. *Science* 2000; 287 (5462): 2474-2476.

25. Alphey N, Alphey L, Bonsall MB. A model framework to estimate impact and cost of genetics-based sterile insect methods for dengue vector control. *PLoS ONE* 2011; 6 (10): e25384.

26. Schliekelman P, Gould F. Pest control by the release of insects carrying a female-killing allele on multiple loci. *J Econ Entomol* 2000; 93 (6): 1566-1579.

27. Labbé GM, Scaife S, Morgan SA, Curtis ZH, Alphey L. Female-specific flightless (fsRIDL) phenotype for control of *Aedes albopictus*. *PLoS Negl Trop Dis* 2012; 6 (7): e1724.

28. Fu G, Lees RS, Nimmo D, Aw D, Jin L, Gray P, et al. Female-specific flightless phenotype for mosquito control. *Proc Natl Acad Sci USA* 2010; 107 (10): 4550-4554.

29. Wise de Valdez MR, Nimmo D, Betz J, Hong-Fei G, James A, Alphey L, et al. Genetic elimination of dengue vector mosquitoes. *Proc Natl Acad Sci USA* 2011; 108 (12): 4772-4775.

30. Harris AF, Nimmo D, McKemey AR, Kelly N, Scaife S, Donnelly CA, et al. Field performance of engineered male mosquitoes. Nat Biotechnol 2011; 29: 1034-1037.

31. Lacroix R, McKemey AR, Raduan N, Kwee Wee L, Hong Ming W, et al. Open field release of genetically engineered sterile male *Aedes aegypti* in Malaysia. *PLoS ONE* 2012; 7 (8): e42771.

32. Carvalho DO, McKemey AR, Garziera L, Lacroix R, Donnelly CA, Alphey L, et al. Suppression of a field population of *Aedes aegypti* in Brazil by sustained release of transgenic male mosquitoes. *PLoS Negl Trop Dis* 2015; 9 (7): e0003864.

33. Caragata EP, Dutra HLC, Moreira LA. Exploiting intimate relationships: Controlling mosquito-transmitted disease with *Wolbachia*. *Trends in Parasitology* 2016; 32 (3): 207-218.

34. Werren JH, Zhang W, Guo LR. Evolution and phylogeny of *Wolbachia*: reproductive parasites of arthropods. *Proc Biol Sci* 1995; 261 (1360): 55-63.

35. Rousset F, Bouchon D, Pintureau B, Juchault P, Solignac M. *Wolbachia* endosymbionts responsible for various alterations of sexuality in arthropods. *Proc Biol Sci* 1992; 250 (1328): 91-98.

36. Jiggins FM, Hurst GDD, Majerus MEN. Sex ratio distortion in *Acraea encedon* is caused by a male-killing bacterium. *Heredity* 1998; 81: 87-91.

37. Hurst GDD. *Wolbachia*, cytoplasmic incompatibility, and the evolution of eusociality. *J Theor Biol* 1997; 184: 99-100.

38. Moreira LA, Iturbe-Ormaetxe I, Jeffery JA, Lu G, Pyke AT, Hedges LM, et al. A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, Chikungunya, and Plasmodium. *Cell* 2009; 24; 139 (7): 1268-1278.

39. Aliota MT, Walker EC, Uribe Yepes A, Dario Velez I, Christensen BM, Osorio JE. The *w*Mel strain of *Wolbachia* reduces transmission of Chikungunya virus in *Aedes aegypti*. *PLoS Negl Trop Dis* 2016; 10 (4): e0004677.

40. Stouthamer R1, Breeuwer JA, Hurst GD. *Wolbachia pipientis*: microbial manipulator of arthropod reproduction. *Annu Rev Microbiol* 1999; 53: 71-102.

41. Ritchie S. Rear and release: a new paradigm for dengue control. *Austral Entomology* 2014; 53 (4): 363-367.

42. Rainey SM, Shah P, Kohl A, Dietrich I. Understanding the *Wolbachia*-mediated inhibition of arboviruses in mosquitoes: progress and challenges. *J Gen Virol* 2014; 95 (Pt 3): 517-530.

43. Joubert DA, Walker T, Carrington LB, De Bruyne JT, Kien DHT, Hoang NLT, et al. Establishment of a *Wolbachia* superinfection in *Aedes aegypti* mosquitoes as a potential approach for future resistance management. *PLoS Pathog* 2016; 12 (2): e1005434.

44. Iturbe-Ormaetxe I, Walker T, O'Neill S. *Wolbachia* and the biological control of mosquito-borne disease, *EMBO Reports* 2011; 12 (6): 508-518.

45. Olson KE, Adelman ZN, Travanty EA, Sánchez-Vargas I, Beaty BJ, Blair CD. Developing arbovirus resistance in mosquitoes. *Insect Biochem Mol Biol* 2002; 32 (10): 1333-1343.

46. Franz AW, Sánchez-Vargas I, Adelman ZN, Blair CD, Beaty BJ, James AA, et al. Engineering RNA interference-based resistance to dengue virus type 2 in genetically modified *Aedes aegypti*. *Proc Natl Acad Sci USA* 2006; 103 (11): 4198-4203.

47. Mathur G, Sánchez-Vargas I, Álvarez D, Olson KE, Marinotti O, James AA. Transgene-mediated suppression of dengue viruses in the salivary glands of the yellow fever mosquito, *Aedes aegypti*. *Insect Mol Biol* 2010; 19: 753-763.

48. Franz AW, Sánchez-Vargas I, Piper J, Smith MR, Khoo CC, James AA, et al. Stability and loss of a virus resistance phenotype over time in transgenic mosquitoes harbouring an antiviral effector gene. *Insect Mol Biol* 2009; 18 (5): 661-672.

49. Sperança MA, Capurro ML. Perspectives in the control of infectious diseases by transgenic mosquitoes in the post-genomic era – a review. *Mem Inst Oswaldo Cruz* 2007; 102 (4): 425-433.

50. Raikhel AS, Kokoza VA, Zhu J, Martin D, Wang SF, Li C, et al. Molecular biology of mosquito vitellogenesis: from basic studies to genetic engineering of antipathogen immunity. *Insect Biochem Mol Biol* 2002; 32 (10): 1275-1286.

51. Frentiu FD, Zakir T, Walker T, Popovici J, Pyke AT, Van den Hurk A, et al. Limited dengue virus replication in field-collected *Aedes aegypti* mosquitoes infected with Wolbachia. *PLoS Negl Trop Dis* 2014; 8 (2): e2688.

52. Van den Hurk AF, Hall-Mendelin S, Pyke AT, Frentiu FD, McElroy K, et al. Impact of *Wolbachia* on infection with chikungunya and yellow fever viruses in the mosquito vector *Aedes aegypti*. *PLoS Negl Trop Dis* 2012; 6 (11): e1892.

53. Glaser RL, Meola MA. The native *Wolbachia* endosymbionts of *Drosophila melanogaster* and *Culex quinquefasciatus* increase host resistance to West Nile virus infection. *PLoS ONE* 2010; 5 (8): e11977.

54. Rance E, Ye YH, Woolfit M, McGraw EA, O'Neill SL. The relative importance of innate immune priming in *Wolbachia*-mediated dengue interference. *PLoS Pathog* 2012: 8(2): e1002548.

55. Segoli M, Hoffmann AA, Lloyd J, Omodei GJ, Ritchie SA. The effect of virus-blocking *Wolbachia* on male competitiveness of the dengue vector mosquito, *Aedes aegypti*. *PLoS Negl Trop Dis* 2014; 8 (12): e3294.

56. Lu P, Bian G, Pan X, Xi Z (2012). *Wolbachia* induces density-dependent inhibition to dengue virus in mosquito cells. *PLoS Negl Trop Dis* 2012; 6 (7): e1754.

57. Blagrove MS, Arias-Goeta C, Failloux AB, Sinkins SP. *Wolbachia* strain *w*Mel induces cytoplasmic incompatibility and blocks dengue transmission in *Aedes albopictus*. *Proc Natl Acad Sci USA* 2012; 109 (1): 255-260.

58. Martínez J, Longdon B, Bauer S, Chan YS, Miller WJ, et al. Symbionts commonly provide broad spectrum resistance to viruses in insects: a comparative analysis of *Wolbachia* strains. *PLoS Pathog 2014;* 10 (9): e1004369.

59. Hoffmann AA, Iturbe-Ormaetxe I, Callahan AG, Phillips BL, Billington K, et al. Stability of the wMel Wolbachia infection following invasion into *Aedes aegypti* populations. *PLoS Negl Trop Dis* 2014; 8 (9): e3115.

60. McMeniman CJ, Lane RV, Cass BN, Fong AW, Sidhu M, Wang YF, et al. Stable introduction of a life-shortening *Wolbachia* infection into the mosquito *Aedes aegypti*. Science 2009; 323 (5910): 141-144.

61. Kambris Z, Cook PE, Phuc HK, Sinkins SP. Immune activation by life-shortening *Wolbachia* and reduced filarial competence in mosquitoes. *Science* 2009; 326 (5949): 134-136.

62. Pan X, Zhou G, Wu J, Bian G, Lu P, Raikhel AS, et al. *Wolbachia* induces reactive oxygen species (ROS)-dependent activation of the Toll pathway to control dengue virus in the mosquito *Aedes aegypti*. *Proc Natl Acad Sci USA* 2012; 109 (1): E23-31.

63. Xi Z, Khoo CCH, Dobson SL. Interspecific transfer of *Wolbachia* into the mosquito disease vector Aedes albopictus. *Proc Biol Sci* 2006; 273 (1592): 1317-1322.

64. Yeap HL, Mee P, Walker T, Weeks AR, O'Neill SL, Johnson P, et al. Dynamics of the "popcorn" *Wolbachia* infection in outbred *Aedes aegypti* informs prospects for mosquito vector control. *Genetics* 2011; 187 (2): 583-595.

65. Ross PA, Wiwatanaratanabutr I, Axford JK, White VL, Endersby-Harshman NM, Hoffmann AA. *Wolbachia* Infections in *Aedes aegypti* differ markedly in their response to cyclical heat stress. *PLoS Pathog* 2017; 13 (1): e1006006.

66. Yeap HL, Axford JK, Popovici J, Endersby NM, Iturbe-Ormaetxe I, Ritchie SA et al. Assessing quality of life-shortening *Wolbachia*-infected *Aedes aegypti* mosquitoes in the field based on capture rates and morphometric assessments. *Parasites & Vectors* 2014, 7: 58.

67. Benedict M, D'Abbs P, Dobson S, Gottlieb M, Harrington L, Higgs S, et al. Guidance for contained field trials of vector mosquitoes engineered to contain a gene drive system: recommendations of a scientific working group. *Vector Borne Zoonotic Dis* 2008; 8 (2): 127-166.

68. James AA. Gene drive systems in mosquitoes: rules of the road. *Trends in Parasitology* 2005; 21 (2): 64 67.

69. Marshall J. The effect of gene drive on containment of transgenic mosquitoes. *J Theor Biol* 2009; 258 (2): 250-265.

70. Reeves RG, Denton JA, Santucci F, Bryk J, Reed FA. Scientific standards and the regulation of genetically modified insects. *PLoS Negl Trop Dis* 2012; 6 (1): e1502.

71. Murray JV, Jansen CC, De Barro P. Risk associated with the release of *Wolbachia*-infected *Aedes aegypti* mosquitoes into the environment in an effort to control dengue. *Front Public Health* 2016; 4: 43.

72. Brown DM, Alphey LS, McKemey A, Beech C, James AA. Criteria for identifying and evaluating candidate sites for open-field trials of genetically engineered mosquitoes. *Vector Borne Zoonotic Dis* 2014; 14 (4): 291-299.

73. Ramsey JM, Bond JG, Macotela ME, Facchinelli L, Valerio L, Brown DM, et al. A regulatory structure for working with genetically modified mosquitoes: lessons from Mexico. *PLoS Negl Trop Dis* 2014; 8 (3): e2623.

74. Lavery JV, Harrington LC, Scott TW. Ethical, social, and cultural considerations for site selection for research with genetically modified mosquitoes. *Am J Trop Med Hyg* 2008; 79 (3): 312-318.

75. Ferguson NM, Kien DT, Clapham H, Aguas R, Trung VT, Chau TN, et al. Modeling the impact on virus transmission of *Wolbachia*-mediated blocking of dengue virus infection of *Aedes aegypti*. *Science Translational Medicine* 2015; 7 (279): 279ra37.

76. Alphey L, McKemey A, Nimmo D, Neira Oviedo M, Lacroix R, Matzen K, et al. Genetic control of Aedes mosquitoes. *Pathogens and Global Health* 2013; 107 (4): 170-177.

77. Benedict MQ, Robinson AS. The first releases of transgenic mosquitoes: an argument for the sterile insect technique. *Trends Parasitol* 2003; 19 (8): 349-355.

78. Wilson A, Lindsay S, et al. *How to design vector control efficacy trials: Guidance on phase III vector control field trial design provided by the Vector Control Advisory Group*. WHO_HTM_NTD_WEM_2017.03. Geneva: World Health Organization; 2017.

79. Bouzid M, Brainard J, Hooper L, Hunter PR. Public health interventions for *Aedes* control in the time of Zikavirus – a meta-review on effectiveness of vector control strategies. *PLoS Negl Trop Dis* 2016; 10 (12): e0005176.

80. Bowman LR, Donegan S, McCall PJ. Is dengue vector control deficient in effectiveness or evidence? Systematic review and meta-analysis. *PLoS Negl Trop Dis* 2016; 10 (3): e0004551.

81. Alvarado Castro V, Paredes Solís S, Nava Aguilera E, Morales Pérez A, Alarcón Morales L, Balderas Vargas NA, et al. Assessing the effects of interventions for *Aedes aegypti* control: systematic review and meta-analysis of cluster randomised controlled trials. *BMC Public Health* 2017; 17 (Suppl 1): 384.

82. Pereira Lima E, Oliveira Fonseca Goulart M, Leite Rolim Neto M. Meta-analysis of studies on chemical, physical and biological agents in the control of *Aedes aegypti*. *BMC Public Health* 2015; 15: 858.

83. Morrison AC, Minnick SL, Rocha C, Forshey BM, Stoddard ST, Getis A, et al. Epidemiology of dengue virus in Iquitos, Peru, 1999 to 2005: interepidemic and epidemic patterns of transmission. *PLoS Negl Trop Dis* 2010; 4 (5): e670.

84. World Health Organization, Special Programme for Research and Training in Tropical Diseases (TDR). *Dengue: guidelines for diagnosis, treatment, prevention and control: new edition*. Geneva: World Health Organization; 2009.

85. Bowman L, Runge-Ranzinger S, McCall P. Assessing the relationship between vector indices and dengue transmission: a systematic review of the evidence. *PLoS Negl Trop Dis* 2014; 8 (5): e2848.

86. Focks DA and Special Programme for Research and Training in Tropical Diseases (TDR). *A review of entomological sampling methods and indicators for dengue vectors*. Geneva: World Health Organization; 2004. http://apps.who.int/iris/bitstream/10665/68575/1/TDR_IDE_DEN_03.1.pdf

87. Focks DA, Chadee DD. Pupal survey: an epidemiologically significant surveillance method for *Aedes aegypti:* an example using data from Trinidad. *Am J Trop Med Hyg* 1997; 56: 159-167.

88. Focks DA, Brenner RJ, Hayes J, Daniels E. Transmission thresholds for dengue in terms of *Aedes aegypti* pupae per person with discussion of their utility in source reduction efforts. *Am J Trop Med Hyg* 2000; 62 (1): 11-18.

89. Focks DA, Alexander N. *Multicountry study of Aedes aegypti pupal productivity survey methodology: findings and recommendations.* Geneva: World Health Organization; 2006. https://extranet.who.int/iris/restricted/bitstream/10665/69354/1/TDR_IRM_DEN_06.1_eng.pdf

90. Brown CA, Lilford RJ. The stepped wedge trial design: a systematic review. *BMC Medical Research Methodology* 2006; 6: 54.

91. Reiner RC Jr, Achee N, Barrera R, Burkot TR, Chadee DD, Devine GJ, et al. Quantifying the epidemiological impact of vector control on dengue. *PLoS Negl Trop Dis* 2016; 10 (5): e0004588.

92. Lambrechts L, Ferguson NM, Harris E, Holmes EC, McGraw EA, O'Neill SL, et al. Assessing the epidemiological effect of *Wolbachia* for dengue control. *Lancet Infect Dis* 2015; 15 (7): 862-866.

93. Lambrechts L. Predicting *Wolbachia* potential to knock down dengue virus transmission. *Annals of Translational Medicine* 2015; 3 (19): 288.

94. Suaya JA, Shepard DS, Siqueira JB, et al. Cost of dengue cases in eight countries in the Americas and Asia: a prospective study. *Am J Trop Med Hyg* 2009; 80 (5): 846-855.

95. Gubler DJ, Meltzer M. Impact of dengue/dengue hemorrhagic fever on the developing world. *Adv Virus Res* 1999; 53: 35-70.

96. Huy R, Wichmann O, Beatty M, Ngan C, Duong S, Margolis HS, et al. Cost of dengue and other febrile illnesses to households in rural Cambodia: a prospective community-based case-control study. *BMC Public Health* 2009; 9: 155.

97. Garg P, Nagpal J, Khairnar P, Seneviratne SL. Economic burden of dengue infections in India. *Trans R Soc Trop Med Hyg* 2008; 102 (6): 570-577.

98. Meltzer MI, Rigau-Perez JG, Clark GG, Reiter P, Gubler DJ. Using disability-adjusted life years to assess the economic impact of dengue in Puerto Rico: 1984-1994. *Am J Trop Med Hyg* 1998; 59 (2): 265-271.

99. Lum LC, Suaya JA, Tan LH, Sah BK, Shepard DS. Quality of life of dengue patients. *Am J Trop Med Hyg* 2008; 78 (6): 862-867.

100. Luz PM, Grinsztejn B, Galvani AP. Disability adjusted life years lost to dengue in Brazil. *Trop Med Int Health* 2009; 14 (2): 237-246.

101. Valdes LG, Mizhrahi JV, Guzman MG. Impacto económico de la epidemia de dengue 2 en Santiago de Cuba, 1997. *Rev Cubana Med Trop* 2002; 54 (3): 220-227.

102. Ferrando J. *Estimate of the costs of the dengue epidemic in 1994 in Nicaragua.* Washington, DC: Pan American Health Organization; 1995.

103. Armien B, Suaya JA, Quiroz E, Sah BK, Bayard V, Marchena L, et al. Clinical characteristics and national economic cost of the 2005 dengue epidemic in Panama. *Am J Trop Med Hyg* 2008; 79 (3): 364-371.

104. Von Allmen SD, López Correa RH, Woodall JP, Morens DM, Chiriboga J, Casta Vélez A. Epidemic dengue fever in Puerto Rico, 1977: a cost analysis. *Am J Trop Med Hyg* 1979; 28 (6): 1040-1044.

105. Undurraga EA, Betancourt Cravioto M, Ramos Castañeda J, Martínez Vega R, Méndez Galván J, Gubler D J, et al. Economic and disease burden of dengue in Mexico. *PLoS Negl Trop Dis* 2015; 9 (3): e0003547.

106. Guzmán MG, Triana C, Bravo J, Kourí G. The estimation of the economic damages caused as a consequence of the epidemic of hemorrhagic dengue in Cuba in 1981. *Rev Cubana Med Trop* 1992; 44 (1): 13-7.

107. Shepard DS. Aggregate economic cost of dengue in Puerto Rico. 58th Annual Meeting of the American Society of Tropical Medicine and Hygiene (ASTMH). Washington, DC; 2009.

108. Morrison AC, Zielinski-Gutierrez E, Scott TW, Rosenberg R. Defining challenges and proposing solutions for control of the virus vector *Aedes aegypti. PLoS Med* 2008; 5 (3): e68.

109. Achee NL, Gould F, Perkins TA, Reiner RC Jr, Morrison AC, Ritchie SA, et al. A critical assessment of vector control for dengue prevention. *PLoS Negl Trop Dis* 2015; 9 (5): e0003655.

Annex

Menu of vector control interventions

Environmental management

The aim of environmental management is to modify the environment where the *Aedes aegypti* mosquito develops and lives.[1,2,3] There are three types of environmental management intervention:[4]

Environmental modification: Permanent structural changes to reduce *Aedes* larvae habitats. For example: installation and distribution of piped drinking water in communities and dwellings.

Environmental manipulation: Temporary physical barriers in *Aedes* habitats. For example: elimination (clean-up) or proper management (by covering, turning over, or washing out) of breeding sites, in addition to the recycling of specific objects that can serve as breeding sites, such as tires. Environmental manipulation is intended to reduce the density and positivity of breeding sites (through clean-up campaigns) and is usually accompanied by community participation strategies and education campaigns that promote the behavioral changes needed to modify household control practices.[5,6,7,8,9]

Changes in housing: Lasting physical barriers for reducing *Aedes*–human contact. For example: installation of mosquito nets or use of insecticide-impregnated materials such as curtains and nets in doorways and windows to achieve more effective protection of housing and people.[10] The use of insecticide-impregnated materials can produce a marked and prolonged reduction in vector populations as measured by Breteau indices and indices of positive houses and pupae per person. However, it is not clear how the use of such materials may influence the abundance of adult mosquito populations or vector–human contact.

Social mobilization and community participation

The ineffectiveness of control programs has shown the need for effective social mobilization and mass communication to bring about changes at the community level, rather than only at the individual level, and the need to promote the sustainability of control strategies.[11] Hence, behavioral change and social mobilization have been proposed as engines of change.[12]

The social and communication sciences have shown that for interventions to be sustainable and successful, the community must be involved from the outset in their design and implementation. It is also now recognized that mobilizing social networks rather than individuals, involving diverse members and segments of the community, and promoting change among operational personnel, in the organization of programs, and in legislation are important elements for success.[14,15,16]

Community participation and social mobilization strategies are vector control alternatives, although the intensity and duration of their use varies, as does the coverage of the target or recipient population. In general, mass mobilization experiences tend to be short-term undertakings (campaigns to clean up and eliminate breeding sites, dengue awareness days, etc.). They may also be of longer duration but selective with regard to the groups involved (schools,[17] health committees, social groups, environmental groups, municipal groups, etc.) or the aspect of vector control targeted (most productive breeding sites, management of tires, etc.). In contrast, traditional programs tend to emphasize the continuity of actions over their sustainability, and they favor repetition over strengthening or feedback.[18]

The lack of appropriate indicators for assessing behavioral change among the members of endemic communities limits the capacity of programs to monitor and evaluate the effectiveness of the strategies employed. Studies differ in terms of type of intervention, behavioral patterns to be modified, target population, and outcome indicators, which makes it difficult to identify the factors acted upon.

In general, conclusions on the impact of community participation measures, whether as a standalone intervention or in combination with other actions, have not been very favorable, especially because it is not clear how human behaviors and their relationship with entomological or epidemiological impact indicators can be measured.[19,20]

Educational interventions

Interventions of this type are intended to modify household and urban environmental practices (waste management) in order to achieve a positive impact on the control of larval densities. However, there is a big difference between what is learned through the educational intervention and what is done with that knowledge, how long the knowledge is retained, and how often it needs to be reinforced. A systematic review suggests that the impact may last up to 24 months when education occurs as part of a community-based intervention, although impact by type of educational material used was not assessed.[21]

Personal protection

Aedes–human contact can be prevented with chemical repellents that are applied individually[22] or spatially, actively (e.g., coils, tablets) or passively (on paper strips).[23,34] The use of passive spatial repellents in combination with lethal ovitraps or insecticide-impregnated materials can be an effective strategy against *Aedes*–human contact, which can be complemented with rational use of insecticides.

Biological control

Biological control consists in introducing pathogens, parasites, or predators into breeding habitats during the early stages of mosquito development in order to reduce populations. Biological control agents include the mosquitocidal bacterium *Bacillus thuringiensis israelensis*, larvivorous fish of the genera *Gambusia* and *Poecilia*,[25] tilapia, and certain species of predatory copepods (*Mesocyclops longisetus*).[26,27,28]

B. thuringiensis israelensis reduces larval densities for up to four weeks, and it is therefore not recommended as an isolated or long-term measure.[29] As for larvivorous fish, their impact on larval densities can be significant (lasting up to two years), but it varies with the type of fish, their longevity in the container (tank, pool), and acceptance of members of the community. Although they have been found to be more effective than B. *thuringiensis* and household spraying, the evidence is not conclusive.[30]

There are also substances that have a selective effect on *Aedes*; normally they resemble an essential metabolite or a toxin and pose a low risk for humans, wildlife, and the environment. Examples include chemical analogs that function as insect growth regulators.

Chemical larval control

The use of larvicides to prevent larval development in water reservoirs is an essential component of the vast majority of national programs in the Region of the Americas.

The traditional approach consists in eliminating (destroying) breeding sites from the environment or neutralizing them with a larvicide, whose application requires a search of broad urban areas for water containers or vessels so that the larvicide can be applied with variable frequency regardless of container size, capacity, or productive potential; seasonal stability; or community acceptance (resistance).

Interventions using temephos reduce larval densities, but they are not sustainable over time. Their effectiveness at the community level is determined by quality of delivery, type of water in the container and its intended use (consumption, cleaning, storage), and environmental factors (temperature, food supply, light exposure). The effectiveness of such interventions is limited by the need for repeated application, community resistance to temephos use in drinking water, and operational problems such as coverage, opportunity, time required, and the costs of extensive application of the insecticide to all potential containers. Furthermore, there is no evidence of effect on transmission.[31,32]

An innovative approach is to target efforts towards the most productive breeding sites, not only of larvae but of the more advanced stages (pupae), as breeding sites vary in terms of size, capacity, permanence, productivity, and control alternatives.[33,34]

As an undesirable effect, the continuous application of chemical substances by vector control personnel strengthens communities' perception that the government is responsible for all aspects of vector control and that residents have little or no responsibility.[35]

Control of adult mosquitos and protection from vector–human contact

Most national dengue prevention and control programs rely on insecticides to control larvae and adult mosquitoes. Chemical control of *A. aegypti* adults involves the use of insecticides with a view to immediately reducing population density and survival and thus halting the transmission of viruses by decreasing vector–human contact.

Normally, most funding under control program budgets is allocated to staff salaries, procurement of chemical products, and the purchase of application equipment. Chemical products have an important role in an integrated prevention and control program, although before any product is applied there should be a critical assessment of the susceptibility of local mosquitoes to the product selected and of how, when, and where each product is to be used. Routine monitoring of mosquito susceptibility to insecticides should be part of any chemical control program.[36]

Adulticides are applied in the following ways:

Spatial spraying. Spatial sprays are applied in a non-residual ultra-low volume (ULV) formulation by means of cold or thermal fogging in open areas and spaces, either on the ground from heavy equipment mounted on vehicles or from the air from light aircraft or helicopters. They can also be applied using motorized backpack sprayers for rapid low-volume spraying inside houses, rooms, buildings, and other enclosed spaces, thereby reaching natural *Aedes* shelters. Spatial spraying alone is not recommended as an effective means of control; in combination with other control measures, it offers variable results.[37,38,39]

Residual treatments. Residual treatment involves the spraying of residual insecticides: (a) with manual compression pumps for traditional spraying, or (b) with backpack sprayers for spraying surfaces (walls and ceilings) in homes and their outbuildings.

It has recently been proposed that residual insecticides should be applied in ovitraps treated with an attractant (as a "lure-and-kill" strategy)[40,41,42] and in insecticide-impregnated materials such as curtains in doors and windows,[43] breeding site covers, and mosquito nets. Theoretically, in both scenarios, adult survival would be affected as a result of contact with insecticide-treated surfaces. The use of permethrin-impregnated nets has also proved effective in reducing *A. aegypti* for several months and has had an impact on dengue transmission.[44]

Most adult control interventions involve the application of insecticide in open spaces, using vehicle-mounted heavy equipment. Manual ULV spraying from a land vehicle or aircraft have shown a high degree of effectiveness (close to 100%) in bioassays.[45,46,47,48,49] However, the effectiveness of this method declines considerably if it is evaluated with entomological indicators based on field collections, for example the presence and the number of eggs collected with ovitraps or the number of resting females inside houses.[50]

Environmental spraying is relatively ineffective as a routine control strategy[51] and should therefore be used only as an emergency response to outbreaks. The effectiveness of this practice is variable, possibly because the spray does not penetrate in interior spaces where adult mosquitoes rest; moreover, the application procedure is costly.[52] However, reducing populations in the peridomestic space indirectly reduces the proportion of adults entering houses to rest.[39] Factors to be borne in mind are that the lethal effect is transitory and that mosquito populations normally recover within one or two weeks

References

1. Pan American Health Organization (PAHO). *Dengue and dengue hemorrhagic fever in the Americas: guidelines for prevention and control.* Washington, DC: PAHO; 1994.
2. World Health Organization (WHO). *Manual on environmental management for mosquito control with special emphasis on malaria vectors.* Geneva: WHO; 1982.
3. Erlanger T, Keiser J, Utzinger J. Effect of dengue vector control interventions on entomological parameters in developing countries: a systematic review and meta-analysis. *Medical and Veterinary Entomology* 2008; 22 (3): 203-221.
4. Keiser J, Singer BH, Utzinger J. Reducing the burden of malaria in different eco-epidemiological settings with environmental management: a systematic review. *Lancet Infect Dis* 2005; 5 (11): 695-708.
5. Leontsini E, Gil E, Kendall C, Clark G. Effect of a community-based *Aedes aegypti* control programme on mosquito larval production sites in El Progreso, Honduras. *Trans Royal Soc Trop Med Hyg* 1993; 87 (3): 267-271 .
6. Fernández EA, Leontsini E, Sherman C, Chan AS, Reyes CE, Lozano RC, et al. Trial of a community-based intervention to decrease infestation of *Aedes aegypti* mosquitoes in cement washbasins in El Progreso, Honduras. *Acta Tropica* 1998; 70 (2): 171-183.
7. De Caires P. *Aedes aegypti* control in the absence of a piped water potable water supply. *American Journal of Tropical Medicine* 1947; 27 (6): 733-743.
8. Winch PJ, Leontsini E, Rigau Pérez JG, Ruiz Pérez M, Clark GG, Gubler DJ. Community-based dengue prevention programs in Puerto Rico: impact on knowledge, behaviour, and residential mosquito infestation. *Am J Trop Med Hyg* 2002; 67 (4): 363-370.
9. Sánchez L, Pérez D, Pérez T, Sosa T, Cruz G, Kourí G, et al. Intersectoral coordination in *Aedes aegypti* control. A pilot project in Havana City, Cuba. *Trop Med Int Health* 2005; 10 (1): 82-91.
10. Ogoma SB, Lweitoijera DW, Ngonyani H, Furer B, Russell TL, Mukabana WR, et al. Screening mosquito house entry points as a potential method for integrated control of endophagic filariasis, arbovirus and malaria vectors. *PLoS Negl Trop Dis* 2010; 4 (8): e773.
11. Gómez Dantés H. Documenting inputs and outputs and learning from ecohealth projects: Dengue, IDRC technical report. Mayo del 2007.
12. Winch PJ. Social and cultural responses to emerging vector-borne diseases. *Journal of Vector Ecology* 1998; 23 (1): 47-53.

13. Winch PJ, Lloyd LS, Hoemeke L, Leontsini E. Vector control at the household level: an analysis of its impact on women. *Acta Tropica* 1994; 56 (4): 327-339.
14. Parks WJ, Lloyd LS, Nathan MB, Hosein E, Odugleh A, et al. International experiences in social mobilization and communication for dengue prevention and control. *Dengue Bulletin* 28; 2004 (supl.): 1-7.
15. Lloyd LS, Winch P, Ortega-Canto J, Kendall C. The design of a community-based health education intervention for the control of Aedes aegypti. *Am J Trop Med Hyg* 1994; 50 (4): 401-411.
16. Rosenbaum J, Nathan MB, Ragoonanansingh R, Rawlins S, Gayle C, Chadee DD, et al. Community participation in dengue prevention and control: a survey of knowledge, attitudes, and practice in Trinidad and Tobago. *Am J Trop Med Hyg* 1995; 53 (2): 111-117.
17. Ávila Montes G, Martínez M, Sherman C, Fernández Cerna E. Evaluación de un módulo escolar sobre dengue y Aedes aegypti dirigido a escolares en Honduras. *Rev Panam Salud Publica* 2004; 16 (2): 84-94.
18. Ribeiro Montiani CH, Gutemberg C. Movilización social de residentes de una ciudad para el control del dengue. En: Lloyd L. *Mejores prácticas para la prevención y el control del Dengue en las Américas*. Environmental Health Project; 2003; pp. 58-65.
19. Heinze C, Garrido MV, Kroeger A. What do community-based dengue control programmes achieve? A systematic review of published evaluations. *Trans R Soc Trop Med Hyg* 2007; 101 (4): 317-325.
20. Elder J, Ballenger-Browning K. Community involvement in dengue vector control. Is effective but the contribution of human behavior needs to be defined. *BMJ* 2009; 338: b1023.
21. Al-Muhandis N, Hunter PR. The Value of Educational Messages Embedded in a Community-Based Approach to Combat Dengue Fever: A Systematic Review and Meta Regression Analysis. *PLoS Negl Trop Dis* 2011; 5 (8): e1278.
22. Katz T, Miller J, Hebert A. Insect repellents: Historical perspectives and new developments. *J Am Acad Dermatol* 2008; 58 (5): 865-871.
23. Kawada H, Yen NT, Hoa NT, Sang TM, Van Dan N, Takagi M. Field evaluation of spatial repellency of metofluthrin impregnated plastic strips against mosquitoes in Hai Phong City, Vietnam. *Am J Trop Med Hyg* 2005; 73 (2): 350-353.
24. Kawada H, Maekawa Y, Takagi M. Field trial on the spatial repellency of metofluthrin impregnated plastic strips for mosquitoes in shelters without walls (beruga) in Lombok, Indonesia. *J Vector Ecol* 2005; 30 (2): 181-185.
25. Seng CM, Setha T, Nealon J, Socheat D, Chantha N, Nathan MB. Community-based use of the larvivorous fish *Poecilia reticulata* to control the dengue vector *Aedes aegypti* in domestic water storage containers in rural Cambodia. *J Vector Ecol* 2008; 33 (1): 139-144.
26. Gorrochotegui Escalante N, Fernández Salas I, Gómez Dantés H. Field evaluation of *Mesocyclops longisetus* (Copepoda: Cyclopoidea) for the control of larval *Aedes aegypti* (Diptera: Culicidae) in northeastern Mexico. *J Med Entomol* 1998; 35 (5): 699-703.
27. Martínez Ibarra JA, Guillén Y, Arredondo Jiménez JI, Rodríguez López MH. Indigenous fish species for the control of *Aedes aegypti* in water storage tanks in southern Mexico. *Biocontrol* 2002; 47 (4): 481-486.
28. Suárez Rubio M, Suárez M. The use of the copepod *Mesocyclops longisetus* as a biological control agent for *Aedes aegypti* in Cali, Colombia. *J Am Mosq Control Assoc* 2004; 20 (4): 401-404.

29. Boyce R, Lenhart A, Kroeger A, Velayudhan R, Roberts B, Horstick O. *Bacillus thuringiensis israelensis* (Bti) for the control of dengue vectors: systematic literature review. *Trop Med Int Health* 2013; 18 (5): 564-577.
30. Han WW, Lazaro A, McCall PJ, George L, Runge-Ranzinger S, Toledo J, et al. Efficacy and community effectiveness of larvivorous fish for dengue vector control. *Trop Med Int Health* 2015; 20 (9): 1239-1256.
31. George L, Lenhart A, Toledo J, Lazaro A, Han WW, Velayudhan R, et al. Community effectiveness of temephos for dengue vector control: a systematic literature review. *PLoS Negl Trop Dis* 2015; 9 (9): e0004006.
32. Mir S. Mulla, Usavadee Thavara I, Tawatsin A, Chompoosri J. Procedures for the evaluation of field efficacy of slow-release formulations of larvicides against *Aedes aegypti* in water-storage containers. *J Am Mosq Control Assoc* 2004; 20 (1): 64-73.
33. Romero-Vivas C, Wheeler J, Falconar A. An inexpensive intervention for the control of larval Aedes aegypti assessed by an improved method of surveillance and analysis. *J Am Mosq Control Assoc* 2002; 18 (1): 40-46.
34. Tun-Lin W, Lenhart A, Nam VS, Rebollar Téllez E, Morrison AC, Barbazan P, et al. Reducing costs and operational constraints of dengue vector control by targeting productive breeding places: a multi-country non-inferiority randomized trial. *Trop Med Int Health* 2009; 14 (9): 1143-1153.
35. Spiegel J, Bennett S, Hattersley L, Hayden M, Kittayapong P, Nalim S, et al. Barriers and bridges to prevention and control of dengue: the need for a social-ecological approach. *EcoHealth* 2005; 2 (4): 273-290.
36. Reiter P, Nathan M. *Guidelines for assessing the efficacy of insecticide space sprays for control of the dengue vector Aedes aegypti*. Geneva: World Health Organization; 2001.
37. Hudson J. The emergency ultra-low-volume spray campaign against *Aedes aegypti* adults in Paramaribo, Suriname, 1982. *Bulletin of the Pan American Health Organization* 1986; 20 (3): 292-301.
38. Manrique-Saide P, Coleman P, Davies C, Rebollar Téllez E, Che Mendoza A, Dzul Manzanilla F. Entomological evaluation of ground-vehicle-mounted ULV spraying on *A. aegypti* in residential areas of Merida, Mexico. 73rd Annual Meeting of the American Mosquito Control Association (AMCA), Orlando (Florida, United States), 1 to 5 April 2007.
39. Esu E, Lenhart A, Smith L, Horstick O. Effectiveness of peridomestic space spraying with insecticide on dengue transmission; systematic review. *Trop Med Int Health* 2010; 15 (5): 619-631.
40. Williams C, Ritchie S, Long S, Dennison N, Russell R. Impact of a bifenthrin treated lethal ovitrap on *Aedes aegypti* oviposition and mortality in north Queensland, Australia. *J Med Entomol* 2007; 44 (2): 256-262.
41. McCall P, Kittayapong P. *Control of Dengue Vectors: Tools and Strategies.* Report of the Scientific Working Group Meeting on Dengue. Geneva: 1 to 5 October 2006; pp. 110-119.
42. Morrison AC, Zielinski-Gutiérrez E, Scott T, Rosenberg R. Defining challenges and proposing solutions for control of the virus vector *Aedes aegypti*. *PLoS Med* 2008; 5 (3): e68.
43. Kroeger A, Lenhart A, Ochoa M, Villegas E, Levy M, Alexander N, et al. Effective control of dengue vectors with curtains and water container covers treated with insecticide in Mexico and Venezuela: cluster randomized trials. *BMJ* 2006; 332 (7552): 1247-1250.

44. Igarashi, Akira. Impact of dengue virus infection and its control. *FEMS Immunology & Medical Microbiology* 1997; 18 (4): 291-300.
45. Echevers G, Moura-Lima M, Miranda-Franco R, Calheiros L. Results of spraying with ultra-low-volume malathion at ground level in Panama City. *Bulletin of the Pan American Health Organization* 1975; 9 (3): 232-237.
46. Uribe L, Garrido G, Nelson M, Tinker M, Moquillaza J. Experimental aerial spraying with ultra-low-volume (ULV) malathion to control *Aedes aegypti* in Buga, Colombia. *Bulletin of the Pan American Health Organization* 1984; 18 (1): 43-57.
47. Perich MJ, Rocha NO, Castro AL, Alfaro AW, Platt KB, Solano T, et al. Evaluation of the efficacy of lambda-cyhalothrin applied by three spray application methods for emergency control of *Aedes aegypti* in Costa Rica. *J Am Mosq Control Assoc* 2003; 19 (1): 58-62.
48. Arredondo Jiménez J, Arvizu H. New technique of space treatments with the use of mist blowers for the control of *Aedes aegypti* in Mexico. 73rd Annual Meeting of the American Mosquito Control Association (AMCA), Orlando (Florida, United States), 1 to 5 April 2007.
49. Arredondo Jiménez J, Rivero N. Space treatments of insecticide for control of dengue virus vector *Aedes aegypti* in southern Mexico. I. Baseline penetration trials in open field and houses. *J Am Mosq Control Assoc* 2006; 22 (2): 301-305.
50. Newton EAC, Reiter P. A model of the transmission of dengue fever with the evaluation of the impact of ultra-low volume (ULV) insecticide applications on dengue epidemics. *Am J Trop Med Hyg* 1992; 47 (6): 709-720.
51. Perich M, Tidwell M, Williams D, Sardelis M, Pena C, Mandeville D, et al. Comparison of ground and aerial ultra-low volume applications of malathion against *Aedes aegypti* in Santo Domingo, Dominican Republic. *J Am Mosq Control Assoc* 1990; 6 (1): 1-6.
52. Gubler D. The emergence of epidemic dengue fever and dengue hemorrhagic fever in the Americas: a case of failed public health policy. *Rev Panam Salud Publica* 2005; 17 (4): 221-4.

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