

Time to empower RIDL and Wolbachia against Zika

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Abstract (Short): RIDL and Wolbachia are two potentially powerful tools in the fight against Zika but their technological advancement is being hampered by policy barriers. Here, we discuss what could be done to overcome these regulatory deadlocks.

After a historical series of localised epidemics within Africa and Oceania (1), the vector-borne Zika virus has emerged as a rising threat in Brazil (2). The World Health Organization (WHO) recently announced that it is expecting 3-4 million cases this year in the Americas due to its rapid spread and transmission potential. Concomitantly, increasing cases of documented infant microcephaly have been linked to maternal Zika infection (3).

Zika is now a global public health emergency (4) with 48 countries reporting confirmed cases of local transmission in the past 9 months (as of 29th April 2016, Source: European Centre for Disease Prevention and Control). It also has the potential to have seasonal incursions to areas such as the US, southern Europe and Australia, where its main reported mosquito vectors, *Aedes aegypti* and *Ae. albopictus*, are present (5) (see Figure 1 for vector distribution and current countries affected). Its vector profile is thought to be shared with dengue but could potentially expand to include other more prevalent mosquitoes. Ongoing research at the Oswaldo Cruz Foundation suggests that *Culex quinquefasciatus* could be a carrier (6), possibly contributing towards the noticeable increase in the rate of Zika spread. Of particular concern is the upcoming Rio Olympic Games in August, which is expected to facilitate disease spread by increasing the number of infectious travelers.

Post-Epidemic vs. Preventative. Although the race for a vaccine is underway, no candidate is available in the foreseeable future, making vector management essential for containing Zika to prevent further epidemics. With an incomplete map of transmission pathways and multiple global genetic strains, which could include many vectors and sexual contact, the true severity of Zika's spread and distribution of past to current outbreaks is difficult to establish. Compounding this is the poor understanding of the public health and economic burden of disease vectors (7), which raises the importance of long term and cost-effective solutions that are safe and preventative.

Traditionally, control methods involved habitat management with the application of larvicides and removal of breeding habitats, or preventative measures such as indoor residual spraying and wide-area fogging (8). Whilst these principal methods show short-term efficacy and should be continued,

by themselves they will have a limited and transient effect on the global spread of Zika, as evidenced by current dengue endemicity. *Ae. aegypti* prefers to breed in small, frequently obscure, habitats, and being both diurnal and anthropophilic, requires considerable human intervention. With issues such as competing application priorities and widespread insecticide resistance, the consequent high accumulated project costs often results in delayed post-epidemic action. Therefore, new complementary approaches that are both longer-lasting and large scale are required. Two promising breakthroughs include the engineering of RIDL (Release of Insects with Dominant Lethality) by OXITEC (9,10) and Wolbachia-infected mosquitoes in the Eliminate Dengue Project (10,11).

Large-Scale Integrated Vector Management. The bacterium Wolbachia naturally occurs in many different insect species and when transferred into *Ae. aegypti*, causes cytoplasmic incompatibility (see Figure 2A for Wolbachia mechanisms). Over a small number of generations, it is able to spread, replace the wild population and reduce reproductive capability. Currently, product development and deployment strategy is being optimised (12). RIDL also offers highly species-specific control, using a lethal gene that causes the premature death of progeny (see Figure 2B for RIDL mechanisms). Unlike Wolbachia, RIDL is a suppression strategy, with dramatic reductions in native mosquito populations observed in a few weeks (13).

These techniques only release males, which do not increase transmission risk as only females bite. Their proponents envision for a targeted yet large-scale campaign, expanding our arsenal of current vector control tools. They face however many obstacles.

Issues include the persistence of their releases, mosquito rearing schemes and community acceptance. Yet other issues are particularly damaging. Stigma created by spurious media claims of a direct link between RIDL and Zika can have a long lasting effect, denormalising the technology to the public, and evoking opposition. This severely undermines the scientific process by disregarding the undergoing thorough safety testing behind these projects. The lack

of structured and objective information in the public forum could stifle the development of policy measures, even when widespread intrinsic opposition does not exist.

Risk Assessments Are In Place. RIDL and Wolbachia have been utilised outside of the laboratory. RIDL has been tested in Grand Cayman, Malaysia and has trials undergoing in Brazil (14) while Wolbachia mosquitoes have been released in small-scale trials in Vietnam, Indonesia, Colombia and Singapore, and expanded trials in Australia and Brazil (15). The vector control community is unanimous that health and safety are the primary concern, and stringent guidelines are in place to ensure public well-being.

The main public concerns revolve around the unintended contamination by these mechanisms in both humans and the environment, and the unknown consequences of such events. Scientists have taken these concerns into account, which has led to engagement with external risk assessment experts and they continue to carry out field safety tests, acknowledging that public concern can be constructive, driving innovation and good working practices. RIDL utilises a fail-safe bio-containment feature with confined mosquito spread risk, and site specific studies including biological assays found no significant ecological impacts of the technology (14,16). For Wolbachia, a panel of national independent experts outside the Eliminate Dengue Project gave a conservative estimate of “negligible risk” in employing the bacterium (17).

Although no negative impacts have been recorded, RIDL has faced a mixed public response with accusations of incomplete risk assessment procedures, lack of transparency regarding results and political agendas. Despite this, RIDL has continued to increase its client base with many visible supporters from the scientific community actively engaging with the public. Recently, WHO has begun backing trials of genetically modified (GM) mosquitoes, showing its support for “the use of both old and new approaches to mosquito control as the most immediate line of defence”. On the contrary, being non-GM, Wolbachia technologies have fewer public relation barriers. Their success is also attributed to the thorough engagement and communication campaigns across their target

sites, and a slow and cautious scaling-up program.

Recommendations. To combat Zika more effectively and quickly, these technologies require more government funding and the highly efficient allocation of resources. This calls for cross-border collaboration between scientists, politicians and the public, integrating bottom-up community led projects with the top-down technological approaches. Initial investment is key for emerging technologies with long-term aspirations at the forefront. Progress can be stifled if bureaucratic barriers are not overcome, having the detrimental effect of creating regulatory deadlocks, draining funds and wasting time. The risks in not using GM technology should be discussed on par with their use on a timescale that allows for containment of Zika before it reaches remote areas and becomes extremely difficult to manage; including conflicts zones, rural segregated populations and stigmatised communities.

GM crops, which are grown in 28 countries (18), can serve as a template for Zika regulatory policies, taking on lessons through years of discussion and debate. The technologies are not the final solution, facing similar problems of resistance to current methods, but can contribute towards a durable and sustainable control outcome if used in a functional regulatory framework that meshes science with policy. This requires continued spatial modelling, examining the effects of different release patterns across time and space, the costs of different schemes, and effective deployment and monitoring strategies. Theoretical exploration continues with field-testing where policymakers can aid scientists to employ the technologies on a larger scale.

Moreover, control efforts should complement the distributional severity of the outbreak. In this case, Brazil should be targeted as it is the current Zika epicentre. The current lack of a global strategy is cost-ineffective as both hosts and vectors are spatially mobile, causing endemic infection and seasonally driven outbreaks. Thus problems of host population re-infection and vector resurgence can continually occur even if local eradication is achieved. It is therefore important to bring large-scale technologies such as RIDL and Wolbachia forward in the policy realm. If

implemented widely, they not only offer long-term cost-saving benefits, but also contribute towards community-level protection by reducing the number of transmissible infection pathways. Implementation of these policies can provide the technology to vulnerable nations, regardless of their wealth, and accelerates innovation as they develop specific strains for their sites. As demand for these products increase, competitive markets will emerge, leading to higher production standards, larger mosquito rearing facilities and regional transport dissemination methods. GM technologies for vector control will mainly be utilised in middle to low-income countries that lack the research infrastructure available elsewhere, and therefore their reliance on international regulatory pathways makes international cooperation critical. If this is not feasible, then it becomes necessary to implement research to commercialisation pipelines within borders so that individual countries will have the power to control localised Zika transmission as effectively as possible. The global route is more appealing however as it shares scientific and labour resources but this requires the enabling of extensive testing at more field sites, and the streamlining of regulation and approval processes.

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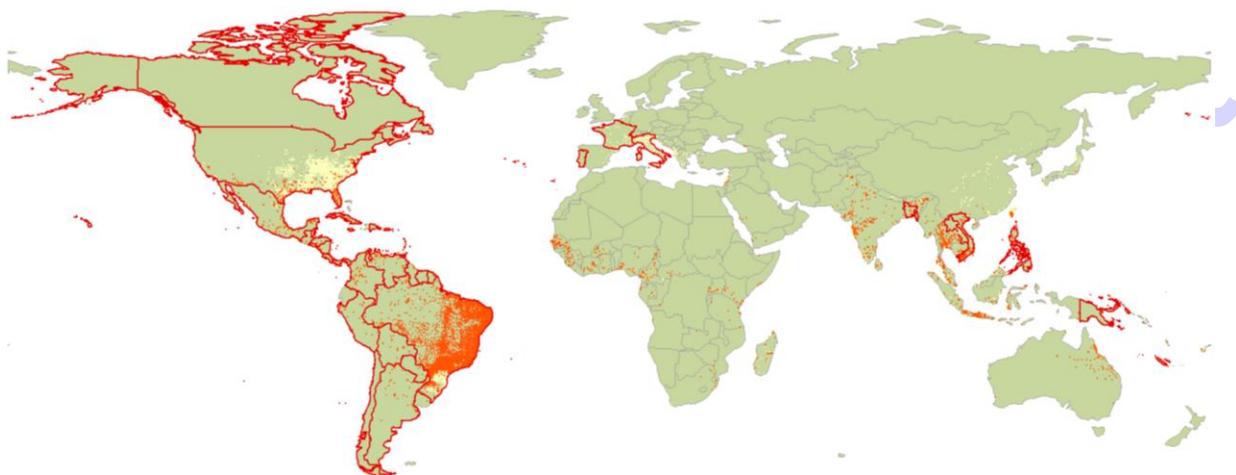


Fig. 1. The distribution of *Ae. aegypti* and *Ae. Albopictus*, and countries with confirmed local Zika transmission. The distribution of the two main Zika vectors in the tropics and subtropics (*Ae. Aegypti* in orange, *Ae. Albopictus* in yellow) overlaid with locations of past and ongoing outbreaks (confirmed local transmission in countries in the last 9 months as of 29th April 2016 shown with red border, Source: European Centre for Disease Prevention and Control).

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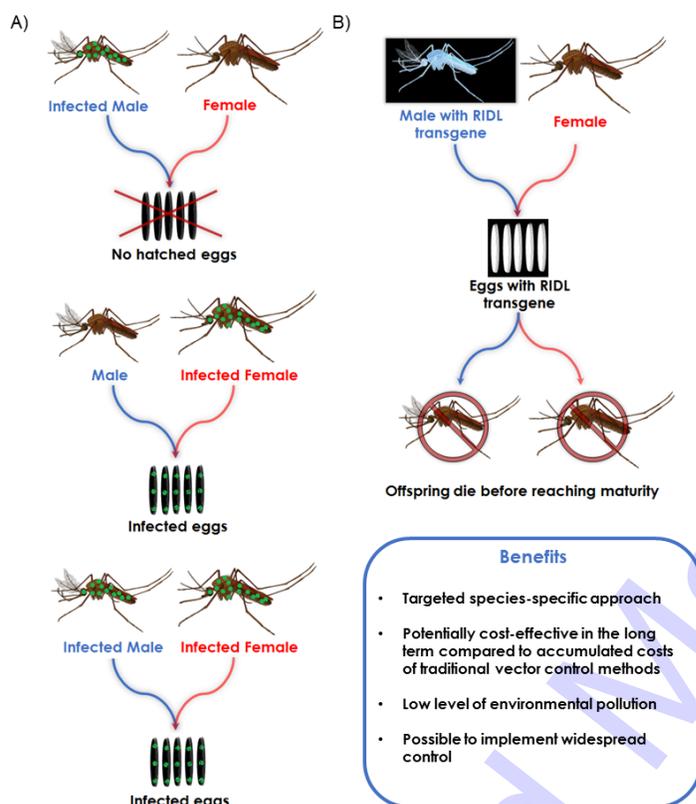


Fig. 2. Mechanisms and Benefits of RIDL and Wolbachia.

2A) Outcomes of different mating combinations with Wolbachia-infected mosquitoes (12): After the release of Wolbachia-infected males, their mating events with wild-type females lead to no eggs hatching. Any wild-type males that mate with Wolbachia-infected females will result in all progeny carrying Wolbachia. Infected males that mate with infected females will also observe this consequence. The pairings highlighted above demonstrate where sterility occurs and how Wolbachia is able to spread down generations.

2B) Effect of the RIDL transgene in mosquito populations (Inset, *Ae. aegypti* mosquito) (13): Mass reared *Ae. aegypti* males carry a lethal mutant gene, which when reared in a laboratory setting, can be controlled allowing them to survive normally. Once released, however, the progeny they create when mating with wild females are unable to survive past the pupal stage.

Their delayed death maintains density dependence, which keeps competition for resources high and maintains natural death rates. By preventing wild females from laying viable young, population reduction occurs at sites of RIDL mosquito release.

Statements

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