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Insect vectors endosymbionts as solutions against diseases

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Viral diseases transmitted by mosquitoes, known as arboviruses, pose a significant threat to human life and are a major burden on many health systems around the world. Currently, arbovirus control strategies rely on insecticides or vector source reduction and, in the absence of effective, accessible and affordable vaccines, mainly on symptomatic based, non-specific treatments. However, insecticides have the potential to interfere with non-target organisms, cause environmental toxicity and insecticide resistance reduces their effectiveness as a sustainable control method. Complementary and sustainable strategies are urgently needed. *Wolbachia*, an invertebrate endosymbiont, has been used as an alternative strategy for arboviral control, through suppression or modification of mosquito populations. Here we discuss the burden that arboviruses impose on human populations and how *Wolbachia* can be used as a sustainable strategy for control, in alignment with the United Nations- 2030 Agenda for Sustainable Development.

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Arboviruses are significant causes of human mortality and morbidity. Although the emergence of arboviruses (arthropod-borne viruses), such as dengue virus (DENV) and yellow fever virus (YFV), have been a threat to human health for centuries, in the last few decades they have become an increased threat to public health, causing a significant exacerbation of the disease burden worldwide [1•,2,3]. Several arboviruses have emerged (or re-emerged) and are dispersing geographically more widely and more rapidly than in the past [3,4]. This is mainly due to an increase in human population, as well as a significant increase in global transportation, greater urbanization, failure of existing vector control methods and land-use changes (particularly the loss of forest areas due to agricultural and urban development) [4–7].

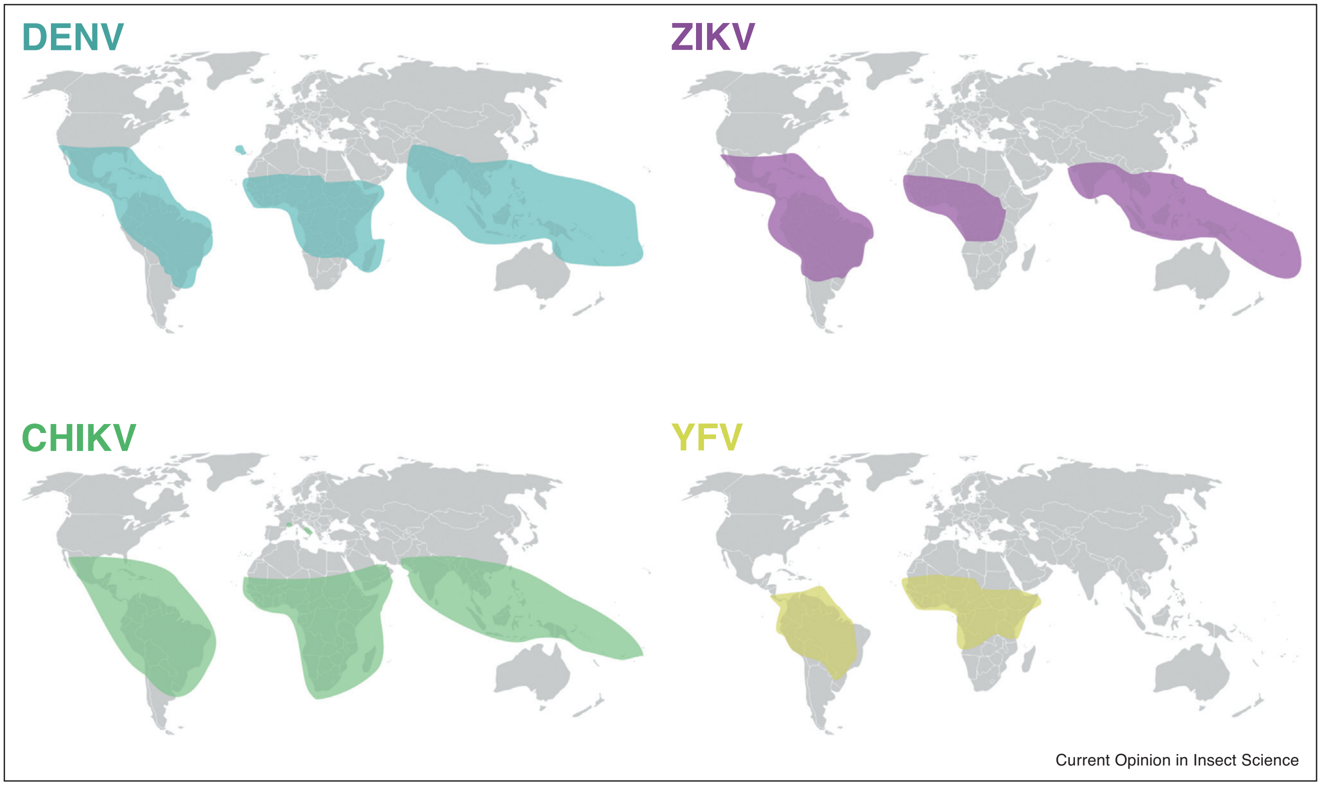
The incidence of dengue has increased greatly in the past two decades, with the number of symptomatic cases more than doubling each decade [8]. At present, approximately half of the world's population lives in dengue endemic areas, where it is estimated that a total of 390 million infections occur (symptomatic and asymptomatic) each year, with more than 13 000 fatal cases [3]. This pandemic-prone scenario prompted the World Health Organization (WHO) to designate dengue fever as one of the top 10 threats to global health in 2019.

Zika virus (ZIKV) is another arbovirus that has recently spread worldwide to approximately 84 countries. Although ZIKV was discovered in Africa in 1947, the first known outbreak occurred only in 2007 on the Yap Island (in the western Pacific). This outbreak was followed by a French Polynesian epidemic in 2013 and culminated in the latest outbreak in Latin America during 2015–2016. Zika virus became a major public health threat globally, due to its association with congenital Zika (CZS) and Guillain-Barré syndromes [1•]. These associations resulted in the WHO declaring the outbreak a Public Health Emergency of International Concern in early 2016 [9].

In addition to dengue virus and Zika virus, other mosquito-borne arboviruses are emerging and/or re-emerging and, in some cases, expanding their geographic distribution. These include chikungunya virus (CHIKV), yellow fever virus (YFV), West Nile virus (WNV), Japanese encephalitis virus (JEV), St Louis encephalitis virus (SLEV), Rift Valleys fever (RVFV), Mayaro virus (MAYV) and Oropouche virus (OROV) (see Figure 1). These arboviruses are pathogenic to humans, with conditions ranging from a subclinical disease, to a severe life-threatening illness [1•,4,10].

Despite the growing public health threat caused by these arboviruses, there are no specific treatments nor efficacious vaccines for most of them. Instead of focusing on blocking arboviral infection of humans, most of the mitigation strategies rely on vector control, with the objective

Figure 1



Contemporary distribution of the most important arboviruses.
Abbreviations: DENV, dengue virus; ZIKV, Zika virus; CHIKV, chikungunya virus; YFV, yellow fever virus.

being to block transmission. Although several strategies have been used to control vector populations, such as synthetic chemical insecticides, chemical and microbial larvicides, insect growth regulators and bacterial toxins, they have not been able to eliminate arbovirus transmission and prevent epidemics, nor to stop the wider spread of the diseases [11].

Conventional vector control strategies primarily approach the insect vectors as 'enemies' to be eliminated. However, new, alternative strategies have been developed that instead use insect vectors as 'allies' and as tools to reduce arboviral transmission. These approaches aim to generate vectors that are actually resistant to arbovirus infection. The use of *Wolbachia* in *Aedes aegypti* to increase resistance to dengue and other arboviruses, including Zika and Mayaro, is one of the most successful examples of how a vector can be used as an 'ally' to combat arboviruses.

Wolbachia pipientis (Rickettsiaceae) is a maternally transmitted α-proteobacterium endosymbiont, infecting at least 40% of terrestrial insect species [12]. Recent re-classification, based mostly on multilocus sequence typing, has identified 18 major phylogenetic lineages, called supergroups (A to R) [13], confirming the abundance of this unique species. Interaction of *Wolbachia* with its host is often associated with reproductive manipulation, with the degree of manipulation depending on the host and the bacterium strain. *Wolbachia* was discovered in the mosquito *Culex pipiens* in 1924, in which caused a phenomenon called cytoplasmic incompatibility (or CI); where uninfected females that mate with infected males become sterile. [14]. This particular manipulation gives advantage to the endosymbiont, allowing *Wolbachia* to quickly spread into uninfected insect populations [15].

Wolbachia is naturally present in several mosquito species including *Aedes albopictus* and *Culex pipiens*, but not in *Aedes aegypti*, the primary vector of dengue worldwide. Infections of *Wolbachia* into *Ae. aegypti* have been achieved in the laboratory via embryo injection of purified *Wolbachia*, or through cytoplasm transfer from another infected donor [16,17]. Once the transinfection in *Ae. aegypti* is obtained, it frequently becomes sustainable by being passed by the mother to offspring, through her eggs [16,17]. This process does not involve any genetic transformation of the mosquito host, nor of the bacterium.

A major breakthrough was the discovery that the insertion of particular *Wolbachia* strains into *Aedes aegypti*, can promote pathogen interference. Several reports demonstrated the potential spectrum of *Wolbachia* to block, in mosquitoes, the replication of dengue virus (DENV), chikungunya (CHIKV), Zika (ZIKV), yellow fever (YFV) and Mayaro viruses (MAYV) [18••,19–23]. Several mechanisms of how *Wolbachia* can reduce pathogen replication and transmission have been suggested, such as upregulation of immune genes, increased production of reactive oxygen species (ROS) upon *Wolbachia* infection, and competition between the virus and *Wolbachia* for cell resources, such as cholesterol [24,25].

Two main *Wolbachia*-based vector control approaches have been used internationally with the objective of ultimately reducing the transmission of arboviruses. The suppression strategy, exclusively uses the CI mechanism and involves the release of *Wolbachia*-infected males into a population of uninfected females, causing sterilization and resulting in mosquito population suppression. This approach, named Incompatible Insect Technique (IIT), involves the ongoing production of millions of mosquitoes, as well as sex separation (male-only releases). Because *Wolbachia* is a 'dead end' in male mosquitoes, this method does not naturally sustain and therefore requires the ongoing release of male releases into the environment [26•].

Recently, a successful field demonstration of this first strategy, combining the incompatible and sterile insect techniques (IIT–SIT), was reported in China. Researchers released *Aedes albopictus* males, with an artificial triple *Wolbachia* infection, and irradiated pupae to prevent unintentionally released triple-infected females. This approach nearly eliminated two field populations of *Ae. albopictus* after a two-year release period [27]. In the case of *Ae. aegypti*, two recent studies in the USA have only used IIT with improved automated sex sorting technologies to achieve local population suppression [28,29•]. In Fresno (CA, USA), during peak mosquito season, the number of female mosquitoes dropped from between 95% and 98%, in release areas, in comparison to areas without the intervention [29•].

The second approach links the pathogen blocking ability of certain *Wolbachia* strains with the CI mechanism. Here, both males and female *Wolbachia*-mosquitoes are released into the environment, with *Wolbachia* maintaining in the wild after approximately four to six months of mosquito releases [30,31•]. This strategy has some natural advantages in comparison to the 'suppression' method, including: a simpler rearing method, without the need to sort and separate males and females; a lower number of insects needing to be released; and finally, and most importantly, there is no need for ongoing mosquito releases, as *Wolbachia* mosquitoes natural self-sustain [26•].

Two cities in Northern Australia have received *Wolbachia* infected mosquitoes, as part of the activities conducted by the World Mosquito Program (formerly known as the Eliminate Dengue Program). In Townsville, a city of approximately 170 000 inhabitants, *Wolbachia* mosquito egg releases took place with the help of community members, including school children, as part of school science education programs. *Wolbachia* (*w*Mel strain) was successfully introduced over an area of 66 km^2 during a 28-month period. Importantly, no local dengue transmission has been confirmed after *Wolbachia* establishment, despite imported cases still occurring [32]. In the second city Cairns, a recent report has also demonstrated that *Wolbachia* frequencies have remained stable for up to eight years. Analysis of dengue case notifications indicate that where the *Wolbachia* method was implemented, there has been almost the complete elimination of local dengue transmission over the past five years, [33••].

In Malaysia, *Ae. aegypti* containing wAlbB have been released in several urban areas with high and stable temperatures (around 36°C). *Wolbachia*-positive mosquitoes remained stable in the release areas (up to 98% when monitored), following the natural mosquito population seasonality. Furthermore, based on passive case monitoring, the authors have detected a 40% reduction in dengue cases, in comparison to control sites. The authors highlighted that in some areas, the introduction of *Wolbachia w*AlbB has reduced dengue cases to the extent that insecticide fogging by the local health authorities was no longer needed [31•].

Although the data presented above is preliminary and based on case notifications by local health authorities, there is increasing evidence that *Wolbachia* can impose a huge impact on the reduction of arboviruses worldwide. This has recently been studied in Indonesia where, through a combination of several models, it was predicted that a national roll-out of *Wolbachia* at 100% coverage could achieve a long-term average of 86.2% (UI 36.2–99.9%) reduction in dengue cases of all severities. Furthermore, it would likely avoid 6.7 million symptomatic cases, 947 000 hospitalisations and 3154 deaths a year, based on 2015 Indonesian Government data [34].

The potential use of *Wolbachia* as an effective new tool for vector control is considerable. In 2018–19, the 'self-sustaining' or 'modification' method was used as case studies in two UN Sustainable Development Goals' Voluntary National Reviews (VNRs), in Fiji and Australia. In the Australian review, the WMP's work was highlighted as an innovative approach being trialed in the Pacific and Asia [35] to help protect an estimated half a million people from arboviruses such as dengue, Zika and chikungunya. Measurable reduction in arboviruses, in particular dengue, is the focus of the WHO in seeking to develop new vector control goals for 2030, as part of the UN Sustainable Development Goals (SDGs) for neglected tropical diseases [36•]. These follow the previous 2012 WHO goals for 2010–2020 which have not been achieved, in part, due to existing interventions proving insufficiently effective in preventing spread [36•]. The development of new technologies focused on reducing disease transmission will be central to tackling future morbidity caused by these viruses, in particular dengue [36•]. *Wolbachia*'s potential contribution to these revised goals, as a new technology, is significant. With indicators showing that the 'self-sustaining' method in particular, can have a major global impact on the dengue burden [37].

Focus on vector control goals as a contribution to the UN SDGs, presents an important opportunity to broaden the international discussion regarding vector control, in particular of arboviruses, such as dengue. Vector control intersects with several SDGs (see Figure 2) but importantly, is also a cross-cutting issue [38] and evidence indicates that there is value in it being identified as one. It should not be viewed in isolation as only a health issue, but instead, with consideration of several important cross-cutting themes, including gender equality and climate action.

Although Goal #3, Good Health and Well-Being, in particular target 3.3, specifically aims to end epidemics of neglected tropical diseases, such as dengue (along with other communicable diseases), setting and achieving revised vector control objectives will also directly contribute to other goals. These include:

Figure 2

Global vector control response 2017–2030.

Goal 1# No Poverty — up to 45% of dengue treatment health costs are borne by families, at three times the cost of monthly incomes [39], medical treatment for mosquito-borne diseases can exacerbate financial hardship and inequalities;

Goal #5 Gender Equality — girls in Vietnam are more likely to develop severe dengue symptoms compared to similarly aged boys [40], with females usually the primary carers for those sick with dengue;

Goal 13# Climate Action — climate change is estimated to dramatically increase the world population at risk of dengue contraction to an additional two billion people by 2080 [41], and;

Goal 17# Partnerships for the Goals — large scale success in arbovirus intervention not only requires the development of new vector control strategies, but also collaborative implementation, involving multiple sectors, partners and geographies. For example, the World Mosquito Program is operating across three continents and 12 countries with health ministries and research institutes sharing field data and diagnostic information and conducting knowledge transfer of optimal research and deployment methods. The global effort to control and eliminate mosquito-borne diseases is one of the largest public health initiatives ever undertaken. Successful mobilization of resources, including technical and financial, will require extensive global partnering.

Development of the new WHO vector control goals represents an opportunity for targeted and measurable action on tackling a substantial public health burden [36•]. Furthermore, they also represent an opportunity to broaden the vector control narrative by highlighting global partnerships and their contribution to cross-cutting issues. In particular, helping to address social and health inequalities for women and girls, and building communities resilient to the increased risk of arboviruses due to climate change. The *Wolbachia* approach serves as an example of innovative new vector control tools and the importance of increased cross-sectoral collaboration and consideration.

For real impact, the rollout of large-scale interventions in all arbovirus endemic countries will require major funding [36•]. To increase interest and the likelihood of significant, long term funding, partnering and intervention commitments from governments, development banks, other donors and the private sector are necessary. This can best be achieved by showing that the approach to vector control going forward is not 'more of the same' and that concerted efforts, and close alignment with several UN SDGs, can have a major impact on one of the world's greatest 2030 challenges.

Conflict of interest

The authors declare no conflict of interest.

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