



# *Wolbachia* in Dengue Control: A Systematic Review

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## Abstract

**BACKGROUND:** Dengue fever outbreaks have been an important public health issue causing high morbidity and mortality, and serious economic effects, particularly in Asia. Control strategies are a challenge to be implemented due to a variety of factors. However, new approaches such as *Wolbachia*-infected *Aedes aegypti* have been shown to successfully lowering the life spans of the mosquito, eggs resistance, and disease transmission capabilities. Field trials are still on-going, and there are data to support its benefit in a large population. This systematic review aims to determine the current progress and impact of using *Wolbachia* in curbing dengue cases in high dengue case locations worldwide.

**METHODOLOGY:** The study uses the Preferred Reporting Items for Systematic reviews and Meta-Analyses review protocol, while the formulation of the research question was based on population of interest, comparison, and outcome. The selected databases include Web of Science, Scopus, PubMed, SAGE, and EBSCOhost. A thorough identification, screening, and included process were done and the results retrieved four articles. These articles were then ranked based on quality using mixed methods appraisal tool.

**RESULTS:** A total of four articles were included from 2019 and 2020 reports in both dengue- and non-dengue-endemic settings. In this review, comparisons in terms of the hierarchy of the study design, community engagement and acceptance, *Wolbachia*-infected *A. aegypti* deployment, entomological outcome, and epidemiological outcomes were detailed. All four studies showed a decrease in dengue incidence in *Wolbachia*-intervention populations.

**CONCLUSION:** *Wolbachia* programs have been shown to be an effective method in combating dengue diseases. Strong community engagement and involvement from multidisciplinary teams are important factors to ensure the effectiveness and good outcomes of the program.

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## Introduction

Dengue fever is a mosquito-borne disease, which is the main infectious disease in tropical and subtropical nations, particularly in urban areas. Dengue fever is caused by the flaviviridae virus that comes in four different serotypes namely dengue virus (DENV)-1, DENV-2, DENV-3, and DENV-4 [1], [2]. Recovery from infection causes a life-long protective immunity to the specific serotype, but only partial and transient protection against other serotypes. Therefore, it is possible to be infected with dengue fever multiple times [3].

Dengue fever has been on the rise around the world, thus has raised a concern. Dengue cases have doubled every decade from 8.3 million cases in 1990 to 58.4 million cases in 2013 [4]. According to another study, there were about 105 million cases of dengue fever that was reported in 2017. The number of death due to dengue also has increased from 16,957 in 1990 to

40,467 in 2017 [5]. Dengue fever outbreaks have already become epidemics causing high morbidity and mortality, as well as serious economic effects, particularly in South Asia and Southeast Asia [2], [6], [7], [8]. The World Health Organization (WHO) estimated there are 390 million cases per year with just 25% of the cases are symptomatic [3]. Nevertheless, a recent study found that many people were exposed to dengue without having had the disease and lived in a dengue hotspot location, thus indicating an unnoticed dengue infection in the population that requires substantial public health efforts to combat the disease [9].

*Aedes aegypti* is a primary vector for dengue, while *Aedes albopictus* is a secondary vector, which usually bites just after sunrise and around sunset [10], [11]. Dengue infection can be controlled in several ways, one of which is vector control [12]. Environmental management, chemical methods, personal protection, and community involvement in the Communication for Behavioural Impact are the options available for vector control [13]. However, as indicated by existing statistics,

current methods and strategies are unable to prevent dengue outbreaks effectively due to a complex variety of factors [14], [15], [16], [17]. Furthermore, there is no exact therapy for dengue fever, while an effective and safe vaccination are not fully ready for general use [18], [19]. This has led to exploration for new methods to manage a more sustainable mosquito population. *A. aegypti* mosquitoes infected with *Wolbachia* have been used to control dengue fever. *Wolbachia* was introduced to the *A. aegypti* mosquito species using a stable approach, and it was maintained in the mosquito species through maternal inheritance. Cytoplasmic incompatibility of *Wolbachia* is an advantage to gain access into the *A. aegypti* population [12].

Early laboratory and small-scale research revealed that *Wolbachia*-infected *A. aegypti* successfully lowered the mosquito life spans, eggs resistance, and disease transmission capabilities [20], [21], [22], [23]. This systematic review aims to identify current progress and the impact of using *Wolbachia* in curbing dengue cases in high dengue case locations worldwide.

## Methodology

### The review protocols

The study was guided by the Preferred Reporting Items for Systematic Reviews and Meta-Analyse (PRISMA) review protocol, which is designed specifically for systematic reviews and meta-analyses [24]. PRISMA aims to prompt researchers so that they will source the right information with an accurate level of detail. Based on this review protocol, this study starts its systematic literature review by formulating appropriate research questions. The systematic search consists of three main sub-processes, namely, identification, screening, and included.

### Formulation of research questions

The formulation of the research question for this study was based on population of interest, comparison and outcome (PICO). PICO is a tool that assists authors to develop a suitable research question for a review. It is based on three mains concepts, namely, Population or Problem, Interest or Intervention, and Context or Comparison and Outcome [25]. Based on these concepts, the three main aspects included in the review namely patient (Population), *Wolbachia* (Interest), and curb and Dengue (Outcome) that guided the authors to formulate the main aim.

### Systematic searching strategies

There are three main processes in the systematic searching strategies process, namely,

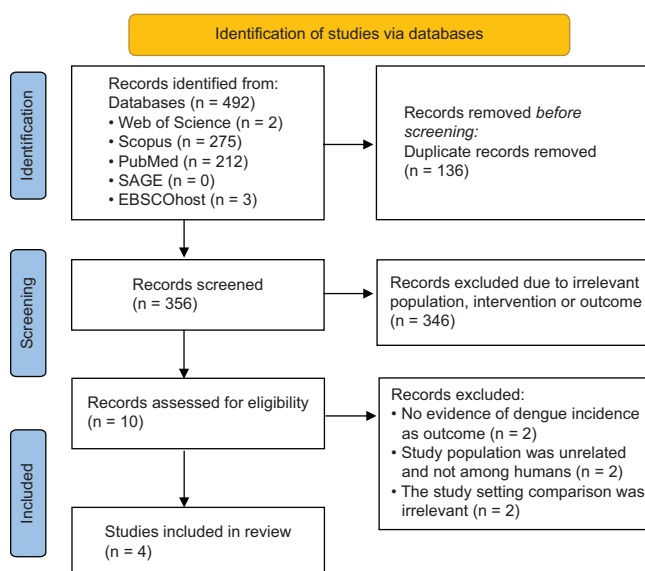


Figure 1: The PRISMA flow diagram [17].

identification, screening, and included (Figure 1).

### Identification

The identification process involves searching for any synonyms, Medical Subject Headings (MeSH) terms, related terms, and variation of the main keywords that include patient, *Wolbachia*, curb, and Dengue (Table 1). This process will provide larger coverage in finding related articles with the selected databases (Web of Science, Scopus, PubMed, SAGE, and EBSCOhost) for the literature search. Some of the distinct features from these databases were a large collection of literature, high-quality articles, and advanced search functions. There were 492 articles obtained from all databases, in which 136 duplicates articles were found and removed. The remaining articles after exclusion were 356 articles.

Table 1: Keywords used in the systematic searching

Population	Intervention	Outcome 1	Outcome 2
Patient	<i>Wolbachia</i>	Curb	Dengue
Human	Bacteria	Restraint	Break-bone fever
Client	Gram-negative small rods	Brake	Classical dengue fever
People	Coccioid forms	Control	Dengue Fever
Community	Wsp protein	Limitation	Classical Dengue
Population		Constraint	Viral Hemorrhagic Fever
Case		Reduce	Flavivirus infections
Victim		Stricture	Arbovirus infections
Sufferer		Damper	
Outpatient		Suppress	
Day Patient		Retard	
In patient		Repress	
		Restriction	
		Barrier	

### Screening

Screening process of the 356 articles was performed with the sorting function from each database. The inclusion criteria of the articles were the journal article, written in the English language, within the year of 2011–2021, observational and interventional studies,

qualitative and quantitative data, and animal studies. Studies that are a systematic review, comment or letter to the editor, abstracts of a conference, and *in vivo* or *in vitro* studies were excluded from the study. Two teams of three or four review authors (NRMN, FSA, MHM, PSNMK, SMA, NIB, and MRH) independently screened the studies for inclusion. Any disagreement at any stage will be led by a discussion to come to a consensus, which was made by a third review author from the other team. Finally, 346 articles were excluded due to irrelevant population, intervention, or outcome.

### **Eligibility**

The eligibility process aims to choose the articles that fulfill the objective of the study from reading the title and abstract of the article. A total of ten articles were manually sorted that satisfy the outcome of reducing dengue incidence or outbreak when comparing treated versus non-treated areas. Studies that are not related to the interest and intended outcome will be excluded. This process excluded six articles due to irrelevant intervention, outcome, and not compatible with the main objective. In the final eligibility process, only four articles were selected (Figure 1).

### **Data extraction and analysis**

Thematic analysis was used in this systematic review as it is considered in synthesizing and integrating mixed research design [26]. The thematic analysis also is a descriptive analysis that allows data to be merged with other data analysis techniques [27]. The selected four articles were read in detail, especially the abstract, method, results, and discussion. Then, the data were extracted if the study was able to answer its research questions and the findings were simplified as tabulated in Appendix A. After these lengthy processes, the authors proceed with the thematic analysis. To generate relevant themes, each author identified the patterns of the extracted data of the reviewed articles and gather them in a group before successfully categorized them into different themes. The theme's accuracy, usefulness, and accurate data representation were reviewed. The developed themes were then submitted to a group of expert panels who are well versed in the systematic review as well as public health-related research. This panel of the expert group then agreed on the generated themes and accurate to the results of the review.

### **Quality appraisal**

The final list of the studies was ranked according to the quality based on the mixed method appraisal tool (MMAT) to ensure the quality of the articles to be reviewed by exploring how the data were extracted for analysis and validation. The MMAT is a recently developed tool that has demonstrated an intraclass correlation of 0.8

based on pilot testing in 2009 and has been proven to be effective and practical for the quality assessment of mixed methods review. The assigned teams extracted the data from the included studies and conducted bias assessment risk. Discussion by a third review author from the other team will be performed if there is any disagreement at any stage to come to a consensus. The authors performed qualitative analysis and appraisal of the included articles by extracting all relevant information using a predesigned and standardized data extraction form. There were five categories of study design utilized in appraising the final studies included in the MMAT, which are qualitative, quantitative randomized controlled trials, quantitative non-randomized, quantitative descriptive, and mixed methods studies. The MMAT quality scoring scale is scored as Yes, Unsure, and No for each criterion. The details of this assessment are reported in Appendix B. The scores of the MMAT met all criteria [28].

## **Results**

The articles included in this study were published in the year 2019 and 2020. There were two studies were done in a dengue-endemic setting, which is the tropical country of Malaysia and Indonesia. The other studies two were conducted in the non-endemic country of Australia, whereby northern Queensland experienced dengue outbreaks during the wet season. All studies were done in an urban setting in northern Queensland. It was done in the Cairns Region, Cassowary Coast Region, Douglas Shire, Charters Towers Region for the first study, and in the city of Townsville for the second study. In Malaysia, it was done in a residential and commercial area of Kuala Lumpur, while in Indonesia, it was done in Yogyakarta involving urban villages or "Kelurahan." The largest deployment scale was done in Northern Queensland in which in the four regions, the total population involved were 157,666 covering 90.1 km<sup>2</sup>. On the other hand, for Townsville, the total population involved was 140,000 covering approximately 66 km<sup>2</sup>. In Indonesia, the area of deployment involved was 64,599 total population with an area of 4.9 km<sup>2</sup>. In Malaysia, information on the total population was missing, but a total of 9966 households were involved within the area of 1.37 km<sup>2</sup>. The longest period of study was done in the four regions of Northern Queensland, which took 8 years to complete followed by a study in the city of Townsville, which took 6 years to complete. Yogyakarta study period was 4 years followed by Kuala Lumpur, which took only 2 years.

### **Study design hierarchy (Comparative study)**

Studies conducted in Kuala Lumpur and Yogyakarta are a comparative study with concurrent

controls [29], [30]. Although the randomization element was absent, the existence of a comparator or control site landed the study design in the level 3 hierarchy of study designs. Level hierarchy is used to reflect the strength of evidence when assessing the efficacy of vector control intervention. In Kuala Lumpur, criteria for the control site are based on a comparable dengue incidence to the release sites in the period before the release within the same district as the release site and similar building type of site (to meet similar mosquito and human population characteristics). For Yogyakarta, the control site is comparable in terms of sociodemographic characteristics and historical dengue incidence. On the other hand, both studies in Northern Queensland are considered comparative studies without concurrent controls, which are the lowest level of the hierarchy in the study designs, which is at level 4 [31], [32].

### **Community engagement or acceptance**

Studies in Northern Queensland and Yogyakarta followed the Public Acceptance Model [33] for their community engagement strategies. A systematic model consisted of four key elements that are (1) raising broad community and stakeholder awareness, (2) quantitative surveys assessing awareness and support, and establishment of issues management system, and (4) community reference [29], [31], [32]. All four key elements were carried out through meetings, community events, displays, house visits, social media, brochures, and others. In Kuala Lumpur, community engagement strategies did not follow any existing systematic or strategic model [30]. Nevertheless, it consists of a consultation with key stakeholders and community groups (religious leaders included). Communication is via meetings, workshops, roadshows, carnivals, home visits, Institute of Medical Research (IMR) laboratory visits, brochures, banners, website, WhatsApp, SMS, feedback surveys before and after release to assess perception, opinion and concerns, and updates on the program. Community engagement in Kuala Lumpur allowed the community members to quickly contact the program coordinator with questions or concerns, thus enabling them to be addressed by the program staff within 24 h. There were community reference groups to independently review and evaluate the program community engagement activities, offer recommendations, feedback on community sentiments toward the program, identify issues that need a proactive response, and keep updated on the latest results of the program. For all studies, the levels of acceptance or approval or comfort ability with the release of the *Wolbachia* infected *A. aegypti* among the population in the intervention area were good (more than 79%).

### ***Wolbachia* infected *A. aegypti* deployment**

Before the deployment or release, the rearing of *Wolbachia* infected *A. aegypti* in the laboratory was done.

wMel strain of *Wolbachia* was used in the studies done in Northern Queensland and Yogyakarta [29], [31], [32], while in Kuala Lumpur, wAlbB strain was used [30]. However, before release, only study in Kuala Lumpur and Yogyakarta performs insecticide testing on the laboratory colonies and the wild mosquitoes from the intervention site during the rearing period. Both studies found that there is an equivalent insecticide resistance profile for laboratory colonies and wild mosquitoes. Both adult and egg release were employed in three studies except in Yogyakarta, which only releases eggs. Staggered deployment was done in all four studies either weekly (for an adult) or 2 weeklies (for the eggs). Adult mosquitoes released in the Kuala Lumpur study were made sure to have wings measurement in the range expected to produce fit and competitive release mosquitoes (average [SD] for males 2.28 [0.10] mm, females 2.96 [0.11] mm). The other three studies only did quality assurance, which measured the emergence rates (of adults from eggs) for each Mosquito Release Container. The approach of community-based deployment supplemented with programmatic targeted deployment was employed in Northern Queensland studies. After release, field monitoring and diagnostics (screening for *Wolbachia* in mosquitoes) were done in all four studies. In Northern Queensland studies, *Wolbachia* infected *A. aegypti* release was done until the frequency of *Wolbachia* in the samples of field-caught mosquitoes was above 50% for 2 consecutive weeks. For the Yogyakarta study, the prevalence of *Wolbachia* in the field-caught mosquitoes needs to be more than 60% for 3 consecutive weeks before release was terminated. In Kuala Lumpur, more than 90% of the *Wolbachia* frequency threshold for three consecutive monitoring periods was used to decide on the release termination [29], [30], [31], [32].

### **Entomological outcome**

After completion of release, all four studies showed that the establishment of *Wolbachia* into the local *A. aegypti* population remain stable since they were released. In Northern Queensland, the trajectory of the wMel strain *Wolbachia* establishment showed that it is predictable and consistent up to 8 years post-release. In Yogyakarta, the wMel strain *Wolbachia* establishment into local *A. aegypti* remained at 100% post-deployment. Meanwhile, the findings of two years of post-deployment in Kuala Lumpur showed only two out of six sites with a high frequency of wAlbB strain *Wolbachia* (more than 90%) [29], [30], [31], [32].

### **Epidemiological outcome**

Studies in Northern Queensland showed a 95% [31] and a 96% [32] reduction in dengue incidence in *Wolbachia*-treated populations. This was based on the regression model estimate of *Wolbachia*

intervention effect from interrupted time series analyses of case notifications data before and after releases. In Yogyakarta, compared to the intervention and control area, 34 dengue cases were notified from the intervention area and 53 from the control area (incidence 26 vs. 79/100,000 person-years) for 24 months following *Wolbachia* deployment. This corresponds to the regression model of interrupted time series to a 73% reduction in dengue incidence associated with the *Wolbachia* intervention [29]. Kuala Lumpur estimation was done using the Bayesian time series model, which showed decreased dengue cases by 40.3% in the intervention sites. Based on the passive case monitoring, reduced human dengue incidence was observed in the release sites compared to the control sites [30].

## Discussion

### ***Effectiveness of Wolbachia as a good biological control***

One of the methods to control dengue transmission is using biological control [34], in which the *Wolbachia* is one of the programs. There are many mechanisms that have been studied in the effectiveness of *Wolbachia* in controlling dengue transmission. The effectiveness of *Wolbachia* in dengue prevention was based on three basic mechanisms, which are direct reduction and or blocking the mosquito's ability to transmit the virus, reduction of the vector density by suppressing mosquito population, and shortening the mosquito lifespan [35].

However, all these results were based on the studies of two different strains of *Wolbachia*, which are the wMel and wAlb. Not all *Wolbachia* strains were effective in blocking the DENV transmission [36]. To date, findings showed that wMel and wAlb strains are the only effective strain in controlling dengue transmission.

### ***Practicability of Wolbachia program***

Several aspects of the *Wolbachia* program have been identified to be a beneficial and practical method in dengue prevention and control, which were sustainability, cost-effectiveness, environmentally friendly, and health safety.

A good prevention program should have good sustainability [37]. Results from this review showed that the *Wolbachia* program is a sustainable method in dengue disease control. All other papers showed that the population of *Wolbachia* can last for several years from the initial release. However, the wMel strain showed a superior level in terms of sustainability compared to the

wAlb strain. This may be due to the wMel strain has been studied earlier than the wAlb strain. Nazni et al. (2019), in a study, used wAlb strain, because the strain has a higher resistance to heat. However, other studies using wMel strain showed better effectiveness in controlling dengue despite the high temperature. wAlb strain requires frequent monitoring after the first release compared to wMel strain. This may be due to the factor of population density. Studies using wMel in Indonesia and Australia involved a larger population compared to the study in Malaysia.

The *Wolbachia* program has been shown to be cost-effective. It is projected that this program can save 50 million per year [31]. In Indonesia, the *Wolbachia* program has been shown to be a highly cost-effective intervention with gross cost-effectiveness below \$1500 per DALY averted [38]. With these advantages, this program can be applied to other countries, especially low-income countries.

Other than that, this program also is an environmentally friendly method. *Wolbachia* release may not cause an impact on the ecosystem. Unlike the conventional methods such as thermal fogging, it may give a negative impact on the ecosystem, in which it may kill other non-targeted insects [39]. This will, in turn, risking biodiversity. Therefore, the application of *Wolbachia* will prevent and minimize this impact.

In terms of the acceptance of the program, health safety is a concern from the community. *Wolbachia* program has been shown to be safe for humans and the environment. The risk of exposure to hazardous chemicals such as pesticides can be avoided using this application. This also will create more confidence in the community in accepting the program to be conducted in their places.

### ***Community engagement***

In the earlier approach of community engagement applied for the *Wolbachia* program in Australia between 2011 and 2014, the approach involved consultation with the stakeholders and community, implementation of program outreach among the community to gain resident permission for the release of *Wolbachia*, and meeting with a reference group of residents and community leaders. Concurrently, advertisements through mass media were also conducted to support the program [32]. Although the system illustrates a mixture of approaches, the *Wolbachia* release program was successfully accepted by the community. This outreach approach was supported by a study conducted in Singapore, whereby the Singaporean scientists and technicians were involved in the door-to-door house visits and roadshows to answer any questions related to the *Wolbachia* technology [40].

There was a similar approach in the *Wolbachia* Release Program in Malaysia. However, the essential measures taken to engage with the public, interactions and public meetings were initiated with the local government, political, religious, and community leaders. There was a total of 40 stakeholders and community engagement activities were conducted in the community halls. In addition, communities and State Assemblyman were also invited to IMR to experience first-hand the science of *Wolbachia*. The involvement of top leaders could enhance the influence of the awareness among the communities. The trust toward the program by the community was strengthened by the information spread by the community leader themselves. The use of online platforms also could be an advantage for the Malaysian *Wolbachia* release program [30]. The website link can easily reach all layers of communities with active Internet usage by all groups of age. The information regarding the benefit of *Wolbachia* release to the selected area can enhance the acceptance of the communities affected by this virus.

This was proved in several studies showing that the approach used to build community engagement for the *Wolbachia* program by applying the Public Acceptance Model [33] has successfully gained the public's trust [29], [31], [32]. This model includes four phases, in which one of the phases involve quantitative surveys in assessing community awareness and support as well as pre-release surveys. These methods have been postulated to be the strength to enhance community understanding concerning the *Wolbachia* release program following the awareness promotion in those studies. Since this successful model has not been applied [30], it was suggested that a quantitative survey and pre-release survey should be conducted among Malaysian communities in *Wolbachia* Release Program.

Despite the successful methods applied by those studies, many other methods can be adapted to *Wolbachia* Releasing Program to get public acceptance. A study conducted in Brazil involved a multidisciplinary team comprised professional experts in communication, biology, environmental science, geography, environmental engineering, and social work to communicate and introduce the *Wolbachia* program to the community [41]. This effort can help improve the knowledge and awareness of the community and lead the community to accept the *Wolbachia* program. Meanwhile, public participation provides an accurate early warning information system about the Asian tiger mosquito (*A. albopictus*) invasion in Spain. This has encouraged the public to systematically report tiger mosquito sightings and raise awareness about steps that can be taken to reduce the risk of mosquito-borne diseases. It illustrates the robust public participation in engaging the mosquito-borne disease surveillance worldwide [42]. In Tanzania, the concept of community

engagement applied was using readily available knowledge and experiences of local community residents to predict density and distribution of disease-transmitting mosquitoes. It was an innovative crowdsourcing approach. Thus, this study provides evidence that we can rely on community knowledge and experiences to identify areas with the most abundant or least abundant mosquitoes even without entomological surveys [43].

### **Vulnerability**

Several factors could detrimentally affect the stability of transinfected *Wolbachia* in *A. aegypti* populations and its ability to inhibit virus transmission. This effect makes the *Wolbachia* more vulnerable. High larval temperatures could decrease *Wolbachia* density and maternal leakage, which means incomplete *Wolbachia* transmission to the eggs laid by the infected females [44]. It can potentially reduce *Wolbachia* frequency following the invasion. Due to this, the studies that were analyzed in this study emphasize the need for a supplementary release of *Wolbachia* mosquito to achieve optimum population to overcome those issues [31].

Nevertheless, the *Wolbachia* strain is also vulnerable to environmental adaptation [44]. This adaptation may subsequently lead to the decrement of their effectiveness. Due to this factor, there is a need for further study in investigating the new strain of *Wolbachia* mosquitoes since the recently available strains are only wMel and wAlbB.

### **Strength, limitation, and recommendation**

To date, not many papers review the effectiveness of *Wolbachia* infected *Aedes* field deployment. The process of reviewing the previous studies was based on validated protocol. All studies in this review involved field trial deployment (natural setting in the community) followed by reduced dengue incidence.

Nevertheless, this review has some limitations. It is not easy to evaluate the effectiveness of the *Wolbachia* program without considering the other confounding factors such as the presence of pre-existing dengue of another method of the dengue control program. This issue can be improved by expanding the program to a bigger scale such as involving the whole state or even become a national program. Meanwhile, the study that has been reviewed is limited to small community trials. Therefore, this limits the information on the effectiveness and the possible barriers to ensure the program's success in managing dengue disease. For example, the geographical or climate factor of a specific study site may play an essential role in implementing the *Wolbachia* release program.

The success of the *Wolbachia* program depends on the ongoing long-term monitoring to confirm the durability of *Wolbachia* and its persistence in local *A. aegypti* populations. The expansion of this program to a larger scale involving multiple countries, especially dengue-endemic countries should be enlightened by the world organization.

## Conclusion

Successful establishment of *Wolbachia* strains, which reduce mosquito longevity or interfere with the pathogen in its vector is predicted to have substantial long-term benefits in terms of reducing disease transmission. Strong community engagement and multidisciplinary participation can ensure the effectiveness of the program. Several vulnerability factors that may influence the effectiveness need further study on a larger scale. Finally, the good practicability of the *Wolbachia* program may change the way in combating dengue disease worldwide in the future.

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## Authors' Contributions

All authors contributed to the design and implementation of the research, analysis of the results, and writing of the manuscript.

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## Appendix

### Appendix A: Data extraction table

Authors/year	Country/ Dengue Endemic Setting	Study Design/ Hierarchy/Period of study	Experimental Unit	Total population/ area size (square kilometre)	Comparator/control site criteria	Community Acceptance/ Engagement	<i>Wolbachia</i> Infected <i>Aedes</i> Aegypti Deployment method	Outcome
Ryan <i>et al.</i> , 2019 [32]	Northern Queensland, Australia/ Not endemic, however there are occurrence of dengue outbreaks, particularly during wet season.	Comparative study without concurrent controls (Interrupted time series)/Level 4/2011-2019	Urban geographical area (Cairns Region, Cassowary Coast Region, Douglas Shire, Charters Towers Region)	Total population: 157,666; area: 90.1km <sup>2</sup>	Nil	2 stage/method of approach. 2011-2014: consultation with key stakeholders and community groups, meetings, display, door knocking, mailouts to assess support and participation, the permission of release, updates on program, random household surveys. 2015-2017: Followed the Public Acceptance Model (33); Raising broad community and stakeholder awareness; quantitative surveys assessing awareness and support; establishment of issues management system; community reference group	Staggered deployment from 2011-2017 of wMel strain <i>Wolbachia</i> infected <i>Aedes aegypti</i> . Both methods of eggs and adults release were used. Release weekly or 2 weeklies, for a duration ranging from 2 to 23 weeks, either 75-150 viable eggs or 25-100 adults or both egg and adult per release. Community and school egg release program also included. Release until the frequency of <i>Wolbachia</i> in samples of field-caught mosquitoes was above 50% for 2 consecutive weeks. Prior to deployment, rearing of <i>Wolbachia</i> infected <i>Aedes</i> <i>aegypti</i> , after release, field monitoring and diagnostics (screening for <i>Wolbachia</i> in mosquitoes) were done. No insecticide resistance assay done.	Entomological: After completion of releases (>23 weeks), median weekly <i>Wolbachia</i> frequencies ranged between 66.0 and 95.0% through until week 52, and were above 80% after that. Predictable and consistent trajectory of <i>Wolbachia</i> establishment in <i>Ae. aegypti</i> populations since release for up to 8 years. Epidemiological: Analysis of dengue case notifications data demonstrates near-elimination of local dengue transmission for the past 5 years in locations where <i>Wolbachia</i> has been established. The regression model estimates of <i>Wolbachia</i> intervention effect from interrupted time series analyses of case notifications data prior to and after releases, indicated a 96% reduction in dengue incidence in <i>Wolbachia</i> treated populations (95% confidence interval: 84–99%)
Nazni <i>et al.</i> , 2019 [30]	Kuala Lumpur, Malaysia/ Endemic setting, area selected have persistent occurrence of dengue over the previous 4 years (2014-2018)	Comparative study with concurrent controls (Controlled Bayesian Time Series)/Level 3/2017-2019	Urban geographical area (Petaling and Gombak District)	No number of populations. Release site: Total of 9966 household (approx.), area: 1.37 km <sup>2</sup> Control site: 38,485 household, 3.61km <sup>2</sup> )	Comparable dengue incidence to the release sites in the period 2013 to the start of release, within same district as release site as possible, building type of "primary" site (to meet similar mosquito and human population characteristics)	Consultation with key stakeholders and community groups (religious leaders included), meetings, workshops, roadshow, carnivals, home visit, IMR lab visit, brochures, banners, website, WhatsApp, SMS, feedback surveys prior to and after release to assess perception, opinion and concerns, updates on the program. >95% gives approval for release project	Staggered deployment in 2017 of wAlbB strain <i>Wolbachia</i> infected <i>Aedes</i> <i>aegypti</i> . Two days prior to release, fogging was done to suppress the wild population. Before release, insecticide susceptibility assays were done on <i>Aedes aegypti</i> from study site and <i>Wolbachia</i> infected <i>Aedes aegypti</i> . Similar susceptibility to pyrethroids, organophosphate fenitrothion and pirimphos. Wing measurements taken from mass-reared adults were in the range expected to produce fit, competitive release mosquitoes (average [SD] for males 2.28 [0.10] mm, females 2.96 [0.11] mm). Both adults and eggs were used. 50 Adults mosquitoes per cup released weekly, 200 eggs left out for 2 weeks for adults to emerge at site. After release, field monitoring and diagnostics (screening for <i>Wolbachia</i> in mosquitoes) were done. Release stops when the <i>Wolbachia</i> frequency reached >90% on three consecutive monitoring periods	Entomological: The frequency of wAlbB has remained high at two sites following invasion (Mentari Court and Commercial Centre). The frequency at Mentari Court is currently still >90%, 2 years after releases were terminated. Epidemiological: Dengue incidence was reduced following releases in all intervention sites. Based on passive case monitoring, reduced human dengue incidence was observed in the release sites when compared to control sites. A Bayesian time series model produced an estimate of dengue case reduction of 40.3% over all intervention sites (95% credible interval 5.06–64.59), with posterior probability of a reduction in intervention sites post-release of 0.985

(Contd...)

## Appendix A: (Continued)

Authors/year	Country/ Dengue Endemic Setting	Study Design/ Hierarchy/Period of study	Experimental Unit	Total population/ area size (square kilometre)	Comparator/control site criteria	Community Acceptance/ Engagement	<i>Wolbachia</i> Infected <i>Aedes</i> Egypti Deployment method	Outcome
Indriani <i>et al.</i> , 2020 [29]	Yogyakarta, Indonesia/ Yogyakarta is one of the highest endemic areas in Indonesia, especially children aged 1–10 years old (68%)	Comparative study with concurrent controls (Controlled Interrupted Time Series)/Level 3/2015-2019	Urban geographical site (villages or Kelurahans in Yogyakarta city)	Release site: total population: 64,599; area: 4.9 km <sup>2</sup> Control Site: total population: 33,535; Area: 3.1 km <sup>2</sup>	comparable sociodemographic characteristics and historical dengue incidence	Extensive community engagement was done following the Public Acceptance Model (33). Key elements of this approach included: meetings with key stakeholders and community leaders; meetings and ongoing regular communication with existing community reference groups at the village, city and provincial level; a communications campaign through social media, traditional media, mobile billboards, and community events; a household-based survey to evaluate awareness and acceptance prior to releases; and a 'stakeholder enquiry system' to receive and respond to any issues arising from stakeholders or community members. 79% public acceptance in intervention area.	Staggered deployment from 2016 to 2017 of wMel strain <i>Wolbachia</i> infected <i>Aedes Aegypti</i> with 13–15 rounds for each "Kelurahan." During rearing, insecticide resistance testing was done, equivalent insecticide resistance profile from the lab colony and wild mosquitoes. <i>Wolbachia</i> -carrying mosquitoes were released as eggs using mosquito release containers (MRCs). Quality assurance done (emergence rates) for each Mosquito Release Containers. After release, field monitoring and diagnostics (screening for <i>Wolbachia</i> in mosquitoes) were done. Release stopped when prevalence of <i>Wolbachia</i> in field caught mosquitoes was >60% for 3 consecutive weeks. Total mosquito estimated release ~128,000	Entomological: Rapid and sustained introgression of wMel <i>Wolbachia</i> into local <i>Ae. aegypti</i> populations was achieved. The median <i>Wolbachia</i> prevalence was 73% (range 67–92%) 1 week after releases stopped, and 100% (96–100%) 2 years post-deployment. In the control areas, single <i>Wolbachia</i> -infected <i>Ae.</i> <i>aegypti</i> mosquitoes were detected on 11 occasions, but there has been no evidence of <i>Wolbachia</i> establishment. Epidemiological: Thirty-four dengue cases were notified from the intervention area and 53 from the control area (incidence 26 vs. 79 per 100,000 person-years) during 24 months following <i>Wolbachia</i> deployment. This corresponded in the regression model to a 73% reduction in dengue incidence (95% confidence interval 49%, 86%) associated with the <i>Wolbachia</i> intervention. Exploratory analysis including 6 months additional post-intervention observations showed a small strengthening of this effect (30 vs. 115 per 100,000 person-years; 76% reduction in incidence, 95%CI 60%, 86%)
O'Neill <i>et al.</i> , 2018 [31]	Northern Queensland, Australia/ Not endemic, however there are occurrence of dengue outbreaks, particularly during wet season	Comparative study without concurrent controls (Interrupted Time Series)/Level 4/2013-2019	Urban geographical area (city of Townsville)	Release site: Total pop: 140,000, area: ~66 km <sup>2</sup>	Nil	Followed the Public Acceptance Model (33); Raising broad community and stakeholder awareness; quantitative surveys assessing awareness and support; establishment of issues management system; community reference group. 87% -95% comfortable with community mosquito release	Staggered deployment from 2013 to 2015 wMel strain <i>Wolbachia</i> infected <i>Aedes aegypti</i> . During rearing, insecticide resistant assay not done. Both eggs and adults were used for deployment. In 28 months of release phase, total of 4 million mosquitoes were release. Approach of community-based deployment supplemented with programmatic targeted deployment were employed. Quality assurance done (emergence rates) for each Mosquito Release Containers. After release, field monitoring and diagnostics (screening for <i>Wolbachia</i> in mosquitoes) were done. Releases continued in each suburb until the frequency of <i>Wolbachia</i> in samples of field-caught mosquitoes from that suburb was above 50% for 2 consecutive weeks	Entomological: <i>Wolbachia</i> was successfully established into local <i>Aedes aegypti</i> mosquitoes across 66 km <sup>2</sup> in four stages over 28 months. In some suburbs, <i>Wolbachia</i> frequencies fluctuated for a number of months before eventually rising to above 80%. In five suburbs, a small number of supplementary releases were undertaken to ensure establishment. In all suburbs, the infection frequency has remained stable without any signs of <i>Wolbachia</i> being lost from the mosquito population Epidemiological: The model-based estimate of intervention effect from the interrupted time series analysis suggests a 95% reduction in dengue incidence in <i>Wolbachia</i> treated populations (95% confidence interval: 84–98%), adjusted for season, imported cases, and allowing for temporal autocorrelation of cases

**Appendix B: Screening for Bias with mixed method appraisal tool (MMAT)**

Quantitative Non-Randomized Studies	3.1 Are the participants representative of the target population?	3.2 Are measurements appropriate regarding both the outcome and intervention (or exposure)?	3.3 Are there complete outcome data?	3.4 Are the confounders accounted for in the design and analysis?	3.5 During the study period, is the intervention administered (or exposure occurred) as intended?
Ryan <i>et al.</i> , 2019	Yes	Yes	Yes	Yes	Yes
Nazni <i>et al.</i> , 2019	Yes	Yes	Yes	Yes	Yes
Indriani <i>et al.</i> , 2020	Yes	Yes	Yes	Yes	Yes
O'Neill <i>et al.</i> , 2019	Yes	Yes	Yes	Yes	Yes