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The Effectiveness of Wolbachia Deployment as a Dengue Control Method: A Systematic Review

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Abstract
As a neglected tropical disease, dengue significantly impacts global health. World Health Organization data shows that approximately 390 million people are infected with the dengue virus each year, with 96 million cases manifesting clinically. Various innovations have been pursued to control dengue virus infections, one of which is the use of *Wolbachia*, a bacterium with high potential to address this issue. *Wolbachia* can reduce and even halt dengue virus transmission by mosquitoes by inhibiting the virus's development and replication within the *Aedes aegypti* mosquito. This study explores the effectiveness of *Wolbachia* in reducing dengue incidence across different regions. The method used in this study follows the PRISMA guidelines. Data sources include PubMed, ScienceDirect, and Cochrane, yielding 585 studies screened and narrowed down to four studies meeting inclusion criteria: one study in Malaysia, one in Brazil, and two in Yogyakarta, Indonesia. Each study's risk of bias was assessed using RoB2 and ROBINS-I according to its methodology. Success rates were evaluated based on the prevalence of *Wolbachia*-infected mosquitoes and the reduction in dengue incidence over a defined period. The results show that *Wolbachia* prevalence can reach 100%, with the highest reduction in dengue incidence reaching up to 77%. This outcome indicates that *Wolbachia* is an effective tool for controlling dengue.

Keywords: *Aedes Aegypti*, Control, Dengue, Neglected Tropical Diseases, *Wolbachia*

1. Introduction

Neglected Tropical Diseases (NTDs) comprise a diverse group of 20 diseases that disproportionately affect impoverished populations, particularly in tropical and subtropical regions (Montresor, 2023). These diseases thrive in areas with inadequate sanitation, limited access to clean water and healthcare, and environments where people live close to animals and vectors of infectious diseases, such as remote rural areas, informal settlements, or conflict zones (Engels & Zhou, 2020). NTDs impose substantial social and economic burdens, impacting over one billion people worldwide and resulting in more than 200,000 deaths per year from snake bites, rabies, and dengue (Montresor, 2023). Addressing NTDs is critical to obtaining universal health coverage. The global response to NTDs aims to support impoverished populations and address their health and economic challenges (Engels & Zhou, 2020).

As previously mentioned, dengue is classified as one of the NTDs. It is an acute viral infection transmitted between humans by the *Aedes aegypti* mosquito. The role of environmental factors in dengue transmission, such as temperature, rainfall, and seasonal variations, cannot be overstated. These factors significantly impact the presence of the *Aedes aegypti* mosquito (Cattarino et al., 2020). Dengue is the most common human arbovirus infection, with an estimated 105 million infections globally each year, of which 51 million are symptomatic (Asish et al., 2023). According to WHO, there are approximately 390 million dengue virus infections annually, with 96 million clinical cases, and about 3.9 billion people are at risk. Dengue is now endemic in over 100 countries in WHO regions, including Africa, America, Eastern Mediterranean, Southeast Asia, and Western Pacific.

The current strategy for controlling dengue infection focuses on reducing adult and immature mosquito populations by using insecticides and educating the public on how to remove breeding grounds. Maintaining low mosquito populations is still challenging despite massive resource efforts, which results in recurring seasonal outbreaks (Pinto et al., 2021). Therefore, research and health innovations are necessary to help reduce and control these dengue outbreaks.

Several studies have been conducted to innovate dengue outbreak control. Research has demonstrated that *Aedes aegypti* mosquitoes infected with *Wolbachia* have a lower potential for transmitting human arboviruses such as dengue, Zika, and chikungunya (Tantowijoyo et al., 2020). *Wolbachia* represents a recent innovation aimed at curbing the spread of dengue within communities. In recent years, *Wolbachia* has become a primary focus of research efforts to mitigate mosquito-borne outbreaks (Caragata et al., 2021).

Wolbachia is a type of bacterium commonly found in insects (Asri Nuraeni, 2020). It is an intracellular endosymbiotic bacterium, naturally present in around 60% of insect species, including several mosquitoes (Dorigatti et al., 2018). *Wolbachia* can inhabit various tissues within its host, including the ovaries and testes, allowing it to be maternally inherited. This bacterium is under continuous study to assess its success and effectiveness in controlling infectious diseases, often spread by arthropods like mosquitoes. *Wolbachia* is an obligate endosymbiotic bacterium that cannot survive outside its host cells and is transmitted vertically through the female (Li & Liu, 2021). *Wolbachia* from *Drosophila melanogaster* has been successfully isolated into the eggs of *Aedes aegypti* mosquitoes, where it establishes a stable symbiosis and is passed on to subsequent generations. *Wolbachia* does not genetically modify mosquitoes, meaning *Aedes aegypti* is not genetically engineered. Research shows that *Wolbachia* inhibits the development and replication of the dengue virus, reducing transmission, with some programs reporting an 86% reduction in dengue spread (Suwantika et al., 2020).

2. Methods

2.1 Guidelines

This systematic review was conducted following the PRISMA guidelines specifically designed for systematic reviews and meta-analyses. PRISMA offers a framework that aids researchers in achieving accurate and reliable data selection for systematic reviews. This systematic review developed a pertinent scientific question using the established guidelines.

2.2 Formulating the Scientific Question

The scientific question for this systematic review was developed using the PICO framework (Population, Intervention, Comparison, Outcome). PICO is a tool that assists researchers in constructing a suitable scientific question based on three core concepts: population or problem, intervention, comparison, and outcome. In this review, the three primary aspects considered are humans (population), *Wolbachia* (intervention), and dengue (outcome).

2.3 Systematic Search Strategy

The systematic search involved three main steps: identification, screening, and inclusion (Figure 1).

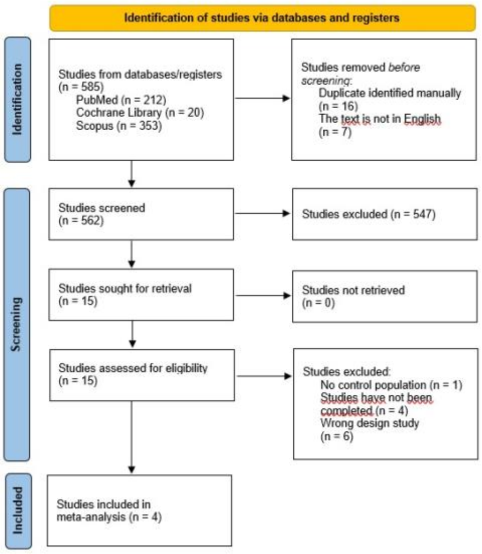

Figure 1: PRISMA Flow Chart

2.4 Identification

The identification process included searching for synonyms, medical subject headings (MeSH), and related terms within the categories of humans, *Wolbachia*, and dengue. Databases used included PubMed, Scopus, and Cochrane. This search yielded a total of 585 articles, with 16 duplicates and 7 non-English articles removed, leaving 562 articles.

2.5 Screening

The screening was conducted for the remaining 562 articles by categorizing them from each database. Articles that were journal articles, written in English, and contained both qualitative and quantitative data were required to meet the Inclusion criteria. Systematic reviews and articles with inconsistent findings were excluded. This process excluded 547 articles, leaving 15 suitable articles. Further manual screening was conducted to assess intervention results, excluding studies without a control population, incomplete studies, and those with incompatible study designs, ultimately resulting in 4 eligible studies.

2.6 Data Extraction

Data extraction involved detailed analysis of each study's abstract, methodology, and results. Extracted data were then organized into a data extraction table for analysis, identifying patterns within the data available.

3. Results

Of the four included studies, one was a cluster-randomized trial (Utarini et al., 2021), and the other three were quasi-experimental trials (Anders et al., 2020; Nazni et al., 2019; Pinto et al., 2021). Two studies were conducted in Indonesia (Anders et al., 2020; Utarini et al., 2021), one in Brazil (Pinto et al., 2021), and one in Malaysia (Nazni et al., 2019). The methods for disseminating *Wolbachia* differed, with two studies using mosquito eggs (Anders et al., 2020; Utarini et al., 2021) and two using adult mosquitoes directly (Nazni et al., 2019; Pinto et al., 2021). All studies showed a reduction in dengue cases, but the percentage decrease varied, ranging from 40.3% to 77%.

Table 1: Data Extraction Results from Four Studies

No	Author	Location (Year)	Method	Release Zone	*Wolbachia* Deployment Technique	*Wolbachia* Prevalence	Results
1	(Utarini A, Indriani C, Ahmad RA, Tantowijoyo W, Arguni E, 2021)	Yogyakarta, Indonesia (2021)	Cluster Randomized Trial	There were 12 intervention clusters where *Aedes aegypti* mosquitoes infected with wMel were released, and 12 control clusters with no releases, covering a total area of 26 km² and a population of 311,700.	Mosquito eggs were placed in intervention clusters from March to December 2017, with each cluster receiving between 9 and 14 deployment cycles.	The average monthly prevalence of wMel per cluster was 95.8% over a 27-month clinical survey period.	Dengue incidence reduced by 77%.
2	(Nazni et al., 2019)	Kuala Lumpur, Malaysia (2019)	Quasi-Experimental Trial	Release of *Wolbachia*-carrying *Aedes aegypti* was conducted in 6 locations across Kuala Lumpur with high endemic dengue transmission.	Mosquitoes were released weekly in the morning across pre-defined grids, with *Wolbachia* frequency monitored approximately 4 weeks post-release.	After releases ended, *Wolbachia* frequency remained stable and high: 98% at Mentari Court and 95% in other areas.	A 40.3% reduction in dengue cases across all intervention areas.
3	(Pinto et al., 2021)	Brazil (2021)	Quasi-Experimental Trial	Release took place in four zones with a total area of 83 km² and a population of 373,000.	*Wolbachia*-infected adult mosquitoes were deployed weekly over 40 km² for 35 months, from February 2017 to December 2019, using mobile vehicles.	Weekly screenings of a minimum sample of 168 mosquitoes showed a wMel prevalence of 100% every week except for 3 screenings, with prevalence never dropping below 97%.	Dengue incidence reduced by 69% with *Wolbachia* intervention.
4	(Anders et al., 2020)	Yogyakarta, Indonesia (2022)	Quasi-Experimental Trial	Intervention area covering 5 km² with an adjacent population of 65,000, and a 3 km² area with a population of 34,000 as the non-intervention zone.	*Wolbachia*-infected mosquito eggs were released using mosquito release containers (MRCs), which were placed outside houses, sheltered from direct sunlight and rain, from August 2016 to March 2017.	Median *Wolbachia* prevalence was 73% (range 67-92%) one week post-deployment and reached 100% (range 96-100%) two years after deployment.	Dengue incidence reduced by 73%.

4. Discussion

4.1 Methods of Deployment of Wolbachia

Based on four selected and analyzed journals, there are two main methods for disseminating *Wolbachia*. The first method involves releasing eggs of mosquitoes infected with *Wolbachia*. This process entails collecting eggs of the *Aedes aegypti* mosquito infected with *Wolbachia* and placing them in a designated intervention area. Two research journals from Yogyakarta using this method reported a prevalence of 67-95.8%. However, one of the journals indicated an increase in prevalence that could reach up to 100% (Anders et al., 2020).

The second method involves directly releasing *Aedes aegypti* mosquitoes infected with *Wolbachia* into the intervention area (Nazni et al., 2019; Pinto et al., 2021). This release can occur at several points within a container or from a moving vehicle. The prevalence in this case ranges from 95% to 100%. This demonstrates that spreading *Wolbachia* through the direct release of adult mosquitoes yields a higher prevalence more quickly than using eggs.

4.2 Data Calculation of Prevalence of Wolbachia-Infected Mosquitoes

In all included studies, *Aedes aegypti* mosquitoes carrying *Wolbachia* exhibited a high prevalence with a relatively diverse range of percentages. The monitored prevalence of *Wolbachia* after the cessation of releases will serve as a reference for controlling the spread of *Wolbachia* in the intervention areas. This prevalence can provide information on whether the mosquito distribution was carried out correctly and successfully and whether there is a need for further release of *Wolbachia* in areas with low prevalence.

This also helps determine the duration of *Wolbachia* screening in *Aedes aegypti* mosquitoes if necessary. For instance, in the second Yogyakarta study, testing was conducted weekly when *Wolbachia* prevalence was <80%, biweekly when ≥80%, and every four weeks when ≥90%. The study showed rapid *Wolbachia* establishment in the intervention area, with continuous increases in infected *Aedes aegypti* prevalence during the first-year post-release. The median *Wolbachia* prevalence was 73% (67-92%) one week after release ceased and 100% (96-100%) two years after placement. Additionally, the average monthly cluster prevalence rate of wMel was 95.8% (interquartile range, 91.5 to 97.8) over 27 months of clinical surveillance (Anders et al., 2020).

A study in Malaysia showed an increase in *Wolbachia* frequency to over 80% at all intervention sites. After the cessation of mosquito releases, the frequency of *Wolbachia* remained stable and high (98% at 12 months after releases stopped). In some areas, the frequency exceeded 95%, but fluctuations occurred over time after the cessation (Nazni et al., 2019). Screening for wMel infection was conducted weekly in the Brazil study using a minimum sample of 168 mosquitoes from the release colony, employing quantitative polymerase chain reaction (qPCR). A prevalence of wMel was found to be 100% at all screening times, except for three screening times, but the results stayed below 97% (Pinto et al., 2021).

4.3 Effectiveness of Wolbachia in Reducing Dengue Incidence

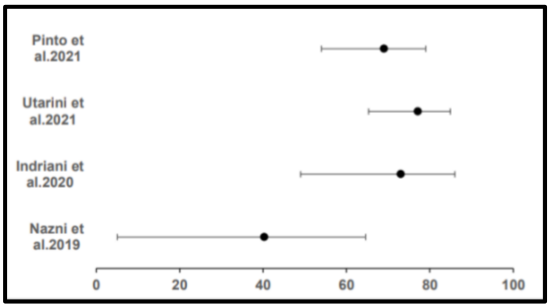

Figure 2: The reduction in dengue incidence (indicated by black dots) was assessed with 95% CI (horizontal lines)

The reduction in dengue incidence in areas with *Wolbachia*-infected mosquito releases has shown positive results. The study in Yogyakarta revealed a significant difference in dengue incidence, with only 67 cases reported among 2905 participants (2.3%) in the intervention area, compared to 318 cases among 3401 participants (9.4%) in the control area. These results indicate a protective efficacy of 77.1% (95% CI, 65.3 - 84.9) (Utarini et al., 2021). The second study in Yogyakarta showed a 73% reduction in dengue cases derived from an interrupted time series (ITS) analysis reporting monthly dengue cases from January 2006 to March 2019. Furthermore, an analysis conducted 6 months after intervention until September 2019 demonstrated an enhancement in reducing dengue incidence to 76% (Anders et al., 2020).

In Brazil, a release of *Aedes aegypti* infected with *Wolbachia* was conducted in Niterói. This area was divided into zones 1, 2, 3, 4, and a control zone. Using an Interrupted Time Series (ITS) analysis, a reduction in dengue cases was observed starting from 46% in zone 3, with the most significant reduction in zone 2 at 75.9% and an average reduction of 69.4% of cases occurring in Niterói (Pinto et al., 2021). Subsequently, a study conducted in Malaysia differed from the other areas as it utilized the wAlbB strain, while the previous three studies used the wMel strain. The results, derived from comparing dengue incidence from 2013 to 2019 in mosquito release and control areas based on data from the Malaysian National Dengue Surveillance System, revealed a reduction in dengue incidence of 40.3% across all intervention areas using a Bayesian time series model (Nazni et al., 2019).

5. Conclusion

There are still many limitations in the studies, particularly regarding some data needing more control cases for comparison, indicating a need for further studies to ensure the accuracy of the analytical data obtained. Both methods used for disseminating *Wolbachia* in *Aedes aegypti* mosquitoes demonstrated similarly positive results. However, the direct dispersal of *Wolbachia* using adult mosquitoes can yield higher results more quickly than releasing through mosquito eggs. The *Wolbachia* prevalence observed after the cessation of releases yielded high outcomes in each intervention area without significant hindrances or disruptions. Analyses conducted on four studies across different countries found that employing *Wolbachia* as a dengue control strategy positively inhibited and reduced the spread of the dengue virus.

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