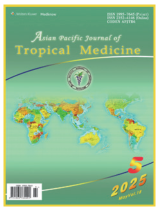

Systematic Review and Meta-analysis
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Wolbachia−infected mosquito to suppress the transmission chain of mosquito−borne virus: A systematic review and meta−analysis of community−based health intervention trials

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ABSTRACT

Objective: To identify the efficacy of *Wolbachia*-based intervention by reviewing community-based trials through meta-analysis and systematic review methods.

Methods: Studies about *Wolbachia*-infected mosquito intervention were collected through a screening process. Records underwent data extraction and quality assessment independently by the authors. The primary outcome of the study was protective efficacy. Quantitative analysis was conducted through meta-analysis and multivariate meta-regression using Rstudio Ver.2024.09.0-375. Qualitative analysis was done by summarizing records' findings.

Results: 10 out of the 10 660 records met the criteria. The evidence was moderate in quality and highly heterogeneous. Intervention consisted of releasing *Wolbachia*-infected *Aedes aegypti* in densely populated settlements. The combined protective efficacy of *Wolbachia* intervention against dengue is highly heterogeneous (79%; 95% *CI* 70-88; I^2=98%). *w*Mel strain is significantly more efficacious compared to *w*AlbB (protective efficacy 84%; 95% *CI* 76-93; I^2=95%) *vs*. 64% (95% *CI* 46-82; I^2=85%); $P<0.01$) in preventing dengue cases. *Wolbachia*-infected mosquito populations were found to be unstable upon release cessation, which necessitated periodic release and monitoring to maintain desired concentration and protective efficacy.

Conclusions: *Wolbachia*-based intervention is effective in suppressing the transmission of mosquito-borne diseases, especially dengue, with an excellent safety profile. However, community acceptance and policy remain as significant barriers to implementation.

KEYWORDS: Dengue; Mosquito-Borne Diseases; Mosquito; Primary prevention; *Wolbachia*

1. Introduction

Mosquito-borne diseases, including dengue, zika, and chikungunya, inflict a heavy and persistent burden in most developing countries in tropical areas, despite eradication efforts

Summary
Question: What is the efficacy of *Wolbachia*-based interventions in reducing the incidence of mosquito-borne infections?
Findings: This meta-analysis demonstrates that *Wolbachia*-based interventions are highly effective in preventing mosquito-borne diseases, particularly dengue, with an overall protective efficacy of 79%. Intervention using the *w*Mel strain showed superior performance compared to the *w*AlbB strain (84% *vs*. 64%). These results were consistent across diverse geographic and climatic conditions, and the protective effects persisted long after the interventions ended in most studies.
Meaning: *Wolbachia*-based strategies offer a sustainable and impactful approach to reducing mosquito-borne disease transmission, with long-term benefits observed globally.

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that have been in motion for decades. Dengue, which is endemic in most tropical countries, was listed as one of the top 10 global mortality threats by the World Health Organization in 2019. Each year, there are an estimated 50-100 million symptomatic cases worldwide[1–3]. Failure to control the *Aedes* population as along with urbanization and uncontrolled population growth, contributes to the high incidence of dengue worldwide. Efforts to reduce the number of mosquitoes through insecticide spraying and community counselling to eradicate mosquito nests are strategies that are still being implemented. However, the goal of reducing the number of mosquitoes has not been achieved and seasonal outbreaks still occur. In addition, the increasing resistance to insecticides in the mosquito population further complicates the eradication efforts. Therefore, a new effective and affordable strategy to control the vector of arbovirus, *Aedes* (*Ae.*) *aegypti*, is needed[3–5].

One of the new strategies is the release of mosquitoes infected with *Wolbachia* intracellular bacteria (*Wolbachia*-infected *Ae. aegypti*). *Wolbachia* is an endosymbiotic bacterium that occurs naturally in several insect species, although not in *Ae. aegypti*, hence the need for a transinfection method to generate *Wolbachia*-infected mosquitoes. Laboratory studies that transinfected the *Wolbachia* strain *w*Mel into *Ae. aegypti* resulted in mosquitoes with reduced transmission potential for dengue and other arboviruses. Female mosquitoes infected with *w*Mel transmit the bacteria to their offspring through infected eggs. In addition, *w*Mel also manipulates mosquito reproduction through the mechanism of cytoplasmic incompatibility resulting in the introgression of *w*Mel into wild mosquito populations. The use of *Wolbachia* in mosquito population control can have two objectives, namely (1) to replace natural mosquito populations with mosquitoes that have lower phenotypic competence, by releasing female mosquitoes or (2) to suppress existing mosquito populations by releasing male mosquitoes that are reproductively incompatible with non-*Wolbachia*-infected females due to cytoplasmic incompatibility induced by *Wolbachia* infection. In addition to requiring the release of a larger number of mosquitoes, the male release method may not maintain the presence of *Wolbachia* in wild mosquito populations. This study aims to assess the efficacy of releasing *Wolbachia*-infected mosquitos in preventing mosquito vector transmission based on community interventional studies that have been conducted[4,6–8].

2. Methods

2.1. Study design and eligibility criteria

The screening process described in this paper was in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analysis guidelines and Cochrane Handbook for Systematic Reviews and Meta-Analysis. The inclusion criteria of this review comprised: (1) a community health intervention trial, (2) the release of *Wolbachia*-infected mosquito in at least community scale, (3) measuring the incidence of mosquito-borne infections (dengue, zika, chikungunya) prior to and post-intervention. However, we excluded studies that were: (1) inaccessible, (2) written in a language other than Indonesian or English, and (3) cross-sectional, in vitro, and in vivo studies. This study protocol was registered on the PROSPERO (CRD42024567748).

2.2. Literature searching strategy and study selection

Literature searching was conducted independently by all the authors in January 2023 on five major databases, namely PubMed, Cochrane, Science Direct, ProQuest, and Google Scholar. Searches were carried out using differing keywords "*Wolbachia*", "dengue", "release" or "deployment", and "trial". The results underwent a selection process in accordance with Preferred Reporting Items for Systematic Review and Meta-Analysis guidelines mentioned above. Records reporting the outcome in unsuitable formats and narrative findings were used for qualitative evidence synthesis.

2.3. Data extraction and risk of bias assessment

Data extraction and validation were performed by three of the authors, MAN, TDG, and HH. In order to comprehensively uncover the relationships between variables, the extracted data comprised the following: (1) study characteristics (author and location), (2) subject characteristics (intervention population size and intervention site size), (3) intervention characteristics (mosquito release method, number of mosquitos released, and measurement endpoints), (4) the primary outcome, which was the protective efficacy of the intervention against mosquito-borne infections (dengue, zika, chikungunya), (5) secondary outcomes, which were the endpoint prevalence of *Wolbachia*-infected mosquito population and the population stability. Any miscellaneous findings worth mentioning were included in the commentary section.

Risk of bias assessments were carried out after data extraction using ROBINS-I for non-randomized interventional studies. The study was assessed by two of the authors, HH and TDG, while MAN resolved conflicts between two assessors. Lastly, the quality of evidence of the records was presented in Grading of Recommendations, Assessment, Development and Evaluation format.

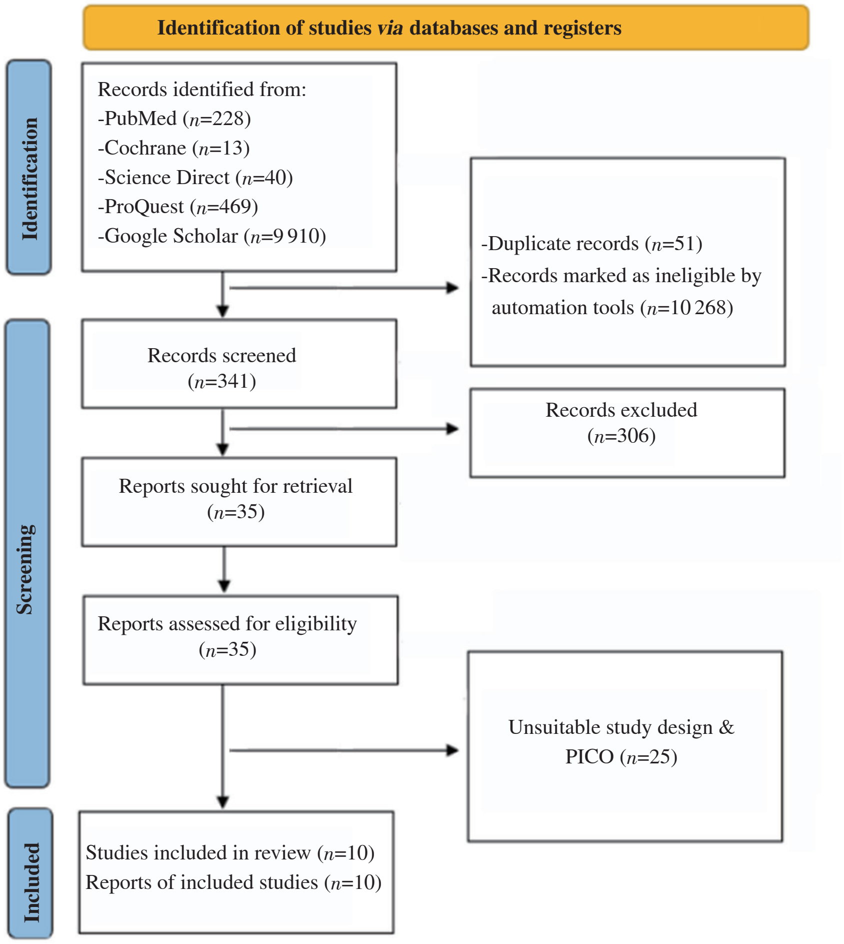


Figure 1. Flowchart of the selection process. PICO: Population, intervention, comparison and outcomes.

2.4. Quantitative analysis

Meta-analysis was carried out using RStudio v.2022.07.2+576, through the "meta" and "metasens" packages. The meta data of the records were computed using the inverse variance method with the effect size and approximated standard error (based on the *CI* range) as the input values. To determine the proportionate reduction of dengue cases in treated areas compared to control areas, across different studies we used protective efficacy (PE), which can be calculated with this formula[2]:

$$PE=100\times(1-RR)$$

where *RR* stands for the relative risk of dengue in treated population. This formula can also be written as follows:

$$PE= 100\times\left(1-\frac{\text{Dengue risk at intervention site}}{\text{Dengue risk at control site}}\right)$$

Due to variable results and many possible confounding variables, the random-effects model was used and heterogeneity of pooled statistics was assessed using *Chi*-square and I^2 statistics. Subgroup analyses were conducted based on different strains, regions, and intervention lengths (endpoints). In order to address the heterogeneity, further meta-regression was performed with secondary outcomes and characteristics as predictors.

3. Results

3.1. Summary and the characteristics of the study

Of the 10 660 records screened, a total of 10 studies were retrieved (Figure 1), all of which were deemed uniform in study design and low to moderate in risk of bias. The majority of the excluded studies

Table 1. Quantitative data from compiled studies.

Author, year	Virus	Location	Strain	ES	95 % *CI*	
					Low	High
Utarini *et al*., 2021[2]	DENV-1	Yogyakarta, Indonesia	*w*Mel	0.71	0.18	0.90
	DENV-2	Yogyakarta, Indonesia	*w*Mel	0.84	0.72	0.91
	DENV-3	Yogyakarta, Indonesia	*w*Mel	0.75	0.05	0.94
	DENV-4	Yogyakarta, Indonesia	*w*Mel	0.74	0.58	0.84
	Total (Pooled analysis)	Yogyakarta, Indonesia	*w*Mel	0.77	0.65	0.85
Pinto *et al*., 2021[4]	Dengue	Niteroi, Brazil	*w*Mel	0.69	0.54	0.79
	Chikungunya	Niteroi, Brazil	*w*Mel	0.56	0.16	0.77
	Zika	Niteroi, Brazil	*w*Mel	0.37	0.01	0.60
O'Neill *et al*., 2019[9]	Dengue	Townsville, Australia	*w*Mel	0.95	0.84	0.98
Indriani *et al*., 2020[1]	Dengue	Yogyakarta, Indonesia	*w*Mel	0.76	0.60	0.86
Ryan *et al*., 2020[10]	Dengue	Cairns, Australia	*w*Mel	0.96	0.84	0.99
Hoffman *et al*., 2024[11]	Dengue	East Malaysia	*w*AlbB	0.62	0.50	0.71
Velez *et al*., 2023[12]	Dengue	Colombia	*w*Mel	0.95	0.94	0.97
Nazni *et al*., 2019[13]	Dengue	Selangor, Malaysia	*w*AlbB	0.40	0.05	0.65
dos Santos *et al*, 2022[14]	Dengue	Rio de Janeiro, Brazil	*w*Mel	0.76	0.64	0.71
Lim *et al*., 2024[15]	Dengue	Singapore	*w*AlbB	0.77	0.76	0.79

ES: Effect size, representing the magnitude of protective efficacy (PE). Low 95%: Lower bound of the 95% confidence interval for the effect size of PE. High 95%: Upper bound of the 95% confidence interval for the effect size of PE. DENV: Dengue virus. *w*Mel, *w*AlbB: *Wolbachia* strains used for the intervention.

(10 268 records) were eliminated through the use of search engine filters, which excluded other study and document types (grey papers, reviews, book sections, *in vitro* and *in vivo* studies, commentaries, and other irrelevant content). Studies were conducted in Australia, Brazil, Malaysia, Indonesia, Colombia, and Singapore, with a total number of subjects in intervention sites reaching 6 million, and covering a total area of approximately 430 km^2. Most of the studies employed *w*Mel strain, while others used the *w*AlbB strain, of which all studies reporting *Ae. aegypti*, both in the form of eggs or adults as the vector. The median follow-up duration is 28 months, ranging from 16-102 months. All studies reported PE against dengue, while only Pinto *et al*. reported on other mosquito-borne viruses, namely chikungunya and zika. Extracted data were tabulated and displayed in Table 1.

Table 1 summarized the quantitative results from studies evaluating the impact of *Wolbachia* interventions on mosquito-borne virus suppression. We included several parameters: (1) *Wolbachia* strain used in field release, (2) reported effect sizes with 95% confidence intervals, and (3) the mosquito-borne viruses of which the PE was measured, such as dengue, chikungunya, or zika.

Table 2 summarizes key characteristics and findings from studies included in the meta-analysis. "Population characteristics" refers to the study data, including information about the populations involved in the intervention and control groups, rather than the geographic regions. The "Comments" column provides interpretations by the authors of this manuscript, based on insights gained from the reviewed studies. These comments include observations about study design, implementation, limitations, and areas for further exploration.

The pooled protective efficacy against dengue of the *Wolbachia*-based intervention in this study is 79% (95% CI 70-80; I^2=98%) as displayed in Figure 2. Due to high variation between studies in intervention approach and conditions capable of affecting the results, the use of a random effect model is more favourable in this meta-analysis. Although the results yielded a rather heterogeneous result, further subgrouping based on the strain (Supplementary Figure 1) and location (Supplementary Figure 2) was performed. Dengue incidence reduction was more pronounced in populations treated with *w*Mel-infected mosquitoes, achieving an 84% reduction in cases compared to a 64% reduction observed with *w*AlbB-infected mosquitoes (84%; 95% *CI* 76-93 *vs*. 64%; 95% *CI* 46-82; *P*<0.01)(Supplementary Figure 1). Studies conducted in Asia demonstrated a protective efficacy of 72%, those in America showed 81%, and studies in Australia exhibited the highest efficacy at 95% (72%; 95% *CI* 64-80 *vs*. 81%; 95% *CI* 65-96 *vs*. 95%; 95% *CI* 90-100; *P*<0.01) (Supplementary Figure 2). These findings illustrate

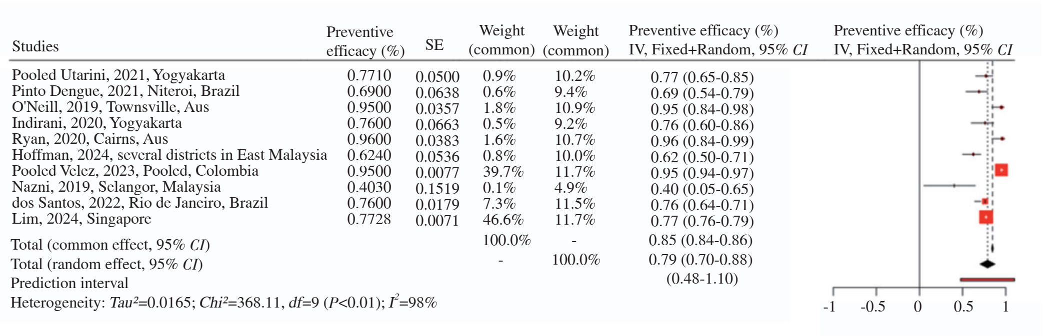
Table 2. Qualitative extraction of each study.

Author, year	Location	Subject characteristics			Population characteristics	Release strategy	Stability at endpoint (%)	Comments
		Cover area (km^2)	Population size (in thousands)	Density (thousands/km^2)				
Utarini *et al.*, 2021[2]	Yogyakarta, Indonesia	26	311.7	11.99	-Age: 3 to 45 years old, with a median of 11.6 years -Sex: 48.8% female -Health status: 4.7% hospitalized during the study period	*w*Mel infected *Aedes aegypti* was released for several months in each cluster during 2017 (March 2017 - December 2017). There were 9 to 14 release rounds, with each round lasting 2 weeks, in each intervention cluster.	95.8	-Potential for dengue virus to evolve resistance to the *Wolbachia* intervention. -Need to explore the ability of the *Wolbachia* intervention to reduce transmission of other arboviruses besides dengue.
Pinto *et al.*, 2021[4]	Niteroi, Brazil	83	373	4.49	-Total population of Niteroi: 484 918 (as of 2010 census) -Niteroi is divided into 7 health districts and 4 release zones plus 1 control zone for the study	*w*Mel infected *Aedes aegypti* is deployed over a 35 months period (February 2017-December 2019), released weekly from a moving vehicle. The initial release points determined on the grids were then distributed to the nearest vehicle-accessible road for vehicle releases. Initial release periods were 10-16 weeks duration, with subsequent re-releases conducted in local areas where *w*Mel prevalence was <40% in 3 consecutive monitoring events as measured at least 4 weeks after the conclusion of releases. This resulted in re-releases being conducted in approximately 30% of the initial release areas.	33-90	-The deployments were not randomized, so there is potential for confounding. -The disease surveillance data has imperfect specificity and sensitivity.
O'Neill *et al.*, 2019[9]	Townsville, Australia	66	140	2.12	-The total area considered 'covered' by *Wolbachia* was 128 km^2 -The residential population in the 'covered' area was 140 000 in 2016	*w*Mel infected *Aedes aegypti* were deployed as eggs using mosquito release containers over the period of 28 months.	93.4	-Potential instability of *w*Mel *Wolbachia* at high temperatures, though field data showed long-term stability. -Potential confounding effects of climate, as *Wolbachia* releases coincided with hot and dry years. -This study highlighted community involvement in mosquito rearing and release which improved feasibility, community acceptance, and possibly, efficacy.
Indriani *et al.*, 2020[1]	Yogyakarta, Indonesia	8	99	12.38	-Intervention area: 7 urban villages with a total population of 64 599 -Control area: 3 urban villages with a total population of 33 535 -Sociodemographic characteristics: Comparable between intervention and control areas	Wolbachia-carrying *Aedes aegypti* mosquitoes were released as eggs using mosquito release containers. Releases occurred between August 2016 and March 2017, with 13-15 rounds of releases in each subdistricts (kelurahan). The release was stopped when the prevalence of *Wolbachia* in field-caught mosquitoes was >60% for three consecutive weeks of releases.	100	-The study measured mosquito susceptibility to insecticides. Although not statistically significant, *w*Mel-infected mosquitoes were slightly more susceptible to insecticides. -The study did not determine the DENV serotypes circulating or the incidence of other *Aedes*-borne diseases like zika and chikungunya.
Ryan *et al.*, 2020[10]	Cairns, Australia	9.3	164.519	17.69	-Population density: Areas with higher human population density were selected -Dengue case history: Areas with historical dengue case reports were selected	Between 2011 and 2015, weekly releases of adult, *w*Mel infected *Aedes aegypti*, were undertaken on a per-household basis, at a density of 1:3 to 1:10 houses. Between 2016 and 2017, releases were undertaken on an area basis, where the target release area was divided into a series of 100 m × 100 m grids, with a single release point located inside each grid. Releases were generally undertaken between 800-1 600 hours each day.	66-95	Mosquitoes infected with *w*Mel also exert a protective effect on the control region.

Table 2 continues in the next page.

Table 2. Continued.

Author, year	Location	Subject characteristics			Population characteristics	Release strategy	Stability at endpoint (%)	Comments
		Cover area (km^2)	Population size (in thousands)	Density (thousands/ km^2)				
Hoffman *et al*., 2024[11]	East Malaysia	1.37	128	93.43	-High-rise residential areas in Selangor, Kuala Lumpur, and Putrajaya, Malaysia -High historical incidence of dengue cases over the previous 5 years	Initially, *Aedes aegypti*-infected mosquito eggs were used in the operational releases but there was a switch to adults later during the COVID -19 period. Mosquitoes were released in over 20 spots across Kuala Lumpur.	82.3	-Inability to randomize control and release sites due to the operationalized nature of the releases and the need to respond rapidly to outbreaks. -Reliance on local health officers with varying levels of experience for releases and monitoring, rather than using the same team across all areas. -Changes to the release methods due to restrictions imposed by the COVID-19 outbreak.
Velez *et al*., 2023[12]	Colombia	135	3 345	24.78	-Large urban population of 3.3 million people across three municipalities (Bello, Medellín, and Itagüí) -Located at elevations between 1 400-2 100 m above sea level -Experience a warm, stable climate year-round with average temperatures between 21.8 ℃-23.1 ℃	Both eggs and adult *Aedes aegypti* mosquitoes infected with *w*Mel were released.	74.91	-Interruptions to releases and monitoring due to COVID-19 pandemic. -Need to optimize monitoring approach for large-scale deployments, potentially focusing on representative areas rather than broad periodic monitoring.
Nazni *et al*., 2019[13]	Selangor, Malaysia	1.37	38.8	28.32	Urban areas of Kuala Lumpur, Malaysia, where dengue is endemic	wAlbB-infected *Aedes aegypti* in adult and egg forms were carried out in 6 sites. Releases were conducted for 20 weeks and terminated when the *Wolbachia* frequency reached > 90% on 3 consecutive monitoring periods. Releases ceased for 4 months, and then a second release period was undertaken for 31 weeks at a lower rate.	98	-The study period was relatively short, and longer monitoring postrelease would further increase the accuracy of the estimated effect size on dengue incidence. -As more areas become *Wolbachia*-positive, the proportion of imported dengue cases will decrease, making the effect of the intervention harder to detect.
dos Santos *et al*., 2022[14]	Rio de Janeiro, Brazil	86.8	890	10.25	-Population in the release area: around 890 000 -The release area was divided into 5 zones covering 86.8 km^2	The *w*Mel-infected *Aedes aegypti* release programme started in the northwest of the city in August, 2017. The release area was subdivided into five zones (RJ1, RJ2, RJ3.1, RJ3.2, and RJ3.3), covering a total area of 868 km^2 with around 890 000 inhabitants, and releases were phased through the different zones.	27-60	The analysis only covers data up to 2019, and extending the analysis to later years would provide insight into the long-term durability of the intervention, but this was not possible due to the impact of the COVID-19 pandemic.
Lim *et al*., 2024[15]	Singapore	10.33	608	58.86	-Age: adolescents (7-20 years), adults (21-60 years), older adults (61 years), and children (0-6 years) -Location: individuals living in intervention sites (607 872) and control sites (3 894 544) in Singapore	The releases of *w*AlbB infected adult *Aedes aegypti* were conducted two times a week (weekdays, between 6:30 and 10:30) at four designated public locations in high-rise public housing estates. To ensure an even distribution of mosquitoes, releases were done at six to 12 equally spaced release locations per apartment block, with half of mosquitoes released on the ground floor and the other half on upper floors, alternating between middle (floors 5-6) and higher floors (floors 10-11).	NA	-The intervention was only tested in high-rise public housing estates, so the results may not generalize to other settings. -The analysis assumed no spillover effects from the intervention sites to the control sites, which could have led to conservative estimates of the intervention's efficacy.



Studies	Preventive efficacy (%)	SE	Weight (common)	Weight (common)	Preventive efficacy (%) IV, Fixed+Random, 95% *CI*
Pooled Utarini, 2021, Yogyakarta	0.7710	0.0500	0.9%	10.2%	0.77 (0.65-0.85)
Pinto Dengue, 2021, Niteroi, Brazil	0.6900	0.0638	0.6%	9.4%	0.69 (0.54-0.79)
O'Neill, 2019, Townsville, Aus	0.9500	0.0357	1.8%	10.9%	0.95 (0.84-0.98)
Indirani, 2020, Yogyakarta	0.7600	0.0663	0.5%	9.2%	0.76 (0.60-0.86)
Ryan, 2020, Cairns, Aus	0.9600	0.0383	1.6%	10.7%	0.96 (0.84-0.99)
Hoffman, 2024, several districts in East Malaysia	0.6240	0.0536	0.8%	10.0%	0.62 (0.50-0.71)
Pooled Velez, 2023, Pooled, Colombia	0.9500	0.0077	39.7%	11.7%	0.95 (0.94-0.97)
Nazni, 2019, Selangor, Malaysia	0.4030	0.1519	0.1%	4.9%	0.40 (0.05-0.65)
dos Santos, 2022, Rio de Janeiro, Brazil	0.7600	0.0179	7.3%	11.5%	0.76 (0.64-0.71)
Lim, 2024, Singapore	0.7728	0.0071	46.6%	11.7%	0.77 (0.76-0.79)
Total (common effect, 95% *CI*)			100.0%	-	0.85 (0.84-0.86)
Total (random effect, 95% *CI*)			-	100.0%	0.79 (0.70-0.88)
Prediction interval					(0.48-1.10)

Heterogeneity: Tau^2=0.0165; Chi^2=368.11, df=9 (P<0.01); I^2=98%

Figure 2. Pooled analysis of the efficacy of *Wolbachia*-based intervention.

Table 3. Grading of recommendations, assessment, development and evaluation for evidence quality assessment.

Evidence	*n*	Effect size	GRADE assessment				Other	GRADE	Importance
			Risk of bias	Inconsistency	Imprecision	Indirectness			
Pooled analysis: *w*Mel & *w*AlbB	10	0.79 (0.70-0.88); I^2=98% High	+	-	-	+	Meta regression was yet capable of explaining the high heterogeneityamong studies	++-- Low	Critical
Subgroup: *w*Mel strain	7	0.84 (0.76-0.93); I^2=95% High	+	-	+	+	The interval is still around the practicallyimportant range, hence we graded the imprecision as such	+++- Moderate	Critical
Subgroup: *w*AlbB strain	3	0.64 (0.46-0.82); I^2=85% Moderate	+	-	-	+	NA	++-- Low	Critical
Subgroup: Asia	5	0.72 (0.64-0.80); I^2=70% Moderate	+	-	+	+		+++- Moderate	High
Subgroup: Latin America	3	0.81 (0.65-0.96); I^2=98% High	+	-	-	+		+++- Moderate	High
Subgroup: Australia	2	0.95 (0.90-1.00); I^2=0% High	+	+	+	+	Due to low amount of study, the authors decided to downgrade the evidence quality by 2 points, accidental results yielding low heterogeneity is still a possibility	++-- Low	High

*w*Mel, *w*AlbB: *Wolbachia* strains used for the intervention.

variations in outcomes based on mosquito strain and geographic location of the intervention.

In order to further investigate the source of heterogeneity, we conducted a multivariate meta-regression with population density, length of intervention, location, and strain used as covariates. However, the resulting model was still unable to account for the heterogeneity between studies. Overall, despite the high heterogeneity, the positive impact of *Wolbachia* implementation in reducing dengue incidence remained undeniable.

3.3. Evidence quality and risk of bias assessment

After performing evidence quality and risk of bias assessments, we concluded that the evidence presented is moderate to low in quality (Table 3), with a notably low to medium risk of bias, as displayed in Supplementary Figure 3. This conclusion affects the reliability of the respective studies' results. We found that the results between studies were moderately heterogeneous, despite repeated sensitivity analyses. Meta-regression also failed to account for any significant heterogeneities, despite multiple permutative attempts to use extracted covariates to build a model suitable for accounting for our heterogeneous results.

4. Discussion

4.1. Efficacy of Wolbachia-infected mosquitoes to suppress the dengue incidence

The deployment of *Wolbachia*-infected *Ae. aegypti* mosquitoes possessed a significant preventive efficacy in reducing dengue incidence, with an overall protective efficacy estimate of 0.79 (95% *CI* 0.70-0.88). Due to variations between studies, such as different premediating factors, different approaches taken by studies, and other interplaying factors that might vary, it was justified to use the random effects model as the method for effect size estimation. This result could be directly translated to associated reduction of approximately 79% in dengue incidence in the treated populations. Moreover, we failed to account for heterogeneity across studies through a rigorous process of multivariate meta-analysis using covariates extracted in this study. This indicates a more complex interplay between factors or covariates, either accounted for or unaccounted for, in this study and its attempts. Between studies diversity was found to be vast, which originated from many factors, including different methodologies used, climate, the number of mosquitoes employed and its population stability, among others.

Reduction in dengue incidence was found to be higher in studies of *w*Mel-infected mosquitoes with an 84% reduction in cases compared to *w*AlbB-infected mosquitoes, which showed a 64% reduction in cases. The establishment of *w*AlbB under elevated temperature conditions, as documented in a study conducted by Nazni *et al.* in Malaysia, suggests its viability for utilization in hotter tropical climates and they opted for the *w*AlbB strain due to its superior thermostability in comparison to *w*Mel during the developmental stages of mosquito larvae. Nevertheless, there is no indication that the establishment of *w*Mel was hindered in the climatic conditions of Yogyakarta, as found by Indriani *et al*. Besides, Velez *et al.* found that the mean daily temperatures in Medellín and Bello (21.8 ℃-23.1 ℃) were relatively moderate when compared with other regions where *Wolbachia* has been introduced, such as Australia and Indonesia, which led to delayed developmental timelines for *Ae. aegypti* mosquitoes, potentially impeding the establishment of *Wolbachia*. In these areas, the turnover of mosquito generations was prolonged, with development durations of 21-25 days at 22 ℃ as opposed to 12-13 days at 28 ℃. Furthermore, the extended gonotrophic cycle (the period between blood meals) further decelerated the turnover rate[1,12,13].

The *Wolbachia* strains *w*AlbB and *w*Mel exhibit variations in several crucial aspects. The *w*AlbB strain demonstrates greater thermostability, maintaining its density and stability over a range of temperatures, including high temperatures. In contrast, the *w*Mel strain is more susceptible to temperature fluctuations, leading to notable reductions in density and maternal transmission fidelity at elevated temperatures. Both strains affect the fitness of *Ae. aegypti* mosquitoes, albeit in different ways. Research indicates that *w*AlbB and *w*Mel have distinct effects on various characteristics related to fecundity, such as egg hatching rates, larval survival, and adult longevity. Observations in field conditions suggest that *w*AlbB maintains its infection frequency and density across diverse temperature ranges, which is crucial for the sustained suppression of the dengue virus in natural populations. Conversely, high temperatures can compromise the performance of *w*Mel, potentially hindering its ability to inhibit the dengue virus. Although both strains have been investigated for their proteome perturbations and antiviral properties, unique disparities in their mechanisms may influence their efficacy in varying environmental conditions[16,17].

Dengue can be acquired outside of a person's residence during the day, which has not yet been targeted using *Wolbachia* replacement. This makes the effect of *Wolbachia* more difficult to detect with passive surveillance. Thus, the overall effect size detected is a conservative estimate. Factors such as people's mobility and the risk of acquiring dengue outside their neighborhoods may explain the non-linear association between neighborhood-level *w*Mel infection prevalence and dengue risk. The geographic scale of the intervention area and the resident population's mobility suggest that some notified cases could have acquired their DENV infection outside the *Wolbachia*-treated area. This results in downward biases in the estimated total intervention efficacies[1,4,13,15].

The included studies were conducted on 3 different continents, namely Asia, America, and Australia, with the following differences in protective efficacy: Asia 72%, America 81%, and Australia 95%. The geographical setting plays a crucial role in the deployment and establishment of *Wolbachia* in mosquito populations. High temperatures can adversely affect the density and stability of *Wolbachia*. A study in Cairns, Australia, during a heatwave showed a temporary reduction in *Wolbachia* infection prevalence, but the levels later recovered to nearly 100%. Similarly, a study in Vietnam found that elevated temperatures were linked to a decrease in *Wolbachia* infection frequency, indicating that temperature can influence the local establishment of *Wolbachia*. The urban environment can also impact the spread and stability of *Wolbachia*. Pinto *et al.* found that the complex urban landscape, with high-rise areas and large informal settlements led to slower *Wolbachia* introgression due to spatial variations in mosquito abundance and limited mosquito dispersal, suggesting that urban landscapes can create barriers to the spread of *Wolbachia*. Furthermore, large-scale

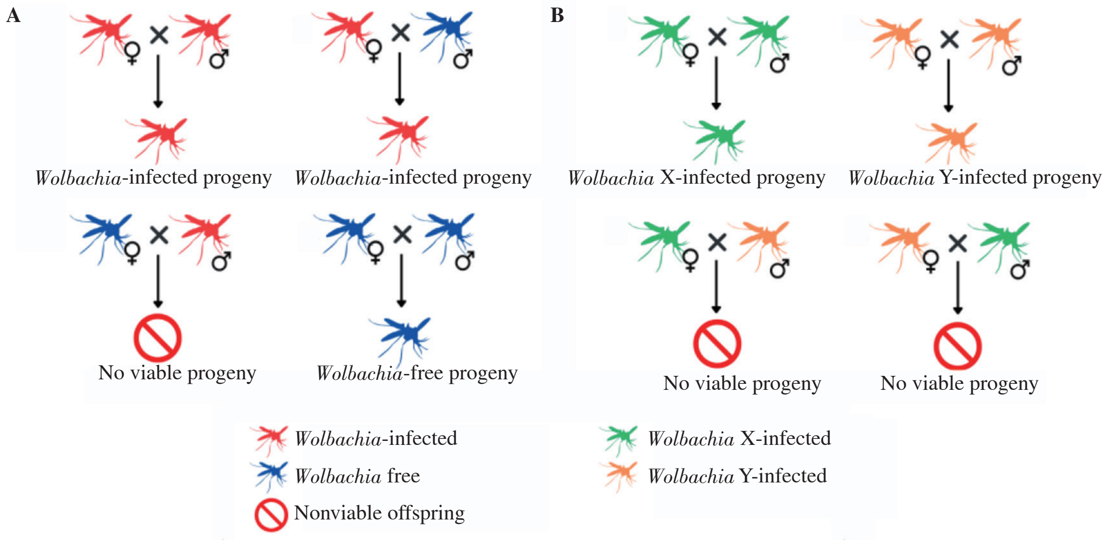


Figure 3. Cytoplasmic incompatibility concept. A. Unidirectional cytoplasmic mechanism. B. Bidirectional cytoplasmic mechanism.

deployments of *Wolbachia* can have undesirable consequences, such as promoting genetic homogenization at large geographic scales, which might have long-term negative effects on mosquito populations. Geographical factors also influence community engagement and acceptance of *Wolbachia* deployment[4,18,19].

4.2. Mechanism of protective effect of Wolbachia pipientis strain wMel against dengue virus transmission

Wolbachia, an intracellular bacterium passed down through the maternal line, resides inside host cells and relies on its host's resources to multiply. Two methods for controlling vectors have been developed: releasing *Wolbachia*-infected male mosquitoes into the environment to mate with uninfected females, resulting in non-viable offspring, or by replacing the natural mosquito population with infected females (Figure 3). Infected female mosquitoes with *Wolbachia* can lessen the spread of dengue, zika, chikungunya, and yellow fever viruses. *Wolbachia* induces reproductive abnormalities in hosts, causing the bacteria to spread in the population through a process known as cytoplasmic incompatibility. Cytoplasmic incompatibility occurs when *Wolbachia*-infected male mosquitoes reproduce with female mosquitoes that are either uninfected by *Wolbachia* (unidirectional) or infected with incompatible types of *Wolbachia* (bidirectional), leading the population to favor *Wolbachia*-infected females[5,20,21].

Wolbachia acts as a shield against viruses, battling for resources within the host. Its impact on viruses results in lower virus levels and reduced harm to the host. Furthermore, it reduces the spread and growth of viruses by lowering infection rates and transmission[20,21].

These antiviral effects have been proven by many studies that directly measured dengue virus viral load. The study by Utarini *et al.* mentioned that there was a significant difference in viral load between wild-type *Ae. aegypti* and *w*Mel-infected *Ae. aegypti*. Statistical calculations in the study revealed a 13% to 30% reduction in vector transmission opportunity. This study also showed the highest viral load reduction for DENV-2 and the lowest for DENV-1.2. However, viral RNA observations by Flores *et al.* proved that the pattern of *Wolbachia* suppression of dengue virus strains is dependent on the *Wolbachia* strain. In addition, in this study, the *w*Mel strain also showed suppression, in contrast to the findings by Utarini *et al.*, indicating heterogeneity in suppression results and the need for further studies to confirm the findings[2,5,22,23].

4.3. Wolbachia-infected mosquito population stability

Based on the search results, introducing *Wolbachia*-infected *Ae. aegypti* mosquitoes can effectively control the incidence of dengue over long periods of time. This is because the growth rate of *Wolbachia* infection tends to remain stable until the end of the study. A study by Ryan *et al*[18] in Cairns, Australia, demonstrated that the level of *Wolbachia* infection in the *Ae. aegypti* population was stable. The median *Wolbachia* infection rate reached 82.4% at week 12 and increased to 92.3% at week 22. Median weekly post-intervention (after 23 weeks) *Wolbachia* infection rates ranged from 66% to 95% through week 52, and infection rates remained above 80%. Monitoring *Wolbachia* infection rates in mosquito populations at several intervention sites in Cairns, Australia showed that *Wolbachia* infection rates remained high, with an average *Wolbachia* infection rate of 94.7%-95.4% after 8 years. Although there were fluctuations in *Wolbachia* infection rates during the initial release period at some locations, *Wolbachia* infections ultimately persisted in the mosquito population without intervention or additional releases. Another study conducted by O'Neill *et al.* in Townsville, Australia, showed that *w*Mel infection rates were stable during and after release. Despite fluctuating for several months, the *w*Mel infection rate ultimately reached more than 80%. In addition, *w*Mel infection rates at all locations remained stable, with no signs of *w*Mel disappearing from the mosquito population. Since the intervention, no local transmission of dengue has been confirmed in the Townsville area. This study has proven that introducing *Wolbachia* into mosquito populations can effectively control the incidence of dengue in the long term because the growth rate of *Wolbachia* infection is relatively high and stable[12,13].

Studies on the stability of the growth rate of the *w*Mel strain of *Wolbachia* infection in local mosquito populations have shown differences between Australia and Brazil. A study by Pinto *et al.* in Niteroi, Brazil revealed spatial heterogeneity in the prevalence of *w*Mel in local mosquito populations. Some regions showed a high prevalence of *w*Mel infection, exceeding 80%, while others demonstrated moderate prevalence (40%-70%). This indicates that the minimum prevalence rate of *w*Mel infection in Brazil is still within the medium category, with variations in different regions ranging from medium to high prevalence. Another study in Rio de Janeiro by dos Santos *et al.* found that 29 months after phased releases began in five areas, the prevalence of *w*Mel in local *Ae. aegypti* mosquitoes ranged from 27% to 60%. It's unclear why *w*Mel failed to establish quickly in Rio de Janeiro despite extensive releases. Factors such as variable dengue incidence, seasonal fluctuations in *w*Mel effectiveness, with lower levels during the hottest period of the year, and challenges in areas with high dengue incidence, including large and unevenly distributed mosquito populations and difficult-to-access locations like favela communities, may have complicated the *w*Mel release program. These findings align with a similar study by Pinto *et al.* in Brazil, highlighting spatial heterogeneity in the results[4,10,18].

A study conducted in Yogyakarta, Indonesia, by Utarini *et al.* demonstrated that two years after the introduction of *w*Mel into the mosquito population, the level of *w*Mel-infected mosquitoes varied between regions, indicating that the population was not static. In contrast, another study in Yogyakarta, Indonesia, by Indriani *et al.* revealed that the prevalence of *Wolbachia* in local *Ae. aegypti* mosquito population in the intervention area remained stable. The prevalence of *Wolbachia* increased over the course of one year, with a median prevalence of 73% (ranging from 67% to 92%) one week after the release was halted. It further reached a median prevalence of 100% (ranging from 96% to 100%) two years after the discontinuation of the release. Consequently, all mosquitoes in parts of the intervention area in Yogyakarta, Indonesia, were successfully infected by *Wolbachia* after two years, resulting in a stable level of *Wolbachia* infection within the mosquito population[1,2].

A study conducted by Nazni *et al.* in Selangor, Malaysia, revealed that the frequency of *Wolbachia* increased rapidly to over 80% at all sites. Following the cessation of releases, the *Wolbachia* frequency remained stable and high, reaching 98% at 12 months after releases ceased in Mentari Court. However, a study by Hoffman *et al.* in East Malaysia showed that across all 20 release sites, the mean *Wolbachia* frequency during the release and post-release monitoring periods was 82.3%, ranging from 56.2% to 96%. At some sites, *Wolbachia* frequencies increased rapidly and remained high, while at others, they increased more slowly before reaching a high frequency. Additionally, there were sites where *Wolbachia* frequencies experienced instability before settling at a high level. It's worth noting that some of this instability may have been a result of small sample sizes, although the data points were based on a minimum of 10 individuals[15,16].

Based on the authors' own observations, the *Wolbachia*-infected *Ae. aegypti* mosquito population in the wild followed a logarithmic growth pattern until the study endpoint or until the release cessation. However, there are variations in *Wolbachia* infection levels in *Ae. aegypti* mosquito populations across different locations. Studies in Australia generally indicate better and more stable *Wolbachia* infection rates compared to studies in Brazil, Indonesia, and Malaysia. This suggests that environmental conditions may affect the stability of *w*Mel infection levels in *Ae. aegypti* mosquito populations. One study by Hien *et al.* found that environmental temperature and annual seasons influence the stability of *Wolbachia* infection levels in *Ae. aegypti* mosquito populations. The prevalence of *Wolbachia* infection tends to decrease with higher environmental temperatures during the dry season and increase with lower temperatures during the winter[10].

4.4. Safety considerations

The deployment of *Wolbachia* is scalable, safe, and long-lasting, and it is linked to a reduction in dengue transmission. In a study by Buchori *et al.* in Yogyakarta, Indonesia, the risks associated with *Wolbachia* deployment were assessed by identifying various hazards to humans and the environment. These hazards included ecological concerns, effectiveness in mosquito management, economic and sociocultural issues, and public health standards. The assessment suggests that while most risks are minimal to low, continuous monitoring and community engagement are essential to prevent adverse effects and ensure the success of this innovative vector control strategy. A similar finding in a study by Murray *et al.* suggested that the risk of "causing more harm" was minimal, with the biggest concern being reduced mosquito control efforts due to the mistaken belief that dengue was no longer a threat. However, this was still considered a low risk[12,22,23].

4.5. Applicability, public acceptance, and policy recommendation

Despite the clear benefits of *Wolbachia*-based intervention, public acceptance of this type of program remains one of the main challenges. While a survey conducted in Puerto Rico by Sánchez-González *et al*., yielded more supportive results. However, in Indonesia, pro and con oponions sparked a heated debate on the implementation of this program. For instance, there are hoaxes about *Wolbachia*-infected mosquito spreading “*LGBT*-gene” to mass-destruction weapons. However, the author believes with a proper community outreach, this obstacle could be easily addressed, as demonstrated by community survey done by Indriani *et al*. This highlights the importance of community engagement strategy prior and after intervention[1,25–27].

Our research suggests a strong support for implementing *Wolbachia*-based interventions in halting mosquito-borne diseases, which extend not only to arboviruses, but also other types, such as zika and chikungunya, as suggested by Pinto *et al.* This would greatly benefit the public health program, as mosquito-borne diseases remain a key challenge to many, especially hot and humid climate countries that lies near the equatorial region. According to the Centers for Disease Control, about 40% of the Earth's population resides in this region, with an annual rate of about 100 to 400 million cases. Implementing this result might yield up to 300 million prevented cases of dengue, and about 14 000-16 500 lives from death attributable to dengue. This might amount to billions of dollars budget saved on healthcare-related cost and productivity loss[4,28,29].

We were unable to recommend any release strategy due to the heterogeneous results and conditions that mediate the protective effect displayed by the study. This would calls for further studies that compares releasing methods. However, we identified several factors related to the protective efficacy of the intervention, namely the population density, intervention length, and strain used, with *w*Mel yielding a consistently better result. Another important factor to consider is the stability of *Wolbachia*-infected population. Longer intervention/release periods offered some benefits in prolonging the effect and stability compared to the shorter ones, with higher positive correlation between the percentage of *Wolbachia* in the population and protective efficacy suggested by several studies. Therefore, we recommend periodical *Wolbachia* release and monitoring, specifically until the desirable protective efficacy reached based on the saturation of the *Wolbachia* strain amongst mosquito population, with intervals of 2-4 weeks.

5. Strengths and limitations

This review's greatest strength lies in its ability to capture the large effect size of the intervention. With such a high effect size, the positive effects of the intervention can be confirmed with high confidence. We also discovered some of key factors influencing the intervention, such as *Wolbachia* dissemination level and the stability of the population, the density of people living in the intervention area, the strain used (*w*Mel yielded the best results), and the length of the intervention. However, we were unable to pinpoint the exact effect of each factor, which calls for more controlled research. Moreover, as the first review that employed quantitative methods in this form of community-based interventions, we believe the newly found effect estimates could be serve as a basis for governmental policy-making. The high quality of our evidence further supports this notion.

However, despite positive results, we still believe that the effects of the release strategy among many more unknown and uncontrolled variables are not yet properly accounted for, and our review failed to reliably offer a new perspective despite comparing studies to each other. Our attempt to account for heterogeneity through multivariate meta-regression failed to produce a model capable of significantly reducing the in-between studies heterogeneity. We believe a more thorough approach to controlling variables, such as temperature and weather fluctuations, humidity, as well as a more uniform releasing strategy in the future would be sufficient to bypass this challenge. The aforementioned study could be conducted in succession with the actual release efforts carried out by the municipal or any local governing bodies in charge.

6. Conclusions

Wolbachia-infected mosquito release interventions is effective in reducing the incidence of mosquito-borne virus infection. Our results strongly support the use of *Wolbachia* in places with mosquito-borne endemicity, with evidence heavily supporting its action against dengue incidence. This action is proven to be efficacious, cost-effective, and environmentally safe. This action is mediated by hindering reproduction of the mosquito and inducing host incompatibility for the virus to reproduce.

However, practicing community-wide *Wolbachia* release comes with several challenges, such as unpredictability of variables such as *Wolbachia* population, climate, temperature, and weather, release strategy, and even the landscape of the intervention sites. This would call for constant monitoring and tailoring of the release strategy to meet the desired saturation. Moreover, policy and community acceptance remain as significant challenges in implementation. However, the author believes that with proper community outreach and stakeholder coordination, these efforts could provide a feasible solution to one of the biggest public health concerns across the area.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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Authors' contributions

NMA: Project leader, conceptualization, administrative, analysis, data collection, software. HH: Data collection, analysis, formatting, publication. GTD: Data collection, analysis, formatting, literature review validator. SR: Validation, advisor & supervision.

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Publisher's note

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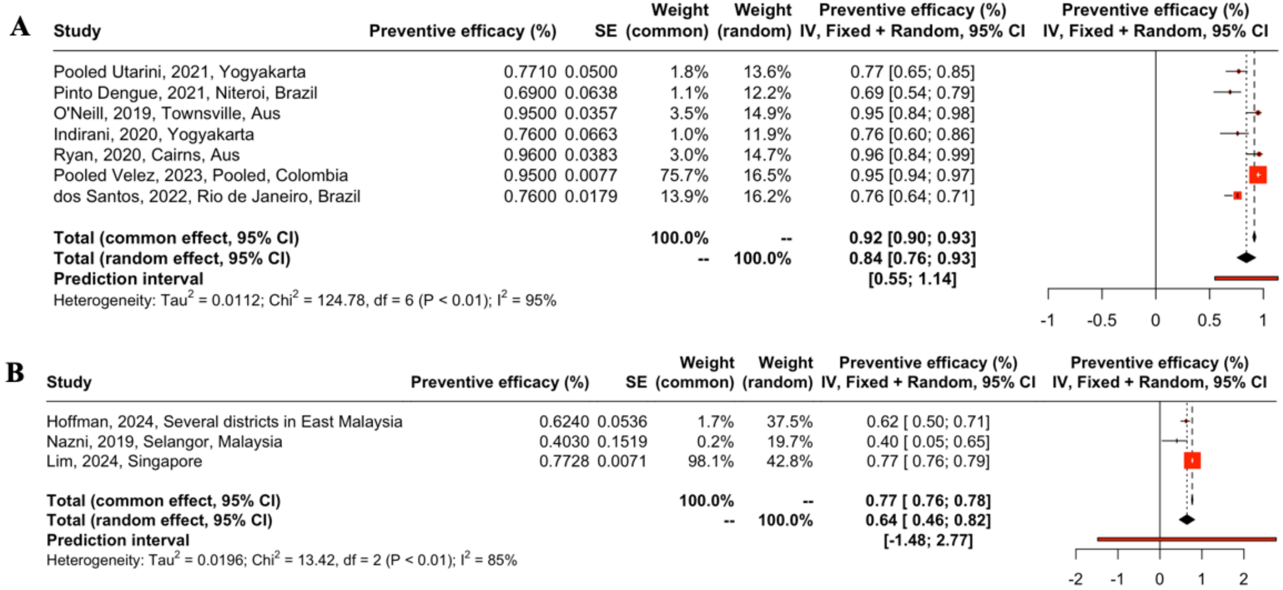
Supplementary Materials



A

Study	Preventive efficacy (%)	SE	Weight (common)	Weight (random)	Preventive efficacy (%) IV, Fixed + Random, 95% CI
Pooled Utarini, 2021, Yogyakarta	0.7710	0.0500	1.8%	13.6%	0.77 [0.65; 0.85]
Pinto Dengue, 2021, Niteroi, Brazil	0.6900	0.0638	1.1%	12.2%	0.69 [0.54; 0.79]
O'Neill, 2019, Townsville, Aus	0.9500	0.0357	3.5%	14.9%	0.95 [0.84; 0.98]
Indirani, 2020, Yogyakarta	0.7600	0.0663	1.0%	11.9%	0.76 [0.60; 0.86]
Ryan, 2020, Cairns, Aus	0.9600	0.0383	3.0%	14.7%	0.96 [0.84; 0.99]
Pooled Velez, 2023, Pooled, Colombia	0.9500	0.0077	75.7%	16.5%	0.95 [0.94; 0.97]
dos Santos, 2022, Rio de Janeiro, Brazil	0.7600	0.0179	13.9%	16.2%	0.76 [0.64; 0.71]
Total (common effect, 95% CI)			**100.0%**	**--**	**0.92 [0.90; 0.93]**
Total (random effect, 95% CI)			**--**	**100.0%**	**0.84 [0.76; 0.93]**
Prediction interval					**[0.55; 1.14]**

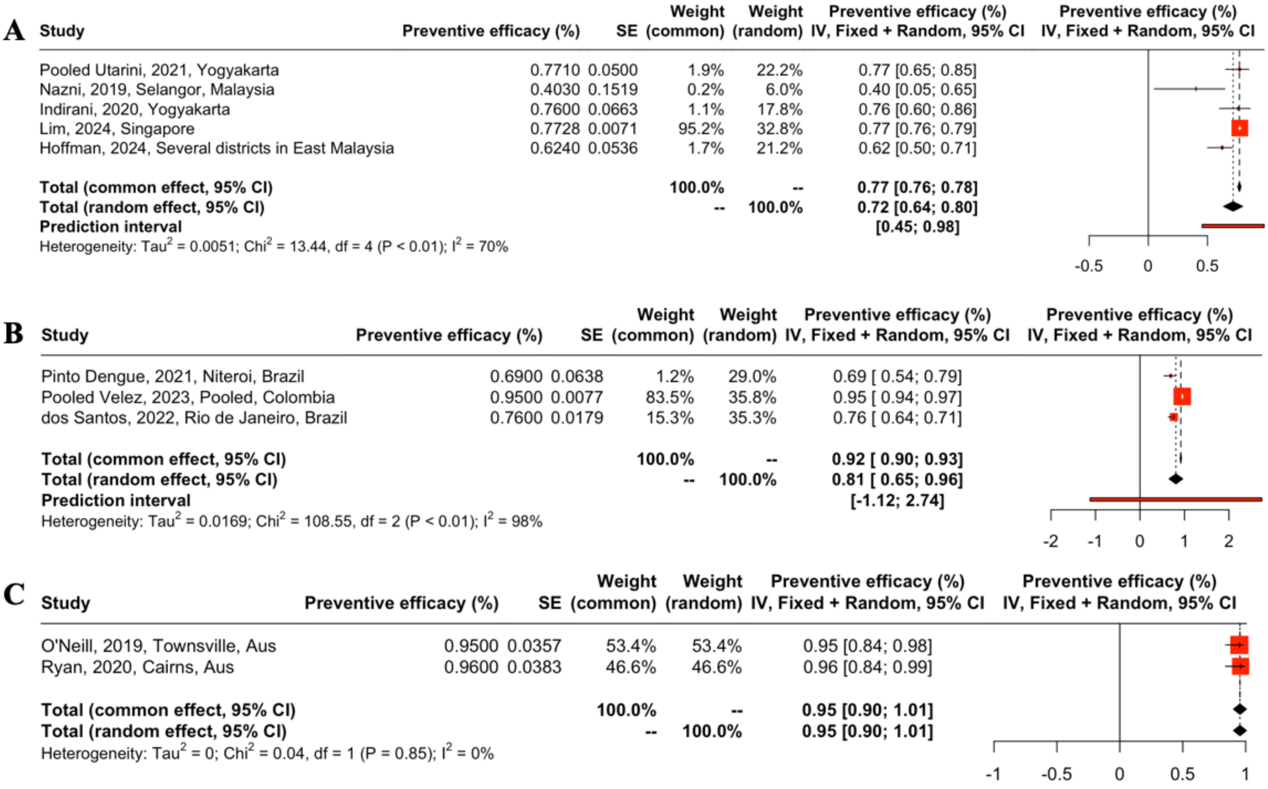
Heterogeneity: $Tau^2 = 0.0112$; $Chi^2 = 124.78$, df = 6 ($P < 0.01$); $I^2 = 95\%$

B

Study	Preventive efficacy (%)	SE	Weight (common)	Weight (random)	Preventive efficacy (%) IV, Fixed + Random, 95% CI
Hoffman, 2024, Several districts in East Malaysia	0.6240	0.0536	1.7%	37.5%	0.62 [0.50; 0.71]
Nazni, 2019, Selangor, Malaysia	0.4030	0.1519	0.2%	19.7%	0.40 [0.05; 0.65]
Lim, 2024, Singapore	0.7728	0.0071	98.1%	42.8%	0.77 [0.76; 0.79]
Total (common effect, 95% CI)			**100.0%**	**--**	**0.77 [0.76; 0.78]**
Total (random effect, 95% CI)			**--**	**100.0%**	**0.64 [0.46; 0.82]**
Prediction interval					**[-1.48; 2.77]**

Heterogeneity: $Tau^2 = 0.0196$; $Chi^2 = 13.42$, df = 2 ($P < 0.01$); $I^2 = 85\%$

Supplementary 1. Pooled analysis of studies employing A. *wMel* strain; B. *wAlbB* strain



A

Study	Preventive efficacy (%)	SE	Weight (common)	Weight (random)	Preventive efficacy (%) IV, Fixed + Random, 95% CI
Pooled Utarini, 2021, Yogyakarta	0.7710	0.0500	1.9%	22.2%	0.77 [0.65; 0.85]
Nazni, 2019, Selangor, Malaysia	0.4030	0.1519	0.2%	6.0%	0.40 [0.05; 0.65]
Indirani, 2020, Yogyakarta	0.7600	0.0663	1.1%	17.8%	0.76 [0.60; 0.86]
Lim, 2024, Singapore	0.7728	0.0071	95.2%	32.8%	0.77 [0.76; 0.79]
Hoffman, 2024, Several districts in East Malaysia	0.6240	0.0536	1.7%	21.2%	0.62 [0.50; 0.71]
Total (common effect, 95% CI)			**100.0%**	**--**	**0.77 [0.76; 0.78]**
Total (random effect, 95% CI)			**--**	**100.0%**	**0.72 [0.64; 0.80]**
Prediction interval					**[0.45; 0.98]**

Heterogeneity: $Tau^2 = 0.0051$; $Chi^2 = 13.44$, df = 4 ($P < 0.01$); $I^2 = 70\%$

B

Study	Preventive efficacy (%)	SE	Weight (common)	Weight (random)	Preventive efficacy (%) IV, Fixed + Random, 95% CI
Pinto Dengue, 2021, Niteroi, Brazil	0.6900	0.0638	1.2%	29.0%	0.69 [0.54; 0.79]
Pooled Velez, 2023, Pooled, Colombia	0.9500	0.0077	83.5%	35.8%	0.95 [0.94; 0.97]
dos Santos, 2022, Rio de Janeiro, Brazil	0.7600	0.0179	15.3%	35.3%	0.76 [0.64; 0.71]
Total (common effect, 95% CI)			**100.0%**	**--**	**0.92 [0.90; 0.93]**
Total (random effect, 95% CI)			**--**	**100.0%**	**0.81 [0.65; 0.96]**
Prediction interval					**[-1.12; 2.74]**

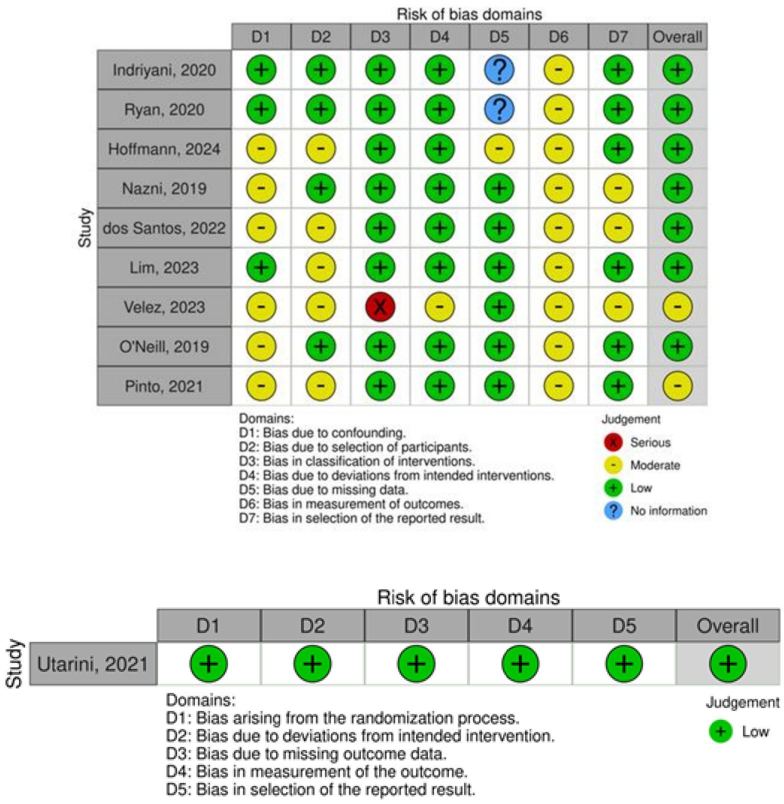
Heterogeneity: $Tau^2 = 0.0169$; $Chi^2 = 108.55$, df = 2 ($P < 0.01$); $I^2 = 98\%$

C

Study	Preventive efficacy (%)	SE	Weight (common)	Weight (random)	Preventive efficacy (%) IV, Fixed + Random, 95% CI
O'Neill, 2019, Townsville, Aus	0.9500	0.0357	53.4%	53.4%	0.95 [0.84; 0.98]
Ryan, 2020, Cairns, Aus	0.9600	0.0383	46.6%	46.6%	0.96 [0.84; 0.99]
Total (common effect, 95% CI)			**100.0%**	**--**	**0.95 [0.90; 1.01]**
Total (random effect, 95% CI)			**--**	**100.0%**	**0.95 [0.90; 1.01]**

Heterogeneity: $Tau^2 = 0$; $Chi^2 = 0.04$, df = 1 ($P = 0.85$); $I^2 = 0\%$

Supplementary 2. Subgroup analysis of studies done in **A.** Asia; **B.** Latin America; **C.** Australia



Risk of bias domains

Study	D1	D2	D3	D4	D5	D6	D7	Overall
Indriyani, 2020	+	+	+	+	?	-	+	+
Ryan, 2020	+	+	+	+	?	-	+	+
Hoffmann, 2024	-	-	+	+	-	-	+	+
Nazni, 2019	-	+	+	+	+	-	-	+
dos Santos, 2022	-	-	+	+	+	-	-	+
Lim, 2023	+	-	+	+	+	-	+	+
Velez, 2023	-	-	X	-	+	-	-	-
O'Neill, 2019	-	+	+	+	+	-	+	+
Pinto, 2021	-	-	+	+	+	-	+	-

Domains:
D1: Bias due to confounding.
D2: Bias due to selection of participants.
D3: Bias in classification of interventions.
D4: Bias due to deviations from intended interventions.
D5: Bias due to missing data.
D6: Bias in measurement of outcomes.
D7: Bias in selection of the reported result.

Judgement
X Serious
- Moderate
+ Low
? No information

Risk of bias domains

Study	D1	D2	D3	D4	D5	Overall
Utarini, 2021	+	+	+	+	+	+

Domains:
D1: Bias arising from the randomization process.
D2: Bias due to deviations from intended intervention.
D3: Bias due to missing outcome data.
D4: Bias in measurement of the outcome.
D5: Bias in selection of the reported result.

Judgement
+ Low

Supplementary 3. Traffic light plot for risk of bias assessment