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Systematic Review

Impact of *Wolbachia*-infected *Aedes aegypti* on dengue incidence in endemic regions: a systematic review and meta-analysis

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Abstract

Dengue remains a major public health challenge in endemic regions, and conventional vector-control strategies have shown limited long-term effectiveness. The release of *Wolbachia*-infected *Aedes aegypti* mosquitoes has emerged as a novel biocontrol strategy to reduce dengue transmission. We conducted a systematic review and meta-analysis to evaluate the effectiveness of *Wolbachia* deployments in reducing dengue incidence. We searched PubMed, Embase, and the Cochrane Library until 30 April 2025 for randomized controlled trials and quasi-experimental studies conducted in dengue-endemic settings. Population-level effectiveness was assessed using pooled incidence rate ratios (IRRs), and individual-level protective efficacy using pooled odds ratios (ORs). Random-effects meta-analysis with restricted maximum likelihood with Hartung–Knapp adjustment was applied. Subgroup analyses examined geographic region and deployment coverage. Nine studies involving >8 million individuals were included. *Wolbachia* deployment was associated with a substantial reduction in dengue incidence (pooled IRR 0.15, 95% CI 0.05–0.39), corresponding to an estimated 85% reduction. Full-area deployments demonstrated greater reductions (IRR 0.046, 95% CI 0.038–0.055) than partial-area deployments (IRR 0.22, 95% CI 0.07–0.74). Exploratory individual-level analyses yielded a pooled OR of 0.29 (95% CI 0.01–8.41) with substantial heterogeneity ($I^2 = 86.5\%$).

Keywords *Aedes aegypti*, dengue, meta-analysis, systematic review, vector control, *Wolbachia*

Introduction

Dengue virus infection remains a major public health challenge in tropical and subtropical regions of the world. Transmitted primarily by *Aedes aegypti*, dengue is among the most prevalent mosquito-borne viral diseases, with an estimated 390 million infections annually across >100 countries, of which >90 million are symptomatic.[1–4] According to the WHO, dengue incidence has increased substantially over recent decades, with the highest burden concentrated in low- and middle-income countries in Southeast Asia, the Americas, and parts of Africa, where rapid urbanization, population growth, climate variability, and constrained vector control capacity sustain endemic transmission and recurrent epidemics.[4,5] Beyond its high incidence, dengue is associated with substantial morbidity, mortality, and economic burden on health systems, with seasonal outbreaks placing significant strain on healthcare services, particularly in hyperendemic settings where all four serotypes co-circulate.[3–5]

Despite extensive control efforts, sustained dengue prevention has proven difficult. Conventional vector-control strategies have shown limited long-term effectiveness in urban environments, while vaccine-based approaches face important constraints related to immunological complexity, serotype-specific risk, and safety considerations.[6] These challenges have driven interest in alternative, sustainable interventions that reduce transmission at the population level rather than relying solely on individual protection.

Wolbachia-based mosquito biocontrol has emerged as a promising approach for dengue prevention. *Wolbachia pipientis* is a maternally inherited intracellular bacterium common in many insects, but is not naturally present in *A. aegypti*.[7,8] When introduced into *A. aegypti*, *Wolbachia* can reduce the transmission

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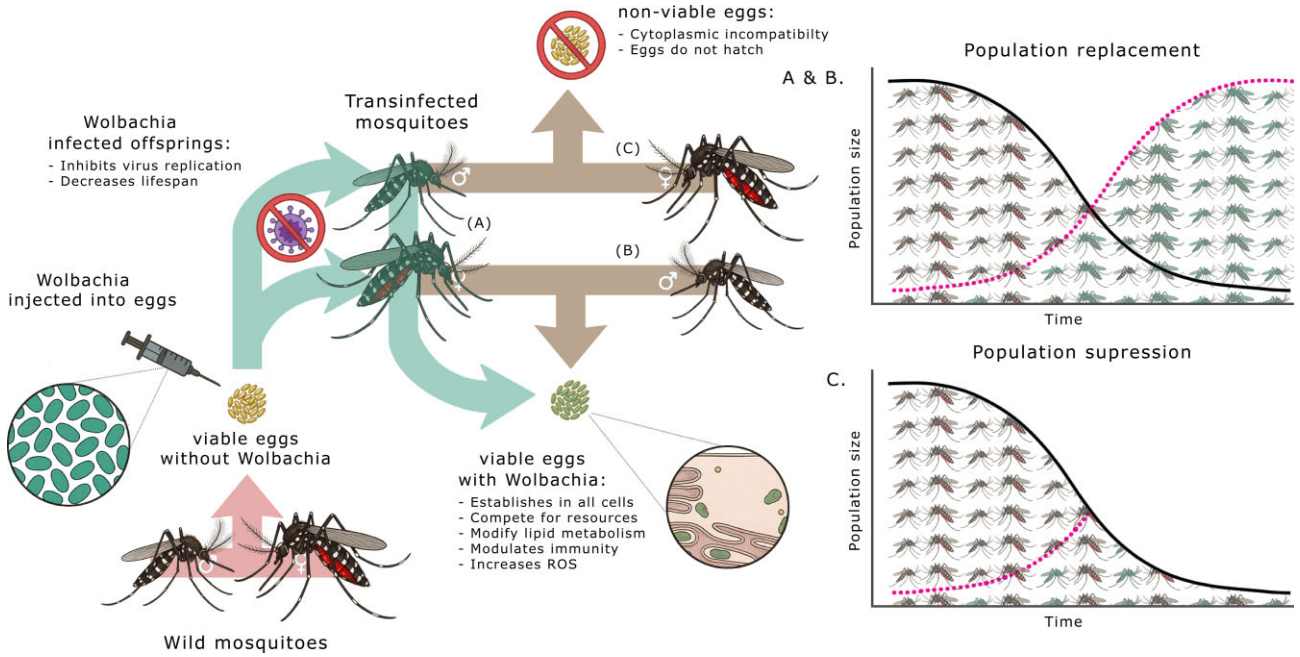

Figure 1 *Wolbachia* vector control strategies (original schematic). *Wolbachia* is introduced into *Aedes aegypti* through transinfection, resulting in mosquitoes that carry the bacterium and transmit it maternally to offspring. *Wolbachia* can reduce dengue transmission potential by lowering vector competence for dengue virus. Cytoplasmic incompatibility (CI) provides a reproductive advantage to *Wolbachia*-infected females: matings involving an infected female × infected male (A) or infected female × uninfected male (B), produce viable *Wolbachia*-infected offspring, whereas infected male × uninfected female matings (C) yield non-viable eggs. These principles underpin two deployment approaches. Population replacement releases infected males and females to drive *Wolbachia* introgression and replace local mosquito populations over time, while population suppression releases infected males only (incompatible insect technique, IIT) to reduce mosquito abundance. ROS: Reactive oxygen species.

of dengue, Zika, chikungunya, and yellow fever viruses, and can spread through mosquito populations via cytoplasmic incompatibility, enabling establishment after release.[8–10]

Two operational deployment strategies are used in practice. Population replacement releases *Wolbachia*-infected males and females to promote *Wolbachia* introgression via maternal transmission, reducing vector competence over time. By contrast, population suppression releases infected males only to induce cytoplasmic incompatibility and reduce mosquito density (the incompatible insect technique), sometimes with sterilization safeguards to minimize unintended establishment.[8–10] Figure 1 provides a schematic overview of these two approaches.

Following the first successful field deployments in Australia, *Wolbachia*-based interventions have been implemented and scaled up across multiple dengue-endemic regions, including Southeast Asia and South America, with numerous studies reporting substantial reductions in dengue incidence. However, the available evidence spans a range of study designs and implementation contexts, including randomized trials and large-scale quasi-experimental evaluations conducted under real-world conditions.[9]

To address limitations in direct comparability across studies, we conducted a systematic review and meta-analysis to evaluate the effectiveness of *Wolbachia*-infected *A. aegypti* deployments in reducing dengue incidence in endemic regions. We synthesized population-level incidence rate ratios (IRRs) from quasi-experimental studies and individual-level protective effectiveness estimates from randomized and test-negative designs.[11,12] By integrating evidence across study designs and settings, this analysis provides a concise, policy-relevant assessment of *Wolbachia*-based dengue control to inform future programme design and scale-up.

Materials and methods

Eligibility criteria

This review follows preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines for reporting systematic reviews,[13] with the review protocol officially registered with PROSPERO under the ID CRD420251041586. Studies considered for inclusion were required to meet the eligibility criteria based on the following PICOTT framework:

(1) Population: Individuals of any age in dengue-endemic areas.
(2) Intervention: The release or established presence of *Wolbachia*-infected *A. aegypti* mosquitoes as a dengue-control intervention.
(3) Comparator: Areas without *Wolbachia*-infected mosquitoes, historical baseline data, areas with standard forms of vector control, or no intervention.
(4) Outcomes: Incidence of dengue infection through either virologically confirmed dengue incidence, clinical diagnosis, or dengue case notifications.
(5) Type of studies (study design): Randomized controlled trials (RCTs), quasi-experimental studies, and prospective or retrospective cohort studies.
(6) Time frame: No restrictions on duration of follow-up or publication date.

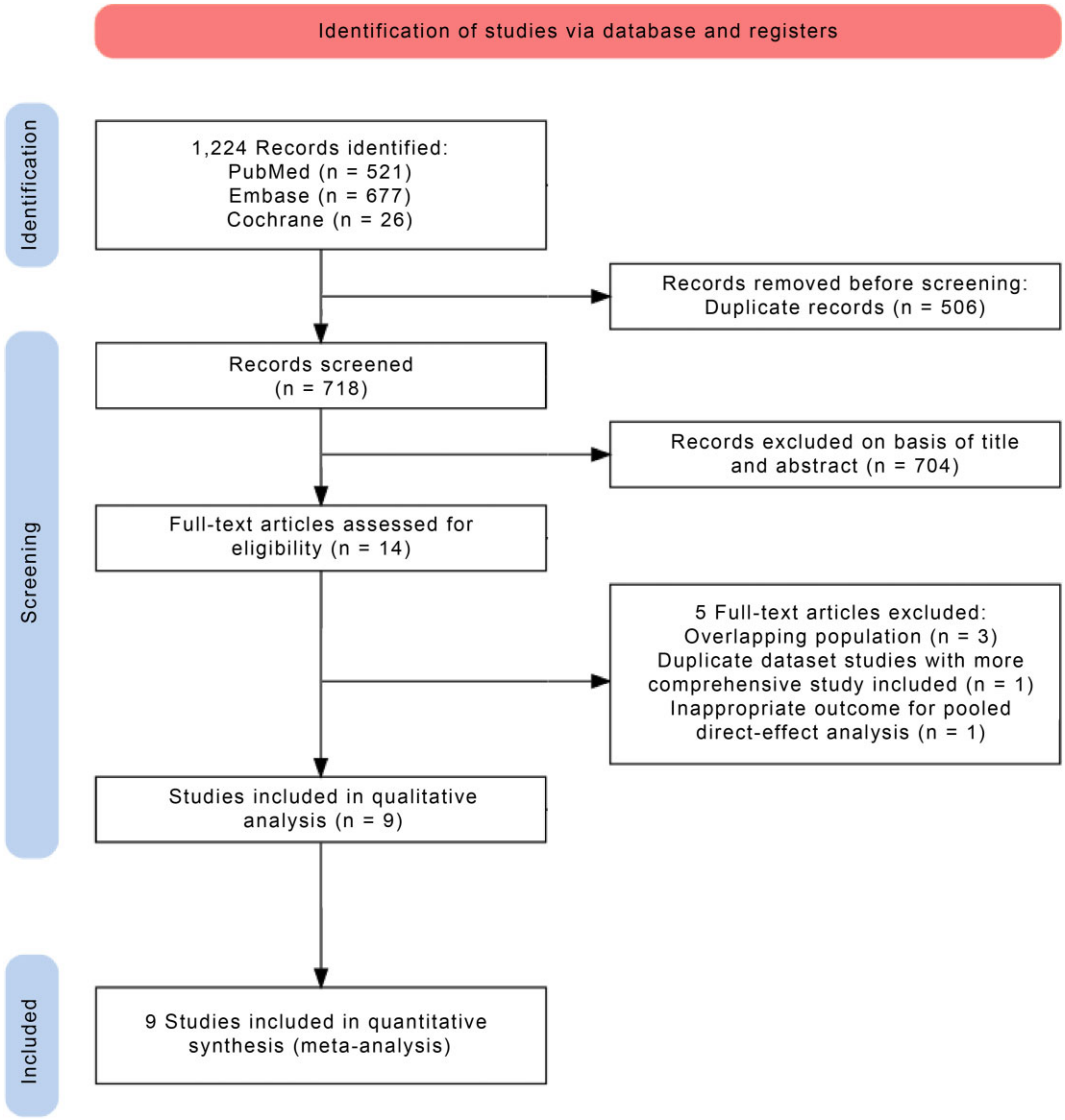

Figure 2 PRISMA diagram. Detailed process of study selection for inclusion in the systematic review and meta-analysis. Details of study identification, screening, eligibility assessment, and inclusion are provided in the Supplementary Appendix.

Studies were excluded if they met any of the following criteria: (i) laboratory or animal-based studies without community implementation; (ii) interventions not involving *Wolbachia*-infected mosquitoes such as chemical-based spatial repellents or other forms of vector-control methods without a *Wolbachia* component; (iii) lack of a comparator or baseline data; (iv) insufficient outcome data for effective estimation; (v) report only entomological outcomes without corresponding clinical endpoints; (vi) reviews, editorials, modelling-only studies, case reports, or conference abstracts without extractable data; (vii) full text not accessible or insufficient methodological detail; (viii) not published in English; or (ix) study design outside the prespecified inclusion criteria above.

Search strategy and study selection

A systematic literature search was conducted in Embase, the Cochrane Library, and PubMed up to 30 April 2025 using terms related to *Wolbachia* and dengue. The full search strategy for each database is provided in Supplementary Table S1.

Titles and abstracts were independently screened by two authors (H.M.Z. and A.A.O.) to identify potentially eligible studies. To enhance accuracy and reproducibility, a second pair of authors (R.S.G. and L.S.R.) independently repeated the search and verified citation counts across databases. All records were imported into a reference management platform with machine learning support (Rayyan AI)[14] for systematic review and consistency checks (Figure 2).

Additional studies were identified through citation tracking, including reference list screening, forward citation searches, and review of related articles. Grey literature sources were also searched to minimize publication bias. Full-text screening was conducted independently by the same two authors (H.M.Z and A.A.O), with disagreements resolved by a third author (R.S.G.).

Data extraction

The main data associated with the desired outcomes were extracted independently by two separate authors (A.A.O. and R.S.G.) from the selected studies. The usable extracted data, reviewed by the main author (H.M.Z.), included population-level dengue incidence and individual-level protective efficacy. Data such as severe dengue and/or dengue hospitalization, dengue-related mortality, introgression rates in mosquito population, Zika and chikungunya incidence and time-based protective efficacy were not uniformly reported by the studies and therefore not pooled in this analysis. Other relevant information included study type and design, country, population at risk, area of control and intervention, coverage type, study dates, *Wolbachia* strain used, sex of the mosquitoes and release methods employed, intervention type, dengue case confirmation methods and interventional periods (including pre- and post-interventions) were also collected and are displayed in Table 1.

As part of data extraction, studies were categorized according to the extent of *Wolbachia* deployment coverage at the time of outcome assessment. We define full-area coverage as interventions implemented across entire cities or regions without contemporaneous untreated areas, aiming for near-universal *Wolbachia* establishment in the local *A. aegypti* population.[15,16] By contrast, partial-area coverage refers to deployments in selected clusters, zones, or high-risk neighbourhoods and high-rise residential areas within a broader urban environment.[11,12,17–20,21] These studies retained contemporaneous untreated comparator areas, such as matched neighbourhoods or zones, and typically represented phased or targeted deployment strategies rather than full city-wide implementation. Our classification reflects the coverage at the time of outcome assessment, during which untreated areas remained and served as comparators.

Risk of bias assessment

Risk of bias was independently assessed by two reviewers (T.S. and A.R-P.) using design-specific, standardized tools (Figure 3). The Cochrane Risk of Bias 2 (RoB 2) tool[22,23] was applied to the RCT by Utarini *et al.*,[12] assessing bias across the domains of the randomization process, deviations from intended interventions, missing outcome data, measurement of outcomes, and selection of the reported result.[22] For non-randomized studies of interventions, the Risk of Bias in Non-randomised Studies - of Interventions (ROBINS-I) tool was used to ensure consistent assessment across observational designs.[24] All assessments were reviewed with the first author (H.M.Z.), who adjudicated disagreements to achieve consensus, and overall risk of bias was classified as low risk, some concerns, or high risk, in accordance with Cochrane guidance.

Statistical analysis

All statistical analyses were performed using R Studio (Version Studio 2025.09.2+418) with the meta and metafor packages. Meta-analyses were conducted using the metagen function (meta package) to pool effect estimates across studies. The primary effect measure was the IRR, used to compare dengue incidence in areas with *Wolbachia* deployment with those without this intervention. Most included studies reported the intervention effect as a relative reduction in dengue incidence, expressed as a percentage with corresponding 95% confidence intervals (CI). These percentage reductions were converted to IRRs using the relationship:

$$\text{IRR} = 1 - (\text{Percentage Reduction}/100)$$

A random-effects model was applied to account for between-study heterogeneity, with between-study variance estimated using the restricted maximum likelihood (REML) method. Confidence intervals for pooled estimates were calculated using the Hartung–Knapp–Sidik–Jonkman (HKSJ) adjustment, in accordance with established methodological recommendations.

For individual-level evidence from test-negative analyses (AWED cluster-randomized trial and Singapore evaluation),[11,12] the primary effect measure was the odds ratio (OR) for virologically confirmed dengue comparing residents in *Wolbachia*-exposed versus unexposed areas/clusters. These ORs were pooled separately from population-level IRRs using the same random-effects framework (REML with HKSJ adjustment). Where reported, protective effectiveness was expressed as 100 x (1–OR) for interpretability, but meta-analysis was conducted on the OR scale.

The certainty of evidence in this systematic review and meta-analysis was assessed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) methodology (see Summary of evidence (GRADE)).

Results

Study selection and characteristics

The literature search identified 1224 records, of which nine studies met the inclusion criteria after screening (Figure 2). These comprised one cluster-RCT and eight quasi-experimental evaluations, collectively covering >8 million individuals across dengue-endemic regions in Southeast Asia, South America, and Australia.[11,12,15,18–21] Individual-level protective effectiveness data were available from the AWED trial in Indonesia and a test-negative study from Singapore.[11,12] Details of the study selection process, including the rationale for study exclusions, are outlined in the Supplementary Appendix (see Supplementary Table S2).

Included studies varied in setting, scale, and implementation strategy. Study populations ranged from ~98 000 to >3.3 million individuals, with follow-up periods spanning 24 to 60 months. Most studies used the wMel *Wolbachia* strain, while a minority deployed wAlbB. Release methods were generally weekly or biweekly and often involved both male and female adult mosquitoes. Interventions were implemented either as city-wide releases or as phased, cluster-based deployments within larger urban areas. Based on deployment strategy at the time of outcome assessment, studies were categorized as full-area or partial-area *Wolbachia* coverage.

Quality of study assessment

All non-randomized studies were judged to have an overall moderate risk of bias using the ROBINS-I tool, reflecting the inherent susceptibility of quasi-experimental designs to confounding in the absence of random allocation. In one study,[16] additional concerns related to deviations from intended interventions and missing outcome data were identified, largely due to COVID-19–related

Table 1 Baseline characteristics of primary and exploratory studies.

	Lim *et al.* 2024 (1) [21,f]	Hoffmann *et al.* 2024 [18,f]	Pinto *et al.* 2021 [19,f]	Indriani *et al.* 2020 [20,f]	Ryan *et al.* 2019 [17,f]	O'Neil *et al.* 2018 [15,f]	Velez *et al.* 2023 [16,f]	Lim *et al.* 2024 (2) [11,g]	Utarini *et al.* 2021 [12,g]
Study type	Quasi-experimental	Quasi-experimental	Quasi-experimental	Quasi-experimental	Quasi-experimental	Quasi-experimental	Quasi-experimental	Quasi-experimental	Cluster-RCT
Study design	SCM	ITS (Bayesian time-series Poisson model)	ITS (negative binomial regression)	ITS (controlled, non-Bayesian)	ITS (controlled)	ITS (uncontrolled, negative binomial regression)	ITS (negative binomial design)	TND (logistic regression)	TND (GEE logistic regression)
Country	Singapore	Malaysia	Brazil	Indonesia	Australia	Australia	Colombia	Singapore	Indonesia
Geographic area	Southeast Asia	Southeast Asia	South America	Southeast Asia	Australia	Australia	South America	Southeast Asia	Southeast Asia
Population at risk[b]	607 872	1 048 849	484 918	98 134	156 156	140 000	3 339 163	2 500 382[a]	311 682
Total area (km^2)	10.3	8.1	134.55	7.97	91.88	128	134.82	Not reported	25.62
No. control sites	4	76	1	3	5	NA	NA	12	12
No. intervention sites	4	20	4	7	69	32	29	4	12
Coverage type[c]	Partial	Partial	Partial	Partial	Partial	Full	Full	Partial	Partial
Study dates	EW1 2014–EW26 2022	EW1 2013–EW26 2022	EW1 2007–EW27 2020	EW1 2006–EW40 2019	EW1 2000–EW13 2019	EW1 2001–EW13 2019	EW1 2008–EW26 2023	EW1 2019–EW26 2022	EW2 2018–EW12 2020
Wolbachia strain	*wAlbB*	*wAlbB*	*wMel*	*wMel*	*wMel*	*wMel*	*wMel*	*wAlbB*	*wMel*
Sex of released mosquitos	Male-only	Both	Both	Both	Both	Both	Both	Male-only	Both
Release method	Adult mosquitoes	Adult mosquitoes and eggs	Adult mosquitoes	Eggs only	Adult mosquitoes and eggs	Adult mosquitoes and eggs	Adult mosquitoes	Adult mosquitoes	Eggs only
Intervention type	IIT-SIT + High-fidelity sex sorting	Introgression	Introgression	Introgression	Introgression	Introgression	Introgression	IIT-SIT + High-fidelity sex sorting	Introgression
Method of confirmation	Routine surveillance	Routine surveillance	Routine surveillance	Routine surveillance	Routine surveillance	Routine surveillance	Routine surveillance	RCT-PCR, NS1 antigen or IgM	RT-PCR, NS1 antigen or IgM
Pre-interventional period[d]	286 weeks	313 weeks	582 weeks	585 weeks	857 weeks	759 weeks	476 weeks	364 weeks	156 weeks
Post-intervention period[d]	156 weeks	166 weeks	109 weeks	144 weeks	148 weeks	169 weeks	163 weeks	182 weeks	115 weeks
Intervention duration[e]	157 weeks	95 weeks	90 weeks	28 weeks	12 weeks	14 weeks	38 weeks	157 weeks	30 weeks
Outcome type reported	Population-level dengue incidence	Population-level dengue incidence	Population-level dengue incidence	Population-level dengue incidence	Population-level dengue incidence	Population-level dengue incidence	Population-level dengue incidence	Individual-level protective effectiveness	Individual-level protective effectiveness

EW: epidemiological week; GEE: logistic regression using generalized estimating equations; IIT-SIT: incompatible insect technique-sterile insect technique (refer to figure 1); ITS: interrupted time series; SCM: synthetic control method; TND: time negative design.

[a]: Estimates based on demographic and available census data as control area(s) population were not explicitly detailed in the study; [b]: includes individuals in both interventional and control areas analysed by the study which are considered at risk for dengue. In Lim *et al.* (1), this population includes only the interventional sites due to study design (emulation cluster-randomized test negative design); [c]: full-area coverage involves city-wide *Wolbachia* deployments without untreated comparators while partial-area coverage targets selected zones while retaining untreated areas for comparison (Results - Study selection and characteristics); [d]: calculated as the average, rounded to the nearest decimal, across all units included in the study when not explicitly stated; [e]: calculated as the average, rounded to the nearest decimal, of all intervention zones; [f]: Studies included in the main analysis evaluating population-level dengue incidence with *Wolbachia* intervention; [g]: Studies included in exploratory analysis evaluating individual-level protective effectiveness with *Wolbachia* intervention.

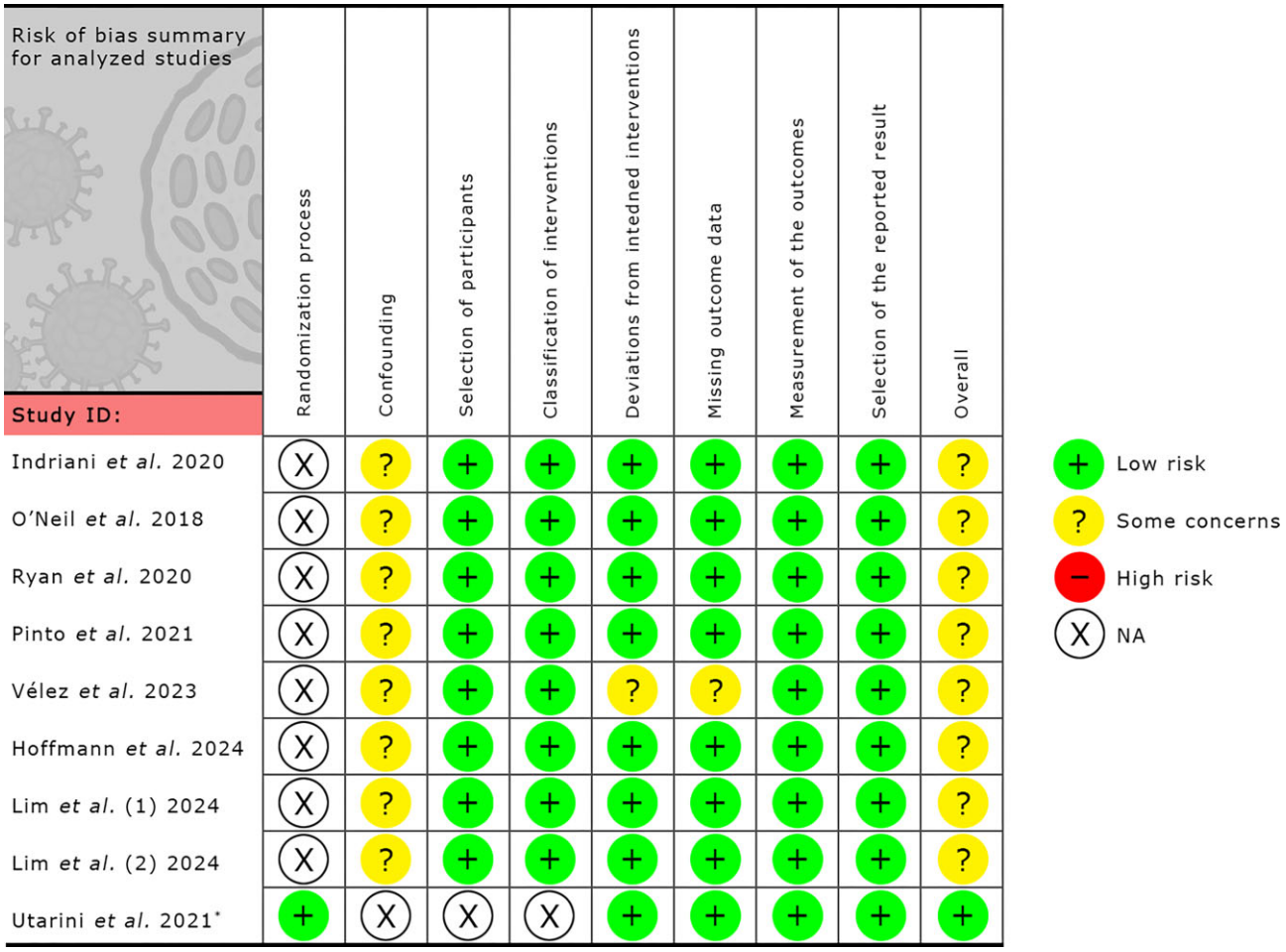

Figure 3 Risk of Bias Assessment for the Included Studies. RoB 2 was used for the randomized controlled trial, and ROBINS-I for non-randomized studies; symbols denote low risk, some concerns, high risk, or not applicable.

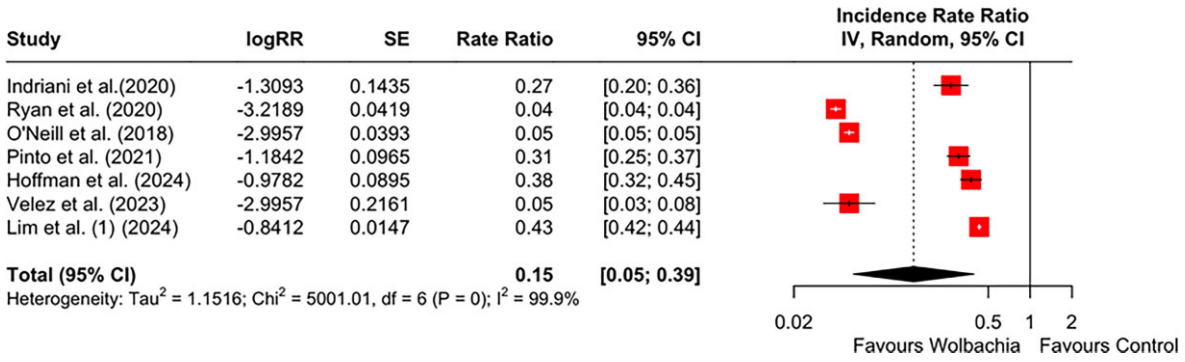

Figure 4 Dengue Incidence Forest Plot. Pooled incidence rate ratios (IRR) comparing *Wolbachia* intervention versus control areas. Estimates were synthesized using an inverse-variance random-effects model, and horizontal lines represent 95% confidence intervals; the diamond indicates the pooled effect. Values <1 favour *Wolbachia*.

disruptions that delayed *Wolbachia* deployment and interrupted dengue surveillance; these limitations may have affected exposure classification and outcome ascertainment but were transparently reported. The sole RCT included[12] was assessed using the RoB 2 tool and judged to be at low risk of bias across all domains. A summary of risk of bias assessments is presented in Figure 3.

Outcomes

Reduction in dengue incidence

Across seven population-based studies, *Wolbachia* deployment was consistently associated with a substantial reduction in dengue incidence across diverse geographic and epidemiological settings (Figure 4). The pooled IRR was 0.15 (95% CI 0.05–0.39),

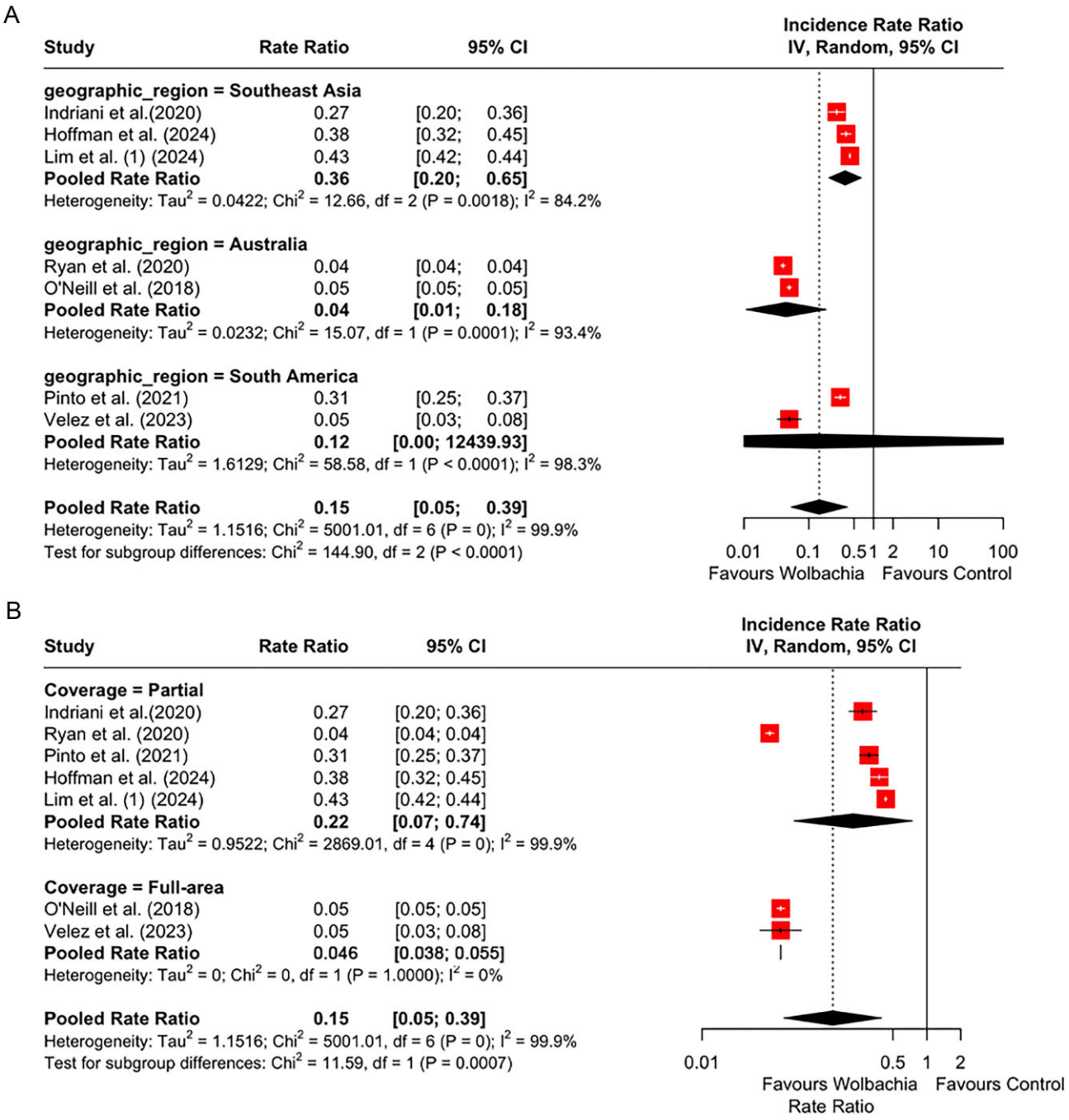

Figure 5 Subgroup analyses of *Wolbachia* deployments on dengue incidence. Random-effects inverse-variance meta-analyses are shown as rate ratios (incidence rate ratios) with 95% confidence intervals. (A) Subgrouped by geographic region (Southeast Asia, Australia, South America). (B). Subgrouped by deployment coverage (partial vs full-area). Diamonds indicate pooled subgroup and overall effects; tests for subgroup differences are shown within panels. Values <1 favour *Wolbachia*.

corresponding to an approximate 85% reduction in dengue incidence compared with untreated areas.

All included studies demonstrated reductions in dengue incidence, with individual IRRs ranging from 0.04 to 0.43. Although the magnitude of effect varied between studies, the direction of association was consistent. Between-study heterogeneity was substantial ($I^2 = 99.9\%$), reflecting differences in study design, geographic context, and deployment strategies.

For studies reporting multiple non-overlapping intervention-area estimates (e.g. separate municipal-level IRRs), a single study-level estimate was derived for meta-analysis using prespecified pooling procedures (see Supplementary Figure S1).

Subgroup analyses

By geographic region

Subgroup analyses by geographic region suggested broadly protective effects of *Wolbachia* deployments across settings (Figure 5A). Pooled estimates indicated substantial reductions in dengue incidence in Southeast Asia (IRR 0.36, 95%

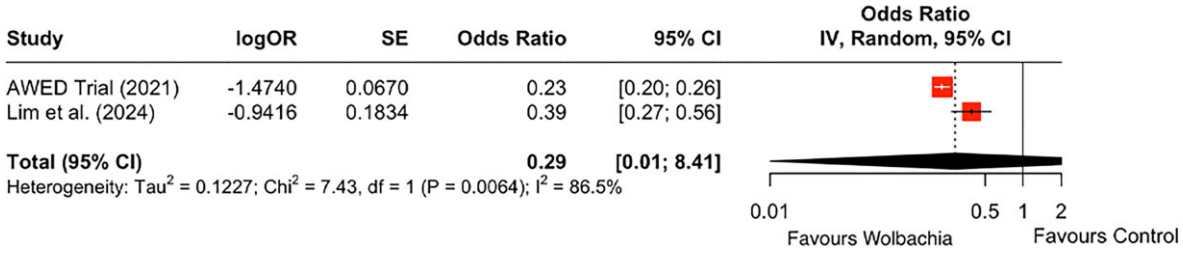

Figure 6 Individual-level protective effect of *Wolbachia* against dengue. Forest plot showing study-specific and pooled odds ratios (ORs) with 95% confidence intervals for individual-level dengue risk comparing *Wolbachia*-exposed versus control areas (random-effects model; squares = study weights, horizontal lines = 95% CIs, diamond = pooled estimate). Values <1 favour *Wolbachia*; heterogeneity was substantial ($I^2 = 86.5\%$).

CI 0.20–0.65) and Australia (IRR 0.04, 95% CI 0.01–0.18). Estimates for South America were highly imprecise due to substantial between-study heterogeneity and the small number of contributing studies and should be interpreted cautiously. Overall, regional differences may partly contribute to the observed heterogeneity.

By coverage

Subgroup analysis by intervention coverage showed that full-area *Wolbachia* deployments were associated with a pooled IRR of 0.046 (95% CI 0.038–0.055), corresponding to a 95% reduction in dengue incidence (Figure 5B). By contrast, partial-area deployments yielded a pooled IRR of 0.22 (95% CI 0.07–0.74), representing a 78% reduction. The difference between subgroups was statistically significant ($P < .0001$).

Exploratory subgroup analysis: individual-level protective efficacy

An exploratory analysis was conducted for studies reporting individual-level protective efficacy (Figure 6). These studies were analysed separately from the main meta-analysis to avoid conflating outcome metrics and to focus specifically on individual infection risk.[11,12]

The AWED cluster-randomized trial[12] reported an intention-to-treat OR of 0.23 (95% CI 0.20–0.26) for virologically confirmed dengue in *Wolbachia*-treated versus control clusters (protective effectiveness 77%). Lim *et al.*,[11] a quasi-experimental test-negative study, reported an OR of 0.39 (95% CI 0.27–0.56) (protective effectiveness of ~61%).

The pooled OR from these studies was 0.29 (95% CI: 0.01–8.41) and there was substantial heterogeneity ($I^2 = 86.5\%$). This imprecision means the pooled result should be interpreted cautiously and is best viewed alongside the consistent direction of effect in both studies rather than a definitive pooled estimate, particularly given differences in study design and implementation context.

Leave-one-out sensitivity analysis

Leave-one-out sensitivity analysis showed that the pooled effect consistently favoured *Wolbachia* deployment and was not driven by any single study (Supplementary Figure S2). Heterogeneity remained high across all iterations, suggesting that variability is primarily attributable to between-study differences rather than the influence of an individual outlier.

A Baujat plot was used to assess each study's contribution to heterogeneity and its influence on the pooled estimate (Supplementary Figure S3). A small number of studies contributed disproportionately to heterogeneity and/or influence, while most studies had minimal impact on either metric. Full study-specific Baujat plot interpretation is provided in the Supplementary Appendix.

Publication bias

Publication bias was not formally assessed because <10 studies were included in the meta-analysis, a threshold below which funnel plots and statistical tests are considered unreliable.[25,26]

Summary of evidence (GRADE)

The certainty of evidence was assessed using the GRADE framework.[27] The primary outcome of dengue incidence reduction was rated as low certainty, downgraded for risk of bias from non-randomized designs and substantial heterogeneity. The exploratory outcome of protective efficacy was rated as low certainty due to risk of bias, inconsistency, and imprecision. Although the direction of effect was consistent, confidence in the precise magnitude remains limited. Detailed GRADE assessments are provided in Supplementary Table S3.

Discussion

This systematic review and meta-analysis demonstrates that *Wolbachia*-based *A. aegypti* interventions are associated with substantial reductions in dengue incidence. Protective effects were observed across multiple geographic regions and study designs. Importantly, despite variability in the magnitude of effect, the direction of impact was consistently protective, supporting both reductions in population-level transmission and individual-level protection in treated settings. These findings underscore the relevance of *Wolbachia*-based approaches in dengue-endemic regions where sustained and scalable vector control remains a clinical and public health priority.

Marked statistical heterogeneity was observed across included studies, an expected finding given the diversity of real-world implementation contexts. *Wolbachia* deployments vary in strategy, scale, achieved coverage, surveillance systems, baseline transmission intensity, and health system capacity, all of which can influence observed epidemiological outcomes. In this setting, heterogeneity likely reflects genuine differences in implementation and context rather than inconsistency of direction. From a translational perspective, the consistent protective direction across

studies is more informative than the precise magnitude of pooled estimates for interventions intended to operate at population scale.

A key contribution of this review is the explicit distinction between full-area and partial-area *Wolbachia* deployment strategies. Larger and more consistent reductions in dengue incidence were observed in studies implementing city-wide or area-wide coverage, whereas partial-area deployments demonstrated protective effects with greater variability. This pattern is biologically and epidemiologically plausible, as incomplete coverage permits ongoing transmission through untreated areas and population movement across intervention boundaries, potentially attenuating measurable impact. These findings support threshold effects in vector replacement and highlight the importance of achieving high and sustained coverage when designing and scaling programmes.

Variation in estimated effectiveness was also observed across geographic regions, with robust protective effects reported in Southeast Asia and Australia, and imprecise estimates in South America. The latter reflects substantial between-study variability and limited data rather than evidence of reduced effectiveness. Differences across regions are likely influenced by variation in programme maturity, operational complexity, achieved *Wolbachia* prevalence, and surveillance quality, as well as the impact of the COVID-19 pandemic. Accordingly, regional subgroup findings should be interpreted cautiously and not viewed as comparative assessments of programme performance.

Evidence from the AWED cluster-randomized trial previously established the efficacy of *Wolbachia*-based vector replacement under controlled conditions. The present meta-analysis extends this evidence by integrating data from large-scale, real-world deployments evaluated using quasi-experimental designs and routine surveillance systems. By synthesizing population-level outcomes across diverse operational settings alongside experimental evidence, this review provides a more comprehensive assessment of effectiveness under conditions relevant to routine clinical and public health implementation.

This review has several strengths. A comprehensive and systematic search strategy was applied across multiple databases, supplemented by citation tracking, minimizing the likelihood of missing relevant studies. The inclusion of both randomized and quasi-experimental designs enabled evaluation of *Wolbachia* effectiveness under controlled trial conditions and large-scale real-world implementation. Population-level incidence outcomes and individual-level protective effectiveness were analysed separately, avoiding inappropriate pooling across outcome scales. Heterogeneity was addressed conservatively through random-effects modelling, programme-informed subgroup analyses, and formal assessment of evidence certainty using the GRADE framework.

Several limitations should be acknowledged. Substantial heterogeneity was present, reflecting differences in implementation strategies, coverage, surveillance systems, and epidemiological contexts. The number of RCTs remains limited, and much of the population-level evidence derives from non-randomized designs with potential for residual confounding. Outcome ascertainment and surveillance quality varied between settings, which may have influenced effect estimates. In addition, insufficient and inconsistent reporting precluded pooled analysis of severe dengue outcomes and dengue-related mortality. Nonetheless, the consistent protective direction observed across diverse settings and study designs supports the robustness of the overall conclusions.

Taken together, these findings support *Wolbachia*-based interventions as an effective population-level strategy for reducing dengue transmission. However, effectiveness is contingent on programme design, particularly deployment scale, achieved coverage, and sustained implementation. The evidence favours city-wide or area-wide rollouts where feasible and highlights the need for careful planning and monitoring in partial or phased deployments. *Wolbachia*-based strategies should be integrated within broader national dengue-control frameworks, alongside strengthened clinical and entomological surveillance and complementary vector-control measures, rather than implemented as a standalone intervention.

Further work integrating cost-effectiveness analyses, longer-term follow-up, and implementation science will be essential to inform optimal deployment strategies and guide sustainable scale-up within national dengue-control programmes. In parallel, studies incorporating individual-level data are needed to evaluate effects on clinically relevant outcomes, including severe dengue, hospitalization, and healthcare utilization.

Conclusions

This systematic review and meta-analysis indicates that *Wolbachia*-based *A. aegypti* interventions are associated with substantial reductions in dengue incidence across diverse settings. While effect sizes varied, the consistently protective direction of effect supports the effectiveness of *Wolbachia* deployments, particularly where wide-area coverage is achieved. These findings support *Wolbachia*-based strategies as an important component of integrated dengue-control programmes in endemic regions.

Author contributions

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Conflicts of interest

The authors declare no conflict of interest regarding this article.

Ethical approval

Ethical approval was not required for this systematic review and meta-analysis, as it involved only previously published data and did not include identifiable individual information.

Data availability

The data underlying this article are available in the article and in its online Supplementary material.

Supplementary material

Supplementary material is available at *Transactions* online.

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