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Wolbachia-carrying *Aedes* mosquitoes for preventing dengue infection (Protocol)

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[Intervention Protocol]

Wolbachia-carrying *Aedes* mosquitoes for preventing dengue infection

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ABSTRACT

Objectives

This is a protocol for a Cochrane Review (intervention). The objectives are as follows:

To assess the efficacy of *w*Mel-, *w*MelPop-, and *w*AlbB-carrying *Aedes* species deployments for preventing dengue virus infection.

BACKGROUND

Description of the condition

Dengue is a mosquito-borne viral infectious disease which is endemic in over 100 countries (Brady 2012). The dengue virus (DENV) is a single positive-stranded ribonucleic acid (RNA) virus of the *Flaviviridae* family, which is spread through the bite of female *Aedes aegypti* (*Ae aegypti*) mosquitoes and, to a lesser extent, *Aedes albopictus* and *Aedes polynesiensis.Aedes* mosquitoes are vectors for several viruses as well as DENV, including yellow fever virus, chikungunya virus, West Nile virus, Japanese encephalitis virus, and Zika virus. The risk of infection is present in all areas inhabited by *Aedes* mosquitoes, particularly in tropical climates.

A study on the prevalence of dengue estimates that 3.9 billion people are at risk of infection, and 70% of the actual burden of disease is in Asia (Bhatt 2013). Since the 1960s, there has been a 30-fold increase in global dengue incidence (WHO 2012). The geographic expansion of DENV infection has resulted in increased frequency and severity of the disease. While 80% of dengue infections are mild and asymptomatic, the number of reported deaths rose from 960 in 2000 to 4032 in 2015, mostly affecting younger age groups (WHO 2022a).

Dengue is caused by four distinct serotypes of DENV that are closely related (DENV-1, DENV-2, DENV-3, and DENV-4). Infection and recovery from a specific serotype provides lifelong immunity to that serotype; however, cross-immunity to other serotypes is partial and temporary (Reich 2013). One in 20 people with dengue can go on to develop severe dengue (Alexander 2011*).* Those who develop severe dengue usually go through three phases: febrile, recovery, and critical. The critical phase is associated with clinically significant plasma leakage leading to shock; haemorrhage due to low platelet count and coagulopathy; and severe organ impairment such as hepatitis, encephalitis, or myocarditis (CDC 2021). Early recognition is crucial to clinical management as it allows for the identification of people who are likely to progress to severe dengue, and the adoption of timely and appropriate interventions (WHO 2009). Managing severe dengue effectively reduces mortality to less than 1% (WHO 2022a).

Description of the intervention

Background

Wolbachia is a genus of gram-negative intracellular bacterial endosymbiont that is found in over 60% of all arthropod species (Hilgenboecker 2008). The bacterium is associated with phenotypic manipulations in host species, meaning it is able to modify characteristics of the host. Specifically, it can decrease vectors' fitness (ability to survive and mate) and reproductive capacities and increase their resistance to arthropod-borne viruses. *Wolbachia* is maternally inherited, and its potential use in vector control is based on two strategies: population suppression and population replacement. Both strategies are driven by one of the most prominent features imposed by *Wolbachia* on their host: cytoplasmic incompatibility, a phenomenon that results in sperm and eggs being unable to form viable offspring. This may be unidirectional (only one *Wolbachia* strain is involved during mating) or bidirectional (two different individuals carry different strains of *Wolbachia*). Unidirectional and bidirectional cytoplasmic incompatibility both drive the population suppression strategy. Unidirectional cytoplasmic incompatibility may also drive the population replacement strategy when *Wolbachia*-carrying females are present. Infected eggs can be fertilized by sperm from any male, whereas uninfected eggs can only be fertilized by sperm from uninfected males. Therefore, infected females will produce a greater number of offspring than uninfected females, and because *Wolbachia* is only inherited maternally, the frequency of infection increases with each generation.

Potential impact on dengue transmission

The introduction of *Wolbachia*-carrying *Aedes* species presents a promising vector control strategy. Studies exploring the properties of different *Wolbachia* infections in insect hosts have identified both life-shortening and antiviral effects of *Wolbachia* in *Drosophila melanogaster* (McMeniman 2009; Min 1997; Moreira 2009). These properties of *Wolbachia* constitute a potential vector control strategy for dengue, as well as for other vector-borne diseases, as they may reduce the ability of vectors to carry viruses that cause vector-borne disease in humans or reduce the fitness of the vectors themselves; in both cases, the result is a reduction in viral transmission. To explore this possibility, experimental studies have investigated transfection of vectors with strains of *Wolbachia* through embryo microinjection and adult microinjection (for details on the process of transfection, see Hughes 2014). In this review, we will focus on strains of *Wolbachia* that have been demonstrated to stably transinfect vectors of DENV with a potential effect on dengue transmission; that is, strains of *Wolbachia* that can infect *Aedes* mosquitoes and be passed on to their progeny and cause a disadvantage in vector fitness or ability to be infected with DENV, with the potential to reduce DENV transmission in humans.

Stable transfection of *Aedes*

Studies have shown that the *Wolbachia* strains *w*MelPop, *w*Mel, and *w*AlbB can achieve stable transfection of *Aedes* mosquitoes with a potential effect on dengue transmission.

Experimental transfection of *Ae aegypti* with *w*MelPop demonstrated the life-shortening effect of *Wolbachia* in the dengue vector, with around a 50% reduction in adult female lifespan (McMeniman 2009). To explore the hypotheses that this *Wolbachia* strain may alter vector competence for arboviruses, investigators transfected *Ae aegypti* with *w*MelPop and exposed them to dengue and chikungunya viruses (Moreira 2009). This demonstrated a reduced ability for arboviruses to establish infection in *w*MelPop-carrying *Ae aegypti,* potentially due to competition for host cell components and upregulation of immune effector genes (Moreira 2009). Experimental investigations continued to explore the properties of *Wolbachia*-carrying *Aedes* mosquitoes to identify the *Wolbachia* strain most suitable for a dengue prevention strategy. According to Walker 2011, *w*Mel *Wolbachia*-carrying *Ae aegypti* displays reproductive phenotype cytoplasmic incompatibility with minimal apparent fitness costs and high maternal transmission, providing optimal phenotypic effects for invasion. The same study demonstrated the ability of *w*Mel *Wolbachia* to provide protection against DENV in *Ae aegypti.* Blagrove 2013 evidenced the infection of *Aedes albopictus* with *w*Mel *Wolbachia* by bidirectional cytoplasmic incompatibility, demonstrating a promising new method to prevent or reduce dengue. Other studies have shown that the *w*AlbB strain of *Wolbachia* can induce a viral inhibitory effect against DENV in *Ae aegypti* and *Aedes polynesiensis* mosquitoes (Bian 2010; Bian 2013). Johnson 2015 provides a detailed summary of the effects

of *Wolbachia* strains on vectors for mosquito-borne disease. Table 1 summarizes evidence of the effect of *Wolbachia* on dengue vectors.

The deployment of *Wolbachia* carrying *Aedes* mosquitoes into dengue-endemic areas is a potential strategy to prevent dengue transmission and infection in humans. Releases of female *Wolbachia*-carrying mosquitoes would facilitate the spread of *Wolbachia* infection throughout the wild *Aedes* population by the mechanism of unidirectional cytoplasmic incompatibility, and reduce the ability of the wild vector population to carry DENV and transmit the infection.

Existing evidence of *Wolbachia* as a vector control strategy

Researchers have piloted vector control strategies utilising *Wolbachia* in several global locations inhabited by *Aedes* mosquitoes, including Singapore, Puerto Rico, Texas, California, Colombia, and Brazil (CDC 2022; NEA 2022; Wellcome 2022).

In Singapore, the National Environment Agency (NEA) has released male *Wolbachia*-carrying *Aedes* mosquitoes and used the population suppression strategy for dengue prevention. *Wolbachia*-carrying male mosquitoes mate with uninfected female mosquitoes, resulting in unhatched eggs and a reduced mosquito population (NEA 2022). In some towns, this has resulted in up to 98% reduction in *Ae aegypti* populations, and sites with at least one year of releases have reported 88% fewer dengue cases than areas with no releases (NEA 2022).

Experimental studies in Australia have demonstrated the effect of the population replacement strategy on *Ae aegypti* using the *w*Mel strain of *Wolbachia*. The number and frequency of *w*Mel *Wolbachia*-carrying mosquito deployments varied from one to two releases per week for a duration of between five and 23 weeks (Ryan 2019). Deployments were typically discontinued when the frequency of *Wolbachia* in field-caught mosquitoes exceeded 50% for a period of more than two weeks, at which point it was expected that the frequency of infection would increase self-sustainably without further deployments. However, some studies implement a higher *Wolbachia* infection threshold before discontinuation of *Wolbachia*-carrying mosquito deployments. Across the experimental sites, short-term releases of between five and 23 weeks with either eggs or adult mosquitoes resulted in the establishment of *Wolbachia* in mosquito populations (Ryan 2019). An analysis of case notifications data prior to and after mosquito deployments indicated a 96% reduction in dengue incidence in *Wolbachia*-treated populations (Ryan 2019). Ovitrapping data after the initial implementation of *w*Mel *Wolbachia*-carrying *Aedes* deployments showed that the frequency of *Wolbachia* infection in the *Ae aegypti* population was above 0.96 at all release areas, meaning infection was stable in the vector population (Ross 2022).

How the intervention might work

Experimental transfection of *Aedes* mosquitoes with certain *Wolbachia* strains has demonstrated strong cytoplasmic incompatibility, shown no effect on egg viability (meaning the strain is more likely to persist in wild populations), and reduced vector competence to carry arbovirus infections (Bian 2010; Bian 2013; Blagrove 2013; Johnson 2015; Walker 2011). *Wolbachia*-carrying *Aedes* mosquitoes can be periodically deployed into populated areas, either as adult mosquitoes or at the larval stage, where they mate with the wild population. The population replacement strategy involves releasing both male and female *Wolbachia*-carrying *Aedes* mosquitoes to pass *Wolbachia* on to *Aedes* offspring, meaning the prevalence of the *Wolbachia* infection in the vector population continuously increases. As levels of *Wolbachia* transfection increase, the capacity of the *Aedes* population to transmit arboviral infections such as DENV infection decreases, and the risk of disease outbreak also decreases. Conservative modelling estimates of *w*Mel *Wolbachia*-carrying *Ae aegypti* deployments in a large human population suggest that *Wolbachia* could lead to an immediate and long-term reduction in dengue, nearing elimination (Dorigatti 2018). Currently, the World Mosquito Program facilitates dengue prevention programmes globally using the *w*Mel *Wolbachia*-carrying mosquito replacement strategy (www.worldmosquitoprogram.org). The population suppression strategy involves releasing non-biting male *Wolbachia*-carrying mosquitoes, resulting in incompatible mating with uninfected females and a reduction in the mosquito population. This is the strategy used in Singapore (NEA 2022).

Why it is important to do this review

Dengue is a rapidly spreading mosquito-borne disease with 60 million cases of infection recorded in 2019, an increase of 30 million since 1990 (Yang 2021). Although the incidence of the disease is growing rapidly in middle-high socio-demographic index (SDI) regions, dengue remains most prevalent and most fatal in low- and middle-income countries (Yang 2021).

Vector control is an important component of dengue prevention programmes, and the WHO recommends integrated vector control strategies, including targeted residual spraying, larval control, and personal protective measures (WHO 2009). Most approaches are expensive and need teams that understand the characteristics of the vector and people in the local area (Knerer 2020; Ritchie 2021; Soh 2021). Methods that do not rely on insecticide are becoming more important, as resistance to all four classes of insecticide has been reported in *Aedes* arbovirus vectors in the Americas, Asia, and Africa (Moyes 2017). Effective integrated vector control is difficult to achieve in resource-limited endemic countries. In urban centres, vector control strategies are hampered by urbanization, building design, and inadequate water supply management (Jansen 2010). The WHO encourages city planners, environmentalists, and engineers to work together in urban environmental mosquito control, but in practice this is difficult to implement (WHO 2022b). Research evidence is limited, and one systematic review found only two randomized controlled trials (RCTs) assessing the efficacy of dengue vector control to reduce dengue incidence (Bowman 2016).

Vaccines for long-lasting protection against all four dengue viruses are in development following the success of a live-attenuated vaccine against closely related Japanese encephalitis virus (Monath 2002). Dengvaxia (CYD-TDV), developed by Sanofi-Pasteur, was the first approved vaccine for dengue, licenced in 2015 for use in individuals aged nine to 45 years living in endemic areas, and currently approved in 20 countries (WHO 2018). Analyses of the long-term safety of this vaccine have demonstrated inconsistent efficacy and safety in seropositive and seronegative individuals, with a lower vaccine efficacy and increased risk of hospitalization and severe dengue in seronegative individuals (Hadinegoro 2015). These results have led to considerable vaccine hesitancy, particularly in the Philippines, which was the first and only country to introduce Dengvaxia to their public vaccination

programme: after 830,000 children had received at least one dose, Philippine policymakers suspended the vaccine (Wilder-Smith 2019). In 2017, a SAGE working group on dengue vaccines recommended that countries considering introducing a dengue vaccination programme should implement a pre-vaccine screening strategy to determine the serostatus of individuals and ensure only seropositive individuals are included in the programme (WHO 2018). As a result, use of vaccines for dengue is currently limited in favour of alternative dengue prevention methods.

Researchers are exploring the possibility of using the endosymbiotic bacteria *Wolbachia* as an innovative dengue prevention strategy (www.worldmosquitoprogram.org). One analysis of early observational studies on *w*Mel-*Wolbachia*-carrying *Ae aegypti* deployments conducted in Australia demonstrated a protective efficacy of more than 95% (95% confidence interval (CI) 84% to 99%; 2 studies) against cases of dengue fever (DF; Cochrane Response 2021). One controlled interrupted time series study conducted in Indonesia also demonstrated an adjusted protective efficacy of 73% (95% CI 49% to 89%) for monthly incidence of dengue haemorrhagic fever (DHF; Cochrane Response 2021).

A systematic review of well-conducted RCTs investigating *Wolbachia*-carrying *Aedes* deployments will provide an evidence-based summary of the efficacy of this intervention for the prevention of DENV infection.

OBJECTIVES

To assess the efficacy of *w*Mel-, *w*MelPop-, and *w*AlbB-carrying *Aedes* species deployments for preventing dengue virus infection.

METHODS

Criteria for considering studies for this review

Types of studies

We will include RCTs, including cluster-RCTs (cRCTs), as they have the best trial design for evaluating the efficacy of interventions (Higgins 2022).

Types of participants

Adults and children living in endemic and epidemic-prone areas where DENV infection is prevalent.

Types of interventions

Intervention

*w*Mel-, *w*MelPop-, and *w*AlbB-carrying *Aedes* deployments plus any local existing mosquito-control measures. Any cointerventions should be balanced across the control and intervention arms. Based on existing evidence on stable *Wolbachia* infections in transinfected hosts, we will only include studies investigating specific combinations of *Wolbachia* and *Aedes*, as outlined in Table 1.

Control

Any local existing mosquito-control measures, including individual-, household-, and community-level interventions. Such interventions may include, but are not limited to, education programmes, reduction in larval source habitats, insecticide spraying, Abate temephos, and bed net use.

Types of outcome measures

We will assess the outcome measures at all time points up to longest follow-up. We will group the time points as short-term (up to 12 months after final deployment) and long-term (more than 12 months after final deployment). The outcomes listed are outcomes of interest and will not be used as criteria for study inclusion.

Primary outcomes

Epidemiological outcomes

- Virologically confirmed dengue (VCD) case incidence (local, imported, or both) confirmed by reverse transcription polymerase chain reaction (RT-PCR) or enzyme-linked immunosorbent assay (ELISA)
- Prevalence of DENV infection

Entomological outcomes

- Prevalence of dengue DNA in the mosquito population
- Mosquito density (for population suppression strategy)
- Prevalence of *Wolbachia*-carrying mosquitoes (for population replacement strategy)

Secondary outcomes

Epidemiological outcomes

- Notified DF or DHF cases (suspected or confirmed, based on self-reporting or clinical examination)

Entomological outcomes

- Spatial distribution of *Wolbachia*-infected mosquitoes

Clinical outcomes

- All-cause mortality
- Hospitalizations due to DF or DHF
- Adverse events potentially related to *Wolbachia*-carrying *Aedes* deployments

Other outcomes (narrative description)

- Community acceptability
- Cost and resources

Search methods for identification of studies

We will identify all relevant studies regardless of language or publication status (published, unpublished, in press, and in progress). We will include studies published from 2009, the year when *w*Mel-*Wolbachia* was first successfully transferred to *Ae aegypti* mosquitoes (Walker 2011).

Electronic searches

We will search the following databases using the search terms and strategy described in Appendix 1.

- Cochrane Infectious Diseases Group Specialized Register
- Cochrane Central Register of Controlled Trials (CENTRAL) in the Cochrane Library
- MEDLINE (Ovid)
- Embase (Ovid)
- Science Citation Index-Expanded (Web of Science)

- Conference proceedings citation index (Web of Science)
- CAB Abstracts (Web of Science)
- CINAHL (EBSCOhost)
- LILACS (BIREME)

We will also search the WHO International Clinical Trials Registry Platform (ICTRP; apps.who.int/trialsearch), and ClinicalTrials.gov (clinicaltrials.gov/ct2/home) for trials in progress, using "Aedes" or "Dengue" or "DENV" and "Wolbachia" or "wMel" or "wMelPop" or "wAlbB" as search terms.

Searching other resources

We will check the reference lists of relevant studies to identify additional references.

Conference proceedings

We will search the proceedings of the Global Sustainable Technological and Innovation (G-STIC) conferences for the past five years.

Data collection and analysis

Selection of studies

We will use standard Cochrane methods for selecting studies (Higgins 2022). Two review authors (TF, YS) will independently screen titles and abstracts of identified records, eliminating those they consider clearly ineligible. We will retrieve the full-text articles of the remaining records and independently assess them against predefined criteria. We will resolve discrepancies by discussion or by involving a third review author (WR), if necessary.

Data extraction and management

Two review authors (TF, DD) will independently extract data using a standardized piloted data extraction form. We will contact the study authors to obtain missing data. At each step of data extraction, we will resolve any discrepancies through discussion between the review authors.

We will extract the following information.

- General information: author, title, publication date, country, study date(s), study location (urban/rural), baseline endemicity of dengue, funding details, conflicts of interest
- Study characteristics: aim, unit of allocation, number of units, adjustment for clustering, length of follow-up
- Participants: number of participants, method of recruitment, withdrawal or loss to follow-up, age, sex, socio-economic status
- Intervention: mosquito life stage (egg, larva, adult), number of deployments, timing/frequency of deployments, location of deployments, aimed percentage vector population replacement, achieved percentage vector population replacement, field monitoring strategies, co-interventions (e.g. insecticide spraying, bed net use, larvicide control)
- Comparator: description of local vector control strategies in place
- Outcome(s): primary outcome(s), secondary outcome(s)

Assessment of risk of bias in included studies

We will assess the risk of bias of the included studies using the Cochrane risk of bias tool RoB 2 (Higgins 2022; Sterne 2019). To assess individually randomized trials, we will use the RoB 2 Excel tool (available at www.riskofbias.info/welcome/rob-2-0-tool/current-version-of-rob-2); for cRCTs, we will use the modified tool with an additional domain for assessing bias arising from randomization of clusters (www.riskofbias.info/welcome/rob-2-0-tool/rob-2-for-cluster-randomized-trials). The effect of interest is the effect of assignment at baseline, regardless of whether the interventions were received as intended (the 'intention-to-treat effect'). We will assess risk of bias for all outcomes specified in the Primary outcomes section, which contribute to the review's summary of findings table.

Two review authors (TF, IAR) will independently assess the risk of bias of all specified results. We will resolve any disagreements through discussion with a third review author (GV).

The RoB 2 tool considers the following domains.

- Bias arising from the randomization of clusters (for cRCTs only)
- Bias arising from the randomization of participants
- Bias due to deviations from the intended interventions
- Bias due to missing outcome data
- Bias in measurement of the outcome
- Bias in selection of the reported result

We will use the recommended signalling questions to assess the RoB 2 domains, responding 'yes', 'probably yes', 'probably no', 'no', or 'no information'. We will use the RoB 2 algorithm to reach an overall risk of bias judgement ('low risk of bias', 'some concerns', or 'high risk of bias') for each domain.

We will reach an overall risk of bias judgement for a specific outcome by combining the judgements for all domains. Any study with low risk of bias for all domains will achieve an overall low risk of bias judgement; some overall concerns is assumed when at least one domain has some concerns, and studies with a high risk of bias for at least one domain obtain an overall high risk of bias judgement (Higgins 2022).

We will store the full RoB 2 data (e.g. completed Excel tool), which will be available on request.

Measures of treatment effect

For dichotomous outcomes, we will use the risk ratio with the corresponding 95% interval (CI) as the effect measure. For count/rate outcomes, we will use the rate ratio with 95% CI as the effect measure. We will use adjusted measures of effect for cRCTs (see Unit of analysis issues). We will not include unadjusted measures of effect for cRCTs in meta-analyses.

Unit of analysis issues

For cRCTs, we will extract measures of effect that are adjusted for clustering where possible. If the study authors have not performed any adjustments for clustering, we will adjust the raw data using an intraclass correlation coefficient (ICC) value. If the study reports no ICC value, we will request this information from the study authors, obtain it from similar studies, or estimate it ourselves. If we estimate the ICC, we will perform sensitivity analyses to investigate the robustness of our results. We will not present results from cRCTs that are not adjusted for clustering.

If we identify multi-arm trials, we will select relevant arms for inclusion in our analyses. If more than two arms are relevant to this review, we will either combine intervention arms so that there is one comparison, or split the control group between multiple comparisons to avoid double-counting of participants in meta-analysis.

Dealing with missing data

We will contact study authors to obtain missing study characteristics, missing outcomes, missing summary data, and missing individual data.

We will assess the risk of reporting bias due to missing studies and missing outcomes as described in the Assessment of reporting biases section.

If we are unable to obtain missing summary data, we will calculate or estimate the required data from other reported statistics using formulas specified in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2022).

If we are unable to obtain missing individual data, we will take this into account when assessing risk of bias (Higgins 2022; Sterne 2019). In the first instance, we will conduct a complete case analysis, and we may perform sensitivity analyses to investigate the impact of missing data. For example, we may vary the event rate within missing individuals from intervention and control groups within plausible limits, or we may exclude studies thought to be at risk of bias from our meta-analyses.

Assessment of heterogeneity

We will assess the extent of clinical and methodological heterogeneity by examining study characteristics (e.g. region, severity of clinical disease, insecticide resistance, dengue serotype, mosquito fitness, retention of cytoplasmic incompatibility).

We will present results of meta-analyses in forest plots, which we will inspect visually to assess statistical heterogeneity (non-overlapping CIs generally signify statistical heterogeneity). We will also use the Chi^2 test with a P value of less than 0.1 to indicate statistical heterogeneity. We will quantify heterogeneity using the I^2 statistic, which describes the percentage of the variability in effect estimates that is due to heterogeneity rather than sampling error. We will interpret this statistic using the following guidance from the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2022).

- 0% to 40%: might not be important
- 30% to 60%: may represent moderate heterogeneity*
- 50% to 90%: may represent substantial heterogeneity*
- 75% to 100%: considerable heterogeneity*

*The importance of the observed value of I^2 depends on magnitude and direction of effects and strength of evidence for heterogeneity (e.g. P value from the Chi^2 test, or a CI for I^2: uncertainty in the value of I^2 is substantial when the number of studies is small).

Assessment of reporting biases

We will search for ongoing trials that meet our eligibility criteria and classify them as 'ongoing' until they are published.

If we include 10 studies in a meta-analysis, we will explore the possibility of small-study biases (a tendency for estimates of the intervention effect to be more beneficial in smaller studies) for the primary outcomes using funnel plots. In the case of asymmetry, we will consider various explanations such as publication bias, poor study design, and the effect of study size.

Data synthesis

We will analyse data using Review Manager Web (RevMan Web 2022), using random-effects models in all cases. Where we consider meta-analysis to be inappropriate due to important clinical, methodological, or statistical heterogeneity, we will summarize data in tables.

Subgroup analysis and investigation of heterogeneity

We intend to conduct subgroup analysis to explore whether the following characteristics constitute sources of heterogeneity in the meta-analysis.

- Endemicity (endemic versus epidemic-prone)
- Age (children under 18 years versus adults 18 years and older)

If there is still substantial unexplained heterogeneity (defined in Assessment of heterogeneity), we may explore the following characteristics.

- Region
- Severity of clinical disease
- Insecticide resistance
- Dengue serotype
- Mosquito fitness
- Retention of cytoplasmic incompatibility

Sensitivity analysis

We may perform sensitivity analyses to investigate the impact of missing data. For example, we may vary the event rate within missing participants from intervention and control groups within plausible limits, or we may exclude studies thought to be at high risk of attrition bias from our meta-analyses.

If we estimate the ICC to adjust data from cRCTs for clustering, we will perform sensitivity analyses to investigate the robustness of our results.

Summary of findings and assessment of the certainty of the evidence

We will present the main results of the review in summary of findings tables, rating the certainty of evidence according to the GRADE approach. We will follow current GRADE guidance as recommended in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2022).

Two review authors (TF, GV) will independently assess the certainty of the evidence, considering risk of bias, inconsistency, imprecision, indirectness, and publication bias.

The summary of findings table will include the following outcomes.

- VCD case incidence (local, imported, or both) confirmed by RT-PCR or ELISA
- Prevalence of DENV infection

- Prevalence of dengue DNA in the mosquito population
- Mosquito density (for population suppression strategy)
- Prevalence of *Wolbachia*-carrying mosquitoes (for population replacement strategy)

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The following people conducted the editorial process for this article.

- Sign-off Editor (final editorial decision): Dr Joseph Pryce, CIDG
- Managing Editor (selected peer reviewers, collated peer-reviewer comments, provided editorial guidance to authors, edited the article): Dr Deirdre Walshe, CIDG
- Copy Editor (copy editing and production): Julia Turner
- Peer-reviewers (provided comments and recommended an editorial decision):
  - Two peer reviewers provided content peer review, but chose not to be publicly acknowledged
  - Mohamed Magzob, University of Gezira (consumer review)
  - Afroditi Kanellopoulou Methods Support Unit Methods and Evidence Synthesis Development team Cochrane, UK (stats review)
  - Ina Monsef Faculty of Medicine and University Hospital Cologne, University of Cologne, Department I of Internal Medicine, Center for Integrated Oncology Aachen Bonn Cologne Düsseldorf, Cochrane Haematology, Cologne, Germany (search review)

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ADDITIONAL TABLES

Table 1. Evidence of stable transfection of dengue vectors with *Wolbachia*

Mosquito species	*Wolbachia* strain
Aedes aegypti	*w*MelPop, *w*Mel, *w*AlbB
Aedes albopictus	*w*Mel
Aedes polynesiensis	*w*AlbB

Table adapted from Johnson 2015

APPENDICES

Appendix 1. Search strategy – MEDLINE (Ovid)

1 Search strategy – MEDLINE (Ovid)

Ovid MEDLINE(R) and In-Process, In-Data-Review & Other Non-Indexed Citations <1946 to present>

1 Dengue Virus/

2 exp Dengue/

3 dengue.tw, kf.

4 DENV*.tw,kf.

5 1 or 2 or 3 or 4

6 Aedes/

7 aedes.tw,kf.

8 mosquito*.tw,kf.

9 (dengue adj2 vector*).tw,kf.

10 6 or 7 or 8 or 9

11 5 or 10

12 Wolbachia/

13 wolbachia.tw,kf.

14 (Wmel or wMelPop or wAlbB).tw.

15 12 or 13 or 14

16 11 and 15

17 Randomized Controlled Trial.pt.

18 controlled clinical trial.pt

19 (randomized or placebo or randomly or trial or groups).ab.

20 drug therapy.fs

21 17 or 18 or 19 or 20

22 exp animals/ not humans/

23 21 not 22

24 16 and 23

This is the preliminary search strategy for MEDLINE (Ovid). It will be adapted for other electronic databases. We will report all search strategies in full in the final version of the review.

CONTRIBUTIONS OF AUTHORS

All authors contributed to the protocol design, including Background and Methods, and approved the final version.

DECLARATIONS OF INTEREST

TF is a CIDG Research Associate, and was not involved in the editorial process. She has no known conflicts of interest to declare.
YS works as a systematic reviewer for Cochrane Response, an evidence services unit operated by Cochrane. Cochrane Response was contracted by the WHO to conduct a systematic review on *w*Mel *Wolbachia*-carrying mosquitoes for the biocontrol of dengue virus infection. She has no known conflicts of interest to declare.
MC is a CIDG Editor, and was not involved in the editorial process. She has no known conflicts of interest to declare.
WR has no known conflicts of interest to declare.
DD has no known conflicts of interest to declare.
IAR has been an employee of the Cochrane Central Executive Team (Cochrane Response/Evidence, Production & Methods Directorate) since 2021. Cochrane Response was contracted by the WHO to conduct a systematic review on *w*Mel *Wolbachia*-carrying mosquitoes for the biocontrol of dengue virus infection. She has no known conflicts of interest to declare.
GV works as senior systematic reviewer for Cochrane Response, an evidence services unit operated by Cochrane. Cochrane Response was contracted by the WHO to conduct a systematic review on *w*Mel *Wolbachia*-carrying mosquitoes for the biocontrol of dengue virus infection. She has no known conflicts of interest to declare.

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