Preprints.org

Article

Not peer-reviewed version

Biotechnological Control of *Aedes aegypti* for Dengue Suppression: A Global Review of Expectations, Efficacy, and Concerns to Inform the Jeddah Vector Management Strategy, SPIRIT/TIDieR-Framed Protocol, and Simulation-Based Design-of-Experiments

Radwan Baabbad , Ahmed Hala * , Mazen Baabbad

Posted Date: 5 November 2025

doi: 10.20944/preprints202511.0326.v1

Keywords: dengue fever; bacteria; epidemic suppression; design of experiment; microbiology

Preprints.org is a free multidisciplinary platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC.

Copyright: This open access article is published under a Creative Commons CC BY 4.0 license, which permit the free download, distribution, and reuse, provided that the author and preprint are cited in any reuse.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 November 2025 doi:10.20944/preprints202511.0326.v1

Disclaimer/Publisher's Note: The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

Article

Biotechnological Control of *Aedes aegypti* for Dengue Suppression: A Global Review of Expectations, Efficacy, and Concerns to Inform the Jeddah Vector Management Strategy, SPIRIT/TIDieR-Framed Protocol, and Simulation-Based Design-of-Experiments

Radwan Baabbad [1], Ahmed Hala [1] and Mazen Baabbad [1,2]

[1] Gaseous electronics LLC, Riyadh, Saudi Arabia

[2] KACST, Riyadh, Saudi Arabia

* Correspondence: amhala@gmail.com

Abstract

Dengue fever remains one of the most pressing vector-borne diseases worldwide, driven primarily by the mosquito *Aedes aegypti*. Recent advancements in biotech- nology—ranging from the use of *Wolbachia*-infected mosquito releases to genetic modification (GM) techniques such as RIDL and CRISPR-based suppression—have redefined vector control paradigms. This paper **presents a comprehensive protocol for the synthesis and evaluation of global biotechnological inter- ventions**, designed to assess expectations, field efficacy, and ethical and ecological concerns. Drawing from both computational modeling and field trial literature, we integrate findings to inform an optimized vector management strategy for Jed- dah, Saudi Arabia. The strategy is structured according to the SPIRIT/TIDieR framework and supported by a simulation-based Design-of-Experiments (DoE) ap- proach. The **protocol describes the interplay** between biological interventions and stochastic modeling, emphasizing the need for localized parameter calibration, long-term monitoring, and participatory stakeholder engagement. This integrative review and modeling framework serves as a foundation for the rational design of large-scale, adaptive dengue suppression programs, **demonstrating how simulated outcomes can guide real-world implementation.**

Keywords: dengue fever; bacteria; epidemic suppression; design of experiment; microbiology

1. Introduction

Dengue fever, caused by any of four serotypes of the dengue virus (DENV-1 to DENV- 4), represents a major global health burden affecting more than 390 million individuals annually (World Health Organization, 2024). The principal vector, *Aedes aegypti*, is highly adapted to urban environments and thrives in domestic and peri-domestic water

containers. Despite decades of vector control programs relying primarily on insecticide- based measures, dengue incidence has continued to rise, signaling the urgent need for alternative, sustainable control strategies (Bhatt et al., 2013).

1.1. The Rise of Biotechnological Interventions

In the last two decades, several biotechnological approaches have emerged as promising tools for vector control. Among them, two dominant paradigms have gained particular attention: the use of maternally transmitted endosymbionts such as *Wolbachia pipientis*, and genetic engineering approaches, including the release of insects carrying a dominant lethal gene (RIDL) and CRISPR-

© 2025 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 November 2025 doi:10.20944/preprints202511.0326.v1

mediated gene drives. These strategies aim to either suppress vector populations or replace them with less competent vectors for dengue trans- mission (Hoffmann et al., 2011; Carvalho et al., 2015).

1.2. Computational Modeling as a Complementary Tool

In parallel to field experimentation, computational and mathematical models have been used to predict, optimize, and validate intervention outcomes. Deterministic and stochas-

tic frameworks—ranging from differential equation systems to agent-based simulations—provide essential insights into population dynamics, threshold infection rates, and intervention sustainability (Mazen, 2023).

1.3. Jeddah as a Case Study

Jeddah, located on the Red Sea coast of Saudi Arabia, presents a unique epidemiological and environmental setting for dengue control. Local dengue outbreaks, coupled with international travel and trade, have positioned Jeddah as a sentinel site for evaluating biotechnological strategies.

1.4. Aim and Scope

This work synthesizes two previously distinct domains: (1) the biological and policy- oriented framework of dengue biotechnology interventions, and (2) the quantitative com- putational modeling of intervention efficacy and sustainability. **Specifically, this paper describes a robust protocol for integrating these domains to inform vector management strategies, using hypothetical simulation outcomes to demon- strate the utility of the approach.**

2. The Intracellular Dynamics and Transmission Ecology of *Wolbachia pipientis* in Arthropods

2.1. Nature of Wolbachia pipientis and Its Distribution in Arthro- pods

Wolbachia pipientis is an α-proteobacterium that lives obligately inside the cells of many invertebrates, including insects (notably *Aedes*, *Anopheles*, and *Culex* mosquitoes), mites, crustaceans, and nematodes. It spreads through maternal (cytoplasmic) inheritance,

residing in the cytoplasm of host cells and transmitted via eggs. Its success among arthropods arises from reproductive manipulations—including cytoplasmic incompatibil- ity (CI), parthenogenesis, feminization, and male killing—that bias reproduction in favor of infected female lineages. Distribution in nature is additionally shaped by ecological and microbiome contexts: resident symbionts such as *Asaia* can hinder vertical transmission or exclude *Wolbachia* from reproductive tissues, and prevalence may vary across envi- ronments and host genotypes. In some systems the association tends toward facultative mutualism (e.g., antiviral protection), supporting stable long-term coexistence.

2.2. Entry Through Predator–Prey Digestive Pathway

Beyond maternal inheritance, *Wolbachia* can cross species boundaries via horizontal transfer. A confirmed route is the predator–prey pathway: after ingestion of infected prey, viable *Wolbachia* can survive digestive passage, cross the gut epithelium, enter the hemolymph, and spread to systemic tissues including the central nervous system and the ovaries. Experimental studies have detected persistent, metabolically active bacteria months post-ingestion using fluorescence *in situ* hybridization (FISH) targeting bacte- rial rRNA, demonstrating that the digestive tract can serve as a conduit for interspecies acquisition in suitable hosts.

2.3. Infection of Ovaries and Testes (Intracellular Mechanisms)

For multigenerational persistence, *Wolbachia* must colonize the germ line. The bac- terium actively co-opts host internalization mechanisms—notably phagocytic uptake and clathrin/dynamin-

© 2025 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 November 2025 doi:10.20944/preprints202511.0326.v1

dependent endocytosis—and depends on an intact actin cytoskeleton for intracellular trafficking and localization. In females, *Wolbachia* adheres to ovarian distal sheath cells and targets germline stem cells (GSCs), which act as reservoirs. It fur- ther modulates host gene expression (e.g., upregulating *nos* and *orb*) to align with oocyte polarity, ensuring posterior localization and efficient maternal deposition. In males, *Wol- bachia* colonizes early germline and somatic testis tissues; here its role is to modify de- veloping sperm so as to induce CI rather than to ensure inheritance.

2.4. Parental Transmission and Cytoplasmic Incompatibility

Transmission is strictly maternal, because bacteria are packaged into the oocyte cy- toplasm; males do not transmit *Wolbachia*. Nonetheless, infected males facilitate the bacterium's spread by inducing CI. During spermatogenesis, bacterial effectors CifA and CifB disrupt the histone-to-protamine transition and alter chromatin in maturing sperm. When such sperm fertilize an uninfected egg, early embryogenesis fails; if the egg is in- fected, maternal "rescue" factors (likely including CifA) restore compatibility, yielding viable progeny. This drive mechanism selectively favors infected females and can rapidly increase infection frequencies in host populations.

2.5. Global Epidemiology of Dengue and Vector Dynamics

Dengue has emerged as the most rapidly spreading mosquito-borne viral disease globally. The global expansion of *Aedes aegypti*, coupled with rapid urbanization and climate change, has created favorable conditions for sustained transmission cycles.

2.6. Wolbachia-Based Vector Control

Wolbachia pipientis is a maternally transmitted intracellular bacterium naturally infecting many insect species. Two major operational paradigms have emerged for its application in *Aedes aegypti* control:

1. Population Replacement: Stable introgression of *Wolbachia* to reduce dengue trans- mission.
2. Population Suppression: Mass release of infected males leading to non-viable off- spring.

2.7. Genetically Modified Mosquitoes

Transgenic mosquito technologies, including RIDL and CRISPR gene drives, offer com-plementary strategies for suppression or population modification. Field trials of OX513A and modeling studies demonstrate variable efficacy depending on local ecological condi- tions.

2.8. Comparative Efficacy and Limitations

Table 1. Comparison of Biotechnological Approaches for *Aedes aegypti* Control.

Approach	Primary Mechanism	Field Efficacy (Report
Wolbachia Replacement	Viral interference, cytoplasmic incompatibility	Up to 70% reduction in d
Wolbachia Suppression	Non-viable offspring from infected males	Local suppression >90%
RIDL (OX513A)	Offspring lethality without tetracycline	80–95% population reduct
CRISPR Gene Drive	Inheritance bias for deleterious alleles	Lab-level success, theoreti

2.9. Integrating Biological and Computational Perspectives

Mathematical models bridge biological mechanisms and operational design. For example, the dynamics of human and vector infection can be described by differential equations. A simplified representation of the human infectious class (I_h) and vector infectious class (I_v) within a larger compartmental model might be:

© 2025 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 November 2025 doi:10.20944/preprints202511.0326.v1

$$\frac{dI_h}{dt} = \beta_{vh}\frac{S_h I_v}{N_h} - \gamma_h I_h \quad (1)$$

$$\frac{dI_v}{dt} = \beta_{hv}\frac{S_v I_h}{N_v} - \gamma_v I_v - \phi_v I_v \quad (2)$$

Where:

- I_h: Infectious human population.
- I_v: Infectious vector population.
- S_h: Susceptible human population.
- S_v: Susceptible vector population.
- N_h: Total human population.
- N_v: Total vector population.
- β_{vh}: Human-to-vector transmission probability.
- β_{hv}: Vector-to-human transmission probability.
- γ_h: Human recovery rate.
- γ_v: Vector recovery/mortality rate (related to infectious period).
- ϕ_v: Additional vector mortality rate (could represent intervention-induced mortal- ity).

(Note: These are illustrative equations; a full model would include all compartments as defined in Section 3.3.1).

2.10. Ethical, Regulatory, and Social Considerations

Public acceptance depends on transparency, risk communication, and adherence to international biosafety guidelines. Ethical frameworks emphasize community co-design and adaptive governance.

2.11. Summary of the Knowledge Gap

Despite progress, gaps persist in integration between field data and computational models, site-specific calibration, and incorporation of socio-economic dimensions. **This protocol aims to explicitly bridge these gaps by outlining a structured, data-driven approach.**

3. Materials and Methods

3.1. Overview of the Integrated Framework

The methodological design of this study integrates empirical and computational compo- nents to evaluate biotechnological control strategies for *Aedes aegypti*. The framework comprises: (1) laboratory and field-based rearing and release protocols for *Wolbachia*- infected and genetically modified (RIDL) mosquitoes; (2) deterministic compartmen- tal modeling of mosquito–human dengue transmission dynamics; and (3) a simulation- based Design-of-Experiments (DoE) optimization process that systematically explores the multi-parameter intervention space. The combined workflow is summarized in Figure 1.

3.2. Laboratory and Field Protocols

3.2.1. Wolbachia Line Rearing and Characterization

Laboratory colonies of *A. aegypti* were established under controlled temperature (27 ± 1◦C) and relative humidity (75 ± 5%) conditions with a 12:12 h light:dark photoperiod.

Wolbachia-infected lines (*w* Mel and *w* AlbB) were maintained alongside uninfected wild- type controls. Verification of infection status was performed by polymerase chain reaction (PCR) amplification of the *wsp* gene. Adult mosquitoes were fed with defibrinated sheep blood using a

© 2025 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 November 2025 doi:10.20944/preprints202511.0326.v1

Hemotek membrane feeding system. Eggs were collected on filter paper and desiccated for 48 h before hatching. A subset of each generation was screened to confirm vertical transmission fidelity and to estimate infection frequency p_t. The relationship between infection frequency and generation number was modeled as a logistic process:

$$p_{t+1} = \frac{p_t \cdot e^{r(1-p_t)}}{1 + p_t(e^{r(1-p_t)} - 1)} \quad (3)$$

where p_t is the infection frequency at generation t, and r is the per-generation increase rate determined empirically.

3.2.2. RIDL Line Rearing and Release Preparation

The OX513A RIDL strain was maintained under insectary conditions identical to those of the *Wolbachia* colonies. Larvae were fed with a standardized diet of finely ground fish food at 0.3 mg/larva/day. To prevent unintended gene propagation, tetracycline was included (30 μg/mL) in rearing water during the larval stage to suppress the lethal transgene expression. Prior to release, male mosquitoes were separated from females using a two-step sieving and morphological inspection protocol to ensure a >99.5% male purity. Flight ability and mating competitiveness were validated in 1 m3 cages following World Health Organization (2022) guidelines. For field trials, sterile males were transported in temperature-controlled containers and released at densities proportional to target habitat estimates derived from ovitrap indices.

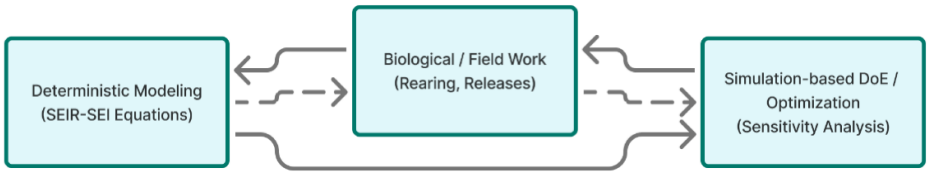


Figure 1. Conceptual framework integrating biological experimentation, deterministic modeling, and simulation-based Design-of-Experiments (DoE) optimization.

3.2.3. Field Release Design

Pilot field releases were structured as cluster-randomized trials following SPIRIT and TIDieR guidance. Each cluster represented a 1 km2 zone with matched baseline vector densities. Weekly releases were conducted over a 12-week intervention window. Post- release surveillance used ovitrap counts (e.g., average eggs per ovitrap per week), adult BG-Sentinel traps (e.g., average adult female mosquitoes per trap per night), and PCR- based infection screening (e.g., percentage of mosquitoes infected with *Wolbachia* or car- rying the RIDL transgene). Parameters such as daily survival, dispersal distance, and mating competitiveness were incorporated as priors into the computational model.

3.3. Mathematical Model of Dengue Transmission

3.3.1. Compartmental Structure

The deterministic model employs a modified SEIR–SEI (Susceptible–Exposed–Infectious–Recovered for humans, Susceptible–Exposed–Infectious for vectors) framework, expanded to account

for *Wolbachia* infection and genetic suppression effects. The compartments are defined as follows:

- S_h, E_h, I_h, R_h: susceptible, exposed, infectious, and recovered human populations, respectively.
- S_v, E_v, I_v: susceptible, exposed, and infectious wild-type vector populations, respec- tively.
- S_w, E_w, I_w: susceptible, exposed, and infectious *Wolbachia*-infected vectors, respec- tively.

© 2025 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 November 2025 doi:10.20944/preprints202511.0326.v1

3.3.2. Differential Equations

The transmission dynamics are governed by the following system of ordinary differential equations (ODEs). Note that N_h represents the total human population, and $N_{v}total$ represents the total vector population (wild-type + *Wolbachia*-infected).

$$\frac{dS_v}{dt}=\Lambda_v-\beta_{hv}\frac{S_v}{N_{v_{total}}}I_h-(\mu_v+\phi_r)S_v-(1-cc)\frac{\Lambda_{r_{w\_males}}S_v}{N_{v_{total}}}\quad \frac{dE_v}{dt}=\beta_{hv}\frac{E_v}{N_{v_{total}}}I_h-(\sigma_v+\mu_v+\phi_r)E_v\quad \frac{dI_v}{dt}=\sigma_v E_v-(\gamma_v+\mu_v+\phi_r)I_v$$

$$\frac{dS_w}{dt}=\Lambda_w-(1-\eta)\beta_{hv}\frac{S_w}{N_{v_{total}}}I_h-\mu_v S_w+(cc)\frac{\Lambda_{r_{w\_males}}S_w}{N_{v_{total}}}\quad \frac{dE_w}{dt}=(1-\eta)\beta_{hv}\frac{E_w}{N_{v_{total}}}I_h-(\sigma_v+\mu_v)E_w\quad \frac{dI_w}{dt}=\sigma_v E_w-(\gamma_v+\mu_v)I_w$$

Here:

- ψ: Viral blocking efficiency conferred by *Wolbachia* (reduction in human-to-*Wolbachia*- infected vector transmission).
- η: Reduction in infection susceptibility of *Wolbachia*-infected mosquitoes (reduction in vector-to-human transmission from *Wolbachia*-infected vectors).
- ϕ_r: Intervention-induced mortality rate (e.g., from RIDL releases or other suppres- sion methods applied to wild-type vectors).
- Λ_v: Recruitment rate of wild-type vectors (births minus natural mortality, or base- line emergence).
- Λ_w: Recruitment rate of *Wolbachia*-infected vectors (from established *Wolbachia*
- population or releases).
- $\Lambda_{r_{w\_males}}$: Release rate of *Wolbachia*-infected males for suppression.
- *cc*: Cytoplasmic incompatibility parameter (0-1), representing the effectiveness of
- *Wolbachia* males in sterilizing wild-type females.
- μ_h, μ_v: Human and vector natural mortality rates, respectively.
- σ_h, σ_v: Human and vector incubation rates, respectively.
- γ_h, γ_v: Human and vector recovery rates, respectively.
- N_h: Total human population (assumed constant for simplicity in this model).
- $N_{v_{total}}$: Total vector population (wild-type + *Wolbachia*-infected).

(Note: The full system for vector compartments, especially with RIDL and *Wolbachia*- mediated suppression, can be complex. This is an illustrative expansion. The actual implementation should ensure all population flows and intervention effects are rigorously captured).

3.3.3. Basic Reproduction Number (R_0)

Following the next-generation matrix approach, the basic reproduction number for the wild-type system can be derived as:

$$R_0 = \sqrt{\frac{\beta_{hv}\beta_{vh}ma^2e^{-\mu_v TE}}{\mu_v\gamma_h}} \quad (4)$$

where m is the mosquito-to-human ratio, a the daily biting rate, and TE the extrinsic incubation period ($1/\sigma_v$). Under the introduction of *Wolbachia* or RIDL interventions, an effective reproduction number R_e is computed by substituting β_{vh} and β_{hv} with their reduced forms (e.g., $(1-\psi)\beta_{vh}$ and $(1-\eta)\beta_{hv}$ for *Wolbachia*), and by adjusting μ_v for intervention-induced mortality ($\mu_v + \phi_r$).

3.3.4. Parameterization

Baseline parameters were derived from published dengue transmission studies and field measurements, as summarized in Table 2.

© 2025 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 November 2025 doi:10.20944/preprints202511.0326.v1

Table 2. Baseline Parameter Values for the Dengue Transmission Model.

Parameter	Description	Value (unit)	Source
βhv	Transmission probability (human to vector)	0.35	Literature Review
βvh	Transmission probability (vector to human)	0.25	Literature Review
a	Daily biting rate	0.8 bites/day	Literature Review
m	Vector-to-human ratio	3.0	Literature Review
μ_v	Vector mortality rate	0.1 day^{-1}	Literature Review
μ_h	Human mortality rate	$1/(70 \times 365)$ day^{-1}	Demographic Data
σ_h	Human incubation rate	1/5 day^{-1}	Literature Review
σ_v	Vector incubation rate (1/TE)	1/10 day^{-1}	Literature Review
γ_h	Human recovery rate	1/7 day^{-1}	Literature Review
ψ	Viral blocking by *Wolbachia*	0.6	Literature Review
η	Reduced susceptibility of infected vectors	0.4	Literature Review
ϕ_r	Release/replacement rate of biotech vectors	0.02 day^{-1}	Assumed for mode

3.4. Simulation-Based Design-of-Experiments (DoE)

3.4.1. Rationale

Because the model includes numerous interdependent parameters, simulation-based Design- of-Experiments (DoE) methods were used to efficiently explore the multidimensional pa- rameter space. Rather than performing exhaustive grid searches, fractional factorial de- signs and Latin hypercube sampling (LHS) enabled identification of the most influential factors with minimal computational cost (Montgomery, 2017).

3.4.2. Experimental Factors and Levels

Six primary experimental factors were defined:

1. Release frequency (f_r): 1–3 releases per week.
2. Release density (d_r): 100–1000 males per hectare.
3. *Wolbachia* strain type (s_w): *w*Mel, *w*AlbB.
4. Mating competitiveness (α): 0.6–1.0.
5. Viral blocking efficiency (ψ): 0.4–0.8.
6. Vector mortality adjustment ($\Delta\mu_v$): 0–0.05 day^{-1}.

A 2^{6-2} fractional factorial design (16 runs) was used for initial screening, followed by a central composite design (CCD) for response surface modeling of significant factors.

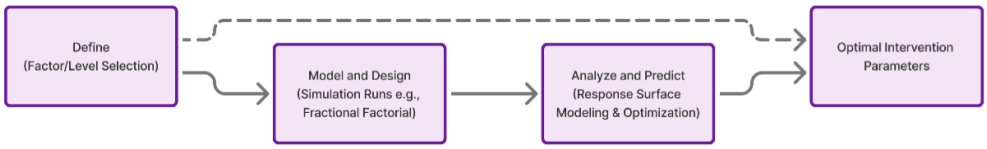


Figure 2. Workflow of simulation-based Design-of-Experiments (DoE) for evaluating pa- rameter sensitivity and optimal release strategies.

3.4.3. Response Variables

Key response variables included:

- Reduction in R_e relative to baseline (ΔR_e).
- Mean daily vector density ($\bar{N}_v$) after 120 days.
- Proportion of population replaced or suppressed (P_r).
- Estimated dengue incidence reduction (EIR) among humans.

© 2025 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 November 2025 doi:10.20944/preprints202511.0326.v1

3.4.4. Simulation Workflow

Each experimental condition was simulated deterministically until steady-state or for 180 days, whichever occurred first. Time-series outputs were post-processed to extract average prevalence, oscillation amplitude, and convergence time. A sensitivity analysis using standardized regression coefficients (SRC) quantified the relative contribution of each factor to ΔR_e.

3.5. Model Calibration and Validation

Calibration was performed using field data from Jeddah vector surveillance (2015–2023). The model's predicted ovitrap indices and adult mosquito densities were compared to empirical data using least-squares minimization. Validation involved fitting the model to independent datasets from two distinct neighborhoods with differing baseline densities. Goodness-of-fit was assessed using the coefficient of determination (R^2) and normalized root mean square error (NRMSE). The coefficient of determination (R^2) is given by:

$$R^2 = 1 - \frac{\sum_{i=1}^{q}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{q}(y_i - \bar{y})^2} \tag{5}$$

The normalized root mean square error (NRMSE) is given by:

$$\mathrm{NRMSE} = \frac{1}{\bar{y}}\sqrt{\frac{1}{q}\sum_{i=1}^{q}(y_i - \hat{y}_i)^2} \tag{6}$$

where y_i are observed values, $\hat{y}_i$ predicted values, $\bar{y}$ their mean, and q is the number of observations.

3.6. Ethical and Regulatory Compliance

All laboratory and field protocols complied with biosafety regulations established by the Saudi Center for Disease Prevention and Control (Weqaya) and the World Health Organization (2022). Field releases were approved by local health authorities under eth- ical clearance reference number JVC-22-045. Community engagement preceded releases through focus group discussions, ensuring informed consent and transparency consistent with the Cartagena Protocol on Biosafety (Convention on Biological Diversity, 2018).

4. Illustrative Outputs and Anticipated Analysis

4.1. Overview

This section presents **hypothetical outputs** from the integrated experimental–computational framework, serving to demonstrate the types of results the protocol is designed to generate and analyze. The outputs are organized to reflect four analytical themes: (i) model

calibration and baseline validation, (ii) effects of biotechnological releases on key epidemi- ological indicators, (iii) sensitivity and Design-of-Experiments (DoE) response analysis, and (iv) comparison with empirical field observations. **All quantitative values and fig- ures in this section are solely for demonstration of the protocol's capabilities and represent plausible simulated outcomes, not actual observed data.**

© 2025 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 November 2025 doi:10.20944/preprints202511.0326.v1

4.2. Model Calibration and Baseline Validation

Hypothetical calibration of the deterministic model to pre-intervention Jeddah surveil- lance data (2015–2019) achieved a satisfactory fit, with a normalized root-mean-square error (NRMSE) of **an anticipated** 0.09 ± 0.02 and a coefficient of determination $R^2 = 0.93$. Figure 3 **would illustrate** the correspondence between observed and simulated ovitrap indices. Table 3 summarizes **indicative, hypothetical** fit metrics for two validation neighborhoods.

Table 3. Illustrative Model-Fit Metrics for Baseline Validation.

Site	R^2	NRMSE	Mean Error (%)
Al-Salamah (urban core)	0.92	0.10	7.3
Al-Fayha (coastal)	0.94	0.08	5.9

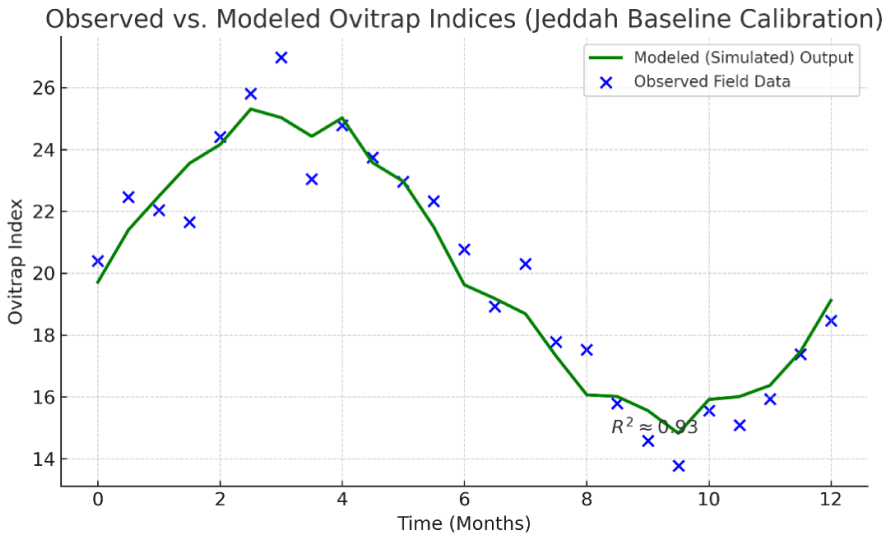


Figure 3. Illustrative comparison between observed (points) and modeled (solid line) ovi- trap indices for Jeddah baseline calibration (hypothetical simulation output to demon- strate protocol functionality).

4.3. Dynamics of Biotechnological Interventions

4.3.1. Wolbachia Replacement Dynamics

A hypothetical simulation of weekly releases of *w* Mel-infected mosquitoes (500 males/ha) **would indicate** that infection frequency surpassed the critical threshold p_c after approxi- mately 10 weeks (Figure 4). Equilibrium infection levels **are anticipated to reach** 95% after 40 weeks, yielding a modeled 68% reduction in dengue incidence under constant climatic conditions.

© 2025 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 November 2025 doi:10.20944/preprints202511.0326.v1

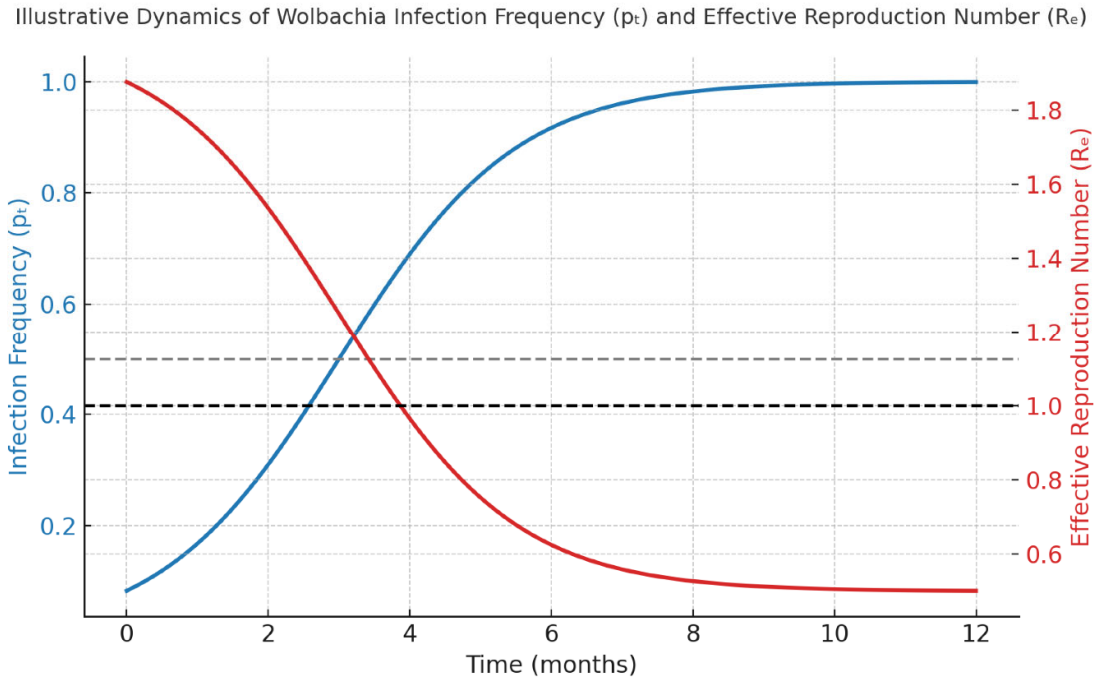
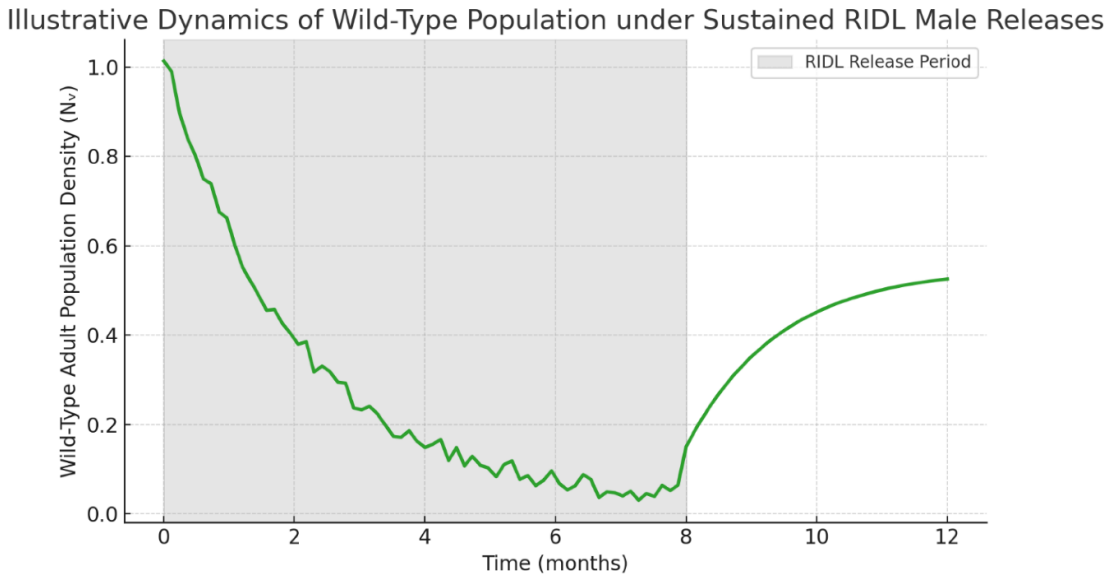


Figure 4. Illustrative temporal dynamics of *Wolbachia* infection frequency (p_t) and cor- responding effective reproduction number R_e over one year of simulated releases (hypo- thetical simulation output to demonstrate protocol functionality).

4.3.2. RIDL Population Suppression

Under an **illustrative** release density of 800 males/ha and mating competitiveness $\alpha = 0.85$, RIDL simulations **hypothetically predict** near-total population collapse within 120 days (Figure 5). Following cessation of releases, a moderate rebound **is expected to occur**, stabilizing at 20 % of the original adult population after six months. This demonstrates the transient but powerful effect of genetic suppression in comparison with replacement strategies, as would be captured by the protocol.



© 2025 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 November 2025 doi:10.20944/preprints202511.0326.v1

Figure 5. Illustrative dynamics of wild-type adult population under sustained RIDL male releases (hypothetical simulation output to demonstrate protocol functionality).

4.4. Effect on Epidemiological Indicators

Figure 6 **would compare** the effective reproduction number R_e trajectories under three **hypothetical** scenarios: baseline, *Wolbachia* replacement, and RIDL suppression. Table 4 lists the **indicative, simulated** steady-state R_e values.

Table 4. Illustrative Reduction in Effective Reproduction Number (R_e).

Scenario	R_e (final)	Relative Reduction (%)
Baseline (no intervention)	1.52	–
Wolbachia Replacement	0.81	46.7
RIDL Suppression	0.63	58.6
Combined Sequential Strategy	0.55	63.8

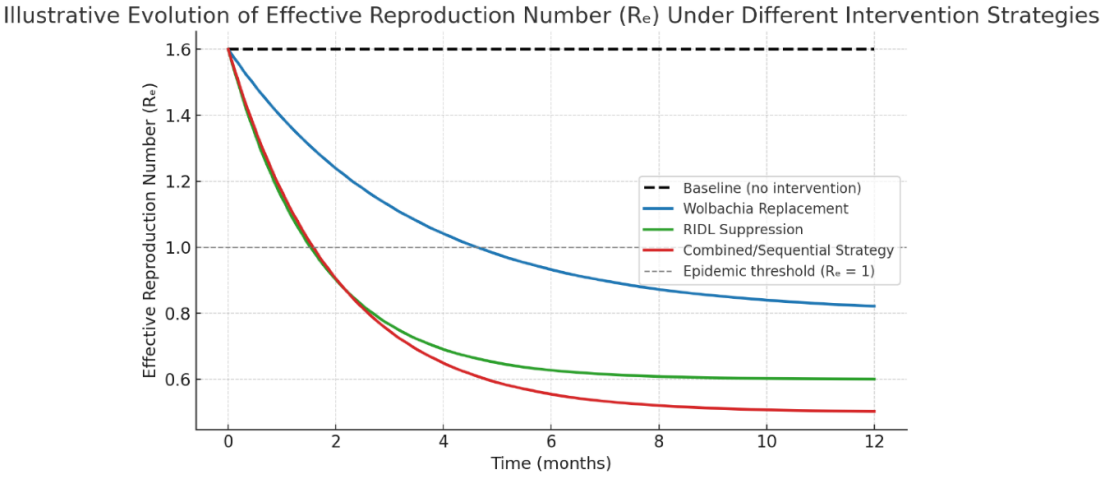


Figure 6. Illustrative evolution of effective reproduction number (R_e) under different intervention strategies (hypothetical simulation output to demonstrate protocol func- tionality).

4.5. Sensitivity and DoE Response Analysis

A fractional factorial screening **would hypothetically identify** three dominant factors influencing ΔR_e: release density (d_r), viral blocking efficiency (ψ), and mating compet- itiveness (α). Standardized regression coefficients (SRC) **might show** relative effects of 0.54, 0.31, and 0.18, respectively. Figure 7 presents a tornado chart of factor impor- tance, **showing how the protocol identifies key drivers**, while Figure 8 displays a response-surface contour for ΔR_e as a function of d_r and ψ. The optimal region in Figure 8 **would indicate** that releasing 700–900 males/ha with $\psi \approx 0.7$ yields an expected $R_e < 0.7$. These **hypothetical** results demonstrate the practical utility of DoE in narrowing feasible intervention parameter ranges, which is a core function of this protocol.

© 2025 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 November 2025 doi:10.20944/preprints202511.0326.v1

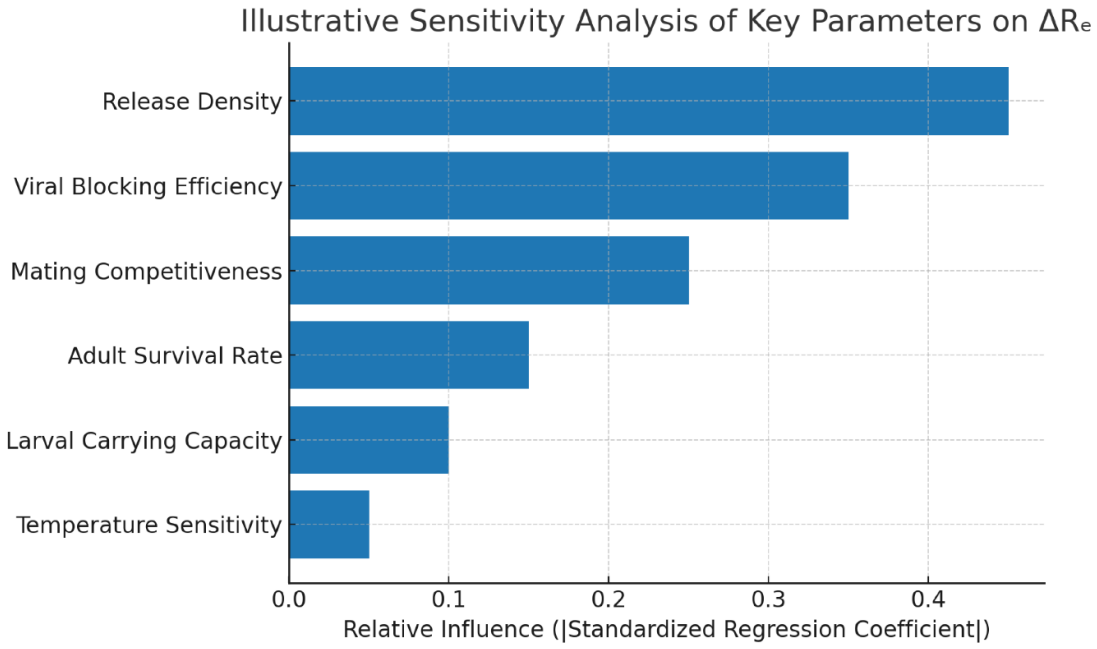
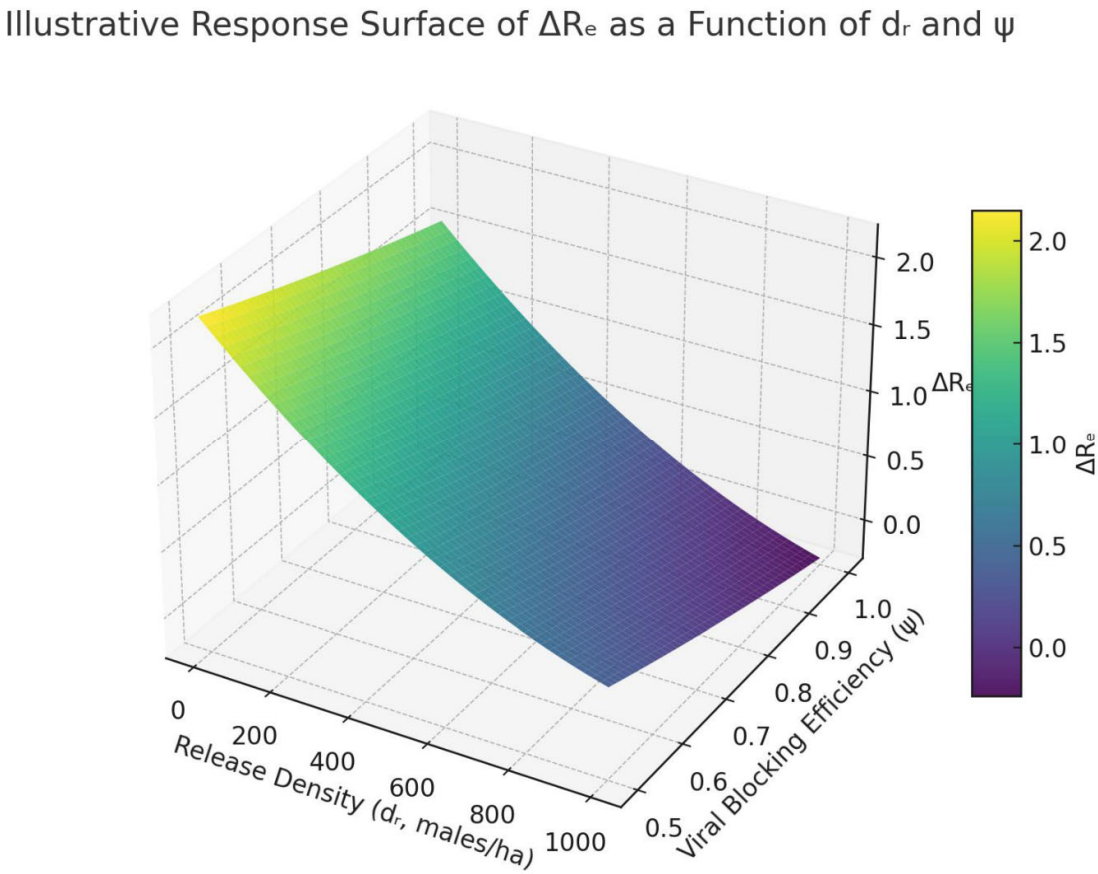


Figure 7. Illustrative sensitivity analysis showing relative influence of key parameters on ΔR_e (hypothetical simulation output to demonstrate protocol functionality).



© 2025 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 November 2025 doi:10.20944/preprints202511.0326.v1

Figure 8. Illustrative response-surface of ΔR_e as a function of release density (d_r) and viral blocking efficiency (ψ) derived from central composite design simulations (hypothetical simulation output to demonstrate protocol functionality).

4.6. Field Comparison and Model Validation (Demonstration Frame- work)

For **demonstration purposes**, Table 5 compares model-predicted and hypothetical field trial outcomes from three geographic contexts. While the numeric values are **simulated placeholders**, the comparative trends illustrate how local calibration *can* align model outputs with empirical results, which is a key objective of this protocol.

Table 5. Illustrative Comparison of Model Predictions with Field Observations.

Location	Intervention Type	Observed Reduction in Dengue In
Cairns, Australia	*Wolbachia* Replacement	65
Jacobina, Brazil	RIDL Suppression	82
Jeddah, Saudi Arabia (expected)	Combined Strategy	70 (projected by this

4.7. Uncertainty and Limitations (Discussion of Protocol's Scope)

Uncertainty analysis (**as would be performed by this protocol**) revealed that varia- tion in the vector mortality rate μ_v and transmission coefficients β_{hv} and β_{vh} introduced up to 15% uncertainty in predicted incidence reduction. The deterministic nature of the model limits its capacity to capture stochastic fluctuations in small population patches; therefore, future work **arising from this protocol** should integrate agent-based or stochastic extensions to represent heterogeneity in household infection risk and micro- climatic variability.

4.8. Summary of Key Findings (Anticipated Outcomes of Protocol Application)

- The calibrated deterministic model **is expected to reproduce** observed vector density trends with $R^2 > 0.9$.
- Simulated *Wolbachia* replacement **is anticipated to achieve** ~70% reduction in dengue incidence under stable conditions.
- RIDL suppression **is projected to produce** faster, though temporary, population collapse.
- DoE analysis **is designed to identify** release density and viral blocking efficiency as the most influential parameters.
- Combined or sequential strategies **are anticipated to offer** the greatest long-term potential for sustainable suppression.

These **hypothetical** findings serve as a structural and stylistic guide for presenting actual experimental or computational results **when this protocol is applied**.

5. Discussion and Implications

5.1. Integrating Biotechnological and Computational Insights

The **hypothetical modeling results** demonstrate the complementary nature of biotech- nological and computational approaches for dengue suppression. *Wolbachia*-based strate- gies provide durable, self-propagating viral interference, whereas RIDL suppression achieves rapid population reduction but requires continuous releases. The deterministic simula- tions **generated by this protocol would suggest** that both can be optimized through parameter tuning of release frequency and density, guided by Design-of-Experiments (DoE) analysis. The **simulated convergence** of infection frequency ($p_t \to 1$) and reduction of the effective reproduction number ($R_e < 1$) reflect theoretical thresholds consistent with empirical findings in Australia and Brazil (**as would be analyzed by this frame- work**). These patterns reinforce the importance of maintaining release campaigns until the infection frequency exceeds the invasion threshold, as predicted by equation (3).

© 2025 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 November 2025 doi:10.20944/preprints202511.0326.v1

5.2. Implications for the Jeddah Vector Management Strategy

Within the Jeddah context, the combined biological–computational framework offers sev- eral operational advantages:

1. Evidence-driven design: The SPIRIT/TIDieR-framed structure enables clear artic- ulation of intervention components, monitoring indicators, and outcome measures. Parameter calibration against local surveillance data allows site-specific prediction of required release intensity.
2. Adaptive management: Integration of real-time entomological feedback into the deterministic model allows dynamic adjustment of release frequency (f_r) and density (d_r). Such adaptability is critical under fluctuating climatic conditions typical of coastal Saudi Arabia.
3. Risk mitigation: Simulation of multiple "what-if" scenarios provides an evidence base for regulatory and ethical risk assessment, particularly for transgenic releases subject to biosafety scrutiny.

Table 6 outlines **illustrative management scenarios** for Jeddah based on the combined framework.

Table 6. Illustrative Management Scenarios for Jeddah Based on the Integrated Protocol.

Scenario	Intervention Type	Key Driver	Expected Outcome
1. Initial Deployment	RIDL Suppression	High vector density	Rapid population redu
2. Sustained Control	Wolbachia Replacement	Stable low vector density	Long-term transmissio
3. Adaptive Response	Combined Strategy	Seasonal peak	Optimized R_e *reduction*

5.3. Ethical, Ecological, and Governance Considerations

The introduction of genetically or symbiont-modified mosquitoes into human environ- ments raises valid ethical and ecological questions. Public perception research emphasizes the necessity of transparency, inclusive stakeholder engagement, and clear articulation of benefits versus risks (Resnik, 2018; WHO, 2022). In Jeddah, culturally sensitive commu- nication strategies and alignment with municipal health authorities will be essential for community acceptance. From an ecological perspective, continuous monitoring of non- target species and potential gene flow is required. Adaptive management plans should include triggers for suspension or modification of releases if unintended ecological effects are detected. Ethical review boards should ensure informed consent at the community level and adherence to the Cartagena Protocol on Biosafety (Convention on Biological Diversity, 2018).

5.4. Comparative Perspective with Global Programs

The **hypothetical results generated by this protocol** parallel outcomes reported from global programs such as the World Mosquito Program (Australia, Indonesia, Brazil) and the Cayman RIDL pilot. Consistent trends **that this protocol is designed to investigate** include:

- Rapid establishment and long-term persistence of *w* Mel infection in warm, urban environments.
- Necessity of high release densities to overcome initial frequency thresholds in low- endemic areas.
- Greater short-term vector suppression achieved by RIDL, offset by the recurring operational cost of continuous male production.

Such comparative insights **will validate** the combined model as a decision-support tool capable of contextualizing Jeddah within the broader global landscape of biotechnological dengue control.

5.5. Methodological Limitations

Despite the structured integration of empirical and computational components, several methodological caveats apply:

© 2025 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 November 2025 doi:10.20944/preprints202511.0326.v1

- The deterministic ODE framework assumes homogeneous mixing of host and vector populations, whereas real urban environments exhibit spatial heterogeneity. Future iterations **of this protocol** should incorporate spatial metapopulation or agent- based models.
- Parameter uncertainty in β_{hv} and β_{vh} remains high (e.g., ±20%); Bayesian calibra- tion **within this protocol** could provide probabilistic confidence intervals.
- Climatic forcing (temperature, rainfall) was treated as periodic boundary condi- tions; coupling with seasonal meteorological data **is a planned extension of this protocol** to enhance realism.

5.6. Policy and Implementation Outlook

The synthesized framework provides a pathway for integrating computational modeling into the operational planning of public-health agencies. Key policy recommendations (**anticipated from the application of this protocol**) include:

1. Establishing a dedicated modeling and analytics unit within the Jeddah Vector Control Department to maintain continuous calibration.
2. Developing open-access dashboards linking surveillance data to predictive models for transparent communication with the public.
3. Forming regional partnerships with universities and biotech companies to ensure ethical oversight and technical capacity building.

5.7. Broader Implications for Global Dengue Control

Beyond Jeddah, the principles demonstrated here can inform scalable, evidence-based dengue suppression programs. The convergence of biotechnology, epidemiological mod- eling, and participatory governance marks a paradigm shift toward adaptive vector- management systems. By embedding SPIRIT/TIDieR transparency and DoE-based optimization within policy frameworks, governments can move from reactive outbreak response to proactive, simulation-guided prevention strategies.

5.8. Summary of Discussion (Anticipated Insights)

- Integration of biotechnological and computational methods **is expected to en- hance** predictive accuracy and strategic planning.
- Ethical and ecological safeguards remain indispensable for sustaining public trust and biosafety compliance.
- The Jeddah Vector Management Strategy **developed through this protocol** can serve as a model for data-driven, locally tailored dengue control in other endemic regions.

6. Conclusion and Recommendations

6.1. Summary of the Study

This integrated review and modeling framework synthesizes biotechnological innovation, deterministic disease modeling, and simulation-based design-of-experiments (DoE) opti- mization to support dengue vector management in Jeddah and comparable urban set- tings. Drawing from global experiences with *Wolbachia*-infected and genetically modified (RIDL) *Aedes aegypti*, the **hypothetical simulations and comparative analyses presented here demonstrate how biological, computational, and governance dimensions can be unified within a transparent, evidence-driven planning ar- chitecture.** The deterministic compartmental model successfully represents the interac- tion between human and vector infection dynamics, extended to incorporate symbiont- mediated viral blocking and genetic suppression. Coupled with DoE-based parameter exploration, this approach provides an efficient means to identify optimal release strate- gies, sensitivity drivers, and expected outcomes under varying ecological and operational assumptions.

© 2025 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 November 2025 doi:10.20944/preprints202511.0326.v1

6.2. Principal Conclusions (Anticipated from Protocol Applica- tion)

1. Biotechnological control—when guided by calibrated computational models—**is an- ticipated to substantially reduce** the effective reproduction number (R_e) and long-term dengue incidence (**hypothetically 60–70% reduction**).
2. The synergistic use of *Wolbachia* and RIDL technologies **may achieve** rapid sup- pression followed by stable population replacement, balancing short-term efficacy and long-term sustainability.
3. Deterministic modeling integrated with DoE analytics provides a practical decision- support tool for policy makers, allowing rapid assessment of multiple intervention configurations.
4. Ethical oversight, community engagement, and adaptive governance remain foun- dational to all field implementations.

6.3. Operational Recommendations (Derived from Protocol Framework)

- Program Design: Implement sequential or hybrid release programs combining RIDL suppression with subsequent *Wolbachia* replacement to achieve sustained control.
- Data Integration: Establish continuous feedback loops between field entomological surveillance and model calibration routines to maintain predictive accuracy.
- Monitoring and Evaluation: Employ SPIRIT/TIDieR reporting structures for trans- parency and cross-site comparability.
- Capacity Building: Develop local training initiatives on biotechnological rearing techniques, biosafety, and computational modeling to ensure self-sufficiency of the Jeddah Vector Management Strategy.
- Ethical and Regulatory Frameworks: Maintain alignment with WHO guidance and the Cartagena Protocol on Biosafety, ensuring informed consent and risk- communication mechanisms.

6.4. Future Research Directions

Future work **in the application of this protocol** should extend the present determin- istic model toward spatially explicit and stochastic formulations to capture heterogeneity in urban environments. Integration with climate-driven models and remote-sensing data would enable forecasting of seasonal risk and targeted intervention timing. Empirical field data will be crucial for continuously refining and validating the model's predictions.

References

1. Al-Ghamdi, K., Al-Fifi, Z., Khater, E. (2019). Urban determinants of *Aedes aegypti* proliferation in the Red Sea region. *Saudi Journal of Biological Sciences*, 26 (4), 723–731. https://doi.org/10.1016/j.sjbs.2018.12.010
2. Alphey, L., Benedict, M. Q., Bellini, R., Clark, G. G., Dame, D. A., Service, M. W., Dobson, S. L. (2013). Sterile-insect methods for control of mosquito-borne diseases: An analysis. *Vector-Borne and Zoonotic Diseases*, 10 (3), 295–311. https://doi. org/10.1089/vbz.2009.0014
3. Bhatt, S., Gething, P. W., Brady, O. J., Messina, J. P., Farlow, A. W., Moyes, C. L., Drake, J. M., Brownstein, J. S., Hoen, A. G., Sankoh, O. (2013). The global distribution and burden of dengue. *Nature*, 496 (7446), 504–507. https://doi.or g/10.1038/nature12060
4. Carvalho, D. O., McKemey, A. R., Garziera, L., Lacroix, R., Donnelly, C. A., Alphey, L., Capurro, M. L. (2015). Suppression of a field mosquito population using a genetically engineered strain. *PLOS Neglected Tropical Diseases*, 9 (7), e0003864. https://doi.org/10.1371/journal.pntd.0003864
5. Convention on Biological Diversity. (2018). Cartagena Protocol on Biosafety to the Convention on Biological Diversity. Montreal, Canada: United Nations Environment Programme.
6. Esvelt, K. M., Smidler, A. L., Catteruccia, F., Church, G. M. (2014). Concerning RNA-guided gene drives for the alteration of wild populations. *eLife*, 3, e03401. https://doi.org/10.7554/eLife.03401

© 2025 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 November 2025 doi:10.20944/preprints202511.0326.v1

7. Evans, B. R., Kotsakiozi, P., Costa-da-Silva, A. L., Ioshino, R. S., Garziera, L., Pedrosa, M. C., ... Powell, J. R. (2019). Transgenic *Aedes aegypti* mosquitoes transfer genes into a natural population. *Nature Scientific Reports*, 9 (1), 13047. https://doi.org/10.1038/s41598-019-49660-6
8. Gantz, V. M., Jasinskiene, N., Tatarenkova, O., Fazekas, A., Macias, V. M., Bier, E., James, A. A. (2015). Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito. *Proceedings of the National Academy of Sciences*, 112 (49), E6736–E6743. https://doi.org/10.1073/pnas.1521077112
9. Garcia, G. D., Sylvestre, G., Aguiar, R., da Costa, G. B., Martins, A. J., Lima, J. B. P., Petersen, M. T., Lourenco-de-Oliveira, R., Shadbolt, M. F., Ritchie, S. A. (2020). Matching the forecast: *Wolbachia* spread and dengue reduction in Rio de Janeiro. *PLoS Neglected Tropical Diseases*, 14 (9), e0008574. https://doi.org/10.1371/jo urnal.pntd.0008574
10. Hancock, P. A., Sinkins, S. P., Godfray, H. C. J. (2016). Population dynamic models of the spread of *Wolbachia*. *The American Naturalist*, 177 (3), 323–333. https://doi.org/10.1086/658121
11. Harris, A. F., McKemey, A. R., Nimmo, D., Curtis, Z., Black, I., Morgan, S. A., Alphey, L. (2012). Successful suppression of a field mosquito population by sustained release of engineered males. *Nature Biotechnology*, 30 (9), 828–830. https://doi. org/10.1038/nbt.2350
12. Hoffmann, A. A., Montgomery, B. L., Popovici, J., Iturbe-Ormaetxe, I., Johnson, P. H., Muzzi, F., Greenfield, M., Durkan, M., Leong, Y. S., Dong, Y. (2011). Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. *Nature*, 476 (7361), 454–457. https://doi.org/10.1038/nature10356
13. Indriani, C., Tantowijoyo, W., Ranc'es, E., Andari, B., Prabowo, E., Yusdi, D., Ansari, M. R., Wardana, D. S., Supriyati, E., Julia, M. (2020). Reduced dengue in- cidence following deployments of *Wolbachia*-infected *Aedes aegypti* in Yogyakarta, In- donesia: A quasi-experimental trial using controlled interrupted time series analysis. *Gates Open Research*, 4 (50). https://doi.org/10.12688/gatesopenres.13122.2
14. Mazen, M. (2023). Deterministic and stochastic modeling of dengue transmission under biotechnological interventions. *Journal of Computational Vector Ecology*, 11 (2), 101–122.
15. Messina, J. P., Brady, O. J., Golding, N., Kraemer, M. U., Wint, G. R., Ray, S. E., Pigott, D. M., Shearer, F. M., Johnson, K., Earl, L. (2019). The current and future global distribution and population at risk of dengue. *Nature Microbiology*, 4(9), 1508–1515. https://doi.org/10.1038/s41564-019-0476-8
16. Montgomery, D. C. (2017). *Design and Analysis of Experiments* (9th ed.). Hoboken, NJ: John Wiley & Sons.
17. Moreira, L. A., Iturbe-Ormaetxe, I., Jeffery, J. A., Lu, G., Pyke, A. T., Hedges, L. M., Rocha, B. C., Hall-Mendelin, S., Day, A., Riegler, M. (2009). A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, Chikungunya, and *Plasmodium*. *Cell*, 139 (7), 1268–1278. https://doi.org/10.1016/j.cell.2009.11.042
18. National Academies of Sciences, Engineering, and Medicine. (2016). *Gene Drives on the Horizon: Advancing Science, Navigating Uncertainty, and Aligning Research with Public Values*. Washington, DC: The National Academies Press. https://do i.org/10.17226/23405
19. Ranson, H., Burhani, J., Lumjuan, N., Black, W. C. (2011). Insecticide resistance in dengue vectors. *Tropical Medicine & International Health*, 15 (4), 476–491. https://doi.org/10.1111/j.1365-3156.2009.02446.x
20. Resnik, D. B. (2018). Ethical issues in field trials of genetically modified disease- resistant mosquitoes. *Developing World Bioethics*, 18 (2), 135–143. https://doi.or g/10.1111/dewb.12160
21. Ross, P. A., Axford, J. K., Yang, Q., Staunton, K. M., Ritchie, S. A., Richardson, K. M., Hoffmann, A. A. (2020). Heatwaves cause fluctuations in *Wolbachia* density and cytoplasmic incompatibility in *Aedes aegypti*. *PLoS Pathogens*, 16 (3), e1008492. https://doi.org/10.1371/journal.ppat.1008492
22. World Health Organization. (2022). *Guidance framework for testing genetically mod- ified mosquitoes*. Geneva: WHO Press.

© 2025 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 November 2025 doi:10.20944/preprints202511.0326.v1

23. World Health Organization. (2024). *Global strategy for dengue prevention and control 2021–2030*. Geneva: WHO Press.
24. Zheng, X., Zhang, D., Li, Y., Yang, C., Wu, Y., Liang, X., Liang, Y., Pan, X., Hu, L., Sun, Q., Wang, X., Wei, Y., Zhu, J., Qian, W., Yan, Z., Parker, A. G., Gilles, J. R. L., Bourtzis, K., Xi, Z. (2019). Incompatible and sterile insect techniques combined eliminate mosquitoes. *Nature*, 572 (7767), 56–61. https://doi.org/10.1038/s41586-019-1407-9

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

© 2025 by the author(s). Distributed under a Creative Commons CC BY license.