Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Persistent Wolbachia-mediated dengue virus blocking in Aedes aegypti five years after deployment in New Caledonia

Benjamin Dupuis

benjamin.dupuis@pasteur.fr

Institut Pasteur, Université Paris Cité, Arboviruses and Insect Vectors Unit

Anna-Bella Failloux

Institut Pasteur, Université Paris Cité, Arboviruses and Insect Vectors Unit

Nicolas Pocquet

Institut Pasteur de Nouvelle Calédonie, Medical Entomology Unit,

Short Report

Keywords: Wolbachia, Aedes aegypti, dengue virus, vector competence, pathogen blocking, vector control

Posted Date: November 23rd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11511197/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Background

Wolbachia infection reduces dengue virus (DENV) transmission by *Aedes aegypti*, but the temporal dynamics underlying this effect remain incompletely characterized in field-relevant mosquito populations. Here, we compare DENV vector competence and viral replication kinetics between *Wolbachia*-infected *Ae. aegypti* from Noumea and a *Wolbachia*-free population from Ouvea, New Caledonia.

Methods

Female mosquitoes were orally infected with DENV-2 and sampled at 3, 7, 14, 21, 28, and 35 days post-infection (dpi). Infection, dissemination, and transmission were assessed, and viral titers were quantified in the abdomen, head-thorax, and saliva. Vector competence endpoints were analyzed using logistic regression, while viral titers were analyzed using linear models. The extrinsic incubation period (EIP) was estimated from transmission kinetics.

Results

Across all time points, DENV infection, dissemination, and transmission were markedly reduced in *Wolbachia*-infected mosquitoes from Noumea compared with *Wolbachia*-free population from Ouvea, with infection detected in 10.1% versus 86.7% of mosquitoes, dissemination in 3.4% versus 75.8%, and transmission in 1.7% versus 31.5%, respectively. After adjustment for dpi, the odds ratio of infection, dissemination, and transmission were strongly reduced in Noumea. The population effect on infection varied significantly over time, whereas no population × dpi interaction was detected for dissemination or transmission. The estimated EIP_{25} and EIP_{50} were delayed in Noumea compared with Ouvea, reaching 23.9 and 24.5 days, respectively, versus 14.4 and 17.6 days in Ouvea. Viral titers were also lower in Noumea, with significant population × dpi interactions in the abdomen and head-thorax. No significant interaction was detected in saliva, although comparisons were limited by the very low number of virus-positive Noumea mosquitoes at several time points.

Conclusions

Wolbachia infection strongly reduced DENV vector competence in the Noumea population five years after field release, with reduced vector competence and reduced viral titers. These findings demonstrate sustained functional pathogen blocking even after long period in field conditions and support the continued effectiveness of *Wolbachia*-based dengue control.

Introduction

Dengue is one of the most widespread mosquito-borne viral diseases worldwide, with *Aedes aegypti* as its main urban vector [1, 2]. Conventional dengue control relies largely on reducing mosquito populations,

notably through insecticide-based interventions [3, 4]. However the widespread emergence of insecticide resistance can compromise their effectiveness [5, 6]. Alternative vector control strategies have therefore gained increasing attention, including the deployment of *Wolbachia*-infected *Ae. aegypti* [7, 8]. *Wolbachia*, a maternally transmitted bacterium can reduce mosquito vector competence for dengue virus (DENV), thereby reducing viral transmission and providing a population-based approach to the control of dengue and, more broadly, other arboviruses [9, 10].

Field deployment of *Wolbachia* has demonstrated its capacity to establish and persist in natural *Ae. aegypti* populations, accompanied by reduction in dengue incidence in several settings [11, 12]. Notably, in Australia, *Wolbachia*-mediated dengue suppression has remained effective approximately 10 years after the initial releases, supporting the long-term durability of this approach [13]. Whether this long-term effectiveness is maintained across different mosquito populations and environmental contexts remains an important question. Following establishment, mosquito genetic background, microbial communities, environmental conditions, and virus-host interactions may influence the effectiveness of *Wolbachia*-based strategies [14–16]. Assessing pathogen blocking directly, rather than relying solely on *Wolbachia* prevalence or persistence, is therefore important to determine whether the functional efficacy of the strategy is maintained over time.

In New Caledonia, where increasing insecticide resistance has challenged conventional vector control [17], *Wolbachia*-infected *Ae. aegypti* were first released in Noumea in 2019 as part of a population-replacement strategy [18]. Initially, the efficacy of the *Wolbachia*-based pathogen blocking had been demonstrated in New Caledonia prior to field release. Five years after the initiation of deployment, *Wolbachia* remains established in the local mosquito population [19], providing an opportunity to assess the persistence of its functional effect on DENV transmission. This setting therefore provides an opportunity to compare the functional phenotype demonstrated before deployment with that observed several years after *Wolbachia* establishment in the field. Here, we compare the vector competence of *Wolbachia*-infected *Ae. aegypti* from Noumea with a *Wolbachia*-free population from Ouvea, New Caledonia. By monitoring DENV infection, dissemination, and transmission at multiple time points following an infectious blood meal, we assessed whether *Wolbachia*-mediated pathogen blocking remains effective five years after the beginning of the field deployment.

Materials and Methods

Mosquito lines

Two *Ae. aegypti* populations were collected at the larval stage in New Caledonia in 2024: a *w*Mel-*Wolbachia*-infected population from Noumea (W+), where *w*Mel had been deployed by the World Mosquito Program, and an uninfected population from Ouvea (W−), a site without *Wolbachia* releases. Details of larval collection and population characteristics are provided in the related manuscript currently under consideration. The mosquitoes tested were from the F4 generation for the Noumea population and the F2 generation for the Ouvea population.

Larvae were reared at 26.5°C and fed *ad libitum* with brewer's yeast supplemented with selenium and vitamins E and B9 (Gayelord Hauser, Ecotone, France). Adults were maintained at 28°C and approximately 75% relative humidity under a 12:12 h light:dark cycle and provided *ad libitum* access to 10% sucrose solution.

Virus

Dengue virus serotype 2 (DENV-2), genotype V (Asian I), strain Bangkok, isolated in 1974 from a human serum sample in Bangkok, Thailand (GenBank accession number MK268692), was used in this study [20]. This strain was selected for its high replicative capacity, providing a robust challenge to assess the ability of *Wolbachia*-infected mosquitoes to restrict DENV-2 infection and dissemination.

Vector competence study and virus titration

All experimental infections were performed under BSL-3 conditions. Groups of 65 5–7-day-old females were offered a DENV-2-infected blood meal at a final concentration of 10^7 FFU/mL using a Hemotek® system (Hemotek Ltd, Blackburn, UK). Engorged females were maintained at 28 ± 1°C and 75% relative humidity under a 12:12 h light:dark cycle and provided 10% sucrose ad libitum. At 3, 7, 14, 21, 28, and 35 days post-infection (dpi), 32 females per time point and lines were sampled. Abdomen and head/thorax were homogenized separately in 300 µL Leibovitz L-15 medium supplemented with 2% fetal bovine serum (FBS) to assess infection and viral dissemination, respectively. Saliva was collected in tips containing 5 µL FBS and transferred to 45 µL Leibovitz L-15 medium to assess the presence of infectious viruses.

Infectious DENV particles were quantified by focus-forming assay (FFA) on *Aedes albopictus* C6/36 cells [21]. Briefly, serial tenfold dilutions of sample were inoculated onto confluent C6/36 monolayers for 1 h at 28°C. Cells were then overlaid with L-15 medium containing 8% FBS, 1% antibiotic-antimycotic, and 4% carboxymethylcellulose and incubated for 5 days at 28°C. Cells were subsequently fixed, permeabilized, and immunostained using a DENV-specific mouse monoclonal antibody (clone D3-2H2-9-21; Merck, Germany) followed by an Alexa Fluor 488-conjugated goat anti-mouse IgG secondary antibody (Invitrogen). Viral foci were visualized by fluorescence microscopy (EVOS FL; Life Technologies), and infectious virus titers were expressed as focus-forming units (FFU) per sample.

Statistical analysis

Statistical analyses were performed in R [22]. DENV infection, dissemination, and transmission were analyzed as binary outcomes using logistic regression models. For each endpoint, models included population (*Wolbachia*-free Ouvea versus *Wolbachia*-infected Noumea), dpi (3, 7, 14, 21, 28, and 35 days), and their interaction. Population effects were assessed after adjustment for dpi, and the population × dpi interaction was used to determine whether differences between populations varied over the course of

infection. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated from the regression models. Firth's penalized logistic regression was used for analyses affected by complete or quasi-complete separation [23]. Extrinsic incubation period (EIP) was estimated from the transmission kinetics using three-parameter log-logistic dose–response models fitted separately for each population, with the number of transmitting mosquitoes relative to the total number tested at each dpi as the response and the corresponding sample size as weights. EIP_{25} and EIP_{50} were defined as the time required to reach 25% and 50% predicted transmission, respectively. Estimates and 95% CIs were obtained using the delta method. EIP_{50} values between populations were estimated using parameter tests implemented in the *drc* package [24].

For quantitative viral titers, only virus-positive mosquitoes were included in the analyses. Viral titers were log10-transformed prior to analysis. Linear models included population, dpi as a categorical variable, and their interaction. The population × dpi interaction was tested by comparing models with the interaction term. Population differences at each dpi were assessed using estimated marginal means, with Holm adjustment for multiple comparisons [25]. Viral titers were analyzed separately for the abdomen, head-thorax, and saliva. All tests were two-sided, and statistical significance was defined as $p < 0.05$.

Results

Reduced DENV vector competence and delayed transmission in the Wolbachia-infected population

Across all sampling time points (3, 7, 14, 21, 28, and 35 dpi), all three components of DENV vector competence were markedly lower in the *Wolbachia*-infected Noumea population than in the *Wolbachia*-free Ouvea population (Fig. 1). Overall, DENV infection was detected in 86.7% (143/165) of Ouvea mosquitoes compared with 10.1% (18/179) of Noumea mosquitoes. Dissemination occurred in 75.8% (125/165) of Ouvea mosquitoes compared with 3.4% (6/179) of Noumea mosquitoes, while transmission was detected in 31.5% (52/165) and 1.7% (3/179), respectively. After adjustment for dpi, the odds of infection were significantly lower in Nouméa than in Ouvea (OR = 0.015, 95% CI: 0.007–0.030, $p < 0.001$). Similarly, dissemination and transmission were strongly reduced in Noumea (Firth logistic regression; OR = 0.0055, 95% CI: 0.0018–0.0141, and OR = 0.028, 95% CI: 0.0074–0.0783, respectively; both $p < 0.001$). We then assessed whether the magnitude of these population differences varied over time. A significant population × dpi interaction was detected for infection (likelihood-ratio $\chi^2_5 = 14.75$, $p = 0.011$), whereas no significant interaction was observed for dissemination (likelihood-ratio $\chi^2_5 = 10.89$, $p = 0.054$) or transmission (likelihood-ratio $\chi^2_5 = 4.05$, $p = 0.543$).

A. Infection efficiency of *Ae. aegypti* from Ouvea and Noumea populations. **B**. Dissemination efficiency of *Ae. aegypti* from Ouvea and Noumea populations. **C**. Transmission efficiency of *Ae. aegypti* from Ouvea and Noumea populations. Infection was assessed in the abdomen, dissemination in the head-thorax, and transmission in saliva at 3, 7, 14, 21, 28, and 35 days post-infection. Blue represents Ouvea mosquitoes naturally free of *Wolbachia*, whereas green represents *Wolbachia*-infected mosquitoes from Noumea. Points indicate the observed proportion of positive mosquitoes at each time point, lines represent the

three-parameter logistic models fitted to this data, and shaded areas represent exact 95% Wilson binomial confidence intervals. Between 25 and 32 mosquitoes were analysed per population and time point, except for Ouvea at 3 days post-infection (n = 19).

The estimated EIP_{25} was 14.4 days (95% CI: 11.6–17.2) in Ouvea and 23.9 days (95% CI: 0.9–47.0) in Noumea. Similarly, the estimated EIP_{50} was 17.6 days (95% CI: 13.3–21.9) in Ouvea and 24.5 days (95% CI: 1.0–48.0) in Noumea. The higher EIP estimates in Noumea were consistent with a delayed transmission dynamic, although the wide 95% CI for Noumea reflect the low proportion of mosquitoes capable of transmitting DENV in this population.

Reduced DENV viral titers in the Wolbachia-infected population

Viral titers were compared between Ouvea and Noumea across the kinetics (Table 1). In the abdomen, titers were significantly lower in Noumea, with a significant population × dpi interaction ($F_{(5,149)} = 15.02$, $p < 0.001$) and lower titers in Noumea from 7 dpi onwards. A significant population × dpi interaction was also observed in the head-thorax ($F_{(4,120)} = 4.46$, $p = 0.002$), whereas no significant interaction was detected in saliva ($F_{(1,49)} = 1.27$, $p = 0.265$). However, quantitative comparisons should be interpreted cautiously given the low number of virus-positive mosquitoes in Noumea at several time points.

Table 1
Viral titers across tissues and days post-infection.

dpi	Abdomen		Head-thorax		Saliva	
	Ouvea	Noumea	Ouvea	Noumea	Ouvea	Noumea
3	**2.08** (1.82–2.56; n = 11)	**2.73** (2.05–3.15; n = 4)	**1.08** (n = 1)	**3.68** (n = 1)	—	—
7	**3.95** (3.76–4.25; n = 24)	**3.19** (n = 3)	**2.89** (2.56–3.15; n = 18)	—	—	—
14	**4.95** (4.56–5.39; n = 28)	**1.08** (1.08–1.08; n = 5)	**4.78** (4.42–5.08; n = 26)	**4.46** (n = 1)	**1.15** (0.78–1.78; n = 5)	—
21	**4.92** (4.56–5.08; n = 27)	**3.03** (n = 1)	**5.19** (5.08–5.37; n = 27)	**4.62** (n = 1)	**0.60** (0.30–1.34; n = 13)	—
28	**4.86** (4.59–5.07; n = 26)	**4.12** (n = 3)	**5.25** (5.09–5.54; n = 26)	**4.74** (n = 2)	**1.20** (0.78–1.78; n = 17)	**0.95** (n = 2)
35	**4.08** (4.08–4.38; n = 27)	**2.53** (n = 2)	**4.78** (4.38–5.06; n = 27)	**4.48** (n = 1)	**1.78** (0.90–2.30; n = 17)	**0.30** (n = 1)

Viral titers are expressed as log10 focus-forming units (FFU) per sample and were calculated among virus-positive mosquitoes only. Values are presented as median with interquartile range (Q1–Q3) for groups with more than 3 virus-positive mosquitoes (n > 3); for groups with 1–3 positive mosquitoes, only the median is reported. *n* indicates the number of virus-positive mosquitoes included in the titer

calculation. A dash (—) indicates that no virus-positive mosquito was detected. DPI, days post-infection; FFU, focus-forming units.

Discussion

Five years after the initiation of *Wolbachia*-infected *Ae. aegypti* releases in Noumea, the pathogen blocking phenotype remained strongly expressed. These mosquitoes showed marked reductions in DENV infection, dissemination, and, most notably, transmission compared with the *Wolbachia*-free population. The magnitude of this reduction was consistent with the strong pathogen blocking previously reported in the New Caledonian line before field deployment [18], supporting the persistence of its functional phenotype following establishment in the field. This finding is in line with long-term observations from other *Wolbachia* deployment sites, where sustained pathogen blocking has been reported several years and even a decade after releases [11, 26, 27][13].

Because environmental and chemical conditions encountered by *Ae. aegypti*, including temperature [28, 29] and insecticide exposure [30], can affect the *Wolbachia*–host interaction and potentially modulate pathogen blocking, the persistence of this phenotype cannot be assumed from pre-deployment observations alone [14]. We therefore assessed DENV vector competence in the current Noumea population, five years after *Wolbachia* deployment, to determine whether the pathogen-blocking phenotype remained effective under field-established conditions.

Before *Wolbachia* deployment, a study reported comparable DENV-1 vector competence in Noumea and Ouvea populations [31]. In this study, we used DENV-2 and showed that the *Wolbachia*-free Ouvea population remained highly competent for DENV, with an estimated EIP_{50} of 17.6 days. In contrast, transmission in Noumea was delayed with higher EIP_{25} and EIP_{50} estimates, although these estimates were associated with wide confidence intervals due to the low number of transmitting mosquitoes. These findings highlight the value of extending vector competence assessments to later time points, as transmission in Ouvea continued to increase beyond 14 dpi, with an EIP_{50} estimated at approximately 18 days. Although the EIP is dependent on the specific virus–vector combination [32], assessing later time points may be important for accurately estimating this parameter, which is a key component of vectorial capacity, particularly when transmission is delayed or develops slowly [33].

The reduction in vector competence was also reflected in viral titers, with significant population × dpi interactions in the abdomen and head-thorax compartments. These interactions indicate that the difference in DENV titers between Noumea and Ouvea varied over the course of infection. No significant interaction was detected in saliva, although quantitative comparisons were limited by the very low number of virus-positive mosquitoes in Noumea at several time points (only three in total).

Importantly, transmission in the *Wolbachia*-infected population was detected only at 28 and 35 dpi. Therefore, studies restricted to early time points, such as 7–14 dpi, could have classified this population as completely non-transmitting and overlooked the residual transmission occurring at later stages.

Beyond describing the phenotype, longitudinal vector competence data provide parameters that can be incorporated into quantitative transmission models. In particular, the timing and probability of becoming infectious are relevant parameters for estimating transmission potential and vectorial capacity, allowing the impact of delayed and low-frequency transmission under *Wolbachia* infection to be better accounted for. However, the epidemiological relevance of transmission occurring at such late time points is expected to be limited because the probability of mosquitoes surviving to these ages decreases with time [34]. The contribution of late transmission to overall transmission potential therefore depends not only on the probability of becoming infectious, but also on the probability of surviving long enough to reach the duration of EIP [35, 36]. This distinction is particularly relevant when interpreting residual transmission detected under *Wolbachia* infection, as rare transmission events at advanced mosquito ages may contribute relatively little to population-level transmission.

In our *Wolbachia*-free population, the estimated EIP_{50} was approximately 17.6 days, while the transmission curve reached its upper plateau only around 30 dpi. For comparisons between mosquito populations or vector control strategies, kinetic designs should therefore cover a sufficiently long period to encompass both the onset and stabilization of transmission. This is particularly relevant for *Wolbachia*-based strategies, as *Wolbachia* infection can lengthen the DENV extrinsic incubation period and reduce the frequency of infectious mosquitoes [37], consistent with the delayed transmission observed in our *Wolbachia*-infected population. The EIP estimates obtained for the *Wolbachia*-infected population should nevertheless be interpreted cautiously. The EIP_{25} and EIP_{50} estimates were associated with very wide confidence intervals, reflecting the small number of transmitting mosquitoes. These estimates should therefore be considered exploratory, whereas the observed transmission proportions provide a more robust measure of the strong reduction in transmission associated with *Wolbachia* infection.

Conclusion

Overall, these findings demonstrate that the *Wolbachia*-mediated pathogen-blocking phenotype remain strongly express in Noumea five years after field deployment. Although residual DENV transmission was detectable at late time points, its low frequency and occurrence at advanced mosquito ages suggest limited contribution to transmission under field conditions. These findings provide evidence of sustained functional pathogen blocking five years after field deployment and support the persistence of the *Wolbachia*-mediated phenotype under field conditions.

Abbreviations

BSL-3
Biosafety Level 3
DENV
Dengue virus
DENV-2

Dengue virus serotype 2
DPI
Day post-infection
EIP
Extrinsic Incubation Period
FBS
Fetal Bovine Serum
FFA
Focus-Forming Assay
FFU
Focus-Forming Units
OR
odds ratio
WMP-NC
World Mosquito Program – New Caledonia
95% CI
95% confidence interval

Declarations

Ethics declarations

Ethics approval and consent to participate

Not applicable. This study did not involve human participants, human data, or identifiable personal information.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

Funding

This work was supported by the Laboratoire d'Excellence "Integrative Biology of Emerging Infectious Diseases" (grant n°ANR-10-LABX-62-IBEID) and the program “Explore” of the Institut Pasteur. B.D.

received a doctoral fellowship from the ANRS Infectious Emerging Diseases. The funders had no role in study design, data collection and interpretation, or decision to submit the work for publication.

Author Contribution

B.D. conceived and designed the study, performed the experiments, conducted the data analysis and interpretation, and wrote the original draft of the manuscript. N.P. contributed to the study conception and design, statistical analysis and interpretation, funding acquisition, and critical review and editing of the manuscript. AB.F. contributed to the study conception and design, funding acquisition, and critical review and editing of the manuscript. All authors have read and approved the final version of the manuscript.

Acknowledgement

Authors thank Malika Hocine and Amandine Breton for their help in rearing mosquitoes.

Data Availability

The data that supports the findings of this study are available in the data source.

References

1. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. Nature. 2013;496:504–7. https://doi.org/10.1038/nature12060
2. Souza-Neto JA, Powell JR, Bonizzoni M. *Aedes aegypti* vector competence studies: A review. Infection, Genetics and Evolution. 2019;67:191–209. https://doi.org/10.1016/j.meegid.2018.11.009
3. Van Den Berg H, Da Silva Bezerra HS, Al-Eryani S, Chanda E, Nagpal BN, Knox TB, et al. Recent trends in global insecticide use for disease vector control and potential implications for resistance management. Sci Rep. 2021;11:23867. https://doi.org/10.1038/s41598-021-03367-9
4. Achee NL, Gould F, Perkins TA, Reiner RC, Morrison AC, Ritchie SA, et al. A Critical Assessment of Vector Control for Dengue Prevention. Halstead SB, editor. PLoS Negl Trop Dis. 2015;9:e0003655. https://doi.org/10.1371/journal.pntd.0003655
5. Al-Amin HM, Gyawali N, Graham M, Alam MS, Lenhart A, Hugo LE, et al. Insecticide resistance compromises the control of *Aedes aegypti* in Bangladesh. Pest Management Science. 2023;79:2846–61. https://doi.org/10.1002/ps.7462
6. Asgarian TS, Vatandoost H, Hanafi-Bojd AA, Nik¬poor F. Worldwide Status of Insecticide Resistance of *Aedes aegypti* and *Ae. albopictus*, Vectors of Arboviruses of Chikungunya, Dengue, Zika and Yellow Fever. JAD [Internet]. 2023 [cited 2026 Sep 30]; https://doi.org/10.18502/jad.v17i1.13198

7. O'Neill SL, Ryan PA, Turley AP, Wilson G, Retzki K, Iturbe-Ormaetxe I, et al. Scaled deployment of *Wolbachia* to protect the community from dengue and other *Aedes* transmitted arboviruses. Gates Open Res. 2019;2:36. https://doi.org/10.12688/gatesopenres.12844.3
8. Achee NL, Grieco JP, Vatandoost H, Seixas G, Pinto J, Ching-Ng L, et al. Alternative strategies for mosquito-borne arbovirus control. PLoS Negl Trop Dis. 2019;13:e0006822. https://doi.org/10.1371/journal.pntd.0006822
9. Moreira LA, Iturbe-Ormaetxe I, Jeffery JA, Lu G, Pyke AT, Hedges LM, et al. A *Wolbachia* Symbiont in *Aedes aegypti* Limits Infection with Dengue, Chikungunya, and Plasmodium. Cell. 2009;139:1268–78. https://doi.org/10.1016/j.cell.2009.11.042
10. Van Den Hurk AF, Hall-Mendelin S, Pyke AT, Frentiu FD, McElroy K, Day A, et al. Impact of *Wolbachia* on Infection with Chikungunya and Yellow Fever Viruses in the Mosquito Vector *Aedes aegypti*. Turell MJ, editor. PLoS Negl Trop Dis. 2012;6:e1892. https://doi.org/10.1371/journal.pntd.0001892
11. Ryan PA, Turley AP, Wilson G, Hurst TP, Retzki K, Brown-Kenyon J, et al. Establishment of *w*Mel *Wolbachia* in *Aedes aegypti* mosquitoes and reduction of local dengue transmission in Cairns and surrounding locations in northern Queensland, Australia. Gates Open Res. 2019;3:1547. https://doi.org/10.12688/gatesopenres.13061.2
12. Pinto SB, Riback TIS, Sylvestre G, Costa G, Peixoto J, Dias FBS, et al. Effectiveness of *Wolbachia*-infected mosquito deployments in reducing the incidence of dengue and other *Aedes*-borne diseases in Niterói, Brazil: A quasi-experimental study. PLoS Negl Trop Dis. 2021;15:e0009556. https://doi.org/10.1371/journal.pntd.0009556
13. Ross PA, Robinson KL, Yang Q, Callahan AG, Schmidt TL, Axford JK, et al. A decade of stability for *w*Mel *Wolbachia* in natural *Aedes aegypti* populations. PLoS Pathog. 2022;18:e1010256. https://doi.org/10.1371/journal.ppat.1010256
14. Ross PA, Ritchie SA, Axford JK, Hoffmann AA. Loss of cytoplasmic incompatibility in *Wolbachia*-infected *Aedes aegypti* under field conditions. Rasgon JL, editor. PLoS Negl Trop Dis. 2019;13:e0007357. https://doi.org/10.1371/journal.pntd.0007357
15. Padde JR, Lu Q, Long Y, Zhang D, Hou M, Chen L, et al. The impact of environmental and host factors on *Wolbachia* density and efficacy as a biological tool. Decoding Infection and Transmission. 2023;1:100006. https://doi.org/10.1016/j.dcit.2023.100006
16. Ser SL, Dennington NL, Engler HI, Harris ML, Jones MJ, Avila FW, et al. Interactions between mosquito genetic background and *Wolbachia* strain affect dengue virus blocking and fitness in South American populations of *Aedes aegypti*. Asgari S, editor. PLoS Negl Trop Dis. 2026;20:e0014403. https://doi.org/10.1371/journal.pntd.0014403
17. Dupuis B, Russet S, Mahossem M, Drain A, Pol M, Kilama S, et al. Past and present resistance status to pyrethroid insecticides of *Aedes aegypti* from New Caledonia. Sci Rep. 2026; https://doi.org/10.1038/s41598-026-70544-z
18. Pocquet N, O'Connor O, Flores HA, Tutagata J, Pol M, Hooker DJ, et al. Assessment of fitness and vector competence of a New Caledonia *w*Mel *Aedes aegypti* strain before field-release. Turell MJ,

editor. PLoS Negl Trop Dis. 2021;15:e0009752. https://doi.org/10.1371/journal.pntd.0009752

19. World Mosquito Program. Success in New Caledonia: *Wolbachia* Eliminates Dengue Epidemics [Internet]. World Mosquito Program. 2025. https://www.worldmosquitoprogram.org/en/news-stories/stories/success-new-caledonia
20. Vazeille-Falcoz M, Mousson L, Rodhain F, Chungue E, Failloux AB. Variation in oral susceptibility to dengue type 2 virus of populations of *Aedes aegypti* from the islands of Tahiti and Moorea, French Polynesia. Am J Trop Med Hyg. 1999;60:292–9. https://doi.org/10.4269/ajtmh.1999.60.292
21. Payne AF, Binduga-Gajewska I, Kauffman EB, Kramer LD. Quantitation of flaviviruses by fluorescent focus assay. Journal of Virological Methods. 2006;134:183–9. https://doi.org/10.1016/j.jviromet.2006.01.003
22. R Core Team. R: A Language and Environment for Statistical Computing [Internet]. Vienna, Austria: R Foundation for Statistical Computing; 2024. https://www.R-project.org/
23. Heinze G, Schemper M. A solution to the problem of separation in logistic regression. Statistics in Medicine. 2002;21:2409–19. https://doi.org/10.1002/sim.1047
24. Ritz C, Baty F, Streibig JC, Gerhard D. Dose-Response Analysis Using R. Xia Y, editor. PLoS ONE. 2015;10:e0146021. https://doi.org/10.1371/journal.pone.0146021
25. Searle SR, Speed FM, Milliken GA. Population Marginal Means in the Linear Model: An Alternative to Least Squares Means. The American Statistician. 1980;34:216–21. https://doi.org/10.1080/00031305.1980.10483031
26. Utarini A, Indriani C, Ahmad RA, Tantowijoyo W, Arguni E, Ansari MR, et al. Efficacy of *Wolbachia*-Infected Mosquito Deployments for the Control of Dengue. N Engl J Med. 2021;384:2177–86. https://doi.org/10.1056/NEJMoa2030243
27. Anders KL, Ribeiro GS, Lopes R da S, Amadeu P, da Costa TR, Riback TIS, et al. Long-Term Durability and Public Health Impact of City-Wide *w*Mel *Wolbachia* Mosquito Releases in Niterói, Brazil, During a Dengue Epidemic Surge. Trop Med Infect Dis. 2025;10:237. https://doi.org/10.3390/tropicalmed10090237
28. Ross PA, Axford JK, Yang Q, Staunton KM, Ritchie SA, Richardson KM, et al. Heatwaves cause fluctuations in *w*Mel *Wolbachia* densities and frequencies in *Aedes aegypti*. PLoS Negl Trop Dis. 2020;14:e0007958. https://doi.org/10.1371/journal.pntd.0007958
29. Ulrich JN, Beier JC, Devine GJ, Hugo LE. Heat Sensitivity of *w*Mel *Wolbachia* during *Aedes aegypti* Development. Kittayapong P, editor. PLoS Negl Trop Dis. 2016;10:e0004873. https://doi.org/10.1371/journal.pntd.0004873
30. Dupuis B, Mousson L, Vazeille M, Bohers C, Kilama S, Pocquet N, et al. *Wolbachia*-mediated dengue control remains robust under insecticide stress in *Aedes aegypti* [Internet]. 2026 [cited 2026 Oct 1]. https://doi.org/10.21203/rs.3.rs-10406736/v1
31. Calvez E, Guillaumot L, Girault D, Richard V, O'Connor O, Paoaafaite T, et al. Dengue-1 virus and vector competence of *Aedes aegypti* (Diptera: *Culicidae*) populations from New Caledonia. Parasites Vectors. 2017;10:381. https://doi.org/10.1186/s13071-017-2319-x

32. Lambrechts L. Quantitative genetics of *Aedes aegypti* vector competence for dengue viruses: towards a new paradigm? Trends in Parasitology. 2011;27:111–4. https://doi.org/10.1016/j.pt.2010.12.001
33. Shapiro LLM, Whitehead SA, Thomas MB. Quantifying the effects of temperature on mosquito and parasite traits that determine the transmission potential of human malaria. Schneider D, editor. PLoS Biol. 2017;15:e2003489. https://doi.org/10.1371/journal.pbio.2003489
34. Dye C. Vectorial capacity: Must we measure all its components? Parasitology Today. 1986;2:203–9. https://doi.org/10.1016/0169-4758(86)90082-7
35. Joubert DA, Walker T, Carrington LB, De Bruyne JT, Kien DHT, Hoang NLT, et al. Establishment of a Wolbachia Superinfection in *Aedes aegypti* Mosquitoes as a Potential Approach for Future Resistance Management. Schneider DS, editor. PLoS Pathog. 2016;12:e1005434. https://doi.org/10.1371/journal.ppat.1005434
36. Bellan SE. The importance of age dependent mortality and the extrinsic incubation period in models of mosquito-borne disease transmission and control. PLoS One. 2010;5:e10165. https://doi.org/10.1371/journal.pone.0010165
37. Ye YH, Carrasco AM, Frentiu FD, Chenoweth SF, Beebe NW, Van Den Hurk AF, et al. *Wolbachia* Reduces the Transmission Potential of Dengue-Infected *Aedes aegypti*. Rasgon JL, editor. PLoS Negl Trop Dis. 2015;9:e0003894. https://doi.org/10.1371/journal.pntd.0003894

Figures

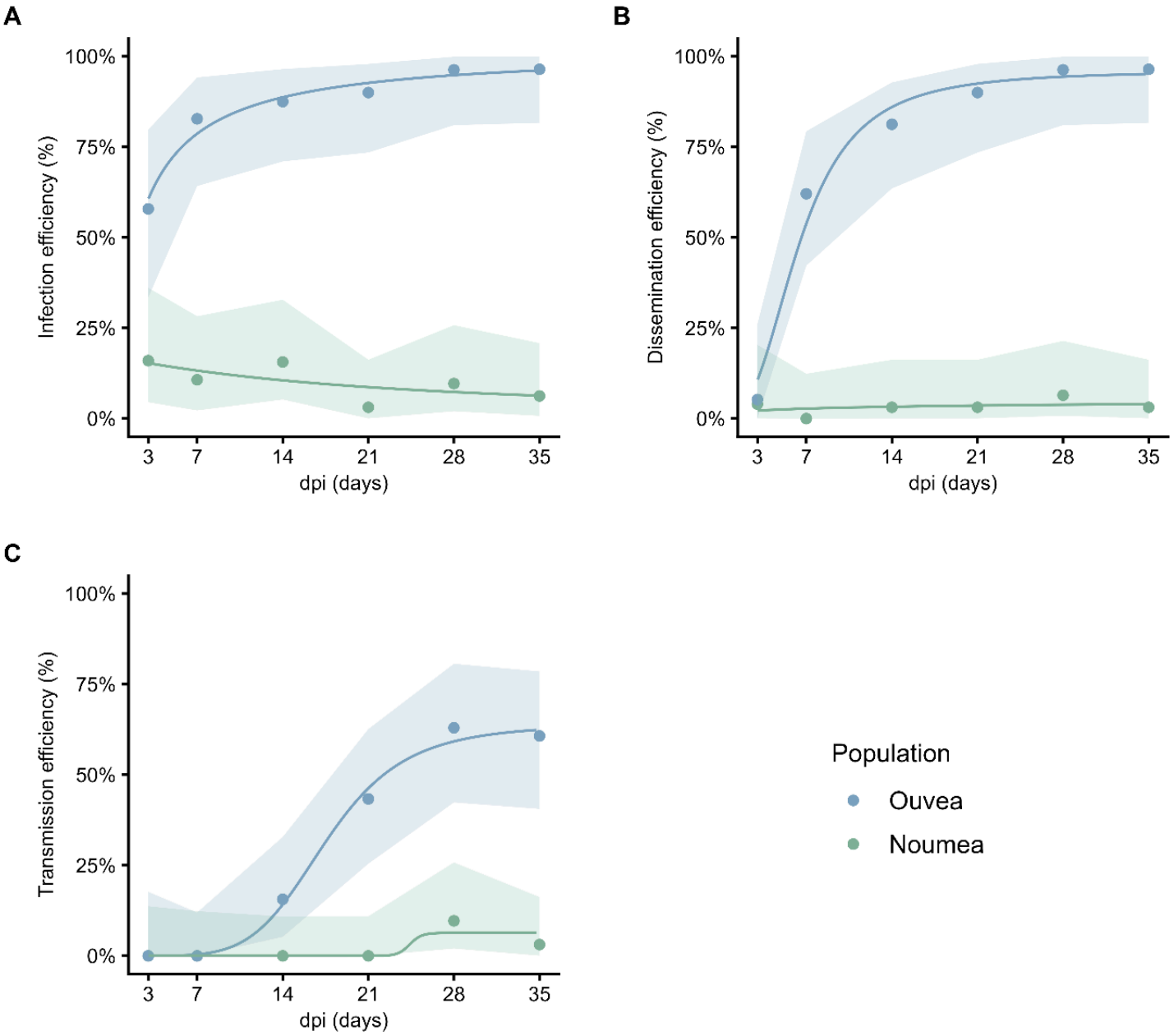

Figure 1

Kinetics of DENV infection, dissemination, and transmission in *Ae. aegypti* from Ouvea and Noumea populations.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Sourcedata.csv
- Graphicalabstract.png