Assessing *Wolbachia*-mediated sterility for dengue control: emulation of a cluster-randomized target trial in Singapore

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BACKGROUND

Matings between male *Aedes aegypti* mosquitoes infected with *w*AlbB strain of *Wolbachia* and wildtype females yield non-viable eggs. We evaluated the efficacy of releasing *w*AlbB-infected *Ae. aegypti* male mosquitoes to suppress dengue.

METHODS & MATERIALS

A cluster-randomized test-negative target trial from 2019 - 2022 was performed to evaluate the efficacy of releasing *Wolbachia*-infected *Aedes aegypti* males for dengue control in Singapore. Infected male mosquitoes were released twice weekly in intervention townships using either targeted or expanding release strategies. Dengue test-positive percentages were obtained from sustained exposure to field intervention after 3, 6 and ≥ 12 months within each year, from 2019 to 2022. These percentages were then compared to the same data set obtained from a *Wolbachia*-unexposed population from 12 pre-randomized control townships in the same time period.

Dengue test data from 133,821 individuals who reported febrile illness were first analyzed, subsequently excluding those with multiple residential addresses, conflicting test results and insufficient Wolbachia exposure (<3 months). Test outcomes were determined using RT-qPCR, NS1 antigen, or IgM assays. Ultimately, results from 76,265 individuals were included in the study.

Robustness checks for the model estimates were carried out via:

- Repetition of analysis without adjustment for covariates
- Conducting placebo in-time and in-space checks on control and intervention sites
- Recomputed intervention efficacies using different methods such as an alternate propensity score matching procedure and logistic regression without weighting

STATISTICAL ANALYSIS

Intervention efficacy was estimated using logistic regression, with dengue test-positive individuals compared to test-negative controls. Covariates were adjusted for, including environmental, socioeconomic, urbanisation and meteorological factors, alongside data on vegetation, housing density, building height and proximity to open drainage.

REFERENCES

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RESULTS

Primary analysis showed *Wolbachia* exposure for ≥ 3, 6 , or 12 months was associated with a **lower risk of testing positive for dengue** (13.6% testing positive in groups exposed for ≥ 3 months vs 21.7% in unexposed groups). **Higher periods of exposure** was associated with **greater levels of protective efficacy**. (Reduction in dengue incidence by 61% in areas receiving ≥ 12 months of sustained intervention.

Table 4 Odds ratios (ORs) and test-positive percentages for different *Wolbachia* exposure categories and across year, age and sex subgroups

Exposure time#	OR (95% CI)	Test positive (%) Exposed	Test positive (%) Unexposed	OR (95% CI)	Test positive (%) Exposed	Test positive (%) Unexposed	OR (95% CI)	Test positive (%) Exposed	Test positive (%) Unexposed	OR (95% CI)	Test positive (%) Exposed	Test positive (%) Unexposed	OR (95% CI)	Test positive (%) Exposed	Test positive (%) Unexposed
By year	2019			2020			2021			2022			Aggregate		
3 months	**0.51** * (0.23–0.85)	7.7% (37/482)	17.2% (3839/22 377)	**0.59** * (0.28–0.83)	21.9% (525/2395)	27.5% (6549/23 852)	0.73 (0.27–1.88)	.4% (147/2294)	9.5% (1121/11 787)	**0.37** * (0.2–0.58)	13.2% (247/1878)	31.0% (3477/11 200)	**0.53** * (0.31–0.75)	13.6% (956/7050)	21.7% (14 986/69 216)
6 months +	**0.49** * (0.13–0.74)	6.1% (19/314)	17.2% (3839/22 377)	**0.58** * (0.31–0.83)	22.4% (376/1679)	27.5% (6549/23 852)	0.59 (0.36–1.09)	6.3% (137/2191)	9.5% (1121/11 787)	**0.41** * (0.24–0.65)	13.4% (235/1751)	31.0% (3477/11 200)	**0.56** * (0.33–0.76)	12.9% (767/5935)	21.7% (14 986/69 216)
12 months +	**0.23** * (0.06–0.4)	3.2% (3/94)	17.2% (3839/22 377)	**0.48** * (0.36–0.58)	15.9% (98/617)	27.5% (6549/23 852)	**0.51** * (0.28–0.87)	6.2% (108/1737)	9.5% (1121/11 787)	**0.4** * (0.23–0.63)	13.4% (219/1635)	31.0% (3477/11 200)	**0.39** * (0.22–0.62)	10.5% (428/4068)	21.7% (14 986/69 216)
By age and gender	0–20			20–65			65+			Male			Female		
3 months +	0.59 (0.24–1.0)	12.4% (93/750)	18.4% (1881/8549)	**0.54** * (0.32–0.79)	16.5% (664/3462)	24.8% (10 427/31 612)	0.54 (0.33–1.01)	8.7% (195/2237)	15.5% (2584/16 637)	0.67 (0.29–1.04)	14.6% (498/3404)	22.1% (7236/32 686)	**0.39** * (0.31–0.53)	11.1% (374/3376)	19.6% (6167/31 389)
6 months	0.75 (0.22–1.1)	12.7% (80/630)	18.4% (1881/8549)	**0.54** * (0.36–0.	15.6% (525/3367)	24.8% (10 427/31 612)	**0.53** * (0.26–0.82)	8.3% (158/1912)	15.5% (2584/16 637)	**0.67** * (0.29–1)	13.7% (393/2865)	22.1% (7236/32 686)	**0.45** * (0.35–0.56)	10.9% (309/2846)	19.6% (6167/31 389)
12 months +	**0.34** * (0.13–0.62)	8.9% (38/415)	18.4% (1881/8549)	**0.38** * (0.24–0.55)	12.5% (283/2271)	24.8% (10 427/31 612)	0.46 (0.22–1.21)	7.7% (105/1358)	15.5% (2584/16 637)	**0.49** * (0.25–0.84)	11.9% (238/1999)	22.1% (7236/32 686)	**0.27** * (0.16–0.41)	8.3% (161/1943)	19.6% (6167/31 389)

*Denotes an OR that is less than 1 with 95% confidence intervals (CIs) that do not contain 1. These ORs show that there a significant protective effect of *Wolbachia* interventions on the risk of dengue. Figures are also bolded to denote statistical significance for clarity. ORs are estimated using doubly robust logistic regression with weights for each individual estimated using inverse probability weighting. Cluster bootstrap at the town resolution was used to obtain CIs to account for town-specific spatial clustering of data and the intervention **Unexposed group taken as the pre-randomized set of 12 controls #An individual testing for febrile illness is considered *Wolbachia*-exposed if the individual resides in a sector with 3, 6 or ≥12 months of sustained *Wolbachia* release ##Unweighted percentages of individuals testing positive in *Wolbachia*-exposed and *Wolbachia*-unexposed sectors

Table 3 Baseline characteristics of study population pre- and post-*Wolbachia* releases in intervention and pre-selected control group, at the sector resolution (the numbers in bracket represent standard deviation for each characteristic)

	Intervention Observed	Intervention Weighted	Control Observed	Control Weighted	SMD**
Pre-intervention (EW1 2010 to EW52 2016)					
Pre-intervention dengue incidence per 100 000*	112.22 (116.53)		113.78 (110.34)		
Post-intervention (EW1 2019 to EW26 2022)					
Post-intervention dengue incidence per 100 000*	158.8 (93.19)		294 (230.72)		
Dengue test positive (%)#	13.6 (0.004)	13.2 (0.001)	21.7 (0.002)	21.2 (0.002)	
Covariates					
Male (%)	50.19 (0.006)	49.49 (0.002)	51.09 (0.002)	51.09 (0.002)	0.022
Age (years)	49.65 (23.8)	45.09 (24.65)	45.25 (23.68)	45.42 (23.69)	−0.02
NDVI (Vegetation Index)	0.33 (0.05)	0.32 (0.05)	0.33 (0.05)	0.33 (0.05)	−0.08
Area within 300 m of a waterbody (%)	0.18 (0.25)	0.29 (0.29)	0.37 (0.41)	0.36 (0.4)	−0.1
Public housing height (m)	31.52 (4.66)	33.99 (4.45)	37.88 (10.21)	37.53 (10.19)	−0.28
Public housing age (years)	32.33 (7.96)	28.56 (11.16)	29.07 (11.07)	29.23 (11)	−0.07
Number of public housing units	722.4 (877.74)	1080.62 (1180.89)	713.71 (711.19)	714.96 (709.31)	0.28
Distance of centroid to drainage network (m)	359.87 (245.88)	354.52 (236.27)	448.77 (322.88)	446.37 (322.08)	−0.23
Length of drainage network (m)	88.08 (190.24)	48.04 (133.81)	43.52 (117.89)	44.61 (119.15)	−0.02
Forest area (%)	0 (0)	0 (0)	0.0004 (0.004)	0.0004 (0.004)	−0.09
Grass area (%)	0.001 (0.006)	0.002 (0.008)	0.008 (0.03)	0.008 (0.03)	−0.18
Total vegetation area (%)	0.02 (0.04)	0.03 (0.05)	0.03 (0.06)	0.03 (0.06)	0.13
Building area (%)	0.26 (0.04)	0.25 (0.04)	0.25 (0.06)	0.25 (0.06)	−0.04
Maximum temperature (°C)[1]	31.87 (1.05)	31.94 (0.99)	31.93 (1.06)	31.98 (1.01)	−0.01
Mean temperature (°C)	27.86 (0.84)	27.90 (0.76)	28.10 (0.84)	28.14 (0.80)	−0.2
Rainfall (mm)	7.36 (5.47)	6.55 (5.30)	6.37 (5.24)	6.38 (5.21)	0.02
Mean wind speed	8.18 (2.14)	9.11 (2.44)	9.28 (2.67)	9.18 (2.61)	0.01
Relative humidity	79.87 (2.83)	79.69 (3.24)	79.52 (3.28)	79.45 (2.87)	0.04

*Pre-intervention period dengue incidence denotes number of dengue cases per 100 000 per sector annually #Post-intervention percentage of dengue test-positives compared to total number of tests per sector. Only data on dengue tests were available 2016 onwards [1]Maximum temperature was calculated by taking maximum of temperature across all sectors within intervention or control groups. Length of drainage network and number of public housing units were calculated by taking sum across all sectors within intervention or control groups. The remaining characteristics were calculated by averaging across all sectors within intervention or control groups. All the calculations were done for the specified time period **Standardized mean differences (SMDs) after inverse probability weighting of intervention (*Wolbachia*-exposed) and controls (*Wolbachia*-unexposed) individuals. Tested individuals were considered *Wolbachia*-exposed here if they reside in a place of residence that has sustained *Wolbachia* interventions for ≥3 months

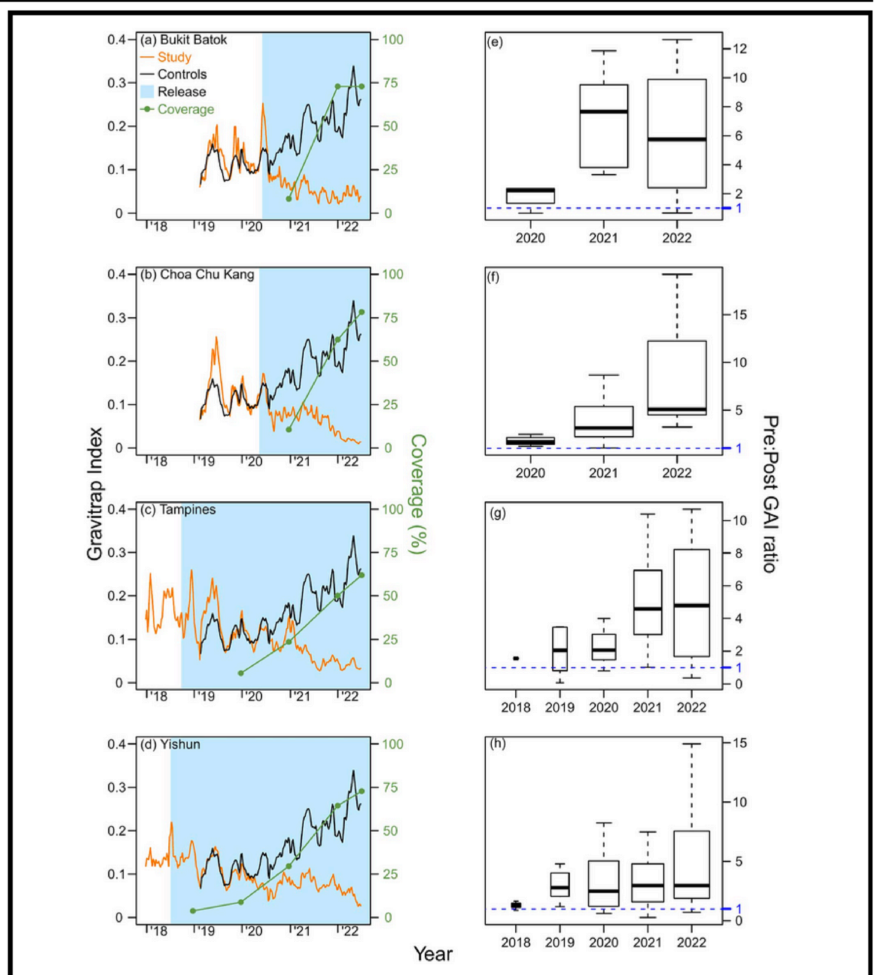

Figure 1 Weekly average, town-level Gravitrap *Aedes aegypti* index (GAI) and *Wolbachia* coverage (%) from 2018 to 2022 in the intervention sites of (a) Bukit Batok, (b) Choa Chu Kang, (c) Tampines and (d) Yishun. The average GAI from the 12 controls is plotted for comparison. GAI is defined as the mean number of female adult *Ae. aegypti* caught per functional Gravitrap per week, hence proxies for adult *Ae. aegypti* abundance in public housing areas in release area of each town. The geographical coverage (%) represents the percentage of areas within the town that is covered by *Wolbachia* interventions for at least 6 months and is calculated at the end of each year. Points represent coverage of *Wolbachia* interventions by the end of each year. The 6-month mark for coverage is based on the time it takes *Wolbachia* release to have ~80% suppressive efficacy on *Ae. aegypti* abundance. The corresponding ratios of pre- and post-intervention GAIs at the same township is also plotted in (e), (f), (g) and (h), per year, on a per-township basis. The threshold of 1 indicates no difference between pre- and post-GAIs for a specific sector in that specific year vs the pre-intervention period, and values >1 indicate lower GAIs in the post-intervention period

DISCUSSION

The use of releasing *Wolbachia*-infected male mosquitoes to suppress wildtype *Ae. aegypti* population has **shown promise** as a **complement to conventional approaches of vector control** in Singapore in its protective efficacy on dengue. Findings align with prior research, offering **potential against other *Aedes*-borne diseases**. with high public acceptance and long-term stability. Despite higher initial costs, this method avoids potential drawbacks such as viral resistance and climate sensitivity.

The test-negative design's assumptions were met with sensitive diagnostics, robust surveillance and confounder adjustments, ensuring reliable results. Despite potential residual confounding and staggered adoption effects, subgroup analysis and placebo validated the findings of this approach to enhance overall reduction of dengue transmission in the long run.

Our future plans pairing this knowledge with other vector control methods to complement mosquito-borne diseases in Singapore. The project can also factor in differences in housing to best adapt to Singapore's populace.