

HARNESSING WOLBACHIA TO REDUCE DENGUE TRANSMISSION - A REVIEW

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ABSTRACT

In this article, we discuss the possible, underlying molecular and biological basis of dengue case reduction by *Wolbachia*-infected mosquitoes, their field releases trial, and mathematical modelling of reduction in transmission of DENV attributable to the presence of *Wolbachia*. This study suggests that *Wolbachia*infected DENV control could reduce dengue incidences up to 40.3 to 96% in the field release sites and A laboratory-based trial reported 100% suppression when *Wolbachia*-infected male mosquitoes were released. Moreover, Mathematical modeling predicted promising results in dengue reduction by *Wolbachia*. Documented evidence in this study support *Wolbachia* as a novel vector control approach to limit dengue transmission.

Key words: *Aedes aegypti*, dengue, *Wolbachia*-infected mosquitoes, *Wolbachia* deployment, DENV reduction, cytoplasmic incompatibility, insect-specific viruses, pelo protein, Toll pathway, mathematical modelling

Dengue is an important arboviral disease affecting over one-third of the global population (Ford et al., 2019). Dengue virus serotypes (DENV 1, DENV 2, DENV 3, and DENV 4) are responsible for dengue infection, which poses a significant threat to human health. It is spread by the bite of female Aedes mosquitoes. To complement the existing approaches to controlling dengue, efforts are being made to use the endosymbiotic bacterium genus Wolbachia to reduce mosquitoes' ability to transmit arboviruses such as zika and dengue. Wolbachia are gram-negative, naturally occurring, maternally transmitted endosymbiotic bacteria that can impact host reproductive systems through various methods, including male-killing, cytoplasmic incompatibility (CI), feminization, and parthenogenesis, which potentially benefit the host in some cases (Dobson et al., 2004; Alexandrov et al., 2007; Brownlie et al., 2009). Wolbachia strains are found naturally in at least 40% of the insect population (Zug and Hammerstein, 2012). It has been detected in a variety of arthropods, but not in Aedes aegypti (the principal dengue vector) (Xi et al., 2005; McMeniman et al., 2009; Kambris et al., 2010; Hughes et al., 2011).

Researchers are working to create novel vector control measures as an adjunct to ongoing control measures. Although *Wolbachia* does not naturally infect *A. aegypti* but nowadays transinfected *A. aegypti* has been used as a viable strategy to limit DENV transmission in various parts of the world. Multiple authors have achieved this stable transfection (O'Neill et al., 2019). Insects carrying Wolbachia had lower viral loads as compared to uninfected ones due to the process known as virus blocking (Hilgenboecker et al., 2008). Importantly, several Wolbachia strains can fight against viral infection in a variety of arthropod hosts. Over the last few decades, several distinct strains from Drosophila melanogaster such as wMelPop-CLA, wAu, wMelCS, wRi, wMel, and wAlbB/wAlbA, wPip from A. albopictus, as well as wMel/wAlbB from both the hosts have been successfully transinfected in A. aegypti, resulting in stable infection (Joubert et al., 2016; McMeniman et al., 2009; Walker et al., 2011; Xi et al., 2005). The strains wAlbB and wMel, which were generated from A. albopictus and Drosophila melanogaster, can protect the host against ZIKA and DENV virus infections (Walker et al., 2011; Aliota et al., 2016). Many field trials are currently underway on Wolbachia-infected A. aegypti mosquitoes as a biocontrol technique to minimize DENV spread (Fraser et al., 2020). The World Mosquito Program (WMP) has released Wolbachia-infected mosquitoes in twelve countries, including Australia, Vietnam, Brazil and Indonesia to reduce dengue fever (Nainu et al., 2019). Wolbachia-infected mosquitoes were bred in specialized facilities before being discharged into dengue affected areas. Wolbachia is then passed on to their progeny by transovarial transmission by these female mosquitoes, restricting arboviral transmission (O'Neill, 2018).

Researchers worldwide have extensively explored the reduction of dengue cases through various mechanisms involving Wolbachia. To gain a comprehensive understanding of the findings, it is imperative to consolidate the diverse perspectives reported by different authors. This review aims to facilitate a thorough examination of the existing literature which will help in identifying the research gaps and draw conclusive insights about the current state of knowledge on Wolbachia's impact on dengue case reduction. The emergence of *Wolbachia* as a dengue control, the molecular and biological basis of dengue reduction by Wolbachia, the evidence from the community-based field trials and mathematical modeling explained by different authors are summarised herein to understand how far Wolbachia mediated approach is successful. This research will aid vector management programs by examining the progress or regress of Wolbachia as a biocontrol tool by the scientific community in the fight against dengue fever.

1. Emergence of Wolbachia as a biocontrol method

Wolbachia was first described in the 1920s, and W. pipientis is a bacterial endosymbiont that was discovered in Culex mosquito ovaries in 1924. It is thought to infect two-thirds of all extant insect species. Until the 1970s, little research had been done on it, till Wolbachia was proposed as a mosquito control method in 1967 documented by Yen and Barr, 1971. Afterward, in 2005, persistent infection of A. aegypti with a Wolbachia strain from the mosquito A. albopictus was established, causing high rates of cytoplasmic incompatibility, and rapidly spreading to high frequencies in experimental populations (Xi et al., 2005). Several years later, the second wave of breakthroughs occurred with the discovery of Wolbachia-induced phenotypes in mosquitoes that directly impacted pathogen transmission. Under laboratory conditions in 2009, stable insertion of a lifeshortening strain of Wolbachia from Drosophila into A. aegypti resulted in halved adult mosquito lifetime, indicating that mosquitoes would not live long enough to spread the dengue virus (McMeniman et al., 2009). Furthermore, this life-shortening Wolbachia strain directly hindered the ability of a variety of diseases to infect and proliferate in A. aegypti, including the dengue virus. In 2011, Wolbachia was established in the Australian A. aegypti population (Hoffmann et al., 2011). With the introduction of transinfection techniques around a decade ago, there was a rebirth of interest in Wolbachia-based vector-borne disease

management strategies. *Wolbachia* infections in naïve hosts can now be created using embryonic microinjections into the developing embryo germline. *Wolbachia* transinfection is more likely to succeed when the donor and recipient animals are closely related, and the expression of *Wolbachia*-induced phenotypes is conserved across hosts.

2. Molecular and biological basis of dengue case reduction

Wolbachia manipulates the host environment to foster a positive symbiotic relationship. The underlying Wolbachia mediated molecular mechanisms are not fully understood, although it may be owing to increased inborn immunity as multiple immune genes such as cecropins, defensins, and other Toll pathway genes were elevated in Wolbachia-infected A. aegypti (Bian et al., 2010). There are two techniques for dealing with Wolbachia in dengue. The first method involves solely releasing modified male mosquitoes. This method has been effectively implemented in Singapore and Guangzhou, China, as well as in portions of the United States such as Miami, Texas, and California since 2015 (Ong, 2021). Because eggs laid by unmodified females that mate with modified males do not hatch, the community's population (mosquito) is drastically reduced this known as population suppression (Ong, 2021). The second strategy is utilized by places including Vietnam, Indonesia, Malaysia, Brazil, and Australia, includes the release of modified mosquitoes of both sexes. Females infected with the bacteria pass it on to their offspring. The modified mosquitoes gradually replace the natural population over time (from months to years, depending on the characteristics of the release site) known as population replacement (Ong, 2021). The mechanism underlying the symbiotic association of A. aegypti with Wolbachia and the longterm consequences of this interaction is still unknown. However, the probable mechanism has been listed in this section as follows:

A. Cytoplamic inheritance (CI)

CI is a process that gives female mosquitoes a reproductive advantage. When an infected male mosquito mates with an uninfected female mosquito or has a different form of *Wolbachia*, a phenomenon known as CI occurs, resulting in embryo death. An uninfected male can mate with infected females and successfully spread *Wolbachia* and any gene it carries. This discovery was initially observed in *Culex* mosquitoes, where the presence of *Wolbachia* causes resistance to several RNA viruses (Hedges et al., 2008; Teixeira et al., 2008). CI confers a reproductive advantage on Wolbachia-infected females because the symbiont is transmitted maternally and the bacterium spreads rapidly through uninfected populations. Wolbachia also makes their hosts less susceptible to a variety of pathogens, including viruses, other bacteria, nematodes, fungi, and the malaria parasite (Terradas et al., 2017). The cytological implications of CI manifest themselves during postfertilization zygotic development. Both directions of a cross are incompatible due to the infection with different strains of CI-inducing this is known as bidirectional CI. Wolbachia-infected males undergo mitosis after mating with an uninfected female or a female infected with an incompatible Wolbachia strain. The absence of similar effects in the embryos of Wolbachia-infected females that mated with Wolbachia-infected males suggests that CI is produced by sperm and rescued within infected female eggs and this rescue factor' is absent in uninfected females (Caragata et al., 2021). Wolbachia, which may infect insect populations through CI, offers a novel approach to lowering tropical arboviral mosquito-borne diseases like dengue fever. The sustained establishment of the wMel strain in A. albopictus decreases the ability of DENV-infected mosquitoes to transmit the virus. This bidirectional CI introduces a method for stable Wolbachia establishment into populations since bidirectionally incompatible crossing types cannot coexist stably. Once the wMel strain has acquired population dominance, a fully bidirectional CI can be formed in A. albopictus, allowing for local fixation.

B. Role of miRNA

The researchers investigated the differential expression of cellular miRNAs in A. aegypti infected with the Wolbachia strain wMelPop-CLA (Leitner et al., 2021). This work discovered differences in cellular miRNA expression and investigated one induced miRNA, aae-miR-2940, which influences the expression of one mosquito host gene, metalloprotease *m41ftsh* (Fig. 1). The wAlbB strain could inhibit viral multiplication in the midgut and reduce viral transmission throughout the mosquito's head and thorax (Leitner et al., 2021). In addition to these findings, researchers also reported that DENV was stopped from propagating by activating the Toll pathway with RNAi-depleted cacti and a REL1 inhibitor (Xi et al., 2008). Wolbachia's transcriptional response to DENV infection was studied in A. aegypti Aag2 cells infected with the wAlbB strain of Wolbachia. According to the 3



Fig. 1. The host - *Wolbachia* interaction: *Wolbachia* induces reproductive abnormalities and increased expression of various cellular miRNA

findings, many genes were upregulated and some were downregulated, primarily during the early stages of host cell infection, demonstrating *Wolbachia's* ability to alter the host's gene expression profile. Essential cellular, transmembrane, endoribonuclease (Rnase HII), stress response functions, a regulator of sigma 70-dependent gene transcription (6S RNA), and primary type 1 and type 2 secretion systems were pre-regulated as a result of DENV infection, but ribosome structure, several *Wolbachia* transport, and binding proteins, and elongation factor associated genes were downregulated. (Leitner et al., 2021).

C. Role of Toll and IMD pathway

According to one study, when the Wolbachia strain wAlbB is injected into A. aegypti, it induces the Toll and Immune Deficiency (IMD) pathway. The silencing of the IMD and Toll pathways by RNAi resulted in a reduction in the wAlbB burden. The Wolbachia strain also increases the expression of the peptidoglycan recognition protein (PGRP)-LE in the carcass of A. aegypti, and when it was silenced, the symbiont load was reduced. Activation of the Toll and IMD pathways in transgenic mosquitoes results in increased wAlbB infection. These findings indicate that the host's innate immunity was used to establish and sustain the hostmicrobial symbiosis. The study's findings will help predict the long-term stability of the A. aegypti and Wolbachia symbiosis, which can be used to combat Zika and Dengue fever infections..

D. Insect specific viruses (ISV)

Over the last decade, advances in sequencing technology (next-generation sequencing and

metagenomics) and phylogenetics have led to the discovery of a diverse set of novel RNA viruses associated with hematophagous insects (Vasilakis and Tesh, 2015). ISVs infect insects and cells from insects, but they do not live or multiply in vertebrates or cells from vertebrates. Some insect-specific viruses isolated from A. aegypti have been shown to control arboviruses like West Nile and Dengue. A. anphevirus (AeAV), a negative-sense RNA virus belonging to the Mononegavirales order, had been studied in A. aegypti. According to the findings, Wolbachia stimulates AeAV replication as compared to a tetracycline-cleared cell line, which inhibits DENV multiplication in vitro. ISV has been found to suppress the antiviral RNA interference (RNAi) response in Culex-Y virus (CYV), a member of the Birnaviridae family, Dezidougou virus from the negevirus taxon, PCLV, A. densoviruses (family Parvoviridae), and the unclassified Humaita-Tubiacanga virus (HTV) have all been found in wild captured and laboratory A. aegypti (Aguiar et al., 2015). The fact that AeAV propagates in A. aegypti and A. albopictus cell lines but not in mammalian cell lines suggests it is an ISV, however, it still needs to be validated in cell lines from other species. The findings indicate that DENV replication was reduced in the presence of AeAV, which was significant at lower MOI (Multiplicity of infection) and could be effective in DENV suppression. (Fig. 2) (Aguiar et al., 2015).

E. Role of microbiota

The role of local microbiota in insect physiology and immunity in DENV blocking was also investigated in one study. This study demonstrated that the introduction of two bacteria species (Proteus sp. and Paenibacillus sp.) into the mosquito midguts separately resulted in a significantly lower level of dengue virus infection, whereas the introduction of other species (including Pantoe sp. and Comamonas sp.) produced no significant difference in dengue virus titre compared to control group (Ramirez et al., 2012) (Fig. 3). The findings of another study showed that while Wolbachia influenced the relative abundance of various microbiota present in the midgut, Elizabethkingia, and unclassified Enterobacteriaceae dominated the microbiome of both Wolbachia-infected and uninfected mosquitos. They also compared Wolbachia (wMel) infected and uninfected A. aegypti mosquitoes using 16s rDNA profiling. Despite a substantial change in the microbiome makeup, antibiotic therapy did not affect DENV blocking by wMel. This study reported that Wolbachia-mediated DENV inhibition appears to be independent of microbiome composition (Audsley et al., 2017).

F. Role of pelo protein

The role of the pelo protein, which is involved in protein translation, in the mosquito-DENV interaction and *Wolbachia* antiviral response was investigated in one study. The findings show that the pelo protein is upregulated during DENV replication and silencing of this pelo protein decreases DENV virion generation, implying that it aids DENV replication. When *Wolbachia* is present in female mosquitoes, the pelo protein is downregulated and its location is altered,



Fig. 2. Molecular and biological basis of Wolbachia-mediated DENV control in the host

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Fig. 3. The molecular and biological basis of Wolbachia-mediated DENV blocking

which may reduce DENV replication in *A. aegypti* (Asad et al., 2018). In this study, the pelo was identified as a host factor for DENV replication, and its suppression by *Wolbachia* resulted in virus blocking. When pelo was knocked out, the number of DENV infectious particles produced and secreted outside the cells were decreased significantly, but there was no effect on DENV total genomic RNA, implying that pelo may play a role in viral genomic RNA translation and/or DENV-2 virions assembly and release (Asad et al., 2018).

G. Role of LincRNA

The sequence and structure of lincRNAs are critical to their function, particularly when they interact with DNA, RNA, or proteins. Extensive base-pairing of lincRNA with target mRNA can stabilise translation, whereas partial base-pairing can accelerate mRNA decay or inhibit translation of the target mRNA. One study conducted by Etebari et al presented a comprehensive list of A. aegypti lincRNAs, which will be added to the other microRNAs and piRNAs. This work also contributes to the current annotation of the A. aegypti genome and identified potential immune-related lincRNAs in A. aegypti. They examined the expression pattern of some selected lincRNAs in response to the microbial challenge, specifically dengue virus serotype 2 (DENV-2) and Wolbachia uninfected and infected adult mosquitoes and Aa20 cells. The number of hosts lincRNAs increased after DENV-2 infection, with some of them suppressing virus replication in mosquito cells. LincRNA 1317 silencing by RNAi increased viral replication, indicating that it may play a role in the host's antiviral defense. The findings of this study may help to develop new strategies to control vectors or prevent virus multiplication in them (Etebari et al., 2016).

H. Host resources

The transmission ability of the virus in *Wolbachia*infected *A. aegypti* was dramatically reduced when compared to wild-type mosquitoes that did not carry *Wolbachia*. Some findings revealed that the level of inhibition varied between tissues and over time. This variation could be related to DENV dynamics and the distribution of *Wolbachia* in mosquitos. The antiviral action of *Wolbachia* may be due to direct rivalry between DENV and *Wolbachia* for the same resources available for metabolic change in the host, which may affect the host environment in a way that is unfavourable to dengue virus replication (Bian et al., 2010).

I. Role of receptors

The viral binding experiments reveal how the *Wolbachia* strain wAlbB affected DENV serotype 2 (DENV-2) and ZIKV attachment to *A. aegypti* Aag-2 cells. In the experiment, RNAi was used to mute the DENV mosquito receptors in Aag-2 cells that were controlled differently by wAlbB to identify the components that inhibited viral binding. The findings

showed that *Wolbachia* inhibits ZIKA and DENV binding to host Aag-2 cells by decreasing the production of the two DENV receptors, tubulin, and dystroglycan (Lu et al., 2020).

J. Role of AeVago1 gene

Suppression of the *AeVago1* gene in *Wolbachia*infected cells significantly increases DENV replication while having little effect on *Wolbachia* density. This study found two Vago homologs in *A aegypti* and discovered that *Wolbachia* significantly induced *AeVago1* in the *Wolbachia*-mosquito-DENV interaction. This *AeVago1* gene may function in the mosquito as a host factor, preventing DENV replication (Asad et al., 2018).

3. Dengue case reduction explained by mathematical modelling

In this paragraph we are summarising information pooled from articles based on mathematical modeling. One mathematical model was introduced to quantify the impact of introducing Wolbachia into the mosquito population on human dengue cases (Ndii et al., 2015). This model is a compartment-based system of firstorder differential equations, with seasonal forcing in the mosquito population provided by the adult mosquito mortality rate. This study focuses on a single dengue outbreak characteristic of a seasonally variable mosquito population and reported that if Wolbachia-carrying mosquitoes compete with mosquitoes that do not carry Wolbachia, a reduction in human dengue cases can be attained. They also compared two different strains of the bacteria and their potential impacts and reported that the strain wMel decreases mosquito's lifetime by no more than 10% while allowing them to compete with non-Wolbachia-carrying mosquitoes. On the other hand, mosquitoes bearing the wMelPop strain are unlikely to survive since it reduces mosquito longevity by up to 50%. Therefore, they concluded that wMel is better than the wMelPop strain (Ndii et al., 2015). Another study creates a deterministic compartment mathematical model to predict the persistence of Wolbachia-infected mosquitos by considering the competition between Wolbachia-infected and non-Wolbachia-infected mosquitos (Ndii et al., 2012). This model used six compartments representing the mosquito population. The model assumes homogeneous mixing and does not account for mosquito dispersal. The numerical solutions, stability analysis, and sensitivity analysis revealed that Wolbachia-infected mosquitoes can eventually dominate the population if they survive.

The steady-state stability is determined by the key parameters in the mosquito life cycle. The findings reveal that the reproduction rate, death rate, maturation rate, and maternal transmission all have a role in the persistence of Wolbachia-infected mosquitoes. This study concluded that Wolbachia dominates the population, for the parameter values where it persists, and hence the introduction of Wolbachia has a high potential to reduce dengue transmission (Ndii et al., 2012). Again, a deterministic model was built and tested in the presence of vaccination and Wolbachia. This compartment-based model separates human and mosquito populations into different compartments based on their disease condition (Ndii, 2020). The results of numerical simulations were given and the consequences for public health were examined. The findings revealed that Wolbachia outperforms vaccination in reducing the number of dengue cases if vaccine efficiency is insufficient; otherwise, vaccination alone is adequate to reduce dengue incidence, and a combination of Wolbachia and vaccine is not required (Ndii, 2020). Another model was developed by integrating human birth and death rates into a mathematical model created by (Ndii et al., 2015) to explore long-term dynamics. This model also focuses on areas where dengue is not endemic but can spread due to a dengue vector and the regular arrival of people with dengue. They investigate the impact of Wolbachia on the time range during which outbreaks can occur and the sensitivity of the findings to transmission rate and seasonal forcing amplitude (Ndii et al., 2016). This deterministic model uses nonlinear first-order ordinary differential equations to classify the human population into separate classes based on their sickness status. Dengue is imported for three months in all scenarios and demonstrated that the possible breakout seasons have been lowered in the presence of Wolbachia-carrying mosquitoes. The reduction of dengue incidences was found as the strength of seasonality increased. Wolbachia reduces overall case counts by up to 80% in low strength of seasonality. The findings of this model suggest that *Wolbachia* can decrease the time it takes for epidemics to begin. In areas with minimal seasonality and a moderate transmission rate, Wolbachia intervention can be beneficial (Ndii et al., 2016). Again, one continuous-time mathematical model was created to evaluate the desirable qualities of the Wolbachia strain to be introduced into the system. This study showed that Wolbachia had a lot of potential for controlling dengue fever in locations where R_o (basic reproduction number) is not too high. Wolbachia strains that diminish but do not eradicate dengue transmission have little effect on endemic stable states or epidemic sizes if R_0 is substantial (Hughes and Britton, 2013). In such instances, it may be worth the extra effort to introduce Wolbachia strains that eradicate dengue transmission unless control methods to reduce R₀ by reducing mosquito numbers are also implemented (Hughes and Britton, 2013). The data were fitted to a mathematical model of DENV transmission that included the kinetics of viral infection in humans and mosquitos (Ferguson et al., 2015). Model estimates predicted that wMel will reduce DENV transmission's basic reproduction number, R₀, by 66-75%. The findings showed that high-frequency introduction of wMelPopinfected A. aegypti in a dengue-endemic context would result in total DENV transmission abatement. The establishment of wMel-infected A. aegypti is also expected to significantly impact transmission, which could be enough to eliminate dengue in low or moderate transmission settings but may not be enough to achieve total control in high R₀ conditions. These findings provide a framework for deciding which Wolbachia strains to release in the field and evaluating their likely impact (Feruguson et al., 2015). An ensemble of transmission models predicted the expected reduction in dengue transmission following a state-wide roll-out of wMel Wolbachia. In Indonesia, it was estimated that 7.8 million symptomatic dengue cases occurred in 2015, with 332,865 disability-adjusted life years lost (DALYs). Non-severe cases that did not seek treatment or were difficult to diagnose in outpatient settings accounted for most dengue cases, resulting in significant underreporting (Ferguson et al., 2015). The estimated burden was concentrated in a few large cities, with 90% of dengue cases occurring in 15.3% of the land area. Over the long term, it was anticipated that implementing a state-wide Wolbachia population replacement program would prevent 86.2% of cases. These findings imply that interventions focused on the cities with the highest dengue burden can disproportionately influence dengue transmission. In such congested surroundings, area-wide therapies such as Wolbachia, which are deployed based on the area covered, could protect people more effectively than individual-focused interventions such as vaccinations.

4. Community based field trial of *Wolbachia* infected *Aedes* mosquitoes

In this paragraph, we summarised the communitybased field releases that have been conducted in countries like Australia, Indonesia, Malaysia, Brazil, and Singapore are listed in Table 1. Based on the results, it was seen that dengue occurrences decreased from 40.3% to 96% when *Wolbachia*-infected *Aedes* mosquitos of various strains were released in the study sites. In all the listed field release studies, sustained transfection was obtained in just a few generations. One research was undertaken in Brazil, which incidentally revealed a 56% decline in chikungunya, a 37% drop in zika prevalence, and a 69% drop in dengue occurrences. We have observed that *Wolbachia's* success rate in lowering DENV incidence was moderate to high. The *w*Mel strain was found to be the most significant strain in lowering dengue incidences (Walker et al., 2011).

CONCLUSIONS

Wolbachia is a promising approach towards controlling some mosquito-borne viral diseases. However, longitudinal studies over many generations are required to assess the sustainability and persistence of Wolbachia in mosquitoes and their role in Dengue control. Therefore, Wolbachia-based control methods need more field and laboratory studies to better understand the molecular and biological phenomena involved in the reduction of Den virus transmission. Moreover, the direct impact of Wolbachia on human health has not been reported yet, but further research on this aspect is also an important parameter. Apart from the Wolbachia mediated control, other novel vector control interventions such as genetically modified mosquitoes (GMM) have been released in different countries. The Sterile Insect Technique (SIT) is a method of controlling insect reproduction; as they do not reproduce due to eggs laid down by the females are sterile and hence the insect population diminishes over time. Due to the diminished performance of sterilized males produced by sterilization, the application of SIT for mosquitoes that transmit human disease has been limited. Another issue for SIT programs targeting Aedes species is the challenge of initially reducing wild population densities before delivering sterile males. We have found various mechanisms described by many researchers across the globe which could lead to DENV reduction by Wolbachia infected A. aegypti such as CI provides a unique method for population replacement and bidirectional CI induces a possible mechanism for population replacement strategy, aae-miR-2940, a cellular miRNA helps in the viral inhibition, in the presence of Wolbachia many toll and IMD pathway genes, antioxidant pathway genes were activated, ISVs such as AeAV have been shown to control DENV virus in A. aegypti, Pelo protein regulates the DENV replication, lincRNA 1317, studies found that it may

Sl.	Country/	Year	Type of study	Type of Wolhachia	Outcome
<u>1</u> NO.	Australia	2009-	Semi-field community-based	wMel wMelPon-	Stable infection within a few
1.	Australia	2010	Semi-neid community-based	CLA	generations and blocks DENV 2 replication (Walker et al., 2011)
2.	Indonesia, Yogyakarta	2014- 2015	Community-based field release	wMel	<i>Wolbachia</i> infection was stable in the population (Tantowijoyo et al., 2020).
3.	Indonesia, Yogyakarta	2016- 2017	A quasi-experimental trial using controlled interrupted time series analysis	wMel	Sustained introgression was found and 73% reduction in dengue incidence (Indriani et al., 2020)
4.	Australia	2014- 2016	Community-based field release	wMel	Effective in a reduction in local dengue transmission (O'Neill et al., 2019)
5.	Brazil	2014- 2016	EVITA Dengue trial design, Cluster-randomised trial	wMel-BH	Stable Wolbachia infection was found after cessation of releases (Collins et al., 2022)
6.	Malaysia	2017	Community-based field release	wAlbB	40.3% reduction in dengue incidence (Nazni et al., 2019)
7.	Brazil	2017- 2019	Community engagement field release	wMel	Reduced incidences of <i>Aedes</i> borne disease (69% dengue, 56 % chikungunya and 37% Zika reduction) (Pinto et al., 2021)
8.	Indonesia, Yogyakarta	2018- 2019	Cluster-randomized test- negative design trial	Wolbachia	60% reductions in treated areas (Anders et al., 2018)
9.	Australia	2011- 2013	Community-assisted method	wMel	96% reduction of dengue transmission (Ryan et al., 2020)
10.	Singapore	2019	Community-based trial	wAlbB-SG	Reduction of dengue incidence from 71 % to 88% (Ng and Consortium, 2021)
11.	Guangzhou CN	2014- 2017	Large-scale deployment (Both Incompatible insect technique and sterile insect technique (IIT/SIT)	wAlbB/wAlbB/ wPip	>94% suppression of target populations (<i>A. albopictus</i>) (Zheng et al., 2019)
12.	Fresno, USA	2018	Large-scale deployment (Both Incompatible insect technique and sterile insect technique (IIT/SIT)	wAlbB (Aedes aegypti)	>95% suppression of target population (Crawford et al., 2020)
13.	Miami. USA	2018	Large-scale deployment (treatment vs control)	wAlbB (Aedes aegypti)	78% decrease in female population (Mains et al., 2019)
14.	Brazil	2015- 2016	Large-scale roll-out strategy	wMelRio (Aedes aegypti)	Long term persistent of <i>Wolbachia</i> infection (Gesto et al., 2021)

Table 1. Community-based field release of Wolbachia-infected Aedes aegypti

help in antiviral defence, the direct competition between the Wolbachia and virus for the same resources in the host environment, it may be possible the existence of the Wolbachia before than the virus may eliminate the virus from the host, decrease the production of DENV binding receptors tubulin and dystroglycan in the presence of Wolbachia etc. Despite this knowledge, it is still unclear how these effects are triggered and why they affect DENV. Understanding the contributions of each of these processes to arboviral suppression in Wolbachiainfected A. aegypti has been difficult, and the antiviral syndrome induced by Wolbachia species in the host is likely multi-layered, with no single mechanisms to blame. Based on the findings of this survey, we believe, there is hope for the use of Wolbachia in dengue vector control programs as well as other vector-borne diseases, as most studies show positive results after minimizing and resolving issues associated with Wolbachia-based strategies.

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AUTHOR CONTRIBUTION STATEMENT

SB prepared, wrote and conceptualised, SAK Conceptualised, Prepared and Reviewed the manuscript, PG conceptualized and reviewed, DK reviewed the manuscript. All authors read and approved the manuscript

CONFLICT OF INTEREST

No conflict of interest.

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