Jurnal Ilmu Kedokteran (Journal of Medical Science), Jilid 20, Nomor 1, Maret 2026, Hal.21-29
https://doi.org/10.26891/JIK.v20i1.2026. 21-29

Literature Review
The Role of *Wolbachia* in DENV Replication as Dengue Control Strategies

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ABSTRACT

Dengue fever (DHF) remains a major public health problem in tropical countries such as Indonesia, with cases continuing to rise in recent years. Conventional vector control strategies are often less effective, creating the need for innovative biotechnology-based approaches. One promising strategy is the utilization of *Wolbachia* bacteria, which has been shown to suppress dengue virus (DENV) replication in *Aedes aegypti* mosquitoes. Although its effectiveness is well supported by empirical evidence, the underlying molecular mechanisms remain incompletely understood. Current findings indicate that *Wolbachia*-mediated inhibition of DENV is multifactorial, involving interactions with the endoplasmic reticulum, competition for cellular nutrients, suppression of pro-viral signalling pathways, regulation of host miRNAs, stimulation of mosquito immune responses, and enhancement of viral RNA degradation. These complex and synergistic mechanisms highlight *Wolbachia*'s potential not only as a tool for vector control but also as a foundation for developing more effective strategies in dengue prevention and management.

***Keywords**: Wolbachia, Aedes aegypti, Virus replication, Dengue*

INTRODUCTION

Dengue hemorrhagic fever (DHF) is still a serious threat to public health in Indonesia, which is one of the endemic countries. Based on data from the Indonesia Ministry of Health, dengue fever (DF) cases have shown a significant increase in the last two years. In 2023, there were 114,720 reported DHF cases with 894 deaths, reflecting a case fatality rate (CFR) of about 0.78%. By week 43 of 2024, the situation had worsened significantly, with 210,644 cases—an 83.6% increase compared to 2023—and 1,239 deaths, corresponding to a CFR of 0.59%. These cases were spread across 259 districts/cities in 32 provinces.[1] Additionally, more than 624,000 suspected DHF cases were reported through the Early Warning and Response System (Sistem Kewaspadaan Dini dan Respons, SKDR), indicating a much broader potential burden. At the regional level, the ASEAN region also continues to face a high burden, with over 219,000 cases and 774 deaths, giving a CFR of around 0.35%. Within ASEAN, Indonesia accounts for the largest share of cases, making it the most affected country in the region his spike in cases generally occurs during the rainy season.[2] Climate change has resulted in shifts in extreme weather patterns, including increased rainfall and temperatures in various tropical regions, including Indonesia. This condition creates an ideal environment for the breeding of *Aedes aegypti* mosquitoes, the main vector of dengue virus.

Dengue virus (DENV) is transmitted through the bite of *Aedes aegypti* and *Aedes albopictus* mosquitoes. Dengue virus infection can cause mild to severe symptoms, and secondary infections with different serotypes can lead to fatal complications such as severe dengue, shock, and organ failure. Each year, severe dengue can account for approximately 25,000 deaths.[3]

There are currently no antiviral drugs available to treat DENV infection. The FDA-approved dengue vaccine (Dengvaxia), which consists of tetravalent live attenuated virus, is of limited use.[3] In the absence of a widely effective therapy or vaccine, current disease control efforts rely heavily on conventional strategies. Most approaches focus on suppressing mosquito populations through nest eradication and the use of insecticides and

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larvicides.[4] However, the increase of insecticide resistance in *Aedes aegypti* populations and the continued occurrence of dengue outbreaks show that this approach has not been effective. This has led to biotechnology-based innovations, such as the release of *Aedes aegypti* mosquitoes infected with *Wolbachia* bacteria.

Stable trans infection of *Aedes aegypti* with several *Wolbachia* strains has been shown to confer resistance to generalized infection by dengue virus and other arboviruses.[5] A number of laboratory studies, field observations, and controlled trials in several countries including Indonesia have shown that *Wolbachia* infection can reduce DENV replication and the risk of transmission to humans.[6] *Wolbachia* is known to have various mechanisms of action in suppressing DENV infection, one of which is by inhibiting DENV replication in the body of the *Aedes aegypti* mosquito. The main advantage of this strategy is its ability to keep mosquitoes from transmitting the virus even though their population numbers do not decrease drastically. However, the molecular mechanism underlying *Wolbachia*'s ability to suppress DENV replication is not yet clearly understood and remains an area of active research. Therefore, there is a need for a literature review that summarizes and analyzes various hypotheses regarding the mechanism of action of *Wolbachia* in inhibiting virus replication, to provide a more complete picture of the direction and development of research in this field.

DENV BIOLOGY

Dengue virus (DENV) is an arbovirus of the genus *Flavivirus*, family *Flaviviridae* that is the leading cause of mosquito-borne viral infections worldwide, with an estimated 390 million cases of infection annually.[6] Dengue virus (DENV) consists of four serotypes (DENV1 to DENV4) with a positive single-stranded RNA genome ~11 kilobases long, with one open reading frame that is translated into polyproteins, then processed into structural (C, prM, and E) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) that play an important role in replication and immune evasion.[7] The transmission of this virus is mediated by the bite of female *Aedes aegypti* mosquitoes, which only rely on the mosquito's innate immune system because of the lack of an adaptive immune system in dealing with arbovirus infections.[8]

After the female *Aedes aegypti* mosquito sucks blood from a viremic human, dengue virus (DENV) enters the mosquito body through the digestive tract and infects midgut epithelial cells. The initial infection process begins with the binding of the virus to cell surface receptors and entry via clathrin-mediated endocytosis. Within about two days after blood collection, infection spreads to roughly 30% of epithelial cells. The virus then continues to spread laterally between cells until it reaches the hemolymph and secondary tissues, including the salivary glands.[9] Dengue Virus (DENV) in mosquito cells is highly dependent on manipulation of intracellular metabolism, especially lipid membrane remodeling. The virus triggers changes in the phospholipid profile and increases the unsaturation level of fatty acids, which is important for the formation of vesicle packets (VP) in the endoplasmic reticulum (ER) membrane as the main site of viral RNA replication. Dengue Virus (DENV) non-structural proteins, such as NS3 and NS5, form replication complexes that synthesize genomic (+) RNA from double-stranded RNA (dsRNA) templates. Lipid biosynthesis, especially fatty acid synthase (FAS) enzyme activity, is locally activated by DENV to optimally support vesicle formation and viral replication.[7] The accumulation of viral proteins in the ER due to intense replication will induce reticulum stress, activate the unfolded protein response (UPR), and cause metabolic dysfunction in host cells. Viruses also utilize host lipids, such as cholesterol and phospholipids to make replication membranes, and target autophagy pathways.[7,9]

The formation of infectious virions in the mosquito body not only depends on RNA replication, but also requires the ubiquitin-proteasome pathway (UPP). Inhibition of UPP was shown not to affect the amount of viral RNA synthesized, but significantly decreased the number of infectious virus particles, indicating that UPP is important in the virion maturation or assembly stage.[10]

MOSQUITO INNATE IMMUNE RESPONSES TO DENV

While DENV manipulates host metabolism to replicate efficiently, mosquitoes are not passive hosts. Instead, they rely on a range of innate immune mechanisms to restrict viral infection. On the other hand, mosquitoes have various molecular defense mechanisms against DENV infection. On the other hand, mosquitoes have various molecular defense mechanisms against DENV infection.

The antiviral state induced by *Wolbachia* is a culmination of multiple synergistic mechanisms. While metabolic competition for cellular resources forms a foundational barrier to DENV replication, the activation of the host's immune pathways provides a critical second layer of defense. One of immune responses in dengue is the activation of the Delta-Notch signaling pathway that triggers DNA endoreplication in midgut cells, increases antiviral gene expression and strengthens local immune responses.[8] Other immune pathways such as RNA interference (RNAi), Toll, Immune Deficiency (IMD), and Janus kinase/Signal Transducers, and Activators of Transcription (JAK/STAT) also play an important role in limiting viral replication through the production of antimicrobial peptides and the induction of apoptosis.[11] Among these immune mechanisms, the Toll pathway is frequently cited as one of the most dominant and consistently upregulated immune responses in *Wolbachia*-infected *Aedes aegypti* mosquitoes, playing a non-redundant role in limiting DENV infection.[12,13] However, the relative importance of each pathway can be context-dependent, varying with the *Wolbachia* strain, mosquito tissue, and viral serotype. For instance, the RNAi pathway remains the mosquito's most potent innate antiviral defense against arboviruses generally, and evidence suggests Wolbachia may enhance its efficiency, while the IMD pathway is also strongly implicated in defense against DENV.[14,15] Therefore, rather than a single dominant immune pathway, it is the concerted and often synergistic upregulation of this suite of immune responses that creates a robust antiviral environment. After successfully bypassing these defenses, the virus migrates to the salivary glands within 7–10 days post-infection. At this stage, mature virions are secreted through mosquito saliva, enabling transmission back to humans.[10]

WOLBACHIA BIOLOGY

Wolbachia is a Gram-negative bacterium that is endosymbiont and lives inside its host cell. It is maternally inherited and is able to persist in host populations by manipulating the reproductive biology of its host. Some insects carrying *Wolbachia* are known to have protection against viral pathogen infection.[16] In addition, *Wolbachia* has also been shown to be effective in inhibiting the replication of various arboviruses, including DENV.[17] This ability is then utilized in biological-based vector control strategies, with the aim of reducing the transmission capacity of mosquitoes as disease vectors. Several strains of *Wolbachia* have been identified as antiviral and non-antiviral, among them, *w*Mel and *w*AlbB strains are the most widely used as biocontrol tools as they both exhibit strong antiviral effects.[18,19]

Wolbachia is naturally found in many insects, but not in *Aedes aegypti* mosquitoes. However, *Wolbachia* can be artificially introduced into *Aedes aegypti* mosquitoes through intrathoracic injection technique, which is a method of direct injection into the mosquito thorax using the Nanoject II device. This method allows consistent and controlled dosing of bacteria or viruses, compared to the oral method (natural infection) which tends to result in high variability infection rates.[16]

The use of *Wolbachia* in inhibiting DENV replication in *Aedes aegypti* mosquitoes has been phenotypically proven, but in-depth understanding of its molecular mechanism is still evolving. The symbiotic interaction between *Wolbachia* and the mosquito host is highly complex and multifactorial, involving various interrelated physiological changes. The inhibitory mechanism does not rely on a single pathway, but through several strategies that work synergistically.[17,20]

MOLECULAR MECHANISMS OF WOLBACHIA INHIBITION ON DENV REPLICATION

Wolbachia Interacting With Vector Endoplasmic Reticulum Prevents DENV From Replicating

Dengue virus (DENV) replicates by utilizing endoplasmic reticulum (ER) organelles in host cells to form replicative vesicle structures (viral packets). This structure is important as it is the site of RNA synthesis and the formation of new viral particles. The presence of *Wolbachia* antiviral strains such as *w*Mel and *w*AlbB has a significant impact on the replication process of DENV by targeting the same area, the ER.[7,9] The interaction between *Wolbachia* and cell organelles in *Aedes aegypti* can be observed using transmission electron microscope (TEM) which shows that about 50% of *w*Mel and *w*AlbB are directly attached to the ER and 20% of the rest are wrapped by the ER, while other strains that do not have antiviral effects are found to be scattered freely in the cytoplasm without special contact with the ER, [17] using special staining showing that *Wolbachia* with antiviral strains appear to collocate with ER membrane.[9]

Although *Wolbachia* and DENV did not physically compete for space, the presence of *Wolbachia* attached to the ER was thought to cause changes in the chemical environment in the ER, thus altering the composition of the ER. These changes prevented the virus from forming replication complexes optimally. This was observed in *Aedes aegypti* mosquito cells infected with *Wolbachia* *w*Mel, where no replication vesicles were found at all when infected with DENV. Whereas in cells infected with *w*AlbB, DENV replication vesicles appeared in very small numbers and were imperfectly shaped. In contrast, cells infected with the non-antiviral strain of *Wolbachia* had similar results to cells without *Wolbachia*, where vesicles were formed in large numbers indicating that the replication process was active. This fact indicates that *Wolbachia* strains with antiviral effects can inhibit viral replication through interactions with ERs, preventing DENV from using ERs and disrupting the function of these organelles in supporting viral replication.[21]

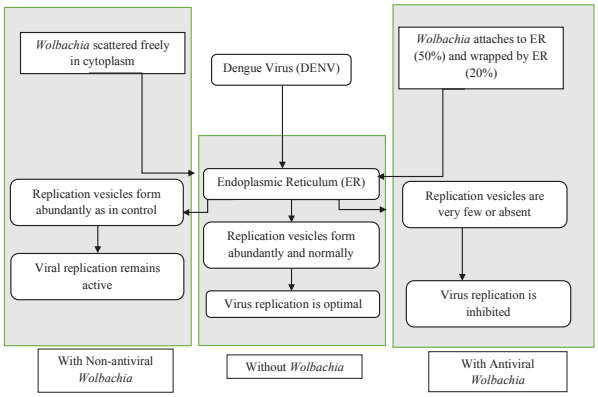

Figure 1. Effects of *Wolbachia* on DENV replication in *Aedes aegypti* cells, showing differences in ER–derived vesicle formation under antiviral, non-antiviral, and no *Wolbachia* conditions.

Wolbachia Modifies Vector Lipid Metabolism in Disrupting DENV Resources

In addition to disrupting the function of ER in DENV replication, *Wolbachia* antiviral strain also modifies lipid metabolism in host mosquito cells as a strategy to inhibit viral replication. It is known that DENV is highly dependent on lipids, such as sphingomyelin, phospholipids (cardiolipin), cholesterol and acyl-carnitine, to construct new viral membranes and fulfill its energy needs.[22] Under normal conditions, DENV infection causes an increase in these lipids, to support the replication process. However, when *Aedes aegypti* mosquitoes are infected with *Wolbachia* especially *w*Mel and *w*AlbB, the lipid profile in the cell changes. Several types of lipids that usually increase during viral infection actually decrease when *Wolbachia* is present, resulting in the virus lacking raw materials and viral replication being disrupted.[7,16]

The metabolic modification effect of *Wolbachia* also stimulates the formation of lipid droplets (LDs) in mosquito cells, which are small fat droplets in the cytoplasm that store fat and energy. The study showed that the number and size of LDs increased significantly in cells containing *Wolbachia* antiviral. When DENV infects these cells, LD formation increases further, indicating that LDs are part of the cell's antiviral response induced by *Wolbachia*.[9] To test the role of LD directly, the researchers used the drug C75 (an inhibitor of LD

synthesis) in *Wolbachia*-containing cells, which was administered with the aim of inhibiting the LD-forming enzyme. As a result, the amount of LD decreased, and the DENV was able to reproduce despite the presence of *Wolbachia*.[9] This suggests that LD is a necessary component in the antiviral effect mediated by *Wolbachia*.

Wolbachia affects the expression of metabolic genes such as decreased expression of insulin receptor and acyl-carnitine levels, which contribute to decreased fat storage and utilization by mosquito cells. As a result, the virus loses access to energy sources and essential raw materials needed to reconstitute itself.[22] Both *Wolbachia* and the virus are unable to make their own lipids and are highly dependent on host cell lipids, leading to competition for resources between the two, but more so, it appears that *Wolbachia* and DENV interfere with vector lipid metabolism in opposite ways.[9,22] Thus, *Wolbachia*-induced changes in lipid metabolism, mainly in the form of specific lipid reduction and LD formation, become a double barrier to the ability of DENV to replicate efficiently in the host cell.

Wolbachia Surface Protein (WSP) Suppresses Pro-Viral Serine/Threonine Kinase (STK)

Wolbachia also inhibits DENV replication through direct interaction between its secreted proteins and host proteins that support the viral replication process. The protein is *Wolbachia* Surface Protein (WSP) which interacts with *Aedes aegypti*'s Serine/Threonine Kinase (STK) protein.[17] STK is a regulatory protein involved in cellular signaling pathways and has been shown to play a role in supporting DENV replication. *STK* gene expression increased in mosquito cells separately infected with DENV and *Wolbachia*. However, its expression was significantly suppressed in cells infected with DENV after *Wolbachia* infection.[17] Silencing of *STK* gene through RNA interference approach leads to a decrease in the number of replicated viruses, while activation of *STK* expression through RNA activation leads to an increase in viral replication. This effect was also confirmed in an *in vivo* assay, where suppression of *STK* expression in live mosquitoes decreased the viral replication rate.[17,23]

WSP directly inhibits STK function through molecular interactions, thereby reducing the availability of STK to the virus. Thus, *Wolbachia* creates unfavorable intracellular conditions for DENV to proliferate. This intervention is specific and efficient as it targets host factors that are essential for viral replication. Therefore, WSP functions not only as structural protein, but also as effector molecule that performs antiviral functions through inhibition of host signaling elements.

Molecular Regulation of miRNAs Amplifies Mosquito Antiviral Immune Responses to DENV

Wolbachia infection of *Aedes aegypti* triggers regulation of host microRNA (miRNA) expression that plays an important role in inhibiting DENV replication. One of the main mechanisms identified is the activation of the cytoplasmic miRNA biogenesis pathway that produces specific miRNAs to suppress viral replication pathways. *Wolbachia* is known to induce the expression of aae-miR-2940, which functions to suppress the expression of *AaDnmt2* gene, a host gene that supports the stability of the viral genome and tends to increase during DENV infection. In addition, aae-miR-2940 increases the expression of metalloprotease genes *m41 ftsh* and *arginine methyltransferase 3 (AaArgM3)*, which play a role in maintaining *Wolbachia* density and disrupting the intracellular environment required for the virus to replicate.[22]

Wolbachia also modulates the expression of other miRNAs such as aae-miR-12 and aae-miR-981. The miRNA aae-miR-12 targets the *MCT1* gene, which is involved in the autophagy process, while aae-miR-981 suppresses the *importin β-4* gene that functions in intracellular transport. Suppression of expression of these genes inhibits the pathways used by the virus for infection and duplication.[19,20] In addition, *Wolbachia* also increases the expression of aae-miR-34-3p, which acts as an activator of the Toll immune pathway through increased expression of *MyD88*, an important adaptor protein in insect innate immune responses. Activation of this pathway significantly increases the expression of antimicrobial peptide genes such as *Defensin A (DEFA)*, *Defensin E (DEFE)*, *Cecropin D (CECD)*, *Cecropin E (CECE)*, *Cecropin F (CECF)*, and *Cecropin N (CECN)*.

These peptides have direct antiviral activity against DENV, thus contributing to enhancing the mosquito defense system.[22,24] Suppression of aae-lnc-2268 long noncoding RNA expression by *Wolbachia* led to increased production of aae-miR-34-3p. Overexpression of aae-miR-34-3p was shown to strengthen the protective effect of *Wolbachia* against DENV infection, while its decreased expression weakened the effect. This confirms that artificially strengthening the expression of aae-miR-34-3p can optimize viral inhibition to the point of total elimination. Through this mechanism, *Wolbachia* creates a direct molecular link between miRNA regulation and activation of the mosquito immune system, making miRNAs a controller of virus replication.[24]

Wolbachia also produces specific miRNAs, such as WsRNA-46, which increase the expression of dynein protein in host cells.[22] Dynein is a motor protein involved in the transportation of vesicles and intracellular particles along microtubules. Increased dynein activity creates two mechanisms of virus inhibition: first, *Wolbachia* absorbs dynein usage for its own use, thereby reducing the accessibility of this protein for virus particles; second, altered microtubule dynamics due to dynein dominance disrupts DENV transport to the replicative site. As a result, DENV fails to reach the site for genome multiplication, and eventually the replication process is halted.[22,24] Thus, *Wolbachia* creates competitive conditions for intracellular resources required by the virus, while initiating miRNA-based immune responses that strengthen mosquito resistance to DENV infection.

Wolbachia RNase HI Contributes to Blocking DENV Replication

wRNase HI, a type I ribonuclease H enzyme encoded by *Wolbachia*, plays an important role in inhibiting the replication of RNA viruses such as DENV through a specific molecular degradation mechanism. wRNase HI selectively targets and cleaves hybrid RNA-DNA molecules, intermediate structures formed during viral replication. In positive-sense RNA viruses such as DENV, replication involves the synthesis of negative-strand RNA as a template to generate a new viral genome. This process can produce hybrid RNA-DNA intermediates that are formed when viral RNA (+ssRNA) interacts with host DNA due to the activation of retrotransposon elements, at this stage, wRNase HI will recognize and degrade viral RNA molecules bound to host DNA (RNA-DNA hybrid), thus disrupting viral RNA synthesis.[25]

The expression of wRNase HI increased rapidly within 30 minutes after DENV infection, while the mosquito's natural RNase HI did not respond, proving that the viral inhibition originated from *Wolbachia*, not the host. When wRNase HI expression was reduced using antisense RNA, DENV replication increased sharply, but *Wolbachia* density and host gene expression were not compromised. In contrast, when wRNase HI was artificially expressed in mosquito cells, DENV RNA levels and virus particles decreased significantly without affecting host genes such as *actin* or mosquito *RNase H1*. This effect was consistent across *Wolbachia* strains, but did not apply to negative-sense RNA viruses such as *Phasi Charoen-like* virus (PCLV), suggesting enzyme specificity to certain viruses.[25] The complexity of this interaction suggests that *Wolbachia* utilizes various aspects of mosquito physiology to create an unfavorable environment for DENV replication. The resume of the *Wolbachia* mechanisms in the cell explained in Figure 2.

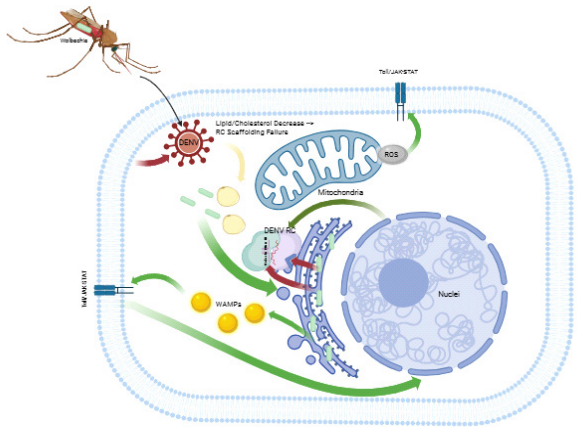

Figure 2. This schematic illustrates the multifaceted strategies employed by *Wolbachia* to suppress DENV replication within the *Aedes aegypti* mosquito host cell. Three primary, interconnected mechanisms contribute to the antiviral state. Green arrow showed the immune priming mechanism, yellow arrow showed resource competition and red arrow showed the direct interference.

FIELD EFFECTIVENESS, SUSTAINABILITY, AND SOCIETAL IMPACT OF THE WOLBACHIA STRATEGY

Despite the overwhelming success in reducing dengue incidence across field trials in Yogyakarta and Townsville—demonstrating significant public health and economic benefits—our understanding of the underpinning mechanisms remains incomplete. The current knowledge, summarized in the preceding sections, focuses on the downstream consequences of *Wolbachia* infection. However, to develop more robust and universally effective strains, the remaining gaps must be addressed at a deeper molecular level. Specifically, future research must focus on clarifying the precise molecular pathways and effectors, as discussed below.

The intervention of *Wolbachia*-infected *Aedes aegypti* mosquitoes has significantly reduced dengue virus transmission. A randomized controlled trial in Yogyakarta, Indonesia, showed that dengue incidence decreased by 77% after the deployment of *Wolbachia*-infected mosquitoes in the community compared to areas without intervention. This effect indicates a potential spillover effect to surrounding areas, not only limited to protecting the release area.[26,27] Similar findings were also observed in Townsville, Australia, where despite an increase in imported dengue cases from overseas, local spread did not occur, which means *Wolbachia* is effective in breaking the chain of transmission locally.[28]

Not only that, the use of *Wolbachia* also provides a sustainable impact in dengue control, not only effective immediately, but also has promising long-term potential. The proportion of *Wolbachia*-infected mosquitoes remains high in the community years after the initial intervention, suggesting that the spread is stable without the need for repeated releases in the short term.[27] Mathematical models estimate that to maintain maximum effectiveness, at least 75% of the mosquito population must carry *Wolbachia*.[19] In this context, the resilience of *Wolbachia* to environmental stresses such as tropical temperatures and seasonal changes is a key factor, and field data suggest that strains such as *w*Mel are quite stable under such conditions.

From an economic perspective, the use of *Wolbachia* is considered efficient and has the potential to reduce the burden of public health costs. Studies in Vietnam showed that although the initial costs for mosquito production, release and monitoring were considerable, the investment resulted in substantial savings in the medium to long term.[19] Dengue treatment costs, national vector control efforts, and losses due to work absenteeism can be significantly reduced. In the context of developing countries with high dengue burden, this cost efficiency is a strong argument for widespread adoption of *Wolbachia* program.

Community acceptance and safety factors are also important to consider in the implementation of this strategy. To date, there is no evidence that *Wolbachia*-infected mosquitoes pose a direct risk to human health.[29] The bacteria are not infectious to humans and do not change the behavior of mosquitoes to become more aggressive. This approach is considered more environmentally friendly than chemical insecticides. Furthermore, the involvement of local communities in every stage of the program from education, training, to monitoring, has been proven to increase community trust and participation. With the combination of high effectiveness, sustainability, cost-efficiency and safety, *Wolbachia*-based strategies appear promising as one of the most impactful innovations in modern dengue control.

Although *Wolbachia*-based approaches have shown promising success in reducing dengue incidence, studies related to the use of *Wolbachia* are still very diverse based on the types of strains that continue to evolve, some studies currently focus on strains such as *w*Mel and *w*AlbB. from this variety of strains, it is still unclear which strain is most consistently effective in various conditions. The differences in effectiveness, stability, and ecological impact of each strain are still debatable, especially because local environmental factors can affect the performance of each strain. In addition, the ongoing dynamics of DENV evolution also add to the complexity in determining the most optimal strategy. This indicates the need for more in-depth studies to identify the most suitable strains to be applied in various geographical and ecological conditions, both in terms of biological effectiveness and sustainability of implementation.

CONCLUSION

The mechanisms by which *Wolbachia* inhibits DENV replication are multifactorial and synergistic. However, within this complex interplay, metabolic competition, particularly the sequestration of essential host lipids, is widely regarded as the most dominant and fundamental mechanism. This competition creates a foundational bottleneck by starving the virus of the crucial resources it needs to build its replication complexes, thereby amplifying the effectiveness of other pathways. Meanwhile, the role of the primed innate immune response (e.g., Toll and IMD pathways), while a powerful reinforcing mechanism, remains the most debated area; its absolute requirement is uncertain as viral blocking can still occur in some immune-compromised cells. Therefore, it can be concluded that resource competition is the central mechanism, while the precise contribution of innate immunity continues to be an active area of research. Although the effectiveness of this approach has been demonstrated in various field studies, the variation among *Wolbachia* strains and the evolutionary dynamics of DENV remain a challenge. Therefore, more in-depth studies are still needed to optimize *Wolbachia*-based dengue control strategies in a sustainable and context-specific manner, ensuring their long-term success against the evolving landscape of arbovirus transmission.

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