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Wolbachia: The selfish Trojan Horse in dengue control

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ABSTRACT

Dengue fever has re-emerged as a major public health challenge. Of late, several promising attempts have been made to control the disease with limited success. An innovative method of biological control of dengue is the use of the bacterium *Wolbachia*. Selected strains of *Wolbachia* have been introduced into *Aedes aegypti* to prevent transmission of dengue viruses by the vector. *Wolbachia* prevents dengue transmission by either directly blocking the virus or by decreasing the lifespan of the vector. The mechanism by which it causes these effects is not clearly understood. The main concern of this technique is the emergence of a new dengue virus serotype which may evade the protection offered by *Wolbachia*. The technique is environment friendly and holds promise for control of other vector borne diseases.

Introduction

Dengue fever has re-emerged as a major public health challenge worldwide; with 2.5 billion people at risk of infection, more than 100 million cases and 25,000 deaths being reported annually.[1] As there is no licensed vaccine or specific treatment against dengue, preventive measures are the best strategy, which consist mainly of environmental management, chemical control, and personal protective measures. However, such measures have met with limited success due to poor/improper implementation.

Besides, as the *Aedes* mosquito is a day biter, individual protection using bednets is easier to preach than bringing into practice, thus accentuating the need for alternate options in dengue control.

Recently, there have been several promising new attempts to control dengue. However, the much awaited vaccine trial in

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Thailand did not meet the expectations; providing only 30% overall effectiveness, though it did provide higher coverage for three of the four dengue virus serotypes.[2]

Biological methods have been used recently with some success. An innovative promising method of biological control of dengue is the use of the bacterium *Wolbachia*.

The novel technique

The new technique is based on the premise that the symbiotic bacterium *Wolbachia* easily infects a wide range of invertebrate hosts including crabs, mites, insects and filarial nematodes. The infection is so common that almost 75% of all arthropod species are infected. Meta-analysis of earlier studies based on a variety of sampling techniques has revealed that out of 3500 mosquito species, 85 have been screened for *Wolbachia*. Of these 85 species, 31.4% have been found to be infected.[3] *Wolbachia* does not infect *Aedes aegypti* in the native form, though the bacterium infects other species of mosquitoes such as *Culex pipiens* and *Aedes albopictus* naturally.

In this technique, selected strains of *Wolbachia pipientis* have been introduced into *A. aegypti* to prevent transmission of dengue viruses by the vector. These strains were initially detected in a laboratory population of the common fruitfly, *Drosophila melanogaster*; wherein it protected the vector from certain RNA viruses.[4] Since the dengue virus also has an RNA genome, the possibility of using *Wolbachia* for protection of *Aedes* mosquitoes from infection with dengue viruses has been explored. The infection is passed vertically from the female mosquito to the offspring transovarially. *Wolbachia* thus introduced are capable of spreading rapidly in wild *A. aegypti* populations.

Why *Wolbachia*?

As a symbiont, *Wolbachia* has several useful effects; thereby making it an ideal candidate for dengue control. The bacterium increases the vigour of the female *Aedes* mosquito thereby enabling the infection to spread to virtual fixation. This is the reason why *Wolbachia* is often referred to as the selfish bacterium. Besides, as *Wolbachia* infects a wide variety of hosts, one strain of the bacterium isolated from a particular species may be introduced into another species. In addition, *Wolbachia* produces a range of effects that can be exploited for disease control.

Mechanism of action

Meta-analysis of studies conducted earlier[4–6] have shown that *Wolbachia* negates the capability of the vector to transmit disease through biological action by either directly blocking transmission of the virus or by decreasing the life span of the vector; thereby nipping the viral infection prematurely. As *Wolbachia* cannot be cultured in vitro, the mechanism by which it causes these effects is poorly understood.

A. albopictus inherits *Wolbachia* infection maternally. In male *A. albopictus* mosquitoes naturally infected with *Wolbachia*, the bacterium modifies the sperm, thereby resulting in failure of karyogamy after fertilisation, leading to death of the embryo. If the *Wolbachia* infected male and infected female mate together, *Wolbachia* retrieves this modification, resulting in normal growth and development of the embryo. Hence, mating between *Wolbachia* infected males and uninfected females will be incompatible and the eggs would not be able to hatch. However, reciprocal mating is compatible, as *Wolbachia* infected female *Aedes* mosquitoes can mate successfully with both *Wolbachia* infected and uninfected males, with the result that all the offspring will have *Wolbachia*. This mechanism of unidirectional cytoplasmic incompatibility[7] provides a disproportionate mating advantage to *Wolbachia* infected females vis-à-vis the uninfected females, thereby promoting maternally inherited *Wolbachia* infection into virgin host populations.

When *A. aegypti* mosquitoes infected with the *wMel* strain of *Wolbachia* were fed with dengue virus contaminated blood along with non-infected mosquitoes, and the degree of dengue infection was analysed after a period of two weeks by quantifying the amount of dengue viral nucleic acid in both groups of mosquitoes. It was observed that the dengue viral nucleic acid in *wMel* – infected mosquitoes was 1500 fold less than in the *Wolbachia* – uninfected mosquitoes; thereby affording protection to the *wMel* – infected mosquitoes against subsequent infection by dengue virus.[8]

Similarly, the mosquito saliva was also analysed for the amount of dengue infection. It was found that dengue was present in only 4.2% of saliva samples taken from the *wMel* – infected mosquitoes vis-à-vis 80.2% of saliva samples taken from non-infected mosquitoes.[8]

The modality through which *wMel* stops the virus from replicating is not very clear. However, there is substantive evidence which suggests that the *Wolbachia* competes with the dengue virus for the limited sub-cellular fatty acid resources required for viral replication.[9]

The blocking achieved by *Wolbachia* is not absolute. *Wolbachia* naturally infects *A. albopictus*, which is a vector for arboviruses including dengue and chikungunya virus. Studies of virus dynamics in *A. albopictus* have shown a decline in *Wolbachia* density as the viral life cycle enters the transmission stage,[10] suggesting the reversal of interference caused by *Wolbachia* with the passage of time.

Protection afforded by *Wolbachia* against the dengue virus depends on the magnitude of *Wolbachia* infection in the mosquito. For example, the *w*AlbB strain of *Wolbachia* does not provide any protection to *A. albopictus* against dengue. However, when *A. aegypti* is infected with the same *w*AlbB strain, it prevents dengue viral infection in *A. aegypti*. The reason for the same is *w*AlbB is capable of surviving in greater numbers in *A. aegypti*, than in *A. albopictus*. This points to the fact that *Wolbachia* infection must reach a minimum critical level before dengue infection can be prevented.

The *wMelPop-CLA* strain of *Wolbachia* shortens the life of the *A. aegypti* mosquitoes. By infecting female mosquitoes with the *Wolbachia* strain *wMelPop-CLA* their lifespan could be reduced to half, thereby eliminating the infected mosquitoes before the virus could reach maturity.

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