Wolbachia Infections and Their Impact on Outbreaks of Yellow Fever Virus

Natalie Bontrager, MPH, School of Public Health, University of Minnesota, Minneapolis, MN

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Abstract

Yellow Fever Virus is responsible for 30,000 human deaths annually in the equatorial regions of Africa and South America. Due to frequent urban outbreaks and a shortage of Yellow Fever vaccines, new control measures are necessary to protect the health of humans. This paper is a review of current research regarding the potential use of *Wolbachia* bacteria as a biocontrol for the largest human vector of Yellow Fever Virus, the *Aedes aegypti* mosquito. *Wolbachia* has been shown to be effective in preventing transmission of other RNA Flaviviridae diseases such as Zika Virus, Dengue Fever and Chikungunya. Due to the cost-effectiveness and ease of implementing *Wolbachia* infections into *Aedes aegypti* populations it is a recommended control effort against Yellow Fever Virus transmission.

Introduction

Yellow Fever Virus

Yellow Fever Virus (YFV) is a flavivirus transmitted by the *Aedes aegypti* mosquitos to non-human primates (NHP) in a sylvatic, or jungle, cycle of transmission versus an urban cycle of transmission. During the sylvatic cycle, humans encounter mosquitoes in the jungle and are infected with Yellow Fever (YFV). Despite having a successful vaccine for the past 70 years, YFV continues to be endemic in some South American countries. YFV cases are a major concern because the lethality of the haemorrhagic fever is 20–50% in humans and has been the cause of 30,000 human deaths annually in the equatorial regions of Africa and South America [1] [2]. Urban outbreaks in the past decade indicate that the sylvatic nature of the disease may be increasing transmission risk in the environment to humans. It is known that rainfall, temperature, humidity, and altitude are correlated with YFV outbreak [3]. Land-use changes and deforestation are also associated with YFV cases, typically occurring in agricultural and cattle grazing areas [4]. There was an increased rate of YFV in Peru in the 1990s that was attributed to humans migrating from coastal and mountainous regions to the Amazon provinces [5]. This is an instance where individuals are moving from low-prevalence areas that have low vaccination rates to high-risk areas. Vaccination programs in Peru against YFV have aimed to address this risk, along with mosquito control efforts such as netting and spraying [5].

Wolbachia

Wolbachia is an alpha-protobacterium that was first identified in Culex mosquitoes in 1924, and is thought to infect upwards of 70% of insects and approximately 28% of mosquito species [6]. *Wolbachia* has been shown to be effective in preventing transmission of other RNA Flaviviridae diseases such as Zika Virus, Dengue Fever and Chikungunya Virus[1], [7]–[9]. *Wolbachia* reduces virus transmission from mosquitos to humans or NHP through maternal transmission within mosquitoes. The bacteria transmit to offspring if *Wolbachia*-infected females mate with an uninfected male or a male infected with a compatible Wolbachia type [10]. Because of the high lethality of YFV in humans, new and novel methods for vector control are necessary to maximize human health. The objective of this paper is to discuss if *Aedes aegypti* mosquitoes infected with *Wolbachia* are a potential biocontrol against YFV transmission.

Main Arguments

Wolbachia-infected mosquitos may be the best candidates for YFV biocontrol in areas that are harder to reach or vaccinate, and they are safer than chemical alternatives [11]. A paper from Kamtchum-Tatuene et.al describes how mosquitos are infected with *Wolbachia* in a lab through a method called transinfection [11]. Since *Aedes aegypti* mosquitoes do not have natural *Wolbachia* infections, they would require this laboratory transinfection to establish colonies. *Wolbachia* would not only inhibit virus transmission within mosquitoes, but it would also reduce the reproductive fitness of *Aedes aegypti* [11].The paper describes how *Wolbachia*-infected female mosquitoes who mate with an uninfected male mosquito produce

infected offspring, and uninfected female mosquitoes who mate with a *Wolbachia*-infected male mosquito produce no offspring. Additionally, if mosquitoes carrying different strains of *Wolbachia* mate, they produce no offspring [11]. Finally, those infected with the same strain of *Wolbachia* will only produce *Wolbachia*-infected offspring. Thus, *Wolbachia* adds a population control measure along with virus disruption within the mosquitoes themselves. [11].

An important feature of *Wolbachia* as a control measure is that it does not transmit from mosquitoes to animals or humans. Because *Wolbachia* is in such a large percentage of insects, humans have been exposed to it for many years without any adverse effects [12]. *Wolbachia* has also not transferred to the environment or animals in any lab trials [12]. Therefore, it is a safer form of vector control than the use of historic pesticides, such as DDT, or other chemical organophosphate efforts that have diminishing effectiveness due to a growing and measurable resistance in *Aedes aegypti* populations [13].

Current prevention efforts through mosquito control and vaccination are not enough in the countries with high prevalence. New prevention efforts need to be developed to control the spread of YFV. Urban outbreaks of the historically sylvatic disease are occurring and could pose a serious threat to densely populated cities [14]. A regular dose of the vaccine confers lifelong immunity and is a successful control measure against YFV [15]. However, because pharmaceutical companies are not making profits from the vaccine, there is a worldwide shortage [16]. As such, the World Health Organization's (WHO) Strategic Advisory Group of Experts (SAGE) decided that fractional doses of 1/5 to 1/10 of the typical dose could be administered in outbreak situations[16]. Yet, this dosage change has not been accepted on regulatory grounds. It is unknown how stable the lower dosage will be, the duration of immunity, or how effective a lower dose is in children [17]. The WHO states that fractional dosing should not be used for routine immunization since the data do not show how long a fractional dose could last [18].

For the following reasons I believe *Wolbachia* infected mosquitoes is the best approach for reducing the incidence of YFV outbreaks. Because of lack of profit, there is currently no commercial incentive to develop a new vaccine and the cost effectiveness of *Wolbachia* outweighs this burden. There are currently only four manufacturers of the YFV vaccine who can make up to 80 million doses per year and creating viable vaccine doses takes time [19]. Doses may take up to 6 months to produce and those doses last only three years. The cost-benefit of continually producing a product that is not always used is difficult for these manufacturers [19].

Since *Wolbachia* has been shown to be effective in preventing transmission of other RNA Flaviviridae diseases such as Zika Virus, Dengue Fever and Chikungunya, there could be a significant reduction in the economic burden diseases inflicted on countries. The benefit to communities far outweighs the cost of infecting mosquitoes in a lab and monitoring their effects. It takes only 10 weeks for a population of *Wolbachia*-infected mosquitoes to establish in the wild [20]. Because *Aedes aegypti* eggs only hatch in ideal environmental conditions it is relatively cost effective and easy to establish colonies by mailing an envelope of eggs to a location for propagation in water later. No special care is required for the eggs i.e. being kept on ice, kept cool, kept wet, etc [20]. *Aedes aegypti* eggs will last up to a year in a dried state, but hatch immediately when submerged in water [21]. A study program conducted in Townsville, Australia from January 2001 – October 2018 states that the cost of undertaking such a program breaks down into 5 major cost categories: 23% community engagement (staff, surveys, overheads), 41% field deployment (staff, transport, mosquito release containers, equipment), 24% monitoring (staff, transport, BGS traps, GIS, supplies), 9% diagnostics (staff, reagents), and 2% production (staff, consumables) [22]. For the four stages of release in Townsville, the cost per person and cost per km^2 are as follows:

Table 3 from the O'Neill study showing Cost per person and cost per km2 for each of the four release stages in Townsville [22].

Cost per person and cost per km ^{2} for each of the four release stages in Townsville.

Stage	Release area km ^{2}	Months required to deploy	Average FTE [1]	Cost per person AUD$	Cost per km ^{2} AUD$
Stage 1	20.3	14	10	29	69,762
Stage 2	18.2	6	12	16	37,268
Stage 3	17.6	4	11	19	23,231
Stage 4	9.7	5	8	13	37,313

[1]Average number of full-time equivalent (FTE) staff used to undertake deployment. It excludes staff required to produce mosquitoes for release or undertake diagnostics.

The O'Neill study further states that the costs could be significantly reduced in denser tropical cities, and that future deployments "should be able to be reduced to less than $1US per person" [22]. Lastly, the costs of using *Wolbachia* as an intervention would not be ongoing because it would remain maintained in the mosquito populations [22]. To contrast these costs, the total annual costs for dengue alone, in Australia, are about $2.7 million US per year since 199 0[23].

Potential Limitations

A study conducted by K. N. Johnson showed that efforts to utilize *Wolbachia* to reduce vector biting-rate drastically reduced disease transmission in Dengue and Zika, however scientists have argued that *Wolbachia* should not be used because the mechanism is unknown [10]. Johnson states, while it is true that *Wolbachia's* mechanism is currently unclear, it has been shown that it likely targets the mosquito's immune genes, or that the interference is due to competition for cell components. It is also known that insects require cholesterol and fatty acids in their diet [10]. Flavivirus and Alphavirus rely on cholesterol for replication, thus *Wolbachia* may be competing with the viruses for resources within the host insect [10].

Despite a finding that *Wolbachia* decreased viral transmission in *Aedes* and *Haemogogus* mosquitoes, it increases viral transmission within *Culex tarsalis* mosquitoes[10]. However, a further study concluded *Culex tarsalis* were only transiently infected with *Wolbachia* "via artificial micro-injection and may not be representative of insects with tissue infections" that contain YFV [24]. Ethical concerns regarding this bioengineering of mosquitoes should be considered thoughtfully and carefully. Members of the public and community who are not involved in a study trial area need to be protected from the negative impacts of potential Culex bites if viral transmission could occur [25].

Conclusion

This paper makes an argument for the use of *Wolbachia* bacteria as a novel method for YFV disease control. Using *Wolbachia* infected mosquitos as a biocontrol for viruses such as YFV reduces infection rates, health impacts on humans and NHP, and reduce the economic toll in endemic countries[1]. This bioengineering control method is currently being used successfully for Dengue and Zika control and works without proven health or environmental risks[6]. Future research should focus on how geographic regions impact cases of YFV and if specific high-risk regions be targeted with *Wolbachia* infected mosquitos without spread beyond these areas.

Author Contact Information

Natalie Bontrager: brownn@umn.edu

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