24 The Use of *Wolbachia* by the World Mosquito Program to Interrupt Transmission of *Aedes aegypti* Transmitted Viruses

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Abstract

The biological control of mosquito transmission by the use of the naturally occurring insect-specific bacterial endosymbiont Wolbachia has been successfully tested in small field trials. The approach has been translated successfully to larger field sites in Townsville, Australia and expanded to more than 10 countries through the Eliminate Dengue Program. The broader application of the program beyond limiting the transmission of dengue and including other *Aedes aegypti* borne mosquitoes has seen the program growing into a global not-for-profit initiative to be known as the World Mosquito Program.

Keywords

World Mosquito Program · Biological mosquito control · Cytoplasmic Incompatibility · wMel *Wolbachia* strain · Randomised control cluster trial

Wolbachia is a naturally occurring bacterial endosymbiont of insects that is estimated to occur in up to 40–60% of all insect species [6, 14]. It has been of interest to basic biologists for many years due to the unusual ways it manipulates host insect reproduction to ensure its efficient transmission into populations. *Wolbachia* is not infectious but instead is maternally inherited through the insect egg cytoplasm. It has evolved mechanisms to transmit itself very efficiently into host populations by either directly or indirectly favouring female insects that carry Wolbachia to leave behind more offspring than uninfected counterparts [12]. One of the best studied of these mechanisms is cytoplasmic incompatibility (CI) in which embryonic development is arrested in *Wolbachia* uninfected embryos that are fertilised by sperm that have matured in the presence of *Wolbachia* (Fig. 24.1), or in embryos fertilized by sperm matured in the presence of a different strain of *Wolbachia* than in the female egg.

The World Mosquito Program (WMP), farmerly known as the Eliminate Dengue Program is a non-profit research consortium operating in a number of countries www.worldmosquitoprogram.org (Fig. 24.2). It aims to develop Wolbachia as an intervention to control mosquito-transmitted viruses such as dengue, zika and chikungunya. The key feature of the Eliminate Dengue Program is the intentional release of *Wolbachia*-infected mosquitoes into target areas that will then transmit *Wolbachia* into wild Aedes mosquito populations [7]. CI provides the mechanism by which

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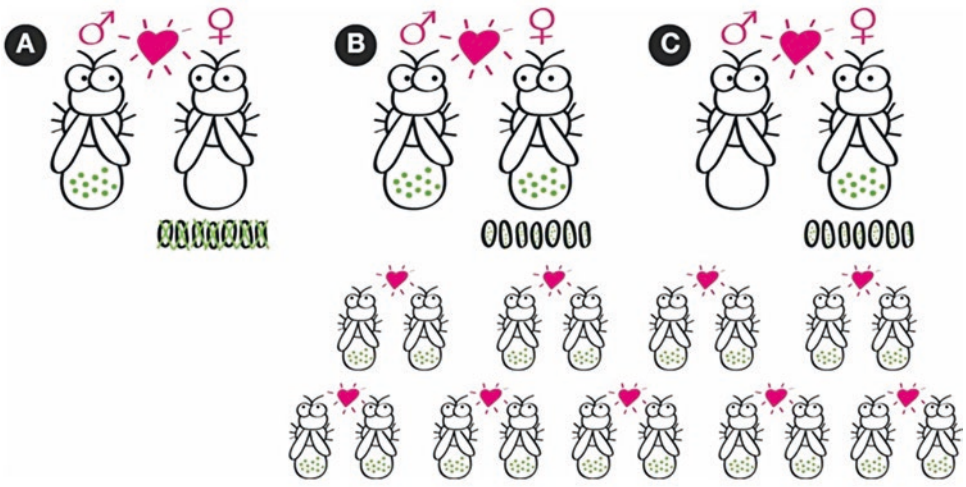

Fig. 24.1 *Wolbachia* infections induce a phenomenon known as cytoplasmic incompatibility in infected hosts that acts as a drive mechanism to push *Wolbachia* into the host populations by indirectly favouring *Wolbachia* infected females. This is done by reducing the reproductive output of *Wolbachia* uninfected females in a population which benefits the maternally transmitted *Wolbachia*

Fig. 24.2 Locations where Eliminate Dengue release activities are being undertaken as of 2016. A number of new sites will be added in 2017

Wolbachia will establish and maintain itself in wild mosquito populations over a number of mosquito generations once released, even if the *Wolbachia* strain places a mild genetic load on the mosquito it infects [8]. Given that *Wolbachia* is quite ubiquitous in the natural environment it is somewhat intuitive that environmental or human health risks associated with its introduction into urban areas should be minimal. This is supported by independent risk analysis [10].

The key attribute of *Wolbachia* that the World Mosquito Program is basing its intervention on is its demonstrated ability to interfere with the replication of human pathogens in *Wolbachia* infected *Aedes* mosquitoes. This includes Flaviviruses like dengue, West Nile and Zika [1, 3, 5, 9, 11], Alphaviruses like chikungunya [2] as well as a range of other viruses and parasites. Analysis of dengue blocking data where mosquitoes have been fed on bloods from dengue patients indicates that the establishment of *Wolbachia* in *Aedes aegypti* populations can be predicted to reduce R0 for dengue by more than 70%, which in most epidemiological settings should completely stop local dengue transmission [4].

To implement a World Mosquito Program intervention it is necessary to release *Wolbachia* infected mosquitoes, both male and female, until the local frequency of *Wolbachia* in wild *Aedes aegypti* mosquitoes surpasses an unstable equilibrium point estimated to be less than 0.3 for the wMel strain of *Wolbachia*. Once this unstable equilibrium point is surpassed it is expected that *Wolbachia* will locally establish and if the establishment area is sufficiently large then start to slowly spread out from the release area. This theory has now been tested in five countries where establishment of wMel has been achieved according to these principles. Typically, quite small numbers of mosquitoes need to be released to surpass the unstable equilibrium point. In Northern Australia in the first release experiments undertaken 10 mosquitoes (both male and female) were released per house per week for 10 weeks and this was sufficient to achieve establishment [7]. In other countries longer release periods have been required if target mosquito populations are larger. Despite the need to release females the experience to date has been that most members of a community undergoing releases do not complain of increased biting pressure, presumably because nuisance biting by other species dominates the personal experience of residents.

Once a series of releases has been undertaken *Wolbachia* is expected to then maintain itself in the local population indefinitely under the action of CI. The deployment is predicted to be robust if it becomes successfully established initially and is demonstrated from data from our earliest release sites in Northern Australia where Wolbachia has sustained itself in local mosquito populations at frequencies above 80–90% since establishment from 10 weeks of releases in 2011 (Fig. 24.3). This is an extremely important attribute of the interventions that WMP is undertaking as costs for implementing the intervention are essentially front loaded during releases and then restricted to periodic monitoring. This avoids the need for ongoing expenditure as is the case for other interventions such as vector suppression technologies or vaccines and makes the WMP approach both sustainable and highly cost-effective.

It can be noted from Fig. 24.3 that while Wolbachia maintains itself at a very high frequency in the wild mosquito population it is rarely at complete fixation. We presume that this is a result of some leakiness in maternal transmission rates of *Wolbachia*, possibly through the action of environmental heat in some breeding sites. Of particular note though is that frequencies of Wolbachia of around 80–90% may be more optimal for disease reduction than complete fixation. At lower infection levels we can expect incompatible crosses generated from Wolbachia via the CI mechanism to put downward pressure on mosquito population sizes that should act in concert with the transmission blocking properties of Wolbachia to enhance the effects of pathogen blocking. Even at frequencies of around 80% in populations the effects of reduced vector competence should still have very large impacts on

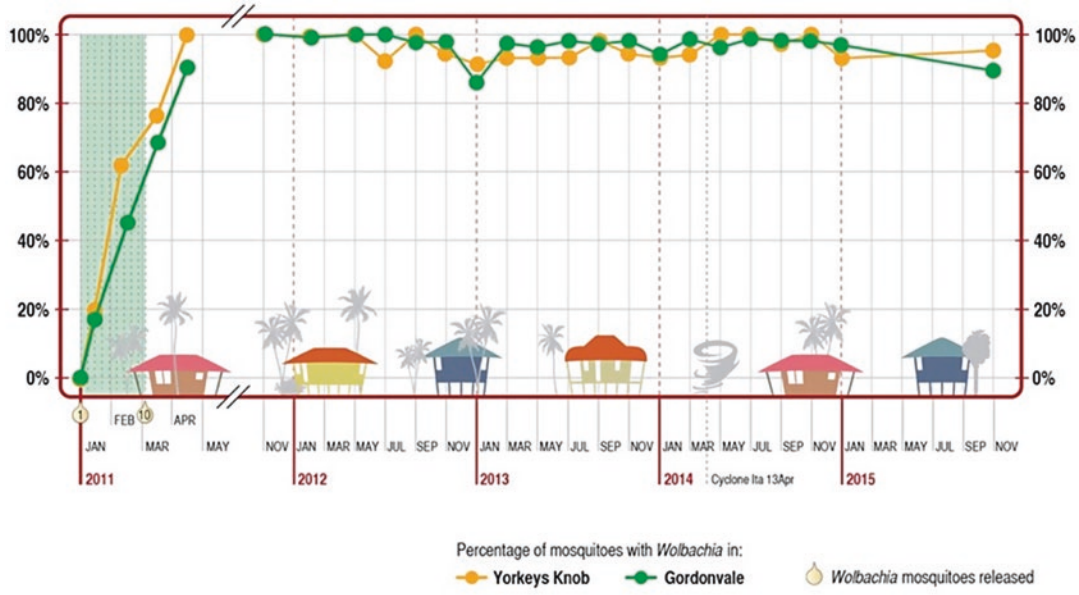

Fig. 24.3 Results of *Wolbachia* monitoring in the first two sites in Northern Australia where wMel releases were undertaken showing the frequency of Wolbachia in the sampled wild mosquito population in both sites. Wolbachia has maintained itself at high frequency since introduction in 2011

transmission, consistent with the observational data gathered so far.

Pilot releases have now been successfully undertaken in five countries including Australia, Indonesia, Vietnam, Brazil and Colombia and indicate that the wMel strain can be deployed successfully in diverse settings both ecologically and culturally. Observational data from these deployments supports large impacts on disease transmission as predicted by modelling. In all areas where *Wolbachia* has now established in these five countries we have not detected any examples of local transmission of dengue to date, defined as clustering of dengue cases in time and space, despite local transmission occurring in neighbouring areas. A key feature of all these deployments is that they have all occurred with strong community support and virtually no opposition. Similarly, there have been no adverse impacts identified in any of these deployments either human related or environmental.

In 2014 the WMP undertook its first scaled release over the entire city of Townsville using a mixture of egg and adult deployments. This scale up required a new form of community engagement requiring community consent rather than individual informed consent. The deployment in Townsville also successfully used community deployments to augment programmatic deployments. Community deployments featured the use of small mosquito release containers supplied with *Wolbachia* mosquito eggs and fish food and required only the addition of water and placement of the container in a suitable shady location for 2–3 weeks until all mosquitoes had emerged. As part of the community release program a targeted program also ran in schools where school students undertook the releases in a citizen science experiment. An area of 95 km^2 was targeted in the city of Townsville (almost the entire city) and the intervention was successfully deployed over three stages in 2.5 years providing the first indications that the method could be scaled effectively over small cities. As per earlier pilot releases there have been no examples of locally transmitted dengue cases in Townsville in any areas where *Wolbachia* has been established at the time of writing.

Within the last 2 years there has been considerable alarm in the international community of

the enormity and difficulty in controlling the South American outbreaks of Zika virus which have now spread to nearly all the countries where dengue transmission occurs. Given the similarity in the ecology of dengue and Zika we can expect ultimately that Zika transmission should co-exist with dengue transmission in the same geographies that have the main transmission vector, *Aedes aegypti*. Since Zika virus is quite closely related to dengue viruses there was an expectation that *Wolbachia* should block Zika transmission in much the same way as dengue viruses and these assumptions have since demonstrated empirically [1, 3]. Indeed the degree of blocking that has been demonstrated for Zika in the laboratory appears stronger if not similar to dengue, which bodes well for using the WMP *Wolbachia* approach to block Zika transmission in the field.

In March 2016 a special advisory group to the WHO made a public recommendation that the Wolbachia interventions being undertaken by WMP should move to pilot deployments over larger scales than previously attempted given the encouraging preliminary evidence for potential impact against Zika [13]. Based on this recommendation two large pilot deployments have commenced in Rio de Janeiro/Niteroi in Brazil and Medellin/Bello in Colombia targeting populations of around 2–2.5 M in each deployment.

At the same time a randomised controlled cluster trial is underway in the city of Yogyakarta which is expected to complete in late 2019 and another randomised trial planned to start in Vietnam by 2018. Together these approaches will provide a basket of evidence to understand the impact of the WMP intervention on arbovirus transmission. The measures include: (1) Laboratory studies showing impaired vector competence, (2) Mathematical modelling predicting large impacts on transmission, (3) Observational time series data capturing before and after impact on dengue cases measured through the existing health surveillance system, (4) Randomised cluster trials and (5) Large pilot deployments over large populations centres.

Over the next 2 years these deployments and measurements of impact will accumulate so that we will have accurate measures of effectiveness. At the same time, we will have learned how to deploy at the scale of very large cities and reduced our costs with a goal of reaching a target of US$1/person protected. If the results on these studies continue to be positive then it will be our goal to collaborate with governments in disease affected areas to make this technology and best practice methods for its deployment available to countries in need.

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