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Original Article

New Vector Control Measures on Dengue Fever: a Literature Review

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Abstract

Dengue fever is a vector-borne infectious disease prevalent in tropical and sub-tropical areas. It is transmitted by *Aedes* mosquito, principally *A. aegypti* and *A. albopictus*. The primary control measure is eliminating the mosquito breeding sites, which suppresses the mosquito density to reduce the risk of dengue fever occurrence. However, because breeding sites can hardly be eliminated and production of new breeding sites is fast, novel technologies and vaccine development should also be incorporated in the program on disease control. According the recent studies have shown that the genetically modified mosquito and rickettsia symbiosis-Wolbachia, has potential for control of *A. aegypti* density and good results in small-scale field studies. The long term effects and impacts on ecology remained uncertain because of the limited study population and relatively short follow-up duration. Hence, continuously educating the public to eliminate the mosquito breeding sites is necessary before dengue vaccines and novel technologies are available.

Keywords: Dengue fever, *A. aegypti*, biological control, genetically modified mosquito

Introduction

Dengue fever (DF) is the most important vector-borne disease caused by Dengue virus, which is mainly transmitted by *A. aegypti* and *A. albopictus*. DF is prevalent in tropical and sub-tropical areas, about 25 billion people (40% of global population) are at risk of getting infection and there are about fifty million cases occurred each year [1]. Because *A. aegypti* usually resides around human residence, and if something interrupts the adult mosquitoes from sucking blood, they leave the original host and bite another one for a full blood meal, they have become the most important vector in DF epidemics [2]. The control measures used in Taiwan, including eliminating the mosquito breeding sites

and distributing larvivorous fishes to prevent mosquito from breeding, focused on reducing the mosquito density and thus decreased the risk of DF occurrence. However, because breeding sites can hardly be eliminated and production of new breeding sites is fast, DF outbreak still occurs every year. Thus, before the dengue vaccines are commercialized and available in the market, novel technologies should be incorporated in the program for control.

Sterile insect technique (SIT) is an eco-friendly and species-specific method of vector control [3]. Briefly, male insects are sterilized using chemicals, radiation or genetic engineering, such as chromosomal translocation. Because female insects which mate with sterile male insects will have no or fewer offspring, releasing large amount of sterile male insects into environment to compete with wild-type male insects will reduce the population of their next generation. Repeated release of sterile insects can eventually wipe out a population, though it is often more useful to consider controlling rather than eradicating it. The technique has been used for more than 50 years and has successfully controlled some vectors, such as Screw-worm fly [4] and Tsetse fly [5]. For DF and Yellow Fever, several sterile insect techniques have been applied on *A. aegypti* [6], but all attempts were failed because the sterile male were probably less competitive or the released amount was too small.

Considering the difficulties that encountered in sterile insect techniques, two strategies, population suppression and population replacement, using the advanced molecular biology and genetic engineering, are under investigation. In population suppression strategy, such as release of insects carrying a dominant lethal gene (RIDL), use the lethal gene to cause their offspring cannot survive. Compared with SIT, female mates with RIDL male can drop eggs capable to hatch and die in the late stage. Their larvae and pupae can compete with wild-type larvae and pupae, compromise the survival and reduce the population of wild-type mosquitoes [7]. Therefore, RIDL can produce a late-acting dominant lethal genetic system. In population replacement strategy, the ability to transmit pathogens is deprived in these genetically modified mosquitoes and their offspring inherit the incapability. Releasing these mosquitoes into fields can gradually replace the wild-type species with new species and thus improve the disease control. For example, *A. aegypti* infected by intra-cellular symbiotic rickettsia, *Wolbachia* spp., will develop immunity against pathogens such as dengue viruses. Because female mosquitoes can transmit the *Wolbachia* spp. to their offspring, the infected mosquitoes resistant to viruses gradually replace wild-type species [7]. Some studies reported another example of population replacement by using a technique called RNA Interference (RNAi). An inverted-repeat RNA sequence is inserted into *A. aegypti* so the mosquitoes will develop resistant to dengue virus serotype 2 (DENV-2). Because their offspring will inherit this immunity, the virus transmission can be controlled [8].

In this article, we reviewed recent literatures about the effects of genetically modified mosquitoes and *Wolbachia* spp. on the control of *A. aegypti*.

Genetically modified mosquitoes

British company Oxitec has successfully created three sterile species of mosquitoes using the technique of RIDL, including *A. aegypti* OX513A, *A. aegypti* OX3604C, and *A. albopictus* OX3688 [9]. Red fluorescence protein was used as a marker of gene selection. In an environment without tetracycline supplementation, female progeny of OX3604C, and OX3688 lose the ability to fly, so they cannot mate, bite, or transmit disease. In the late-acting dominant lethal genetic system, the progeny are not only more susceptible to predators' attack or natural death, but also able to compete resources with wild-type species and compromise their survival. In terms of OX513A, or LA513A [10], a lethal system using tetracycline operator (tetO) - tetracycline-repressible transcription activator protein (tTAV), was applied to control *A. aegypti* [11] as shown in Figure 1.

1. Mechanism

A. aegypti LA513A uses tetO promoter to control downstream tTAV gene, and the overexpression of tTAV will result in mosquito death. If tetracycline was given to larvae of LA513A, the antibiotics taken by the larvae would bind to tTAV so that the tTAV could not bind to tetO promoter and the larvae would survive. Once LA513A was released to natural environment and mate with wild-type *A. aegypti*, their offspring without tetracycline supplementation, tTAV would bind to tetO promoter and express more tTAV which could bind to tetO promoter and then express more tTAV, eventually result in mosquito death. In addition to the lethal system, Oxitec also differentiated between LA513A male and female pupae according size difference. The selected LA513A male pupae grew up to adult mosquitoes were released to achieve further control of wild-type *A. aegypti* .

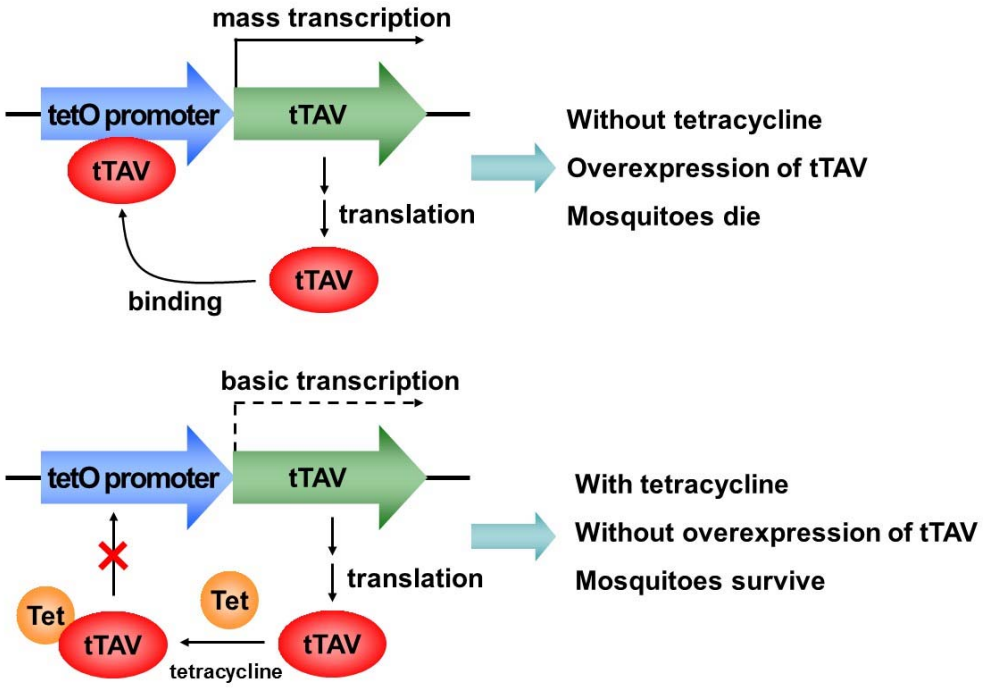

Figure 1.The tetO - tTAV lethal system [11]

2. Performance in the field studies

According to the reports by Science and Development Network on November 11, 2010, Oxitec has released the *A. aegypti* OX513A (the same species as aforementioned LA513A) in Grand Cayman island of Cayman Islands in 2009 [12]. The preliminary results were presented in the 59th annual conference of American Society of Tropical Medicine and Hygiene, ASTMH, on November 4, 2010. That was the first field study of releasing genetically modified mosquitoes -3,000,000 male adult *A. aegypti* OX513A were released to mate with wild-type female *A. aegypti*. Because the progeny could not survive without tetracycline, the population of wild-type *A. aegypti* was significantly reduced by 80% in about one half year. Based on this study, genetically modified mosquitoes should be incorporated with other vector control measures in response to the aggravating DF epidemics.

Rickettsia symbiosis with insects - *Wolbachia* spp.

Wolbachia spp. is an intracellular symbiotic Rickttsia; it can be found in many arthropods. The reproductive system of host insect changes after getting W*olbachia* spp. infection - it develops cytoplasmic incompatibility (CI), parthenogenesis, and feminization [13]. O'Neill et al. isolated the *Wolbachia* spp. (wMelPop) from a fruit fly, *Drosophila melanogaster*. To infect *A. aegypti* with wMelPop, which was subcultured in cell culture of *A. aegypti* for 3 years was successfully tamed and was able to replicate in *A. aegypti*. The wMelPop was micro-injected into embryonic cells of wild-type *A. aegypti*; some of them survived. The existences of wMelPop in survived adult *A. aegypti* were confirmed by polymerase chain reaction (PCR) and wMelPop-infected female *A. aegypti* were selected, named as PGYP1. PGYP1 were subsequently proved to carry wMelPop for more than 33 generations [14], which can be used in control of *A. aegypti* with beneficial effects listed below.

1. Reduce the life span of mosquitoes

Most pathogens transmitted by mosquitoes need some time, or extrinsic incubation period (EIP), to replicate inside the host and become infectious. The EIP was about 1-2 weeks, so if the life span of mosquitoes could be reduced, the risk for disease transmission would be decreased. According to previous studies, the life span of *Drosophila* would be reduced to half by wMelPop [15]. Similar results were found in the life span of PGYP1 female, it would be reduced to 27 days under 25℃ temperature and 80% humidity level, significantly shorter than the 61 days of wMelPop-uninfected female *A. aegypti*. It would be further reduced to 25 days under 30℃ temperature and 80% humidity level, shorter than the 43 days of wMelPop-uninfected female *A. aegypti*. It was suggested that wMelPop infection would reduce the life span of *A. aegypti* significantly[14].

2. Cytoplasmic incompatibility (CI)

wMelPop-infected *A. aegypti* produce cytoplasmic incompatibility was shown in

Figure 2. O'Neill et al. found that more than 2,500 ova produced by PGYP1 male and wild-type female *A. aegypti* were unable to become larvae because of cytoplasmic incompatibility, and therefore reduce the proportion of wild-type male *A. aegypti* in the environment [14]; they also found about 75% ova produced by PGYP1 male and PGYP1 female could become larvae, indicating that PGYP1 female could prevent production of cytoplasmic incompatibility. In addition, because wMelPop could be found in most progeny of PGYP1 female, wMelPop should be maternal transmitted and could be spread rapidly among wild-type *A. aegypti*.

3. Prevent dengue virus infection

Based on previous studies, *Wolbachia* spp.-infected Drosophila fruit fly was found to be resistant to RNA virus [17], O'Neil et al. designed a study to compare the differentiation of DENV-2 infection between PGYP1 and wild-type *A. aegypti*. When the infected DENV-2 viral load was at Log5.3-6/mL, the proportions of DENV-2-infected wild-type *A. aegypti* on day 7 and day 14 were 30-100% and 48-97% respectively, while none of the PGYP1 could be detected with DENV-2 virus infection [18]. In addition, by using immunofluorescence staining, DNEV-2 virus could not infect the wMelPop-infected PGYP1 cell, and it was suggested that wMelPop could interfere with the co-infection of dengue virus to the same host. The phenomenon was demonstrated by analyzing the viral protein synthesis of DENV-2 or Chikungunya virus in PGYP1 cells was significantly slower.

4. Performances in the field studies

O'Neil et al. selected Wolbachia (wMel), similar to wMelPop structurally and genetically, was used in field study to evaluate the infection rate among *A. aegypti* [19]. A semi-field cage study was conducted first in an environment simulating Queensland, Australia [20]. The amount of wMel-infected *A. aegypti* (MBYP2)and wild-type *A. aegypti* were differentiated between 65% and 35%, respectively. The infection rate of wMel increased rapidly, which was over 90% by the end of 30 days, indicating that wMel could effectively spread among *A. aegypti*. To test the susceptibility of MGYP2 female to dengue virus, MGYP2 female were fed with blood containing DENV-2. On day 14, the susceptibility of MGYP2 female was 10,000 times less than wild-type female *A. aegypti*. The result is supported that wMel-infected *A. aegypti* could effectively prevent getting dengue virus infection.

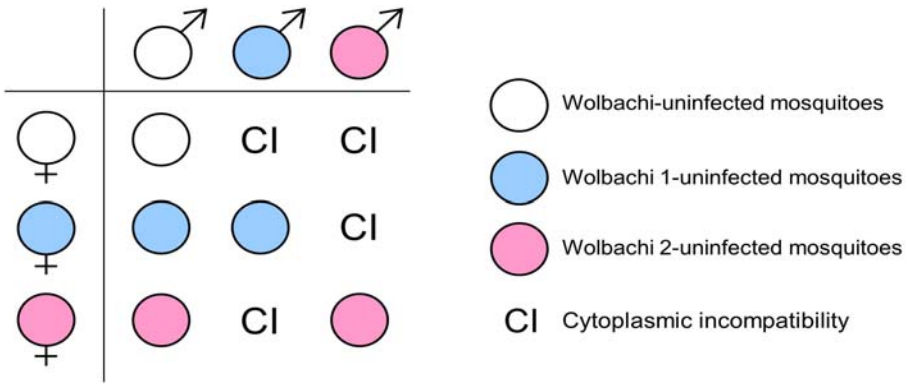

Figure 2. The Wolbachi-induced cytoplasmic incompatibility [16]

An open field study was conducted in Yorkeys Knob and Gordonvale near Carins in northeastern Australia from January to May 2011 [21]. The study was approved by Australian Pesticides and Veterinary Medicines Authority (AVPMA). During the study period, 10,000 to 22,000 MGYP2 were released weekly for 9 to 10 weeks, and ovitraps were also distributed to the same field. The ovitraps were collected every two weeks to detect if the *A. aegypti* larvae carry the wMel and to estimate the overall wMel infection rate. The wMel infection rate was more than 15% on the second week, and it further increased to over 90% on the fifth weeks after stop releasing MGYP2. The study showed that wMel could effectively infect the wild-type *A. aegypti* in Australia and the wMel infection peaked and sustained on third and fourth months after MGYP2 were released. The authors concluded that using Wolbachia could be potential in control of the spread of dengue virus, and they would work on evaluating the actual efficiency in control of DF epidemics.

Discussion

The phase 3 clinical trial of live-attenuated tetravalent dengue vaccine, manufactured by Sanofi-Aventis, has been initiated in Australia and Malaysia in 2010, yet the timing of commercialization remains unpredictable. To achieve a better mosquito control, novel techniques including genetically modified mosquitoes and symbiotic rickettsia were under investigation and the initial results were remarkable. According to the preliminary data, O'Neill et al. have successfully infected the wild-type *A. aegypti* in Australia with wMel and made them resistant to dengue virus infection. The Wolbachia could be vertically transmitted to offspring, so the protection against dengue virus infection could be transmitted too. Although this would be very helpful in control DF epidemics, the safety impacts on our natural environment and eco-system have not been evaluated yet.m and the wMel-infected *A. aegypti* affect hosts of other unknown pathogens remained uncertain. While the study conducted by Oxitec in Carin, it was short in duration and less open to the public. Without ecological evaluation, releasing the genetically modified mosquitoes, unlike infertile mosquitoes treated by radiation, might result in transmission of the translocated genes to other species because of gene shift. The risk would be difficult to monitor and control, leading to new public health or medical issues.

The second field study was initiated in a remote forest area in Pahang state in Malaysia on December 21, 2010. According to the report of Medical Research Center of Malaysia on January 26, 2011, 6,000 genetically modified mosquitoes produced by Oxitec were released [22]. The purpose of this study was to evaluate the flying distance and the survival rate, not the effects on the population of the wild-type *A. aegypti*. Although the biosafety authorities have approved this study and all genetically modified mosquitoes were claimed to be killed using pesticide in early January 2011.

However, many domestic, international experts and environmental groups were still very concerned and discontented with the study. They used the unopened study in Carins as a reference and asked for a more comprehensive safety evaluation about releasing genetically modified mosquitoes.

Carins is a small isolated island in Caribbean. The surface area was only about 200 km^2, even smaller than Taipei City (about 270km^2). The study results could be very different in a densely populated area or a big city. Control of *A. aegypti* in a small area had been successful in Taiwan. Between 1989 and 1996, integrated control measures have been taken to eliminate *A. aegypti* in Liuqiu Island in Pingtung County (about 6.8km^2). By releasing larvivorous fishes in mosquito breeding sites, spreading larvicidesin water storage system of vegetable gardens, improving of water storage systems in household and drinking water accessibility, removing of discarded water containers and tires, the Breteau index of *A. aegypti*. in Liuqui Island was decreased from 53.9 in 1989 to 1.2 in 1996, and the proportion of *A. aegypti*. was decreased from 65% in 1988 to 0-22% in 1996 [23]. Even with the experience in Liuqui Island, the similar measures used to deal with *A. aegypti* in cities at high risk of DF outbreaks, such as Tainan City and Kaohsiung City, were not successful. The difference between densely populated cities and small remote area could make the outcome different.

According to the molecular epidemiological study, the source of indigenous DF outbreak in Taiwan these years could be traced back to an imported DF case. The dengue virus always became untraceable after the outbreaks and thus DF was not considered as an indigenous infectious disease in Taiwan [24]. However, the existence of *A. aegypti* in counties and cities southern to Budai Town of Chiayi County could make the risks of DF outbreak remarkably increased, and novel technologies such as genetically modified mosquitoes and rickettsia symbiotic should be incorporated into our control measures, if a comprehensive environmental assessment and risk evaluation could be done. The acceptability of general population and legal issues should also be considered. Hence, continuously educating the public to eliminate the mosquito breeding sites is necessary before dengue vaccines and novel technologies are available.

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Outbreak Investigation Express

A Pertussis Outbreak at a Hospital in Southern Taiwan, January 2012

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Abstract

Pertussis (whooping cough) is a respiratory disease mainly spread by droplets. In the past, without available vaccine, pertussis was contagious with high incidence and fatality. Nowadays, as Taiwan has high coverage on routine vaccines, more than thirty percent of the pertussis cases during 2008 to 2009 were among non-immunized infants under one year of age. The common source of infection is transmitted by caregivers at home or older siblings. In January 2012, a hospital in southern Taiwan notified a confirmed case of pertussis (index case) affecting a two month-old infant. Further investigation found one asymptomatic carrier among health care staff who had been working and taking care of the index case in the neonatal wards during index case' last admission. The index case and the staff had been contacted with another confirmed case of three month-old infant in the same neonatal ward in December 2011. This was the first nosocomial pertussis outbreak occurred at a neonatal ward in southern Taiwan. Fortunately, after local department of health and the hospital made efforts to take relevant control measures, the outbreak was confined from spreading to the community.

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