Review Article

Dengue vector control in Malaysia: Are we moving in the right direction?

Vythilingam, I.* and Wan-Yusoff, W.S.
Parasitology Department, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur
*Corresponding author e-mail: indrav@um.edu.my; indra.vythilingam@gmail.com

Abstract. Dengue is a major public health problem across more than 123 countries. Vector control has been the hallmark of the dengue control programme in many countries in Southeast Asia since there are no anti-dengue drugs available, and the most recent dengue vaccine is partly efficacious. House-to-house larval surveys, source reduction, larviciding, fogging, ULV which have been carried out since the inception of the dengue control programme in the 1970s are no longer practicable and need to be augmented by more targeted but less ambitious outbreak responses that focus on a few tools that might justify expense of deployment. However, according to recent reports these tools have not really been evaluated for their effectiveness in dengue control. Novel techniques such the release of genetically modified mosquitoes (RIDL) and the use of the bacterium *Wolbachia* to control the populations of the *Ae. aegypti* are still under trial. In this review proactive methods to detect epidemics have been suggested. Tools based on adult mosquitoes is an important strategy for dengue vector surveillance and control. The outbreak response may be more efficient when timely vector control measures are implemented after the immediate detection of an infected mosquito.

INTRODUCTION

Dengue fever was first reported in Malaysia in 1902 (Skae, 1902). Dengue has now become a major public health problem with about 50-100 million cases reported in over 123 countries (Brady *et al.*, 2012). Of late cases of dengue have increased 30 folds globally compared to 50 years ago (Achee *et al.*, 2015). The first major epidemic of dengue in Malaysia occurred in 1974 (7.3 per 100,000) followed by outbreaks in 1982 (14.8 per 100,000) (Shekhar & Huat, 1992) and subsequently every 4 to 5 years (Bakar & Shafee, 2002). Dengue became a notifiable disease in Malaysia in 1971 and in 1975 the Destruction of Disease Bearing Insect act (DDBIA) was introduced. In the 1980s and 1990s dengue cases were under control and epidemics only occurred as cyclical events (Teng & Singh, 2001).

However, in the new millennium cases have increased beyond control and mortality due to dengue has also increased. While an increase 47% was reported in the number of reported dengue cases in Malaysia during 2012 and 2013, about 62% increase was reported during 2013 and 2014 (Mudin, 2015). There are no drugs to treat dengue and only recently a dengue vaccine became available but is not so effective against all sero types and it falls short of the levels of protection required for a standalone intervention (Capeding *et al.*, 2014). Thus, vector control became the hallmark of the dengue control programme (Chang *et al.*, 2011). The primary vector is *Ae. aegypti* and the secondary vector is *Ae. albopictus*

(Rudnick, 1965). These are container breeding mosquitoes and the eggs can withstand desiccation for at least six months.

House-to-house larval surveys were conducted to compute the house index, Breteau index or container index. This has been useful and there is evidence to show that the house index has been reduced form 58.8% in the 1980s (Ho & Vythilingam, 1980) to 6.9% in the 1990s (Sulaiman *et al.*, 1996), and between 1.5 and 2% in recent years (Mudin, 2015). Although currently the house index reported is below 2% (Mudin, 2015) epidemics of dengue are on the increase. This shows that new strategies are needed for the dengue control programmes.

An infected person or an asymptomatic person can easily travel from one country to another in a matter of hours and bring along the virus with him or her. All it takes is for one *Ae. aegypti* to have a full blood meal on that person. After 8 to 10 days the mosquito becomes infective and during a single blood meal is able to transmit the virus to multiple persons (Gubler & Rosen, 1976). This is because *Ae. aegypti* is easily disturbed while feeding and thus finds another source to feed on. Studies in Thailand, using DNA analysis have shown that a single mosquito has had blood meals from multiple people (Harrington *et al.*, 2014). The study also showed that there were instances where the blood meal did not match the DNA profile of the household or neighbouring community (Harrington *et al.*, 2014). This shows that the mosquito may have taken a blood meal from a visitor to the area. It is also known that asymptomatic people are more infectious to the mosquitoes compared to a person showing signs and symptoms of dengue (Duong *et al.*, 2015). Therefore this shows that how quickly the virus can spread from one place to another.

Dengue vector control measures like fogging or ULV are only carried out when cases of dengue are reported. This is a reactive method because when one case has been reported, there will be many more cases to follow due to movement of people and the presence of the *Ae. aegypti*. However, the community is always involved only when

epidemics occur. At that point in time it is too late to implement preventive measures that would impact transmission.

In this review we discuss the promising new tools that are available and proactive measures that can be used to reduce epidemics of dengue. In view of dengue epidemics occurring yearly and also the increasing mortality due to dengue it is timely for new proactive measures to be introduced so as to work towards the elimination of dengue.

AEDES SURVEILLANCE

Larval surveys

House-to-house larval surveys were introduced in Malaysia and Singapore in the 1970s for the control of container breeding mosquitoes mainly *Ae. aegypti* and *Ae. albopictus* (Chan, 1985, Cheong *et al.*, 1986). From these surveys the *Aedes* house index, Breteau index and container index can be computed. These indices were used as a surveillance tool and also during surveys health personnel were able to provide health education to the people. House owners would be advised to get rid of unwanted containers and those that were needed to store water should be properly covered. Temephos (abate sand granules) an organophosphate was also provided either free of charge or sold very cheaply at about RM1/- in the 1980s. Abate can effective for a period of 6 to 24 weeks (Bang & Pant, 1972).

Larval surveys have been effective and that was proved by the successful program that was implemented in Singapore along with public education and law enforcement (Ooi *et al.*, 2006). The *Aedes* house index reduced form 16% to 2% (Ooi *et al.*, 2006). It is a known fact that source reduction is a good surveillance strategy because it gets rid of the breeding sites of the vectors. However, it is now known that there are many cryptic sites where *Aedes* breeds and thus it is difficult to destroy those sites. Roof gutters are one good example where these mosquitoes breed and are seldom checked for breeding.

Another problem with house-to-house larval surveys is that in urban areas the number of houses and especially high rise apartments have increased over the years while the number of health staff needed to carry out the surveys remain static. In addition most of the houses are also locked during the day time as the occupants are at work and some consider it as intrusive (Azil et al., 2015). Since studies have shown that there is no correlation between larval indices and dengue cases (Morrison et al., 2008), it is timely to focus on new tools for dengue surveillance.

Pupal index

It was also suggested that the pupal index is more closely related to the adult density compared to the larval indices (Focks et al., 2000). However, the survey to find pupae is more time consuming and thus this index will not be applicable in the present situation. Thus in high dense urban areas replacing larval surveys with pupal surveys will not be a solution.

USE OF INSECTICIDES FOR DENGUE VECTOR CONTROL

Temephos

As mentioned above Temephos is the insecticide of choice for Aedes larval control, since it is cheap and has long residual activity (Bang & Pant, 1972). The operational dosage used is 1mg/L, while the WHO diagnostic dosage is 0.012mg/L (Chen et al., 2005). The operational dose is effective as shown by studies that 100% mortality was obtained within two hours (Chen et al., 2005). However, when tested against the recommended diagnostic dosage, studies have shown that Ae. aegypti was showing resistance to Temephos with RR ratio ranging from 0.68 to 1.82 (Chen et al., 2005). Development of resistance at various levels have also been reported in the neighbouring countries like Thailand (Ponlawat et al., 2005), Indonesia (Mulyatno et al., 2012), Singapore (Koou et al., 2014a) and Cambodia (Polson et al., 2001). However, since the dosage used is

about 84 times higher than recommended it would be still effective.

Fogging and ULV

When cases of dengue are reported fogging and ULV will be carried out to kill the infected adult mosquitoes so as to break the chain of transmission. Pyrethroid is the insecticide of choice used in fogging while malathion 96% concentrate (an organophosphate) is used in ULV (Vythilingam & Panart, 1991). House-to-house fogging is carried out in the case house and 200 meters surrounding the case house for each case reported. While if there is an epidemic then ULV is used so that a larger area can be covered. However, studies have shown that ULV is not effective to kill the adult mosquitoes, since droplets only get carried as far as the sitting room of the houses and if the mosquitoes are hiding in the closets or under beds they will not get killed (Reiter et al., 1997, Perich et al., 2000, Tanrang & Vythilingam, 2004). There was a study conducted to evaluate the efficacy of ULV using malathion and pyrethroids and it was found that malathion was more effective compared to pyrethroids (Tanrang & Vythilingam, 2004).

A more economical method of fogging was proven where only case house and 50 meters surrounding the case house was fogged while ULV was conducted for the rest of the 150 meters (Omar et al., 2011). By this method the health staff will be able to cover more areas and will cost the government less money. Mark release recapture studies have shown that Ae. aegypti generally do not move far from where they are released. In Thailand they were either found in the same house or in the neighbouring house within 50 meters (Harrington et al., 2005). Perhaps more studies should be conducted to evaluate the efficacy of fogging only case house and 50 meters surrounding the case house. From reviews it has been shown space spraying have not been effective in reducing dengue epidemics (Esu et al., 2010). Since there is a limited class of insecticides in our armamentarium for control of vector borne diseases we need to be judicious in the use of insecticides. Aedes aegypti mosquitoes

have developed resistance to most of the pyrethroids (Ponlawat *et al.*, 2005; Jirakanjanakit *et al.*, 2007; Wan-Norafikah *et al.*, 2010; Koou *et al.*, 2014b; Koou *et al.*, 2014a; Ishak *et al.*, 2015) however, they are still susceptible to organophosphates (Huong & Thi Bach Ngoc, 1999; Jirakanjanakit *et al.*, 2007; Koou *et al.*, 2014b; Hasan *et al.*, 2016).

Outdoor residual spraying

Indoor residual spraying has been effective for control of malaria vectors (Pluess *et al.*, 2010). All house in malarious areas had to be sprayed to ensure that there was more than 90 % coverage to provide good results. It is known that *Anopheles* mosquitoes rest on the walls before and after feeding so that they pick enough insecticides to kill themselves. However, due to behavioural changes the *Anopheles* mosquitoes entered houses to bite and exited houses after biting without resting on the walls. It was then that insecticide treated bednets were introduced. Thus, for dengue control before carrying out outdoor residual spraying (ORS) studies should be conducted to show that the *Aedes* mosquitoes are indeed resting outdoors on the walls.

Preliminary studies have shown that the efficacy of the ORS is effective for six weeks (Rozilawati *et al.*, 2005), it also showed that there was no significant difference between treated and control areas in terms of ovitrap index (Rozilawati *et al.*, 2005). There are a few factors that have to be considered before ORS can be introduced. The insecticide used should be effective for at least 9-12 months since in urban areas large number of houses need to be covered and thus if ORS has to be carried out every 3 months it would not be practical and economical. Secondly would people in urban areas allow their houses to be sprayed? Experience in malaria control have shown that one needs good coverage for spraying to be effective. Thus, if many house owners refuse to allow houses to be sprayed then the impact would not be good. Finally we also know that *Aedes* mosquitoes are becoming resistant to pyrethroids as mentioned above, thus we need to weigh this procedure carefully before embarking on it.

Bacillus thuringiensis israelensis **(*Bti*)**

Bacillus thuringiensis var. *israelensis* (*Bti*) is a gram positive bacteriium that has shown larvicidal activity against mosquito larvae (Mulla, 1990) and is not easy for this bacteriium to develop resistance to the larvae. A systematic review was recently carried out to determine the effectiveness of *Bti* against dengue vectors and control of dengue fever (Boyce *et al.*, 2013). In general it has been shown that *Bti* is effective against immature stages of *Aedes* when applied directly to water storage containers and the mortality has been between 70 and 95% and provided residual efficacy between 1–3 months (Sumanadasa *et al.*, 2011). However, further evidence through cluster randomised control trials are needed to link ento-mological evidence to dengue transmission measures (Boyce *et al.*, 2013).

In Malaysia, in a randomised control trial with space spraying of *Bti* (Vectobac WG) was carried out either using truck mounted ULV or mist blower in a residential area of about 300 houses. The spraying was conducted biweekly for seven weeks followed by weekly cycles for seven weeks and finally biweekly cycles for four weeks (Tan *et al.*, 2012). It was implied that *Bti* application was able to suppress *Ae. aegypti* and *Ae. albopictus* population at a dengue endemic site and potentially interrupt dengue transmission in humans (Tan *et al.*, 2012). If one application is effective for 1-3 weeks what is the rational of applying biweekly for seven weeks? What is the cost effectiveness of such an operation? Can the Ministry of Health afford to carry out such operations throughout the country where epidemics occur? Studies in Singapore showed that when misting operations were carried out residual activity was effective for about 1-3 weeks depending on the environmental conditions and also exposed cups had higher mortality compared to those that were hidden (Sumanadasa *et al.*, 2011).

However, in Cambodia it was shown that two applications of *Bti* to all containers was able to suppress the dengue cases compared to the previous year (Setha *et al.*,

2016). With all these contrasting results proper randomised control trials should be carried out in order to evaluate the effectiveness of space spraying *Bti*. It is known that reduction in vector densities does not correlate well with reduction in dengue cases (Reiner Jr *et al.*, 2016).

Insect growth regulator

An insect growth regulator that has been commonly tested against *Aedes* larvae is Pyriproxyfen. This IGR inhibits the development of the adult mosquito. Studies have shown that the adult mosquito is able to pick up the IGR from one container while ovipositing or resting and transfer to another container during subsequent visit (Devine *et al.*, 2009). Only low doses of pyriproxyfen are required to prevent emergence of adult mosquitoes and has residual activity for 11-15 week (Vythilingam *et al.*, 2005) in Malaysia. In Cambodia where slow release formulation was used it was effective for six months (Seng *et al.*, 2008). However, there is only weak evidence to show that dengue cases have been suppressed in areas where pyriproxyfen has been used (Maoz *et al.*, 2017). More randomised control trials are needed to prove its effectiveness.

ENVIRONMENTAL FACTORS IN RELATION TO *AEDES*

Numerous studies have been conducted to show correlation between entomological indicators, dengue cases and environmental factors like rainfall, temperature and humidity as proactive measures for dengue surveillance (Li *et al.*, 1985; Thanh & Giao, 1996; Hii *et al.*, 2009; Barrera *et al.*, 2011; Xu *et al.*, 2014) with varying results. However, current results are of great contrast when compared to studies conducted in the 1980s. One such study was in Malaysia where it was shown that the lag time between heavy rainfall and increase in dengue cases and *Ae. aegypti* was about two-three months (Li *et al.*, 1985). However, a recent study showed that the increase in *Ae. aegypti* will be lag three weeks after rainfall (Lau *et al.*, 2017). The shorter lag could be due to higher human density and perhaps more breeding sites were created after the rainfall. In general it has been postulated that in areas where rainfall is uniformly distributed there is no correlation between rainfall and *Aedes* density, however, where rainfall was seasonal there was positive correlation with *Aedes* density and dengue cases (Scott *et al.*, 2000). In Singapore it was found that absolute humidity was the best indicator for dengue cases between years 2001 to 2009 compared to rainfall, temperature and relative humidity (Xu *et al.*, 2014). Thus, it is clear that the environmental factors are localised and thus it is very difficult to use these factors in a general plan of action for a particular district, state, or country.

TARGETING ADULT *AEDES* MOSQUITOES

For decades the surveillance for dengue has always been targeted towards the larvae. This has been due to the fact that adult *Aedes* mosquitoes are not attracted to light traps. Back pack aspirators can be used for collecting resting mosquitoes inside houses but this is very labour intensive and intrusive and thus not a suitable method for surveillance. The collection is also very dependent on the skill and diligence of each worker.

In recent years the BG-Sentinel (BGS) trap has been introduced which is effective for the collection of both *Ae. aegypti* and *Ae. albopictus* (Maciel-de-Freitas *et al.*, 2006; Williams *et al.*, 2006). In a randomised control trial (RCT) carried out in Brazil it was found that BGS did not significantly reduce the dengue in the intervention area. Only houses that were using the trap had no cases (Degener *et al.*, 2014) and there were also people who refused to have traps set up in their houses. Thus, it does not seem to be an efficient tool for surveillance and it would be very expensive to deploy these traps to each and every house.

Sticky traps to capture adult *Aedes* mosquitoes

Sticky traps are simple tools that have been used to capture gravid adult *Aedes* mosquitoes when they come to oviposit (Ritchie *et al.*, 2004; Gama *et al.*, 2007; Chadee & Ritchie 2010; Resende *et al.*, 2012). The traps come in different designs and sizes but they serve the same function. It has been demonstrated in Puerto Rico that the use of AGO trap (also a sticky trap) was able to reduce the density of *Ae. aegypti* by 50-70% in the area that had traps compared to the control areas (Barrera *et al.*, 2014). AGO trap cost US12.50 and four traps were set per house. It was also felt that these traps could be used along with surveillance measures such as larviciding and adulticiding (fogging and ULV) and would also be compatible with other control measures such as release of sterile male mosquitoes, and RIDL male mosquitoes as these traps do not efficiently capture male mosquitoes (Barrera *et al.*, 2014). Since adult *Aedes* mosquitoes can now be easily captured using sticky oviposition traps, perhaps it is timely to change strategies for surveillance from larvae to adults.

NEW PARADIGMS FOR MOSQUITO SURVEILLANCE AND CONTROL

Genetically modified mosquitoes

RIDL (Release of Insects carrying Dominant Lethal) is a genetically modified mosquito and only male mosquitoes are released. When a RIDL male mosquito mates with a wild female her progeny will not survive if there is no tetracycline in the water (Thomas *et al.*, 2000). It is species specific and release of these males will be able to suppress the natural population of the *Ae. aegypti* population as shown in studies carried out in Cayman Islands (Harris *et al.*, 2012). RIDL female mosquito is susceptible to dengue virus and female bites. It will be argued that only male mosquitoes are released, but when thousands of mosquitoes are released it is possible that females too would be released as there is no fool-proof method to separate them. As pointed out by (McNaughton, 2012), for this kind of technology to be introduced

we need to have public engagement to dispel the myth about their concerns over the contamination of environment and risk to humans. This is where there needs to be a collaborative effort between the public health sector and the academia for these kinds of projects to be successful. Studies have shown that with the release of the OX513A male *Ae. aegypti* in Cayman islands they managed to reduce the *Ae. aegypti* population by 82% (Harris *et al.*, 2012) while in Brazil they reduced the population by 95% (Araújo *et al.*, 2015) compared to the non-treated areas. In Malaysia field release of OX513A and a wild type of *Ae. aegypti* was carried out in the jungle area of Pahang. It was found that the OX513A males dispersed to a mean distance of 52 m compared to the wild type which was 100 m. However, the life expectancy was almost similar for both which was 2.0 *vs* 2.2 (Lacroix *et al.*, 2012). Although a lot of time and effort was spent on this transgenic mosquito as a possible control tool for dengue vectors this project came to a sudden halt in Malaysia. Thus, the proposed use of the RIDL *Ae. aegypti* for dengue control in Malaysia has been shelved.

Wolbachia* infected *Ae. aegypti

Wolbachia a group of intracellular bacteria is found in most arthropods and nematodes (Werren, 1997; Stouthamer *et al.*, 1999). It is found naturally occurring in *Ae. albopictus* but is not found in *Ae. aegypti. Wolbachia* from *Drosophila melanogaster* or *Ae. albopictus* was introduced into *Ae. aegypti* to suppress dengue in its new host (Moreira *et al.*, 2009; Bian *et al.*, 2010). When *Wolbachia* infected male *Ae. aegypti* mates with wild female *Ae. aegypti* all her offspring will not be viable due to cytoplasmic incompatability (CI). However, when a *Wolbachia* infected female *Ae. aegypti* mates with wild male *Ae. aegypti* all her progeny will carry the *Wolbachia. Wolbachia* infected *Ae. aegypti* has low susceptibility to dengue virus (Frentiu *et al.*, 2014). Laboratory studies have shown that at least 14% of *Ae. aegypti* with *Wolbachia* were susceptible to Zika virus compared to 100% without *Wolbachia* (Tan *et al.*, 2017).

Different strains of *Wolbachia* have been introduced into *Ae. aegypti* and are being tested in many parts of the world. It was shown that wAlbB has been most effective under higher temperatures (26-37ºC), and *Wolbachia* is able to spread to the wild population of *Ae. aegypti* (Ross *et al.*, 2017). However, wMel and wMelpop were not transmitted to the next generation when the different stages of mosquito life cycle were exposed to high temperatures (26-37ºC) (Ross *et al.*, 2017). This shows that some strains of *Wolbachia* do not induce CI at high temperature, thus, it is important to select the most suitable *Wolbachia* strain before it can be used for field releases. In Australia *Wolbachia* (*w*Mel) infected *Ae. aegypti* have been released, they have been able to invade the wild population of *Ae. aegypti* to near fixation within few months of release (Hoffmann *et al.*, 2011). However, it is important that randomised control studies be conducted to show that dengue epidemics can be reduced using *Wolbachia* infected *Ae. aegypti*.

THE WAY FORWARD

It will take some years before the new techniques like RIDL or *Wolbachia* infected mosquitoes can be adopted and used as control measures for dengue vectors. Will all countries affected by dengue be able to have the infrastructure to breed these mosquitoes in large quantities for field release? It will take years before these tools can be implemented on a large scale. Thus, other tools and paradigms that require less expenditure should be tested and used for the surveillance and control of dengue vectors.

Although *Aedes* larval surveys have been effective in the past years, currently it has been shown that there is no correlation between larval indices and dengue cases (Morrison *et al.*, 2008). Fogging and ULV are carried out when cases of dengue are reported. It is known that asymptomatic cases of are more infectious to mosquitoes (Duong *et al.*, 2015), thus when fogging is carried out cases are reported, it will not be effective as can be seen in current epidemics.

Thus, what is needed is an early warning system so that action can be taken before cases are reported. One such proactive method is detecting dengue virus in mosquitoes and instituting control measures before cases are reported. In Thailand it was shown that positive *Aedes* mosquito was obtained before a dengue case was reported (Yoon *et al.*, 2012). They used RT-PCR to detect the dengue virus in mosquitoes and of course it is difficult for public health workers to adopt these methods for regular surveillance. Recently, it has been demonstrated that the NS1 antigen test kit (used for detecting dengue virus antigen in patients) can be used to detect the dengue virus antigen in mosquitoes (Tan *et al.*, 2011; Lee *et al.*, 2013; Voge *et al.*, 2013). This is a method that can be used by public health workers for the surveillance of dengue vectors.

Studies have also shown that dengue cases occur lag of one week after infected mosquitoes are obtained (Lau *et al.*, 2017). The health staff are able to cover a larger number of premises setting up GOS traps (less than US$1) to collect mosquitoes compared to carrying out larval surveys (Lau *et al.*, 2017). The reasons why epidemics of dengue are not being controlled may be due to the fact that manpower is not sufficient to cover all houses during larval surveys, secondly many of the houses are locked during the surveys carried out during working hours, thirdly many cryptic breeding sites are not found during larval surveys. In bigger towns the Local Council is in charge of the vector borne diseases and there is lack of expertise to implement proper vector control measures in these areas (Kumarasamy, 2006).

Therefore, it is timely that a randomised control trial should be carried out on successful research projects to show that they would be effective to reduce dengue epidemics. One such project is the use of the GOS trap and NS1 kit which is a proactive measure to detect dengue virus in mosquitoes (Lau *et al.*, 2017). Control measures should be instituted once positive mosquitoes are obtained and not wait for cases to occur. Thus, it is important that a randomised control trial be conducted on this paradigm. If this

paradigm of GOS trap and NS1 can be used successfully for the surveillance and control of dengue it will be useful in many countries and the retraining of the staff will not be too difficult compared to some of the newer technology. This method is simple and more cost effective compared to larval surveys. Now that international travel has become very cheap and people are moving from country to country within a few hours, dengue has also spread to many countries. We always say that due to unplanned urbanisation dengue has become a serious public health problem. What about Putra Jaya? It is a well-planned city but dengue epidemics do occur (Mulligan *et al.*, 2012). Thus, it is timely that academia, public health institutions and communities work together to eliminate dengue. If we seriously want to eliminate dengue we need to change strategy and be more proactive in our surveillance and control methods. By controlling dengue we will also be able to control Zika and chikungunya to a certain extent as they are spread by the same vector. Just like there was a change in strategy from indoor residual spraying to insecticide treated bednets for malaria vector control, it is timely to adopt changes for dengue surveillance and control.

REFERENCES

Achee, N.L., Gould, F., Perkins, T.A., Reiner, Jr, R.C., Morrison, A.C., Ritchie, S.A., Gubler, D.J., Teyssou, R. & Scott, T.W. (2015). A critical assessment of vector control for dengue prevention. *PLoS Neglected Tropical Diseases* **9**: e0003655.

Araújo, H.R., Carvalho, D.O., Ioshino, R.S., Costa-da-Silva, A.L. & Capurro, M.L. (2015). *Aedes aegypti* control strategies in Brazil: incorporation of new technologies to overcome the persistence of dengue epidemics. *Insects* **6**: 576-594.

Azil, A.H., Ritchie, S.A. & Williams, C.R. (2015). Field worker evaluation of dengue vector surveillance methods: Factors that determine perceived ease, difficulty, value, and time effectiveness in Australia and Malaysia. *Asia Pacific Journal of Public Health* **27**: 705-714.

Bakar, S.A. & Shafee, N. (2002). Outlook of dengue in Malaysia: A century later. *Malaysian Journal of Pathology* **24**: 23-27.

Bang, Y. & Pant, C. (1972). A field trial of Abate larvicide for the control of *Aedes aegypti* in Bangkok, Thailand. *Bulletin of the World Health Organization* **46**: 416.

Barrera, R., Amador, M. & MacKay, A.J. (2011). Population dynamics of *Aedes aegypti* and dengue as influenced by weather and human behavior in San Juan, Puerto Rico. *PLoS Neglected Tropical Diseases* **5**: e1378.

Barrera, R., Amador, M., Acevedo, V., Caban, B., Felix, G. & Mackay, A.J. (2014). Use of the CDC autocidal gravid ovitrap to control and prevent outbreaks of *Aedes aegypti* (Diptera: Culicidae). *Journal of Medical Entomology* **51**: 145-154.

Bian, G., Xu, Y., Lu, P., Xie, Y. & Xi, Z. (2010). The endosymbiotic bacterium *Wolbachia* induces resistance to dengue virus in *Aedes aegypti. PLoS Pathogens* **6**: e1000833.

Boyce, R., Lenhart, A., Kroeger, A., Velayudhan, R., Roberts, B. & Horstick, O. (2013). *Bacillus thuringiensis israelensis* (*Bti*) for the control of dengue vectors: Systematic literature review. *Tropical Medicine & International Health* **18**: 564-577.

Brady, O.J., Gething, P.W., Bhatt, S., Messina, J.P., Brownstein, J.S., Hoen, A.G., Moyes, C.L., Farlow, A.W., Scott, T.W. & Hay, S.I. (2012). Refining the global spatial limits of dengue virus transmission by evidence-based consensus. *PLoS Neglected Tropical Diseases* **6**: e1760.

Capeding, M.R., Tran, N.H., Hadinegoro, S.R.S., Ismail, H.I.H.M., Chotpitaya-sunondh, T., Chua, M.N., Luong, C.Q., Rusmil, K., Wirawan, D.N. & Nallusamy, R. (2014). Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: a phase 3, randomised, observer-masked, placebo-controlled trial. *The Lancet* **384**: 1358-1365.

Chadee, D.D. & Ritchie, S.A. (2010). Efficacy of sticky and standard ovitraps for *Aedes aegypti* in Trinidad, West Indies. *Journal of Vector Ecology* **35**: 395-400.

Chan, K.L. (1985). Singapore's dengue haemorrhagic fever control programme: a case study on the successful control of *Aedes aegypti* and *Aedes albopictus* using mainly environmental measures as a part of integrated vector control, Tokyo: Southeast Asian Medical Information Center.

Chang, M.S., Christophel, E.M., Gopinath, D. & Abdur, R.M. (2011). Challenges and future perspective for dengue vector control in the Western Pacific Region. *Western Pacific Surveillance and Response Journal: WPSAR* **2**: 9.

Chen, C., Nazni, W., Lee, H. & Sofian-Azirun, M. (2005). Susceptibility of *Aedes aegypti* and *Aedes albopictus* to temephos in four study sites in Kuala Lumpur City Center and Selangor State, Malaysia. *Tropical Biomedicine* **22**: 207-216.

Cheong, W., Rudnick, A. & Lin, T. (1986). The vectors of dengue and dengue hemorr-hagic fevers in Malaysia. Dengue fever studies in Malaysia. *Institute Medical Research Bulletin* **23**: 155-167.

Degener, C., Eiras, A., Azara, T., Roque, R., Rösner, S., Codeço, C., Nobre, A., Rocha, E., Kroon, E. & Ohly, J. (2014). Evaluation of the effectiveness of mass trapping with BG-sentinel traps for dengue vector control: a cluster randomized controlled trial in Manaus, Brazil. *Journal of Medical Entomology* **51**: 408-420.

Devine, G.J., Perea, E.Z., Killeen, G.F., Stancil, J.D., Clark, S.J. & Morrison, A.C. (2009). Using adult mosquitoes to transfer insecticides to *Aedes aegypti* larval habitats. *Proceedings of the National Academy of Sciences* **106**: 11530-11534.

Duong, V., Lambrechts, L., Paul, R.E., Ly, S., Lay, R.S., Long, K.C., Huy, R., Tarantola, A., Scott, T.W. & Sakuntabhai, A. (2015). Asymptomatic humans transmit dengue virus to mosquitoes. *Proceedings of the National Academy of Sciences* **112**: 14688-14693.

Esu, E., Lenhart, A., Smith, L. & Horstick, O. (2010). Effectiveness of peridomestic space spraying with insecticide on dengue transmission; systematic review. *Tropical Medicine & International Health* **15**: 619-631.

Focks, D.A., Brenner, R.J., Hayes, J. & Daniels, E. (2000). Transmission thresholds for dengue in terms of *Aedes aegypti* pupae per person with discussion of their utility in source reduction efforts. *American Journal of Tropical Medicine and Hygiene* **62**: 11-18.

Frentiu, F.D., Zakir, T., Walker, T., Popovici, J., Pyke, A.T., van den Hurk, A., McGraw, E.A. & O'Neill, S.L. (2014). Limited dengue virus replication in field-collected *Aedes aegypti* mosquitoes infected with *Wolbachia. PLoS Neglected Tropical Diseases* **8**: e2688.

Gama, R.A., Silva, E.M., Silva, I.M., Resende, M.C. & Eiras, Á.E. (2007). Evaluation of the sticky MosquiTRAP™ for detecting *Aedes* (*Stegomyia*) *aegypti* (L.)(Diptera: Culicidae) during the dry season in Belo Horizonte, Minas Gerais, Brazil. *Neotropical Enomology* **36**: 294-302.

Gubler, D.J. & Rosen, L. (1976). A simple technique for demonstrating trans-mission of dengue virus by mosquitoes without the use of vertebrate hosts. *The American Journal of Tropical Medicine and Hygiene* **25**: 146-150.

Harrington, L.C., Scott, T.W., Lerdthusnee, K., Coleman, R.C., Costero, A., Clark, G.G., Jones, J.J., Kitthawee, S., Kittayapong, P. & Sithiprasasna, R. (2005). Dispersal of the dengue vector *Aedes aegypti* within and between rural communities. *American Journal of Tropical Medicine and Hygiene* **72**: 209-220.

Harrington, L.C., Fleisher, A., Ruiz-Moreno, D., Vermeylen, F., Wa, C.V., Poulson, R.L., Edman, J.D., Clark, J.M., Jones, J.W. & Kitthawee, S. (2014). Heterogeneous feeding patterns of the dengue vector, *Aedes aegypti*, on individual human hosts in rural Thailand. *PLoS Neglected Tropical Diseases* **8**: e3048.

Harris, A.F., McKemey, A.R., Nimmo, D., Curtis, Z., Black, I., Morgan, S.A., Oviedo, M.N., Lacroix, R., Naish, N. & Morrison, N.I. (2012). Successful suppression of a field mosquito population by sustained release of engineered male mosquitoes. *Nature Biotechnology* **30**: 828-830.

Hasan, H.A., Jaal, Z., Ranson, H. & McCall, P. (2016). Pyrethroid and organophosphate susceptibility status of *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse) in Penang, Malaysia. *International Journal of Entomological Research* **3**: 91-95.

Hii, Y.L., Rocklöv, J., Ng, N., Tang, C.S., Pang, F.Y. & Sauerborn, R. (2009). Climate variability and increase in intensity and magnitude of dengue incidence in Singapore. *Global Health Action* **2**: 2036.

Ho, T. & Vythilingam, I. (1980). A preliminary survey of *Ae. aegypti* in Selangor Peninsular Malaysia. *Medical Journal Malaysia* **24**: 409-414.

Hoffmann, A., Montgomery, B., Popovici, J., Iturbe-Ormaetxe, I., Johnson, P., Muzzi, F., Greenfield, M., Durkan, M., Leong, Y. & Dong, Y. (2011). Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. *Nature* **476**: 454.

Huong, V.D. & Thi Bach Ngoc, N. (1999). Susceptibility of *Aedes aegypti* to Insecticides in South Vietnam. *Dengue Bulletin* **23**: 85-89.

Ishak, I.H., Jaal, Z., Ranson, H. & Wondji, C.S. (2015). Contrasting patterns of insecticide resistance and knockdown resistance (kdr) in the dengue vectors *Aedes aegypti* and *Aedes albopictus* from Malaysia. *Parasites & Vectors* **8**: 181.

Jirakanjanakit, N., Rongnoparut, P., Saengtharatip, S., Chareonviriyaphap, T., Duchon, S., Bellec, C. & Yoksan, S. (2007). Insecticide susceptible/resistance status in *Aedes* (*Stegomyia*) *aegypti* and *Aedes* (*Stegomyia*) *albopictus* (Diptera: Culicidae) in Thailand during 2003–2005. *Journal of Economic Entomology* **100**: 545-550.

Koou, S.Y., Chong, C.S., Vythilingam, I., Ng, L.C. & Lee, C.Y. (2014a). Pyrethroid Resistance in *Aedes aegypti* Larvae (Diptera: Culicidae) From Singapore. *Journal of Medical Entomology* **51**: 170-181.

Koou, S.Y., Chong, C.S., Vythilingam, I., Lee, C.Y. & Ng, L.C. (2014b). Insecticide resistance and its underlying mechanisms in field populations of *Aedes aegypti* adults (Diptera: Culicidae) in Singapore. *Parasites & Vectors* **7**: 471.

Kumarasamy, R. (2006). Dengue fever in Malaysia: time for review? *Medical Journal Malaysia* **61**: 1-3.

Lacroix, R., McKemey, A.R., Raduan, N., Wee, L.K., Ming, W.H., Ney, T.G., Siti Rahidah, A.A., Salman, S., Subramaniam, S. & Nordin, O. (2012). Open field release of genetically engineered sterile male *Aedes aegypti* in Malaysia. *PLoS One* **7**: e42771.

Lau, S.M., Chua, T.H., Sulaiman, W.Y., Joanne, S., Lim, Y.A.L., Sekaran, S.D., Chinna, K., Venugopalan, B. & Vythilingam, I. (2017). A new paradigm for *Aedes* spp. surveillance using gravid ovipositing sticky trap and NS1 antigen test kit. *Parasites & Vectors* **10**: 151.

Lau, S.M., Vythilingam, I., Doss, J.I., Sekaran, S.D., Chua, T.H., Sulaiman, W.Y., Chinna, K., Lim, Y.A.L. & Venugopalan, B. (2015). Surveillance of adult *Aedes* mosquitoes in Selangor, Malaysia. *Tropical Medicine & International Health* **20**: 1271-1280.

Lee, C., Vythilingam, I., Chong, C.S., Razak, M.A.A., Tan, C.H., Liew, C., Pok, K.Y. & Ng, L.C. (2013). Gravitraps for management of dengue clusters in Singapore. *American Journal of Tropical Medicine and Hygiene* **88**: 888-892.

Li, C.F., Lim, T.W., Han, L.L. & Fang, R. (1985). Rainfall, abundance of *Aedes aegypti* and dengue infection in Selangor, Malaysia. *The Southeast Asian Journal of Tropical Medicine and Public Health* **16**: 560-568.

Maciel-de-Freitas, R., Eiras, Á.E. & Lourenço-de-Oliveira, R. (2006). Field evaluation of effectiveness of the BG-Sentinel, a new trap for capturing adult *Aedes aegypti* (Diptera: Culicidae). *Memórias do Instituto Oswaldo Cruz* **101**: 321-325.

Maoz, D., Ward, T., Samuel, M., Müller, P., Runge-Ranzinger, S., Toledo, J., Boyce, R., Velayudhan, R. & Horstick, O. (2017). Community effectiveness of pyriproxyfen as a dengue vector control method: A systematic review. *PLoS Neglected Tropical Diseases* **11**: e0005651.

McNaughton, D. (2012). The importance of long-term social research in enabling participation and developing engagement strategies for new dengue control technologies. *PLoS Neglected Tropical Diseases* **6**: e1785.

Moreira, L.A., Iturbe-Ormaetxe, I., Jeffery, J.A., Lu, G., Pyke, A.T., Hedges, L.M., Rocha, B.C., Hall-Mendelin, S., Day, A. & Riegler, M. (2009). A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, Chikungunya, and *Plasmodium*. *Cell* **139**: 1268-1278.

Morrison, A.C., Zielinski-Gutierrez, E., Scott, T.W. & Rosenberg, R. (2008). Defining challenges and proposing solutions for control of the virus vector *Aedes aegypti*. *PLoS Medicine* **5**: e68.

Mudin, R.N. (2015). Dengue incidence and the prevention and control programme in Malaysia. *International Medical Journal Malaysia* **14**: 5-9.

Mulla, M.S. (1990). Activity, field efficacy, and use of *Bacillus thuringiensis israelensis* against mosquitoes, pp. 134-160, Bacterial control of mosquitoes & black flies. Springer.

Mulligan, K., Elliott, S.J. & Schuster-Wallace, C. (2012). The place of health and the health of place: Dengue fever and urban governance in Putrajaya, Malaysia. *Health & Place* **18**: 613-620.

Mulyatno, K.C., Yamanaka, A. & Konishi, E. (2012). Resistance of *Aedes aegypti* (L.) larvae to temephos in Surabaya, Indonesia. *Southeast Asian Journal of Tropical Medicine and Public Health* **43**: 29-33.

Omar, M., Zaliza, S., Mariappan, M., Zainal, A. & Chua, K.B. (2011). Field evaluation on the effectiveness of a modified approach of chemical fogging against the conventional fogging in controlling dengue outbreak. *The Malaysian Journal of Pathology* **33**: 113.

Ooi, E.E., Goh, K.T. & Gubler, D.J. (2006). Dengue prevention and 35 years of vector control in Singapore. *Emerging Infectious Diseases* **12**: 887-893.

Perich, M., Davila, G., Turner, A., Garcia, A. & Nelson, M. (2000). Behavior of resting *Aedes aegypti* (Culicidae: Diptera) and its relation to ultra-low volume adulticide efficacy in Panama City, Panama. *Journal of Medical Entomology* **37**: 541-546.

Pluess, B., Tanser, F.C., Lengeler, C. & Sharp, B.L. (2010). Indoor residual spraying for preventing malaria. The Cochrane Library. Reviews 2010, Issue 4. Art. No.: CD006657. DOI: 10.1002/14651858. CD006657.pub2.

Polson, K.A., Curtis, C., Seng, C.M., Olson, J.G., Chantha, N. & Rawlins, S.C. (2001). Susceptibility of Two Cambodian population of *Aedes aegypti* mosquito larvae to temephos during 2001. *Dengue Bulletin* **25**: 79-83.

Ponlawat, A., Scott, J.G. & Harrington, L.C. (2005). Insecticide susceptibility of *Aedes aegypti* and *Aedes albopictus* across Thailand. *Journal of Medical Entomology* **42**: 821-825.

Reiner Jr, R.C., Achee, N., Barrera, R., Burkot, T.R., Chadee, D.D., Devine, G.J., Endy, T., Gubler, D., Hombach, J. & Kleinschmidt, I. (2016). Quantifying the epidemiological impact of vector control on dengue. *PLoS Neglected Tropical Diseases* **10**: e0004588.

Reiter, P., Gubler, D.J., Gubler, D. & Kuno, G. (1997). Surveillance and control of urban dengue vectors, CAB International, New York.

Resende, M.C. de., Ázara, T.M.F. de., Costa, I.O., Heringer, L.C., Andrade, M.R. de., Acebal, J.L. & Eiras, Á.E. (2012). Field optimisation of MosquiTRAP sampling for monitoring *Aedes aegypti* Linnaeus (Diptera: Culicidae). *Memórias do Instituto Oswaldo Cruz* **107**: 294-302.

Ritchie, S.A., Long, S., Smith, G., Pyke, A. & Knox, T.B. (2004). Entomological investigations in a focus of dengue transmission in Cairns, Queensland, Australia, by using the sticky ovitraps. *Journal of Medical Entomology* **41**: 1-4.

Ross, P.A., Wiwatanaratanabutr, I., Axford, J.K., V.L. White, J.K., Endersby-Harshman, N.M. & Hoffmann, A.A. (2017). *Wolbachia* infections in *Aedes aegypti* differ markedly in their response to cyclical heat stress. *PLoS Pathogens* **13**: e1006006.

Rozilawati, H., Lee, H.L., Mohd Masri, S., Mohd Noor, I. & Rosman, S. (2005). Field bioefficacy of deltamethrin residual spraying against dengue vectors. *Tropical Biomedicine* **22**: 143-148.

Rudnick, A. (1965). Studies of the ecology of dengue in Malaysia: a preliminary report. *Journal of Medical Entomology* **2**: 203-208.

Scott, T.W., Morrison, A.C., Lorenz, L.H., Clark, G.G., Strickman, D., Kittayapong, P., Zhou, H. & Edman, J.D. (2000). Longitudinal studies of *Aedes aegypti* (Diptera: Culicidae) in Thailand and Puerto Rico: population dynamics. *Journal of Medical Entomology* **37**: 77-88.

Seng, C.M., Setha, T., Nealon, J., Socheat, D. & Nathan, M.B. (2008). Six months of *Aedes aegypti* control with a novel controlled-release formulation of pyriproxyfen in domestic water storage containers in Cambodia. *Southeast Asian Journal of Tropical Medicine and Public Health* **39**: 822-826.

Setha, T., Chantha, N., Benjamin, S. & Socheat, D. (2016). Bacterial larvicide, *Bacillus thuringiensis israelensis* strain AM 65-52 water dispersible granule formulation impacts both dengue vector, *Aedes aegypti* (L.) population density and disease transmission in Cambodia. *PLoS Neglected Tropical Diseases* **10**: e0004973.

Shekhar, K.C. & Huat, O.L. (1992). Epidemiology of Dengue/Dengue Hemorrhagic Fever in Malaysia-A Retrospective Epidemiological Study. 1973-1987. Part II: Dengue Fever (DF). *Asia Pacific Journal of Public Health* **6**: 126-133.

Skae, F. (1902). Dengue fever in Penang. *British Medical Journal* **2**: 1581.

Stouthamer, R., Breeuwer, J.A. & Hurst, G.D. (1999). *Wolbachia pipientis*: microbial manipulator of arthropod reproduction. *Annual Reviews in Microbiology* **53**: 71-102.

Sulaiman, S., Pawanchee, Z.A., Arifin, Z. & Wahab, A. (1996). Relationship between Breteau and house indices and cases of dengue/dengue hemorrhagic fever in Kuala Lumpur, Malaysia. *Journal American Mosquito Control Association* **12**: 494-496.

Sumanadasa, S.D.M., Lee, C., Lam-Phua, S.G., Lu, D., Chiang, L.-P., Koou, S.Y., Tan, C.H., Pang, S.C., Maideen, N., Ng, L.C. & Vythilingam, I. (2011). Misting of *Bacillus thuringiensis israelensis* (*Bti*) to control *Aedes albopictus* in an industrial area-the Singapore experience. *Dengue Bulletin* **35**: 181-193.

Tan, A.W., Loke, S.R., Benjamin, S., Lee, H.L., Chooi, K.H. & Sofian-Azirun, M. (2012). Spray application of *Bacillus thuringiensis israelensis* (*Bti* strain AM65-52) against *Aedes aegypti* (L.) and *Ae. albopictus* Skuse populations and impact on dengue transmission in a

dengue endemic residential site in Malaysia. *Southeast Asian Journal of Tropical Medicine and Public Health* **43**: 296.

Tan, C.H., Wong, P.S.J., Li, M.Z.I., Vythilingam, I. & Ng, L.C. (2011). Evaluation of the Dengue NS1 Ag Strip® for Detection of Dengue Virus Antigen in *Aedes aegypti* (Diptera: Culicidae). *Vector-Borne and Zoonotic Diseases* **11**: 789-792.

Tan, C.H., Wong, P.J., Li, M.I., Yang, H., Ng, L.C. & O'Neill, S.L. (2017). *w*Mel limits Zika and chikungunya virus infection in a Singapore *Wolbachia*-introgressed *Ae. aegypti* strain, *w*Mel-Sg. *PLoS Neglected Tropical Diseases* **11**: e0005496.

Tanrang, Y. & Vythilingam, I. (2004). Field trial to determine the efficacy of pyrethroid Fendona 10SC application using ultra low volume for the control of *Aedes* mosquitoes. *Tropical Biomedicine* **21**: 57-65.

Teng, A.K. & Singh, S. (2001). Epidemiology and new initiatives in the prevention and control of dengue in Malaysia. *Dengue Bulletin* **25**: 7-12.

Thanh, N. & Giao, P. (1996). Epidemiology and clinical feature findings of dengue haemorrhagic fever in Ho Chi Minh city and Centre for Tropical Diseases-Vietnam. *Tropical Medicine* **36**: 177-186.

Thomas, D.D., Donnelly, C.A., Wood, R.J. & Alphey, L.S. (2000). Insect population control using a dominant, repressible, lethal genetic system. *Science* **287**: 2474-2476.

Voge, N.V., Sánchez-Vargas, I., Blair, C.D., Eisen, L. & Beaty, B.J. (2013). Detection of dengue virus NS1 antigen in infected *Aedes aegypti* using a commercially available kit. *The American Journal of Tropical Medicine and Hygiene* **88**: 260-266.

Vythilingam, I. & Panart, P. (1991). A field trial on the comparative effectiveness of malathion and Resigen by ULV application on *Aedes aegypti*. *Southeast Asian Journal of Tropical Medicine and Public Health* **22**: 102-107.

Vythilingam, I., Luz, B.M., Hanni, R., Beng, T.S. & Huat, T.C. (2005). Laboratory and field evaluation of the insect growth regulator pyriproxyfen (Sumilarv 0.5G) against dengue vectors. *Journal of the American Mosquito Control Association* **21**: 296-300.

Wan-Norafikah, O., Nazni, W.A., Lee, H.L., Zainol-Ariffin, P. & Sofian-Azirun, M. (2010). Permethrin resistance in *Aedes aegypti* (Linnaeus) collected from Kuala Lumpur, Malaysia. *Journal of Asia-Pacific Entomology* **13**: 175-182.

Werren, J.H. (1997). Biology of *Wolbachia*. *Annual Review of Entomology* **42**: 587-609.

Williams, C.R., Long, S.A., Russell, R.C. & Ritchie, S.A. (2006). Field efficacy of the BG-Sentinel compared with CDC Backpack Aspirators and CO_2-baited EVS traps for collection of adult *Aedes aegypti* in Cairns, Queensland, Australia. *Journal of the American Mosquito Control Association* **22**: 296-300.

Xu, H.Y., Fu, X., Lee, L.K.H., Ma, S., Goh, K.T., Wong, J., Habibullah, M.S., Lee, G.K.K., Lim, T.K. & Tambyah, P.A. (2014). Statistical modeling reveals the effect of absolute humidity on dengue in Singapore. *PLoS Neglected Tropical Diseases* **8**: e2805.

Yoon, I.-K., Getis, A., Aldstadt, J., Rothman, A.L., Tannitisupawong, D., Koenraadt, C.J., Fansiri, T., Jones, J.W., Morrison, A.C. & Jarman, R.G. (2012). Fine scale spatiotemporal clustering of dengue virus transmission in children and *Aedes aegypti* in rural Thai villages. *PLoS Neglected Tropical Diseases* **6**: e1730.