



Amrita Haikerwal¹, Madan LB Bhatt¹ and Shailendra K Saxena^{1,2*}

¹Center for Advanced Research (CFAR)-Stem Cell/Cell Culture Unit, King George's Medical University (KGMU), Lucknow 226003, India

²CSIR-Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500007, India

Dates: Received: 04 April, 2017; **Accepted:** 06 July, 2017; **Published:** 07 July, 2017

***Corresponding author:** Shailendra K Saxena, Center for Advanced Research (CFAR)-Stem Cell/Cell Culture Unit, King George's Medical University (KGMU), Lucknow 226003, India; Tel: 91 522 2257450; Fax: 91522 2257450; E-mail: shailen@kgmcindia.edu

Keywords: Dengue virus; Preventions; Therapeutics; Dengue vaccines

<https://www.peertechz.com>

Review Article

Reducing the Global Burden of Dengue: Steps toward Preventive Methods

Abstract

The epidemiological data for Dengue has validated the alarming situation of dengue across the world, which in turn brings a huge population at the risk of dengue infection. Dengue virus (DENV) has four serotypes, thus the generation of a single therapeutic to target all the 4 viruses can be challenging. The severity of this infection ranges from dengue fever and in extreme condition causes fatal haemorrhagic fever. Significant methods for preventing dengue transmission were vector control and dengue vaccination, both these methods will help in minimizing the quantum of disease. Novel techniques like using *Wolbachia*, Entomopathogenic fungi and genetically modified mosquitoes are successfully advancing in the direction for eliminating the *Aedes aegypti* and *Aedes albopictus* population. Moreover, clinical trials of dengue vaccines such as CYD-TD, LATV Δ 30 and DENVax are also showing promising results.

Abbreviations

DENV: Dengue Virus; DF: Dengue Fever; DHF: Dengue Haemorrhagic Fever

Introduction

With an estimated count of 390 million cases annually around the world, resurgence and increasing global distribution of dengue is undoubtedly a threat to the public health [1]. The probable reason behind escalating incidence rate of Dengue virus (DENV) infection reported by leading Health organizations depend on factors such as urbanization, trading and transport, favourable climatic conditions, high population density, unavailability of vector control approaches, and the growing viral evolution. The lack of effective antivirals, and the limited applications of the approved vaccine at the time of high risk, has unfortunately lowered the opportunities of eradicating the DENV infection from the core. Nevertheless, implementation of several management programs to prohibit the vector-borne transmission of DENV in humans might ameliorate the current strenuous situation.

The Epidemiological surveillance of dengue infection reported to the pioneer health organizations determines the actual burden of the infection. Pan America Health Organization (PAHO) has recorded 2,338,848 number of dengue cases in America, where 1,496,282 cases were from Brazil during 2016 [2]. In 2015, South-East Asia Regional Office (SEARO) of World

Health Organization (WHO) has estimated 1,000,000 cases of dengue in this region [3]. Among South Asian countries, India has National Vector Borne Disease Control Programme (NVDCP) which has proclaimed 111,880 cases across the country in 2016 [4]. Another organization of WHO, West-Pacific Regional Office (WPRO) has reported around 1,500,000 cases of dengue [5]. The severity of dengue infection is increasing across the whole world. Even though the reported cases of fatality are very low of dengue infection but the number of cases are spiking every year. These organizations play a significant role in determining the authentic level of risk associated with such infectious outbreaks.

Life cycle and transmission of dengue virus

DENV is a prominent member of *Flaviviridae* family, carried majorly by *Aedes aegypti* while in some regions dengue is also transmitted by *Aedes albopictus*. DENV is a + sense ss RNA virus comprising of 10.7kb genome that translates into a single polyprotein which encodes for both structural and non-structural proteins [6]. DENV constitutes of 4 serotypes such as DENV 1-4 and these serotypes are consistent habitants of tropical and sub-tropical regions, hence, the generation of a single therapeutic to target all the 4 viruses can be challenging. Capsid, pre-M and envelope proteins assemble and form the structure of DENV which along with the ssRNA generates viral particles whereas NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5, participates in RNA replication.

From female mosquito's saliva, virus enters the body of human host where DENV chiefly infects dendritic cells and monocytes. DENV can bind with different receptors like DC-SIGN, GRP78/BiP and heparan sulphate present on the cell surface [7]. Viral attachment initiates clathrin-mediated endocytosis where pH dependent fusion of DENV envelope with the endosome occurs. Subsequently, uncoating and release of ssRNA into the cytoplasm commences the translation that in turn, initiates replication of viral genome. Translation of ssRNA takes place on the rough endoplasmic reticulum (RER) that generates structural and non-structural proteins whereas replication of the ssRNA occurs in the invagination of the Endoplasmic Reticulum (ER) by NS5 proteins [8]. The assembly of capsid, envelope, pr-M and RNA constitute immature viral particles. Furthermore, maturation of viral particles is manifested in the Golgi complex where pH dependent cleavage of pr-M to M leads budding off from the organelle. The infectious mature virions are finally egressed from the host cell [7].

Pathogenesis

Dengue infection is asymptomatic which begins with dengue fever (DF) and in some extreme cases cause fatal dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS) [9]. DF constitutes headache, body pain, joint pain and nausea during the first 7-14 days of infection. During later stages of viremia, capillary leakage with thrombocytopenia (less than $100,000 \text{ mm}^{-3}$), profuse vomiting occurs which establishes the onset of severe dengue. DHF and DSS manifest bleeding of multiple organs and respiratory failure. The severity of infection depends on the age and nutritional status of individuals where mostly infants and older generation individuals are affected. Neutralizing antibodies restricts the viral population during primary infection. In an endemic region, primary infection can provide protection only for a short period as the secondary infection could be from other serotypes. Thus, initiating antibody dependent enhancement (ADE) [10] of dengue infection due to the cross-reactivity between the neutralizing antibodies of primary infection and the DENV serotype of secondary infection causes generation of DENV-Ab complex which further binds with the FC- γ receptor and infects the macrophages [11].

Preventing DENV transmission

According to the report from WHO, dengue infection is an endemic disease in more than 100 countries around the globe. The number of cases and distribution of dengue is escalating rapidly, thus, prevention of dengue is the need of the hour. Various appropriate measures to restrain this catastrophic dengue infection are discussed below.

Vector control

The primary mode of eradication of DENV will be controlling the spread of its vector by targeting female *A. aegypti* and *A. albopictus* mosquitoes or inhibiting the contact between the human host and the vector. Numerous vector control methods are discussed below as well as in the Table 1.

Household personal protection: At household level, protection against dengue is crucial as children and infants are prime targets of dengue thus, use of mosquito repellents, impregnated nets, mosquito killer coils, wearing full-sleeved shirts and pants, changing water in vase and pots on regular basis, avoiding accumulation of stagnant water in cooler, drains and terrace will provide protection from mosquitoes. The active ingredient used in mosquito repellents are N, N-diethyl-meta toluamide (DEET), dimethyl phthalate (DMP), picaridin and p-methane-3,8-diol (PMD). DEET can effectively

Table 1: Vector control methods for preventing dengue.

Modes of control	Types	Examples
Household personal Protection	Mosquito repellent	<ul style="list-style-type: none"> DEET (N,N- diethyl-meta-toluamide) PMD (p-methane-3,8-diol) Picaridin
	Wear protective clothes	<ul style="list-style-type: none"> Full sleeved shirts, long pants, light coloured clothes
	Impregnated mosquito nets	<ul style="list-style-type: none"> Insecticide treated nets (Pyrethroids) Long lasting Insecticide treated bed nets (α-cypermethrin, Deltamethrin)
Biological	Larvivorous fish	<ul style="list-style-type: none"> <i>Gambusia affinis</i> <i>Poecilia eticulate</i> (Guppy)
	Entomopathogenic fungi	<ul style="list-style-type: none"> <i>Lageidium giganteum</i> <i>Coelomomyces stegomyiae</i> <i>Entomophthora muscae</i>
	Predatory bacteria	<ul style="list-style-type: none"> <i>Bacillus thuringiensis</i> var. <i>israelensis</i> (Bti)
	Endosymbiotic bacteria	<ul style="list-style-type: none"> <i>Wolbachia pipientis</i> (reduces mosquito population by inducing sterility)
	Copepods	<ul style="list-style-type: none"> <i>Cyclops vernalis</i> <i>Mesocyclops thermocyclopoides</i> <i>Mesocyclops guangxiensis</i>
Chemical	Oils	<ul style="list-style-type: none"> Cinnamon Any biodegradable oil
	Space sprays	<ul style="list-style-type: none"> Thermal fogs (vaporized formulations of insecticides) Cold fogs (Ultra Low Volume of insecticides are sprayed)
	Insecticides	<ul style="list-style-type: none"> Organophosphate insecticides (Malathion, Fenitrothion) Pyrethroids (Permethrin, Deltamethrin)
	Toxins	<ul style="list-style-type: none"> <i>Bacillus thuringiensis</i> var. <i>israelensis</i> (Bti)
Environmental maintenance	Prevent water logging	<ul style="list-style-type: none"> Proper dispensation of water during heavy rainfall
	Destroying breeding habitats of mosquitoes	<ul style="list-style-type: none"> Cleaning of stagnant water on a regular basis Disinfecting pits and gutters
	Urbanization	<ul style="list-style-type: none"> Planned construction of infrastructures restricts larval habitats
	Solid waste management	<ul style="list-style-type: none"> Non-biodegradable wastes are potential breeding sites.
Genetically modified	Introducing a dominant lethal gene to sterilize male population	<ul style="list-style-type: none"> Offspring die at larval stage

repel arthropods and especially mosquitoes in a range of 38cm from the human host [12]. The molecular targets of these compounds are still not known but DEET prevents the behavioural attraction of *Aedes aegypti* mosquitoes towards the lactic acid present in human sweat thus blocking the electrophysiological activity of olfactory neurons found on the antennae of mosquito [13]. Plant-based mosquito repellents are another growing area which will overcome the drawbacks of inorganic chemical compounds presently applied in the mosquito repellents. Lemon eucalyptus (*Corymbia citriodora*) extracts is used to isolate PMD as a natural mosquito repellent [14]. The other preventive method could be use of impregnated nets treated with insecticides such as pyrethroids and deltamethrin. A prominent instance of adopting impregnated nets in Sub-Saharan Africa had decreased clinically 40% number of cases of Malaria during 2000–2015 [15].

Biological methods: The advantages of biological methods are incomparable to other known methods for preventing these parasitic mosquitoes. The lifecycle of mosquito constitutes of stages such as egg to larvae to pupa and finally to adult. These biological treatments can be introduced to kill the mosquito at different stages [16]. Larvivorous fishes, entomopathogenic fungi, predatory bacteria, endosymbiotic bacteria and copepods are conveniently used methods. Larvivorous fishes such as *Gambusia affinis*, *Poecilia eticulate* (Guppy) prey on the larval stages of mosquitoes [17]. The merits of introducing Larvivorous fishes in a breeding site of mosquitoes will decrease the larval count, can be easily grown in shallow water, and are cost effective as well as environmental friendly [18]. The long term feasibility and reliability of using larvivorous fishes for the larval control seems to be limited in the regions where breeding sites are few and easily identifiable [19]. Several researches conducted using variety of larvivorous fishes were incapable of getting a significant and consistent results in showing their role in reduction of larval density [20].

Entomopathogenic fungi like *Lageidium giganteum*, *Coelomomyces stegomyia* and *Entomophthora muscae* could kill both larval and adult populations of mosquito, further only

once the fungus has to be applied at the breeding sites and it could be transmitted to other uninfected breeding sites by the female mosquitoes themselves. Entomopathogenic fungus particularly kills mosquitoes by releasing toxins and these fungi do not affect any other species or humans [21]. DENV infected *Aedes aegypti* mosquito becomes immuno-compromised, thus making it susceptible to fungal attack, therefore, in a recent study it was examined that coupling fungi *Metarhizium anisopliae* with Imidacloprid, an insecticide could kill *Aedes aegypti* mosquitoes [22]. Predatory bacteria such as *Bacillus thuringiensis var. israelensis* (Bti) are another approach to decimate the larval stage of *Aedes aegypti* and *Aedes albopictus* by releasing toxins and virulence factors which acts as insecticidal [23].

Endosymbiotic bacteria such as *Wolbachia pipientis* induces sterility specifically in the male mosquito population by using technique like cytoplasmic incompatibility (CI) where on mating with the normal female mosquito the survival rate of offspring is very low. Incompatible Insect technique (IIT) is another opportunity to diminish the mosquito population by releasing the *Wolbachia* infected male mosquitoes to compete with the normal male, thus, inducing sterility [24]. Copepods like *Cyclops vernalis*, *Mesocyclops thermocyclopoides*, *Mesocyclops longisetus* and *Mesocyclops guangxiensis* could be used to kill *Aedes aegypti* and *Aedes albopictus* population growing in flower pots, tires, vases or pits. These crustaceans are very useful in managing the mosquito community in urban areas where planting sapling inside the house and keeping flowers in the vase are a common practice [25].

Chemical methods: The use of insecticides, oils and fogging for killing the *Aedes aegypti*, *Aedes albopictus* and other mosquitoes species were frequently used, however, after considering the environmental affects the use of inorganic chemicals was minimized (Figure 1). Generally, oils are applied on the surface of ditches to suffocate the larvae and pupa population [26]. Biodegradable oils like cinnamon oil, soybean oils are used separately or in conjugation with other mosquito repellents to acts as a potent inhibitor of

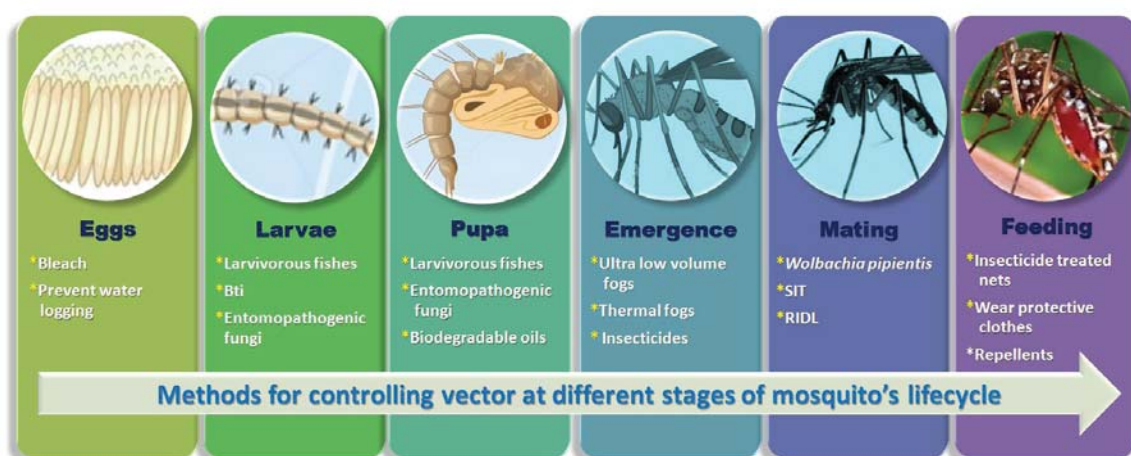


Figure 1: Methods for controlling vector population. The appropriate methods targeted for limiting the mosquito populations are estimated based on different stages of mosquito's lifecycle.

mosquitoes. Furthermore, practice of such organophosphate insecticides such as malathion, fenitrothion, permethrin and deltamethrin are prohibited in order to prevent hampering of human health, ecology and environmental strata [27]. Space sprays are recommended by WHO to be used at the time of extreme situations. They are classified into two types as thermal fogs and cold fogs. Thermal fogs are generated by applying high temperature to release vapours of insecticide in oil. The frequently used insecticides are 4% malathion and 1% fenitrothion for killing the adult mosquitoes. Cold fogs or ultra low volume (ULV) fogs produces fine droplets, containing definite amount of insecticides, necessary for killing these mosquitoes. This lethal dose is very effective in eliminating mosquitoes from the endemic regions [28].

Environmental: Dengue is a tropical and sub-tropical disease, where climatic conditions for breeding mosquitoes are favourable because of optimum humidity and temperature. Thus, the maintenance of environment plays a significant role in restricting the population of mosquitoes. During heavy rainfall, collection of water in pots, in unused utensils, furniture kept on the terrace, ditches or gutters are the suitable habitats for breeding mosquitoes. Regular removal of stagnant water is necessary, pits and ditches should be covered, and water should not be stored for long time in containers, moreover, use of disinfectants in household ditches are fundamental approaches for preventing the spread of *Aedes*. Well-planned construction of houses and infrastructures will enhance the opportunities to limit the larval habitats [29].

Genetically modified: The principle of Sterile Insect Technique (SIT) and Release of Insects carrying a Dominant Lethal gene (RIDL) is to bring genetic alterations in the male population of *Aedes aegypti* mosquitoes (Figure 1) for controlling the number of offspring produced [16]. In SIT, the adult males are reared in enormous number and treated with radiation, furthermore these sterilized males are introduced in the target wild population where they mate with wild female and suppress the generation of progeny. The repeated use of this method in an area will effectively eliminate the population of the mosquitoes. The other genetically modified approach is RIDL which induces a dominant lethal gene in the male population and when they mate with normal female mosquitoes, produce progeny that expresses the lethal gene and dies at larval stage. Thus, various effective methods are developed to defeat dengue by combating with its vector [30].

Dengue vaccines

The alarming increase of number cases of dengue infection throughout the globe strongly needs dengue vaccine that could produce effective immune response for all serotypes. The development of effective robust dengue vaccine will face numerous hurdles such as factors like virulence of the attenuated virus should be regulated to activate the host immune response, furthermore, the tetravalent dengue vaccine should uniformly generate the immune response for the 4 serotypes [31]. These vaccines could be given to individuals already exposed to dengue virus as well as to the naive individuals. WHO recommended, the first dengue vaccine is Dengvaxia

or CYD- tetravalent dengue vaccine (CYD-TDV) developed by Sanofi Pasteur. CYD-TDV is a chimeric vaccine, constitutes of yellow fever 17D vaccine with *prM* and *E* genes of yellow fever virus replaced by dengue virus (1-4 serotypes). The CYD-TDV is under Phase III trial in Latin America where 3 vaccinations are given to individuals of age 9-16 years [32]. Likewise, National Institute of Health has developed live attenuated tetravalent vaccine (LATV) Δ 30 where 30 nucleotides were deleted from the 3' UTR of c-DNA of dengue virus. LATV was tested in USA and has undergone Phase I trial for individuals of age 18-50. A single dose of LATV comprised of four distinctive serotypes of DENV (TV001-TV004) [33]. Similarly, other dengue vaccine is DENVax, developed by Takeda, constitutes 4 recombinant dengue virus serotypes with a backbone of DENV2 genome along with modification in *prM* and *E* gene of the DENV2, reconstituted with particular *prM* and *E* gene of DENV serotypes. DENVax has undergone Phase 1 trial in Colombia and was tried in individuals of age 18-45 years [34]. With the result shown in clinical trials, further research is required to develop effective vaccine. The efficacy and side effects of dengue vaccines should be analysed before delivering them to the market.

As the numbers of dengue cases are increasing at a rapid pace thus, various integrated vector control strategies should be implemented to reduce the size of mosquito population. The techniques involving larvivorous fishes, entomopathogenic fungi, endosymbiotic bacteria, biodegradable oils and genetically modified mosquitoes are cost effective, environmental friendly and effective against killing the mosquitoes at different stages of their life cycle; however the feasibility and reliability of these methods is still to be estimated at large. The regular maintenance and implementation of these strategies is necessary for being a sustainable solution. To validate the efficacy of these biological methods several research surveys needs to be conducted on the basis of large number of breeding sites in urban and rural areas. Nevertheless, other preventive methods such as the dengue vaccines are more promising in terms of restricting the number of cases of dengue because they are feasible and easily accessible.

Conclusions and Future Perspectives

Dengue being a global economic burden has created a state of alert, yet there is no completely effective approach to prevent this catastrophic disease. With the advancement in integrated vector control methods and development of potent dengue vaccines, there are possibilities to eliminate dengue. The virtuous strategies such as use of impregnated mosquito nets, larvivorous fish, entomopathogenic fungi, *Bacillus thuringiensis var. israelensis* (Bti) and *Wolbachia pipiensis* have shown promising outcomes in killing *Aedes aegypti* and *Aedes albopictus* mosquitoes. These methods are targeted selectively towards the specific stage of the mosquito's life cycle. Genetically modified strategies for controlling the mosquito populations is a competent method, however, it requires undergoing various trials for authenticating its efficacy. The application of insecticides was once a very popular mode of wiping out mosquitoes but due to its harmful effects on flora and fauna, presently its use has been limited to thermal and colds fogs

where insecticides are added in very low concentration. Despite of having potential vector control strategies, generation of a robust vaccine could be the ultimate solution in preventing dengue infection in endemic regions.

Acknowledgments

The authors are grateful to the Vice Chancellor, King George's Medical University (KGMU), Lucknow and Director, Centre for Cellular and Molecular Biology, Council of Scientific and Industrial Research (CSIR-CCMB), India for the encouragement and support for this work. SK Saxena is also supported by CCRH, Government of India, and US NIH grants: R37DA025576 and R01MH085259. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

References

- Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, et al. (2013) The global distribution and burden of dengue. *Nature* 496: 504-507. [Link: https://goo.gl/1UNEpQ](https://goo.gl/1UNEpQ)
- (2017) Pan America Health Organization Number of reported cases of dengue and severe dengue (SD) in the Americas, by country: Figures for 2016. *Epidemiological Week PAHO/WHO*. [Link: https://goo.gl/sUaPiU](https://goo.gl/sUaPiU)
- (2017) South- East Asia Regional Office Dengue data application. *Epidemiology / WHO*. [Link: https://goo.gl/oUXBd5](https://goo.gl/oUXBd5)
- (2016) National Vector Borne Disease Control Programme Dengue cases and deaths in the country since 2010. [Link: https://goo.gl/wqyA8L](https://goo.gl/wqyA8L)
- (2017) West-Pacific Regional Office Dengue data application. *Epidemiology / WHO*. [Link: https://goo.gl/nLyEZg](https://goo.gl/nLyEZg)
- de Armas-Rillo L, Valera MS, Marrero-Hernández S, Valenzuela-Fernández A (2016) Membrane dynamics associated with viral infection. *Rev Med Virol* 26: 146-160. [Link: https://goo.gl/8ZmskP](https://goo.gl/8ZmskP)
- Diamond MS, Pierson TC (2015) Molecular insight into dengue virus pathogenesis and its implications for disease control *Cell* 162: 488-492. [Link: https://goo.gl/WYX9yZ](https://goo.gl/WYX9yZ)
- Mlera L, Melik W, Bloom ME (2014) The role of viral persistence in flavivirus biology. *Pathog Dis* 71: 137-163. [Link: https://goo.gl/9jHP1E](https://goo.gl/9jHP1E)
- Screaton G, Mongkolsapaya J, Yacoub S, Roberts C (2015) New insights into the immunopathology and control of dengue virus infection. *Nat Rev Immunol* 15:745-759. [Link: https://goo.gl/gYTRkB](https://goo.gl/gYTRkB)
- Halstead SB (2003) Neutralization and antibody-dependent enhancement of dengue viruses. *Adv Virus Res* 60:421-467. [Link: https://goo.gl/cqrByi](https://goo.gl/cqrByi)
- Dejnirattisai W, Jumnainsong A, Onsirirakul N, Fitton P, Vasanawathana S, et al (2010) Cross-reacting antibodies enhance dengue virus infection in humans. *Science* 328: 745-748. [Link: https://goo.gl/BSZnaR](https://goo.gl/BSZnaR)
- Ditzen M, Pellegrino M, Vossell LB (2008) Insect odorant receptors are molecular targets of the insect repellent DEET. *Science* 319: 1838-1841. [Link: https://goo.gl/JVtD8a](https://goo.gl/JVtD8a)
- Pickett JA, Birkett MA, Logan JG (2008) DEET repels ORNery mosquitoes. *Proc Natl Acad Sci* 105: 13195-13196. [Link: https://goo.gl/WZCJpG](https://goo.gl/WZCJpG)
- Phasomkusolsil S, Soonwera M (2010) Insect repellent activity of medicinal plant oils against *Aedes aegypti* (Linn.), *Anopheles minimus* (Theobald) and *Culex quinquefasciatus* Say based on protection time and biting rate. *Southeast Asian J Trop Med Public Health* 41: 831-840. [Link: https://goo.gl/gu8q7p](https://goo.gl/gu8q7p)
- Bhatt S, Weiss DJ, Cameron E, Bisanzio D, Mappin B, et al. (2015) The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. *Nature* 526: 207-211. [Link: https://goo.gl/xpytwj](https://goo.gl/xpytwj)
- Benelli G, Jeffries CL, Walker T (2016) Biological Control of Mosquito Vectors: Past, Present, and Future *Insects* 7: 52-70. [Link: https://goo.gl/DsV6nv](https://goo.gl/DsV6nv)
- (2003) Regional Office for the Eastern Mediterranean Use of fish for mosquito control. World Health Organisation. [Link: https://goo.gl/Mga85P](https://goo.gl/Mga85P)
- (2005) National Vector Borne Disease Control Programme Guidelines on the use of Larvivorous fish for vector control. [Link: https://goo.gl/FJCT1w](https://goo.gl/FJCT1w)
- Chandra G, Bhattacharjee I, Chatterjee SN, Ghosh A (2008) Mosquito control by larvivorous fish. *Indian J Med Res* 127: 13-27. [Link: https://goo.gl/MNxWA9](https://goo.gl/MNxWA9)
- Walshe DP, Garner P, Abdel-Hameed Adeel AA, Pyke GH, Burkot T (2013) Larvivorous fish for preventing malaria transmission. *Cochrane Database Syst Rev* 12: 1-67. [Link: https://goo.gl/jjp7ec](https://goo.gl/jjp7ec)
- Scholte EJ, Knols BG, Samson RA, Takken W (2004) Entomopathogenic fungi for mosquito control: a review. *J Insect Sci* 4: 19. [Link: https://goo.gl/RkWm8Z](https://goo.gl/RkWm8Z)
- Paula AR, Carolino AT, Paula CO, Samuels RI (2011) The combination of the entomopathogenic fungus *Metarhizium anisopliae* with the insecticide Imidacloprid increases virulence against the dengue vector *Aedes aegypti* (Diptera: Culicidae). *Parasit Vectors* 4: 8. [Link: https://goo.gl/gMHq8L](https://goo.gl/gMHq8L)
- Ritchie SA, Rapley LP, Benjamin S (2010) *Bacillus thuringiensis var. israelensis* (Bti) provides residual control of *Aedes aegypti* in small containers. *Am J Trop Med Hyg* 82: 1053-1059. [Link: https://goo.gl/VeLgff](https://goo.gl/VeLgff)
- Brelsfoard CL, Dobson SL (2011) *Wolbachia* effects on host fitness and the influence of male aging on cytoplasmic incompatibility in *Aedes polynesiensis* (Diptera: Culicidae). *J Med Entomol* 48: 1008-1015. [Link: https://goo.gl/22axks](https://goo.gl/22axks)
- Soumare MK, Cilek JE (2011) The effectiveness of *Mesocyclops longisetus* (Copepoda) for the control of container-inhabiting mosquitoes in residential environments. *J Am Mosq Control Assoc* 27:376-383. [Link: https://goo.gl/rQycdp](https://goo.gl/rQycdp)
- (2009) World Health Organization Dengue Guideline for Diagnosis, Treatment, Prevention and Control. [Link: https://goo.gl/pgML6R](https://goo.gl/pgML6R)
- Achee NJ, Gould F, Perkins TA, Reiner RC, Morrison AC, et al. (2015) A critical assessment of vector control for dengue prevention. *PLoS Negl Trop Dis* 9: 1-19. [Link: https://goo.gl/Xy8Fd2](https://goo.gl/Xy8Fd2)
- Gratz NG (1999) Space sprays for the control of *Aedes aegypti* in South-East Asia and the Western Pacific. *Dengue Bulletin* 23: 80-84. [Link: https://goo.gl/4XhtL5](https://goo.gl/4XhtL5)
- Thammapalo S, Chongsuvivatwong V, Geater A, Dueravee M (2008) Environmental factors and incidence of dengue fever and dengue haemorrhagic fever in an urban area, Southern Thailand. *Epidemiol Infect* 136: 135-143. [Link: https://goo.gl/PA8bvX](https://goo.gl/PA8bvX)
- Yakob L, Funk S, Camacho A, Brady O, Edmunds WJ (2017) *Aedes aegypti* control through modernized, integrated vector management. *PLoS Curr* 9: 1-34. [Link: https://goo.gl/h6M2RP](https://goo.gl/h6M2RP)
- Kirkpatrick BD, Durbin AP, Pierce KK, Carmolli MP, Tibery CM, et al. (2015) Robust and immune responses to all 4 dengue virus serotypes following administration of a single dose of a live attenuated tetravalent dengue vaccine to healthy, Flavivirus-naïve adults. *J Infect Dis* 212: 702-710. [Link: https://goo.gl/jPqvVM](https://goo.gl/jPqvVM)



32. Guy B, Barrere B, Malinowski C, Saville M, Teyssou R, et al. (2011) From research to phase III: preclinical, industrial and clinical development of the Sanofi Pasteur tetravalent dengue vaccine. *Vaccine* 29: 7229-7241. [Link: https://goo.gl/XEEyRD](https://goo.gl/XEEyRD)

33. Durbin AP, Kirkpatrick BD, Pierce KK, Elwood D, Larsson CJ, et al. (2013) A single dose of any of four different live attenuated tetravalent dengue vaccines is safe and immunogenic in flavivirus-naive adults: a

randomized, double-blind clinical trial. *J Infect Dis* 207:957-965. [Link: https://goo.gl/qmZmvH](https://goo.gl/qmZmvH)

34. Osorio JE, Velez ID, Thomson C, Lopez L, Jimenez A, et al. (2014) Safety and immunogenicity of a recombinant live attenuated tetravalent dengue vaccine (DENVax) in flavivirus-naive healthy adults in Colombia: a randomised, placebo-controlled, phase 1 study. *Lancet Infect Dis* 14: 830-838. [Link: https://goo.gl/KHsbey](https://goo.gl/KHsbey)