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Aedes: What Do We Know about Them and What Can They Transmit?

Biswadeep Das, Sayam Ghosal and Swabhiman Mohanty

Abstract

Mosquitoes thrive mostly in the tropics and act as the vectors of some of the most debilitating human diseases caused by bioagents. Among the plethora of mosquitoes, *Aedes* transmit arboviruses, which have caused large-scale outbreaks throughout the world. *Stegomyia* is the most important subgenus of *Aedes*, which includes *Ae. aegypti* and *Ae. albopictus* vectors that are widespread and transmit a wide variety of arbovirus belonging to *Togaviridae* with the genus *Alphavirus* (Sindbis virus, equine encephalitis viruses, chikungunya virus), *Flaviviridae* with the genus *Flavivirus* (yellow fever virus, dengue 1–4 viruses, West Nile virus, Japanese and St. Louis encephalitis/SLE-viruses) and the *Bunyaviridae* with the genera *Bunyavirus* (California Group), and *Phlebovirus* (Rift Valley fever). In India, dengue and chikungunya are the most important arboviral diseases transmitted by *Ae. aegypti* and *Ae. albopictus* in recent time. Chikungunya and dengue are acute debilitating arthritogenic and hemorrhagic (dengue) disease, caused by enveloped single-stranded RNA virus belonging to *Alphavirus* and *Flavivirus*, respectively. In this chapter, we will comprehensively delineate the taxonomy of *Aedes* mosquitoes, their geographical distribution, evolutionary biology of chikungunya and dengue viruses, mechanism of transmission, and proposed vector control strategies against *Aedes* mosquitoes.

Keywords: *Aedes*, taxonomy, vector borne disease, chikungunya, dengue, phylogeny, *Wolbachia*

1. Introduction

1.1 *Aedes* mosquito: overview

1.1.1 Brief account on mosquitoes

Mosquitoes are one of the most important groups of insects, because of their significance to humans and animals as vectors of some of the most debilitating diseases. They are small, two-winged insects and found mostly living in the humid tropics and subtropics. Mosquitoes are widely investigated by the researchers because they act as the vectors for a variety of pathogens and parasites including viruses, bacteria, protozoans and nematodes.

1.1.2 Mosquito systematics and classification of Aedes

Mosquitoes are placed in the family Culicidae, suborder Nematocera of the order Diptera (the two-winged flies or true flies). The Culicidae family contains over 3200 species and are divided into 3 subfamilies: Anophelinae, Culicinae, and Toxorhynchitinae [1]. Subfamily Toxorhynchitinae comprises a single genus, *Toxorhynchites* comprising about 76 species. *Toxorhynchites* are not considered as medically important, because both sexes of Toxorhynchitinae possess a proboscis which curves backwards, thereby making them incapable of piercing the skin and transmitting disease in comparison to Anophelinae and Culicinae. There are three genera Anophelinae subfamily, however; only *Anopheles* is of medical importance [2]. There about 60 species of *Anopheles* mosquitoes which are known to be vectors of malaria [3]. Culicinae are the major vectors of arboviruses and filariasis. Medically most important genera in subfamily Culicinae are *Culex*, *Aedes*, *Mansonia*, *Haemagogus*, and *Sabethes* [3, 4].

There are more than 2500 species of Culicinae, with *Aedes* being the major genus, belonging to tribe Aedini [4]. Aedini is the largest tribe of family Culicidae with currently comprising 1240 recognized species. The traditional classification of Aedini is based on the concept of identifying few genera and numerous subgenera [5, 6]. The tribe Aedini was considered as a natural group; however, it was noted that some members showed affinities with all other higher-level taxa of subfamily Culicinae [6]. Species of the tribe Aedini vary extremely and are difficult to identify at the genus level because of overlapping suites of similar morphological features. Hence, different combinations of attributes are required to clarify the majority of the genera, subgenera and species. General characteristics of the tribe include the presence of toothed unguis (tarsal claws) and a pointed abdomen in most females. The traditional classification of Aedini prior to the end of the twentieth century comprised nine genera and 50 subgenera [7, 8]. *Aedes* was by far the largest genus comprising about 1000 species and further subdivided into 41 subgenera. Reclassification of genus *Aedes* began with the elevation of *Verrallina* and *Ayurakitia* to generic status [9, 10], followed by subsequent separation of the remaining subgenera into genera *Aedes* and *Ochlerotatus* [11]. Huge controversies

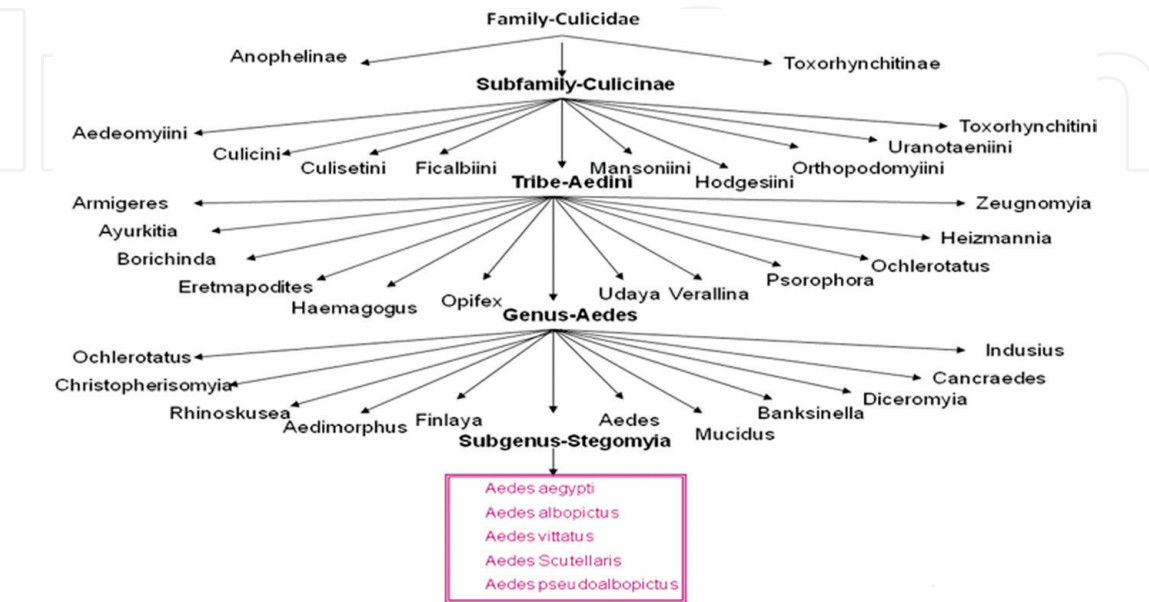


Figure 1. Classification of *Aedes* up to the subgenus level showing few important species of the medically important subgenus *Stegomyia*.

have aroused after the reclassification, especially after upgrading *Ochlerotatus* group to generic status, due to which correct classification of *Aedes* still remains a paradigm [12]. Genus *Aedes* is further subdivided into several subgenera comprising over 900 species (**Figure 1**). Subgenus *Stegomyia* comprises most of the medically important *Aedes* species which are best known vectors of yellow fever, dengue fever, chikungunya fever and some forms of filariasis and other viral diseases [3].

1.1.3 The life cycle of *Aedes mosquito*

Like all other Dipterans, *Aedes* mosquitoes are holometabolous insects, meaning that they undergo a complete metamorphosis process, starting with an egg, larva, pupa, and adult stage.

The adult life span can range from 2 weeks to a month depending on environmental conditions [13]. Most species are unautogenous, that means after copulation the females have to take a blood meal to complete egg development: *Eggs*: after taking a complete blood meal, females produce on an average 100–200 eggs per batch; however, the number of eggs produced is dependent on the size of the blood meal. Eggs are laid on damp surfaces in areas likely to temporarily flood, such as tree holes and man-made containers, and are laid singly, rather than in a mass. Generally, eggs are positioned at varying distances above the water line, and the female mosquito does not lay the entire clutch at a single site; instead, it spreads out the eggs over two

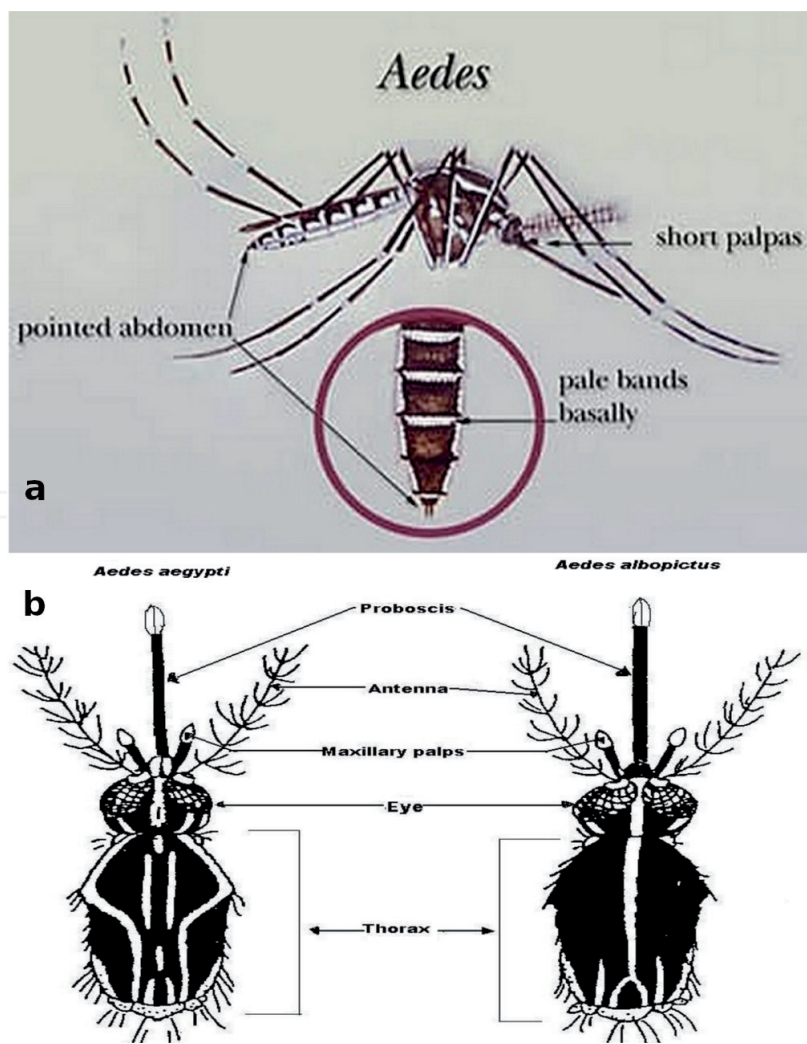


Figure 2.
(a) General morphological parts of an *Aedes* mosquito and (b) thorax of adult female *Ae. aegypti* and *Ae. albopictus* mosquito.

or more sites [14]; *Larvae*: mosquito larvae are often called “wrigglers” or “wigglers,” because they appear to wiggle sporadically in the water upon disturbance. *Aedes* larvae breathe oxygen through a posteriorly located siphon that is held above the water surface, whereas the rest of the body hangs vertically. Larvae are generally found around homes in puddles, pots, cement tanks, tree holes, tires, or within any receptacle retaining water. Larval development is dependent on temperature. The larvae transition through four instars, spending a small duration in the first three instars, and up to 3 days in the fourth instar. Fourth instar larvae are approximately eight millimeters long and are vigorous feeders. Males generally pupate earlier because they develop faster than females. If the environmental temperatures are cool, *Ae. aegypti* can remain in the larval stage for months until the water supply is sufficient [14]; *Pupae*: after the fourth instar, *Aedes* larvae enter the pupal stage. Pupae, also called “tumblers,” do not feed and take around 2–3 days to develop. Adults emerge by ingesting air to expand the abdomen thus splitting open the pupal case and emerge head first; *Adult*: *Aedes* adults can be remarkably distinguished from other mosquitoes by observing the whole body, which is striped, and so called “decorative mosquito” which is more distinct on the legs and scutellum, with short palpi and more or less pointed abdomen with pale bands. For example, adult *Ae. aegypti* and *Ae. albopictus* are often differentiated by the white scale bands on the dorsal part of the thorax. In case of *Ae. aegypti*, the pattern comprises two straight lines surrounded by curved lyre-shaped lines on the side. In contrast, a single broad line of white scales in the middle of the thorax is present in *Ae. albopictus* [15] (Figure 2).

1.1.4 Arbovirus and *Aedes* mosquitoes

Arboviruses (arthropod-borne-viruses) are defined as the viruses, which multiply within arthropods and can be transmitted by the arthropods to vertebrates. Usually, the arthropod gets infected by feeding on the blood from an infected vertebrate during viremia (virus circulation in the peripheral blood vessels), and then the virus can be transmitted to another vertebrate-host (horizontal transmission) after proliferation in the vector. Arboviruses can also be passed from one arthropod generation to another by transovarian transmission (vertical transmission). Several species of *Aedes* transmit arbovirus, which have caused large scale outbreaks throughout the world. *Stegomyia* is the most important subgenus of *Aedes* from medical point of view, followed by subgenus *Finlaya*, *Aedimorphus* and *Diceromyia*. *Ae. aegypti*, *Ae. albopictus*, *Ae. vittatus*, *Ae. scutellaris*, *Ae. pseudoscutellaris*, *Ae. polynesiensis*, *Ae. bromeliae* and *Ae. africanus* are the important vectors of subgenus *Stegomyia* that transmit several arboviral diseases across the world, out of which *Ae. aegypti* and *Ae. albopictus* are the most important vectors that are widespread and transmit a wide variety of arbovirus belonging mainly to three families: the Togaviridae comprising the genus *Alphavirus* (e.g., Sindbis virus, equine encephalitis viruses, chikungunya virus), Flaviviridae with the genus *Flavivirus* (e.g., yellow fever virus, dengue 1–4 viruses, West Nile virus, Japanese and St. Louis encephalitis/SLE-viruses) and the Bunyaviridae comprising the genera *Bunyavirus* (e.g., California Group), and *Phlebovirus* (Rift Valley fever) [16]. *Ae. poicilius*, *Ae. togoi*, *Ae. kochi*, *Ae. niveus*, and *Ae. harinasutai* of subgenus *Finlaya* are important vectors of arboviruses and microfilariae in the Oriental Region. *Ae. vexans* of the subgenus *Aedimorphus* has an extensive distribution in tropical Africa, Central America, Southeast Asia, and temperate regions of the Nearctic and Palaearctic Regions. Two species of the subgenus *Diceromyia*, *Ae. taylori* and *Ae. furcifer* have been implicated in the transmission of yellow fever virus and the spread of chikungunya virus in Africa [17].

In India, dengue and chikungunya are the most important arboviral diseases that are transmitted mainly by *Ae. aegypti* and *Ae. albopictus* and have caused massive and unprecedented outbreaks in recent times, thereby causing huge loss of lives and economic burden to the nation [18–20].

1.2 Chikungunya: overview

1.2.1 History and phylogenetics of CHIKV evolution

CHIKV was first isolated and characterized in 1953 during an epidemic of febrile polyarthritides in Tanzania (formerly Tanganyika) [21]. The word “chikungunya” comes from ChiMakonde, the language spoken by the Makonde people, an ethnic group in southeast and northern Mozambique meaning “that which contorts or bends up” and refers to the stooping posture of infected patients due to severe joint pain.

Since the 1953 Tanzania outbreak, CHIKV has caused outbreaks in various parts of Africa. The re-emergence of CHIKV epidemic in Africa was documented in 1999–2000 in Kinshasa where an estimated 50,000 persons were infected. The first documented Asian outbreak took place in 1958 in Bangkok, Thailand. Since then, many outbreaks have been recorded from Cambodia, Vietnam, Laos, Myanmar, Malaysia and Indonesia. There is historical evidence that chikungunya virus originated in Africa and subsequently spread to Asia [22]. Phylogenetic analysis of CHIKV virus sequences originally identified three distinct clades separated primarily by geography designated the West African, Central/East African (ECSA) and Asian genotypes. The Asian genotypes have a high degree of sequence identity among themselves whereas the African strains exhibit wider sequence diversity and have been shown to undergo genetic microevolutions even during the course of an epidemic [23]. Recent phylogenetic studies showed that the Indian Ocean and Indian subcontinent outbreaks were caused by virus strains of the Indian Ocean lineage (IOL), which evolved from the ECSA genotype [19, 24]. This lineage first emerged in Kenya in 2004, and subsequently spread to several Indian Ocean islands, India and Southeast Asia. The IOL strains involved in the Indian Ocean and Indian outbreaks possessed the initial adaptive mutation, E1-A226V [25], which is a major genetic determinant of adaptation of CHIKV to *Ae. albopictus* vector species and provides a plausible explanation for how this mutant CHIKV caused epidemics in regions lacking the more typical urban vector, *Ae. aegypti* [26]. Introduction of new viral strains, viz. IOL strains inevitably leads to the question whether particular genotypes of CHIKV are associated with higher virulence or severe disease. In addition, the lack of a suitable animal model for CHIKV makes it difficult to verify such hypotheses [27]. On the other hand, association of the re-emergence of endemic strains with the outbreaks leads to a different question that can be clarified only by a combination of classic epidemiology and comparative genomics: whether the viruses re-emerged due to environmental, population immunity and/or vectorial factors, or whether outbreaks were triggered by adaptive evolution of the virus that endowed it with an increase in fitness and virulence? Therefore, knowledge of the complete genetic blueprint of CHIKV is essential for clarifying these crucial questions.

1.2.2 Chikungunya virus (CHIKV): genome structure and organization

Chikungunya is an acute debilitating arthritogenic disease, caused by chikungunya virus (CHIKV) belonging to *Alphavirus* genus; family *Togaviridae*, which consists of *Alphavirus* and *Rubivirus* genera. Approximately, 40 *alphaviruses* can infect vertebrates such as humans, rodents, birds, and horses, along with invertebrates. Mosquito vectors are responsible for the transmission between species,

thereby rendering *alphaviruses* to be considered as arboviruses. CHIKV is an enveloped particle and has a single-stranded RNA genome of positive polarity. The genome is approximately 11.8 kb in length [28]. Under electron microscopy in green monkey kidney (Vero) cells CHIKV particles reveal a characteristic *Alphavirus* morphology.

CHIKV genome consists of polyadenylated RNA and is capped, which encodes two open reading frames (ORFs). Two-third of the genome includes the 5' ORF that encodes four nonstructural proteins, which are involved in genome replication, capping of RNA, polyprotein cleavage, etc., essential for viral replication. This region is expressed as an nsP1-3 or nsP1-4 polyprotein via cap-dependent translation, which is further cleaved by an nsP2-encoded protease. In context to the structural protein ORF, it is embedded in a subgenomic mRNA, and is translated into proteins via a cap-dependent mechanism. This structural ORF polyprotein is finally cleaved into capsid, envelope glycoproteins E1, and glycoprotein E2. The mature virion comprises 240 heterodimers of E2/E1, which are arranged as trimeric spikes on its surface, and has a diameter of 70 nm (**Figure 3**). After being translocated by the secretory pathway, these heterodimer spikes penetrate into the plasma membrane of infected cells, and cytoplasmic nucleocapsids containing the genomic RNA and 240 copies of the capsid protein bud from the cell surface for assembly of the virion envelope and envelope protein spikes [28].

During early infection, the nonstructural proteins are synthesized directly from the two third of genomic RNA as a P1234 polypeptide that is further cleaved to form Nsp1, Nsp2, Nsp3, and Nsp4 nonstructural proteins. Nsp1 protein is involved in the synthesis of minus-strand RNA, in addition to building association of the replication complex with cellular membranes. Nsp2 protein acts as a helicase and proteinase that cleaves the nonstructural polyprotein to form the individual

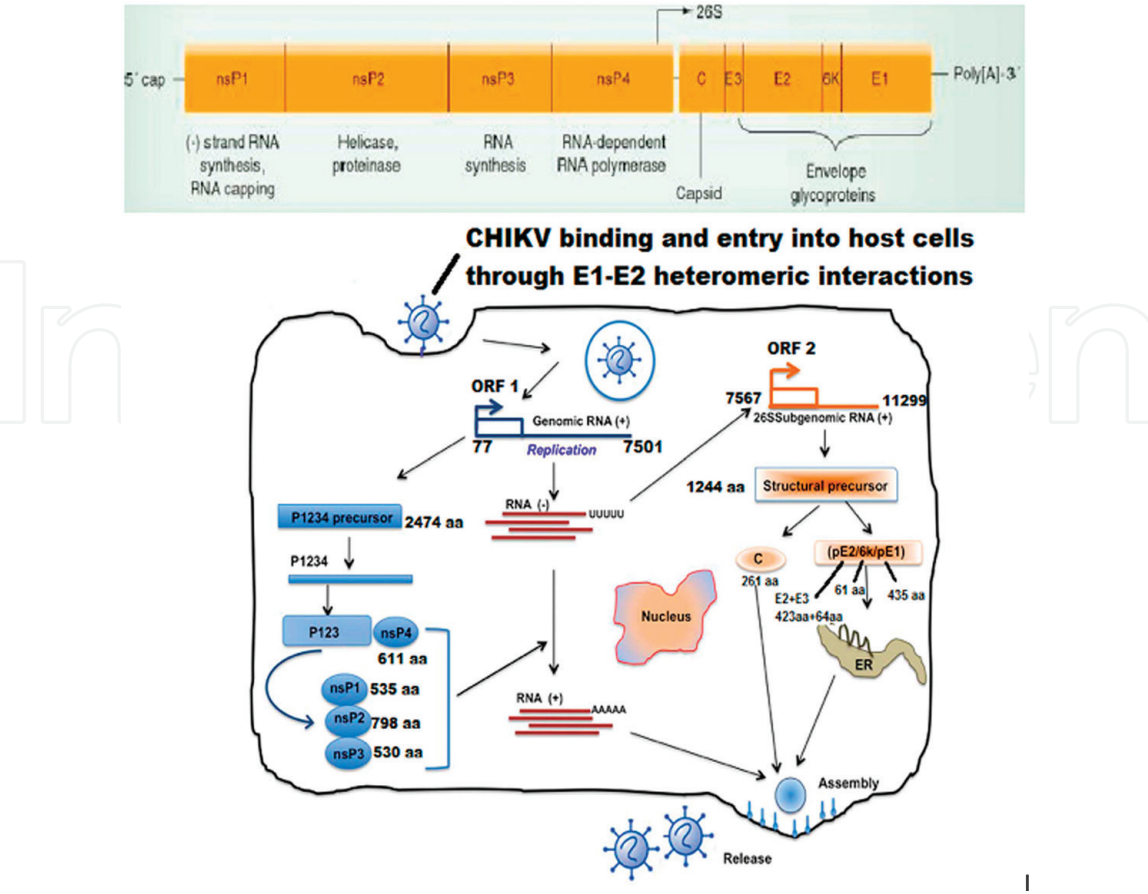


Figure 3.
CHIKV genome, binding, and entry into host cells followed by replication.

nonstructural proteins [30]. The function of Nsp3 in viral replication is largely unknown, however, it is probably involved in RNA synthesis [28]. Nsp4 interacts with the N terminal region and other nonstructural proteins and host factors, and acts as the viral RNA polymerase. The sub-genomic mRNA (26S) synthesizes single polypeptide that comprises the structural proteins, such as capsid, E3, E2, 6K and E1. These proteins are then cleaved co-translationally and post translationally to form the functional structural proteins. These structural proteins have important functions during virus replication and particularly, in the interaction with the host. Such interaction with the host was first presented by the production of antibodies that played important roles in the recovery from infection [31].

The region between the nonstructural and structural domains is called the “junction region,” which enhances the transcription of an intracellular subgenomic 26S RNA. There are two other untranslated regions, along with the junction region; one at the 5'-end, which is required for the synthesis of the plus-strand [25], and the other at the 3' end between the stop codon of the E1 gene and the poly (A) tail. This region is mainly involved in translation of viral proteins rather than in replication of the genomic RNA [28, 32].

1.2.3 Replication cycle of CHIKV

The interaction between the envelope proteins of CHIKV and receptors of host cells is required to penetrate into vertebrate cells. The cellular receptors for CHIKV are still unknown; however, in other Alphavirus the laminin receptor, glycosaminoglycans and DC-SIGN (CD209) molecules are involved in viral uptake [33]. The virus is transported into the cell by endocytosis of clathrin-coated vesicles. The activation of E1 protein from the E1-E2 complex is initiated because of the pH reduction of the vesicle, thereby initiating fusion of viral and endosomal membranes, resulting in the release of the nucleocapsid into the cytoplasm. Replication of CHIKV occurs in the cytoplasm. The first event is P1234 precursor polyprotein translation and RNA replication. P1234 polyproteins are directly translated from the viral genome, followed by the initiation of RNA replication through the synthesis of a complete minus-strand RNA, which serves as the template for the synthesis of the viral genome and for the transcription of 26S subgenomic plus-strand RNA from the internal promoter of the junction region. As both processes are inter-linked, Nsp4 associates with P123 and other host factors to regulate the synthesis of minus-strand RNA, after cleavage from the P1234 polyprotein. This switching from genome replication to transcription of sub-genomic 26S positive-strand RNA is also regulated by the nonstructural proteins that were cleaved from the P123 polyproteins [34]. The 26S subgenomic RNA that serves as the mRNA translates the structural protein precursor, and further undergoes co-translational cleavage to become mature (C-E3-E2-6 k-E1). Autocatalytic cleavage of the N-terminal region of structural polyprotein precursor generates the capsid protein, followed by encapsidation of the viral genomic RNA, thereby, resulting in the rapid assembly of nucleocapsid cores in the cytoplasm. In parallel, E2 and E1 are transferred to the plasma membrane after being cleaved from the envelope polyprotein precursor. Finally, the packaging of the virus is performed in the cytoplasm by the assembly of nucleocapsid cores along with glycoproteins, and the virus is released by budding through the cellular membrane to form an enveloped virion [34].

1.2.4 Clinical presentations of CHIKV

The most common symptom in chikungunya disease is painful polyarthralgia, mainly bilateral, symmetrical and culminates within few days usually affecting

peripheral joints like ankles, toes, fingers, elbows, wrists and knees. The joints exhibit extreme tenderness and swelling with patients frequently reporting incapacitating pain that lasts for weeks or months. Other typical signs for CHIKV infection include fever, headache, retro-orbital pain, chills, weakness, lumbar back pain, joint stiffness, malaise, nausea and a rash that may or may not be accompanied by other signs and symptoms of the disease [35]. The acute illness lasts 3–5 days, with recovery in 5–7 days. The incubation period following the bite of an infected mosquito is short (2–6 days) and ends with a sudden onset of fever reaching as high as 104°F that may last up to 10 days. The fever almost always precedes the rash and joint pain and only very rarely has been reported as biphasic with recurrence noted on the fourth or fifth day of illness. The rash, appearing primarily on the trunk, face, and limbs of the body is visible on day 2–5 postinfection, and may last up to 10 days. Older patients with an history of rheumatism exhibit more severe symptoms in comparison to younger patients [36].

1.2.5 Pathogenesis and diagnosis of CHIKV

Detailed studies on the pathogenesis of the chikungunya fever are rare. It is hypothesized that after inoculation, primary viral multiplication occurs in lymphoid and myeloid cells. The arthropod vectors acquire the virus by sucking blood during this period. The virus, then spreads to the targeted organs and immune system starts functioning at this stage, leading to the activation of both humoral and cellular immunity. This response of the body leads to the development of clinical features of the disease [36].

The probable diagnosis of chikungunya fever can be made on the basis of the presence of the virus in the community, and a clinical trial of fever, rashes and arthralgia, which are suggestive of the illness. The virus produces neutralizing and haemagglutination inhibiting (HI) antibodies, which helps in serological diagnosis. HI test is the simplest diagnostic test; however, it identifies the group rather than specific virus. Confirmation of the illness is done by detection of the antigen or antibody to the analyte in the blood sample of patient [37]. Reverse transcriptase polymerase chain reaction (RT-PCR) is a confirmatory test for the identification of CHIKV. IgM capture ELISA is the most sensitive serological assay, and can distinguish the chikungunya from dengue. All virus isolation procedures need to be done under bio safety level 3 (BSL-3) precautions, although such precautions may not be necessary in the countries where CHIKV is endemic.

1.2.6 Transmission cycles of CHIKV

CHIKV is transmitted by mosquitoes belonging to genus *Aedes*. The mosquitoes considered to be the main vectors for CHIKV are *Ae. albopictus* and *Ae. aegypti* [3]. Continuous variations in the geographic distribution of these vectors have been documented in several studies. *Ae. aegypti* was considered to be the primary vector of CHIKV in most parts of the globe [38], whereas *Ae. albopictus* (common name Asian tiger mosquito) was considered to be the secondary vector and was restricted to Asia [38]. However, the recent reemergence of CHIKV in many parts of the world has been mainly associated with *Ae. albopictus* vector [19, 38]. Furthermore, reports indicate *Ae. albopictus* to replicate and transmit the old African genotype of CHIKV as well as the recent Indian Ocean strain of CHIKV better than those of *Ae. aegypti* and other *Aedes* species [39]. CHIKV is endemic in tropical regions of Africa and Asia, where the mechanisms of CHIKV transmission and maintenance appears to be very complex and vary significantly depending on the particular region where virus activity is detected.

1.2.7 CHIKV in African mosquito vectors

In Africa, CHIKV is believed to be maintained in a sylvatic as well as with urban/rural cycle involving wild nonhuman primates and forest-dwelling *Aedes* mosquitoes (**Figure 4**). Several field studies conducted in Senegal, Nigeria, Uganda, Tanzania, Cote d'Ivoire, Central African Republic and South Africa concluded that the main sylvatic vectors of CHIKV are probably *Ae. furcifer*, *Ae. taylori*, *Ae. africanus*, *Ae. luteocephalus* and *Ae. Neoafricanus* [32]. Based on the isolation frequencies, it appears that *Ae. furcifer* and *Ae. taylori* are more important in southern and western Africa, while *Ae. africanus* is the chief vector in central regions [35]. Laboratory studies have confirmed vector competence of *Aedine* sylvatic mosquitoes in Africa. In South Africa, the oral infectious dose of *Ae. furcifer* was 50% (OID₅₀)—the titer of the virus in the blood meal sufficient to infect 50% of mosquitoes was found to be less than 6.2 log/ml resulting in a transmission rate of 25–32%. This is sufficient to sustain CHIKV transmission from velvet monkeys and baboons, which develop viremia up to 7–8 log/ml [41].

1.2.8 CHIKV circulation in Asian mosquito vectors

In contrast to Africa, only urban/rural CHIKV transmission cycle has been described in Asia (**Figure 4**). *Ae. aegypti* is the main vector of CHIKV and *Ae. albopictus* is believed to play a secondary role in several outbreaks. CHIKV epidemics in humans seem to be disconnected from zoonotic transmission, however; the recent study of seroprevalence to CHIKV infection among wild monkeys in the Philippines showed presence of anti-CHIKV IgG in 59.3% of animals tested, suggesting existence of a possible sylvatic transmission cycles [42]. Currently, it is believed that persistence of CHIKV in Asia results from viral migration back and forth among different locations sustained by the human-*Ae. aegypti* cycle [43].

The vectors responsible for viral transmission during these epidemics have not been definitely characterized. Both *Ae. aegypti* and *Ae. albopictus* are present in India and their epidemiologic significances for CHIKV transmission probably vary

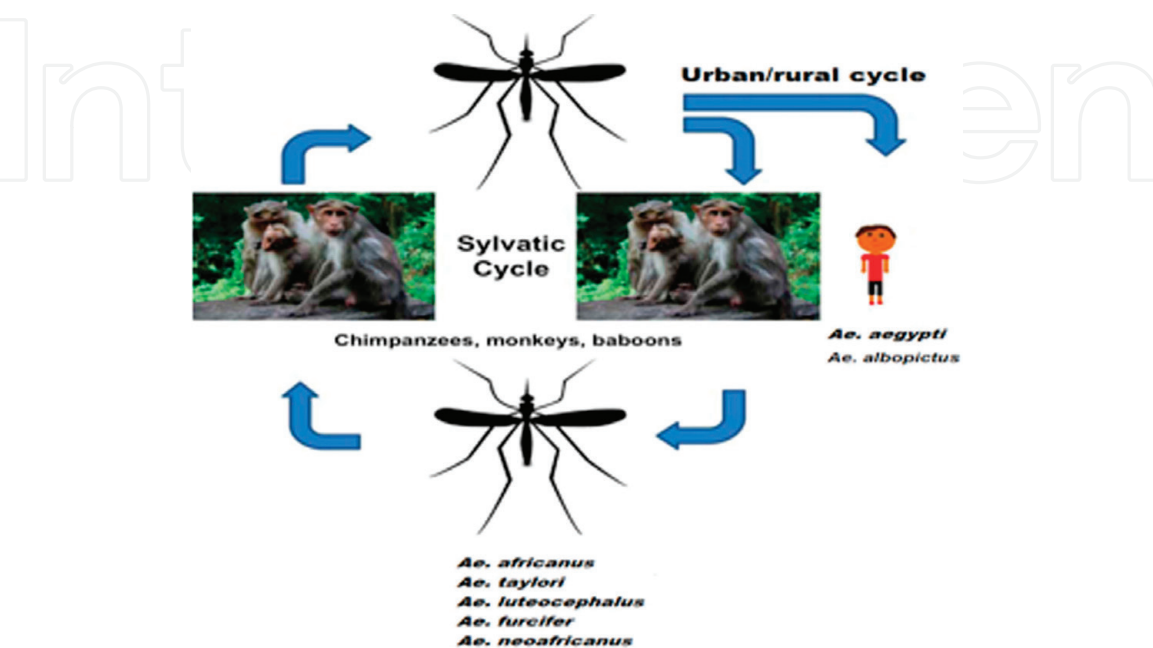


Figure 4.
CHIKV transmission cycle in Asia and Africa (modified from Thiboutot et al. [29, 40]).

dependent on the geographic location. Another intriguing feature of the 2006–2008 CHIKV epidemic in India, beside the magnitude, is the fact that this epidemic was caused by virus of the ECSA genotype. All previous outbreaks were caused by Asian genotype of CHIKV. It was proposed that this shift in viral genotype was the major factor in the re-emergence of Chikungunya in an unprecedented outbreak in India after a gap of 32 years [44].

1.3 Dengue: overview

1.3.1 History and geographic distribution of DENV

DENV is found in tropical and subtropical areas throughout the world, with prevalence in both urban and suburban areas. DENV is endemic in more than one-hundred countries with more than two-and-a-half billion people and around 40% of the world's population living in areas at risk for infection. The World Health Organization estimates that there are between fifty and one-hundred million DENV infections each year, causing hospitalization of five-hundred thousand people, and a death rate of two-and-a-half percent [45]. The earliest report of disease with dengue-like symptoms dates back to a Chinese encyclopedia of disease symptoms and remedies that was published from 265 to 420 A. D during the Chin Dynasty [46]. It is speculated that DENV was the etiological agent during disease outbreaks in the French West Indies in 1635, in Panama in 1699, and the Philadelphia epidemic of 1780 [47]. Reported cases of dengue disease were seen in 1779 and 1780 in Africa, Asia, and North America [48]. The first verified dengue epidemic occurred from 1953 to 1954 in the Philippines followed by a quick global spread of epidemics of DF/DHF. In the 1980s and 1990s, DENV continued to expand, and reached areas with mosquito vectors [49].

The very first report of the existence of dengue fever in India was in 1946 from US soldiers in Kolkata [50]. Since then, there was no significant dengue activity reported anywhere in the country for the next 18 years. In 1963–1964, an epidemic of dengue fever was reported from the Eastern Coast of India, further spreading northwards and reached Delhi in 1967 and Kanpur in 1968. Simultaneously, the DENV epidemic also engulfed the southern part of the country and gradually the whole country was affected by wide spread epidemics followed by endemic/hyperendemic prevalence of all the four serotypes of DENV. However, most dengue outbreaks in India were simple dengue fever with very rare cases of DHF/DSS epidemics. The first major wide spread epidemics of DHF/DSS occurred in 1996 in India, involving areas around Delhi and Lucknow, further spreading across the country [51, 52]. Since then, the epidemiology of DENV and its prevalent serotypes has been frequently changing in India.

1.3.2 Dengue virus (DENV)

Dengue virus (DENV) is a member of enveloped, positive-strand RNA viruses of the flaviviridae family. The flaviviridae family also includes West Nile virus, yellow fever virus, Japanese encephalitis virus, hepatitis C virus, and tick-borne encephalitis virus. Flaviviruses are transmitted to humans by arthropod vectors such as mosquitoes or ticks [49].

1.3.3 Dengue viral structure

There are four phylogenetically and genetically distinct, but antigenically related serotypes classified as DENV-1, DENV-2, DENV-3 and DENV-4. The dengue virion is

a spherical particle, existing as either a 50 nm diameter immature particle or a mature 60 nm diameter particle with a lipopolysaccharide envelope. DENV genome is about 11 kb with a single ORF encoding three structural proteins: capsid (C), membrane (M), and envelope (E) and seven viral encoded nonstructural proteins: NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5 (Halstead, 2008). DENV RNA contains a type I cap structure (m⁷GpppAmpN₂), located at its 5'-end, and lacks the poly (A) tail at its 3' end. The DENV genome is surrounded with C proteins and forms the inner core. The structural proteins E and M are surface proteins on the virion envelope and the conformations of these proteins are used to distinguish between immature and mature virus. The immature virus is referred to as "spiky" as M proteins bound to a precursor membrane protein (pr) form heterodimers with E proteins that appear as "spikes" on the viral surfaces. In mature virions, the soluble pr is cleaved from M protein by furin, anchoring the M proteins and causing the pr protein to be absent in the mature viral membrane [53].

1.3.4 Dengue viral replication

DENV enters a variety of cells including macrophages, monocytes, and dendritic cells through cell endocytic vesicles. The first step in DENV infection is binding to the cellular receptors on the surface of the target cell like ubiquitous receptor (DC-SIGN). This interaction leads to the internalization of the virion via receptor-mediated endocytosis, resulting in the fusion of the virus with the endosomal membrane because of acidification, and finally releasing the viral genome into the cytoplasm. DENV genome is associated with rough ER (site of its translation), because the viral RNA can act as mRNA. Viral replication occurs in two steps: first, the positive-polarity RNA is copied to RNA of negative polarity that serves as a template for the synthesis of multiple strands of RNAs of positive polarity (amplification process); the positive-polarity RNA can then translate into proteins, for further RNA synthesis of negative polarity, or can become associated with structural proteins C, E, and M to form the viral progeny. Second, the immature virus particles travel to the Golgi apparatus in vesicles, where they undergo glycosylation, and are finally transported through secretory vesicles outside the cell (**Figure 5**). During the latter process, the furin cleaves prM in M to generate mature virions, which is the final step of viral morphogenesis [55]. The three main elements that are necessary for DENV replication are: *cis*-acting elements, *trans*-acting factors, and viral induced membranes.

1.3.4.1 *Cis*-acting elements

The *cis*-acting elements are mainly located at both ends of DENV genome in the 5'- and 3'-UTR. The cyclization sequences, as well as the upstream UAG region located at both ends of DENV genome and the downstream AUG region induce circularization of DENV genome. For an efficient negative-strand RNA synthesis, the secondary structure of the stem loop at the 3'-end (3'-SL), along with the secondary structure of SL structures within the 5'-UTR are essential. The initiation of viral replication occurs with the binding of NS5 (RNA dependent RNA-polymerase) to the 5'-UTR.

Trans-acting factors are of two types: viral *trans*-acting factors and cellular *trans*-acting factors.

Viral *trans*-acting factors: NS3 and NS5 (multifunctional and multidomain proteins, respectively) are the only proteins encoded by DENV possessing catalytic activities. NS5 has two main activities: RNA-dependent RNA-polymerase and methyltransferase. NS3 has protease, helicase, and nucleoside triphosphatase activities. NS3 functions by regulating its association with other viral proteins. NS1 and the small nonstructural proteins are required for anchoring the viral replication complex to the membranes of the endoplasmic reticulum.

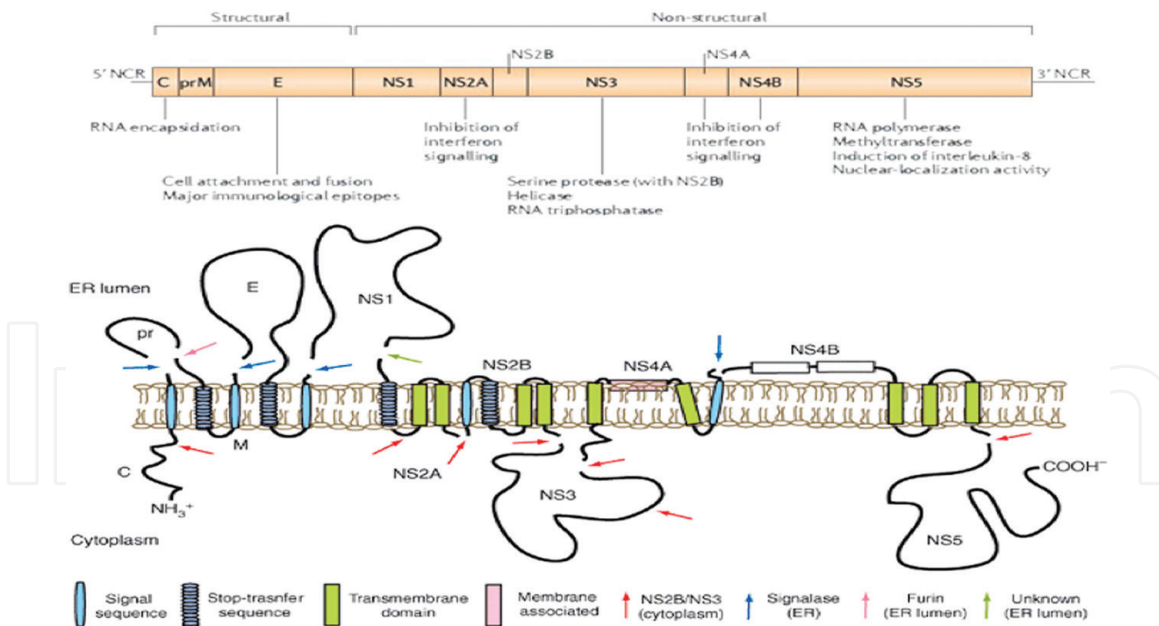


Figure 5. Picture demonstrating: gene organization in DENV RNA genome (top), membrane topology and proteolytic cleavage sites of the transcribed polyprotein (bottom). Arrows denote the cellular and viral proteases, which process the immature polyprotein into ten separate proteins (modified from Perera and Kuhn [54]).

Cellular *trans*-acting factors: several cellular proteins, such as elongation factor 1a (EF1a), polypyrimidine tract binding protein (PTB), LA, calreticulin, PDI, and the heterogenous nuclear factors A1, A2/B1 and Q, have been found to bind to the 5'- or 3'-UTR of DENV. During DENV infection, PTB and La proteins translocate from the nucleus to the cytoplasm and act as the positive and negative regulators of viral replication, respectively. The YB-1 protein might participate in the switching from viral translation to replication or might have a role as an antiviral factor [54, 55].

1.3.4.2 Viral induced membranes (replication complex)

For the formation of the replication complex, proliferation and the generation of invaginations of the ER membranes are required initially, which are probably induced by NS4A and NS3 in conjunction with cellular and other viral proteins. Invaginations are mainly considered as the site for viral replication. The DENV RNA is exported to the convoluted membranes that might potentially store proteins and lipids required for DENV replication. Viral morphogenesis is initiated by the association of the RNA and the C protein generating nucleocapsids. The C protein accumulates around the lipid droplets in the ER. Accumulation of immature viral particles occurs in the lumen of dilated ER cisternae, which are then transported to the *cis*-Golgi for maturation (**Figure 6**) [56].

1.3.5. Classification and symptoms of dengue

Cases of symptomatic dengue have historically been classified by severity according to WHO guidelines first published in 1975, which differentiate between cases of dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome [3] (**Figure 7**). However, dengue epidemiology has changed considerably since these guidelines were first published, leading to difficulties with the use of this classification system in a clinical setting. Examples of severe dengue that do not follow WHO criteria of DHF stratification are dengue with hemorrhage but without evidence of plasma leakage; dengue with shock syndrome without fulfilling all four DHF criteria; and severe dengue accompanied with organ dysfunction and a low level of plasma

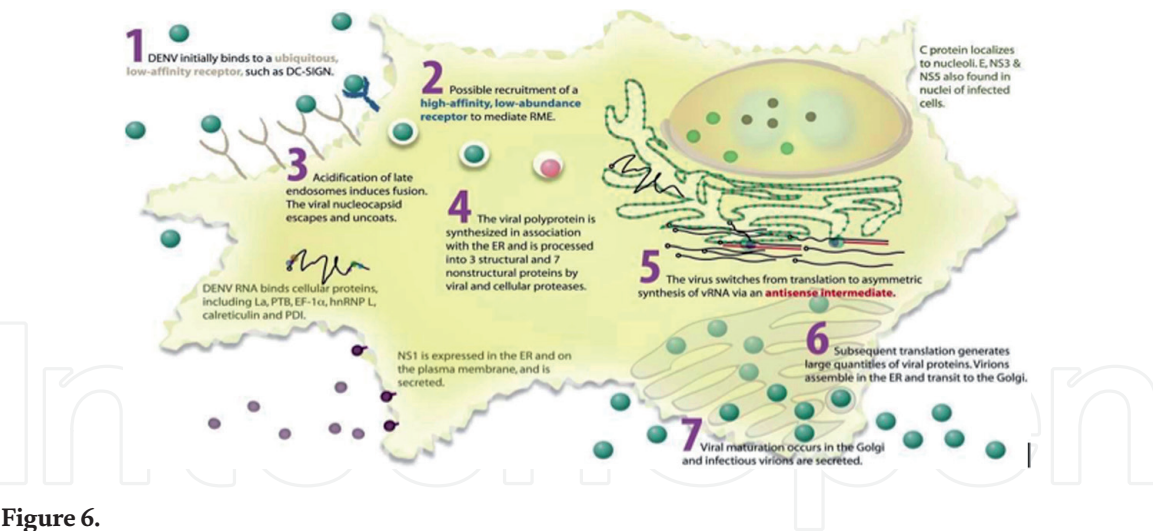


Figure 6.
Dengue replication cycle. Dengue enters a susceptible cell through receptor-mediated endocytosis. In endosomal vesicles, dengue virions are uncoated and release the genome into the ER. Viral RNA is translated into a polypeptide and processed to form viral proteins. Replication and viral assembly occurs in the ER, and the virions travel to the Golgi for modification and is exported via exocytic vesicles (adapted from Clyde et al. [56]).

leakage. In view of the above facts, recently the WHO Tropical Disease Research (TDR), 2009 [57] proposed a new classification of dengue, i.e., dengue (D), dengue with warning signs (DW) and severe dengue (SD) in order to re-evaluate the current classification for better management of high case fatalities. However, the previous

Dengue fever

Acute febrile illness with two or more of the following:

- headache
- retro-orbital pain
- myalgia
- arthralgia
- rash
- haemorrhagic manifestations
- leukopenia

and either supportive serology or proximity to laboratory confirmed cases.

Dengue haemorrhagic fever

The following indications must all be present:

- fever or a history of fever
- haemorrhagic tendencies evidenced by one or more of the following:
 - a positive tourniquet test
 - petechiae, ecchymoses or purpura
 - bleeding from the mucosa, gastrointestinal tract, injection sites or other locations
 - haematemesis or melaena
- thrombocytopenia (100,000 cells per mm³ or less)
- evidence of plasma leakage due to increased vascular permeability, manifested by at least one of the following:
 - a rise in the haematocrit equal to or greater than 20% above average for age, sex and population
 - a drop in the haematocrit following volume-replacement treatment equal to or greater than 20% of baseline
 - signs of plasma leakage such as pleural effusion, ascites and hypoproteinaemia

Dengue shock syndrome

All four of the above DHF criteria must be present, plus evidence of circulatory failure manifested by either of the following:

- rapid, weak pulse and narrow pulse pressure (<20mmHg)
- hypotension for age (systolic pressure <80mmHg for those less than 5 years of age, or <90mmHg for those greater than or equal to 5 years of age) and cold, clammy skin and restlessness

Figure 7.
Dengue case classification [3]. Dengue cases are classified as dengue fever (DF), dengue hemorrhagic fever (DHF), or dengue shock syndrome (DSS) according to the clinical observations shown in the figure.

classification of dengue [3] is still being followed in most countries for rapid diagnosis and prompt treatment of most cases with aggravated symptoms.

1.3.6 Pathogenesis and diagnosis of DENV

For the establishment of DENV infection in the patient, an incubation period generally occurs ranging between 3 and 14 days after a patient gets infected with DENV through the bite of an infected female *Aedes* mosquito. The patient subsequently experiences the painful febrile period when viremia is at its peak, which recedes in about 5–7 days after the onset of fever, coincident with defervescence. DHF/DSS usually develops during this time and the patient may develop intense clinical manifestations. If DHF develops, the patient may rapidly go into a state of shock and die within 12–24 hours if left untreated. After defervescence, laboratory diagnosis is based on IgG and IgM antibody detection. The disease progression for dengue is presented in the schematic form in **Figure 8**. The most commonly used diagnostic laboratory tests for DENV detection include those that detect DENVs, such as isolation by tissue culture and RT-PCR, and those that detect antibodies against the virus, such as enzyme-linked immunosorbent assay (ELISA), neutralization tests, haemagglutination inhibition (HI), and immunofluorescence (IF). Although the gold standard laboratory diagnosis of any flavivirus infection is isolation, and further characterization of the virus (for example by antigen detection) from the patient sample, it is a lengthy process and requires over a week for completion. In contrast, RT-PCR could be performed within hours and could therefore improve patient care. These detection methods are mainly used within approximately 10 days of the onset of symptoms because the virus is present in the sera typically till the duration of fever [3]. After this period, antibodies generated against DENV can be detected using serological methods. The antibodies neutralize DENV, and therefore, it is not possible to detect or culture the virus once the immune response is significantly underway.

1.3.7 Immune response and antibody-dependent enhancement (ADE)

Throughout his/her lifetime, a person can suffer from dengue infection four times (once for each of the four DENV serotypes). Both primary (first) and secondary (subsequent) infections by any DENV serotype can result in any of the two clinical manifestations: less severe DF or more severe DHF. A life-long immunity is conferred against the infecting serotype if primary infection occurs in a patient, along with a

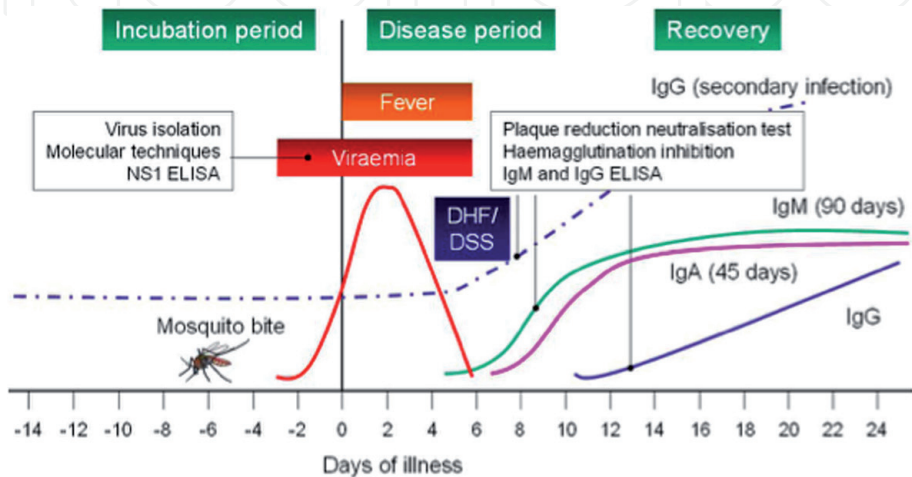


Figure 8.
 Course of dengue infection and the timings and choices of diagnostic methods.

brief protection against infection by other DENV serotypes in the recovered patient. However, epidemiological data and some studies suggest that the immunity thus gained, after the lapse of the temporary cross-serotypic protection, increases the probability of an individual to develop DHF when infected by a second heterologous DENV serotype. A hypothesis that can explain this phenomenon is the antibody-dependent enhancement (ADE), which states that immunocomplexes are formed between the preexisting sub-neutralizing antibodies from the primary infection and the second infecting DENV serotype, which bind to the cells bearing Fcγ receptor (FcγR) (monocytes and B cells), thereby, leading to increased virus uptake and replication [58] (**Figure 9**).

1.3.8 Vectors and transmission cycles of DENV

Dengue is transmitted from person to person through the bites of infected female *Aedes* mosquitoes. DENV is believed to have been maintained in sylvatic/enzootic transmission cycles involving nonhuman primate hosts and vector species living in forests. The virus was first transmitted to humans when the two hosts (humans and nonhuman primate) came into contact, and was, thereafter established in continuous human-mosquito cycles in and/or around human population centers. Several species of the genus *Aedes* are known to transmit DENV; the principal vector is *Aedes aegypti*. The Australian naturalist, Thomas Lane Bancroft in 1906 first suggested that *Ae. aegypti* is the carrier of dengue fever based on epidemiological grounds [59]. *Ae. aegypti* is a day-biter that prefers to breed in domestic and peridomestic water containers. Its adaptation to human habitats and its desiccation-resistant eggs have allowed it to flourish in urban centers.

Ae. albopictus, commonly known as the Asian tiger mosquito is considered as the secondary vector of DENV. Koizumi et al., 1917 first identified its role as the dengue vector in semi-tropical regions in Taiwan [60]. *Ae. albopictus* serves as the primary vector for dengue in countries where *Ae. aegypti* is absent and as a maintenance vector in rural areas where both species coexist [61]. Moreover, ecology changes and global urbanization have caused major changes in the vectorial behavior of the two species, rendering *Ae. albopictus* to be the major vector of arboviral diseases like dengue and chikungunya in many countries, like India [19, 20]. In the Pacific islands, *Ae. polynesiensis* has been suggested as the primary dengue vector, whereas *Ae. scutellaris* was identified as the “jungle” vector for dengue [62]. In the continued absence of vaccines and specific treatment, effective vector control (either through fogging that kills adult mosquitoes, application of larvicides that target the aquatic stage of mosquitoes, source reduction that reduces their breeding habitat or biological control methods employing *Wolbachia* to hinder the fertility of mosquitoes) is currently the only practical method available for reducing the incidence of dengue disease [63].

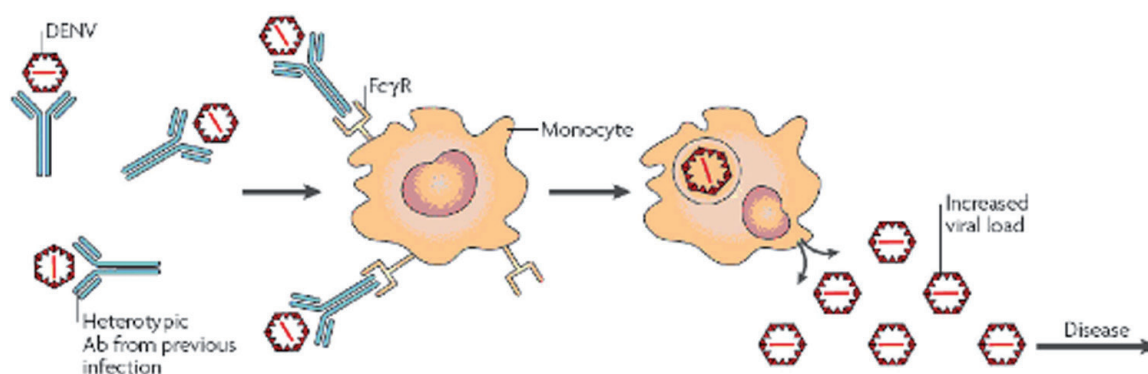


Figure 9.
 Model for antibody-dependent enhancement (ADE) of dengue virus replication.

1.3.9 Role of phylogenetics in DENV evolution

DENV serotypes have been classified into multiple genotypes based on their genomic diversity. Genotype classification can often unveil the geographical origin of the dengue virus strains, except for the sylvatic genotypes. This has assisted in the temporal and spatial tracking of the virus transmission routes, which has served as the basis of molecular epidemiological studies, focusing on determining the causative agents of dengue epidemics, such as the introduction of new viruses and the result of re-emergence of endemic strains (**Figure 10**). Moreover, DENV has a tendency to evolve rapidly due to factors such as inter and intra serotypic recombination, mutation and ecological changes, thereby resulting in generation of new genotypes, which are more virulent, resistant and can cause massive outbreaks affecting large number of people. Introduction of such new viral genotypes inevitably leads to the question whether particular genotypes of DENV are associated with higher virulence or severe disease. To date, several diseases have often been associated with several DENV genotypes originating in Southeast Asia. The lack of a suitable animal model for the dengue disease poses challenges in confirming such hypothesis [64].

1.4 Vector control strategies

1.4.1 Conventional vector control

Vector control programs greatly depend on the use of chemicals such as insecticides like DDT, pyrethroids, organophosphates, and temephos. The annual demand of the insecticides amounts to more than 50,000 tons, with DDT being the most commonly used insecticide in the past. DDT, which is mainly used in indoor spraying for the control of vectors of malaria and visceral leishmaniasis, is forbidden in most of the countries today after the Stockholm Convention in 2001, when it was discovered to be dangerous to wildlife and the environment as it can remain in the environment and food chain for a considerably long time. Regarding other insecticides, most of them have undesirable effects besides their life-saving benefits. For example, vectors can become resistant and the nonbiodegradability of the chemical frequently causes environmental damage. Although efforts have been conducted to develop a suitable vaccine against arboviral diseases like dengue and chikungunya,

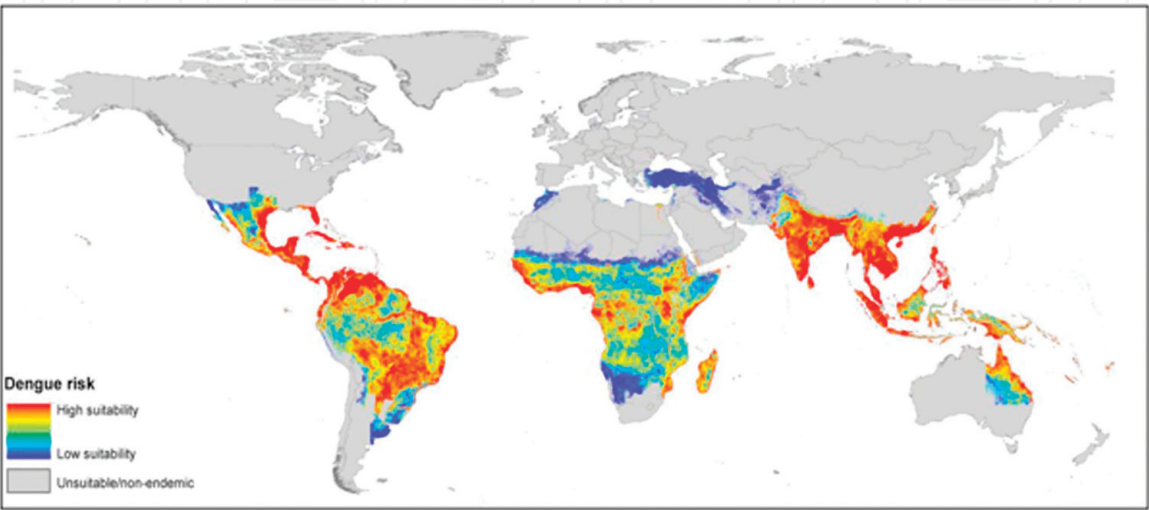


Figure 10. Dengue risk map showing the highly suitable dengue epidemic areas around the world, depicting India to be a high-risk zone for dengue outbreaks (adapted from Simmons et al. [63]).

no vaccine has been developed with 100% efficacy to date. Therefore, the only means of reducing case fatality rate is early diagnosis and proper case management. The chief mode of controlling the disease is by eliminating the vector. It is of course much cheaper to prevent an outbreak of the disease than to diagnose and to treat the cases.

The major strategies for controlling *Aedes* vectors are: (1) reduction of *Aedes* breeding sites through environmental sanitation by the elimination of all nonessential water-containing receptacles. This is by far the most effective method in terms of long-term reduction of the mosquito population; (2) protection of water-containing receptacles by putting lids or covers to prevent egg laying by the mosquitoes; (3) release of larvivorous fish or other biological organisms as predators/parasites of larvae; (4) observation of a “Weekly Dry Day,” i.e., the containers can be emptied at least once a week through generating awareness among the local population; (5) cleaning the containers before and after the rainy season can also contribute in reducing the mosquito populations and (6) space spraying, for example, with malathion against adult mosquitoes and larviciding with temephos.

1.4.2 *Wolbachia*: potential biocontrol agent

Wolbachia is a bacterium belonging to the tribe *Wolbachia* and family Rickettsiaceae and order Rickettsiales. They are a widespread group of bacteria commonly found in the reproductive tissues of arthropods. *Wolbachia* have attracted much attention by virtue of its ability to manipulate the reproduction of its arthropod hosts. Mosquito vectors such as *Aedes*, *Culex*, and *Anopheles* transmit a variety of diseases like dengue, filaria, Japanese encephalitis, and malaria. The vectors have gained resistance against insecticide and pesticides due to their variant mutation in genetic constitution. The continuous use of insecticides for control strategies increasingly faces the problems of high cost, increasing mosquito resistance and negative effects on nontarget organisms. *Wolbachia* have attracted scientific interest due to their ability to manipulate host reproduction, leading to distinct phenotypic effects in the host such as parthenogenesis, feminization, male killings and cytoplasmic incompatibility [65]. These modifications typically confer a reproductive advantage to infected individuals and allow the rapid spread of *Wolbachia* through a population [66, 67]. The most common effect of *Wolbachia* infection in mosquitoes is cytoplasmic incompatibility, which was first described in *Culex pipiens*, when infected male mosquitoes mated with uninfected female mosquitoes of the same species. The ability of *Wolbachia* to manipulate its host biology enables it to increase in frequency in host populations without the need for horizontal transmissions [68]. Hence *Wolbachia* can be used as a potential weapon against pests and the diseases they can carry.

Molecular phylogeny represents a great source of information for better understanding the evolutionary relationships among *Wolbachia* to analyze changes occurring in different organisms during evolution. The strain variation, of any *Wolbachia* species in mosquito populations is necessary for understanding the evolutionary mechanisms of *Wolbachia* genotypes in vector mosquitoes. Phylogenetic analysis of *Wolbachia* using different molecular markers is important to understand the evolution, pathogenesis and strain typing in areas having abundant arboviral vectors. Several molecular phylogenetic studies have been reported using 16S rRNA gene, *ftsZ* cell cycle gene, *wsp* *Wolbachia* surface protein gene, out of which *wsp* gene has been the most preferred for phylogenetic analysis [69].

Therefore, further studies of the natural occurrence and diversity of *Wolbachia* in the major *Aedes* vectors are of high interest. Ultimately, this will be useful for making strategies for vector control programmes by determining the specific strain of *Wolbachia* that is present in *Aedes* followed by artificial infection of *Wolbachia* into the major *Aedes* vectors that will effectively reduce their life span thereby reducing disease transmission.

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Author details

Biswadeep Das*, Sayam Ghosal and Swabhiman Mohanty
KIIT School of Biotechnology, KIIT University, Bhubaneswar, India

*Address all correspondence to: biswadeep.das@kiitbiotech.ac.in

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