

Dengue and other flavivirus infections

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Summary

Flaviviruses are responsible for yellow fever, Zika fever and dengue, all of which are major human diseases found in tropical regions of the globe. They are zoonoses with a transmission cycle that involves primates as reservoirs and mosquitoes of the genus *Aedes* as vectors. The recent upsurge of urban epidemics of yellow fever, Zika fever and dengue has involved human-to-human transmission with mosquitoes as the vector. This paper is primarily concerned with dengue, which has become the pre-eminent arbovirolosis in terms of public health.

Keywords

Arbovirus – Dengue – Flavivirus – Vaccines – Vector transmission – Virus diagnosis – Yellow fever – Zika fever.

Introduction

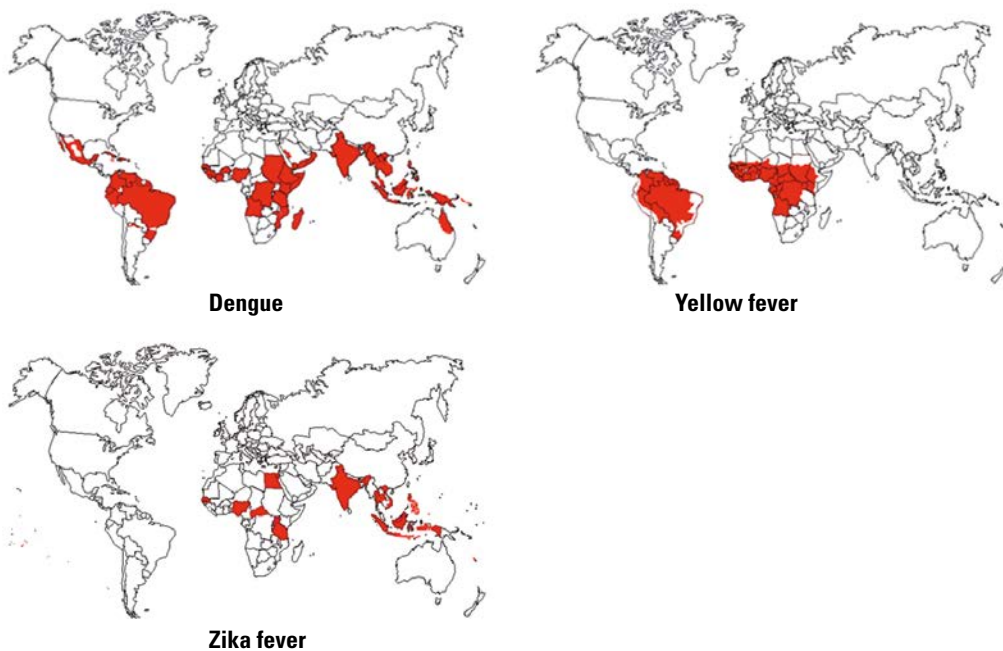
The flaviviruses responsible for yellow fever, Zika fever and dengue are zoonotic arboviruses of great importance for human health (1). Their global distribution is shown in Figure 1. The flaviviruses that cause these three diseases are transmitted to humans mainly by haematophagous mosquitoes of the genus *Aedes* (*Stegomyia*).

In Africa and South America, the sylvatic transmission cycle of the yellow fever virus uses primates as the reservoir, while urban yellow fever epidemics involve the anthropophilic peri-urban mosquito *Aedes aegypti* in a human–mosquito transmission cycle (2). The World Health Organization (WHO) estimates that there are at least 200,000 cases of yellow fever a year and the haemorrhagic manifestations of the disease are responsible for 30,000 deaths, with a fatality rate of up to 50%. The yellow fever vaccine 17D has been available since the late 1930s and a single dose confers immunity for at least ten years, with very few undesirable side effects for vaccinated patients (3, 4).

The Zika virus circulates in Africa and Asia, where an enzootic transmission cycle appears to involve primates, with the mosquitoes *Ae. aegypti* and *Ae. albopictus* as vectors

(5). The epidemic capacity of the Zika virus, including in urban areas, was recently documented during episodes in Gabon in 2007, then in Micronesia in 2007 and French Polynesia in 2013 (5, 6, 7). The recent outbreaks of Zika fever in the South Pacific and Brazil lead us to consider this mosquito-borne disease as a newly emerging arbovirolosis that should be monitored very closely.

Within a few decades dengue has become the pre-eminent arbovirolosis in most tropical regions of the world (8, 9). The dengue virus is found in forested areas of West Africa and Asia, where primates and *Aedes* mosquitoes support its sylvatic transmission cycle. At present, dengue is hyper-endemic in Southeast Asia, South and Central America, the Caribbean region and the South Pacific, where there are more than 100 million symptomatic cases a year out of an estimated 400 million infected individuals. Severe dengue, the serious form of the disease, affects 500,000 individuals a year, with a mortality rate of up to 20% (10, 11, 12). Dengue epidemics occur mainly in large urban areas in tropical regions where *Ae. aegypti* acts as the vector. In view of the major threat it poses to human health, this review will focus on dengue as the major arbovirolosis of the early 21st Century.

**Fig. 1****Global distribution of dengue, yellow fever and Zika fever**

Countries where the virus circulates and/or human cases have been reported are shown in red

Dengue

Vector transmission of dengue

The dengue virus is transmitted to humans mainly by the mosquitoes *Ae. aegypti* and *Ae. albopictus* (1, 7, 8, 13). The viral contamination route is usually via the saliva of an infected mosquito during the probing phase (Fig. 2), which precedes the feeding phase (Fig. 3). Vector transmission relies on the feeding habits of the female mosquito, which has become haematophagous in order to sustain the reproduction cycle. After approximately three to seven days, infected individuals experience a sharp rise in temperature accompanied by viraemia, which lasts on average from four to six days.

During the acute viral infection phase, a female *Aedes* mosquito is able to ingest the dengue virus while feeding on the blood of a vertebrate host. The virus spreads progressively to all mosquito organs, where it replicates actively without significantly affecting the insect's lifespan. The virus finally accumulates in the salivary glands, which probably play a key role in determining the efficiency with which the pathogen is transmitted from vector to host (14). The infected female *Aedes* mosquito becomes capable of transmitting the dengue virus within seven to ten days of the start of infestation. Its vectorial capacity is maintained

throughout its life, which averages from one to two months, with blood meals at intervals of two to four days, as in the case of *Ae. albopictus*. A vertebrate vector that has acquired the dengue virus is potentially capable of transmitting the infectious agent to less than ten individuals over a radius of around 200 metres. Vertical transmission of the dengue virus by *Aedes* mosquitoes cannot be ruled out.

Traditional entomological strategies to prevent and control dengue require the use of insecticides, larvicides or insect repellents against the mosquitoes that carry the disease. The most recent strategies involve the release of insects carrying a dominant lethal gene (Released Insect with a Dominant Lethal, or RIDL, method) or intracellular *Wolbachia* bacteria (15). The presence of *Wolbachia* in arthropods has been successfully used to control the vector-borne spread of dengue.

Epidemiology of dengue

Circulating viral strains can be broken down into distinct genotypes, classified as serotypes DEN-1, DEN-2, DEN-3 and DEN-4, which co-circulate in the majority of hyper-endemic zones (1, 8, 9). Today dengue is a remarkable example of an arbovirolosis that no longer requires the intervention of primates as reservoirs or amplifying hosts to maintain epidemic transmission to humans.

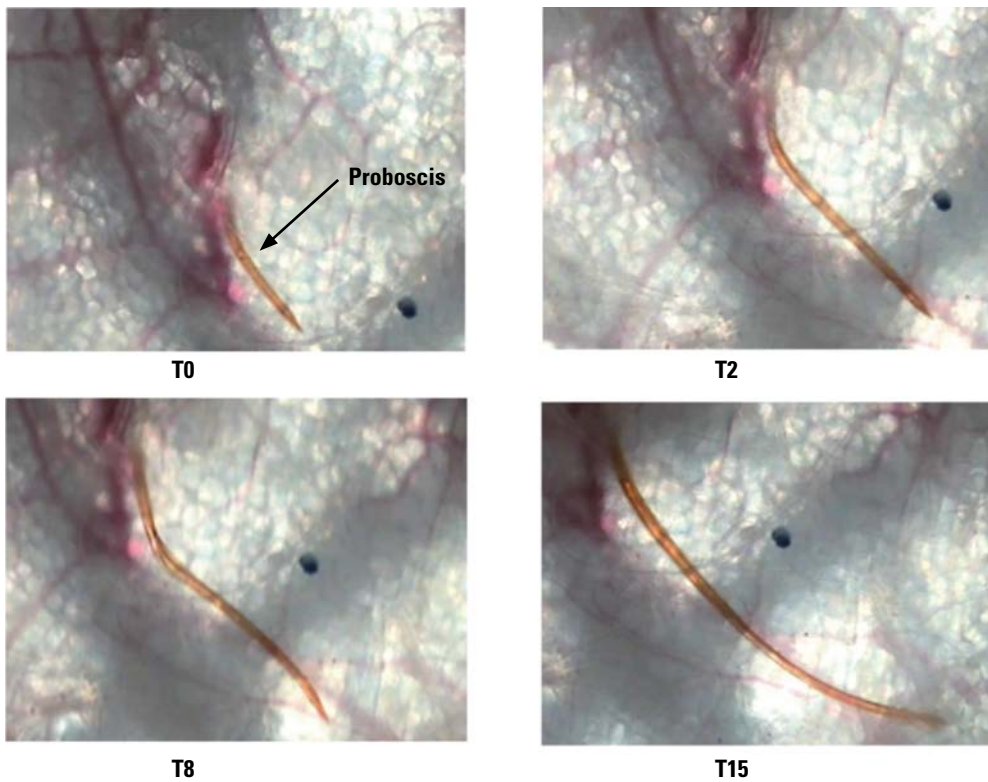


Fig. 2
Probing phase of bloodsucking by the *Aedes aegypti* mosquito
The proboscis of the female mosquito penetrates the dermis of the vertebrate host as far as a blood vessel (the time is given in seconds). Salivation takes places during the probing process

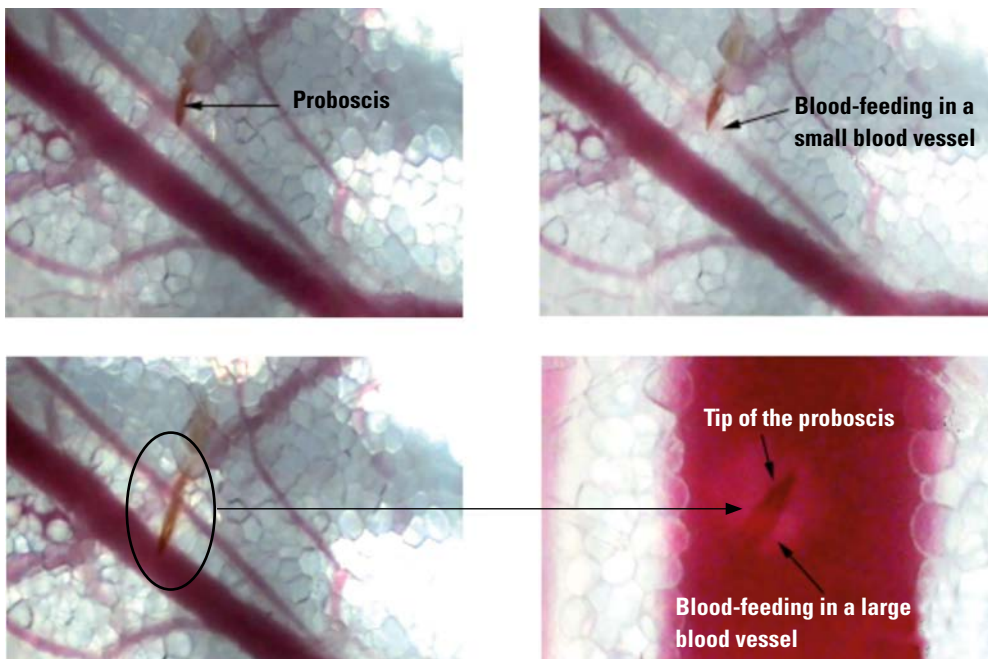


Fig. 3
Blood-feeding phase of the *Aedes aegypti* mosquito
The proboscis of the female mosquito is inserted perpendicular to the blood vessel. The vector is capable of feeding from different calibre blood vessels during the same blood meal

The geographical spread of dengue in the past 30 years is primarily a consequence of the abandonment or failure of eradication campaigns against the vector mosquito *Ae. aegypti*. The rapid progression of this disease, with an increasing number of severe dengue cases, is explained in part by the active circulation of different dengue serotypes and viral strains pathogenic to humans, coupled with the intensification of global trade and constant flows of people, and exacerbated by the spread of *Ae. aegypti* and the proven ability of *Ae. albopictus* to transmit the dengue virus. Rapid urban growth in Latin America and Southeast Asia is highly conducive to inter-human transmission via *Ae. aegypti*, thereby increasing the risk of often large-scale epidemics. The climatic changes observed on the various continents are also likely to be conducive to the spread of the disease vector. The risk of dengue transmission has now become a reality in temperate regions, including Europe, where the *Ae. albopictus* mosquito has been spreading inexorably since its recent introduction.

Dengue and severe dengue

The vast majority of dengue virus infections are inapparent, and the impact of these inapparent infections on the epidemiology of the disease, including their effect on the transmission of the pathogen by its vectors, is currently a matter of investigation (16). A wide range of symptoms are displayed in cases of symptomatic infection with dengue virus (10, 11, 12). The commonest form of the disease is dengue, which usually takes the form of a fever with few suggestive signs. Retro-orbital and abdominal pain, myalgia and a maculopapular rash with petechiae are frequently observed clinical signs following infection with dengue virus. In the vast majority of cases, signs of infection and the associated symptoms subside with no other significant complications. In countries where dengue is endemic, the disease may be confused with other fever syndromes, such as chikungunya, measles, typhoid or leptospirosis (17). A small percentage of symptomatic individuals infected with the dengue virus develop severe forms of the disease. Young children remain most at risk of severe forms of dengue, with a mortality rate of up to 5%. Dengue haemorrhagic fever and dengue shock syndrome are the most serious forms of viral infection. Increased vascular permeability can cause severe haemoconcentration, with peritoneal and pleural effusions indicating that the disease is serious and that there is a risk of a fatal hypovolaemic shock within 24 hours. WHO has recently proposed the adoption of a new classification of the severity of dengue which simply describes the clinical signs of dengue and severe dengue and identifies warning signs (18, 19). The mechanisms causing severe dengue are still poorly understood, but are certainly dependent on intrinsic aspects of the vector and host, as well as on virulence determinants of the infecting viral strain (20). In humans, dengue virus infection results in long-term adaptive immunity that only protects against the infecting

serotype. Consequently, individuals are susceptible to infection by all four dengue virus serotypes (DEN-1 to DEN-4) throughout their life. Epidemiological data suggest that the risk of developing severe dengue increases when there is secondary infection with a dengue virus serotype other than that responsible for the primary infection (10, 11, 12). Predictors of severe dengue have still not been identified. The number of cases of severe dengue is increasing in South and Central America and Southeast Asia, but the African continent, for reasons yet to be explained, has remained virtually untouched by severe forms of the disease despite the fact that the virus is actively circulating. Genetic factors of dengue virus susceptibility may contribute to the severity of the disease among the populations of regions where the disease is endemic.

Diagnosis of dengue

Laboratory tests are essential for diagnosing dengue because the clinical signs vary significantly within the infected population (21, 22, 23, 24). Serum, whole blood or plasma are often used for confirmation of dengue virus infection. During the acute phase of the disease, viraemia is identified by the real-time polymerase chain reaction (RT-PCR) method using pan-dengue primers and/or RNA-specific primers for each virus (DEN-1 to DEN-4). Virus isolation from a positive serum is possible on cultures of mammalian cell lines (usually Vero or BHK-21) or invertebrate cell lines (usually C6/36), and positive amplification of the dengue virus confirms viral infection in a patient. A recent alternative to molecular diagnosis is to identify the NS1 virus protein, which circulates in substantial quantities in the bloodstream during the acute phase of the disease. WHO recommends the use of early and rapid dengue diagnostic kits based on NS1 antigenaemia by enzyme-linked immunosorbent assay (ELISA) using the capture method or strips on whole blood. Serological tests based on immunoglobulin M (IgM) and immunoglobulin G (IgG) seroconversion are commonly used to diagnose dengue, while the standard reference method remains haemagglutination inhibition and neutralisation. ELISA serological testing, using strips or cassettes to detect IgM and IgG seroconversion, are routinely used for dengue diagnosis and many kits are commercially available. IgM capture using the MAC-ELISA method is the preferred method for diagnosing a recent infection with dengue virus. It is performed on serum, capillary blood deposited on blotting paper or saliva. A significant rise in IgG levels on paired sera over one to two weeks confirms the diagnosis of a recent dengue virus infection. However, cross-antigenicity between the dengue virus and other flaviviruses, including the Zika virus, is a limiting factor for the specificity of the commercially available serological tests, including those targeting dengue virus IgM antibodies. The development of new diagnostic tools that are more sensitive and specific to the dengue virus remains a priority for prevention of the disease.

Dengue vaccines under development

The World Health Organization has set a target of halving the mortality rate of dengue by 2020. There are still no effective treatments or vaccines against the disease on the market. Several vaccine candidates are in pre-clinical or clinical development (25). The most successful so far is the recombinant tetravalent vaccine CYD-TDV, which will be mainly for paediatric use. This produces four chimeric yellow fever/dengue viruses in which the genome of yellow fever vaccine 17D has been recombined by genetic engineering to expose the surface of the viral particle to the major antigens of all four dengue virus serotypes (DEN-1 to DEN-4). Clinical phase studies show the CYD-TDV vaccine candidate to be effective enough to protect against dengue after three doses at six-month intervals.

Concluding remarks

Medically important flaviviruses in particular dengue, are arboviruses of great importance for human health. Prevention and control of yellow fever, dengue, and now Zika fever, are driving the development of effective control strategies against mosquitoes, while intensifying research programmes to improve laboratory diagnosis. The development of a paediatric vaccine against dengue that protects against severe forms of the disease remains a public health priority.

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