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## Dengue: a continuing global threat

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### Abstract

Dengue fever and dengue haemorrhagic fever are important arthropod-borne viral diseases. Each year, there are ~50 million dengue infections and ~500,000 individuals are hospitalized with dengue haemorrhagic fever, mainly in Southeast Asia, the Pacific and the Americas. Illness is produced by any of the four dengue virus serotypes. A global strategy aimed at increasing the capacity for surveillance and outbreak response, changing behaviours and reducing the disease burden using integrated vector management in conjunction with early and accurate diagnosis has been advocated. Antiviral drugs and vaccines that are currently under development could also make an important contribution to dengue control in the future.

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Dengue is the most important arthropod-borne viral infection of humans. Worldwide, an estimated 2.5 billion people are at risk of infection, approximately 975 million of whom live in urban areas in tropical and sub-tropical countries in Southeast Asia, the Pacific and the Americas<sup>1</sup>. Transmission also occurs in Africa and the Eastern Mediterranean, and rural

communities are increasingly being affected. It is estimated that more than 50 million infections occur each year, including 500,000 hospitalizations for dengue haemorrhagic fever, mainly among children, with the case fatality rate exceeding 5% in some areas<sup>1-4</sup>.

The annual average number of dengue fever/dengue haemorrhagic fever (DF/DHF) cases reported to the World Health Organization (WHO) has increased dramatically in recent years. For the period 2000–2004, the annual average was 925,896 cases, almost double the figure of 479,848 cases that was reported for the period 1990–1999. In 2001, a record 69 countries reported dengue activity to WHO and in 2002, the Region of the Americas alone reported more than 1 million cases. Although there is poor surveillance and no official reporting of dengue to WHO from countries in the African and Eastern Mediterranean regions, in 2005–2006 outbreaks of suspected dengue were recorded in Pakistan, Saudi Arabia, Yemen, Sudan and Madagascar<sup>1-4</sup>, and a large outbreak of dengue involving >17,000 cases was documented in the Cape Verde islands in 2009<sup>5</sup>. Travellers from endemic areas might serve as vehicles for further spread<sup>6-9</sup>. Dengue epidemics can have a significant economic and health toll. In endemic countries in Asia and the Americas, the burden of dengue is approximately 1,300 disability-adjusted life years (DALYs) per million population, which is similar to the disease burden of other childhood and tropical diseases, including tuberculosis, in these regions<sup>10</sup>.

The geographical areas in which dengue transmission occurs have expanded in recent years (FIG. 1), and all four dengue virus serotypes (DENV-1–4) are now circulating in Asia, Africa and the Americas, a dramatically different scenario from that which prevailed 20 or 30 years ago (FIG. 2). The molecular epidemiology of these serotypes has been studied in an attempt to understand their evolutionary relationships<sup>11</sup>.

This Review will provide an update on our understanding of the pathogenesis of this successful pathogen, how we diagnose and control infection and the progress that has been made in vaccine development.

## Dengue virus pathogenesis

Dengue viruses belong to the genus flavivirus within the *Flaviviridae* family. DENV-1–4 evolved in non-human primates from a common ancestor and each entered the urban cycle independently an estimated 500–1,000 years ago<sup>12</sup>. The virion comprises a spherical particle, 40–50 nm in diameter, with a lipopolysaccharide envelope. The positive single-strand RNA genome (FIG. 3), which is approximately 11 kb in length, has a single open reading frame that encodes three structural proteins — the capsid (C), membrane (M) and envelope (E) glycoproteins — and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5). Important biological properties of dengue viruses, including receptor binding, haemagglutination of erythrocytes and the induction of neutralizing antibodies and the protective immune response, are associated with the E glycoprotein. Each DENV shares around 65% of the genome, which is approximately the same degree of genetic relatedness as West Nile virus shares with Japanese encephalitis virus. Despite these differences, each serotype causes nearly identical syndromes in humans and circulates in the same ecological niche<sup>13</sup>.

The mosquito vectors, principally *Aedes aegypti*, become infected when they feed on humans during the usual five-day period of viraemia. The virus passes from the mosquito intestinal tract to the salivary glands after an extrinsic incubation period, a process that takes approximately 10 days and is most rapid at high ambient temperatures<sup>14</sup>. Mosquito bites after the extrinsic incubation period result in infection, which might be promoted by mosquito salivary proteins<sup>15</sup>. In the skin, dengue viruses infect immature dendritic cells through the non-specific receptor dendritic cell-specific ICAM3-grabbing non-integrin (DC-SIGN)<sup>16</sup>. Infected dendritic cells mature and migrate to local or regional lymph nodes where they present viral antigens to T cells, initiating the cellular and humoral immune responses. There is also evidence of abundant replication of DENVs in liver parenchymal cells and in macrophages in lymph nodes, liver and spleen, as well as in peripheral blood monocytes<sup>17</sup>. Both *in vitro* and *in vivo*, macrophages and monocytes participate in antibody-dependent enhancement (ADE)<sup>18–20</sup>. ADE occurs when mononuclear phagocytes are infected through their Fc receptors by immune complexes that form between DENVs and non-neutralizing antibodies. These non-neutralizing antibodies result from previous heterotypic dengue infections or from low concentrations of dengue antibodies of maternal origin in infant sera<sup>21</sup>. The co-circulation of four DENV serotypes in a given population might be augmented by the ADE phenomenon<sup>22</sup>.

DENVs produce several syndromes that are conditioned by age and immunological status. During initial dengue infections, most children experience subclinical infection or mild undifferentiated febrile syndromes. During secondary dengue infections the pathophysiology of the disease changes dramatically, particularly sequential infections in which infection with DENV-1 is followed by infection with DENV-2 or DENV-3, or infection with DENV-3 is followed by infection with DENV-2<sup>23–25</sup>. Such infections can result in an acute vascular permeability syndrome known as dengue shock syndrome (DSS). The severity of DSS is age-dependent, with vascular leakage being most severe in young children, a phenomenon that is thought to be related to the intrinsic integrity of the capillaries<sup>26,27</sup>. In adults, primary infections with each of the four DENV serotypes, particularly with DENV-1 and -3, often results in DF. Some outbreaks of primary DENV-2 infections have been predominantly subclinical<sup>24</sup>. Nonetheless, dengue infections in adults are often accompanied by a tendency for bleeding that can lead to severe haemorrhages.

Dengue infections can be life-threatening when they occur in individuals with asthma, diabetes and other chronic diseases<sup>28–30</sup>. Host factors that increase the risk of severe dengue disease include female sex, several human leukocyte antigen (HLA) class I alleles, a promoter variant of the DC-SIGN receptor gene, a single-nucleotide polymorphism in the tumour necrosis factor (TNF) gene and AB blood group<sup>31–36</sup>. Host factors that reduce the risk of severe disease during a second dengue infection include race, second or third degree malnutrition, and polymorphisms in the Fc $\gamma$  receptor and vitamin D receptor genes<sup>37–42</sup>. Secondary dengue infections in adults can produce the classical DSS or severe disease complicated by haemorrhages. The severity of secondary dengue infections has been observed to increase from month-to-month during island outbreaks<sup>43</sup>; the longer the interval between the first and second infection the more severe is the accompanying disease<sup>44,45</sup>. Tertiary dengue infections can cause severe disease, but only rarely<sup>25</sup>.

*In vitro* studies demonstrate that the infection of human monocytes and mature dendritic cells results in increased virus replication as a result of the suppression of the interferon system<sup>45</sup>. Type I interferon-associated genes are less abundantly activated in peripheral blood mononuclear cells taken from patients with severe dengue disease compared with milder disease<sup>46</sup>. Subsequently, the increased number of infected cells present targets for CD4<sup>+</sup> and CD8<sup>+</sup> T cells, resulting in large quantities of interleukin (IL)-10, IL-2, interferon (IFN)- $\gamma$  and TNF that, singly or in combination, might contribute to endothelial damage and altered haemostasis. Virions released from infected cells might also directly damage endothelial cells and the uptake of the non-structural protein NS1 by hepatocytes might promote viral infection of the liver<sup>47–49</sup>. During DHF, the complement cascade is also activated and the levels of the complement activation products C3a and C5a correlate with the severity of illness<sup>49</sup>. Soluble and membrane-associated NS1 have been demonstrated to activate human complement. The levels of the terminal SC5b–9 complement complex and plasma NS1 correlated with disease severity, suggesting links between the virus, complement activation and the development of DHF/DSS<sup>50</sup>. Alternative hypotheses of dengue pathogenesis include the suggestions that secondary T-cell responses are blunted because stimulation of T-cell memory results in the production of heterotypic CD4<sup>+</sup> and CD8<sup>+</sup> cells that have a diminished capacity to kill but nonetheless release inflammatory cytokines that contribute to disease severity<sup>51</sup>; that severe disease is caused by DENVs of increased virulence<sup>52</sup>; and the suggestion that cross-reactivity between NS1 and human platelets and endothelial cells raises antibodies that damage these cells<sup>53</sup>.

One working hypothesis of dengue pathogenesis that is consistent with the available evidence is that severe disease in infants with primary infections and in older individuals with secondary infections is the result of ADE of infection of mononuclear phagocytes. Infection by an antibody–virus complex suppresses innate immune responses, increasing intracellular infection and generating inflammatory cytokines and chemokines that, collectively, result in enhanced disease. Liver infection and a pathogenic role for NS1 add to the complexity. In patients with DF, IFN production and activated natural killer cells can limit disease severity.

## Clinical signs and immunological response

Dengue-associated deaths are usually linked to DHF/ DSS. Even though no vaccines or drugs are available, severe disease can be successfully managed by careful monitoring of the warning signs and early initiation of aggressive intravenous rehydration therapy. During the early febrile stage (the symptoms of which include fever, malaise, headache, body pains and rash), clinicians cannot predict which patients will progress to severe disease. Later, during defervescence, symptoms such as bleeding, thrombocytopenia of  $<100,000$  platelets  $\text{mm}^{-3}$ , ascites, pleural effusion, haematocrit  $>20\%$  and clinical warning signs, such as severe and continuous abdominal pain, restlessness and/or somnolence, persistent vomiting and a sudden reduction in temperature (from fever to subnormal temperature) associated with profuse perspiration, adynamia (loss of strength or vigor) and sometimes fainting, can be indicative of plasma extravasation and the imminence of shock. At this point, patients should receive fluid replacement (crystalloids) to avoid haemodynamic instability, narrowness of blood pressure and hypotension. Early resuscitation can prevent other

complications, such as massive haemorrhage, disseminated intravascular coagulation, multiple organ failure, and respiratory failure due to non-cardiogenic pulmonary oedema<sup>54–57</sup>. Treatment of uncomplicated dengue cases is only supportive, including plenty of oral fluids during the febrile period and paracetamol (acetaminophen), the daily dosage of which should not be exceeded to prevent intoxication mainly related to liver function. When dengue shock becomes prolonged or recurrent, intravenous fluids should be given carefully according to age and dosage to prevent fluid overload as this can result in pulmonary oedema.

Recent publications have suggested that the WHO syndromic case definition of DHF/DSS should be evaluated for clinical utility<sup>58–62</sup>. A prospective multi-centre study in several Latin-American and Southeast Asian countries is planned that will provide standardized descriptions of dengue clinical presentations in the context of the current WHO case definitions.

The acquired immune response to dengue infection consists of the production of antibodies that are primarily directed against the virus envelope proteins. The response varies depending on whether it is a primary or secondary infection<sup>63,64</sup>. A primary antibody response is seen in individuals who are not immune to dengue and a secondary immune response is observed in patients who have had a previous dengue infection (FIG. 4). A primary infection is characterized by a slow and low-titre antibody response. Immunoglobulin (Ig)M antibodies are the first isotype to appear, by day 3–5 of illness in 50% of hospitalized patients and by day 6–10 of illness in 93–99% of cases. The IgM levels peak ~2 weeks after the onset of fever and then generally decline to undetectable levels over the next 2–3 months<sup>54,55,65</sup>. Dengue-specific IgG is detectable at low titre at the end of the first week of illness and slowly increases. By contrast, during a secondary infection, high levels of IgG antibodies that crossreact with many flaviviruses are detectable even in the acute phase and rise dramatically over the following 2 weeks<sup>65</sup>. The kinetics of the IgM response are more variable; as IgM levels are significantly lower in secondary dengue infections, false-negative test results for dengue-specific IgM have been reported during secondary infections<sup>55,66,67</sup>. Following a dengue infection, IgG can be lifelong, which complicates the serodiagnosis of past, recent and current infections<sup>65,67</sup>. IgA and IgE responses have also been documented but the utility of detecting these immunoglobulins as markers for dengue serodiagnosis requires further study<sup>68</sup>.

In areas where two or more flaviviruses are circulating, multiple and sequential flavivirus infections make differential diagnosis difficult owing to the presence of pre-existing antibodies and the phenomenon of original antigenic sin (during sequential flavivirus infections, B-cell clones responding to the first infection synthesize antibodies with higher affinity for the first infecting virus than for the second infecting virus)<sup>69</sup>.

## Laboratory diagnosis of dengue infection

Laboratory confirmation of dengue infection is crucial as the broad spectrum of clinical presentations, ranging from mild febrile illness to several severe syndromes, can make accurate diagnosis difficult. Among the methods available for dengue diagnosis, virus

isolation provides the most specific test result. However, facilities that can support viral culture are not always available. The detection of the viral genome or viral antigens also provides evidence of infection.

Seroconversion of IgM or IgG antibodies is the standard for serologically confirming a dengue infection. The presence of IgM or high levels of IgG in acute serum collected from a suspected dengue case suggests a probable dengue infection<sup>54,55</sup>. BOX 1 shows the laboratory criteria for confirmed and probable dengue infections.

## Virus isolation

The *Aedes albopictus* mosquito C6/36 cell line is the method of choice for DENV isolation, although other mosquito (such as *Aedes pseudoscutellaris* AP61) and mammalian (including Vero cells, LLC-MK2 cells and BHK21 cells) cell lines can also be used<sup>70,71</sup>. Sera that have been collected from suspected dengue cases in the first 3–5 days of fever (the viraemic phase) can be used for virus isolation. After an incubation period permitting virus replication, viral identification is performed using dengue-specific monoclonal antibodies in immunofluorescence and PCR assays<sup>63,64,72,73</sup>. Serum is often used for virus isolation but plasma, leukocytes, whole blood and tissues obtained at autopsy can also be used<sup>63,74,75</sup>.

## Serological testing

Serological assays are most commonly used for diagnosis of dengue infection as they are relatively inexpensive and easy to perform compared with culture or nucleic acid-based methods. When a dengue infection occurs in individuals who have experienced a previous dengue infection, a secondary immune response occurs, which generates high levels of IgG through the stimulation of memory B cells from the previous infection as well as an IgM response to the current infection. Because high levels of IgG compete with IgM for antigen binding, an IgM capture assay can be used.

## MAC-ELISA

The Armed Forces Research Institute of Medical Sciences (AFRIMS) developed an IgM antibody-capture enzyme-linked immunosorbent assay (MAC-ELISA) for dengue in regions where dengue and Japanese encephalitis virus co-circulate<sup>65</sup>. Today, many groups have developed their own in-house MAC-ELISAs. Dengue-specific IgM in the test serum is detected by first capturing all IgM using human-specific IgM bound to a solid phase. The assay uses a mixture of four dengue antigens (usually derived from dengue virus-infected cell culture supernatants or infected suckling mouse brain preparations)<sup>76</sup>. Compared to the haemagglutination inhibition assay as the gold standard, MAC-ELISA shows a sensitivity and specificity of 90% and 98%, respectively, in samples collected after 5 days of fever<sup>55</sup>. In addition to serum, dengue-specific IgM can be detected in whole blood on filter paper (sensitivity 98.1% and specificity 98.5%)<sup>77,78</sup> and in saliva (sensitivity 90.3% and specificity 92.0%)<sup>79</sup>, but not in urine<sup>68</sup>. More than 50 commercial kits are available with variable sensitivity and specificity<sup>65,80–82</sup>. False-positive results due to dengue-specific IgG and crossreactivity with other flaviviruses is a limitation of the MAC-ELISA, mainly in

regions where multiple flaviviruses co-circulate. Some tests also show non-specific reactivity in sera from patients with malaria and leptospirosis<sup>82</sup>.

### **IgG ELISA**

An ELISA for dengue-specific IgG detection can be used to confirm a dengue infection in paired sera. It is also widely used to classify primary or secondary infections<sup>53,54,63,64</sup>. Some protocols use serum dilutions to titre dengue-specific IgG<sup>83</sup> and others use the ratio of IgM to IgG<sup>66,84</sup>. The assay uses the same dengue antigens as MAC-ELISA and it correlates with results from the haemagglutination inhibition assay. In general, an IgG ELISA lacks specificity within the flavivirus serocomplex groups, however it has been demonstrated that the IgG response to the prM membrane glycoprotein is specific to individual flaviviruses as no crossreactivity was observed in sera collected from individuals infected with dengue or Japanese encephalitis virus<sup>85</sup>. Similarly, it has been demonstrated that IgG specific for the NS5 protein can potentially discriminate between infections caused by West Nile, dengue and St Louis encephalitis viruses<sup>86</sup>. Finally, dengue-specific IgG was shown to have high specificity in an assay using a recombinant polypeptide located in the N-terminal region of the envelope protein<sup>87</sup>. IgG assays are also useful for sero-epidemiological studies to identify past dengue infection.

### **IgM:IgG ratio**

A dengue virus E and M protein-specific IgM:IgG ratio can be used to distinguish primary from secondary dengue virus infections. IgM capture and IgG capture ELISAs are the most common assays for this purpose. According to this method, a dengue infection is defined as a primary infection if the IgM:IgG OD ratio is greater than 1.2 (using patient sera at 1:100 dilution) or 1.4 (using patient sera at 1:20 dilution), or as a secondary infection if the ratio is less than 1.2 or 1.4 (REFS 88,89). However, in a recent publication the authors indicated that the IgM:IgG ratio varies depending on whether the patient has a serologically non-classical or classical dengue infection, and redefined the ratios<sup>84</sup>. Hence the cut-off for the IgM:IgG ratio is not well defined.

### **Neutralization assays**

The plaque reduction neutralization technique (PRNT) and the micro-neutralization assay are used to define the infecting serotypes following a primary infection. These tests are mainly for research and vaccine studies<sup>90-94</sup>.

### **Nucleic acid amplification tests**

Many nucleic acid amplification tests (NAATs) have been developed for the diagnosis of dengue infection. Some techniques are quantitative and others can be used for serotyping. However, none has been commercialised to date and quality assurance materials are not widely available to ensure the quality of the results.

### **Reverse transcriptase PCR (RT-PCR)**

Many dengue RT-PCR assays have been described in the past 10 years. These in-house assays target different genes and use different amplification procedures. The most

commonly used NAATs are based on a single RT-PCR assay<sup>95,96</sup>, a nested RT-PCR assay<sup>96</sup> or a one-step multiplex RT-PCR assay<sup>97</sup>. The nested PCR reaction involves an initial reverse transcription and amplification step using dengue primers that target a conserved region of the virus genome followed by a second amplification step that is serotype specific. The products of these reactions are separated by electrophoresis on an agarose gel, which allows the dengue serotypes to be differentiated on the basis of size. The sensitivity of RT-PCR assays in comparison to virus isolation in mosquito cell culture varies between 25% and 79%<sup>98</sup>.

### Real-time RT-PCR

The real-time RT-PCR assay is a one-step assay that allows virus titre to be quantified in approximately 1.5 hours. The detection of the amplified target by fluorescent probes replaces the need for post-amplification electrophoresis. Many real-time RT-PCR assays have been developed that are either 'singleplex', detecting one single serotype per reaction, or 'multiplex', identifying all four serotypes from a single sample<sup>99-101</sup>. One advantage of this assay is the ability to determine viral titre early in dengue illness, which is believed to be an important predictor of disease severity<sup>102</sup>.

### Nucleic acid-sequence based amplification assay (NASBA)

The NASBA assay is an isothermal RNA-specific amplification assay that has been adapted for dengue virus. Its performance is comparable to that of other NAATs<sup>103</sup>.

### Antigen detection

Dengue antigens can be detected in tissues such as liver, spleen and lymph nodes as well as tissues from fatal cases (slides from paraffin-embedded, fresh or frozen tissues) using an enzyme and a colorimetric substrate with antibodies that target dengue-specific antigens<sup>104-106</sup>.

### NS1 antigen and antibody detection

NS1 is a glycoprotein produced by all flaviviruses and is essential for viral replication and viability. Because this protein is secreted into the bloodstream, many tests have been developed to diagnose DENV infections using NS1. These tests include antigen-capture ELISA, lateral flow antigen detection and measurement of NS1-specific IgM and IgG responses. NS1 antigen detection kits are now commercially available. As yet, these kits do not differentiate between the different DENV serotypes. Additional independent studies are needed to confirm the performance of these kits and to further validate the diagnostic and prognostic significance of NS1 and NS1-specific antibody detection<sup>107-109</sup>.

### Dengue control and prevention strategies

A global strategy for dengue prevention and control was promulgated more than 10 years ago and comprises five major elements (BOX 2).

Efforts have since been made to focus on three fundamental aspects: surveillance for planning and response, reducing the disease burden and changing behaviours to improve

vector control<sup>110</sup>. The 2002 World Health Assembly Resolution urged greater commitment among Member States and WHO to implement this strategy<sup>111</sup>. Of particular significance is the 2005 revision of the International Health Regulations<sup>112</sup>, which includes mention of DF (and yellow fever) as an example of a health ‘event that may constitute a public health emergency of international concern’ and which, under such circumstances, should be notified to WHO.

In recent years several new, improved or validated tools and strategies for dengue control and prevention have been developed and are available to public health practitioners and clinicians (BOX 3).

### Vector control

To reduce or prevent dengue virus transmission there is currently no alternative to vector control. Most endemic countries have a vector control component in their dengue control and prevention programmes but its delivery by public health practitioners is frequently insufficient, ineffective or both.

Given its behaviour and generally close association with humans, the principal vector *A. aegypti* requires the use of a combination of vector-control methods, notably environmental management methods and chemical control methods based on the application of larvicides and adulticide space sprays<sup>113</sup>. Chemical controls typically must be added to water stored for domestic use, including drinking water. The active ingredients of four larvicides have been assessed by the International Programme on Chemical Safety (IPCS) to determine their safety for use as mosquito larvicides in drinking water at dosages that are effective against *Aedes* spp. larvae. Since the early 1970s the organophosphate temephos has been widely used, but increasing levels of resistance<sup>114,115</sup>, householders’ rejection of the treatment of their drinking water, and difficulties in achieving high and regular levels of coverage are important technical and operational constraints.

Biological control agents, including larvivorous fish and copepods, have had a demonstrable role in controlling *A. aegypti*<sup>116,117</sup>, but operational difficulties — particularly the lack of facilities and expertise in mass rearing, and the need to frequently re-introduce these agents into some container habitats — have largely precluded their widespread use.

Environmental management is generally considered to be an essential component of dengue prevention and control, particularly when targeting the most productive container habitats of the vector<sup>118</sup>. Source reduction, ‘cleanup’ campaigns, regular container emptying and cleaning (targeting not only households but also public spaces such as cemeteries, green areas and schools), installation of water supply systems, solid waste management and urban planning all fall under the rubric of environmental management. However, huge investments in infrastructure are needed to increase access to safe and reliable water supplies and solid waste disposal systems. In addition to overall health gains, such provision would clearly have a major impact on vector ecology, although the relationship is complex. For instance, cost recovery mechanisms, such as the introduction of metered water, might actually encourage the household collection and storage of roof catchment rainwater, which can be harvested at no cost. Although not studied carefully, the construction of community water

distribution services to rural townships and villages might be contributing to the rural spread of dengue in Southeast Asia and elsewhere by facilitating domestic water storage. When decisions on such infrastructure development are being made, the views of Ministers of Public Health and municipal health departments are seldom voiced loudly, even when the economic and public health burden of diseases linked to water and sanitation are recognized, including those associated with dengue.

Most efforts in vector control are centred at the household and community levels, but with few exceptions, the achievements to date have been largely unspectacular and there have been difficulties in scaling up from the project level<sup>119</sup>. Nevertheless, such community-based interventions are widely seen as the most promising way of improving delivery and achieving long-term control of the vector through behaviour change. Towards this end, a TDR/WHO guide for planning social mobilization and communication for dengue fever prevention and control has been developed<sup>113</sup>. Additionally, new 'consumer-friendly' tools such as window curtains and water container covers treated with long-lasting insecticide are being tested<sup>120</sup> as well as controlled release larvicides that provide several months of control following a single application to targeted containers.

Products for personal and household protection have a huge potential for household pest control. Generally speaking, these commercial products tend to be used by consumers not so much in response to any perceived public health concerns, but to alleviate the nuisance of biting mosquitoes and in some settings households are prepared to spend substantial amounts of money on these products<sup>121</sup>.

With the increased political recognition of dengue as a public health problem and commitment to prevention and control, better organized control services using new tools and partnership strategies, based on the principles of integrated vector management, are likely to have a major impact on dengue transmission<sup>2</sup>.

### **Vaccine development**

As a result of the failure of vector control, the continuing spread and increasing intensity of dengue has renewed interest and investment in dengue vaccine development, making a safe, effective and affordable tetravalent dengue vaccine a global public health priority<sup>122</sup>. Dengue vaccine development has been in progress for several decades, however the complex pathology of the illness, the need to control four virus serotypes simultaneously and insufficient investment by vaccine developers have hampered progress<sup>122</sup>.

The observation that DHF/DSS is associated with DENV secondary infection poses a special challenge to the development of a dengue vaccine, leading to a requirement that such vaccines should induce a robust immune response against the four serotypes in naive as well as previously immune individuals. Animal models are only partially useful for vaccine evaluation. The poor understanding of the mechanisms involved in inducing protective immunity against dengue infection poses additional challenges<sup>123</sup>. Finally, cases of DHF/DSS have recently been documented 20 or more years after primary dengue infection, which adds a new dimension to the problem<sup>25,44</sup>.

The available data suggest that neutralizing antibodies are the major contributors to protective immunity<sup>124,125</sup>, however the role of the cellular immune response requires further study<sup>123</sup>. In this context, clinical trials are crucial for vaccine development owing to the unique information they provide on immune responses and reactogenicity. Also, long-term observations of vaccinated populations will be required to demonstrate the absence of ADE or severe disease.

The ideal dengue vaccine should be free of important reactogenicity, induce life-long protection against infection with any of the four DENV serotypes and be affordable<sup>126,127</sup>. Vaccine candidates should be evaluated in population-based efficacy trials in several at-risk populations in different geographical settings including Asia and the Americas, which experience different patterns of dengue transmission intensity and dengue virus circulation<sup>122</sup>. Vaccine developers are working with the Pediatric Dengue Vaccine Initiative (PDVI) to establish suitable field sites. Developers are also working with the WHO Initiative for Vaccine Research (WHO/IVR) to define the immunological correlates for protection and clinical trial design. Because of the important role of neutralizing antibodies as surrogates of protection, the validation of neutralization tests is a priority<sup>128</sup>. Current approaches to vaccine development involve using live attenuated viruses, inactivated viruses, subunit vaccines, DNA vaccines, cloned engineered viruses and chimeric viruses using yellow fever vaccine and attenuated dengue viruses as backbones<sup>129–134</sup>. TABLE 1 summarizes the most advanced vaccine candidates.

Significant progress in the development of dengue vaccine candidates has been achieved lately<sup>135,136</sup>. An Acambis/Sanofi Pasteur yellow fever–dengue chimeric vaccine is in advanced Phase II testing in children in Thailand and others are in Phase I or advanced preclinical evaluation. It is expected that a licensed vaccine will be available in less than 10 years.

## Conclusions

Dengue is now a global threat and is endemic or epidemic in almost every country located in the tropics. While we wait for new tools such as vaccines, antiviral drugs and improved diagnostics, better use should be made of the interventions that are currently available. The challenge that awaits us in the near future will be how to scale up to deploy these new tools.

In recent years, several partnerships such as the PDVI, the Innovative Vector Control Consortium, the Asia-Pacific Dengue Prevention Partnership and the European union's DENFRAME and DENCOC projects have come into existence, receiving funding from the Bill and Melinda Gates Foundation, regional Development Banks and the private sector. These partnerships are working with WHO and national governments to develop new tools and strategies to improve diagnostics and clinical treatments and to achieve a successful vaccine.

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**Box 1****Laboratory diagnosis of a dengue virus infection****Confirmed dengue infection**

- Virus isolation
- Genome detection
- Antigen detection
- IgM or IgG seroconversion

**Probable dengue infection**

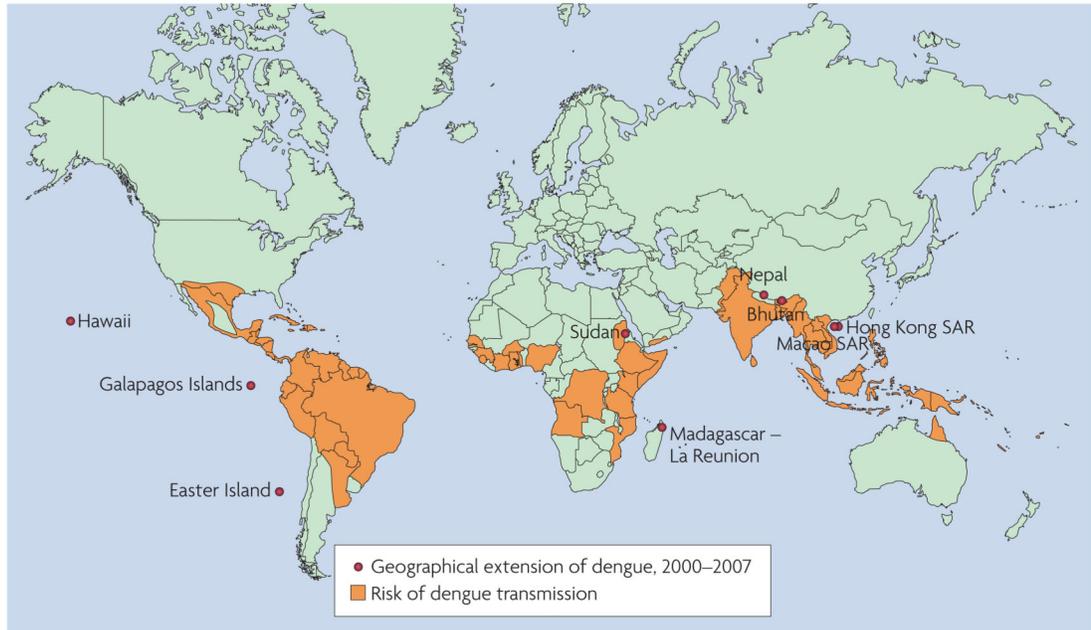
- IgM positive
- Elevated IgG titre (that is, 1,280 or greater by haemagglutination inhibition test)

**Box 2****The global strategy for dengue prevention and control**

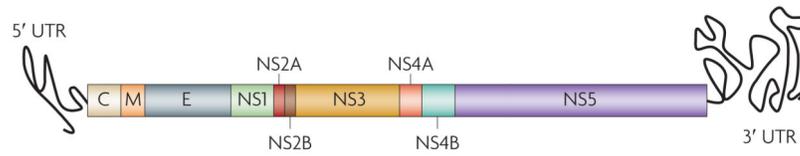
- Vector control, based on the principles of integrated vector management
- Active disease surveillance based on a comprehensive health information system
- Emergency preparedness
- Capacity building and training
- Vector control research

**Box 3****Tools and resources for dengue control and prevention**

- Rapid commercial diagnostic tests in use in endemic countries
- *Pocket Book of Hospital Care for Children* (inclusion of dengue in the management of fever)<sup>137</sup>
- An audiovisual guide and transcript for health care workers responding to outbreaks<sup>138</sup>
- Guidelines for planning social mobilization and communication<sup>139</sup>
- Global strategic framework for integrated vector management<sup>140</sup>
- TDR–Wellcome Trust CD-ROM. Topics in International Health Series: dengue
- Entomological survey to identify the most productive container habitats of the vector(s)<sup>116</sup>
- Seven insecticide products evaluated by WHO as mosquito larvicides (five insect growth regulators and two bacterial larvicides), four of which are approved for use in drinking water and three for space spray applications to control mosquitoes
- Advances in the development and operational deployment of DengueNet (<http://apps.who.int/globalatlas/default.asp>) for global dengue surveillance
- International Health Regulations 2005 (REF. 112): voluntary compliance in effect
- Planning Social Mobilization and Communication for Dengue Fever Prevention and Control: A Step-by-Step Guide<sup>139</sup>

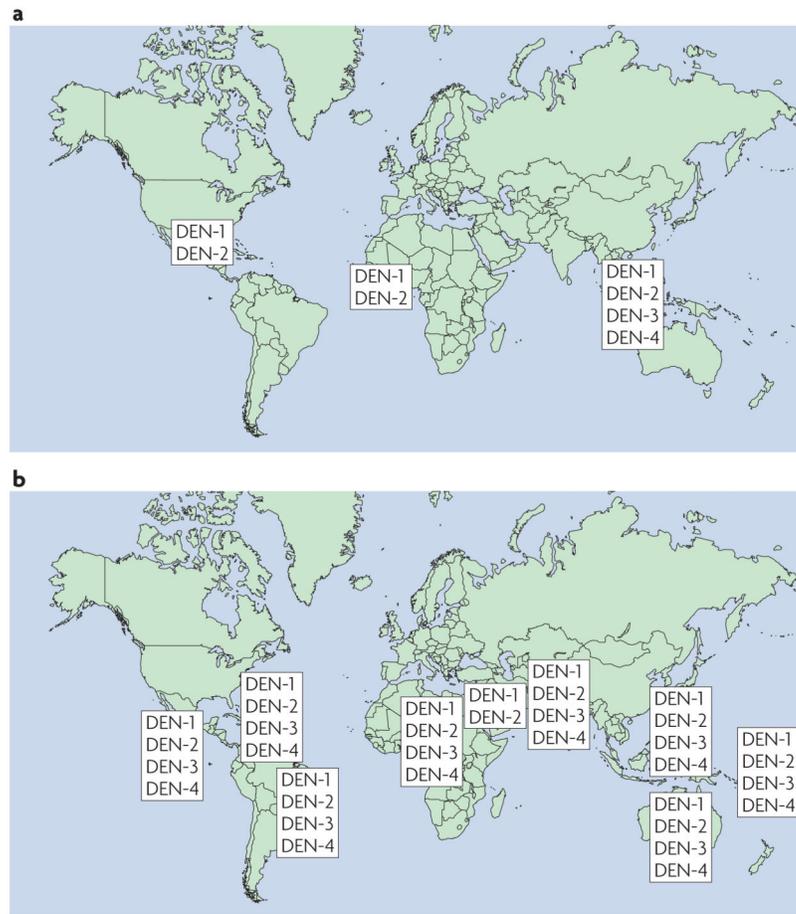


**Figure 1. Countries and areas at risk of dengue transmission, 2007**  
Data from WHO.



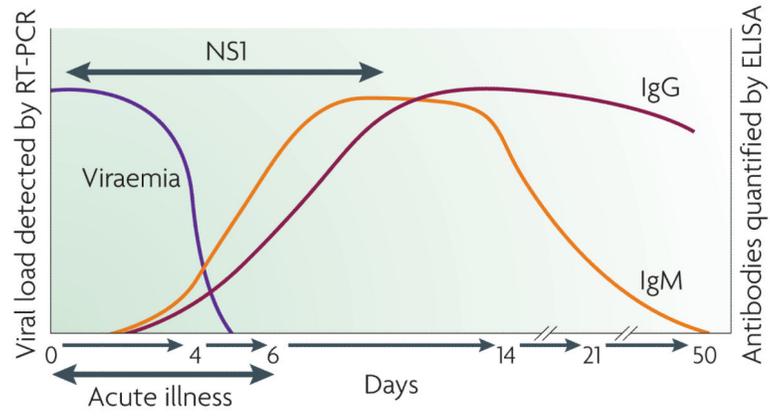
**Figure 2. The dengue virus genome**

The single open reading frame encodes three structural proteins (the capsid (C), membrane (M) and envelope (E) glycoproteins) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5).



**Figure 3. The change in distribution of dengue serotypes**

The figure shows the distribution in 1970 (a) and 2004 (b). Reproduced with permission from REF. 141.



**Figure 4. Dengue virus, antigen and antibody responses used in diagnosis**  
 Ig, immunoglobulin; NS, non-structural.

**Table 1**  
**Selected dengue vaccine candidates**

<b>Vaccine approach</b>	<b>Developer</b>	<b>Status</b>
Live attenuated tetravalent chimeric YF–DEN vaccine	Sanofi Pasteur	Phase II
Live attenuated tetravalent viral isolate vaccine	WRAIR and GSK	Phase II
Live attenuated chimeric DEN2–DEN vaccine	CDC and Inviragen	Phase I
Recombinant E subunit vaccine	Merck	Phase I
Live attenuated tetravalent vaccine comprising 3' deletion mutations and DEN–DEN chimeras	US NIH LID and NIAID	Phase I
Subunit recombinant antigen (domain III) vaccine	IPK/CIGB	Preclinical
Live attenuated chimeric YF–DEN vaccine	Oswaldo Cruz Foundation	Preclinical
Tetravalent DNA vaccine	US NMRC and GenPhar	Preclinical
Purified inactivated tetravalent vaccine	WRAIR and GSK	Preclinical

CDC, Centers for Disease Control and Prevention; CIGB, Center for Genetic Engineering and Biotechnology; GSK, GlaxoSmithKline; IPK, Pedro Kouri Tropical Medicine Institute; NIAID, National Institute for Allergy and Infectious Diseases; US NIH LID, United States National Institutes of Health Laboratory of Infectious Diseases; US NMRC, United States Naval Medical Research Center; WRAIR, Walter Reed Army Institute of Research; YF, yellow fever.