



## Dengue: Need an Entomological and Virological Surveillance for Disease Control

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### ABSTRACT

Dengue infection is the most important *arbo-virus* infection of humans and the most important tropical infectious disease after malaria. This disease is characterized by headache, arthralgia, myalgia, rash, nausea, vomiting. Four serotypes of DENV exist, and severe illness is usually associated with secondary infection by a different serotype. *Aedes aegypti* is the vector for transmission of this disease and the only effective way to prevent epidemic dengue fever/dengue hemorrhagic fever (DF/DHF) is to control growth of the mosquito vector i.e *Aedes aegypti*. A global approach aimed at increasing the capacity for surveillance and outbreak response, changing behaviors and reducing the disease burden using included vector management in conjunction with early and precise diagnosis has been advocated. This review confers how effectively the Virological-entomological laboratory-based surveillance systems in endemic areas furnish information for effective vector control measures.

**Keywords:** Dengue, Epidemics, Pathogenesis, Vector Born Diseases.

### INTRODUCTION

Dengue is the most important arthropod-borne viral infection of humans. Recently this disease becomes a major international public health concern. Worldwide, an estimated 2.5 billion people are at risk of infection and two fifths of the world's population is now at risk for dengue annually.<sup>1,3</sup> Dengue fever is an acute febrile illness with sudden onset of fever, followed by development of generalized symptoms and sometimes a macular skin rash. It is known as "break bone fever" because of severe muscular pains. Dengue fever/dengue hemorrhagic fever/dengue shock syndrome (DF/DHF/DSS) is caused by the dengue viruses (DENVs), members of the *Flaviviridae* family including small enveloped viruses.<sup>2</sup> This flavivirus carries a single-stranded RNA genome of relatively simple organization. A single open reading frame in the RNA directs the synthesis of a long poly-protein that is processed by viral and host cell proteases to produce ten viral proteins, including three structural proteins (core [C]; membrane [M], produced as a precursor protein; envelope [E]) and seven nonstructural (NS) proteins. The DENV complex encompasses four closely related serotypes: DENV-1, DENV-2, DENV-3, and DENV-4 are transmitted to humans by the domestic mosquitoes *Aedes aegypti* and *Aedes albopictus*. There is no direct person-to-person spread. Monkeys acts as a reservoir host in the Southeast Asia and West Africa.

The aspects responsible for the Global resurgence of DF/DHF are unprecedented population growth, unplanned and uncontrolled urbanization, and increased air travel, absence of an effective mosquito control programme and deterioration of the Public Health infrastructure. The risk factors for infection with dengue virus are the increased density of the mosquito vector,

reinfestation with *Ae. aegypti* of a new geographical area, warm and humid climate, increased population density, water storage pattern in houses, storage of junk in open spaces, including tyres, coconut shells etc that trap rain water and introduction of new serotype of the virus, etc. Vaccines or antiviral drugs are not available for dengue viruses; the only effective way to prevent epidemic DF/DHF is to control the mosquito vector, *Ae. aegypti* and avert its bite and therefore this review acknowledge how the Virological-entomological laboratory-based surveillance systems can efficient in endemic areas and to furnish information for effective vector control measures.

### Historical Prospective

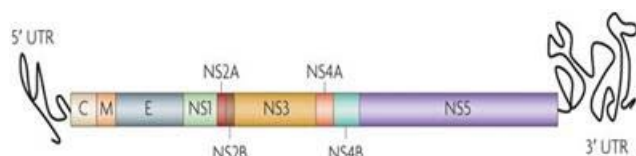
Dengue fever caused major epidemics from the 17th to the early 20th centuries.<sup>4</sup> In most Central and South American countries, effective disease prevention was achieved by eliminating the principal epidemic mosquito vector, *Aedes aegypti*, during the 1950s and 1960s. In Asia, however, effective mosquito control was never achieved, and a severe dengue hemorrhagic fever (DHF) emerged following World War II. In the latter region, the *Aedes aegypti* eradication program had been disbanded in the early 1970s; by the 1980s, this species had re-infested most tropical countries of the region.

### Global Scenario

Globally, DHF has emerged as a major cause of hospitalization and death. Dengue is the second most important tropical disease (after malaria) with approximately 50 to 100 million cases of dengue fever and 500,000 cases of DHF each year. The geographical areas in which dengue transmission occurs have expanded in recent years including all four dengue virus serotypes (DENV-1–4) are now circulating in the Asia, Africa and the America, a dramatically different scenario



from that which prevailed 20 or 30 years ago.<sup>5</sup> The molecular epidemiology of these serotypes has been studied in an effort to understand their evolutionary relationships (Figure 1).<sup>6</sup> The geographical distribution of Dengue virus is widespread in the tropical and subtropical regions of the Central America, South America, South-East Asia and Africa (Figure 2). According to World Health Organization's 40% of the world's population are now at risk from dengue. Approx 50-100 million dengue infections occur worldwide annually. Before 1970, only nine countries had experienced severe dengue epidemics. The disease is endemic in more than 100 countries. Cases across the Americas, Southeast Asia and western Pacific have exceeded 1.2 million cases in 2008 and over 2.2 million in 2010. In 2010, 1.6 million cases of dengue were reported in the Americas alone, of which 49,000 were severe dengue cases. An estimated 500,000 people with severe dengue require hospitalization each year, a large proportion of who are children. About 2.5% of those affected die. According to the Union health ministry note, dengue has been identified as one of the 17 neglected tropical diseases by the UN body.



**Figure 1:** 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 2:** Countries and areas at risk of Dengue transmission, 2013. Data from WHO.

### Indian Scenario

The first epidemic of clinical dengue-like illness was recorded in the Madras in 1780. Gupta et.al summarized the occurrence of the epidemics of dengue like illness in India in 2012.<sup>7</sup> The first virologically provided evidence of epidemic DF in India occurred in Kolkata and Eastern Coast of India in 1963–1964.<sup>8-10</sup> In the North India a

severe epidemic of febrile illness associated with the hemorrhagic manifestation occurred in 1968 at Kanpur after the rains.<sup>11</sup> The first major widespread epidemics of DHF/DSS occurred in India in 1996 involving areas around Delhi, Lucknow and then it spread to all over in the country.<sup>12-13</sup> Between September 2001 and January 2002, an epidemic of dengue occurred in Chennai, Tamil Nadu. Following this another outbreak was reported in the year 2003 (Delhi and its adjoining states) in the Northern India. Several serotypes of Dengue virus has resulted in concurrent infection in some patients with multiple serotypes of Dengue virus.<sup>14</sup>

### Mode of Transmission

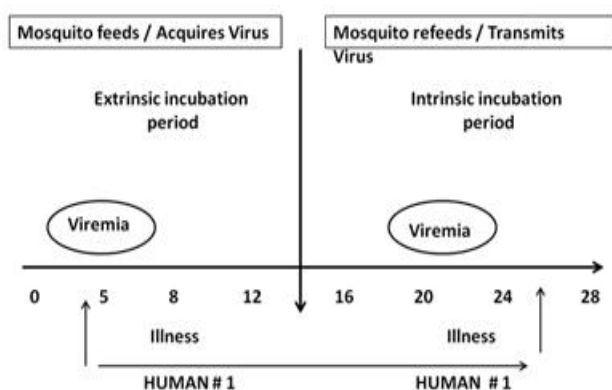
DENV serotypes transmit between humans in nature by mosquitoes of the genus *Aedes*, principally *Aedes aegypti*, which is highly domesticated and has a preference for biting humans. Dengue can follow two types of life cycles; sylvatic and epidemic depending on the type of strain. Sylvatic strains are antagonistically different than epidemic strain and mostly maintain transmission between nonhuman primates and forest dwelling *Aedes aegypti*. Human infection by a sylvatic strain is rare.

Epidemic dengue virus strains infect and replicate within humans and domesticated *Aedes* species.<sup>15</sup> Epidemic dengue has become endemic in tropical urban slums where the main cause of rise in vector population is poor sanitation and stagnant water. High vector density combined with high population density in urban areas has allowed the dengue virus to get a strong foothold in many of the world's over-crowded tropical cities. The dengue virus life cycle begins with infection of a human or nonhuman primate host via an infected mosquito vector. Hosts begin to experience symptoms starting from four to seven days post infection, which usually lasting to ten days followed by a three week recovery period. The virus initially replicates in target organs like the liver, spleen, and thymus but eventually make its way into the lymphatic system where it replicates in lymph tissue and white blood cells. The lymphatic system serves as a gateway to the bloodstream where the virus continues to replicate. Eventually there are very high levels of virus in the blood (viremia) and disease transmission can occur (Figure 3). Transmission of dengue virus arises when an uninfected mosquito bites an infected host and takes up the virus with its blood meal. The virus replicates in the midgut of the mosquito then infects its hemocoel (body cavity). From the hemocoel the virus eventually makes its way to the salivary glands. Once the salivary glands are infected the mosquito can infect other human hosts and the cycle continues. The length of time required for the virus to reach the salivary glands of a mosquito after the ingestion of an infected blood meal is about eight to ten days depending on both viral and host factors.<sup>16-17</sup>

### Laboratory Diagnosis

Laboratory diagnosis 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. Sero-conversion of IgM or IgG antibodies is the standard for serological confirmation of 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>18-19</sup>



**Figure 3:** Transmission cycle of Mosquito in humans

### Virus isolation

The C6/36 cell line from *Aedes albopictus* mosquito is used in DENV isolation method, 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 for virus isolation.<sup>20-21</sup> Sera that have been collected from the 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. Serum is often used for virus isolation but plasma, leukocytes, whole blood and tissues obtained at autopsy can also be used.

### 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 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

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>22</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>19</sup> In addition to serum, dengue-specific IgM can be detected in whole blood on filter paper (sensitivity 98.1% and specificity 98.5%) and in saliva (sensitivity 90.3% and specificity 92.0%), but not in urine.<sup>23-26</sup> An ELISA for dengue-specific IgG detection can be used to confirm a dengue infection in sera. It is also widely used to classify primary or secondary infections. Some protocols use serum dilutions to titre dengue-specific IgG and others use the ratio of IgM to IgG.<sup>27-29</sup> The assay uses the same dengue antigens as MAC-ELISA and it correlates with the results of 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 cross reactivity was observed in sera collected from individuals infected with dengue or Japanese encephalitis virus.<sup>30</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>31</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>32</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.<sup>33,34</sup> However, in 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>35</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>36-39</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 commercialized 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, a nested RT-PCR assay or a one-step multiplex RT-PCR assay.<sup>41,42</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>43</sup>

### Real-time RT-PCR

The real-time RT-PCR assay is a one-step assay that allows virus titer 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>44-46</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>47</sup>

### Immunity

Several reports of large-scale serological surveys have been published in the last 5 years.<sup>48-54</sup> Although such studies may aid understanding of overall dengue activity in the area, few have attempted to use these data to guide vector-control operations. Serological surveys could be useful in elucidating the roles played by *A. aegypti* and *A. Albopictus* during epidemic and inter-epidemic periods. Such a study would be entirely feasible in places like India where the geographical distribution of *A. aegypti* is different from that of *A. albopictus*.

### Surveillance systems

A common theme that appears in surveys of the areas where research on dengue is needed is good surveillance system. Literature suggests that a lot can be done to improve the sensitivity and specificity of surveillance programs. Many countries continue to monitor dengue cases by using a passive surveillance approach. An update on the major DF/DHF endemic countries in the world with subjective evaluation of the status and efficiency of their surveillance systems, whether they have laboratory capabilities, and whether their systems have an early warning predictive capability for epidemic transmission, was summarized by Gubler in 2002. Passive surveillance

relies on disease notification by healthcare professionals who have a duty to report all suspected cases to public health authorities. However, passive surveillance systems are uniformly insensitive because of the low index of suspicion for dengue during inter-epidemic periods.<sup>55-58</sup>

### Entomological Surveillance

The entomological surveillance is an index to assess vector population density that may be predictive of epidemic dengue transmission. Since eradication is not feasible, the goal of public-health, preventive measure is to maintain a vector population density that is too low to support sustained viral transmission. Environmental factors and human activities play an important role in determining of mosquito population differentiation. Number of methods is available for the detection or monitoring immature and adult populations. Selection of appropriate sampling methods depends on the surveillance objectives, levels of infestation, available funding and skills of personnel. Several indices have been described and are currently used to monitor *Ae. aegypti* population for dengue virus transmission. These are as follows as Table 1.

**Table 1:** Indexing Pattern for monitoring of Entomological Surveillance

House Index	The percentage of housing infested with larvae or pupae.
Container Index	The percentage of water holding containers infested with larvae or pupae.
Breteau Index	The number of Positive container per 100 houses inspected.

Different collection methods are used for trapping of mosquito population for monitoring and control of dengue transmission in these subtropical areas: Adult resting collection studies performed with backpack aspirator are an efficient and effective means of evaluating adult mosquito densities. The methods for collection of adult mosquitoes also lead to be labor intensive and depend on the proficiency of collection skills. For larval surveys Ovitrap can be used as a complementary surveillance method. These are especially useful for the early detection of new infestation in areas from which the mosquitoes had been eliminated.<sup>18</sup>

### Vector control

In most Central and South American countries, effective disease prevention was achieved by eliminating the principal epidemic mosquito vector, *Aedes aegypti*, during the 1950s and 1960s. In Asia, however, effective mosquito control was never achieved, and a severe dengue hemorrhagic fever (DHF) emerged following World War II. During the 1950s, 1960s, and 1970s, this new form of dengue occurred as periodic epidemics in few countries. During the 1980s, however, incidences increased dramatically, expanding distribution of the virus



and the mosquito vector to the Pacific islands and tropical America. In the latter region, the *Aedes aegypti* eradication program had been disbanded in the early 1970s; by the 1980s, this species had reinfested in most tropical countries of the region. Increased disease transmission and frequency of epidemics caused by multiple virus serotypes in Asia, Pacific Islands, the Caribbean, and South America. A complicating factor is the role of herd immunity. Clearly, the vector-population densities required for epidemic transmission are lower in regions with low herd immunity.<sup>59</sup>

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. Various reports have shown that the inclusion of community participation along with the use of old and new vector-control tools as well as biological control have had positive effects on preventing disease transmission.<sup>60-63</sup> However, sustainability remains a problem, and there is a need to establish proactive, laboratory-based disease and vector surveillance programmes that provide a trigger for intensified vector control to prevent epidemic transmission. Experience gained in India suggests that keeping the density of the vector population below a threshold for epidemic transmission is a moving target. Lowered herd immunity after implementation of effective control measures may paradoxically lead to a rise in the number of cases of dengue, which in turn, requires more intensive vector-control measures to prevent an epidemic.<sup>64-66</sup>

Identification of predictive entomological thresholds or markers for epidemic dengue, in combination with population susceptibility would thus be a useful area of research. 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 larvicide and adulticide space sprays.<sup>18</sup> The role of *A. aegypti* is obvious, but the role of *A. albopictus* in maintaining dengue endemicity is less clear. Although *A. albopictus* is an excellent host and experimental vector for dengue viruses, it has not been frequently associated with epidemic dengue.<sup>67-69</sup> The reason for this is thought to be related to this species ecology and blood-meal seeking behavior; *A. albopictus* has a broader host range, and usually bites only once in obtaining a blood meal. However, in most countries where dengue is endemic, both species of vectors can be found. Moreover in the India, *A. albopictus* outnumbered and *A. aegypti* are more widespread geographically.

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* species larvae. Since the early 1970s the organophosphate temephos has been widely used, but increasing levels of resistance, 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>70-73</sup>

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. 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. Most efforts in vector control are centered 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>74,75</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 behavior change. Towards this end, a TDR/WHO guide for planning social mobilization and communication for dengue fever prevention and control has been developed. 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>18</sup>

#### **Application of remote sensing and geographic information system**

An effective surveillance tool is required to identify the population at risk. Strategic assessment of areas at risk of a vector borne disease will facilitate rapid action and control initiatives, such as targeted pesticide application or distribution of medication.

The science and technology associated with remote sensing and geographic information system (GIS) are suitable for identifying these environmental targets. Vector control strategies require knowledge of the ecology of breeding and resting habitats and behavior of various mosquito species. Periodical surveys are essential to develop a suitable vector control strategy. Remote sensing is the observation and measurement of objects from a distance, i.e. instruments or recorders are not in direct contact with objects under investigation. High attitude color –infrared photography was also used to produce a detailed map of *Aedes* breeding sites in a newly formed mosquito abatement district in Michigan. These habitat maps were subsequently used to direct control measures. All together, capability of synthetic aperture

radar (SAR) and the sensitivity of surface moisture conditions and target geometry are identified as the primary advantages of radar remote sensing for this application.<sup>76</sup>

In recent years, the development of powerful mathematical and computer tools allows for more sophisticated modeling of outbreaks of infectious disease. Such models also allow for a theoretical assessment of the ecological determinants of epidemic transmission, effectiveness of disease control and preventive measures.<sup>77-79</sup> Emergency control measures could perhaps benefit from the use of such a tool to assess their efficacy. They might allow for various control modalities to be assessed for their effectiveness in reducing virus transmission, given a range of likely scenarios. However, for such a tool to be practically useful, validation of the mathematical assumptions need to be carried out with actual epidemiological and entomological data.

## CONCLUSION

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. Virological surveillance of cases that present with mild viral syndrome may yield such pre-epidemic isolates for comparative analysis. Although more work is needed to be done before such data is used for epidemic prediction, the key to understanding dengue epidemiology lies in better virological surveillance during the inter-epidemic periods. With the combination of active virological and entomological surveillance, such studies may be helpful for better understanding of the dynamics of dengue transmission as well as factors that contribute to endemic versus epidemic transmission. A significant area of research is therefore needed to determine the influence of viral factors on disease transmission dynamics and disease severity. 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 Denco 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. One of the important step against disease control is the interruption of viral transmission. This will require a sensitive and cost-effective disease- and vector-surveillance, coupled with a community based larval-control programme. Few countries where dengue is endemic have such public-health infrastructure in place.<sup>57</sup> In developing countries like India the problem of dengue is mammoth, that is compounded by the huge population, poor medical and diagnostic facilities,

inadequate mosquito control and all the ground conditions that favor expansion of the vector. Developing countries needs a large number of virus laboratories that may provide quick and reliable diagnosis. Efforts should be done to develop improve, proactive, entomology based good laboratory surveillance systems, that can forecast impending dengue epidemics. In addition to this, for the evaluation of vector densities and distribution, integrated vector management is also required.

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