

Urgent need for a permanent dengue surveillance system in India

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Dengue is one of the most important mosquito-borne viral diseases in the world, and is endemic in approximately 120 countries. It has been estimated that there are 50–100 million cases of dengue fever and 3.6 billion people are at risk of infection¹. It is emerging and re-emerging in the tropics and currently poses the most significant arboviral threat to humans. What was once a disease with low attack rates, moderate pathogenicity, and infrequent epidemics has become, in the last two decades, the leading arboviral cause of illness and death in humans². The frequency of dengue outbreaks is increasing and spreading, with Asian countries experiencing such outbreaks every three to five years³. Unplanned urban expansion, leading to high densities of humans exposed to anthropophilic mosquito vectors, as well as globalization and travel, have contributed to hyperendemic transmission of all four dengue serotypes throughout the tropics^{4,5}.

Dengue fever has been known in India for over two centuries. It has been endemic in several parts of the country with interspersed random epidemics being reported from various places throughout India. After 2000, dengue and dengue hemorrhagic fever (DHF) have become diseases of serious concern due to thousands of reported cases and hundreds of deaths every year⁶. There are two patterns of dengue virus (DENV) transmission: epidemic dengue and hyperendemic dengue. Epidemic dengue transmission occurs when the DENV is introduced into a region as an isolated event that involves a single viral strain. If the number of vectors and susceptible hosts is sufficient, explosive transmission can occur, with an infection incidence of 25–500. Hyperendemic dengue transmission is characterized by the continuous circulation of multiple viral serotypes in an area where a large pool of susceptible hosts and a competent vector (with or without seasonal variation) are constantly present. This is the predominant pattern of global transmission and in hyperendemic transmission appears to be a major risk for dengue haemorrhagic fever⁷.

Outbreak investigations conducted in several areas reveal that dengue surveil-

lance is essential in each state so as to forecast the outbreaks well in advance. The presently used entomological indices are mainly based on immature survey, and they are neither qualitative nor informative with regard to forecasting dengue outbreak. During dengue outbreak study, it has become customary to collect serological and entomological samples so as to assess whether the epidemic has occurred or not. It is difficult to confirm the origin and spread of dengue in a particular area through epidemiological enquiries made during dengue outbreak study. Unlike other infectious diseases such as cholera and malaria, the number of dengue-affected cases in each area is less. Only few mosquitoes are generally found infected with the DENV. So, the scope for undertaking epidemiological and transmission studies in such areas is limited.

Where does the DENV come from?

Where does the DENV come from? Humans or mosquitoes? This seems to be a complicated question if we consider many dengue outbreaks in different parts of India simultaneously. There may be two possibilities to explain the cause for the outbreak of dengue in an area. One possibility of spread of infection is from an imported case (human) and by the bite of infected mosquitoes (*Aedes aegypti* or *Ae. albopictus*). Once infected, the female *Aedes* mosquito carries the virus during its lifespan. Dengue is transmitted to other persons and the infection spreads to adjacent areas but within 100–500 m, as the vector mosquitoes are generally weak fliers⁸. DENV transmission is spatially and temporally focal and local. Focal transmission is associated with recent DENV introductions and that one or a few mosquitoes are likely responsible for all the transmission in each cluster. *Ae. aegypti* vectors are nervous feeders, disrupting the feeding process at the slightest movement, only to return to the same or a different person to continue feeding moments later. Due to this behaviour, *Ae. aegypti* females often feed on several persons during a single blood meal and, if infectious, may infect multi-

ple persons in a short time⁹. But it has not been the case in areas where dengue outbreak has occurred so far. Infection usually spreads to one or two person. In fact, infection must spread to many other susceptible persons in a locality due to the feeding behaviour of the vector mosquitoes, but this is not so in reality. So, there may be some other factors such as herd immunity, which may influence the transmission dynamics of dengue.

In most countries where dengue is endemic, both species of vectors can be found. Furthermore, in many places such as Singapore, *Ae. albopictus* outnumber *Ae. aegypti*, and are more widespread geographically¹⁰. Although *Ae. albopictus* may not be an efficient vector for epidemic dengue; it may play an important role in endemic transmission, maintaining DENVs in the population until the population immunity is sufficiently lowered and the *Ae. aegypti* population is sufficiently dense to support epidemic transmission¹¹.

The other possibility for dengue outbreak is due to the activity of DENV which may be influenced by seasonality. DENV may be circulating in a particular proportion in the population. The virus that causes dengue fever comes in several forms and can lie dormant in the body for years until reactivated by a new infection, or by the weakening of the immune system from other causes. During its dormant stages, it remains 'hidden' and does not cause any symptoms of illness¹². The DENV may be circulating either in humans or mosquitoes or in some 'hidden reservoirs', or between any of these two hosts and mosquitoes. In the case of humans, DENV may be in the lyzogenic phase in certain supportive organs such as the liver, spleen, brain, thymus, etc. It is active and undergoes replication but without producing any pathogenic effects in the host. This may be possible in humans who have acquired partial immunity to DENV.

The virus initially replicates in target organs like the liver, spleen and thymus, but eventually makes its way to the lymphatic system where it replicates in the lymph tissue and white blood cells¹³. Eventually virus titres are high enough in the bloodstream of the host for disease

transmission to occur. Viruses can evolve and gain mutations over time resulting in variations. These variations may help the virus hide from the immune system. The transmission from vector to host for a particular DENV strain is determined by viral infectivity of mosquito cells and the rate of viral replication in these cells. Strains belonging to more virulent genotypes have fast rates of viral replication as well as higher virus titres in human and vector hosts¹⁴.

Dengue has been particularly sensitive to both periodic fluctuations and sustained changes in global and local climates, because vector biology and viral replication are temperature and moisture-dependent^{6,15,16}. With the advent of the favourable season, DENV is again activated and starts circulating in the peripheral blood of the human. The DENV is taken up by vector mosquitoes while they feed on dengue viraemic human host and further development of DENV takes place in the midgut of the vector mosquitoes. Now DENV becomes infective units and reaches the salivary glands of the vector mosquitoes and subsequently spreads infections among human hosts. Although environmental factors are important, they are not the only ones critical to dengue transmission. The virus must be present, there must be sufficient number of humans still susceptible, or non-immune, to the virus, and there must be contact between those susceptible humans and the mosquito vectors¹⁷.

The second possibility is that during non-transmission season, DENV may be maintained in nature by vector mosquitoes through transovarial transmission (TOT). In TOT, the virus passes through egg, larva, pupa and adult¹⁸ stages. During this process, the number of virus particles may be lost but not considerably. Thus TOT is helpful in maintaining the DENV in at least some vector mosquitoes during non-transmission season. Vertical transmission of DENV was observed in males of *Ae. aegypti* collected mainly in summer months when dengue infections in humans were low, suggesting that DENVs adopt a novel strategy of surviving adverse climatic conditions¹⁹. Male *Ae. albopictus* can transmit DENV sexually during mating and females can transmit it vertically more efficiently than can *Ae. aegypti* females¹¹. When the favourable season occurs, these mosquitoes become the initial source of infection and the typical

transmission is effected with the build-up of vector mosquito density along with other congenial factors. *Aedes* vectors could also serve as reservoirs of the DENV during the dry season²⁰.

The third possibility is that there must be some hidden reservoirs around human dwellings. In addition to humans as the primary reservoir for DENV, domestic animals such as cat, rat, dog, cow, buffalo, goat, monkey, etc. may also serve as a 'hidden reservoir' for DENV. They may also contribute the virus to humans during the transmission season^{21,22}. A silent transmission may be going on within the triad of human-mosquito-hidden reservoirs, but with less intensity. Transmission may also be maintained with different DENV strains mostly between sylvatic non-human primates and *Ae. aegypti*. These primates may also transfer DENV to domestic 'hidden reservoirs' while they visit nearby villages for food.

The very purpose of dengue surveillance is to forecast the impending outbreak in a suspected area or wherever it may be of concern. This cannot be accomplished by just undertaking entomological and epidemiological surveys after the dengue outbreak. The pattern of biennial epidemics more closely resembles the observed multi-annual cycle of dengue epidemics in endemic countries²³. A more severe biennial epidemic implies that the virus, during the epidemic, is circulating among a greater proportion of the population, thus causing the number of secondary infections to increase. Indeed, there is relative increase in the number of primary infections. Secondary infections have a greater likelihood of resulting in DHF²⁴. Though a country may be endemic for dengue, no specific area in a country is endemic for dengue for a long time. Infection slowly spreads from one village to another, one town to another town and one district to another district. In fact, the presence of dengue vectors and antibodies to DENV in a community does not necessarily reflect on re-emergence of dengue in that area.

Necessity of a permanent dengue surveillance system

Unfortunately there are no anti-viral drugs for DENV infection. The only treatment is supportive care; fluid replacement and

pain management. In such a situation, interruptions of the vector-host transmission cycle are the only option for disease control²⁵. To stop transmission from host to vector, those infected only need to be prevented from further exposure to mosquitoes. Surveillance is fundamental for setting goals and evaluating success. In an attempt to find a more efficient and cost-effective form of vector control, researchers are trying to understand the complexities of DENV replication in mosquito vectors. Co-infection of the mosquitoes with multiple DENV strains or the bacterium *Wolbachia*, and interactions between DENV and the innate immune system of mosquitoes all modulate viral replication¹⁴. The replication kinetics of DENV in the mosquito-host has overarching effects on the epidemiology of the disease. Infection of *Wolbachia* decreases the ability of mosquito vectors to transmit DENV by shortening the lifespan of the vector²⁶. With a better understanding of how each of these factors affects the overall viral load and rate of viral replication within the vector, intentional infection of vector populations with *Wolbachia* is currently being considered as a novel biocontrol strategy in vector control²⁷. Only a permanent dengue surveillance system could give better insights to understand virus-host-vector interactions. Then it would be possible to come up with better strategies for vector transmission control to mitigate and prevent future DENV epidemics.

For dengue control programmes to be effective, information on the occurrence of infection and disease in the population is essential. However, as most dengue infections are asymptomatic or unapparent, presenting themselves as non-differential fevers of unknown etiology, surveillance systems based on the monitoring and notification of symptomatic cases have low sensitivity and are not capable of detecting low or sporadic transmission. When dengue transmission is absent or silent in a locality or it is the season of low disease incidence, surveillance efforts should be directed to the early detection and characterization of disease activity. Early in the epidemic, passive rather than active surveillance should be emphasized in areas where disease activity and dengue serotypes have already been defined. The accurate and early detection of incipient dengue epidemics is a critical goal for effective dengue prevention and control. The surveillance system must be

able to differentiate transient and seasonal increases in disease incidence from activity seen at the beginning of the outbreak.

Disease surveillance

Passive surveillance relies on disease notification and passive surveillance systems are uniformly insensitive because of the low index of suspicion for dengue during inter-epidemic periods. Two main problems are encountered in passive surveillance for dengue. Dengue infection leads to a wide range of disease manifestations, completely asymptomatic, undifferentiated viral syndrome, DF, DHF, dengue shock syndrome, and other severe manifestations such as neurotropic disease and hepatic failure. Passive surveillance using case definitions lacks specificity since many other infectious diseases, such as influenza, chikungunya fever, viral haemorrhagic fevers, enterovirus infections, leptospirosis, malaria, typhoid fever, etc. all present with symptoms and signs that are similar to those seen in patients with dengue in the acute phase of illness²⁸. The use of passive surveillance alone also ignores patients who present with undifferentiated febrile illness or viral syndrome. This group of patients may represent a large proportion of those with symptomatic dengue infection, depending on the age of the patient and the strain of the infecting virus. Any attempt to carry out passive surveillance among this group of cases will not be feasible. However, mild viral syndrome may be of particular use in monitoring dengue transmission during inter-epidemic periods when the incidence of classical DF and DHF is low. In countries where dengue circulates perendemically, it is likely that emergence of genetic variants with greater epidemic potential may be partially responsible for the cyclical outbreaks, since certain viral clades appear to be more closely associated with increased transmission and severe disease outcomes²⁹. Virological surveillance on cases that present with mild viral syndrome may yield such pre-epidemic isolates for comparative analysis. Although more work will need to be done before such data can be used for epidemic prediction, the key to understanding dengue epidemiology lies in better virological surveillance during the inter-epidemic periods³⁰.

Passive surveillance alone will not generate sufficient information needed for the prediction of dengue outbreaks. An active laboratory-based surveillance system and a better understanding of the epidemiology of dengue are needed for more effective prevention³¹. Active dengue surveillance is a powerful tool for understanding the underlying evolutionary, ecological and epidemiologic mechanisms of emergence. Passive surveillance overlooks asymptomatic infections, which can be high in dengue, and between outbreaks (or even between seasonal highs). However, adding active surveillance elements to a well-functioning passive surveillance system improves sensitivity; adding laboratory elements to the system improves specificity³². A given DENV serotype often falls below detectable levels. The virus does not go locally extinct, because phylogenetic data indicate that strains usually persist and/or evolve locally in the Puerto Rico (PR) rather than by reintroduction from other places^{33,34}. Epidemiologists, mathematicians and entomologists should interact on more aspects of the dengue transmission cycle to find better approaches for measuring and interpretation of the transmission dynamics of dengue.

Entomological surveillance

The clear need in entomological surveillance is an index or a measure of vector population density that may be predictive of epidemic dengue transmission. Since eradication is not feasible, the goal of public health preventive measures, in the absence of a vaccine, is to maintain a vector population density that is too low to support sustained viral transmission. It was thought from experience in Singapore in the 1970s that a premises index (the percentage of premises where *Ae. aegypti* larvae are found) of less than 5% was sufficient to prevent epidemic dengue. However, since the 1990s, it is obvious that in Singapore, dengue incidence has increased dramatically, despite an overall premise index of 2% and below. Despite low premise and national indices, there are places in Singapore where the density of the *Ae. aegypti* population is high³⁵. Thus many of the existing immature indices of dengue vectors such as Breteau and Container indices are not sufficiently sensitive to forecast or prevent the dengue epidemics. The use of

various immature indices in surveillance is considered generally uninformative, and the refinement or development of more accurate indicators of risk and the means of measuring them is being emphasized. Monitoring natural infectivity of mosquitoes carried through the vertical route of the virus, may serve as an important surveillance tool for risk prediction as well as for the prevention of dengue emergence in an endemic setting³⁶. The adult traps that are being advocated for use in the surveillance of dengue vectors are not satisfactory. Though BG-Sentinel traps are claimed to be more efficient than other existing traps in the collection of dengue vectors, they collect mostly nulliparous females and frequently males outnumber the total collection. Though MosquiTRAP is better than Adultrap and backpack aspirator, it has the serious disadvantage of acting as a breeding site for dengue vectors. Till now, no trap has been designed and evaluated for collecting all the sub-populations of dengue vectors, and such a tool could be useful in the surveillance of dengue vectors³⁷. Beliefs such as 'no water = no mosquitoes = no dengue = no deaths' do not hold good nowadays. It is likely that dengue vectors are continually responding or adapting to environmental change. 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.

A major problem with emergency control operations is that because of poor surveillance they are usually implemented near peak epidemic transmission, too late to have any impact on the epidemic. We need a large number of virus laboratories that may provide quick and reliable diagnosis. Efforts should be made to develop improved, proactive, laboratory-based surveillance systems that can forecast impending dengue epidemics³⁸.

A simplified model for dengue surveillance

Ten to 20 endemic sentinel villages may be selected in each district for undertaking vertical dengue vector surveillance. Month-wise random collection of human serum samples and vector mosquitoes (both immature and adults) should be done simultaneously. These samples

need to be subjected to appropriate serological/molecular tests to confirm the presence of the concerned antibodies (IgM)/antigen (Ns1) in blood samples and DENV in mosquitoes preferably by RT-PCR using previously established procedures^{39,40}. Each village should have sentinel clinics/physicians, fever alert and sentinel hospital for dengue surveillance. A laboratory with all facilities for isolation and serotyping of the DENV should be set up at each district. If the human community and vector mosquitoes are free from dengue antibodies and DENV respectively, it is understood that there is no dengue transmission. If they are positive for dengue antibodies and DENV respectively, it indicates that transmission is going on in that area. If the human community is negative for dengue antibodies and the vector mosquitoes are positive for DENV, there is an impending dengue outbreak in that area. Whenever we get dengue-positive samples, serotyping should also be carried out.

Conclusion

The spread of dengue and DHF has been increasing due to the ever-increasing population, unplanned urbanization with inadequate public health system, poor vector control, viral evolution, increasing international recreational activities, and business and military travel to endemic areas. The only solution to reduce the intensity of further spread of dengue is to have a permanent dengue surveillance system. Such a system in each state of India could indicate the spread of the DENV and its serotypes in space and time. A greater understanding of the interactions between mosquitoes and the different serotypes of dengue will be possible. This would give us a better picture of transmission dynamics and would potentially lead to better prediction of epidemics, and thus better control of the disease. We shall be able to spot the season and the area that need more attention for monitoring, preventing and forecasting the impending outbreaks to an appreciable extent. With existing technologies such as geographical information system, polymerase chain reaction, rapid antigen test kits and sequencing and bioinformatics, we have the opportunity to take a holi-

stic approach in an attempt to suppress the resurgence of dengue epidemics. A separate dengue surveillance system (DengueNet-India) for India needs to be developed in partnership with the Ministry of Health, Government of India; research centres and laboratories around the country in collaboration with WHO. The database should be continuously updated to share current and historical data on dengue cases with DengueNet-India and DengueNet-WHO. The goals of DengueNet-India are to standardize the reporting of dengue cases and to provide early warning prior to dengue epidemics, which may help reduce fatality rates.

Note: The views expressed here are those of the authors only and do not reflect the views of the organization, to which they belong.

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