Hindawi Publishing Corporation
ISRN Virology
Volume 2013, Article ID 207580, 5 pages
http://dx.doi.org/10.5402/2013/207580

Research Article

A Comprehensive Study on the 2012 Dengue Fever Outbreak in Kolkata, India

Bhaswati Bandyopadhyay,[1] Indrani Bhattacharyya,[1] Srima Adhikary,[1] Jayshree Konar,[1] Nidhi Dawar,[1] Jayeeta Sarkar,[1] Saiantani Mondal,[1] Mayank Singh Chauhan,[2] Nemai Bhattacharya,[1] Anita Chakravarty,[2] Asit Biswas,[3] and Bibhuti Saha[1]

[1] *Calcutta School of Tropical Medicine, 108 C. R. Avenue, Kolkata 700073, India*
[2] *Maulana Azad Medical College, Bahadur Shah Zafar Marg, New Delhi, Delhi 110002, India*
[3] *NRHM, Government of West Bengal, Swasthya Bhavan Sector V, Salt Lake, Kolkata 700091, India*

Correspondence should be addressed to Bhaswati Bandyopadhyay; drbhaswatib@gmail.com

Received 13 June 2013; Accepted 17 July 2013

Academic Editors: A. Mastino and J. Stephenson

Background. Dengue viruses (DV) belong to the family Flaviviridae, with four serotypes referred to as DV-1, DV-2, DV-3, and DV-4. A large-scale outbreak of dengue fever occurred in 2012 involving several districts of West Bengal. *Objective*. To present a comprehensive picture of the dengue fever outbreak in 2012 and to identify the prevailing serotypes. *Materials and Methods*. Serum samples were collected from suspected dengue fever cases. Samples from fever cases <5 days duration were tested for dengue NS1 antigen employing Pan Bio (Australia) NS1 ELISA kit. Serum samples of ≥5 days fever were tested for dengue-specific IgM by MAC ELISA test kit prepared by the National Institute of Virology Pune, India. Serotyping of dengue samples was done by dengue-specific reverse transcriptase polymerase chain reaction (RT-PCR). *Results*. The number of dengue cases in 2012 clearly outnumbered the dengue cases in 2010 and 2011. The majority of the cases were in the age group 11–30 years with a male preponderance. Outbreak occurred during the months of Aug.–Nov. indicating increased vector transmission in the monsoon and postmonsoon periods. The prevailing serotypes in this outbreak were Den1, Den3, and Den4.

1. Introduction

Dengue viruses (DV) belong to the family Flaviviridae, and there are four serotypes of the virus referred to as DV-1, DV-2, DV-3, and DV-4 [1]. DV is a positive-stranded encapsulated RNA virus and is composed of three structural protein genes, which encode the nucleocapsid or core (C) protein, a membrane-associated (M) protein, an enveloped (E) glycoprotein, and seven nonstructural (NS) proteins. It is transmitted mainly by *Aedes aegypti* mosquito and also by *Ae. albopictus*. All four serotypes can cause the full spectrum of disease from a subclinical infection to a mild self-limiting disease, the dengue fever (DF), a severe disease that may be fatal, and the dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS).

Dengue is the most common and widespread arboviral infection in the world today. It is an increasingly prevalent tropical arbovirus infection with significant morbidity and mortality [1]. dengue infection has been known to be endemic in India for over two centuries as a benign and self-limited disease. In recent years, the disease has changed its course manifesting in the severe form as DHF and with increasing frequency of outbreaks [2]. dengue infection in a previously nonimmune host produces a primary response of antibodies characterized by a slow and low-titer antibody response. IgM antibody is the first immunoglobulin isotype to appear. In a suspected case of dengue, the presence of antidengue IgM antibody suggests recent infection. Anti-dengue IgM detection using enzyme-linked immunosorbent assay (ELISA) represents one of the most important advances and has become an invaluable tool for routine dengue diagnosis [3]. Specifically, MAC ELISA (IgM antibody capture ELISA) diagnosis is based on detecting dengue-specific IgM [4].

Dengue fever is a recurrent problem in West Bengal. Dengue was first documented in Kolkata (Calcutta) in 1824, and several epidemics took place in the city during the years 1836, 1906, 1911, and 1972 (affecting 40% of the city people) [5]. In India, DHF was first reported in Kolkata in 1963-64 [6, 7]. Thereafter, several outbreaks occurred in India including Kolkata [8–13]. Following the last large-scale dengue outbreak in 2005 [14], recently there was a major outbreak in 2012 involving several districts of West Bengal. The nature and extent of the outbreak of dengue was thoroughly investigated. The purpose of this paper is to present a comprehensive report on the diagnosis of dengue fever cases, available from January to December 2012 and also to identify the serotype/s presently circulating in the region. Although outbreaks did not occur, a higher number of cases of suspected dengue infection were reported to our referral laboratory in the similar months in 2010 and 2011. In this study, we have compared the serological and virological profiles of the confirmed dengue cases in the years 2010, 2011, and 2012. An attempt has also been made to evaluate the newly introduced dengue NS1 antigen detection kit in comparison to the MAC ELISA kit supplied by the National Institute of Virology, Pune.

2. Materials and Methods

The epidemiological data were collected from different sources, but the main pool of patients was those who attended the referral Virology Laboratory at Calcutta School of Tropical Medicine. dengue fever patients typically develop sudden onset of high-grade fever. Hence, fever cases of all age groups and either sex attending the referral Virology Laboratory (all the year round) of Calcutta School of Tropical Medicine were selected as per WHO criteria (An acute febrile illness with ≥2 of the following manifestations: headache, retro-orbital pain, myalgia, arthralgia, rash, and haemorrhagic manifestation).

For the detection of dengue-specific IgM antibodies, blood was collected from each patient suspected to be suffering from dengue, at least 5 days after onset of fever starting from January 2010 to December 2012, and age and sex of each patient were recorded.

However, in 2012, blood samples were also collected from clinically suspected acute dengue fever cases (fever duration ≤ 4 days), due to the availability of dengue NS1 antigen detection kits supplied by Integrated Disease Surveillance Programme (IDSP). A portion of the dengue NS1-positive sera were preserved in −80°C freezer for further serotyping.

2.1. Serology. The samples were screened for the presence of dengue-specific IgM antibodies by IgM antibody capture enzyme-linked immunosorbent assay (MAC-ELISA): using a kit prepared by the National Institute of Virology, Pune, India (as an integral part of the National Vector Borne Disease Control Programme), strictly following the manufacturer's protocol [15].

For detection of the presence of dengue NS1 antigen in the acute sera, Pan Bio (Australia) NS1 ELISA kit was used.

2.2. Molecular Serotyping

2.2.1. RNA Extraction. Dengue viral RNA was isolated directly from the serum samples using the High Pure Viral RNA kit (Roche, Germany) according to the manufacturer's instructions.

2.2.2. Detection and Serotypic Characterization of Dengue Virus by PCR. The detection and serotypic characterization of dengue virus was performed using previously published primers by Lanciotti et al. [16]. The RT-PCR assay employed in this study could distinguish the 4 dengue serotypes by the size of the products as described by Lanciotti et al. [16]. This includes a step of RT-PCR using a highly conserved primer pair, D1 (forward) and D2 (reverse), and a step of second-round PCR using the primers D1 and 4 serotype-specific primers, TS1, TS2, TS3, and TS4. The expected size of the RT-PCR products is 511 bp (D1 and D2) (external PCR product) and 482 bp (D1 and TS1 for dengue-1), 119 bp (D1 and TS2 for dengue-2), 290 bp (D1 and TS3 for dengue-3), and 392 bp (D1 and TS4 for dengue-4). The products were electrophoresed through 2% agarose gel, stained with ethidium bromide, and examined under ultraviolet light using a digital gel documentation system.

3. Results

A comprehensive picture of dengue epidemic that occurred in West Bengal state in 2012 is given in Table 1.

Although dengue cases were detected from 13 districts of West Bengal, clearly Kolkata was the worst affected, and it alone shared 74.32% of the dengue cases.

Figure 1 shows the distribution of the percentage of the dengue cases in various age groups in either sex. It clearly reveals that the highest number of cases belonged to the age group 11–30 yr and males clearly outnumbered the females.

As the outbreak of dengue mainly occurred in the months of August to November of 2012, Figure 2 shows the weekly distribution of the cases; the highest number of cases was reported from the 1st week of September to almost mid-October for both seromarkers of dengue (IgM and the NS1 antigen).

Fever cases with suspected dengue-like illness are tested in our referral Virology Laboratory all the year round. Table 2 reveals that only 11.5% of the fever cases were due to dengue fever in the year 2010. But in 2011 and 2012, the numbers escalated steeply to more than double.

Figure 3 shows the month wise distribution of total dengue cases in 3 consecutive years 2010, 2011, and 2012. The number of positive dengue cases in 2010 and 2011 was comparatively lower than that of 2012. However, in all the three years the highest number of cases were recorded during the monsoon and postmonsoon periods. The number of affected cases declined with the onset of winter.

In an attempt to evaluate the performance of the newly introduced dengue NS1 antigen detection kit, 62 cases clinically suggestive of dengue fever and who reported early just after the onset of fever were followed up to a period of 14 days (Table 3). 38 of the fever cases were dengue NS1-positive

Table 1: District wise distribution of dengue confirmed cases in West Bengal in 2012.

Districts	Dengue-positive cases
Kolkata	382
Howrah	19
Hooghly	19
Birbhum	1
Malda	1
S 24 pgs	24
N 24 pgs	38
Murshidabad	1
Nadia	5
East Midnapore	8
West Midnapore	5
Bankura	5
Burdwan	6

Table 2: Dengue confirmed cases in each year from 2010 to 2012.

Year	Total fever cases	Total dengue cases
2010	763	88 (11.5%)
2011	1343	328 (24.4%)
2012	1518	514 (33.86%)

Table 3: Comparison of the results between dengue MAC ELISA kits and dengue NS1 antigen kits in 62 fever cases.

Total dengue follow-up cases: $n = 62$			
Sample tested on ≤4 days fever		Sample tested on ≥7 days fever	
NS1 positive	38	IgM positive	24
		IgM negative	14
NS1 negative	24	IgM positive	6
		IgM negative	18

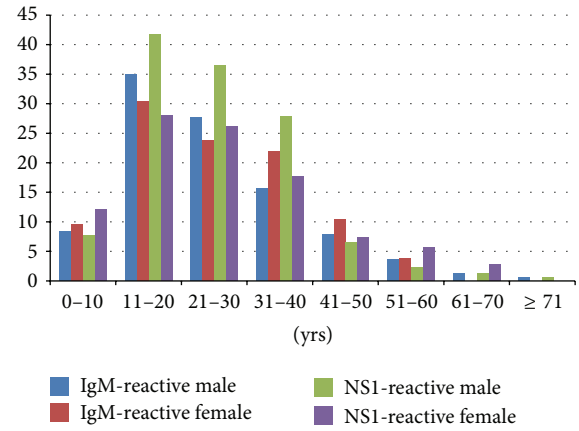

Figure 1: Age and sex gradation according to IgM and NS1% positivity.

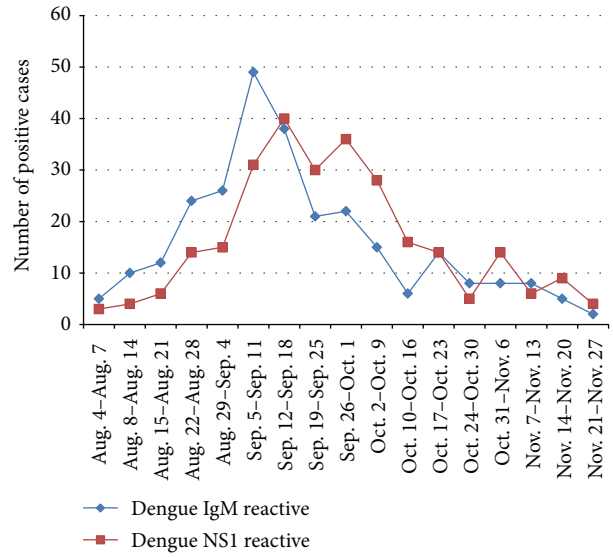

Figure 2: Week wise distribution of the NS1- and IgM-positive dengue cases—Aug. to Nov. 2012.

and 24 were negative. However, on follow-up testing, for the presence of dengue IgM antibodies (7 days or after), 24 out of 38 dengue NS1-positive cases were also found to be positive for dengue IgM Antibodies. Dengue IgM negativity among the 14 dengue NS1 antigen-positive cases could be accounted for either a false positive dengue NS1 antigen test or the cases which were of secondary dengue fever where the IgM antibody was below the detection limit. Among the 24 dengue NS1 antigen-negative cases, 6 cases showed the presence of dengue IgM antibodies on subsequent followup. These findings warrant to undertake a study with a bigger sample size and the serological findings corelated with dengue PCR and dengue IgG antibody test. It is also important to identify any cross-reactivity of Den NS1 antigen with other causes of fever during the same season. However, NS1 antigen positivity should be correlated clinically for accurate diagnosis.

An attempt was also made to identify the circulating serotypes of dengue virus in the city of Kolkata. Six, acute serum samples (duration of fever < than 4 days), collected immediately after the onset of the dengue fever outbreak, were transferred to the referral Virology Laboratory of Maulana Azad Medical College, New Delhi, maintaining cold chain. The identified serotypes were Den1 in 3 samples (two female patients, 16 and 46 years of age, and one male patient of age 13 years), Den3 in 2 samples (two male patients of age 18 and 15 years), and Den4 in one sample from an 18-year female patient. All the patients were from Kolkata district. Clinical recovery was observed in all the patients affected with Den1 serotype. Another patient with Den3 serotype also had uneventful recovery. A 15-year-old male patient (affected with Den3 serotype) and an 18-year-old female patient affected with Den4 serotype did not turn up for followup. However, no adverse report was obtained from these two patients.

4. Discussion

Dengue is emerging as a major public health problem in India. It is one of the major public health threats in Kolkata

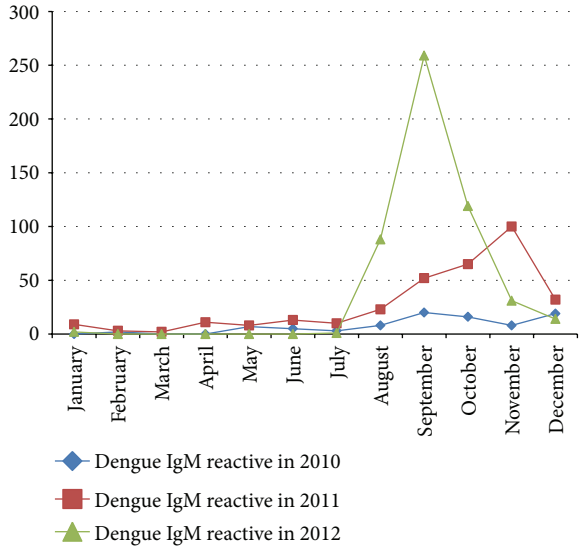

Figure 3: Monthly distribution of the positive dengue cases in 2010, 2011, and 2012.

[17]. Since the first epidemic in Kolkata during 1963-64, many places in India have been experiencing dengue infection [6–13]. India witnessed widespread dengue fever outbreak in the year 2012. Tamil Nadu reported the highest number of cases in the country being 9,249, followed by West Bengal which reported 6,067 cases. The other states which also reported increased number of dengue cases were Maharashtra, Kerala, Karnataka, Odisha, Delhi, Gujarat, Puducherry, Haryana, and Punjab [18].

The 2012 dengue fever outbreak in West Bengal was widespread in nature. Although majority of the cases (74.3%) belonged to Kolkata, 12 other districts of West Bengal were also involved. This corroborates with the findings of Hati [14], Taraphdar et al. [19], and Sarkar et al. [20] that dengue is gradually spreading to the rural areas of West Bengal.

The highest numbers of cases were recorded in the age group 11–30 yrs and males clearly outnumbered the females. Gupta et al. [2] and Chakravarti and Kumaria [21] also reported maximum cases in the age group 21–30 years with male preponderance. Sarkar et al. [17], however, reported maximum cases in the age group 0–10 years with female preponderance.

The majority of the cases were reported during the monsoon and postmonsoon seasons, in accordance with the reported patterns of dengue transmission [22].

According to published reports, all four serotypes of the dengue virus are cocirculating in India [23]. There was evidence of cocirculation of multiple serotypes (Den1, Den3, and Den4) in the city of Kolkata. The identified serotypes were Den1 in 3 samples, Den3 in 2 samples, and Den4 in one sample. Den2 serotype was not found in samples collected during the early part of the outbreak. Den3 outbreaks were also previously reported in Kolkata in 1983 [13] and in 2005 [3]. Sarkar et al., however, reports the cocirculation of all the four dengue serotypes with predominance of Den2 in 2010 [17]. These findings indicate that Kolkata is a hyperendemic zone for dengue virus. Attention is therefore required for effective vector control measures, as the distribution of stray cases throughout the year points to the perennial transmission of dengue virus. As, during epidemic and nonepidemic years, dengue infections are mostly seen in postmonsoon season, hence preventive measures should be in full swing at the very onset of the monsoons.

Conflict of Interests

All the authors declare that there is no financial conflict of interests.

Acknowledgments

The authors express their heartfelt gratitude and sincere thanks to the Director of School of Tropical Medicine and officials of Public Health and Communicable Disease Department of Government of West Bengal for their active support and cooperation throughout the study period. The authors specially extend their gratitude to the staff of Virology Unit and all participants in our study.

References

[1] D. J. Gubler, "Dengue and dengue hemorrhagic fever," *Clinical Microbiology Reviews*, vol. 11, no. 3, pp. 480–496, 1998.

[2] E. Gupta, L. Dar, G. Kapoor, and S. Broor, "The changing epidemiology of dengue in Delhi, India," *Virology Journal*, vol. 3, article 92, 2006.

[3] A. K. Hati, "Studies on dengue and dengue haemorrhagic fever(DHF) in West Bengal State, India," *Journal of Communicable Diseases*, vol. 38, no. 2, pp. 124–129, 2006.

[4] D. W. Vaughn, S. Green, S. Kalayanarooj et al., "Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity," *Journal of Infectious Diseases*, vol. 181, no. 1, pp. 2–9, 2000.

[5] H. H. Scott, "Dengue," in *A History of Tropical MedicIne*, vol. 11, pp. 808–819, Edward Arnold & Co, London, UK, 1937-1938.

[6] B. K. Aikat, N. R. Konar, and G. Banerjee, "Haemorrhagic fever in Calcutta area," *The Indian Journal of Medical Research*, vol. 52, pp. 660–675, 1964.

[7] J. K. Sarkar, S. K. Chakravarty, and R. K. Sarkar, "Sporadic cases of haemorrhage and/or shock during dengue epidemics," *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 66, no. 6, pp. 875–877, 1972.

[8] S. Balaya, S. D. Paul, L. V. D'Lima, and K. M. Pavri, "Investigations on an outbreak of dengue in Delhi in 1967," *The Indian Journal of Medical Research*, vol. 57, no. 4, pp. 767–774, 1969.

[9] F. M. Rodrigues, M. R. Patankar, K. Banerjee et al., "Etiology of the 1965 epidemic of febrile illness in Nagpur city, Maharashtra State, India," *Bulletin of the World Health Organization*, vol. 46, no. 2, pp. 173–179, 1972.

[10] V. S. Padbidri, C. N. Dandawate, and M. K. Goverdhan, "An investigation of the aetiology of the 1971 outbreak of febrile illness in Jaipur city, India," *The Indian Journal of Medical Research*, vol. 61, no. 12, pp. 1737–1743, 1973.

[11] P. V. Karamchandani, "Study of 100 cases of dengue fever in Madras Penitentiary," *Indian Medical Gazette*, vol. 72, pp. 532–534, 1973.

[12] J. K. Sarkar, S. N. Chatterjee, and S. K. Chakravarty, "Three-year study of mosquito-borne haemorrhagic fever in Calcutta," *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 61, no. 5, pp. 725–735, 1967.

[13] K. K. Mukherjee, S. K. Chakravarti, P. N. Dey, S. Dey, and M. S. Chakraborty, "Outbreak of febrile illness due to dengue virus type 3 in Calcutta during 1983," *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 81, no. 6, pp. 1008–1010, 1987.

[14] A. K. Hati, "Dengue serosurveillance in Kolkata, facing an epidemic in West Bengal, India," *Journal of Vector Borne Diseases*, vol. 46, no. 3, pp. 197–204, 2009.

[15] D. Cecilia, M. B. Kakade, A. B. Bhagat et al., "Detection of dengue-4 virus in Pune, Western India after an absence of 30 years—its association with two severe cases," *Virology Journal*, vol. 8, article 46, 2011.

[16] R. S. Lanciotti, C. H. Calisher, D. J. Gubler, G. J. Chang, and A. V. Vorndam, "Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction," *Journal of Clinical Microbiology*, vol. 30, no. 3, pp. 545–551, 1992.

[17] A. Sarkar, D. Taraphdar, and S. Chatterjee, "Molecular typing of dengue virus circulating in Kolkata, India in 2010," *Journal of Tropical Medicine*, vol. 2012, Article ID 960329, 5 pages, 2012.

[18] V. Varshney, "Dengue cases in 2012 highest in four years," Down to Earth, 2012, http://www.downtoearth.org.in/content/dengue-cases-2012-highest-four-years.

[19] D. Taraphdar, A. Sarkar, M. K. Bhattacharya, and S. Chatterjee, "Sero diagnosis of dengue activity in an unknown febrile outbreak at the Siliguri Town, District Darjeeling, West Bengal," *Asian Pacific Journal of Tropical Medicine*, vol. 3, no. 5, pp. 364–366, 2010.

[20] A. Sarkar, D. Taraphdar, and S. Chatterjee, "Investigations of recurrent outbreaks of unknown fever, establish rural dengue activity in West Midnapore, a costal district in West Bengal, India," *Archives of Clinical Microbiology*, vol. 1, no. 4, 2010.

[21] A. Chakravarti and R. Kumaria, "Eco-epidemiological analysis of dengue infection during an outbreak of dengue fever, India," *Virology Journal*, vol. 2, article 32, 2005.

[22] P. Reiter, "Climate change and mosquito—borne disease," *Environmental Health Perspectives*, vol. 109, supplement 1, pp. 141–161, 2001.

[23] N. Gupta, S. Srivastava, A. Jain, and U. C. Chaturvedi, "Dengue in India," *The Indian Journal of Medical Research*, vol. 136, no. 3, pp. 373–390, 2012.