

Co-Circulation of Dengue Virus Serotypes in Delhi, India, 2005: Implication for Increased DHF/DSS

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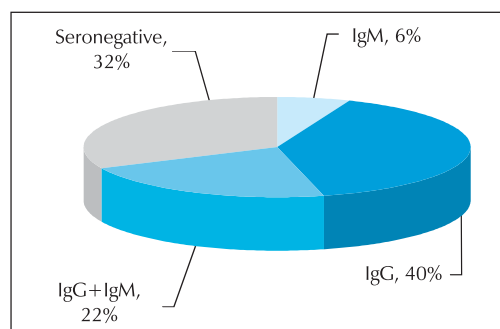
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Dengue is now re-emerging as one of the most important public health problems in India. Outbreak of dengue during the post-monsoon period is a regular feature and major outbreaks have been reported from most parts of country, including Delhi, since 1996.^[1] We have been associated with investigation of various dengue epidemics in Delhi^[2] and adjoining areas in north India since 1996. We have been following the trends of dengue epidemic in Delhi since then with regard to the prevalence of serotypes and genotypes of the circulating viruses through molecular epidemiological studies employing virus isolation, genome amplification, sequencing of the amplicon and phylogeny.^[3,4,5]

We report here the co-circulation of DENV serotypes from the samples collected from suspected dengue patients admitted to various hospitals of the Municipal Corporation, Delhi (MCD), during the post-monsoon period from September to November 2005. A total of 174 clinical samples were thoroughly investigated for the presence of virus-specific antibodies, genomic RNA by RT-PCR, serotyping by one-step single-tube multiplex PCR, virus isolation and genotyping. The serological analysis using the in-house NC membrane-based indirect dipstick ELISA employing purified cell culture cocktail antigen

kit revealed overall 68% seropositivity.^[6] The antibody profile revealed 6% IgM, 40% IgG and 22% IgM and IgG antibodies, indicating that most of the patients had carryover antibodies from past infections (Figure 1). Further analysis of these samples by RT-PCR for demonstration of viral genome indicated 12% (21/174) positivity, confirming the lower percentage of the incidence of dengue infection in 2005 (Figure 2). The serotyping of the RT-PCR-positive samples by multiplex PCR revealed the presence of mixed serotypes. A total of 15 samples were found positive for DENV-2, and 6 samples were found positive for DENV-3 virus.

Figure 1: Antibody profile of dengue suspected patients in Delhi during September to November 2005



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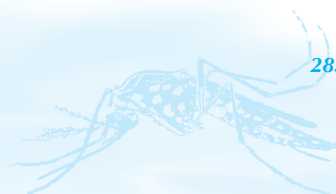
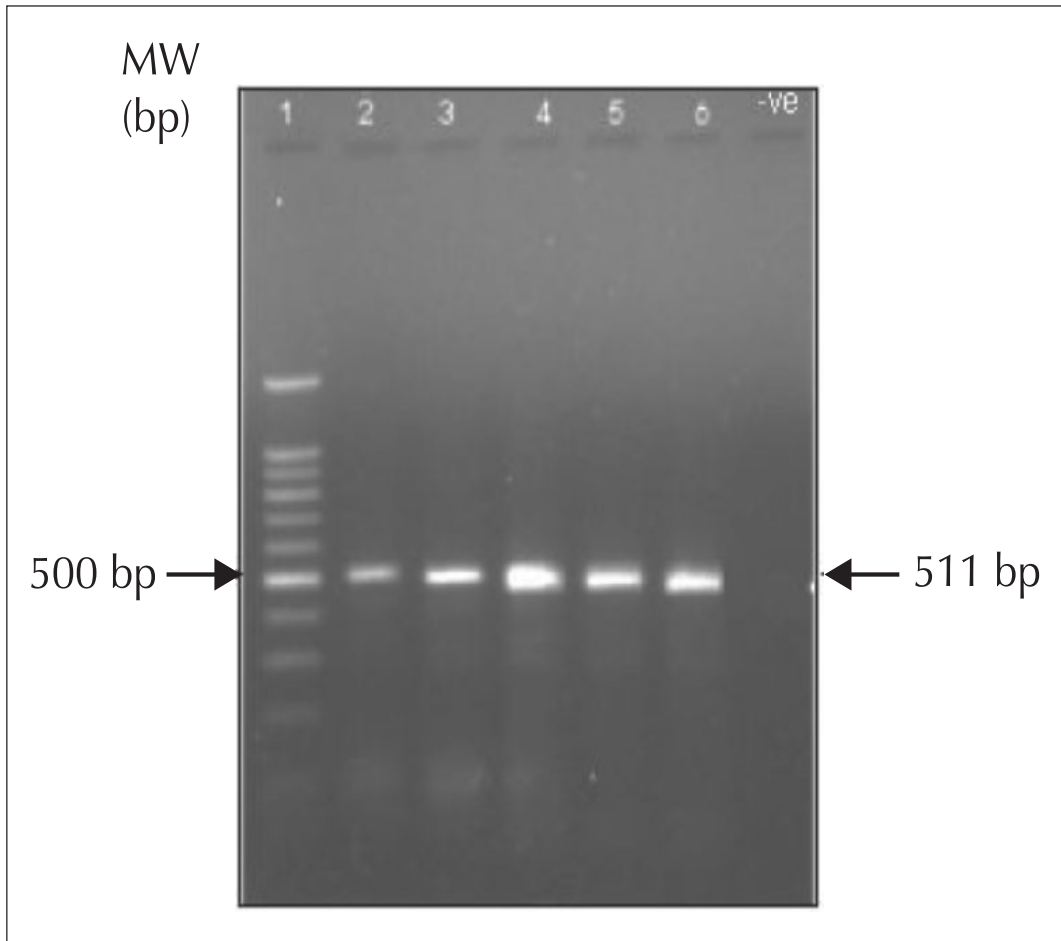


Figure 2: RT-PCR analysis of dengue suspected patient serum samples (lanes 2-5), showing the presence of dengue virus-specific 511 bp amplicon, lane 1: 100 bp DNA ladder (Promega, USA); lane 6: DENV-3-positive control; lane 7: showing absence of dengue-specific amplicon (negative control)



We made similar observations in Mumbai, where there was a sudden increase in the number of cases of dengue haemorrhagic fever following unprecedented floods during August–September 2005. A total of 111 serum samples from dengue-suspected patients were collected from various hospitals in Mumbai. The RT-PCR analysis employing dengue complex-specific consensus primers revealed

a total of 7 serum samples to be positive for the presence of dengue-specific amplicon (511 bp). Further serotyping by multiplex PCR with serotype-specific primers revealed 230 bp amplicon characteristic of DENV-2 in 6 samples and 390 bp amplicon characteristic of DENV-3 in 1 sample, which were later confirmed by nucleotide sequencing.

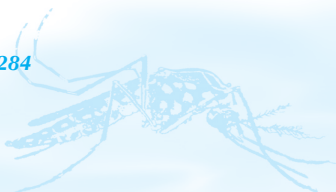


Figure 3: Dendrogram generated by neighbour-joining method showing the genotyping clustering of DENV-2 viruses. Representative strains of DENV-1, DENV-3 and DENV-4 were used to root the tree. Each strain is abbreviated with strain designation followed by first four letters of country of origin and last two digits of the year of isolation

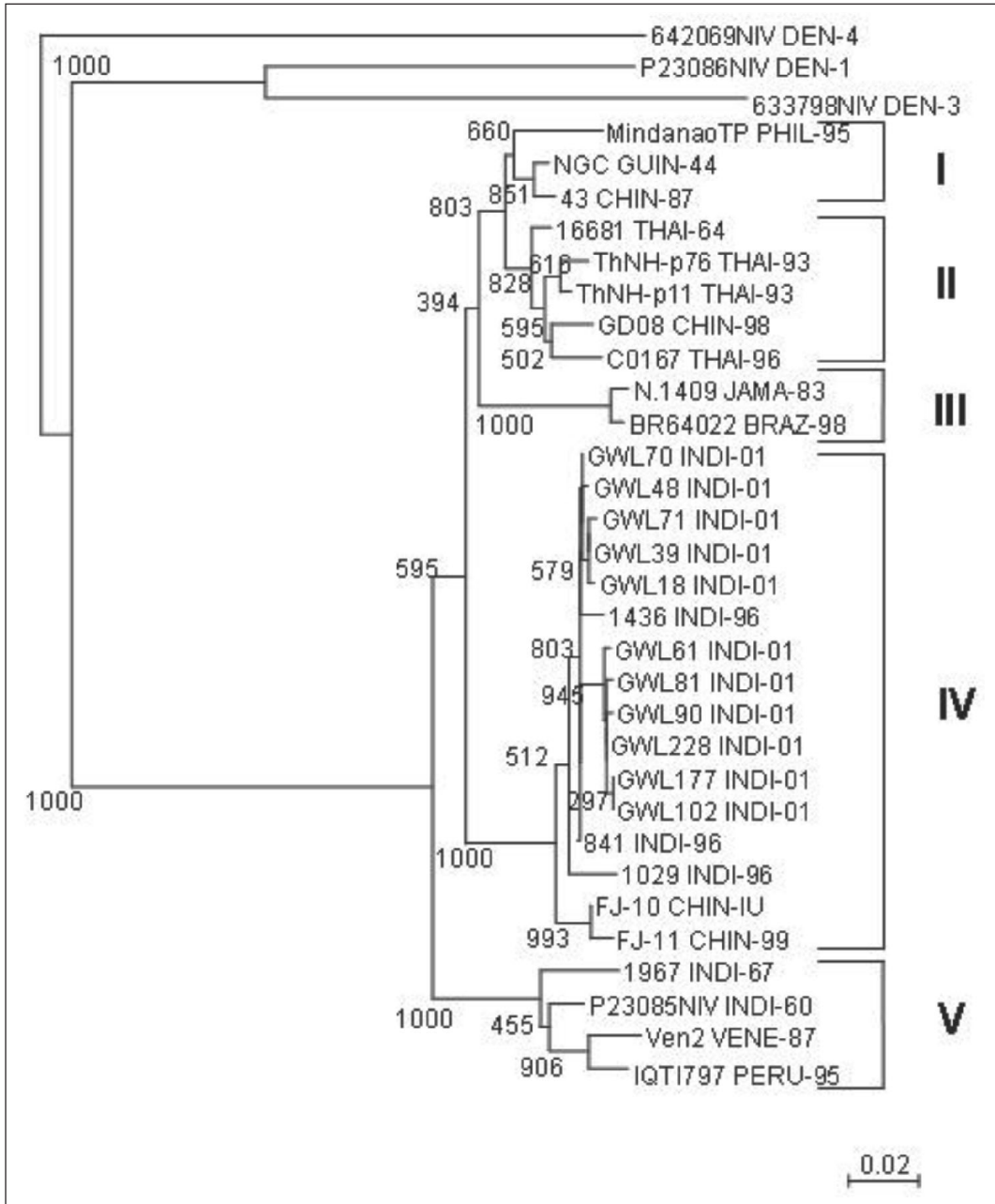
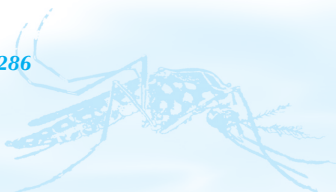
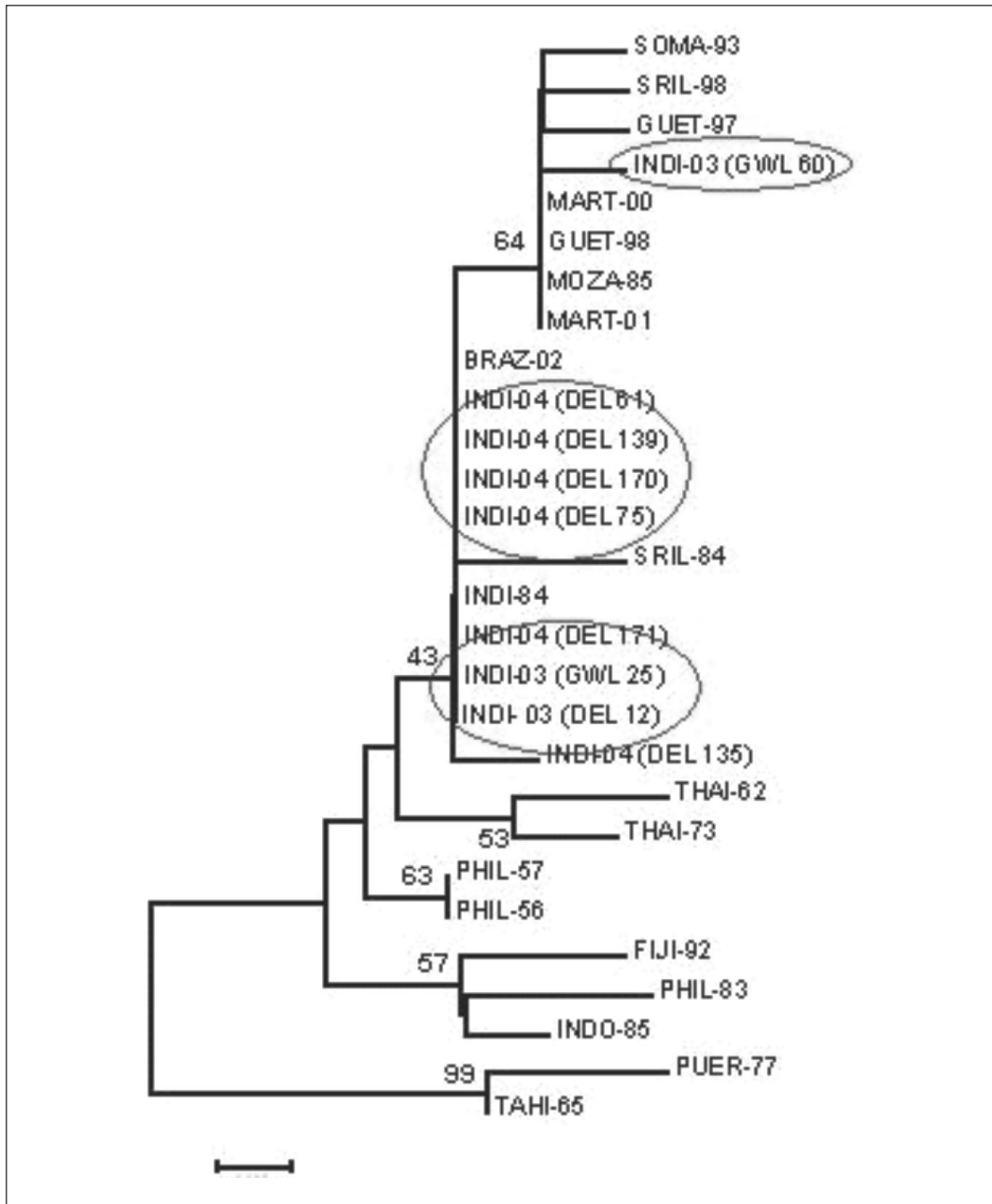


Figure 4: Dendrogram generated by neighbour-joining method showing the genotyping clustering of DENV-3 viruses. Each strain is abbreviated with strain designation followed by first four letters of country of origin and last two digits of the year of isolation



Further genotyping by nucleotide sequencing and phylogenetic analysis revealed that in the case of DENV-2 there was genotypic shift, wherein the previously circulating genotype V had been replaced by genotype IV. The DENV-3 serotype that has been predominant since 2003 still belongs to the genotype III, which is also circulating in neighbouring South-East Asian countries like Sri Lanka, Bangladesh, Viet Nam, Indonesia, etc. (Figures 3 and 4). The genotype IV of DENV-2^[4] and genotype III of DENV-3^[7] viruses are the most virulent among all genotypes, are cosmopolitan in nature and are responsible for fatal outbreaks of DHF/DSS in most parts of the tropics.

The last 10 years of the follow-up study of dengue epidemics has revealed the predominance of a single serotype. All the four serotypes of DENV (1, 2, 3 & 4) have been

isolated during previous dengue outbreaks in Delhi, but a particular type has always predominated. DENV-2 was predominant up to 2002 and was subsequently replaced by DENV-3 in 2003 and 2004.^[3-5] This trend of circulation of DENV-2 and DENV-3 in 2005 indicated that dengue virus has already established its endemicity in Delhi.

Conclusion

The co-circulation of more than one serotype of dengue will enhance the risk of more incidences of DHF/DSS through the proposed antibody-dependent enhancement (ADE) theory.^[8] This is a point of major concern for public health authorities, which calls for implementation of strict control measures to prevent future epidemics.

References

- [1] Gore MM. Need for constant monitoring of dengue infections. *Indian J Med Res* 2005 Jan;121(1):9-12.
- [2] Dar L, Broor S, Sengupta S, Xess I, Seth P. The first major outbreak of dengue hemorrhagic fever in Delhi, India. *Emerg Infect Dis* 1999 Jul-Aug;5(4):589-90.
- [3] Parida MM, Dash PK, Upadhyay C, Saxena P, Jana AM. Serological & virological investigation of an outbreak of dengue fever in Gwalior, India. *Indian J Med Res* 2002 Dec;116:248-54.
- [4] Dash PK, Parida MM, Saxena P, Kumar M, Rai A, Pasha ST, Jana AM. Emergence and continued circulation of dengue-2 (genotype IV) virus strains in northern India. *J Med Virol* 2004 Oct;74(2):314-22.
- [5] Dash PK, Saxena P, Abhyankar A, Bhargava R, Jana AM. Emergence of dengue virus type-3 in northern India. *Southeast Asian J Trop Med Public Health* 2005 Mar;36(2):370-7.
- [6] Parida MM, Upadhyay C, Saxena P, Dash PK, Jana AM, Seth P. Evaluation of a dipstick ELISA and a rapid immunochromatographic test for diagnosis of dengue virus infection. *Acta Virol* 2001;45(5-6):299-304.
- [7] Dash PK, Parida MM, Saxena P, Abhyankar A, Singh CP, Tewari KN, Jana AM, Sekhar K, Rao PV. Reemergence of dengue virus type-3 (subtype-III) in India: implications for increased incidence of DHF & DSS. *Virol J* 2006 Jul 6;3:55.
- [8] Halstead SB. Neutralization and antibody-dependent enhancement of dengue viruses. *Adv Virus Res* 2003;60:421-67.

