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Dengue virus serotype-3 (subtype-III) in Port Blair, India

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Epidemics of dengue fever (DF)/dengue hemorrhagic fever (DHF) have been occurring in the South East Asian Region (SEAR)[1] and are considered to be an emerging public health problem world over[2]. Dengue is an *Aedes* transmitted viral infection caused by any of the four dengue virus serotypes (DENV 1–4) which belong to the genus *Flavivirus*. This virus causes spectrum of manifestations ranging from DF to DHF and dengue shock syndrome (DSS), which is fatal and usually associated with secondary infections[1].

In India, DF, DHF and DSS have been reported from different parts of the country[3–4]. Port Blair is the capital of Andaman and Nicobar Islands, an archipelago of >500 islands and islets situated in the Bay of Bengal, at a distance of 1200 km from the peninsular India. People are constantly moving between mainland of India and these islands. Dengue was not reported from these islands[5]. However, patients with febrile DF, DHF and DSS were reported for the first time in 2009[4]. Subsequently, circulation of dengue serotypes 1 and 2 was established[6].

From mid-July 2013, medical and public health professionals observed an increase in the number of febrile cases with retro-orbital pain among the Wharf (a structure adjoining the harbour comprising a barrack housing the security personnel) staff of Haddo area adjoining the harbour in Port Blair. The Directorate of Health Services, Andaman and Nicobar Administration requested to investigate the cause for the sudden increase in number of febrile cases. In view of the clinical features suggestive of dengue and widespread prevalence and infestation of vector mosquitoes, dengue infection was suspected[7–8]. We report the findings of the investigations undertaken among the suspected cases (security personnel) residing in barrack adjoining the harbour area.

A case definition for suspected DF was made based on the symptoms/signs listed in WHO guidelines[9]. We used a more inclusive case definition that mandated presence of any one of the symptoms/signs such as sudden onset of fever, headache, retro-orbital pain, and myalgia for the diagnosis of probable case of dengue fever with an aim to increase the sensitivity.

A total of 23 blood samples were collected from the suspected patients after obtaining informed consent. All the samples were tested for the presence of anti-DENV, anti-chikungunya virus (CHIKV) IgM antibodies by IgM capture ELISA kits developed by National Institute of Virology (NIV), Pune[10], and anti-leptospiral antibodies by in-house developed microscopic agglutination test (MAT) with a panel of known endemic antigens[11]. All samples were also assessed for IgG antibodies by using IgG capture ELISA (PanBio, France).

RNA was extracted from all the serum samples using Qiagen viral RNA extraction kit (Qiagen, USA) following manufacturer's instructions. RT-PCR analysis was carried out for the presence of DENV and CHIKV RNA

Table 1. IgM, IgG and RT-PCR results of the suspected and confirmed patients

S.No.	Lab. ID	Age/Sex	IgM ELISA	IgG ELISA	RT-PCR
1.	HDW-01	40/M	Positive	Positive	Negative
2.	HDW-02	35/M	Negative	Negative	Negative
3.	HDW-03	55/M	Negative	Negative	Negative
4.	HDW-04	39/M	Negative	Negative	Positive
5.	HDW-05	42/M	Negative	Negative	Positive
6.	HDW-06	36/M	Negative	Negative	Positive
7.	HDW-07	25/M	Negative	Positive	Positive
8.	HDW-08	25/M	Negative	Negative	Positive
9.	HDW-09	24/M	Negative	Negative	Positive
10.	HDW-10	41/M	Negative	Negative	Positive
11.	HDW-11	31/M	Positive	Positive	Negative
12.	HDW-12	35/M	Negative	Negative	Positive
13.	HDW-13	40/M	Negative	Negative	Negative
14.	HDW-14	25/M	Positive	Positive	Negative
15.	HDW-15	42/M	Negative	Positive	Negative
16.	HDW-16	50/M	Negative	Negative	Negative
17.	HDW-17	24/M	Negative	Negative	Negative
18.	HDW-18	25/M	Negative	Negative	Negative
19.	HDW-19	53/M	Negative	Negative	Positive
20.	HDW-20	24/M	Negative	Positive	Negative
21.	HDW-21	44/M	Negative	Negative	Positive
22.	HDW-22	24/M	Negative	Positive	Negative
23.	HDW-23	49/M	Negative	Negative	Negative

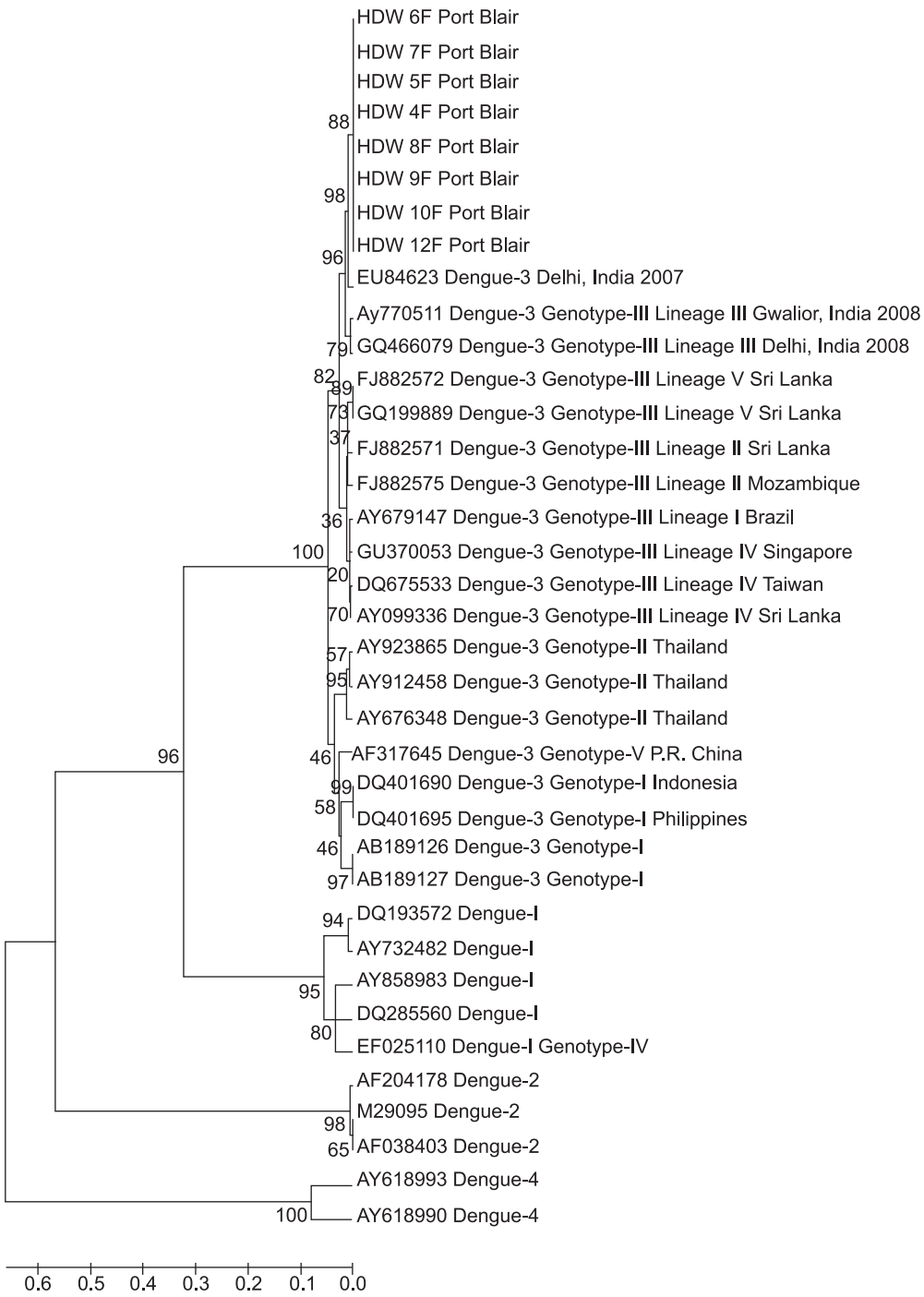

Fig. 1: Phylogenetic neighbour-joining tree showing eight Port Blair DENV sequences (HDW6F, HDW7F, HDW5F, HDW4F, HDW8F, HDW9F, HDW10F and HDW12F) that have been grouped with DENV serotype-3 and genotype-III.

in all the samples by adopting standard protocol[12–13]. Further, PCR product was purified and subjected to DNA sequencing analysis. RT-PCR positive samples were further subjected for virus isolation using Vero-E6 cell lines[14].

Of the 23 serum samples, 3 (13%) were found positive for DENV specific IgM antibodies. None of the samples were positive for CHIKV IgM and leptospiral antibodies. Of the 23 samples, 7 (30.4%) were positive for IgG antibodies against dengue virus, 12 (52.1%) samples were positive by RT-PCR analysis, amplified PCR products with the size of 511 bp (Table 1). These findings indicate that the aetiology of the infection was due to DENV. No severe cases of DHF/DSS were observed during the investigations. Patients confirmed to be IgM positives and RT-PCR positive, met the criteria

of dengue fever case definition. The nucleotide sequencing of the 511 bp amplicon confirmed that the virus sequence was homologous with DENV-3. The sequences were submitted to the NCBI (Accession numbers KF915030, KF915031, KF915032, KF915033, KF915034, KF915035, KF915036 and KF915037). All samples were negative for CHIKV RT-PCR analysis. Four RT-PCR positive samples were subjected to culture, for virus isolation but it was not successful.

Further, analyses were carried out by using maximum composite likelihood model[15] and MEGA5[16]. The mean pairwise genetic distance (K2P) between the nucleotide sequences of dengue virus from Port Blair was zero percent. The genetic distance between the dengue virus reported in Delhi (EU846234; 2007) was K2P=0.015%. The mean genetic distance between dengue reported during the current investigation in Port Blair and serotype dengue-3 from worldwide was smaller (0.070%) than that of dengue-1 (0.642%), dengue-2 (1.138%) or dengue-4 (1.356%) serotypes. The neighbour joining phylogenetic tree analysis indicated that the dengue virus associated with the sudden increase in febrile cases clustered with genotype-III and the lineage III (Fig. 1).

Dengue outbreaks with different serotypes have been reported in the mainland, India and also from Andaman Nicobar Islands[3, 6]. Emergence of dengue serotype-3 in north and south India has been reported in the past[17–18]. Earlier studies showed the existence of dengue serotype 1 and 2 in these islands[6]. The investigations from the present study indicate circulation of third serotype of dengue virus in the urban Port Blair. The current findings are in consonance with the investigations reported elsewhere[18]. Thus, it is evident that three serotypes are now in circulation within the urban agglomeration of Port Blair[6]. At this juncture, it is pertinent to mention that as per the antibody-dependent enhancement hypothesis, secondary DENV infections are risk factors for DHF/DSS[19]. Therefore, existence and circulation of multiple serotypes in these islands could precipitate the frequency of occurrences with DSS/DHF. The detection of dengue-3 serotype could be due to introduction of a new serotype; else it could be due to coincidental observation of the circulating serotype. The current scenario warrants constant vigil to monitor the situation coupled with a robust surveillance component integrated with meteorological information and management of *Aedes* spp breeding sites could prevent outbreaks in the future.

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