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SHORT REPORT
Dengue: a newly emerging viral infection in Andaman and Nicobar Islands, India

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SUMMARY

Prior to 2009 dengue fever had not been reported in the Andaman and Nicobar archipelago. In 2009, a few patients with dengue fever-like illness were reported, some of whom tested positive for dengue antibodies. In 2010, 516 suspected cases were reported, including some with dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS); 80 (15·5%) were positive for dengue antibodies. DENV RNA was detected in five patients and PCR-based typing showed that three of these belonged to serotype 1 and two to serotype 2. This was confirmed by sequence typing. Two clones of dengue virus, one belonging to serotype 1 and the other to serotype 2 appeared to be circulating in Andaman. Emergence of severe diseases such as DHF and DSS might be due to recent introduction of a more virulent strain or because of the enhancing effect of sub-neutralizing levels of antibodies developed due to prior infections. There is a need to revise the vector-borne disease surveillance system in the islands.

Key words: Andaman and Nicobar Islands, dengue fever, dengue serotypes.

Dengue has emerged as a global health problem, as evidenced by a series of epidemics throughout the tropical and subtropical regions of the world [1]. Dengue fever (DF) and dengue haemorrhagic fever (DHF) are the most important arboviral diseases in terms of both morbidity and mortality. Dengue virus (DENV) belongs to the genus *Flavivirus*, family Flaviviridae, with four serologically related but antigenically distinctive serotypes (DENV-1, -2, -3, -4) [2]. These serotypes induce a spectrum of illness, ranging from uncomplicated febrile illness to severe and fatal syndromes such as DHF and dengue shock syndrome (DSS) [3].

Andaman and Nicobar Islands is an archipelago of more than 500 islands and islets, stretching over 700 km from north to south, in the Bay of Bengal. There are 38 inhabited islands with a population of about 380 000 [4]. Prior to 2009 dengue had not been reported in Andaman and Nicobar archipelago [5], although antibodies against dengue virus (DENV-2) have been detected in the population [6]. In 2009, a few clinical suspected cases were reported by the health services, some of which tested positive for IgM anti-DENV antibodies. An entomological survey in

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1999 showed widespread infestation of *Aedes* mosquitoes in Port Blair [7]. A large outbreak of another *Aedes* mosquito-borne disease, chikungunya fever, occurred in South Andaman in 2006 [5].

During June–August 2010, a number of patients with DF-like febrile illness attended G. B. Pant Hospital, the only referral hospital in the Islands, and other healthcare facilities in Port Blair town situated in South Andaman Island. Investigations were conducted to identify and characterize the aetiological agent. A case definition for suspected DF was made based on the symptoms/signs listed in World Health Organization's guidelines [8]. According to the case definition, any patient with acute febrile illness associated with headache, retro-orbital pain, rash, muscle pain or pain in the joints was considered as a suspected case of DF. Although the WHO case definition mandates the presence of at least two from a list of symptoms/signs including the above-mentioned ones, as well as haemorrhagic manifestations and leucopenia for a diagnosis of a probable case, we used a more inclusive case definition that mandated presence of any one of the symptoms/signs with the objective of increasing the sensitivity. All patients who attended G. B. Pant Hospital and a childcare hospital in Port Blair and fulfilled the case definition were included in the study. Blood specimens were collected from these patients on the day of reporting to the hospital.

The study was approved by the institutional ethics committee of the Regional Medical Research Centre (RMRC).

All the samples were tested for the presence of anti-DENV and anti-chikungunya virus (CHIKV) IgM antibodies by IgM capture ELISA kits developed by the National Institute of Virology (NIV), Pune [9]. A subsample of the patients who reported to the hospital within 4 days of onset of symptoms and were negative for IgM anti-DENV antibodies was also tested for DENV RNA by reverse transcription – polymerase chain reaction (RT–PCR) followed by nested PCR for detection of serotype. RNA was extracted from the serum samples using Qiagen Viral RNA Extraction kit (Qiagen, USA) and RT–PCR was conducted following the standard protocol [10]. The first-round PCR targeted a 511-bp fragment covering the capsid-protein C and pre-M regions of the viral genome followed by nested multiplex PCR to differentiate between the serotypes of DENV as the size of the amplified product was specific to each of the four serotypes of DENV.

The 511-bp fragments amplified in the first-stage PCR were purified with QIAquick PCR purification kit (Qiagen) and sequenced using the Big Dye Terminator Cycle Sequencing kit (Applied Biosystems, USA) according to the manufacturer's instructions. All DNA sequences were assembled using SeqMan II version 5.03 (DNASTAR, USA). These sequences were then aligned with previously published sequences of DENV strains belonging to various combinations of serotypes and genotypes by the Clustal W multiple alignment and pair-wise alignment program of MEGA software suit (version 4). Genetic distances were estimated using Kimura's two-parameter algorithm and a phylogenetic tree was constructed by the neighbour-joining method [11]. The statistical significance of the relationships obtained was estimated by bootstrap resampling analysis (1000 repetitions). In addition, the aligned DNA sequences were BLAST-searched to assess their identity with previously characterized sequences of the DENV genome.

During the period between March and December 2010, a total of 516 patients fulfilling the case definition criteria attended the two hospitals. Blood samples of all these patients were tested by DENV IgM capture ELISA and 80 (15·5%) were positive. Distribution of cases by month of reporting showed a large peak in July–August, when 321 (62·2%) of the 516 suspected cases and 60 (75%) of the 80 confirmed cases occurred. None of the samples was positive for CHIKV IgM antibodies. Of the 80 confirmed cases, 26 (32·5%) were aged <5 years and 48 (60·0%) were aged <15 years. Although suspected patients were diagnosed at the hospitals at Port Blair, some were referred from other islands such as Middle Andaman ($n=5$), Little Andaman ($n=1$) and Car Nicobar ($n=2$). None of the confirmed cases had a history of travel outside the islands.

One hundred and nine samples negative by IgM capture ELISA were processed by RT–PCR and five (4·6%) showed amplification of the 511-bp fragment common to all serotypes of DENV. All five of the samples were from patients residing in the main island of South Andaman. Three of these also showed amplification of the 482-bp fragment specific to DENV-1 and the remaining two showed amplification of the 119-bp fragment specific to DENV-2, indicating that both DENV serotypes 1 and 2 exist in the islands. The NCBI BLAST search result showed that the sequences of the three strains that showed amplification of the 482-bp fragment specific to DENV-1 (DG/PB 18, DG/PB 06, DG/PB 15) had 96–98% homology with DENV serotype 1 sequences while the

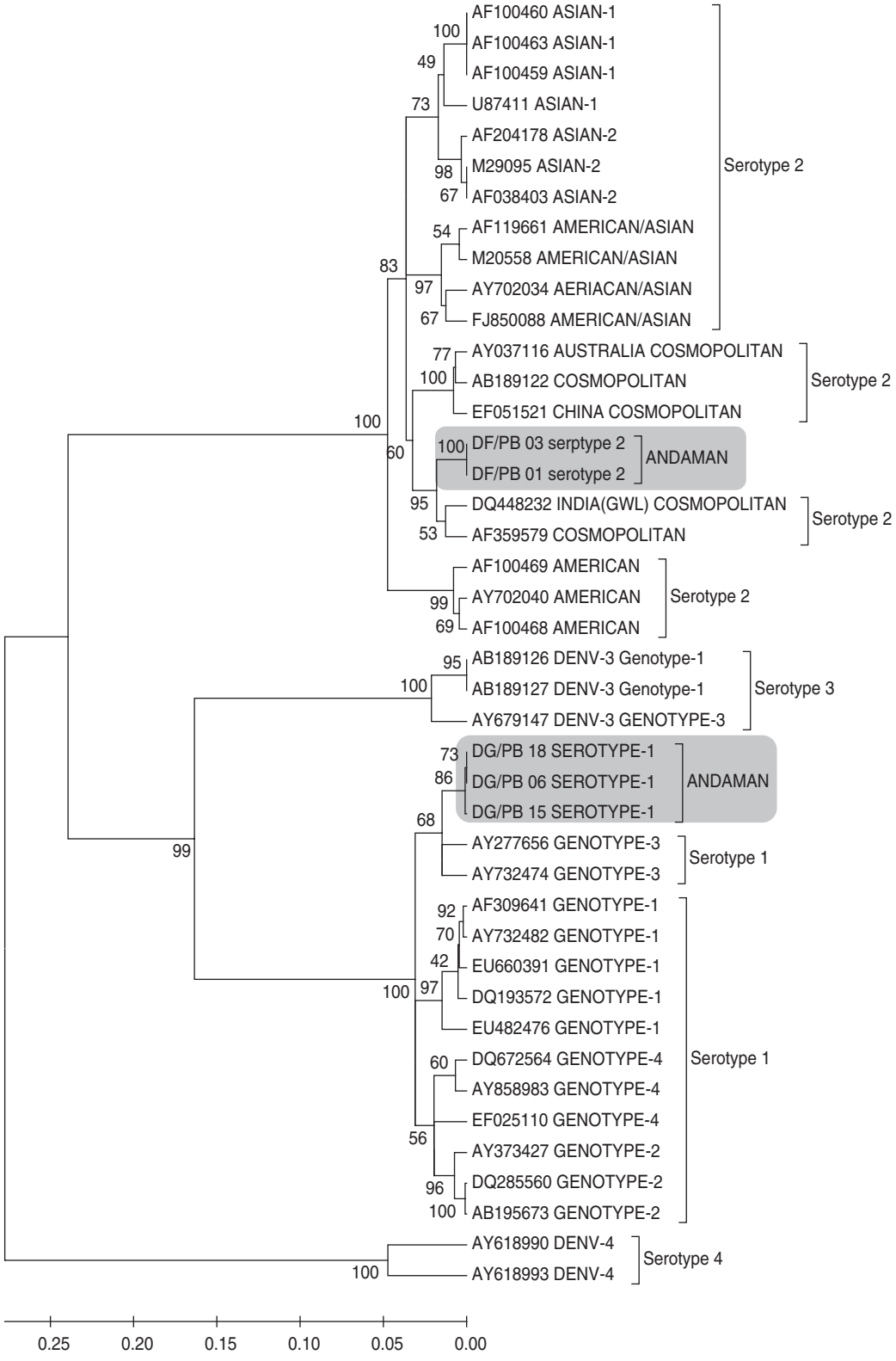

Fig. 1. Phylogenetic neighbour-joining tree showed three Andaman DENV sequences (DG/PB 18, DG/PB 06, DG/PB 15) have been grouped with DENV serotype 1 as well as genotype 3 and the other two sequences of Andaman DENV (DF/PB 03, DF/PB 01) grouped with DENV serotype 2 of genotype 1.

sequences of the two strains that showed amplification of the 119-bp fragment specific to DENV-2 (DF/PB 03, DF/PB 01) had 92–98% homology with the DENV serotype 2 sequences available in the database, thus further confirming the existence of dengue serotypes 1 and 2 in the islands.

In the phylogenetic tree (Fig. 1), our DENV-1 sequences (DG/PB 18, DG/PB 06, DG/PB 15) and DENV-2 sequences (DF/PB 03, DF/PB 01) formed subclusters under the DENV serotype 1 cluster and the DENV serotype 2 cluster, respectively. The mean genetic distances between Andaman DENV-1 and worldwide DENV-1 (0·035) as well as between Andaman DENV-2 strains and worldwide DENV-2 (0·069) were very small. The Andaman DENV-1 subcluster showed close relatedness to genotype 3 while the Andaman DENV-2 subcluster showed close relatedness to the cosmopolitan genotype.

Dengue occurrence in India has shown a substantial increase from previous years with more than 28 000 cases reported by the National Vector-Borne Disease Control Programme (NVBDCP). Outbreaks have been reported in many places including Delhi and Tamilnadu in July–August [12]. The present study conclusively shows the presence of DENV serotypes 1 and 2 in Andaman. Antibody-dependent enhancement (ADE) of infection has been postulated to cause severe and fatal complications in dengue viral infections such as DHF and DSS [13]. ADE occurs when a patient who was previously infected with one serotype of the virus and has sub-neutralizing levels of antibodies, becomes re-infected with a different serotype. The existence of multiple serotypes in the region, therefore, may pose a high risk of DHF and DSS.

Viral factors including structural differences [14] have also been implicated in the development of DHF and DSS [13]. The introduction of the South East Asian genotype of DENV-2 is believed to have caused epidemics of DHF in the Americas in the 1980s [15]. Intra-epidemic evolution of the circulating virus has been postulated to increase the severity of the disease as epidemics progress. Research so far appears to indicate that viral virulence factors and detrimental host responses are collectively responsible for the increased vascular permeability that occurs in DHF and DSS [16].

Serologically confirmed DF was first detected in the islands in 2009, although dengue antibodies were detected in an earlier population-based study [6]. In 2010 a few clinically suspected cases of DHF and DSS were reported. Dengue viral infection could have been occurring in Andaman Islands silently during the past few years as has been reported to have occurred in the South Pacific Islands [16]. There is a possibility that a new viral strain has been introduced to the islands recently and because of either a higher virulence of the strain or the enhancing effect of the sub-neutralizing levels of antibodies present in the population due to prior unnoticed infection in the past, or both, severe clinical forms are now starting to emerge.

The present study is a strong signal that DF is emerging as an important public health problem in these islands. *Aedes* mosquito infestation is widespread in the islands and there is a strong likelihood that outbreaks will emerge in the future. The existence of multiple serotypes of the virus in the islands increases the risk of DHF and DSS and therefore, future dengue outbreaks could lead to increased morbidity and mortality. The vector-borne diseases surveillance system in these islands needs to be revised and vector surveillance should be given top priority as vector-control measures are the only available preventive measures for dengue. The public health system including patient care facilities needs to be prepared to respond to any future dengue outbreak where a larger proportion of cases might develop severe complications such as DHF and DSS.

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DECLARATION OF INTEREST

None.

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