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ORIGINAL ARTICLE

Identification of Dengue Type 1 Virus (DENV-1) in Koreans Traveling Abroad

Young Eui Jeong, Yeon Hee Kim, Jung Eun Cho, Myung Guk Han, Young Ran Ju*

Division of Arboviruses, Korea National Institute of Health, Osong, Korea.

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Abstract
Objectives: To date, no indigenous dengue virus (DENV) transmissions have been reported in Korea. However, imported dengue infections have been diagnosed in travelers returning from endemic areas. This study presents the first virological evidence of travel-associated DENV importation into South Korea.
Methods: From January 2004 to June 2006, a total of 278 serum samples from 245 patients with suspected dengue fever were tested using the Panbio Dengue Duo IgM/IgG Rapid Strip Test. We selected 11 of the early symptomatic-phase sera that were negative for IgM and retrospectively studied them by virus isolation and reverse transcription-polymerase chain reaction.
Results: All 11 serum samples were found to be DENV positive by reverse transcription-polymerase chain reaction and viruses were successfully isolated from seven of the 11 serum samples. All the isolates were identified as DENV serotype-1.
Conclusion: We successfully isolated seven DENV serotype-1 strains for the first time in South Korea from imported infections. Considering that the vector mosquito, *Aedes albopictus*, already exists in South Korea, we propose that a vector surveillance program for dengue is urgently needed.

1. Introduction

Dengue virus (DENV) is a single-stranded, positive-sense RNA virus belonging to the genus *Flavivirus*, family *Flaviviridae* [1]. The four antigenically distinct serotypes (DENV-1, -2, -3, and -4) cause various forms of illness, ranging from inapparent infection or classic dengue fever (DF) to severe and life-threatening dengue hemorrhagic fever/dengue shock syndrome [2]. DENV infections are now endemic in more than 100 countries in tropical and subtropical regions, with an estimated 50 million infections annually [3,4].

With increasing international air travel, DENV infection is a potential risk for travelers to tropical areas where dengue is endemic or epidemic. As the numbers of imported cases grow, dengue is increasingly recognized as a serious public health problem in non-endemic countries [5—8]. In South Korea, the diagnosis of

*Corresponding author.
E-mail: juyran@nih.go.kr

dengue began in 2001 at the Division of Arboviruses, Korea National Institute of Health using a commercial immunochromatographic test kit. To date, no indigenous DENV transmissions have been reported in Korea. However, cases of imported dengue infection have been diagnosed in travelers returning from endemic or epidemic areas. Studies of the DENV isolated from travelers have provided useful information about the strains circulating in tropical regions, especially in countries that do not promptly analyze their domestic isolates, and have revealed the emergence of novel DENV strains and a genotype shift in countries [9,10].

Although a positive result using a commercial serologic kit is one of the indications of DENV infection, it should be confirmed using other diagnostic tools. For this reason, since July 2006, laboratory testing in Korea has been followed up using a reverse transcriptase-polymerase chain reaction (RT-PCR) and virus isolation. In this study, we isolated DENV serotype-1 (DENV-1) strains for the first time in Korea and analyzed the phylogenetic relationships of these DENV-1 isolates.

2. Materials and Methods

2.1. Serum samples

Serum samples from patients with dengue-like symptoms were forwarded to the Division of Arboviruses at Korea National Institute of Health for serologic diagnosis. Between January 2004 and June 2006, a total of 278 serum samples were collected. The specimens were tested using the Panbio Dengue Duo IgM/IgG Rapid Strip Test (PanBio, Queensland, Australia) [11,12]. Of these, 11 early symptomatic-phase sera that were negative in the serologic test (but in convalescent-phase serum were positive) were further tested by virus isolation and RT-PCR (Table 1).

2.2. Virus isolation

Invertebrate C6/36 cells (CRL 1660; American Type Culture Collection) were grown in 25 cm^2 tissue culture flasks at 30°C in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin. The cells were treated with 1 mL of each of the 11 sera diluted 1:10 with MEM containing 2% FBS and antibiotics. After adsorption for 2 hours, the cells were washed once with phosphate-buffered saline and cultured in maintenance medium at 30°C. The culture supernatants were collected seven days after infection, clarified by centrifugation, and stored at −70°C for analysis. The remaining cells were suspended in phosphate-buffered saline, spotted onto Teflon-coated slides, and tested for the presence of viruses before virus typing by immunofluorescence assay (IFA). In the IFA, the cells were stained with commercially available monoclonal antibodies (mAbs; Chemicon International, CA, USA) that were either reactive with all DENV serotypes or were specific for individual serotypes [13]. When viruses could not be typed with these mAbs, other mAbs (D2-1F1-3 for DENV-1, 3H5-1-21 for DENV-2, D6-8A1-12 for DENV-3, and 1H10-6-7 for DENV-4) donated by the Centers for Disease Control and Prevention (CDC, USA) were used instead [14]. Three more passages were performed if the IFA and RT-PCR were negative. Specimens that gave no positive result after the third passage were regarded as negative.

2.3. Multiplex RT-PCR

To type the DENV, multiplex RT-PCR was performed using the primer pairs reported by Harris et al [15]. Viral RNA was extracted from serum samples and culture supernatants using the QIAamp viral RNA mini kit (Qiagen, Hilden, Germany). The RNA was reverse transcribed and amplified using the Qiagen One Step RT-PCR kit and the Multiplex PCR kit (Qiagen). RNA

Table 1. Summary of the patient information

			Virus isolation[b]		
Patient code/yr	Travel history	Serotype by RT-PCR[a]	IFA	RT-PCR	Designation
32/2004	IU	2	−	−	
38/2004	IU	3	−	−	
47/2004	India, Singapore	1, 2	1	1	DenKor-01
09/2005	Indonesia	1	1	1	DenKor-02
10/2005	Indonesia	1	1	1	DenKor-03
12/2005	Indonesia	1	1	1	DenKor-04
35/2005	Thailand	1	1	1	DenKor-05
51/2005	Philippines	4	−	−	
108/2005	India	1	−	−	
115/2005	Thailand	1	1	1	DenKor-06
64/2006	Philippines	1	1	1	DenKor-07

[a]*Tested with original serum samples;* [b]*Virus isolation and typing.*

IU = information unavailable; the dash (−) = negative; RT-PCR = reverse transcriptase-polymerase chain reaction; IFA = immunofluorescence assay.