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Laboratory-based Dengue Surveillance in Taiwan, 2005: A Molecular Epidemiologic Study

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Abstract. We present the results of laboratory-based dengue surveillance in Taiwan for 2005. A phylogenetic study showed that multiple dengue epidemics were caused by three different imported dengue virus (DENV) strains. A strain of DENV-3 (genotype I) imported from the Philippines first appeared in the southern part of Kaohsiung City and later spread to Kaohsiung County from August to December, which resulted in 77 cases of dengue. Another strain of DENV-3 (genotype II) imported from Vietnam first appeared in the central part of Kaohsiung City and later spread to Kaohsiung County from September to December, which resulted in 35 cases of dengue. A strain of DENV-2 (American/Asian genotype) imported from Vietnam first appeared in Tainan City and later spread to Kaohsiung City/County from October to December, which resulted in 60 cases of dengue. This study provides molecular epidemiologic evidence that most dengue in Taiwan is caused by imported strains of the virus.

INTRODUCTION

Dengue viruses (DENVs) are mosquito-borne flaviviruses and are the most prevalent arboviruses in tropical and subtropical regions of Asia, the South Pacific, Africa, and Central and South America.[1] They produce a spectrum of illness ranging from inapparent infection to moderate febrile illness, dengue fever (DF), to severe and fatal hemorrhagic disease, dengue hemorrhagic fever (DHF), and to dengue shock syndrome (DSS).[2,3] It has been estimated that approximately 2.5 billion people live in areas at risk of dengue infection and it is expected that this number will increase because of the enlarging habitat of the competent dengue vectors *Aedes aegypti* and *Ae. albopictus*, growing numbers of susceptible human hosts, and increasing spread of DENV through rapid and frequent global travel. The dramatic global spread of epidemic DF/DHF has shifted many southeast Asian and Central and South American countries from non-endemic to hypoendemic to hyperendemic status in the past 60 years.[4,5]

There are four distinct DENV serotypes, designated DENV-1, DENV-2, DENV-3, and DENV-4. Infection induces a lifelong protective immunity to the homologous serotype, but only brief protection against heterologous serotypes.[6] All four DENV serotypes have been associated with DF and DHF. The exact mechanisms responsible for DHF/DSS are not fully understood, but it is widely accepted that secondary infection is the main risk factor.[7] Thus, the cocirculation of several different DENV serotypes in a geographic area favors DHF in that area. Other risk factors may include viral virulence,[8,9] the host genetic background,[10] and cross-reactive T cells.[11,12]

Global movement of DENV between different geographic areas has been regarded as an important factor leading to a dramatic increase in DF, and to the emergence of DHF as a significant public health problem in the Americas and Asia. Currently, all four DENV serotypes are endemic in both regions. In southeast Asia, activities associated with World War II were important factors in the increase of DF and DHF. Since the detection of DHF in Manila in 1953, there has been a dramatic increase in the number of DHF patients and the countries reporting this severe form of dengue.[4] In the Americas, the 1981 Cuban epidemic was the first documented DHF epidemic, which coincided with the introduction of the new genotype of DENV-2, the Southeast Asian genotype.[13] In addition, an epidemic of DF/DHF in the Americas was attributed to genotype III of DENV-3 that originated on the Indian sub-continent.[14]

Epidemics of dengue in Taiwan were documented in 1902, 1915, and 1922 in Penghu Islet, 1924 and 1927 in southern Taiwan, 1931 in Tainan, and island-wide in 1942–1943.[15] The virus was then silent in Taiwan for almost four decades until 1981, when the DENV-2 epidemic recurred on the islet of Liuchiu Township in Pingtung County and infected approximately 80% of the inhabitants.[15] This was followed by an over-winter outbreak of DENV-1 infection in 1987–1988 in Kaohsiung City/County and Pingtung County. This was the largest epidemic since World War II and was estimated to have involved more than 100,000 dengue cases.[16,17] Since that time, local outbreaks have been recorded almost every year in southern Taiwan. Table 1 summarizes major dengue outbreaks (more than 100 laboratory-confirmed cases) in Taiwan during the last three decades. The designations of DENV genotypes are based on the classification of Goncalvez and others,[18] Twiddy and others,[19] Lanciotti and others,[20] and Klunthong and others[21] for DENV-1, DENV-2, DENV-3, and DENV-4, respectively. Although Taiwan health authorities have required reports to distinguish between DF and DHF since 1994, relatively few cases of DHF have been reported. In this report, we present the results of laboratory-based dengue surveillance in Taiwan for 2005.

MATERIALS AND METHODS

Human serum samples. Dengue fever and DHF are category 2 reportable infectious diseases in Taiwan, and suspected cases must be reported within 24 hours of clinical diagnosis. Clinical diagnoses of DHF were made according to the criteria of the World Health Organization.[22] To provide effective surveillance, both passive (hospital-based reporting system) and active (such as fever screening at airports, self-reporting, expanded screening for contacts of confirmed cases, patients with fever of unknown origin, school-based

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TABLE 1
Major dengue outbreaks in Taiwan between 1981 and 2005*

Year(s)	Epidemic area	Serotype	Genotype	Confirmed cases	DHF cases
1981	Liuchiu Township, Pingtung County	DENV-2	Asian genotype 2	> 8,000	–
1987–1988	Kaohsiung City/County, Pingtung County	DENV-1	Genotype I	4,916	–
1991	Kaohsiung City/County	DENV-1	Genotype I	149	–
1994	Kaohsiung City/County	DENV-3	Genotype I	222	10
1995	Taipei County	DENV-1	Genotype I	329	5
1998	Tainan City	DENV-3	Genotype II	238	23
	Kaohsiung City/County	DENV-2	Asian genotype 1		
2000	Tainan City	DENV-4	Genotype II	113	1
2001–2002	Southern Taiwan	DENV-2	Cosmopolitan genotype	5,610	227
2004	Pingtung County	DENV-1	Genotype IV	336	4
2005	Kaohsiung City/County	DENV-3	Genotype I	202	3
	Kaohsiung City/County	DENV-3	Genotype II		
	Tainan City	DENV-2	American/Asian genotype		

* DENV = dengue virus; DHF = dengue hemorrhagic fever.

reporting) surveillance systems were established by central and local health departments in Taiwan. Human serum samples of suspected dengue cases were submitted to the Research and Diagnostic Center, Centers for Disease Control, Taiwan (Taiwan CDC), for confirmation of DENV infection. Two central dengue laboratories were set up for routine diagnosis, the Kun-Yang Laboratory in Taipei City in northern Taiwan and the Fifth Branch Laboratory in Kaohsiung City in southern Taiwan. Human serum samples used in this study were derived from confirmed dengue cases submitted to the Taiwan CDC in 2005. Serum samples collected 1–7 days after the onset of symptoms were referred to as acute-phase samples. Serum samples collected after day 7 were referred to as convalescent-phase samples.

Laboratory diagnosis. Infection with DENV was defined as a febrile illness associated with the detection of DENV-specific IgM and IgG antibodies, isolation of DENV, or detection of DENV RNA by reverse transcription–polymerase chain reaction (RT-PCR).[23] A one-step SYBR Green I real-time RT-PCR (QuantiTect SYBR Green RT-PCR kit; Qiagen, Hilden, Germany) was performed in the Mx4000™ quantitative PCR system (Stratagene, La Jolla, CA) to detect and differentiate DENV serotypes in acute-phase serum samples as described.[24] A real-time RT-PCR was performed using two sets of consensus primers, one primer set targeting a region of the nonstructural protein 5 (NS5) genes to detect all flaviviruses, and the other primer set targeting a region of the capsid (C) gene to detect all DENV serotypes. Positive samples were then confirmed by DENV serotyping using four sets of serotype-specific primers targeting the C gene to differentiate the DENV serotypes. For RT-PCR–positive cases, partial NS5 gene sequencing (153 nucleotides in length) was routinely performed using RT-PCR products of the one-step SYBR Green I real-time RT-PCR to determine the DENV serotypes and genotypes.

For the detection of DENV-specific IgM and IgG antibodies, envelope (E)/membrane (M)–specific capture IgM and IgG enzyme-linked immunosorbent assays were used to detect and differentiate primary and secondary DENV infections in acute-phase and convalescent-phase serum samples as described.[25] Isolation of DENV was performed using a mosquito cell line (clone C6/36 of *Ae. albopictus* cells). For each acute-phase serum sample, 50 μL of 1:50, 1:100, 1:200, 1:400 diluted serum sample (diluted in RPMI 1640 medium containing 1% fetal calf serum; Gibco/BRL Life Technologies, Gaithersburg, MD) was added to a 96-well microtiter plate, 10^5 cells/100 μL/well of C6/36 cells were added to the microtiter plate, and the plate was incubated for 7 days at 30°C. Cells were harvested and infection was confirmed by an immunofluorescence assay using dengue serotype-specific monoclonal antibodies. The viruses were passaged in C6/36 cells and harvested for nucleotide sequencing after the first or second passage. Viruses were identified using the nomenclature of serotype/country/strain/year of isolation.

Virus isolates selected for complete E gene sequencing. For phylogenetic analyses, nucleotide sequences of complete E genes of DENV isolates from all imported cases and representative indigenous cases were determined. Representative indigenous cases were selected based on their place of residence, date of onset of symptoms, and serotype of DENV recovered. From all imported dengue cases identified in Taiwan in 2005, 53 strains (11 DENV-1, 18 DENV-2, 15 DENV-3, and 9 DENV-4) were isolated. Analysis of the representative indigenous cases enabled isolation of 5 DENV-2 (American/Asian genotype), 18 DENV-3 (genotype I) and 7 DENV-3 (genotype II).

Preparation of viral RNA, RT-PCR amplification, and nucleotide sequencing. Viral RNA was extracted from either acute-phase serum samples or culture supernatant of C6/36 cell line infected with each of the isolated DENV strains using the QIAamp Viral RNA Mini kit (Qiagen). Primers used for RT-PCR and nucleotide sequencing in this study are shown in Table 2. Primers were designed to amplify and sequence C, premembrane (prM), and E gene sequences. Two sets of primers, D2-14F/D2-1572R and D2-1157F/D2-2610R, were used for RT-PCR to amplify DENV-2 sequences. Primer sets DN5UTRF/D3-1268R and D3-1186F/ D3-2621R were used for RT-PCR to amplify DENV-3 sequences. All primers were used for sequencing. The Titan one-tube RT-PCR system (Roche, Mannheim, Germany) was used for RT-PCR. After an initial denaturation at 90°C for 3 minutes, RT was carried out at 50°C for 45 minutes, followed by PCR at 94°C for 3 minutes, 35 cycles of 94°C for 20 seconds, 50°C for 30 seconds, and 68°C for 2 minutes with 5 seconds/cycle added to elongation step after the first 15 cycles, and a prolonged elongation at 68°C for 7 minutes. The PCR products were purified using the Qiagen QIA quick Gel Extraction kit (Qiagen). Nucleotide sequences were determined with the ABI Prism

TABLE 2
Primers used for RT-PCR and DNA sequencing of DENV-2 and DENV-3*

Primer	Sequence (5′ → 3′)	Genomic region
Primers used for RT-PCR and DNA sequencing of C-prM-E gene of DENV-2		
D2-14F	ACG TGG ACC GAC AAA GAC AGA TTC	5′UTR (13–36)†
D2-556F	GAC CTT GGT GAR TTG TGT GAA G	PrM (556–577)†
D2-642R	GCA CCA ACA ATC TAT GTC TTC	PrM (622–642)†
D2-1157F	GCC CAA CAC AAG GRG AAC CCA	E (1156–1177)†
D2-1232R	TGT CTA CCA TGG AGT GTT TGC AG	E (1209–1231)†
D2-1541F	ARA YAA AGC TTG GCT GGT GCA	E (1552–1562)†
D2-1572R	CAT TGC CTG TGC ACC AGC CAA GC	E (1559–1571)†
D2-1644R	TGT CTC TTT CTG TAT CCA ATT TGA	E (1621–1644)†
D2-2028F	AGT CAA CAT AGA AGC AGA ACC	E (2028–2048)†
D2-2162R	GCT CCY CTC ATT GTT GTC TC	E (2143–2162)†
D2-2610R	CAR TCT TGT TAC TGA GCG GA	NS1 (2591–2610)†
Primers used for RT-PCR and DNA sequencing of C-preM-E gene of DENV-3		
DN5UTRF	AGT TGT TAG TCT ACG TGG ACC G	5′UTR (1–22)‡
D3-541F	CAA CAT GTG CAC ACT CAT AGC	PrM (529–549)‡
D3-636R	CAG CAG TCA ATG TCT TCA GG	PrM (617–636)‡
D3-1186F	GGA GCA GGA CCA GAA CTA C	E (1185–1204)‡
D3-1268R	TCC CTT GCC AAA CAA ACC AC	E (1258–1267)‡
D3-1568F	TTT GAC CTA CCY CTA CCA TGG	E (1568–1588)‡
D3-1695R	TGC GAT CCA AGG ACT ACT ACT TCT TG	E (1669–1694)‡
D3-2036F	GAA CCT CCT TTT GGG GAA AG	E (2036–2055)‡
D3-2149R	TCT GGC AGT GGC CTC GAA C	E (2131–2149)‡
D3-2621R	GCT TCC ACA AGA GGT TCT CCA TTC	NS1 (2598–2621)‡

* RT-PCR = reverse transcription–polymerase chain reaction; DENV = dengue virus; C = capsid; prM = premembrane; E = envelope; UTR = untranslated region.
† Numbering from GenBank accession number AF038403.
‡ Numbering from GenBank accession number M93130.

automated DNA sequencing kit and the ABI Prism 3700 DNA sequencer (Applied Biosystems, Foster City, CA) according to the manufacturer's protocols. Overlapping nucleotide sequences were combined for analysis and edited with the Lasergene software package (DNASTAR Inc., Madison, WI). Nucleotide sequences of complete E genes of DENV strains described in this study were submitted to GenBank and their accession numbers (DQ518630–DQ518679 and EF540856) are shown in Figure 1.

Phylogenetic analysis. A total of 31 DENV-3 and 30 DENV-2 E gene sequences were used in this study for phylogenetic analyses (Figure 1). These data included 26 DENV-3 and 25 DENV-2 sequences of Taiwan isolates selected from representative strains of imported cases and indigenous cases from major local DENV-3 and DENV-2 outbreaks, and combined with 5 DENV-3 and 5 DENV-2 global reference sequences of different genotypes available in GenBank. The nucleotide sequences were aligned using Clustal W software.[26] Phylogenetic and molecular analyses were conducted using MEGA version 3.1[27] or PHYLIP version 3.6.[28] Genetic distances were calculated using the Kimura two-parameter distance algorithm with 1,000 bootstrap replicates. The neighbor-joining method[29] was used to generate phylogenetic trees.

RESULTS

Imported dengue cases in Taiwan, 2005. A total of 104 laboratory confirmed imported dengue cases were identified in Taiwan during 2005. Table 3 summarizes the countries of origin and DENV serotypes of these imported cases. Among them, 46 (44.2%) cases were identified by fever screening at airports. All of these imported cases were introduced from Asian countries, with the exception of one case from the Central American country of Belize. Similar to the findings of our previous study, Indonesia, Vietnam, the Philippines and Thailand were the most frequent importing countries.[30]

Multiple dengue epidemics in southern Taiwan, 2005. A total of 202 laboratory confirmed indigenous dengue cases were recorded in Taiwan during 2005. Among them, 12 cases were infected with DENV-1 between January and February. These cases represented the last wave of the 2004 outbreak in the regions of Pingtung County and Kaohsiung City/County. The remaining 190 cases were infected with either DENV-3 or DENV-2 between August and December 2005. There were three DHF cases and no deaths. Molecular epidemiologic study showed that three different strains of DENV, two DENV-3 (D3/Taiwan/812KH0508a/2005 and D3/Taiwan/807KH0509a/2005) and one DENV-2 (D2/Taiwan/704TN0510a/2005) had been newly imported and co-circulated in the regions of Kaohsiung City/County, Tainan City, between August and December 2005.

Transmission dynamics of the three DENV strains. Table 4 summarizes the transmission dynamics, areas infected, and the total cases estimated for each of the three DENV strains. The first dengue outbreak began in the southern part of Kaohsiung City on August 12 and was caused by a strain of DENV-3, D3/Taiwan/812KH0508a/2005. Later, the same strain of virus spread to Kaohsiung County. Another outbreak caused by a different strain of DENV-3, D3/Taiwan/807KH0509a/2005, began in Sanmin District of Kaohsiung City on September 13 and later spread to Kaohsiung County. A third outbreak caused by a strain of DENV-2, D2/Taiwan/704TN0510a/2005, began in North District of Tainan City on October 4 and later spread to Kaohsiung City/County.

Molecular epidemiologic study of DENV in Taiwan, 2005. The complete E gene sequences of three distinct DENV isolates (D3/Taiwan/812KH0508a/2005, D3/Taiwan/807KH0509a/2005, and D2/Taiwan/704TN0510a/2005) from indigenous index cases were determined and compared with the sequences

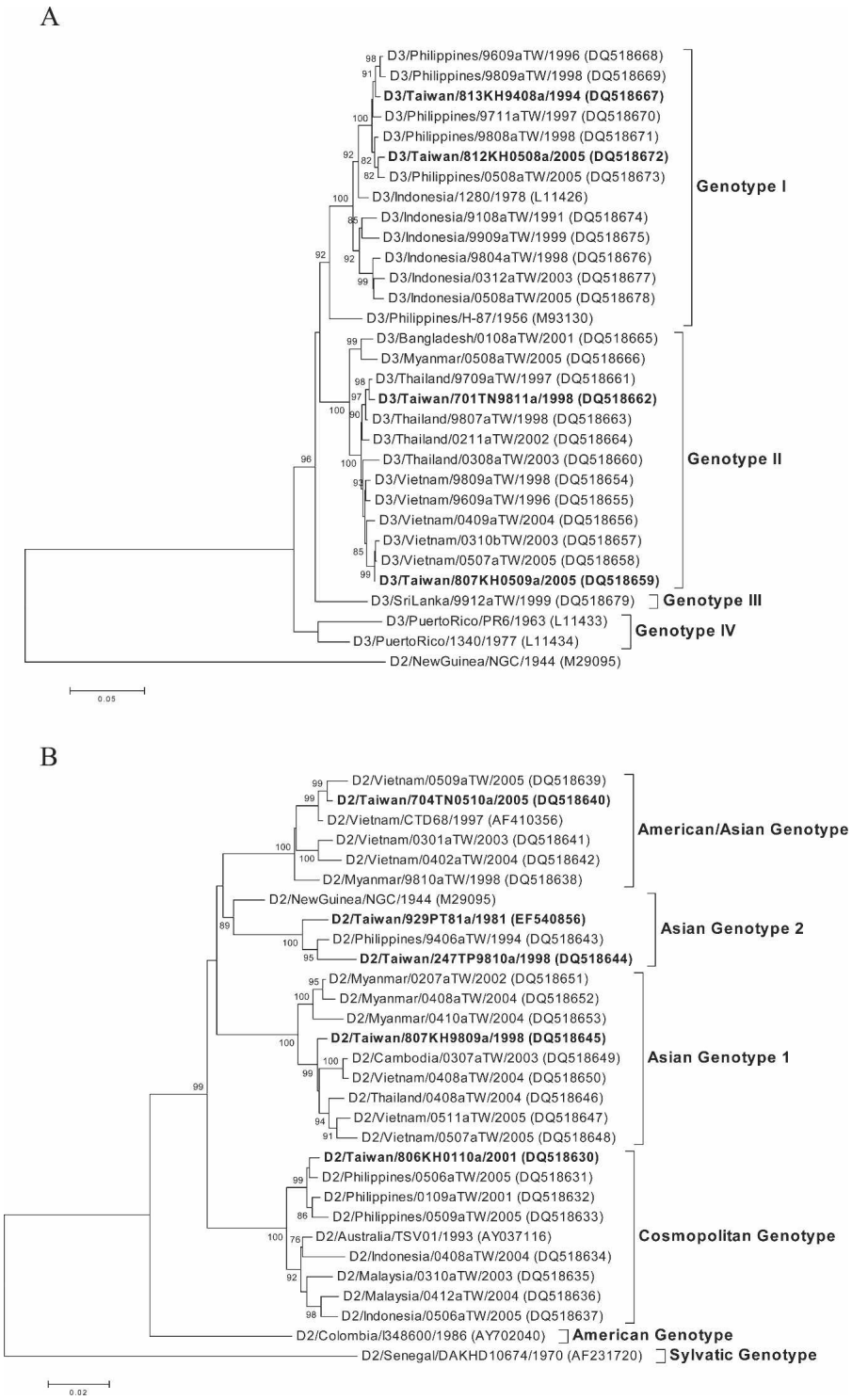

FIGURE 1. Phylogenetic trees based on the complete envelope gene sequences of **A**, 31 strains of dengue virus type 3 (DENV-3) and **B**, 30 strains of DENV-2. The trees were constructed by the neighbor-joining method. Bootstrap support values greater than 75 are shown. The epidemic strains isolated from major dengue outbreaks in Taiwan are designated in **bold**. Phylogenetic trees constructed with the neighbor-joining and maximum-likelihood methods produced similar topologies, differing only within a few terminal groupings in DENV-3 and DENV-2. Viruses were identified using the nomenclature of serotype/country/strain/year of isolation. GenBank accession numbers are shown in the parentheses. The scale bars on the left indicate substitutions per site.

available from GenBank and the dengue database of the Taiwan CDC. Figure 1A shows the phylogenetic tree of complete E gene sequences of 31 strains of DENV-3. DENV-3 Genotype I contains viruses from southeast Asia and South Pacific islands, including virus isolates from cases imported from the Philippines and Indonesia collected by Taiwan CDC. The index strain, D3/Taiwan/812KH0508a/2005 is most closely related to the isolate D3/Philippines/0508aTw/2005 and was

TABLE 3
Countries of origin and DENV serotypes of imported dengue cases in Taiwan, 2005*

Country of origin	No. imported cases (no. RT-PCR positive)			Serotype†				
	Fever screening	Non-fever screening‡	Total	DENV-1	DENV-2	DENV-3	DENV-4	Unknown
Indonesia	16 (15)	21 (8)	37 (23)	9	5	7	2	14
Vietnam	9 (9)	13 (6)	22 (15)	1	11	3	0	7
The Philippines	6 (6)	4 (1)	10 (7)	1	2	1	3	3
Thailand	6 (6)	3 (0)	9 (6)	0	0	0	6	3
Myanmar	4 (4)	3 (1)	7 (5)	0	0	5	0	2
Cambodia	1 (1)	5 (0)	6 (1)	0	0	0	1	5
Malaysia	1 (0)	4 (1)	5 (1)	1	0	0	0	4
Singapore	2 (2)	3 (0)	5 (2)	1	0	1	0	3
Bangladesh	1 (1)	0 (0)	1 (1)	0	1	0	0	0
Belize	0 (0)	1 (0)	1 (0)	0	0	0	0	1
India	0 (0)	1 (0)	1 (0)	0	0	0	0	1
Total	46 (44)	58 (17)	104 (61)	13	19	17	12	43

* DENV = dengue virus; RT-PCR = reverse transcription–polymerase chain reaction.
† DENV serotypes were identified by real-time RT-PCR.
‡ Imported cases from non-fever screening were reported from passive (hospital) surveillance and other active surveillance systems.

likely introduced from the Philippines. DENV-3 Genotype II comprises viruses from Asia, including virus isolates from cases imported from Vietnam, Thailand, Myanmar, and Bangladesh. The index strain, D3/Taiwan/807KH0509a/2005, belongs to this genotype and is most closely related to D3/Vietnam/0507aTw/2005 and was likely introduced from Vietnam. Figure 1B shows the phylogenetic tree of the complete E gene sequences of 30 strains of DENV-2. The American/Asian genotype contains viruses from Latin America and Asia including viruses isolated from cases imported from Myanmar and Vietnam. The index strain, D2/Taiwan/704TN0510a/2005, belongs to this genotype and is most closely related to the isolate D2/Vietnam/0509aTw/2005 and was most likely introduced from Vietnam.

DISCUSSION

Dengue is not considered endemic in Taiwan, which suggests that constant importation of multiple DENV from the neighboring southeast Asian countries through close commercial links and air travel is responsible for the local outbreaks that occur each year. To reduce the introduction of emerging infectious diseases and prevent local outbreaks, the Taiwan CDC established a laboratory-based dengue surveillance system to identify febrile patients at the airports by an infrared thermal scanner.[30] The peak months for imported dengue cases were July to September, and fewer cases were detected in January–April 2005. Most (44 of 46) of the confirmed cases identified by airport fever screening were in the viremic stages with positive real-time RT-PCR and negative IgM and IgG results. Among these cases in the viremic stages identified by airport screening, 34 cases were identified on days 1–3 after onset of illness. In contrast, the imported cases reported from passive (hospital) surveillance systems were evenly distributed 1–20 days after the onset of illness.

Phylogenetic analyses suggested that the three epidemic strains, D3/Taiwan/812KH0508a/2005 (DENV-3, genotype I), D3/Taiwan/807KH0509a/2005 (DENV-3, genotype II), and D2/Taiwan/704TN0510a/2005 (DENV-2, American/Asian genotype), which co-circulated in southern Taiwan in 2005, were recently imported from the Philippines, Vietnam, and Vietnam, respectively. It should be emphasized that the

TABLE 4
Summary of the three dengue epidemics in southern Taiwan, 2005*

Dengue virus strain	Serotype genotype	Epidemic area infected	First case	Last case	Total cases
D3/Taiwan/812KH0508a/2005	DENV-3 genotype I	Siaogang District, Kaohsiung City	August 12	August 13	2
		Cijin District, Kaohsiung City	August 12	September 25	> 31†
		Cianjhen District, Kaohsiung City	August 16	December 4	9
		Lingya District, Kaohsiung City	September 17	November 21	3
		Fongshan City, Kaohsiung County	September 29	November 15	19
		Zuoying District, Kaohsiung City	November 7	December 5	11
		Sanmin District, Kaohsiung City	November 8	November 10	2
D3/Taiwan/807KH0509a/2005	DENV-3 genotype II	Sanmin District, Kaohsiung City	September 13	December 7	> 25†
		Niaosong Township, Kaohsiung County	November 4	December 5	5
		Fongshan City, Kaohsiung County	November 13	November 17	5
D2/Taiwan/704TN0510a/2005	DENV-2 American/Asian Genotype	North District, Tainan City	October 4	December 9	> 46†
		Annan District, Tainan City	October 21	December 16	8
		Sanmin District, Kaohsiung City	November 13	November 13	2
		South District, Tainan City	November 18	November 24	3
		Fongshan City, Kaohsiung County	November 30	November 30	1

* DENV = dengue virus.
† Number indicates the minimal estimate of dengue cases determined by sequence analysis and epidemiologic investigation.

three most closely related imported DENV strains (D3/Philippines/0508aTw/2005, D3/Vietnam/0507aTw/2005, and D2/Vietnam/0509aTw/2005) are different from the three index epidemics strains (D3/Taiwan/812KH0508a/2005, D3/Taiwan/807KH0509a/2005, and D2/Taiwan/704TN0510a/2005) with 12, 3, and 13 nucleotide differences in E gene sequences, respectively.

Overall nucleotide sequence analyses of the E protein gene from the 30 DENV isolates collected from the beginning to the end of the outbreak show that 17, 8, and 5 isolates were grouped into three different clusters derived from each of the three index strains D3/Taiwan/812KH0508a/2005 (genotype I), D3/Taiwan/807KH0509a/2005 (genotype II), and D2/Taiwan/704TN0510a/2005 (American/Asian genotype), respectively.

Our results also provided strong evidence that dengue is not an endemic disease in Taiwan because different serotypes, genotypes, and/or strains were found to be responsible for the yearly outbreaks, and the epidemic strains disappeared with the ending of each local outbreak (Table 1 and Figure 1). In Taiwan, geographic location (subtropical island in Southeast Asia) and vector distribution (coexistence of both *Ae. aegypti* and *Ae. albopictus*) provide an ideal environment for outbreaks of dengue. However, the weather in southern Taiwan (rainy and hot temperatures averaging 28°C in summer versus dry and relatively cold temperatures averaging 20°C, occasionally with temperatures of 10–15°C in winter) could be a bottleneck for continuous transmission of DENV.

It was interesting to note that only DENV-3 and DENV-2 caused major outbreaks in 2005 despite many cases of imported DENV-1 and DENV-4. Furthermore, DENV-3 strains spread mainly in Kaohsiung City/County, whereas DENV-2 strain affected mainly in Tainan City in 2005. This is most likely attributable to protective herd immunities to DENV-1 (1987-1988 outbreak) and DENV-2 (2001-2002 outbreak) in the Kaohsiung area. In contrast, all four DENV serotypes may have the potential to transmit in Tainan City because of the lack of large epidemics during the last two decades. This study illustrates the continuing risk to countries where dengue is not endemic, but which have not eradicated mosquito vectors or introduction of dengue viruses by travelers.

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