Chikungunya virus (CHIKV) by SYBR-Green I-based real-time RT-PCR and nucleotide sequence analysis of the RT-PCR product. The CHIKV was isolated and the total nucleotide sequences were determined. Phylogenetic analysis showed that this CHIKV is similar to LR-2006_OPY1 Reunion strain.

Purpose:

Emergence of pathogenic microorganisms is an increasing concern. Infection by mosquito-borne viruses is a foremost problem in Asia. Understanding the epidemiological situations of the diseases and the phenotypic and genotypic characteristics of viruses contributes to the development of new strategies for control and prevention. In order to promote communication and exchange of the information of dengue and other mosquito-borne viruses, laboratory network between Asia and Pacific Rim should be developed and strengthened.

Methods:

1 Clinical samples and laboratory diagnosis

Human serum samples from clinically suspected dengue cases were submitted to the Arbovirus laboratory of Taiwan CDC, Department of Health, for confirmation of DENV infection. Two central dengue laboratories were set up for routine diagnosis, a Kun-Yan Laboratory in Taipei City of northern Taiwan and a fifth branch laboratory in Kaohsiung City of southern Taiwan. A rapid diagnostic system was developed to detect and differentiate various flavivirus infections using one-step real-time reverse transcriptase polymerase chain reaction (RT-PCR) and envelope membrane (E/M)-specific capture IgM and IgG enzyme-linked immunosorbent assay (ELISA) (1-3). One-step SYBR Green I RT-PCR amplification was performed in the Mx4000^{TM} quantitative PCR system (Stratagene) to detect and differentiate the DENV serotype in the acute-phase serum samples as previously described (1). In addition, all acute phase serum samples submitted to the Kun-Yan Laboratory from fevered patients identified at the Taoyuan international airport were screened for Flavivirus and Alphavirus by one-step real-time RT-PCR method using group-specific and virus-specific primers (4).

2 Virus isolation and identification

DENV were isolated from the acute phase serum samples of confirmed cases. The isolation of DENV was performed using mosquito cell line (clone C6/36 of *Aedes albopictus* cells). For each acute phase serum, 4 μl of serum sample was diluted in 200 μl cultured medium (RPMI, Gibco/BRL, Life Technologies, containing 1% FCS) and added to a 96-well microtiter plate, 50 μl/well in

quadruplicate. Then, 10^5 cells/100 μl/well of C6/36 cell line were added into the microtiter plate and incubated at 37℃ for 7 days. Cells were harvested and virus isolates were identified by the indirect fluorescent antibody test with DENV group-specific and serotype-specific monoclonal antibodies.

3. Primers used for RT-PCR and nucleotide sequencing of DENV

PCR primer pairs used for RT-PCR and nucleotide sequencing of DENV were designed based on the consensus sequences of DENV available from the GenBank database and Taiwan CDC dengue virus database.

4. Preparation of viral RNA, RT-PCR amplification and nucleotide sequencing

Viral RNAs were extracted from either acute phase serum samples or culture supernatant of C6/36 cell line infected with each of the isolated DENV strain using the QIAamp viral RNA mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Nucleotide sequences of partial NS5 gene fragment were determined directly from acute phase serum samples using RT-PCR product of one-step SYBR Green I quantitative RT-PCR (1). Partial NS5 gene sequencing was routinely performed to detect and differentiate serotype and genotype of the newly identified DENV. For full-length structure gene sequencing, extracted viral RNA from culture supernatant of C6/36 cell line infected with each of the isolated DENV strain was used as a template for cDNA synthesis, which subsequently was used for PCR amplification. Two overlapping PCR products spanning the full-length structure gene were purified from agarose gels and directly sequenced in both directions using ABI Prism automated DNA sequencing kit and ABI Prism 3700 DNA sequencer (Applied Biosystems) according to the manufacturer's protocol. Overlapping nucleotide sequences were combined for analysis and edited with the Laser software package (DNASTAR Inc, Madison, WI).

5. Phylogenetic analysis

Phylogenetic analyses were conducted using PHYLIP (version 3.6) or MEGA version 3.1. Genetic distances were calculated by using Kimura 2-parameter distance algorithm with 1,000 bootstrap replicates. Neighbor-Joining method was used to generate the phylogenetic trees.

6. Chikungunya virus identification and characterization

We started to screen acute phase serum samples of fevered patients for potentially imported Flavivirus and Alphavirus in 2006. A set of primers with consensus or specific sequences to various Flavivirus and Alphavirus were designed and used for routine screening of fevered patients identified at the Taoyuan international airport. Table 1 showed the primer sets used for real-time

RT-PCR screening of CHIKV and other Alphavirus (5). The first CHIK imported case in Taiwan was identified by this active surveillance system in 2006.

Results:

1. Imported dengue cases in Taiwan, 2006

A total of 109 laboratory confirmed imported dengue cases were identified in Taiwan in 2006. Among these patients, 47 (43.1%) were diagnosed through the airport fever screening surveillance system. Table 2 showed the summary of countries of origin and DENV serotypes of imported cases. Almost all of these imported cases were introduced from countries of Southeast Asian countries with two exceptions, which were introduced from Madagascar and El Salvador. Similar to our previous report during the 2002-2005 periods, Indonesia, Vietnam, the Philippines and Thailand are on the top list of importing countries (4). The results reflected the frequency of air travel between Taiwan and these nations, as well as the intensity of massive dengue outbreaks in the country origin during the same period. From the acute phase serum samples of all imported cases, 20 DENV-1, 21 DENV-2, 16 DENV-3, and 4 DENV-4 strains were isolated. The nucleotide sequences of partial NS5 gene fragment and full-length structure gene region of DENV strains isolated from all imported cases were determined and deposited in Taiwan Pathogenic Microorganism Genome Database for molecular epidemiological analysis.

2. Multiple dengue epidemics in southern Taiwan, 2006

For local dengue outbreaks in Taiwan, a total of 963 dengue patients were laboratory confirmed with 19 cases of DHF and 4 deaths in 2006. The nucleotide sequences of partial NS5 gene fragment were routinely determined from the acute phase serum samples of confirmed cases. The full-length structure genes of DENV strains isolated from representative indigenous cases were determined. Representative indigenous cases were selected based on the information of epidemiological investigation and preliminary results of partial NS5 gene sequences showing different infection time, infection place, and DENV serotypes and genotypes of these patients. From more than 200 DENV isolates obtained from acute phase serum samples of corresponding indigenous cases, 1 DENV-1, 36 DENV-2, and 123 DENV-3 were selected for full-length structure genes sequencing. The results showed that 5 different DENV strains (3 DENV-2, and 2 DENV-3) were co-circulated in Kaohsiung City and Kaohsiung County with limited overlap in the transmission areas. The transmission dynamics of these five DENV strains were determined. The only DENV-1 strain isolated was from a single indigenous case in Pintung County without other reported cases. It is interesting to note, however, one of his neighbors was found to be an imported dengue case coming back from

Vietnam one month ago after extensive serological investigation.

3. Nucleotide sequencing and phylogenetic analysis

Figure 1 illustrated the evolutionary relationship of DENV-2 strains using E genes sequences available from Taiwan CDC dengue genomic database and GenBank. Phylogenetic tree was constructed by neighbor-joining method and five genotypes were identified. Bootstrapping with 1000 replicates was used to place confidence value on groupings within the trees. The DENV strains responsible for the three major DENV-2 epidemics in 2006 were highlighted. It was interesting to note that all of these three DENV-2 strains showed highest homologies to Vietnam DENV-2 strains, which were designated in the Asian 1 genotype.

4. Chikungunya virus identification and characterization

In order to reduce the importation of emerging infectious diseases into Taiwan, we decided to expand the surveillance program by screening Flavivirus and Alphavirus from the acute phase serum samples of fevered patients identified at the Taoyuan international airport in 2006. We successfully identified the first imported CHIK case in Taiwan through this surveillance system on 20 November 2006. The patient is a 13-yr-old Taiwanese student who was detected with high fever at the airport when coming back from Singapore. The acute phase serum sample was found to be positive for CHIKV by SYBR-Green I-based real-time RT-PCR and nucleotide sequence analysis of the RT-PCR product. The CHIKV was isolated from the acute phase serum sample and the full genome sequences were determined. Phylogenetic analysis showed that this CHIKV is similar to LR-2006_OPY1 Reunion strain (Figure 2).

Discussion:

In a world of global village, it is a real challenge to effectively control imported infectious diseases. The successful application of fever screening at the airport in Taiwan during the period of 2003-2006 had so far identified more than 300 confirmed cases of imported fever patients with dengue, enteric bacteria, and malaria diseases. To monitor potential Arbovirus importation, we started to screen Flavivirus and Alphavirus from the acute phase serum samples of fever patients in 2006. A total of 47 imported dengue cases were identified from this surveillance system. In addition, a first CHIK case was identified at the Taoyuan international airport. The multiple dengue epidemics occurred each year in southern Taiwan in recent years demonstrated that many dengue virus strains were silently imported. It is interesting to note that only DENV-3 and DENV-2 caused major outbreaks in southern Taiwan despite similar numbers of DENV-1 was imported. Furthermore, DENV-3 strains caused far more dengue cases than DENV-2 strains. This is most likely due to protective herd immunities to DENV-1 and DENV-2 since more than 20%

individuals in Kaohsiung City had been infected with DENV-1 and/or DENV-2 from previous large DENV-1 and DENV-2 outbreaks. The only DENV-1 strain isolated was from an indigenous case in Pintung County, where no other cases were identified. Our experiences suggested that local outbreak could be prevented if indigenous dengue case(s) could be identified in the first transmission cycle. Since emergence control measures could be effectively launched to prevent the spread of imported virus by local mosquitoes. Accordingly, local outbreaks were usually occurred through delayed clinical diagnosis and reporting to allow further spread of imported virus in a environment suitable for mosquito breeding. Consequently, the index imported dengue cases could not be traced for most local dengue outbreaks.

Genetic phylogenies are the "gold standard" for defining viral genealogy, transmission, and molecular evolution. By combined genetic and geographic data, it is possible to know the virus evolution, geographic distribution, and global movement. The genomic database can be used to compare different strains of the virus, identify the genetic factors that determine their virulence, and look for new therapeutic, vaccine and diagnostic targets. To reach this goal, nucleotide sequences of more dengue virus strains should be determined and made available in public database together with related epidemiologic data.

During 2006, several Asian countries including Hong Kong, Taiwan, Japan, and Singapore had reported the introduction of CHIKV, which was not endemic in these countries during the last 50 years. The repeated introduction of CHIKV into many European countries and other continents is an alert to remind us that we live in a global village. Current status suggested that CHIKV strains originally transmitted in Indian Ocean had emerged in many Southeast Asian countries and other continents. With rapid travel, viruses can be quickly introduced to remote areas, usually within 24 hours. With the global warming effect, expanding ranges of vectors and increased introduction of Arbovirus, such as DENV and CHIKV would be expected in the near future. Enhanced surveillance should be implemented to monitor its global movement and to prevent its introduction and establishment in these regions.

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1. **Publication list for this work:**
None

Table 1. Primer sets used for real-time RT-PCR screening of chikungunya virus

Primer	Sequence(5'→ 3')	Amplicon size
Alphavirus (Taiwan CDC)		
AL-2	TAA TGC CAG AGC GTT TTC GCA	414 b.p.
AL-3	GTG GTG TCA AAC CCT ATC CA	
Chikungunya virus (ref. 2)		
CHIKV-F	AAG CTY CGC GTC CTT TAC CAA G	209 b.p.
CHIKV-R	CCA AAT TGT CCY GGT CTT CCT	

Table 2. Countries of origin and DENV serotypes of imported cases in Taiwan, 2006

Country of origin	No. of imported cases		Serotype				
	Total	Fever screening	DENV-1	DENV-2	DENV-3	DENV-4	Unknown
Vietnam	36	17	8	12	3	1	12
Indonesia	20	10	5	4	3	1	7
The Philippines	16	6	1	5	7	0	3
Thailand	11	8	2	4	0	3	2
Cambodia	9	1	0	0	4	0	5
Malaysia	6	2	4	0	1	0	1
Bangladesh	4	1	0	0	2	0	2
India	3	2	1	1	0	0	1
Myanmar	2	0	0	0	1	0	1
Madagascar	1	0	1	0	0	0	0
El Salvador	1	0	1	0	0	0	0
Total	109	47	23	26	21	5	34

Figure 1. Phylogenetic tree derived from full-length E gene sequences (1485 b.p.) of DENV-2 strains using Neighbor-Joining method.

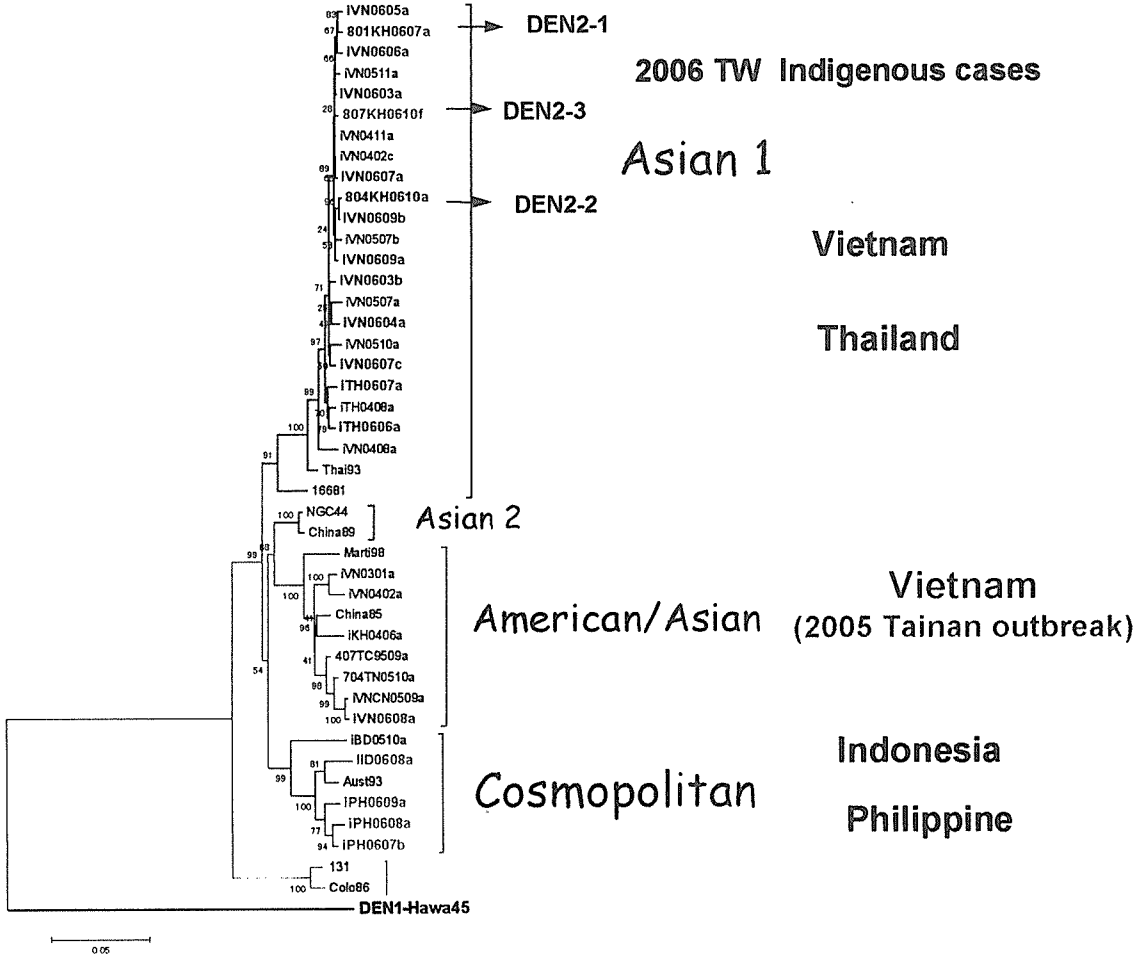
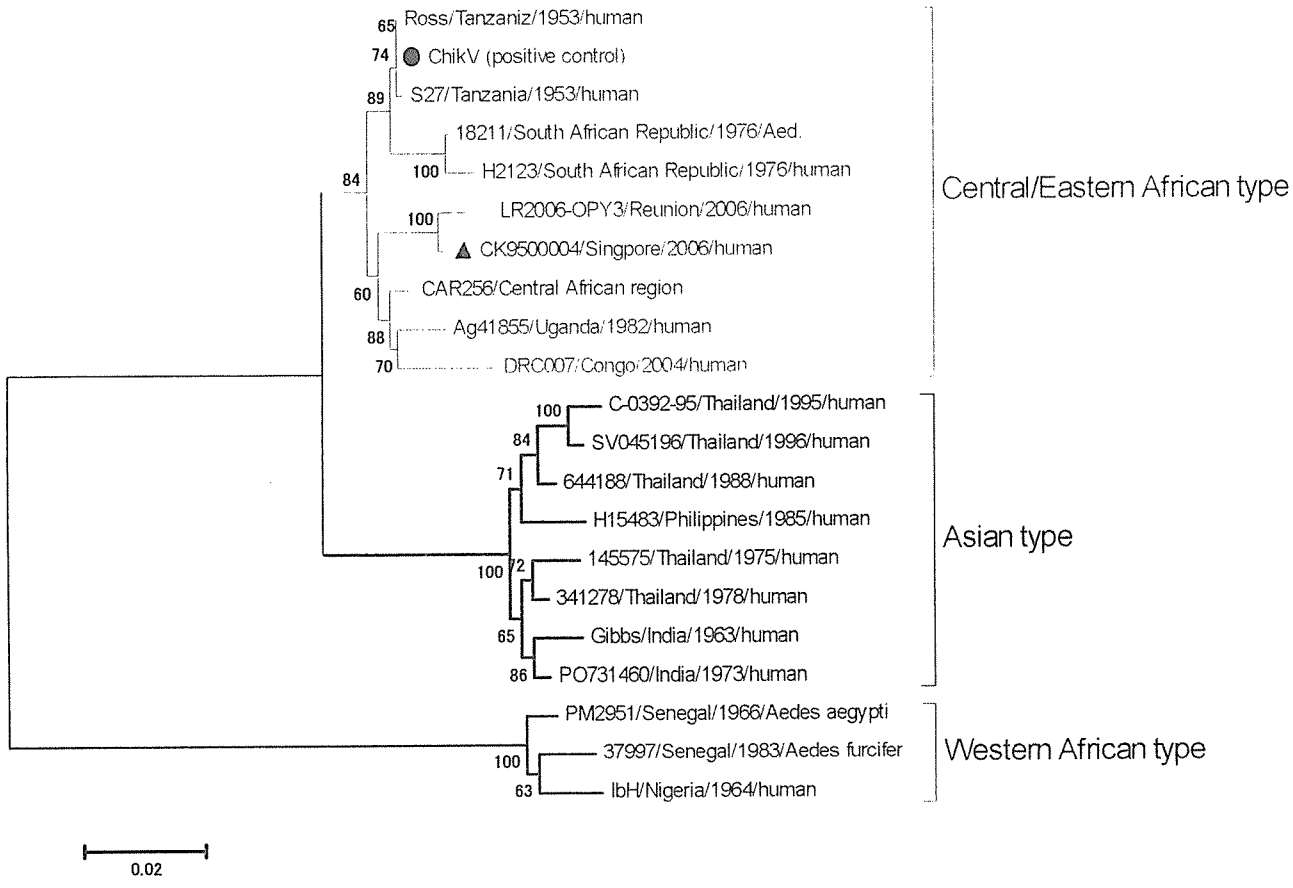


Figure 2. Phylogentic tree derived from E1 gene sequences (1050 b.p.) of Chikungunya virus using Neighbor-Joining method.



Characterization of dengue viruses prevalent in Indonesia

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Although dengue epidemic occurres at regular intervals in Indonesia since it was first recognized in Java (Pratana L, 1970), there have been limited reports of the diversity of dengue viruses isolated in Indonesia. Analysis of the viruses isolated from DHF patients in Indonesia from 1975 to 1978 demonstrated that all 4 dengue viruses were endemic in Jakarta, but dengue-3 virus (DV-3) was the most predominant (Gubler, 1979). Dengue 3 virus was also the most frequently isolated virus outside Jakarta and had the widest distribution in Indonesia (Gubler, 1979; Corwin, 2001)

In this three-year study we are planning to do the molecular and phenotype characterization to four dengue serotypes from patients with different disease severities and more strains will be analyzed to get a better picture on the amino acid substitutions. We hope this study can give us better understanding on the molecular epidemiology of dengue viruses and can further contribute to the development of new strategies for control and prevention of dengue infection

Here we report our results in the 2006 which focused on specimen collection and sequencing of several strains of DEN-3.

OBJECTIVES:

Emergence of pathogenic microorganisms is an increasing concern. Infection by mosquito-borne viruses is a foremost problem in Asia, including Indonesia. Understanding the epidemiologic situations of the diseases and the phenotypic and genotypic characteristics of viruses contributes to the development of new strategies for control and prevention. In order to promote communication and exchange of the

information of dengue and other mosquito-borne viruses, laboratory network among Asia and Pacific Rim should be developed and strengthened.

METHODS:

1.. Source of the viruses, serology and RT-PCR.

Specimens were collected from hospitalized patients with suspected dengue infection in Jakarta within the period of February 2006 to December 2006. Serum samples were serologically examined by Hemaglutination inhibition test and rapid immunochromatographic assay (PanBio Inc., Brisbane, Australia). The sera were also tested by RT-PCR (Lanciotti, 1992) to see the presence and type of virus.

2. Virus isolation

Virus isolation was done by inoculating 50 ul of 1:10 dilution serum samples onto C6/36 cell line in 24 well plates (Yamada et al, 2002). Dengue viruses were propagated in C6/36 cells at room teperature for 7 days. The culture supernatants were harvested and centrifuged at 900 g for 5 minutes and then filtered through the syringe driven millex GV with 0.22 um filter unit (Millipore, Co. Bedford MA USA). Culture supernatants were collected and checked for the presence of dengue virus by Hemagglutination assay and RT-PCR (Lanciotti, 1992).

3. Determination of viral nucleotide sequences

3.1. *RT-PCR and DNA sequencing*

To genotypically characterize the isolated viruses, nucleotide sequencing will be done. Sequencing were done to 4 strains of DEN-3 for envelop region and 3 of them were also sequenced for NS1. RNA were extracted from 140 ul of plasma using Viral RNA Isolation Kit (Qiagen, GmbH, Germany) according to the manufacturer's instruction. Complementary DNA (cDNA) strands were reverse-transcribed using Super Script II First Strand Synthesis System with Random hexanucleotide primer according to the manufacturer's instructions (Invitrogen). Amplification of DNA fragments were

performed according to the annealing temperature corresponding primers used and length of the expected product. After amplification and purification, the DNA were sent to the DNA sequencing facility at the Eijkman Institute, Jakarta. The sequences were determined using Taq Big Dye Deoxy Terminator Cycle sequencing kits

Results

Patient characterization:

141 patient plasma were collected from March to December 2006, consisting of 22 plasma from children (< 14 years old) and 119 plasma from adult (≥ 14 years old)
To confirm dengue infection and to find out the immunologic status of the patients, HI test were done. From 141 patients, only 116 had paired samples, and most of the paired sera had interval less than 7 days. From HI results (table 1) can be seen that most of the patients had secondary infection (66.4%), 2 (1.7%) patients were negative, and 7 patients (7.7%) were indeterminate because the interval of their sera is less than 7 days.

Tabel 1. Hemaglutination inhibition test result

Infection	**N**	**%**
Primary	5	4,3
Secondary	77	66,4
Primary/secondary	9	7,7
Presumptive	14	12,1
Indeterminate	9	7,7
Negative (not dengue)	2	1,7
Total	**116**	**100**

Viral isolation and RT-PCR results

Viral isolation using C6/36 cell line was attempted, but for unknown reason the results were unsatisfying.

From 155 samples tested, 86 samples were RT-PCR positive. Among the positives, the most frequent was DEN-2 (50.0%), followed by DEN-3 (29.1%), DEN-1 (17.4%), and rarely DEN-4 (3,5%).

Tabel 2. Age and infecting dengue serotype

Age	RT-PCR Positive				n positive/ n tested	%
	DEN-1	DEN-2	DEN-3	DEN-4		
< 14 y.o.	4	1	4	2	11/22	50.0%
≥ 14 y.o.	11	42	21	1	75/119	63.0%
Total	15	43	25	3	86/141	61.0%

Sequencing of DEN-3.

Sequencing of envelope region 4 strains DEN-3 and NS1 region of 3 DEN-3 strains (table 3) were done directly from cDNA prepared from viral RNA from plasma (QIAmp viral RNA – Qiagen).

The sequencing data were analysed using Genetyx 2.0 dan BioEdit 7.0 (http://www.mbio.ncsu.edu.BioEdit/bioedit.html). For pairwise comparison and phylogenetic analysis the sequence data from this study were compared to data from our previous study and from GenBank.

To analyse envelop region, 15 strains were compared. We found that envelope nucleotide identity was from 92.4% to 99.9%, and polypeptide identity was between 96.5% to 100%. The 100% polypeptide identity can be seen in strains Indonesia DS 029/06 (DHF) and strain 98901517 (DHF); 98901403 (DSS) and 98901437 (DSS); strain 98901517 (DHF), strain 98901403 (DSS) and 98901437 (DSS), as well as strain 98901403 (DSS) strain 98901437 (DSS). Lowest polypeptide identity was seen in strains CO360/94 (Thai, DF) compared to DS 002/06 (Indonesia, DF) dan DSA 02/06 (Indonesia,DSS) (Table 6). However, no specific amino acid change in the envelope region can be seen attributed to certain grade of dengue infection.

To analyse NS1 region, 14 strains of DEN-3 were compared. The identity of NS1 gene was from 99.1% to 99.9%; while the identity of polypeptide was from 97.1% to 100%. A 100% protein identity could be seen in strains Indonesia DS 002/06 (DF), and DSA 02/06 (DSS); in strains 98901640 (DHF) and 98901403 (DSS); and in Thai strains. CO360/94 (DF), KPS/551 (DF) and KPS/207 (DSS). The lowest polypeptide identity was seen in strain KPS/551 (DF) compared to DS 029/06 (DHF) and 98901517 (DBD) (Table 7). In NS1 region also no specific amino acid change can be seen attributed to certain grade of dengue infection.

Phylogenetic analysis of DEN-3 based envelop gene on was done with strains sequenced in this study and data obtained from Genbank. The results showed that the strains sequenced in this study belonged to subtype I.

Conclusion

From March to December 2006, 141 patient plasma were collected, consisting of 22 plasma from children (< 14 years old) and 119 plasma from adult (≥ 14 years old).
From 141 patients, only 116 had paired samples, HI test showed that most of the patients had secondary infection 77 (66.4%), 2 (1.7%) patients were negative, and 7 patients (7.7%) were indeterminate because the interval of their sera is less than 7 days. From 141 samples tested, 86 samples were RT-PCR positive. Among the positives, the most

frequent was DEN-2 (50.0%), followed by DEN-3 (29.1%), DEN-1 (17.4%), and rarely DEN-4 (3,5%). Four DEN-3 strains were sequenced for envelop region and 3 of them were also sequenced for NS-1 region. Phylogenetic tree based on the envelop region showed that all 4 strains sequenced in this study belonged to subtype I.

Table 3 : Dengue virus isolated in Jakarta which were sequenced in this study

No	Strain	Year isolated	Clinical manifestation	Serotype
1	DS 002/06	2006	DF	DEN-3
2	DS 029/06	2006	DHF	DEN-3
3	DSA 02/06	2006	DSS	DEN-3
4	17/04	2004	DHF	DEN-3

Tabel 4. Primers used for PCR and sequencing

Primers	Nucleotide sequence
D3-846s	CCCTATTTCTTGCCCATTACA
D3-1204s	CGTGTGTAAGCATACATACG
D3-1715s	GCACTGACAGGAGCTTACAGA
D3-1996s	AGTGGTGACCAAGAAGGA
D3- NS1 1s	CCGCTGGGATCCGACATGGGGTGTGTCATA
D3-2698s	GGTCCTAGAGCAAGGGAA
D3-3078s	CTGCACATGGCCAAAATCAC
D3-1056c	TTCTCGAGGAATTCTGCTGAGGCTAGAGACTTTA
D3-1192c	CTGCTCCTCAGGTAGAAT
D3-1911c	CCTTTGTACTCAACCTTAATGA
D3-2716c	TTTCCCTTGCTCTAAGACCC
D3-3367c	AAGTGTGCACGAGCGGCAAC
D3-3833c	AGCAAATTCCATTCGCCATTTG

Table 5 : DEN-3 strains analysed for pairwise comparison

No	Strain	Origin	Year	Clinical maifestation	Source/ GenBank accession #
1	DS 002/06	Jakarta, Indonesia	2006	DD	This study
2	DS 029/06	Jakarta, Indonesia	2006	DBD	This study
3	DSA 02/06	Jakarta, Indonesia	2006	DSS	This study
4	17/04*	Jakarta, Indonesia	2004	DBD	This study
5	CH53489	Thailand	1973	DBD	Raekiansyah, M. *et al.* 2005
6	KPS/551	Thailand	1998	DD	Raekiansyah, M. *et al.* 2005
7	CO360/94	Thailand	1994	DD	AY923865
8	98902890	Sumatra, Indonesia	1998	DD	AB189128
9	98901640	Bandung,Indonesia	1998	DBD	AY912455
10	98901590	Bandung,Indonesia	1998	DBD	AY912454
11	98901517	Sumatra, Indonesia	1998	DBD	AB189127
12	CO331/94	Thailand	1994	DSS	AB189126
13	KPS/207	Thailand	1998	DSS	AY912458
14	98901403	Sumatra, Indonesia	1998	DSS	AB189125
15	98901437	Sumatra, Indonesia	1998	DSS	AB189126

* : envelop region only.

Table 6. Pairwise comparison of envelope region of dengue virus type 3 strains

% identity of Nucleotide	% identity of polypeptide														
	CH 53489 (DHF)	DS 002/06 (DF)	DS 029/06 (DHF)	DSA 02/06 (DSS)	17/04 (DHF)	CO360 /94 (DF)	KPS/ 551 (DF)	9890 2890 (DF)	9890 1640 (DHF)	9890 1590 (DHF)	9890 1517 (DHF)	CO331 /94 (DSS)	KPS/ 207 (DSS)	9890 1403 (DSS)	9890 1437 (DSS)
CH53489 (DHF)		97,7	97,9	97,7	98,1	97,7	98,1	98,5	97,9	97,9	97,9	98,1	97,9	97,9	97,9
DS 002/06 (DF)	93,5		99,7	99,5	99,5	96,5	97,3	99,1	97,7	97,9	99,7	97,7	97,1	99,7	99,7
DS 029/06 (DHF)	93,3	98,3		99,7	99,7	96,7	97,5	99,3	97,9	98,1	100	97,9	97,3	100	100
DSA 02/06 (DSS)	93,4	99,1	98,5		99,5	96,5	97,3	99,1	97,7	97,9	99,7	97,7	97,1	99,7	99,7
17/04 (DHF)	93,5	99,4	98,2	98,9		96,7	97,5	99,5	97,9	98,1	99,7	97,9	97,3	99,7	99,7
CO360/94 (DF)	97,1	92,5	92,4	92,4	92,5		98,7	97,1	98,5	98,5	96,7	97,9	98,5	96,7	96,7
KPS/551 (DF)	97,0	92,6	92,6	92,6	92,6	99,3		97,9	99,3	99,3	97,5	98,7	99,7	97,5	97,5
98902890 (DF)	93,9	97,2	97,2	97,4	97,2	93,0	93,1		97,9	98,1	99,3	98,3	97,7	99,3	99,3
98901640 (DHF)	95,8	94,5	94,8	94,5	94,5	97,2	97,5	94,0		99,5	97,9	98,7	99,1	97,9	97,9
98901590 (DHF)	95,8	94,7	94,9	94,6	94,7	97,3	97,6	94,3	99,6		98,1	98,5	99,1	98,1	98,1
98901517 (DHF)	93,9	99,0	98,6	98,8	98,9	92,9	93,1	97,7	94,8	94,9		97,9	97,3	100	100
CO331/94 (DSS)	97,3	93,0	93,1	92,9	93,0	98,5	98,7	93,5	96,8	96,8	93,4		98,5	97,9	97,9
KPS/207 (DSS)	97,0	92,7	92,7	92,6	92,7	99,2	99,7	93,2	97,4	97,5	93,1	98,7		97,3	97,3
98901403 (DSS)	93,7	99,0	98,9	98,9	98,9	92,8	92,9	97,7	94,8	94,9	99,3	93,4	93,0		100
98901437 (DSS)	93,9	99,1	98,7	98,9	98,9	93,0	93,1	97,7	94,9	95,0	99,9	93,5	93,2	99,3	

Fig. ... Pairwise comparison of NS1 region of dengue virus type 3 strains

% identity of nucleotide	% identity of Polypeptide													
	CH 53489 (DHF)	DS 002/06 (DF)	DS 029/06 (DHF)	DSA 02/06 (DSS)	CO360 /94 (DF)	KPS/ 551 (DF)	9890 2890 (DF)	9890 1640 (DHF)	9890 1590 (DHF)	9890 1517 (DHF)	CO331 /94 (DSS)	KPS/ 207 (DSS)	9890 1403 (DSS)	9890 1437 (DSS)
CH53489 (DHF)		98,2	97,7	98,2	98,5	98,5	98,0	98,0	98,8	97,7	98,2	98,5	98,0	98,0
DS 002/06 (DF)	94,1		99,4	100	98,0	98,0	98,5	99,7	99,4	99,4	97,7	98,0	99,7	99,7
DS 029/06 (DHF)	92,8	97,3		99,4	97,4	97,4	98,0	99,7	98,8	98,8	97,1	97,4	99,7	99,1
DSA 02/06 (DSS)	94,2	99,3	97,6		98,0	98,0	98,5	99,7	99,4	99,4	97,7	98,0	99,7	99,7
CO360/94 (DF)	97,5	93,5	92,4	93,6		100	97,7	97,7	98,5	97,4	99,7	100	97,7	97,7
KPS/551 (DF)	97,0	93,6	92,1	93,3	99,4		97,7	97,7	98,5	97,4	99,7	100	97,7	97,7
98902890 (DF)	94,2	96,6	95,9	96,9	93,6	93,5		98,2	99,1	98,0	97,4	97,7	98,2	98,2
98901640 (DHF)	93,6	98,1	99,0	98,3	93,1	92,8	96,4		99,1	99,1	97,4	97,7	100	99,4
98901590 (DHF)	94,6	97,2	96,3	97,5	94,1	93,8	98,1	97,0		98,8	98,2	98,5	99,1	99,1
98901517 (DHF)	93,8	98,4	97,9	98,7	93,2	92,9	96,8	98,6	97,4		97,1	97,4	99,1	99,7
CO331/94 (DSS)	97,2	93,6	92,7	93,7	99,3	98,7	93,5	93,2	94,0	93,3		99,7	97,4	97,4
KPS/207 (DSS)	97,3	93,5	92,2	93,4	99,8	99,6	93,6	92,9	93,9	93,0	99,1		97,7	97,7
98901403 (DSS)	93,7	98,3	98,5	98,6	93,2	92,9	96,7	99,3	97,3	98,9	93,3	93,0		99,4
98901437 (DSS)	93,9	98,5	98,0	98,8	93,3	93,0	96,9	98,7	97,5	99,9	93,4	93,1	99,0	