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Table 1. Summary of genotype distributions of DENV strains isolated from imported cases in Taiwan, 2009

Serotype	DENV-1			DENV-2				DENV-3		DENV-4		Total
Genotype	I	II	III	Asian1	Asian2	Asian/ American	Cosmopo litan	I	II	I	II	
Indonesia	6*	3					13	9			8	39
Vietnam	26			5					4			35
Thailand	6			1					1			8
Philippines							7	4		1		12
Cambodia				4								4
Singapore							2					2
Myanmar									1			1
Malaysia	2							1				3
Pakistan										1		1
India							1					1
Bangladesh									1			1
Total	40	3	0	10	0	0	23	14	7	2	8	107

Table 2. Imported chikungunya viruses in Taiwan, 2009

Patient No.	Seq name	Location	E1-226	Genotype
CK9800001	0901aTw	Malaysia	V	East/Central/South African
CK9800002	0904aTw	Singapore	V	East/Central/South African
CK9800003	0904bTw	Indonesia	A	Asian
CK9800009	0905aTw	Thailand	V	East/Central/South African
CK9800020	0908aTw	Indonesia	A	Asian
CK9800028	0909aTw	Indonesia	A	Asian
CK9800045	0911aTw	Thailand	V	East/Central/South African
CK9800046	0912aTw	Indonesia	V	East/Central/South African
CK9800047	0912bTw	Malaysia	V	East/Central/South African

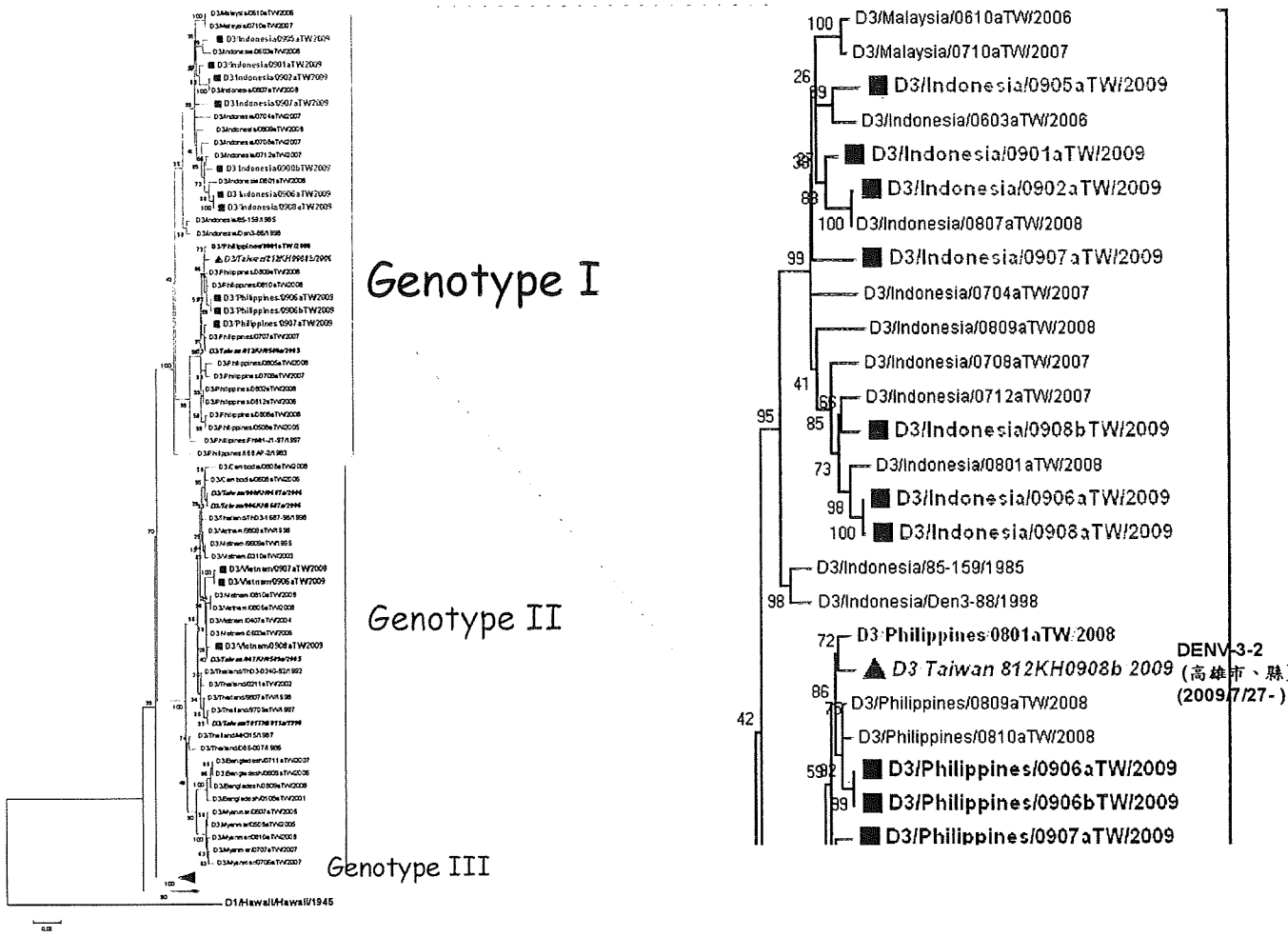

Figure 1. Phylogenetic tree of E genes from DENV-3 strains isolated from indigenous and imported dengue cases in Taiwan, 2009. The trees were constructed by the neighbor-joining method. Viruses were identified using the nomenclature of serotype/country/strain/year of isolation. GenBank accession numbers are shown in the parentheses.

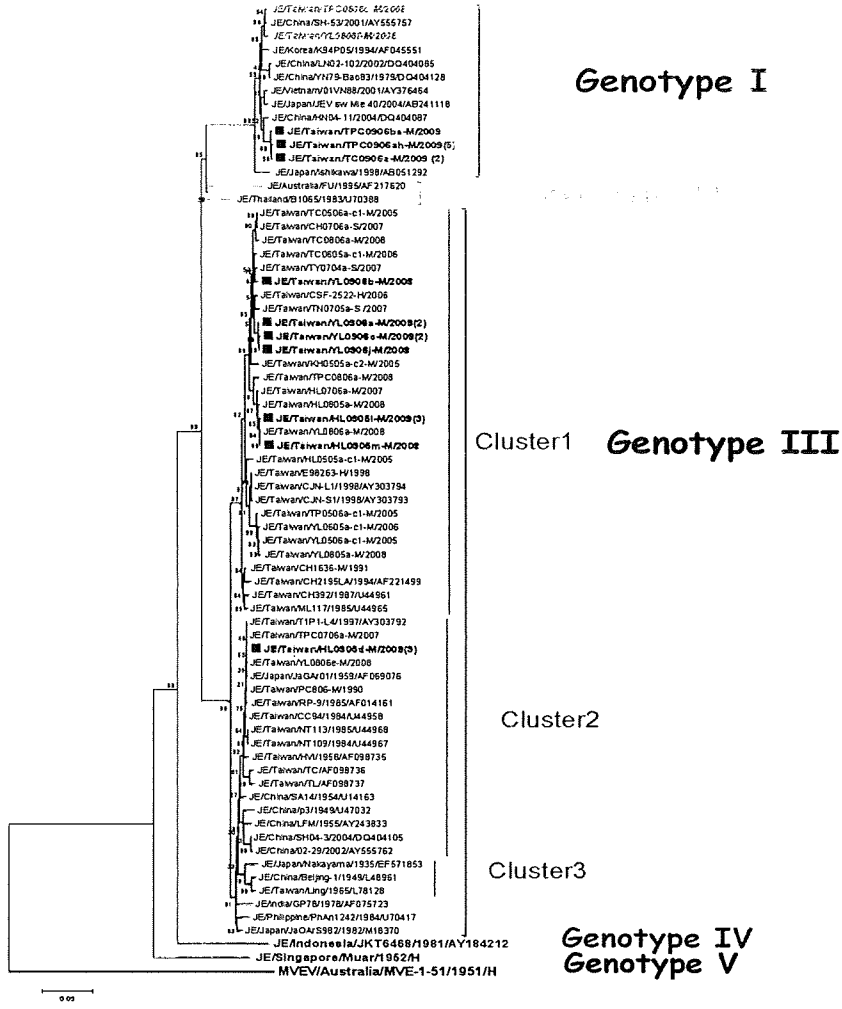

Figure 2. Phylogenetic tree showing the genetic relationship among Japanese encephalitis virus isolates. The tree was constructed on the basis of complete envelope nucleotide sequences (1500bp) of Japanese encephalitis virus strains isolated in Taiwan together with reference sequences available from GenBank.

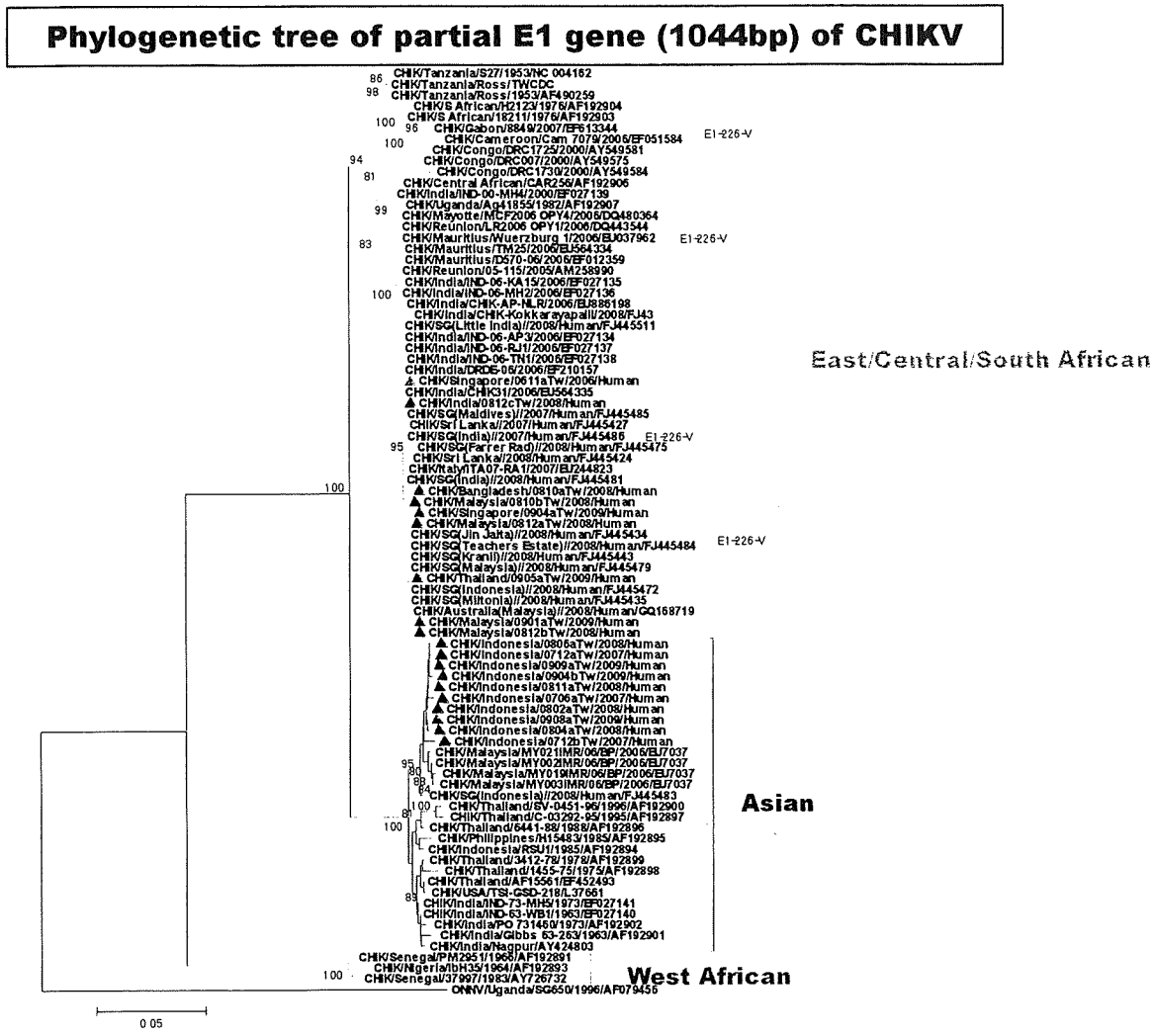

Figure 3. Phylogenetic relationships of chikungunya virus (CHIKV) isolates from 19 imported cases in Taiwan. The tree was constructed by the neighbor-joining method using partial nucleotide sequences of envelope protein 1 (E1) gene (1044 bp) of various CHIKV strains. O'nyong-nyong (ONN) virus sequence was used as the outgroup virus. The 19 imported CHIKV strains in Taiwan are designated by triangles. Viruses were identified using the nomenclature of virus/country/strain/year of isolation/GenBank accession number. The scale bar on the left indicates substitutions per site.

ANALYSIS OF ENVELOPE, NS1 AND NS3 GENE AND POLYPEPTIDE SEQUENCES OF DENGUE VIRUSES IN JAKARTA

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INTRODUCTION

Since first reported in 1968 (Sumarmo, 1987) dengue remain a major problem in Indonesia. During Jan-Nov 07, 127,687 cases with 1296 death were reported, which is 20% increase in morbidity compared to the same period in 2006 (WHO/SEARO). In Indonesia all serotypes are endemic. DHF have been reported in all provinces. (Setiati, 2007).

As mentioned in previous report, in this study we were more focus to continuing molecular epidemiology study based on env genes, as well as proteins which is the target of diagnostic test (NS-1), and the potential target of antiviral drugs and vaccine, i.e. NS-3 protein. By studying those genes, we hope we can be able to evaluate use of NS-1 based diagnostic kits, and in the future develop diagnostic kits based on recent, indigenous, Indonesian strains.

METHODS:

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

Specimens were collected from hospitalized patients with suspected dengue infection in Dr. Cipto Mangunkusumo Central Hospital, Jakarta within the period of 2006 to 2009. Serum samples collected in 2006 were serologically examined by Hemaglutination inhibition test. Rapid immunochromatographic assay (PanBio Inc., Brisbane, Australia) were done to year 2006 and 2009 specimens. The sera were also tested by RT-PCR (Lanciotti, 1992; Reynes J-M, 2003) to see the presence and type of

virus. To the year 2009 samples, NS-1 ELISA (Panbio Inc, Brisbane, Australia) were also done.

2. *Determination of viral nucleotide sequences*

RT-PCR and DNA sequencing

RNA were extracted from 140 µl of plasma using Viral RNA Isolation Kit (Qiagen, GmbH, Germany or Roche) according to the manufacturer's instruction. Complementary DNA (cDNA) strands were reverse-transcribed using Super Script III 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 to primers used (Table 1) and length of the expected product. After amplification and purification, the DNA was sent to the DNA sequencing facility at the Eijkman Institute, Jakarta or Microbiology Laboratory of the Faculty of Siences, University of Indonesia. The sequences were determined using Taq Big Dye Deoxy Terminator Cycle sequencing kits. Table 1 shows the primers used for PCR and sequencing. The nucleotide sequences were analysed using software Genetyx-Win version 5.1.

RESULTS AND DISCUSSION

Specimen characterization

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 February to April 2007, 97 patient plasma samples were collected, consisting of 47 plasma from children and 50 plasma from adult. During 2009, 112 samples, consisting of 60 samples from adults and 52 samples from children. From 2006 to 2009, a total of 350 specimens were collected, consisting of 141, 147 dan 112 specimens in 2006, 2007 and 2009 respectively. Of those, 229 specimens were from adults and 121 specimens were from children. (Table 1)

Dengue serotypes

As shown in table 1, all four serotypes could be found in Jakarta, with the dominance of DENV-2 (year 2006), DENV-3 (year 2009), and in 2007 DENV-2 and DEN-3. DENV-4 was rarely found in the hospitalized patients examined. Detection rate of dengue viral RNA was between 25% to 63 %.

In this study we found all dengue serotypes in hospitalized patients in the Dr. Cipto Mangunkusumo Hospital in Jakarta. DENV-2 and DENV-3 were more often found than the other serotypes. This is in agreement with previous report by Setyati et al. DENV-4 was rarely found.

Table 1. Dengue serotypes in patients samples collected in 2006, 2007 and 2009

		RT-PCR positive					
Year	Age	**DEN-1**	**DEN-2**	**DEN-3**	**DEN-4**	**n pos/ n tested**	**%**
2006	< 14 y.o.	4	1	4	2	11/22	50.0%
	≥ 14 y.o.	11	42	21	1	75/119	63.0%
2007	< 14 y.o.	0	13	10	0	23/47	48.9%
	≥ 14 y.o.	2	7	10	1	20/50	40%
2009	< 14 y.o.	5	4	10	1	20/52	38.4%
	≥ 14 y.o.	4	5	6	0	15/60	25%
Total		26	72	61	5	164/350	

Sequencing of envelope

From 2006 to 2009 envelope genes of one DENV-1. six DENV-2 and six DENV-3 strains were sequenced (table 2). As reported previously, the genotype of viruses sequenced in this study were as follow: DENV-1 were genotype IV(Goncalves, 2002), DENV-2 were identified as cosmopolitan genotype and Asian genotype I (Huang, 2007), DENV-3 belong to genotype I (Zhang, 2005).

Table 2 : Dengue virus strains sequenced in this study

No	Strain	Year isolated	Clinical manifestation	Serotype	Genes sequenced
1.	DS27/06	2006	DHF-I	DENV-1	Env, NS1
2	DS31/06	2006	DHF-I	DENV-2	Env, NS1
3	DS24/06	2006	DF	DENV-2	Env
4	DS20/06	2006	DHF-II	DENV-2	Env
5	DSA06/07	2007	DHF-III	DENV-2	Env
6.*	DSA25/07	2007	DHF-I	DENV-2	Env
7	DS 002/06	2006	DF	DENV-3	Env, NS1
8.	DS 029/06	2006	DHF-II	DENV-3	Env, NS1
9.	DSA 02/06	2006	DSS	DENV-3	Env, NS1, NS3
10.	17/04	2004	DHF-II	DENV-3	Env, NS1
11.*	DS22/07	2007	DHF-1	DENV-3	Env, NS1, NS3
12.*	DS46/07	2007	DHF-1	DENV-3	Env, NS1, NS3
13.*	DS18/09	2009		DENV-2	Env

Analysis of NS1 region

In addition to previous report, we analyzed the NS-1 region of two DENV-3 year 2007 strains. In all strains analysed, the four B-cell epitopes reported by Roehrig et al.(1997) and Falconar (1997, 2008), For DENV-3 the. Epitopes analysed were LD2 (aa 25-33 : VHTWTEQYK), epitope 24A (aa 61-69: TRMENLLWKQ), epitope LX1 (aa113-119: YSWKTWGK) and epitope 24C (301-309: TTVSGKLIH) were conserved (Figure 1)

NS-3 region of DENV-3

We analysed NS3 region of three strains, i.e. DSA 002/06 (DSS), DS22/07 (DHF-1), and DS46/07 (DHF-1), and looked at the sites reported to be involved in the function of NS3 protein. The three strains were analysed together with 12 other strains reported in Genbank.

Tabel 3. DENV-3 strains analysed on NS3 gene

No	Strain	Origin of Strain	Year	Clinical manifestation	Source/ *Gen bank accession no.*
1	DS 46/07	Jakarta, Indonesia	2007	DBD	This study
2	DS 22/07	Jakarta, Indonesia	2007	DD	This study
3	DSA 02/06	Jakarta, Indonesia	2006	DSS	This study
4	98901403	Sumatra, Indonesia	1998	DSS	AB189125
5	D3-MY00-22366	Kualalumpur, Malaysia	2000	DBD	FN429905
6	D3-MY00-22447	Kualalumpur, Malaysia	2000	DBD	FN429901
7	DENV-3/VE/BID-V1114/2001	Venezuela	2001	DD	FJ182015
8	98902890	Sumatra, Indonesia	1998	DD	AB189128
9	D3-MY05-33927	Kualalumpur, Malaysia	2005	DD	FN429917
10	98901590	Bandung, Indonesia	1998	DBD	AY912454
11	98901517	Sumatra, Indonesia	1998	DBD	AB189127
12	CO331/94	Thailand	1994	DSS	AY876494
13	D3-MY05-34640	Kualalumpur, Malaysia	2005	DD	FN429918
14	PF92/2956	Tahiti	1992	DSS	AY744682
15	98901437	Sumatra, Indonesia	1998	DSS	AB189126
16	PF89/320219	Tahiti	1989	DSS	AY744678

Helicase Motif I - VI , Catalytic Triad, and Cytotoxic T cell epitope of NS3 protein

In all strains analysed, helicase motives I, II. IV, V (Lin et al, 2006; Matusan et al., 2001) were well conserved. Differences were only found in the motif III at the amino acid D324E (strain CO331/94,D3-MY00-2236) and in the motif VI at the amino acid V452A (DENV-3/VE/BID/V1114/2001, CO331/94,D3-MY-0022366). The catalytic Triad (Lin et al, 2006), i.e., His 51, Asp 75, Ser 135 were well conserved in all strains analysed.

Ramapraba et al. Reported in 2007 that cytotoxic T cell response to T cell epitope $NS3_{422-431}$ (RVIDPRRCLK) increased in DHF patient compared to DF. This amino acid region (422-431) were well conserved in all strains analysed. List of peptide changes in NS3 protein are shown in table 4.

DENV-3 NS3 Nucleotida and amino acid homology

Analysis were done to 16 DENV-3 strains from Asia and America from the year 1989 to 2007. The results showed that the homology ranged from 92.8% (D3-MY05-33927 from Malaysia 2005 vs strain DENV-3/VE/BID/V1114/2001 from Venezuela 2001) to 99.9% (strain 98901517 vs strain 98901437 – both from Indonesia-, and strain D3-MY05-33927 vs strain D3-MY05-34640 – both from Malaysia). However, peptide analysis showed that this protein is highly conserved. The homology of peptides were between 98,2% to 100%. The lowest homology were between DS2207 (Indonesia, 2007) and strain CO331/94 (Thailand, 1994) as well as DS2207 with D3-MY00-22366 (Malaysia, 2000) (Table 5). These results with teh limited number of samples suggest that the NS3 nucleotide and peptide difference was not related to the disease severity but more to the geographical and year of isolation.

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Fig.1 : Multialignment of NS1 protein of DENV-1, DENV-2, DENV-3 Indonesian strains sequenced in this study. The B-cell epitopes are in bold.

```
D1 DS 27-06 ns1      1:DSGCVINWKGRELKCGSGIFVTNEVHTWTEQYKFQADSPKRLSAAIGKAWEEGVCGIRSA 60
D2 DS 31-06 ns1      1:DSGCVVSWKNKELKCGSGIFITDNVHTWTEQYKFQPESPSKLASAIQKAHEEGICGIRSV 60
D3 17-04  NS1        1:DMGCVINWKGKELKCGSGIFVTNEVHTWTEQYKFQADSPKRLATAIAGAWENGVCGIRST 60
d3 DS 002-06 NS1     1:DMGCVINWKGKELKCGSGIFVTNEVHTWTEQYKFQADSPKRLATAIAGAWENGVCGIRST 60
d3 DS 029-06 NS1     1:DMGCVINWKGKELKCGSGIFVTNEVHTWTEQYKFQADSPKRLATAIAGAWENGVCGIRST 60
d3 DSA 02-06 NS1     1:DMGCVINWKGKELKCGSGIFVTNEVHTWTEQYKFQADSPKRLATAIAGAWENGVCGIRST 60
d3 DS 22-07  NS1     1:DMGCVINWKGKELKCGSGIFVTNEVHTWTEQYKFQADSPKRLATAIAGAWENGVCGIRST 60
d3 DSA 02-06 NS1     1:DMGCVINWKGKELKCGSGIFVTNEVHTWTEQYKFQADSPKRLATAIAGAWENGVCGIRST 60
                        *.***..**..*********.*..**********..**..*..**..*.*.*.*****.

d1 DS 27-06         61:TRLENIMWKQISNELNHILLENDMKLTVVVGDVSGILAQGKKSIRPQPMEHKYSWKSWGK 120
D2 DS 31-06 ns1     61:TRLENLMWKQITPELNHILSENEVKLTIMTGDIKGIMQAGKRSLRPQPTELKYSWKTWGK 120
D3 17-04  NS1       61:TRMENLLWKQIANELNYILWENNIKLTVVVGDIIGVLEQGKRTLTPQPMELKYSWKTWGK 120
d3 DS 002-06 NS1    61:TRMENLLWKQIANELNYILWENNIKLTVVVGDIIGVLEQGKRTLTPQPMELKYSWKTWGK 120
d3 DS 029-06 NS1    61:TRMENLLWKQIANELNYILWENNIKLTVVVGDIIGILEQGKRTLTPQPMELKYSWKTWGK 120
d3 DSA 02-06 NS1    61:TRMENLLWKQIANELNYILWENNIKLTVVVGDIIGVLEQGKRTLTPQPMELKYSWKTWGK 120
d3 DS  22-07 NS1    61:TRMENLLWKQIANELNYILWENNIKLTVVVGDIIGILEQGKRTLTPQPMELKYSWKTWGK 120
d3 DSA 02-06 NS1    61:TRMENLLWKQIANELNYILWENNIKLTVVVGDIIGVLEQGKRTLTPQPMELKYSWKTWGK 120
                        **.**..****..***.**.**..***...**..* ...**....***.*.*****.***

d1 DS 27-06        121:AKIIGADVQNTTFIIDGPNTPECPDDQRAWNIWEVEDYGFGIFTTNIWLKLRDSYTQVCD 180
D2 DS 31-06 ns1    121:AKMLSTELHNHTFLIDGPETAECPNTNRAWNSLEVEDYGFGVFTTNIWLKLKERQDVFCD 180
D3 17-04  NS1 aa   121:AKIVTAEIQNSSFIIDGPNTPECPNASRAWNVWEVEDYGFGVFTTNIWLKLREMYTQLCD 180
d3 DS 002-06 NS1   121:AKIVTAEIQNSSFIIDGPNTPECPNASRAWNVWEVEDYGFGVFTTNIWLKLREMYTQLCD 180
d3 DS 029-06 NS1   121:AKIVTAEIQNSSFIIDGPNTPECPNASRAWNVWEVEDYGFGVFTTNIWLKLREMYSQLCD 180
d3 DSA 02-06 NS1   121:AKIVTAEIQNSSFIIDGPNTPECPNASRAWNVWEVEDYGFGVFTTNIWLKLREMYTQLCD 180
d3 DS  22-07 NS1   121:AKIVTAEIQNSSFIIDGPNTPECPNASRAWNVWEVEDYGFGVFTTNIWLKLREMHTQLCD 180
d3 DS  46-07 NS1   121:AKIVTAETQNSSFIIDGPNTPECPNASRAWNVWEVEDYGFGVFTTNIWLKLREMYTQLCD 180
                        **.......*..*.****.*.***...****..********.*********.......**

d1 DS 27-06        181:HRLMSAAIKDSKAVHADMGYWIESEKNETWKLARASFIEVKTCIWPKSHTLWSNGVLESE 240
D2 DS 31-06 ns1    181:SKLMSAAIKDNRAVHADMGYWIESALNDTWKIEKASFIEVKSCHWPKSHTLWSNGVLESE 240
D3 17-04  NS1 aa   181:HRLMSAAVKDERAVHADMGYWIESQKNGSWKLEKASLIEVKTCTWPKSHTLWSNGVLESD 240
d3 DS 002-06 NS1   181:HRLMSAAVKDERAVHADMGYWIESQKNGSWKLEKASLIEVKTCTWPKSHTLWSNGVLESD 240
d3 DS 029-06 NS1   181:HRLMSAAVKDERAVHADMGYWIESQKNGSWKLEKASLIEVKTCTWPKSHTLWSNGVLESD 240
d3 DSA 02-06 NS1   181:HRLMSAAVKDERAVHADMGYWIESQKNGSWKLEKASLIEVKTCTWPKSHTLWSNGVLESD 240
d3 DS  22-07 NS1   181:HRLMSAAVKDERAVHADMGYWIESQKNGSWKLEKASLIEVKTCTWPKSHTLWSNGVLESD 240
d3 DS  46-07 NS1   181:HRLMSAAVKDERAVHADMGYWIESQKNGSWKLEKASLIEVKTCTWPKSHTLWSNGVLESD 240
                        ..******.**..*************..*..**...**.*****.*.***************.

d1 DS 27-06        241:MIIPKIYGGPISQHNYRPGYFTQTAGPWHLGKLELDFDLCEGTTVVVDEHCGNRGPSLRT 300
D2 DS 31-06 ns1    241:MIIPKNFAGPVSQHNYRPGYHTQTAGPWHLGRLEMDFDFCEGTTVVVTEDCGNRGPSLRT 300
D3 17-04  NS1 aa   241:MIIPKSLAGPISQHNYRPGYHTQTAGPWHLGKLELDFNYCEGTTVVITENCGTRGPSLRT 300
d3 DS 002-06 NS1   241:MIIPKSLAGPISQHNYRPGYHTQTAGPWHLGKLELDFNYCEGTTVVITENCGTRGPSLRT 300
d3 DS 029-06 NS1   241:MIIPKSLAGPISQHNYRPGYHTQTAGPWHLGKLELDFNYCEGTTVVITENCGTRGPSLRT 300
d3 DSA 02-06 NS1   241:MIIPKSLAGPISQHNYRPGYHTQTAGPWHLGKLELDFNYCEGTTVVITENCGTRGPSLRT 300
d3 DS  22-07 NS1   241:MIIPKSLAGPISQHNYRPGYHTQTAGPWHLGKLELDFNYCEGTTVVITENCGTRGPSLRT 300
d3 DS  46-07 NS1   241:MIIPKSLAGPISQHNYRPGYHTQTAGPWHLGKLELDFNYCEGTTVVITENCGTRGPSLRT 300
                        ******...**.**********.**********.**.**..*******..*.**.*******

d1 DS 27-06        301:TTVTGKIIHEWCCRSCTLPPLRFKGEDGCWYGMEIRPVKEKEENLVKSMVSA         352
D2 DS 31-06 ns1    301:TTASGKLITEWCCRSCTLPPLRYRGEDGCWYGMEIRPLKEKEENLVNSLVTA         352
D3 17-04  NS1 aa   301:TTVSGKLIHEWCCRSCTLPPLRYMGEDGCWYGMKLDP                      337
d3 DS 002-06 NS1   301:TTVSGKLIHEWCCRSCTLPPLRYMGEDGCWYGMEIRPINEKEENMVKSLVSA         352
d3 DS 029-06 NS1   301:TTVSGKLIHEWCCRSCTLPPLRYMGEDGCWYGMEIRPINEKEENMVKSLVSA         352
d3 DSA 02-06 NS1   301:TTVSGKLIHEWCCRSCTLPPLRYMGEDGCWYGMEIRPINEKEENMVKSLVSA         352
d3 DS  22-07 NS1   301:TTVSGKLIHEWCCRSCTLPPLRYMGEDGCWYGMEIRPINEKEENMVKSLVSA         352
d3 DS  46-07 NS1   301:TTVSGKLIHEWCCRSCTLPPLRYMGEDGCWYGMEIRPINEKEENMVKSLVSA         352
                        **..**.*.**************..*********...*  ..... .......
```

Table 4. NS3 peptide sequence of DENV-3 strains

Strain	NS3-20	NS3-31	NS3-40	NS3-60	NS3-68	NS3-71	NS3-84	NS3-90	NS3-115	NS3-255	NS3-283	NS3-399	NS3-434	NS3-444	NS3-452	NS3-568	NS3-578	NS3-589
98902890 DD	D	F	V	H	N	S	R	Q	I	K	I	K	I	L	V	E	M	K
DENV-3/VE/BID/V1114/2001 DD	E	F	V	Y	N	S	R	Q	T	R	I	K	I	L	A	Q	M	K
DS2207 DD	E	L	I	Y	N	S	R	K	I	K	I	K	I	L	V	E	M	K
D3-MY05-33927 DD	E	F	V	Y	N	S	R	Q	I	K	I	K	I	L	V	E	M	K
D3-MY05-34640 DD	E	F	V	Y	N	S	R	Q	I	K	I	K	I	L	V	E	M	K
DS4607 DBD	E	F	V	Y	N	S	R	K	I	K	I	K	I	E	V	E	M	K
98901517 DBD	E	F	V	Y	S	S	R	Q	I	K	I	K	I	L	V	E	M	K
CO331/94 DBD	E	F	V	H	N	S	R	Q	I	R	V	R	I	L	A	E	M	R
D3-MYOO-22447 DBD	E	F	V	Y	N	S	R	Q	I	K	I	K	I	L	V	E	M	K
D3-MYOO-22366 DBD	E	F	V	H	N	S	R	Q	I	R	V	R	I	L	A	E	M	R
DSA0206 DSS	E	F	V	Y	N	S	R	K	I	K	I	K	S	L	V	E	M	K
98901437 DSS	E	F	V	H	N	N	K	Q	I	R	I	K	I	L	V	E	M	K
PF89/320219 DSS	E	F	V	Y	N	S	R	Q	I	K	I	K	I	L	V	E	M	K
PF92/2956 DSS	E	F	V	H	N	N	K	Q	I	R	I	K	I	L	V	E	M	K
98901403 DSS	E	F	V	Y	S	S	R	Q	I	K	I	K	I	L	V	E	V	K

Table 5. NS3 Nucleotide and amino acid sequence similarity among strains of DENV-3

Nucleotide	peptide														
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
A. DS4607	~	99.4	99.5	99.4	99.7	99.5	99.2	98.7	98.7	98.4	98.9	99.7	98.4	99.7	99.7
B. DSA0206	99.5	~	99.5	99.4	99.7	99.5	99.2	98.7	98.7	98.4	98.9	99.7	98.4	99.7	99.7
C. DS2207	99.0	99.3	~	99.2	99.5	99.4	99.0	98.5	98.5	98.2	98.7	99.5	98.2	99.5	99.5
D. 98901403	98.9	99.1	99.0	~	99.7	99.8	99.2	98.7	98.7	98.4	98.9	99.7	98.4	99.7	99.7
E. 98901437	99.1	99.4	99.2	99.6	~	99.8	99.5	99.0	99.0	98.7	99.2	100.0	98.7	100.0	100.0
F. 98901517	99.1	99.3	99.1	99.6	99.9	~	99.4	98.9	98.9	98.5	99.0	99.8	98.5	99.8	99.8
G. 98902890	97.5	97.7	97.6	97.8	98.0	97.9	~	99.2	99.2	98.9	99.0	99.5	98.9	99.5	99.5
H. PF89/320219	96.2	96.4	96.2	96.6	96.8	96.8	96.7	~	100.0	98.7	98.9	99.0	98.7	99.0	99.0
I. PF92/2956	96.0	96.2	96.0	96.4	96.7	96.6	96.6	99.7	~	98.7	98.9	99.0	98.7	99.0	99.0
J. CO331/94	93.0	93.4	93.0	93.4	93.5	93.4	93.6	94.0	93.8	~	98.9	98.7	100.0	98.7	98.7
K. DENV-3/VE /BID/V1114/2001	92.8	93.0	92.9	93.2	93.3	93.2	93.5	93.4	93.4	93.6	~	99.2	98.9	99.2	99.2
L. D3-MYOO-22447	98.8	99.0	98.8	99.5	99.4	99.4	97.9	96.6	96.4	93.4	93.1	~	98.7	100.0	100.0
M. D3-MYOO-22366	92.9	93.1	92.9	93.2	93.3	93.2	93.4	93.9	93.7	98.9	93.2	93.2	~	98.7	98.7
N. D3-MY05-33927	98.3	98.5	98.5	99.0	99.0	98.9	97.5	96.1	96.0	93.2	92.8	99.6	92.9	~	100.0
O. D3-MY05-34640	98.3	98.5	98.5	99.2	99.0	98.9	97.5	96.1	96.0	93.1	92.9	99.6	92.9	99.9	~

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The research title: Investigation of arbovirus in Jiangxi province, China, 2009

Abstract: An investigation was conducted to identify mosquito species and mosquito-borne arboviruses at three sites in Jiangxi province (Jiujiang, Shangrao and Jingdezhen cities), on the border of Fujian, Zhejiang, Hubei and Hunan provinces. Eastern of China. A total of 5905 mosquitoes representing 4 genera (Culex, Anopheles, Armigeres and Aedes) and 5 species were collected. *Culex tritaeniorhynchus* was the dominant mosquito species (75%(4575/5905)) in this collection, *Armigeres subalbatus* was 16% (961/5905).*Anopheles sinensis* was 6%(345/ 5905) and 23 *Aedes vexans* and 1 *Aedes albopictus* were collected. The mosquitoes were pooled by species and location, were homogenized, and the supernatant was inoculated into C6/36 cells and BHK21 cells. 4 strains of viruses were isolated from *Cex. tritaeniorhynchus*, and all of 4 virus strains is Japanese Encephalitis Virus, based on serology and molecular biology. The JEVs isolated in Jiangxi in 2009 are genotype I of JEV based on sequence of E gene of JEV. This investigation suggests that there are mosquitoes vectors of JEV and JEVs circulating in Jiangxi province still.

In additional
A paper has been published in Chinese in 2009, which was supported by last fiscal year of this program as following(Electronic version was attached):
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Review

Japanese Encephalitis in Mainland China

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CONTENTS:

SUMMARY: Japanese encephalitis (JE) is a seasonal epidemic disease with a 50-year recorded history in China. Its characteristics can be summarized as follows: (i) it is a seasonal epidemic disease; approximately 90% of cases are recorded in July, August, and September each year. The peak of JE onset is 1 month earlier in South China than in the north of the country; (ii) the disease is highly sporadic. It is rare for more than two cases to appear simultaneously in one family; (iii) most affected children are under 15 years old; (iv) the disease is widely distributed in all areas of the nation except Qinghai Province, Xinjiang Uygur Autonomous, and Tibet. Due to widespread application of the JE vaccine, the number of JE cases has decreased significantly nationwide, from 174,932 cases of morbidity in 1971 to 5,097 cases in 2005.

1. Introduction

Japanese encephalitis (JE) is an acute epidemic disease of the central nervous system (CNS) caused by infection with the Japanese encephalitis virus (JEV). JE mainly affects children and adolescents. According to the World Health Organization (WHO) statistics, approximately 35,000 cases of JE are reported each year, causing approximately 5,000 deaths—a mortality rate of 5-40%. Approximately 50% of JE patients present neurological and mental sequelae (1). JEV is transmitted by mosquitoes and the genus *Culex*, which is major vector. It is a perennial disease in tropical areas, but is clearly seasonal in temperate zones, with a peak incidence period between June and October each year. At present, JE is endemic in over 20 countries and areas, including the Pacific coastal areas of Far East Russia, Japan, China, North and South Korea, India, Vietnam, Laos, Myanmar, Thailand, Cambodia, the Philippines, Malaysia, Singapore, Bhutan, Indonesia, Nepal, Sri Lanka, Guam, Papua New Guinea, and Australia (2,3). The traditional endemic areas of JE are mainly distributed in the countries and regions of Asia, where epidemics are often reported. For example, an outbreak of JE in India in 2005 involved 5,737 reported cases and resulted in 1,344 deaths (4). At present, the areas of JE endemism are tending to expand. For instance, in 1995, an outbreak of JE occurred in Papua New Guinea and among the original inhabitants of the northern islands of Australia, and even appeared in the northern areas of Australian mainland (5,6). JE has consequently become one of the most prominent public health issues in the world.

China is the main region of JE endemism. Our data reveal the occurrence and epidemic outbreaks of JE in all provinces except Xinjiang Uygur Autonomous, Tibet, and Qinghai. JE is one of four arbovirus diseases currently prevalent in China (7). In this paper, we summarize the epidemics of JE in mainland China in recent years and the prevention measures taken there.

2. Incidence and mortality

JE case reporting is currently mandated by law in China. Since the establishment of a case reporting system in 1951, the incidence of JE has been recorded annually in China. Further, since 2004, each JE case has been electronically recorded and the data collected at the national level by the Chinese Center for Disease Control and Prevention (China CDC); the case reporting system has thus become both more sensitive and more efficient.

Historically, there have been two major JE epidemics. The first, in 1966, had an annual incidence of >15/100,000 nationwide, whereas the second, in 1971, was associated with 174,932 cases of morbidity and an incidence of 20.92/100,000 (8-10). Since the 1980s, JE vaccination has been widely used in our country and, as a consequence, the number of morbidities has declined gradually year by year. Prior to the 1990s, the annual morbidities numbered between 20,000 and 40,000.

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Table 1. The incidence and death of JE in 1996 - 2005

Year	No. of JE cases	Incidence (1/100,000)	No. of death cases	Mortality (1/100,000)	Case fatality (%)
1996	10,308	0.8660	379	0.0318	3.677
1997	10,060	0.8343	370	0.0307	3.678
1998	12,490	0.9977	510	0.0407	4.083
1999	8,556	0.6889	348	0.0280	4.067
2000	11,779	0.9489	375	0.0302	3.184
2001	9,795	0.7707	246	0.0194	2.511
2002	8,769	0.6548	229	0.0171	2.611
2003	7,860	0.5829	366	0.0271	4.6565
2004	5,422	0.4171	200	0.0154	3.6887
2005	5,097	0.3898	214	0.0164	4.1985
Total	90,136	–	3,237	–	–

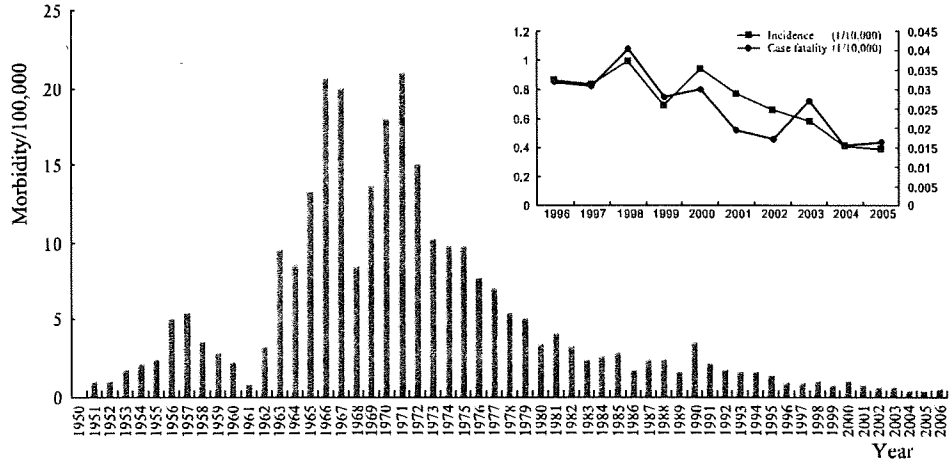

Fig. 1. JE incidence in 1951 - 2006, China.

However, the number of JE morbidities decreased from 11,779 to 5,097 between 2000 and 2005, the annual incidence declined from 0.9489/100,000 to 0.3898/100,000, the number of mortalities declined from 375 to 214, and the mortality rate also decreased from 0.0302/100,000 to 0.0164/100,000. Over this 6-year period, the annual mortality rate ranged from 2.51 to 4.66% (11,12). The trends in the incidence and mortality of JE in mainland China in recent years are illustrated in Figure 1 and Table 1.

3. Seasonal distribution of cases

JE cases are reported from January to December each year nationwide and reveal a low incidence in the periods from November to May. However, the number of morbidities in June is typically double that occurring in May. The number of morbidities occurring in July and August each year are generally pooled. The number of morbidities in August accounts for 41.14% of the total annual morbidities. The relatively high morbidity level is maintained in September, declines in October, and then decreases significantly in November. The number of morbidities between June and October accounts for 97.42% of the total annual morbidity. A summary of 10 years' data reveals that the monthly distribution trend of morbidity is consistent (Figure 2 and Table 2). Thus, the incidence of JE exhibits a clear seasonal distribution.

In addition, the monthly distribution data shows that both the incidence and mortality rates increase significantly from June, peak in July and August, and then decline from October onwards, indicating a consistent annual pattern in the incidence and mortality of JE (13).

The occurrences of JE cases in the north and south region of China have been shown to be slightly different. In the south, JE cases start to increase in July and decrease significantly in August, whereas in the north, JE cases begin to increase in August and clearly decrease in September (13-15).

4. Geographic distribution of cases

A total of 31 provinces in mainland China have reported JE cases; the exceptions including Qinghai Province, Xinjiang Uygur Autonomous, and Tibet. The cases are scattered in various endemic localities with no obvious aggregation of the disease. Based on the average annual incidence of JE between 1996 and 2005, the regions of endemism in mainland China can be classified into the following four groups (Figure 3).

4-1. Highly endemic areas

The average incidence in these areas is considered to be approximately >1/100,000. The areas include Sichuan Province, Guizhou Province, Chongqing City, Shaanxi Province, and Yunnan Province. Annually, the number of morbidities in these five areas accounts for 50% of the total cases nationwide, comprised as much as 74.1% of the total cases in 2002. The combined population of these areas, however, represents only 26% of the national population (10,11,13-16).

4-2. Moderately endemic areas

The average incidence in these areas is considered to be between 0.5/100,000 and 1/100,000. The areas include the