

# Distribution of Japanese Encephalitis Virus, Japan and Southeast Asia, 2016–2018

## Appendix

**Appendix Table 1.** List of primers for JEV\*

Primer name	Sequence	Sense/Antisense
JEV136F	5'- ATCAATATGCTGAAACGCGG –3'	Sense
JEV197R	5'- ACTACCCTCTTCACTCCCAC –3'	Antisense
JEV541F	5'- GTCATCGTGATCCCCACCTC –3'	Sense
JEV898F	5'- CTTGGMTGGATGCTTGGCAG –3'	Sense
JEV922R	5'- TGTTGCTGCCAAGCATCCA –3'	Antisense
JEV980R	5'- AAAGTGTATGCCGGAGCGACC –3'	Antisense
JEV1306F	5'- AAAGGAAGCATTGACACATG –3'	Sense
JEV1684F	5'- AGAGAACTTCTCATGGAATTTG –3'	Sense
JEV2335F	5'- TTTGGTGGAAATGCTCTGGAT –3'	Sense
JEV3135R	5'- CTCTCCGAAGACDGCCCTCTCAAG –3'	Antisense
JEV3635F	5'- TTCCTGCGGTTTTGGGGGCCCT –3'	Sense
JEV3689R	5'- GTGTAAGTGATGCCCCAAGCATCAG –3'	Antisense
JEV4330F	5'- ATGGCAGTGTCTTACGTGGT –3'	Sense
JEV5035F	5'- AATGGAGACATCATAGGC –3'	Sense
JEV5026F	5'- CTGGACTCCAATGGAGACAT –3'	Sense
JEV5950F	5'- TCTCCCATAACCAGTGCAAG –3'	Sense
JEV6113R	5'- GGCATGTGTATGTTGTCTA –3'	Antisense
JEV7115R	5'- GATGTGGTCACGTATTC –3'	Antisense
JEV7381F	5'- ACTGATGTGCCYGAAGTGGAAAG –3'	Sense
JEV7670R	5'- AAGGAGGGCTTGTGAGCYTTCTT –3'	Antisense
JEV7930F	5'- GGAGGCTGGAGCTACTACGC –3'	Sense
JEV8689F	5'- GCCAATGTCACCACAATGGC –3'	Sense
JEV9454F	5'- ATGGACGTGATATCAAGAGAAGA –3'	Sense
JEV10490R	5'- GTCTACCCAGTATCCGGTGG –3'	Antisense
JEV10527F	5'- GACTGGGTTAACAAATCTGACAA –3'	Sense

Primer name	Sequence	Sense/Antisense
JEV10564F	5'- CCTCAGAACCGTCTCGGAAG –3'	Sense
JEV10970R	5'- AGATCCTGTGTTCTTCCTCACCA –3'	Antisense
MAMD†	5'- AACATGATGGGRAARAGRGARAA –3'	Sense
cFD2‡	5'- GTGTCCCAGCCGGCGGTGTCATCAGC –3'	Antisense

\*JEV, Japanese encephalitis virus.

†Obtained from Scaramozzino et al. (1).

‡Obtained from Kuno et al. (2).

**Appendix Table 2.** Information on pig sera used for the isolation of JEV in this study\*

Site of sample collection	Month of sample collection	No. of tested		Isolates of JEV
		RT-PCR	Isolation	
<b>Thailand</b>				
Nakorn Nayok	May-August 2017	100	20	1
Prachinburi	July 2017	100	–	–
Sakon Nakhon	August 2017	51	12	0
Lop Buri	August 2017	40	–	–
Saraburi	August 2017	60	–	–
Tak	February 2018	256	7	0
Ratchaburi	February 2018	122	–	–
<b>The Philippines</b>				
Butuan, Agusan del Norte	March 2018	56	12	2
Santo Tomas, Davao del Norte	March 2018	12	–	–
Tagum, Davao del Norte	March 2018	6	–	–
Digos, Davao del Sur	March 2018	11	–	–
<b>Indonesia</b>				
Bengkulu, Sumatera (wild boar)	May 2017	26	–	–
Denpasar, Bali	October 2017	105	5	2
<b>Total</b>		<b>945</b>	<b>56</b>	<b>5</b>

\*JEV, Japanese encephalitis virus; RT-PCR, reverse transcription PCR.

**Appendix Table 3.** Information on mosquitoes used for the isolation of Japanese encephalitis virus in this study

Site (place, month/year)	Cx.																		
	<i>Culex</i>	<i>Cx.</i>	<i>Cx.</i>	<i>Cx.</i>	<i>pipiens</i>	<i>Cx.</i>	<i>Cx.</i>	<i>Cx.</i>	<i>Cules</i>	<i>Anopheles</i>	<i>A.</i>	<i>A.</i>	<i>Anopheles</i>	<i>Aedes</i>	<i>Ae.</i>	<i>Ae.</i>	<i>Armigeres</i>	<i>Mansonia</i>	
	<i>tritaeniorhynchus</i>	<i>pseudovishnui</i>	<i>vishnui</i>	<i>quinquefasciatus</i>	<i>pallens</i>	<i>gelidus</i>	<i>hutchinsoni</i>	<i>fsocephala</i>	sp.	<i>sinensis</i>	<i>vagus</i>	<i>campestris</i>	sp.	<i>albopictus</i>	<i>vexans</i>	<i>japonicus</i>	<i>sublbatus</i>	<i>uniformis</i>	
Yamaguchi, Japan (cowshed, 2014 total)	3457*	–	–	–	29	–	–	–	–	770	–	–	–	7	3	15	9	–	
Yamaguchi, Japan (cowshed, 2015 total)	1701	477	–	–	56	–	–	–	–	1323	–	–	–	33	12	6	–	–	
Yamaguchi, Japan (cowshed, 2016 total)	7218†	822	–	–	36	–	–	–	–	1424	–	–	–	33	11	1	–	–	
Khlong Luang, Thailand (cowshed, Nov 2015)	635	–	47	–	–	608	–	–	–	–	–	2	160	2	–	–	1	8	
Thanyaburi, Thailand (pigpen, Sep 2016)	1121	–	78	59	–	1180	–	–	–	–	18	–	–	–	–	–	–	13	
Mindanao, the Philippines (cowshed, Jul 2015)	63	–	13	–	–	21	3	3	–	–	5	–	–	–	–	–	–	–	
Mindanao, the Philippines (cowshed, Mar 2017)	176	–	63	–	61	41	–	74	4	–	–	–	–	–	–	–	–	–	
Bogor, Indonesia (cowshed, Oct 2015)	2	–	–	10	–	–	–	–	–	–	–	–	3	–	–	–	4	–	
Bogor, Indonesia (cowshed, Jun 2016)	24	–	28	1	–	22	13	–	32	–	96	–	2	6	–	–	6	–	
Bogor, Indonesia (cowshed, Nov 2016)	73	–	–	–	–	19	5	–	–	–	26	–	–	–	–	–	5	–	
<b>Total</b>	<b>14470</b>	<b>1299</b>	<b>229</b>	<b>70</b>	<b>182</b>	<b>1891</b>	<b>21</b>	<b>77</b>	<b>36</b>	<b>3517</b>	<b>143</b>	<b>2</b>	<b>165</b>	<b>81</b>	<b>26</b>	<b>22</b>	<b>25</b>	<b>21</b>	

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	<i>Culex</i>	<i>Cx.</i>	<i>Cx.</i>	<i>Cx.</i>	<i>Cx.</i>	<i>Cx.</i>	<i>Cx.</i>	<i>Cx.</i>	<i>Cx.</i>	<i>Cules</i>	<i>Anopheles</i>	<i>A.</i>	<i>A.</i>	<i>Anopheles</i>	<i>Aedes</i>	<i>Ae.</i>	<i>Ae.</i>	<i>Armigeres</i>	<i>Mansonia</i>
Site (place, month/year)	<i>tritaeniorhynchus</i>	<i>pseudovishnui</i>	<i>vishnui</i>	<i>quinqüefasciatus</i>	<i>pallens</i>	<i>gelidus</i>	<i>hutchinsoni</i>	<i>fscocephala</i>	sp.	<i>sinensis</i>	<i>vagus</i>	<i>campestris</i>	sp.	<i>albopictus</i>	<i>vexans</i>	<i>japonicus</i>	<i>sublbatus</i>	<i>uniformis</i>	

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\*The number of *Cx. tritaeniorhynchus* and *Cx. pseudovishnui* were mixed.

†Two Japanese encephalitis virus isolates were obtained.



**Appendix Figure.** Maximum-likelihood phylogeny of 1143 strains of Japanese encephalitis virus (JEV) based on the envelope (E) gene sequences (1500 nt from each viral genome). The tree was reconstructed using MEGA6 with 50 bootstraps under the generalized time reversible model. In this tree, blue, green, yellow, and purple indicate the JEV strains that were isolated from Japan, Thailand, the Philippines, and Indonesia, respectively. Arrows indicate the JEV strains obtained in the present study.

This analysis revealed that the new JEVs isolated from Japan, Thailand, the Philippines, and Indonesia belong to GI-a, GI-b, GIII, and GIV, respectively. Origin, year, and country for each JEV strain are provided in parentheses. bo, bovine; eq, equine; hu, human; G, genotype; MQ, mosquito; NA, data not available; sw, swine; wb, wild boar.

#### **Appendix References**

1. Scaramozzino N, Crance JM, Jouan A, DeBriel DA, Stoll F, Garin D. Comparison of flavivirus universal primer pairs and development of a rapid, highly sensitive heminested reverse transcription-PCR assay for detection of flaviviruses targeted to a conserved region of the NS5 gene sequences. *J Clin Microbiol.* 2001;39:1922–7. [PubMed](#)  
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2. Kuno G, Chang GJ, Tsuchiya KR, Karabatsos N, Cropp CB. Phylogeny of the genus *Flavivirus*. *J Virol.* 1998;72:73–83. [PubMed](#)