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Serotype distribution of dengue virus in Trang Province, Southern Thailand, 2024

Suwandee Sapcharoen[1*], Ativet Sawetadul[1], Tipattaraporn Panich[2], Khatayut Nigapruke[1]

[1]Regional Medical Sciences Center 12/1 Trang, Department of Medical Sciences, Trang Province, Thailand.
[2]National Institute of Health, Department of Medical Sciences, Nonthaburi Province, Thailand.

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ABSTRACT

Background: Dengue fever is a significant public health concern in Thailand, which consists of *Aedes* mosquitoes as a vector. The incidence of dengue fever has increased, positioning it among the top 10 infectious diseases affecting health in the Southern region of Thailand.

Objectives: This study aimed to examine serotypes and genotypes of the dengue virus causing dengue fever in Trang province in 2024.

Materials and methods: From April to August 2024, a total of 125 acute serum or plasma samples were collected from patients with dengue NS1 antigen screening test positive results and/or were clinically diagnosed with dengue fever. The samples were tested for dengue viral RNA detection and serotyping using real-time RT-PCR. Genotype identification was performed using whole-genome sequencing.

Results: Of these, 73 samples (58.4%) were detected for dengue viral RNA. The predominant serotype was DENV-3 (N=31, 42.5%), followed by DENV-2 (N=27, 37.0%), DENV-4 (N=13, 17.8%), and DENV-1 (N=2, 2.7%). By analyzing and comparing the whole genome sequence of 3 samples (TR-22, TR-37, and TR-38), all results were DENV-4 Genotype I. The percent of identity for genotype classification ranged from 95.1% to 99.3%.

Conclusion: The findings would be useful for establishing effective control and prevention measures for the outbreaks and could contribute to the development of diagnostic tests and vaccines in the future.

Introduction

Dengue fever is a major public health issue in many countries worldwide, with the *Aedes aegypti* mosquito serving as the primary vector for transmitting the disease to humans.[1,2] It is commonly found in tropical and subtropical regions of the Americas, the eastern Mediterranean, Africa, the western Pacific, and Southeast Asia.[3] Globally, the World Health Organization (WHO) estimates that more than 390 million people are infected with the dengue virus annually, with approximately 20,000 deaths attributed to the infection. Seventy percent of all cases have occurred in countries within Asia.[3,4,5,6]

Dengue virus (DENV) is a single-stranded RNA virus with a genome size of approximately 10.7 kilobases.[5] The viral structure includes a lipid envelope and various structural proteins, such as premembrane/membrane, capsid and envelope proteins, as well as non-structural proteins, including NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5.[6] The Dengue virus belongs to genus Flavivirus and family Flaviviridae, with four distinct serotypes: DENV-1, DENV-2, DENV-3 and DENV-4.[5,6] These serotypes

∗ **Corresponding contributor.**
Author's Address: *Regional Medical Sciences Center 12/1 Trang, Department of Medical Sciences, Trang Province, Thailand.*
E-mail address: suwandee.s@dmsc.mail.go.th
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are different based on epitopes, which are small units or positions on the viral surface that interact with antibodies, and genetic differences. Each serotype can be further subdivided into multiple genotypes based on nucleotide sequences, with no more than 6% variation within a single genotype.[6] DENV-1 includes six genotypes (genotype I, II, III, IV, V and VI), DENV-2 includes five genotypes (Asian I, Asian II, Asian/American, American, Cosmopolitan, and sylvatic genotypes), DENV-3 includes five genotypes (genotype I, II, III, IV and V), and DENV-4 includes five genotypes (genotype I, IIA, IIB, III and sylvatic genotypes).[7] All four serotypes are in circulation in Thailand.[3] Clinical symptoms of dengue fever may range from mild dengue fever (DF) to severe dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). DF presents with fever, including headache, bone or joint and muscular pains, nausea, vomiting, and small petechial hemorrhages on the skin. In severe cases, it can lead to severe fever (DHF and DSS), which is a result of circulatory failure, shock, and death.[8] Individuals who have been infected once develop lifelong immunity to the serotype that they were exposed to, and short-term immunity (approximately 3 to 12 months) to other serotypes, which means that they can be infected multiple times by different serotypes. Currently, rapid diagnostic tests that detect the NS1 antigen protein are used for screening dengue virus infections, and real-time RT-PCR assays are used for detecting viral RNA with high sensitivity and specificity, allowing confirmation of infection and serotype identification. Additionally, antibody testing for IgM and IgG specific to the dengue virus using the ELISA method can also confirm the presence of infection.[9]

The global incidence of dengue fever has risen, making it one of the top 10 global health concerns in 2019.[10.] In Thailand, continuous outbreaks of dengue fever have occurred, with a major outbreak occurring in 2013, when 154,773 cases were reported.[11] The high mortality rate was recorded in 80 cases, accounting for 0.13% in 2018.[12] According to the Epidemiology Surveillance Report 506 from the Department of Disease Control, the dengue situation from fiscal years 2018 to 2023 showed a significant outbreak in 2019, followed by a downward trend in 2020. However, in 2022, the number of infected cases and deaths started to rise again. In 2023, the highest number of deaths was reported with an outbreak comparable to 2019.[13] In 2023, there were 158,620 reported cases and 181 deaths (0.11%), while these cases were 3.5 times higher than in 2022.[14] DENV-2 serotype was the most predominant, accounting for 31.3% of cases, followed by DENV-1 at 29.9%. However, DENV-3 had a 5 to 16-fold increase compared to the last five years.[15] The shifting predominant serotypes over the years could be a significant factor contributing to the dengue outbreak, as patients lack immunity to the changing serotypes. Dengue fever exhibits seasonal variation with an increase in cases starting in April, peaking between June and August during the rainy season. The number of cases tends to decrease in September, but if the number of cases remains high toward the end of the year, it may lead to continued outbreaks in the following year.

The Department of Disease Control reported the situation for the year 2023 from January 1 to December 20, in regional health area 12, which includes seven provinces: Songkhla, Satun, Trang, Phatthalung, Pattani, Yala, and Narathiwat. The cumulative number of cases was 15,096 with an incidence rate of 301.64 per 100,000 people and resulted in 23 deaths.[16] In Trang, there were eight districts affected by the outbreak: Mueang Trang, Kantang, Yan Ta Khao, Palian, Huai Yot, Wang Wiset, Na Yong, and Ratsada.[16] According to the 2023 surveillance data, the total reported number of dengue fever cases in Trang was 1,089 cases. However, there is currently lack of data on dengue virus serotypes and genotypes of infected patients in Trang. Therefore, this study aimed at investigating serotypes and genotypes of dengue virus in Trang in 2024, to utilize this data for establishing effective control and prevention measures for the outbreaks. Additionally, the findings could contribute to the development of diagnostic tools and vaccines in the future.

Materials and methods

Sample Size

Sample size was calculated using Cochran's formula:

$n_o = (Z^2 \times p \times q) / e^2$

$n_o = (1.96^2 \times 0.5 \times 0.5) / 0.05^2$

Finite Population Correction

$n = n_o / (1 + ((n_o - 1) / N))$

$n = n_o / (1 + ((n_o - 1) / 1,089))$

$= 284.6 \approx 285$

Where:

n_0 = required sample size

Z = Z-score corresponding to the desired confidence level

p = estimated proportion of the population

q = 1 − p

e = acceptable margin of error

n = adjusted sample size

N = total population size

According to the 2023 surveillance data, the total reported number of dengue fever cases in Trang was 1,089. Based on this formula, assuming a confidence level of 95% (Z=1.96), an estimated population proportion (p) of 0.5, and a margin of error (e) of 0.05, the calculated sample size for a one-year study period was 285 participants. Since the current study spans only five months, the sample size was proportionally adjusted to 119 participants.

Specimen collection

From April to August 2024, acute serum or plasma was collected from 125 patients in Trang who tested positive using the NS1 antigen screening test using the careUS Dengue Combo NS1 & IgM/IgG kit (Wells Bio Inc., Republic of Korea) and/or those diagnosed with dengue fever by physicians. Inclusion criteria were an acute illness for 5 days post-symptom, including two or more of the following: fever, headache, nausea, vomiting, red

eyes, rash, joint pain, muscle pain. The age of patients ranged from 8 months to 75 years old. All specimens were confirmed to have dengue virus infection by quantitative reverse transcription polymerase chain reaction (qRT-PCR) at the Regional Medical Sciences Center 12/1 Trang and kept at -20°C until further use.

Nucleic acid extraction

Following the manufacturer's instructions, total nucleic acids were extracted from 200 µL of each serum or plasma using Zybio nucleic acid extraction kit (Magnetic bead method) with automated Zybio EXM3000 nucleic acid isolation system (Zybio Inc., China) with an elution volume of 80 µL. For whole genome sequencing, nucleic acids were extracted from 150 µL of each cell culture or serum or plasma using QIAamp Viral RNA Mini Kit (QIAGEN, Germany) with an elution volume of 30 µL.

Dengue virus serotyping

For the detection of dengue virus serotypes, one-step real-time RT-PCR was performed using a VIASURE Real-Time PCR Detection kit (Certest Biotec S.L., Spain) that targets the conserved region of the NS5 gene (DENV-1), envelope gene (DENV-2), prM gene (DENV-3) and NS2A gene (DENV-4) with qTOWER^{3}G Real-Time PCR (Analytik Jena, Germany) in 20 µL PCR reaction (5 µL of each extracted sample). A result is considered positive when the cycle threshold (Ct) value is less than 40, according to the manufacturer's instructions. The remaining serums were stored at -20 °C and transported under cold chain conditions to the National Institute of Health, Department of Medical Sciences.

Dengue virus isolation and amplification

Samples that tested positive for dengue virus serotyping via real-time RT-PCR with Ct values below 25 were cultured to propagate the virus for whole genome sequencing using next-generation sequencing. Dengue virus was cultured from 5 µL of serum or plasma samples in C6/36 cells (derived from *Aedes albopictus* mosquitoes) using Leibovitz's 15 cell culture medium (Life Technologies, USA) supplemented with Fetal Bovine Serum (FBS) (Sigma, Paraguay), Tryptose Phosphate Broth (TPB) (Himedia, USA) and Penicillin-Streptomycin 10,000 units/mL (Life Technologies, USA). The culture was incubated at 28±2 °C for 7 days. Cytopathogenic effects (CPE) were observed every 1-2 days. The culture supernatant containing the virus was collected, and the dengue virus serotype was confirmed by infecting the cells and performing the in-house Indirect Immunofluorescent Antibody (IFA) test. Positive results were indicated by the presence of green fluorescence in the cells under a fluorescence microscope (Nikon, Japan).

Library preparation and whole genome sequencing

Dengue virus whole genome nucleotide sequencing using Next Generation Sequencing (NGS). Five microliters of RNA were prepared for cDNA synthesis using the TURBO DNA-free™ Kit (Thermo Fisher Scientific Inc., USA). The RNA was then combined with one µL of Random Hexamers and used for first-strand cDNA synthesis with SuperScript™ IV Reverse Transcriptase and dNTP Mix (10 mM each) (Thermo Fisher Scientific Inc., USA). Second-strand cDNA synthesis was subsequently performed using Sequenase Version 2.0 DNA Polymerase (Thermo Fisher Scientific Inc., USA), followed by cDNA cleanup using Agencourt AMPure XP (Beckman Coulter Inc., USA). Twelve microliters of the eluted sample were used to prepare a DNA library (DNA preparation) with the Illumina DNA/RNA Prep Tagmentation Kit (Illumina, USA) and Illumina DNA/RNA UD Indexes Set D. Afterward, the library concentration was measured using a Qubit 2.0 fluorometer (Invitrogen, USA), and the samples were pooled with 0.2N NaOH and diluted with Hyb buffer (Illumina, USA). Finally, 600 µL of sample volume was loaded onto MiSeq Reagent Kit v3 (150 Cycles) and sequenced using the MiSeq sequencer platform (Illumina, USA), with single-end 150 bp read configuration. The sequenced data were in FASTQ format.

Data analysis of whole genome sequencing

The data obtained were analyzed for dengue virus genotypes using the BLAST (Basic Local Alignment Search Tool) program. The nucleotide sequence results from each sample were compared with reported nucleotide sequences of dengue virus available in the GenBank database, which can be accessed at https://blast.ncbi.nlm.nih.gov/Blast.cgi. The comparison of the results was based on the percent of identity. Additionally, the nucleotide sequence relationships based on the phylogeny of the dengue virus were constructed using MEGA 12 software (https://www.megasoftware.net). The TN+G+I model with the Maximum Likelihood method was applied, and the robustness of the analysis was evaluated using 1,000 bootstrap replicates. The nucleotide sequence of Zika virus (NC_012532) was designated as the outgroup.

Results

Patients who passed the screening using the NS1 antigen test and/or were diagnosed with dengue fever by physicians in Trang were enrolled. The number of suspected dengue cases during the study period by month (Figure 1). The highest number of patients was recorded in July 2024. A total of 125 samples were subsequently tested for real-time RT-PCR with serotyping. Dengue viral RNA was detected in 73 samples using real-time RT-PCR, accounting for 58.4%. The most common serotype was DENV-3 (N=31, 42.5%), followed by DENV-2 (N=27, 37.0%), DENV-4 (N=13, 17.8%), and DENV-1 (N=2, 2.7%), respectively (Figure 2).

It can be observed that the average rainfall has been steadily increasing since April, reaching a peak in August 2024. The average rainfall from April to August 2024 in Trang, reported by Trang's meteorological station, which was shown as 61.6, 227.6, 222.5, 238.8, and 514.8 millimeters, respectively, as illustrated in Figure 1. Due to limitations in the available data, statistical analysis of the relationship between rainfall and patient numbers was not conducted.

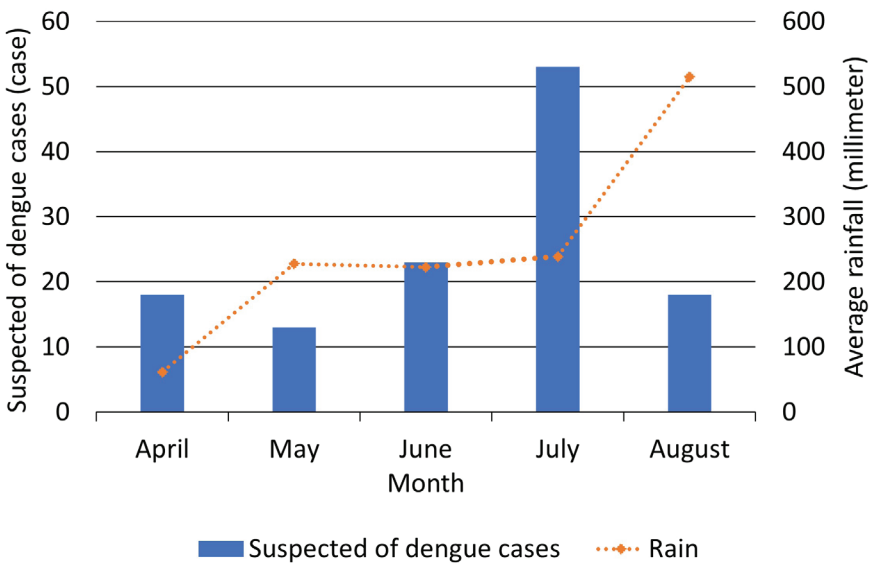
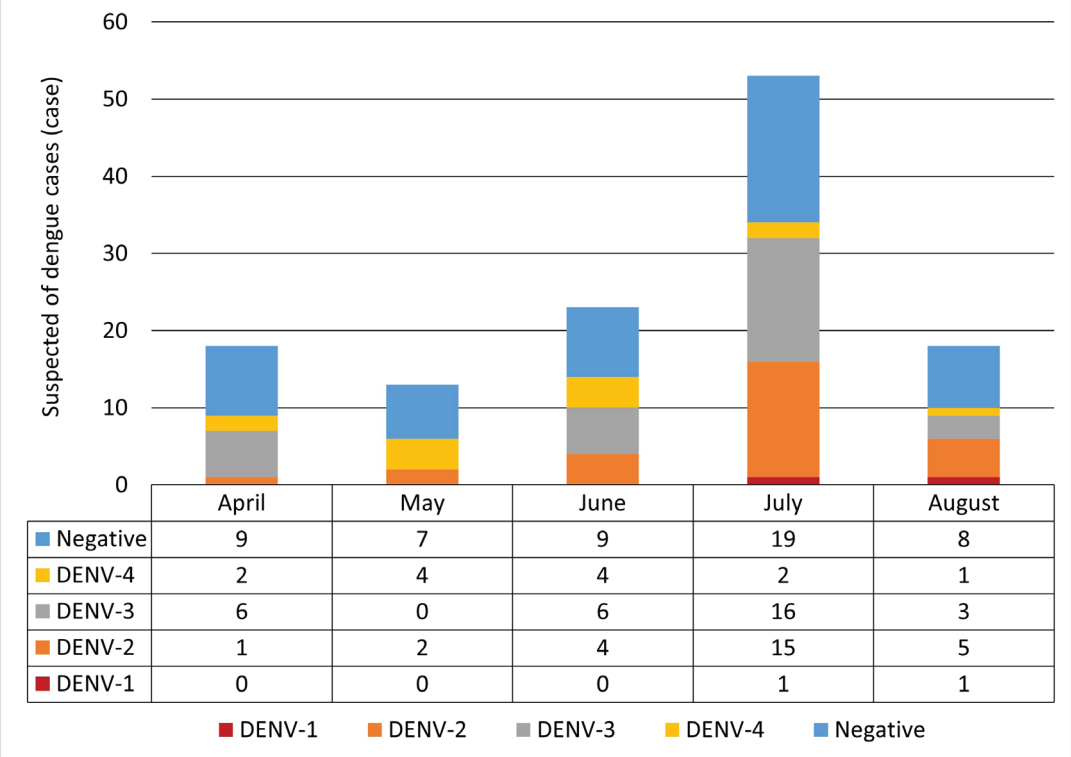


Figure 1. *Correlation between the number of suspected dengue cases and average rainfall during the study period in Trang.*



	April	May	June	July	August
Negative	9	7	9	19	8
DENV-4	2	4	4	2	1
DENV-3	6	0	6	16	3
DENV-2	1	2	4	15	5
DENV-1	0	0	0	1	1

Figure 2. *Monthly distribution of dengue cases from April to August 2024.*

Among the patients, the number of females (N=38) was slightly higher than males (N=35), accounting for a 1.1:1 ratio. Most infected individuals were in the age group 15-24 years (N=31), followed by the age group 10-14 years (N=21) and the age group 25-34 years (N=20), as shown in Table 1. The ages of the 73 dengue-infected patients ranged from 3 to 72 years old (mean 23.2 years old).

A total of 98 samples were screened with the NS1 antigen test, but only 69 samples showed concordant results with the detection of dengue viral RNA and serotype classification using real-time RT-PCR, including 55 samples with NS1 antigen positive and 14 samples with NS1 antigen negative. Additionally, 23 patients who were diagnosed with dengue fever tested positive for NS1 antigen but negative by real-time RT-PCR, while 6 patients were tested negative for NS1 antigen but positive by real-time RT-PCR, as shown in Table 2. Dengue NS1 antigen detection revealed a sensitivity of 90.2% (55/61) and specificity of 37.8% (14/37) when compared to the qRT-PCR results.

Table 1. Demographic characteristics of dengue patients, categorized by real-time RT-PCR positive and negative results.

Characteristics	Dengue virus positive, N (%)				Dengue virus negative N (%)	Total N (%)
	DENV-1	DENV-2	DENV-3	DENV-4		
Gender						
Male	0 (0.0)	12 (20.3)	18 (30.5)	5 (8.5)	24 (40.7)	59 (47.2)
Female	2 (3.0)	15 (22.7)	13 (19.7)	8 (12.1)	28 (42.4)	66 (52.8)
Total	2 (1.6)	27 (21.6)	31 (24.8)	13 (10.4)	52 (41.6)	125 (100.0)
Age (years)						
<1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	1 (0.8)
1-4	0 (0.0)	0 (0.0)	4 (80.0)	0 (0.0)	1 (20.0)	5 (4.0)
5-9	0 (0.0)	4 (22.2)	6 (33.3)	1 (5.6)	7 (38.9)	18 (14.4)
10-14	0 (0.0)	7 (33.3)	6 (28.6)	4 (19.1)	4 (19.1)	21 (16.8)
15-24	0 (0.0)	6 (19.4)	7 (22.6)	3 (9.7)	15 (48.4)	31 (24.8)
25-34	1 (5.0)	3 (15.0)	2 (10.0)	2 (10.0)	12 (60.0)	20 (16.0)
35-44	0 (0.0)	4 (40.0)	1 (10.0)	3 (30.0)	2 (20.0)	10 (8.0)
45-54	0 (0.0)	1 (11.1)	2 (22.2)	0 (0.0)	6 (66.7)	9 (7.2)
55-64	1 (25.0)	0 (0.0)	3 (75.0)	0 (0.0)	0 (0.0)	4 (3.2)
>64	0 (0.0)	2 (33.3)	0 (0.0)	0 (0.0)	4 (66.7)	6 (4.8)
Total	2 (1.6)	27 (21.6)	31 (24.8)	13 (10.4)	52 (41.6)	125 (100.0)

Table 2. Correlation between the NS1 antigen test and real-time PCR detection of dengue virus.

Parameter	Real-time RT-PCR positive, N (%)				Real-time RT-PCR negative, N (%)	Total, N (%)
	DENV-1	DENV-2	DENV-3	DENV-4		
NS1 antigen positive	2 (2.6)	16 (20.5)	27 (34.6)	10 (12.8)	23 (29.5)	78 (79.6)
NS1 antigen negative	0 (0.0)	4 (20.0)	2 (10.0)	0 (0.0)	14 (70.0)	20 (20.4)
Total	2 (2.0)	20 (20.4)	29 (29.6)	10 (10.2)	37 (37.8)	98 (100.0)

***Note**: Among the enrolled samples, there was no initial NS1 antigen test data for 27 samples. Of these, 12 samples were real-time RT-PCR positive (7 samples for DENV-2, 2 samples for DENV-3, and 3 samples for DENV-4), while 15 samples were real-time RT-PCR negative.*

Samples that tested positive for dengue virus serotyping using real-time RT-PCR with Ct values of less than 25 were cultured to obtain the virus for whole genome sequencing using NGS. The data was then analyzed and compared to determine the dengue virus genotype. A total of 12 samples were processed for culturing, but only 3 samples were cultured positive for further testing: TR-22, TR-37, and TR-38, all of which were DENV-4 serotype. The genotype analysis revealed that all 3 samples were identified as DENV-4 Genotype I, with the percent of identity ranging from 95.11% to 99.29%, as shown in Table 3.

By analyzing and comparing the nucleotide sequence data from samples with more than 80% genome coverage of the dengue virus using a phylogenetic tree, one sample (TR-38) was identified as DENV-4 of Genotype I, supported by a bootstrap value of 98%, as shown in Figure 3.

Table 3. Characterization of dengue virus using the BLAST program.

Sample number	Serotype	Genotype	% Identity	Similar GenBank Access Number
TR-22	DENV-4	Genotype I	95.11	KY451945
TR-37	DENV-4	Genotype I	98.85	LC410197
TR-38	DENV-4	Genotype I	99.29	LC410196

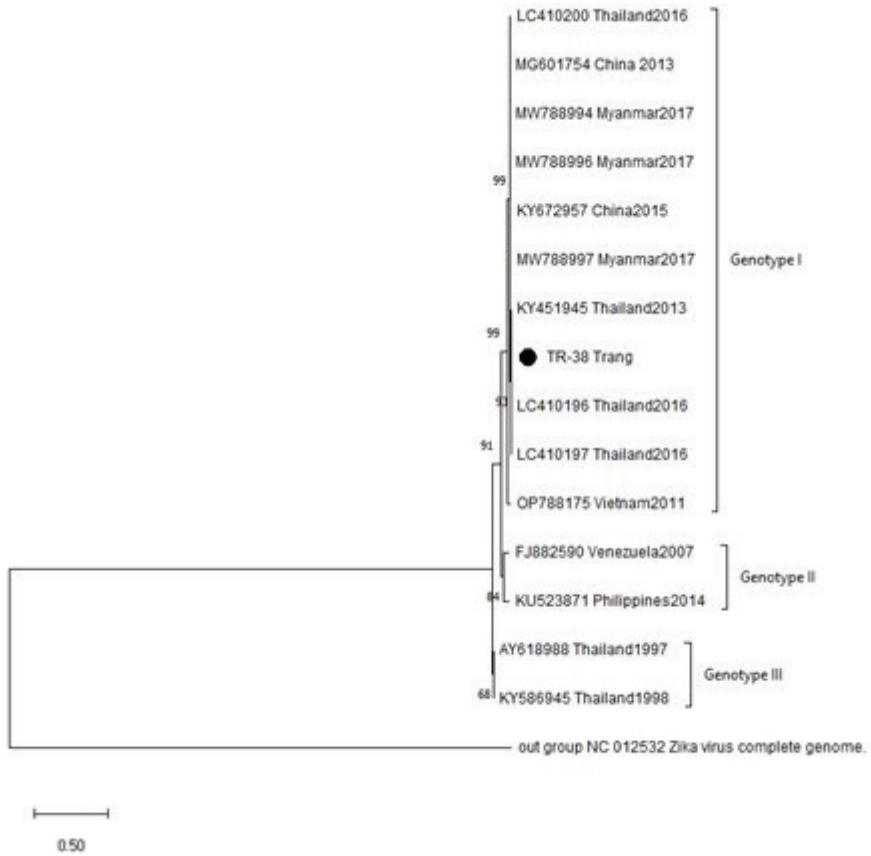


Figure 3. *Phylogenetic tree showing genotypic classification of DENV-4 dengue virus.*

Discussion

The outbreak of dengue fever often occurs in the rainy season. According to an epidemiological survey of dengue fever in Thailand between 2000 and 2011, the number of cases typically increases from June to August. Dengue prevention and control programs in Thailand are mainly based on hospital case reports within 24 hours in order to prevent transmission by spraying insecticides within 100 meters of the patient's house.[17] This study was limited to laboratory-confirmed dengue cases, and the incompleteness of patient data may have affected the accuracy of correlations drawn with provincial average rainfall data, particularly in Trang. All serotypes of dengue virus were found in the Southern region between 2005 and 2010, with DENV-1 being the most prevalent from 2005 to 2009. However, the trend in the epidemiological data of dengue serotypes shifted in 2010, and DENV-2 became the most common.[3] This study also found that

DENV-2 was the most prevalent, like the findings in 2010. The study in other regions of Thailand between 2005 and 2010 by Kriengsak Limkittikul *et al*., Central, Northern and Northeastern regions reported DENV-1 as the most prevalent serotype from 2005 to 2009, with DENV-2 being predominant in 2010.[3] Only few data of dengue serotype distribution in the upper Southern region of Thailand during from 2014 to 2021 revealed that 1,220 samples were positive accounting for 50.4%. The highest prevalent dengue serotype was DENV-1, accounting for 40.3%.[18] A study by Aranya Pinyorattanachot *et al*. revealed that epidemiological data on dengue fever in Thailand from 2014 to 2021 showed that the highest incidence rate occurred in 2018, followed by 2019, with a subsequent decline in 2020 and 2021, potentially due to the impact of the COVID-19 pandemic. Data from the Department of Medical Sciences on dengue virus serotype surveillance between 2005 and 2019 indicated that all four dengue virus serotypes (DENV-1 to DENV-4) have been co-circulating in Thailand. Notably, during the outbreak years, a distinct shift in the predominant circulating serotype was observed: DENV-3 was predominant in 2013, DENV-3 again in 2015, and DENV-1 became dominant in 2018.[18] Jacqueline Kyungah Lim *et al*. showed that dengue epidemics occur every 2-4 years in Ratchaburi province, Thailand.[19]

The study from other countries in Asia, Jean Claude Balingit *et al*. found an outbreak in Bali, Indonesia in 2022 where the ratio of female to male patients was 5:3, indicating a higher infection rate among females, like the findings in this study. Analysis of 48 patient samples revealed the following distribution: 28 samples of DENV-3, 14 samples of DENV-2, 5 samples of DENV-1, and 1 sample of co-infection between DENV-1 and DENV-3, with no cases of DENV-4 reported in this outbreak.[20] The study of Yu Kie Chem *et al*. on the molecular epidemiology of dengue virus in Malaysia from 2015 to 2021 analyzed 42,763 patient samples for serotype detection. The study found that DENV-2 and DENV-1 were the predominant serotypes, with detection rates of 35.9% and 33.4%, respectively, while DENV-3 and DENV-4 were also found in Malaysia.[21] A molecular characterization from dengue patients from 2019-2020 in northern Vietnam resulted in predomination of DENV-2.[22] In the Lao People's Democratic Republic, DENV-1 was predominant in 2010-2011 and in 2015, DENV-3 in 2012-2013 and DENV-4 in 2014 and 2016.[23] As previously described, the distribution of dengue virus serotypes appears to be influenced by the geographic localization of affected populations, correlates with variations in clinical disease severity, and is supported by laboratory-based diagnostic data.

Clinical symptoms of dengue fever range from mild flu-like symptoms to severe conditions, such as bleeding or shock, which can lead to death. Laboratory diagnosis during the acute phase is crucial for timely identification, and molecular techniques for detecting the viral genetic materials, such as qRT-PCR and RT-PCR, are the standard diagnostic methods. In developing countries with limited molecular diagnostic tools, serological tests are often used as alternatives, such as methods detecting NS1 antigen and antibodies (IgM and IgG) against the virus, including ELISA. For diagnosing dengue fever using rapid diagnostic tests, detecting the NS1 antigen is also a preliminary screening method that is fast and able to identify the NS1 antigen in patients with both primary and secondary infections. The NS1 protein can be detected up to 9 days after the onset of symptoms. However, the study by Mya Myat Ngwe Tun *et al*. in 2024 evaluated the newly developed Fujifilm Dengue non-structural antigen diagnostic kit and compared it to the SD Bioline NS1 antigen test kit with 140 samples. The rapid diagnostic test kit showed sensitivity, ranging from 88.6% to 94.3% and specificity of 100%. Nonetheless, real-time PCR remains the gold standard for diagnosis.[24] In this study, the careUS Dengue Combo NS1 & IgM/IgG kit showed 90.2% sensitivity and 37.8% specificity. The timing of sample collection may contribute to discrepancies between NS1 antigen and PCR results. These results may represent false-positive NS1 antigen detections since the samples were negative for DENV RNA. However, longitudinal studies show that NS1 antigen can persist longer than viral RNA, likely due to its extended half-life.[25] The study by Danielle Gyurech *et al*. reported a false-positive dengue NS1 antigen test in a traveller with an acute Zika virus infection.[26] However, this study did not include the detection of Chikungunya and Zika viruses, which are other co-circulating pathogens; there may have been cases of these infections.

This study could analyze the full genome nucleotide sequence of dengue virus using NGS for only 3 samples, probably due to the poor storage and/or transportation conditions of the samples. One potential limitation of this study is the possibility of viral inactivation during specimen transport from the Medical Science Center 12/1 Trang to the National Institute of Health, Nonthaburi, as the considerable distance and transit duration could have affected sample integrity. The study by Caio Santos de Souza *et al*. used samples with high viral genomic concentration for whole genome sequencing, whereas samples with insufficient viral genomic concentration were analyzed for the nucleotide sequence of the envelope (E) gene.[6] Patcharaporn Nonyong *et al*. suggested that the shorter fragments of PCR products have been more suitable for sequencing.[17] This method is beneficial for determining genotypes in future studies. Three samples from Trang were found to be DENV-4 Genotype I, while DENV-4 Genotype II has been reported in Indonesia.[27] In the Americas, DENV-4 Genotype IIb has been identified in Panama.[28] Alejandra Rojas *et al*. also revealed a DENV-4 Genotype II outbreak in Paraguay.[29] Thus, dengue genotype varies by geographical regions.

Limitation

None

Conclusion

Dengue fever is considered a significant public health issue, particularly in the Southern region of Thailand, which experiences substantial rainfall throughout the

entire year. It is important to annually monitor trends in the changing epidemiological patterns of dengue serotypes. By performing the viral RNA detection and serotype classification of dengue virus using real-time RT-PCR of 73 patient samples in Trang in 2024, all four dengue virus serotypes were identified, while the proportions of DENV-3, DENV-2, DENV-4, and DENV-1 were 42.5%, 37.0%, 17.8% and 2.7%, respectively. Furthermore, the whole genome sequencing of dengue virus from 3 samples using NGS revealed DENV-4 Genotype I. However, this study has limitations in analyzing genotypes due to the small sample size, and further studies are still needed. The findings from this study would be useful for establishing effective control and prevention measures for the outbreaks, and the data could also contribute to the development of diagnostic tools and vaccines in the future.

Ethical approval

The study was approved by the Institutional Review Board of the Department of Medical Sciences, the Ministry of Public Health, Thailand (DMSc-EC011).

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Conflict of interest

The authors declare no conflicts of interest.

CRedit authorship contribution statement

Suwandee Sapcharoen: conceptualization, investigation, data curation, writing original draft, review and editing; **Ativet Sawetadul**: investigation; **Tipattaraporn Panich**: conceptualization, investigation, data curation; **Khatayut Nigapruke**: supervision.

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