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Case Report

A cluster of dengue cases in travelers: A clinical series from Thailand

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Abstract: Dengue is an overlooked tropical disease for which billions of people are at risk. The disease, caused by a *Flavivirus* with four distinct serotypes, is transmitted primarily by urbanized *Aedes* mosquito species. The infection leads to a spectrum of clinical manifestations, with the majority being asymptomatic. Primary dengue and, to a greater extent, subsequent infection, mainly secondary dengue infection, are associated with increased severity. Increased global travel and recreational tourism expose naïve individuals to dengue, the most common arboviral infections in travelers. We describe a cluster of possible primary acute dengue infections in a group of 12 individuals who presented to Bangkok Hospital for Tropical Diseases in 2017. Infection was confirmed by dengue NS1 antigen and multiplex real-time RT-PCR. Nine individuals required hospitalization, and four developed dengue warning signs. The mean arterial pressure was significantly lower in the group with dengue warning signs. The period from the day of arrival in Thailand and the first day of symptoms was significantly shorter in adolescents with warning signs. Leukocytes, neutrophils, and platelets declined significantly at defervescence and were negatively correlated with day of illness. Six clinical isolates were identified as dengue serotype-1, with identical sequences suggesting that these patients were infected with the same virus.

Keywords: Dengue; *Flavivirus*; serotype-1; primary infection; dengue warning signs

1. Introduction

Dengue remains one of the leading causes of hospitalization in endemic regions.[1] Seventy percent of dengue infections occur in Asia, with annual estimates of over 390 million infections and 96 million cases.[2] Earlier descriptions of dengue reflected the morbidity of the illness. For example, in Africa, fatalities from dengue were described as "ka dinga pepo," which translates into "devil's disease."[3] In Philadelphia, Pennsylvania, USA, dengue was referred to as break-bone fever. Europeans spoke the word dengue during the trans-Atlantic slave trade.[4-6]

The causative agent of dengue is a single-stranded RNA *Flavivirus*, which was first isolated during World War II in Japan and Hawaii.[7,8] This is an arbovirus of great concern to public health, similar to other *Flaviviridae* such as the yellow fever virus, Japanese en-

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cephalitis virus, Zika virus, and West Nile virus.[9] The dengue virus (DENV) has four distinct serotypes (DENV1-4) and two to six genotypes within each serotype. Persons infected with one serotype mount a life-long immunological response to the same serotype but only a transient protection lasting several months against the remaining serotypes.[10] Serotype-specific severity has been previously described.[11-13]

The majority of individuals infected with DENV do not develop symptoms and serve as a reservoir of the virus.[14-16] Serological surveys reported that 80% of individuals in populations living in endemic regions are seropositive.[17] Humans develop symptoms after an average incubation period of 3 days.[18] These symptoms include high-grade fever, loss of appetite, headache, gastrointestinal symptoms, myalgia, rash, and bleeding.[19] The clinical course is self-limiting, and symptomatic patients recover within 10 days after the onset of symptoms. Subsequent infections with heterogeneous serotypes are associated with endothelial dysfunction that causes intravascular plasma to leak into the extravascular spaces.[20] This disequilibrium of intravascular volume peaks at defervescence and causes hypotension.[21] Without intervention to correct low blood pressure, the clinical course can rapidly deteriorate, leading to profound shock and multiple organ failure. To date, no approved anti-viral agent exists for dengue. Although several candidate vaccines have shown promising results, only one vaccine, Dengvaxia®, has been approved for ages 9-45 years. However, use of this vaccine is limited to individuals with a prior dengue infection; it is not recommended for dengue-naïve individuals.

In Thailand, vaccination against dengue is approved, and dengue is holo-endemic in the country, with an observed trend of large outbreaks occurring every 4 years. All four serotypes are in circulation at any given time of the year. Bangkok, a metropolitan city located in the tropical region of Thailand, is habitat for the Asian tiger mosquito (*Aedes albopictus*) and *Aedes aegypti*.[22] These particular species of mosquitoes are highly urbanized, nest in close proximity to humans, and have heterogeneous biting trends. These behaviors facilitate dengue transmission in an environment without proper measures against mosquito bites, leading to sporadic cases that are reported between outbreaks. Case rates are highest in the last quarter of the year. The predominant circulating serotype in Thailand from 2007 to 2017 was DENV-1.[23] In 2017, there were 6,107 cases reported from Bangkok through the end of October, and 53,190 cases were reported by the end of the year in the entire country. As Thailand is a top travel destination due to its diversity of landscapes and cultures, the country attracts millions of travelers annually, and some fall victim to tropical diseases such as dengue.[24-29]

Considerable research has focused on secondary dengue infection due to its association with disease severity.[13] Literature on primary dengue infection is limited, especially in regard to reports concerning infections in regions with decreasing dengue-naïve populations. Why only some individuals are symptomatic during primary infection while others remain asymptomatic is unknown.[30] Primary symptomatic infections can be severe, leading to hospitalization and sometimes death.[31-33]

This report examines a cluster outbreak of dengue in a group of travelers (n=12) who visited Bangkok in October 2017 for recreational tourism (training in martial arts, Muay-Thai kick-boxing). These travelers were from three different provinces of mainland China, Hainan, Guizhou, and Zhejian. None of the affected individuals had a history of symptomatic dengue infection.

2. Materials and Methods

2.1 Patient cohort

We retrospectively reviewed the de-identified clinical data for a group of 12 Chinese travelers who presented to Bangkok Hospital for Tropical Diseases with symptoms of fever during mid-October through mid-November. Dengue infection was diagnosed using a commercially available lateral flow kit (Biosynex, Swiss S.A, Fribourg, Switzerland) that detects the non-structural protein 1 (NS1) antigen. In addition, six aliquots of

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120 µL of serum corresponding to six patients were collected from the available left-over specimens of routine investigations for further serological and molecular analyses. For the remaining six cases, there were no left-over acute-phase specimens available for molecular analysis. The patients reported no travel history outside of Bangkok, and all patients stayed in the dormitories where the recreational activities took place. Some tropical diseases, such as murine typhus and leptospirosis, were not considered in the differential diagnosis due to negative history of exposure to rodents or flash floods in Bangkok. There was no clinical suspicion of co-infections with other arboviruses circulating in Bangkok or cosmopolitan viruses.

2.2 Dengue detection, serotyping, and envelope nucleotide sequencing

Dengue antigen testing was performed using a commercially available lateral flow kit that detects the NS1 antigen (Biosynex). For dengue serology, a commercially available kit was used to detect IgM and IgG (SD, Bioline, Sankt Germany) in accordance with the manufacturer's protocol. Dengue serotype was determined using a Genesig dengue subtyping multiplex kit, a commercial one-step reverse transcriptase multiplex real-time PCR assay conducted according to the manufacturer's instructions (Primerdesign, Chandler's Ford, United Kingdom) using a CFX96TM real-time PCR cycler (Bio-Rad, Hercules, CA, USA). Viral RNA was extracted from serum using a QIAamp Viral RNA mini kit and then further amplified using a One-step RT-PCR kit (Qiagen, Germany) with specific primers for position 935–2419 of the envelope region, as published in a previous study.[34] The sequencing reaction was prepared using BigDye terminator v1.1 (Applied Biosystems, USA) and run on an ABI 3130XL sequence analyzer. The obtained sequences were manually aligned to reference sequences of DENV-1 Mochizuki (AB074760) using AliView.[35] The newly generated sequences were deposited in GenBank (accession numbers LC642557 - LC642562).

2.5 Phylogenetic analysis

Dengue genotype identification and epidemiological characteristics were analyzed using a phylogenetic method. A nucleotide sequence dataset was prepared including the newly obtained sequences, NCBI Blast result sequences exhibiting sequence identity >99.60, recent epidemic strains from 2015-present, and the reference genotype sequence. The sequence dataset was aligned using AliView,[35] and a maximum-likelihood (ML) tree was constructed in IQ-TREE under TIM2+F+4, with 1000 ultrafast bootstrap replicates.[36, 37] The ML tree was visualized using Figtree.

2.6 Data analysis

Clinical and laboratory data were retrieved anonymously using a standardized case record form. All information was transferred onto an electronic data sheet using Microsoft Excel and analyzed using Statistical Package for the Social Sciences (SPSS) software and Graph Pad Prism software. Exposure duration was estimated by calculating the number of days from the time of arrival in Bangkok until symptoms developed. Time of presentation referred to the duration from the first symptom to hospital arrival. Acute phase was defined as the febrile period with a body temperature ≥38.5°C and presenting to the hospital within 1 to 3 days after onset of symptoms. Defervescence was estimated based on the time at which body temperature decreased below 37.7°C and the patient remained afebrile. Relative bradycardia was identified when a dissociation of pulse and temperature was observed. A leukocyte count less than 3,500 cells/µL was defined as leukopenia, mild neutropenia when neutrophils were in the range 1,000 to 1,500 cells/µL, and severe neutropenia when there were <500 neutrophils/µL. To segregate the patients into groups based on dengue with or without warning signs of severe dengue, we re-

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ferred to the WHO 2009 dengue clinical classification guideline.[38] Further, we compared adolescents (10 to 19 years of age) and adults (20 years and older) by grouping them into Group A = with warning signs, and Group B = without warning signs. Distributive frequencies of clinical findings were estimated using all-group analysis. All categorical variables were tested for observed frequencies using the Fisher's exact test. The Wilcoxon and Mann-Whitney tests were used for non-parametric testing. Non-parametric Spearman's rank correlation coefficients were calculated to explore correlations among the hematological indices, clinical data, and days of illness.

3. Results

The patient group included eight male adolescents, three male adults, and one female adult. The median age was 18 years, with an interquartile range (IQR) of 16.25-22.25 years. Ten patients became symptomatic within a window of less than 4 weeks of arrival (Figure A1). The median time of presentation to the hospital was 2 days, with an IQR of 1.25-2.75 days. All patients presented during the febrile phase, except for a single patient who presented at the time of defervescence.

Nine of the 12 patients required hospitalization, and four of the nine hospitalized patients developed dengue warning signs, which included hepatomegaly. The frequency of headache was significantly higher among patients requiring hospitalization (Table A1). The median recorded body temperature was 39.1°C (IQR: 38.5-39.5°C), the median heart rate was 82 beats per minute (IQR: 68.5-95.5 beats per minute), and 83.3% of the patients had relative bradycardia. Other common symptoms among the 12 patients were headache (58%), nausea (42%), myalgia, rash, and loss of appetite (33%), dizziness, vomiting, diarrhea, and bleeding (17%), and abdominal pain and fatigue (8%). No patient in this group reported retro-orbital pain or arthralgia. The median leukocyte count was 3,900/μL (IQR: 2,250-4,800/μL). The median neutrophil, lymphocyte, and platelet counts were 2,730/μL, 489/μL, and 173,000/μL, respectively. The mean arterial blood pressure was significantly lower in the group with warning signs (p=0.001) compared with the group without warning signs (Table A2).

At the time of presentation, all 12 cases were positive for dengue NS1, but serology was available only for two cases. A primary-type antibody response (positive IgM and negative IgG) was detected in the first case, and neither anti-IgM nor IgG were detected in the latter case. Molecular multiplexing analysis revealed that six patients were infected with the DENV-1 serotype. The median cycle threshold among these six cases was 19.88 (IQR: 18.86-25.44).

3.1. Descriptive analysis of warning signs by age group

We did not observe any statistically significant differences in the clinical parameters between adolescents and adults. However, the level of alanine transaminase was significantly elevated in adolescents compared with adults (p=0.024), as shown in Table A3. Among the adolescents, the duration of exposure was significantly shorter in the group with warning signs (Group A) compared with the group without warning signs (Group B), with a median of 10 days versus 16 days (p=0.046). Similarly, in the adolescents, the body mass index was significantly lower in Group A (p=0.025). All adolescents in Group A had relative bradycardia, as did 80% of those in Group B. Among adults, there were no significant differences observed between those who developed warning signs and those who did not, as shown in Table 1.

Table 1. Comparison of patients with and without dengue warning signs by age group.

	Adolescents (8)		p value	Adults (4)		p value
	Group A (3)	Group B (5)		Group A (1)	Group B (3)	

	Exposure duration, days	10 (9)	16 (12)	**0.046**	8 (8)	20 (4)	0.655
	Body mass index kg/m^2	21.0 (17.6)	22.7 (22.0 – 26.2)	**0.025**	23.1 (23.1)	25.5 (22.9)	0.655
	Temperature °C	39.3 (39.0)	39.0 (38.3 – 39.5)	0.539	37.3 (37.3)	39.2 (38.4)	0.180
	Heart rate, beats per minute	82.0 (82.0)	82.0 (60.0 – 98.5)	0.878	68 (68)	80 (70)	0.180
	Mean arterial pressure, mmHg	73.3 (68.0)	79.3 (73.0 – 85.0)	0.101	70.3 (70.3)	97 (90.6)	0.180
	Day of presentation, days	2 (2)	2 (1.5 - 2.5)	0.491	5 (5)	1 (1)	0.157
	Hospitalization, n (%)	3 (100)	3 (60)	0.464	1 (100)	2 (66.7)	0.505
	Fever, n (%)	3 (100)	5 (100)	NA	1 (100)	3 (100)	NA
	Dizziness, n (%)	0	2 (40)	0.464	0	0	NA
	Relative bradycardia, n (%)	3 (100)	4 (80)	1.000	0	3 (100)	0.250
	Hepatomegaly, n (%)	1 (33.3)	0	0.375	1 (100)	0	0.250
	Headache, n (%)	2 (66.7)	3 (60)	1.000	0	2 (66.7)	1.000
	Myalgia, n (%)	1 (33.3)	1 (20)	1.000	0	2 (66.7)	1.000
	Arthralgia, n (%)	0	0	NA	0	0	NA
	Rash, n (%)	0	2 (40)	0.464	1 (100)	1 (33.3)	1.000
	Bleeding, n (%)	1 (33.3)	0	0.375	1 (100)	0	0.250
	Loss of appetite, n (%)	2 (66.7)	1 (20)	0.464	1 (100)	0	0.250
	Nausea, n (%)	2 (66.7)	1 (20)	0.464	1 (100)	1 (33.3)	1.000
	Vomiting, n (%)	1 (33.3)	1 (20)	1.000	0	0	NA
	Abdominal pain, n (%)	1 (33.3)	0	0.375	0	0	NA
	Diarrhea, n (%)	1 (33.3)	1 (20)	1.000	0	0	NA
	Fatigue, n (%)	0	1 (20)	1.000	0	0	NA

The data are in median (IQR) or countThe data are presented as median IQR and actual numbers with percent. In some only the median and the 25th percentile are provided due to low number of cases. Day of presentation represents the day the patient visited the hospital after developing symptoms. Group A refers to cases with warning signs. Group B refers to cases without warning signs.

3.2. Hematological profile during illness

The median hemoglobin level and hematocrit percentage were within the normal ranges, and no significant differences were observed during the febrile phase or at defervescence. However, the median leukocyte, neutrophil, and platelet counts had significantly declined at defervescence. There were no other statistically significant differences observed with regard to other white blood cell counts, as shown in Table 2.

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Table 2. Hematological profile during the febrile and defervescent phases of dengue infection.

Parameters	Febrile phase	Defervescence	p value
Hemoglobin, g/dL	14.70 (13.20 – 14.90)	15.15 (13.85 – 15.70)	0.121
Hematocrit, %	43.30 (39.60 – 44.00)	44.45 (42.93 – 45.35)	0.130
Leukocytes /μL	3,900 (1,900 – 3,900)	1,900 (1,750 – 2,100)	**0.010**
Neutrophils /μL	2,730 (1,540 – 3,640)	1,330 (1,225 – 1,470)	**0.010**
Lymphocytes /μL	546 (234 – 720)	642 (516 – 797)	0.260
Monocytes /μL	156 (75 – 265)	130 (60 – 209)	0.231
Eosinophils /μL	0 (0 – 26)	26 (0 – 66)	0.138
Basophils /μL	216 (136 – 420)	292 (210 – 402)	0.481
Platelets /μL	174,000 (140,000 - 214,000)	103,000 (63,250 – 172,750)	**0.015**

The data are provided as median (IQR).

3.3 Analysis of correlations between clinical parameters

We observed some correlations within the hematological profile in this cohort. Heart rate at presentation was positively correlated with recorded body temperature (r_s=0.696, p=0.014). A positive correlation was also observed between heart rate and leukocyte count (r_s=0.606, p=0.039) and neutrophil count (r_s=0.606, p=0.039). Similarly, the monocyte count was positively correlated with leukocyte count (r_s=0.711, p=0.0002), neutrophil count (r_s=0.711, p=0.0002), and lymphocyte count (r_s=0.510, p=0.015), as shown in the Supplementary Material in Appendix A (Figures A2-A3).

Leukocyte, neutrophil, and platelet counts were negatively correlated with the day of illness, whereas lymphocyte count and hemoglobin and hematocrit levels were positively correlated with day of illness, as shown in Figure 1.

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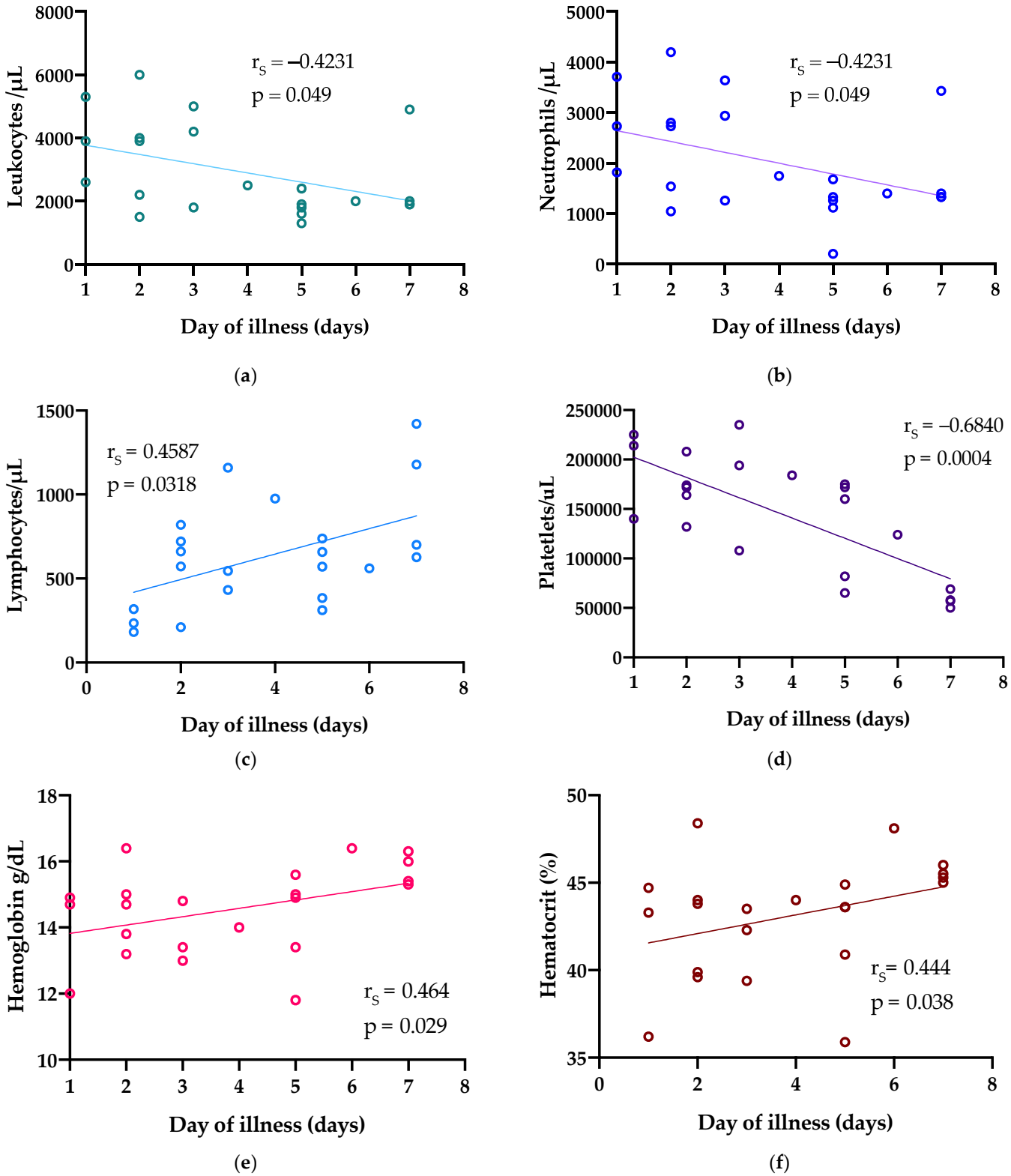

Figure 1. Spearman's correlation analysis of hematological profile kinetics during the acute phase. Trends observed with leukocytes **(a)**, neutrophils **(b)**, lymphocytes **(c)**, platelets **(d)**, and hemoglobin **(e)** and hematocrit **(f)** levels.

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3.4 Phylogenetic analysis

Samples from six cases were subjected to envelope gene sequencing. All sequences were identical. The obtained sequences were searched for similarity to other DENV-1 sequences using the NCBI Blast database. Our sequences had the highest nucleotide identity (99.87%) to a DENV-1 strain from China collected in November 2017 (MT856285.1) and a strain isolated from a traveler returning to China after visiting Thailand in January 2019 (MN923098.1). Our strain shared 99.60-99.80% similarity with China, Hong Kong, and Thailand strains detected in 2018-2019. Myanmar strain 2015 (MG894863) was isolated the earliest among these highly related strains.

The phylogenetic tree revealed our DENV-1 sequences were genotype I and clustered together with China, Hong Kong, and Thailand strains detected in 2018-2019 (Figure 2). This cluster formed a clade with strains from Thailand, Myanmar, China, Singapore, and Malaysia isolated in 2015-2017, apart from strains from India and Vietnam that circulated during the same period. Interestingly, six patients were infected with DENV-1, which shared identical envelope region sequences. This cluster was further identified as genotype I, which was first recognized in Thailand in 1981 and has continuously circulated for over 30 years.[39] Furthermore, this genotype has dynamically circulated all over Southeast Asia for decades.[40-42] The specimens were collected in November 2017, which was the early phase of the Thailand DENV-1 wave lasting from 2018 to the present. The sequences related to strains including Thailand, China, and Myanmar strains isolated from 2015-2017 were consistent with the epidemic reported in this region.[43, 44] Moreover, the sequences related to Thailand and China strains were isolated later, in 2018-2019. Although the patients in the present study exhibited symptoms and then recovered while in Thailand, travel-associated transmission must be considered, especially between holo-endemic and other endemic areas.[45]

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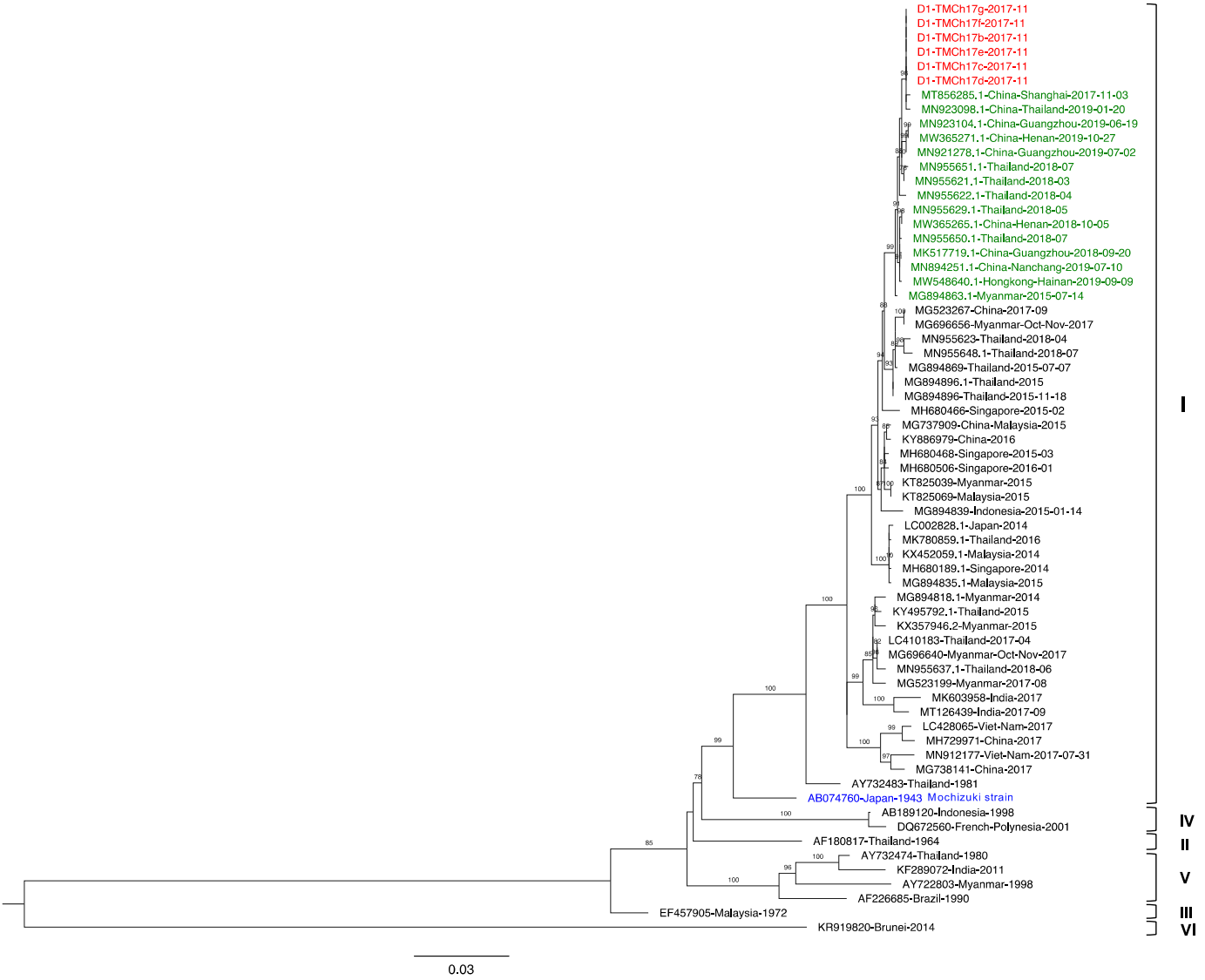

Figure 2. Maximum-likelihood tree of DENV-1 based on the 1485-bp envelope region sequence. The tree was constructed using TIM2+F+4 with 1,000 bootstrap replications. Bootstrap values >75 indicate relationship to the adjacent branch. Sequences obtained in the present study, related sequences sharing nucleotide identity >99.60%, and the Mochizuki strain are indicated in red, green, and blue, respectively. DENV-1 genotypes are indicated by the brackets to the right.

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4. Discussion

Here, we described the clinical manifestations, including blood cell kinetics, during acute dengue infection in a group of travelers in Thailand. All patients arrived in Bangkok via a direct flight from mainland China, and dengue infections were detected at the hospital within a 4-week window period. In estimating the median exposure period, we excluded two patients whose arrival dates were uncertain. As a result, the shortest exposure duration was 4 days, and the longest was 23 days. In previously described studies, seroconversion occurred in 2.9% of 447 travelers after traveling for a month in endemic regions and in 6.7% in 104 travelers after traveling for 6 months.[46, 47]

Clusters of dengue have been defined as two or more cases within a perimeter of 200 meters in which onset of symptoms occurred within 3 weeks.[48] In this cohort, the majority of patients (9/12) became symptomatic within 3 weeks. In travelers, dengue is suspected if an individual becomes symptomatic within 2 weeks after returning from an endemic region. Some patients in this cohort were from provinces in China with a history of dengue outbreaks.[49] Six were symptomatic within 2 weeks of arrival, whereas the remaining patients became symptomatic more than 2 weeks after arrival. However, the first two patients who fell ill remained in Thailand for an extended duration beyond 2 weeks and likely had acquired the virus in Bangkok.

An earlier description of primary dengue infection among children living in endemic regions was reported to be mild, and hospitalization was infrequent.[15] Others have reported observations of primary dengue infections in up to 15% of children (median±SD age: 7.9±2.9 years) and 34% of adults (median±SD age: 26.6±9.9 years).[50, 51] Previous studies have demonstrated that age is associated with symptomatic illness in dengue.[30] Further, viremia was demonstrated to be lower in asymptomatic cases compared to symptomatic cases.[16] Animal studies have suggested that interferons alpha, beta, and gamma are crucial at various time points for controlling the dengue virus within the host.[52] Interferons are cytokines with anti-viral properties, and the non-structural viral proteins of the dengue virus may potentially inhibit the interferon response in order to evade the innate anti-viral immune response. Interferons also induce cells to express the major histocompatibility complexes, and some of these human leukocyte antigen alleles have demonstrated some degree of protection from infection. All patients in our cases were healthy adolescents and young adults; this age group might be more likely to develop symptoms during dengue infection.

In Central America, Nicaragua 36% of children with primary dengue due to DENV-1 infection required hospitalization.[53] Primary dengue infections reportedly occur in as many as 97% of adults from non-endemic regions visiting endemic regions.[54] Although we were unable to determine the sequence of infection in some cases in this cohort, 75% of the patients required hospitalization. The prototype DENV-1 serotype is known as the Mochizuki strain, which later diverged to genotypes I, II, III, IV, and V in different geographic regions.[55] In animal studies, DENV-1 has been demonstrated to have an extended duration of viremia compared with other serotypes. DENV-1 virulence has also been described elsewhere.[56] We suspect that patients infected with the DENV-1 serotype have a higher chance of becoming symptomatic during primary infection.

All patients in the present study described a history of high-grade fever and were febrile, except for a single patient who presented at the time of defervescence. In this cohort, headache was the predominant symptom along with fever and associated with hospitalization (Table A1). Other typical features of dengue-like rash and bleeding were present in a subset of cases. No patients exhibited lymphadenopathy, retro-orbital pain, or arthralgia. In addition, there was no clinical evidence of plasma leakage, which can occur primarily with secondary dengue infection. A striking observation was that 80% of patients had relative bradycardia, which was reported in up to 60% of patients in a previous prospective study.[57]

This phenomenon of relative bradycardia, known as the Liebermeister's rule, has been described as occurring in several tropical diseases caused by intracellular, atypical gram-negative bacteria, parasites, or viruses.[58-60] In resource-limited settings without

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available point-of-care rapid diagnostic kits, this clinical finding can be useful when considering the differential diagnosis, especially in regions where other arboviruses such as chikungunya virus or Zika virus co-circulate, as these other viruses are not known to cause relative bradycardia.[61] Furthermore, bradycardia in dengue-infected patients can result from direct virus-associated myocardial injury, which often is benign, transient, and self-limiting.[62] Whether relative bradycardia is associated with primary infection is unknown. In this cohort, there was no difference in having bradycardia with and without warning signs.

Hepatomegaly—one of the seven dengue warning signs—was not exhibited in a majority of the adolescents and adults. Liver involvement in dengue is associated with increased severity.[63] In this cohort, the liver enzymes were not analyzed in all cases. We observed some mild to moderate elevation of transaminase. Transaminitis in dengue often is transient, either due to direct viral injury of liver cells or irreversible hypoxic insult from shock.[64] Impairment of liver function in dengue infection contributes to the hemorrhagic features characteristic of dengue.[65, 66] We previously demonstrated that increased expression of interleukin-8 is associated with hepatitis and bleeding in dengue infection.[67] A predominant increase in aspartate aminotransferase compared to alanine transaminase is a typical pattern of transaminitis in dengue, but this is often confounded by increased consumption of acetaminophen to treat the common symptoms.[68] In this cohort, gastrointestinal symptoms were reported in some adolescents but rarely in adults. A predominance of gastrointestinal symptoms after primary dengue infection was reported in a previous dengue cohort in Chinese patients naïve to dengue in Singapore.[54] Diarrhea is a common problem associated with travel and can be misleading as gastroenteritis, potentially leading to an unnecessary course of antimicrobials.[69] Other causes like dengue should be considered when a new onset of diarrhea occurs in conjunction with high-grade fever in tropical regions.[70] Similarly, in this cohort, diarrhea occurred earlier during the illness, overlapping symptoms of gastroenteritis.

Some limitations in this study include not being able to demonstrate primary infections in all cases or identify the serotype in all cases. As all patients presented promptly to the hospital following the onset of symptoms, diagnostics focused mainly on antigen detection, and anti-IgM and IgG levels were only available in two cases, which indicated a primary infection. We also were only able to obtain serum from six patients. In addition, due to the retrospective nature of this report, we were unable to directly interview the patients to obtain information regarding their knowledge, attitudes, and practices regarding dengue and its prevention while visiting endemic regions. We loosely associated age and infecting serotype as playing a role in whether patients become symptomatic during primary infection. Further research is required to understand what factors are in play in asymptomatic dengue patients that could help in the development of new therapeutics against dengue infection.

Advocacy to promote awareness about dengue infection and transmission of the virus will help travelers visiting endemic regions take all necessary precautionary steps to prevent dengue by preventing mosquito bites. In conclusion, dengue remains a potential problem in travelers to endemic regions. In addition, cluster outbreaks can occur in groups traveling together in the absence of adequate precautionary measures.

Supplementary Materials: Figure A1: Exposure duration in endemic region. Figure A2: Analysis of correlations between heart rate and temperature, leukocyte count, and neutrophil count. Figure A3: Correlations between monocyte count and other white blood cell indices. Table A1: Descriptive analysis of the patient cohort. Table A2: Descriptive analysis of cases involving dengue warning signs. Table A3: Descriptive analysis by age group.

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W.M., W.P., and T.S.; visualization, H.I., J.P., E.E.N., W.N., and T.S.,; supervision, W.N., W.P., E.E.N., and T.S.; project administration, H.I., W.M., P.P., and L.C.; funding acquisition, W.N., E.E.N., and T.S. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University (MUTM 2019-017-01, approved on 15 March 2019)

Informed Consent Statement: Written informed consent has been obtained from the patients to publish this paper.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available to ensure the privacy of the study participants.

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Appendix A

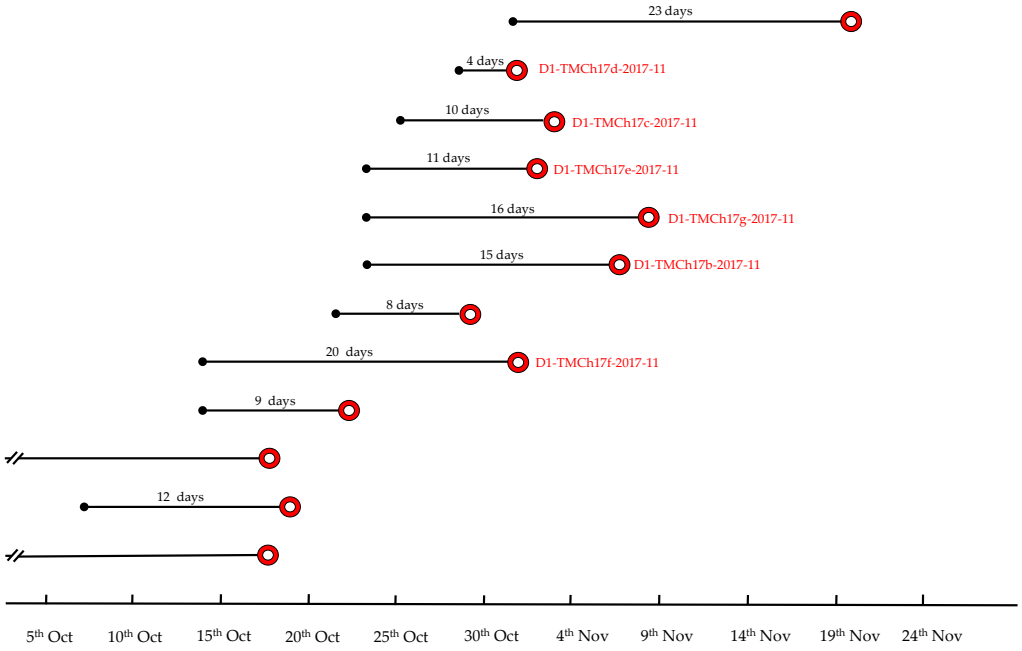
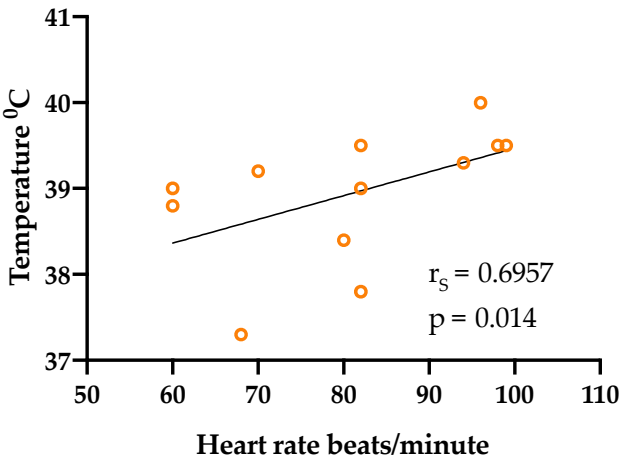
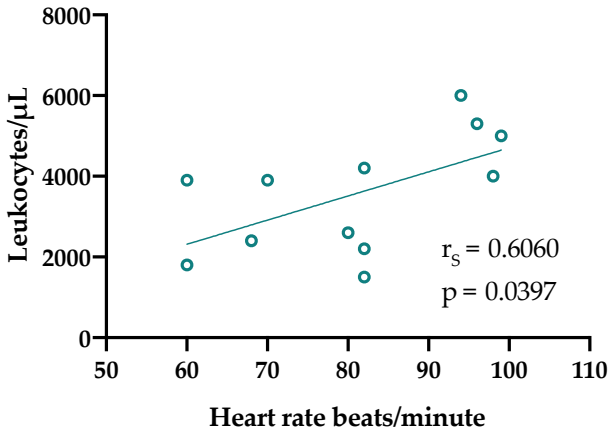


Figure A1. Time from date of arrival in Bangkok until symptoms developed, indicated in red with the sequence reference number.





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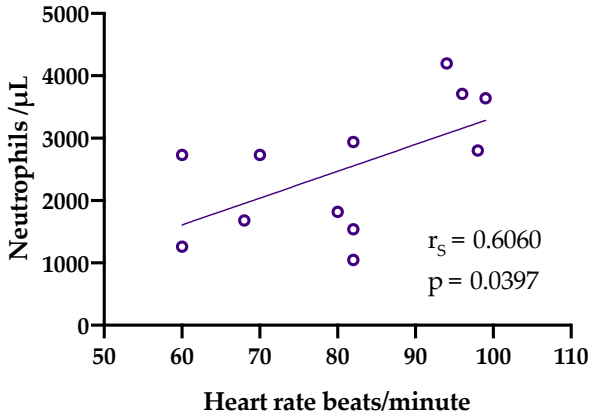
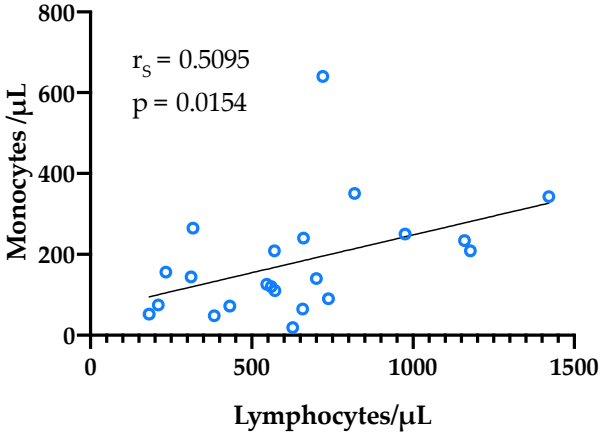
(a) (b)

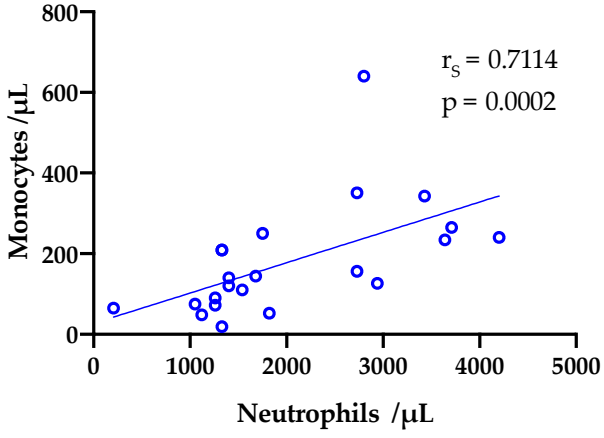


(c)

Figure A2. Spearman's correlation analysis of heart rate and temperature, leukocyte count, and neutrophil count. (**a**) Temperature and heart rate; (**b**) Leukocyte count and heart rate; (**c**) Neutrophils and heart rate.



(a)



(b)

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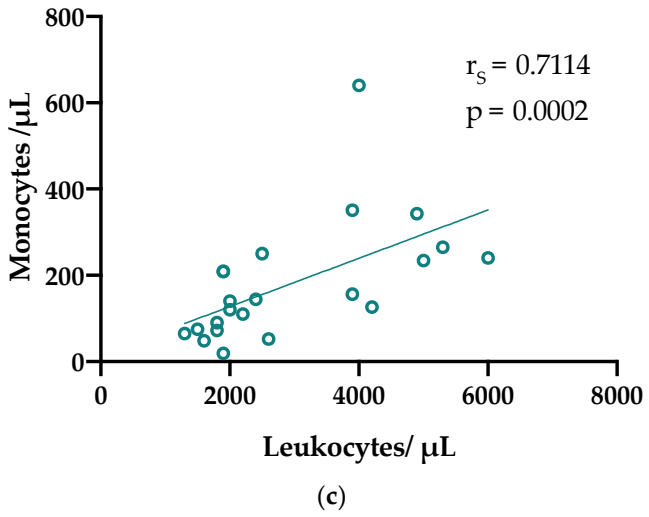

Figure A3. Spearman's correlation of monocyte count with other white blood indices (**a**) Monocytes with lymphocytes; (**b**) Monocytes with neutrophils; (**c**) Monocytes with leukocytes.

Table A1. Descriptive analysis of the patient cohort.

	Dengue infection (12)	Inpatient (9)	Outpatient (3)	p value
Exposure duration, days	11.5 (8.7 – 17.0)	13.50 (9.25 – 19.0)	8 (4)	0.295
Age, years	18.0 (16.2 – 22.2)	17.0 (15.5 – 21.5)	19 (16)	0.576
Body mass index kg/m^2	22.7 (21.2 – 25.2)	21.9 (21.0 – 24.1)	24.68 (22.60)	0.229
Temperature °C	39.1 (38.5 – 39.5)	39.3 (38.7 – 39.5)	38.80 (37.80)	0.225
Hear rate, beats per minute	82.0 (68.5 – 95.5)	82.0 (74.0 – 97.0)	70 (60.00)	0.192
Mean arterial pressure, mmHg	77.1 (71.5 – 90.0)	74.6 (70.6 – 89.3)	82.00 (79.33)	0.166
Day of presentation, days	2.0 (1.2 – 2.7)	2.0 (1.5 – 3.0)	2 (1)	0.370
Dizziness	2 (16.7)	2 (22.2)	0	1.000
Relative bradycardia	10 (83.3)	8 (88.9)	2 (66.7)	0.455
Hepatomegaly	2 (16.7)	2 (22.2)	0	1.000
Headache	7 (58.3)	7 (77.8)	0	**0.045**
Myalgia	4 (33.3)	3 (33.3)	1 (33.3)	1.000
Rash	4 (33.3)	4 (44.4)	0	0.491
Bleeding	2 (16.7)	2 (22.2)	0	1.000
Loss of appetite	4 (33.3)	4 (44.4)	0	0.491
Nausea	5 (41.7)	5 (55.6)	0	0.205
Vomiting	2 (16.7)	1 (11.1)	1 (33.3)	0.455
Abdominal pain	1 (8.3)	1 (11.1)	0	1.000
Diarrhea	2 (16.7)	2 (22.2)	0	1.000
Fatigue	1 (8.3)	1 (11.1)	0	1.000
Hemoglobin, g/dL	14.70 (13.25 – 14.97)	14.70 (13.30 – 14.95)	13.80 (13.00)	0.781
Hematocrit, %	43.40 (39.67 – 43.95)	43.50 (40.95 – 44.35)	39.90 (39.40)	0.309
Leukocytes /µL	3900 (2250 – 4800)	3900 (2400 – 4800)	2400 (1500)	0.308

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Neutrophils /μL	2730 (1575 – 3465)	2730 (1680 – 3675)	1680 (1050)	0.308
Lymphocytes /μL	489 (253 – 705)	572 (276 – 769)	312 (210)	0.229
Monocytes /μL	150 (83 – 258)	234 (91 – 308)	126 (75)	0.309
Eosinophils /μL	0 (0 – 46)	0 (0 – 39.50)	0 (0 – 0)	0.613
Basophils /μL	68 (137 – 417)	216 (138.45 – 426)	408 (120)	0.926
Platelets /μL	173000 (134000 – 212500)	174000 (136000 – 219500)	164000 (82000)	0.309
AST /IUL	36.50 (31.50 – 45.25)	33 (22.50 – 40.50)	40 (39)	0.101
ALT /IUL	16.50 (13.25 – 59.00)	17 (11 – 45)	16 (14)	0.653
Ct value	19.88 (18.86 – 25.44)	19.95 (18.04 – 25.62)	19.82 (19.82)	0.770

The data are in median (IQR), including frequencies with percentages. In some variables, only the median and the 25th percentile are provided due to low number of cases. Inpatient refers to cases hospitalized and outpatient refers to cases not requiring hospitalization.

Table A2. Descriptive analysis of cases with dengue warning signs.

	Warning signs (4)	No warning signs (8)	p value
Exposure duration, days	9.5 (8.2 – 10.7)	16.0 (10.0 – 20.7)	0.087
Age, years	15.5 (11.0 – 24.5)	19.0 (17.0 – 22.2)	0.230
Body mass index kg/m^2	21.1 (18.4 – 22.6)	23.7 (22.6 – 27.1)	**0.042**
Temperature °C	39.1 (37.7 – 39.4)	39.1 (38.5 – 39.5)	0.797
Hear rate, beats per minute	82.0 (71.0 – 91.0)	81.0 (62.0 – 97.0)	1.000
Mean arterial pressure, mmHg	71.8 (68.5 – 74.3)	85.0 (76.0 – 95.4)	**0.017**
Day of presentation, days	2.5 (2.0 – 4.5)	2.0 (1.0 – 2.0)	0.082
Hospitalization, n (%)	4 (100)	5 (62.5)	0.491
Dizziness	0	2 (25)	0.515
Relative bradycardia	3 (75)	7 (87.5)	1.000
Hepatomegaly	2 (50)	0	0.091
Headache	2 (50)	5 (62.5)	1.000
Myalgia	1 (25)	3 (37.5)	1.000
Rash	1 (25)	3 (37.5)	1.000
Bleeding	2 (50)	0	0.091
Loss of appetite	3 (75)	1 (12.5)	0.067
Nausea	3 (75)	1 (12.5)	0.222
Vomiting	1 (25)	1 (12.5)	1.000
Abdominal pain	1 (25)	0	0.333
Diarrhea	1 (25)	1 (12.5)	1.000
Fatigue	0	1 (12.5)	1.000
Hemoglobin, g/dL	14.05 (12.35 – 14.92)	14.75 (13.35 – 15.42)	0.444
Hematocrit, %	43.05 (37.72 – 43.95)	43.40 (39.67 – 44.42)	0.865
Leukocytes /μL	3050 (1900 – 3900)	4100 (2450 – 5225)	0.173
Neutrophils /μL	2135 (1330 – 2730)	2870 (1715 – 3692)	0.173

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Lymphocytes /μL	502 (283 – 757)	432 (235 – 705)	0.734
Monocytes /μL	133 (81 – 302)	189 (87 – 258)	0.734
Eosinophils /μL	0 (0 – 43)	0 (0 – 46)	0.762
Basophils /μL	303 (156 – 444)	266 (132 – 417)	0.734
Platelets /μL	191000 (124500 – 212500)	168000 (134000 – 217250)	0.734
AST /IUL	30.5 (14)	36.5 (32.5 – 154.25)	0.739
ALT /IUL	41 (9)	16.5 (13.75 – 97)	0.737
Ct value	25.62 (25.62)	19.75 (17.22 – 19.92)	0.064

The data are in median (IQR), including frequencies with percentages. In some variables, only the median and the 25th percentile are provided due to low number of cases.

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Table A3. Descriptive analysis by age group.

	Adolescent (8)	Adults (4)	p value
Exposure duration, days	11.5 (9.7 – 16.0)	14.0 (52.0 – 2.2)	1.000
Age, years	17.0 (14.5 – 18.5)	25.0 (20.7 – 30.7)	**0.006**
Body mass index kg/m^2	21.9 (21.0 – 24.1)	24.3 (22.9 – 29.5)	0.062
Temperature ^{o}C	39.1 (38.8 – 39.5)	38.8 (37.5 – 39.8)	0.607
Hear rate, beats per minute	82.0 (65.5 – 97.0)	75.0 (68.5 – 92.0)	0.493
Mean arterial pressure, mmHg	74.8 (71.5 – 81.3)	93.8 (75.4 – 104.7)	0.126
Day of presentation, days	2.0 (2.0 – 2.7)	1.5 (1.0 – 4.2)	0.522
Hospitalization, n (%)	4 (100)	5 (62.5)	1.000
Dizziness	2 (25)	0	0.515
Relative bradycardia	7 (87.5)	3 (75)	1.000
Hepatomegaly	1 (12.5)	1 (25)	1.000
Headache	5 (62.5)	2 (50)	1.000
Myalgia	2 (25)	2 (50)	0.547
Rash	2 (25)	2 (50)	0.547
Bleeding	1 (12.5)	1 (25)	1.000
Loss of appetite	3 (37.5)	1 (25)	1.000
Nausea	3 (37.5)	2 (50)	1.000
Vomiting	2 (25)	0	0.515
Abdominal pain	1 (12.5)	0	1.000
Diarrhea	2 (25)	0	0.515
Fatigue	1 (12.5)	0	1.000
Hemoglobin, g/dL	14.75 (13.25 – 14.97)	14.25 (12.45 – 15.97)	0.799
Hematocrit, %	43.55 (40.27 – 43.95)	41.60 (37.12 – 47.12)	0.610
Leukocytes /μL	3250 (2500 – 4800)	4100 (2450 – 5225)	0.932
Neutrophils /μL	2275 (1575 – 3465)	2870 (1715 – 3692)	0.932
Lymphocytes /μL	559 (342 – 779)	432 (235 – 705)	0.308
Monocytes /μL	135 (81.50 – 223.50)	189 (87 – 258)	0.308
Eosinophils /μL	0 (0 – 50.37)	0 (0 – 46)	0.613
Basophils /μL	312 (137.47 – 451.50)	266 (132 – 417)	0.396
Platelets /μL	157000 (114000 – 204500)	168000 (134000 – 217250)	0.396
AST /IUL	39 (32.50 – 272)	36.5 (32.5 – 154.25)	0.297
ALT /IUL	17 (16.5 – 205)	16.5 (13.75 – 97)	**0.024**
Ct value	22.61 (17.29 – 25.80)	19.75 (19.68)	0.355

The data are in median (IQR), including frequencies with percentages. In some variables, only the median and the 25th percentile are provided due to low number of cases.

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