DENGUE VIREMIA AND DENGUE SEROPREVALENCE IN BLOOD DONORS, BANGKOK, THAILAND

Aumnad Khongsup[1], Parichart Permpikul[1], Panisadee Avirutnan[2,3,4], Adisak Songjaeng[2,3], Nattaya Tangthawornchaikul[4], Tanapan Prommool[4], Nuntaya Punyadee[2,3], Dumrong Mairiang[2,3,4] and Chunya Puttikhunt[2,3,4]

[1]Department of Transfusion Medicine, [2]Division of Dengue Hemorrhagic Fever Research, [3]Siriraj Center of Research Excellence in Dengue and Emerging Pathogens, Faculty of Medicine Siriraj Hospital, Mahidol University; [4]Molecular Biology of Dengue and Flaviviruses Research Team, Medical Molecular Biotechnology Research Group, National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Bangkok, Thailand

Abstract. Dengue virus (DENV) infection is a major public health problem worldwide. Dengue clinical manifestations may range from asymptomatic, to mild symptomatic, and to a life-threatening shock syndrome. In endemic countries including Thailand blood donations from asymptomatic dengue-infected persons pose a risk for DENV transmission. We investigated the prevalence of dengue viremia and dengue immunity among Thai blood donors (n = 400) from August 2015 to March 2016 using nested RT-PCR and anti-DENV IgM/IgG capture ELISA. Only one donor was positive for dengue virus serotype 2 (DENV2) viremia as revealed by RT-quantitative PCR and virus isolation. After a two-month follow up, the donor did not develop symptoms related to DENV infection and anti-DENV IgG and IgM levels remained just above and below cut-off values, respectively and were unchanged from the day of blood donation. In dengue serological studies, anti-DENV antibody ELISA was positive for IgG in 246 (61%) and both IgG and IgM in 24 (6%) of blood donors, respectively. In conclusion, the prevalence of detectable dengue (DENV2) viremia in blood donors in Bangkok was 0.25% but there still exists concern in dengue endemic regions of a finite risk of infection from blood transfusions. Further studies with a larger sample size will be required to evaluate the cost-benefit of an additional measure to detect dengue viremia in blood donations in Thailand.

Keywords: blood donor, dengue, seroprevalence, Thailand, viremia

Correspondence: Parichart Permpikul, Department of Transfusion Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, 2 Wanglang Road, Bangkok Noi, Bangkok 10700, Thailand
Tel: +66 (0) 2419 8081 ext. 103, +66 (08) 1374 1293 E-mail: parichart.per@mahidol.ac.th

INTRODUCTION

Dengue virus (DENV) infection is one of the major public health concerns as widespread infection is reported globally in tropical and sub-tropical regions where mosquito vectors breed, in particular *Aedes* spp (WHO, 2020). Clinical manifestations vary from asymptomatic or mild clinical presentation to a life-threatening severe shock syndrome (Teo *et al*, 2009). Several studies suggested DENV could be transmitted via blood transfusions from asymptomatic donors (Ranjan *et al*, 2016; Karim *et al*, 2017; Tsai *et al*, 2018; Kulkarni *et al*, 2019; Slavov *et al*, 2019). However, there are a limited number of documented cases of symptomatic DENV infection transmitted through blood transfusion, eg in Brazil (Levi *et al*, 2015), Hong Kong (Chuang *et al*, 2008), Puerto Rico (Matos *et al*, 2016; Stramer *et al*, 2012) and Singapore (Oh *et al*, 2015; Tambyah *et al*, 2008).

The presence of DENV infection in donor blood is confirmed by detection of viremia and by serology. A large epidemiological study in Brazil revealed a prevalence of dengue viremia of 0.54% (87/16,241) in healthy blood donors, but no evidence of post-transfusion DENV infection (Busch *et al*, 2016). A similar prevalence of dengue viremia in blood donors (0.3%; 9/2,994) was reported from Honduras (Linnen *et al*, 2008), but very low prevalence of 0.07% (12/16,521 donors) in Puerto Rico (Mohammed *et al*, 2008), 0.013% (1/8,000 donors) in Taiwan (Tsai *et al*, 2018), and none among 5,879 donors in Australia (Linnen *et al*, 2008).

Thailand has been a dengue endemic region since 1958 (Gubler, 2002). However, to the best of our knowledge, there has been no investigation in the country into the prevalence of DENV in blood donors or its transmission via blood transfusion. Here, the prevalence of asymptomatic DENV infection and seroprevalence among seemingly healthy blood donors in Bangkok were determined. The findings should provide baseline data to assist in deciding the need to add a DENV viremia test of donated blood.

MATERIALS AND METHODS

Study population

Healthy volunteers (n = 400) were recruited from an equal number of routine blood donors at Siriraj Hospital, Mahidol University and the National Blood Centre, Thai Red Cross Society (NBC), Bangkok from August 2015 to March 2016. A binary randomization sequence was computer-generated to determine which donor, with respect to order of visit, would be invited to participate in the study. Every week, participants (n = 10) per study site were enrolled. All donors were selected based on Thailand national blood donor selection criteria, which followed WHO guidelines on assessing donor suitability for blood donation (WHO, 2012).

The study protocol was approved by the Siriraj Institutional Review Board (Approval No. Si 338/2015) and the Thai Red Cross Society Review board (Approval No. NBC 1/2015) in accordance with the Declaration of

Helsinki. Prior written informed consent was provided by each participant.

DENV detection and serotyping by nested RT-PCR

A three ml aliquot of blood was collected in an EDTA tube (Vacuette, Greiner Bio-One GmbH, Kremsmünster, Austria) and plasma and buffy coat were stored at -70°C until use. RNA was extracted from a 150 µl aliquot of plasma or buffy coat using a TRIzol extraction reagent (Ambion, Life Technologies, Carlsbad, CA). RNA was precipitated with isopropanol containing 1 µg/µl carrier RNA (QIAGEN, Hilden, Germany), suspended in 50 µl of nuclease-free water and stored at -70°C until use. DENV viral RNA was detected by nested RT-PCR as previously described (Yenchitsomanus *et al*, 1996). In brief, first-strand cDNA synthesis was performed in a reaction volume of 12 µl containing 5 µl of RNA template, 1X PCR reaction buffer, 4.2-mM MgCl2, 10 U of rRNasin RNase inhibitor, 6 U of reverse transcriptase enzyme (Promega, Madison, WI), 0.8 mM dNTP (New England BioLabs, Ipswich, MA) and 0.8 µM of DENV group-specific reverse primer (DEUR; 5'-GCTGTGT-CACCCAGAATGGCC-3') at 42°C for 45 minutes. First round of PCR was performed using DENV-specific outer primers (DEUR and DEUL; 5'-TGGCT-GGTGCACAGACAATGGTT-3') in 50-µl reaction solution containing 12-µl of cDNA template, 1X GoTaqFlexi polymerase buffer, 3-mM MgCl2, 1 U of GoTaqFlexi DNA polymerase (Promega, Madison, WI), 0.2 mM dNTP (New England BioLabs, Ipswich, MA), 0.2 µM of DENV group-specific primers (DEUR and DEUL), and the following thermocycling conditions [in a Bio-Rad thermal cycler T100 (Bio-Rad, Hercules, CA)]: 95°C for 5 minutes; 35 cycles of 95°C for 30 seconds, 45°C for 30 seconds and 72°C for 30 seconds; with a final step of 72°C for 5 minutes. In the second round PCR, DENV-specific multiplex inner primers (DENV1; D1L/D1R, DENV2; D2L/D2R, DENV3; D3L/D3R and DENV4; D4L/D4R primers) were used in a 25-µl reaction solution containing 1-µl of first round PCR template, 1X GoTaqFlexi polymerase Green buffer, 3-mM MgCl2, 0.5 U of GoTaqFlexi DNA polymerase (Promega, Madison, WI), 0.2 mM dNTP (New England BioLabs, Ipswich, MA), 0.4 µM of DENV-specific multiplex inner primers,), and the following thermocycling conditions: 95°C for 5 minutes; 45 cycles of 95°C for 30 seconds, 62°C for 30 seconds and 72°C for 30 seconds; with a final step of 72°C for 5 minutes. Amplicons [DENV1 (506 bp), DENV2 (346 bp), DENV3 (196 bp), and DENV4 (143 bp)] were analyzed by 2% agarose gel-electrophoresis and Ethidium bromide (Sigma-Aldrich, St Louis, MO) dye staining.

Dengue IgM and IgG capture ELISA

DENV IgM or IgG detection was performed using an in-house capture ELISA modified from that of Innis *et al* (1989). Pooled sera from non-dengue patients (PND) and convalescent sera (PCS) were used as negative and positive controls, respectively. Pooled sera of known IgG/IgM titers from

dengue patients were diluted 1:100 and used as a "Weak positive serum" for the assay. IgG or IgM in donor plasma (2-µl plasma diluted in 198-µl of diluent buffer) was bound on ELISA plates coated with anti-human IgM or IgG (Dako, Glostrup, Denmark) by incubating at room temperature for 2 hours. Antibody-antigen complexes were then detected by addition of murine monoclonal anti-flavivirus (4G2) antibody, followed by rabbit secondary anti-mouse IgG horseradish peroxidase-conjugated IgG and then chromogenic substrate ortho-phenylenediamine dihydrochloride (OPD) (Sigma-Aldrich, St Louis, MO). After incubation at room temperature for 15 minutes, the reaction was stopped by adding 2M H_2SO_4 and $A_{492\ nm}$ measured using an ELISA plate reader (Biochrom Anthos 2010; Cambridge, UK). IgG or IgM units were calculated using the formula:

$$IgM\ or\ IgG\ unit = \frac{A_{492nm}(test) - A_{492nm}(PND)}{A_{492nm}(weak\ positive) - A_{492nm}(PND)} \times 100$$

Sample is interpreted as positive for dengue-specific antibody when IgG and IgM units are >8 and >16 respectively. Cut-off values were determined based on IgG and IgM units in plasma samples of healthy donors (n = 15) that showed negative neutralizing antibodies by standard plaque reduction neutralization test (WHO, 2007).

Verification of DENV-positive blood donor

Any positive plasma samples from the nested RT-PCR screening were verified by six different techniques: (i) two sets of nested RT-PCR; first, the serotype-specific primers for envelope (E) gene following Yenchitsomanus *et al* (1996) (outer pair; DEUR-DEUL and inner pair; D2R-D2L primers) and second, the in-house specific-primers for 3' UTR region based on the conserved sequences of all four DENV serotypes including outer primers (OF 5'-GAGYAARCYRTGCWGCCTGTRGC-3' and OR 5'-TCCATTYYTSYGGCGYTCT-GTGCC-3' where R = A or G S = G or C, W = A or T, and Y = C or T base) and inner primers (IF 5'-GGTTA-GAGGAGACCCCTCCC-3' and IR 5'-GGCGYTCTGTGCCTGGA-3' where Y = C or T base; (ii) DENV capsid-specific RT-qPCR SYBR-Green I assay (Shu *et al*, 2003); (iii) DENV serotype-specific RT-qPCR hydrolysis probe assay (Johnson *et al*, 2005); (iv) NS1 ELISA as previously described (Puttikhunt *et al*, 2011); (v) viral genome RT-qPCR assay using two hydrolysis probes, one specific to a gene described by Johnson *et al* (2005) and the other to the conserved 3' UTR region of all 4 serotypes using FP 5'-GGTTAGAGGAGACCCCTCCC-3', RP 5'-GGCGYTCTGTGCCTGGA-3' and probe FAM-5'-CAGGATCTCTGGTC-TYTCCCAGCGT-3'-BHQ where Y = C or T base (Mairiang *et al*, 2021); and (vi)

virus isolation as previously described (Yamada *et al*, 2002). For method (vi), in brief, DENV-positive plasma sample was inoculated onto monolayers of Baby hamster kidney (BHK) and C6/36 mosquito cell lines (both transformed cell lines were obtained from the American Type Culture Collection) for infectious virion recovery. Culture media and cells were collected at Day 10 and Day 30 post-inoculation of BHK and C6/36 cells, respectively. DENV in media and cells were detected using both nested RT-PCR and RT-qPCR. Amplicons from a positive sample also were sequenced (Macrogen, Seoul, Korea) (MW999831, MZ004433, MZ007506) which was analyzed using a Clustal Omega Blast (https://www.ebi.ac.uk/Tools/msa/clustalo/) (Madeira *et al*, 2019) and Bioedit free alignment software (Hall *et al*, 1999) to confirm DENV identity. In addition, virus culture supernatants were used to confirm the presence of infectious DENV through a focus forming assay (FFA) as previously described (Punyadee *et al*, 2015).

Limit of detection (LOD) of nested-RT-PCR with serotype-specific primers, capsid RT-qPCR SYBR Green I and RT-qPCR hydrolysis probe assay was 2, 10 and 2.5 focus forming units (ffu)/ml virus titer, respectively. In viral genome quantification assay, limit of quantification (LOQ) of DENV2 Envelope gene-specific RT-qPCR hydrolysis and RT-qPCR 3' UTR hydrolysis probes were 700 genome copies/ml, based on detection efficiency for in vitro transcribed RNA standards. In NS1 ELISA, the LOD was 1 ng/ml.

RESULTS

Asymptomatic dengue viremia in Thai blood donors

Four hundred healthy blood donors were enrolled in this study. The donors' age ranged from 19 to 64 years (mean 37 years) (Table 1). There were 213 male and 187 female blood donors. Among 400 blood donors, 330 (82.5%) were repeat donors who had donated blood at least once before donation at enrollment.

Nested RT-PCR screening was positive in one blood donor and identified as positive for DENV2 infection (Fig 1A). The donor, a 41-year-old male, was contacted and monitored for signs and symptoms compatible with dengue fever or dengue hemorrhagic fever at intervals of one week, two weeks and two months after blood donation, but presented with no such signs or symptoms. However, several cases of acute DENV infection were reported in this donor's neighborhood at the time of donation (data not shown).

Confirmation for DENV2 in citrate-dextrose blood of the donor obtained one week after the original blood donation was conducted on a plasma sample by a nested RT-PCR assay using in-house specific-primers targeting DENV 3' UTR region (Fig 1B), and the specific-primers targeting DENV2 Envelope (E) gene in buffy coat (Fig 1C) and plasma (Fig 1D). However, negative results were obtained with the plasma sample using capsid RT-qPCR SYBR Green I, serotype-specific DENV2 E gene RT-qPCR hydrolysis probe assays, but a positive result using

Table 1

Demographic profile of blood donor participants recruited at Siriraj Hospital, Mahidol University and the National Blood Centre, Thai Red Cross Society, Bangkok, Thailand (August 2015 to March 2016)

Parameter	Number (%)* (n = 400)
Mean age, years, (range)	37 (19-74)
Gender	
Male	213 (56)
Female	187 (44)
Donation status	
First-time donor	70 (18)
Repeat donor	330 (82)

*unless specified

RT-qPCR in-house 3' UTR hydrolysis probe assay (although RNA amount could not be quantified) (data not shown).

In view of the contradictory RT-PCR results, the presence of DENV2 in donor blood was confirmed by inoculating plasma obtained on the day of blood donation onto monolayers of BHK and C6/36 cells. On Day 10 and Day 30 post-inoculation of BHK and C6/36 cells respectively, DENV2 E gene and E mRNA 3' UTR were detected using RT-PCR in both cell lines (Figs 2A and B), but DENV2 E gene was not detected in both cell line culture media (Fig 2C). Partial DENV2 E gene fragment (300 bp) amplified from donor plasma sample and infected cell lines were sequenced and compared using Multiple Alignment Clustal Omega Blast and Bioedit alignment software packages with DENV2 reference strain sequences and those from Thailand present in Genbank database, revealing blood donor DENV2 E gene fragment with 91-98% identity with DENV2 reference strains and 92-100% identity with 96 DENV2 sequences from Thailand (1995-2019).

NS1 ELISA and FFA results were negative (data not shown). However, based on the overall results, it was estimated that asymptomatic dengue (DENV2) viremia among the blood donors was 0.25% (95% confidence interval (CI): 0.01-1.61)).

Dengue seroprevalence in Thai blood donors

The same set of plasma samples as well as plasma collected at Week 1, Week 2 and Month 2 post-blood donation from

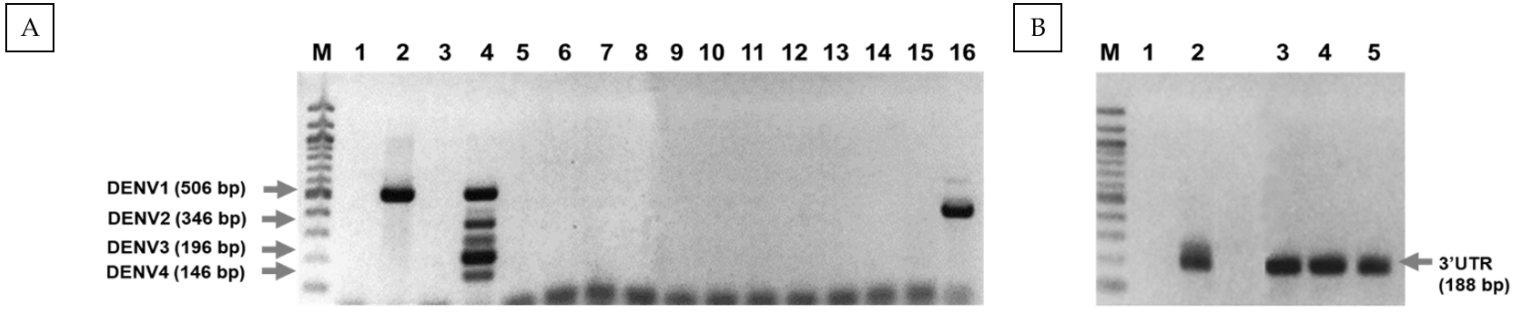

Fig 1 - Representative RT-PCR dengue virus (DENV) amplicon profile from screening of plasma/cell from blood donor participants recruited at Siriraj Hospital, Mahidol University and the National Blood Centre, Thai Red Cross Society, Bangkok, Thailand (August 2015 to March 2016)

(A) Multiplex nested RT-PCR. Lane 1, negative control; lane 2, DENV1 strain Hawaii; lane 3, non-template negative control; lane 4, reference DENV serotypes 1-4; lanes 5-16, blood donors' plasma.

(B) RT-PCR employing DENV 3' UTR specific primers. Lane 1, a negative control; lane 2, DENV1 strain Hawaii; lane 3 and 4, RNA samples from two separate plasma aliquots of blood donor (ID 1-168; panel A, lane 16) collected on the same day; lane 5, RNA sample from plasma of blood donor ID 1-168 collected a week later.

Remark: Lane 4 of Panel A (reference serotype DENV1-4) contains a non-specific band between expected DENV2 and DENV3 bands generated by a multiplex PCR reaction.

Fig 1 - cont.

(C) Nested RT-PCR employing DENV2 E gene specific primers. Lane1, a negative control; lane 2, DENV1 strain Hawaii; lane 3, reference DENV serotypes 1-4; lane 4, non-template negative control; lane 5, buffy coat of donor ID 1-168.

(D) CPD blood specimen was also used to verify the positive DENV2 detection in the blood donor (ID 1-168) by nested RT-PCR employing DENV2 E gene specific primers. Lane1, DENV1 strain Hawaii; lane 2, culture media negative control; lane 3, reference DENV serotypes 1-4; lane 4, citrate-phosphate-dextrose blood plasma of donor ID 1-168; lane 5, non-template negative control. Lane M, DNA 100 bp markers.

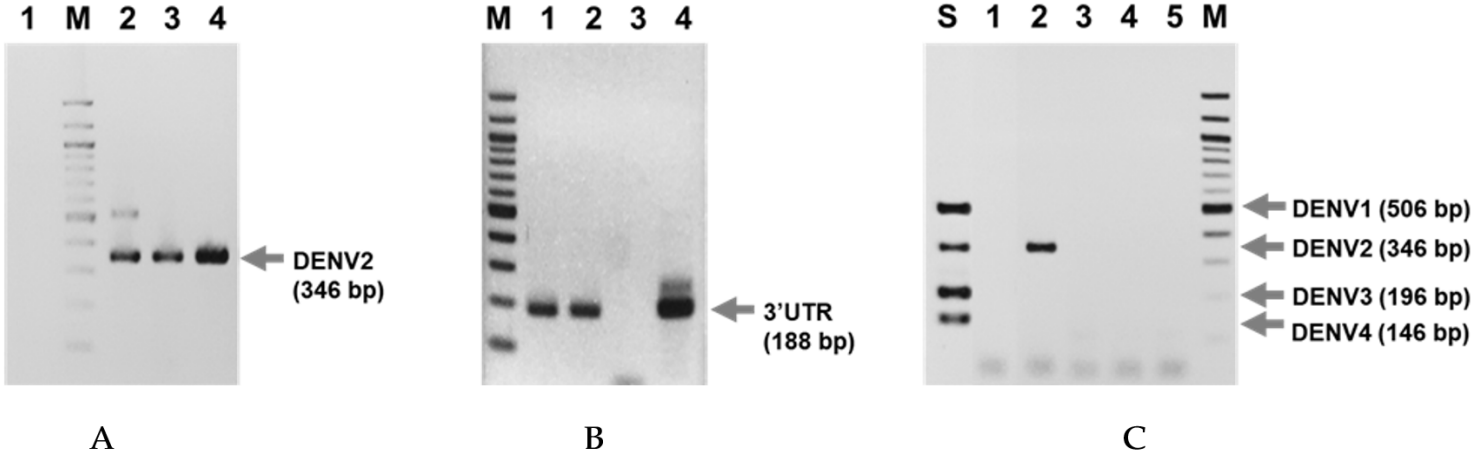

Fig 2 - Nested RT-PCR amplicon of dengue virus DENV2 isolated from blood donor plasma-inoculated BHK and C6/36 cell lines. DENV-positive plasma sample (donor ID 1-168) was inoculated onto monolayers of BHK and C6/36 cells, and culture media and cells were collected at day 10 and day 30 post-inoculation of BHK and C6/36 cells, respectively for DENV identification.

(A) Using primers targeting DENV envelope (E) gene. Lane 1, negative control; lane 2, plasma inoculated-C6/36 cells; lane 3, plasma inoculated-BHK cells; lane 4, DENV2 strain 16681 (positive control).

(B) Using primers targeting DENV 3' UTR region. Lane 1, plasma inoculated-C6/36 cells; lane 2, plasma inoculated-BHK cells; lane 3, a negative control; lane 4, DENV2 strain 16681 (positive control).

(C) Using primers targeting DENV E gene. Lane S, reference strains of DENV; lane 1, negative control; lane 2, DENV2 strain 16681 (positive control); lane 3 and 5, culture media from plasma inoculated-C6/36 cells collected on day 10 and day 30 post-inoculation, respectively; lane 4, culture media from plasma inoculated-BHK cells collected on day 10 post-inoculation. Lane M, 100 bp DNA size markers.

DENV2-positive donor were subjected to anti-DENV IgG and IgM capture ELISA, resulting in border-line anti-DENV IgG positivity (9-11 units; cut-off value >8 units) and negligible anti-DENV IgM positivity (0-4 units; cut-off value >16 units). Seroprevalence of anti-dengue IgG/IgM in blood donors (n = 400) recruited in the study were also assessed, demonstrating median (interquartile range (IQR): 25-75%) unit of anti-dengue IgG and IgM was 17.0 (5.71-34.27) and 1.68 (1.00-5.05) respectively, with 246 (61%) and 24 (6%) donors having higher than cut-off units of anti-dengue IgG and IgM were 26.94 (17.00-46.71) and 23.82 (18.55-38.13) respectively, *ie* a 67% seroprevalence of anti-DENV antibodies in blood donors in the Bangkok from August 2015 to March 2016.

DISCUSSION

Thailand is a dengue endemic country, and the majority of DENV infections are asymptomatic (Duong *et al*, 2015). Therefore, the risk of DENV transmission through blood transfusion is expected to be substantial. In addition, transfusion-transmitted severe dengue cases have been documented in Singapore (Tambyah *et al*, 2008). The present study found that blood donors with asymptomatic dengue viremia were uncommon (0.25%). Blood donors were recruited in Bangkok between August 2015 and March 2016, when the country's dengue infection rate was at its peak and declining, but the study still covered a major period of the nation's dengue outbreak (Fig 3).

The single dengue infected donor was positive for DENV2, the serotype found in 17% of dengue hemorrhagic fever patients in Bangkok during the outbreak season between 2015 and 2016 (Liulak *et al*, 2017). Dengue NS1 antigen and anti-DENV IgM antibodies were not detected in this donor during two months of follow-up. However, a blood sample from the donor collected one week after the blood donation was still positive for DENV2 detected using an RT-PCR assay of DENV2 E mRNA 3' UTR. This might reflect the presence of viral genome fragments as detection by an in-house 3′ UTR hydrolysis probe was positive while detection of E gene was negative. Previously, Li *et al* (2011) reported the existence of short fragments of DENV RNA (regulatory elements at the 3′ and 5′ ends of the genome) in sera of dengue patients. More recently, Tsai *et al* (2018) identified one individual among 8,000 Taiwanese blood donors with DENV using RT-qPCR. This individual was infected with DENV2 as determined by both virus isolation in C6/36 cells and virus genome sequencing but serology assays were negative for both NS1 antigen and DENV-specific antibodies. Titer of isolated virus was extremely low [<50 plaque forming units (pfu)/ml] similar to our study in which virus genome was found specifically in cells but not in culture media. Blood components of this Taiwanese donor were distributed prior to dengue detection and the Taiwan Centers for Disease Control reported no recipients developed signs and symptoms associated with DENV infection up to seven months.

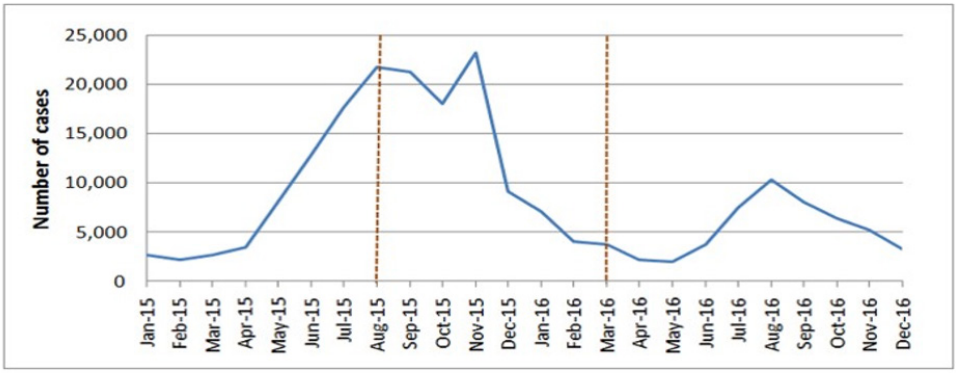

Fig 3 - Number of dengue cases in Thailand (2015-2016)

Data were obtained from the Department of Disease Control, Ministry of Public Health (http://odpc3.ddc.moph.go.th/login/sys/content/showmem/jul_show.php). Vertical dash lines demarcate the period during which blood donors were recruited for our study.

The prevalence of asymptomatic DENV-infected blood donors varies from country to country. Among 200 blood donors in India (Ranjan *et al*, 2016), 1,685 donors in Guanxi, China (Gao *et al*, 2018) and 5,879 donors in Australia (Linnen *et al*, 2008), no donor showed dengue viremia, but DENV-infected blood donors were identified in three studies in Brazil [3/2,858 donors reported by Linnen *et al* (2008), 2/500 donors by Dias *et al* (2012) and 87/16,241 donors by Busch *et al* (2016)], in Guangzhou, China (2/3,000 donors) (Zeng *et al*, 2018), in Honduras (9/2,994 donors) (Linnen *et al*, 2008), in Saudi Arabia (50/910 donors) (Ashshi *et al*, 2017), and in Taiwan (1 of 8,000 donors) (Tsai *et al*, 2018). These variations could be attributed to differences in dengue prevalence at the time and location of each study as well as the sensitivity of the detection technique and whether pooled or individual sample were used.

In the present study, both anti-dengue IgG and IgM were detected in 6% of the donors (24 of 400 donors), among whom 12% (3 of 24 donors) had high anti-DENV IgM levels (>50 units). The presence of anti-DENV IgM indicates an infection within the previous three months (WHO, 2011), but detailed interviews of donors' medical history revealed no signs or symptoms related to dengue infection during the two weeks prior to blood donation, suggesting that the infection was asymptomatic. The majority of blood donors (67%) were found anti-DENV IgG positive, indicative of past infection. Vongpunsawad *et al* (2017) reported 79.2% seroprevalence in four different provinces of Thailand in 2014, comparable with those in dengue

endemic countries, including Brazil, Malaysia and Singapore. The prevalence of anti-DENV IgM and IgG in Brazil is 2.8-8.8 and 88.7-90.9%, respectively (Busch *et al*, 2016), in Malaysia, 4.2 and 39.12%, respectively, with 2.8% of the study population positive for both IgG and IgM (Harif *et al*, 2014), in Singapore, 2.83 and 52.0%, respectively (Low *et al*, 2015), and in Taiwan, 0.21 and 13%, respectively, with 0.16% prevalence for both anti-DENV IgM and IgG (Tsai *et al*, 2018); however, in non-endemic countries, such as China, prevalence is 0.007-0.36 and 0.42-3.4%, respectively (Gao *et al*, 2018).

In summary, the findings demonstrate a small but significant proportion of recent asymptomatic dengue infection among healthy blood donors in Bangkok as the study took place during a waning period of dengue outbreaks in the country. Further studies with a larger sample size will be required to assess the cost-benefit of implementing additional measures for screening blood donations to prevent possible blood transfusion-transmitted dengue in Thailand.

ACKNOWLEDGEMENTS

The authors thank staff of Siriraj Blood Bank and the National Blood Centre, Thai Red Cross Society for their assistance in recruiting blood donors for the study and of the Division of Dengue Hemorrhagic Fever Research (DHFR), Siriraj Center of Research Excellence in Dengue and Emerging Pathogens, Faculty of Medicine Siriraj Hospital, Mahidol University for the anti-DENV IgG and IgM assays. The study was funded by a Siriraj Research Grant and forms a part of the requirements of Aumnad Khongsup's Master of Science Degree, Mahidol University.

CONFLICTS OF INTEREST DISCLOSURE

The authors declare no conflicts of interest.

REFERENCES

Ashshi AM, Alghamdi S, El-Shemi AG, *et al*. Seroprevalence of asymptomatic dengue virus infection and its antibodies among healthy/eligible Saudi blood donors: findings from Holy Makkah City. *Virology (Auckl)* 2017; 8: 1-5.

Busch MP, Sabino EC, Brambilla D, *et al*. Duration of dengue viremia in blood donors and relationships between donor viremia, infection incidence and clinical case reports during a large epidemic. *J Infect Dis* 2016; 214: 49-54.

Chuang V, Wong TY, Leung YH, *et al*. Review of dengue fever cases in Hong Kong during 1998 to 2005. *Hong Kong Med J* 2008; 14: 170-7.

Dias LL, Amarilla AA, Poloni TR, Covas DT, Aquino VH, Figueiredo LT. Detection of dengue virus in sera of Brazilian blood donors. *Transfusion* 2012; 52: 1667-71.

Duong V, Lambrechts L, Paul RE, *et al*. Asymptomatic humans transmit dengue virus to mosquitoes. *Proc Natl Acad Sci USA* 2015; 112: 14688-93.

Gao Z, Zhang Y, Yang Y, *et al*. Dengue virus infections among blood donors in Guangxi of China, 2013-2014. *Transfus Med* 2018; 28: 236-42.

Gubler DJ. Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. *Trends Microbiol* 2002; 10: 100-3.

Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucleic Acids Symp Ser* 1999; 41: 95-8.

Harif NF, Kader ZS, Joshi SR, Yusoff NM. Seropositive status of dengue virus infection among blood donors in North Malaysia. *Asian J Transfus Sci* 2014; 8: 64.

Innis B, Nisalak A, Nimmannitya S, *et al*. An enzyme-linked immunosorbent assay to characterize dengue infections where dengue and Japanese encephalitis co-circulate. *Am J Trop Med Hyg* 1989; 40: 418-27.

Johnson BW, Russell BJ, Lanciotti RS. Serotype-specific detection of dengue viruses in a fourplex real-time reverse transcriptase PCR assay. *J Clin Microbiol* 2005; 43: 4977-83.

Karim F, Nasir N, Moiz B. Transfusion transmitted dengue: one donor infects two patients. *Transfus Apher Sci* 2017; 56: 151-3.

Kulkarni R, Tiraki D, Wani D, Mishra AC, Arankalle VA. Risk of transfusion-associated dengue: screening of blood donors from Pune, western India. *Transfusion* 2019; 59: 458-62.

Levi JE, Nishiya A, Felix AC, *et al*. Real-time symptomatic case of transfusion-transmitted dengue. *Transfusion* 2015; 55: 961-4.

Li D, Lott WB, Lowry K, Jones A, Thu HM, Aaskov J. Defective interfering viral particles in acute dengue infections. *PLoS One* 2011; 6: e19447.

Linnen JM, Vinelli E, Sabino EC, *et al*. Dengue viremia in blood donors from Honduras, Brazil, and Australia. *Transfusion* 2008; 48: 1355-62.

Liulak W, Saichaer P, Waidab W, *et al*. Prospective study of dengue in Bangkok during 2015-2016. *Southeast Asian J Trop Med Public Health* 2017; 48 (Suppl 1): 33-8.

Low SL, Lam S, Wong WY, Teo D, Ng LC, Tan LK. Dengue seroprevalence of healthy adults in Singapore: serosurvey among blood donors, 2009. *Am J Trop Med Hyg* 2015; 93: 40-5.

Madeira F, Park YM, Lee J, *et al*. The EMBL-EBI search and sequence analysis tools APIs in 2019. *Nucleic Acids Res* 2019; 47: W636-41.

Mairiang D, Songjaeng A, Hansuealueang P, *et al*. Application of one-step reverse transcription droplet digital PCR for dengue virus detection and quantification in clinical specimens. *Diagnostics (Basel)* 2021; 11: 639.

Matos D, Tomashek KM, Perez-Padilla J, *et al*. Probable and possible transfusion-transmitted dengue associated with NS1 antigen-negative but RNA confirmed-positive red blood cells. *Transfusion* 2016; 56: 215-22.

Mohammed H, Linnen JM, Munoz-Jordan JL, *et al*. Dengue virus in blood donations, Puerto Rico, 2005. *Transfusion* 2008; 48: 1348-54.

Oh HB, Muthu V, Daruwalla ZJ, Lee SY, Koay ES, Tambyah PA. Bitten by a bug or a bag? Transfusion-transmitted dengue: a rare complication in the bleeding surgical patient. *Transfusion* 2015; 55: 1655-61.

Punyadee N, Mairiang D, Thiemmeca S, *et al*. Microparticles provide a novel biomarker to predict severe clinical outcomes of dengue virus infection. *J Virol* 2015; 89: 1587-607.

Puttikhunt C, Prommool T, U-thainual N, *et al*. The development of a novel serotyping-NS1-ELISA to identify serotypes of dengue virus. *J Clin Virol* 2011; 50: 314-9.

Ranjan P, Natarajan V, Bajpai M, Gupta E. High seroprevalence of dengue virus infection in blood donors from Delhi: a single centre study. *J Clin Diagn Res* 2016; 10: DC08-10.

Shu PY, Chang SF, Kuo YC, *et al*. Development of group-and serotype-specific one-step SYBR green I-based real-time reverse transcription-PCR assay for dengue virus. *J Clin Microbiol* 2003; 41: 2408-16.

Slavov SN, Ciliao-Alves DC, Gonzaga FAC, *et al*. Dengue seroprevalence among asymptomatic blood donors during an epidemic outbreak in Central-West Brazil. *PloS One* 2019; 14: e0213793.

Stramer SL, Linnen JM, Carrick JM, *et al*. Dengue viremia in blood donors identified by RNA and detection of dengue transfusion transmission during the 2007 dengue outbreak in Puerto Rico. *Transfusion* 2012; 52: 1657-66.

Tambyah PA, Koay ES, Poon ML, Lin RV, Ong BK, Transfusion-Transmitted Dengue Infection Study G. Dengue hemorrhagic fever transmitted by blood transfusion. *N Engl J Med* 2008; 359: 1526-7.

Teo D, Ng LC, Lam S. Is dengue a threat to the blood supply? *Transfus Med* 2009; 19: 66-77.

Tsai JJ, Lin PC, Tsai CY, Wang YH, Liu LT. Low frequency of asymptomatic dengue virus-infected donors in blood donor centers during the largest dengue outbreak in Taiwan. *PLoS ONE* 2018; 13: e0205248.

Vongpunsawad S, Intharasongkroh D, Thongmee T, Poovorawan Y. Seroprevalence of antibodies to dengue and chikungunya viruses in Thailand. *PLoS One* 2017; 12: e0180560.

World Health Organization (WHO). Blood donor selection: guidelines on assessing donor suitability for blood donation, 2012 [cited 2020 Jun 30]. Available from: URL: https://apps.who.int/iris/bitstream/handle/10665/76724/9789241548519_eng.pdf?sequence=1&isAllowed=y

World Health Organization (WHO). Comprehensive guidelines for prevention and control of dengue and dengue haemorrhagic fever, 2011 [cited 2020 Jun 30]. Available from: URL: https://apps.who.int/iris/bitstream/handle/10665/204894/B4751.pdf?sequence=1&isAllowed=y

World Health Organization (WHO). Dengue and severe dengue, 2020 [cited 2020 Jun 30]. Available from: URL: https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue

World Health Organization (WHO). Guidelines for plaque reduction neutralization testing of human antibodies to dengue viruses, 2007 [cited 2020 Jun 30]. Available from: URL: https://apps.who.int/iris/bitstream/handle/10665/69687/who_ivb_07.07_eng.pdf?squence=1&isAllowed=y

Yamada KI, Takasaki T, Nawa M, Kurane I. Virus isolation as one of the diagnostic

methods for dengue virus infection. *J Clin Virol* 2002; 24: 203-9.

Yenchitsomanus PT, Sricharoen P, Jaruthasana I, *et al*. Rapid detection and identification of dengue viruses by polymerase chain reaction (PCR). *Southeast Asian J Trop Med Public Health* 1996; 27: 228-36.

Zeng P, Liao Q, Gao Z, He M, Rong X. Sero-prevalence and viremia status of dengue virus among asymptomatic blood donors post epidemic outbreak in Chinese Guangzhou in 2015. *Transfus Med* 2018; 28: 468-9.