Dengue viremia in blood donors identified by RNA and detection of dengue transfusion transmission during the 2007 dengue outbreak in Puerto Rico

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BACKGROUND: In 2007, a total of 10,508 suspected dengue cases were reported in Puerto Rico. Blood donations were tested for dengue virus (DENV) RNA and recipients of RNA-positive donations traced to assess transfusion transmission.

STUDY DESIGN AND METHODS: Blood donation samples from 2007 were maintained in a repository and tested individually for DENV RNA by transcription-mediated amplification (TMA); a subset was further tested by an enhanced TMA (eTMA) assay. TMA-reactive samples were considered confirmed if TMA (including eTMA) was repeat reactive (RR). All TMA-RR samples were tested by quantitative, DENV type–specific reverse transcriptase–polymerase chain reaction (RT-PCR) and for anti-DENV immunoglobulin (Ig)M by enzyme-linked immunosorbent assay. Samples positive by RT-PCR were further tested for infectivity in mosquito cell culture. Patients receiving components from TMA-RR donations were followed.

RESULTS: Of 15,350 donation samples tested, 29 were TMA-RR for a prevalence of 1 per 529 (0.19%). DENV Types 1, 2, and 3 with viral titers of 10^5 to 10^9 copies/mL were detected by RT-PCR in 12 samples of which all were infectious in mosquito culture. Six TMA-RR samples were IgM positive. Three of the 29 recipients receiving TMA-RR donations were tested. One recipient in Puerto Rico transfused with red blood cells containing 10^8 copies/mL DENV-2 became febrile 3 days posttransfusion and developed dengue hemorrhagic fever. The recipient was DENV-2 RNA positive by RT-PCR; both the donor and the recipient viruses had identical envelope sequences.

CONCLUSIONS: High rates of viremia were detected in blood donors in Puerto Rico coupled with the first documented transfusion transmission of severe dengue disease, suggesting that further research on interventions is needed.

Dengue is a disease caused by four related RNA viruses of the genus *Flavivirus*, dengue virus (DENV)-1, -2, -3, and -4.[1] However, not all DENV infections result in clinically apparent disease. Approximately 75% of all DENV infections are asymptomatic, including those among adults.[2-6] Each DENV type is capable of causing the full spectrum of disease from nonspecific, acute febrile illness to severe disease including dengue hemorrhagic fever (DHF) and dengue shock syndrome. Approximately 5% of patients with dengue develop severe disease, which is thought to occur more commonly among those with second or subsequent infections.[7] Infection with one DENV-type produces lifelong immunity against that DENV-type and short-term (≤2 months) cross-protection against

ABBREVIATIONS: ARC = American Red Cross; DENV(s) = dengue virus(-es); DHF = dengue hemorrhagic fever; ED = emergency department; eTMA = enhanced transcription-mediated amplification; IR = initially reactive; MAC-ELISA = immunoglobulin M–capture enzyme-linked immunosorbent assay; PDSS = passive dengue surveillance system; RR = repeat reactive; S/CO = signal to cutoff; TMA = transcription-mediated amplification.

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infection with the other three DENVs.[1,3,7] Therefore, an individual may have up to four DENV infections in their lifetime.

DENVs are primarily transmitted from person to person through the bite of an infected *Aedes aegypti* or *Aedes albopictus* mosquito.[8] DENV replicates in humans for 3 to 14 days before symptom onset. Infected persons can transmit DENV to mosquitoes as early as 1 to 2 days before symptoms develop and throughout the approximately 7-day viremic period.[9] Because of this, and the fact that viremia can be high titer (in excess of 10^7 viral RNA copies/mL) even among those who remain asymptomatic, DENV may be transfusion transmitted.[10-12] Cases of dengue after receipt of blood products or donor organs or tissue and after occupational exposure in a health care setting have been reported.[13-17] However, the true incidence of transfusion-transmitted dengue is unknown because many infections are asymptomatic or result in mild, nonspecific febrile illness that may not be recognized as transfusion acquired, and if a case is suspected, transfusion transmission (vs. vector-borne transmission) is difficult to prove in recipients in dengue-endemic countries. Moreover, there is no surveillance for such events, and diagnostic services to investigate infections and their sources are often not widely available in many endemic countries.[18]

Dengue is a major public health problem in the tropics; an estimated 50 million cases occur annually and 40% of the world's population lives in areas with DENV transmission.[19-22] Dengue is not endemic in the continental United States, Hawaii, or Alaska;[23-25] however, several dengue outbreaks with local transmission have occurred in Texas,[26,27] Hawaii,[28,29] and Florida[30,31] in the past decade. Dengue is endemic in the US territories of Puerto Rico, the Virgin Islands, and American Samoa, and millions of US travelers are at risk as dengue is the leading cause of febrile illness among travelers returning from the Caribbean, Latin America, and South Central/Southeast Asia.[32,33]

In 2007, there was a large, islandwide dengue outbreak in Puerto Rico with 10,508 reported cases.[34] It was the largest outbreak in Puerto Rico in nearly a decade and only the second outbreak to involve the simultaneous transmission of all four DENVs (although DENV-3 predominated followed by DENV-2). The 2007 outbreak was notable for the reappearance of DENV-1 and DENV-4 after nearly a decade of absence and an increase in disease severity compared with the 1994 to 1995 and 1998 outbreaks. It was in this context that we tested blood donations for DENV RNA to determine the rate of donors presenting with DENV RNA positivity and viremia as assessed by infection in mosquito cells; we also evaluated recipients of RNA-positive units to determine if transfusion transmission could be documented.

MATERIALS AND METHODS

General approach

Over 28,000 EDTA plasma samples collected in plasma preparation tubes (PPT, Becton Dickinson, Franklin Lakes, NJ) from blood donations to the Puerto Rico region of the American Red Cross (ARC) during the dengue outbreak in 2007 (June-December) were retained frozen in a repository. After the dengue season, and the number of available samples by week were assessed relative to the epidemic, selected samples (focusing on the peak weeks of the epidemic) were batch tested for DENV RNA using transcription-mediated amplification (TMA; Gen-Probe, San Diego, CA). Samples were TMA tested individually with initially reactive (IR) samples retested by TMA in duplicate. TMA repeat-reactive (RR) samples were considered positive.[10] TMA-RR samples were diluted 1 to 16 in plasma screened negative for all infectious disease markers including DENV RNA, and the dilutions were retested using the same TMA assay in singlet. All DENV RNA testing was performed during 2008 at Gen-Probe. Virologic, infectivity, and serologic testing performed on all TMA-RR samples at the Dengue Branch of the Centers for Disease Control and Prevention (CDC) in Puerto Rico included qualitative and quantitative DENV type-specific real-time, reverse transcriptase–polymerase chain reaction (RT-PCR), mosquito (*A. albopictus*) cell culture (C6/36 cells), and anti-DENV immunoglobulin (Ig)M-capture enzyme-linked immunosorbent assay (MAC-ELISA).[35-37] Hospitals receiving components from TMA-RR donations were contacted for recipient follow-up including elicitation of a history of illness, administration of a risk factor questionnaire, and submission of a serum sample to the Dengue Branch, CDC, for diagnostic testing for evidence of DENV infection including RT-PCR, MAC-ELISA, and anti-DENV IgG ELISA. Due to the retrospective nature of the study, recipient contact only occurred at 1 year or more after transfusion. The institutional review board of the ARC approved the study.

DENV TMA assay

The DENV TMA assay used for this study is based on the same technology as blood screening assays (PROCLEIX, Novartis Vaccines and Diagnostics, Emeryville, CA) for the RNA components of the human immunodeficiency virus (HIV) and hepatitis C virus (HCV) TMA assay (Ultrio assay, Gen-Probe, San Diego, CA; and Novartis Vaccine and Diagnostics) and that of the West Nile virus (WNV) assay (Gen-Probe/Novartis), both of which have been licensed by the US Food and Drug Administration. The DENV TMA assay is a research qualitative nucleic acid test for the detection of DENV RNA, which includes target capture and TMA,

followed by chemiluminescent detection of DENV RNA. The assay design most closely resembles the PROCLEIX WNV assay including the same base reagent formulations (with dengue-specific oligonucleotides) and processed on the automated system (TIGRIS, Novartis) utilizing software that performed all cutoff calculations and validity criteria using the same interpretative algorithms as the WNV assay. In a comparative study of DENV TMA and RT-PCR, TMA was 10 to 100 times more sensitive than RT-PCR and could detect RNA in up to 80% of clinical cases that were RT-PCR negative.[38] The DENV TMA assay can detect all four DENV types to below 20 copies/mL.[11,39] A subset of donations that tested TMA nonreactive (n = 8684) was retested by an enhanced TMA (eTMA). Based on internal Gen-Probe results, the eTMA assay is more sensitive than the routine TMA used in this study. The eTMA assay showed 95% detection at 14.9, 18.3, 13.0, and 16.4 copies/mL DENV-1 (95% confidence interval [CI], 11.7-20.4), DENV-2 (95% CI, 14.4-24.7), DENV-3 (95% CI, 10.3-17.6), and DENV-4 (13.0-22.2), respectively.

Recipient tracing

After hospital or transfusion service notification of the distribution of potentially infectious DENV RNA–containing components, recipients of TMA-RR donations were traced, consented, and tested for evidence of DENV infection after transfusion. Evidence of current or past DENV infection required the presence of DENV RNA and/or IgM and IgG antibodies in follow-up samples from the recipient with signs and symptoms consistent with dengue infection from the recipient's chart review. Consenting recipients also completed the questionnaire regarding DENV clinical history and risk factors. Serum samples from RNA-positive recipients and their respective donations were inoculated into cultured C6/36 cells and the presence of virus was confirmed by RT-PCR and indirect immunofluorescence. Isolates were further propagated and viral RNA was extracted from culture supernatant using the Universal BioRobot 16 System (Qiagen, Valencia, CA). The BioRobot Universal System automates and integrates all the instrumentation, software, purification and enzyme-related steps required for high-throughput molecular applications including RNA purification from blood. The envelope glycoprotein (E) gene was amplified and sequenced; sequence data were restricted to the E gene open reading frame (1485 bp). GenBank accession numbers were obtained. Evolutionary distances were computed and several E gene sequences from GenBank were included in the phylogenetic tree to support tree topology. Multiple sequence alignment was performed using ClustalW. Evolutionary distances were inferred using neighbor-joining trees.

RESULTS

2007 dengue outbreak in Puerto Rico

During the 2007 dengue season in Puerto Rico, 10,508 suspected cases of dengue, or 2.9 cases per 1000 population, were reported to the passive dengue surveillance system (PDSS). The PDSS is collaboratively operated by the Puerto Rico Department of Health and the CDC, Dengue Branch. By law, dengue fever, DHF, and/or dengue shock syndrome are reportable conditions in Puerto Rico and suspected cases are reported via PDSS along with submission of a serum sample for free dengue diagnostic testing. All four DENV types were in circulation in 2007 with a total of 3293 (33%) processed samples confirmed positive for DENV. DENV-3 and DENV-2 were detected most often (62 and 31%, respectively). More than 50% (52.5%) of reported cases were hospitalized, one-third (31.8%) had hemorrhage, 2.2% had DHF, and there were 44 reported deaths.[34] A repository of 28,277 samples from blood donations collected in Puerto Rico from June 1 to December 31, 2007, was created during this outbreak.

DENV TMA repeat reactivity and overall prevalence of DENV RNA among blood donations

Of 15,350 samples randomly selected from Peak Weeks 32 to 49 for DENV RNA testing by TMA, 28 were TMA-IR and 25 were TMA-RR for a positive rate of 1 per 614 (0.16%; Fig. 1). The 25 TMA-RR samples included DENV-1, -2, and

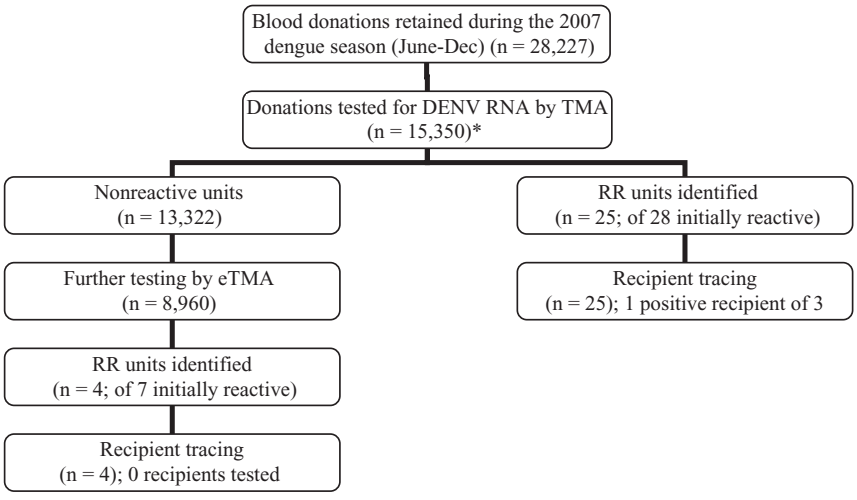

Fig. 1. DENV blood donation screening algorithm and TMA screening results including the 29 TMA RR blood donations. *TMA specificity based on IR samples that did not repeat as reactive = 15,315/15,321 = 99.96% (95% CI: 99.93-99-99).

TABLE 1. DENV TMA-RR blood donations collected in Puerto Rico in 2007

	S/CO by TMA*			S/CO by eTMA			CDC testing			
Unit	Initial	Retest	1:16	Initial	Retest	1:16	Serotype†	Viral load (copies/mL)	C6/36‡	Anti-DENV IgM
1	**27.75**	**38.99**	**38.91**	**87.16**	**88.52**		DENV-2	1.12×10^9	**Pos**	Neg
2	**32.34**	**33.30**	**31.14**				DENV-2	5.08×10^8	**Pos**	**Pos**
3§	**33.30**	**37.38**	**35.39**	**91.10**	**83.09**		DENV-2	1.35×10^8	**Pos**	Neg
4	**37.66**	**39.16**	**40.26**	**87.13**	**89.32**		DENV-3	7.25×10^7	**Pos**	Neg
5	**40.29**	**27.03**	**36.10**	**82.29**	**92.04**		DENV-3	1.37×10^7	**Pos**	Neg
6	**32.73**	**35.03**	**34.99**				DENV-3	1.18×10^7	**Pos**	Neg
7	**33.91**	**32.87**	**33.89**				DENV-3	7.67×10^6	**Pos**	Neg
8	**31.97**	**30.59**	0.17				DENV-1	4.49×10^6	**Pos**	Neg
9	**19.14**	**13.94**	0.21				DENV-2	2.82×10^6	**Pos**	**Pos**
10	**33.10**	**38.68**	**40.31**	**87.86**	**89.91**		DENV-3	6.39×10^5	**Pos**	Neg
11	**31.25**	**33.56**	**27.75**				DENV-3	3.50×10^5	**Pos**	Neg
12	**5.68**	**20.55**	**1.16**	**29.48**	**21.59**		DENV-3	1.00×10^5	**Pos**	Neg
13	**34.81**	**37.21**	**32.97**	**76.16**	**32.72**			$<10^3$	Neg	Neg
14	**23.38**	**31.07**	**13.29**	**31.25**	**31.18**			$<10^3$	Neg	Neg
15	**14.23**	**23.26**	**7.32**	**28.59**	**3.28**			$<10^3$	Neg	**Pos**
16	**13.14**	**25.77**	0.07	**29.26**	**12.51**			$<10^3$	Neg	Neg
17	**11.51**	**5.63**	0.04					$<10^3$	Neg	Neg
18	**8.17**	**16.58**	0.03					$<10^3$	Neg	Neg
19	**6.64**	**8.91**	0.20					$<10^3$	Neg	**Pos**
20	**5.06**	**4.12**	**1.37**	**29.96**	**8.61**			$<10^3$	Neg	Neg
21	**3.37**	**4.95**	0.83					$<10^3$	Neg	**Pos**
22	**2.95**	**25.28**	0.03					$<10^3$	Neg	**Pos**
23	**8.20**	**1.40**	0.13					$<10^3$	Neg	Neg
24	**4.46**	0.01	0.21	**24.80**	0.06			$<10^3$	Neg	Neg
25	**1.02**	**2.29**	0.13	**28.01**	0.01			$<10^3$	Neg	Neg
26‖	0.45			**26.38**	**27.55**	0.02		$<10^3$	Neg	Neg
27‖	0.17			**26.18**	**30.99**	0.02		$<10^3$	Neg	Neg
28‖	0.30			**25.31**	**29.11**	0.03		$<10^3$	Neg	Neg
29‖	0.50			**24.34**	**17.85**	0.05		$<10^3$	Neg	Neg

* TMA reactive when the S/CO ratio is 1.00 or greater.
† Serotype-specific, real-time RT-PCR.
‡ C6/36 = the mosquito cell line used for infectivity studies.
§ Unit 3 was involved in a transfusion transmission.
‖ Four TMA nonreactive samples were eTMA reactive.
Bold text indicates positive values.

-3 detected by DENV type–specific RT-PCR. Of the 25 TMA-RR units, 14 (56%) were reactive at a 1-to-16 dilution and 12 (48%) had RNA titers of 10^5 to 10^9 copies/mL (Table 1). All 12 samples with quantifiable RNA infected mosquito cell cultures of which nine (75%) were detectable at a 1-to-16 dilution. Six of 25 TMA-RR units were IgM positive of which only two of the six had quantifiable virus and infected mosquito cells in culture.

Seven of 8684 TMA-nonreactive donations were eTMA IR and four were eTMA RR (Fig. 1 and Table 1). In addition, 13 of 25 TMA-RR donations with sufficient volume were retested by eTMA and all were reactive (Table 1) with high signal-to-cutoff (S/CO) ratios. Of the four additional eTMA-RR donations that tested nonreactive by TMA, none was confirmed by PCR, all were eTMA nonreactive at a 1-to-16 dilution, none infected mosquito cells in culture, and none contained IgM; however, all of the confirmatory methods have lesser sensitivity than TMA.[39] Thus, the four eTMA-RR donations were combined with the 25 TMA-RR donations for a total study yield of 29 RNA-reactive donations (further referred to as TMA-RR) of which nearly 80% lacked IgM.

Combined, 35 IRs and 29 RRs were identified from 15,350 tested samples, resulting in a DENV RNA prevalence during the 2007 outbreak season of 1 per 529 (0.19% or 18.9 per 10,000) and an overall TMA specificity based on IR samples that did not repeat of 99.96% (15,315/15,321; 95% CI, 99.93-99.99; Fig. 1). TMA-RR (including eTMA-RR) donors were detected between July and November, which encompassed the majority of the outbreak period (Fig. 2). Figure 2 also provides the number of cases reported by week of illness onset to the PDSS and the laboratory diagnoses of these cases.

Recipient tracing

Information on all 29 recipients of TMA-RR donations was obtained but serum samples for diagnostic testing were available from only three recipients (Fig. 1 and Table 2); pretransfusion samples were not available from any recipient. Two recipients consented to be tested and both had testing done nearly 2 years posttransfusion. MAC-ELISA was negative for anti-DENV IgM and anti-DENV IgG ELISA was also negative. These two additional recipients had received red blood cells (RBCs) prepared from a

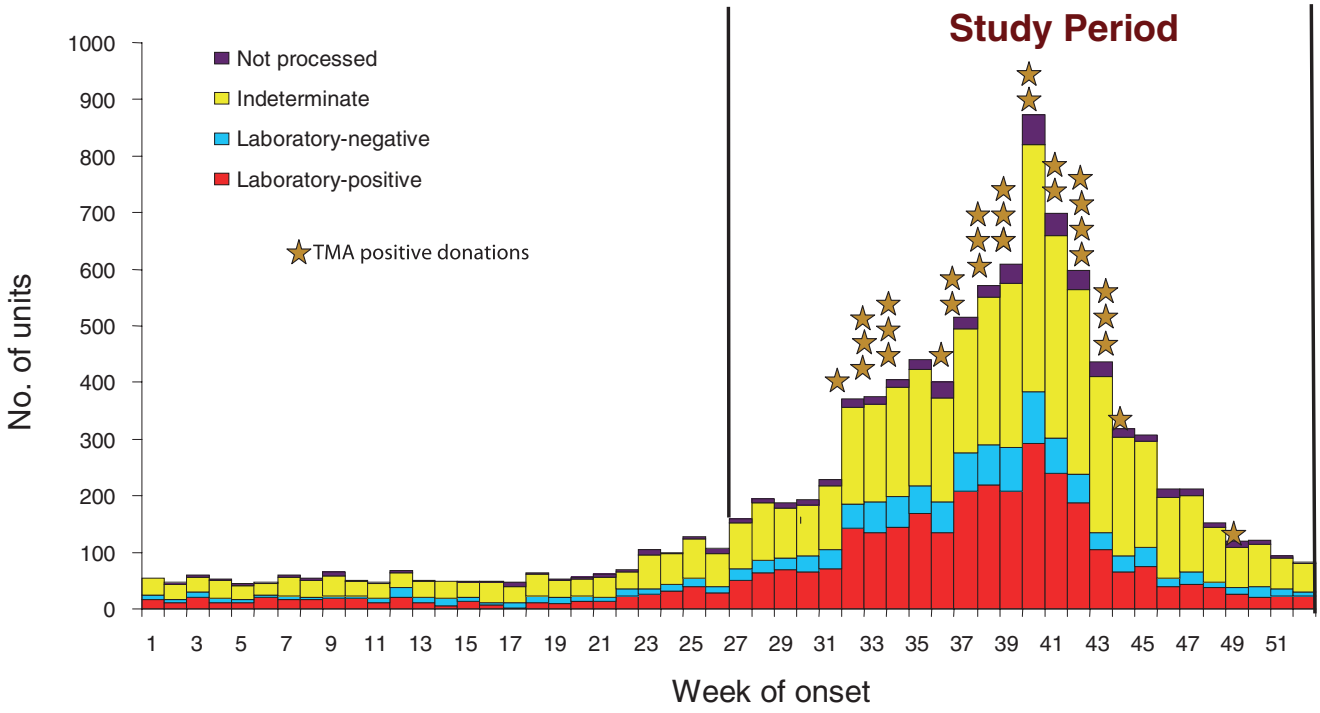

Fig. 2. Number of suspected dengue cases by laboratory outcome reported weekly during 2007 and the week in which TMA-RR blood donors were identified; the study period is indicated as that between the vertical lines.

TABLE 2. Recipient tracing results for 29 recipients who received DENV TMA-RR donations

Unit	Serotype	Viral load (copies/mL)	Component type	Recipient information
1	DENV-2	1.12×10^9	PP	Unit discarded
2	DENV-2	5.08×10^8	RBCs	Died within 3 weeks after transfusion, unrelated to dengue
3*	DENV-2	1.35×10^8	RBCs	DHF 3 days after transfusion; donor-recipient sequencing confirmed
4	DENV-3	7.25×10^7	RBCs	None
5	DENV-3	1.37×10^7	RBCs	Followed for 6 weeks; no s/s suggestive of dengue
6	DENV-3	1.18×10^7	RBCs	Died same day as transfusion
7	DENV-3	7.67×10^6	RBCs	None
8	DENV-1	4.49×10^6	RBCs	None
9	DENV-2	2.82×10^6	RBCs	None
10	DENV-3	6.39×10^5	RBCs	None
11	DENV-3	3.50×10^5	RBCs	Died within 7 months after transfusion, unrelated to dengue
12	DENV-3	1.00×10^5	RBCs	Followed for 2 months; no s/s suggestive of dengue
13		$<10^3$	RBCs	None
14		$<10^3$	RBCs	Died without s/s suggestive of dengue
15		$<10^3$	RBCs	None
16		$<10^3$	RBCs	None
17		$<10^3$	RBCs	Died 1 day after transfusion; no s/s suggestive of dengue
18†		$<10^3$	RBCs	Antibody (IgM/IgG) negative on follow-up 26 months after transfusion
19†		$<10^3$	RBCs	Antibody (IgM/IgG) negative on follow-up 23 months after transfusion
20		$<10^3$	RBCs	Unit discarded
21		$<10^3$	RBCs	None
22		$<10^3$	RBCs	Died within 3 weeks after transfusion, unrelated to dengue
23		$<10^3$	RBCs	None
24		$<10^3$	RBCs	Unit discarded
25		$<10^3$	RBCs	Discharged 6 days posttransfusion; no s/s suggestive of dengue
26‡		$<10^3$	RBCs	None
27‡		$<10^3$	RBCs	None
28‡		$<10^3$	RBCs	None
29‡		$<10^3$	RBCs	None

* Unit 3 was involved in a transfusion transmission.
† Units 18 and 19 went to recipients who were subsequently tested for DENV antibody (IgG/IgM).
‡ Donations detected as RR by eTMA.
PP = plateletpheresis unit; s/s = signs/symptoms.

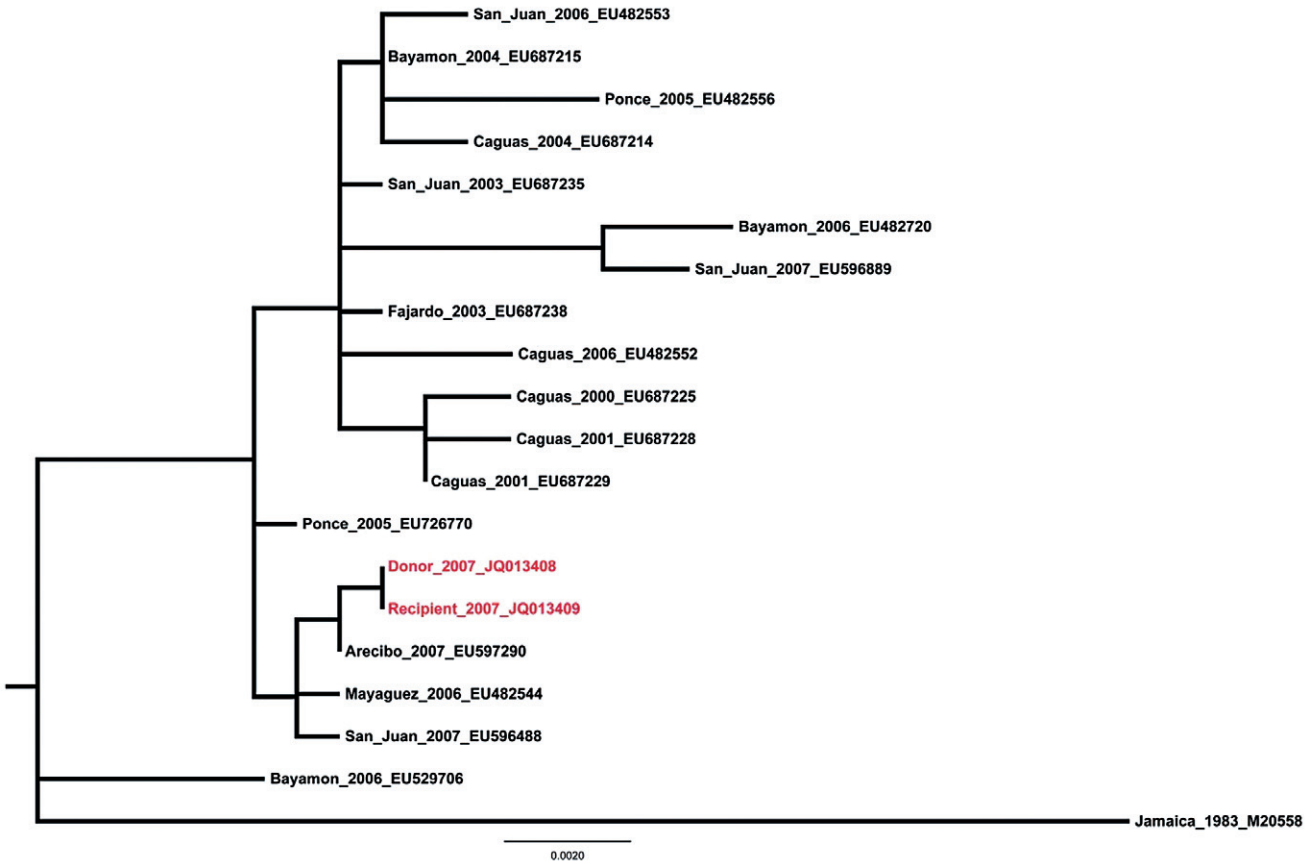

Fig. 3. Maximum likelihood phylogeny of complete E gene sequences (1485 bp) obtained from 19 endemic clinical isolates obtained between 2000 and 2007. Taxa label indicates the geographical region, collection date, and GenBank accession number. Caguas is the region where the city of Cidra is located, which is where the recipient resided. Isolates marked in red represent the sequences obtained from the blood donor and recipient. Pairwise distance shows a 100% similarity between these two sequences and less than 99% from the other sequences represented in the tree. The ML tree was rooted using the Jamaica 1983 E gene sequence.

blood unit in which the plasma contained fewer than 1000 DENV RNA copies/mL, one of which was from an IgM-positive donor (Tables 1 and 2; Donor Samples 18 and 19). In addition, neither recipient developed signs or symptoms consistent with DENV infection after transfusion. A third recipient in Puerto Rico who was transfused with RBCs from a unit containing 10^8 copies of DENV-2/mL of plasma became febrile 3 days posttransfusion and developed DHF (see Case report). The attending physician suspected dengue in the recipient and sent a serum sample for diagnostic testing on Day 5 after onset of illness that was positive for DENV-2 by RT-PCR. No other transmissions were detected.

The implicated donor unit was from a 31-year-old female who did not report any dengue-related symptoms before or after donation and was healthy on the day of donation. She had donated once previously in 2002 without event. Her unit was collected on September 13, 2007; testing showed that her plasma contained 1.35×10^8 copies/mL of DENV-2 and was IgM negative (Tables 1 and 2). RBCs prepared from the unit were transfused into a recipient who subsequently tested DENV-2 positive by RT-PCR (see Case report).

Frozen aliquots of the donation and recipient samples were available for further study. The sequence of 1485 bp corresponding to the DENV-2 envelope gene confirmed DENV-2 in both the donor and the recipient viruses and showed 100% sequence identity between the two viruses. Figure 3 shows the maximum likelihood analysis of the donor-recipient pair along with other DENV-2 isolates from various geographic areas representing different dengue outbreaks. Sequencing of viral isolates focused on the Caguas region of Puerto Rico including Cidra where the recipient resided. The sequencing results demonstrate that the virus from the donor and the recipient were identical and differed from other viruses found in the region where the recipient resided.

Case report

On September 26, 2007, an 80-year-old man with bronchial asthma, chronic hypertension, chronic obstructive

pulmonary disease, moderate tricuspid regurgitation, and myelodysplastic syndrome characterized by refractory anemia with ringed sideroblasts was admitted for symptomatic anemia. He was given 2 units of RBCs early in the morning of September 27, 2007. During the second transfusion, the patient became confused and pulled the line out of his arm, contaminating the floor and resulting in loss of half of the unit (i.e., he received 160 of 291 mL of the DENV-2 TMA-RR unit). The transfusions were otherwise uncomplicated; there were no transfusion-associated reactions and the patient's vital signs and electrocardiogram remained stable throughout. The patient was discharged to his home in central Puerto Rico that same evening.

On September 30, 2007, the recipient returned to the hospital's emergency department (ED) with complaints of general malaise and "not feeling good" since hospital discharge. The patient reported having chills, polyarthralgia, dry cough, headache, and fever since that morning (approx. 72 hr after transfusion). His hematocrit (Hct) was 35.5% and it had been stable since discharge but his creatinine and blood urea nitrogen were slightly elevated from baseline at 1.8 and 25.5 mg/dL. In the ED triage, the recipient had a temperature of 37.8°C, heart rate of 83 bpm, respiratory rate of 20 bpm, a blood pressure of 117/56 mmHg, and SaO_2 of 88% on room air. He appeared to be acutely ill but was alert, active, oriented, and not in any acute respiratory distress. The physical exam was unremarkable except for dry mucous membranes, minimal coarse rhonchi over the right lung field and bibasilar crackles, and a 2/6 systolic ejection murmur at the left sternal border. The recipient was given 0.9% normal saline intravenously, 3 L of oxygen by nasal canula, and respiratory treatments with ipratropium bromide and a β2-adrenergic agonist. He was readmitted with a presumptive diagnosis of health care–associated pneumonia for which he was given vancomycin and cefepime for 7 days. Blood and urine cultures collected in the ED and the initial chest radiograph were negative. A repeat chest radiograph on October 2 showed a right upper lung infiltrate.

Despite treatment with antibiotics, the recipient continued to have fever until the early morning of October 3, during which time his platelet (PLT) count and white blood cell count progressively declined from 183,000 and 4600 cells/mm^3 respectively, at admission to 40,000 and 1800 cells/mm^3. As a result, the diagnosis of dengue was considered and a serum sample was sent to the CDC's Dengue Branch for diagnostic testing where it tested DENV-2 positive by RT-PCR. In response to his low absolute neutrophil count, filgastrim, a granulocyte–colony-stimulating factor, was added to his treatment regimen. In the 48 hours after defervescence, the patient was noted to have episodes of hypotension (i.e., systolic blood pressure <90 mmHg) even though he had not had any antihypertensive medications since admission. In response, the patient was given intravenous volume replacement with 0.9% normal saline. At the same time, his serum albumin declined from 4.1 to 3.0 g/dL, and he developed large hematomas at injection sites. Even though the patient had no clinically significant bleeding detected, he met the criteria for DHF, namely, he had a fever for 5 days, thrombocytopenia, hemorrhagic manifestations, and plasma leakage as evidenced by development of hypotension and hypoalbuminemia after defervescence. The recipient received 1 unit of pheresis PLTs for a PLT count of 10,000 cells/mm^3 on October 6 and 1 unit of RBCs for a Hct of 24.6%. The remainder of the hospital course was uneventful and he fully recovered from DHF. He received 1 unit of RBCs before being discharged to home on October 11, 2007.

DISCUSSION

This study demonstrates a high frequency of blood donations with plasma DENV TMA-RR (1:529) during the 2007 dengue season in Puerto Rico. Of the 29 TMA-RR units, nearly 80% lacked IgM; nearly half had high viral loads and were capable of infecting mosquito cells in culture, proving that these donations were viremic and could pose a risk to recipient safety. However, fewer than half of the TMA-RR units could be detected in a 1-to-16 dilution, the common pool size used for TMA for other viruses (HIV, HCV, HBV, and WNV); predictably, those detected at a 1-to-16 dilution also had high viral loads. Since the infectious dose of DENV by transfusion is not known, and underlying susceptibility of recipients will vary, all RNA-positive units should be considered potentially infectious. Transfusion transmission was documented in this study, which was the first to document transfusion-transmitted DENV resulting in significant clinical illness.

Studies in Brazil, Honduras, and Puerto Rico have demonstrated the presence of DENV RNA and viremia among blood donations using TMA to detect viral RNA.[10,11] In one study, 9 of 2994 (0.37%) plasma specimens from Honduras in 2004 to 2005 and three of 4858 (0.06%) archived plasma specimens from Brazil in 2003 tested positive although none of 5879 archived plasma specimens collected by the Australian Red Cross Blood Services in 2005 was positive.[11] In a prior study in Puerto Rico, 12 of 16,521 (0.07%) archived unlinked plasma specimens collected by the ARC between September 20 and December 4, 2005, were TMA-RR in a year where 6039 cases of dengue-related disease were reported versus 10,508 reported cases in 2007.[10] In that study, as in our study, fewer than half of the TMA-RR samples confirmed by type-specific RT-PCR or were viremic as demonstrated by mosquito culture. However, both RT-PCR and mosquito cell culture are less sensitive than TMA.[39]

Modeling studies estimating the DENV transfusion transmission risk in the absence of testing have been per-

formed in various geographic areas. These include an estimated average risk during a dengue outbreak in 2004 in Cairns, Queensland, Australia, of 0.5 per 10,000;[40] a range of risk of 1.6 to 6 per 10,000 during 2005 in Singapore;[41] and, most recently in Puerto Rico, an average estimated risk of viremic donations of 7.0 per 10,000 over a 16-year period from 1995 to 2010.[42] Of note, the modeled estimated risk of viremic donations in Puerto Rico in 2007 was identical to the 29 TMA-RR donations observed in this study, with a 95% tolerance interval for the modeled estimate of 29 of 11 to 52. The modeled finding may be an overestimate based on the fact that not all RNA-positive donors will be viremic and infectious.[42]

There have been reports of DENV transmission through transfusion or transplantation.[12-14] The first published case of transfusion-transmitted dengue occurred in Hong Kong in 2002. The donor became symptomatic 1 day after donation and one recipient of RBCs developed dengue-related illness 3 days after transfusion; the patient subsequently seroconverted. Both the donor and the recipient had DENV-1 RNA identified in their blood by RT-PCR.[13] More recently, a second cluster of DENV transfusion transmission was identified in Singapore in which the donor became symptomatic 1 day after donation and two recipients (one of RBCs and the other of fresh-frozen plasma) developed dengue-related illness and seroconverted; the third recipient (of PLTs) was asymptomatic but developed IgM and IgG antibodies. The donor and the two symptomatic recipients were positive for DENV-2 RNA.[14] In addition, DHF was reported 5 days after receipt of a kidney transplant from an infected donor in Singapore[43] and dengue was reported in a bone marrow recipient in Puerto Rico in which DENV-4 was isolated from blood and tissues 4 days after transplant.[44] Moreover, seven instances of nosocomial transmission of dengue have been reported: six through needle stick injuries[45-49] and one through contact of infectious blood with the mucous membranes of a laboratory worker.[50]

Based on the results from this and the earlier studies,[10,11] it is clear that DENV RNA–containing donations occur and interventions should be considered. One intervention that the ARC implemented for collections during the 2009 dengue season in Puerto Rico included the use of a predonation question regarding dengue-related symptoms coupled with the use of an enhanced postdonation information sheet encouraging donors to call back if dengue-like symptoms developed (persistent fever and any of the following: headache, eye pain, muscle aches, joint or bone pain, new rash, bleeding from the nose or gums, or bruising easily). However, these measures would be predicted to be ineffective due to the fact that 53% to 87% of DENV infections are asymptomatic;[51,52] in fact, during the time of use, only one donor reported postdonation symptoms. Due to the fact that TMA has not been available for blood donation screening, serologic testing for DENV using a commercial NS1 antigen ELISA (Bio-Rad, Paris, France)[53] was implemented in March 2010; however, the clinical sensitivity of the NS1 antigen assay has been demonstrated to be 3- to 10-fold less sensitive than TMA by testing blood donations from the same DENV outbreak year.[54,55] For screening of donated blood, assays targeting DENV RNA are the preferred approach.

There may be several reasons why only a very limited number of dengue transfusion transmissions have been reported including: 1) recipient immunity from homotypic serotypes or recent heterotypic serotype immunity; 2) the infectious dose required for transfusion transmission may be higher than expected; and 3) clinical illness after transfusion may not be recognized as dengue, or if recognized, it may be incorrectly assumed to be mosquito acquired.[42] In any event, in an endemic area, the focus of public health is mosquito control versus the investigation of potential DENV transfusion transmission. Undoubtedly there are more DENV transfusion transmissions than have been documented, our case only being the third cluster reported. It seems likely that more infections resulted from the TMA-RR units identified by this study because not all recipients of such units were tested. Further, not all donations were tested during the 2007 dengue season in Puerto Rico. Therefore, the transmission of DENV-2 to one recipient through transfusion that was confirmed through this study represents the minimum level of transfusion transmission that occurred during the 2007 season in this dengue-endemic area. Based on the results of infecting mosquito cells in culture, in which a viral load of 10^5/mL was able to cause infection, 12 of 29 TMA-RR units contained infectious virions and hence were a risk to recipients. Since these 12 units were identified from 15,350 donations screened, this translates to a transfusion transmission risk of 1 per 1279 or approximately 0.1% of donations during the epidemic season in a dengue-endemic area. Results from this study indicate the need for additional research into the best strategies for preventing dengue transmission via blood transfusion in endemic areas and determining how such strategies should be implemented in nonendemic areas where dengue has recently been introduced.

CONFLICT OF INTEREST

None of the authors had a conflict of interest.

REFERENCES

1. Stramer SL, Hollinger FB, Katz LM, Kleinman S, Metzel PS, Gregory KR, Dodd RY. Emerging infectious disease agents and their potential threat to transfusion safety. Transfusion 2009;49(Suppl 2):1S-29S. Dengue virus fact sheet; updated Dec 2010. [cited 2011 Oct 16]. Available from: URL: http://www.aabb.org/resources/bct/eid/Pages/default.aspx

2. Porter KR, Beckett CG, Kosasih H, Tan RI, Alisjahbana B, Rudiman PI, Widjaja S, Listiyaningsih E, Ma'Roef CN, McArdle JL, Parwati I, Sudjana P, Jusuf H, Yuwono D, Wuryadi S. Epidemiology of dengue and dengue hemorrhagic fever in a cohort of adults living in Bandung, West Java, Indonesia. Am J Trop Med Hyg 2005;72:60-6.
3. Rothman A, editor. Dengue virus. Current topics in microbiology and immunology. Vol. 338. Berlin Heidelberg: Springer-Verlag; 2010.
4. Tan PC, Rajasingam G, Devi S, Omar SZ. Dengue infection in pregnancy: prevalence, vertical transmission, and pregnancy outcome. Obstet Gynecol 2008;111:1111-7.
5. Waterman SH, Novak RJ, Sather GE, Bailey RE, Rios I, Gubler DJ. Dengue transmission in two Puerto Rican communities in 1982. Am J Trop Med Hyg 1985;34:625-32.
6. Guzman MG, Kouri G, Valdes L, Bravo J, Alvarez M, Vazques S, Delgado I, Halstead SB. Epidemiologic studies on dengue in Santiago de Cuba, 1997. Am J Epidemiol 2000;152:793-9.
7. Halstead SB. Antibodies determine virulence in dengue. Ann N Y Acad Sci 2009;1171(Suppl 1):E48-56.
8. Halstead SB. Dengue virus-mosquito interactions. Annual Rev Entomol 2008;53:273-91.
9. Nishiura H, Halstead SB. Natural history of dengue virus DENV-1 and DENV-4 infections: reanalysis of classic studies. J Infect Dis 2007;195:1007-13.
10. Mohammed H, Linnen JM, Muñoz-Jordán JL, Tomashek K, Foster G, Broulik AS, Petersen L, Stramer SL. Dengue virus in blood donations, Puerto Rico, 2005. Transfusion 2008;48: 1348-54.
11. Linnen JM, Vinelli E, Sabino EC, Tobler LH, Hyland C, Lee TH, Kolk DP, Broulik AS, Collins CS, Lanciotti RS, Busch MP. Dengue viremia in blood donors from Honduras, Brazil, and Australia. Transfusion 2008;48:1355-62.
12. Wilder-Smith A, Schwartz E. Dengue in travelers. N Engl J Med 2005;353:924-32.
13. Chuang VW, Wong TY, Leung YH, Ma ES, Law YL, Tsang OT, Chan KM, Tsang IH, Que TL, Yung RW, Liu SH. Review of dengue fever cases in Hong Kong during 1998 to 2005. Hong Kong Med J 2008;14:170-7.
14. Tambyah PA, Koay ES, Poon ML, Lin RV, Ong BK. Dengue hemorrhagic fever transmitted by blood transfusion. N Engl J Med 2008;359:1526-7.
15. Petersen LR, Busch MP. Transfusion-transmitted arboviruses. Vox Sang 2010;98:495-503.
16. Tomashek KM, Margolis HS. Dengue: a potential transfusion-transmitted disease. Transfusion 2011;51:1654-60.
17. Rodríguez Rodríguez D, Garza Rodríguez M, Chavarria AM, Ramos-Jiménez J, Rivera MA, Taméz RC, Farfan-Ale J, Rivas-Estilla AM. Dengue virus antibodies in blood donors from an endemic area. Transfus Med 2009;19:125-31.
18. Teo D, Ng LC, Lam S. Is dengue a threat to the blood supply? Transfus Med 2009;19:66-77.
19. Thomas SJ, Strickman D, Vaughn DW. Dengue epidemiology: virus epidemiology, ecology, and emergence. Adv Virus Res 2003;61:235-89.
20. WHO. Dengue and dengue haemorrhagic fever. Geneva: World Health Organization; 2008.
21. DengueNet. WHO's internet-based system for the global surveillance of dengue fever and dengue haemorrhagic fever (dengue/DHF). [cited 2011 Oct 16]. Available from: URL: http://www.who.int/denguenet Dengue/DHF–global public health burden. Wkly Epidemiol Rec 2002;77:300-4.
22. World Health Organization. Impact of dengue. [cited 2011 Oct 16]. Available from: URL: http://www.who.int/csr/disease/dengue/impact/en/index.html
23. Morens DM, Fauci AS. Dengue and hemorrhagic fever: a potential threat to public health in the United States. JAMA 2008;299:214-6.
24. Ehrenkranz NJ, Ventura AK, Cuadrado RR, Pond WL, Porter JE. Pandemic dengue in Caribbean countries and the southern United States—past, present and potential problems. N Engl J Med 1971;285:1460-9.
25. Centers for Disease Control and Prevention (CDC). Dengue fever at the U.S.-Mexico border, 1995-1996. MMWR Morb Mortal Wkly Rep 1996;45:841-4.
26. Ramos MM, Mohammed H, Zielinski-Gutierrez E, Hayden MH, Lopez JL, Fournier M, Trujillo AR, Burton R, Brunkard JM, Anaya-Lopez L, Banicki AA, Morales PK, Smith B, Muñoz JL, Waterman SH. Epidemic dengue and dengue hemorrhagic fever at the Texas-Mexico border: results of a household-based seroepidemiologic survey, December 2005. Am J Trop Med Hyg 2008;78:364-9.
27. Brunkard JM, Loez JL, Ramirez J, Cifuentes E, Rothenberg SJ, Hunsperger EA, Moore CG, Brussolo RM, Villarreal NA, Haddad BM. Dengue fever seroprevalence and risk factors, Texas-Mexico border, 2004. Emerg Infect Dis 2007;10:1477-83.
28. Effler PV, Pang L, Kitsutani P, Vorndam V, Nakata M, Ayers T, Elm J, Tom T, Reiter P, Rigau-Perez JG, Hayes JM, Mills K, Napier M, Clark GG, Gubler DJ. Dengue fever, Hawaii, 2001-2002. Emerg Infect Dis 2005;11:742-9.
29. Fujimori L. Dengue fever hits Pearl City. Star Advertiser 2011. March 25, 2011; Sect. 7.
30. Florida Department of Health. Florida arbovirus surveillance. [cited. 2011 Oct 16]. Available from: URL: http://www.doh.state.fl.us/environment/medicine/arboviral/index.html
31. Trout ABG, Rodriguez M, Barber J, Leal A, Radke E, Weis K, Stanek D, Stark L, Blackmore C, Gallagher G, Hunsperger E, Tomashek K, Gregory G, Sauber-Schatz E. Locally acquired dengue—Key West, Florida, 2009-2010. MMWR Morb Mortal Wkly Rep 2010;59:577-81.
32. Freedman DO, Weld LH, Kozarsky PE, Fisk T, Robins R, von Sonnenburg F, Keystone JS, Pandey P, Cetron MS. GeoSentinel Surveillance Network. Spectrum of disease and relation to place of exposure among ill returned travelers. N Engl J Med 2006;354:119-30.
33. Mohammed HP, Ramos MM, Rivera A, Johansson M,

Muñoz-Jordan JL, Sun W, Tomashek KM. Travel-associated dengue infections in the United States, 1996 to 2005. J Travel Med 2010;17:8-14.
34. Tomashek KM, Rivera A, Muñoz-Jordan JL, Hunsperger E, Santiago L, Padro O, Garcia E, Sun W. Description of a large island-wide outbreak of dengue in Puerto Rico, 2007. Am J Trop Med Hyg 2009;81:467-74.
35. 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.
36. Martin DA, Muth DA, Brown T, Johnson AJ, Karabatsos N, Roehrig JT. Standardization of immunoglobulin M capture enzyme-linked immunosorbent assays for routine diagnosis of arboviral infections. J Clin Microbiol 2000;38: 1823-6.
37. Rosen L, Gubler D. The use of mosquitoes to detect and propagate dengue viruses. Am J Trop Med Hyg 1974;23: 1153-60.
38. Muñoz-Jordan JL, Collins CS, Vergne E, Santiago GA, Petersen L, Sun W, Linnen JL. Highly sensitive detection of dengue virus nucleic acid in samples from clinically ill patients. J Clin Microbiol 2009;47:927-31.
39. Carrick JM, Knight J, Lontoc-Bugay C, Motta C, Wellbaum JB, Fleischer C, Muñoz JL, Stramer SL, Linnen JM. Highly sensitive and equivalent detection of dengue virus serotypes 1, 2, 3, and 4 with an enhanced transcription-mediated amplification assay. Transfusion 2009;49:29A.
40. Seed CR, Kiely P, Hyland CA, Keller AJ. The risk of dengue transmission by blood during a 2004 outbreak in Cairns, Australia. Transfusion 2009;49:1482-7.
41. Wilder-Smith A, Chen LH, Massad E, Wilson ME. Threat of dengue to blood safety in dengue-endemic countries. Emerg Infect Dis 2009;15:8-11.
42. Petersen LR, Tomashek K, Biggerstaff BJ. Estimated prevalence of dengue viremic blood donors in Puerto Rico, 1995-2010. Transfusion 2012;52:1647-51.
43. Tan FL, Loh DL, Prabhakaran K, Tambyah PA, Yap HK. Dengue haemorrhagic fever after living donor renal transplantation. Nephrol Dial Transplant 2005;20:447-8.
44. Rigau-Perez JG, Vorndam AV, Clark GG. The dengue and dengue hemorrhagic fever epidemic in Puerto Rico, 1994-1995. Am J Trop Med Hyg 2001;64:67-74.
45. De Wazieres B, Gil H, Vuitton DA, Dupond JL. Nosocomial transmission of dengue from a needle stick injury. Lancet 1998;351:498.
46. Wagner D, De With K, Huzly D, Hufert F, Weidmann M, Breisinger S, Eppinger S, Kern WV, Bauer TM. Nosocomial acquisition of dengue. Emerg Infect Dis 2004;10: 1872-3.
47. Hirsch JF, Deschamps C, Lhuillier M. Metropolitan transmission of dengue by accidental inoculation at a hospital. Ann Intern Med 1990;141:629.
48. Langgartner J, Audebert F, Schölmerich J, Glück T. Dengue virus infection transmitted by needle stick injury. J Infect 2002;44:269-70.
49. Nemes Z, Kiss G, Madarassi EP, Peterfi Z, Ferenczi E, Bakonyi T, Ternak G. Nosocomial transmission of dengue. Emerg Infect Dis 2004;10:1880-1.
50. Chen LH, Wilson ME. Transmission of dengue virus without a mosquito vector: nosocomial mucocutaneous transmission and other routes of transmission. Clin Infect Dis 2004;39:e56-e60.
51. Rodriguez-Figueroa L, Rigau-Perez JG, Suarez EL, Reiter P. Risk factors for dengue infection during an outbreak in Yanes, Puerto Rico in 1991. Am J Trop Med Hyg 1995;52: 496-502.
52. Burke DS, Nisalak A, Johnson DE, Scott RM. A prospective study of dengue infections in Bangkok. Am J Trop Med Hyg 1988;38:172-80.
53. Bessoff K, Phoutrides E, Delorey M, Acosta LN, Hunsperger E. Utility of a commercial nonstructural protein 1 antigen capture kit as a dengue virus diagnostic tool. Clin Vacc Immunol 2010;17:949-53.
54. Stramer SL, Linnen J, Carrick JM, Bentsen C, Krysztof DE, Hunsperger E, Muñoz-Jordan J, Dodd RY. Dengue donor viremia determined by RNA and NS1 antigen and detection of dengue transfusion transmission during the 2007 dengue outbreak in Puerto Rico. 2010. Vox Sang 2009; 99(Suppl 1):32. (JE-S1-02).
55. Stramer SL, Foster GA, Boucher C, Miller D, Dickson S, Lenes B, Carrick JM, Linnen J, Lanteri MC, Bentsen C. Comparing the yield of dengue viremic blood donations using NS1 antigen (Ag) and NAT. Transfusion 2011; 51(Suppl S2-101A):4A.