資料４－４　②
（日本赤十字社）

平成 26 年 9 月 24 日開催
薬事・食品衛生審議会
血液事業部会運営委員会
提　　出　　資　　料

日本赤十字社血液事業本部

デングウイルスの国内感染が確認されたことに伴う対応について

【対応策】
①デング熱の国内感染例の周知に係るポスターの掲示
②献血受付及び問診時の発熱等の確認徹底
③献血者健康情報の申告のお願い
（献血後 14 日以内の急な発熱・頭痛・皮膚の発疹等）
④献血者への対応（感染発生地域に行かれた方の 4 週間の献血制限）

	対応地域			
実施日	対応策①	対応策②	対応策③	対応策④
平成 26 年 8 月 27 日	東京都 埼玉県	東京都 埼玉県	東京都 埼玉県	
平成 26 年 8 月 28 日	全国	全国	東京都 埼玉県 神奈川県 千葉県	
平成 26 年 9 月 5 日	全国	全国	全国	全国 1)
平成 26 年 9 月 11 日	全国	全国	全国	全国 2)

【感染発生地域】
1）東京・代々木公園等、厚労省の発表した感染発生地域に行かれた方
2）東京・代々木公園周辺、新宿中央公園、外堀公園

（5 年保存）

血 総 第 １２４ 号
平成 26 年 9 月 11 日

日本赤十字社
各血液センター所長　様

日本赤十字社
血液事業本部長
（公印省略）

デングウイルスの国内感染例が確認されたことに伴う対応について（その４）

標記対応については、平成 26 年 9 月 5 日付血総第 120 号をもって通知したところですが、その後、代々木公園周辺以外の場所においてデング熱に感染したと考えられる患者が発生したことが明らかとなりました。

こうした状況を踏まえて、血総第 120 号による対応を下記のとおり変更することとしたので、速やかに対応願います。

記

1. 対象施設
全血液センター

2. 献血者への対応について
(1) 別紙 1 を掲示して献血者に周知し、代々木公園周辺、新宿中央公園及び外濠公園に行かれた方には最後に行かれてから 4 週間献血をご遠慮いただくこと。
(2) 掲示方法に関しては各施設の運用によること。
※1　上記 2.(1)の下線部（以下「献血制限対象地域」という。）については、今後変更する場合があること。また、その際には別途連絡すること。
※2　献血制限対象地域の定義
当該地域が市町村より狭い範囲に特定されており（○○公園等）、次の①または②の少なくとも一方に該当する地域とする。
① 当該地域で 2 名以上の感染が確認されている、または疑われていること。
② 当該地域で感染蚊が確認されていること。

3. 献血受付及び問診等の対応について

(1) 献血希望者に対して発熱等の状況の確認を徹底し、必要に応じて非接触型体温計等を用いて体温測定を実施し、検診医師に情報を引き継ぐこと。

(2) 検診医師は、献血希望者に対して感染発生地域への訪問歴を適宜確認し、最後に行かれてから4週間経過していない場合は、献血をご遠慮いただくよう説明すること。

(3) 献血された血液の品質確保等に資するため、献血後14日以内に急な発熱（頭痛、皮膚の発疹等を伴う）があった場合は、申告をお願いすることとし、献血者全員に別紙2を配布して周知すること。

なお、得られた情報については、献血者健康情報により対応し、詳細な手順については別紙3に従うこと。

4. その他

(1) 関係職員に本件に関する内容を周知徹底すること。

(2) 別紙4の本社ホームページで掲出した情報にリンクを貼り、自社ホームページ上に掲出すること。

(3) 本件にかかる問合せについては、厚生労働省の通知にある「デング熱に関するQ&A」及び別紙5の「デング熱による献血制限に関するQ&A」を参照して各血液センターで対応すること。

ただし、報道機関から全国的な取り組み、方針などに関する問合せがあり、各血液センターにおける対応が困難な場合は血液事業本部経営企画課で対応すること。

(3) 不明な点は献血推進課・医務採血課・安全管理課に問い合わせること。

5. 添付資料

(1) 掲示物 ・・・・・・・・・・・・・・・・・・・・・・・・ 別紙1

(2) 献血にご協力いただいた方へ ・・・・・・・・・・・・ 別紙2

(3) 血液センターの対応 ・・・・・・・・・・・・・・・・ 別紙3

(4) デング熱の国内感染例を受けて（第四報） ・・・・・ 別紙4

(5) デング熱による献血制限に関するQ&A ・・・・・・・・ 別紙5

以上

別紙 1

「デング熱」の国内感染が発生しています。

以下の場所に行かれた方は４週間献血をご遠慮ください。

東京・代々木公園周辺

新宿中央公園

外濠公園

デング熱の症状には、**急な発熱、頭痛、皮膚の発疹**等があります。

最近、上記症状があった方は職員へお申し出ください。

◆ デング熱とは

・デングウイルスに感染した蚊からうつる病気です。
・日常生活で人から人へ直接うつる病気ではありませんが、**輸血用血液から感染した例**が海外で報告されています。
・感染後症状がでるまでの期間は約２日～１４日ですが、**感染しても症状が出ない場合もあります**。
・詳細は、厚生労働省、国立感染症研究所のホームページをご覧ください。

輸血用血液の安全のためご理解とご協力をお願いいたします。

○○赤十字血液センター

別紙２

献血にご協力いただいた方へ

デング熱に関連するお願い

献血後 14 日以内に、急な発熱（頭痛、皮膚の発疹等を伴う場合もあります。）があった場合は、献血日、氏名、生年月日を、できるだけ早く血液センターまでご連絡をお願いします。

※ご連絡をいただいた方のプライバシーは確実に守られますのでご安心下さい。

〇〇赤十字血液センター

電話◇◇◇－◇◇◇－◇◇◇◇

献血にご協力いただいた方へ

デング熱に関連するお願い

献血後 14 日以内に、急な発熱（頭痛、皮膚の発疹等を伴う場合もあります。）があった場合は、献血日、氏名、生年月日を、できるだけ早く血液センターまでご連絡をお願いします。

※ご連絡をいただいた方のプライバシーは確実に守られますのでご安心下さい。

〇〇赤十字血液センター

電話◇◇◇－◇◇◇－◇◇◇◇

Dengue Viruses

(with comments on Zika virus, another mosquito-borne flavivirus)

Disease Agent:

- Dengue viruses (DENV-1, DENV-2, DENV-3, DENV-4)

Disease Agent Characteristics:

- Family: *Flaviviridae*; Genus: *Flavivirus*
- Virion morphology and size: Enveloped, ~50 nm in diameter
- Nucleic acid: Linear, positive-sense, single-stranded RNA, ~10.7 kb in length
- Physicochemical properties: Susceptible to 70% ethanol, 1% sodium hypochlorite, 2% glutaraldehyde and quaternary ammonium compounds; sensitive to heat; low pH inactivates virus
- Dengue viruses are stable in dried blood and exudates up to several days at room temperature.

Disease Name:

- Dengue, dengue fever (DF), dengue hemorrhagic fever (DHF), dengue shock syndrome (DSS)
  - New WHO classification categorizes dengue into dengue, with and without warning signs, and severe dengue, rather than DHF and DSS
- Sometimes referred to as "break-bone fever" because of the nature of the symptoms

Priority Level:

- Scientific/Epidemiologic evidence regarding blood safety: Low in the continental US; priority is related to asymptomatic viremia that has been shown to result in transfusion-transmitted disease and potential for emergence in those parts of the continental US where the vector exists but disease is not endemic. This risk is mitigated by the low rate of autochthonous transmission in the continental US, and by existing malaria deferrals that would exclude much travel from areas of the world where dengue and malaria coexist. Concern is moderate to high in Puerto Rico and non-US endemic areas.
- Public perception and/or regulatory concern regarding blood safety: Moderate in the continental US; moderate/high in Puerto Rico where the virus is endemic and non-US endemic areas
- Public concern regarding disease agent: Very low in the continental US; moderate/high in Puerto Rico where the virus is endemic and non-US endemic areas

Background:

- Dengue is among the most important mosquito-borne viral disease in the world. The disease is caused by four serologically and genetically distinct viruses termed dengue virus (DENV) -1, -2, -3 and -4. Each DENV has four or more genetic groups (or genotypes).
- In the last 50 years, dengue incidence has increased 30-fold, worldwide.
- Dengue is highly endemic in most tropical and subtropical areas of Asia and the Americas; dengue exists in Africa but degree of endemicity is not known.
- In the US, dengue is endemic in Puerto Rico, Virgin Islands and most US-affiliated Pacific Islands; incidence increases during the wet, hot season of each year. The intensity of annual epidemics is cyclical.
- Throughout the continental US, dengue is the most frequently diagnosed cause of fever in travelers returning from Asia and the Americas.
- Along the US-Mexico border, outbreaks of dengue have been identified in the US when there are epidemics along the Mexican side of the border. CDC serological surveys have demonstrated prevalence of 40% along the US side of the Texas-Mexico border and 78% on the Mexico side. This high rate suggests endemic transmission.
- During 2009-2010, a locally transmitted dengue outbreak was identified in Key West, FL, and isolated autochthonous cases were found farther north in FL.
- During the summer of 2013, a very localized outbreak of dengue occurred among residents of Martin and St. Lucie Counties in Florida. Twenty-two cases were eventually reported. The major blood collection organization briefly suspended collections in the involved counties. This response was made possible by their relatively few collections in the epidemic area and their ability to enhance collections elsewhere. Whether this approach would be feasible in more extensive outbreaks without jeopardizing the blood supply is unknown and dependent on multiple considerations.
- Evidence exists for additional circulation of genetic variants in nonhuman primates.

Common Human Exposure Routes:

- Vector-borne; transmission occurs through a mosquito-human cycle
- The importance of transmission from the sylvatic mosquito-nonhuman-primate cycle is minimal

Likelihood of Secondary Transmission:

- Isolated cases of parenteral transmission; aerosol transmission does not occur.

At-Risk Populations:

- Tropical areas of Asia, Oceania, Africa, and the Americas usually in the monsoon or rainy season, especially among persons residing in substandard living conditions
- Outbreaks in Queensland Australia are occasional, small and quite tightly restricted to Cairns and Townsville.
- Travelers to endemic region (e.g., 3.4 cases/1000 Israeli travelers to Thailand) with highest proportionate morbidity for travelers to Southeast Asia and the Caribbean

- Secondary infections with a new virus type have been associated with an increased risk of severe dengue.

Vector and Reservoir Involved:

- *Aedes* species mosquitoes
- Both urban (human-mosquito) and sylvatic (primate-mosquito) cycles are observed.

Blood Phase:

- Asymptomatic viremia is recognized. Viremia typically begins 2-3 days before the onset of symptoms, and it continues for 4-5 days during acute illness.
- Viremia for dengue 1, 2, and 3 infections ranges from barely detectable to 10^8 virions per mL for 2-12 days (median of 4-5 days); titers for dengue 4 are about 100-fold lower.
- NAT prevalence studies among blood donors in endemic areas (Brazil, Puerto Rico, and Honduras) have shown rates of 0.06%, 0.07-0.2%, and 0.40%, respectively. Virus was cultured from some of these donors.

Survival/Persistence in Blood Products:

- Frozen plasma, red cells and platelets have been associated with transfusion transmission; one case involved a 38-day old red cell unit.

Transmission by Blood Transfusion:

- The first documented transfusion-associated case of dengue occurred in 2002 during a local outbreak in Ma Wan, Hong Kong, an area that is not endemic for dengue. The index recipient was a 76-year-old seronegative woman who developed fever without rash 2 days after receiving a unit of packed red blood cells collected from a 17-year-old donor who was diagnosed with dengue (generalized rash) 7 days postdonation. The blood had been stored at 4-8°C for 38 days prior to transfusion. RT-PCR testing of the recovered donor plasma and archived specimens from the donor and recipient were found to be positive for DENV-1. IgM-specific antibody also developed in the recipient posttransfusion.
- The second documented transfusion transmission was a cluster reported from Singapore, an area endemic for dengue in which three cases occurred from one donation from an infected donor. The donor was a 52-year-old male whose components were transfused to three recipients. The donor reported fever the day following donation, and a stored serum sample was positive for DENV-2 by PCR. Both the RBC and FFP recipients reported fever 1-2 days posttransfusion and tested positive by PCR for DENV-2. Direct sequencing of PCR amplified products from the donor and two recipients showed alignment of envelope segments for DENV-2. The platelet recipient was asymptomatic for dengue. All three recipients tested antibody positive for IgM and/or IgG with documented seroconversion in the RBC recipient 11 days posttransfusion.
- Another report of transfusion transmission was in Puerto Rico in 2007 following tracing of a patient who received an RNA-positive packed red cell unit from a donor in which greater than 10^8 copies/mL were detected. Donor screening was part of a research study investigating the frequency of donor viremia in Puerto Rico during the 2007 dengue outbreak. Recipient samples were available from the CDC as part of a repository of all clinical cases reported on the island. Both the donor and recipient samples had identical sequences of DENV-2 covering 1500 nucleotides in the envelope region. The recipient developed DHF 4 days posttransfusion.
- Transmission also has been observed after needle stick exposure and in bone marrow and kidney transplant recipients.

Cases/Frequency in Population:

- The incidence is variable, but can be as high as 1-8% during epidemic periods in endemic countries. Worldwide, the WHO estimates 50 to 100 million cases of infection and 500,000 cases of life-threatening severe dengue occur annually (0.5-1.0% of those infected).
- In 2010, the global burden of dengue was estimated at 390 million total infections, of which 96 million were symptomatic.
- In endemic areas, over 90% of the population may be antibody positive.

Incubation Period:

- 3-14 days (usually 4-7 days)

Likelihood of Clinical Disease:

- Low; the US CDC estimates that one-half or more of all dengue-infected individuals are asymptomatic, that is, they have no clinical signs or symptoms of disease. Other reports indicate significant variability with documented asymptomatic to symptomatic ratios of 2:1 to 13:1 depending on circumstances surrounding the acquisition of the data.
- Homologous immunity to a single serotype is complete and probably lifelong, but cross-protection between serotypes lasts less than 12 weeks.

Primary Disease Symptoms:

- Dengue fever presents as an abrupt onset of fever sustained for up to 5-7 days, accompanied by a transient maculopapular rash that occurs in up to 50% of the patients around day 4, severe headache, retrobulbar pain, lumbosacral aching pain ("break-bone fever"), conjunctivitis, and facial flushing followed by myalgia or bone pain, anorexia, nausea, vomiting, weakness, and prostration.
- The rash begins on the legs and trunk and spreads centripetally but spares the soles and palms. It may desquamate. In some cases, a biphasic course may occur.
- Severe dengue (DHF, DSS) is quite rare with less than 0.5% of secondary cases progressing to this outcome. It occurs more often in persons previously infected with another serotype of DENV. The distinctive feature of severe dengue is the rapid onset of capillary leakage (pleural effusion, ascites, or

hypoproteinemia) leading to hypotension and hemoconcentration. This results in shock with a subset presenting with hemorrhagic manifestations (petechiae, epistaxis, gastrointestinal bleeding and menorrhagia) that occurs 4-7 days after onset of the disease often following a period of defervescence.
- Central neurologic disorders (encephalopathy, peripheral mononeuropathy, polyneuritis, etc.) may occur in severe dengue although this relationship is not fully accepted by all experts.
- Convalescence may be prolonged.
- Differentiation from chikungunya is sometimes difficult, although excruciating, symmetrical joint pain, as opposed to myalgia, is more consistent with chikungunya.

Severity of Clinical Disease:

- Moderate to high

Mortality:

- High with severe dengue in many endemic regions (5-20% mortality rate if untreated), but lower death rate (0.2%) with staff experienced in the management of the disease
- WHO estimates 20,000 deaths annually worldwide.

Chronic Carriage:

- None

Treatment Available/Efficacious:

- Supportive treatment only

Agent-Specific Screening Question(s):

- No specific question is in use; however, the current questions related to travel outside US and Canada for malaria deferral will result in deferral for travel to many dengue endemic areas.
- Travel questions could be broadened to include areas where malaria is not present and dengue outbreaks are occurring.
- Some authorities in the European Union and elsewhere have implemented temporary deferral periods following any travel to the tropics that are not malaria endemic. This would mitigate risk from dengue, chikungunya and other acute pathogens; e.g., Zika virus, by preventing donation until resolution of asymptomatic viremia. It would be associated with a significant operational burden and loss of donors. The utility of any such intervention is speculative even for known transfusion-transmitted agents as no transfusion-transmitted dengue from travelers has been reported in the US.
- Another strategy is the use of information sheets to enhance postdonation symptom reporting to facilitate quarantine and withdrawal of potentially infectious components.

Laboratory Test(s) Available:

- No FDA-licensed blood donor screening test exists; however, research NAT assays have been used for blood donor prevalence studies and the detection of virus in asymptomatic individuals. Data from one NAT-reactive donor with RNA reactivity of at least 29 days and whose index donation was infectious in mosquito cell culture indicates that the duration of DENV RNA by a transcription-mediated amplification (TMA) assay may be longer than previously demonstrated with less sensitive PCR assays. However, associated infectivity in the presence of neutralizing antibody is expected to be considerably shorter than the duration of the RNA detection interval.
- NS1 antigen is not as sensitive as virus-specific RNA (at least by TMA) for blood donor screening, but its detection correlates with high RNA titers.
- DENV-specific IgM antibody can be detected by EIA 4-5 days after onset of symptoms and remains detectable for 3-6 months. The detection rate is lower in secondary DENV infection than in primary infection.
- In blood donors studied retrospectively in Puerto Rico in 2005 and in 2007 and confirmed positive for dengue RNA, 1/12 (3.4%) and 6/29 (20.6%) were IgM positive (using the CDC MAC ELISA), respectively. Variability was likely related to the extent of the dengue outbreaks (2007 > 2005).
- Differential diagnosis for Zika virus (see below) should be considered if travel to French Polynesia or other endemic areas has occurred.

Currently Recommended Donor Deferral Period:

- No FDA Guidance or AABB Standard exists.
- The appropriate deferral period for clinical dengue is unknown but would likely be on the order of several weeks after the resolution of symptoms.
- Current available scientific data do not support a temporary deferral for donors living in nonendemic or nonoutbreak areas of the US who have traveled to an outbreak and/or endemic area.

Impact on Blood Availability:

- Agent-specific screening question(s): Not generally considered applicable due to concerns of donor loss without demonstrated efficacy
- Laboratory test(s) available: Not applicable; data collected using research tests indicate impact would be low

Impact on Blood Safety:

- Agent-specific screening question(s): Not generally considered applicable due to questions of sensitivity and specificity
- Laboratory test(s) available: Not applicable; potential impact of NAT may be significant in dengue endemic areas but minimal in continental US.

Leukoreduction Efficacy:

- No data available. Plasma viremia makes a clinically significant impact unlikely.

Pathogen Reduction Efficacy for Plasma Derivatives:

- Multiple pathogen reduction steps used in the fractionation process have been shown to be robust in the removal of enveloped viruses.

Other Prevention Measures:

- Mosquito control and avoidance
- Vaccines are in clinical trials.

Comments Related to Zika Virus:

- In 2007, an outbreak of Zika virus, another mosquito-borne flavivirus that has never before been reported outside of Africa or Asia, occurred on Yap Island, a group of four closely grouped islands in Micronesia. Zika virus was originally isolated in 1947 from a rhesus monkey in the Zika forest in Uganda. The virus is believed to be transmitted to humans by infected *Aedes* species mosquitoes. The outbreak was characterized by rash, conjunctivitis, and arthralgia with most infected individuals having only mild symptoms. Although some patient sera had cross-reacting IgM antibody against dengue virus, especially among patients with evidence of previous dengue infections which is common to Micronesia, the illness was clinically distinct from dengue, and Zika RNA was isolated from 15 cases with no other arboviral RNA. A total of 49 Zika virus cases were confirmed of the 185 suspect cases; serosurveys estimated that approximately three quarters of the islands' population (or ~900 people) had illness attributable to Zika virus infection. This outbreak highlights the risk of further expansion of mosquito-borne flaviviruses and the need for robust epidemiologic and laboratory surveillance systems.
- In October 2013, epidemic Zika virus infection recurred on the islands of French Polynesia. By January 13, 2014, 361 laboratory-confirmed and 7156 clinically suspect cases had been reported; however, estimates are that there are more than 35,000 cases as part of this outbreak. This includes the first autochthonous cases identified in New Caledonia where up to 19 autochthonous cases have been reported and 30 in travelers to French Polynesia (February 7, 2013; http://www.promedmail.org/direct.php?id=20140210.2268533). Acute fever with a mild headache is common to most cases along with a maculopapular rash that covers most of the body (median of 6 days with range of 2-14 days), arthritis or arthralgia (median of 3.5 days with range of 1-14 days), and conjunctivitis. The US CDC has issued a travel notice reinforcing standard mosquito avoidance measures. There have been no reported cases of transfusion-transmitted Zika virus. An in-house RT-PCR assay was introduced in January 2014 in French Polynesia.

Suggested Reading:

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ORIGINAL ARTICLE

Review of dengue fever cases in Hong Kong during 1998 to 2005

CME

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Objective To describe the epidemiology, clinical and laboratory findings, and outcomes of patients presenting locally with dengue.

Design Retrospective review of case records.

Setting Public hospitals, Hong Kong.

Patients Medical records of all laboratory-confirmed dengue patients admitted to public hospitals during 1998 to 2005 were reviewed retrospectively.

Results A total of 126 cases were identified, 123 (98%) being dengue fever and three (2%) dengue haemorrhagic fever. One patient who had blood transfusion–acquired dengue fever was highlighted. A total of 116 (92%) cases were 'imported', while 10 (8%) were local. Among the 56 dengue cases confirmed by reverse transcription–polymerase chain reaction, dengue virus type 1 was the most common accounting for 48% of them, followed by type 2, type 3, and type 4 responsible for 23%, 16%, and 13%, respectively. Only type 1 and type 2 were present in locally acquired infections. The median age of the patients was 38 years and the mean duration of hospitalisation was 6 days. There was no mortality, and nearly all patients (98%) presented with fever. Other symptoms at presentation included: myalgia (83%), headache (65%), fatigue (59%), and skin rash (60%). More than one third of patients had gastro-intestinal and upper respiratory complaints. Maculopapular skin rash was the most common physical finding. Thrombocytopenia, neutropenia, and lymphopenia were present in 86%, 78%, and 69% of the patients, respectively. In only 29% of the patients was dengue fever included in the initial differential diagnosis. The demographic, clinical, and laboratory findings as well as outcomes did not differ significantly among the four dengue serotypes, but the lowest lymphocyte counts of type 3 was lower than the other serotypes (P=0.004).

Conclusion When physicians encounter patients with a relevant travel history, presenting with fever and skin rash, and having compatible haematological findings, dengue fever should be included in the differential diagnosis.

Key words
Dengue; Dengue hemorrhagic fever; Serotyping

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Introduction

Dengue is the most common and widespread arthropod-borne viral infection in the world today. It is recognised in over 100 countries throughout the tropics and subtropical areas and threatens the health of approximately 40% of the world's population, of nearly 2.5 billion people.[1] The highest burden of disease occurs in South-East Asia and the Western Pacific, where it is one of the 10 leading causes of hospitalisation and childhood mortality.[2]

In Hong Kong, dengue fever was made notifiable since March 1994 and all infections reported to the Department of Health (DH) are investigated to establish their source. The number of cases reported is showing an increasing trend in recent years; the vast majority being imported from other countries. Hong Kong experienced its first local dengue case in September 2002.[3] Thereafter, several others were encountered in Ma Wan and local cases were subsequently identified sporadically in 2002 and 2003.

The epidemiology, clinical manifestations, and laboratory findings of dengue fever infections and its complications have been extensively described in the medical literature,[4,5] but comprehensive review is lacking for our local patients.

回顧1998至2005年間香港的登革熱病症

目的 對本地登革熱病症的流行病學情況、臨床發現、化驗結果和治療結果作出描述。

設計 病例個案回顧。

安排 香港的公立醫院。

患者 1998至2005年間，入住公立醫院並由化驗確診為登革熱病人的醫療紀錄作回顧。

結果 126宗病例中，123人(98%)患上登革熱，3人(2%)患上出血性登革熱，1人因輸血而染病。116宗(92%)病例是在外地感染回港後發病，10宗(8%)病例在本港感染。由反聚合酶連鎖反應確定的56宗病例中，以「登革病毒一型」最常見，佔同類病症的48%，其次為二型(23%)、三型(16%)和四型(13%)。本地感染個案中只發現一型和二型病毒。病人的年齡中位數為38歲，平均住院期為6日。沒有病人因感染登革熱而死亡。幾乎所有病人(98%)都出現發燒病徵，其他病徵包括肌肉痛(83%)、頭痛(65%)、疲倦(59%)和皮膚出疹(60%)。超過三分一病人出現腸胃和上呼吸道不適。皮膚出疹是最常見在肢體上的病徵。血小板減少、白血球減少和淋巴細胞減少的出現比率分別為86%、78%和69%。只有29名病人在初步診斷時有將登革熱考慮在內。四種登革血清在人口分佈、臨床狀況、化驗發現和治療結果上都沒有明顯分別，但「登革病毒三型」的最低淋巴球數目較其他類型少(P=0.004)。

結論 醫療人員如發現病人有相關旅行歷史，有發燒和皮膚出疹，和出現相似的血液學結果，便應把登革熱作為鑒別診斷。

The objective of this review was to describe the epidemiology and explore the clinical characteristics and laboratory findings of dengue fever and dengue haemorrhagic fever (DHF) cases admitted to Hong Kong public hospitals during the period 1998 to 2005. We also compared the clinical and laboratory features of the four dengue serotypes identified by the polymerase chain reaction (PCR) technique.

Methods

We included patients admitted to public hospitals during 1998 to 2005 by using selective criteria "any diagnosis ICD9CM code" starting with "061 dengue" through the Clinical Data Analysis and Reporting System. A patient list was retrieved and matched with the laboratory-confirmed dengue cases notified to the DH. A case was defined as confirmed by detection of viral genomic sequences in autopsy tissue, serum or cerebrospinal fluid samples by PCR; a four-fold or more rise in immunoglobulin G (IgG) or IgM antibody titres to one or more dengue virus antigens in paired serum samples; or a positive IgM antibody titre in late acute or convalescent phase serum specimens (obtained between September 2003 and July 2004). The epidemiological data and virological results were provided by the Surveillance and Epidemiology Branch, Centre for Health Protection, DH. The clinical presentations, laboratory findings, and outcomes of all the confirmed cases were retrospectively reviewed through medical records.

The dengue cases were categorised into dengue fever, DHF, and dengue shock syndrome. In this paper, the definition of DHF was based on the World Health Organization's criteria and defined as: fever lasting 2 to 7 days, haemorrhagic tendencies (a positive tourniquet test; petechiae, ecchymoses or purpura; bleeding from the mucosa, gastro-intestinal tract, injection sites or other locations; haematemesis or melaena), thrombocytopenia (with platelet counts ≤100 x 10^9 /L) and evidence of plasma leakage due to increased vascular permeability (a rise in haematocrit ≥20% above average for age, sex in the population, a drop in the haematocrit following volume-replacement treatment of ≥20% from baseline, and features consistent with plasma leakage such as pleural effusion, ascites, and hypoproteinaemia). Dengue shock syndrome was defined as DHF together with direct evidence of circulatory failure or indirect evidence manifested as a rapid and weak pulse, narrow pulse pressure (20 mm Hg or hypotension for age) or cold, clammy skin and altered mental status.

Statistical analysis was carried out to compare the epidemiological, clinical, and laboratory findings among the four dengue serotypes. The categorical variables were compared by the Chi squared and Fisher's exact tests. Normally distributed data were compared by analysis of variance and data with skewed distributions by the Kruskal-Wallis test.

Results

Disease trend

In all, 126 patients with laboratory-confirmed dengue fever were admitted to public hospitals from 1998 to 2005. Only three (2%) patients suffered from DHF, while the remaining 123 (98%) had dengue fever; no dengue shock syndrome was reported. The number of patients encountered showed an upward trend from 1998 (2 cases) to 2003 (35 cases), and subsequently remained more or less constant in 2004 (20 cases) and 2005 (24 cases). A total of 116 (92%) were imported, while in 10 (8%) the infection was locally acquired (Fig 1).

No locally acquired disease was reported until in 2002, when nine patients were identified. Among them, six cases were confirmed to be epidemiologically related to the Ma Wan outbreak. Another patient acquired the infection through blood transfusion from one of the Ma Wan cases. The remaining two locally acquired cases in 2002 and one

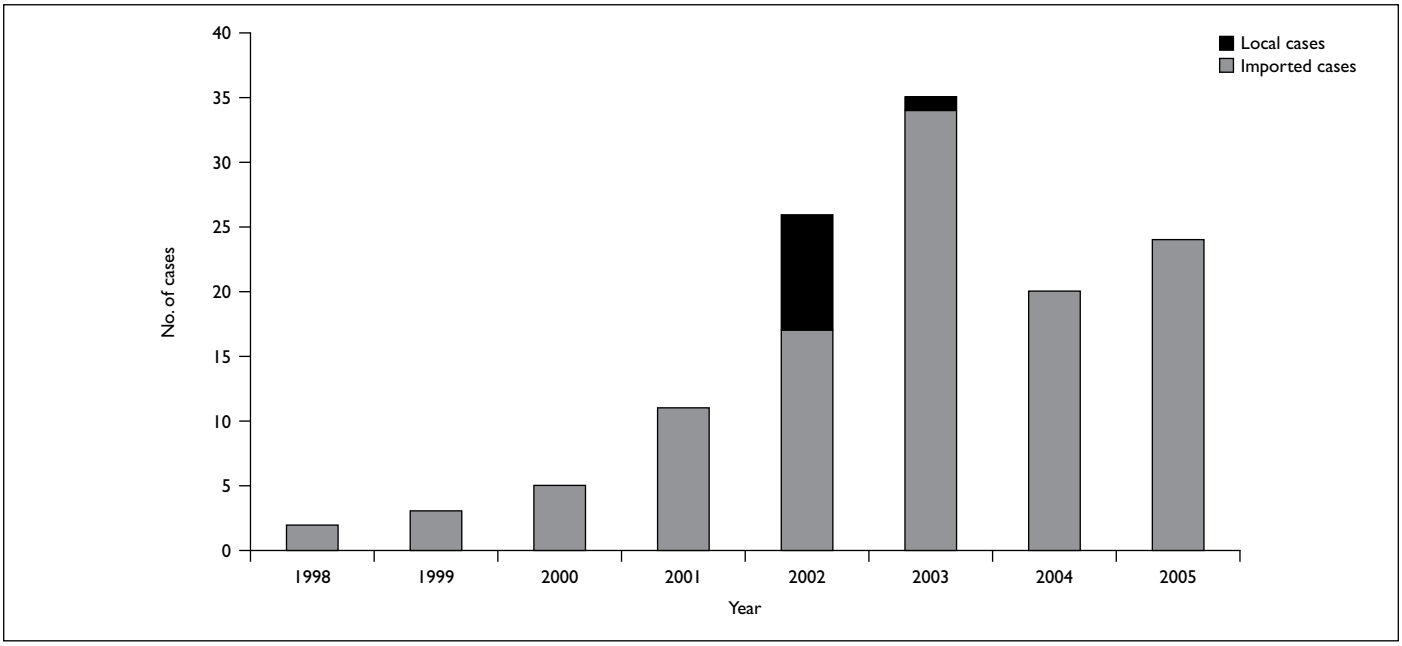

FIG 1. Numbers of dengue fever cases admitted to public hospitals in Hong Kong, 1998-2005

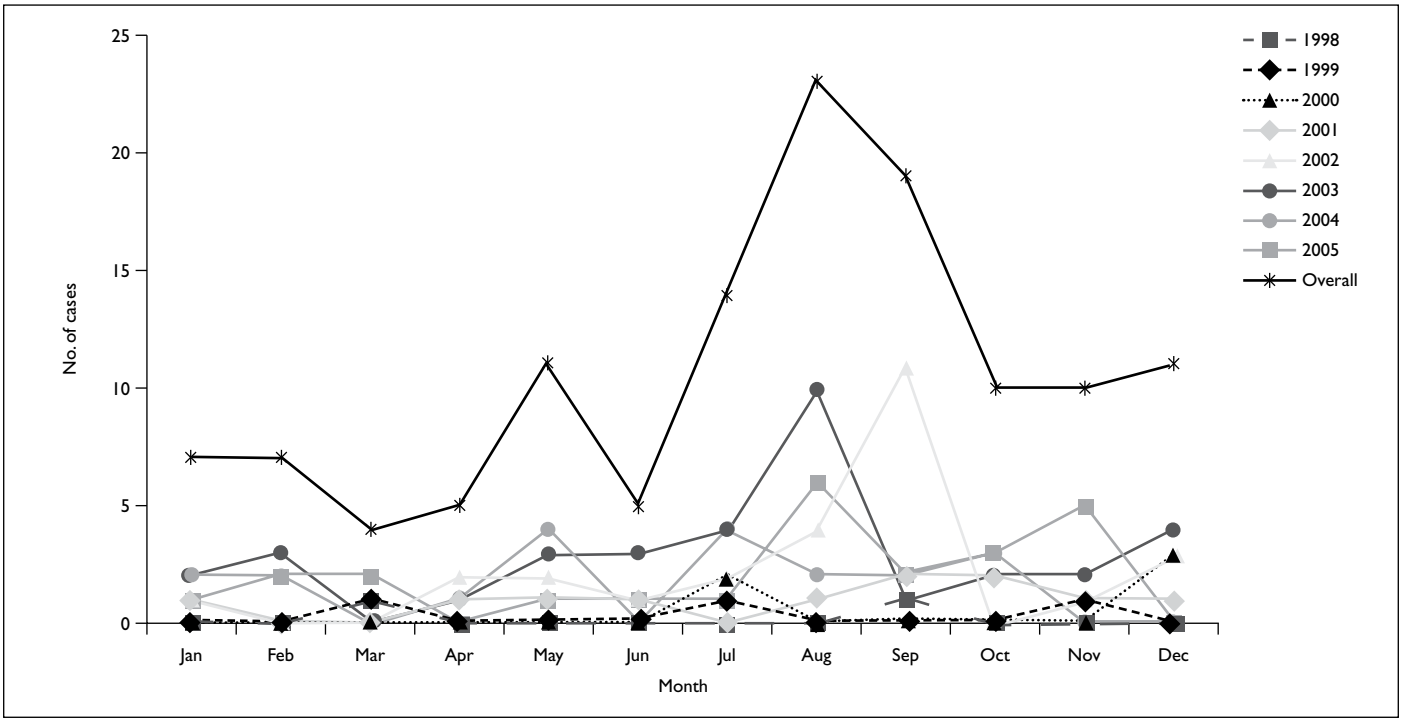

FIG 2. Seasonal variation of dengue fever cases admitted to public hospitals in Hong Kong

in 2003 were sporadic.

Seasonality

In Hong Kong, dengue cases were reported all year round. Figure 2 demonstrates the seasonal variation of cases, with a peak from July to September.

Country of origin for infection

Among the 116 imported cases, 106 (91%) were acquired in South-East Asian countries (Indonesia, Thailand, the Philippines, Vietnam, Singapore, Malaysia, Cambodia, Macau, and the Pacific Islands), eight (7%) originated from South Asia (India, Pakistan, Bangladesh, Sri-Lanka, and Nepal), and one (1%)

from Pitcairn island. Data for one case could not be determined as the patient had recently travelled to more than one country where the infection was endemic.

Patient demographics

The median age of the patients was 38 (range, 5-72) years and the female-to-male ratio was 1:1.2; five (4%) were paediatric patients (aged under 16 years); 114 (90%) were Hong Kong residents. A small proportion of the patients were migrant workers or tourists (4% and 5%, respectively). Among the Hong Kong residents, 86 (75%) were Chinese, 11 (10%) were from other Asian nations (Indonesia, the Philippines, Myanmar, Thailand), three (3%) were White and two (2%) belonged to the Pakistani/Nepalese group. Data on the origin of the remaining 12 patients were missing.

Serotype prevalence

Laboratory data on reverse-transcription PCR serotyping were available since 2002 and the serotypes of the corresponding 56 cases are shown in Figure 3.

All four serotypes, DEN-1, DEN-2, DEN-3 and DEN-4 were present among imported cases; while only DEN-1 (n=6) and DEN-2 (n=1) were present in local cases. Overall, DEN-1 was the most prevalent dengue serotype, responsible for nearly half (48%, 27/56) of all cases, followed by DEN-2 which accounted for about one quarter (23%, 13/56).

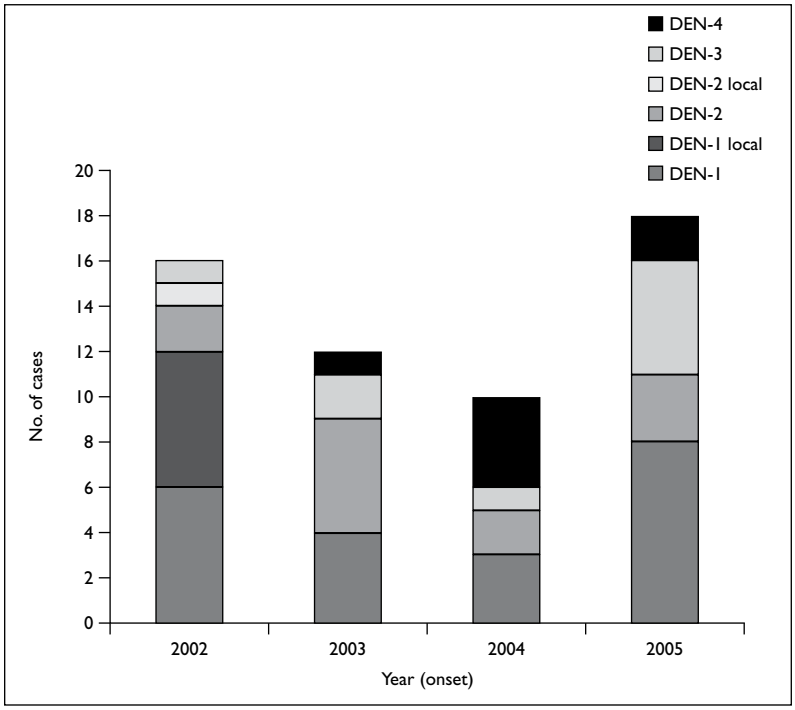

FIG 3. Distribution of serotypes among the dengue fever cases identified from 2002 to 2005

DEN-1 denotes dengue virus type 1, DEN-2 dengue virus type 2, DEN-3 dengue virus type 3, and DEN-4 dengue virus type 4

Clinical presentations and outcome

Approximately 98% (122/124) of patients presented with fever; the mean value for the highest temperature being 38.2°C (standard deviation, 1.0°C) [Table 1]. The second commonest presenting symptom was myalgia, 83% (75/90). Two thirds of patients had headache, fatigue, and skin rashes. One third of the patients (24/71) complained of retro-orbital pain. The chief presenting complaints in more than one third of the patients were gastro-intestinal (nausea, vomiting and/or diarrhoea) or upper respiratory tract (dry cough and/or sore throat) or both. Over one quarter of patients (28/108, 26%) complained of abdominal pain, and one complained of blurred vision. Except for petechiae which were present in 45% (47/105) of the patients, other spontaneous bleeding was uncommon. Maculopapular skin rash was the commonest physical finding; in 70% of those with a rash it occurred predominately on the trunk. Lymphadenopathy was uncommon, which was only elicited in 16% of the patients. No patient demonstrated biphasic fever. Only one patient had clinical and radiological features of plasma leakage (pleural effusion), and was confirmed to be due to DHF as the final diagnosis. The mean duration of hospitalisation for these patients was 6 days, and there was no mortality.

Laboratory findings

Thrombocytopenia was the most common haematological finding, which affected 107 (86%) of the 124 patients with available platelet counts (Table 1). The mean value of the lowest platelet counts was 64 x 10^9 /L. Among those with available results, neutropenia, atypical lymphocytes, and lymphopenia were present in 78%, 75%, and 69% of the patients respectively; half had prolonged activated partial thromboplastin time values. Corresponding proportions with deranged liver function tests and hypoalbuminaemia are also shown in Table 1. Mean values for aspartate aminotransferase and alanine aminotransferase were 212 IU/L and 169 IU/L, respectively.

Clinical differential diagnosis

Dengue infection was included as an initial clinical differential diagnosis in only 29% of the patients. Other differential diagnoses included: viral infection, upper respiratory tract infection, gastroenteritis, typhoid fever, chest infection, malaria, scarlet fever, scrub typhus, influenza, and fever for investigation.

TABLE 1. Recorded clinical symptoms, physical and laboratory findings of dengue cases

Symptoms/findings	No. of patients (%)	Remarks (reference range for laboratory tests)
Clinical symptoms		
Fever	122/124 (98)	
Myalgia	75/90 (83)	
Headache	68/105 (65)	
Skin rash	72/121 (60)	
Fatigue	50/85 (59)	
Dizziness	20/44 (45)	
Retro-orbital pain	24/71 (34)	
Gastro-intestinal tract (nausea, vomiting, and/or diarrhoea)	39/112 (35)	
Upper respiratory tract (non-productive cough, sore throat)	32/112 (29)	
Bleeding manifestations		
Epistaxis	7/67 (10)	
Gum bleeding	8/66 (12)	
Haematemesis	1/65 (2)	Dengue haemorrhagic fever
Tarry stool	1/69 (1)	Dengue haemorrhagic fever
Petechiae	47/105 (45)	
Clinical signs		
Skin rash	86/124 (69)	
Lymphadenopathy	19/116 (16)	
Laboratory findings		
Thrombocytopenia	107/124 (86)	Platelets: 145-370 x 10^9/L
Lymphopenia	79/114 (69)	Lymphocytes: 1.0-3.1 x 10^9/L
Neutropenia	89/114 (78)	Neutrophils: 1.7-5.8 x 10^9/L
Atypical lymphocytes	92/123 (75)	
Prolonged activated partial thromboplastin time	49/97 (51)	Activated partial thromboplastin time: 27.5-40.5 sec
Elevated aspartate aminotransferase	29/32 (91)	Aspartate aminotransferase: <38 IU/L
Elevated alanine aminotransferase	98/123 (80)	Alanine aminotransferase: 3-36 IU/L
Hypoalbuminaemia	34/123 (28)	Albumin: 35-52 g/L

Comparison of epidemiological, clinical, and laboratory findings among the four dengue virus serotypes

There were no statistically significant differences in terms of disease severity between the four virus types, patient gender, age and duration of hospitalisation, headache, myalgia, arthralgia, retro-orbital pain, skin rash, fatigue, gastro-intestinal and respiratory symptoms (Table 2). The percentages of patients with bleeding tendencies were 50%, 67%, 63%, and 33% for DEN-1, DEN-2, DEN-3, and DEN-4 virus type infections, respectively. Further analysis of the haemorrhagic manifestations was conducted by categorisation into epistaxis, gum bleeding, haematuria, and petechiae; 75% of these patients exhibited petechiae only, with no statistically significant difference between virus types (P=0.58). Nor was there any statistically significant difference between patients having different virus subtype infections for laboratory variables, except that the lowest lymphocyte counts of patients infected by serotype 3 was lower than the other serotypes (P=0.004).

Dengue haemorrhagic fever

Of the 126 patients under study, three (2%) were classified as DHF; all were imported from South-East Asian countries, and none could recall a previous history of dengue infection. Their demographic, clinical, and laboratory findings are shown in Table 3. They all received intravenous fluid replacement and platelet transfusions, recovered uneventfully without progression to dengue shock syndrome, and were discharged on day 6 or day 7 after hospital admission. Although these three patients did not recall prior infection, in one it was likely, as evidenced by respective acute and convalescence antibody titres.

Discussion

This is a comprehensive review of dengue fever patients admitted to Hong Kong public hospitals over the past 8 years. Epidemiological data showed that more than 70% of the patients were local Chinese residents with a travel history to neighbouring South-East Asian countries, where dengue fever is more endemic.[6] The most prevalent serotype was DEN-1, followed by DEN-2, DEN-3, and DEN-4, which was consistent with the serotype patterns in the countries from which such infections were imported.[7,8] The outbreak in Ma Wan was the first local one in Hong Kong; only DEN-1 and DEN-2 virus subtypes were encountered in local patients during 2002 and 2003.

Seasonal variations in dengue infections should be interpreted with cautions. Dengue fever is a travel-related arthropod-borne viral disease in Hong Kong; disease activity is closely related to and depends on the seasonal and weather conditions of countries from which the virus is imported. It is difficult to determine the seasonal patterns of dengue fever acquired locally based on the few reported cases. Monthly ovitrap surveillance in Hong Kong showed that the density of *Aedes albopictus* increases from April and peaks in June.[9] It is important to alert the public to keep vigilance against this mosquito-borne viral disease during this peak period.

We report here the first blood transfusion–transmitted dengue in the literature. The patient was a 76-year-old woman, with a history of hypertension and bronchiectasis. She was admitted in 2002 because of progressive malaise. Blood tests revealed

TABLE 2. Comparison of demographic, clinical, and laboratory findings in patients infected with the four dengue serotypes*

	PCR† type 1 (n=27)	PCR type 2 (n=13)	PCR type 3 (n=9)	PCR type 4 (n=7)	Overall (n=56)	P value
Gender (M:F)	13:14	6:7	5:4	3:4	27:29	1.0000
Age, median (IQR)	36.0 (24.0-52.0)	54.0 (33.0-66.0)	28.0 (23.5-61.0)	35.0 (21.0-63.0)	36.0 (26.3-57.8)	0.3559
Duration of hospitalisation, median (IQR) [days]	5.0 (4.0-7.0)	6.0 (3.0-7.5)	7.0 (4.5-8.5)	5.0 (4.0-6.0)	5.0 (4.0-7.0)	0.4589
Retro-orbital pain	9/18 (50)	3/10 (30)	0/4 (0)	1/6 (17)	13/38 (34)	0.2297
Rash—symptom	15/25 (60)	8/13 (62)	2/9 (22)	2/7 (29)	27/54 (50)	0.1332
Rash—sign	17/26 (65)	10/13 (77)	6/9 (67)	1/7 (14)	34/55 (62)	0.0509
Abdominal pain	3/25 (12)	2/12 (17)	1/8 (13)	1/7 (14)	7/52 (13)	1.0000
Diarrhoea	11/25 (44)	5/12 (42)	2/8 (25)	1/6 (17)	19/51 (37)	0.5956
Bleeding manifestation (epistaxis, gum bleeding, petechiae, haematuria)	13/26 (50)	8/12 (67)	5/8 (63)	2/6 (33)	28/52 (54)	0.5775
Hepatomegaly	2/26 (8)	2/13 (15)	0/9 (0)	1/7 (14)	5/55 (9)	0.5883
Leukopenia	25/26 (96)	10/13 (77)	9/9 (100)	5/7 (71)	49/55 (89)	0.0529
Lymphopenia	20/22 (91)	9/13 (69)	8/9 (89)	4/6 (67)	41/50 (82)	0.2550
Atypical lymphocyte	18/26 (69)	10/13 (77)	7/9 (78)	6/7 (86)	41/55 (75)	0.8848
Thrombocytopenia	26/26 (100)	11/13 (85)	8/9 (89)	6/7 (86)	51/55 (93)	0.0931
Elevated aspartate aminotransferase	8/9 (89)	3/4 (75)	4/4 (100)	2/2 (100)	17/19 (89)	1.0000
Elevated alanine aminotransferase	23/26 (88)	11/13 (85)	7/9 (78)	6/7 (86)	47/55 (85)	0.8954
Hypoalbuminaemia	10/26 (38)	5/13 (38)	5/9 (56)	4/7 (57)	24/55 (44)	0.6658
Highest temperature, mean (SD)	38.6 (1.0)	38.2 (1.1)	38.6 (1.3)	38.7 (0.6)	38.5 (1.0)	0.6893
Transfusion	4/23 (17)	2/12 (17)	1/8 (13)	2/6 (33)	9/49 (18)	0.8548

* Data are shown in No. (%), except otherwise stated
† PCR denotes polymerase chain reaction

TABLE 3. Demographic, clinical, and laboratory findings in patients with dengue haemorrhagic fever

Sex/age (years)	Ethnicity	Fever	Haemorrhagic manifestations	Lowest platelet count (x 10^9/L)	Plasma leakage	Laboratory findings	
						Serotype	Serology titer
M/38	Thai	37.2°C	Petechiae, bloody diarrhoea	9	Pleural effusion	Not done	Immunoglobulin M +ve
M/46	Chinese	38.4°C	Petechiae, bruises	9	Ascites	DEN 2	Immunoglobulin M +ve
F/49	Thai	38°C	Coffee ground vomitus, petechiae	8	Hypoalbuminaemia, haemoconcentration	DEN 1	4-fold increase*

* 1st titre: 640 (DEN-1), 5120 (DEN-2), 1280 (DEN-3), 1280 (DEN-4); 2nd titre: 5120 (DEN-1), 10 240 (DEN-2), 10 240 (DEN-3), 10 240 (DEN-4)

macrocytic anaemia and pancytopenia. She was diagnosed to have vitamin B12 deficiency anaemia, which was treated by vitamin B12 replacement and received a blood transfusion on 24 August 2002. On day 2 post-transfusion, she developed low-grade fever, but no skin rash, headache, myalgia, arthralgia, or retro-orbital pain. The patient was treated with antibiotics as for a urinary tract infection, based on the microbiological findings. The fever subsided 3 days later and the patient recovered uneventfully. The blood product she received was donated by a 17-year-old asymptomatic patient living in Ma Wan, during his viremic phase on 17 July 2002. On 24 July 2002, he developed generalised skin rash and attended the Accident and Emergency Department of Yan Chai Hospital. In October, he was subsequently picked up as one of the dengue cases based on serology results during the active case finding exercise in Ma Wan. Molecular testing performed on the donated blood product was positive for dengue virus type 1. The woman who had received the blood transfusion was recalled for blood testing on 7 October 2002, and was found to be positive for corresponding IgM antibodies and had a haemagglutination-inhibition titre of 1:2560. This incident was the first documented cases of such transmission in the literature, and since October

2002, the Hong Kong Red Cross Blood Transfusion Service (BTS) has intensified its donor deferral systems to counter this possibility. Specifically, it now asks about symptoms of dengue fever in the Blood Donor Registration Form (Supplement) by reminding all prospective donors to inform the BTS staff of all instances for flu, fever, headache, eye pain, muscle/joint pain, vomiting, and skin rash experienced 2 weeks before or after blood donation.

In our study, dengue fever was far more common than DHF and dengue shock syndrome, which were rare events. Our patients only manifested mild bleeding with good clinical outcomes and no fatalities. The clinical presentations of dengue fever, such as fever, myalgia, headache, and arthralgia, were comparable to findings reported in other studies.[10-12] Our patients (35%) presented with fewer gastroenteritis symptoms compared to those of others (50-98%).[11,12] Lymphadenopathy was documented in only 16% of our patients, which is much lower than the figure of 50% reported elsewhere.[13] This difference may be accounted for by less-than-adequate physical examination. Gum bleeding and epistaxis were reported in 12% and 10% of our patients respectively, which was also much lower than that reported previously.[11,12] Such differences could be due to the populations studied; patients recruited in endemic countries were mainly encountered during outbreaks in which both dengue fever and DHF were common. Previous studies showed dengue disease severity correlated with high viremia titres, secondary infection, and DEN-2 serotype infection.[14,15] Our findings showed that the haemorrhagic tendencies and duration of hospitalisation were not related to specific serotypes. Although some of our patients did receive platelet transfusions, the efficacy of such treatment in speeding recovery remains controversial. According to Thai experts, platelets are almost immediately destroyed by immune lysis after administration.[16]

Our study had several limitations. First, the target patients were limited to those with laboratory-confirmed dengue admitted to public hospitals. During 1998 to 2005, DH received notification of 203 dengue cases, including 77 who were admitted to private hospitals or consulted general practitioners only. The disease burden might also be underestimated, because some patients might have recovered, without seeking medical attention, while others might not have undergone serological testing. Second, statistical analysis could not be carried out to compare clinical and laboratory parameters in patients with dengue fever and DHF, as there were too few of the latter. Third, laboratory results before 2002 were not available in the Public Health Laboratory Information System. Fourth, not all clinical symptoms and signs listed in Table 2 could be retrieved from the medical records, as some may not have been specifically asked for or looked for.

In conclusion, dengue fever should be considered in the differential diagnosis of febrile patients with or without a travel history. Health care providers should therefore have an understanding of the infection, the spectrum of its clinical features, and methods of diagnosis and appropriate treatment. Until the *Aedes* mosquito can be effectively controlled or a cost-effective vaccine is developed, dengue fever will remain a public health concern, especially in South-East Asia. Control at source is one of the keys to combating dengue fever and requires active participation from all sectors of the community.

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amounts of several enzymes, including cytochrome P-450 (CYP) 1A1, CYP1B1, CYP2B6, CYP2E1, and CYP3A5, as well as members of the glutathione S-transferase family. These enzymes may generate allergenic metabolites of various drugs, including azathioprine.[4]

We have demonstrated, by patch testing and enzyme-linked immunosorbent spot assay, a case of indirect conjugal azathioprine-induced allergic contact dermatitis. The mechanistic basis for the husband's idiosyncratic drug hypersensitivity has not yet been determined, but there may be an aberrant pathway of detoxification within his skin, resulting in the production of an immunogenic compound.

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Dengue Hemorrhagic Fever Transmitted by Blood Transfusion

TO THE EDITOR: Dengue, the most common vectorborne viral infection worldwide,[1] is predominantly transmitted by the *Aedes aegypti* mosquito. We describe a well-documented cluster of blood transfusion–associated dengue infections in Singapore, a country in which the disease is endemic.

A 52-year-old, asymptomatic, repeat blood donor gave blood on July 15, 2007. An investigation of all recipients of his blood products was initiated after he informed the blood bank that he had had a fever the day after donation. The stored serum sample was positive for dengue virus type 2, as ascertained by means of a polymerase-chain-reaction (PCR) assay.[2]

The recipient of the donor's red cells had fever and myalgia 2 days after transfusion. The recip-

Table 1. Characteristics of the Donor and Recipients.

Patient	Age	Sex	Coexisting Conditions	Symptoms of Dengue Fever	Signs of Capillary Leak	Results of Serologic Testing	Findings on PCR Assay*	Outcome
	yr							
Donor	52	M	None	Fever and myalgia after donation (not hospitalized)	None	Not done	Dengue virus type 2	Full recovery
Recipient of fresh-frozen plasma	64	M	Diabetes mellitus, hypertension, ischemic heart disease, recent coronary-artery bypass graft, chronic renal impairment	Day 2 after transfusion (hospital day 12): fever, jaundice, malaise, and worsening thrombocytopenia	Worsening of bilateral pleural effusions	Seroconversion (on July 19, negative for IgG and IgM; on July 31, positive for both)	Dengue virus type 2	Discharged in good health
Recipient of packed red cells	72	M	Diabetes mellitus, hypertension, ischemic heart disease, peptic ulcer disease	Day 2 after transfusion (hospital day 6): fever, myalgia, malaise	Small right pleural effusion	IgG-positive on follow-up	Dengue virus type 2	Discharged in good health
Recipient of platelets	74	M	Hepatocellular carcinoma	None	None	Positive for both IgG and IgM on follow-up	Not done	Discharged in good health

* PCR denotes polymerase chain reaction.

ient of the donor's fresh-frozen plasma had fever and worsening pleural effusions the day after transfusion. Both recipients were positive for dengue virus type 2 as detected with the use of a PCR assay, with serologic evidence of secondary dengue infections, and received supportive care and were discharged in good health. The recipient of the donor's platelets was asymptomatic but had serologic evidence of a recent secondary dengue infection on follow-up. Clinical and laboratory details of the patients are shown in Table 1.

We cloned the PCR-amplified products from the donor and two recipients, by using a cloning protocol (Topo TA, Invitrogen). Direct sequencing of all available envelope glycoprotein gene-cloned segments 78 bp in length, by means of a sequencing kit (ABI PRISM 3100, Applied Biosystems), showed alignment with published sequences for dengue virus type 2 in the GenBank database that are highly conserved in local circulating strains; we were unable to perform whole-genome sequencing for definitive confirmation. Given the timing of the infections — soon after transfusion and, in the plasma recipient, during a prolonged stay in an air-conditioned mosquito-free intensive care unit — the evidence for transfusion-related transmission is convincing.

To our knowledge, transfusion-associated dengue is quite rare; there was a report from Hong Kong, where the disease is not endemic.[3] Although it is transient, asymptomatic dengue viremia is a potential risk to the blood supply.[4] Nucleic-acid testing has greatly improved blood safety; for example, the potential risk of transfusion-associated transmission of West Nile virus in the United States has been markedly reduced through stratified molecular screening.[5] Although screening is expensive, confidence in the blood supply could outweigh cost-effectiveness considerations.

In our patients, prompt recognition through a donor callback system led to favorable clinical outcomes despite the advanced age and multiple coexisting conditions of the patients. This case illustrates the difficulties encountered when attempting to ensure a safe blood supply in the face of emerging flavivirus threats worldwide.

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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

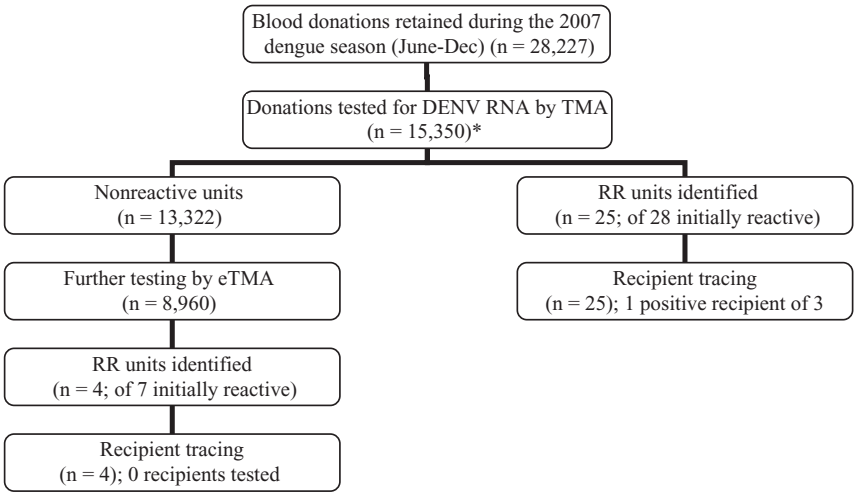

Fig. 1. DENV blood donation screening algorithm and TMA screening results including the 29 TMA RR blood donations. *TMA specificity based on IR samples that did not repeat as reactive = 15,315/15,321 = 99.96% (95% CI: 99.93-99-99).

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

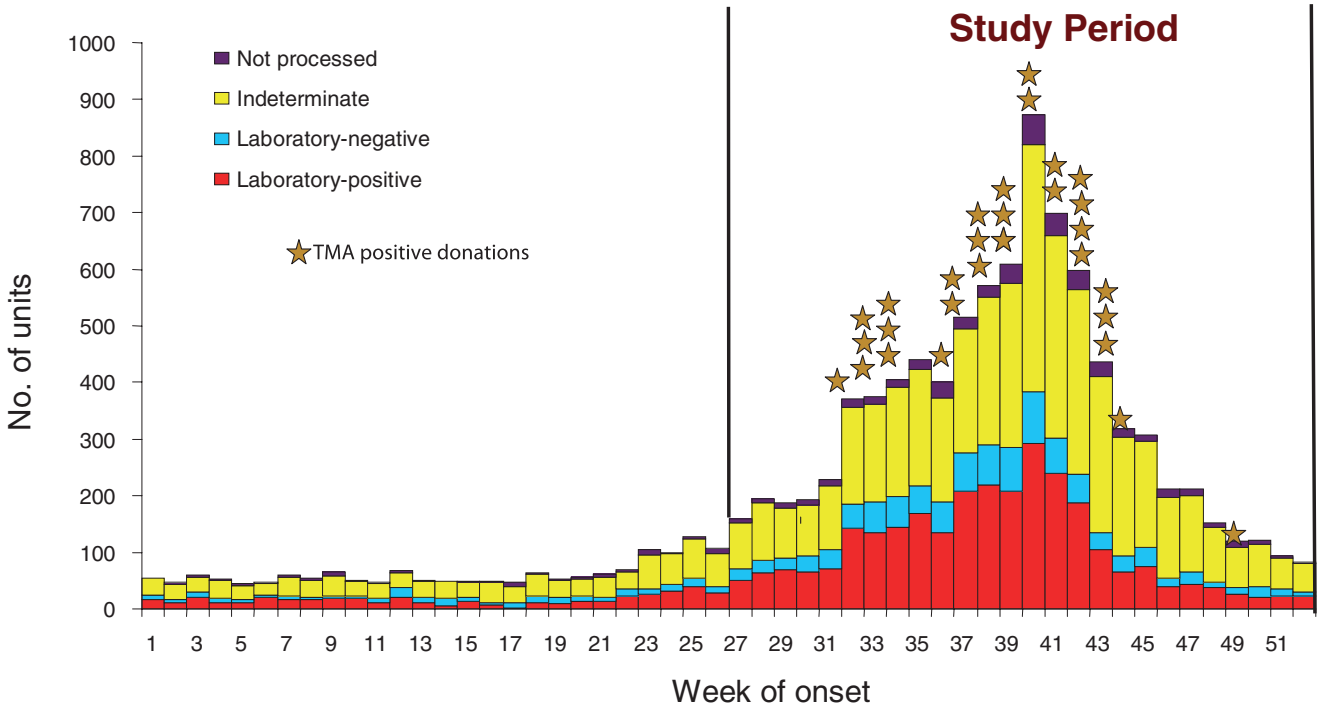

Fig. 2. Number of suspected dengue cases by laboratory outcome reported weekly during 2007 and the week in which TMA-RR blood donors were identified; the study period is indicated as that between the vertical lines.

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.

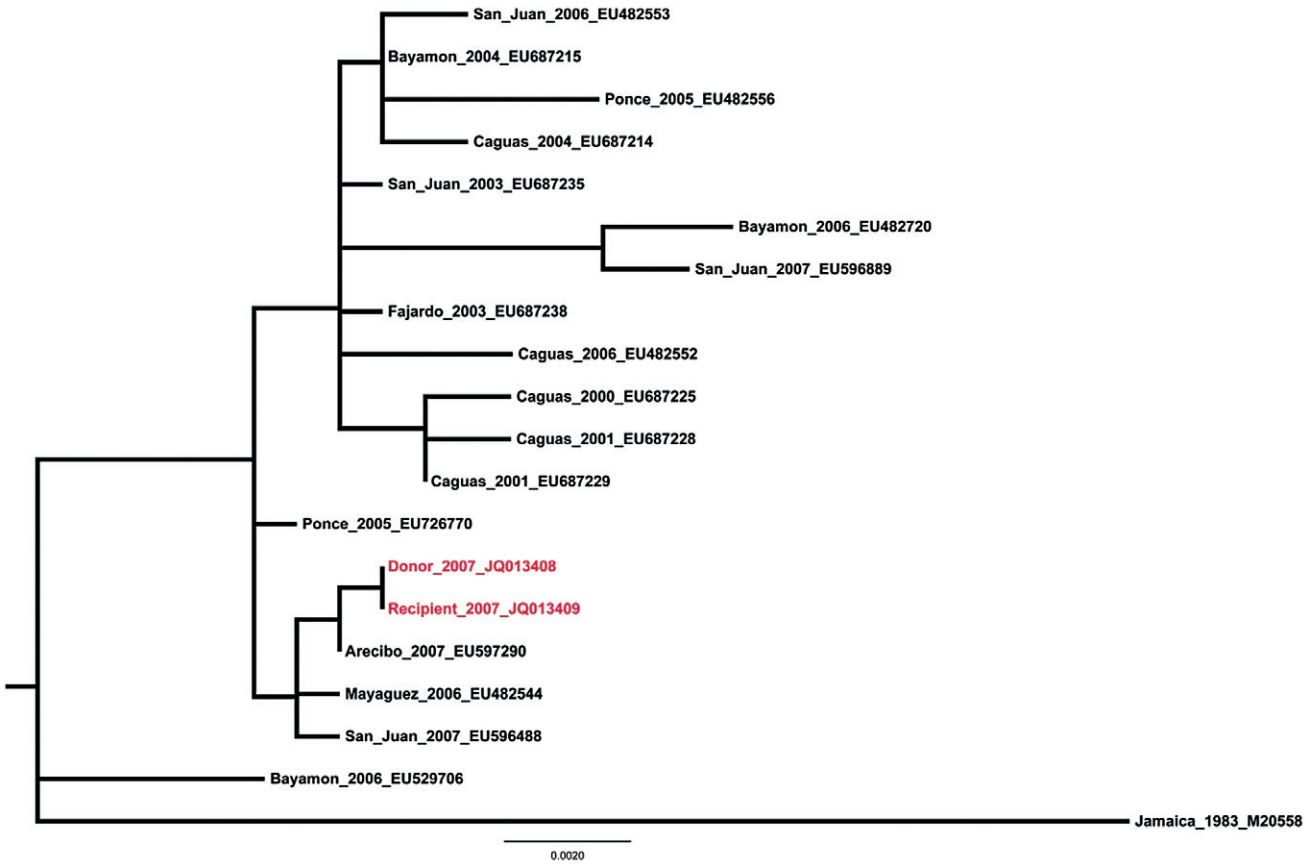

Fig. 3. Maximum likelihood phylogeny of complete E gene sequences (1485 bp) obtained from 19 endemic clinical isolates obtained between 2000 and 2007. Taxa label indicates the geographical region, collection date, and GenBank accession number. Caguas is the region where the city of Cidra is located, 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.

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