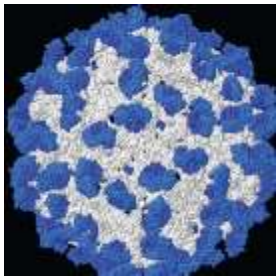
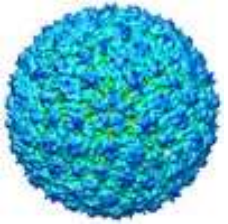


Dengue Virus
Is it an emerging threat to blood safety?

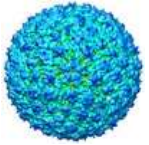
Dr.P.Arumugam,MD

The TN Dr MGR Medical University

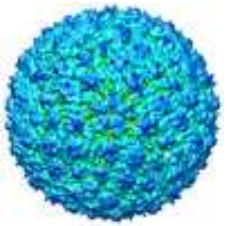
Chennai



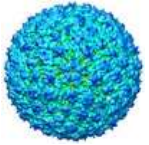
- **Why do you consider Dengue virus as a potential threat to blood safety?**



1. The viremia is frequently **asymptomatic** & usually lasts **for 2 to 7 d**
2. The **viral load** may be **relatively high** (from 10^4 to 10^8 copies/mL by NAT) in blood
3. The **disease** can **occur as** important **outbreaks**
4. The competent mosquitoes have a large distribution in the considered area
5. The viral infection has a **high seroprevalence in** populations boarding the **considered area**
6. **Infected blood products** could be **imported** from **epidemic/ endemic areas**



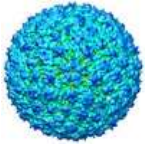
- **Has transfusion transmissible Dengue been demonstrated in recipients world wide?**



The **first** report concerned

- a **76 year-old woman** who received a blood transfusion in **2002** in a **Hong-Kong** hospital following a severe anaemia;
- **2 days later**, she developed low-grade fever that resolved spontaneously (she received antibiotics for a suspicion of urinary infection).
- The case was secondarily related to dengue because the **donor presented a typical dengue** infection documented by serology.
- **Molecular testing** performed **on the donated blood** product was **positive for DEN-1**.
- 2 months after transfusion, the recipient exhibited IgM antibodies confirmed by seroneutralisation assay.
- The case **was published only 6 years later**

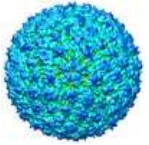
Chuang V, Wong TY, Leung YH, Ma E, Law YL, Tsang O, Chan KM, Tsang I, Que TL, Yung R, et al. Review of dengue fever cases in Hong Kong during 1998 to 2005. Hong Kong Med J. 2008;14:170–177. [PubMed]



The **2nd** study,

- involved a **cluster of 3 cases** contaminated in **Singapore** by the **same donor** who developed fever and myalgia after blood donation.
- **2 days after transfusion**, **2 of the 3** recipients developed a symptomatic infection that resolved spontaneously.
- **Recipients demonstrated** serological evidence of acute **dengue infection**.
- A **PCR assay** performed **on blood specimens** from the donor and the 2 symptomatic recipients was **positive for DEN-2**.

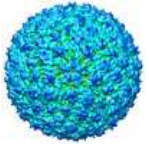
Tambyah PA, Koay ES, Poon ML, Lin RV, Ong BK. Dengue hemorrhagic fever transmitted by blood transfusion. N Engl J Med. 2008;359:1526–1527. [PubMed]



The **3rd** observation,

- was documented from the outbreak of dengue that occurred in **Puerto-Rico** **in 2007**.
- Of **15350 donation samples tested retrospectively**, **29** were found **positive** for DENV genome by **TMA assay**.
- **3 of the recipients** of these contaminated samples tested by NAT & **1 of them**, who received red blood cells containing **10^8 copies/mL** DEN-2, was found positive.
- **3 days after transfusion**, he developed **DHF**.
- Both **donor and recipient** were shown to **harbour** viruses with the **same envelope sequence**.
- This is the **1st case of severe dengue** infection **transmitted by blood products.**

Stramer SL, Linnen JM, Carrick JM, Foster GA, Krysztof DE, Zou S, Dodd RY, Tirado-Marrero LM, Hunsperger E, Santiago GA, et al. Dengue viremia in blood donors identified by RNA and detection of dengue transfusion transmission during the 2007 dengue outbreak in Puerto Rico. Transfusion. 2012;52:1657–1666. [PubMed]



Transfusion transmitted dengue: One donor infects two patients

Dengue virus can be transmitted via blood transfusion. We report an interesting case where **two surgical patients developed possible transfusion transmitted dengue when transfused blood components of the same donor**. Dengue remains a threat to blood supply especially in endemic region.

Farheen Karim, Nadia Nasir, Bushra Moiz Transfusion and Apheresis Science, Vol. 55, Issue 2, October 2016 Section of Hematology and Transfusion Medicine, Department of Pathology and Laboratory Medicine, The Aga Khan University Hospital, Karachi, Pakistan

Real-time symptomatic case of transfusion-transmitted dengue

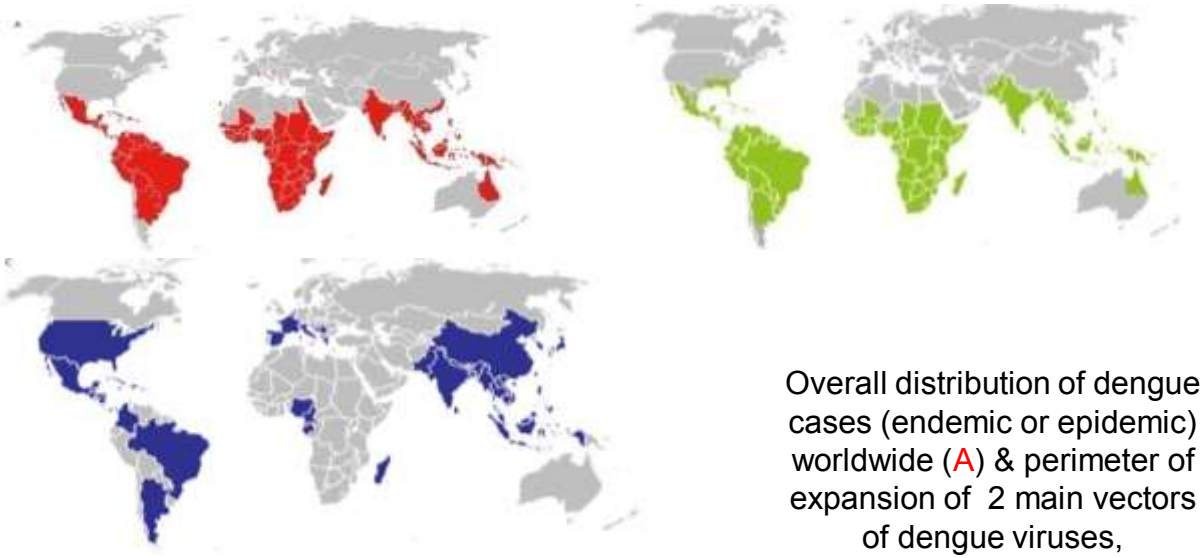
José Eduardo Levi,[1,2] Anna Nishiya,[1] Alvina Clara Félix,[2] Nanci Alves Salles,[1] Luciana Ribeiro Sampaio,[1] Fátima Hangai,[1] Ester Cerdeira Sabino,[3] and Alfredo Mendrone Jr[1]

BACKGROUND: Dengue virus transmission by blood transfusion is a rarely reported event.
CASE REPORT: During a dengue outbreak in São Paulo city, a regular plateletpheresis donor informed the blood bank of being diagnosed a few days after donation. The recipient was hospitalized and displayed symptoms and laboratory evidence of dengue after transfusion.
RESULTS: The donor was immunoglobulin (Ig)G, IgM, and polymerase chain reaction nonreactive on the index sample, seroconverting 20 days later. The platelet units were transfused into two patients. One of them developed fever 3 days after transfusion, with high viral load. His pretransfusion sample was negative for IgG, IgM, and dengue RNA, while the second recipient did not show any symptoms nor laboratory evidence of dengue infection.
CONCLUSIONS: This case brings additional evidence that dengue is indeed transmissible by blood transfusion and clinical manifestations, although rare, do occur.

de São Paulo; and the [3]Department of Infectious and Parasitic Diseases, Faculty of Medicine, University of São Paulo, São Paulo, Brazil.

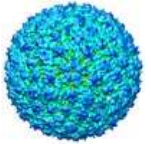
TRANSFUSION 2015;55:961–964

- **Why there is a limited number of transfusion –transmitted documented cases compared to the higher incidence of disease among general population?**



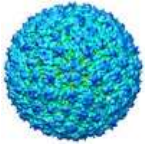
[World J Virol. 2015 May 12; 4(2): 113–123.](#)
Published online 2015 May 12.

Overall distribution of dengue cases (endemic or epidemic) worldwide (A) & perimeter of expansion of 2 main vectors of dengue viruses, *Aedes aegypti* (B) & *Aedes albopictus* (C).



Different arguments put forward for such a paradox:

1. **Absence of documented inquiry** between donor and recipient ,
2. It is often difficult to differentiate infection transmitted by mosquitoes and blood products;
3. The disease is **frequently asymptomatic or mild in donor, recipient or both**, with spontaneous resolution within a few days;
4. **Most of transfusion-transmitted cases** are intended to occur in areas where **dengue is endemic**, However, **most recipients would already** have been **exposed to mosquito-transmitted DENV early in their** life, which prevents them from being infected again *via* infected blood products.



Are there any studies related to transfusion transmissible dengue in India?

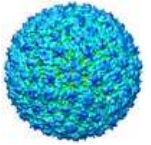
- A study conducted among 110 voluntary blood donors in **Chennai** revealed IgG seroposivity of 91.8% (101/110 donors), none were positive for NS1 antigen, anti-DENV IgM

Nonstructural protein 1 (**NS1) antigen test** was done on **1709 donations** during dengue outbreak in the months August to November 2013

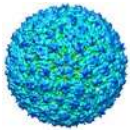
Conclusions: Majority cases were whole blood, replacement, male donors with 76.10% donors in <35 years age group. About 17.85% were single donor platelet donations. **NS1 antigen in all donors was negative**. In the past, dengue affected mainly children who do not donate blood. With the changing trend,

Mangwana S. Dengue viremia in blood donors in Northern India: Challenges of emerging dengue outbreaks to blood transfusion safety. Asian J Transfus Sci 2015;9:177-80

Department of Blood Transfusion Services, Sri Balaji Action Medical Institute, Paschim Vihar, New Delhi, India Date of Web Publication 12-Aug-2015

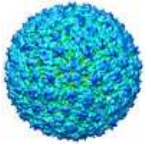


- **How long DENV is viable in stored blood components?**

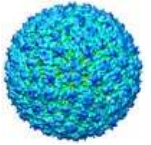


- Analysis of the RBCs by plaque assays showed **viable DENV throughout the 42 day storage period**, consistent with stabilization of the virus at 1-6^{o}C
- The presence of DENV1-4 was confirmed by quantitative RT-PCR, which showed that the **DENV RNA genome** was **replicated** with a **peak at Day 3** by as much as **7-fold, with continued detection throughout the 7 day platelet storage period**

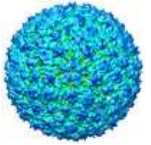
Persistence Of Dengue Virus In Stored Platelets and Red Blood Cells
Michael R Sutherland, Ayo Y Simon, Katherine Serrano, Peter Schubert, Jason Acker and Ed L.G. Pryzdial **Blood 2013** 122:2397;



- **What is the minimum number of copies per ml is needed to cause infection in the recipient?**

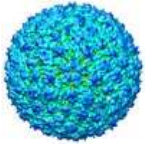


- **If virus positive blood components are transfused, what would be the clinical outcome in the recipients?**



- **Would dengue fever remain as chronic infection?**
- **Does it have an asymptomatic carrier state?**

- **There is no chronic infection with dengue virus or carriage state known.**



- **What are the laboratory investigations that can confirm the diagnosis both in donors and recipients?**

Lab Diagnosis

- **Direct Diagnosis**
- Cell culture
- Antigen detection
- NAT

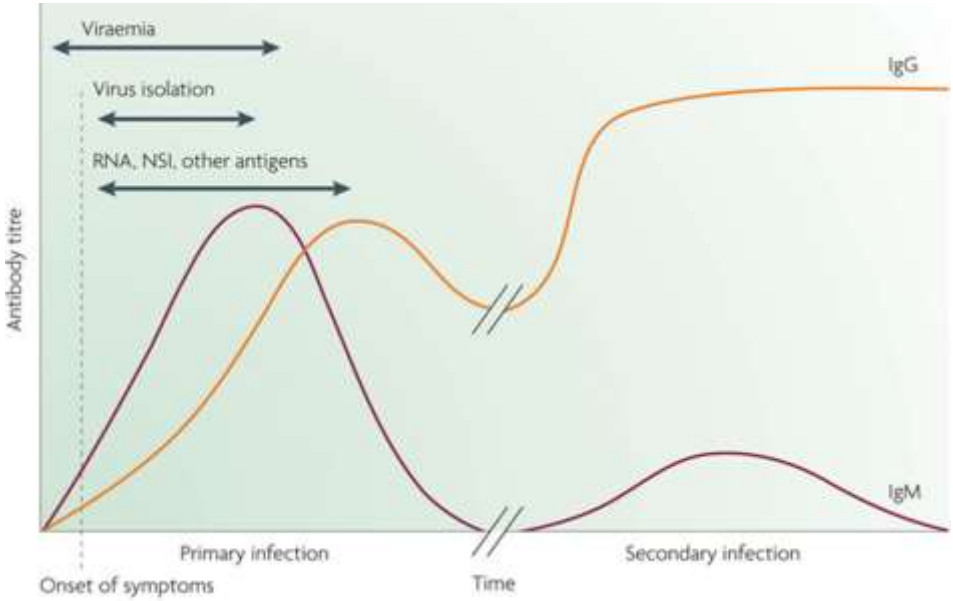
- **Indirect Diagnosis**
- Serological tests (IgM)

- **No FDA licensed blood donor screening tests exists**
- However, in research NAT assays have been used for blood donor prevalence studies and the detection of virus in asymptomatic individuals

(Feb 1 **2014 AABB** https://www.aabb.org>eid>documents)

Laboratory Diagnosis - Serology

- Antigen test detecting DENV NS1 protein is positive during 1st 5 d following initial symptoms
- Sensitivity of this test is optimal for primary infection
- Negative test does not exclude the diagnosis in case of secondary infection (ref)

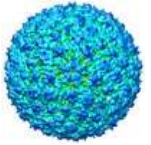


Marker	Detectable from…	To…
NS1 antigen	Day1	Day 18
IgM antibody	Day 5 (80% of cases) Day 6 to 10 (93% to 99% of cases)	Day 90
IgG antibody	Day 8 (1st flavivirus infection) Day 1 (2nd flavivirus infection)	Years

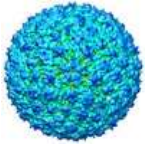
Nature Reviews | Microbiology

Laboratory Diagnosis - NAT

- Detection of DENV genome by NAT has become **gold standard** for the diagnosis of recent infection
- Positive **within 1st 5 d** of disease
- **Very sensitive and specific**
- RT-PCR & TMA (Different molecular technologies for diagnosis)
- Choice of the primers apply either on highly conserved parts of RNA genome within the 4 serotypes (or)
  - on a combination of sequences specific of each of the 4 serotypes
- **95% detection level at 14.9 geq/ml**



- **What are the preventive measures that can be taken to reduce the risk of transfusion transmissible dengue?**

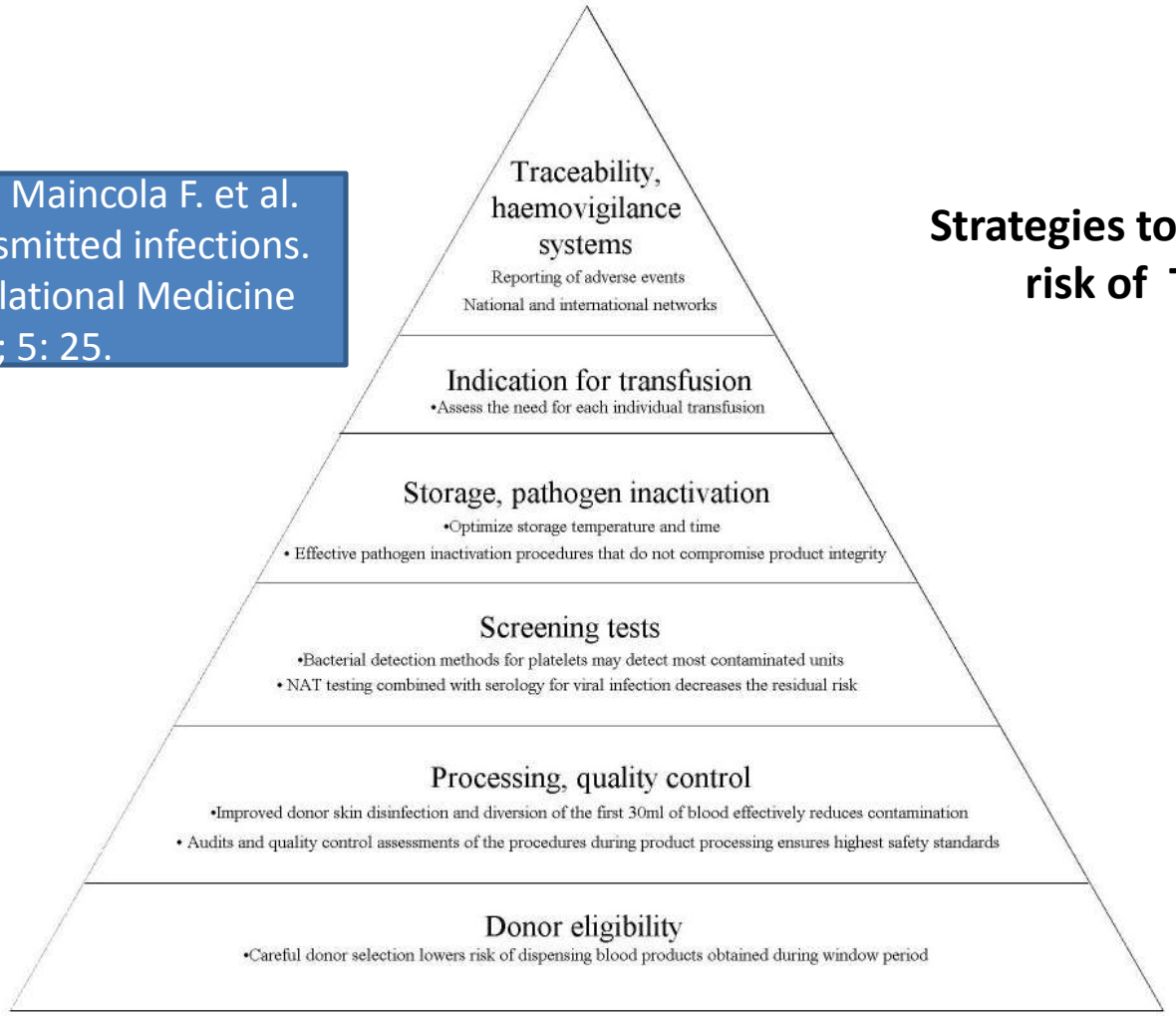


Measures available for reducing the risk of Transfusion-transmitted Dengue

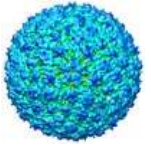
- clinical **selection of donors**
- Implementation of **screening tests** specific for dengue
- Non-specific **reduction or inactivation of pathogens** by the use of physical or chemical treatments applied to blood products.

Measures available for reducing the risk of Transfusion-transmitted Dengue

Bihl F, Castelli D, Maincola F. et al. Transfusion-transmitted infections. Journal of Translational Medicine 2007; 5: 25.



Strategies to reduce risk of TTI

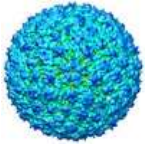


Clinical selection of donors

- presence of fever in blood donors is a general contra-indication

In **non-endemic areas**, the clinical selection of donors consists of

- excluding travelers returning from endemic regions for a period of **4 wk**

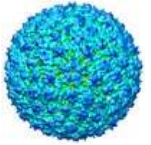


Donor deferral for dengue

Country	Donor deferral for dengue	
	H/O dengue infection	H/O Fever
Singapore	6 months	3 weeks
Hong Kong	6 months	2 weeks
Srilanka	No specific deferral	2 weeks
Australia	4 weeks	
New Zealand	4 weeks	
UK	2 weeks	
US	4 weeks	

Is dengue a threat to the blood supply?

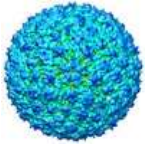
Teo D, Ng LC, Lam S. Transfusion Medicine 2009;19:66-77



Screening Tests

- **Views about implementation of screening tests to prevent transfusion transmissible dengue infection**

- Only **NAT** is the **Gold standard** investigation for detection of dengue virus. It is **very specific and sensitive**
- **No cost-effectiveness study** has been conducted **to evaluate the economic burden** of the implementation of a molecular screening targeting DENV **neither in endemic or non endemic areas**
- Studies demonstrated that **targeted donor screening** seems to be more cost-effective than **mass donor screening**



Pathogen Inactivation

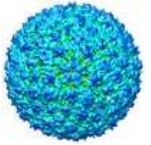
- **Physical/ chemical treatments applied to blood products for non-specific reduction or inactivation of pathogens**

Pathogen Inactivation Techniques

- Solvent-detergent treatment
- Dyes containing phenothiazine
- Nanofiltration based on photo activation
- The Intercept® system
- Mirasol® system
- Theraflex UV® system

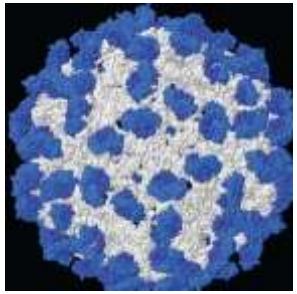
For red blood concentrates

- riboflavin
- Inactine®
- alkylating agent



- **Are there any licensed dengue vaccine available ?**

- **Yes (** WHO website stated that one dengue vaccine has been licensed, Dengvaxia® (CYD-TDV), developed by Sanofi Pasteur
- Approximately 5 additional dengue vaccine candidates are in clinical development, with 2 candidates (developed by Butantan and Takeda) expected to begin Phase III trials in early 2016.
- **What is Dengvaxia® (CYD-TDV)?**
- It was **first licensed in Mexico in December 2015** for use in individuals 9-45 years of age living in endemic areas. CYD-TDV is a **live recombinant tetravalent dengue vaccine** developed by Sanofi Pasteur (CYD-TDV), given as a 3-dose series on a 0/6/12 month schedule.
- **INDIA may require more clinical trials for Sanofi's dengue vaccine**



- Cost – effective Targeted donor screening programme can be implemented in endemic areas during seasonal outbreaks since no carrier state or chronic infection known
- Donor deferral of 2 weeks with H/O Fever in endemic areas like India during seasonal outbreaks can be implemented?
- In non-endemic areas exclude travelers returning from endemic regions for a period of 4 wk

Recommended studies on larger number donor population in endemic areas like India during seasonal outbreaks and follow-up of recepients to understand further about TTDengue?

Dengue Fever
Is it an emerging threat to blood safety?

Yes

selection of donors

screening tests

Pathogen reduction or inactivation

Vaccine.

Vector Control


61
Pākāla
Puttur
SH-5A
Ponneri
Thaavanampalle
Tiruvottiyur
Ambattur
Arakkonam
Pallavaram
Chennai
Madras
Arcot
Kanchipuram
Ārani
Chengalpattu
Mamallapuram
Tiruvettipuram
Mamallapuram
Polur
Vandavasi
Gingee
Tindivanam
Tiruvannāmalai
Puducherry
Pondicherry
Villupuram
Panruti
Cuddalore


நன்றி
Thank You.