Virologica Sinica 37 (2022) 774–777

Contents lists available at ScienceDirect

Virologica Sinica

journal homepage: www.keaipublishing.com/en/journals/virologica-sinica
www.virosin.org

Letter

Screening for arboviruses in healthy blood donors: Experience from Karachi, Pakistan

Moiz Ahmed Khan [a], Kehkashan Imtiaz [a], Humaira Shafaq [a], Joveria Farooqi [a], Mohammad Hassan [b], Afia Zafar [a], Maureen T. Long [c], Kelli L. Barr [d], Erum Khan [a,*]

[a] Clinical Microbiology Laboratory, Department of Pathology & Lab Medicine, Aga Khan University, Karachi 74800, Pakistan
[b] Blood Bank, Haematology & Transfusion Medicine, Department of Pathology & Lab Medicine, Aga Khan University, Karachi 74800, Pakistan
[c] Comparative Diagnostic and Population Medicine, University of Florida, Gainesville FL32611, USA
[d] Center for Global Health and Infectious Disease Research, University of South Florida, Tampa FL33620, USA

Dear Editor,

Arboviruses of medical importance are maintained in nature in enzootic cycles between haematogenous vectors and susceptible vertebrate hosts (Huang et al., 2019). The rapid increase in human populations around the globe and the associated urbanization are creating irreversible damage to the ecosystem, giving rise to many problems including emergence and intensification of the vector borne diseases (Sutherst, 2004). Among vector borne diseases, mosquito-borne arboviruses including dengue virus (DENV), West Nile virus (WNV), Japanese Encephalitis virus (JEV) and Zika virus (ZIKV) are rapidly emerging in the affected regions of the world (Palmer et al., 2011). The primary route of infection of these arboviruses is through the bite of infected mosquitoes, however, in the endemic regions, the concern for other routes of virus transmission remains, including transfusion-related transmission. This risk is especially high during outbreak season when viruses are actively circulating in the community and donors with asymptomatic infection are capable of donating blood contaminated with arboviruses (Stramer et al., 2009).

This concern for transfusion-related transmission of arboviruses is not new, and cases of such transmission have been reported. Endemic countries, like Singapore, China, Puerto Rico and Brazil, have shown evidence of DENV transmission by this route (Tambyah et al., 2008; Chuang et al., 2008; Stramer et al., 2012; Sabino et al., 2016). Cases of transfusion-related transmission of WNV was reported in 23 patients who were transfused different blood components during the 2002 epidemic in the United States of America (Pealer et al., 2003). China reported transfusion-related transmission of JEV among immunocompromised patients who received donation from infected asymptomatic blood donors (Cheng et al., 2018).

While there is one report of transfusion-related transmission of DENV from Karachi, Pakistan, the risk posed by other arboviruses is not known (Karim et al., 2017). Here, we performed a cross-sectional study in the blood bank and the clinical microbiology sections of the Department of Pathology and Laboratory Medicine, Aga Khan University, Karachi to investigate the potential transfusion-related transmission of DENV, WNV and JEV. From 1st July 2018 to 31st December 2018, blood donors who presented to the blood bank were consecutively enrolled. Blood donors who met any of the following criteria were deferred and hence, excluded from the study: less than 18 years of age, presence of fever, history of anaphylaxis, anaemia (haemoglobin level of less than 13.5 g/dL for males and less than 12.0 g/dL for females), history of any antibiotic intake in the past two weeks, blood donation within past four months, blood recipient within past one year and history of any coagulation factor deficiency. Informed and written consent was obtained on a consent form if the potential donors fulfilled the inclusion criteria, after they were explained regarding all the tests that shall be performed on their blood samples.

Finally, a total of 360 healthy blood donors samples were collected. All the samples obtained were tested for the presence of serum IgM antibodies and viral nucleic acids using ELISA method and reverse transcriptase polymerase chain reaction (RT-PCR) respectively (Supplementary file). Of the 360 study subjects tested, 99.2% (n = 357) were males and 0.8% (n = 3) were females (Table 1). Median age of blood donors was 28 years (IQR: 24–34). Majority of the donors screened were found to be the residents of the urban regions of Sindh Province of Pakistan (n = 302). The overall donor seropositivity for arboviruses was n = 26 (7.2%).

The most frequently detected IgM antibodies were for DENV and WNV, with 3.9% (n = 14) for each virus type. Among the DENV/WNV positive cases, one was a 42-year-old male resident of the Malir district in whom IgM antibodies were detected against both DENV and WNV, while one donor was a 25-year-old male resident from east district who was positive for DENV, WNV and JEV IgM antibodies (Supplementary

* Corresponding author.
E-mail address: erum.khan@aku.edu (E. Khan).

https://doi.org/10.1016/j.virs.2022.07.008
Received 15 December 2021; Accepted 13 July 2022
Available online 19 July 2022

Table 1
Demographic characteristics of the donors (n = 360) screened for arboviruses at Aga Khan University Karachi Sindh July–December 2018.

Characteristic	Total sample n (%)	Total positive rate n (%)
Gender:		
Male	357 (99.2)	25 (96.2)
Female	3 (0.8)	1 (3.8)
Age (years):		
19–30	217 (60.3)	15 (57.7)
31–40	108 (30)	7 (26.9)
41–50	30 (8.3)	3 (11.5)
>51	5 (1.4)	1 (3.9)
Residence:		
Rural region	58 (16.1)	0 (0)
Urban region	302 (83.9)	26 (100)
Distribution among city districts of Karachi:		
East District	82 (22.8)	10 (38.5)
West District	21 (5.8)	1 (3.9)
South District	21 (5.8)	0 (0)
Central District	63 (17.5)	6 (23)
Malir District	9 (2.5)	3 (11.5)
Not known	96 (26.7)	5 (19.2)
Other regions (beyond Karachi)	68 (18.9)	1 (3.9)

Table S1). Although plaque reduction neutralization test (PRNT) was not performed in this study, a separate study on arbovirus surveillance in the same geographical region showed that 25 of the 28 JEV-WNV-DENV IgM positive patients had at least 80% neutralizing activity against at least one DENV serotype at antibody titers of 1:40 (Khan et al., 2018).

Fig. 1 shows that most of the blood donors who screened positive for IgM antibodies, belonged to Karachi (n = 25), the southwestern port city of Sindh, with the majority belonging to the city's east (40%) and central (24%) districts (Table 1, Supplementary Table S2). About 30.8% of the IgM positive blood donors were residents of Gulshan Town in the east district of Karachi (n = 8), of which five were positive for DENV IgM, two for WNV IgM and one for DENV/WNV/JEV IgM. The north Karachi town in the central district of Karachi showed the second highest positive rate in the city (14.3%, n = 4), of which three were positive for WNV IgM and one for DENV IgM. Table 2 shows the association of baseline, demographic data and information of transfusion reactions in recipients of

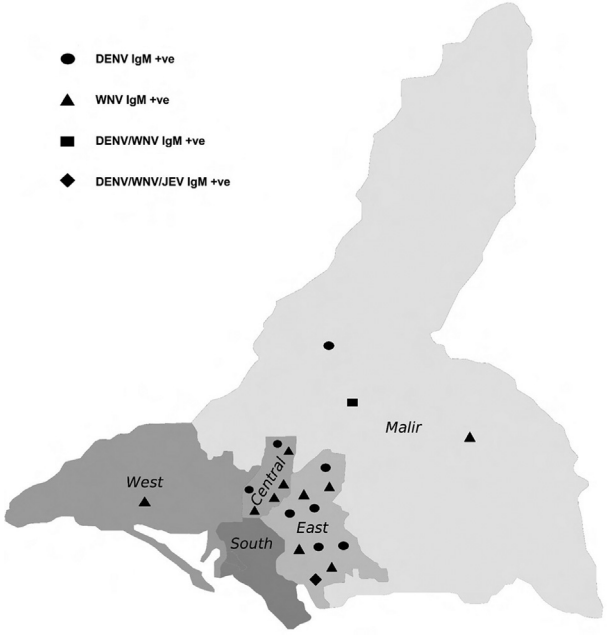

Fig. 1. Distribution of seropositive blood donors among different districts of Karachi.

blood products from study subjects with ELISA positivity. DENV and WNV seropositivity was significantly associated with residence in Malir District of Karachi [OR = 8.1 (1.5–43.03), $P = 0.015$)]. This association can be linked to Malir River that cuts across this district and provides breeding ground for the vector. Furthermore, DENV IgM seropositivity was significantly associated with blood group B negative [OR = 5.6 (1.1–28.6), $P = 0.04$)].

Our findings provide evidence of potential acute subclinical infection to DENV (3.9%), WNV (3.9%) and JEV (0.28%) viruses in healthy blood donors who presented to the blood bank at the Aga Khan University Hospital. DENV infection has been endemic in Pakistan since 2004; the country suffers from seasonal epidemics each year with increasing severity. Evidence of co-circulation of all four serotypes has been reported and severe DENV infections are commonly encountered (Chan et al., 1995; Khan et al., 2020). The most severe DENV outbreak occurred in 2013. There were approximately 17,000 cases, of which 6,000 cases reported from Sindh region (Rasheed et al., 2013). Blood donor screening reports from Pakistan have primarily been focused on DENV only. Most of these studies are limited in terms of the type of screening tests. For example, one such study from Lahore, Punjab region of Pakistan used IgG antibody test for screening, which does not necessarily imply risk for transfusion transmission. Nevertheless, this study showed a significant population of 39.4% of healthy donors have been exposed to the DENV infection during the outbreak season (Mahmood et al., 2013). To the best of our knowledge, our study is first study from Pakistan that has sought for broaden arbovirus screening in blood donors using both IgM antibodies and PCR based nucleic acid tests.

Most of the IgM positive cases in our study belonged to the younger age group (under 30 years old), although no statistical significance was noted. The rate of arbovirus related infections in the younger population is likely reflective of the overall age demographics of the region, because 69.42% of the population of Pakistan is under 30 years old (Pakistan Bureau of Statistics, 2022). In this study, 57.7% of donors who tested positive for arbovirus IgM were between 19 and 30 years of age (Table 1). This could be due to the fact that 60.3% of our overall study population belonged to this age group. Furthermore, the younger age group is likely to be more social and attend crowded places such as colleges, and parks, and hence, may be more prone to the acquisition of these infections. In the recent decades, the world has witnessed an increase in arbovirus infection rates especially in urban settings (Sabino et al., 2016; Linnen et al., 2008; Al-Azraqi et al., 2013). There are several reasons accounting for this rise, including overcrowding of large cities, underdeveloped housing societies, improper sewage disposal, ineffective cleaning, and inadequate arbovirus control strategies which account most for urban settings in lower income countries (Viennet et al., 2016). Karachi is one such city that faces all the risk factors mentioned above and consequently suffers from yearly outbreaks of DENV and dengue-like illness. In our study, almost all the blood donors screened positive (96.2%) were from Karachi with the majority belonging to the city's east (40%) and central (24%) districts, with higher population density than west and Malir district (Table 1). Gulshan Town in the city's east district had the highest number of positive subjects followed by central district of Karachi.

The association of DENV and WNV seropositivity (22.2%) with the city's Malir district was found to be statistically significant ($P = 0.015$). The reason for this finding is possibly the close proximity of Malir district with the Malir River, which has been affected by serious flooding due to heavy rainfalls in the past. In addition to being the site for disposal of the district's sewage, it has been found to be the active breeding site of mosquitoes harbouring potentially disease-causing arboviruses (Kamimura et al., 1986; Saeed and Piracha, 2016).

The presence of IgM antibodies indicates recent infection in a donor, providing evidence of active virus circulation locally and a heightened risk of transfusion-based transmission. All IgM antibody positive blood donors in our study were asymptomatic at the time of screening. This finding is consistent with blood donor related studies from Pakistan conducted in the past (Khan et al., 2018; Barr et al., 2018). None of the

Table 2
Association of baseline, demographic data and information of transfusion reactions in recipients of blood products from study subjects with ELISA.

	DENV IgM n (%) OR (95% CI)	WNV IgM n (%) OR (95% CI)	JEV IgM n (%) OR (95% CI)
Overall positivity	14 (3.9%) 0.9 (0.02–50.3) ($P = 1$)	14 (3.9%) 0.9 (0.02–50.3) ($P = 1$)	1 (0.28%) 0.05 (0.0008–3.7) ($P = 0.2$)
Blood group			
A+	2 (2.6%) 0.6 (0.1–2.7) ($P = 0.49$)	3 (3.8%) 1.0 (0.3–3.6) ($P = 0.98$)	0
B+	7 (6.5%) 2.5 (0.8–7.2) ($P = 0.1$)	4 (3.7%) 0.9 (0.3–3.1) ($P = 0.92$)	0
AB+	1 (4.9%) 1.1 (0.1–9.1) ($P = 0.91$)	1 (4.9%) 1.1 (0.1–9.1) ($P = 0.91$)	0
O+	2 (1.7%) Reference	6 (5.2%) Reference	1 (0.9%) Reference
B-	**2 (16.7%)** **5.6 (1.1–28.6)** **($P = 0.04$)**	0	0
O-	0	0	0
Rh+	12 (3.7%) Reference	14 (4.3%) Reference	1 (0.3%) Reference
Rh-	2 (5.6%) 1.5 (0.3–7.1) ($P = 0.59$)	0	0
Age group			
19–30 y	6 (2.8%) Reference	10 (4.6%) Reference	1 (0.5%) Reference
31–50 y	7 (5.1%) 1.6 (0.6–4.8) ($P = 0.36$)	4 (2.9%) 0.6 (0.2–2.1) ($P = 0.45$)	0
> 50 y	1 (20%) 6.6 (0.7–63.04) ($P = 0.1$)	0	0
Gender			
Female	1 (33.3%) 5.7 (0.5–64.3) ($P = 0.16$)	0	0
Male	13 (3.6%) Reference	14 (3.9%) Reference	1 (0.3%) Reference
Karachi City Districts			
Central	2 (3.2%) 0.8 (0.2–3.6) ($P = 0.75$)	4 (6.4%) 1.95 (0.6–6.4) ($P = 0.27$)	0
East	6 (7.3%) Reference	5 (6.1%) Reference	1 (1.2%) Reference
South	0	0	0
West	0	1 (4.8%) 1.25 (0.2–10.07) ($P = 0.83$)	0
Malir	**2 (22.2%)** **8.1 (1.5–43.03)** **($P = 0.015$)**	**2 (22.2%)** **8.1 (1.5–43.03)** **($P = 0.015$)**	0
Not known	3 (1.8%) 0.3 (0.09–1.1) ($P = 0.08$)	**2 (1.2%)** **0.2 (0.04–0.9)** **($P = 0.03$)**	0
Transfusion reactions	0	0	0

donors found positive for IgM antibodies in our study showed evidence of viremia using real time PCR assays. There are limited studies on arboviral antibody kinetics and relation with viremia. The WNV experimental infection model using Rhesus macaque monkeys has provided some insight into antibody kinetics and duration of viremia (Ratterree et al., 2004). It has shown that viremia can be detected by viral culture and viral nucleic acid detection within 4–10 days post-infection, but the IgM antibodies would rise concomitantly and remain elevated up to 45 days. In our study, none of the blood donors were found to be viremic at the time of donation and PCR signals remained negative for all samples tested. This is most likely due to short lived viremic phase of the disease.

Ratterree's study also emphasized the sensitivity of the PCR test used to assess viremia. Using two different PCR methods, this study found that nested PCR was more sensitive in detection of WNV, 26 out of 28 samples verses 13 when using conventional real time PCR assay (Ratterree et al., 2004). Thus, the negative PCR test in our donor population could be due to low sensitivity of PCR assay as we used real time PCR assay. Similarly, reports of blood donor studies showing IgM positive and PCR negative results for DENV have been reported from other countries. A report from New Delhi, India found that 143 of the 200 healthy blood donors were screened positive for DENV IgM but none were found to be positive for viral nucleic acid detection using convention RT-PCR assay (Ranjan et al.,

2016). Further studies using more robust nucleic acid testing methods are required to assess true risk of transfusion transmission of these viruses.

The association of blood group B negative (B-) with DENV IgM seropositivity was found to be statistically significant in our study ($P = 0.04$). There have been similar association of DENV infections with ABO blood groups. Ravichandran et al. noted association of dengue fever with AB blood group compared to other blood groups (Ravichandran et al., 2019). Similarly, another study has linked the severity of dengue fever with AB blood group. The genetic factors such as HLA and ABO blood group have been implicated as an effect on the individual's resistance or susceptibility to infectious diseases (Kalayanarooj et al., 2007). Whether or not B negative blood group in our population predisposes to increased risk of dengue virus infection needs to be further explored.

One limitation of this study is that it is a single centre study. Nevertheless, the blood bank of the Aga Khan University Hospital is one of the largest transfusion banks in Pakistan. Our results are generalizable for age and gender demographics. Another important limitation is the type of PCR assay used. Although we have used conventional real time PCR assay protocol and reported PCR positive signals from symptomatic patients in the past (Barr et al., 2018), for the donor screening purpose, low viral load samples may be missed in conventional PCR assay. Multiplexed PCR assay would have been more sensitive.

Regular follow-up of the recipients for the development of arbovirus related symptoms and timely lab testing are essential to assess and provide evidence of true transfusion related transmission of infection. In this study, we did not actively follow up the patients who received DENV/WNV/JEV IgM positive blood after their discharged from the hospital. However, as part of standard blood transfusion protocol of our blood bank, all recipients were instructed to report any febrile or other symptomatic reaction post transfusion to the unit through its helpline number.

To the best of our knowledge, this is the first report of arbovirus screening of blood donors from Pakistan. We conclude that due to endemic circulation of arboviruses in Pakistan, the risk of transmission of DENV, WNV and JEV through blood transfusion exists, especially during the outbreak season. Further multi-centre studies using more sensitive nucleic acid-based testing and recipient follow-ups are required to confirm this risk and true burden of transfusion-based transmission. That will provide evidence for regular donors screening for these viruses.

Footnotes

This work was supported by the Pathology Research Committee of the Department of Pathology and Lab Medicine at the Aga Khan University, which awarded the Resident Research Grant to Moiz Ahmed Khan. The committee didn't have any role in the study design, in the collection, analysis and interpretation of data, in the writing of the draft or in the decision to submit the article for publication. The authors declare that they have no competing interests. The study was exempted from ethical approval by the Institutional Review Board of Aga Khan University (Ref # 2019-0488-2928).

Most of the data generated and analysed during this study are included in this published article and its supplementary information files. The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.virs.2022.07.008

References

Al-Azraqi, T.A., El Mekki, A.A., Mahfouz, A.A., 2013. Seroprevalence of dengue virus infection in Aseer and Jizan regions, Southwestern Saudi Arabia. Trans. R. Soc. Trop. Med. Hyg. 107, 368–371.

Barr, K.L., Khan, E., Farooqi, J.Q., Imtiaz, K., Prakoso, D., Malik, F., Lednicky, J.A., Long, M.T., 2018. Evidence of chikungunya virus disease in Pakistan since 2015 with patients demonstrating involvement of the central nervous system. Front. Public Health 6, 186.

Chan, Y.C., Salahuddin, N.I., Khan, J., Tan, H.C., Seah, C.L., Li, J., Chow, V.T., 1995. Dengue haemorrhagic fever outbreak in Karachi, Pakistan, 1994. Trans. R. Soc. Trop. Med. Hyg. 89, 619–620.

Cheng, V., Sridhar, S., Wong, S., Wong, S., Chan, J., Yip, C., Chau, C.H., Au, T., Hwang, Y.Y., Yau, C., Lo, J., Lee, C.K., Yuen, K.Y., 2018. Japanese encephalitis virus transmitted via blood transfusion, Hong Kong, China. Emerg. Infect. Dis. 24, 49.

Chuang, V.W., Wong, T., Leung, Y., Ma, E.S., Law, Y., Tsang, O.T., Chan, K.M., Tsang, I., Que, T.L., Yung, R., Liu, S.H., 2008. Review of dengue fever cases in Hong Kong during 1998 to 2005. Hong Kong Med. J. 14, 170–177.

Huang, Y.S., Higgs, S., Vanlandingham, D.L., 2019. Arbovirus-mosquito vector-host interactions and the impact on transmission and disease pathogenesis of arboviruses. Front. Microbiol. 10, 22.

Kalayanarooj, S., Gibbons, R.V., Vaughn, D., Green, S., Nisalak, A., Jarman, R.G., Mammen Jr., M.P., Perng, G.C., 2007. Blood group AB is associated with increased risk for severe dengue disease in secondary infections. J. Infect. Dis. 195, 1014–1017.

Kamimura, K., Takasu, T., Ahmed, A., Ahmed, A., 1986. A survey of mosquitoes in karachi area, pakistan. J. Pakistan Med. Assoc. 36, 182–188.

Karim, F., Nasir, N., Moiz, B., 2017. Transfusion transmitted dengue: one donor infects two patients. Transfus. Apher. Sci. 56, 151–153.

Khan, E., Barr, K.L., Farooqi, J.Q., Prakoso, D., Abbas, A., Khan, Z.Y., Ashi, S., Imtiaz, K., Aziz, Z., Malik, F., Lednicky, J.A., Long, M.T., 2018. Human West Nile virus disease outbreak in Pakistan, 2015–2016. Front. Public Health 6, 20.

Khan, E., Prakoso, D., Imtiaz, K., Malik, F., Farooqi, J.Q., Long, M.T., Barr, K.L., 2020. The clinical features of co-circulating dengue viruses and the absence of dengue hemorrhagic fever in Pakistan. Front. Public Health 8, 287.

Linnen, J.M., Vinelli, E., Sabino, E.C., Tobler, L.H., Hyland, C., Lee, T.H., Kolk, D.P., Broulik, A.S., Collins, C.S., Lanciotti, R.S., Busch, M.P., 2008. Dengue viremia in blood donors from Honduras, Brazil, and Australia. Transfusion 48, 1355–1362.

Mahmood, S., Nabeel, H., Hafeez, S., Zahra, U., Nazeer, H., 2013. Seroprevalence of dengue IgG antibodies among healthy adult population in Lahore, Pakistan. Int. Sch. Res. Notices 1–6, 2013.

Pakistan Bureau of Statistics, 2022. Population by 5 Year Age Group – Pakistan. https://www.pbs.gov.pk/. (Accessed 15 December 2020).

Palmer, S.R., Soulsby, L., Torgerson, P.R., Brown, D.W., 2011. In: Oxford Textbook of Zoonoses. Biology, Clinical Practice, and Public Health Control. Oxford University Press, Oxford.

Pealer, L.N., Marfin, A.A., Petersen, L.R., Lanciotti, R.S., Page, P.L., Stramer, S.L., Stobierski, M.G., Signs, K., Newman, B., Kapoor, H., Goodman, J.L., Chamberland, M.E., West Nile virus transmission investigation team, 2003. Transmission of West Nile virus through blood transfusion in the United States in 2002. N. Engl. J. Med. 349, 1236–1245.

Ranjan, P., Natarajan, V., Bajpai, M., Gupta, E., 2016. High seroprevalence of dengue virus infection in blood donors from Delhi: a single centre study. J. Clin. Diagn. Res. 10, DC08–DC10.

Rasheed, S.B., Butlin, R.K., Boots, M., 2013. A review of dengue as an emerging disease in Pakistan. Publ. Health 127, 11–17.

Ratterree, M.S., Gutierrez, R.A., Travassos da Rosa, A.P., Dille, B.J., Beasley, D.W., Bohm, R.P., Desai, S.M., Didier, P.J., Bikenmeyer, L.G., Dawson, G.J., Leary, T.P., Schochetman, G., Phillippi-Falkenstein, K., Arroyo, J., Barrett, A.D., Tesh, R.B., 2004. Experimental infection of rhesus macaques with West Nile virus: level and duration of viremia and kinetics of the antibody response after infection. J. Infect. Dis. 189, 669–676.

Ravichandran, S., Ramya, S., Kanungo, R., 2019. Association of ABO blood groups with dengue fever and its complications in a tertiary care hospital. J. Lab. Physicians 11, 265–269.

Sabino, E.C., Loureiro, P., Lopes, M.E., Capuani, L., McClure, C., Chowdhury, D., Di-Lorenzo-Oliveira, C., Oliveira, L.C., Linnen, J.M., Lee, T.H., Gonalez, T., Brambilla, D., Kleinman, S., Busch, M.P., Custer, B., 2016. International component of the NHLBI recipient epidemiology and donor evaluation study-III, 2016. Transfusion-Transmitted dengue and associated clinical symptoms during the 2012 epidemic in Brazil. J. Infect. Dis. 213, 694–702.

Saeed, U., Piracha, Z.Z., 2016. Viral outbreaks and communicable health hazards due to devastating floods in Pakistan. World J. Virol. 5, 82–84.

Stramer, S.L., Hollinger, F.B., Katz, L.M., Kleinman, S., Metzel, P.S., Gregory, K.R., Dodd, R.Y., 2009. Emerging infectious disease agents and their potential threat to transfusion safety. Transfusion 49, 1S–29S.

Stramer, S.L., Linnen, J.M., Carrick, J.M., Foster, G.A., Krysztof, D.E., Zou, S., Dodd, R.Y., Tirado-Marrero, L.M., Hunsperger, E., Santiago, G.A., Muñoz-Jordan, J.L., Tomashek, K.M., 2012. Dengue viremia in blood donors identified by RNA and detection of dengue transfusion transmission during the 2007 dengue outbreak in Puerto Rico. Transfusion 52, 1657–1666.

Sutherst, R.W., 2004. Global change and human vulnerability to vector-borne diseases. Clin. Microbiol. Rev. 17, 136–173.

Tambyah, P.A., Koay, E.S., Poon, M.L., Lin, R.V., Ong, B.K., 2008. Dengue hemorrhagic fever transmitted by blood transfusion. N. Engl. J. Med. 359, 1526–1527.

Viennet, E., Ritchie, S.A., Williams, C.R., Faddy, H.M., Harley, D., 2016. Public health responses to and challenges for the control of dengue transmission in high-income countries: four case studies. PLoS Neglected Trop. Dis. 10, e0004943.