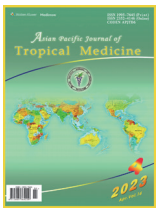

Letter to Editor

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Dengue positivity among blood donors in hyper-endemic region of southern India

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Transfusion-transmitted infections (TTIs) impose meticulous screening protocols in blood banks. Emerging or re-emerging viruses with altered pathogenicity remains debatable whether or not to include those in TTI screening panel. Dengue infection is known to transmit *via Aedes aegypti* mosquito; however, the route of infection through blood transfusion is recently of greater importance. India with hyper-endemic and endemic dengue regions must guarantee the safety in transfusion-related products. The donor with asymptomatic dengue infection during viremic phase can act as a reservoir for transmission. The acute infection in dengue is ascertained by detecting either nonstructural-1 protein (NS1) or IgM antibody whereas the prevalence of dengue can be monitored by the presence of IgG.

The presence of viremia in blood related products need to be assessed. However, reports on transfusion transmissible dengue infection are still lacking in this part of India. Therefore, the present study aims to determine the dengue NS1 protein and IgM status among blood donors collected at a tertiary care hospital in Southern India. Fortunately, we receive samples for routine dengue testing from patients residing in and around camp area. Hence, the latter part of this study is designed to substantiate the active circulation of dengue virus in and around camp area during camp period.

A single blinded randomized pilot study was designed for which serum samples those were negative to TTIs were collected from Department of Transfusion Medicine. The remaining serum of non-reactive samples collected during blood donation camp organized at Surulipatti village (9.7059 ° N, 77.3016 ° E) of Theni district on 30th September 2017 was taken for this study. The samples were de-identified and assigned a donor number from one to 95. Once collected, the samples were aliquoted into small vials and kept in deep freezers at 20 ℃.

NS1 protein and dengue specific IgM detection was carried out by Panbio™ Dengue Early ELISA (Abbott Diagnostics, Korea) and IgM capture ELISA (National Institute of Virology, India) respectively. Samples positive to either one of the above tests were subjected to Panbio™ IgG ELISA (Abbott Diagnostics, Korea). All the tests were performed and the interpretation of results was done as per the manufacturer's instructions provided in the kit. Appropriate controls from the kits and the in-house controls prepared from positive samples were added in each run.

The active circulation of the dengue virus in and around the study area was evidenced by two factors: duration and location. Villages near the blood camp were identified by point-radius method using Maptive software (www.maptive.com)[1]. The duration for data stratification was calculated on the basis of maximum incubation period of dengue virus (*i.e*) 10 days. The data pertaining to hospitalized dengue cases were downloaded in the form of Microsoft Excel sheet from NIE server (http://112.133.207.124:82/vdln/) maintained by National Institute of Epidemiology, Chennai, Tamilnadu. The sorting was done based on the closest villages identified earlier and the date on which the sample was tested. Furthermore, the resultant samples those were tested only for IgM was retrospectively tested for NS1 antigen using stored serum.

Once dengue testing was completed, the positive and negative status of individual donor for each assay was represented as D

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(donor number) followed by NS1+/-/IgM+/e/-/IgG+/-. The positivity was expressed in percentage and their combinations were depicted in proportional Venn diagram using Biovenn online software with some modifications[2]. The dengue cases filtered from hospital-based data were tabulated with their respective positives.

We tested 95 blood donor samples for dengue infection. Of these, 8 (8.4%) samples were positive to NS1 (2, 2.1%) and/or IgM (6, 6.3%) as per kit cut off values (Table 1). Our study revealed that all NS1 positive samples (D25/NS1+/IgM-/IgG- and D50/NS1+/IgMe/IgG-) were IgG negative. Out of six IgM positives, two donor samples (D46 and D81) turned out to be IgG negative (Figure 1).

Table 1. Individual donor status of dengue positivity for different ELISA assays.

Sample ID	IgM	OD	Cut off	NS1	Panbio units	IgG	Panbio units
D25	-	0.187	0.222-0.333	+	30.01	-	5.50
D34	+	0.422	0.215-0.323	-	1.11	+	26.00
D38	+	0.427	0.260-0.390	-	0.99	+	23.90
D46	+	0.621	0.208-0.312	-	3.35	-	3.32
D48	+	0.296	0.187-0.280	-	0.82	+	24.80
D50	#	0.385	0.269-0.404	+	42.70	-	9.55
D59	#	0.28	0.269-0.404	-	0.62	-	1.75
D64	+	0.358	0.223-0.335	-	7.80	+	23.40
D81	+	0.495	0.237-0.355	-	0.44	-	15.00

The results were expressed in terms of optical density (OD) for IgM ELISA whereas the OD was converted into Panbio Units for NS1 and IgG ELISA as per manufacturer's instruction. The sign (+), (-) and (#) represented positive, negative and equivocal respectively.

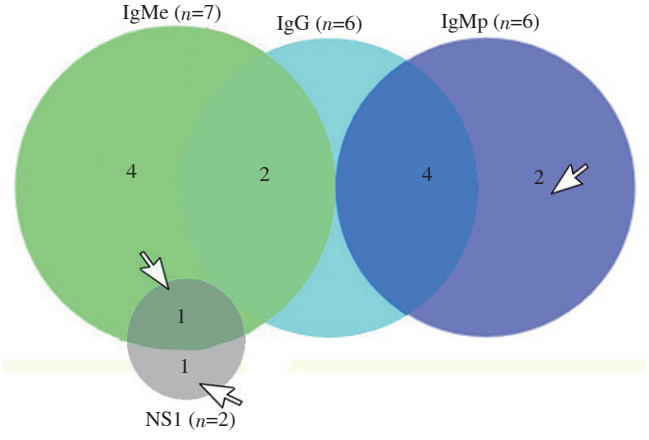

Figure 1. Proportionate Venn diagram depicted combinatorial positivity in dengue cases among blood donors (*n*=95). Each circle depicts each ELISA assay and the intersection area represents the number of samples positive for both bio-analytes. White arrow indicates number of donors with probable acute dengue infection.

A total of 293 records were downloaded between 20th September and 10th October 2017. Point radius method found that four villages were close to the camp area. Using this, filter applied to 'village column' sorted 16 dengue suspected cases, of which, seven (43.7%) had dengue infection. Two positive NS1 cases were identified and one belonged to the village of Surulipatti.

Among flaviviruses, the screening of DENV among donors is highly emphasized due to persistence virus in blood of asymptomatic donor for about seven days[3]. During the viremic phase, there would be greater chance for the transmission of virus to recipients or patients. Though the acute circulation of DENV is sensitively detected by Real Time Polymerase Reaction (RT-PCR), NS1 antigen is more stable in blood and blood-related products compared to RNA[4].

Dengue NS1 antigen was detected in 5.3% of healthy Saudi blood donors[5] whereas 2.1% in the present study suggesting asymptomatic viremia in those donors. The low positivity observed is probably due to stringent donor selection followed in our blood bank. This result was in line with 0.09% DENV positivity among donors of Puducherry[6]. Furthermore, validation with blood donors from multiple camps and the geographical seasonal variation[7] in dengue incidence are yet to be concerned.

Earlier studies from this district concluded that the study region is hyper-endemic to dengue infection[8] and so, IgM and IgG ELISA was also performed to rule out past dengue infection. Especially, samples (D59 NS1-/IgMe/IgG-) those where IgM equivocal but negative for IgG might be a recent infection. In our study, two donor samples fall into the above category. The two NS1 positive samples obviously showed the recent dengue infection and in that, D3360/NS1+/IgMe/IgG- further authenticate its acute state infection. Pilot studies with these kind of combinatorial positivity among blood donors provides better understanding on the phase of infection.

Furthermore, the asymptomatic dengue positivity among blood donors reported in this study was in accordance with the dengue cases got admitted in hospital during that period. There are cases with acute dengue infection from villages that are close proximity to the blood camp. These hospital-based data augment the active circulation of DENV in the study area and during the study period.

To address the asymptomatic dengue cases, samples from blood bank can be considered as an alternative collection site. The knowledge on past or present dengue infection of donor and recipient must be interrogated well in advance to avoid the adverse immunological reaction. The hidden proportion of asymptomatic dengue infections is also noteworthy in the context of sequential infection with other dengue serotypes. This population may unknowingly donate blood and/or travel to less endemic areas and thus spreading the disease. Hence, identification of the asymptomatic dengue infections by stringent surveillance in order to ensure blood safety and in addition, it aids to project the true incidence of dengue infection.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Ethical approval

The research protocol was reviewed and approved by the Institutional Ethical Committee (IEC. No: 884/MEIII/19).

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Authors' contributions

MG conceived of the research hypothesis. MG involved in data collection, performed statistical analysis and manuscript preparation. RS supported the study through critical comments. GST and SL contributed on verifying the analytical methods and verified the manuscript. GST and SL both supervised the findings of this work. SL approved the manuscript for submission and publication.

References

[1] Wieczorek J, Guo Q, Hijmans R. The point-radius method for georeferencing locality descriptions and calculating associated uncertainty. *Int J Geo Inf Sci* 2004; **18**: 745-767.

[2] Hulsen T, de Vlieg J, Alkema W. BioVenn-a web application for the comparison and visualization of biological lists using area-proportional Venn diagrams. *BMC Genomics* 2008; **9**: 488.

[3] Dias LL, Amarilla AA, Poloni TR, Covas DT, Aquino VH, Figueiredo LTM. Detection of dengue virus in sera of Brazilian blood donors. *Transfusion* 2012; **52**: 1667-1671.

[4] Low SL, Lam S, Wong WY, Teo D, Ng LC, Tan LK. Dengue seroprevalence of healthy adults in Singapore: Serosurvey among blood donors, 2009. *Am J Trop Med Hyg* 2015; **93**: 40-45.

[5] Ashshi AM, Alghamdi S, El-Shemi AG, Almdani S, Refaat B, Mohamed AM, et al. Seroprevalence of asymptomatic dengue virus infection and its antibodies among healthy/eligible Saudi blood donors: Findings from holy Makkah city. *Virology (Auckl)* 2017; **8**: 1-51.

[6] Remakanth R, Basavarajegowda A, Dhodapkar R. Prevalence of dengue NS1 antigenemia among healthy blood donors in a tertiary care hospital in Southern India. *Asian J Transfus Sci* 2021; **15**: 140-145.

[7] Amudhan M, Sekar R, Sivashankar M, Raja GA, Ganesan S, Mythreyee M. Higher incidence of dengue in Theni district, South India. *Indian J Med Microbiol* 2015; **33**: 458-459.

[8] Murugesan A, Aridoss D, Senthilkumar S, Sivathanu L, Sekar R, Shankar EM, et al. Molecular diversity of dengue virus serotypes 1-4 during an outbreak of acute dengue virus infection in Theni, India. *Indian J Med Microbiol* 2020; **38**: 401-408.

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