Research Square

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Epidemiological Survey and Screening Strategy for Dengue Virus in Blood Donors from Yunnan Province

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Abstract

BACKGROUND Dengue virus (DENV) can be transmitted through blood transfusion. DENV was not screened regularly in Xishuangbanna Blood Center. This study was conducted in Xishuangbanna Blood Center with an attempt to develop DENV screening strategies in one of China's high-incidence areas.

METHODS Blood samples were collected randomly between June 2019 and August 2019. These samples were first screened for dengue IgG and IgM antibodies using enzyme-linked immunosorbent assay (ELISA). All reactive samples and some randomly-chosen non-reactive samples were used to detect DENV RNAs using real time polymerase-chain-reaction (RT-PCR) assay. After RT-PCR assay, these samples were further tested for soluble nonstructural protein 1 (NS1) using colloidal gold method. The demographic data of DENV positive donors were collected.

RESULTS A total of 2,254 donor samples were collected and tested for dengue IgG and IgM antibodies by ELISA between June 2019 and August 2019. ELISA testing revealed that 598 donor samples were anti-IgG and/or anti-IgM reactive, with a serological prevalence rate of 26.53%. Among all the donor samples, 26 were RT-PCR positive and/or NS1 positive. Moreover, there were significant differences in the prevalence rate of DENV in terms of occupation (*P=0.001*), education(*P<0.001*) and ethnicity (*P=0.026*).

CONCLUSION The prevalence of DENV in Xishuangbanna Blood Center was higher than most other blood centers that have implemented DENV donor screening. Our study provides the first-hand data about the prevalence of DENV and allows development of a screening strategy for clinical use.

Background

The rapid expansion of dengue fever is an evolving worldwide public health threat over the past decade. It is estimated that about 5 billion infections occur in about 100 countries each year, and this situation is likely to become worse [1]. At the heart of the dengue fever problem is the spread of mosquito vectors in most of the tropical and subtropical areas. One of the main vectors is Aedes aegypti, which has adapted to urban environments and is widely distributed in tropical and subtropical latitudes. Historically, this vector emerged from Africa during the slave trade between the 15th and 19th centuries, arrived in Asia through commercial exchanges in the 18th and 19th centuries, and spread across the globe with the increase in travel and trade over the past 50 years [2]. In addition, the geographical range of the second vector Aedes albopictus has also expanded significantly in recent years [3]. Dengue infection in Africa is largely unquantified, but recent outbreaks suggest that large parts of the continent are at increasing risk of the dengue spread [4].

After infection, DENV replicates in the human body for 3 to 14 days before symptoms appear [5]. Since more than 75 per cent of people infected with DENV are asymptomatic, the prevalence of people actively infected with dengue fever during outbreaks increases the risk of DENV infection among adult blood donors. At present, dengue virus (DENV) is listed as a transfusion transmitted disease (TTD) by the American Association of Blood Banks (AABB) [6]. In order to reduce the risk of transfusion transmission

of DENV, routine nucleic acid test (NAT) in blood donor samples is recommended by several high prevalence countries and regions, such as Honduras [7].

In China, there have been three outbreaks of dengue hemorrhagic fever in history, which spread to Guangdong Province, with 6,024 confirmed cases and 6 deaths [8,9]. The outbreak of DENV has brought about increasing concerns about DENV infection throughout the country. In China, DENV has not been included in the routine screening of routine blood donors. However, with the rapidly increased flow of population due to tourism, DENV has been spreading rapidly in some areas of China. In particular, DENV has a high serological prevalence in Guangdong and Guangxi provinces [10,5].

Since no effective antiviral agents to treat dengue infection are available, and transmission of DENV by blood donors in the Mainland China is rising, it is imperative to investigate the prevalence of DENV in high risk areas, analyze the demographic characteristics of DENV-positive blood donors, and evaluate whether wide range DENV antibody screening is needed. In Xishuangbanna Dai Autonomous Prefecture in Yunnan Province, a region close to Laos and other Southeast Asian countries, people live closely, outbreaks take place every other year. The potentially infected blood donors in this area pose a threat to blood safety nationwide. However, there has been no data on dengue virus infection among blood donors in Yunnan Province. In this study, we cooperated with Xishuangbanna Blood Station in Yunnan Province to investigate the prevalence of dengue virus among blood donors. Our study aids the development of China's DENV screening strategy to reduce the risk of transfusion transmission of DENV.

Materials And Methods

Ethics statement

The study was approved by the Ethics Committee of the Institute of Blood Transfusion, Chinese Academy of Medical Sciences & Peking Union Medical College. Written informed consent was obtained from each study participant.

Sample collection

This study was a collaboration between the Institute of Blood Transfusion (IBT) of Chinese Academy of Medical Sciences and Xishuangbanna Blood Center, located in Yunnan province of China. The samples were collected randomly from Xishuangbanna Blood Center between June 2019 to October 2019. Donor history questionnaires were collected as routine practice at the time of donation in blood center. Donor samples were collected for ELISA assay (in EDTA) and NAT assay (in EDTA and separation gel) and were centrifuged and separated within 4 hours. All blood donations were tested for Human immunodeficiency virus (HIV), Hepatitis C (HCV), Hepatitis B (HBV) by ELISA and NAT, and syphilis was tested by ELISA.

Anti-IgG and anti-IgM screening testing for DENV

All collected donor samples were screened for anti-IgG and anti-IgM antibodies, using Dengue virus IgM ELISA and Dengue virus IgG ELISA kits (ELISA, IBL International, Germany), respectively. Any reactive

(anti-IgG and/or anti-IgM) plasma samples in NAT tubes were then transferred to storage tubes (no EDTA) and stored at less than -20^{o}C. Some non-reactive (anti-IgG and/or anti-IgM) samples were selected randomly in NAT tubes, then transferred to storage tubes (no EDTA) and stored at less than -20^{o}C. These samples were then shipped on dry ice to the clinical transfusion center laboratory of IBT.

DENV supplementary testing

Further supplementary testing was performed on samples that were reactive on the DENV screening to confirm the presence of DENV, using RT-PCR (Shanghai ZJ Bio-Tech Co., Ltd, Shanghai,China) and NS1 testing (Colloidal gold method, Wondfo, Guangzhou, China). A blood donation sample was confirmed to be positive if the supplemental testing was also positive. Some non-reactive (anti-IgG and/or anti-IgM) samples were selected randomly and tested for RNA and NS1 antigen by PCR and colloidal gold method. *Fig.1* shows the algorithm of DENV testing. The demographic characteristics of DENV positive donors tested by PCR and/or colloidal gold method were collected.

Statistical Analysis

Serological prevalence was calculated by the number of all reactive (anti-IgG and/or anti-IgM) samples divided by the number of total samples in each group. Pearson's chi-square test was applied to evaluate the difference of serological prevalence between the donor sets (SPSS 17.0, IBM, Armounk, USA). Significance level α was set at $P<0.05$.

Results

The summary of DENV screening

We tested 2,254 donor samples for anti-DENV-IgG and anti-DENV-IgM antibodies by ELISA between June 2019 and October 2019 at Xishuangbanna Blood Center. Out of these samples, 598 were anti-DENV-IgG and/or IgM reactive by ELISA. *Table 1* shows the demographic characteristics and DENV testing results of all donors in Xishuangbanna Blood Center.

Table 1. The Demographic Characteristics and serological prevalence of DENV in each group

Characteristics	Only Anti-IgG reactive	Only Anti-IgM reactive	Anti-IgG And Anti-IgM reactive	Non-reactive	Total	Serological prevalence (%)	χ^2 and P values
Donors Status							
First-Time	267	28	31	809	1135(50.35%)	28.72	5.635, 0.018
Repeat	217	37	18	847	1119(49.65%)	24.30	
Gender							
Male	331	39	29	1033	1432(63.53%)	27.86	3.577, 0.059
Female	153	26	20	623	822 (%)	24.21	
Age Group							
18~25	84	10	6	294	394(17.48%)	25.38	9.337, 0.053
26~35	196	24	18	567	805(35.71%)	29.57	
36~45	124	18	19	441	602(26.71%)	26.74	
46~55	78	13	6	344	441(19.57%)	22.00	
56~60	2	0	0	10	12(0.53%)	16.67	
Occupation							
Farmers	76	9	12	188	285(12.64%)	34.03	25.335, 0.001
Workers	34	3	3	116	156(6.92%)	25.64	
Students	8	1	1	25	35(1.55%)	28.57	
Soldiers	3	0	0	8	11(0.49%)	27.27	
Teachers	4	0	0	34	38(1.69%)	10.53	
Civil Servants	13	3	0	86	102(4.52%)	15.69	
Medical staff	27	3	2	122	154(6.83%)	13.01	
Employee	44	6	5	191	246(10.91%)	22.36	
Others	275	40	26	886	1227(54.44%)	27.79	
Education							
BHS§	239	30	33	639	941(41.75%)	32.09	31.3, <0.001
HSAD*	66	9	4	210	289(12.82%)	27.33	
Bachelor	43	8	1	227	279□12.38%□	18.64	
Master	3	0	0	10	13(0.57%)	23.08	
Others	133	18	11	570	732(32.48%)	22.13	
Ethnicity							
Han	240	35	27	885	1187(52.66%)	25.44	14.36, 0.026
Bai	2	1	0	17	20(0.89%)	15.00	
Miao	6	0	0	12	18(0.80%)	33.33	
Dai	73	7	8	188	276(12.24%)	31.88	
Buliang	14	1	1	18	34(1.51%)	47.06	
Hani	70	11	3	248	332(14.73%)	25.30	
Other	79	10	10	288	387(17.17%)	25.58	
Total	484	65	49	1656	2254	26.53	

§BHS = Below High School

*HSAD = High School and Associate Degree

Serological prevalence of DENV

The total serological prevalence rate was 26.53%, and the serological prevalence were calculated in each group. The prevalence rate of the first-time and repeat donors were 28.72% and 24.30%, respectively.

According to the Pearson's chi-square test, there was significant difference in the prevalence rate of DENV in first-time donors and repeat donors (P=0.018, χ^2= 5.635). The prevalence rate was higher in male donors (27.86%) than female donors (24.21%), with 26~35 age group having the highest prevalence rate. In contrast, there was no significant difference in terms of gender (P=0.059, χ^2= 3.577) and age group (P=0.053, χ^2= 9.337). Further analysis revealed that there were significant differences in the prevalence rate of DENV in terms of occupation (P=0.001, χ^2= 25.335), education(P<0.059, χ^2= 31.30) and ethnicity (P=0.026, χ^2= 14.358). In particular, the prevalence rate was the highest among farmer donors (34.03%), and there were significant differences in the prevalence rate of DENV between farmers and teachers (P=0.003, χ^2= 8.622), civil servants (P<0.001, χ^2= 12.233) and medical staff (P=0.004, χ^2= 8.466). The prevalence rate in donors with below-high school (BHS), high school and associate (HSAD), bachelor, and master degrees, and others were 32.09%, 27.33%, 18.64%, 23.08%, 22.13%, respectively. There were significant difference in bachelor with BHS (P<0.001, χ^2= 18.916) and HSAD (P<0.014, χ^2= 6.052). The prevalence rate is the highest in Buliang nationality (47.06%), followed by Miao nationality (33.33%) and Dai nationality (31.88%); then by Han (25.44%), Bai (15.00%), Hani (25.30%) and other nationalities (25.58%). There were significant differences between Buliang and Bai (P=0.017, χ^2=5.675), Hani (P=0.007, χ^2= 7.353) and Han (P=0.005, χ^2= 8.019). *Table 1* displays the detailed information and serological prevalence rate of DENV in different groups.

DENV RNA testing by PCR and NS1 antigen testing by colloidal gold method

ELISA reactive samples

We detected 598 DENV reactive donor samples using ELISA. These samples were then tested for DENV RNA by PCR. Eight out of the 598 samples were tested positive. Then these 8 PCR positive samples and 142 PCR negative samples (choosing randomly from 590 PCR negative samples) were tested for NS1 antigen by colloidal gold method. Six out of the 8 samples were NS1 positive, whereas 2 out of the 142 samples were NS1 positive, with 6 out of the 142 samples being weakly positive (Their band is weaker than the control band).

ELISA non-reactive samples

We randomly chose 340 of the 1656 samples for RNA test by PCR, 5 were RNA positive. Then these 5 RNA positive samples were tested for NS1 antigen by colloidal gold method. Three out of 5 were NS1 positive. We then chose 50 of the 335 RNA negative samples to test NS1 antigen using colloidal gold method, 4 out of the 50 samples were weakly positive, only 1 out of 50 was positive.

Demographic characteristics of DENV positive donors

Table 2 showed the detailed information of donors who were both ELISA reactive and PCR and/or NS1 positive. There were 16 DENV screening reactive donor samples by ELISA, and these samples were PCR and/or NS1 positive. Of the 16 donors, 8 were females, the other 8 were males, with a range of age

between 21 and 44 years old. Ten of 16 were first-time donors. Most of their education (11 out of 16) were high school and associate degree or below high school.

Table 2. The detailed information of donors who were ELISA reactive and PCR and/or NS1 Positive

Assigned Donor Number	Education	Occupation	Number of Blood Donation	Ethnicity	Blood Group	Results of Anti-IgG	Results of Anti-IgM	Results of NAT	Results of NS1
1	Bachelor	Medical staff	1	Dai	B	Reactive	Non-reactive	positive	Positive
2	High school	Others	2	Han	B	Reactive	Non-reactive	Positive	Positive
3	HSAD*	Employee	7	Dai	B	Reactive	Non-reactive	Positive	Positive
4	HSAD	Employee	1	Han	B	Reactive	Non-reactive	Positive	Positive
5	BHS§	Others	6	Han	B	Reactive	Non-reactive	Positive	Negative
6	BHS	Farmer	1	Dai	B	Reactive	Non-reactive	Positive	Positive
7	HSAD	Others	1	Dai	B	Reactive	Reactive	Positive	Positive
8	BHS	Others	1	Dai	O	Reactive	Reactive	Positive	Negative
9	HSAD	Others	1	Han	O	Reactive	Non-reactive	Negative	Positive
10	BHS	Others	2	Han	A	Reactive	Non-reactive	Negative	Positive
11	Bachelor	Employee	5	Han	O	Reactive	Non-reactive	Negative	WP▲
12	Bachelor	Employee	1	Lagu	A	Reactive	Non-reactive	Negative	WP
13	BHS	Others	5	Dai	A	Reactive	Non-reactive	Negative	WP
14	High school	Others	1	Yi	A	Reactive	Non-reactive	Negative	WP
15	BHS	Others	1	Hani	B	Reactive	Non-reactive	Negative	WP
16	BHS	Others	1	Han	A	Reactive	Non-reactive	Negative	WP

§BHS = Below High School
*HSAD = High School and Associate Degree
▲WP = Weakly Positive

Table 3 showed the detailed information of donors who were ELISA non-reactive but PCR and/or NS1 positive. There were 10 DENV screening non-reactive donor samples by ELISA, but these samples were and PCR and/or NS1 Positive. There were 5 female blood donors and 5 male blood donors. They aged from 26 to 48 years old. There 5 of 10 were first-time donors. Most of their education (8 out of 10) were high school and associate degree or below high school.

Table 3. The detailed information of donors who were ELISA non-reactive but PCR and/or NS1 Positive

Assigned Donor Number	Education	Occupation	Number of Blood Donation	Ethnicity	Blood Group	Results of Anti-IgG	Results of Anti-IgM	Results of NAT	Results of NS1
1	Unkown	Medical staff	5	Dai	B	Non-reactive	Non-reactive	positive	positive
2	BHS[§]	Farmer	1	Dai	B	Non-reactive	Non-reactive	Positive	Positive
3	HSAD*	Others	2	Hani	B	Non-reactive	Non-reactive	Positive	Positive
4	HSAD	Farmer	5	Han	B	Non-reactive	Non-reactive	Positive	Negative
5	Bachelor	Others	1	Han	A	Non-reactive	Non-reactive	Positive	Negative
6	BHS	Farmer	2	Han	AB	Non-reactive	Non-reactive	Negative	WP▲
7	BHS	Others	1	Lahu	B	Non-reactive	Non-reactive	Negative	WP
8	BHS	Others	7	Han	O	Non-reactive	Non-reactive	Negative	WP
9	BHS	Employee	1	Hani	O	Non-reactive	Non-reactive	Negative	WP
10	BHS	Others	1	Han	A	Non-reactive	Non-reactive	Negative	Positive

[§]BHS = Below High School
*HSAD = High School and Associate Degree
▲WP = Weakly Positive

Discussion

In China, blood donation samples are routinely tested for HBV, HCV, HIV, and syphilis, but DENV has not been included in the routine donor testing. In the laboratory, dengue has been tested for using anti-IgG/IgM ELISA, NS1 using a colloidal gold method, and specific RNA using RT-PCR. The diagnostic sensitivity of NS1 test in the febrile phase can exceed 90% for primary infections in persons who have not been infected previously, and antigenemia may persist for several days after the resolution of fever [11,12].

In this study, we studied the DENV infection in blood samples from Xishuangbanna, a high-risk area of mainland China. The total serological prevalence rate was as high as 26.53% (598 out of 2,254) using anti-DENV-IgG and/or IgM ELISA. This prevalence rate may represent the highest serological prevalence ever reported [5,9-10]. *Table 1* showed that the serological prevalence rate was significantly different between first-time and repeat donors, consistent with the serological prevalence of other pathogens among blood donors, such as HBV [13,14]. The results from *Table 1* showed that there existed a significant difference in serological prevalence rate among occupation and education groups. The serological prevalence rate for farmers and lower education-level donors is higher. We speculate that this occupation-related phenotype has something to do with the donors' living environment where the risk of DENV transmission is high. In fact, DENV is mainly transmitted by the aedes aegyptis, which are often found in residential areas, containers (such as tanks, basins, discarded tires, etc.), plant containers (such

as bamboo tubes, tree holes, etc.), and stone pools. It is most likely that residential areas where farmers live are more suitable for aedes aegypti.

A few donors were RNA and/or NS1 testing positive among anti-IgG and/or anti-IgM reactive donors. This may be due to the overlapping of time period of various markers appear in plasma after DENV infection [15]. RNA and NS1 of DENV are the earliest markers appearing in plasma, they may occur on the first day of infection, and last 5 to 7 days [15]; while anti-IgG (secondary infection) and anti-IgM appear in plasma after the 4^{th} day of infection, and last 6 days [15]. In our study, there were a few donors who were RNA and/or NS1 positive but anti-IgG and/or anti-IgM non-reactive, supporting that notion that different viral markers appear in the plasma in a temporal sequence. Our findings, together with others, indicate that to increase the likelihood of DENV detection and reduce the risk of blood transfusion-induced DENV transmission, it may be important to perform a joint test for of DENV RNA, NS1 and IgG/IgM antibodies.

It can be seen from *Table 2* and *Table 3* that the donors were RNA and/or NS1 positive, who are mostly farmers or other occupations with higher exposure to standing water. Efforts at mosquito control in the surrounding environment will aid to reduce DENV infection but multilayer testing will be necessary to decrease blood transfusion-caused DENV transmission.

Conclusion

The prevalence rate of DENV in Xishuangbanna Blood Center is higher than most other blood centers that have implemented DENV donor screening. To reduce the risk of transfusion-transmitted DENV, it may be necessary to conduct DNEV screening for blood donors in high prevalence seasons and in high prevalence areas.

Abbreviations

TTD: Transfusion-Transmitted disease

DENV: Dengue virus

HBsAg: Hepatitis B surface Antigen

HCV : Hepatitis C

HIV: Human immunodeficiency virus

NAT: Nucleic Acid Tests

ELISA: Enzyme-Linked Immuno Sorbent Assay

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Institute of Blood Transfusion, of the Chinese Academy of Medical Sciences & Peking Union Medical College. Written informed consent was obtained from each study participant before the interview, sample collection and testing.

Consent to publish

Not Applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing Interests

The authors declare that they have no competing interests.

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Authors' contributions:

LL designed the experiments and tested NS1 of DENV and wrote and reviewed the manuscript. YL collected plasma samples and tested DENV for anti-IgG and anti-IgM. ZL and HJ reviewed, revised, and edited the manuscript. All other authors participated in the study design, performed the experiments, and collected and analyzed the data. All authors read and approved the final manuscript.

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Figures

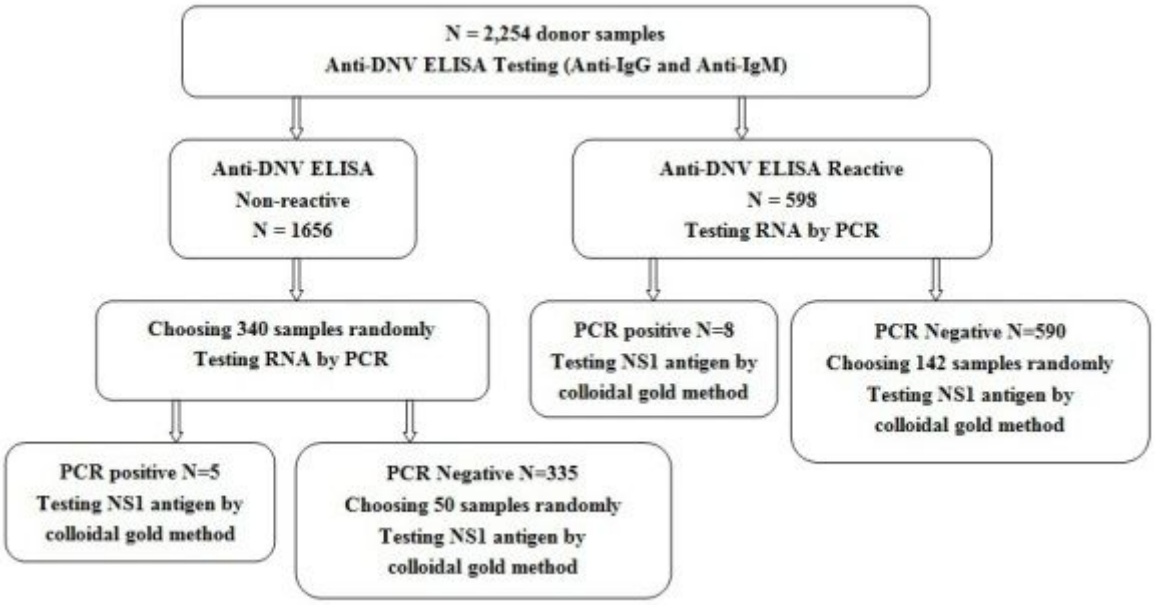

Figure 1

Testing algorithm