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 anti-DENV IgG and IgM 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 the colloidal gold method. The demographic data of donors were collected.

RESULTS

A total of 2,254 donor samples were collected and tested for anti-DENV IgG and IgM by ELISA between June 2019 and August 2019. ELISA testing revealed that 598 donor samples were anti-DENV IgG and/or IgM reactive, with a serological prevalence 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 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 the 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 3.9 billion infections occur in 128 countries in the regions of Africa, South-East Asia, the Eastern Mediterranean, the Western Pacifc and the Americas according to the World Health Organization (WHO), and about 96 million (25%) clinically manifest the disease among 390 million of dengue infection occur each year in people worldwide [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 mostly unquantified, but recent outbreaks suggest that large parts of the continent are at increased risk of the dengue spread [4].

Dengue virus enters the human body through the bite of Aedes mosquitoes, and replicates in capillary endothelial cells and mononuclear macrophage systems, then enters the bloodstream and forms viremia. After infection, DENV replicates in the human body for 3 to 14 days before symptoms appear [5]. Since more than 75 percent 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, the dengue virus (DENV) is listed as a transfusion- transmitted disease (TTD) by the American Association of Blood Banks (AABB) [6]. 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 blood donors. However, with the rapidly increased population flow due to tourism, DENV has been spreading quickly in China's areas. In particular, DENV has a high serological prevalence in Guangdong and Guangxi provinces [5,10].

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. This includes analyzing DENV-positive blood donors' demographic characteristics and evaluating 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 near, and outbreaks occur 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 center in Yunnan Province to investigate the prevalence of dengue virus among blood donors, and development a DNEV screening strategy based on this findings in high prevalence seasons and in high prevalence areas.

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 the Chinese Academy of Medical Sciences and Xishuangbanna Blood Center, located in the Yunnan province of China. The samples were collected randomly from Xishuangbanna Blood Center between June 2019 to October 2019. Donor history questionnaires were obtained as a routine practice at the time of donation at the 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.

Testing algorithm

Firstly, all collected samples were tested for anti-DENV IgG and IgM by ELISA.

Secondly, choosing some samples (including reactive and non-reactive samples of ELISA) randomly to screened for NAT and NS1. Because anti-DENV IgG and IgM can be tested by ELISA later than NAT and NS1, and at the same time, the results of ELISA may be false positive.

Anti-DENV IgG and IgM screening testing

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

Statistical Analysis

Serological prevalence was calculated by the number of all reactive (IgG and/or 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, NY 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 IgM 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.

Serological prevalence of DENV

The total serological prevalence was 26.53% (598 out of 2,254), and the serological prevalence was calculated in each group. The first-time and repeat donors' prevalence was 28.72% (326 out of 1,135) and 24.30% (272 out of 1,119), respectively. According to Pearson's chi-square test, there was a significant difference in the prevalence of DENV in first-time donors and repeat donors (P=0.018, χ^2= 5.635). The prevalence was higher in male donors (27.86%, 399 out of 1,432) than female donors (24.21%, 199 out of 822), with 26~35 age group having the highest prevalence. In contrast, there was no significant difference in gender (P=0.059, χ^2= 3.577) and age group (P=0.053, χ^2= 9.337). Further analysis revealed that there were substantial differences in the prevalence 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 was the highest among farmer donors (34.03%, 97/285), and there were significant differences in the prevalence 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 in donors with below-high school (BHS), high school and associate (HSAD), bachelor, and master degrees, and others were 32.09% (302/941), 27.33% (79/289), 18.64% (52/279), 23.08% (3/13), 22.13% (162/732), respectively. There wwa a significant difference in bachelor with BHS (P<0.001, χ^2= 18.916) and HSAD (P<0.014, χ^2= 6.052). The prevalence is the highest in Buliang nationality (47.06%, 16/34), followed by Miao nationality (33.33%, 6/18) and Dai nationality (31.88%, 88/276); then by Han (25.44%, 302/1187), Bai (15.00%, 3/20), Hani (25.30%, 84/332) and other races (25.58%, 99/387). 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 of DENV in different groups.

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

ELISA reactive samples

We detected 598 anti-DENV reactive donor samples using ELISA. These samples were then tested for DENV RNA by PCR. Eight out of the 598 samples were positive. There 8 PCR positive samples and 142 PCR negative samples (choosing randomly from 590 PCR negative samples) were tested for NS1 antigen by the colloidal gold method. Six out of the eight 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 the colloidal gold method. Three out of 5 were NS1 positive. We then chose 50 of the 335 RNA negative samples to test the NS1 antigen using the colloidal gold method, 4 out of the 50 samples were weakly positive, only 1 out of 50 was positive.

Demographic characteristics

Table 1 displayed demographics of the entire study population. In all 2,254 donors, 1,135 out of 2,254 (50.35%) were First-Time donors, and 1,119 out of 2,254 (49.65%) were Repeat donors. There were 1,432 (63.53%) were males and 822 (36.47%) were females. The educational level of most people (941 out of 2254, 41.75%) were Below High School and more than half (1187 out of 2254, 52.66%) were Han nationality.

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 ages 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 an associate degree or below high school.

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 PCR and/or NS1 Positive. There were five female blood donors and five 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 an associate degree or below high school.

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 IgG/IgM using ELISA, NS1 using a colloidal gold method, and specific RNA using RT-PCR. The diagnostic sensitivity of the 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 was as high as 26.53% (598 out of 2,254) using anti-DENV-IgG and/or IgM ELISA. This prevalence may represent the highest serological prevalence ever reported [5,9-10]. *Table 1* showed that the serological prevalence was significantly different between first-time and repeat donors, consistent with other pathogens' serological prevalence among blood donors, such as HBV [13,14]. The results from *Table 1* showed that there existed a significant difference in the serological prevalence among occupation and education groups. The serological prevalence for farmers and lower education-level donors was higher. We speculate that this occupation-related phenotype has something to do with the donors' living environment where the risk of DENV transmission was high. In

fact, DENV is mainly transmitted by the Aedes aegyptis, 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 positive among IgG and/or IgM reactive donors.This may be due to the overlapping of various markers' time period 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 when the patient noted any symptom during this illness, and last 5 to 7 days [15]; while IgG (secondary infection) and IgM appear in plasma after the 4th day when noting any symptom , and last 6 days [15]. This result shows that NS1 can be detected at the same time as viral RNA. However, the detection of NS1 might be limited during secondary infection because of pre-induced adaptive immunity. Generally, the window period of nucleic acid detection is shorter than that of antibody detection. However, for dengue virus, the risk of missing the test was high if there is only a nucleic acid test to screen for dengue virus prevalence because the nucleic acid quickly disappear from plasma, 590 out of 598 (98.66%) ELISA reactive samples were PCR negative. Our findings, together with others [16], indicate that to increase the likelihood of DENV detection and reduce the risk of blood transfusion-induced DENV transmission, it may be essential 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 help 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. In order to reduce the risk of transfusion-transmitted DENV, it may be necessary to propose a DNEV screening strategy based on these findings in high prevalence seasons and 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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Tables

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 (36.47%)	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

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. 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

Figures

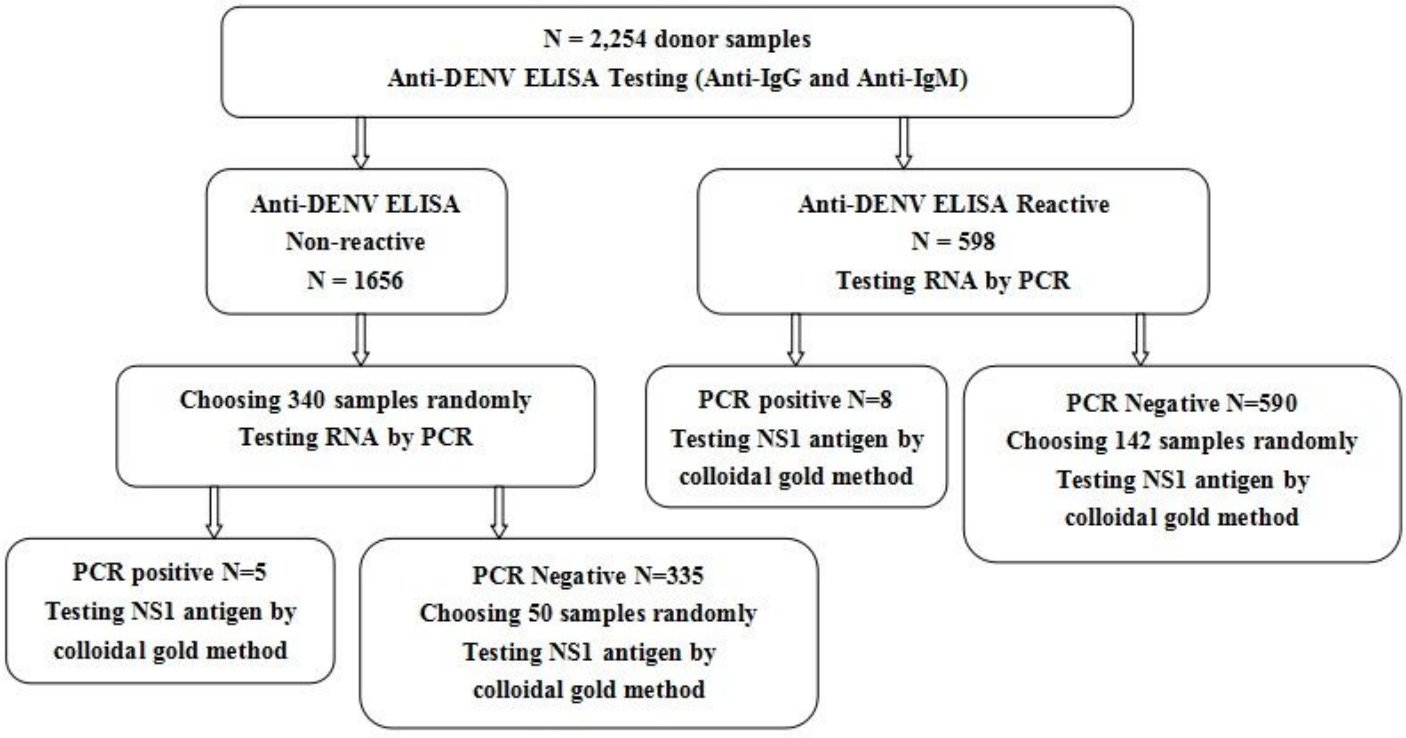

Figure 1

Testing algorithm