Parvovirus B19 DNA and antibodies in Chinese plasma donors, plasma pools and plasma derivatives

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Background : Human parvovirus B19 (B19V) is a frequent contamination of plasma pools and plasma derivatives. Previous studies were mainly focused on limited aspects, further assessment of prevalence of B19 DNA and antibodies in plasma donors, pooled plasma and plasma derivatives should be performed in China.

Study design and methods: Individual plasma donor's samples from four plasmapheresis areas and pooled plasma from four Chinese blood products manufacturers were screened by B19V DNA diagnostic kits from October 2018 through May 2020. The positive samples were confirmed by nested PCR and subjected to sequence analysis and alignment for phylogenetic studies. Moreover, samples from 11 B19V DNA-positive donors who had frequent plasma donation, 20 batches of plasma derivatives produced by pooled plasma with viral load of B19V DNA exceeding 10^4IU/mL were also tested for B19V DNA.

Results: A total of 17187 plasma donors were analyzed, 44 (0.26%) specimens were found positive for B19V DNA. The quantitative DNA levels ranged from 1.0 1×10^1 to 5.09×10^{12} IU/mL. 44 DNA-positive specimens were also investigated for the seroprevalence of B19V antibodies, among which 2.3% specimens were seropositive for B19V IgG and IgM antibodies. A total of 75% of these samples were positive for B19V IgG. The phylogenic analyses showed that the prevalent genotypes in four provinces plasma donors belong to B19V Genotype 1. 11 individual plasma donors who were B19V DNA positive at the index time were followed for a period. During this period, the DNA levels of B19V were gradually decreased. Moreover, 64.8% (259/400) of pooled plasma were contaminated by B19V, with the concentrations of 1.05×10^0-3.36×10^9 IU/mL. Approximately 72.6% of the DNA-positive plasma pools were only moderately contaminated ($<10^4$ IU/mL), while 27.4 % contained $>10^4$ IU/mL. 20 batches of plasma derivatives which were produced by pooled plasma with viral load of B19V DNA exceeding 10^4IU/mL were also tested. B19V was detected in 5/5 PCC samples, 5/5 factor VIII samples, but not in intravenous immune globulin and albumin samples. The DNA levels of B19V in these samples were ranged from 1.90×10^1 to 3.59×10^7 IU/mL. In samples with B19V DNA $>10^4$ IU/mL, B19V-specific IgG and IgM were not found.

Conclusions The contamination of B19V in pooled plasma and plasma-derived clotting factor

concentrates was serious. Whether B19V NAT screening of plasma and plasma derivatives will be launched in China, Chinese plasma fractionation industries should be encouraged to spontaneously perform B19V NAT screening in plasma donors and mini-pool plasma. These measures can ensure these samples with high titer B19V DNA were discarded at a crucial juncture of prevention and control of this transfusion transmitted virus.

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Abstract

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Study design and methods: Individual plasma donor's samples from four plasmapheresis areas and pooled plasma from four Chinese blood products manufacturers were screened by B19V DNA diagnostic kits from October 2018 through May 2020. The positive samples were confirmed by nested PCR and subjected to sequence analysis and alignment for phylogenetic studies. Moreover, samples from 11 B19V DNA-positive donors who had frequent plasma donation, 20 batches of plasma derivatives produced by pooled plasma with viral load of B19V DNA exceeding 10^4IU/mL were also tested for B19V DNA.

Results: A total of 17187 plasma donors were analyzed, 44 (0.26%) specimens were found positive for B19V DNA. The quantitative DNA levels ranged from 1.01×10^1 to 5.09×10^{12} IU/mL. 44 DNA-positive specimens were also investigated for the seroprevalence of B19V antibodies, among which 2.3% specimens were seropositive for B19V IgG and IgM antibodies. A total of 75% of these samples were positive for B19V IgG. The phylogenic analyses showed that the prevalent genotypes in four provinces plasma donors belong to B19V Genotype 1. 11 individual plasma donors who were B19V DNA positive at the index time were followed for a period. During this period, the DNA levels of B19V were gradually decreased. Moreover, 64.8% (259/400) of pooled plasma were contaminated by B19V, with the concentrations of 1.05×10^0-3.36×10^9 IU/mL. Approximately 72.6% of the DNA-positive plasma pools were only moderately contaminated ($<10^4$ IU/mL), while 27.4 % contained $>10^4$ IU/mL. 20 batches of plasma derivatives which were produced by pooled plasma with viral load of B19V DNA exceeding 10^4IU/mL were also tested. B19V was detected in 5/5 PCC samples, 5/5 factor VIII samples, but not in intravenous immune globulin and albumin samples. The DNA levels of B19V in these samples were ranged from 1.90×10^1 to 3.59×10^7 IU/mL. In samples with B19V DNA $>10^4$ IU/mL, B19V-specific IgG and IgM were not found.

Conclusions

The contamination of B19V in pooled plasma and plasma-derived clotting factor concentrates was serious. Whether B19V NAT screening of plasma and plasma derivatives will be launched

in China, Chinese plasma fractionation industries should be encouraged to spontaneously perform B19V NAT screening in plasma donors and mini-pool plasma. These measures can ensure these samples with high titer B19V DNA were discarded at a crucial juncture of prevention and control of this transfusion transmitted virus.

Introduction

Human parvovirus B19 (B19V) is a non-enveloped virus with a liner single-stranded DNA genome. It belongs to Erythroparvovirus of the *Parvoviridae* family (Cotmore & Tattersall 1984). B19V infection causes variety of illnesses, including fifth disease in children, aplastic crisis in patients with hemolytic disorders, and fatal hydrops in pregnant women, arthropathy, cardiomyopathy and inflammation of other various tissues(Servant et al. 2002). Moreover, the epidemiology of B19V shows a wide geographic distribution around the world and seasonal variations, the peak season of infection is spring and winter. The transmission of B19V is primarily through the upper respiratory route, organ transplantation and blood transfusion (Ganaie & Qiu 2018; Parsyan & Candotti 2007). If the prevalence of B19V in blood donors is about 1%, the plasma pools will be contaminated with a high probability. DNA in the blood donor population reaches 1%, their plasma could easily contaminate the entire pooled plasma. Once the single plasma donor with extremely high DNA concentrations exceeding 10^{14} IU/ml, the B19 viral DNA in the pooled plasma could reach 10^{9} IU/ml. Because of its special structure and small size, B19V is highly resistant to all commonly used inactivation methods. Our preliminary research also indicated that the plasma-derived clotting factor concentrates such as fibrinogen and PCC were found to be highly contaminated with B19V DNA（53.7%-85.7%）, IVIG and albumin were moderately contaminated (0-38.9%) (Zhang et al. 2012). Thus the safety of plasma products need to be further evaluated.

In China, there have been no specific documentation and technical guidelines for monitoring B19V. For better ensuring the safety of plasma donors, many governments request plasma manufacture to perform the screening for B19V in blood and plasma products. For example, the US Food and Drug administration guidelines, U.S. Pharmacopoeia, Plasma Protein Therapeutics Association, and the European regulatory requirements recommend that the viral load of B19V in the manufacturing of pooled plasma should not exceed 10^{4} IU/mL (2020a; 2020b; Jin et al. 2010). However there is no regulation to ensure the safety of pooled plasma and plasma

derivatives for B19V in China. Although the B19V-DNA prevalence among Chinese plasma donors is relatively low, once asymptomatic plasma donors with high levels of B19V, up to 10^{12} IU/mL, may present a greater risk in plasma derivatives (Jia et al. 2019; Li et al. 2020; Marano et al. 2015; Schmidt et al. 2001; Siegl & Cassinotti 1998; Zhang et al. 2012).This study's major objective was to comprehensively and systematically determine the frequency and the levels of B19V DNA in plasma donors, pooled plasma and plasma derivatives from four Chinese blood products manufacturers. Besides, reports of the change of B19V DNA in single –plasma donors are rare, so we also follow-up evaluated the level change of B19V DNA in those B19V DNA-positive donors who was first confirmed after their subsequent donation. And we continued to track and investigate the contamination in the plasma derivatives produced by plasma pools with viral load of B19V DNA exceeding 10^4 IU/mL. Moreover, B19V-specific immunoglobulin (IgG/IgM) antibodies in all of B19V DNA-positive samples were also determined. We expect that this study will provide a series of robust evidences on promoting the quality standards for plasma derivatives in China.

Materials & Methods

Sample collection

From October 2018 through May 2020, 17380 individual plasma samples from 4 provinces (Shandong province, Guangxi province, Sichuan province and Guizhou province) and 400 plasma pools (approximately 17000 single-plasma mixed a pool) were collected from four Chinese blood products manufacturers. Furthermore, the samples from 11 B19V DNA-positive donors who had frequent plasma donation were also collected after their first DNA-positive donation. Also 5 batches of albumin, 5 batches of intravenous immunoglobulin, 5 batches of prothrombin-complex concentrate (PCC) and 5 batches of Factor VIII produced by plasma pools with viral load of B19V DNA exceeding 10^4IU/mL were also collected. The study was approved by the Research Ethics Committee of the Institute of Blood Transfusion (No.202029). The study was based on the plasma samples stored in place collected before and the researchers had no direct contact with the donors. The Ethics Committee waived the need for written consent.

B19V DNA quantitation

Individual plasma samples were tested by pools of 48 individuals. The samples in the B19V positive pools and the pooled plasma were tested separately with 960μL plasma. The virus DNA/RNA kit (Beijing Wantai Biological Pharmacy Enterprise Co., Ltd) was used for nucleic

acid extraction according to the manufacturer's instructions. The DNA extracts were stored at -80℃ prior to PCR analysis. Screening of samples for the B19V DNA was performed with human parvovirus B19V DNA diagnostic kits (PCR-fluorescence probing) (Beijing Wantai Biological Pharmacy Enterprise Co., Ltd). This kit can detect all the three Genotypes of B19V with sensitivity of 20 IU per mL. The Q-PCR assays were performed on a Bio-Rad CFX96 real-time PCR platform (Bio-Rad Laboratories, Hercules, CA).

B19V DNA-positive samples were confirmed by nested PCR (nPCR), the conserved primers located in the NS1 region was used. Detailed nPCR procedures have been described previously(Ke et al. 2011).

Phylogenetic analysis

The phylogenetic information was analyzed using the sequence from NS1-VP1-U region. The sequence was amplified upon the previous description (Servant et al. 2002). The PCR products were purified using NucleoSpin Extract Ⅱ kit (Macherey-Nagel GmbH & Co. KG, Duren, Germany) according to the instructions. The second cycle sequencing reactions were performed using the purified products. The sequences were then read with ABI 3730 (Applied Biosystems). Sequences were determined for both directions. The sequences were aligned using ClustalX 1.83. The neighborhood joint (NJ) and maximum parsimony (MP) analyses were used to detect the phylogenetic position of the samples in this study with the reference sequences using software MEGA 7.0.

B19V serologic assays

B19V-specific antibodies in B19V DNA-positive specimens were investigated using commercial assay kits (Virion-Serion, Würzburg, Germany) according to the manufacturer's instructions.

Statistical methods

Analysis of the data was performed using SPSS 16.0 statistics software (SPSS, Inc., Chicago, IL) and Microsoft Excel 2011.

Results

Prevalence of B19V DNA and antibodies in plasma donors

In this study, 17187 individual samples of plasma donors living in four provinces were collected and tested in China. Of 17187 plasma donors, 44 (0.26%) specimens were found positive for B19V DNA. The quantitative DNA levels ranged from 1.01×10^{1} IU/mL to 5.09×10^{12} IU/ml. As shown in Table 1, 1 of 2000（0.05%）individual plasma sample from company A were positive

for B19V DNA. 18 of 3898 (0.46%) individual plasma sample from company B were positive for B19V DNA, and 2 positive (0.05%) samples contained $>10^4$ IU/ml. The B19V DNA positive samples' percent for company C and company D was 0.33% (13/3985) and 0.16% (12/7304) respectively. Moreover, the distribution of ABO blood type of B19-postive donors was different (blood type A was 18.2%, blood type B was 22.7%, blood type AB was 6.8%, blood type O was 52.3%). The anti-B19V IgG and IgM titers were also tested in B19V DNA positive samples. The presence of anti-B19 antibodies was associated with lower levels of viraemia. 28 out of 44 (63.6%) samples were positive for IgG, with titers in the range of 7.03-3800 IU/mL. Only one sample of 44 (2.3%) was contained IgM, with the titer of 30.91 IU/mL. 11 individual plasma donors who were B19V DNA positive at the index time were followed for a period. The B19V DNA, IgG and IgM were always monitored during the following donations until the COVID-19 pandemic outbreak in 2020. The follow-up tests were shown in Table 2 (typical example), 10 of 11 (83.3%) donors were positive for B19V DNA for no more than 2 times during the following period. 2 donors were positive for B19V DNA all the following period. The donor with the highest B19V DNA (5.09×10^{12} IU/mL) was negative for IgG/IgM at the first donation after index time, then IgG and IgM reached the peak titers at the second donation, IgM became negative half month later, while IgG kept positive for the following period.

B19V DNA and antibodies in plasma pools for fractionation

A total of 400 plasma pools were tested for B19V DNA, IgG and IgM. Of these, 259 pools (64.8%) contained B19V DNA, and 76 out of 259 (29.3%) contained B19V DNA at a level higher than 10^4 IU/mL. The prevalence of B19V DNA in plasma pools differed in different companies. Company A had a prevalence of 27%. However the other three companies had much higher prevalence of B19V DNA, company B 90%, company C 81%, and company D 61%. Meanwhile the number of plasma pools with a viral load higher than 10^4 IU/mL differed between the four companies. All the B19V DNA positive plasma pools were positive for B19V IgG, with titers in the range of 5.755-27.73 IU/mL. No sample was positive for B19V IgM. The results are shown in Table 3.

B19V DNA and antibodies in plasma derivatives

Plasma derivatives which were made from company B plasma pools with viral load of B19V DNA exceeding 10^4 IU/mL were also collected and tested. Table 4 summarized B19V DNA and antibodies in the plasma derivatives. B19V DNA was not detected in albumin and IVIG.

However, all of the PCC and factor were contained with B19V DNA. 80% (4 of 5 batches) of PCC were highly contaminated (higher than 10^4 IU/mL), and the B19V DNA concentration was up to 3.59×10^7 IU/mL. Of all the plasma derivatives only 2 batches of IVIG were positive for B19V IgG.

Phylogenetic relationships among different B19V isolates

17 1200-bp sample sequences were obtained within NS1-VP1-U region. They were aligned with 35 reference sequences to reconstruct the phylogenetic tree under the NJ method. The tree is shown in Fig.1 with the NJ bootstrap values depicted on the branches. All of the samples studied in this article formed a monophyletic group, and they fell in Genotype 1A.

Discussion

In China, the commercial plasma pools collection system is independent of the voluntary non-remunerated whole blood banking system. All of human plasma derivatives are produced only by plasma pools from thousands of healthy plasma donors. Once the plasma donors were infected by B19V, this higher virus titer is sufficient to contaminate plasma pools. Moreover, B19V has no envelope, this virus is resistant to inactivation treatment by heating or solubilizes. Therefore, it can be transmitted by plasma derivatives. Plasma derivatives factor products are important therapies for people with VWD, hemophilia inhibitors and rarer factor deficiencies. Once those plasma derivatives were contaminated with a high titer of B19V, they can cause serious problems in patients with an increased risk of infection. There are few clinical data on the transmission of B19V through these products. Previous studies had illustrated that the plasma-derived factor replacement products usually led to the transmission of B19V in bleeding disorder patients, such as hemophilia. In the earlier report, they found that the seroprevalence of IgG antibodies to B19V was much higher among very young (age 2-7 years) hemophilia patients exposed to plasma-derived products compared to those not exposed (Azzi et al. 1999; Santagostino et al. 1997; Soucie et al. 2004). Another study also found that young children with bleeding disorders exposed only to plasma-derived factor concentrates were 70% more likely to have antibodies to B19V than those unexposed to any products (Soucie et al. 2013). It is well known that patient safety is an important issue (Di Minno et al. 2016).Due to B19V's pathogenicity and risk of transmission through plasma derivatives, great concerns have been raised on it. Japanese, Germany and Netherlands screen for B19V DNA or B19V specific

antibodies in blood donors (2002; Sakata et al. 2013; Schmidt et al. 2007). Regarding to plasma, U.S. Food and Drug Administration (FDA), European Pharmacopia has proposed a limit of 10^4 IU/mL for levels of B19V DNA in plasma pools for manufacturing all kinds of plasma derivatives (2020a; 2020b; Jin et al. 2010). There are no regulations or recommendations for monitoring B19V DNA in China yet. In our study, we monitored the contamination in plasma deviates produced by plasma pools with viral load of B19V DNA exceeding 10^4 IU/mL. Considering to business privacy, only one cooperation company was willing to continue study in this study. Therefore there were only total of 20 batches plasma deviates were continually collected and monitored. These products were factor VIII, PCC, IVIG and albumin made from plasma pools with high B19V DNA (>10^4 IU/mL). The results indicated that B19V DNA was not detected in any batch of albumin and IVIG, except for plasma-derived clotting factor concentrates. These contaminated products must be imposed risks to the patients who received them. Although it would have been interesting to note that how many of the recipients of these plasma derivatives factor products turned positive for B19V. There are many difficulties to perform this assay in China.

Moreover, the safety of plasma pools with regard to B19V has been also a major concern. In 2015, Yuyuan Ma and her team demonstrated that the contamination of B19V in plasma pools was serious in China. In her study, 71.91 % (169/235) of plasma pools were contaminated by B19V, with the concentrations of 5.18×10^2–1.05×10^9 IU/mL. Approximately 31.95 % of the DNA-positive plasma pools were only moderately contaminated (<10^4 IU/mL), while 68.05 % contained >10^4 IU/mL (Jia et al. 2015). These data are consistent with our study. So the data demonstrates a relatively high prevalence of B19V in Chinese plasma pools. According to the limit standard of 10^4 IU/ml for levels of B19V DNA in plasma pools from U.S. FDA, European Pharmacopoeia and the Plasma Protein Therapeutics Association (PPTA), more than 60% plasma pools should be discarded in China. For using plasma resource well, we advise that the B19V DNA screening for individual plasma donor would be better than plasma pools and plasma derivatives.

Previous studies demonstrated that the prevalence of B19V DNA in Chinese plasma donors differed between 0.03% and 0.09% (Han et al. 2015). However, we found that 0.29% (42/14187) specimens were positive for B19V DNA. It was higher than that mentioned before, but it was lower than 0.58% which Ke *et al*. found in whole blood donors(Ke et al. 2011). The results may

be related with plasma donors' geographic differences and methodological differences in diagnostic procedures. Besides, only two plasma donors from Guangxi province were infected with B19V at levels higher than 10^4 IU/mL (5×10^{12} IU/mL). In some reports, the peak of the virus titer might reach to 10^{13} IU/mL (Frickhofen & Young 1989). Thus B19V NAT testing for single Chinese plasma donor screening is necessary. In addition, 11 plasma donors who were B19V DNA positive at the first screening time were followed up until the Covid-19 pandemic. One of the plasma donors was a classic example. In his first donation, B19V DNA was positive with virus titer of 5.09×10^{12} IU/mL. After his second and third donation (about 14 days later), B19V DNA was still positive with lower virus titer (1.86×10^5 IU/mL, 1.09×10^4 IU/mL), IgG and IgM were positive yet. This plasma donor might be in viremia stage at his first donation. After 36 days later, B19V DNA and IgG of the donors were still positive, IgM was negative yet. Those varies were in accordance with epidemiological trends of B19V in blood phase. IgM antibody develops 10-14 days post-infection followed by the development of IgG antibodies directed toward viral capsid components. Meanwhile, this result also indicated that the plasma donor who was after viremia stage can continue to donate plasma, not be permanent refused.

A survey reported that the seroprevalence of B19V-specific IgM antibodies was commonly below 2% in health people. While the IgG antibodies was reported approximately 2% in children under the age of 5 to 80% in blood donors 18-65 years of age (Ke et al. 2011; Kelly et al. 2000; Manaresi et al. 2004). In our study, of the B19V DNA positive individual plasma donors, 62.5% B19V DNA positive individual plasma donors demonstrated the presence of B19V specific IgG, while 100% plasma pools were positive for B19V specific IgG. The IgG presence is a sigh of past infection and gives protective immunity. The prevalence of IgG antibodies increases with age. In most studies, about 30% of 18- to 30- year-old donors have detectable IgG, while about 60% of around 50-year-old donors are seropositive (Zaaijer et al. 2004). A study in our laboratory on characteristics of Chinese plasma donors showed that most plasma donors (78.5%) were aged 46-55 years old (Sun et al. 2021). This may explain the prevalence of B19V specific IgG in Chinese plasma donors. Health Council of Netherlands considers that blood with persistent anti-B19V IgG (B19V specific IgG have been detected in two separate blood samples, one taken at least 6 months after the other) might be B19V-safe blood (2002). B19V specific IgM antibodies are detectable 10 to 14 days after infection and can generally persist for 5 months. The prevalence of B19V IgM serves for the assessment of the rate of donors who were

infected with B19V recently. In this study only one individual plasma donor of B19V DNA positive was detected with B19V specific IgM. There was no association of levels of B19V DNA content and the titer of IgM/IgG.

During the last several years, great efforts have been made to investigate the epidemic and characterization of B19V in plasma donors, plasma pools and plasma derivatives in China. Those data recommended the implementation of B19V screening for plasma donors and plasma pools in order to contract the transmission of B19V via plasma derivatives. Moreover, China's National Medical Products Administration had prepared the national reference standard for B19V DNA detection, and the quantitative real-time detection of B19V kits are being explored. Whether B19V NAT screening of plasma and plasma derivatives will be launched in China, Chinese plasma fractionation industries should be encouraged to spontaneously perform B19V NAT screening in plasma donors and mini-pool plasma. These measures can ensure these samples with high titer B19V DNA were discarded at a crucial juncture of prevention and control of this transfusion transmitted virus.

Our study had some limitations that the plasma derivatives are distributed all around the country and administrated to different recipients, it is really difficult to integrate so many resources to perform such a large-scale systematic research on the recipients of these blood products. Moreover, there may be other uncertainties. For example, participants may be dropped out from the program at any time, and they may be treated with other blood products. Thus those above unfavorable factors may greatly limit the study. In view of this, B19V NAT screening is recommended in plasma donors and mini-pool plasma to better protect the recipients of plasma derivatives.

Conclusions

In this study, we found that the contamination of B19V in pooled plasma and plasma-derived clotting factor concentrates was serious in China. Further follow-up study on the recipients of these blood products was difficult to perform for us. B19V NAT screening is recommended in plasma donors and mini-pool plasma to ensure these samples with high titer B19V DNA were discarded at a crucial juncture of prevention and control of this transfusion transmitted virus.

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Table 1(on next page)

B19 DNA prevalence and levels in plasma donors from four Chinese provinces during 2018 to 2021

1

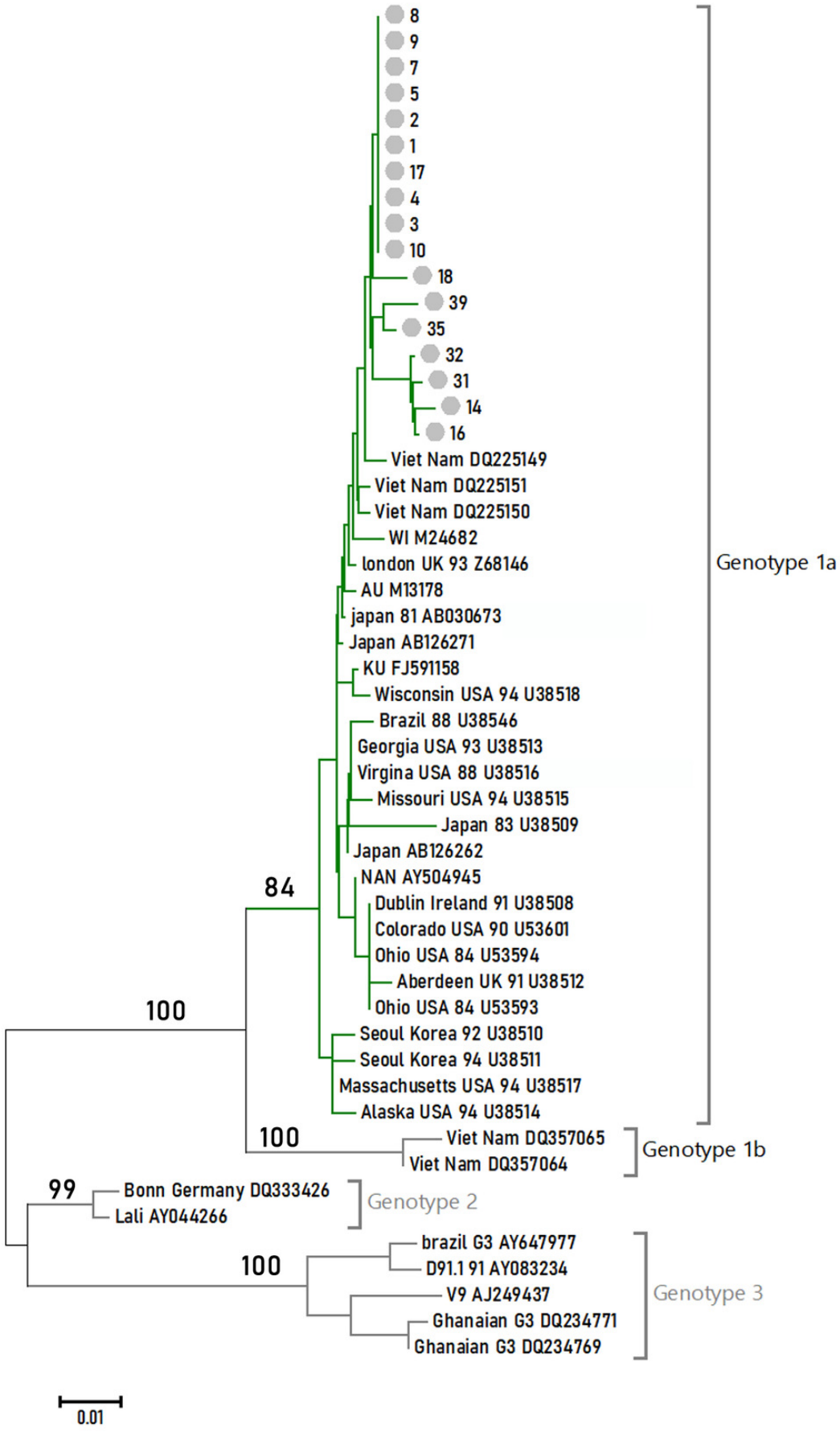
Blood products manufactures	Areas of plasma samples	Samples detection (n)	B19 DNA positive (n)	Prevalence of B19V DNA (%)	95% confidence interval [CI]
A	Sichuan	2000	1	0.05	0.00～0.15
B	Guangxi	3898	18	0.46	0.24～0.66
C	Shandong	3985	13	0.33	0.15～0.50
D	Guizhou	7304	12	0.16	0.07～0.26
Total	/	17187	44	0.26	0.18～0.33

2

Figure 1

Fig.1 Phylogenetic relationships of NS1-VP1-U region of different clones.

Values on the nodes indicate NJ/MP bootstrap values. 24 sequences from this study (labeled with Arabic numerals) and a set of reference sequences downloaded from GenBank (labeled with their GenBank accession number) were analyzed


8
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Viet Nam DQ225151
Viet Nam DQ225150
WI M24682
london UK 93 Z68146
AU M13178
japan 81 AB030673
Japan AB126271
KU FJ591158
Wisconsin USA 94 U38518
Brazil 88 U38546
Georgia USA 93 U38513
Virgina USA 88 U38516
Missouri USA 94 U38515
Japan 83 U38509
Japan AB126262
NAN AY504945
Dublin Ireland 91 U38508
Colorado USA 90 U53601
Ohio USA 84 U53594
Aberdeen UK 91 U38512
Ohio USA 84 U53593
Seoul Korea 92 U38510
Seoul Korea 94 U38511
Massachusetts USA 94 U38517
Alaska USA 94 U38514
Genotype 1a
84
100
100
Viet Nam DQ357065
Viet Nam DQ357064
Genotype 1b
99
Bonn Germany DQ333426
Lali AY044266
Genotype 2
100
brazil G3 AY647977
D91.1 91 AY083234
V9 AJ249437
Ghanaian G3 DQ234771
Ghanaian G3 DQ234769
Genotype 3
0.01

Table 2(on next page)

Varies of viral load and antibody level in a typical B19 DNA-positive donor during his different plasma donation

1

Donor	Sex	Age	Blood group	Donation date	Viral load (IU/mL)	IgM	IgG
1	Male	18	O	10/18/2019	5.09×10^{12}	-	-
				11/04/2019	1.86×10^{5}	*	-
				11/18/2019	1.09×10^{4}	*	#
				12/02/2019	5.15×10^{3}	-	#
				12/16/2019	4.96×10^{3}	-	#
				12/31/2019	3.34×10^{3}	-	#
				01/15/2020	3.04×10^{3}	-	#

2 Not:* B19 I gM positive, # B19 I gG positive

Table 3(on next page)

B19 DNA and antibodies in plasma pools

1

Blood products manufactures	Sample type	Samples detection (n)	B19V DNA-positive samples							
			B19 DNA positive (n)	Prevalence of B19 DNA (%)	95% confidence interval [CI]	B19 viral load ≥1×10⁴IU/mL (n)	Prevalence of B19 viral load ≥1×10⁴IU/mL (%)	95% confidence interval [CI]	IgG positive (%)	IgM positive (%)
A	plasma pools	100	27	27	18.15～5.85	4	4	0～6.40	100	0
B	plasma pools	100	90	90	84.02～95.98	21	21	12.88～29.12	100	0
C	plasma pools	100	81	81	73.18～88.82	17	17	13.74～30.26	100	0
D	plasma pools	100	61	61	51.27～70.73	29	29	19.95～38.05	100	0
Total	plasma pools	400	259	64.8	60.05～69.45	71	17.8	14.91～22.59	100	0

2
3

Table 4(on next page)

Prevalence and levels of B19V DNA and antibodies in plasma derivatives produced by start plasma pools with viral load $\geq 10^4$IU/mL

1

Start plasma pools				Plasma derivatives produced by start plasma pools with viral load ＞10^4IU/mL				
Numbers	**B19V DNA titers ((IU/mL)**	**B19V IgG-positive**	**B19V IgM-positive**	**Names**	**Bathes**	**B19V DNA titers ((IU/mL)**	**B19V IgG- positive**	**B19V IgM-positive**
1	5.25×10^8	#	-	Albumin	A-1	N/A	-	-
2	9.12×10^7	#	-		A-2	N/A	-	-
3	3.02×10^4	#	-		A-3	N/A	-	-
4	1.15×10^8	#	-		A-4	N/A	-	-
5	1.60×10^8	#	-		A-5	N/A	-	-
6	9.12×10^7	#	-	Intravenous immunoglobulin (pH4)	I-1	N/A	#	-
7	3.02×10^4	#	-		I-2	N/A	#	-
8	1.15×10^8	#	-		I-3	N/A	#	-

9	1.60×10^{8}	#	-	Intravenous immunoglobulin (pH4)	I-4	N/A	#	-
10	3.40×10^{7}	#	-		I-5	N/A	#	-
11	8.56×10^{4}	#	-	PCC	P-1	3.59×10^{7}	-	-
12	9.12×10^{7}	#	-		P-2	4.60×10^{6}	-	-
13	3.02×10^{4}	#	-		P-3	6.48×10^{3}	-	-
14	1.15×10^{8}	#	-		P-4	1.47×10^{6}	-	-
15	1.60×10^{8}	#	-		P-5	1.40×10^{6}	-	-
16	5.65×10^{7}	#	-	Factor VIII concentrate	F-1	3.72×10^{1}	-	-
17	9.12×10^{7}	#	-		F-2	1.90×10^{1}	-	-
18	1.15×10^{8}	#	-		F-3	1.35×10^{2}	-	-
19	1.60×10^{8}	#	-	Factor VIII concentrate	F-4	1.65×10^{2}	-	-

20	3.40×10^{7}	#	-		F-5	3.57×10^{2}	-	-

Note:* B19 IgM positive, # B19 IgG positive