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Incidence and estimated risk of residual transmission of hepatitis a virus and parvovirus B19 by blood transfusion in the state of Rio De Janeiro – Brazil: a retrospective study

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Abstract

Background Nonenveloped viruses, such as hepatitis A virus (HAV) and parvovirus B19 (B19V), are not inactivated by detergents and solvents commonly used to manufacture plasma derivatives. Cases of transfusion-transmitted HAV and B19V have already been described in several countries. This study aimed to determine the incidence of HAV and B19V asymptomatic infections in blood donors from Rio de Janeiro and evaluate the residual risk of transmission to blood derivative recipients.

Methods From 2018 to 2019, 1,204 plasma samples were tested by PCR and real-time PCR. HAV and B19V genotypes were determined through sequencing and phylogenetic analysis. The risk of transfusion transmission was determined using a Bayesian statistical approach.

Results HAV-RNA and B19V-DNA were detected in 1.66 (95% CI 0.26–5.48) and 3.32 (95% CI 1.00-7.81) per 1000 donors, respectively. For HAV, all positive samples were classified as subgenotype IB. For B19V, the sequenced samples belonged to genotype 1A. The estimated numbers of infectious blood bags with HAV and B19V were 587 (95% CI 92-1936) and 880 (95% CI 355–2759), respectively.

Conclusion This study originally assessed the incidence of both Hepatitis A Virus (HAV) and B19 Virus (B19V) among Brazilian blood donors, as well as the potential risk of residual transmission of these infections through blood transfusions. Our findings can contribute to future cost-effective studies aimed at implementing screening methods

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for these viruses, which could enhance discussion on surveillance in blood banks and protective measures for blood recipients.

Keywords Hepatitis A, Parvovirus B19, Blood donors, Residual transmission, Incidence

Introduction

Human parvovirus B19 (B19V) and hepatitis A virus (HAV) are small-sized, nonenveloped viruses, resistant to the virus inactivation process commonly used to manufacture plasma derivatives, such as heating, solvent-detergent and even nanofiltration [1–3]. The currently available methods for pathogen inactivation in blood components – Amotosalen + UV-A irradiation and riboflavin + UV-B irradiation – are not enormously effective against HAV and B19V [4]. The physicochemical properties of these viruses pose relevant concerns about the risk of blood transfusion and plasma-derivate transmission.

Although HAV is primarily transmitted by the fecal-oral route, mainly through person-to-person contact, transfusion-transmitted cases of HAV (TT-HAV) infection have been reported since the 1970s [5–8]. In Brazil, two cases of TT-HAV were reported in 2015 by blood products from a single infected donor. It is worth noting that one of these recipients had chronic hepatitis C and died a few days after transfusion due to acute liver failure (ALF) [9]. Hepatitis A (HA) is often asymptomatic in children, whereas acute HA is a moderate to severe disease in adults. HA occasionally leads to liver damage ranging from mild illness to ALF, constituting 0.35% of all cases of ALF [9, 10]. HAV is a single-stranded positive-sense RNA genome of approximately 7.5 kb that belongs to the Picornaviridae family, Hepatovirus genus, and Hepatovirus A species. The viral particle has an icosahedral capsid with a diameter of 27 to 32 nm [11, 12].

B19V is a nonenveloped, single-stranded DNA virus, classified into the Erythroparvovirus genus of the Parvoviridae family with a marked tropism for erythroid progenitor cells in the human bone marrow [13, 14]. The virion has an icosahedral symmetry with a diameter ranging from 18 to 26 nm [15].

Although B19V is primarily transmitted by the respiratory route, there are many reports of B19V transfusion-transmitted (TT-B19V), as well as by administration of plasma products, especially coagulation factors [16–22]. B19V can cause a range of diseases, including erythema infectiosum, transient aplastic crisis, and poliarthropathy [23, 24]. However, most B19V infections present no symptoms in immunocompetent individuals [25, 26]. TT-B19V infections are usually asymptomatic, like most cases of B19V infection in adults, and as such, have historically been neglected. Nevertheless, given the high seroprevalence of B19V among the general population and the fact that TT-B19V infection can cause severe clinical consequences, especially among immunocompromised patients with hematological disorders and pregnant women, the incidence of TT-B19V has gradually received more attention as an important public health problem. Transmission of B19V through blood products is facilitated by two important characteristics: persistent infection in the bone marrow of asymptomatic individuals and prolonged replication after the initial infection, which can occur for several years [16, 17].

The risk of HAV and B19V transmission through blood transfusion mainly occurs during the viremic period, when many viral particles circulate in the blood before the onset of symptoms, making it possible for donors to harbor the circulating virus in their blood at the time of donation without being aware of the infection [1].

Overall, the transmission of infectious diseases through blood transfusion has been reduced in blood banks due to rigorous clinical screening and advancements in serological and molecular methods for detecting the causative agents of such diseases. Considering the potential risk of HAV and B19V transmission through blood components and plasma derivates, these viruses raised relevant concerns about the safety of plasma products.

In some European countries, since 2000, all blood donations have started to be screened by a B19V mini pool real-time nucleic acid test (NAT; German Red Cross Centre and four areas in Austria) [27]. Since 2004, Polish blood donors have also been tested for B19V DNA [28]. In 2008, to reduce the risk of B19V transmission through contaminated blood for transfusion and plasma-derived medicinal products, Japanese Red Cross Blood Centers introduced B19V antigen screening by chemiluminescent enzyme immunoassay for all donated blood [29]. In the United States, it is also recommended that all pools be tested for B19V. No country requires HAV blood donation screening; however, some countries require donation screening to manufacture plasma-derived products or pool plasma for transfusion [30]. Nevertheless, in Brazil, HAV and B19V are not included in the screening tests conducted on blood donors. The screening is only intended for Human Immunodeficiency virus (HIV), Hepatitis B virus (HBV), Hepatitis C virus (HCV), Human T-lymphotropic virus (HTLV), the etiological agents of Syphilis and Chagas disease, and more recently for Malaria, in some hemocenters of the country.

Data on HAV RNA and anti-HAV IgM prevalence in blood donors are limited worldwide. The prevalence of HAV RNA varies from 0.24% among Indian blood donors (between 2004 and 2005) [31] to 0.005% (1/20,000) of HAV RNA among blood donors from the United States

[32]. Data on the prevalence of HAV RNA among Brazilian blood donors, as well as the impact of the hepatitis A outbreak on this group of individuals, remain scarce. Regarding B19V DNA prevalence, few studies show a heterogeneous distribution according to different regions of Brazil: 0.21% in the Midwest [33], 1.0% in the Southeast [34], and 1.9% in the South [35].

Knowledge of the current regional epidemiological scenario of these infections is an essential contribution to blood transfusion safety and to implementing measures for mitigating transfusion-transmitted risks. Thus, this study aimed to determine the incidence of hepatitis A and Parvovirus B19 in blood donors from Rio de Janeiro and estimate the residual risk of transfusion-transmission of these viruses.

Methods

Study population and ethical aspects

This is a retrospective cross-sectional study that investigated HAV and B19V infection in 1,204 plasma samples from blood donors of Instituto Estadual de Hematologia Arthur de Siqueira Cavalcanti (HEMORIO) in Rio de Janeiro, Brazil. HAV and B19V infections were also investigated in the serum samples collected on the day of blood donation.

Among this total of donors, one hundred plasma samples were obtained individually from bags of fresh frozen plasma. However, many of these samples showed qPCR inhibition (detected by RNAse P evaluation), so we tested only 100 samples and evaluated 1,104 samples obtained through plasma mini pools, composed of samples from six different donors (collected in EDTA tubes with separator gel), totaling 184 mini pools, previously prepared at the HEMORIO blood center.

The sampling was stratified between July 2017 and December 2019, aiming to include epidemic and non-epidemic periods of hepatitis A and B19V, according to the seasonality. Plasma samples were selected from blood donors who donated at HEMORIO's blood therapy service, who were negative for parenterally transmitted infectious diseases, routinely tested in Brazil (HIV-1 + 2, HCV, HBV, HTLV-I/II, syphilis, and Chagas disease).

The study protocol was approved by the Ethics Committee of Oswaldo Cruz Institute (CAAE: 48376015.2.0000.5248, number: 1.610.747).

Sample size

A sample size of 742 plasma samples from blood donors was established to estimate B19V DNA incidence in blood donors between 2018 and 2019 using the following parameters: B19V DNA prevalence (average of 0,47%) in blood donors from different studies around the world during the same period [33–40], an accepted margin of error of 0.5% and 95% of confidence level. The prevalences of HAV RNA in other regions are smaller than those for B19V. The sample size for B19V was considered for HAV [1, 41].

Pooling of plasma specimens and RNA/DNA extraction

One thousand one hundred four plasma specimens were pooled into 184 mini pools, each comprising six samples. Aliquots of 110µL from 6 donor plasma specimens were pooled by JANUS® Automated Workstation (Perkin Elmer®, Massachusetts, EUA), and 600µL of the pooled specimen was used for RNA and DNA extraction.

B19V DNA and HAV RNA were extracted from 600µL of plasma pool using the NucliSens easyMAG (bioMérieux, Marcy l' Etoile, France) extraction kit, according to the manufacturer's instructions, to yield an eluted volume of 50µL. Extraction and inhibition controls were included in all extraction runs to control for the extraction process. The 50µL nucleic acid eluates were stored at − 80 °C before and after viral load testing.

Validation of mini pools

This experiment aimed to assess how pooling six samples would impact the ability of the assays to detect B19V DNA through dilution. Highly B19V-viremic plasma samples (10^9 IU/mL) were serially diluted by a factor of 10. Each point of the serial dilutions was used to spike six mixed plasma samples that were initially negative for B19V DNA at a dilution of 1:20. Each spiked pool was extracted and amplified in triplicates to determine the lower limit of detection in pooling at a 1:20 dilution. Statistical comparison between the samples in single or pool detection was made using the Mann–Whitney U test. The $p < 0.05$ was considered statistically significant.

Resolution of pools with a detectable viral load

Mini pools with a detectable viral load were resolved, and individual specimens were extracted and amplified. To clarify the procedures, Fig. 1 represents the algorithm of diagnosis.

Molecular and serologic tests for HAV

HAV RNA was reverse-transcribed and amplified by a single-step Real-time PCR (qRT-PCR) using the AgPath One Step Real-Time PCR kit (Thermo Fisher, Massachusetts, USA) using the TaqMan system (Applied Biosystems 7500 Real-Time PCR System, Applied Biosystems, USA). For absolute quantification, a synthetic oligo-ultramerer for the HAV 5'NCR region (IDT, USA) was used (nt 86–218, Strain: HAF-203). Primers for the 5'NCR region (nt 86F and 240R) and a single labeled 5' FAM probe (nt 198–218, Strain: HAF-203) were used, as previously described [42]. Primer sequences are listed in Table 1.

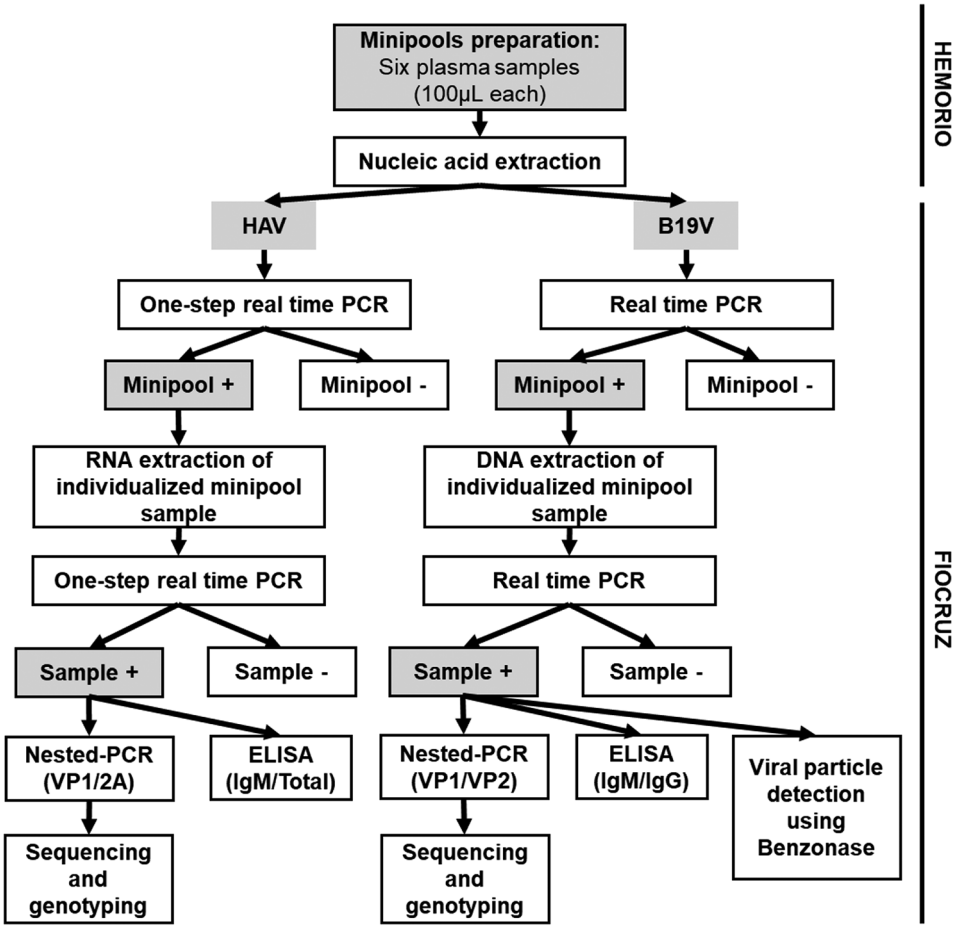

Fig. 1 Algorithm of tests for determining HAV RNA and B19V DNA prevalence and seroprevalence. Ig, immunoglobulin. HAV: Hepatitis A Virus; IU/mL: international units per mL; IgM: Immunoglobulin M; IgG: Immunoglobulin G; SWI: sample with inhibitor for qPCR

Table 1 Primers and probe sequences for HAV RNA and B19V DNA

ID	Sequence (5'-3')	Amplicon	Reference
	HAV		
5'NCR (F)	CTGCAGGTTCAGGGTTCTTAAATC	89 bp	[42]
5'NCR (R)	GAGAGCCCTGGAAGAAAGAAGA		
5'NCR (Probe)	FAM-ACTCATTTTTCACGCTTTCTG		
VP1/2A – 1 (F)	CTATTCAGATTGCAAATTAYAAT	391 bp	[43]
VP1/2A – 1 (R)	AAYTTCATYATTTCATGCTCCT		
VP1/2A – 2 (F)	TATTTGTCTGTYACAGAACAATCAG	244 bp	
VP1/2A – 2 (R)	AGGRGGTGGAAGYACTTCATTTGA		
	B19V		
NS1-E1905 (F)	TGCAGATGCCCTCCACCCA	102 bp	[46]
NS1-E1987 (R)	GCTGCTTTCACTGAGTTCTTC		
NS1 (Probe)	FAM-ACCTCCAAACCACCCCAATTGTCACA		
P12 (F)	CAGCCATACCACCACTGGGACA	563 bp	[47]
P16 (R)	AGTACATATGGTAAATGACCTGCTG		
P13 (F)	GACAAAGAGTATCAGCAAGGAGTG	476 bp	
P16 (R)	AGTACATATGGTAAATGACCTGCTG		

F = Primer forward; R = Primer reverse;

Each sample included in a positive plasma pool was investigated individually for HAV genotyping. For this, a nested PCR using the primer pairs VP1/2A first round (nt 2987–3288, Strain: HAF-203) and VP1/2A second round (nt 2949–3196, Strain: HAF-203) was performed to amplify the 247 bp fragment of the VP1/2A capsid gene, as previously described [43]. The Nested-PCR products were purified using the QIAquick gel extraction (Qiagen, Germany) and then sequenced directly using the BigDye terminator v.1.1 cycle sequencing kit (Applied Biosystems, USA) and an ABI Prism® 3730 DNA analyzer (Applied Biosystems, USA). Both strands of each amplicon were sequenced. For phylogenetic analysis, sequences were aligned using the BioEdit Sequence Alignment Editor v7.2.5 [44]. A maximum likelihood tree was constructed based on the Kimura-2 parameter + G distances available in the program MEGA v.10.0 software [45]. The HAV genotype was determined by including reference sequences of genotypes IA, IB, IIA, and IIB, which are available in GenBank.

According to the manufacturer's instructions, all HAV-RNA-positive serum samples from blood donors were investigated for anti-HAV IgM and anti-HAV total antibodies through an enzyme-linked immunosorbent assay (Biokit, S.A. Barcelona, Spain).

Molecular and serologic tests for B19V

B19V infection was investigated in the serum samples collected on the day of blood donation using serological and molecular assays. Firstly, B19V DNA was also investigated in mini pools, and then individual testing was carried out on each sample included in a positive plasma pool. Real-time PCR (qPCR) was carried out using the TaqMan system (Applied Biosystems 7500 Real-Time PCR System, Applied Biosystems, USA). For absolute quantification, a synthetic standard curve of the B19V NS1 region (IDT, USA) was designed (nt 1905–1987, GenBank: NC_000883.2). Primers for the NS1 region (nt 1905F and 1987R) and a single labeled 5' FAM probe (nt 1925–1948, GenBank: NC_000883.2) were used, as previously described [46]. Primer sequences are listed in Table 1.

The presence of qPCR inhibitors was evaluated in some samples that were positive in conventional PCR and negative in qPCR using an endogenous internal positive control. This control comprises oligonucleotides and a probe labeled at the 5' end by a VIC™ fluorescence for a human genome target called RNAseP (Applied Biosystems, California, USA, Catalog number 4316844). For this commercial internal control, 5µL of extracted DNA was added, which allowed simultaneous amplification of the target sequence of this internal control and the B19V genome.

For B19V genotyping, a semi-nested PCR using the primer pairs VP1/VP2 first round (nt 4127–4665, GenBank: NC_000883.2) and VP1/VP2 second round (nt 4214–4665, GenBank: NC_000883.2) were performed to amplify the 476 bp fragment of the VP1/VP2 capsid gene, as previously described [47]. The semi-nested PCR products were purified using the QIAquick gel extraction (Qiagen, Germany) and then sequenced directly using the BigDye terminator v. 1.1 cycle sequencing kit (Applied Biosystems, USA) and an ABI Prism® 3730 DNA analyzer (Applied Biosystems, USA). Both strands of each amplicon were sequenced.

For phylogenetic analysis, sequences were aligned using the BioEdit Sequence Alignment Editor v7.2.5 [44]. A maximum likelihood tree was constructed based on Tamura-Nei + G + I parameter distances available in the program MEGA v.10.0 software [45]. The B19V genotype was determined by including reference sequences of genotypes IA, IB, II, IIIA, and IIIB, which are available in GenBank.

According to the manufacturer's instructions, all B19V-DNA-positive serum samples from blood donors were investigated for anti-B19V IgM and IgG antibodies through an enzyme-linked immunosorbent assay (VirionSerion, Germany).

Assessment of transfusional transmission risk and statistical analysis

Statistical modeling was applied to estimate the number of blood components infected with B19V or HAV released by the Rio de Janeiro blood bank for donation between 2018 and 2019 and the risk of transfusional transmission of these viruses. The expected number of blood bags infected with B19V or HAV released by HEMORIO during 2018 and 2019 was calculated by multiplying the estimated incidence for B19V or HAV with the total number of blood bags released by HEMORIO (353,302) between the years 2018 and 2019 (180,466 and 172,836, respectively) [48]. The incidence of infected blood bags for B19V and HAV was calculated using a posterior Beta conjugated distribution derived from a Binomial model for the number of infected blood components in a sample of blood bags and a uniform prior distribution for HAV and B19V incidences. The estimated incidences were then set as the mode of the posterior distribution, and the uncertainty was represented by the 95% highest posterior density interval.

In the same way, the rate of infectious donations was estimated by multiplying the rate of infected blood bags by the rate of infectious blood bags. For B19V, according to parameters based on the literature, blood bags whose viral loads showed values > 10^4 IU/mL [16, 36] and which were negative for anti-B19V IgG were considered infectious. There is no consensus in the literature for HAV,

Table 2 Profile of serological and molecular markers of samples positive for HAV-RNA from blood donors in Rio De Janeiro

Samples ID	Year	PCR (VP1-2 A)	Viral load (IU/mL)	RNAseP (control)	Anti-HAV		Genotype
					IgM	Total	
DS49	2019	+	NDI	ND	Negative	Negative	IB
DS62	2019	+	1.58×10^5	NTV	Negative	Negative	IB

NT = Non-tested; NA = Non-applied; ND = Non-detected; NTV = Non-tested due to insufficient volume; NDI = Non-detected due to PCR inhibitors in the sample

Table 3 Profile of serological and molecular markers of samples positive for B19V-DNA from blood donors in Rio De Janeiro

Samples ID	Year	PCR (NS1)	PCR (VP1/VP2)	Viral load (IU/mL)	RNAseP (control)	Anti-B19V		Genotype
						IgM	IgG	
DS190-241	2019	NT	+	3.02×10^7	NA	+	-	1 A
DS190-346	2019	NT	+	2.60×10^3	NA	-	+	1 A
DS195-200	2019	NT	+	3.07×10^7	NTV	-	-	NDI
DS75	2019	+	+	2.84×10^5	NA	+	-	1 A

NT = Non-tested; NA = Non-applied; ND = Non-detected; NTV = Non-tested due to insufficient volume; NDI = Non-detected due to PCR inhibitors in the sample

only case reports of transmission from viral loads > 10^3 IU/mL [8]. Thus, blood bags with viral loads > 10^3 IU/mL, negative for anti-HAV, were considered infectious. In this study model, a 100% transmissibility was considered for donations that met these criteria.

All statistical analyses were performed with R software version 4.4.0 [49], and the highest posterior intervals were calculated using the HDInterval package version 0.2.4 [50].

Table 4 Evaluation of B19V infectious viral particles in serum samples through pre-treatment of the samples with endonuclease followed by qPCR

Samples ID	Without pre-treatment	With pre-treatment
	Viral load B19V (IU/mL)	Viral load (IU/mL)
DS190-241	3.02×10^7	2.70×10^7
DS190-346	2.60×10^3	ND
DS195-200	3.07×10^7	NTV
DS75	2.84×10^5	NTV

ND = Non-detected; NTV = Non-tested due to insufficient volume of the sample

Results

Detection of HAV-RNA and B19V-DNA among blood donors

A total of 1204 plasma samples from blood donors were initially screened for HAV RNA and B19V DNA by qRT-PCR and qPCR, respectively. HAV RNA was detected in two mini pools, while B19V DNA was detected in four. Each HAV or B19V-positive pool contained only one positive individual sample, which was confirmed by RT-qPCR and qPCR, respectively (Tables 1 and 2). These findings indicate an incidence of 0.17% (2/1,204) and 0.33% (4/1,204) of HAV and B19V, respectively, which corresponds to HAV and B19V incidence rates of 1.66 per 1,000 donors and 3.33 per 1,000 donors, respectively.

HAV RNA-positive samples contained a viral load of 1.58×10^5 IU/mL, and B19V DNA-positive samples contained a viral load from 2.60×10^3 to 3.07×10^7 IU/mL. The viral load of one sample positive for HAV-RNA could not be quantified due to the presence of PCR inhibitors, which were confirmed by endogenous RNAseP control (Table 2).

Detection of Hepatitis A and B19V antibodies in blood donors with PCR-positive

All B19V-DNA or HAV-RNA positive serum samples from blood donors were investigated for the presence of specific antibodies (IgM and IgG) to identify the status of the infection as acute, past, immunological window, or suggestive of persistent infection (considered only for B19V infection). The two samples, HAV RNA positive, were negative for anti-HAV antibodies (IgM and total), indicating a period of immunological window before the acute phase of the infection. Two of the four B19V DNA-positive samples were IgM positive/IgG negative, indicating acute infections. The other two samples were negative for IgM. However, one was also negative for IgG, indicating a period of immunological window, while the other was IgG positive, suggesting a persistent infection (Tables 2 and 3).

B19V infectious viral particles in serum samples

Among the four samples with viral load detected, two were evaluated regarding the presence of infectious viral particles (DS190-241 e DS190-346). The other two samples were not evaluated for the presence of infectious particles due to the insufficient volume for this complementary analysis. After treatment with endonuclease, one of the samples positive for B19V-DNA (DS190-241), showed a reduction of viral load, which suggests that part of the viral DNA was encapsidated in viral particles. For the second sample (DS190-346), the viral load became undetectable, indicating the presence of "naked DNA" and, therefore, the absence of viral particles (Table 4).

Characterization of HAV and B19V genotypes detected in blood donors

HAV genotyping was based on the prototype sequences HAS-15 (genotype IA), HAF-203, and HM-175 (genotype IB), SLF88 (genotype II A), and CF-53 (genotype II B), deposited in Genbank. All HAV isolated from blood donors' serum samples were classified as subgenotype IB (Fig. 2). All samples from this study were deposited in the GenBank database under accession numbers PP941961 to PP941962.

B19V genotyping was based on the prototype sequences for all the genotypes (1a, 1b, 2, 3a, and 3b) deposited in Genbank. All B19V isolated from serum blood samples were classified as genotype 1a (Fig. 3). All samples from this study were deposited in the GenBank database under accession numbers PP941963 to PP941965.

Sensitivity experiment

All minipools were consistently detected (Table 5), confirming that a minipool containing an individual specimen with a viral load of 4.8×10^8 IU/mL to 5.2×10^4 IU/mL would be detected by the assay used. There was no statistical difference between these two groups ($p > 0.05$).

Risk evaluation of transfusion transmission

The expected number of blood bags contaminated with HAV or B19V was calculated by the product between the rate of positive samples and the number of blood components released for use during 2018–2019 by Hemorio ($n = 353{,}302$ blood bags). Incidences of 1.66 and 3.32

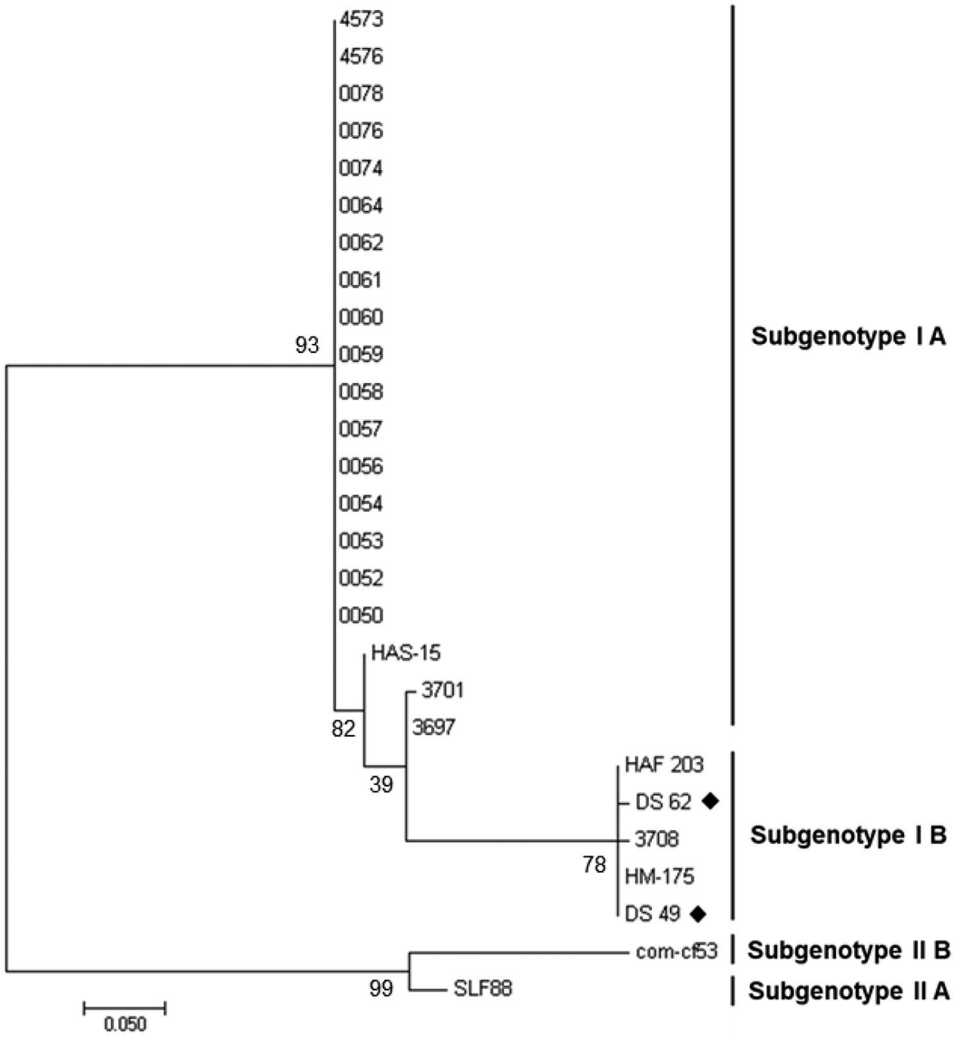

Fig. 2 Phylogenetic analysis of HAV sequences detected from blood donors. The phylogenetic tree was constructed using the Maximum Likelihood method using the Kimura-2 parameter + G model based on sequences of 247 bp of the VP1/2A region of the HAV genome. Samples with black diamonds were from this present study. (*) Sequence HAS-15 is a prototype of subgenotype IA, sequences HAF-203 and HM-175 are prototypes of the subgenotype IB, sequence CF-53 is a prototype of subgenotype IIB, and sequence SLF88 is a prototype of subgenotype IIA. DS49 equals PP941961, and DS62 is PP941962 in the GenBank database

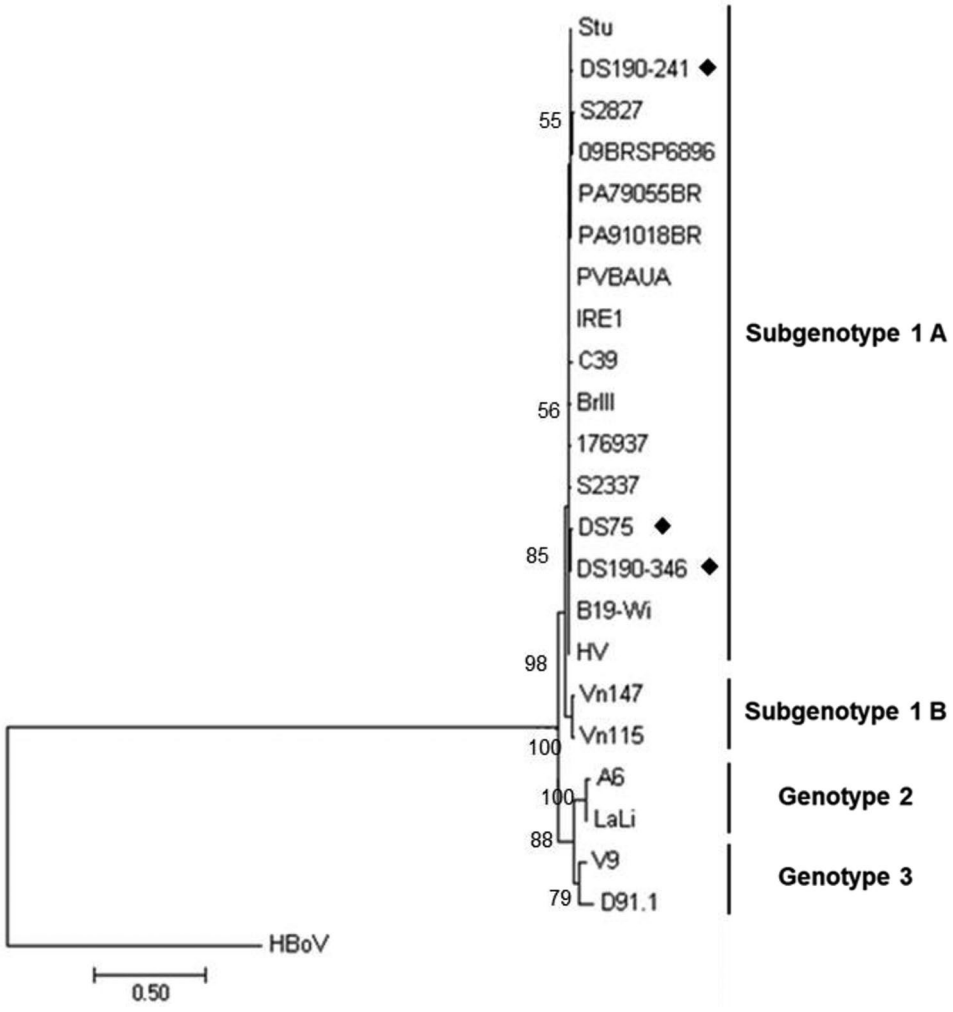

Fig. 3 Phylogenetic analysis of B19V sequences detected from blood donors. The phylogenetic tree was constructed using the Maximum Likelihood method using the Tamura-Nei + G + I model based on sequences of 476 bp of VP1/VP2 do B19V. Samples of black diamonds were from this present study. Sequences Stu, S2827, 09BRSP6896, PA79055BR, PA91018BR, PVBAUA, IRE1, C39, BrIII, 176,937, S2337, B19-Wi, HV were from subgenotype 1a prototypes. Sequences Vn147 and Vn115 were from subgenotype 1b prototypes. Sequences A6 and LaLi were from genotype 2 prototypes. Sequences V9 and D91.1 were from genotype 3 prototypes. DS190-241 equals PP941963, DS75 is PP941964, and DS190-346 is PP941965 in the GenBank database

cases per 1000 blood bags were found for HAV RNA and B19V DNA, respectively. Considering that the total number of released blood components was 353,302 in these two years, the expected numbers of components contaminated with HAV and B19V were 587 and 1,174, respectively (Table 6).

Expected number of potentially infectious blood components for HAV and B19V blood donations

Regarding B19V, three blood bags were considered infectious since they showed viral load above 10^4IU/mL and were negative for anti-B19V IgG, being one of these samples also confirmed by endonuclease treatment (DS190-241), which indicates an infectious donation rate of 3 in 1,204 (0.25%, 95% CI 0.06–0.66). For HAV, two blood bags with a viral load above 10^3 IU/mL and negative for total anti-HAV were detected, indicating an infectious donation rate of 2 in 1,204 (0.17%, 95% CI 0.03–0.53). Considering that the total number of released blood components in the period 2018–2019 was 353,302 and that the infectious donation rate of HAV-RNA and B19V DNA were 0.17% and 0.25%, respectively, we estimated that 587 (95% CI 90-1880) blood bags were potentially transmissible for HAV and 880 (95% CI 210–2334) bags were potentially transmissible for B19V (Table 6).

Discussion

In this study, a retrospective analysis of plasma samples was obtained between July 2018 and December 2019 in the Rio de Janeiro Blood Bank, and the incidence of HAV RNA was 1.66 per 1,000 donors. Although few data are available regarding the incidence of HAV RNA in blood donors worldwide, the prevalence rate observed in this study was higher than those observed, for example,

Table 5 Profile of serological and molecular markers of positive samples for B19V-DNA from blood donors in Rio De Janeiro

Sample dilution	Single		Pool	
	Cq	Viral load (IU/mL)	Cq	Viral load (IU/mL)
10 – 1	19.2	4.8×10^{8}	21.1	1.6×10^{8}
10 – 2	22.8	6.0×10^{7}	24.7	2.0×10^{7}
10 – 3	26.6	6.9×10^{6}	27.8	3.5×10^{6}
10 – 4	28.7	2.0×10^{6}	30.3	8.6×10^{5}
10 – 5	28.2	2.9×10^{6}	30.4	7.8×10^{5}
10 – 6	30.0	9.9×10^{5}	31.6	3.9×10^{5}
10 – 7	35.2	5.2×10^{4}	36.9	1.9×10^{4}
10 – 8	36.6	2.3×10^{4}	ND	ND
10 – 9	36.8	2.1×10^{4}	ND	ND
10–10	ND	ND	ND	ND
10–11	37.0	1.8×10^{4}	ND	ND
10–12	ND	ND	ND	ND

Cq = Cycle of quantification; IU/mL: International units per milliliter; ND: non-detected

Table 6 Estimated number of plasma bags contaminated with HAV and B19V in blood donors during 2018–2019

Period (Years)	Plasma bags	
	N	Mode (95% HPD)
Geral (2018 e 2019)		
HAV		
Total	1.204	-
PCR-Positive (per 1000 plasma bags)	2	1.66 (0.25–5.47)
Expected number of contaminated bags		587 (92-1932)
B19V		
Total	1.204	-
PCR-Positive (per 1000 plasma bags)	4	3.32 (1.00-7.81)
Expected number of contaminated bags		1,174 (355–2759)

in USA blood banks, whose prevalence rates ranged from 0% (0/100,360) in fresh frozen plasma to 0.005% (6/1,297,945) in whole blood bags [51]. In China, recent findings showed an incidence of 0% (0/5,030 and 0/728) in blood donors [1]. These differences may be due to the different sample sizes evaluated, variations in study conditions, and the demographic and epidemiologic characteristics of the population.

There is no data on HAV RNA incidence in blood donors in Brazil. The higher percentage found in this study may be justified by the period in which the samples were obtained, as outbreaks of hepatitis A were reported in neighborhoods of Rio de Janeiro between 2017 and 2018. Similarly, Gallian et al. [52] recently presented similar data in a study conducted in France in 2018, which reported an increase in the prevalence of HAV RNA in blood donors from 0.86/106 to 4.5/106 donors following an outbreak of hepatitis A in the country.

Of the HAV RNA-positive individuals blood donors, 75% were male and between 30 and 60 years old. According to the Brazilian viral hepatitis epidemiological bulletin for 2018, 68.2% of hepatitis A cases in the southeastern region of Brazil occurred in men aged between 20 and 39 years [53]. In 2018, Amado et al. demonstrated an outbreak of hepatitis A in Rio de Janeiro, with a higher incidence also in men aged between 20 and 29 years [54]. This increase in men, particularly in this age group, has also been reported in other countries, especially in men who have sex with men [55, 56].

In a clinically asymptomatic person, a high HAV load (1.58×10^{5} copies/mL) was found in combination with the absence of anti-HAV antibodies (IgM and total). After HAV infection, viremia peaks 1 to 20 days before ALT peaks and decreases during and after the icteric phase. HAV RNA is detectable in the icteric phase and becomes undetectable 2–6 months later, while IgG antibodies are first detected in the recovery phase and remain detectable for life. Thus, the presence of HAV RNA in the absence of anti-HAV IgM and IgG in the blood indicates a recent HAV infection, whose viral incubation period which lasts on average 28 days and precedes the symptoms [57]. Although it was not possible to detect the HAV load of the other positive donor, the absence of anti-HAV antibodies also indicates a period of the immunological window. This data raises concerns because the long incubation period of HAV is a factor that can influence the risk of transfusion transmission, as donors may have the virus circulating in their blood at the time of donation and are unaware of this condition.

The incidence of B19V DNA in blood donors found in the present study (0.33%) was similar to the one from a previous study conducted in central-west Brazil, which showed a prevalence of 0.26% [33]. However, a higher prevalence was reported in Southeast (1.0%, 1/91) and in Southern Brazil (1.9%, 9/480) in peripheral blood samples from volunteer blood donors by the same group [34]. These different rates of B19V DNA in Brazil probably reflect the great diversity of geographical regions due to the large size of the country, seasonal variations of the viral circulation, and/or a possible ongoing B19V outbreak in the state of Rio Grande do Sul, as hypothesized by the authors, since some clusters of B19V isolated appeared in the phylogenetic tree.

Nonetheless, the prevalence of B19V was found to be higher in Brazil compared to countries like Japan (0.01%) [58], Austria (0.26%) [27], and The Netherlands (1/30829 donations in 2003–1/10319 donations in 2004) [59], which routinely screen donated plasma for B19V DNA to exclude donations with a high B19V load before fractionation. This highlights the need to review the surveillance measures for Parvovirus B19 among blood banks in Brazil. B19V DNA prevalence was also higher than in Korea (0.1%) [60], China (0.06-0.58%) [1, 61], Denmark (0.006%) [58], and Portugal (0.18%) [62]. These differences could be due to geographic or seasonal differences in B19V circulation.

Three highly viremic (10^5 and 10^7IU/mL), recently infected, B19V IgG-negative blood donors were detected in this present study. Regarding infectivity, blood products and plasma derivatives prepared from pools with B19V DNA levels below 10^4IU/mL are safe [63]. Therefore, these samples were potentially infectious. In one of these samples, it was possible to perform pre-treatment with endonuclease to evaluate the infectious potential of such samples. The result obtained was corroborated with the hypothesis of acute infection due to the slight reduction in viral load from 3.02×10^7IU/mL to 2.70×10^7IU/mL, showing that this value is due to the presence of infectious particles resulting from active viral replication and indicating the transmissive potential of B19V from this positive blood donor. The other B19V DNA-positive sample was low viremic and B19V IgG-positive. With the pre-treatment with endonuclease of these samples, it was possible to observe a reduction in viral load from 10^3IU/mL to a non-detectable load. This result indicates that the DNA detected was obtained from "naked DNA", indicating the absence of infectious viral particles, suggesting a persistent infection, and ruling out the transmissive potential of this sample. In 2017, Reber et al. [64] also demonstrated the usefulness of this assay for the correct interpretation of diagnosis of persistent infection with B19V. Other authors have also demonstrated persistent low B19V DNA in blood donors [63]. However, although the presence of a very low persistent viral load in the blood donations seems not to affect the process of plasma manufacturing as usually, the presence of anti-B19V IgG seems to neutralize the virus, B19V infection can have serious consequences depending on the clinical condition of the patients (or their anti-B19V IgG status) treated with hemoderivatives.

Based on the incidence of HAV-RNA observed in this study, we estimated 587 contaminated blood components within the total number of bags released for clinical use (n = 353,302) during the studied period (2018–2019), using statistical modeling. This number was the same for blood components with transmissibility potential parameters adopted in this study. There is no consensus about the HAV viral load cut-off for defining the blood product infectivity threshold. Case reports indicate that relatively low viral loads may be capable of infecting recipients, even generating severe conditions, depending on the health conditions of these individuals [7].

The incidence of B19V DNA observed in this study (0.33%) reflects 1,174 potentially contaminated blood components from 2018 to 2019. However, considering the rate of infectious donations, 880 blood bags with B19V transmissive potential were estimated for this period. The adoption of selective screening measures for the B19V in blood products, especially during periods of infectious erythema epidemics [19], and/or for use in patients with hematological diseases like sickle cell disease and beta-thalassemia, where the B19V infection can be fatal and evolve to extensive bone marrow necrosis.

The limitation of the study was the insufficient volume of serum samples for serological tests and the assessment of infectivity in all cases.

This study originally assessed the incidence of both Hepatitis A Virus (HAV) and Parvovirus B19 (B19V) among Brazilian blood donors, as well as the potential risk of residual transmission of these infections through blood transfusions. Our findings can contribute to future cost-effective studies aimed at implementing screening methods for these viruses, which could enhance discussion on surveillance in blood banks and protective measures for blood recipients.

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Author contributions
A.D.R.A. and L.A.A. were involved in the conception and design of this study and in the analysis and interpretation of the data. A.D.R.A., M.M., A.C.S.d.M., J.I.F.L., and C.A.d.C.S. were involved in performing the tests. C.M. and L.B. perform the statistical analysis. All authors were involved in the drafting of the paper and agreed to be accountable for all aspects of the work. All authors have read and agreed to the published version of the manuscript.

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Data availability
All positive samples from this study were deposited in the GenBank database under accession number PP941961 to PP941965.

Declarations

Ethics approval and consent to participate
This study included blood donors who donated at HEMORIO's blood therapy service and were negative for parenterally transmitted infectious diseases routinely tested in Brazil (HIV-1 + 2, HCV, HBV, HTLV-I/II, syphilis, and Chagas disease). The study protocol was approved by the Ethics Committee of Oswaldo Cruz Institute (CAAE: 48376015.2.0000.5248, number: 1.610.747).

Competing interests
The authors declare no competing interests.

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