Genotype 1 of human parvovirus B19 in clinical cases

Maria Isabel de Oliveira[1]*, Ana Maria Sardinha Afonso[2], Suely Pires Curti[1], Patrícia Evelin Silva[2], Tamyris Fernanda Barbosa[2], Elian Reis Silva Junior[2], Cristina Adelaide Figueiredo[1]

[1]PhD, Scientific Researcher, Núcleo de Doenças Respiratórias, Instituto Adolfo Lutz, São Paulo, SP, Brazil
[2]MSc Student, Núcleo de Doenças Respiratórias, Instituto Adolfo Lutz, São Paulo, SP, Brazil

Study conducted at Núcleo de Doenças Respiratórias, Instituto Adolfo Lutz, São Paulo, SP, Brazil

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***Correspondence:**
Instituto Adolfo Lutz,
Núcleo de Doenças Respiratórias
Address: Av. Dr. Arnaldo, 355
São Paulo, SP – Brazil
Postal code: 01246-902
olive40@uol.com.br

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Summary

Introduction: Virus surveillance strategies and genetic characterization of human parvovirus B19 (B19V) are important tools for regional and global control of viral outbreak. In São Paulo, Brazil, we performed a study of B19V by monitoring the spread of this virus, which is an infectious agent and could be mistakenly reported as a rash and other types of infection.
Method: Serum samples were subjected to enzyme immunoassay, real time polymerase chain reaction, and sequencing.
Results: From the 462 patients with suspected cases of exanthematic infections, the results of the 164 serum samples were positive for B19V immunoglobulin M. Among these cases, there were 38 patients with erythema infections and B19--associated with other infections such as encephalitis, hydrops fetalis, chronic anemia, hematological malignancies. These samples were sequenced and identified as genotype 1.
Conclusion: This study showed patients with infections caused by B19V and sequencing genotype 1. Continuous monitoring is necessary to detect all known genotypes, and the emergence of new genotypes of these viruses for case management in public health control activities.

Keywords: human parvovirus B19, B19V genotype, surveillance.

Introduction

Human parvovirus B19 (B19V) is a member of the Parvoviridae family. It is further classified into the subfamily Parvovirinae, genus *Erythrovirus*, and species *Parvovirus* B19. B19V causes erythema infectiosum, a clinical form most commonly seen in children and adolescents, although it may occur at any age in pregnant women, causing serious or even fatal complications to the fetus. Damage in the erythroid progenitor cells caused by B19V can result in severe anemia, particularly in immunocompromised patients. In addition, B19V infection has been associated with many clinical symptoms, such as neurological and myocardial infections, among others.[1-3] B19V infection is often associated with fever and rash, and many times can be mistakenly reported as measles, rubella or other very common exanthema infections.[4]

Based on the phylogenetic analysis of the B19V, it was proposed into three distinct genotypes: 1; 2; and 3 groups. To determine which genotypes of B19V are present in São Paulo city, clinical samples were analyzed, and carried out the genetic characterization of the circulating strains causing exanthematic diseases. That are important tools for regional and global control of viral outbreak.

Method

This study was conducted between 2009 and 2012 at Adolfo Lutz Institute, São Paulo, Brazil, with surveillance specimens collected from 462 patients with suspected B19V. The ages of the patients ranged from 1 month to 55 years of age. Blood samples were collected from individuals with erythema infectiosum, and B19-associated conditions such as encephalitis, hydrops fetalis, severe and chronic anemia, hematological malignancies, and aplastic anemia. Samples were tested using the B19V immunoassay kit and specific immunoglobulin M and G for identification of the VP2 protein (Biotrin International Ltd., Dublin, Ireland). The same samples were processed by real time qPCR (quantitative polymerase chain reaction)

and probes were designed to sequence the fragment region of the *NS1-VP1* gene, encoding for the B19V non-structural protein (position 1399 to 1659), as previously described by Takano & Yamada,[6] using QIAamp® DNA Blood Mini Kit (Qiagen, Hilden, Germany). The samples were sequenced by nested-PCR using primers described by Servants.[5] The amplified products were purified with PureLink PCR Purification Kit (Invitrogen/Life Technologies, Carlsbad, CA) and sequenced using the ABI Prism Big Dye Terminator v3.1 Cycle Sequencing Kits (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol. The sequences obtained were analyzed by CLUSTAL X and BioEdit version 7.0 phylogenetic analysis applications. The sequence generated from the erythrovirus HPV B19 sample was submitted to Blast searches in the GenBank accession numbers, sample from, locations, isolation years, and genotype of the sequences utilized as presented in Table 1. Alignment was used with prototype sequences of genotype 1: strain Pvbua (Acc.Number M13178), genotype 2: prototype strain Lali (Acc. Number AY044266), and genotype 3: prototype strain V9 (Acc. Number AY647977) by the Clustal X program (http://www-igbmc.u-strasbg.fr/BioInfo/ClustalX/) and analyzed by the Megalign, DNAstar, Inc. software.

Results

In order to detect the presence of B19V, an immunoassay of the 462 suspected cases of exanthematic infections was done: 164 (36%) serum samples were positive for B19V IgM and 298 (64%) were positive for B19V IgG. Of the 498 samples analyzed by qPCR, 148 (32%) were found to be positive for B19V protein expression. The infection was equally distributed between male and female patients under the age of 20.

Among the positive samples, 38 with erythema infection and B19-associated were sequenced. The results of the immunoassay (IgG and IgM antibodies) showed 21 samples were positive for both IgM/IgG antibodies, six were negatives IgM/IgG antibodies, seven were positive for IgG antibodies only, and one was positive for IgM only (Table 1). Among the three samples collected from the cerebrospinal fluid (CSF), one was positive while the remaining two were negative for both IgM/IgG antibodies. The 15 samples clinically negative for IgM and/or IgG antibodies, but positive for the same according to DNA analysis, were collected from patients within 3-5 days after the onset of symptoms. In total, 38 sequences were obtained (eight from samples collected in 2009, 18 from 2010, eight from 2011, and four from 2012). All 38 sequences belonged to genotype1 of B19V (Figure 1). A comparison of the nucleotide sequences of the different strains showed a high level of identity. Nucleotide pairwise identity among the different strains ranged from 97 to 99.1% in genotype 1, 86.8 to 88% in genotype 2, and 87.2 to 88.5% in genotype 3. The sequences within genotype 1 were found to be similar (statistical deviation within group, 1.7%).

Discussion

In our study, we observed that 36% of the serum samples were positive for B19V IgM and 64% were positive for B19V IgG. In the article described by Candotti et al.,[7] the percentage of positivity for IgG and IgM was similar to our findings. Clinical symptoms of B19 infection in 39.8% of the patients exhibiting B19-specific IgM were found in patients from Rio de Janeiro and the same results were obtained.[8]

B19V infection causes a benign disease, and neurological complications have been seen but are not usually investigated in encephalitis.[3] Among the cases where the genotype was analyzed, three cases of encephalitis were observed. This was particularly evident in a young man, from whose B19V from CSF was amplified and showed genotype 1.

The incidence of acute B19V infection in pregnancy is approximately 1-2% during the endemic period. However, during the time of an epidemic, the infection rate may rise to > 10%.[9] The peak incidence of B19V-associated hydrops fetalis is between 17 and 24 weeks of gestation.[10,11] Testing maternal serum for IgM/IgG antibodies against parvovirus B19, and DNA detection by PCR can confirm maternal infection. Among the total number of samples analyzed in our study, three were diagnosed with hydrops fetalis, and all three were positive for infection by parvovirus B19. The rapid correction of anemia by in uterus transfusion of packed erythrocytes largely prevents fetal death. Among the cases analyzed, the majority were associated with hematological disorders. Patients with hemoglobinopathies can be vulnerable to B19V persistence due to shortened red blood cell survival.[12] These persistent infections have been reported to be associated with chronic anemia, thrombocytopenia and pancytopenia, arthralgia, or arthritis.[13] Our results showed high positivity for B19V infection from IgM/G assay and qPCR, in patients with hematological malignancy (Table1).

Our findings are consistent with the results reported by other groups. Studies have reported high positivity for B19V IgG in hematological malignancy cases, aplastic anemia, and chronic hematological disorders.[14,15] These results are relevant when clinical symptoms are first observed in conditions with a more prolonged evolution of disease with no specific etiological diagnosis and where the disease may be identified by the presence of IgG.

TABLE 1 Characteristics of B19V subjected to genetical analyses.

Case no.	GeneBank	Symptoms	Month/ Year	Specimen collection	Age (days/ years)	Sex	Serology		RT-PCR	Group
							IgG	IgM		
1	JX 559664	Hydrops fetalis	01/2009	Sera	1	M	Pos	Neg	Pos	1
2	JX 559665	Erythema infectiosum	02/2009	Sera	14	F	Pos	Pos	Pos	1
3	JX 559661	Transient anemia	08/2009	Sera	14	M	Pos	Pos	Pos	1
4	JX 559660	Aplastic anemia	09/2009	Sera	28	F	Neg	Neg	Pos	1
5	JX 559656	Severe anemia	10/2009	Sera	23	F	Pos	Pos	Pos	1
6	JX 559654	Hydrops fetalis	12/2009	Sera	7 D	F	Pos	Pos	Pos	1
7	JX 559658	Transient anemia	12/2009	Sera	2	M	Neg	Neg	Pos	1
8	JX 559652	Chronic anemia	12/2009	Sera	40	F	Neg	Neg	Pos	1
9	JX 559673	Severe anemia	01/2010	Sera	34	F	Neg	Neg	Pos	1
10	JX 559663	Encephalitis	01/2010	CSF	47	M	Pos	Pos	Pos	1
11	JX 559655	Aplastic anemia	01/2010	Sera	20	M	Pos	Pos	Pos	1
12	JX 559653	Erythema infectiosum	01/2010	Sera	25	F	Pos	Pos	Pos	1
13	JX 559659	Hydrops fetalis	01/2010	Sera	3 D	M	Pos	Pos	Pos	1
14	JX 465351	Erythema infectiosum	01/2010	Sera	20	M	Pos	Pos	Pos	1
15	JX 559657	Encephalitis	02/2010	CSF	26	F	Neg	Neg	Pos	1
16	JX 267259	Encephalitis	03/2010	CSF	2	M	Neg	Neg	Pos	1
17	JX 559662	Erythema infectiosum	04/2010	Sera	45	F	Neg	Neg	Pos	1
18	JX 267261	Severe anemia	05/2010	Sera	55	M	Pos	Neg	Pos	1
19	JX 559650	Severe anemia	05/2010	Sera	7	M	Pos	Neg	Pos	1
20	JX 559651	Chronic anemia	06/2010	Sera	30	F	Pos	Neg	Pos	1
21	JX 267260	Chronic anemia	06/2010	Sera	19	M	Pos	Pos	Pos	1
22	JX 267257	Aplastic anemia	06/2010	Sera	19	M	Pos	Neg	Pos	1
23	JX 559674	Severe anemia	08/2010	Sera	2	F	Pos	Neg	Pos	1
24	JX 559648	Hematological malignancies	08/2010	Sera	20	M	Pos	Pos	Pos	1
25	JX 559649	Severe anemia	08/2010	Sera	4	F	Pos	Pos	Pos	1
26	JX 267258	Erythema infectiosum	09/2010	Sera	1	M	Pos	Neg	Pos	1
27	JX 267252	Erythema infectiosum	04/2011	Sera	18	M	Pos	Pos	Pos	1
28	JX 267254	Transient anemia	04/2011	Sera	6	F	Neg	Neg	Pos	1
29	JX 267256	Erythema infectiosum	07/2011	Sera	8	M	Pos	Pos	Pos	1
30	JX 267255	Severe anemia	07/2011	Sera	8	M	Pos	Pos	Pos	1
31	JX 267253	Severe anemia	07/2011	Sera	9	F	Pos	Pos	Pos	1
32	JX 559670	Erythema infectiosum	09/2011	Sera	5	M	Pos	Pos	Pos	1
33	JX 559669	Severe anemia	10/2011	Sera	5	F	Neg	Pos	Pos	1
34	JX 559668	Severe anemia	11/2011	Sera	9	F	Pos	Pos	Pos	1
35	JX 559667	Erythema infectiosum	01/2012	Sera	12	M	Pos	Pos	Pos	1
36	JX 559666	Erythema infectiosum	01/2012	Sera	5	F	Pos	Pos	Pos	1
37	JX 559671	Chronic anemia	01/2012	Sera	50	F	Pos	Pos	Pos	1
38	JX 559672	Severe anemia	03/2012	Sera	21	F	Pos	Pos	Pos	1

CSF: cerebrospinal fluid; Pos: positive; Neg: negative; RT-PCR: reverse transcription-polymerase chain reaction.

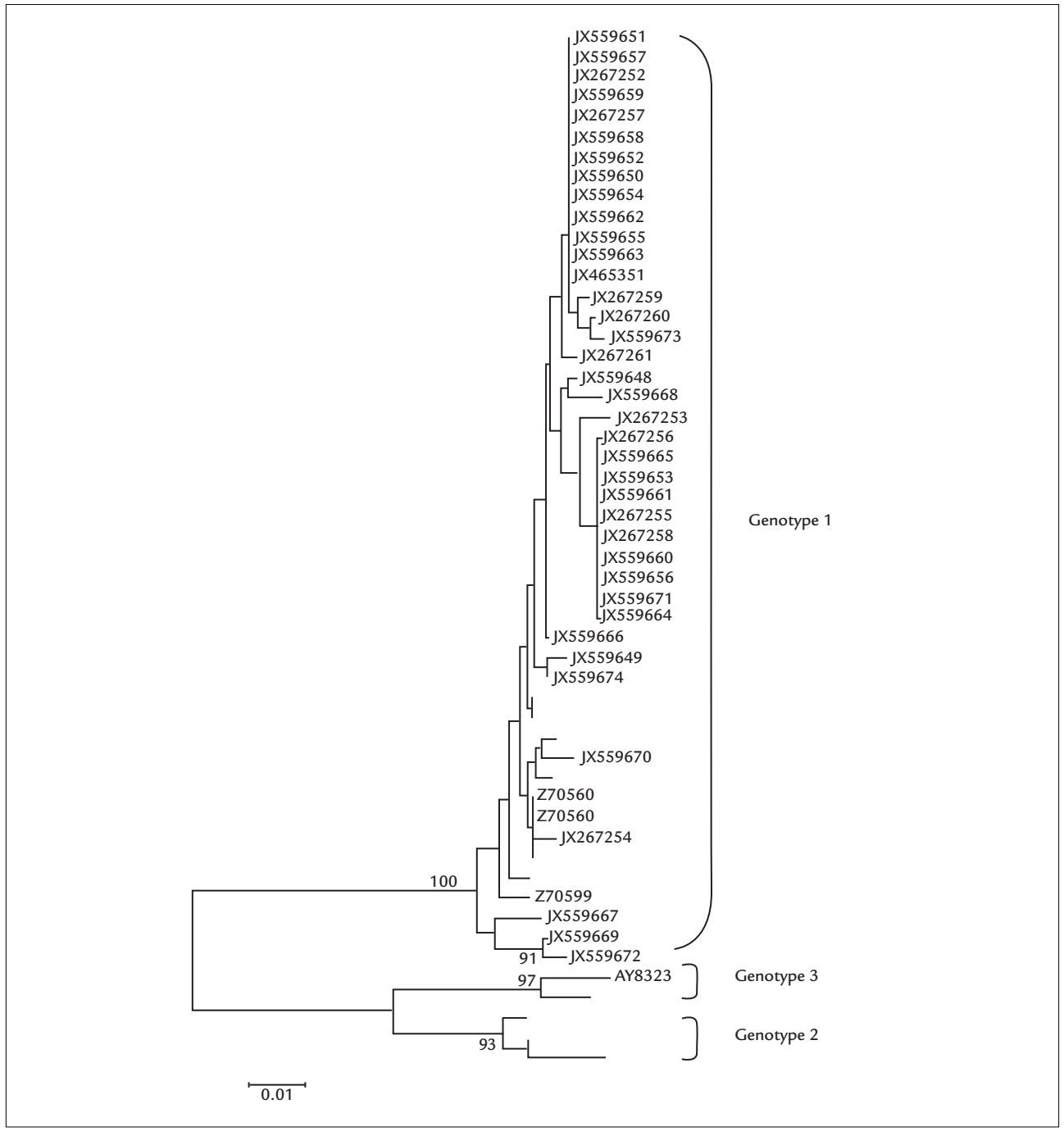

FIGURE 1 Phylogenetic relationships of the nucleotide sequences of B19V strains circulating São Paulo, Brazil, 2009–2012. The tree constructed with the NJ method was evaluated by the bootstrap method with 1,000 replications using MEGA package version 4.0.1.

Currently, there are three B19V genotypes with associated subtypes, genotype 1a, 1b, 2, 3a, and 3b.[16] The genotypes show ~10% nucleotide divergence on the whole genome.[5] The most prevalent B19V genotype in Northern Europe is genotype 1a. Genotype 2 appears to be restricted to people born before 1970, and genotype 3 predominantly occurs in people from West Africa, France and Brazil.[17] Some studies have shown that B19V genetic diversity may depend on the geographical location and the year of isolation in patients. In this study, all isolates belonged to genotype 1.[17] In Brazil, all three genotypes have been identified, although genotype 1 is the most common.[14] Genotype 1 has also been isolated in Vietnam, with a higher frequency of occurrence in children and adults.[18]

In addition, genotype 1 is predominant in the Midwest, South and Southeast regions of Brazil.[19,20]

This work shows the presence of B19V genotype 1. Continuous monitoring is necessary to detect all known genotypes, and the emergence of new genotypes related to infection as the cause of virus spread.

Conflict of interest

The authors declare no conflict of interest.

Resumo

Genótipo 1 do parvovírus humano em casos clínicos

Introdução: Estratégias de vigilância para o parvovírus humano B19 e caracterização genética são ferramentas importantes para o controle regional e global do surto viral. Em São Paulo, Brasil, foi realizado um estudo de parvovírus B19, monitorando a disseminação desse vírus, que é um agente infeccioso e poderia ser erroneamente relatado como uma erupção cutânea e outros tipos de infecções.
Método: As amostras de soro foram submetidas ao ensaio imunoenzimático, PCR quantitativo em tempo real e sequenciamento.
Resultados: Dos 462 pacientes com casos suspeitos de infecções exantemáticas, os resultados das 164 amostras de soro foram positivos para parvovírus B19 imunoglobulina M. Entre eles, 38 pacientes com eritema infeccioso apresentaram B19 associado com outras infecções, como encefalite, hidropisia fetal, anemia crônica, doenças hematológicas malignas. Essas amostras foram sequenciadas e identificadas como genótipo 1.
Conclusão: Os pacientes foram infectados com parvovírus B19 e apresentaram genótipo 1. Monitoração contínua é necessária para detectar todos os genótipos conhecidos e o surgimento de novos genótipos para o controle de casos em saúde pública.

Palavras-chave: parvovírus humano B19, genótipo B19V, vigilância.

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