Revista da Sociedade Brasileira de Medicina Tropical
Journal of the Brazilian Society of Tropical Medicine
Vol.:52:e20190304: 2019
doi: 10.1590/0037-8682-0304-2019

Major Article

Human parvovirus B19 genotype 1 in suspected dengue patients of Tefé, Amazonas State, Brazil

***Regina Maria Pinto de Figueiredo*[1], *Victor Costa de Souza*[2], *Valdinete Alves do* Nascimento[2] *and Felipe Gomes* Naveca[2]**

[1]. Fundação de Medicina Tropical Doutor Heitor Vieira Dourado, Gerência de Virologia, Manaus, AM, Brasil.
[2]. Fundação Oswaldo Cruz Amazônia, Instituto Leônidas e Maria Deane, Manaus, AM, Brasil.

Abstract

Introduction: Human parvovirus B19 (B19V) is a common pathogen, which on infection causes variety of clinical conditions from benign self-limiting exanthematous disease and other similar pathologies to fetal death. **Methods:** We collected 341 serum samples between the first and fourth day after the onset of symptoms from all patients suspected of dengue fever who were attended at Regional Hospital of Tefé. Initially, patients were screened for malaria by blood smear test and negative samples were sent to Fundação de Medicina Tropical Doutor Heitor Vieira Dourado (FMT-HVD) situated in Manaus (AM) for dengue testing using semi-nested multiplex PCR. Further, we investigated 44 malaria and dengue-negative samples of children for B19V DNA by nested-PCR. Positive samples were analyzed by BLAST against entire public non-redundant nucleotide database and genotyped by phylogenetic analyses using neighbor-joining clustering method. **Results:** Eight samples (18.2%) were found to be PCR positive. Fever, headache, ocular pain, and/or muscle pain were reported as the most frequent symptoms by the patients and none were diagnosed with rash at the time of sample collection. Phylogenetic analysis of major capsid protein 2 (*VP2*) and *VP3* coding region showed high similarity with B19V genotype 1. **Conclusions:** Our results reveal the spread of B19V genotype 1 in Tefé. Moreover, our results emphasize the significance of laboratorial differential diagnosis using molecular techniques in patients with acute febrile, and thereby aid the health surveillance system in improving patient care even in the remote areas of Amazon.

Keywords: Acute febrile syndrome. Human parvovirus B19. Molecular detection. Amazonas State. Brazil.

INTRODUCTION

First discovered in 1975[1], B19V is a common human pathogen responsible for many diseases ranging from benign childhood condition such as erythema infectiosum, also known as fifth disease[2] to fetal death[3]. B19V is a single-stranded DNA virus which belongs to the *Parvoviridae* family, genus *Erythrovirus* and is predominantly transmitted via respiratory secretions such as saliva, sputum, or nasal mucus. The virus is recognized by only one serotype which can be further divided into three genotypes with relevant biological properties. B19V prototype-like isolates are known as genotype 1, LaLi and A6 variants as genotype 2, while V9 and D91.1-like isolates are grouped as genotype 3[4,5].

Corresponding Author: Dra. Regina Maria Pinto de Figueiredo.
e-mail: figueiredormp@yahoo.com.br
Orcid: 0000-0003-0222-1934
Received 19 June 2019
Accepted 02 August 2019

At present, B19V genotype 1 is predominantly found worldwide[5]. On the other hand, genotype 2 is nearly extinct and can only be found in subjects born before 1972[6]. Genotype 3 is prevalent in West Africa, and occasionally detected in France and United States[5,7]. Furthermore, all the three genotypes have already been identified in Brazil[8].

Many viral agents such as rubella virus, measles virus, and arboviruses like dengue usually cause symptoms similar to B19V infection which includes rash and joint pain[3,9,10,11]. However, it is unknown what percentage of acute febrile illness cases among children from arboviral endemic areas may be due to B19V infection. Accordingly, during the first dengue epidemics in Manaus, Amazonas State of Brazil; IgM antibodies against B19V were detected in the serum of patients with skin rash but negative for dengue[12]. Thus, laboratorial validation is critical for precise diagnosis of patients suffering from acute febrile illness with rash, particularly when multiple viruses are reported to co-circulate in the same region.

In this study, we investigated B19V infection in children with acute febrile illness that were negative for malaria and dengue, and belonged to a mid-size city, Tefé, in the Amazonas state of Brazil. Furthermore, we performed molecular characterization of the B19V in order to determine the genotypes that are in circulation in Tefé.

METHODS

Study Area

The city of Tefé (03° 21' 14" S; 64° 42' 39" W) is located 523 km from Manaus with approximately 62,000 inhabitants of which about 88% reside in urban areas. Routinely, a considerable number of people migrate from nine other different municipalities to Tefé for work, commerce, and further travel purposes. Tefé is the main fishing port in the Amazonas State region and is an important route in the Solimões river during transport. The busiest airport of this region is also located in this city[13] (https://cidades.ibge.gov.br/brasil/am/tefe/panorama).

Sample collection, molecular testing, and sequence analysis

Patients enrolled in this study had been attended at Regional Hospital of Tefé (HRT) between January and July 2013 and were initially evaluated for malaria by blood smear test. All 341 malaria-negative serum samples from patients aging from six months to 83 years old were collected between the first and fourth day after the onset of symptoms. Further, the serum samples were transferred to the reference hospital for infectious diseases Fundação de Medicina Tropical - Dr. Heitor Vieira Dourado (FMT-HVD) situated in Manaus, the capital of the Amazonas State.

At FMT-HVD, RNA was extracted from the samples using QIAamp viral RNA Mini-Kit (Qiagen, Hilden, Germany) followed by semi-nested multiplex PCR protocol for DENV RNA detection[14]. Furthermore, all 44 samples belonging to children between 1 year and 15 years old that were found to be negative for malaria and dengue were assessed for B19V DNA. Total nucleic-acid was isolated from the samples using DNA PureLink viral RNA/DNA Mini-Kit (Invitrogen, Carlsbad, CA, USA) followed by nested-PCR protocol for the detection of B19V DNA[15]. Briefly, the first PCR reactions was performed with partial major capsid protein 1 (*PVP1*) (5′- ACAAGCCTGGGCAAGTTAGC-3′) as forward primer and *PVP2* (5′- CTGCACCAGTGCTGGCTTCT-3′) as reverse primer. Further, 10% (by volume) of the first PCR reaction product was used for the nested-PCR reaction with primers, PVP2 and PVP3 (5′- TGGGCCTGGCAATGAGCTAC-3′). All nested-PCR products were electrophoresed on agarose gel with ethidium bromide and visualized under UV light. Amplicons were precipitated with solution containing 20% w/v polyethylene glycol (PEG) 8000 (Promega, WI, USA) and 2.5 M NaCl as described by Lis and Schleif [16]. Nucleotide sequencing was performed using the same primers of the nested-PCR reaction and BigDye v3.1 (Applied Biosystems, USA) at the genomics platform of Instituto Leônidas e Maria Deane (ILMD), Fiocruz Amazônia.

All trace files were inspected for quality, trimmed for primer removal, and used for contig assembly using human parvovirus B19 RefSeq Genome (NC_000883.2) as reference. BLAST analysis was performed on the consensus nucleotide sequence obtained from the patient samples of Tefé (Tefé samples)[17] using MegaBLAST algorithm against entire non-redundant nucleotide collection (nr/nt) of GenBank + EMBL + DDBJ + PDB + RefSeq sequences with few exclusions.

Further, the final nucleotide sequence of each sample in this study was aligned using MAFFT v7.222[18] representing each B19V genotype (genotype 1 or G1, 24 sequences; genotype 2 or G2, 7 sequences; genotype 3 or G3, 15 sequences) and a simian parvovirus sequence (U26342) as negative control. Lastly, this dataset was used for viral genotyping based on phylogenetic analysis using neighbor-joining clustering method and 2,000 bootstrap replicates. All bioinformatic analyses were performed with Geneious software version 9.1.5[19]. The final edition of the phylogenetic tree that was made using FigTree v1.4.3 (http:// bio.ed.ac.uk/software/figtree/) and GIMP v2.8.10 (www.gimp.org).

Ethics

Written informed consent was obtained from all patients or their guardians, and their personal data was anonymized. The study was approved by the ethics committee of FMT-HVD under the registration number 118.411.

RESULTS

Among the 44 samples evaluated for B19V, eight (18.2%) samples that were collected between March and July 2013 were found to be positive by nested-PCR. The samples belonged to patients who primarily reported symptoms such as fever, headache, ocular pain, and/or muscle pain; and none of them were diagnosed with rash at the time of sample collection (**Table 1**). The pediatric patients belong to the age group of 6 to 14 years and all of them residing in Tefé with no travel history within 15 days before the onset of the symptoms.

After performing quality trimming and primer removal, a fragment consisting of 285 nucleotides from each sequence was analyzed. Among the B19V nucleotide sequences of Tefé, seven out of eight were found to be identical. TF195 was an exception which had an adenine instead of cytosine (silent mutation) at position 3,047 when compared to the B19V reference sequence (NC_000883). BLAST analysis returned 22 identical sequences (query cover = 100%, identity = 100%). Among the obtained sequences, 15 sequences corresponded to Brazilian patients with leukemia between 2007 and 2010[20] while one was from a fatal case of a 12 year-old boy[21]. One sequence was from a sample collected in USA in 1994 from a dead fetus[22]. We could not find complete records for the other six sequences.

Further, all Tefé sample sequences were aligned with other 46 B19V sequences available at GenBank representing the three genotypes and one simian parvovirus sequence, strain B20 obtained from *Macaca fascicularis*, that was used as an outgroup. Phylogenetic analyses showed that all eight sequences from Tefé samples belonged to genotype 1 (**Figure 1**).

TABLE 1: Clinical characteristics and demographics of B19V-positive patients

Sample ID	Gender	Age (years)	Symptoms Onset	Fever	Headache	Ocular pain	Muscle pain	Vomit
TF111	Male	14	2013-03-20	Yes	Yes	Yes	Yes	No
TF119	Female	14	2013-03-31	Yes	Yes	No	Yes	No
TF175	Female	13	2013-05-20	Yes	Yes	No	No	No
TF195	Male	14	2013-04-20	Yes	Yes	Yes	Yes	No
TF230	Male	7	2013-06-03	Yes	Yes	Yes	No	No
TF 231	Female	7	2013-06-06	Yes	No	No	No	Yes
TF 235	Female	6	2013-06-03	Yes	No	No	No	No
TF311	Male	8	2013-07-06	Yes	NI	NI	NI	NI

TF: Tefé.

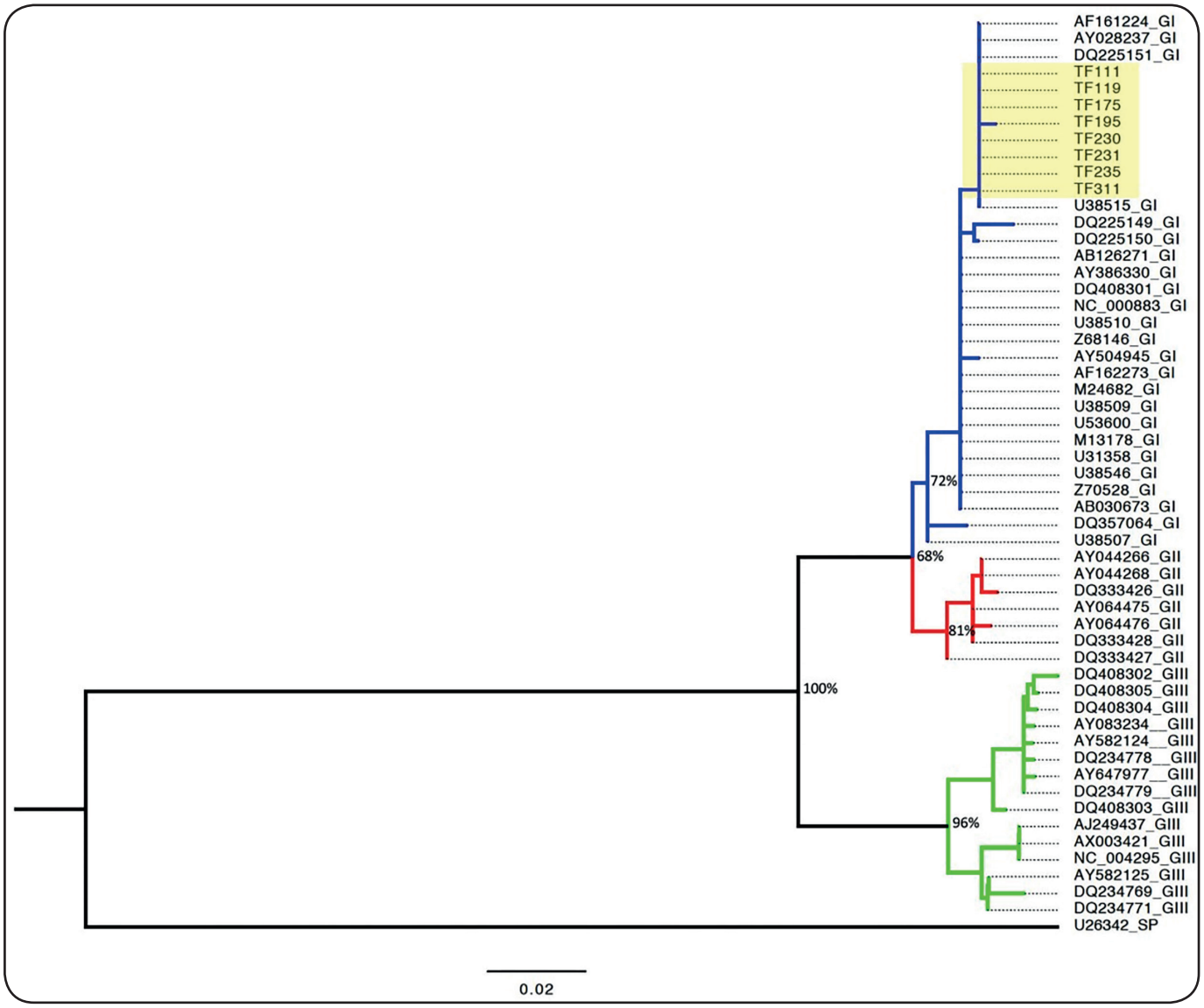

FIGURE 1: Phylogenetic tree of B19V sequences from Tefé, AM, Brazil. Mid-point rooted tree with increasing ordered nodes. B19V clades representing each genotype (G1-G3) are colored in blue (G1), red (G2), or green (G3). Tefé sample sequences (TF111, TF119, TF175, TF195, TF230, TF231, TF235, and TF311) are highlighted in yellow. The bootstrap values of the main nodes are shown. Bar represents nucleotide substitutions per site.

DISCUSSION

In the present study, we performed nested-PCR assay to detect B19V DNA in serum samples of patients with acute febrile syndrome from Tefé, a mid-sized city located in the countryside of the Amazonas state, Brazil. From the 44 patient samples evaluated, we found eight children in the range of 6 to 14 years to be positive for B19V. Several other studies investigating B19V in the Brazilian population living in other different regions found that a prevailing number of B19V positive cases were associated with the same age group[10]. Accordingly, we aimed our present study to investigate the samples from this pediatric (6 to 14 years old) group. Furthermore, the first study to assess B19V-specific antibodies in the samples (collected between January 1999 and December 2003) from patients residing in the Amazonas state predominantly included pediatric patients under 15 years[23]. Consequently, our results suggest that B19V detection should be considered as a differential diagnosis at least in pediatric patients with acute febrile illness.

We detected B19V infections in Tefé between March and July which is also the middle and end phase of monsoon in this region. Although our results were consistent with the previously published data by Figueiredo et al. (2005), we were unable to reveal the seasonal pattern as samples were not collected throughout the year. In regions with temperate climate, B19V infections occur mainly in seasonal periods which includes late winter and early spring[5]. In Brazil, a study performed in Belém, Pará State between 1988-1989 showed that most cases of B19V infection occur in the dry season[24]. Different studies conducted in Rio Janeiro[9] and Espírito Santo[25] have revealed variation in B19V infection with season. However, such observation was not reported by Wermelinger et al. (2002). Thus, further longitudinal studies covering all the regions of the country are required to determine the seasonal pattern of B19V transmission in Brazil. Such a study is highly significant as it will help determine the specific seasons that are at high-risk for parvovirus in each region. Further, this would assist physicians in diagnosing precisely when laboratorial confirmation is unavailable.

Nucleotide sequence analysis of B19V-positive Tefé samples showed eight highly similar sequences, at least in the analyzed target sequence, with only one variation that was observed in sample, TF195. Furthermore, BLAST analysis showed 22 identical sequences that were obtained from patients with varying illness in Brazil and other countries. Phylogenetic analysis of the genome region coding for VP2 and VP3 showed that Tefé sample sequences from Tefé samples were associated with genotype 1, the most common genotype worldwide and among all age groups[5]. Moreover, the sequences of the present study were found to be closely associated with the subtype 1A. However, the relative small length of the analyzed sequence region was not enough to support the findings.

Initial clinical symptoms caused by B19V and other viruses can be easily misdiagnosed with malaria, dengue, and other arbovirus related diseases that are endemic in the Amazon region. Thus, laboratorial confirmation in such cases is highly significant and much needed[26,27]. Often, rash is not found in adult patients infected with B19V. However, arthropathy which can be symmetrical and involve multiple joints, is prevalent specifically among infected middle-aged women[28]. Other clinical conditions such as acute or persistent arthropathy, transient aplastic crisis, hydrops fetalis, and fetal death can occur due to infections during the second trimester of pregnancy[3]. Thus, differential diagnosis of B19V infections during gestation preferably by molecular methods should be recognized as to evaluate the real burden of this disease especially in cases involving Zika virus endemicity. Moreover, in this study, B19V detection was performed in samples collected during the acute phase of illness and before the emergence of rash. Most common symptoms reported by patients in our study was fever, headache, and ocular globe pain. Thus, without specific laboratory assessments, such cases could be easily misdiagnosed as dengue, Zika, or other type of acute febrile illness.

In summary, our results emphasize the importance of considering B19V in differential diagnosis of cases with acute febrile illness. Moreover, the unique epidemiological setting in the Amazon region with several pathogens that can cause acute febrile illness strengthens the necessity of reliable differential diagnosis to include other less-known viruses endemic in the region such as Mayaro virus and Oropouche virus[29]. Furthermore, due to difficulty in accessing laboratories in the remotes areas of the Amazon, the development and use of more field-friendly molecular tests like Loop mediated isothermal amplification (LAMP)[30] should be encouraged.

Nucleotide sequence accession numbers

All nucleotide sequences used in this study have been deposited at GenBank under the accession numbers, MG973208 to MG973215.

ACKNOWLEDGMENTS

The authors are thankful to Programa de Desenvolvimento Tecnológico em Insumos para a Saúde - PDTIS-FIOCRUZ; Secretaria Municipal de Saúde de Tefé and Aileen Chang from George Washington University for her valuable comments and reviewing the manuscript for English.

Conflict of Interest

The authors declare that there is no conflict of interest.

Financial Support

This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (http://www.cnpq.br, grant 475740/2011-4, edital Universal 2011). FGN thanks Fundação de Amparo à Pesquisa do Estado do Amazonas - FAPEAM (www.fapeam.am.gov.br, call 001/2013 - PPSUS / 062.00656/2014 and call 001/2014 - PROEP / 062.01939/2014); Conselho Nacional de Desenvolvimento Científico e Tecnológico (http://www.cnpq.br, grant 440856/2016-7) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (http://www.capes.gov.br, grants 88881.130825/2016-01 and 88887.130823/2016-00) call MCTIC/FNDCT -CNPq / MEC-CAPES/ MS-Decit 14/2016 - Prevenção e Combate ao vírus Zika. FGN is a CNPq fellow.

REFERENCES

1. Cossart YE, Field AM, Cant B, Widdows D. Parvovirus-like particles in human sera. Lancet. 1975;1(7898):72-3.
2. Anderson MJ, Jones SE, Fisher-Hoch SP, Lewis E, Hall SM, Bartlett CL, et al. Human parvovirus, the cause of erythema infectiosum (fifth disease)? Lancet. 1983;1(8338):1378.
3. Bonvicini F, Bua G, Gallinella G. Parvovirus B19 infection in pregnancy-awareness and opportunities. Curr Opin Virol. 2017;27:8-14.
4. Servant A, Laperche S, Lallemand F, Marinho V, De Saint Maur G, Meritet JF, et al. Genetic diversity within human erythroviruses: identification of three genotypes. J Virol. 2002;76 (18):9124-34.
5. Qiu J, Söderlu;nd-Venermo M, Young NS. Human Parvoviruses. Clin Microbiol Rev. 2017;30(1):43-113.
6. Norja P, Hokynar K, Aaltonen L-M, Chen R, Ranki A, Partio EK, et al. Bioportfolio: lifelong persistence of variant and prototypic erythrovirus DNA genomes in human tissue. Proc Natl Acad Sci EUA. 2006;103(19):7450-3.
7. Molenaar-de Backer MWA, Lukashov VV, Van Binnendijk RS, Boot HJ, Zaaijer HL. Global co-existence of two evolutionary lineages of parvovirus B19 1a, different in genome-wide synonymous positions. (N Vasilakis, Ed.). PLoS One. 2012;7(8):e43206.
8. Sanabani S, Neto WK, Pereira J, Sabino EC. Sequence variability of human erythroviruses present in bone marrow of Brazilian patients with various parvovirus B19-related hematological symptoms. J Clin Microbiol. 2006;44(2):604-6.
9. Cubel RC, Garcia AG, Pegado CS, Ramos HI, Fonseca ME, Clewley JP, et al. Human parvovirus B19 infection and hydrops fetalis in Rio de Janeiro, Brazil. Mem Inst Oswaldo Cruz.1996; 91(2):147-51.
10. Wermelinger MC, Oelemann WM, Lima de Mendonça MC, Naveca FG, Von Hubinger MG. Detection of human parvovirus B19 infection: a study of 212 suspected cases in the state of Rio de Janeiro, Brazil. J Clin Virol. 2002;25(2):223-30.
11. Kang JH. Febrile Illness with Skin Rashes. Infect Chemother. 2015;47(3):155-66.
12. Figueiredo RMP, Thatcher BD, Lima ML, Almeida TC, Alecrim WD, Guerra MVF. Exanthematous diseases and the first epidemic of dengue to occur in Manaus, Amazonas State, Brazil, during 1998-1999. Rev Soc Bras Med Trop. 2004;37(6): 476-9.
13. Instituto Brasileiro de Geografia e Estatística [Internet]. Manaus-AM; 2018 [updated 2018 September 2; cited 2018 October 6]. Available from: https://cidades.ibge.gov.br/brasil/am/tefe/panorama
14. Lanciotti RS, Calisher CH, Gubler DJ, Chang GJ, Vorndam AV. Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. J Clin Microbiol. 1992;30(3):545-51.
15. Mendonça MC, Amorim AMF, Santos MG, Barros JJ, Von Hubinger MG, Santos JNSC. Heteroduplex mobility assay and single-stranded conformation polymorphism analysis as methodologies for detecting variants of human erythroviruses. J Virol Methods. 2008; 148(1-2):40-7.
16. Lis JT, Schleif R. Size fractionation of double-stranded DNA by precipitation with polyethylene glycol. Nucleic Acids Res. 1975; 2(3):383-9.
17. Zhang Z, Schwartz S, Wagner L, Miller W. A greedy algorithm for aligning DNA sequences. J Comput Biol.2000; 7(1-2):203-14.
18. Katoh K, Misawa K, Kuma K-I, Miyata T. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. 2002;30(14):3059-66.
19. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, et al. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 2012;28(12):1647-9.
20. Costa AC, Bendit I, Oliveira ACS, Kallas EG, Sabino EC, Sanabani SS. Investigation of human parvovirus B19 occurrence and genetic variability in different leukaemia entities. Clin Microbiol Infect. 2013;19(1):E31-E43.
21. Conteville LC, Zanella L, Marín MA, Filippis AMB, Nogueira RMR, Vicente ACP, et al. Parvovirus B19 1A complete genome from a fatal case in Brazil. Mem Inst Oswaldo Cruz. 2015;110(6):820-1.
22. Erdman DD, Durigon EL, Wang QY, Anderson LJ. Genetic diversity of human parvovirus B19: sequence analysis of the VP1/VP2 gene from multiple isolates. J Gen Virol. 1996; 77(Pt 11):2767-74.
23. Figueiredo RMP, Lima ML, Almeida TMC, Bastos MS. Occurrence of parvovirus B19 in Manaus, AM. Rev Soc Bras Med Trop. 2005;38(5):396-8.
24. Linhares AC, Miranda M, Freitas RB. Parvovírus B19, eritema infeccioso e outras manifestações clínicas. An Bras Delmatol. 1991;66(6):281-2871.
25. Cubel RC, Siqueira MM, Santos EO, Pires MF, Cruz CM, Nascimento JP. Human parvovirus B19 infections among exanthematic diseases notified as measles. Rev Soc Bras Med Trop. 1997;30(1):15-20.
26. Mourão MPG, Bastos MS, Figueiredo RMP, Gimaque JBL, Galusso EDS, Kramer VM, et al. Mayaro fever in the city of Manaus, Brazil, 2007-2008. Vector Borne Zoonotic Dis. 2012;12(1):42-6.
27. Magalhães BML, Alexandre MAA, Siqueira AM, Melo GC, Gimaque JBL, Bastos MS, et al. Clinical profile of concurrent dengue fever and *Plasmodium vivax* malaria in the Brazilian Amazon: case series of 11 hospitalized patients. Am J Trop Med Hyg. 2012; 87(6):1119-24.
28. Weir E. Parvovirus B19 infection: fifth disease and more. CMAJ. 2005; 172(6):743.
29. Naveca FG, Nascimento VAD, Souza VC, Nunes BTD, Rodrigues DSG, Vasconcelos PFDC. Multiplexed reverse transcription real-time polymerase chain reaction for simultaneous detection of Mayaro, Oropouche, and Oropouche-like viruses. Mem Inst Oswaldo Cruz. 2017;112(7):510-3.
30. Morris U, Khamis M, Aydin-Schmidt B, Abass AK, Msellem MI, Nassor MH, et al. Field deployment of loop-mediated isothermal amplification for centralized mass-screening of asymptomatic malaria in Zanzibar: a pre-elimination setting. Malar J. 2015;14(205):1-6.