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Original Article

Molecular and serological detection of human parvovirus B19 in Amazon cities-Brazil, 2013-2018

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ABSTRACT

Parvovirus B19 (B19V) was first detected in Manaus, Amazonas, in 2005 through serological testing. This study reports the occurrence of B19V in samples from patients suspected of having dengue infection from Itacoatiara, Manacapuru, and Tefé. We used molecular and serological techniques to examine samples from patients negative for malaria and dengue from municipalities in Amazonas between 2013 and 2018. B19V DNA was detected in 104 samples, with more positive cases between January and July in the age groups of 0-19 and 20-59 years; B19V equally infected male and female patients. Twenty patients were positive for anti-IgM B19V antibodies. The largest number of positive cases was identified in adults aged 20 to 50 years. Among these, 10 patients also had IgM/DNA B19V antibodies. Our findings confirm B19V as an etiological agent of febrile syndrome in adults and children, emphasizing the importance of differential diagnosis. The simultaneous presence of anti-IgM antibodies and viral DNA highlights the need for additional clinical and laboratory studies of these patients.

KEYWORDS: Amazonas, Brazil, differential diagnosis, IgM, parvovirus B19, viral DNA.

INTRODUCTION

Parvovirus B19 (B19V) was accidentally discovered in 1974 by Cossart *et al*. [1] when trying to detect an HBsAg protein in blood donors. It is a single-stranded non-enveloped DNA virus of 5,596 nucleotides belonging to the Parvoviridae family in genus *Erythrovirus* [2]. It has a high mutation rate among DNA viruses, and may evolve at rates similar to those of many RNA viruses [3]. Its genetic diversity is represented by three genotypes with more than 10% variability between them, described as genotypes 1, 2, and 3 [3]. B19V infection can lead to various clinical conditions ranging from asymptomatic forms, benign self-limited disease similar to other human pathologies, persistent arthropathy, typical skin manifestations such as infectious erythema (fifth disease) that are more common in childhood, or papular-purpuric eruptions in the hands and feet ("gloves and socks" syndrome) in adults [4, 5].

After its discovery, B19V was detected worldwide without any ethnic or geographical distinction but with some regional differences [6]. It is considered a global infectious agent transmitted mainly through the respiratory tract. Moreover, it is transmitted *via* blood transfusions, transplantation of hematopoietic precursor cells and organs, and by vertical transmission from the mother to the fetus, causing hydrops fetalis or intrauterine death in infected fetuses [7, 8]. After an incubation period of 4-14 days lasting up to 21 days, the acute phase is marked by nonspecific symptoms (fever, runny nose, headache, and nausea) that are rarely attributed to the virus [4, 9], as similar symptoms are found in measles, rubella, and dengue [10]. However, clinical manifestations in

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the second phase, such as infectious erythema, arthropathy, transient aplastic crisis, pure red cell aplasia, papular-purpuric eruptions in the hands and feet, and fetal hydrops, allow for clinical diagnosis [11]. In this phase, the clinical conditions caused by B19V are associated with greater morbidity, including epilepsy, meningitis, myocarditis, dilated cardiomyopathy, and autoimmune hepatitis [9, 11].

Although diagnosis is especially important in immunocompromised patients where these complications may have other causes, it is also necessary in regions endemic for several viral agents owing to the clinical similarity between various infectious agents. The detection of viral DNA using polymerase chain reaction (PCR) is the recommended test in patients with transient aplastic crisis or in immunocompromised patients with chronic infection, as these patients do not |test positive for IgM or IgG. Nonetheless, in immunocompetent individuals, the presence of B19V can be confirmed by the detection of IgM antibodies, which indicate acute infection [4].

After the discovery of B19V in 1975 [1], molecular biology studies identified single-stranded viral DNA and palindromic sequences that are characteristic of the Parvoviridae family [12]. B19V was first considered a human disease in the 1980s, when electron microscopy identified viral particles that were morphologically compatible with the Parvoviridae family in febrile patients [13]. Subsequently, several studies reported the association of B19V with transient aplastic crisis, infectious erythema, acute arthropathy, chronic anemia, and cases of intrauterine and perinatal death [14, 15].

In Brazil, B19V was first associated with infectious erythema in 1987 in Belém–Pará. In the following year, anti-B19 antibodies were detected in blood donors in Rio de Janeiro [16, 17]. After these first reports, several clinical epidemiological studies were conducted, expanding the knowledge on B19V infection in Brazil.

In Amazonas, B19V infection was reported for the first time with laboratory evidence in 2005, where IgM was detected in pediatric patients suspected of having dengue [18]. Years later, a study conducted in the municipality of Tefé-AM detected B19V DNA in samples from children suspected of having dengue [19]. This study was conducted in two other municipalities, Manacapuru-AM and Itacoatiara-AM, and viral DNA and IgM antibodies were detected in adult patients [20].

This study reports the occurrence of B19V in samples from patients suspected of having dengue infection from Itacoatiara, Manacapuru, and Tefé. The results of the molecular and serological tests are presented, as well as the clinical and epidemiological characteristics of the positive cases.

MATERIALS AND METHODS

Study area

Manacapuru (03° 17' 59" S; 6° 37' 14" W), Itacoatiara (03° 08' 35" S; 58° 26' 39" W), and Tefé (03° 21' 14" S; 64° 42' 39" W) are municipalities in the state of Amazonas. These municipalities are mainly characterized by their dependence on the local urban network and their connection to nearby cities, with which dependent relationships are established, and displacement of the population occurs in search of goods, services, and work. Moreover, there is a large daily flow of people from other different municipalities [21].

Sample collection and molecular tests

Patients with acute febrile syndrome between the first and fourth day after symptom onset enrolled in this study had visited the Hospital Geral José Mendes of Itacoatiara, Hospital Lazaro Reis of Manacapuru, or Regional Hospital of Tefé between January of 2013 and March 2018. Initially, the patients were tested for malaria by blood smear examination. All 1,001 malaria-negative serum samples from patients of three months to 86 years of age were collected between the first and fourth day after symptom onset. Then, the samples were transferred to the reference hospital for infectious diseases, Fundação de Medicina Tropical—Dr. Heitor Vieira Dourado (FMT-HVD) situated in Manaus (AM). The samples were first tested for dengue virus (DENV) using semi-nested multiplex PCR [22]. In total, 316 negative samples for DENV were randomly chosen and tested for human B19V.

DNA extraction from B19V and PCR

The PureLink viral DNA RNA/DNA Mini-Kit (Invitrogen, Carlsbad, CA, USA) was used for viral DNA extraction followed by nested PCR for the detection of B19V DNA using primers that amplify the genomic region encoding the structural proteins VP1 and VP2 [23]. The first PCR was performed containing: 12.5 µL of Hot Start Master Mix (Qiagen, Hilden, Germany), 0.5 µL of PVP1 forward primers (ACAAGCCTGGGCA AGTTAGC) and reverse PVP2 primers (CTGC ACCAGTGCTGGCTTCT), 2 µL of DNA, and water to a final volume of 25 µL.

The genomic region was amplified in a Veriti thermocycler (Applied Biosystems, Foster City, CA, USA) under the following conditions: denaturation at 95 °C/10 min; followed by 40 cycles of 95 °C/15 s, 60 °C/1 min, and 72 °C/ 1 min; and final extension at 72 °C/5 min. The second round of amplification was conducted with 5 µL of the product of the first PCR diluted 1:100 with PVP2/PVP3 primers (TGGGCCTGGCA ATGAGCTAC) and under the same cycling conditions. The 300 bp products generated in the second round of amplification were subjected to electrophoresis on an agarose gel with ethidium bromide and analyzed under ultraviolet light.

Serological test

In 92 samples, the anti-parvovirus B19 ELISA kit (Euroimmun, Lübeck, Germany) was used to detect IgM antibodies according to the manufacturer's instructions.

Ethics

The patients received information and signed a consent form approved by the Ethical Committee of the Dr. Heitor Vieira Dourado Tropical Medicine Foundation (FMT-HVD), number 700.915.

RESULTS

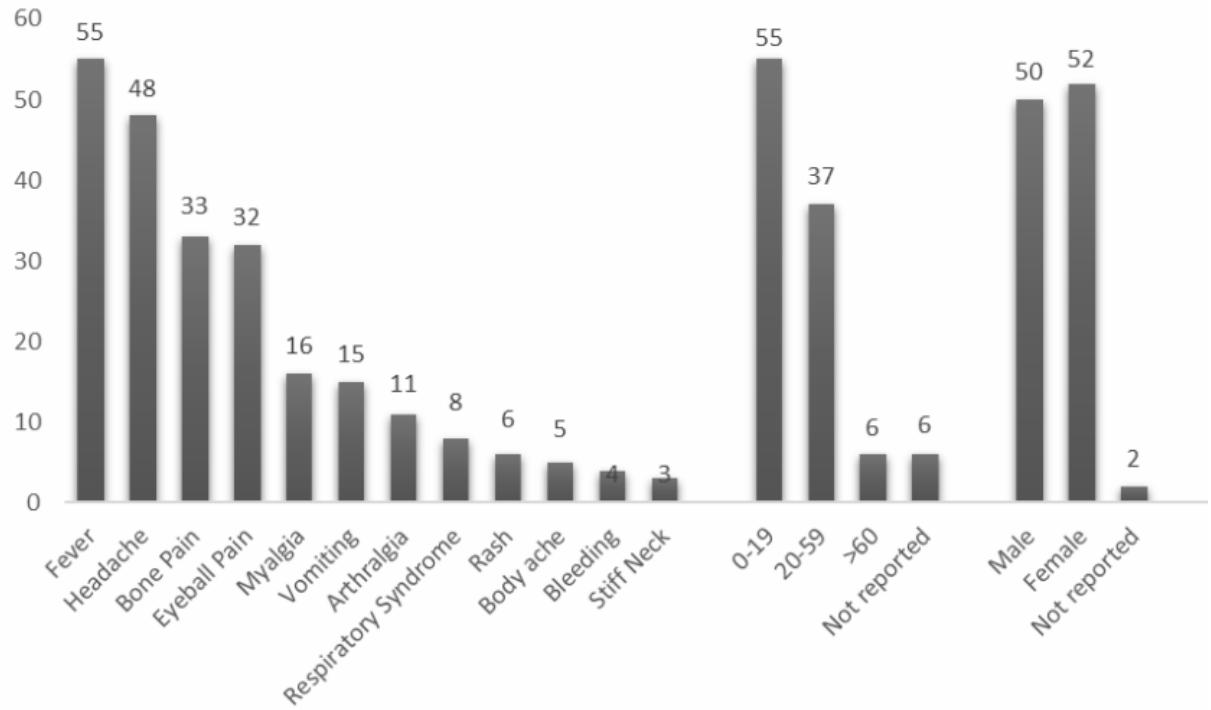
Among the 316 samples analyzed for B19V, 104 samples were determined to be positive using nested PCR. Most positive samples were from Manacapuru, followed by Itacoatiara and Tefé; samples were collected from these hospitals between February to May, January to August, and February to August, respectively. According to the information recorded at the time of collection, 10 patients were ill for more than five days, and the date of symptom onset was not reported in some epidemiological records. Fever, headache, joint pain, eyeball pain, myalgia, vomiting, and arthralgia were the most frequent clinical manifestations, followed by respiratory syndrome, rash, body pain, bleeding, and stiff neck. Patients' age ranged from 1 to 76 years, with most cases occurring in the age groups of 0-19 and 20-59 years; male and female patients were equally infected (Graph 1). We presented 104 cases of B19V, with more positive cases between January and July (Graph 2).

Among the 92 samples tested, 20 were positive for anti-IgM B19V antibodies, only two had more than 5 days of illness, and female adults between 20-50 years old accounted for the largest proportion of positive cases (Graph 3). The simultaneous presence of IgM antibodies and B19V DNA was detected in 10 samples (Table 1).

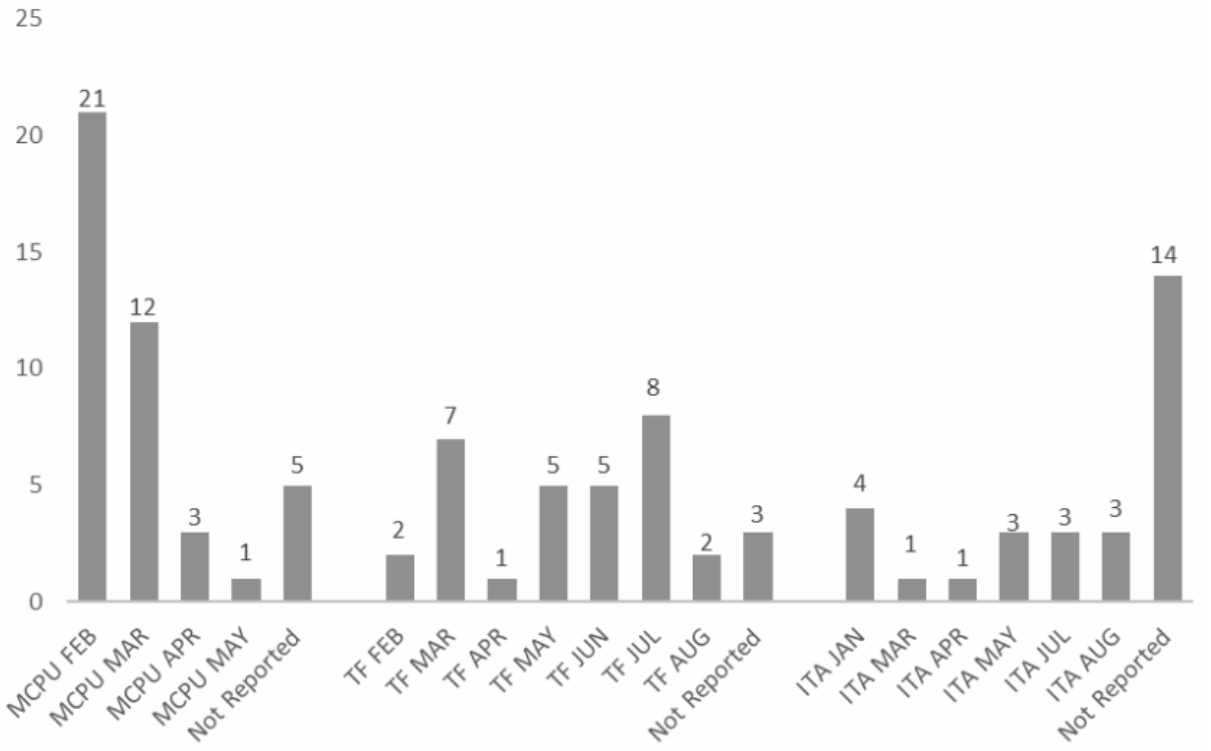
DISCUSSION

B19V DNA was first identified in Amazonas in samples from patients negative for DENV from Tefé (AM) [19], and children of both sexes were infected. In the present study, in addition to Tefé, we present the results obtained in samples from adult and child patients of either sex in the municipalities of Itacoatiara and Manacapuru. Our results corroborate the results of studies conducted in Amazonas and other Brazilian regions that indicated B19V as the cause of unknown fever in adults and children of either sex who were negative for dengue [20, 24, 25].

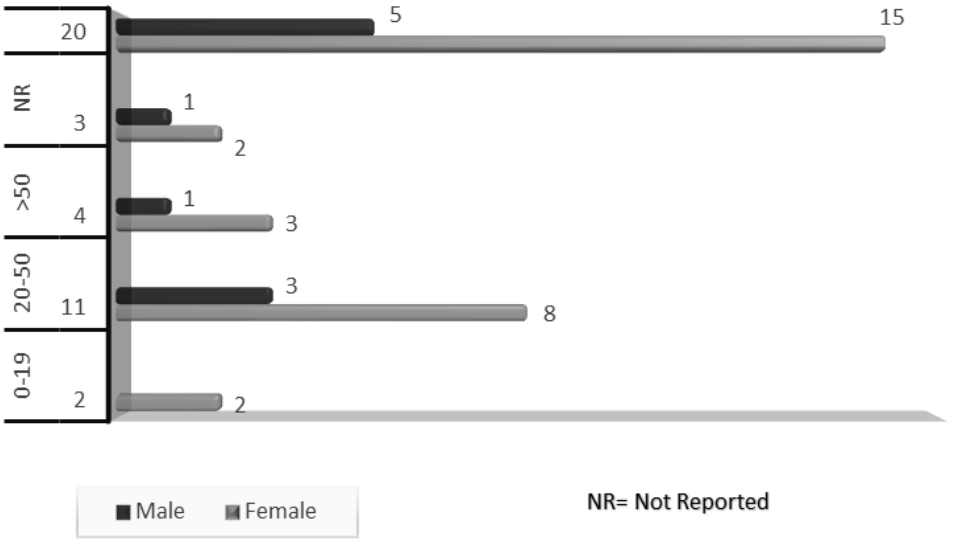
Although B19V infection in temperate regions is more common in winter and spring [14] and B19V is considered a seasonal agent, we have observed that since the first case of B19V recorded in Amazonas, B19V transmission does not seem to have a seasonal pattern. However, it is more frequent during the rainy season between December and May, coinciding with outbreaks of dengue in the region [26]. We also observed that it is underreported, similar to other dengue-like diseases that are clinically diagnosed as dengue in the absence of laboratory confirmation [27, 28].



Graph 1. Distribution of B19V cases by symptoms, age group, and gender.



Graph 2. Monthly distribution of B19V cases by municipality.
MCPU = Manacapuru; TF = Tefé; ITA = Itacoatiara.



Graph 3. Distribution of anti-IgM B19V samples by age and sex.

Table 1. Positive samples for IgM and DNAB19V antibodies.

Sample ID	Age/Gender	Year of collection	Days of illness	IgM/DNA
MCPU 19	33/F	2013	**3**	**POSITIVE**
MCPU 49	NR/ M	2013	NR	**POSITIVE**
MCPU 70	18/F	2013	**5**	**POSITIVE**
MCPU 71	38/F	2013	**2**	**POSITIVE**
MCPU 72	40/F	2013	**6**	**POSITIVE**
TF 256	11/F	2013	**1**	**POSITIVE**
ITA 129	68/M	2017	**5**	**POSITIVE**
ITA 144	34/M	2017	**2**	**POSITIVE**
ITA 176	23/F	2017	NR	**POSITIVE**
ITA 183	45/F	2017	NR	**POSITIVE**

MCPU = Manacapuru; TF = Tefé; ITA = Itacoatiara; NR = Not reported.

We detected IgM B19V antibodies in 20 samples from men and women with a predominance of young adults. Studies on serum prevalence have shown that the serological evidence of past infection for B19V increases with age, being detected in 40% of young adults in contrast to 15% of pre-school children [8, 29]. This variation in younger individuals can be explained by the 3- to 4-year epidemic cycles reported for B19V [8]. In Brazil, the first studies on human B19V infection occurred in the 1980s, when antibodies were identified in blood donors in the city of Rio de Janeiro. IgG antibodies were also found in the sera of pregnant women [11, 16]. Recent studies conducted in women of childbearing age who donated blood demonstrated an anti-B19V IgG seroprevalence of 60.7%, indicating the circulation of B19V in this group [30].

In our study, anti-B19V IgM and viral DNA were detected simultaneously in 10 positive IgM samples,

which were collected at 0-6 days of illness. Literature indicates that IgM antibodies appear one week after infection and persist for 2-3 months [7], resulting in the disappearance of viremia. Nevertheless, several studies have detected viral DNA in various tissues for months and even years in immunocompetent individuals, raising the hypothesis of B19V latency and suggesting that its infectious activity is reactivated in moments of stress or immunosuppression. Further studies are needed to determine why viral DNA persists and whether it is active and can integrate with the human chromosome [31, 32].

One of the limitations of our study is that the patients were not followed-up; thus, some questions, including whether B19V DNA can still be found months after its first identification using nested PCR, and if so, did the patients develop any symptoms, remain unanswered. However, our results corroborate the conclusions of studies indicating the underreporting of B19V infections because their initial clinical manifestations are similar to those of other viruses, such as DENV, Zika, and rubeola, greatly impairing the screening of B19V and the determination of the true percentage of infection of this pathogen in regions with different endemic viruses [33].

CONCLUSION

Our findings confirm the following: (i) B19V is as an etiological agent of febrile syndrome in children and adults of either sex who are negative for dengue; (ii) anti-IgM antibodies and viral DNA can be detected simultaneously, highlighting the need for further clinical and laboratory studies of these patients; and (iii) B19V infection occurs in the months where dengue outbreaks are more frequent, thus leading to underreporting of cases of human parvovirus. These findings emphasize the importance of differential diagnosis, especially in endemic regions where different viral agents with similar clinical manifestations circulate.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

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