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Encephalopathy Caused by Human Parvovirus B19 Genotype 1 Associated with Haemophilus Influenzae Meningitis in a Newborn

Noely E. Ferreira [1,2,#], Antonio C. da Costa [1,2,#], Esper G. Kallas [1], Cassia G. T. Silveira [3], Ana Carolina S de Oliveira [3], Layla Honorato [1,2], Heuder G. O. Paião [1,2], Silvia H. Lima [1,2], Dewton Vasconcelos [2], Marina Farrel [1,2], Silvia F Costa [1,2], Tania R. T. Mendoza [1,2], Hélio R Gomes [4], Steven S. Witkin [2,5] and Maria C. Mendes-Correa [1,2,§]

[1] Departamento de Molestias Infecciosas e Parasitarias da Faculdade de Medicina da Universidade de São Paulo, 05403-000, Brazil; noely.ferreira@gmail.com (N.E.F.); charlysbr@yahoo.com.br (A.C.C); esper.kallas@usp.br (E.G.K); layla.honorato@usp.br (L.H); heuder.paiao@gmail.com (H.G.O.P); silvialima.lima@usp.br (S.H.L);

[2] Instituto de Medicina Tropical, Faculdade de Medicina da Universidade de São Paulo, São Paulo, 05403-000, Brazil; dmvascon@usp.br (D.V); marinafarrel23@gmail.com (M.F); Silviacosta@usp.br (S.C); tozetto@usp.br (T.R.T.M);

[3] Division of Clinical Immunology and Allergy, School of Medicine, University of São Paulo, São Paulo, 05403-000, Brazil; terrassani@gmail.com (C.G.T.S); soares.acso@gmail.com (A.C.S.O);

[4] Laboratório de Investigação Médica LIM 15, Hospital da Clinicas da Faculdade de Medicina da Universidade de São Paulo, 01246903, Brazil; helio.gomes@hc.fm.usp.br (H.R.G);

[5] Department of Obstetrics and Gynecology, Weill Cornell Medicine, 1300 York Avenue, New York, NY 10065, USA; switkin@med.cornell.edu (S.S.W);

* Correspondence: Maria Cássia Mendes-Correa. Av. Dr. Enéas Carvalho de Aguiar, n. 470, São Paulo, 05403-000, Brazil. Email: cassiamc@uol.com.br .

[#] Contributed equally

Abstract: Parvovirus B19 infection is associated with a wide range of clinical manifestations, from asymptomatic to severe neurological disorders. Its major clinical symptoms, fever and rash, are common to multiple viruses, and laboratory tests to detect B19 are frequently not available. Thus, the impact of B19 on public health remains unclear. We report the case of a 38-day old girl admitted to São Paulo Clinical Hospital, Brazil, with an initial diagnosis of bacterial meningitis, seizures, and acute hydrocephalus. Antibiotic therapy was maintained for one week after admission and discontinued after negative laboratory results were obtained. Nine days after symptoms onset, a cerebral spinal fluid (CSF) sample revealed persistent pleocytosis. The complete B19 complete genome was subsequently identified in her CSF by a metagenomic next-generation sequencing approach. This report highlights the possible involvement of B19 in the occurrence of acute neurological manifestations, and emphasizes that its possible involvement might be better revealed by the use of metagenomic technology to detect viral agents in clinical situations of unknown or uncertain etiology.

Keywords: human parvovirus B19; encephalopathy; metagenomic next-generation sequencing

1. Introduction

The human parvovirus B19 (B19) is a linear, non-enveloped DNA virus, classified in the Parvoviridae family, genus Erythroparvovirus [1] with three distinct genotypes: 1, 2 and 3 [2]. It is a highly infectious virus, disseminated by respiratory secretions, transfusion of infected blood products, and vertically from mother to child [3,4]. It has a selective tropism for cells in the erythroid lineage in the bone marrow. Virus binding to the P antigen can induce severe hemolytic reactions such as chronic anemia, aplastic crisis and hydrops fetalis [5]. Most cases of B19 infection tend to be asymptomatic, are more frequent in childhood and have a variable seroprevalence from 40% to 87% [6–8].

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B19 is associated with a wide range of clinical manifestations, from asymptomatic forms to severe neurological disorders [8–10]. Encephalitis and encephalopathy are the most common consequences of parvovirus B19 infection in the central nervous system (CNS) and accounts for 38.8% of the total B19- associated neurological sequelae [11].

B19 is clinically indistinguishable from several other viruses; they co-circulate with measles virus, rubella virus, and arboviruses. In remote areas of Brazil several emerging and re-emerging viruses are present in the population and differential diagnostic tests are scarce. Due to the similarity of B19 clinical symptoms with other viruses, the true B19 clinical and epidemiological impact on public health remains unknown. The percentage of cases due to B19 infection are often mistakenly reported [12,13]. In Brazil, neurological consequences of a parvovirus B19 infection are considered rare, but laboratory tests are not always available to rule out their occurrence[14].

Metagenomic next-generation sequencing (mNGS) technology is a high-throughput approach that can simultaneously investigate all genetic material in a biologic sample. It has been utilized as a very useful exploratory method for the identification of viral infections of undetermined etiology when specific agents are not detected by standard diagnostic tests [15–18].

Although B19 has previously been reported to cause a variety of clinical symptoms in patients with different immune disorders, B19 infection in patients younger than 1 year of age is not common. Here, we report the use of mNGS for the diagnosis of an unusual case of acute viral meningitis in a one-month-old newborn, admitted to the Hospital das Clinicas da Faculdade de Medicina, Sao Paulo University São Paulo, Brazil, with fever, Haemophilus Influenzae meningitis, and hydrocephalus secondary to the infection. Using metagenomics as an alternative method to aid in the diagnosis of meningitis, we detected and characterized the entire genome of B19 from the cerebrospinal fluid (CSF) of this newborn. Metagenomics analysis of CSF in a patient with a severe neurological disorder may be an effective tool for investigating the presence of sequence diversity in a biological sample and represents a novel protocol that may yield a more precise insight into viral evolution dynamics.

2. Setting and case report

The present case report evolved from an analysis of CSF samples collected from patients with suspected CNS infection enrolled in a hospital-based surveillance study conducted in Sao Paulo, Brazil, from March 2018 to November 2019. A total of 600 CSF samples were collected at the Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo, a public hospital (300 samples) and from other different private hospitals in the same city (300 samples), from individuals (both genders and any age group) with clinical suspicion of acute infectious encephalitis or meningitis.

After collection, as per the study protocol, all CSF samples were tested for a range of pathogens using the diagnostic work-up performed in the clinical study which initially included the latex agglutination test, India ink staining, and bacterial, fungi and tuberculosis cultures. The metagenomic approach was performed as a complementary exploratory analysis to determine the etiology of cases of meningitis, encephalitis, or acute meningoencephalitis in which no cause was identified after this previous screening. A specific infectious etiology was defined in 300 cases but remained unknown in the remaining 300 cases.

The remaining volume of the CSF samples were stored at −80°C for further testing in the Virology Laboratory at the Tropical Medicine Institute at Sao Paulo University Medical School where a molecular screening by real time PCR for Human Enterovirus, Parvovirus B19 , Parechovirus [19,20] and Herpesvirus was performed in a viral panel as with some modifications [21] [22]. Among all 600 samples tested, only one sample (0.17%) was positive for Parvovirus B19 by real time PCR.

2.1. Case report

A 38-day old girl was admitted to our hospital with a diagnosis of bacterial meningitis, seizures, and acute hydrocephalus on May 28th, 2019. She had been delivered by cesarean section without complications and achieved normal growth until the 30th day of life, when she presented with fever and vomiting. She was immediately referred for additional analysis. In the hospital unit, meningitis due to Haemophilus Influenzae was diagnosed by a Polymerase Chain Reaction (PCR) assay and

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antibiotic therapy was initiated. Her symptoms worsened to persistent seizures requiring orotracheal intubation (OTI) and sedation. Computer tomography (CT) performed 2 days after symptom onset revealed supratentorial hydrocephalus, in addition to diffuse cerebral edema. The baby was then referred to our hospital for hydrocephalus management, where she was admitted to the neonatal intensive care unit on May 28, 2019. Blood, urine, and CSF samples were collected to confirm the diagnosis of bacterial meningitis and to rule out other co-morbidities.

Routine analyses and cultures of CSF, blood, throat swabs and urine yielded no pathogenic growth. Antibiotic therapy was maintained for an additional one week after admission and was discontinued after negative laboratory tests were obtained. On admission to our hospital (nine days after symptoms onset) a CSF sample indicated pleocytosis (217 leucocytes/ml, with 18% lymphocytes and 67% neutrophils, monocytes 10%, plasma cells 2%, eosinophils 2%, basophils 1%), along with 1 red blood cell/ml, protein content 247 mg/ dL (60-80mg/dL), lactate level 36.9 mg/dL (10.0 to 22.0mg/dL) and glucose concentration 40 mg/dL (2/3 glycemia rate).

During her hospitalization (May 2019 to June 2019), other CSF samples were collected, revealing persistence of cytological changes, particularly an increase in the number of red blood cells. These alterations persisted for about 40 days after the onset of symptoms, in the absence of identification of any etiological agent. The patient's initial CSF sample was subsequently subjected to a more complete etiological investigation.

Computer tomography performed 10 days after the onset of symptoms showed supratentorial hydrocephalus, diffuse cerebral edema, diffuse subarachnoid hemorrhage and multiple hypodense areas affecting the cortex and white matter of the frontal, temporal, parietal, and occipital lobes. An External Ventricular Drain (EVD) was inserted, and the patient remained hospitalized for three months for seizure control. She was discharged after stabilization of her general condition. During the clinical evolution, there was no skin rash described by the attending physicians or the patient's relatives. A serological test for B19 was not performed.

Three years after the initial diagnosis of bacterial meningitis, the child is still being followed up at our hospital, with neurologic sequelae and a diagnosis of cerebral palsy.

3. Materials and Methods

3.1. Assessment of sample

Briefly, 500 µL of the CSF was centrifuged at 12.000 × g for 10 min, and total nucleic acids were extracted from 200 µl of the supernatant using the Extracta 96 Fast Kit (Loccus SP, SP, Brazil). Afterwards, cDNA synthesis was performed with Superscript IV Reverse transcription according to the manufacturer's protocol (Thermo Fisher Scientific Inc.). A second strand of cDNA was obtained using DNA Polymerase I Large (Klenow) Fragment (Promega, WI, USA) and viral nucleic acid dosage was determined according to Quantus (Promega, WI, USA). Subsequently, the sample was submitted to a Nextera XT Sample Preparation Kit (Illumina, CA, USA) to construct a DNA library, identified by means of double barcodes. For size range selection, Pippin Prep (Sage Science, Inc.) was used to select a 400 bp insert (range 400-700 bp). The library was deep sequenced using the Nova Seq 6000 Sequencer (Illumina, CA, USA) with 250 bp ends.

The bioinformatic analysis was performed according to the protocol described by Deng et al [23]. That shared a percent nucleotide identity of 95% or more were assembled from obtained sequence reads by reassembly. The complete B19 genome were mapped with Geneious R9.

4. Results

4.1. Laboratory and clinical epidemiological characterization

The CSF sample was negative for all other etiological agents tested as described above by the latex agglutination test, India ink staining, bacterial, fungi and tuberculosis cultures and real time PCR for Human Enterovirus, Parechovirus and Herpesvirus. Parvovirus B19 was detected with 3.4

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log10 copies/mL by real time PCR. Table 1 presents a summary of characteristics of the case and demographic data.

Table 1. Evolution of CSF and Blood analyses throughout case report.

Date	28/may	29/may	22/jun	**06/jun**	08/jun	12/jun	16/jun	18/jun	24/jun		
Collect CSF	CSF1 Admission	CSF2	CSF3	**CSF4 Case**	CSF5	CSF6	CSF7	CSF8	CSF9	Reference	Viral Load
Cells (mm³)	217	43	395	**213**	80	170	72	47	41	Until 4 cells	N/A
Red cells (mm³)	1	906	221	**225**	118	1	2	0	1360	0 Red Cells	N/A
Lymphocytes (%)	18	56	5	**19**	33	28	47	28	35	70 - 80	N/A
Monocytes (%)	10	22	31	**47**	40	15	18	12	7	60 - 80	N/A
Plasmocytes (%)	2	1	0	**3**	7	37	32	58	51	N/A	N/A
Neutrophils (%)	67	17	52	**21**	14	17	1	2	5	N/A	N/A
Eosinophils (%)	2	*	2	**3**	1	2	1	*	1	N/A	N/A
Basophils (%)	1	3	0	**5**	4	1	1	*	1	N/A	N/A
Macrophages (%)	*	1	10	**1**	1	*	1	*	*	N/A	N/A
Total protein (mg/dL)	247	188	384	**301**	214	114	273	228	93	Lumbar - until 40	N/A
Glucose (mg/dL)	40	27	21	**21**	31	30	31	34	55	50 - 70	N/A
Lactate (mg/dL)	36.9	31.5	36.9	**36**	28.8	22.5	29.7	25.2	22.5	10.0 - 22.0	N/A
Fungus	Negative	Negative	Negative	**Negative**	Negative	Negative	Negative	Negative	Negative	N/A	N/A
Mycobacteria	Negative	Negative	Negative	**Negative**	Negative	Negative	Negative	Negative	Negative	N/A	N/A
Microbiological	Negative	Negative	Negative	**Negative**	Negative	Negative	Negative	Negative	Negative	N/A	N/A
Aerobic culture	Negative	Negative	Negative	**Negative**	Negative	Negative	Negative	Negative	Negative	N/A	N/A
Bacterium test	Negative	Negative	Negative	**Negative**	Negative	Negative	Negative	Negative	Negative	N/A	N/A
qPCR Herpes 1,2	*	*	*	**Undetectable**	*	*	*	*	*	N/A	Undetectable
qPCR Herpes VZV				**Undetectable**	*	*	*	*	*	N/A	Undetectable
qPCR Herpes EBV	*	*	*	**Undetectable**	*	*	*	*	*	N/A	Undetectable
qPCR Herpes CMV	*	*	*	**Undetectable**	*	*	*	*	*	N/A	Undetectable
qPCR Herpes HHV-6	*	*	*	**Undetectable**	*	*	*	*	*	N/A	Undetectable
qPCR Herpes HHV-7	*	*	*	**Undetectable**	*	*	*	*	*	N/A	Undetectable
qPCR Enterovirus sp	*	*	*	**Undetectable**	*	*	*	*	*	N/A	Undetectable
qPCR Parechovirus	*	*	*	**Undetectable**	*	*	*	*	*	N/A	Undetectable
qPCR Parvovirus B19	*	*	*	**Detectable**	*	*	*	*	*	N/A	**3.4 log_{10} copies/mL**

BLOOD

Date	28/may	29/may	30/may	03/jun	**05/jun**	
Blood count	Blood1 Admission	Blood2	Blood3	Blood4	**Blood5 Case**	Reference
Erythrocytes (x10⁶/L)	3.99	3.88	3.65	3.38	**3.56**	3.2 - 4.9
Hemoglobin (g/dL)	11	10.8	10.3	9.6	**9.9**	10.3 - 13.7
Hematocrit (%)	32.2	32.1	30.4	28.1	**29.7**	34 - 48
MCV (fL)	80.7	82.7	83.3	83.1	**83.4**	80 - 114
MCH (pg)	27.6	27.8	28.2	28.4	**27.8**	24 - 34
MCHC (g/dL)	34.2	33.6	33.9	34.2	**33.3**	28 - 32
RDW (%)	17.0	17.4	17.3	16.9	**16.7**	11.6 - 14.8
Leukocytes (x10³/mm³)	22.33	*	13.83	16.84	**13.84**	6 - 18
Rod neutrophils (%)	8	4	2	*	*	3.8 - 4.4
Segmented neutrophils (%)	70	65	75	38	**46**	28 - 30
Lymphocytes (%)	16	17	10	32	*	57 - 61
Monocytes (%)	6	5	5	12	**4**	4.8 - 5.9
Platelets (K/L)	487	466	108	814	**1212**	150 - 450

Abbreviations: * = Not tested; N/A=Not Applicable; CSF= Cerebrospinal Fluid; VZV= Varicella zoster virus; EBV= Epstein-Barr virus; CMV=Cytomegalovirus; HHV-6=Human herpesvirus-6; HHV-7=Human herpesvirus 7;MCV=Mean Corpuscular Volume; MCH=Mean Corpuscular Hemoglobin; MCHC=Mean Corpuscular Hemoglobin Concentration;RDW= Red Cell Distribution Width;

4.2. Metagenomic analysis of the CSF samples

The Parvovirus B19 complete genome was recovered from the CSF sample by a metagenomic approach (Table 2). This complete genome sequence (sample ID 63660) has been deposited in GenBank under the accession number pending.

Table 2.

Assembly				
Sample ID	Mapped reads	Genome length	%B19 genome	Coverage
63660	546.585	5427	100	34.214

4.3. Phylogenetic analysis

Phylogenetic analyses demonstrated similarity/clustering with genotype 1 B19 sequences compared to GenBank sequences. Figure 1 presents the phylogenetic tree found.

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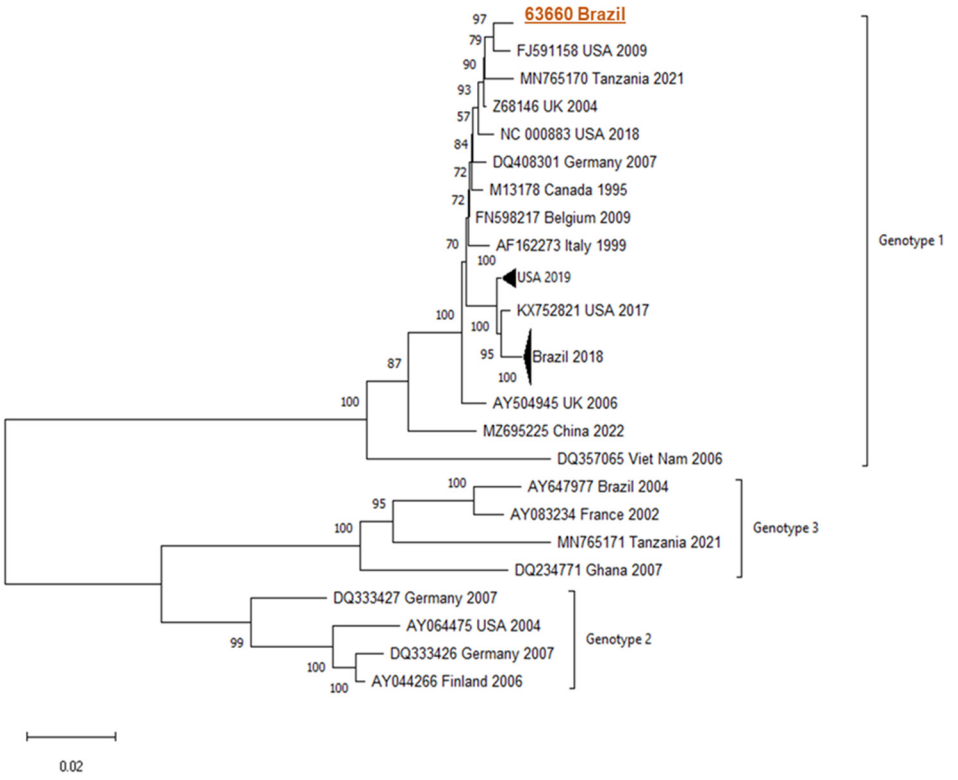

Figure 1. Evolutionary analysis by Maximum Likelihood method and Tamura-Nei model. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value. This analysis involved 29 nucleotide sequences. Evolutionary analyses were conducted in MEGA11 [24] [25].

5. Discussion

Parvovirus B19 is common worldwide and in addition to its classic manifestations, has been associated with a variety of neurological symptoms in children, including encephalitis and encephalopathy. However, B19 infection is not usually investigated in cases of symptomatic neurological diseases in newborns. In most reported cases, this virus was only sought after a negative finding of other pathogens commonly involved in meningoencephalitis.

In our series of 600 CSF samples from acute CNS infections B19 was identified in only a single sample (0.17%). In other case series, this virus has been identified as a pathogen associated with undiagnosed meningoencephalitis, reaching a prevalence rate of up to 4.3% [14],[26],[27] , a frequency higher than that obtained in our series.

The present case report describes a patient of 38 days old with meningitis and encephalopathy associated with Haemophilus Influenzae and Human Parvovirus B19. Her CSF also revealed pleocytosis with increased protein and lactate, and normal glucose levels. These findings are in agreement with prior descriptions of Parvovirus B19 CNS infection [14],[28],[29],[30]. In blood tests, a decrease in hemoglobin with levels below 10 g/dL, and anisocytosis was observed , as reported in the literature [14],[31,32]. The absence of a skin rash in this patient during the clinical course is consistent with previous reports in patients with neurological manifestations caused by B19 infection [14].

Ten days after the onset of symptoms, imaging (CT) showed diffuse cerebral edema, diffuse subarachnoid hemorrhage, and multiple hypodense areas affecting the cortex and white matter in the frontal, temporal, parietal, and occipital lobes. All these alterations have been described among children acutely infected by either Parvovirus B19 or Haemophilus influenza [29],[33,34]. Also, hydrocephalus and seizures are frequently described among the various neurological complications associated with both conditions [33,35].

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The precise mechanism involved in the pathogenesis of B19 is still very controversial. Non-productive infection of B19 virus has been described in various tissues, including the CNS. It has been postulated that infection initiation stimulates cellular responses in the tissues against the virus which could culminate in a myriad of pathologies, such as meningoencephalitis [3],[36]. B19 infection or the expression of viral proteins could modulate the immune response in the infected tissues by dysregulation of cytokines and production of autoantibodies, or by the cytotoxic effect of the B19 viral non-structural protein (NS1) damaging cerebrovascular endothelium [9],[37–43]

It has also been postulated that when patients are under immunosuppression or during concomitant infection with other pathogens, the B19 viral load increases, which could evoke a more potent inflammatory disease [3],[44]. In this regard the immature immune response of the newborn could have also contributed to the severe clinical outcome observed in the present case. The overlapping infections of Haemophilus influenzae and Parvovirus B19, as described in the present case, could also be a determining factor involved in the severity of neurological manifestations observed.

Parvovirus B19 concomitant infection with other infectious agents have been described, such as Influenza virus, staphylococcus, and mumps virus. All cases of B19 concomitant infection with other pathogens described in the literature have in common the fact that they are extremely severe and have evolved with serious sequelae or death.

To the best of our knowledge, this is the first case report of encephalopathy associated with Haemophilus Influenzae and Human Parvovirus B19.

It is relevant to comment on the possibility of vertical parvovirus B19 transmission during pregnancy. Parvovirus B19 infects 1-5% of pregnant women, generally with normal pregnancy outcomes [3], [45]. However, transmission of parvovirus B19 across the placenta can lead to fetal infection [45]. The risk of fetal complications depends largely on the gestational age at the time of maternal infection with parvovirus B19. However, fetal abnormalities associated with parvovirus B19 are rare. Whenever present they may result from injuries to different fetal organs including the brain, and as a result may also cause neurodevelopmental problems.

In the case reported here, according to information from the child´s parents and medical records, pregnancy and delivery were uneventful, and the newborn developed normally until the 30th day of life, when Haemophilus influenzae meningitis was diagnosed. It is plausible to suppose that in case an asymptomatic parvovirus B19 infection occurred during pregnancy or just after delivery, the co-infection with Haemophilus influenzae may have triggered a more potent immune-mediated reaction which led to the observed neurological complications.

The Parvovirus B19 complete genome was recovered from the CSF and the genotypic showed, through phylogeny, sequences similar to B19 genotype 1. Genotype 1 is more prevalent in most parts of the world including Brazil [12,13],[46–50], showing a close relationship with the American sequence (Genebank #FJ591158). The other Brazilian sequences available in the reconstructions were relatively distant, suggesting a distant ancestor. Assessing the nucleotide composition, we observed a variation of approximately 2% in sequences of the same genotype. This diversity is observed in single stranded DNA viruses due to their high mutation rate. According to our findings, there was no evidence of possible modifications in the analyzed Parvovirus B19 genome that would justify its presence in the CSF or the severity of the clinical manifestation. Further studies are necessary to evaluate the role of host's genetic factors or other viral factors which could be involved in the pathogenesis of Parvovirus B 19 in the CNS.

6. Conclusions

Based on the case analyzed here we hypothesize that: 1- Metagenomics analysis of a CSF sample would be useful in the diagnosis of severe meningoencephalitis cases as a complement to traditional diagnostic protocols; 2- B19 could be involved more frequently than has been described so far, since it is not regularly tested for in cases of acute neurological manifestations.

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preparation, N.E.F., and L.H.; data investigation, N.E.F and H.G.O.P.; writing—review and editing, T.R.T.M., S.S.W., M.C.M.C., D.V., S.F.C.; All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

Ethics: This study was approved by the local ethics committee (CONEP) , protocol No. CAAE: 67203417.0.0000.0068.

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