ORIGINAL ARTICLE

Flaviviruses as agents of childhood central nervous system infections in Brazil

P. E. Silva Marinho and E. G. Kroon

Laboratório de Vírus, Departamento de Microbiologia, Universidade Federal de Minas Gerais, Minas Gerais, Brazil

Abstract

Flaviviruses are agents of a major emerging human public health issue, and members of the *Flavivirus* genus have been associated with central nervous system (CNS) infections. In Brazil, a country endemic for some arboviruses, the most clinically relevant neurotropic flaviviruses include dengue virus and Zika virus. Flaviviruses cause diseases ranging from mild or subclinical infections to severe cases as CNS infections. There is a lack of data about the incidence of flaviviruses in the CNS of children in Brazil. In this review, we provide a general overview of several flaviviruses that cause CNS infections in Brazilian children and explore the importance of epidemiologic surveillance of CNS infections in cases of *Flavivirus* infections.

Keywords: Brazil, central nervous system, children, *Flavivirus*
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Corresponding author: E. G. Kroon, Laboratório de Vírus, Departamento de Microbiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Antônio Carlos 6627, Caixa Postal 486, 31270-901, Belo Horizonte, MG, Brazil.
E-mail: kroone@icb.ufmg.br

Introduction

Aseptic meningoencephalitis is an important causes of mortality and morbidity in children. The virus most frequently associated with central nervous system (CNS) infections may vary depending on the geographic region [1]. Enteroviruses and herpesviruses are the viruses most associated with infections in the CNS of children in the United States, while in Asia the Japanese encephalitis virus is most often found [2,3].

Just like the Japanese encephalitis virus, some representatives of the *Flavivirus* genus (family *Flaviviridae*) are transmitted by mosquitoes and are classically classified as neurotropic viruses, including West Nile virus (WNV) and St Louis encephalitis virus (SLEV) [4]. However, in recent years, other members of this genus, primarily dengue virus (DENV) and Zika virus (ZIKV), have been associated with CNS infections [4–6].

In Brazil, flaviviruses considered neurotropic agents, such as WNV and Rocio virus, have been described as circulating, but without cases being reported in children [7,8]. Some other potential neurotropic flaviviruses, including DENV and ZIKV, circulate in epidemics; however, there are few reports of these viruses as aetiologic agents of CNS infections in children. Therefore, the incidence of flaviviruses causing this type of infection in children is unknown.

Our goal was to review information about childhood CNS infections caused by the main flaviviruses circulating in Brazil.

Transmission

Flaviviruses are transmitted to humans primarily through the bite of infected mosquitoes. Some flaviviruses, including ZIKV and DENV, have two distinct transmission cycles: an enzootic, sylvatic cycle, where the virus circulates in *Aedes* spp. mosquitoes and nonhuman primates; and a human or urban cycle, between humans and peridomestic/domestic *Aedes* spp [9].

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WNV and SLEV are transmitted to humans and other mammals via mosquitoes of *Culex* spp. [10], and humans are usually dead-end hosts, as they generally do not generate sufficient viraemia to infect mosquitoes. WNV can also be transmitted between humans via organ transplants, blood transfusion and infected maternal milk [11].

Some reports have suggested that ZIKV can be transmitted from mother to foetus during pregnancy [12], and an ability to cross the placental barrier was also previously suggested for WNV [13]. However, the mechanism by which these viruses cross the placental barrier remains unclear.

Flaviviruses as Aetiologic Agents of CNS Infections

Dengue virus

DENV causes high morbidity and mortality in children living in tropical and subtropical areas of the world [14]. This virus is endemic in Brazil; up to October 2018, a total of 203 157 probable cases of DENV infection had been notified in that year [15]. The number of cases in children is unknown.

In 2017, the Brazilian Ministry of Health reported 7237 notified cases of meningitis probably caused by viruses [16]. Our group investigated 299 children with suspected meningoencephalitis from 2014 to 2018 in Minas Gerais State, Brazil. By analysing cerebrospinal fluid (CSF), DENV RNA was detected in approximately 6% (unpublished data). Viruses commonly detected in childhood CNS infections, such as enteroviruses and herpesviruses, were found at frequencies of 4% and 3%, respectively, similar to those of flaviviruses (unpublished data). Therefore, our data demonstrate the importance of these viruses as causes of childhood CNS infections in Brazil.

Few studies in Brazil have related DENV to CNS infections in children. Marinho et al. [17] reported seven cases where DENV RNA was found in CSF samples from children with meningoencephalitis tested during the 2014–2015 epidemic in Minas Gerais State, Brazil (Table 1). A previous study (2010–2013) using 70 samples from children from the same hospital detected DENV by PCR or CSF serology in 11.7% of cases [18]. A study of 209 cases of suspected viral meningitis or meningoencephalitis in Ceará State from 2005 to 2008 found three children positive for DENV in CSF by serologic testing [19]. Other studies in Rio de Janeiro [20] and Espírito Santo [21] analysed samples from patients with suspected DENV infection. The study carried out in Rio de Janeiro involved 13 IgM-seropositive patients who exhibited neurologic manifestations during the course of their infections. One of these patients was an 11-year-old girl with a diagnosis of encephalitis and positive serum IgM [20]. In Espírito Santo State, Domingues et al. [21] described the cases of two children infected with DENV and with CNS involvement; in both cases, DENV-3 serotype was detected by PCR in serum and CSF.

A case of postinfection acute disseminated encephalomyelitis was reported in a child by Miranda de Sousa et al. [22] in 2006. The patient was an 11-year-old girl born in Porto Velho (Rondônia, Brazil), who presented with antidengue IgM antibodies in the serum and CSF, indicating that neuromyelitis optica may be caused by DENV.

A recent study carried out in the Amazon region looking for DENV in CSF samples found five children positive for DENV by serology or PCR who were diagnosed with meningitis/encephalitis. All children presented cytologic CSF characteristics of viral meningoencephalitis [23].

Zika virus

The circulation of ZIKV has been described in Brazil since 2015 [24], and the Brazilian Ministry of Health was notified of 7071 cases in 2018 [15].

CNS infections related to ZIKV have been described, and not only in foetuses [4,6,25]. In Brazil, a single case of encephalitis was described in an 8-year-old child with ZIKV infection and reactivation of varicella zoster virus. All CSF parameters were normal; the sample was positive for ZIKV by PCR [26].

TABLE 1. Main flaviviruses associated with CNS infections in children in Brazil

Virus	No. of cases	Mode of diagnosis	CNS manifestation	State	Reference
DENV	1	Serology	Encephalitis	Rio de Janeiro	Soares, 2006 [20]
	1	Serology	Acute disseminated encephalomyelitis	Rondônia	Miranda de Sousa, 2006 [22]
	2	PCR	Encephalitis	Espírito Santo	Domingues, 2008 [21]
	3	Serology	Meningitis	Ceará	Araújo, 2012 [19]
	7	PCR and serology	Meningoencephalitis	Minas Gerais	Marinho, 2017 [17]
	5	PCR and serology	Meningitis	Minas Gerais	De Oliveira, 2017 [18]
	5	PCR and serology	Encephalitis and meningitis	Amazonas	Bastos, 2018 [23]
SLEV	3	PCR	Meningoencephalitis	São Paulo	Mondini, 2007 [32]
ZIKV	1	PCR	Encephalitis	—	Vieira, 2018 [26]

CNS, central nervous system; DENV, Dengue virus; SLEV, St Louis encephalitis virus; ZIKV, Zika virus.

In our study of 299 CSF samples, 3% of CSF samples from children with suspected meningoencephalitis were ZIKV positive by PCR (unpublished data). Although studies have primarily focused on congenital infections caused by ZIKV, acquired childhood infections should also be carefully monitored because our data suggest that the virus is an important causative agent of meningoencephalitis in children.

St Louis encephalitis virus

SLEV was first isolated in 1933 during a major epidemic in St Louis, Missouri, USA. Currently this virus is widespread in the Americas and has been detected from Canada to Argentina [27]. In Brazil, SLEV was first isolated in 1969 in the northern region from a pool of *Sabethes belisarioi* mosquitoes in Pará State [28], and it was first isolated from a human sample in São Paulo in 2004 [27].

No outbreaks of SLEV have been described in Brazil; however, serologic evidence has demonstrated its circulation in buffaloes in Pará State [29] and equines in Minas Gerais and São Paulo [30,31].

SLEV RNA has been detected in Brazil; during a large dengue outbreak in 2006, 54 serum samples that were negative for DENV and yellow fever virus were analysed, and SLEV was found in three samples from paediatric patients. All had diagnoses of viral meningoencephalitis. In two of these children, virus RNA was found in the CSF, but in one of them only in the patient's serum [32].

Neuropathogenesis

Studies using mouse and hamster models have helped elucidate how flaviviruses may enter the CNS. However, the pathways used by these neurotropic flaviviruses remain to be elucidated. Petersen et al. [33] reviewed some of the potential pathways for WNV entry into the CNS, including direct infection of the vascular endothelium; virus passage through the vascular endothelium due to disruption of blood–brain barrier integrity by vasoactive cytokines; and a Trojan horse mechanism whereby infected monocytes are trafficked into the CNS.

Neurons have been described as the main targets for *Flavivirus* infection, such as Japanese encephalitis virus in CNS in a mouse model; the activation of astrocytes and microglia may further contribute to neuronal damage [34]. Ramos et al. [35] identified, via a human brain autopsy from a fatal case of dengue haemorrhagic fever, immunoreactivity in neurons, astrocytes, microglia and endothelial cells.

Studies suggest that the *Flavivirus* neuropathogenesis is related to the apoptosis of infected neuronal cells and/or the immune response generated by the cells of the immune system, as microglia can produce and release factors that may be toxic to neurons [34,36]. Souza et al. [37], in a 2013 study of DENV-3 neurovirulence in mice, found that increased levels of nitric oxide synthase 2 (NOS2) could be the cause of the death because it correlated with increased NOS2 and cytokine expression and virus in brain. In $NOS2^{-/-}$ mice, no clinical signs of infection were observed, and cytokines were expressed at low levels, with the exception of interferon gamma [37].

Clinical Manifestations

Approximately 80% of individuals infected with flaviviruses are asymptomatic. Most of the signs and symptoms of these viral infections, such as skin rash, fever, arthralgia, myalgia, headache, retro-orbital pain and conjunctivitis, are common to other arbovirus infections. Symptoms appear to be similar in both children and adults [11,23], with rash observed in approximately 50% of patients, particularly children [11].

Neurologic manifestations associated with DENV and ZIKV have been reviewed [14]. The neurologic manifestations described in children were seizures or tonic-clonic seizures, convulsions [5,14,26], meningitis signs with acute onset of fever, headache, vomiting and nuchal rigidity [19], encephalitis signs of cerebral involvement with altered consciousness or cognition [23,14], increased intracranial pressure [18,19], drowsiness and tremors [19].

Paediatric patients with CNS *Flavivirus* infections often do not exhibit the classic disease presentation [6]. Hence, laboratory diagnosis is of paramount importance for identification of the aetiologic agent.

Laboratory Diagnosis

Diagnosis of CNS viral infections such as *Flavivirus* is a combination of clinical findings, molecular and serologic assays in CSF, neuroimaging and chemocytologic analysis of CSF [1].

The diagnosis of neuroinvasive disease caused by flaviviruses can be made by detection of RNA in the CSF (as described for WNV, for example); however, this technique can be of low sensitivity unless it is performed early in the course of infection. Serology for the detection of virus-specific IgM in CSF is used as an adjunct method [10]. Serology is commonly used to diagnose viruses in the CNS, including flaviviruses, because virus-specific IgM in CSF shows intrathecal synthesis and is considered to reflect viral target antigens within the CNS, as IgM does not cross the blood–brain barrier [4,33].

Chemocytologic analysis of CSF can accurately distinguish between a wide range of CNS diseases that can otherwise be

difficult to diagnose. In case of CNS viral infections, characteristics such as pleocytosis with cell counts up to 200 cells/mm^3, predominance of lymphocytes and high level of protein could be detected [1,14]. However, these parameters will not always be altered in cases of CNS infections by *Flavivirus*. Soares et al. [20] found 57% of the cases of meningitis caused by DENV had normal chemocytologic CSF. Other studies also found positivity for DENV and SLEV without changes in the CSF [17,18,21,32], showing that CSF chemocytologic analysis is not enough to characterize CNS infection by *Flavivirus*.

Samples such as urine and saliva have been used to detect flaviviruses, including ZIKV and WNV. Such sample collection is noninvasive, and may improve tests for routine surveillance and research involving infants and young children [38]. Further, ZIKV RNA has been detected in urine at higher levels and for an extended period of time—up to 36 days after disease onset [39]. The choice of diagnostic test depends on the time of sample collection and the goal of the assay.

Although for some flaviviruses such as DENV no specific changes are seen by imaging modalities such as magnetic resonance imaging or computed tomography, such imaging is important for a general analysis of the areas affected by CNS viral infection [40].

Conclusions

Flaviviruses have a large public health impact across the world, including in Brazil. Although congenital cases, primarily caused by ZIKV, have been the focus of much attention in recent years, cases of flaviviruses acquired in childhood should not be neglected, particularly during outbreaks in endemic areas. These viruses cause CNS infections and should be considered in differential diagnoses of children presenting with aseptic meningoencephalitis. Studies of the incidence of these viruses in the CNS of children are of paramount importance in order to provide an accurate idea of their impact on public health in Brazil, as well as to facilitate monitoring of the emergence of other flaviviruses that are circulating in the country and could cause outbreaks. To ensure proper understanding of the contribution of flaviviruses to childhood CNS disease in Brazil, these viruses must be considered in all relevant clinical contexts.

Conflict of Interest

None declared.

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