Acute neurologic emerging flaviviruses

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Abstract: The COVID-19 pandemic has shed light on the challenges we face as a global society in preventing and containing emerging and re-emerging pathogens. Multiple intersecting factors, including environmental changes, host immunological factors, and pathogen dynamics, are intimately connected to the emergence and re-emergence of communicable diseases. There is a large and expanding list of communicable diseases that can cause neurological damage, either through direct or indirect routes. Novel pathogens of neurotropic potential have been identified through advanced diagnostic techniques, including metagenomic next-generation sequencing, but there are also known pathogens which have expanded their geographic distribution to infect non-immune individuals. Factors including population growth, climate change, the increase in animal and human interface, and an increase in international travel and trade are contributing to the expansion of emerging and re-emerging pathogens. Challenges exist around antimicrobial misuse giving rise to antimicrobial-resistant infectious neurotropic organisms and increased susceptibility to infection related to the expanded use of immunomodulatory treatments. In this article, we will review key concepts around emerging and re-emerging pathogens and discuss factors associated with neurotropism and neuroinvasion. We highlight several neurotropic pathogens of interest, including West Nile virus (WNV), Zika Virus, Japanese Encephalitis Virus (JEV), and Tick-Borne Encephalitis Virus (TBEV). We emphasize neuroinfectious diseases which impact the central nervous system (CNS) and focus on flaviviruses, a group of vector-borne pathogens that have expanded globally in recent years and have proven capable of widespread outbreak.

Keywords: neuroinfectious disease, flavivirus, neurotropism, neurovirulence, CNS

Introduction

The COVID-19 pandemic has shed light on the challenges we face as a global society in preventing and containing emerging and re-emerging pathogens. Multiple intersecting factors, including environmental changes, host immunological factors, and pathogen dynamics, are intimately connected to the emergence and re-emergence of communicable diseases.1–3 There is a large and expanding list of communicable diseases that can cause neurological damage, either through direct or indirect routes. Novel pathogens of neurotropic potential have been identified through advanced diagnostic techniques, including metagenomic next-generation sequencing, but there are also known pathogens which have expanded their geographic distribution to infect non-immune individuals. Factors including population growth, climate change, the increase in animal and human interface, and an increase in international travel and trade are contributing to the expansion of emerging and re-emerging pathogens. Challenges exist around antimicrobial misuse giving rise to antimicrobial-resistant infectious neurotropic organisms and increased susceptibility to infection related to the expanded use of immunomodulatory treatments. In this article, we will review key concepts around emerging and re-emerging flaviviruses, a group of vector-borne pathogens that have expanded globally in recent years and have proven capable of widespread outbreak.4–6 These viruses are transmitted through arthropod vectors, primarily mosquitoes and ticks. Notable mosquito-borne neurotropic flaviviruses include Japanese Encephalitis Virus (JEV), Dengue Virus (DENV), West Nile Virus (WNV), Zika Virus (ZIKV), St Louis Encephalitis Virus (SLE), and Murray Valley Encephalitis Virus (MVEV).
Virus (MVE). Meanwhile, Tick-Borne Encephalitis Virus (TBEV) and Powassan Virus (POWV) are prominent tick-borne neuroinfectious flaviviruses.7 We highlight several neurotropic flaviviruses of interest and discuss factors associated with neurotropism and neuroinvasion, emphasizing diseases which impact the central nervous system (CNS).

Emergence of neurological infections: the pathogen and the host

Close interactions between humans, animals, and the environment contribute substantially to the emergence of infectious pathogens.8 Most human infections, including those which are neurotropic, have origins in animals, which are estimated to contribute approximately 60%.9 To be precise, neurotropic pathogens can directly infect cellular populations of the nervous system including neurons and glial cells.10 Neurovirulence refers to a pathogen’s ability to cause disease specifically in the nervous system.11,12 More broadly speaking, a pathogen may have ‘neurological properties’ if causes neurological dysfunction secondary to the infection without directly invading the nervous system.12 A pathogen’s ability to infect the nervous system and ensuring disease course also depends on host factors, primarily the response of the immune system and destruction of vascular and brain parenchyma by both the infection itself and the inflammatory response.13

Overview of clinical findings of neurotropic infectious diseases

Encephalitis, or infection of the brain parenchyma, classically presents with acute or subacute onset of fever, headache, altered mental status, personality changes, perceptual changes, and disorientation.14 Patients will also present with specific neurological signs that localize to the infected region of the brain, which can vary widely.14 Meningoencephalitis, rhombencephalitis (infection of the brainstem), and encephalomyelitis (infection of the brain and spinal cord) can be seen.15 Certain neuroinvasive infections may affect the peripheral nervous system (PNS), including the muscle nerve and neuromuscular junction, sometimes in combination with CNS phenomena. Neurological signs and symptoms often are not specific to a particular pathogen, making systemic manifestations important in narrowing the differential diagnosis – for example, upper respiratory symptoms, rash, and history of tick bites.15 Cerebrospinal fluid (CSF) studies typically show lymphocytic or neutrophilic pleocytosis (10–500 cells/µl), moderately elevated protein (0.5–1.5 g/l), elevated IgG synthesis rate, and elevated CSF: serum oligoclonal bands.15 Immunocompromised patients may show other abnormalities, such as acellularity or high CSF white blood cell count.15 Imaging studies are helpful to identify clinical encephalitis but are often nonspecific, such as hyperintensity on FLAIR/T2-weighted images and enhancement with gadolinium.15 Some viruses are known to cause specific patterns on magnetic resonance imaging (MRI), such as periventricular enhancement seen in infants with Cytomegalovirus (CMV) encephalitis.15 The hallmark features of viruses that will be discussed in this review are summarized in Table 1. Important considerations based on recent literature related to neurological manifestations of these viruses are also presented.

JEV

General overview

JEV is a vector-borne, enveloped flavivirus that is considered one of the most important encephalitis-causing flaviviruses globally. The severe neurologic sequelae associated with the disease have established JEV as the arthropod-borne virus causing the most disability-adjusted loss of life years annually.19 JEV is transmitted via Culex mosquito vectors and exists in an enzootic cycle in which pigs act as the amplifying host and aquatic birds serve as the maintenance host.20 Humans with JEV are ‘dead-end’ hosts as they do not develop a high enough viral load to infect other species.21 Currently, JEV is endemic in 24 countries across Asia, particularly in rural and agricultural areas of Southeast Asia and the Western Pacific during the summertime and rainy seasons.21 According to the World Health Organization (WHO), the incidence in these regions ranges from 1 to over 10 cases per 100,000 and outbreaks occur every 2–15 years, with most cases occurring in children less than 14 years of age.22

Currently, five different genotypes of JEV have been identified (G1–5) and are thought to derive from a common ancestor in Southeast Asia.
Table 1. Summarized findings of emerging flavivirus neuroinvasive disease.

| Most prevalent regions | Mode of transmission | Neurological complications | Important considerations |
|------------------------|----------------------|---------------------------|-------------------------|
| JEV                    | Culex mosquito through an enzootic cycle with humans, pigs, and water birds | Severe acute manifestations (1% of cases) | Anti-NDMAR encephalitis should be considered in patients with recent JEV infection and new onset behavior or movement disorders |
|                        |                      | • Encephalitis          | Vaccine-preventable and recommended in endemic regions |
|                        |                      | • Aseptic meningitis    |                                                       |
|                        |                      | • Meningoencephalitis   |                                                       |
|                        |                      | Post-acute (10–28 days) manifestations |                                                       |
|                        |                      | • Anti-N-Methyl-D-aspartate receptor (NMDAR) encephalitis |                                                       |
| WNV                    | Culex mosquitoes through an enzootic cycle with birds and humans | West Nile Fever (20–25%) | Neurological sequelae may be permanent, but functional improvement over time has been demonstrated |
|                        | Rare: organ transplant, blood transfusion, breast milk, and vertical transmission (one case) | Severe (1%) | Children appear more likely to present with meningitis than encephalitis |
|                        |                      | • Encephalitis          |                                                       |
|                        |                      | • Meningitis            |                                                       |
|                        |                      | • Myelitis              |                                                       |
|                        |                      | • Acute flaccid paralysis |                                                       |
| ZIKV                   | Aedes mosquito       | Adults                 | Adults with neurological symptoms during acute infection may have long-term sequelae |
|                        | Blood                | • Guillain–Barré syndrome | Infants of mothers with ZIKV during pregnancy may develop neurological symptoms several months to years after birth, even if asymptomatic initially |
|                        | Sexual encounters    | • Neuropathy            | Close monitoring of infants with intrauterine ZIKV exposure is recommended |
|                        | Vertical transmission | • Transverse myelitis   |                                                       |
|                        |                      | • Encephalomyelitis     |                                                       |
|                        |                      | • Meningoencephalitis   |                                                       |
|                        |                      | • Encephalopathy        |                                                       |
|                        |                      | • Chronic inflammatory demyelinating polyneuropathy |                                                       |
|                        |                      | • Ophthalmic abnormalities |                                                       |
|                        |                      | • Congenital Zika Syndrome |                                                       |
|                        |                      | o Spontaneous abortion  |                                                       |
|                        |                      | o Microcephaly          |                                                       |
|                        |                      | o Hypertonicity         |                                                       |
|                        |                      | o Seizures              |                                                       |
|                        |                      | o Irritability          |                                                       |
|                        |                      | o Ophthalmic abnormalities |                                                       |
| TBEV                   | Ixodes ricinus and Ixodes persulcatus, among other tick species | Meningitis | TBEV typically follows a biphasic course, and patients with monophasic illness may have more severe illness |
|                        | Unpasteurized milk   | • Severe encephalitis   | Post-acute cognitive deficits are common and should be monitored |
|                        |                      | o Chronic, progressive encephalitis found in 3% of cases of the TBEV-Sib strain | Vaccine-preventable and recommended in endemic areas |
|                        |                      | • Spinal paralysis      |                                                       |
|                        |                      | o 5–10% of cases: monoparesis, paraparesis, or tetraparesis |                                                       |
|                        |                      | • Asymmetrical cranial nerve dysfunction |                                                       |
|                        |                      | • 40–50% develop post-encephalitic syndrome |                                                       |

(Continued)
| Most prevalent regions | Mode of transmission | Neurological complications | Important considerations |
|------------------------|----------------------|----------------------------|--------------------------|
| **DENV** | *Aedes* mosquito | • Encephalitis  
• Encephalopathy  
• Meningitis  
• Stroke  
• Cerebellar syndrome  
• Transverse myelitis  
• Acute disseminated encephalomyelitis  
• Cerebellitis  
• Ophthalmologic abnormalities | • DENV-associated encephalopathy has a 50% mortality rate  
• Secondary infection with a different DENV serotype is associated with more severe disease  
• Vaccination against DENV is recommended only in the setting of a previous DENV infection |
| Africa  
North and South America  
Eastern Mediterranean  
South-East Asia  
Western Pacific | | | |
| **POWV** | *Ixodes* and *Dermacentor* ticks  
Small/medium mammals are reservoirs | • Headache  
• Altered mental status  
• Cerebellar dysfunction  
• Hemiplegia  
• Encephalitis | • 50% of infections have long-term neurologic sequelae |
| North America | | | |
| **SLE** | *Culex* mosquitoes  
Enzootic cycle between wild birds  
Domesticated animals and humans | • Altered mental status  
• Headache  
• Agitation/confusion  
• Coma  
• Meningitis  
• Encephalitis | • Encephalitis is a common cause of death in first 2 weeks of infection  
• In patients with neurologic disease, approximately 30% develop syndrome of inappropriate antidiuretic hormone secretion (SIADH) |
| North America (primarily United States)  
Sporadic cases in Caribbean, Mexico, and Central America | | | |
| **MVE** | *C. annulirostris* & *Aedes normanensis* mosquitoes  
Enzootic cycle with waterbirds and humans | • Encephalitis  
• Spinal cord dysfunction leading to flaccid paralysis  
• Cranial nerve palsy  
• Tremor | • 30–50% of survivors have long-term neurological sequelae |
| Australia  
Papua New Guinea | | | |

DENV, dengue virus; JEV, Japanese Encephalitis Virus; MVE, Murray Valley Encephalitis Virus; NMDAR, anti-N-methyl-D-aspartate receptor; POWV, Powassan Virus; SLE, St Louis Encephalitis Virus; TBEV, Tick-Borne Encephalitis Virus; WNV, West Nile Virus; ZIKV, Zika Virus.

Table 1. (Continued)
However, with rising global temperatures from climate change, there is growing concern that JEV will become endemic globally with potentially catastrophic effects. Geographic trends have suggested that typical genotype distributions are evolving to become more widespread with infections increasing in non-traditional regions. JEV-G1, which was typically endemic to Southeast Asia, has expanded its regional spread to parts of South, Central, and East Asia, and has now become the most dominant genotype in Asia above the previously dominant G3. JEV-G3 RNA has been found in birds in Europe, and the first case of sequence-confirmed JEV-G3 in Africa was reported in Angola during the 2016 Yellow Fever epidemic. A 19-year-old male without a history of foreign travel presented with fever, jaundice, headache, and blood sample positive for yellow fever, and was subsequently found to be JEV-positive via RNA sequencing. The emerging presence of JEV in non-endemic areas indicates that the strain has potential to amplify the current JEV disease burden in traditionally dormant regions. After a 60-year silent period following the initial detection of JEV-5 in 1952, a novel strain of JEV-G5 emerged in Tibet, China in 2009 and has since been detected in multiple areas of South Korea in following years. With over 3 billion people already at risk for JEV in epidemic areas, these patterns of re-emergence and spread in novel areas emphasize that continual monitoring of JEV in both traditional and non-traditional endemic areas is critical for controlling future disease burden.

Neurotropism of JEV
As with most flaviviruses, the viral glycoprotein E binds to host cell receptors and is endocytosed with clathrin-coated vesicles (endocytosis). Following a bite from an infected mosquito, JEV first enters the epidermis and infects dendritic cells, then travels to lymphoid organs where it replicates, enters circulation, and disseminates to other organs. The mechanism of JEV entry into the CNS remains poorly understood, with recent studies suggesting that inflammatory cytokine and protease-driven breakdown of the blood–brain barrier (BBB) allow for paracellular invasion of neuroinvasive JEV. In vitro models suggest that JEV is capable of transcellular migration through endothelial cells allowing it to breach the BBB and may also be able to replicate in the endothelium. JEV-specific cellular receptors have been proposed but not clearly identified to date. JEV infection has been found primarily in the gray matter of the hypothalamus, thalamus, hippocampus, and substantia nigra, including in pericytes, astrocytes, microglia, and developing neurons. JEV has been reported to have preference for neuronal cell injury, allowing for virus spread through inhibition of neuronal cell proliferation. On infection, there is an increased production of microglial nodules, and it has been suggested that microglia contribute to the neuronal cell death caused by JEV. Neuropathological features of JEV include neuronal necrosis predominantly of the thalamus and brainstem, cerebral astrocytosis, perivascular cuffing by mononuclear cells, and focal gliosis.

Neurological features of JEV
Most patients are asymptomatic or have limited, mild symptoms including fever, headache, nausea, and vomiting. Before the onset of JEV encephalitis, patients may experience nonspecific symptoms, such as diarrhea or coryza. Neuroinvasive complications develop in an estimated 1% of infected individuals and are associated with a 20–30% mortality rate. Of the patients who survive up to 50% experience chronic neurological sequelae of the disease. Patients with encephalitis or meningoencephalitis can present with rapid onset of high fever, headache, altered mental status, seizure, paralysis, speech changes, psychosis, cranial nerve palsies, and parkinsonian features. Damage to the anterior horn cells can cause a poliomyelitis-like flaccid paralysis, while parkinsonian symptoms, such as rigidity, tremors, and mask-like facies, indicate involvement of the basal ganglia. Studies have shown that up to 45–50% of patients have seizure activity during the acute phase of illness, and in children, these rates are even higher.

Unique features of JEV course
While JEV illness is usually monophasic, a secondary phase of immune-mediated anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis has been reported in children and adults. Ma et al. reports three cases of unvaccinated children with immunoglobulin M (IgM)-confirmed JEV who developed symptoms of choreoathetosis, irritability, sleep disorders, agitation, mutism, and rigidity 25–29 days after their initial illness phase. The patients were found to
have anti-NDMAR IgG in CSF samples and made full recoveries with immunoglobulin. Pastel et al., Shaik et al., and Tian et al. similarly each report a case of pediatric anti-NMDAR encephalitis 10 days and 4 weeks, respectively, after confirmed JEV infection. In a prospective study of 31 pediatric patients with clinically diagnosed JEV, five developed autoimmune encephalitis within 2 weeks to 2 months after their acute infection phase: two with anti-NMDAR antibodies, one with anti-GABABR antibodies, and two with other unidentified anti-neuronal surface antibodies. JEV RNA was not found in the CSF of all five patients during their second phase of illness. The study also found that compared to patients who only had one phase of illness, patients with autoimmune encephalitis suffered more long-term neurological deficits. Adult cases of JEV-induced anti-NMDAR encephalitis have also been reported, but the presentation tends to vary from pediatric cases; declining cognition and abnormal behaviors are the most common signs in adults, while chorea is most common in children and adolescents. Importantly, Wang reports an anti-NMDAR encephalitis case in a 2-year-old patient after receiving the JEV vaccine. Patients with a recent history of JEV infection who present with new onset behavioral or movement disorders should be evaluated for anti-NMDAR antibodies.

**Diagnosis, treatment, and prognosis**

A diagnosis of JEV should be considered in patients presenting with encephalitis with a history of recent travel or residence in endemic areas. JEV-specific IgM presence in CSF by enzyme-linked immunosorbent assay (ELISA) is best for diagnostic confirmation because the JEV viral load is typically low in humans, and the test has >95% specificity after 1 week of systemic illness. Neuroimaging will often show symmetric thalamic, basal ganglia, and brainstem lesions on computed tomography (CT) and MRI. Other reported findings include T2 hyperintensities in the hippocampus, cerebellum, and cerebral hemispheres. A recent case study of nine cases of JEV in China revealed that abnormal MRI lesions were most located in the thalamus, hippocampus, midbrain, temporal lobe, basal ganglia, and insula, respectively. However, these findings may not be a sufficient diagnostic tool for JEV encephalitis compared to serological tests: one comparison found that thalamic lesions had 100% specificity for JEV, but only 23% sensitivity.

Treatment of JEV is limited to supportive care as there are no approved antiviral medications. Recent analyses have suggested minocycline may have some clinical efficacy during the acute infectious period; however, these therapies have not been validated for use. Vaccines against JEV are recommended for those who live or plan to travel to endemic regions for longer than 1 month. In the United States, the inactivated vero cell-derived vaccine is given as two-dose series 28 days apart with the last dose given at least 1 week prior to travel to a high-risk region. A booster is also recommended in the setting of continued JEV exposure or if the initial series was received more than 1 year prior. JEV vaccination may also provide cross-protection against ZIKV.

Although JEV encephalitis has proven to be vaccine preventable, recent epidemiological shifts in traditional JEV genotype distribution have spurred the development of targeted monoclonal antibody therapies allowing for inhibition of infection from several strains. One study group testing a panel of anti-JEV mAbs on animal models found that antibodies specifically mapped to protein E domains I and III (JEV-31, JEV-169), exhibited the strongest neutralizing activity against multiple JEV genotypes likely as a result of inhibition of viral fusion. These therapies have not been tested in humans but show promise in identifying potent molecular targets for future drug development as prevalence of additional JEV strains rises.

While neurological symptoms typically improve slowly over 6 months to 1 year, 20–50% of patients who develop encephalitis have permanent neurological sequelae, including paralysis, seizures, and difficulty speaking. A recent 10-year prospective study of Lao patients with severe JEV found that one-fifth of patients died and two-thirds of survivors had neurological sequelae for a
median duration of 4.5 years. Importantly, these sequelae were more common in children than adults.

**Neuroinvasive WNV**

**General overview**

WNV is another enveloped flavivirus with single-stranded, positive-sense RNA. Like other flaviviruses, its genome codes for three structural and seven nonstructural proteins and enters cells via endocytosis. Current evidence suggests that the viral glycoprotein E infects cells by attachment to cellular glycosaminoglycans, C-type lectins, or integrins, but the direct mechanism is still unknown. The first known human case of WNV, a vector-borne pathogen occurred in Uganda in 1937. It is currently endemic in Africa, and has spread worldwide to Europe, the Middle East, West Asia, Australia, and North America. The first North American case was reported in 1999, precipitating a US epidemic that lasted through 2010 and is now the most common arboviral infection in the continent. While several subtypes of WNV have been identified, Lineages 1 and 2 are most widespread and implicated in severe cases. WNV exists in a cycle between mosquitoes and birds, where birds are the primary viral reservoirs and mosquitoes are the primary vector. Humans are infected by the bites of infected Culex mosquitoes. Warmer temperatures are associated with increased incidence, with the highest rates of disease occurring between July and September in North America. Humans are dead-end hosts; the viral load is insufficient to transfer back to mosquitoes. WNV transmission has been reported through organ transplant, blood transfusion, and breast milk, but these reports are rare. 

**Neurological features associated with WNV**

An estimated 80% of WNV cases are asymptomatic. 20–25% of patients may develop West Nile Fever, a self-limited condition characterized by high temperature, headache, body aches, maculopapular rash, nausea, and vomiting lasting for 3–6 days. About 1% of patients develop serious neurological complications including encephalitis, meningitis, myelitis, and acute flaccid paralysis. In addition to headache and fever, these patients present with neck stiffness, altered mental status, flaccid paralysis, and photophobia. Some patients have also been reported to experience acute respiratory distress secondary to diaphragmatic paralysis caused by WNV. The risk of neuroinvasive disease is greater with advanced age, as individuals above 65 years of age are at least 15 times more likely to develop neurological complications. Neurological presentation of WNV in children is similar to adults. A review of WNV cases from the Centers for Disease Control and Prevention from 1999 to 2009 found that children were more likely to present with meningitis than encephalitis.

Up to 75% of patients with neuroinvasive disease present with encephalitis, with presentation ranging from mild confusion to personality changes to coma. Autoimmune encephalitis, which has been associated with related JEV, may also be triggered by WNV. A case of an 84-year-old male who presented with encephalitis during the initial phase of WNV infection, recovered, and then declined at day 10 with worsened mental status, difficulty speaking, extrapyramidal signs, and workup revealing anti-glycine receptor antibodies. Extrapyramidal signs, such as upper extremity coarse tremor, myoclonus, and bradykinesia, have also been commonly reported. Flaccid
paralysis typically occurs as a result of anterior horn cell destruction typically presenting with proximal asymmetric weakness within 48h. Progressive paralysis can lead to respiratory failure, and some patients with extensive spinal cord involvement may develop quadriplegia.\textsuperscript{80} Notably, Chan et al.\textsuperscript{82} reported a case of acute flaccid myelitis without altered mental status secondary to WNV that presented similarly with poliomyelitis, suggesting that even without encephalitis, WNV may be an important differential in asymmetric paralysis.

**Diagnosis, Treatment, and Prognosis**

Diagnosis is confirmed by reverse transcription polymerase chain reaction (RT-PCR), cell culture, or WNV-specific IgG and IgM antibodies in serum or CSF. IgM antibodies are detected with IgM antibody-capture enzyme-linked immunosorbent assay (MAC-ELISA) and may be found within 8 days of illness onset with persistence in serum for over a year.\textsuperscript{61,83} In immunocompetent hosts, RT-PCR may be insensitive due to the transient viral presence in the CSF, therefore serological IgM is the test of choice.\textsuperscript{84} However, it is important to note that in immunocompromised patients, the opposite approach may be more effective due to delayed or absent antibody response causing false seronegative results, and enhanced viral persistence in CSF allowing for prolonged detection by RT-PCR.\textsuperscript{85} In patients with neurological complications, lumbar puncture often shows elevated protein levels ($<150\text{ mg/dL}$) and leukocytosis ($<500\text{ cells/\mu L}$), first with predominantly neutrophils and later lymphocytes. Neuroimaging may exhibit leptomeningeal enhancement, parenchymal spinal cord signal abnormalities, periventricular inflammation, and focal lesions in the pons, basal ganglia, anterior horn, and thalami on MRI.\textsuperscript{80,86} Even though MRI is the most useful imaging for WNV, only 20–70% of patients exhibit imaging abnormalities.\textsuperscript{87}

Treatment is currently limited to supportive care. Although there have been several investigated therapeutic agents, there are no WNV-specific antiviral medications and no vaccines that are licensed for humans. Some therapies that have shown variable effectiveness against WNV include IVIG, interferon, and ribavirin. While human immunoglobulin with high titers of WNV IgG (Omr-IgG-am) developed by a study team in Israel showed promise in enhancing both prophylaxis and acute treatment of neuroinvasive WNV, a subsequent clinical trial conducted by the National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group found no statistically significant difference in outcomes between patients receiving treatment with high-titered anti-WNV antibody IVIG compared to standard IVIG or normal saline.\textsuperscript{88,89}

Monoclonal antibodies (mAbs) have also emerged as a potential therapeutic against WNV. The developed mAb E16 directed against flavivirus surface E glycoprotein showed \textit{in vitro} and \textit{in vivo} inhibition of viral replication through blockade of WNV release from endosomes into the cytoplasm.\textsuperscript{90} Recent work has analyzed the efficacy of monoclonal antibodies against epitopes on envelope E protein domain II (mAb WNV-86), which demonstrates enhanced neutralization against mature virions and a 50% inhibitory concentration three times lower than mAb E16.\textsuperscript{91} In addition, antibodies against flavivirus nonstructural protein 1 (NS1), a glycoprotein involved in recruitment of viral replication factors, have shown protection against WNV through triggering complement-mediated clearance of infected cells.\textsuperscript{92} Although these targeted therapies have shown initial effectiveness in enhancing clearance of WNV in animal models, there is still concern over effectively mitigating the risk of antibody-dependent enhancement of infection and they have yet to be tested for therapeutic efficacy in humans.\textsuperscript{93}

There are vaccines currently authorized for use in veterinary practice in Europe, and emerging research suggests that E protein-based vaccines may be effective.\textsuperscript{94} Prevention of the condition is focused on community mosquito control measures and diligent surveillance of vector populations. In the setting of increased vector mosquito populations, low-volume organophosphate or insecticide application may be indicated.\textsuperscript{86} Most patients with WNV have an uncomplicated recovery; however, mortality among those with neuroinvasive disease is 10%. Survivors often present with permanent complications from the viral neurological sequelae. Documented chronic deficits include neuropsychological impairment, fatigue, and cognitive decline even years after resolution of the infection.\textsuperscript{77,95} Along with chronic deficits, patients with WNV have elevated long-term mortality risk. A cohort study assessing the outcomes of patients hospitalized with WNV found that
there was a twofold increase in mortality of these patients for up to 3 years after illness onset, and factors associated with increased mortality in these patients included older age, encephalitis during acute infection, and need for endotracheal intubation during hospital course. However, a 2018 single-center retrospective review found that on long-term follow-up, most patients who had been hospitalized with neurological WNV manifestations had improved modified Rankin scores (improved from average scores of 3–5 during hospitalization to 0–2), indicating that functional impairment may improve over time. Recent studies suggest there is an association between WNV and future development of neurodegenerative disease due to the impaired BBB causing persistent microglial activation and impaired clearance of amyloid aggregates.

Neuroinvasive ZIKV infection

General overview

ZIKV is a mosquito-borne flavivirus. While the Aedes mosquito is the primary source of spread, ZIKV also spreads through blood, sexual encounters, and vertical transmission. The primary vectors are Aedes aegypti and Aedes albopictus mosquitoes. ZIKV was first detected in monkeys in Uganda in 1947; the first-known spread to humans occurred in 1952. For several decades, ZIKV cases were contained within Africa and Asia, but recent outbreaks in Micronesia (2007), French Polynesia (2013–2014), and most significant, Brazil (2015) spread the virus around the world. In response to the outbreak in Brazil in 2016, the WHO deemed ZIKV a public health emergency of international concern and outlined its potential to spread through sexual transmission. Like other flaviviruses, viral glycoprotein E and precursor protein M are most implicated in the infection mechanism of ZIKV. Following endocytosis, viral RNA is released into the cytoplasm which serves to both allow the virus to replicate and trigger the host cell’s innate immune response. The incubation period can last from 3 to 18 days, but most infected individuals never develop symptoms. In those who do, symptoms are usually mild, such as fever, rash, conjunctivitis, myalgia, joint pain, fetal malaise, and headache, and last for 2–7 days, on average. However, in some cases, neurological complications may develop, including Guillain-Barré syndrome (GBS), neuropathy, myelitis, and ophthalmic abnormalities. Furthermore, the neurotropic potential of ZIKV has been confirmed through the detection of the ZIKV and anti-ZIKV antibodies in the CSF of patients with neurological disease. Perhaps most worrisome – especially given the asymptomatic nature of most ZIKV cases – is the association of infection with spontaneous abortion and congenital abnormalities, such as microcephaly (termed ‘Congenital Zika Syndrome’ or CZS).

Neurological complications of ZIKV

Since the Brazil outbreak of 2015, CZS resulting from vertical transmission has become the most well-known neurological manifestation of ZIKV. The most reported neurological signs in affected infants include microcephaly, hypertonicity, seizures, irritability, and ophthalmologic changes, including optic nerve, retinal, and vision abnormalities, nystagmus, and strabismus. A case-control study of microcephalic infants found higher levels of ZIKV RNA and Zika-specific IgM antibodies in the CSF and serum of cases versus non-microcephalic infants. Fetal CNS anomalies were found to be higher in women who had confirmed Zika infections during pregnancy versus not. A study comparing CNS and eye defects in infants born in Columbia found abnormalities were more common during a ZIKV outbreak (from 2015 to 2016) than in the period before and after. Interestingly, prior to the Brazil outbreak, most studies showed only 0.3–0.5% of ZIKV-infected mothers gave birth to infants with microcephaly. However, during the epidemic, 46% of infected mothers had children with microcephaly, suggesting that the severity of the infection evolves based on multiple factors. Mortality for infants diagnosed with CZS ranges 4–7%, and surviving children have a variety of chronic neurological impairment, such as hearing loss, developmental delay, and vision difficulty. In addition, within children of mothers infected with Zika that showed no anomalies at birth, up to 9% exhibited some neurodevelopmental delay before the age of 2 years.

In adults, Guillain-Barré syndrome (GBS) is the most frequently reported neurological sequela of Zika infection and has been linked to several outbreaks in different countries. It has been postulated that this autoimmune complication occurs secondary to molecular mimicry between ZIKV structural proteins and gangliosides, which are glycolipids.
containing sialic acid found in the nervous system. Patients who develop GBS in the context of Zika usually exhibit symptoms 5–10 days after the onset of acute illness. A case–control study of GBS patients during the ZIKV outbreak in 2013–2014 in French Polynesia found that 100% of cases had Zika antibodies versus only 56% of the control group composed of patients presenting to the same hospital with a non-febrile illness. Similarly, 27 out of a sample of 29 patients diagnosed with GBS in Brazil in 2016 were found to be Zika-positive; another prospective study of 71 GBS patients in Brazil from 2014 to 2017 found that half of the patients with recent evidence of an arbovirus infection had ZIKV. It has been suggested that individuals with Zika-associated GBS have higher morbidity and more severe chronic neurological sequelae, such as, cranial nerve palsies. Other neurological complications associated with adult Zika infection include encephalomyelitis, meningoencephalitis, transverse myelitis, encephalopathy, and chronic inflammatory demyelinating polyneuropathy (CIDP).

**Diagnosis, treatment, and prognosis**

Diagnosis of acute ZIKV is made with RT-PCR or nucleic acid amplification tests. However, since ZIKV is asymptomatic in most patients and often only transiently present in blood and serum samples, RT-PCR is not a consistently reliable diagnostic measure. For this reason, serology testing for Zika antibodies using MAC-ELISA is usually preferred for the detection of Zika in all phases. Zika IgM antibodies usually appear in the CSF 4–7 days after the onset of illness and persist for up to 12 weeks. In addition, Zika can be detected in urine and saliva, as viral shedding in these mediums persists longer than serum and can be found 7–14 days after onset of illness. It is important to recognize that Zika can serologically cross-react with other flaviviruses, which can lead to potential false-positive or equivocal results. For samples exhibiting cross-reactive results, the plaque reduction neutralization test (PRNT) has shown diagnostic utility; however, this test is often limited by the need for more developed infrastructure as it is both time- and resource-intensive. There is still a significant need for accessible and inexpensive testing for Zika despite the major global burden of the disease it causes. Currently, treatment of ZIKV is supportive as there are no FDA-approved treatments or vaccines for the disease.

**Long-term outcomes: adults.** In the post-acute period, adults with Zika infection may continue to experience neurological complications. In one study of 34 Zika-associated GBS cases, patients with GBS during their acute Zika infection were more likely to report disability and depression after 1 year compared to patients who had uncomplicated ZIKV. In vitro studies of human and mouse brain tissue suggest that ZIKV can replicate within human and mouse brain cells and may lead to memory and synaptic changes via virus-induced inflammation and disrupted neuronal–glial communication. This suggests that patients with a history of Zika infection may be at a higher risk of neurodegeneration and need to be monitored for clinical signs.

**Long-term outcomes: children.** Recently, it has become clear that congenital neurodevelopmental complications of vertical Zika transmission may manifest later in development, even if infants appear asymptomatic at birth. A study of children with intrauterine Zika infection who were normocephalic at birth performed worse in several neurodevelopmental domains than those without intrauterine infection at 6-month follow-up, in addition to having shorter attention spans and longer processing times of visual stimuli. Data from the Dominican Republic outbreak found that children of women who had been infected during pregnancy developed post-natal developmental abnormalities, including post-natal microcephaly, hypotonia, hypertonia, hearing issues, and transient developmental delay. A 18-month longitudinal study of Columbian mothers with ZIKV during pregnancy and their infants found that newborns without CZS showed declines over time in neurodevelopmental outcomes using two validated assessments of infant development. Hcini et al. report similar findings that both symptomatic and asymptomatic children are at risk of neurodevelopmental delay at least as far as 3 years old. A recent study analyzing neuroimaging of children diagnosed with CZS at 3-year follow-up revealed delayed myelination, persistent intracranial calcifications, ventriculomegaly, cerebellar hypoplasia, and cortical abnormalities on CT. All children included in this analysis displayed severe neurodevelopmental impairment.

While the mechanism for these delayed effects is unclear, murine models suggest that Zika infection of oligodendrocytes and subsequent cell death in the post-natal period leads to secondary immune demyelination.
persistent replicating ability in the CNS, which could increase the risk of neurological issues in children with intrauterine Zika exposure. Together, these data present a strong case that clinically normal infants at birth whose mothers had a known Zika infection during pregnancy should be closely followed for the signs of neurodevelopmental delays years after initial diagnosis.

TBEV

General overview
TBEV is a flavivirus common in Europe and parts of Asia. TBEV is typically caused by one of the three strains: European (TBEV-Eu), Siberian (TBEV-Sib), and Far Eastern (TBEV-FE). Recent studies have also shown the emergence of the Baikalian (TBEV-Bkl) and the Himalayan (TBEV-Him) subtypes. TBE-FE is associated with the most severe disease course, while TBE-Eu and TBE-Sib have been documented to have a milder presentation. The virus can occasionally be transmitted after the intake of unpasteurized milk products from viremic livestock. TBEV-infected ticks are likely to become more abundant as a result of emerging climatic changes.

Neurological complications of TBEV
The median duration of the first stage of illness is 5 days (range 2–10) with a 7-day symptom-free interval to the second phase (range 1–21). In the first viremic stage, the virus replicates in the Langerhans cells of the skin, travels to draining lymph nodes, and causes common systemic signs of infection as it spreads through the bloodstream. In the second stage, the virus crosses the BBB and replicates in the neurons of the anterior horn, medulla oblongata, pons, cerebellum, dentate nucleus Purkinje cells, and striatum. The clinical spectrum ranges from mild meningitis to severe encephalitis with or without myelitis and spinal paralysis. Importantly, TBEV-Sib is associated in up to 3% of cases with a chronic, progressive encephalitis that is thought to develop over years. A flaccid poliomyelitis-like paralysis may occur as well during the febrile phase of the infection, and in about 5–10% of cases, monoparesis, paraparesis, and tetraparesis can develop, and paralysis of respiratory muscles. Some patients also present with an asymmetrical cranial nerve dysfunction predominantly affecting the ocular, facial, pharyngeal, and vestibular nerves. It has also been suggested that individuals experiencing monophasic rather than typical biphasic presentation have a more severe course of disease.

DENV

General overview
DENV is one of the most common Aedes mosquito-borne viruses worldwide, particularly in tropical and subtropical regions, and is responsible for an estimated 390 million infections yearly – nearly 100 million of which are symptomatic. There are four known serotypes, DENV-1 through DENV-4; while infection with one serotype provides lifetime protection against re-infection of that serotype, it provides only mild and
temporary protection against infection with other serotypes. In fact, secondary infection with a different serotype is associated with more severe disease.⁴ DENV has spread from just nine countries in 1970 to over 100 today, with the highest burden in Asia. While Europe has not seen a DENV epidemic, cases are now seen regularly, and an epidemic is now considered a possibility.¹³⁹

The disease spectrum of DENV can vary widely, from subclinical to severe dengue characterized by acute capillary leakage, thrombocytopenia, and hemorrhage leading to hypovolemic shock and organ failure.⁴,¹⁴⁰ Symptomatic cases commonly present first with acute onset of fever with headache, retroorbital pain, rash, myalgia and arthralgia, anorexia, abdominal pain, and nausea. As of 2009, the WHO classifies DENV infection in three ways: dengue (1) with or (2) without warning signs and (3) severe dengue. Warning signs include abdominal pain or tenderness, persistent vomiting, clinical fluid accumulation, mucosal bleeding, lethargy, liver enlargement of greater than 2 cm, and increasing hematocrit with rapid and simultaneously decreasing platelets.¹⁴⁰ Severe dengue is defined as severe plasma leakage, severe bleeding, or severe organ involvement.¹⁴⁰ It is associated with secondary DENV infection with a different serotype and time between infections is associated with increased disease severity.⁴,¹⁴¹ If untreated, severe dengue has a mortality of up to 20%.¹⁴¹

Neurotropism and neurological manifestations of DENV

DENV was initially considered to be non-neurotropic, and neurological sequelae of DENV infection were attributed to systemic, immune-mediated, or metabolic complications. In recent decades, an increasing body of evidence, including the presence of virions within the CSF, suggests that DENV can invade the nervous system, most likely hematogenously via the BBB.¹⁴² Infection with DENV has been implicated as the cause of encephalitis and encephalopathy in patients from an endemic region, without evidence of other viruses.¹⁴³ Genomic comparison of serologic and CSF samples of DENV patients with neurologic manifestations showed 99.7% similarity, further suggestive of the ability of the virus to cross the BBB.¹⁴¹ In particular, serotypes DENV-2 and DENV-3 are most commonly associated with neurologic manifestations.¹⁴⁴

The most common neurological manifestations of DENV infection are encephalitis and encephalopathy. DENV-associated encephalopathy, which is often seen in the cases of severe dengue, has a 50% mortality rate.¹⁴⁵ Other CNS complications include meningitis, stroke, cerebellar syndrome, transverse myelitis, and acute disseminated encephalomyelitis, and cerebellitis.¹⁴⁶–¹⁴⁹ Ophthalmologic complications have also been widely reported in association with DENV infection, including retinopathy, maculopathy, and choroiditis.¹⁵⁰–¹⁵³

DENV virus in children can manifest differently than adults. Children under the age of 1 year and between 4 and 9 years of age are at the highest risk of developing severe dengue. Mortality ranges 2.5–5% but can be as high as 44% if disease advances to shock.¹⁴⁵ Neurological manifestations reported in children include acute disseminated encephalomyelitis (ADEM), hepatic encephalopathy, parkinsonism, epilepsy, transverse myelitis, subarachnoid hemorrhage, and stroke.¹⁴⁵

Diagnosis, treatment, and prognosis

DENV has an incubation period of 2–7 days followed by a symptomatic period of 4–10 days. Diagnosis via RT-PCR or viral NS1 protein levels is most useful during the febrile phase of infection. Rapid NS1 tests are commercially available and are particularly useful in endemic regions.¹³⁹ However, ELISA for dengue-specific antibodies is the preferred method for definitive diagnosis; IgM levels are detectable within 1 week and are at their highest levels 2–4 weeks following infection.¹⁴¹ In primary infections, IgG antibodies typically appear around day 10 of infection but will rise rapidly within the first week in the setting of secondary infection.¹³⁹ Because DENV and JEV are common in similar regions and thevirions are serologically cross-reactive, DENV encephalitis can be difficult to distinguish clinically from JEV and careful evaluation is the key.¹⁵⁴

To date, no specific treatment for DENV infection exists. Severe dengue and hypovolemic shock must be managed carefully with intravenous rehydration. For milder symptoms, acetaminophen and paracetamol are recommended while NSAIDS should be avoided due to the risk of hemorrhage associated with DENV infection.¹³⁹ Effective and safe vaccine development for dengue has proved challenging. To date, one live-attenuated
recombinant tetravalent vaccine, CYD-TVD (Dengvaxia, manufacturer: Sanofi Pasteur) is commercially available. However, it has been associated with an increased risk of severe dengue if the first natural infection of DENV comes after vaccination and is only recommended for those with a confirmed previous infection of DENV.\textsuperscript{139,155,156}

**Conclusion**

Neurotropic infectious diseases are a growing concern to the global population, with significant associated morbidity and mortality. Ongoing environmental changes are increasing the population’s susceptibility to emerging and re-emerging flaviviruses. Enhancing early detection of neuroinvasive infections, optimizing prevention strategies and public health surveillance systems in the years ahead will be critical to decrease the impact of neurotropic infectious diseases.

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Ethics approval and consent to participate was not required given the review nature of this manuscript.

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**Author contribution(s)**

**Marissa Caldwell:** Writing – original draft; Writing – review & editing.

**Abhilasha P. Boruah:** Writing – original draft; Writing – review & editing.

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