The neurological manifestations of Zika and chikungunya viruses

Master of Philosophy

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Abstract

During 2015-16 Brazil experienced the largest epidemic of Zika virus ever reported. This arthropod-borne virus (arbovirus) has been linked to Guillain-Barré syndrome (a disorder of the peripheral nervous system) in adults but other neurological associations are uncertain. I designed and performed a retrospective clinical study in Rio de Janeiro, Brazil, investigating patients presenting with an acute neurological disorder and suspected recent Zika virus infection. I found a wider spectrum of neurological disease associated with Zika than reported previously, including that of the central nervous system. This has implications for clinical diagnostic pathways and public health measures. The study also highlighted some of the diagnostic challenges associated with arbovirus-associated neurological disease, and showed an unexpected role of chikungunya virus, another arbovirus that has spread rapidly through the Americas since 2013 and continues to affect millions in explosive outbreaks throughout the tropics. This led me to perform a systematic review of neurological disease associated with chikungunya virus; I summarise all described neurological manifestations, highlighting the wide spectrum of disease in adults and children, its importance in vertical transmission in neonates, comparison with Zika and dengue viruses and recent insights into disease mechanisms. The review will be a useful reference tool for clinicians, researchers and public health officials involved in managing complications of this emerging pathogen. Looking forward, this thesis also discusses the important unanswered questions relating to arbovirus-associated neurological disease, including establishing causality, defining the burden of disease and considerations for vaccines, and how we might approach these challenges.

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Presentations:

The spectrum of neurological disease associated with Zika and chikungunya viruses in adults in Rio de Janeiro, Brazil: a case series. NIHR Health Protection Research Unit in Emerging and Zoonotic Infections (HPRU EZI) Annual Conference (2017)

Chapter 1: General Introduction

1.1 Zika

Zika virus is an arthropod-borne virus (arbovirus) first isolated in 1947 in the Zika forest, Uganda. Over the next five decades, the virus was of minimal clinical significance, with only 14 documented cases of human infection.[1-5] In April 2007, physicians on Yap Island, Federated States of Micronesia, noted an outbreak of rash, conjunctivitis, fever and arthralgia; Zika virus was eventually identified as the cause.[6] The next reported major outbreak was in French Polynesia in October 2014, which preceded the explosive spread throughout Latin America from 2015.[7] By December 2015, it had caused an estimated 0·4-1·3 million cases in Brazil alone.[8]

Phylogenetic analyses have shown two major distinct lineages, the African and Asian strains (Figure 1A); the Asian strain has been responsible for the above dissemination throughout the tropics (Figure 2A).[9] Like the related dengue viruses, Zika is a flavivirus (genus *Flavivirus*, family *Flaviviridae*) that causes a fever-arthralgia-rash syndrome and is transmitted principally by *Aedes* mosquitoes and from mother to foetus. Transmission can also occur sexually (including male-female, male-male and female-male) and via blood transfusion.[10]

The most commonly reported symptoms of acute Zika infection are a maculopapular rash, pruritus, fatigue, mild fever and arthralgia, following an incubation period of 3-14 days.[6,7,11] The rate of asymptomatic infection is unknown, although one study from the Yap Island outbreak estimated it to be approximately 80%.[6] In the majority of cases, the virus does not cause complications and the above symptoms resolve without intervention within one week.[7] Rare cases of non-neurological complications have been reported, including transient myocarditis and thrombocytopenia. [12-16]

An apparent association between Zika virus and an increase in severe congenital disease and other neurological disorders, particularly Guillain-Barré syndrome (GBS) (see Table 1 for description),[17-20] prompted the World Health Organisation to declare Zika virus a public health emergency of international concern in February 2016.[21] The association between Zika virus and microcephaly, part of the congenital Zika syndrome, has been the subject of much research over the past two years. The temporal correlation between Zika outbreaks and increased rates of microcephaly (defined as: “a head circumference of more than two standard deviations below the median for age and sex”),[22] observed in Brazil and French

Polynesia, as well as the increased rate of microcephaly in mothers with confirmed Zika infection, and the detection of Zika RNA in brain tissue and amniotic fluid of microcephalic neonates and foetuses together provide a compelling argument in favour of a causative role for Zika in microcephaly.[23-29] Other forms of congenital disease have been described under the umbrella of the congenital Zika syndrome, including arthrogryposis, cerebellar hypoplasia, and damage to the thalamic and corpus callosum.[30-32] However, there still remains much to be explained regarding the association between Zika and congenital disease. For example, following the first wave of Zika infections in Brazil in 2015, there was unexplained regional variance in the incidence of ensuing microcephaly; furthermore, the second wave of Zika in 2016 was not associated with the expected corresponding increase in cases of microcephaly in any part of the country.[33] The reasons underlying these observations will need to be sought from future research.

Table 1: Summary of major acute neurological disorders.

Neurological disorder	Description
Central nervous system	
Encephalitis	Inflammation of the brain, often caused by a viral infection; can present with fever, confusion, seizure
Meningitis	Inflammation of meninges (membranes surrounding brain and spinal cord); can present with headache, photophobia, fever, neck stiffness
Myelitis	Inflammation of spinal cord; can present with weakness, sensory loss, bowel/bladder dysfunction
Acute disseminated encephalomyelitis	Autoimmune demyelinating condition involving white matter of brain and spinal cord; can present with polyfocal neurological deficit
Epilepsy	Disturbance of brain electrical activity leading to seizures
Migraine	Primary headache disorder, often throbbing and unilateral
Stroke	Sudden onset loss of brain function secondary to ischaemia or haemorrhage
Peripheral nervous system	
Guillain-Barré syndrome (GBS)	Autoimmune insult to motor and/or sensory nerves leading to weakness +/- autonomic dysfunction, can lead to respiratory muscle paralysis
Miller-Fisher syndrome	GBS variant with ophthalmoplegia, ataxia and areflexia
Myasthenia gravis	Autoimmune insult to acetylcholine receptors at neuromuscular junction, leading to weakness
Cranial nerve palsy	Loss of function of cranial nerve(s); can present with ophthalmoplegia, diplopia, ptosis, facial weakness

Neurological disease resulting from horizontally transmitted Zika virus infection is the other major clinical concern. Zika-associated GBS has been the most widely reported manifestation of such disease. GBS is an autoimmune disease where peripheral nerves and their spinal roots are targeted, typically following an infection. It results in weakness, autonomic dysfunction and can lead to death, usually from paralysis of respiratory muscles and ensuing dyspnoea and chest infection. Different GBS phenotypes exist; in acute inflammatory demyelinating polyneuropathy, the myelin sheath surrounding the neuron is affected, and in acute motor axonal neuropathy, the target is the axonal membrane itself.[34] A case-control study of the French Polynesian outbreak showed an association between Zika

virus infection and GBS,[18] although prior dengue exposure made interpretation of the virology results challenging because of serological cross reactivity between flaviviruses in current commercially available antibody assays.[35] Dengue, like other flaviviruses including Japanese encephalitis and West Nile viruses, can also cause both peripheral and central nervous system (CNS) disease.[36] Secondary dengue infections are associated with more severe dengue disease, and some have postulated that prior dengue may predispose to more severe Zika infection. More recently, a study from Colombia showed a strong temporal association between GBS and Zika virus, with viral RNA detected in samples from 17 patients.[19] Further evidence for a temporal association comes from an epidemiological analysis that found a close correlation between the incidences of Zika infection and GBS in seven Latin American countries.[37] A few case reports have described Zika virus-associated myelitis (disease of the spinal cord),[38] encephalitis (brain),[39,40] meningoencephalitis,[41] acute disseminated encephalomyelitis,[42] Miller-Fisher syndrome (a GBS variant),[43] and myasthenia gravis (neuromuscular),[44] suggesting that the spectrum of neurological disease may be broader than initially thought. A preliminary epidemiological report from the French Polynesian outbreak indicated a possible increase in other neurological manifestations associated with Zika virus, but gave few details.[45] The spectrum of neurological disease associated with Zika is clearly still undefined, and forms the primary research question addressed in Chapter 2.

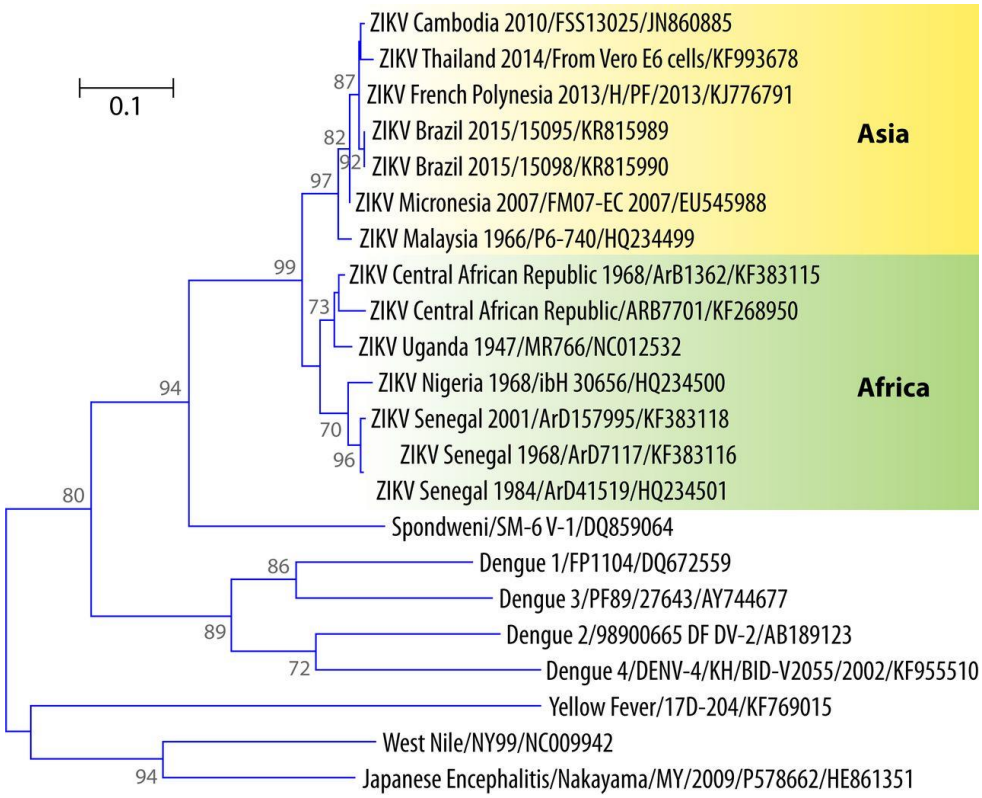
1.2 Chikungunya

Chikungunya is a distinct arbovirus also identified in Africa in the 1950s that has spread to cause epidemics in the tropics in recent years.[46] Historical accounts suggest that it may have caused outbreaks as early as the 18th century,[47] although the virus and its disease were first isolated and documented respectively in 1952-3, in Tanzania.[48,49] Since then, two lineages, namely West African and East/Central/South African (ECSA), have been shown to circulate in sub-Saharan Africa in a sylvatic cycle between mosquitoes and non-human primates.[50] The first documented human outbreaks were in southern Asia during the 1960s-70s[51,52] and were caused by the Asian strain, a descendent of the ECSA strain.[53] After decades of low transmission, an ECSA divergent re-emerged in 2004, having undergone two successive mutations of its envelope E1 glycoprotein.[54] This new lineage, renamed the Indian Ocean Lineage (IOL, Figures 1B and 2B) spread from Kenya to cause explosive outbreaks throughout islands of the Indian Ocean, India and Southeast Asia, affecting millions.[55] In late 2013, the emergence of the Asian strain was reported in the Caribbean,[56] marking its first documented appearance in the Americas. It has since rapidly spread throughout 48 American countries and territories, causing over two million suspected cases

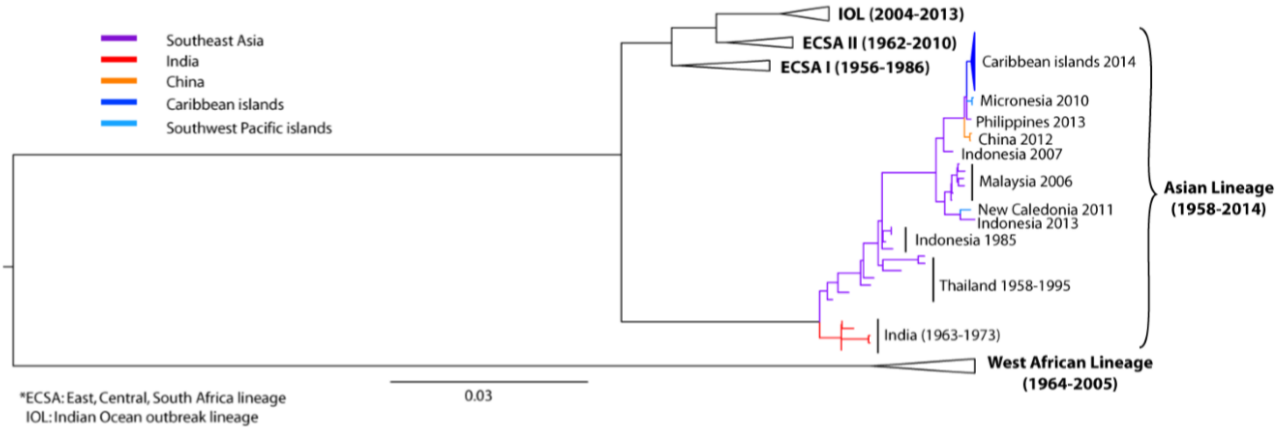
to date.[57] Of note, local circulation of the ECSA strain has also recently been reported in Bahia state, Brazil.[58]

Figure 1: Phylogenetic trees of Zika and chikungunya viruses.

A. Zika



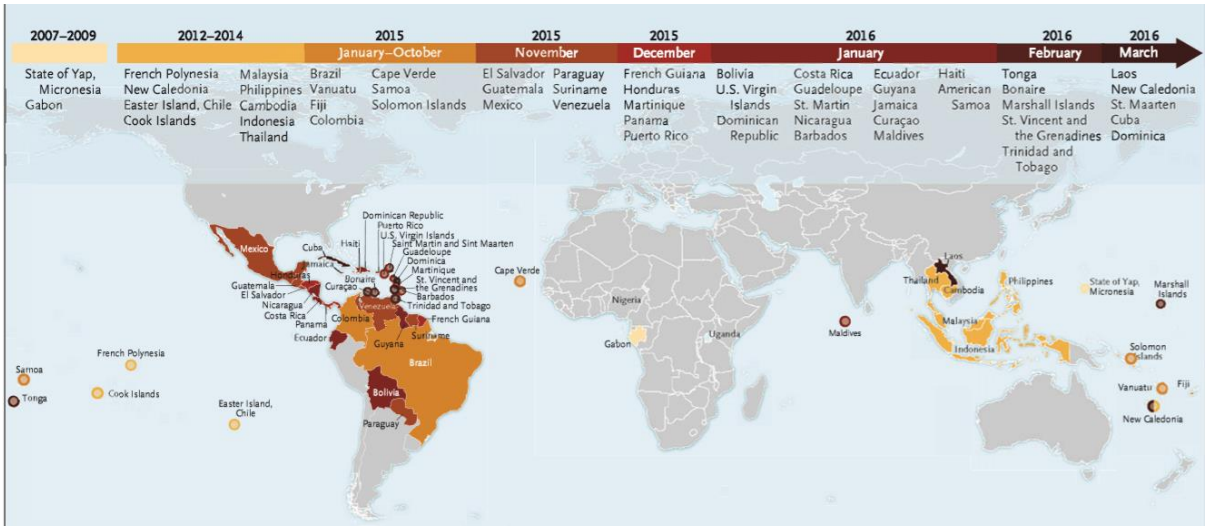
B. Chikungunya



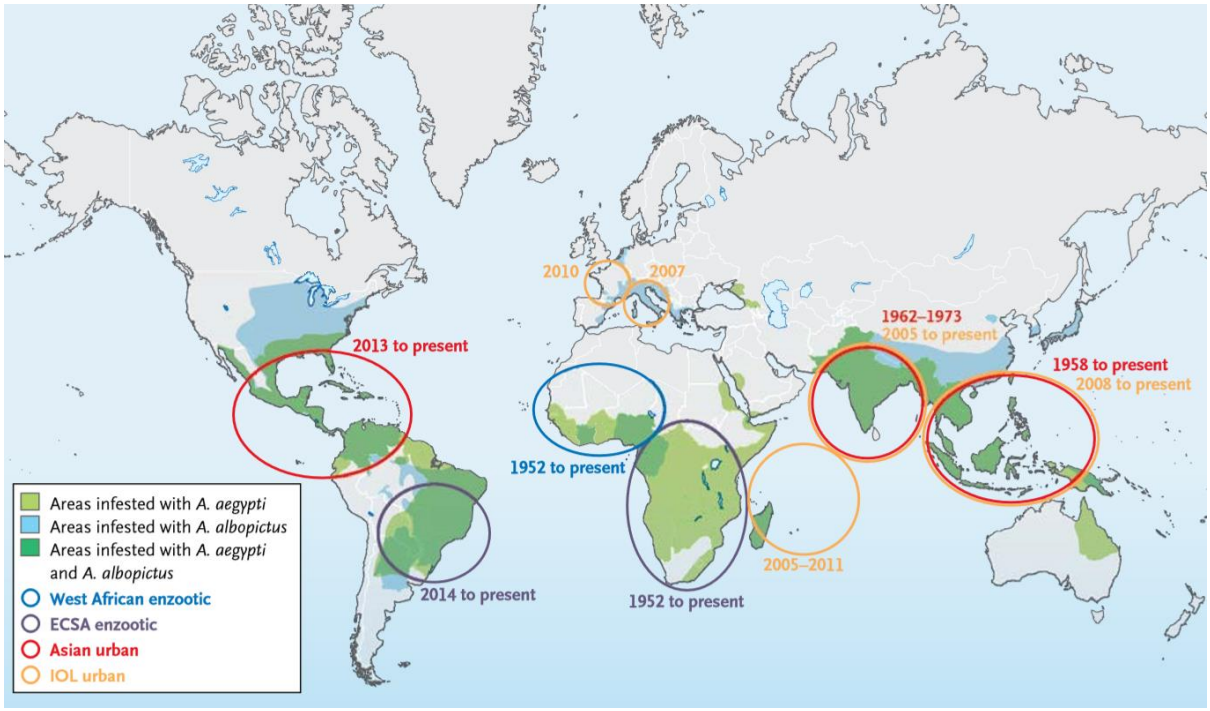
Phylogenetic trees showing A. Zika's relationship to other flaviviruses and divergence into the Asian and African strains (obtained from Musso and Gubler, Clinical Microbiology Reviews)[7] and B. The divergence of chikungunya into four strains (obtained from Weaver, New England Journal of Medicine).[46]

Figure 2: Global spread of Zika and chikungunya viruses.

A. Zika



B. Chikungunya



World maps showing the global spread of Zika (obtained from Peterson *et al.*, NEJM)[59] and chikungunya (obtained from Weaver and Lecuit, NEJM)[46] viruses.

The word “chikungunya” originates from the Makonde language, spoken in Tanzania and Mozambique, meaning “that which bends up”;[60] this refers to the debilitating arthralgia often occurring in the acute phase of infection, along with fever, myalgia, headache and rash. Like Zika and dengue viruses, it is primarily transmitted by *Aedes* mosquitoes and primarily causes a fever-arthralgia-rash syndrome; transmission can also occur vertically from mother

to foetus in the peri-partum period.[61] Chikungunya also occasionally presents with neurological disease, including GBS, encephalitis and myelitis.[62]

Unlike Zika, chikungunya is an alphavirus (genus *Alphavirus*, family *Togaviridae*). Classically, alphaviruses are described in two groups – the 'old world' viruses, including Sindbis, O'Nyong-Nyong and Ross River viruses, which cause a predominantly arthritic syndrome, and the 'new world' viruses, including Eastern, Western and Venezuelan equine encephalitis viruses, which are responsible for outbreaks of encephalitis.[63] Chikungunya virus is now recognised as a cause of both arthritic and neurological disease throughout the tropics.

Seroprevalence studies have reported a range of asymptomatic rates of chikungunya infection, from 3-47%.[64] In acute symptomatic infection, following an incubation period of approximately 3 days,[65] there is an abrupt onset of fever, headache, rash, arthralgia and myalgia which typically last for 1-2 weeks.[66] After this, seroconversion likely confers life-long immunity.[67] As well as neurological manifestations, chikungunya virus is associated with complications of the cardiovascular, renal, respiratory, hepatic, gastrointestinal and adrenal systems, sometimes collectively referred to as "atypical features".[68-70]

1.3 Demonstrating causality

As for other arboviruses, proving that Zika or chikungunya has caused neurological disease can be challenging.[63,71] Traditionally, a causal relationship between microbe and disease was based on Koch's postulates, namely[72]:

1. The agent must be demonstrable in every case of the disease
2. The agent is not present in other diseases
3. After isolation in culture, the agent must be able to produce the disease in experimental animals
4. The agent can be recovered from the experimental animal

There are clear limitations to these in modern microbiology. For example, we know now that certain pathogens can cause multiple diseases, and indeed certain diseases can be caused by more than one pathogen (postulates 1 and 2). Furthermore, modern technologies such as polymerase chain reaction (PCR) assays have increased our detection rate of certain pathogens over isolation in culture (postulate 3). We therefore adapt these postulates today in studies regarding causality. Neurological disorders associated with arbovirus infection have an added layer of complexity, due to the different samples used for testing for the presence of the viruses. The strongest evidence of causality comes from demonstrating that

the virus is in the central nervous system (CNS), which is most often shown by detecting viral RNA in the cerebrospinal fluid (CSF) by PCR; alternatively, the virus may be cultured. In fatal cases, autopsy material may be positive by PCR. For many patients the virus has cleared from the CSF by the time they present; in which case the detection of CSF IgM antibody by ELISA is considered diagnostic. Interpretation is complicated in flavivirus infections because a positive Zika-IgM test can result from known cross-reactivity of serological tests with dengue virus.[35] Because chikungunya is an alphavirus, there is no serological cross reactivity with the flaviviruses, making diagnosis more straightforward (where other alphaviruses are not circulating). It is not known for how long virus, RNA, or IgM remain detectable in Zika- or chikungunya-associated neurological disease, and whether this differs for the different neurological disorders. By analogy with similar arboviruses, one might expect virus to be detectable for the first few days of illness, at which point it is replaced by antibody, which remains for weeks to months.

Both Zika and chikungunya virus infections can be diagnosed by detecting virus in the blood by PCR or culture, and evidence of recent infection can also come from detection of a positive IgM antibody. However, a positive blood test in a patient with neurological disease does not necessarily mean the virus cased the disease; infection may be coincidental, and care must be taken to exclude other possible causes. Both viruses can also be detected in urine, saliva, semen and milk,[73-77] but the same caveats apply.

A further caveat applies to Zika antibody kinetics and detection in patients presenting with neurological disease in areas with endemic dengue. Following a primary dengue virus infection, a second exposure to a different dengue serotype can trigger a secondary immune response, whereby an early IgG response with an undetectable IgM response can be seen.[78] Given the structural similarity between the two flaviviruses, a primary Zika infection in a patient with previous dengue (in essence, a secondary flavivirus infection) could theoretically induce a similar pattern (although this was not observed in a recent animal model, where dengue-immune macaques challenged with Zika still produced an IgM followed by IgG response).[79] The absence of a Zika-IgM response in the CSF or serum of a patient presenting with neurological disease might thus not necessarily indicate an absence of recent, potentially causative Zika infection, because the patient may have produced a secondary IgG response instead. Detection of CSF IgG alone is not a particularly useful tool to investigate a causal relationship between virus and neurological disease, given that it may simply reflect an infection at any point in the past, with passive diffusion of IgG from serum to CSF. However, the use of the CSF antibody index, which compares CSF and serum IgG

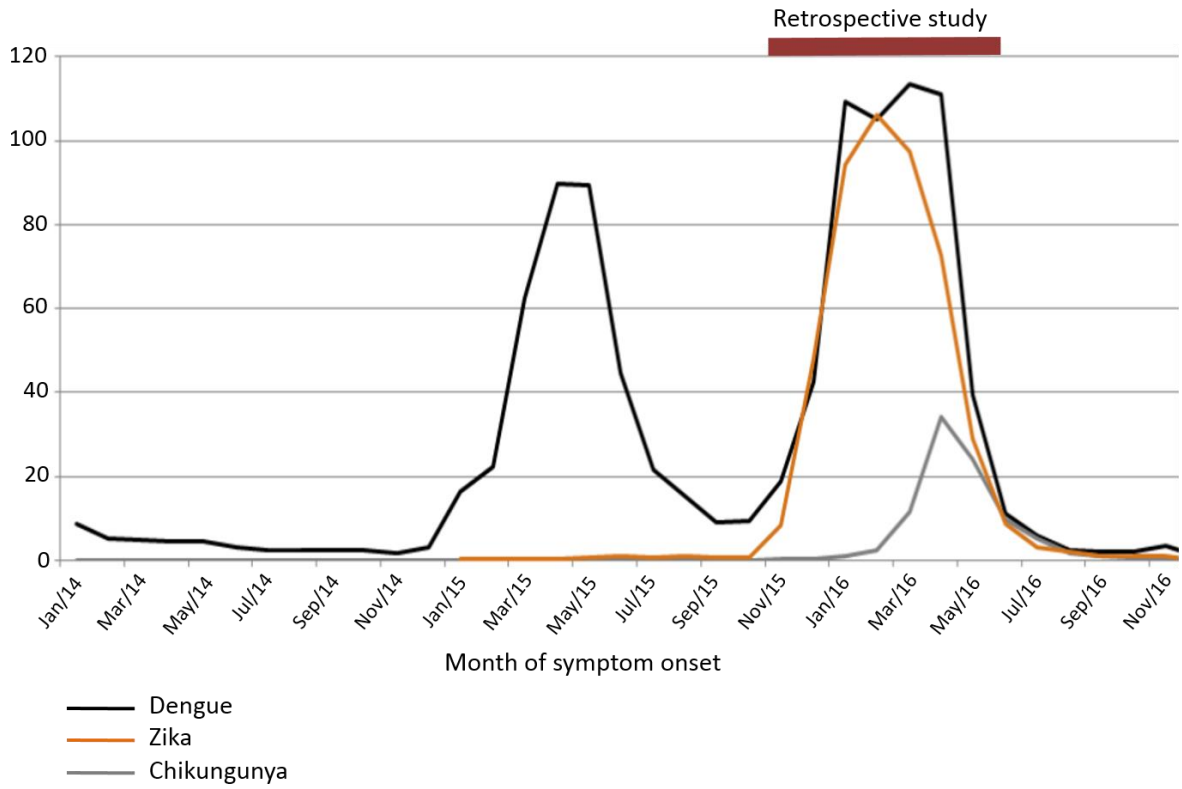
and albumin, is a measure of CSF-specific IgG production[80] and can thus suggest a more causal relationship if positive.

It should be stressed that the isolation of virus, presence of genetic material or detection of antibody help provide evidence for causality, but do not prove it. Further evidence that can help build up the case for causality comes from epidemiological studies, in vitro and in vivo models, and animal models. There are various frameworks incorporating criteria for causality, two of which (Bradford Hill and Shepard's criteria) were used in an article examining the association between Zika virus and birth defects, which argued for a causal association.[24] However, such criteria also have limitations. The Bradford Hill criteria, a set of nine criteria often regarded as the underlying basic principles of inferring causality in epidemiology, were proposed in 1965 – since then, science and technology have of course advanced considerably and helped elucidate the mechanisms linking exposure and disease, not known of the time the criteria were proposed. Today, there are no hard and fast criteria for causality, but rather a mixture of the use of the above tools and common sense scientific deduction is required.

1.4 Co-circulation and co-infection

Co-circulation of Zika, chikungunya and dengue viruses has been reported in much of South America,[81] and is a potential problem in all areas of the world where *Aedes* mosquitoes are endemic. In Rio de Janeiro, the location for the study in Chapter 1, all three viruses were circulating during the time period of investigation (Figure 3). *Aedes albopictus* mosquitoes have the ability to deliver more than one arbovirus in their saliva, raising the possibility of simultaneous transmission of the viruses.[82]

Figure 3: Incidence of notified cases of Zika, chikungunya and dengue in the state of Rio de Janeiro, 2014-2016.



Zika, chikungunya and dengue viruses were all circulating in Rio de Janeiro during the investigation period of the retrospective study (Chapter 1).[83]

Furthermore, co-infection of arboviruses has rarely been detected in patients presenting with neurological disease.[84,85] Given that all three arboviruses are known to be neurovirulent, it is unclear whether in these patients, their neurological disease is associated with one or more of their infections. Co-infection has also been reported in pregnant women,[23,86] the significance of which for the foetus is unclear. The most common co-infection to be reported in all patients is with chikungunya and dengue viruses, though this may be due to the greater number of epidemics of these viruses so far compared to Zika. Albeit rarely, co-infection with all three viruses has been reported, including in patients with neurological disease.[84,87] The differences in disease pathogenesis, presentation and severity between mono- and co-infections are currently unknown.

1.5 Scope of thesis

The recent introduction of both Zika and chikungunya to Latin America and ensuing large-scale epidemics brought the complications of both viruses to the attention of the world.

The spectrum of neurological disease caused by Zika virus was, and still remains, a significant void in our understanding of this emerging pathogen. Therefore, my aim was to characterise this spectrum by investigating cases from the 2015-2016 Zika epidemic in Rio de Janeiro, Brazil, which is described in Chapter 2. As well as delineating clinical features of Zika-associated neurological disease, the results showed an unexpected role of chikungunya virus, which was not well-characterised in Latin America at the time. Although chikungunya-associated neurological disease has been reported far more frequently and for longer than Zika-associated disease, there is a clear lack of consensus and understanding of the conditions amongst clinicians, researchers and in the literature. This led me to perform a systematic review of all neurological disease associated with chikungunya virus globally, which is described in Chapter 3.

Chapter 2: The spectrum of neurological disease associated with Zika and chikungunya viruses in adults in Rio de Janeiro, Brazil: a case series.

2.1 Introduction

During 2015-16, Brazil experienced the largest outbreak of Zika virus ever reported and saw a subsequent increase in cases of Guillain-Barré syndrome (GBS). As discussed in the general introduction, clinical evidence for a causal association between Zika and GBS comes from a number of studies from French Polynesia and Latin America.[18,37,88] Furthermore, a handful of case reports describe Zika associated with other neurological disorders, including central nervous system and ocular disease.[38-44,89] The full spectrum of neurological disease associated with Zika virus remains to be elucidated and will be vital information for the global response to this emergent pathogen.

Brazil has been endemic for dengue virus for over 30 years and since 2014, has also experienced outbreaks of chikungunya virus. In Rio de Janeiro, where the study was conducted, local transmission of Zika and chikungunya viruses were first reported in January and November 2015, respectively. During the study, the city was experiencing outbreaks of all three arboviruses. Dengue virus is a well-recognised cause of neurological disease,[71] with reports from most countries where the virus circulates, including Brazil.[90] Chikungunya virus can also present with neurological disease, although at the time of the study, there were few such reports from South America and its role had not been assessed.[91]

To assess the spectrum of neurological disease associated with Zika virus, I studied adults in Rio de Janeiro with acute neurological syndromes following suspected Zika virus infection. Given their similarities and co-circulation, the cases were also investigated for chikungunya and dengue viruses.

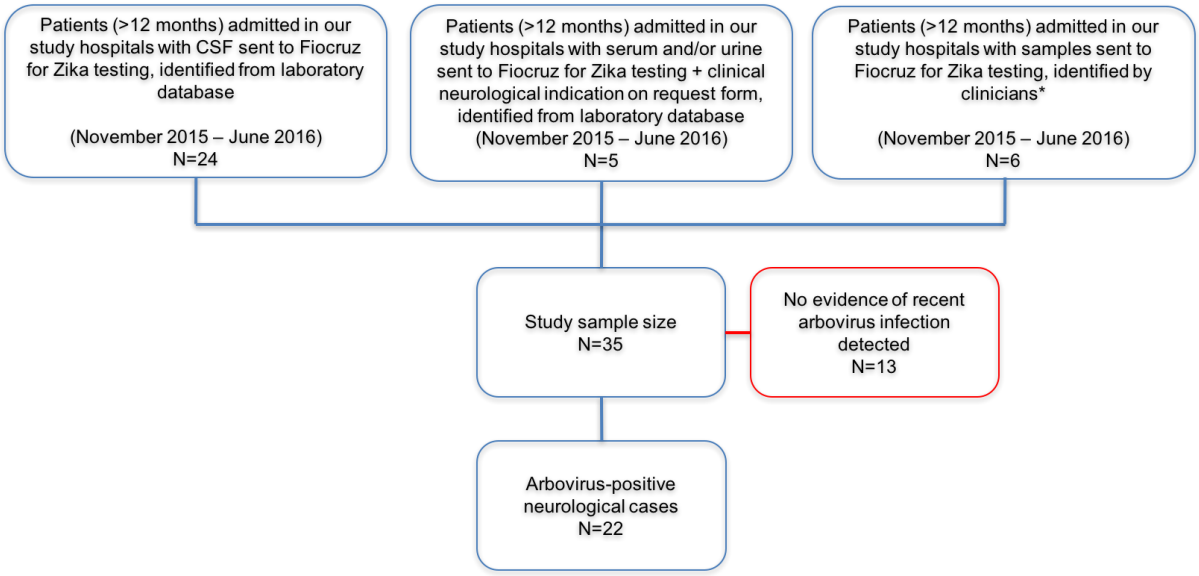
2.2 Methods

I studied patients who had developed a new neurological condition associated with suspected Zika virus infection, whose samples had been submitted to the Flavivirus Reference Laboratory of the Instituto Oswaldo Cruz (Fiocruz), Rio de Janeiro. The study protocol was approved by the Comitê de Ética em Pesquisa do Instituto Nacional de Infectologia Evandro Chagas (reference 59254116.0.1001.5262). Patient-identifying data was anonymised.

2.2.1 Study population

The study included patients admitted in 11 hospitals (appendix) in Rio de Janeiro from 1st November 2015 to 1st June 2016, who had presented with an acute neurological condition associated with a suspected Zika virus infection, as identified by fever, arthralgia or rash illness in the preceding three months. This time period was arbitrarily chosen to be able to capture as many cases as possible of arboviral-associated neurological disease, whilst at the same time not leaving too great a gap in time such that an association becomes less credible. In this evolving epidemic situation three approaches were used to identify patients: using the laboratory database, I retrospectively identified 29 patients who had had either cerebrospinal fluid (CSF) regardless of indication, or serum and/or urine in the context of neurological disease, sent to the laboratory for Zika virus diagnostics; additionally, clinicians from the study hospitals identified six further patients, whose CSF sample was not on the database and whose serum and/or urine request forms did not have an indication (Figure 4). Patients under the age of 12 months were excluded. The study itself was started at the end of June 2016, by which point four patients already had a positive Zika diagnosis via PCR performed by the reference laboratory.

Figure 4: Study population of patients with neurological disease associated with suspected Zika virus infection.



*These patients did not appear in the laboratory database search because their CSF sample was not recorded on the database and no clinical information was included in request forms for serum and/or urine. They were identified by the clinicians who had previously managed their care in the study hospitals.

Clinical information was obtained from case notes and discussion with the patients' clinicians and documented on standardised case report forms by a member of the study team. The

information obtained included demographics, past medical history, admission history, examination, investigations, diagnosis, and management. Investigations included brain and spine imaging for patients with suspected central nervous system infection, and nerve conduction studies with or without electromyography for those with peripheral disease. Nerve conduction study results were reviewed by an independent expert neurophysiologist to ensure consistency. The Brighton criteria were used to indicate the level of certainty for diagnosing GBS and similar criteria were applied for radiculitis, encephalitis, myelitis, and meningitis (appendix). It was determined whether patients had peripheral nervous system disease (GBS, radiculitis), CNS disease (encephalitis, myelitis, meningitis) or both.

2.2.2 Laboratory testing

CSF, serum, and urine samples were tested for evidence of Zika, chikungunya, and dengue virus infection at the Fiocruz Flavivirus Laboratory. The detection of viral RNA and/or IgM-specific antibody in the CSF was considered as evidence of recent CNS infection as previously;[71] IgM antibody in the serum, or RNA in the serum or urine was taken as evidence of systemic infection. All PCR (apart from the initial four samples that were already positive at the time of starting the study) and serological testing was performed by another student, Raquel Medialdea-Carrera.

An expanded protocol based on the interim recommendations from the WHO for laboratory testing for Zika virus was followed:[92] RNA was extracted from 140µl of CSF, serum, and urine samples and eluted in 50µl using the Qiamp Mini Elute Virus Spin Kit from Qiagen (Brazil). The CSF, serum, and urine samples were tested by qRT-PCR for detection of Zika, chikungunya, and dengue virus RNA as described previously (threshold cycle [Ct] cut-off was 38 for all three viruses).[93] Controls included a negative extraction control, a negative water control and a positive control. For all three viruses, primers were used as previously (ZIKV 1086/1162c/1107-FAM; CHIKV 874/961/899-FAM; DENV1 8936/9023/8961, DENV2 1426/1482/1454, DENV3 701/749/722, DENV4 884/953/939 [4-in-1 DENV assay]).[93-95] Samples positive for chikungunya PCR were re-tested because of the unexpected result. If two positive results were found, the result was deemed positive. If the second test was indeterminate, the sample was re-extracted and re-tested, and the result was only deemed positive if the subsequent test was positive. If the second test was negative, the result was deemed negative. Serum IgM and IgG antibodies to Zika virus NS1 antigen and serum and CSF IgM and IgG antibodies to chikungunya virus were measured using commercial ELISAs (Euroimmun, Luebeck, Germany), according to the manufacturer's protocol.[96-98] CSF IgM antibodies to Zika virus were measured using a recommended capture ELISA based on the US Centers for Disease Control and Prevention (CDC) emergency use authorization

protocol (CDC Fort Collins, CO, USA).[99] Serum and CSF IgM and IgG antibodies to dengue virus were measured using commercial ELISAs (Panbio, Brazil). For serum samples with sufficient volume remaining, anti-ganglioside antibodies, which are associated with GBS and other autoimmune neuropathies, were tested by ELISA (Bühlmann-Gangliocombi, Schönenbuch, Switzerland) following the manufacturer's instructions.

2.2.3 Statistical analysis

The median time from illness onset to the development of neurological symptoms was compared for those with CNS and peripheral nervous system disease, and for those with or without CNS Zika virus infection, using the Wilcoxon-Mann-Whitney U-test.

2.3 Results

The study identified 35 patients with new neurological disease associated with a suspected Zika virus infection. Evidence for recent arbovirus infection was found in 22 (63%) of them. Table 2 and Figure 5 show the virological diagnosis for each patient, taking into account any potential serological cross-reactivity between flaviviruses.

Table 2: Virological evidence for Zika, chikungunya and/or dengue virus infection in 22 patients presenting with acute neurological disease, ordered by date of admission.

Patient	Days between infection and sample collection			Zika					Chikungunya					Dengue					Other CSF investigations	Virological Diagnosis
	CSF	Serum	Urine	CSF PCR	CSF IgM	Serum PCR	Serum IgM	Urine PCR	CSF PCR	CSF IgM	Serum PCR	Serum IgM	Urine PCR	CSF PCR	CSF IgM	Serum PCR	Serum IgM	Urine PCR		
1*	11	11	11	-	+	-	-	+	-	-	-	-	-	-	+	-	+	-	Neg: MCS, HSV, VDRL, CRAG	Zika-CNS +/- Dengue-CNS †
2	25	25	25	-	+	-	-	-	-	-	-	+	-	-	+	-	+	-	na	Zika-CNS or Dengue-CNS or both, Chik-Syst
3	4	6	4	-	+	+	-	+	-	-	-	-	-	-	-	-	+	-	Neg: HSV	Zika-CNS +/- Dengue-Syst †
4	20	20	na	+	+	-	-	na	+	-	+	-	na	-	-	-	-	na	Neg: MCS	Zika/Chik-CNS
5	23	20	20	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	Neg: MCS, VDRL	Zika-CNS
6	na	33	33	na	na	-	-	-	na	na	-	+	-	na	na	-	+	-	Neg: MCS, VDRL	Chik/Dengue-Syst
7	18	18	19	-	+	-	+	-	-	-	-	-	-	-	-	-	+	-	na	Zika-CNS or Dengue-Syst or both
8	29	29	29	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	Neg: MCS	Chik-CNS
9	10	na	na	-	+	na	na	na	+	+	na	na	na	-	-	na	na	na	Neg: HSV	Zika/Chik-CNS
10	15	15	70	-	+	-	+	+	-	-	-	-	-	-	na	-	-	-	Neg: MCS	Zika-CNS
11	26	26	26	-	-	-	-	-	+	+	-	+	+	-	-	-	-	-	Neg: MCS, VDRL	Chik-CNS
12	12	na	12	-	-	na	na	-	+	+	na	na	-	-	-	na	na	-	na	Chik-CNS
13	6	9	9	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	Neg: MCS, VDRL, CRAG, CMV/VZV/HSV	Chik-CNS
14	31	na	na	-	-	na	na	na	+	-	na	na	na	-	-	na	na	na	na	Chik-CNS
15	7	na	na	-	-	na	na	na	+	-	na	na	na	-	-	na	na	na	Neg: HSV	Chik-CNS
16	23	na	23	+	+	na	na	-	+	+	na	na	-	-	-	na	na	-	na	Zika/Chik-CNS
17	na	71	72	na	na	-	-	+	na	na	+	-	-	na	na	-	-	-	Pos: VDRL	Zika/Chik-Syst
18	23	na	23	-	-	na	na	+	+	+	na	na	-	-	-	na	na	-	Neg: HSV	Chik-CNS, Zika-Syst
19	13	13	13	-	-	-	-	-	+	+	+	+	-	-	-	-	+	-	na	Chik-CNS, Dengue-Syst
20	35	25	36	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	na	Chik-Syst
21	72	72	72	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	Neg: MCS, VDRL	Chik-Syst
22	66	60	60	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	na	Zika-CNS

Key: "+" =positive, "-" =negative, "na" =sample not available or inadequate volume; PCR=polymerase chain reaction; CSF=cerebrospinal fluid; MCS=microscopy, culture and sensitivity; HSV=herpes simplex virus, CMV=cytomegalovirus, VZV=varicella zoster virus, VDRL=venereal disease research laboratory (syphilis), CRAG=cryptococcal antigen, Chik=chikungunya; CNS=virus detected in central nervous system, Syst=virus detected systemically (i.e. outside CNS) only. Zika virus PCR primers used: 1086-1102, 1107-1137.[93] See Table 3 for antibody levels and IgG results. *Preliminary information for this patient has previously been published. †These patients had PCR evidence of Zika virus infection, with serological evidence of dengue infection potentially secondary to cross-reactivity.

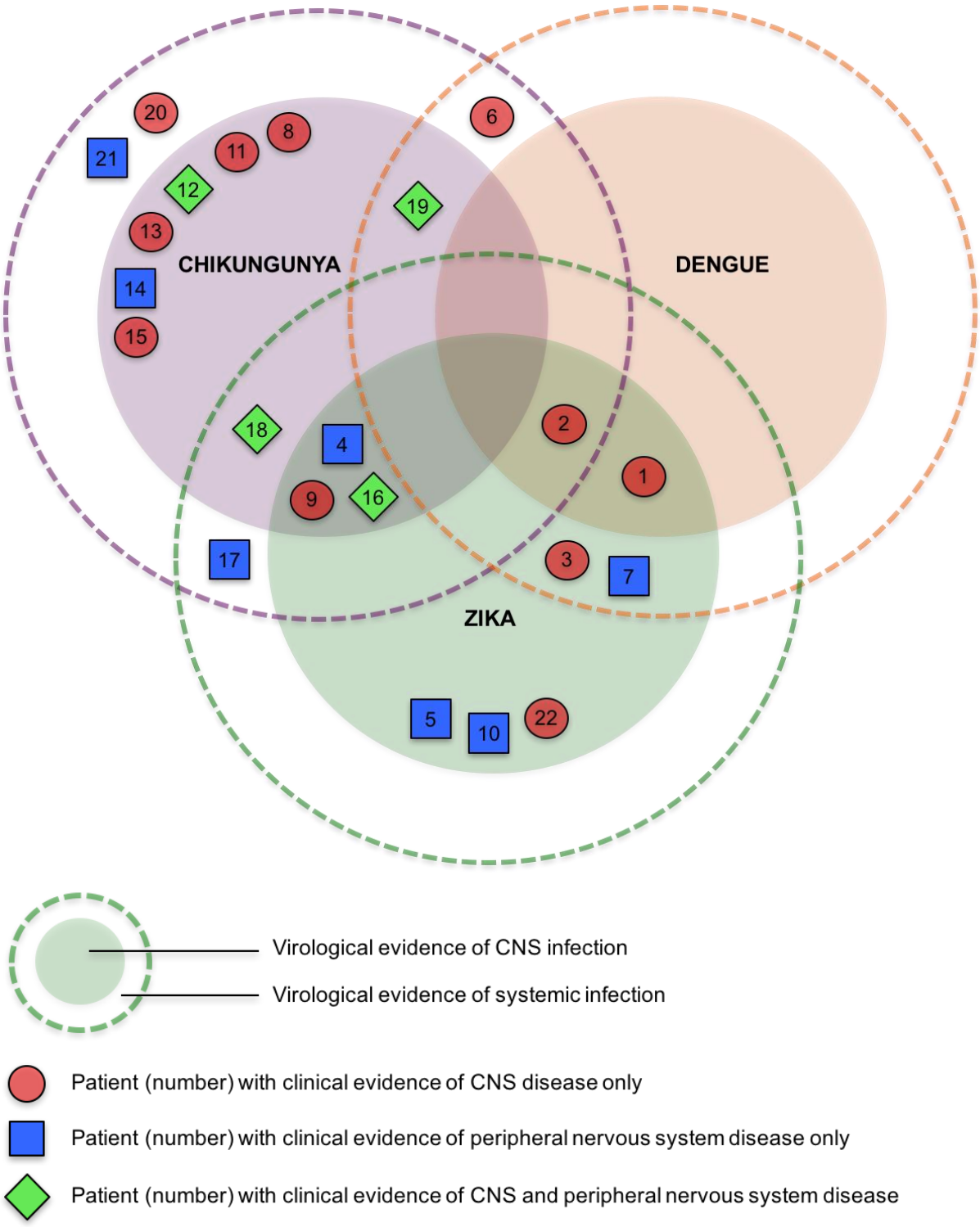
Table 3: Immunological assay values for 22 patients presenting with arboviral-associated neurological disease, ordered by date of admission.

Patient	Days between infection and sample collection			Zika				Chikungunya				Dengue			
	CSF	Serum	Urine	CSF IgM (P/N)	CSF IgG (RU/ml)	Serum IgM (Ratio)	Serum IgG (RU/ml)	CSF IgM (Ratio)	CSF IgG (RU/ml)	Serum IgM (Ratio)	Serum IgG (RU/ml)	CSF IgM (PU)	CSF IgG (PU)	Serum IgM (PU)	Serum IgG (PU)
1	11	11	11	5·5	-	-	111·5	-	-	-	-	21·1	63·2	23·8	60·5
2	25	25	25	35·6	-	-	>200	-	-	1·2	-	17·7	16·0	43·6	62·7
3	4	6	4	7·1	-	-	>200	-	-	-	-	-	21·8	13·7	66·2
4	20	20	na	3·1	40·3	-	>200	-	-	-	-	-	15·1	-	69·4
5	23	20	20	27·7	-	-	>200	-	-	-	-	-	na	-	60·5
6	na	33	33	na	na	-	>200	na	na	1·2	124·0	na	na	47·7	65·0
7	18	18	19	41·1	369·3	1·2	55·4	-	-	-	-	-	21·6	12·2	62·3
8	29	29	29	-	-	-	>200	-	-	-	-	-	-	-	57·1
9	10	na	na	8·7	155·8	na	na	2·5	92·0	na	na	-	-	na	na
10	15	15	70	25·1	na	1·5	na	-	na	-	-	na	na	-	55·2
11	26	26	26	-	-	-	114·3	1·1	-	1·6	-	-	32·6	-	57·7
12	12	na	12	-	-	na	na	3·1	75·8	na	na	-	23·8	na	na
13	6	9	9	-	-	-	40·6	-	na	6·9	73·2	-	na	-	60·0
14	31	na	na	-	-	na	na	-	-	na	na	-	-	na	na
15	7	na	na	-	-	na	na	-	-	na	na	-	-	na	na
16	23	na	23	3·5	67·5	na	na	3·0	56·3	na	na	-	17·1	na	na
17	na	71	72	na	na	-	22·0	na	na	-	-	na	na	-	59·3
18	23	na	23	-	37·2	na	-	1·5	35·9	na	na	-	16·0	na	na
19	13	13	13	-	151·6	-	>200	1·8	58·4	4·4	112·5	-	25·3	14·1	64·5
20	35	25	36	-	-	-	-	-	-	6·8	89·2	-	-	-	53·6
21	72	72	72	-	-	-	86·4	-	-	2·1	-	-	-	-	54·3
22	66	60	60	4·5	-	-	24·6	-	-	-	-	-	-	-	65·7

Key:

"na" =sample not available/not enough volume; "-" =negative; CSF=cerebrospinal fluid. Units and cut-off values: Zika CSF IgM (P/N = ratio of patient optical density to negative control value); Pos: P/N >3. Zika serum IgM and chikungunya IgM (ratio = patient sample/cut-off value); Pos: ratio ≥1·1. Zika IgG and Chikungunya IgG (relative units/ml, RU/ml as described by the manufacturer); Pos: RU/ml ≥22. Dengue IgM and IgG (Panbio Units, PU = ratio of patient optical density to cut-off value x10); Pos: PU >11.

Figure 5: Venn diagram for 22 patients showing virological evidence of CNS or systemic infection with Zika, chikungunya and/or dengue, and clinical presentation with CNS or peripheral nervous system disease.



One can distinguish *virological* evidence of CNS or systemic infection (based on PCR/antibody detection in CSF or serum/urine, respectively) from *clinical* evidence of CNS or peripheral nervous system disease (based on clinical features). Patients in the inner darker circles have evidence of CNS +/- systemic infection with the respective virus. Those in the outer paler circles have evidence of only systemic infection with the respective virus. Note that patients 1 and 3 had confirmed Zika, +/- dengue; patients 2 and 7 had Zika or dengue or both.

Twelve (34%) had evidence of Zika virus infection. Three (9%) with evidence of Zika virus infection alone presented with GBS (two patients) or encephalitis (one). Nine (26%) with evidence of Zika also had evidence for another arbovirus; five with chikungunya, three with dengue, one with both chikungunya and dengue. Three of them presented with GBS, including one with the facial diplegia and paraesthesia variant, and six had CNS infection, predominantly encephalitis and/or myelitis. Ten patients (29%) negative for Zika had evidence for another arboviral infection; eight with chikungunya and two with chikungunya and dengue. Two of these presented with GBS and eight with CNS disease.
The remaining 13 patients (37%), with no evidence of a recent Zika, chikungunya, or dengue virus infection, presented with GBS (six patients) or CNS disease (seven). Their diagnoses are given in the appendix, but they are not considered further here.

2.3.1 Clinical features

All 22 patients with evidence of recent arbovirus infection were Brazilian nationals with no recent travel history, from 18 different districts of Rio de Janeiro. The median (range) age was 51·5 (17-84) years. The male:female ratio was 1:1. Their individual clinical features are summarised in Table 4 and a detailed example of a clinical case, patient 9, is described in the Panel.

Table 4: Individual clinical features of 22 patients presenting with neurological disease associated with Zika, chikungunya and/or dengue virus infection, ordered by date of admission.

Patient	Virological Diagnosis	Systemic Features	Prodrome Length (days)	Neurological Features*	CSF WCC	CSF Protein	Anti-ganglioside Antibody	Neurological Diagnosis (levels of certainty)	Management	Progress and Outcome
1 (F/47)	Zika-CNS +/- Dengue-CNS	Rash, arthralgia, malaise WCC 8·0	4	Confusion, dysarthria, drowsiness; paraparesis; GCS 3; CT head - diffuse white matter hypodensities	10	1·11	GM1	Encephalitis (I)	Mannitol	ICU; intubated; patient rapidly deteriorated and died
2 (F/59)	Zika-CNS or Dengue-CNS or both, Chik-Syst	Fever, rash, arthralgia WCC 8·1	7	UL and LL paraesthesia; spastic quadraparesis; extensor plantars; impaired UL and LL LT, PP, Vi, Pr; urinary retention; GCS 15; MRI spine – intramedullary signal abnormality involving cervical and thoracic cord; MRI brain - normal; NP - normal	4	0·36	GM1, GD1a, GD1b	Myelitis (I)	IVIG; steroids x 2	Developed pulmonary oedema on IVIG; responded to steroids, mRS 3 at 4 months
3 (M/26)	Zika-CNS +/- Dengue-Syst	Fever	1	Confusion; truncal, UL and LL paraesthesia and numbness; spastic hyperreflexic quadraparesis; T5 sensory level; LMN facial nerve and supranuclear gaze palsies; impaired UL and LL LT, PP, Vi, Pr; urinary incontinence; GCS 13; MRI brain and spine - signal abnormality involving cerebellar peduncles, medulla and intramedullary cervical cord	100	1·12	GM1, GD1a, GD1b	Encephalo-myelitis (I,I)	IVIG; steroids	ICU; intubated; improved, mRS 1 at 4 months
4 (M/34)	Zika/Chik-CNS	Rash	12	UL and LL paraesthesia; mild ataxia; bilateral LMN facial nerve palsy; hyperreflexic LL; GCS 15; MRI brain - gadolinium enhancement of bilateral facial nerves; NP - normal	2	0·69	-	GBS variant (facial diplegia with paraesthesia)	IVIG	Improved, full recovery at 2 months
5 (F/41)	Zika-CNS	Fever, rash, malaise WCC 21·8	5	LL and peri-orbital paraesthesia; normotonic areflexic paraparesis; impaired LL LT, PP, Vi; GCS 15; CT brain - normal	0	2·07	na	GBS (II)	IVIG	Improved (extent unknown)
6 (F/30)	Chik/Dengue-Syst	Fever, rash, malaise WCC 9·3	27	LL paraesthesia; normoreflexic paraparesis; urinary retention; GCS 15; MRI brain and spine - normal; NP - normal	0	0·21	-	Myelitis (II)	IVIG; steroids	Improved (extent unknown)
7 (F/66)	Zika-CNS or Dengue-Syst or both	Fever, arthralgia, malaise WCC 7·7	13	UL and LL paraesthesia; flaccid areflexic quadraparesis; bilateral LMN facial nerve palsy; impaired UL and LL LT, PP, Vi; GCS 15; NP - AMSAN	0	0·95	GD1a	GBS (I)	IVIG	ICU; intubated; improved at 1 week (extent unknown)
8 (M/20)	Chik-CNS	Fever WCC 15·4	0	R UL and LL paraesthesia and triparesis (L UL spared), hyperreflexic LL; impaired LL LT, PP, Vi, Pr; C6 sensory level; urinary retention; GCS 15; MRI spine - signal abnormality involving cervical and thoracic cord; MRI brain normal; NP - normal	0	0·29	-	Myelitis (I)	Steroids x 2	Improved, mRS 2 at 3 weeks
9 (F/80)	Zika/Chik-CNS	Fever, rash, arthralgia, malaise PLT 134 WCC 7·5	5	Headache, confusion; flaccid hyporeflexic quadraparesis; GCS 14; MRI brain and spine - signal abnormality involving anterior medulla, anterior cervical and thoracic cord, temporal lobes, amygdala, small area adjacent to temporal horn of L lateral ventricle, pachymeningeal enhancement	117	1·74	na	Encephalo-myelitis (I,I) with subclinical meningitis	IVIG	ICU; developed sacral osteomyelitis; improved, mRS 4 at 2 months
10 (M/38)	Zika-CNS	Fever, rash, malaise PLT 220 WCC 14·0	10	LL paraesthesia; flaccid areflexic quadraparesis; L LMN facial nerve palsy; impaired LL Pr; GCS 15	1	1·72	-	GBS (II)	IVIG; antivirals	ICU; intubated, ventilator-associated pneumonia; improved (extent unknown)
11 (M/76)	Chik-CNS	Rash, arthralgia PLT 254 WCC 13·0	0	2 seizures; confusion, dysarthria, headache, neck stiffness; spastic paraparesis; extensor plantars, palmomental reflex;	80	1·45	GD1a	Meningo-encephalo-myelitis (III,I,III)	Antivirals; antibiotics	Unknown outcome

				LL neuropathic pain; T2-3 sensory level; urinary incontinence; GCS 14						
12 (M/63)	Chik-CNS	Fever, rash, arthralgia, malaise PLT 116 WCC 6·5	2	LL paraesthesia; flaccid areflexic paraperesis; T4 sensory level; urinary retention; fell with intracranial injury; GCS 15 on admission, 12 after fall; CT brain normal, MRI brain and spine normal; NP - AMSAN	0	0·92	-	Myeloradiculitis (II)	IVIG	ICU, intubated (after fall and head injury); no improvement; mRS 5 at 2 months
13 (F/51)	Chik-CNS	Fever, arthralgia, malaise	6	Confusion, 1 x seizure, drowsiness, dysarthria; GCS 3; CT head normal	11	0·45	GQ1b	Encephalitis (I)	Antivirals	Improved, full recovery at 2 months
14 (M/45)	Chik-CNS	Fever, arthralgia, malaise, diarrhoea PLT 203 WCC 8·7	29	UL and LL paraesthesia; flaccid areflexic quadraparesis; impaired UL and LL LT, PP; GCS 15	0	0·75	na	GBS (II)	IVIG	ICU; improved mRS 3
15 (M/84)	Chik-CNS	Fever, rash, arthralgia, malaise, diarrhoea PLT 80 WCC 16·3	4	Confusion, impaired speech and swallow; flaccid hyporeflexic quadraparesis; myalgia; GCS 8; MRI brain - focal areas of hyperintensity likely related to microangiopathy; NP - inflammatory myopathy	42	1·11	na	Encephalitis (I), Myositis	IVIG; antivirals; antibiotics; antifungals	Ventilator-associated pneumonia; no improvement, mRS 5 at 6 weeks
16 (M/65)	Zika/Chik-CNS	Fever, rash, arthralgia, malaise PLT 130 WCC 9·9	0	LL paraesthesia; flaccid areflexic paraparesis; T11 sensory level; impaired LL sensation LT, PP, Vi, Pr; urinary retention; GCS 15; MRI brain and spine normal; NP - AMSAN	10	1·08	na	Myeloradiculitis (I)	IVIG; steroids	No improvement at 3 weeks
17 (F/19)	Zika/Chik-Syst	Rash PLT 537 WCC 9·0	41	UL and LL paraesthesia; quadraparesis, hyporeflexic LL; GCS 15; CT brain normal; NP - AMAN; patient 19 weeks pregnant at admission, foetus diagnosed with Dandy-Walker syndrome	2	0·23	-	GBS (II)	IVIG	No improvement at 3 weeks
18 (F/56)	Chik-CNS, Zika-Syst	Fever, rash, malaise PLT 340 WCC 15·0	4	LL paraesthesia; flaccid areflexic quadraparesis; impaired UL & LL LT, PP, Vi, Pr; C7 sensory level; urinary retention; GCS 15; MRI brain and spine normal; NP - AMSAN	0	0·71	na	Myeloradiculitis (II)	IVIG; steroids	No improvement, mRS 5 at 1 month
19 (M/62)	Chik-CNS, Dengue-Syst	Fever, rash PLT 680 WCC 18·8	8	LL paraesthesia; normotonic hyperreflexic paraplegia; extensor plantars; impaired LL Vi; T6-8 sensory level; urinary incontinence; GCS 15; CT brain normal, MRI brain and spine normal; NP - AIDP	16	1·09	-	Myeloradiculitis (I)	IVIG; steroids	Improved, mRS 2 at 1 month
20 (M/17)	Chik-Syst	Fever, rash PLT 362 WCC 6·3	16	L sided numbness (LL, UL, truncal); L hemiparesis; L UMN facial nerve palsy; headache; impaired L sided UL & LL LT, PP; GCS 15; MRI brain - demyelinating lesions R parietal lobe & thalamus consistent with ADEM	na	0·44	na	ADEM	IVIG; steroids	Improved, mRS 2 at 3 weeks
21 (F/67)	Chik-Syst	Fever, arthralgia PLT 397 WCC 8·9	7	Flaccid areflexic quadraparesis; dysphagia; palatal weakness; dyspnoea; impaired UL & LL Pr, LL Vi; GCS 15; CT brain normal; NP - AMSAN	10	0·71	-	GBS (I)	IVIG x 2	ICU; intubated; no improvement, mRS 5 at 2 weeks
22 (F/52)	Zika-CNS	Fever PLT 412 WCC 10·2	0	LL and R facial numbness, impaired co-ordination; headache, diplopia; GCS 15; MRI brain - signal abnormality involving frontal and parietal lobes, pons and right cerebellar peduncle	6	0·37	GD1a	Encephalitis**	Steroids	Improved, mRS 0 at 2 months

Key: Prodrome length=interval between the systemic features of disease and onset of neurological illness; Chik=chikungunya; CNS=virus detected in central nervous system, Syst=virus detected systemically (i.e. outside CNS) only; PLT=platelet count $x10^9$/L, WCC=white cell count (systemic $x10^9$/L, CSF /µL); L=left, R=right, LL=lower limb, UL=upper limb, CSF=cerebrospinal fluid, LMN=lower motor neuron, UMN=upper motor neuron, LT=light touch, PP=pinprick, Vi=vibration, Pr=proprioception, GCS=Glasgow coma scale; MRI=magnetic resonance imaging, CT=computed tomography, NP=neurophysiology; AMSAN=acute motor and sensory axonal neuropathy; AMAN=acute motor axonal neuropathy; AIDP= acute inflammatory demyelinating polyneuropathy; GBS=Guillain-Barré syndrome, ADEM=acute disseminated encephalomyelitis; IVIG=intravenous immunoglobulin; ICU=intensive care unit admission, mRS=modified Rankin Scale; "na" =not available. For neurological diagnoses the levels of diagnostic certainty are indicated I-III (highest to lowest), as per the Brighton and other criteria (appendix). The time post-onset of neurological symptoms is given for outcomes, where known. *Note the different neurological features presented at different times, e.g. patient 8 initially presented with sensory symptoms, which progressed after two days to triparesis); **Although the GCS score was 15, the clinical features and imaging indicated focal encephalitis.

Panel: Clinical presentation of encephalomyelitis (with subclinical meningitis) associated with Zika and chikungunya virus infections (patient 9).

An 80-year-old Brazilian female with a past medical history of hypertension, obesity and osteoarthritis presented with a one-day history of headache, confusion, and symmetrical weakness in all four limbs. Five days earlier, she had fever, rash, arthralgia and general malaise. There was no preceding history of respiratory or gastrointestinal infection, or recent vaccination, and she did not have any bowel or bladder symptoms.

On examination, she had a diffuse, centrifugal, erythematous rash, with mild joint swelling of the knees. She was mildly confused with a Glasgow Coma Scale of 14. She had a flaccid tetraparesis, worse proximally (Medical Research Council grade 1 in all four limbs) than distally (Medical Research Council grade 3 in all four limbs). Her reflexes were present but depressed throughout. As far as assessment would allow, light touch, vibration, temperature and proprioception appeared normal. There was no sensory level apparent, or cranial nerve involvement.

A lumbar puncture revealed a predominantly lymphocytic CSF pleocytosis of 117 leucocytes/µl, elevated protein of 1.74 g/dL and glucose of 3.9 mmol/L. PCR for HSV 1 and 2 was negative. She had a mild thrombocytopenia of 134 x 10^9/L and raised creatinine of 160 µmol/L. An MRI scan of her brain and spine showed signal abnormality involving the anterior medulla, anterior cervical and thoracic cord, temporal lobes, amygdala and a small area adjacent to temporal horn of her left lateral ventricle, with pachymeningeal enhancement (Figure 6C, D, E). On the basis of the clinical and imaging findings she was diagnosed with encephalomyelitis (with subclinical meningitis) and admitted to the intensive care unit, where her upper limb power marginally improved without treatment. A course of intravenous immunoglobulin therapy did not have any further effect. The patient was discharged to a care home and despite physiotherapy was still unable to walk after seven months.
Her CSF was positive by IgM ELISAs for Zika and chikungunya viruses. PCR was also positive for chikungunya, but not Zika. There was with no evidence of dengue virus infection.

The initial symptoms of arboviral infection included fever (82% of the patients), rash (68%), malaise (55%) and arthralgia (50%). Data was available regarding when these initial symptoms resolved for 11 of 22 patients only (patients 4, 6, 12, 14, 15, 16, 17, 18. 19, 20, 22) – of these, in four patients (12, 15, 16, 22), the neurological symptoms started during or the period of initial symptoms of infection, and in the other seven patients, the neurological

symptoms started (days) after the end of the initial symptoms of infection. Two patients had diarrhoea in the month preceding their neurological illness (Table 4); no patient reported a preceding lower respiratory tract infection or conjunctivitis. Five (patients 11, 13, 15, 16, 22) reported previous dengue. One (patient 22) reported prior vaccination against yellow fever. The median (range) time between infective symptoms and onset of neurological disease was 6·5 (0-41) days. Patients presented with a range of neurological syndromes affecting the CNS, peripheral nervous system, or both, as detailed in Table 4.

The seven patients with GBS all had a preceding febrile and/or rash syndrome, which was a median (range) 12 (5-41) days before the neurological presentation; there was no statistically significant difference in prodrome length between those with and without CNS Zika infection. The presentations for six patients were similar - typically paraesthesia (five patients) with a rapidly ascending symmetrical flaccid paralysis, involving all four limbs in five patients, or the legs only in one (patient 5). Another (patient 4) presented with a GBS variant, with bilateral lower motor neuron facial nerve palsies and paraesthesia in all four limbs. Fifteen patients presented with CNS disease, including encephalitis and/or myelitis, with or without involvement of the meninges or peripheral nerves. All had a febrile or rash syndrome, many with arthralgia and malaise. The median (range) time delay between this systemic illness and neurological disease was 4 (0-27) days; for these patients with CNS disease, there was no statistically significant difference in prodrome length between those with and without CNS Zika infection.

The eight patients with encephalitis (with or without other CNS disease) had a median (range) Glasgow Coma Scale score of 13·5 (3-15); six were confused, and two had seizures. One patient had a supranuclear gaze palsy; two had facial weakness and four had difficulties with speech or swallowing. The ten patients with myelitis (with or without encephalitis) comprised five with paraparesis (one spastic, two flaccid, and two with normal tone), four with quadraparesis (two spastic, two flaccid) and one with a triparesis. Seven of these patients had a sensory level, six had urinary retention and three had urinary incontinence. Three patients with flaccid paresis had signs and symptoms compatible with transverse myelitis, namely a sensory level and urinary retention. However, one patient with encephalomyelitis with flaccid areflexic quadraparesis (patient 9) had extensive imaging changes in the anterior of the cord consistent with poliomyelitis-like anterior horn cell damage. Neurophysiological studies (see below) confirmed the involvement of lower motor neurons for four patients with myelitis: two of those with flaccid paraparesis, one with flaccid quadraparesis, and one with paraparesis and normal tone. The clinical characteristics of the

patients with central, peripheral, and mixed nervous system disease are compared in Table 5.

Table 5: Clinical characteristics of 22 patients presenting with neurological disease associated with Zika, chikungunya and/or dengue virus infection.

	n (%) or median (IQR)			
	All patients (n=22)	**CNS disease (n=11)**	**PNS disease (n=7)**	**CNS & PNS disease (n=4)**
Age (years)	51·5 (35-64·5)	51 (28-67·5)	41 (35-55·5)	62·5 (60·5-63·5)
Males	11 (50%)	5 (45%)	3 (43%)	3 (75%)
Previous yellow fever vaccination (of 12 patients)	1 (8%)	1 of 4 (25%)	0 of 4 (0%)	0 (0%)
Previous dengue (of 20 patients)	5 (25%)	4 (36%)	0 of 5 (0%)	1 of 3 (33%)
Co-morbidity (of 20 patients)	10 (50%)	5 (45%)	2 of 6 (33%)	3 of 3 (100%)
Diabetes mellitus (type II)	2 (10%)	2 (18%)	0 of 6 (0%)	0 of 3 (0%)
Stroke	2 (10%)	0 (0%)	2 of 6 (33%)	0 of 3 (0%)
Hypertension	6 (30%)	3 (27%)	0 of 6 (0%)	3 of 3 (100%)
Hypercholesterolaemia	2 (10%)	1 (9%)	1 of 6 (17%)	0 of 3 (0%)
Cancer	2 (10%)	1 (9%)	0 of 6 (0%)	1 of 3 (33%)
Asthma	1 (5%)	1 (9%)	0 of 6 (0%)	0 of 3 (0%)
Cardiac disease	1 (5%)	0 (0%)	1 of 6 (17%)	0 of 3 (0%)
Neurological disease (Tourette's syndrome)	1 (5%)	1 (9%)	0 of 6 (0%)	0 of 3 (0%)
Systemic features				
Fever	18 (82%)	9 (82%)	5 (71%)	4 (100%)
Rash	15 (68%)	7 (64%)	4 (57%)	4 (100%)
Arthralgia	11 (50%)	6 (55%)	3 (43%)	2 (50%)
Malaise	12 (55%)	5 (45%)	4 (57%)	3 (75%)
Diarrhoea	2 (9%)	1 (9%)	1 (14%)	0 (0%)
Prodrome length (days)	5·5 (2·5-11·5)	4 (0·5-6·5)	12 (8·5-21)	3 (1·5-5)
Neurological symptoms				
Weakness	19 (86%)	9 (82%)	6 (86%)	4 (100%)
Sensory disturbance	17 (77%)	7 (64%)	6 (86%)	4 (100%)
Neurological examination (of 21 patients)				
Cranial nerve involvement	6 (29%)	2 of 10 (20%)	4 (57%)	0 (0%)
Sensory level	8 (38%)	4 of 10 (40%)	0 (0%)	4 (100%)
GCS<15 on admission	8 (38%)	6 of 10 (60%)	1 (14%)	1 (25%)
Diminished or absent reflexes	11 (52%)	2 of 10 (20%)	6 (86%)	3 (75%)
Brisk reflexes	6 (29%)	4 of 10 (40%)	1 (14%)	1 (25%)
Lumbar puncture results				
CSF white cell count (of 21 patients; cells/μL)	4 (0-11)	10·5 (4·5-70·5)	1 (0-2)	5 (0-11·5)
CSF protein (g/dL)	83·5 (44·25-111)	45 (36·4-111·5)	75 (70-133.2)	100 (86·8-108·3)
Treatment				
IVIG	17 (77%)	6 (55%)	7 (100%)	4 (100%)
Steroids	9 (41%)	6 (55%)	0 (0%)	3 (75%)
Outcome				
Responded to treatment (of 21 patients)	14 (67%)	7 of 10 (70%)	5 (71%)	2 (50%)
Admitted to ICU	8 (36%)	3 (27%)	4 (57%)	1 (25%)
Intubated	6 of 21 (29%)	2 of 10 (20%)	3 (43%)	1 (25%)
Died	1 (5%)	1 (9%)	0 (0%)	0 (0%)

Key: CNS=central nervous system; PNS=peripheral nervous system. For certain parameters I did not have data for all patients; the number of patients for whom data was available is indicated in brackets.

2.3.2 Virology and serology

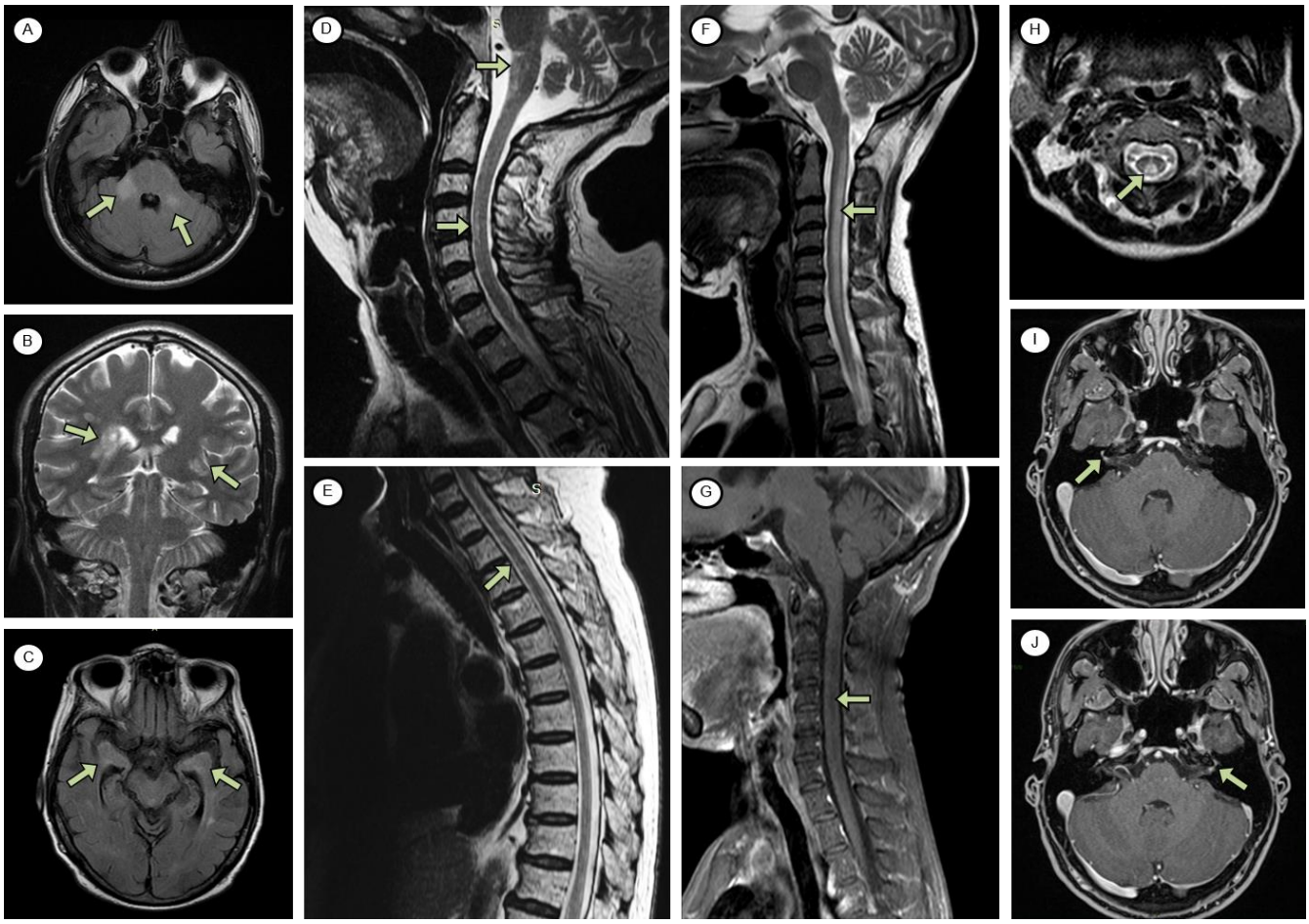
Zika virus RNA was detected in two CSF, one serum and six urine samples (from eight patients); Zika IgM was detected in 10 CSF and two serum samples (10 patients). Chikungunya virus RNA was detected in 11 CSF, six serum, and three urine samples (14 patients); chikungunya IgM was detected in six CSF and seven serum samples (11 patients). Dengue virus RNA was not found; dengue IgM was found in two CSF and six serum samples (six patients).

As expected, many patients had serum IgG against dengue virus, consistent with prior exposure (appendix). Of the 13 patients with no evidence of recent arbovirus infection, serum Zika virus IgG was detected in four. Anti-GM1, GD1a, GD1b and GQ1b antibodies were found in the serum of patients with both peripheral and central nervous system disease (Table 4).

2.3.3 Investigations

Ten out of the 22 patients had a CSF pleocytosis (≥5/μL, all showing predominantly lymphocytes/monocytes) and 15 had a CSF protein above 0·45 g/L. Four patients had a mild thrombocytopenia and seven had a peripheral leucocytosis, but none had leucopenia. Imaging studies are detailed in Table 4, with examples shown in Figure 6. High signal changes were found in the cervical (four patients) and thoracic (two) cord, brainstem (two), cerebellar peduncles (two) and cortex (three); demyelination of the parietal cortex and thalamus was reported in patient 20 and pachymeningeal enhancement in patient 9. Three different neurophysiological patterns were seen in patients with peripheral involvement - acute motor axonal neuropathy (AMAN), acute motor and sensory axonal neuropathy (AMSAN) and acute inflammatory demyelinating polyneuropathy (AIDP) (Table 6). AMAN and AMSAN were seen in patients both with and without probable Zika virus infection; AIDP was seen only in one patient with CNS chikungunya virus infection.

Figure 6: Central nervous system (CNS) imaging abnormalities in patients with evidence of Zika, chikungunya and/or dengue virus infection.



A: Encephalomyelitis in a patient with CNS Zika and systemic dengue infection (patient 3). Fluid attenuation inversion recovery [FLAIR] signal abnormality involving the middle cerebellar peduncles, more marked on the right (axial scan).

B: Acute disseminated encephalomyelitis in a patient with systemic chikungunya infection (patient 20). Confluent areas of T2 signal abnormality suggesting neuroinflammation consistent with demyelination (coronal scan).

C, D, E: Encephalomyelitis with subclinical meningitis in a patient with CNS Zika + chikungunya infection (patient 9). FLAIR signal abnormality involving the medial temporal lobes, amygdala, and a small area of abnormality adjacent to the temporal horn of the left lateral ventricle (C, axial scan). High signal intensity on T2-weighted images in the anterior medulla, and anterior cervical and thoracic cord (D and E, sagittal scans).

F, G, H: Myelitis in a patient with CNS Zika + dengue and systemic chikungunya infection (patient 2). Extensive intramedullary signal abnormality of the cervical cord, without evidence of contrast enhancement (F, sagittal T2-weighted scan; G, sagittal T1-weighted scan with gadolinium; H, axial T2-weighted scan).

I, J: Facial diplegia with paraesthesia in patient with CNS Zika + chikungunya infection (patient 4). Bilateral facial nerve enhancement on T2-weighted images with gadolinium (axial scan).

Table 6: Neurophysiology studies for patients with abnormal neurophysiology, compound muscle action potentials.

Patient number		**7**		**12**		**15**		**16**		**17**		**18**		**19**		**21**	
Neurophysiological diagnosis		**AMSAN**		**AMSAN**		**Inflammatory myopathy**		**AMSAN**		**AMAN**		**AMSAN**		**AIDP**		**AMSAN**	
Clinical diagnosis		**GBS**		**Myelo-radiculitis**		**Encephalitis + myelitis**		**Myelo-radiculitis**		**GBS**		**Myelo-radiculitis**		**Myelo-radiculitis**		**GBS**	
Right/Left		**R**	**L**	**R**	**L**	**R**	**L**	**R**	**L**	**R**	**L**	**R**	**L**	**R**	**L**	**R**	**L**
								MOTOR									
MEDIAN	Amp	2.8	7.1	4.2	3.6	Data unavailable		7.4	11.1	15.2	15	2.5	3.4	7.18	9.2	abs	0.8
	DML	5.3	0.9	3.5	3.8			3.8	3.7	3.3	3.2	4	2.8	4.9	4.5	abs	5
	CV	38.2	44.8	47	-			53.8	55.9	57.7	51.6	52.4	50.1	46.4	49	abs	44
ULNAR	Amp	4.4	-	3.7	3.7			8	9.7	11.1	10.5	3.5	5.1	10.9	11	abs	0.6
	DML	2.8	-	2.5	3.2			2.7	2.3	2.5	2.3	2.7	2.7	3.8	3.6	abs	2.9
	CV	47.2	-	50.8	52.6			46.7	54.1	61.9	60	59.5	63.3	57.4	51	abs	43.9
PERONEAL	Amp	abs	4.3	0.5	0.7			1.3	10.6	1.9	1.4	abs	abs	7.5	1.35	abs	abs
	DML	abs	8.3	5.9	5.8			4.8	4.7	5.2	5.6	abs	abs	4.7	7.4	abs	abs
	CV	abs	39	43.7	47.6			36.7	37	39.8	38.8	abs	abs	35.8	41.5	abs	abs
TIBIAL	Amp	2	0.6	2.4	-			2.1	0.8	3	3.3	-	abs	13.4	12.4	0.6	0.7
	DML	6.4	10.6	4.7	-				7.5	4.7	4.9	-	abs	6	6	6.8	7.5
	CV	33	35	51.2	-				34.4	31.6	38.8	-	abs	37.3	40.2	34.5	40.6
								SENSORY									
MEDIAN	Amp	abs	abs	abs	9.1			abs	-	30.7	46.3	2.2	6.2	3.0	5.2	abs	abs
	Onset lat	abs	abs	abs	2.4			abs	-	2.7	2.7	2.8	2.5	3.88	3.66	abs	abs
	CV	abs	abs	abs	50.6			abs	-	57	55.1	45.8	55.3	43.8	41	abs	abs
ULNAR	Amp	22.3	-	abs	abs			7.7	11.9	29.4	31.9	11.7	3.5	2.2	5.2	abs	abs
	Onset lat	1.9	-	abs	abs			2.5	2.2	2.3	2.2	2	2.2	2.94	2.8	abs	abs
	CV	56.7	-	abs	abs			45.6	50.5	51.7	55.6	54.5	53.6	40.8	42.6	abs	abs
SUPERFICIAL PERONEAL	Amp	25.1	9	-	-			-	-	5.7	11.6	abs	-	13.9	10.7	abs	abs
	Peak lat	4.2	4.1	-	-			-	-	3.2	3.5	abs	-	3.3	2.7	abs	abs
	CV	48.4	43.7	-	-			-	-	61.9	56.5	abs	-	36.4	43.2	abs	abs
SURAL	Amp	-	-	abs	1			7.5	3.3	-	-	abs	-	8	12.2	-	-
	Peak lat	-	-	abs	2.2			5.8	3.3	-	-	abs	-	4.4	3.5	-	-
	CV	-	-	abs	63.3			27.4	42.8	-	-	abs	-	31.5	39.3	-	-

Key:

Amp=distal amplitude, DML=distal motor latency, CV=conduction velocity, abs=absent, "-"=test not done, red=abnormal result

2.3.4 Outcome

Eight (36%; four with confirmed Zika) of the 22 patients required admission to an intensive care unit; six (27%) needed intubation (including half the patients with GBS). Ten patients were treated with IgG intravenous immunoglobulin (IVIG), two with corticosteroids, and seven with both; four with encephalitis received aciclovir as presumptive treatment for herpes simplex virus encephalitis. Of those treated with IVIG, fourteen had a documented start date of their IVIG course and of these, four (patients 5, 6, 7 and 10) had their serological testing done one day after commencing (the other ten had their serological tests taken before IVIG was commenced). Four patients developed hospital acquired infections, including ventilator-associated pneumonia and sacral osteomyelitis secondary to immobility. Fourteen (of 21 with outcome data, 67%; six with confirmed Zika) patients improved; one with evidence of Zika +/- dengue infection of the CNS deteriorated rapidly and died.

2.4 Discussion

With 84 countries or territories now affected by Zika virus,[100] increasing reports of associated neurological disease other than GBS,[38-41,43,44] and growing concern about coinfections with other arboviruses,[84] there is an urgent need to determine the full spectrum of Zika's neurological complications and its relationship with other arboviruses. In this study, which begins to address these questions, over half of the patients with Zika virus infection had presentations other than GBS, suggesting that these complications may be more important than recognised previously. Patients had involvement of the meninges, brain parenchyma, spinal cord, and peripheral nerves in various combinations, as evidenced by the clinical features, imaging and neurophysiological findings, and as has been described for other flaviviruses.[36]

Four patients with evidence of Zika virus infection also had CNS infection with chikungunya virus. Ten further patients negative for Zika tested positive for chikungunya. In South America, reports of neurological disease associated with chikungunya virus are scarce, which may reflect a lack of awareness among clinicians about the potential to affect the nervous system, or the relatively recent arrival of the virus. Interestingly, in one patient who was pregnant at presentation, (patient 17) RNA was detected 30 days after the onset of neurological disease, suggesting a persistent infection or a late coincidental infection. Of note, this patient also had the longest prodrome period (time between first symptoms of infection and onset of neurological disease) of the series at 41 days, with the rest of the cohort presenting at less than one month after initial symptoms; it is worth considering the possibility that this patient's pregnancy may have contributed to this delayed presentation. In

this study of patients from Rio de Janeiro, chikungunya virus was as important a cause of neurological disease as Zika virus. Its importance in other settings where Zika virus is assumed to be the cause of febrile illness, with or without neurological disease, needs to be assessed urgently.

We are only now beginning to understand the full spectrum of neurological syndromes associated with both Zika and chikungunya infections. For example, patient 19 showed clinical signs of myeloradiculitis and had neurophysiological evidence of AIDP, which is also consistent with simultaneous diagnoses of both myelopathy and a form of GBS. Another patient (15) had an unusual combination of encephalitis, cerebral microangiopathy demonstrated on MRI and inflammatory myopathy based on neurophysiological studies. We saw evidence of extensive intramedullary myelitis in some patients, and anterior myelitis in another (e.g. patients 2 and 9 respectively, Figure 6); the latter is consistent with anterior horn cell disease seen in other flavivirus infections.[101] In the French Polynesian study, neurophysiological investigations showed that all Zika patients with GBS had the AMAN subtype;[18] whereas in the Colombian study, almost all had AIDP.[19] This study additionally identified AMSAN, confirming involvement of the sensory axons in Zika virus-associated GBS.

Antigangliosides are a subtype of glycolipid that are present in the neuronal cell membrane, and are involved in cell adhesion, intracellular signalling, myelin-axon interactions and inflammation; they can be targeted by antibodies in GBS and GBS variants, which are thought to result in nerve injury via the complement cascade and/or voltage gated sodium channels.[102] In this study, one patient with AMSAN had anti-GD1a antibodies, which is more normally associated with AMAN. Anti-GD1a antibodies were also detected in Zika and chikungunya patients with central nervous system disease (Table 4). Anti-GM1, anti-GD1b, and anti-GQ1b antibodies were also found in patients with central nervous system disease. Anti-GM1 antibodies are associated with AMAN, anti-GD1b with sensory ataxic neuropathy, and anti-GQ1b with Miller Fisher syndrome and Bickerstaff's brainstem encephalitis.[103] The significance of detecting these antibodies in patients with encephalitis and myelitis is not certain. However, it is hoped that the results included in this series can be used in comparison with future studies, with the view that a higher volume of data may shed light on associations with certain neurological presentations and/or pathophysiological mechanisms of disease.

Although in this study six patients had serological evidence of recent systemic dengue virus infection, five of them also had evidence of CNS infection with a different virus, suggesting

that dengue on its own was less likely to be the cause of the neurological disease (or that some dengue results were false positives as discussed below). One might have expected to see more dengue-associated neurology, given that the virus is circulating widely in Rio de Janeiro[104] and is a well-recognised cause of neurological disease.[71] Whether this in some way reflects the fact that it has been circulating for over 30 years in this city,[90] but Zika and chikungunya viruses have been newly introduced, is not known. Alternatively, this may be due to differing neurovirulence of the three viruses.

For dengue, secondary infection is a risk factor for more severe systemic dengue disease, a phenomenon thought to be mediated by antibody-dependent enhancement.[105] Interestingly, in a recent in vitro study, plasma immune to dengue virus induced potent antibody-dependent enhancement of Zika virus.[106] In the 22 arbovirus-positive cases, all available serum samples were positive for dengue IgG antibody, indicating prior flavivirus exposure, a pattern also seen in 86% of tested patients in the Colombian report on GBS.[19] Larger prospective studies are needed to investigate whether such dengue exposure is a risk factor for developing neurological disease after Zika virus infection.

Combined infection of arboviruses has not been well described in those with neurological presentations. Patients in this series with evidence of dual infections, whether the neurological disease was caused by one arbovirus or the other, or by a combination of the two is unclear. The fact that so many patients had evidence of dual infection may indicate that combined infections are responsible for severe disease, as has been seen in other settings.[107]

Flaviviruses can cause neurological disease by attacking the nervous system directly or indirectly via immune-mediated processes; the latter tend to occur some time after the acute infection, making virological diagnosis especially challenging. Detection of virus in the CSF is usually taken as the strongest evidence of causality, but it has often cleared by the time patients present, making us reliant on detection of virus systemically or demonstration of CSF or serum IgM antibody. Of note, in this series, the higher detection rate of Zika IgM in the CSF versus serum may have been due to the greater sensitivity of the CDC ELISA used for the CSF, as compared to the Euroimmun ELISA used in the serum (evaluation data from private communication, Medialdea-Carrera, R.). An elevated CSF IgG antibody index can also help provide causal evidence, but its use in investigating Zika-associated neurological disease is currently hampered by the lack of a commercially available assay specific enough to distinguish Zika- from dengue-IgG. For both Zika and chikungunya, whether testing urine

for RNA increases the window of detection compared to serum is debated. For Zika, most studies report prolonged shedding in urine compared to serum,[108-110] however one study has reported the opposite.[76] Data for chikungunya excretion in urine is scarce, one study of 48 patients did not find prolonged shedding in the urine;[75] a separate case report, however, reported a patient in whom RNA was detected in urine but not serum 30 days after symptoms.[74] In this series, six patients (two with CNS disease) with no virus in the CSF or serum had virus detected in the urine (five Zika, one chikungunya). This suggests that testing urine in both Zika and chikungunya can be extremely useful and detect viral RNA after clearance from other body fluids, but given the issues surrounding chikungunya PCR in this study (see limitations below), future studies will have to further investigate this effect in chikungunya.

With regard to the potential mechanism by which the associated viruses may have caused neurological disease in these patients, various possibilities exist. Five patients (3, 8, 11, 16, 22) with evidence of one or more of all three viruses had a short prodromal window of 0-1 days, and all presented with CNS disease (patient 16 also had peripheral nerve involvement). The short prodrome period may lend evidence to one of three potential mechanisms: firstly, that the virus is directly neuroinvasive, infecting cells of the nervous system directly; secondly, that the virus triggers a cell-mediated response (causing damage via molecular mimicry) before its systemic symptoms surface; and thirdly, that the virus is associated with a rapid, hyperacute cell-mediated response. In the first scenario, one would expect to detect viral RNA in the CSF, found in patients 8, 11, and 16 but not in patients 3 and 22. In the second and third scenarios, one might expect to detect CSF antibody – IgM was detected in patients, 3, 11, 16 and 22 but not in patient 8. Consistent with these findings, it may be the case that different mechanisms exist for the different viruses and/or type of CNS disease. More studies are certainly needed to help further elucidate these mechanisms. With regard to GBS, there was a range of prodrome lengths from 5-41 days, consistent with the series from French Polynesia that also described a post-infectious temporal profile of disease.[18] This argues against a direct neuroinvasive pathophysiological mechanism, and for autoimmune damage potentially via molecular mimicry. Two of the seven patients (14 and 17) did not have a positive IgM detected, but had samples missing. Furthermore, whether IgG may have a part to play in this process is not known. Of note, the series from Colombia[88] reported patients with both post-infectious and para-infectious disease onsets; the latter of whom may have had disease due to one or more of the three potential pathophysiological mechanisms detailed above.

The results must be interpreted in the context of the study's limitations. First, cross-reactivity between flaviviruses makes distinguishing Zika from dengue by serological tests challenging.[18,35] Four patients (two of whom were positive for Zika by PCR) had elevated IgM antibody to both dengue and Zika, which may represent cross reactivity. Even in cases where CSF IgM was detected for Zika but not dengue virus, given the unknown specificity and sensitivity of the available flavivirus serological tests, caution must be applied. Newer assays in development and plaque reduction neutralization testing will help in the future. Differentiating infections clinically was also difficult; conjunctivitis is commonly seen in Zika[7] and not often reported for dengue or chikungunya, but I did not find conjunctivitis or any other clinical features that could distinguish the infections in this series. I did not look for West Nile virus because it had not been shown to circulate in Rio de Janeiro at the time of the study, but this may be important in other settings. In addition, regarding serology, there is a small but theoretical chance that results can be confounded by administration of IVIG before a serological test is taken. In these patients, this does not affect IgM serology, as the IVIG used was made up of polyclonal IgG only. Furthermore, only four patients (of the 14 patients in whom the date of IVIG administration was known) had serology taken (one day) after IVIG was administered, and all four had IgM detected against their respective viral infection; their diagnoses were thus not affected. Second, the IgM ELISA assays for dengue (Panbio) and chikungunya (Euroimmun) have not been validated for testing in CSF. The assay for detecting IgM against Zika in CSF has been used as a marker of CNS replication in published studies,[111,112] but it has only been 'validated' using three CSF samples.[113] Validating such assays for use with CSF samples is challenging, given that it would require one to assemble a series of Zika cases with neurological complications (cases without neurological disease would not warrant having a lumbar puncture for CSF collection). These cases would need to be virologically confirmed by PCR; this panel could then be used to determine the sensitivity and specificity of the IgM assay. Given that these cases are often not investigated during the window for PCR positivity because of the delayed timing of neurological symptoms after acute symptoms (as in several cases in this series), this makes formal validation difficult. Alternatively, PRNT could be used as the gold standard to confirm recent or past infection, but this procedure also has issues with specificity and sensitivity and would be difficult to interpret for rare sequelae following a common infection. Therefore, we are left with presuming validity of these assays in CSF, which has been used widely for neurological infections, but is a limitation of the study. One test that could be done to strengthen this presumption is to look at the IgG antibody index, as mentioned earlier, to confirm intrathecal antibody (regardless of class) production. Third, given the retrospective nature of the study, I did not have all CSF, serum and urine samples for each patient, thus

potentially under-diagnosing arboviral infections in the cohort; in addition, the timing of sample collection was not standardised. Fourth, only patients who had symptoms consistent with Zika infection were studied. Whether Zika virus can case neurological disease in patients with no febrile illness will need to be addressed in future studies. Fourth, this study included a relatively small number of patients, thus the spectrum of neurology and role of chikungunya described may be even more extensive.

A detailed look at the timing of the samples and comparison between PCR and serological data raises some issues. Due to the retrospective nature of the study, sample timing was not standardised and a there was a range of time post onset of the fever-arthralgia-rash syndrome that samples were collected (0-72 days). In some patients, RNA was detected by PCR a relatively long time after fever-arthralgia-rash syndrome. For example, cases 4, 5, 8 10, 11, 14, 16, 17, 18, 20 and 21 all have RNA detected at or over three weeks after the initial fever-arthralgia-rash syndrome. In some of these cases (5, 10 and 17), Zika virus RNA was detected in urine. As discussed, prolonged shedding of viral RNA in urine has been previously described in Zika virus infection,[76] and so this may not come as a surprise. However, regarding chikungunya, cases 4, 8, 11, 14, 16 and 18 had chikungunya RNA detected in CSF taken more than three weeks after the initial onset of systemic features. Various possibilities exist to explain this. Firstly, it is unknown whether the CNS can act as a reservoir site for chikungunya virus, as may be the case for other viruses such as HIV,[114] which could lead to prolonged viral replication and RNA shedding. However, in these cases, as well as cases 17 and 20 with late detection of chikungunya RNA in serum, one would expect to see a positive corresponding serological response, with detection of either chikungunya-specific IgM or IgG antibodies. This is present in cases 11, 16, 18 and 20 but not in cases 4, 8, 14 and 17, whose samples were negative for IgM or IgG in the available samples (case 14 did not have serum available, making further interpretation difficult). Various explanations exist for these inconsistencies: firstly, these may represent false positive chikungunya PCR results. Various steps were taken to try and avoid this, including using negative extraction and water controls during the PCR process and using separate rooms for extraction and amplification; however, contamination and false positive PCR results are commonplace in laboratories, and this cannot be completely excluded. Another explanation could be that we are seeing false negative results in serological testing; the sensitivities of the chikungunya Euroimmun IgM and IgG assays were reported as 85% and 88% respectively in an evaluation.[98] Thirdly, although an unlikely coincidence, there remains a small chance that patients may have been infected with chikungunya in between the initial fever-arthralgia-rash syndrome (due, therefore, to another pathogen) and sample collection.

Does this affect the conclusions? If one were to assume the first explanation, discarding the chikungunya PCR results and relying on serology alone, the virological diagnoses would change in patients 4, 8, 14, 15 and 17. This would leave patients 2, 6, 9, 11, 12, 13, 16, 18, 19, 20, and 21 with a positive, recent infection with chikungunya preceding their neurological presentation. This subset of patients includes patients with CNS, peripheral nervous system and mixed CNS and peripheral nervous system disease, thus not affecting the conclusion regarding the unexpected association of chikungunya virus with a wide range of neurological disease. However, conclusions regarding co-infection may be affected. Although patients 2, 9, 16, and 18 would still (if chikungunya PCR data were discarded) show evidence of more than one recent infection, this would represent a lower tier in the hierarchy of evidence described earlier, where PCR detection or isolation of virus gives better evidence of infection associated with neurological disease.

In summary, this work adds to the growing body of evidence arguing for a wide spectrum of neurological disease associated with Zika virus infection, including central nervous system disease. Some patients in whom a Zika virus-associated neurological disorder was suspected were actually infected with chikungunya virus, and many were infected with more than one arbovirus. This work also highlights the difficulties and limitations that accompany studies investigating arbovirus-associated neurological disease, especially in the retrospective setting. To understand fully the disease burden of Zika virus, clinicians and public health officials need to look beyond GBS, and also to investigate for other arboviruses that may cause similar neurological disease, particularly chikungunya.

Despite the recognition of chikungunya-associated neurological disease in multiple published case reports and series globally, the disease spectrum was relatively unheard of by researchers and clinicians in Brazil, and indeed at the time of writing there was no comprehensive review in the literature. Given this and the unexpected role of chikungunya identified in this series, I decided to perform a systematic review of the neurological complications of chikungunya, in an attempt to summarise what is known of the diseases and provide an accessible resource to researchers and clinicians continuing to face epidemics of the virus.

Chapter 3: The neurological complications of chikungunya virus: a systematic review.

3.1 Introduction

Chikungunya virus is an alphavirus (genus *Alphavirus*, family *Togaviridae*) that is primarily transmitted to humans by *Aedes* mosquitoes, and occasionally from mother to child. Although the first outbreaks were described in the 1960s, the virus was not considered a major public health problem until 2004, when it caused explosive outbreaks in the tropics. Severe complications of chikungunya infection, including neurological disease, are being recognised increasingly.

Dengue and Zika are also arboviruses that, like chikungunya, are transmitted by *Aedes* mosquitoes. All three arboviruses cause an initial fever-arthralgia-rash syndrome, and are associated with neurological complications[71,115,116] Given increasing reports of co-circulation and co-infection of the three arboviruses in the Americas,[81] the underlying viral aetiology in patients presenting with arbovirus-associated neurological disease is not always clear. Therefore, understanding the similarities and differences between chikungunya, Zika and dengue-associated neurological disease is of importance, and is addressed in this review.

Chikungunya virus is associated with many different complications, but a disorder of the nervous system appears to be the most common severe. In two studies investigating patients with chikungunya infections requiring intensive care, a neurological disorder was the primary issue in 61% (11 of 18)[117] and 79% (15 of 19)[118] of patients with specific chikungunya-related complications.

Unlike Zika, neurological complications of chikungunya virus have been described in many studies, particularly since the 2004 outbreaks. Despite this, there is a lack of awareness and understanding of the disease spectrum amongst clinicians and researchers globally, which may be due partially to a paucity of summation and appraisal of available data. Therefore, I performed a systematic review of the evidence for neurological disease associated with chikungunya virus. A well-designed review was recently published on this topical subject by Cerny *et al.*;[119] the following review approaches the disease spectrum in a different manner. Firstly, I use a less restrictive search strategy, including articles published before 2000, epidemiological studies which do not necessarily report all clinical characteristics, and data on perinatally-acquired chikungunya infection, whereas the review by Cerny *et al.* had a

more stringent restriction process, leading to fewer but more reliably diagnosed cases identified. This review is also structured differently, choosing to give an overview of neurological complications followed by an individual assessment of each major associated neurological disease (the Cerny *et al.* review provides the overview only). Where provided, I present all data detailing the clinical information, investigations, management and outcome for all cases described in the literature. The Cerny *et al.* review presents this information for nine cases with the highest diagnostic certainty only, but goes further in other aspects, such as contacting authors for missing information and assessing overall patient characteristics. Overall, the reviews found similar patterns of disease, such as encephalitis being the most common neurological presentation, and peaks of incidence in elderly and infant age groups. Many analyses could not be compared due to differing methodology; for example, outcome data in Cerny *et al.* was presented firstly for all neurological complications and secondly for presumed direct viral versus autoimmune disease, whereas here it is presented per specific major neurological syndrome. A major difference between the reviews is that Cerny *et al.* categorised the different neurological syndromes into direct viral and autoimmune diseases, based on whether the therapy given was immunosuppressive or not. I did not classify cases as such, because it is not accurate to infer pathophysiological mechanism simply from treatment delivered. Of the other comparable analyses, there were no contrasting conclusions drawn.

3.2 Methods

I searched PubMed and Scopus for articles published up to 29th October 2017 using the following criteria: “chikungunya” AND (“neurolog*” OR “encephal*” OR “meningoencephalitis” OR “guillain-barré syndrome” OR “myelitis” OR “myelopathy” OR “stroke” OR “ocular” OR “optic neuritis” OR “severe” OR “unusual manifestations” OR “neonatal” OR “congenital” OR “perinatal” OR “fatal”) (Figure 7). There were no language restrictions. All published studies describing patients with neurological complications of chikungunya were considered eligible for inclusion, comprising case reports and series, and case-control, cohort, and cross-sectional studies; the information from pathogenesis studies is summarised in the discussion. Articles that did not mention neurological complications of chikungunya were excluded. I identified 14 further publications by ‘hand search’ (see Figure 7) i.e. publications that did not appear in the systematic search but were found either through citations in other articles or incidentally. Data extracted included the numbers of patients with neurological complications, and where available, the time between systemic symptoms of infection and neurological disease, CSF findings, a summary of the clinical presentation, the author’s diagnosis, treatment given and outcome. Each study (and individual case, where possible)

was assigned one of three outcomes based on the data provided (as per the algorithm in Figure 8): A = probable; B = possible; C = interpretation difficult, disputable. Where no diagnosis was given, the diagnostic certainty was recorded as 'not applicable'. The number of patients described per distinct neurological syndrome and aggregate data on diagnosis, treatment and outcome were summated. Information from pathogenesis studies identified in the search is discussed in Chapter 4.

Figure 7: Search strategy to identify publications on neurological complications of chikungunya.

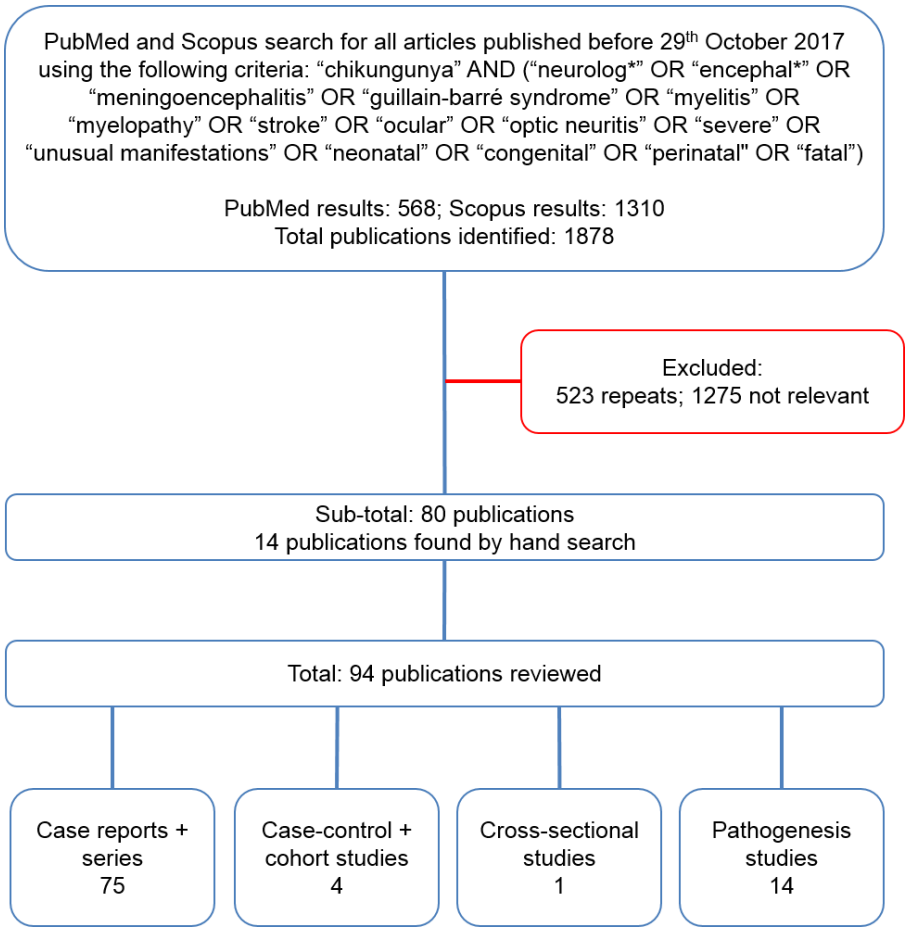
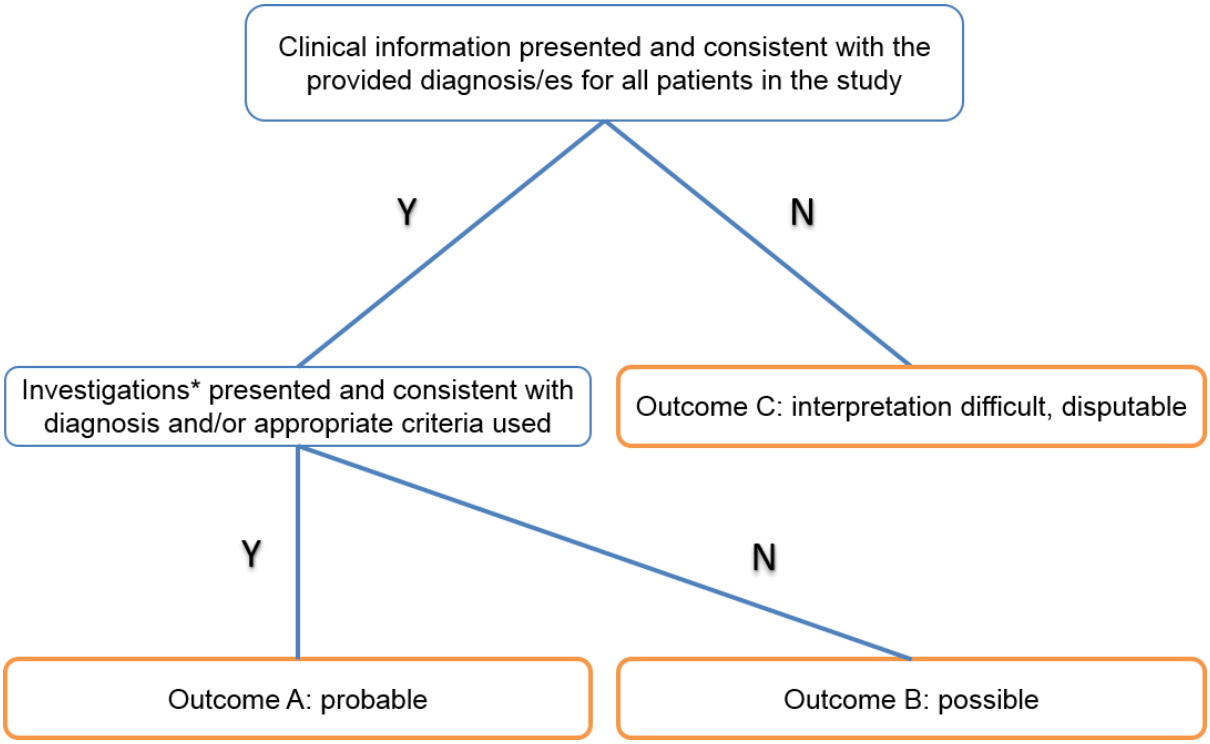


Figure 8: algorithm to classify each study on its neurological diagnostic certainty.



*A bespoke set of results from investigations was required for each diagnosis:

Encephalitis and myelitis: CSF WCC>5 cells/µl and/or neuroimaging compatible with encephalitis/myelitis

GBS: nerve conduction studies consistent with GBS

For encephalopathy and myelopathy, the ophthalmological disorders and cranial nerve palsies, which are clinical diagnoses, results from investigations were not required for outcome A.

If there was uncertainty regarding data provided, the outcome was demoted to outcome C.

3.3 Results

3.3.1 Clinical findings

Seroprevalence studies have reported a range of asymptomatic rates of chikungunya infection, from 3-47%.[64] In acute symptomatic infection, following an incubation period of approximately 3 days,[65] there is an abrupt onset of fever, headache, rash, arthralgia and myalgia which typically last for 1-2 weeks.[66] After this, seroconversion likely confers life-long immunity.[67] As well as neurological manifestations, chikungunya virus is associated with complications of the cardiovascular, renal, respiratory, hepatic, gastrointestinal and adrenal systems, sometimes collectively referred to as “atypical features”.[68-70] However, a disorder of the nervous system appears to be the most common severe complication of chikungunya

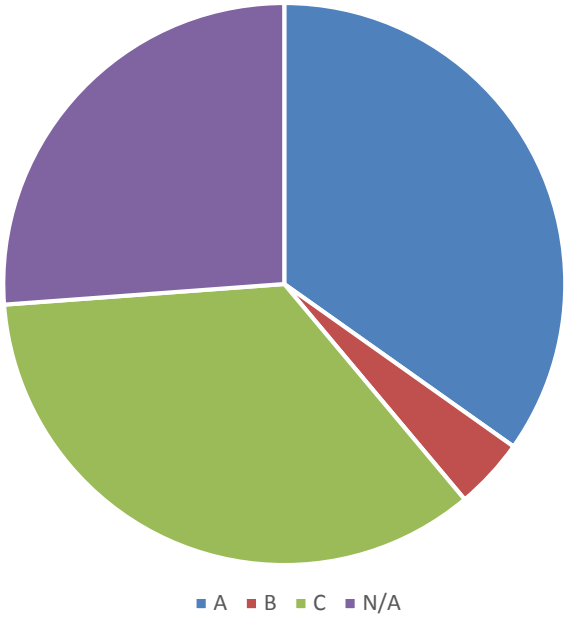
infection. In two studies investigating manifestations of chikungunya in patients requiring intensive care, a neurological disorder was the primary issue in 61%[117] and 79%[118] of chikungunya-infected patients.

In chikungunya-associated neurological disease, the clinician must be vigilant for other complications in the same patient, a pattern also described in dengue but rarely in Zika infection. Amongst 99 cases of chikungunya-associated neurological disease described in a study in India, 69 also had other complications involving, for example, the renal, hepatic and respiratory systems.[69] These patients should be managed using a multi-disciplinary approach.

3.3.2 Neurological manifestations

The literature on neurological manifestations of chikungunya reflects that of the disease activity itself, with 5 publications between 1964-71, and 89 since 2005, including 27 case reports, 48 case series, 1 case-control study, 3 cohort studies, 1 cross-sectional study and 14 pathogenesis studies (Figure 7); autopsy data were included in one report found.[120] Table 7 provides a summary of all cases of reported chikungunya-associated neurological disease. In total, 856 cases of chikungunya-associated neurological disease were found in the literature; 796 (93·0%) were in adults and children infected directly via mosquito, 60 (7·0%) were in neonates infected vertically from mother to child (Figure 10). Because patients were investigated to variable extents, I have categorised them according to the presenting clinical syndromes of encephalopathy, myelopathy, neuropathy, combinations of these, and neuro-ocular disease; I then provided diagnoses, treatment and outcome where it was available. A limitation of this study was that I could not incorporate neurological diagnostic criteria, given the range of information available amongst all the cases published. However, using the algorithm in Figure 8, I was able to classify each study on the basis of diagnostic certainty as A = probable, B = possible, C = disputable and N/A (where no diagnosis was given). In the adult and child cases, 278 (34.9%) were classified as A, 35 (4.4%) as B, 277 (34.8%) as C, and 206 (25.9%) as N/A. In the perinatal cases, 20 (33.3%) were classified as A, 0 (0.0%) as B, 22 (36.7%) as C, 18 (30.0%) as N/A. The total values are presented in Figure 9. The primary reason for studies falling into category C was a lack of clinical information provided, as opposed to inconsistency between clinical information and given diagnosis. Although the qualitative aspect of the review (discussed in the sections below) has been derived from cases in groups A and B, it should be noted that the quantitative aspect must be interpreted with caution, due to the significant contribution from cases falling into group C.

Figure 9: Category of diagnostic certainty for all cases.

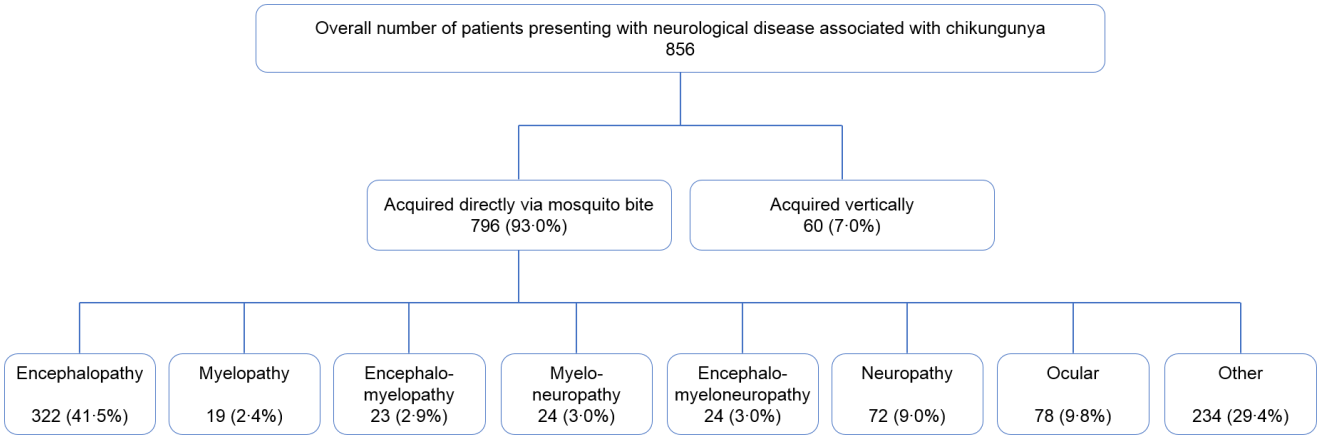


Key:

Diagnostic certainty categories: A = probable (34.8%); B = possible (4.1%); C = interpretation difficult, disputable (34.9%). Where no diagnosis was given, the diagnostic certainty was recorded as N/A = not applicable (26.2%).

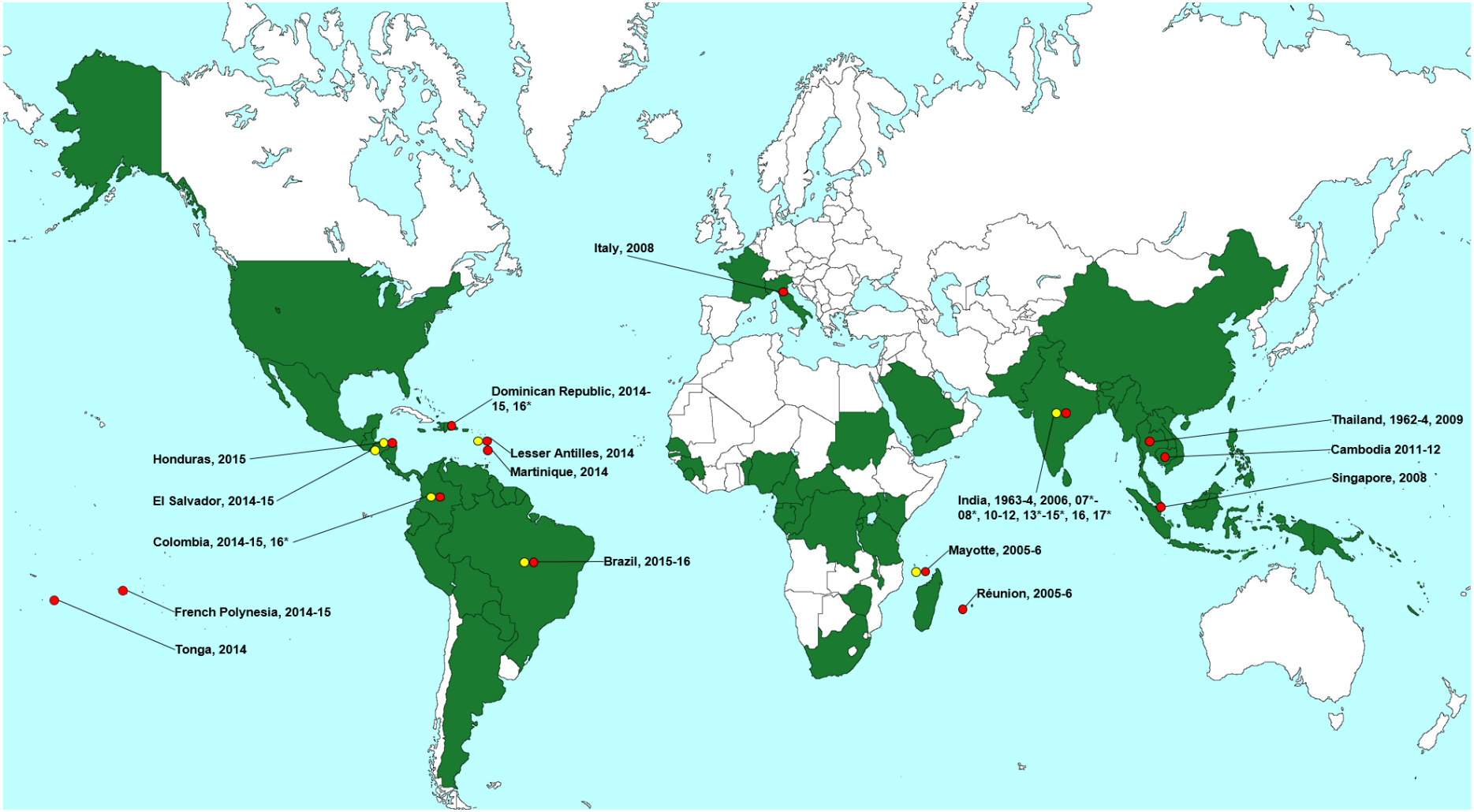
The most common clinical presentation of neurological disease associated with adult and child chikungunya infection was encephalopathy; it accounts for 322 (40·5%) of the 781 patients described. Excluding ocular disease, 474 (77·3%) of all adult and child cases had a pure CNS disorder, 82 (13·4%) had a pure peripheral nervous system disorder, and 57 (9·3%) had a disorder of both the central and peripheral nervous systems. Cases in which co-infection of chikungunya with another neurovirulent arbovirus was detected are not included in Table 7 but are further discussed below.

Figure 10: Presentations of nervous system disease associated with chikungunya infection.



Neurological disease following chikungunya virus infection was first reported during an outbreak in Madras, India, in 1964.[121] Four cases with chikungunya confirmed serologically or by viral isolation were described. Two presented with a meningo-encephalitic picture, ("delirium or coma, and signs of meningeal irritation with nuchal rigidity and Kernig's sign, sluggish pupillary reaction etc"), one with acute flaccid paralysis and elevated cerebrospinal fluid (CSF) protein, suggestive of Guillain-Barré syndrome, and one with transient dysarthria. Since then, neurological manifestations have been reported throughout the Indian ocean, South Asia, the Pacific islands, Southern Europe, the Caribbean and South America, ranging from mild behavioural disorders to severe acute syndromes of both central and peripheral nervous systems (Figure 11).

Figure 11: Global distribution of chikungunya virus and countries/territories with reported associated neurology.



Key:

- adult and child neurological disease associated with chikungunya infection
- vertically acquired neurological disease in the neonate associated with chikungunya infection

*unclear date of case(s), year refers to year of publication

Data regarding global distribution of chikungunya virus acquired from Centers for Disease Control and Prevention website.[122]

Given the large spectrum of neurological disease and scarce epidemiological data, estimating the incidence of neurological disease amongst all systemically symptomatic chikungunya infections is difficult. In one study from the 2006 Indian outbreak,[123] 18 (4·4%) of 405 suspected chikungunya cases attending the recruiting hospital over three months developed neurological complications; this study did not, of course, include the many people with uncomplicated chikungunya infection who did not seek hospital attention. An epidemiological study of the 2005-6 Réunion Island outbreak, found approximately 0·3% of all chikungunya infections resulted in 'atypical' cases,[68] of which 24·1% of the adults presented with abnormal neurology. Thus approximately 0·1% (1 case per 1000) of all chikungunya infections developed neurological disease.

It has been suggested that severe complications of chikungunya infection typically arise in those with co-morbidities.[46] The above epidemiological study describing 610 atypical cases of chikungunya infection showed that underlying respiratory disease, cardiac disease and hypertension were all independently associated with severe complications, including neurological disease.[68] However, a study from India of 124 atypical chikungunya cases did not identify co-morbidity as a significant risk factor for systemic complications or fatality;[69] similarly, in a case series of chikungunya-associated GBS, six (67%) of nine cases did not have any co-morbidities.[124] Thus, although underlying co-morbidities may play a role in neurological and other complications of chikungunya, they are not an indispensable requisite. Interestingly, age has been consistently reported as a significant risk factor for severe manifestations of chikungunya infection, both in the elderly (>60-65),[68,69,125] and in infants.[125]

Future studies will need to determine whether initially asymptomatic chikungunya infections, i.e. without a primary fever, arthralgia or rash syndrome, can cause neurological disease. Although none have been reported to date, the possibility has not yet been investigated in adults. In vertical transmission, a retrospective study from the Réunion Island outbreak found that of 38 symptomatic neonates (with combinations of fever, rash and oedema), two mothers had been asymptomatic (with a positive chikungunya PCR or IgM).[126] However, mild symptoms may have been missed by the mothers due to poor recall, and whether their neonates developed neurological manifestations was unclear.

Co-circulation of chikungunya, Zika and dengue viruses has been reported in much of South America,[81] and is a potential problem in all areas of the world where *Aedes* mosquitoes are endemic. *Aedes albopictus* mosquitoes have the ability to deliver more than one arbovirus in their saliva, raising the possibility of simultaneous transmission of the viruses.[82] Furthermore,

co-infection of arboviruses has been detected in patients presenting with neurological disease.[84,85] Given that all three arboviruses are known to be neurovirulent, it is unclear whether in these patients, their neurological disease is associated with one or more of their infections. Co-infection has also been reported in pregnant women[23,86], the significance of which for the neonate is unclear. The most common co-infection to be reported in all patients is with chikungunya and dengue viruses, though this may be due to the greater number epidemics of these viruses so far compared to Zika. Albeit rarely, co-infection with all three viruses has been reported, including in patients with neurological disease.[84,87] The differences in disease pathogenesis, presentation and severity between mono- and co-infections are currently unknown. It is clear, however, that in endemic areas, pregnant patients with a fever-arthralgia-rash syndrome and all patients presenting with acute neurological disease (regardless of previous viral symptoms) should be investigated for all three of chikungunya, Zika and dengue viruses.

The following sections evaluate the evidence for each of the neurological syndromes that has been described in association with chikungunya virus infection.

Table 7: Reports of neurological disease associated with chikungunya virus in adults and children.

Year of case(s), location	No.	Laboratory evidence for chikungunya	PL	CSF	Neurological features	Diagnosis	Diagnostic certainty	*Treatment* and outcome
Encephalopathy (n=322)								
1963-4, India[127]	2	HI	-	-	Unknown	Encephalitis	C	1 died, 1 unknown
1962-4, Thailand,[52]	1	HI/isol ser	-	-	Unknown	Meningoencephalitis	C	Unknown
2005-6, Mayotte[128]	2	PCR/IgM CSF/ser	-	-	Unknown	Meningoencephalitis	C	Unknown
2005-6, Réunion[118]	14	PCR CSF (2/12), PCR serum (4/10), IgM CSF (11/13), IgM serum (12/13)	-	WCC↑ (5/12) prot↑ (8/12)	Headaches, seizures, focal neurology, altered GCS	Encephalopathy	A	4 died
2005-6, Réunion[68]	84	PCR/IgM CSF/ser/BL	-	-	Unknown	Encephalitis (69), meningoencephalitis (15)	C	6 died
2005-6, Réunion[125]	57 ‡	PCR CSF (40/52), PCR serum (31/37), IgM CSF (21/52), IgM serum (32/37)	-	WCC↑ (21/57) prot↑ (37/55)	International Encephalitis Consortium criteria used to classify patients	Encephalitis (24), Non-encephalitic CHIKV-associated CNS disease (33)	A	7 died, 12 disabled, 16 recov
2006, India[129]	11	IgM CSF/ser	-	-	Headaches, altered sensorium, ataxia, rigidity, opsoclonus; abnormal brain MRI (?no.)	Encephalopathy	A	3 died
2006, India[69]	37	Isol/PCR/IgM CSF/ser§	-	-	Unknown	Encephalitis	C	7 died
2006, India[123]	11	Isol/PCR/IgM/HI CSF/ser (6)	-	-	Unknown	Encephalitis	C	2 died
2006, Réunion[130]	16	PCR/IgM CSF/ser	-	-	Drowsiness, seizures, focal neurological signs; abnormal MRI brain (5) & EEG (?no.)	Encephalitis (12), encephalopathy (4)	A(9) B(7)	2 died, 5 disabled, 9 no neur
2006, India[131]	27	PCR CSF (4)	‖	WCC↑(6/20) prot↑ (14/20)	59% abnormal behaviour; 22% drowsiness, extrapyramidal; 11% seizures; abnormal MRI brain (1/4)	Encephalitis	A(6) B(21)	21 improved, 4 no improven
2007, Italy[132]	1	PCR CSF & ser, HI	5d	WCC↑, prot↑	83y M; confusion, drowsiness	Encephalitis	A	Died after 3d
2008, Singapore[133]	1	IgM ser	3d	-	45y M; drowsiness, headache; abnormal MRI brain	Encephalitis	A	*Antimicrobials*. Full recovery
2009, Thailand[134]	2	IgM CSF	3d	WCC↑, prot↑	27y F; drowsiness; abnormal MRI brain	Meningoencephalitis	A	*Aciclovir*. Full recovery at 6m
		HI	0d	WCC↑, prot↑	85y M; drowsiness, jerky movements; abnormal MRI brain	Meningoencephalitis	A	No improvement
2010-11, India[135]	4	PCR & IgM CSF, IgM ser	4d	WCC↑, prot↑	32y F; seizure, disorientation, neck stiffness	Encephalitis	A	Improved over 10d
		PCR CSF & ser	-	WCC↑, prot↑	50y M; headache, disorientation, drowsiness, meningism	Encephalitis	A	Improved over 7d
		PCR CSF & IgM ser	3d	WCC↑, prot↑	23y M; seizure, dysarthria, hiccups, quadriparesis, CN involvement; abnormal MRI brain; *MP*	Meningoencephalopathy	A	Improved over 3w, mild wea
		IgM CSF & ser	7d	prot↑	29y F; headache, neck stiffness, quadriparesis, drowsiness; abnormal MRI brain and spine	ADEM	A	*MP*. Recovered at 1m, some
2011*, India[136]	1	IgM CSF & ser	10d	NAD	55y M; weakness, vertigo, ↓GCS, nystagmus, bulbar weakness; abnormal MRI brain and spine	Brainstem encephalitis (ADEM)	B	*MP*. Near-complete recovery
2011-12, Cambodia[137]	11	PCR/Isol CSF	-	-	<16y; unknown	Meningoencephalitis	C	Unknown
2012*, India[138]	1	IgM ser	7d	WCC↑, prot N	32y M; seizures, stimulus sensitive myoclonus	Meningoencephalitis	A	*Anticonvulsants*. Fully recove
2014*, India[139]	1	PCR & IgM CSF & ser	2d	-	12y M; seizure, vomiting, altered sensorium, weakness, ↑UL tone & tremors, unequal pupils; abnormal CT	Encephalitis	A	Died after 6d
2014, Tonga[140]	1	PCR ser, IgM CSF & ser	7d	WCC N, prot↑	57y M; altered mental status, seizure; abnormal MRI brain & EEG	Encephalitis	A	*IVIG, anticonvulsants*. Impro
2014, L. Antilles[117]	3	PCR/IgM CSF/ser	-	-	Met Venkatesan[141] criteria for encephalitis	Encephalitis	A	Unknown
2015, Honduras[142]	18	PCR ser (11)◊	-	WCC↑, prot↑	<12mo (11); seizures/lethargy/bulging fontanelle/irritability/hyperalgesia; abnormal MRI brain (5/5), abnormal EEG (7/14)	Meningoencephalitis	C	1 died, remaining unknown
2015*, India[143]	3	IgM CSF/ser	-	NAD	19d F; tonic seizures, poor feeding	Encephalopathy	A	Unknown
				WCC↑, prot N	23d M; multifocal clonic seizures	Encephalopathy	A	
				NAD	25d F; multifocal clonic seizures, poor feeding	Encephalopathy	A	
2015*, Colombia[144]	1	IgM CSF/ser	4d	WCC↑†, prot↑	23d M; seizures, stupor, severe thrombocytopenia; abnormal MRI brain	Encephalitis	A	↓hearing, ↓tone, motor dela
2016*, India[145]	1	PCR CSF & ser	-	NAD	55y M; altered sensorium, GCS 12; abnormal MRI brain	Mild encephalitis with a reversible lesion of the splenium (MERS)	A	Full recovery at 5d

Year of case(s), location	No.	Laboratory evidence for chikungunya	PL	CSF	Neurological features	Diagnosis	Diagnostic certainty	*Treatment* and outcome
2016, Brazil[146]	2	PCR CSF & ser, IgM ser	6d	WCC↑, prot N	51y F; confusion, seizure, drowsiness, dysarthria	Encephalitis	A	*Aciclovir.* Full recovery
		PCR CSF	4d	WCC↑, prot↑	84y M; confusion, dysarthria, dysphagia, quadriparesis; NP: myositis	Encephalitis	A	*IVIG, aciclovir, antibiotics, an*
2016, India[147]	3	PCR ser	-	-	Children; seizures/altered sensorium	Meningoencephalitis	B	Recovered at 3-4d (2), died
2016*, Brazil[148]	2	IgM CSF/ser	4d	WCC↑, prot↑	74y M; confusion, drowsiness, paraparesis; abnormal MRI brain; NP: AMSAN	Encephalitis	A	*IVIG, plasmapheresis.* Improv
			6d,	WCC↑, prot↑	83y M; confusion, lethargy, required ventilation	Encephalitis	A	*Aciclovir, IVIG.* Complete rec
2016, Brazil[149]	3	IgM ser	1d	-	<5y M; headache, seizures, GCS 3, areflexia; abnormal CT brain	Not given	N/A	Died 23d after admission
		IgM ser	10d	WCC N, prot↑	65y M; seizures, GCS 9, required ventilation; abnormal CT brain	Not given	N/A	*Aciclovir.* Died 1d after admi
		IgM ser	9d	-	92y F; ↓GCS, LL involuntary movements, required ventilation	Not given	N/A	Died 10d after admission
2017*, Brazil[150]	1	PCR CSF & ser & urine & saliva	13d	WCC↑, prot↑	57y M; confusion	Meningoencephalitis, anterior uveitis//	A	*Aciclovir, steroids po & top, t*
Myelopathy (n=19)								
2006, India[129]	4	IgM CSF/ser	-	WCC↑, prot↑	UR followed by paraparesis	Myelopathy	A	Improvement (unclear exter
2006, India[123]	5	Isol/PCR/IgM/HI CSF/ser (2)	-	-	Unknown	Myelitis	C	Unknown
2006, India[131]	7	PCR CSF (2), isol (1)	||	WCC↑(2/6), prot↑(2/6)	Para/quadriparesis (7), UR (6); abnormal MRI spine (1)	Myelopathy	A	5 improved, 2 no improvem
2015*, India[151]	1	IgM CSF/ser	2w	WCC N, prot↑	18y M; quadriparesis, ↓sensation, UR, areflexia, myositis (↑creatine kinase); abnormal MRI C1-C6	Myelitis	A	*MP.* Slow, partial improveme
2016, Brazil[91]	1	IgM ser	8d	-	Paraparesis, T10 sensory level	Myelitis	B	*Steroids po.* Fully recovered
2016, Brazil[146]	1	PCR CSF	0d	NAD	20y M; paraesthesia, triparesis, hyperreflexia, C6 sensory level, UR; abnormal MRI spine; NP NAD	Myelitis	A	*MP.* Partially improved
Encephalomyelopathy (n=23)								
2005-6, Réunion[68]	1	PCR/IgM CSF/ser/BL	-	-	Unknown	Myelomeningoencephalitis	C	Unknown
2006, India[129]	7	IgM CSF/ser	-	-	Unknown	Encephalomyelopathy	C	Unknown
2006, India[69]	14	Isol/PCR/IgM CSF/ser§	-	-	Unknown	Encephalomyelitis (11), encephalomyelopathy (3)	C	5 died
2016, Brazil[146]	1	PCR ser & urine, IgM CSF & ser	0d	WCC↑, prot↑	76y M; seizures, confusion, dysarthria, headache, neck stiffness, spastic paraparesis, T2-3 sensory level, UI	Encephalomyelitis	A	*Antivirals, antibiotics.* Unkno
Myeloneuropathy (n=24)								
2006, India[129]	13	IgM CSF/ser			Unknown	Myeloneuropathy	C	Unknown
2006, India[131]	7	PCR CSF (1)	<5 to 10-20d	WCC N (6/6), prot↑ (5/6)	Quadriparesis (6), UR (1); abnormal MRI spine (3); NP AIDP (7)	Myeloneuropathy	A	4 improved, 2 no improvem
2009, Thailand[134]	1	IgM CSF	2w	WCC N, prot↑	44y F; quadriparesis, dysphonia/phagia, facial diplegia, areflexia; abnormal MRI C4-5; NP AMSAN	Myeloneuropathy	A	*IVIG.* Rapid improvement, fu
2012*, India[152]	1	IgM ser	20d	WCC↑, prot↑	56y M; weakness and sensory loss; abnormal MRI spine C2-3 T5-7	Myeloradiculopathy	A	Improved
2014, Dom Rep[153]	1	IgM & IgG ser	10d	WCC↑, prot↑	47y F; L LL weakness, R LL pain, T12 sensory level; abnormal MRI T12-L1+cauda equina	Myeloradiculopathy	A	*MP.* Recovered at 6m, residu
2016, Brazil[146]	1	PCR & IgM CSF	2d	WCC N, prot↑	63y M; paraesthesia; flaccid areflexic paraparesis; T4 sensory level; UR; NP AMSAN	Myeloradiculitis	B	*IVIG.* No improvement
Encephalomyeloneuropathy (n=24)								
2006, India[129]	9	IgM CSF/ser	-	-	Unknown	Encephalomyeloneuropathy	C	Unknown
2006, India[69]	12	Isol/PCR/IgM CSF/ser	-	-	Unknown	Encephalomyeloneuritis (9), encephalomyeloneuropathy (3)	C C	1 died
2008*, India[120]	2	IgM CSF & ser	-	WCC↑#, prot↑	65y M; drowsiness, neck stiffness, weakness; abnormal MRI brain & nerve root; NP AMAN	Encephalomyeloradiculitis	A	*MP.* No improvement
				WCC↑, prot↑	74y M; drowsiness, weakness; abnormal MRI brain & nerve root; NP "generalised sensorimotor peripheral neuropathy"		A	*Dexamethasone.* Died; brain haemorrhages, small foci de
2009, Singapore[154]	1	PCR CSF, ser, urine, skin	2d	WCC N, prot↑	54y M; weakness, confusion, vomiting, sensory level, shock, rhabdomyolysis, UR; NP AIDP; EEG encephalopathy	AIDP	A***	*IVIG.* Full recovery
Neuropathy (n=72)								
1963-64, India[121]	1	HI ser	-	WCC↑Δ, prot↑	Quadriparesis, facial diplegia, ↓R visual acuity	GBS	B	Complete slow recovery ove

Year of case(s), location	No.	Laboratory evidence for chikungunya	PL	CSF	Neurological features	Diagnosis	Diagnostic certainty	*Treatment* and outcome
2005-6, Réunion[68]	4	PCR/IgM CSF/ser/BL	-	-	Unknown	GBS	C	Unknown
2005-6, Réunion[118]	1	IgM CSF & ser	-	WCC N, prot↑	55y M; weakness, hyporeflexia, facial palsy; NP "suggestive" of GBS	GBS	A	Moderately disabled at 6m
2005-6, Réunion[155]	2	IgM CSF	-	WCC N, prot↑	NP sensory-motor deficit (2)	GBS	C	*IVIG*. Rapid improvement
2006, India[131]	7	PCR CSF (1)	||	prot↑(5/6) WCC N (6/6)	Quadriparesis with AIDP (7)	Peripheral neuropathy	A	6 improved; 1 no improvem
2006, Réunion[156]	3	IgM ser	2w	WCC N, prot↑	51y F; quadriparesis, areflexia, facial diplegia; NP AIDP	GBS	A	*IVIG*. Partial recovery at 1m
		PCR ser	3d	NAD	60y M; quadriparesis, L facial palsy, hypoaesthesia, areflexia; NP AIDP	GBS	A	*IVIG*. Good recovery at 1m, r
		IgM & IgG ser	1w	WCC N, prot↑	49y F; paraparesis, proprioceptive ataxia, areflexia, facial diplegia, dyspnoea; NP AIDP	GBS	A	*IVIG*. Good recovery at 1m, r
2006, India[129]	13	IgM CSF/ser	-	-	Unknown	Neuropathy	C	Unknown
2006, Réunion[157]	2	IgM CSF & ser	1w	WCC N, prot↑	51y F; areflexia, facial diplegia, dyspnoea requiring ventilation; NP AIDP	GBS	A	*IVIG*. Good recovery at 2m
		IgM ser	2w	WCC N, prot↑	48y F; weakness, paraesthesia, areflexia, dyspnoea; NP "peripheral neuropathy, conduction block"	GBS	A	*IVIG*. Good recovery
2006, India[123]	2	Nil	-	-	Unknown	GBS	C	Unknown
2006, India¶[158]	4	IgM CSF/ser	-	-	Progressive, symmetrical, ascending quadriparesis with areflexia (4), required ventilation (1)	Acute flaccid paralysis	A	*MP*. Improved: rapidly (3), b
2014, L. Antilles[117]	6	PCR/IgM CSF/ser	-	-	Unknown	GBS	C	Unknown
2014-15, Fr Poly[124]	9	PCR/IgM CSF/ser	-	WCC N, prot↑	Sensorimotor deficit (8), facial diplegia (1); NP mixed axonal & demyelinating (9)	GBS	A	*IVIG (9).* NP returned to ~no
2014-15, L. Antilles[159]	13	PCR CSF (3)/ser (3) IgM ser (13)	1-22d	WCC N, prot↑	Mean age 61; severe (6), autonomic dysfunction (5), required ventilation (5)	AIDP (7), AMSAN (2), MFS (2), pharyngeal-cervical-brachial weakness (1), Bickerstaff's brainstem encephalitis (1)	A	*IVIG (12), plasma exchange (* symptoms, 1 CIDP, 1 unknov
2016, Brazil[146]	1	PCR urine, IgM ser	7d	WCC↑, prot↑	67y F; flaccid areflexic quadriparesis, dysphagia, impaired sensation; NP AMSAN	GBS	A	*IVIG*. No improvement
2016*, Colombia[160]	1	PCR & IgM ser	-	WCC N, prot↑	77y F; paraesthesia, bilateral hemiparesis, impaired sensation, hyporeflexia; NP AIDP	GBS	A	*IVIG*. Fully recovered at 8w
2016, Brazil[149]	1	IgM ser	15d	WCC↑, prot↑	51y F; quadriparesis, neck stiffness	Not given	N/A	*Antibiotics.* Died after 38h
2016*, India[161]	2	IgM CSF/ser	17d	-	18y M; areflexic quadriparesis, dysphagia, dyspnoea; NP AMAN	GBS	A	*Plasmapheresis*. Partial impr
			12d	-	20y M; flaccid quadriparesis, facial & bulbar weakness; NP AIDP	GBS	A	*Plasmapheresis*. Partial impr
Ocular disease (n=78)//								
2006, India[162]	14	IgM ser	11.0d **	-	Visual field defect (14), ↓visual acuity (13), pain (1), floaters (1), diplopia (1), RAPD (9), disc oedema (9), VII CN palsy (2), delayed VEP	Papillitis (6), neuroretinitis (3), retrobulbar neuritis (3), demyelination optic tract (2)	A	*MP 3d, steroids po 2w*. 10 im
2006, India[163]	37	IgM ser	33.2d **	-	Primary presenting complaint ↓visual acuity, unilateral (30), bilateral (7)	Anterior uveitis (11), panuveitis (5), optic neuritis (4), lagopthalmos & VIth nerve palsy (3), retrobulbar neuritis (3), retinitis & vitritis (2), bilateral neuroretinitis (1), keratitis (3), CRAO (1), choroiditis (2), retinal detachment (2)	A	Visual acuity of 26 followed worsened
2006, India[129]	2	IgM CSF/ser	-	-	↓visual acuity	Bilateral retinal haemorrhage (1), branch retinal artery occlusion (1)	A	*Steroids intravitreal.* Minima
2006, India[164]	9	IgM ser	4-12w	-	↓visual acuity/pain/red eye	Episcleritis (1), anterior uveitis (5), retinitis (3)	A	*Indomethacin po*; *steroids, h aciclovir iv/po, steroids po.* F
2007, India[165]	10	IgM ser	1-6w	-	↓visual acuity (10), pain (10), bilateral (3), visual field defects (10), disc oedema (10), RAPD (7), delayed VEP	Papillitis (7), retrobulbar neuritis (1), perineuritis (1), neuroretinitis (1)	A	*MP 3d, steroids po 2w*. Rapid RAPD/visual field/colour vis
2007*, India[166]	1	PCR & IgM ser	2w	-	48y F; bilateral ↓visual acuity, bilateral centrocaecal scotoma, retinal haemorrhage	Bilateral neuroretinitis	A	*Steroids po.* Visual acuity 20/
2009*, India[167]	1	PCR aqueous humour	1w	-	20y F; L ↓visual acuity, tripod dendritic pattern of keratic precipitates	Fuchs' heterochromic iridocyclitis & cataract	A	*Cataract surgery.* Recovered
2010, India[168]	1	IgM ser	4w	-	27y F; bilateral ↓visual acuity, R RAPD, bilateral retinitis posterior pole, macular oedema, serous detachment	Anterior uveitis & retinitis	A	*Steroids po.* Gradual recover
2011*, India[169]	1	PCR ser	1w	-	65y M; bilateral ↓visual acuity, neuroretinitis, cotton wool spots, retinal haemorrhages	Bilateral neuroretinitis	A	*Steroids, aciclovir po.* Partial

Year of case(s), location	No.	Laboratory evidence for chikungunya	PL	CSF	Neurological features	Diagnosis	Diagnostic certainty	*Treatment* and outcome
2015*, Dom Rep[170]	1	IgM & IgG ser	4d	NAD	47y F; bilateral ↓visual acuity, photophobia, optic nerve head oedema, hyperpigmented scars, serous detachment temporal macula, represented with floaters; abnormal MRI orbits	Panuveitis, retinal detachment	A	*Steroids po & top, cyclopento*
2016*, Dom Rep[171]	1	IgM & IgG ser	20d	-	44y F; ↓visual acuity, floaters, keratic precipitates, anterior chamber cells, Koeppe nodules	Intermediate uveitis	A	*Steroids po & top.* Rapid imp
Other focal neurology (n=233)								
1962-4, Thailand[52]	1	HI/isol CSF/ser	-	-	Febrile convulsions	Febrile convulsions	N/A	Unknown
1963-64, India[121]	2	HI	14d	-	Limb paresis and slurring of speech	Not given	N/A	Recovered
			7d		Vocal hoarseness & nasal regurgitation	Not given	N/A	
1964, India[121]	1	HI/isol CSF/ser	4d	-	12y M; Bilateral total ophthalmoplegia, loss of accommodation reflex	CN palsy	A	Complete recovery at 1w
	3	HI/isol CSF/ser	-	NAD	Delirium, coma, meningism, sluggish pupils, dysarthria	Not given	N/A	Unknown
1964, India[172,173]	12	PCR/IgM CSF/ser	-	-	Seizures in infants (3), children (9); associated with fever (12), focal (2), ↓GCS (4)	Seizures	N/A	Died (1), residual neurologic
2005-06, Réunion[155]	21	PCR/IgM CSF/ser	-	WCC N, prot↑ (12)	Confusion (20), headache (7), epilepsy (6), meningism (1), motor deficit (1), sensory deficit (2); EEG diffuse slowing (13), epileptic activity (3), NAD (2)	Not given	N/A	Died (5), generally good out
2005-6, Réunion[68]	12	PCR/IgM CSF/ser/BL	-	-	Seizures	Seizures	N/A	Unknown
2005-6, Réunion[68]	5	PCR/IgM CSF/ser/BL	-	-	Unknown	Stroke (2), cerebellitis (3)	C	Unknown
2006, Réunion[130]	14	PCR/IgM CSF/ser	-	-	Nuchal rigidity, Kernig / Brudzinski's sign, photophobia, tense fontanelle (4)	Meningeal syndrome Febrile seizures (10)	N/A	Mild/no neuro sequelae (1), Asthenia (1), no neuro seque
2006, India[174]	20	IgM ser	-	WCC↑ (9), prot↑ (20)	Altered mental status (20), psychosis (6), seizures (15), CN deficit (20), hemiparesis (1), LMN paraparesis (3), involuntary movements (4), optic neuritis (2)	Not clear	N/A	13 gradual full improvemen
2006, India[175]	8	PCR CSF/ser	-	WCC↑ (2/5)	Altered mental status, meningism, seizures, status epilepticus, aphasia	Not given	N/A	At discharge, normal GCS (6
2006, Réunion[176]	25	PCR CSF (8), IgM/PCR ser	-	WCC↑ (1/17) prot↑ (3/17)	Paediatric cohort: convulsion, confusion, behavioural disorders, meningism; abnormal MRI (2/8), abnormal EEG (8/10)	Not given	N/A	Unknown
2006, India[129]	18	IgM CSF/ser	-	-	Unknown	Encephaloneuropathy (8), Carpal tunnel syndrome (10)	C C	Unknown
2007*, India[177]	1	IgM CSF/ser	13d	WCC N, prot↑	45y M; asymmetric quadriparesis, dysphagia, clonus, dystonia; abnormal MRI brain	ADEM	A	*MP.* Walking independently
2007*, India[178]	1	IgM ser	2-3d	-	15y F; sudden-onset profound L sided hearing loss, tinnitus	Sensorineural hearing loss	A	No improvement at 1m
2011*, India[179]	1	IgM ser	5d	WCC↑, prot↑	26y F; spastic quadriplegia, impaired sensation, UR; abnormal MRI brain & spine	ADEM	A	*MP.* Good clinical & radiolog
2013*, India[180]	1	IgM CSF/ser	9d	WCC↑, prot↑	8y M; flaccid quadriparesis, R UMN facial palsy, seizure, UR; abnormal MRI brain & spine	ADEM	A	*MP.* Minimal improvement a
2014, Martinique[181]	1	IgM & IgG ser	2d	-	62y M; isolated unilateral third nerve palsy	CN palsy	A	Improved at 6m
2014, L. Antilles[117]	2	PCR/IgM CSF/ser	-	-	Diffuse brain ischaemia leading to brain death	Not given	N/A	Died
2014, Fr Poly[119]	1	IgM ser	6d	WCC↑, prot↑	74y M; hypoesthesia, flaccid quadriplegia, dyspnoea, GCS 3, CN palsies, required ventilation; abnormal MRI brain, abnormal EEG, NP axonal polyneuropathy	Bickerstaff's brainstem encephalitis-MFS-GBS overlap	A	*IVIG, MP.* Normal mental fun
2015, Honduras[142]	59	PCR ser	-	-	Seizures	Seizures	N/A	Unknown
2016, India[147]	2	PCR ser (1), IgM ser (1)	<7d	-	Children; hyperactivity, insomnia, aggressive behaviour, hallucinations, behaviour changes	Not given	N/A	Recovered at 4d (1), persist
2016, Brazil[91]	21	IgM ser	-	-	Seizures, altered consciousness, weakness, impaired sensation, sphincter dysfunction, persecutory delusions, suicidal/aggressive behaviour, insomnia, headache	Not given	N/A	Unknown
2016, Brazil[146]	1	PCR ser & urine, IgM ser	16d	prot N	17y M; L hemiparesis & numbness, facial palsy, impaired sensation; abnormal MRI brain	ADEM	A	*MP.* Improved
2017*, India[182]	1	IgM ser	10d	-	5y F; bilateral ophthalmoplegia, blurring of vision	Bilateral ophthalmoplegia	A	*Steroids po.* Unknown

Key:

/ = “or”; e.g. IgM CSF/ser = not specified whether IgM detected in CSF or serum; PL = prodrome length (time between initial infection and onset of neurology); “-“ = data unavailable; PCR = polymerase chain reaction; IgM = immunoglobulin M; Isol = viral isolation; HI = haemoagglutination-inhibition; CSF = cerebrospinal fluid; Ser = serum; BL = bullous lesions; d = days; w = weeks; WCC = white cell count (↑ = >5cells/µl); Prot = protein (↑ = >0.4g/L adults, >1.5g/L neonates); N = normal; NAD = no abnormality detected; F = female; M = male; L/R = left/right; UL/LL = upper/lower limb; UMN/LMN = upper/lower motor neuron; CN = cranial nerve; UR/I = urinary retention/incontinence; AMAN = acute motor axonal neuropathy; AMSAN = acute motor and sensory axonal neuropathy; AIDP = acute inflammatory demyelinating polyneuropathy; RAPD = relative afferent papillary defect; VEP = visual evoked potential; EEG = electroencephalogram; GBS = Guillain-Barré syndrome; MFS = Miller Fisher syndrome; CRAO = central retinal artery occlusion; MP = methylprednisolone; CIDP = chronic inflammatory demyelinating polyneuropathy; Diagnostic certainty: A = probable, B = possible, C = disputable, N/A = not applicable; Dom Rep = Dominican Republic; Fr Poly = French Polynesia; L. Antilles = Lesser Antilles

Footnotes:

* date of article submission, date of case unclear; † WCC reported as “>5”, normal neonatal WCC range 0-30 cells/µl; # WCC reported as “few cells”, unclear actual number/µl; Δ WCC mildly elevated, 8 lymphocytes/field; ‖ range of prodrome lengths: encephalitis: <5 to 10-20d (24), >30d (3); myelopathy: <5 to 20-30d; GBS: <5 to 10-20d; § neurological sequelae in cases with positive DENV IgM as well as CHIKV were attributed to CHIKV; ‡ five vertically transmitted cases; ◊ seven of these patients did not have laboratory evidence of chikungunya infection and were not described further, their age and whether they were vertically transmitted cases was not reported; // one patient with both encephalitis and anterior uveitis has not been included in the ocular disease section; ¶ Andaman & Nicobar Islands; ** mean; *** the given diagnosis of “AIDP” is consistent with only part of the clinical phenotype, which also consists of encephalopathy

3.3.3 Encephalopathy and Encephalitis

Encephalopathy, defined by the International Encephalitis Consortium as "a clinical state of altered mental status, manifesting as confusion, disorientation, behavioural changes or other cognitive impairment,"[141] is one of the most common neurological presentations for arboviral infections. Whilst in some patients this may be due to encephalitis – i.e. brain inflammation associated with direct viral infection – in others it may be a non-specific manifestation of severe systemic disease, for example due to hypoperfusion of the brain.[71,115,183] Strictly speaking, encephalitis is a pathological diagnosis, but for practical purposes it can be diagnosed in an encephalopathic patient if there is surrogate evidence of brain inflammation, for example from a CSF pleocytosis, brain imaging, or focal changes on electroencephalogram.[63,71]

In one study from Réunion Island, where the authors were careful to accurately define encephalitis using the international criteria, the estimated cumulative incidence rate for chikungunya-associated encephalitis was 8.6 per 100,000 people during the 2005-6 outbreak, which lead to a two-fold incidence of all encephalitis in the region.[125] Twenty-four patients with encephalitis were reported (five of whom were neonates infected perinatally), presenting with altered mental status, as well as seizures and focal neurological signs in some. When compared to encephalopathic patients who did not meet criteria for encephalitis, the encephalitic patients had more severe CNS disease and required more intensive care support; this was despite there being no significant difference between chikungunya viral loads or IgM titres in the serum or CSF, which may indicate a role for predisposing host factors in chikungunya virus neurovirulence. Overall, the literature review revealed that 251 of the 322 (78·0%) patients who presented with an isolated encephalopathy syndrome had a diagnosis of encephalitis, whereas 66 (20·5%) and 2 (0·6%) had a diagnosis of encephalopathy and ADEM, respectively. Additionally, involvement of the meninges was also reported in 55 (17·1%) cases.

Symptoms of encephalitis begin between zero and thirteen days following the onset of systemic features of infection (see Table 7). As is the case in other arboviral encephalitides, a CSF pleocytosis is not always seen.[130,131] Unlike encephalitis caused by other CNS pathogens such as herpes simplex virus and cytomegalovirus, which have characteristic imaging abnormalities, chikungunya encephalitis in adults and children does not appear to show a distinct pattern. Described abnormalities include oedema or non-specific haemorrhage on CT, and increased T2 +/- fluid-attenuated inversion recovery (FLAIR) signal (Figure 12) or restricted diffusion signal in several areas of the cerebrum on MRI.[120,130,134] Many cases do not show any imaging abnormalities at all.[125,130] Although there are non-

specific slowing of brain waves in some patients, there is no specific electroencephalogram pattern.[130,140]

In the Réunion Island study, seven of the 57 patients (aged 4 days-88 years) with either encephalopathy or encephalitis died in hospital or shortly after discharge; of the ten adults followed up after three years, three had persistent neurological sequelae in the form of epilepsy, post-infectious dementia and cognitive disorder.[125] Taking into account the attrition in the follow-up cohort, a range from 18-43% of patients were estimated to have neurological sequelae. Similarly, a paediatric series from the same outbreak reported five (31%) of 16 children with chikungunya-associated encephalopathy or encephalitis had residual neurological deficit, whilst two (13%) died.[130] From the literature review, of the 127 patients cases for whom follow-up data was reported, 62 (48·8%) had complete or near-complete recovery, 25 (19·7%) had residual neurological deficit, and 40 (31·5%) died.

3.3.4 Myelopathy and myelitis

Chikungunya virus can cause myelopathy, symptoms of spinal cord disease, which may present with limb weakness, sensory changes, hyperreflexia, bowel and bladder disturbances, depending on the level of the lesion, and extent to which cord is involved. If cord inflammation is confirmed by MRI, a CSF pleocytosis, or elevated CSF IgG index, showing local immunoglobulin production, then it is classified as myelitis.[184]

The incidence of spinal cord disease after chikungunya infection is not known, but it is likely to be less than that of encephalopathy, given that the 90 cases described in the literature comprise less than a third of the 322 cases of encephalopathy. Myelopathy and myelitis usually occur as part of more widespread neurological disease. Of the 90 patients identified, 47 had myelopathy as part of more widespread CNS disease, with encephalopathy or encephalitis; for 48 there was also peripheral nervous system disease in the form or radiculopathy or neuropathy; just 19 patients had a pure myelopathy syndrome. Spinal cord disease typically presents zero days to 3 weeks after the first clinical feature of infection (fever, arthralgia or rash).[131,146] Patients present with weakness in two, three or four limbs, sometimes accompanied by one or more of paraesthesia in the limbs, a sensory level, or urinary retention. In the literature review, of the 12 patients with a pure myelopathy where CSF data was provided, six (50%) had a CSF pleocytosis. Similarly, MRI abnormalities are variable. They may range from changes “suggestive of demyelinating pathology” to extensive T2/FLAIR hyperintensity from the cervicomedullary junction to the level of C6.[151]

No deaths have been reported for chikungunya-infected patients with a pure myelopathy syndrome; follow-up data were reported for 13 patients, 11 of whom improved, though the extent of this was often unclear.

3.3.5 Acute disseminated encephalomyelitis (ADEM)

Like other acute viral infections, chikungunya can trigger an acute inflammatory syndrome involving the brain parenchyma and spinal cord, which is thought to be an immune-mediated response to infection, rather than due to direct viral invasion. The diagnosis of this monophasic illness is usually based on finding focal or multifocal, poorly demarcated white-matter demyelinating lesions on MRI.[185,186]

Six cases of ADEM (5 adults, 1 child) have been described, with the disease starting 5-16 days after the initial fever-arthralgia-rash symptoms of chikungunya infection. Patients presented with a variety of neurological features, including headache, drowsiness, cranial nerve involvement such as facial nerve palsy, vertigo, nystagmus, and bulbar weakness; limb weakness, sensory disturbance, and urinary retention. MRI of the brain and/or spine suggested demyelinating pathology. All six were treated with intravenous methylprednisolone; the outcome varied between good clinical and radiological recovery[179] and permanent neurological disability with confinement to a wheelchair and long-term urinary catheterisation.[180]

3.3.6 Guillain-Barré syndrome (GBS)

Chikungunya-associated peripheral neuropathy without CNS disease has been described for 72 patients in case reports or series, the majority of whom were described as having GBS. In one series of four patients with acute flaccid paralysis, no CSF or neurophysiology results were reported, making diagnosis difficult.[158] Other causes of acute flaccid paralysis, such as anterior myelitis, have not yet been reported in association with chikungunya virus.

Two studies from Réunion Island showed an increased incidence of GBS following a large outbreak of chikungunya. One from the 2014-15 outbreak reported nine patients with chikungunya-associated GBS, representing a four- to nine-fold increase in the island's annual GBS incidence.[124] The increase in the incidence of GBS following the 2006 chikungunya virus outbreak on Réunion Island was estimated to be ~22% compared to the year before.[157] A study from Martinique and Guadeloupe also showed an increase in incidence of GBS following the 2014 chikungunya virus outbreak, but to a lesser extent (two-fold).[159]

Clinically, chikungunya-associated GBS resembles GBS associated with other infections such as *Campylobacter jejuni*, presenting with symmetrical, bilateral flaccid weakness, often with paraesthesia and/or cranial nerve palsy.[34] The four reports in the literature detailing the time interval between chikungunya infection and onset of neurological features describe a prodrome of 3-17 days, compatible with a para- or post-infectious syndrome (Table 7).[156,157,161]

Unlike infections such as *C. jejuni*, which is associated with a more severe pure motor variant of GBS,[187] chikungunya appears to be associated with the full range of GBS variants, as determined by neurophysiological studies, including disorders of motor and sensory axons, and myelin sheaths, sometimes in combination (see Table 7). Further investigation is required to determine risk factors and markers for the different GBS variants, including anti-ganglioside antibodies.

For the 36 cases where treatment was described, 28 (78%) received intravenous immunoglobulin, 2 (6%) intravenous immunoglobulin and plasma exchange, 4 (11%) intravenous methylprednisolone and 2 (6%) plasmapheresis. 40 (87%) of the 46 cases for whom follow-up data were available improved. In some cases this was rapid; in most it had occurred by 3 months.

3.3.7 Ocular complications

Although photophobia and conjunctivitis are associated with the acute phase of chikungunya infection,[188] many later ocular complications have been described up to 12 weeks after infection, which may require emergency management. These include disease of the uvea, retina and optic nerve. As well as inflammation, other pathology has been described, including retinal detachment, intra-retinal haemorrhage and branch retinal artery occlusion (Table 7).[129,163] The literature review found 78 cases of ocular complications of chikungunya infection.

In a retrospective study from India of 37 Indian patients with acute ocular manifestations and IgM-confirmed chikungunya infection, uveitis was the most common diagnosis, occurring in 16 (43%) of patients;[163] forty-eight controls from the chikungunya-endemic area, selected from patients attending the hospital for non-acute problems including cataract and refractive error, were all negative for chikungunya IgM. Recovery was variable – of the 26 patients followed up, the visual acuity improved in 11 (42%), remained the same in 12 (46%), and worsened in three (12%). Overall, of the 67 patients with ocular disease identified for whom

follow-up data was reported, 46 (69%) recovered well, whereas 21 (31%) showed minimal or no improvement.

Although most of the serious ocular complications occur days to weeks after the acute chikungunya infection, in one report five of 14 optic neuritis cases occurred simultaneously with the systemic disease onset.[162] This suggests a more direct viral effect may be important, as well a post-viral immune response.

Disease relapse has been reported in one report, which described bilateral uveitis and retinal detachment, with loss of visual acuity starting four days after symptoms of chikungunya infection.[170] Having received a week's course of oral and topical steroids and recovered within six weeks, the patient re-presented three months later with floaters and keratic precipitates; clinicians should be vigilant for relapse in such cases.

3.3.8 Disease affecting multiple components of the nervous system

As well as the distinct syndromes described mentioned above, chikungunya infection is associated with complex disease involving multiple parts of the nervous system causing, for example, encephalomyelopathy (23 patients identified in the literature review), myeloneuropathy (24) and encephalomyeloneuropathy (24). Where follow-up data was available, unlike in pure myelopathy, more of these patients had an unfavourable outcome (eight deaths, four no improvement) compared to those who improved (eight).

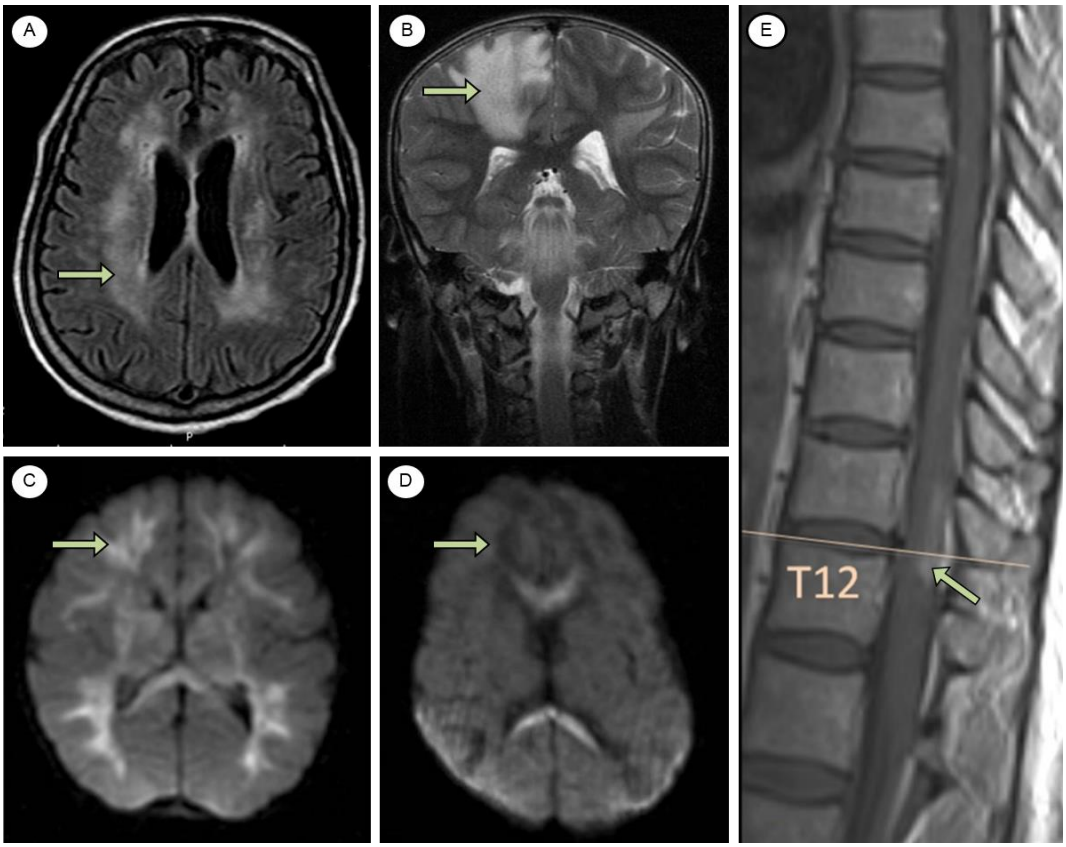
3.3.9 Other

A handful of other neurological disorders have been associated with chikungunya, albeit in smaller numbers. A study from the Réunion outbreak described 32 patients presenting with behavioural changes including attention disorders, irritability and memory issues.[125] Other reports have described febrile seizures, isolated cranial nerve palsies, stroke and hearing loss (Table 8), and one report describes the possibility of an association with chronic fatigue syndrome.[189] Although it is difficult to definitively associate these isolated disorders with chikungunya infection, the full spectrum of chikungunya-associated neurological disease appears to be broad.

Table 8: List of neurological diseases associated with chikungunya virus.

Described frequently	Described occasionally
Encephalopathy	Seizures with or without fever
Myelopathy	Behavioural changes
Encephalomyelopathy	Sensorineural hearing loss
Myeloneuropathy	Stroke
Encephalomyeloneuropathy	Cerebellitis
Guillain-Barré syndrome	Meningism
Acute disseminated encephalomyelitis (ADEM)	Third nerve palsy
Neonatal hypotonia	Encephaloneuropathy
Neuro-ocular disease (uveitis, retinitis, optic neuritis)	Carpal tunnel syndrome
	Bilateral total ophthalmoplegia
	Mild encephalitis with a reversible lesion of the splenium (MERS)
	Bickerstaff's brainstem encephalitis-MFS-GBS overlap

Figure 12: Central nervous system imaging abnormalities in patients with chikungunya infection.



A – Signal abnormality involving the periventricular white matter in an 85-year-old patient with encephalitis (axial fluid attenuated inversion recovery)[134]

B – Confluent areas of signal abnormality consistent with demyelination in an 8-year-old patient with acute disseminated encephalomyelitis (coronal T2)[180]

C – Signal abnormality (hyperintense) involving the corpus callosum and the frontal and parietal lobes in neonate A with vertically acquired encephalopathy (day 6, axial diffusion weighted imaging)[61]

D – Signal abnormality (hypointense) involving the frontal and parietal lobes in neonate B with vertically acquired encephalopathy (day 21, axial diffusion weighted imaging)[61]

E – Signal abnormality at T12 in a 47-year-old patient with myeloradiculopathy (sagittal T1)[153]

3.3.10 Perinatally acquired neurological disease

Most of the evidence on chikungunya causing neonatal disease relates to transmission in the intrapartum period, rather than earlier in pregnancy. A wide range of severe manifestations has been described affecting neonates whose mothers had acute chikungunya infection near the time of delivery (Table 9). A case series from Colombia from 2014-15 described eight infants who required admission to an intensive care unit after contracting chikungunya infection perinatally.[70] All mothers and neonates were PCR and IgM positive for chikungunya in serum; the neonates presented with severe disease including meningoencephalitis, respiratory distress, sepsis, necrotising enterocolitis, myocarditis and pericarditis.

One study from Réunion Island described 739 mothers who experienced symptoms of chikungunya infection during pregnancy, 39 of whom were symptomatic in the intrapartum period (between two days before and two days after delivery).[61] Of these 39 mothers, all infants were asymptomatic at birth, but 19 developed acute disease a median four (range three-seven) days after delivery, giving a vertical transmission rate of approximately 50% for mothers symptomatic in the intrapartum period. The initial clinical features in the affected neonates included fever, distress, poor feeding, petechiae and a maculopapular rash. Nine cases (47%) were reported to have developed encephalopathy. CSF for all nine showed normal biochemistry and white cell counts, and chikungunya PCR was detected in five. MRI imaging data from this study, combined with a later follow-up study[190] shows that among neonates developing encephalopathy or encephalitis after perinatally acquired chikungunya, severe white matter injury is well-characterised in a three-stage pattern: cytotoxic brain oedema (ischaemia), vasogenic oedema (reperfusion) and mass reduction (demyelination).[61,190]

In addition to these severe features, hypotonia has been described in 17 neonates with chikungunya infection from Réunion Island[126], as well as intracerebral haemorrhage secondary to clotting abnormalities.[61,191] Interestingly, neonatal spinal cord and peripheral nervous system disease have not been reported.

With regard to prevention of transmission, no studies have found a protective effect of caesarean section.[61,192] The risk factors for vertical transmission are not understood, although in one study the viral load of chikungunya in the placentas of the 19 transmitters was found to be significantly higher than in 13 non-transmitters.[61] Interestingly, one of the transmitters gave birth to dizygous twins, of whom one acquired infection but the other did not.

In addition to overt disease in some perinatally-infected neonates soon after delivery, there is also evidence for impacts on longer term development. In one study comparing the neurocognitive function at approximately two years of age for 33 children with and 135 without perinatal chikungunya infection, significant differences in development quotients were found, including movement, coordination, language and sociability.[190] Importantly, even those infected at birth without obvious clinical features of neurological disease, such as encephalopathy, had significantly worsened neurocognitive function than uninfected children. Thus, the neurological effects of vertically transmitted chikungunya may not be obvious at birth, emphasising the importance of follow-up of this cohort. The 12 cases with encephalopathy at birth showed still more severe developmental deficit, including cerebral palsy and microcephaly.

Maternal chikungunya virus infection earlier in pregnancy does not appear to affect the foetus; in the above study investigating 739 mothers with chikungunya infection, 700 were symptomatic outside of the intrapartum period, and none of these infants developed symptoms of chikungunya.[61]

Of note, in three out of seven miscarriages occurring before 22 weeks, chikungunya RNA was detected in amniotic fluid for all three, and placenta and foetal brain for two. However, no significant increase in antepartum foetal deaths was seen during the chikungunya outbreak as compared with previous years. Another study of 1400 mothers from Réunion Island[193] found no effect of antepartum chikungunya infection on pregnancy outcomes. Together, these data would suggest that although antepartum congenital infection has been detected in miscarried foetuses, given the lack of epidemiological evidence for a causal association between infection and miscarriage, this may have been an incidental finding.

Zika virus is also now known to cause devastating neurological disease in neonates, which is of global concern.[24] Whereas chikungunya appears to be most damaging around the time of birth, Zika has been associated with neurological sequelae in infections at all stages of pregnancy.[23] Another important difference is in the initial presentation of neonatal disease – in perinatal chikungunya infections, fever-rash and neurological symptoms are only seen at approximately 4 days post-partum. In the Zika congenital syndrome, the damage is done *in utero*, thus neonates can be born with clear evidence of infection, such as microcephaly. Despite the magnitude of outbreaks throughout the tropics, there is a paucity of data on the effects of dengue virus in pregnancy. A recent large retrospective study from Brazil reported an increased risk of preterm birth associated with dengue infection, with no difference in the rate of congenital malformations.[194] Adverse outcomes in neonates following perinatal

dengue infection, such as thrombocytopenia, dengue haemorrhagic fever, and dengue shock syndrome, have been described in a handful of case reports and series in neonates;[195-197] but unlike chikungunya and Zika, apart from one case of anoxic encephalopathy,[198] neurological complications have not been reported.

Table 9: Reports of perinatally acquired neurological disease associated with chikungunya virus.

Year of case(s), location	No.	Evidence for chikungunya	PP	CSF	Neurological features	Diagnosis	Diagnostic certainty	*Treatment* and outcome
Perinatal encephalopathy (n=35)								
2005-6, Réunion[125]	5	Ne: PCR CSF	-	-	Fulfilled International Encephalitis Consortium criteria for encephalitis	Encephalitis	A	Cerebral palsy & blindness (1), poor neurodevelopmental performance (1)
2005-6, Mayotte[128]	3	Ne&M: PCR/IgM CSF/ser	-	-	Unknown	Meningoencephalitis	C	Unknown
2005-6, Réunion[191]	4	Ne&M: PCR/IgM ser	3-7d	-	Seizures; EEG consistent with encephalitis	Meningoencephalitis	A	Survived
2010, India[199]	2	Ne&M: PCR ser	5d	NAD	Altered sensorium, apnoeic seizures	Encephalopathy	A	Spastic diplegia, epilepsy, mental retardation
			3d	NAD	Apnoeic seizures, lethargy	Encephalopathy	A	↓tone, cerebral palsy, ↓vision, mental retardation
2014-15, Colombia[70]	2	Ne&M: PCR/IgM ser	-	-	Unknown	Meningoencephalitis	C	Unknown
2014-15, El Salvador, Colombia & Dom Rep[192]	12	Ne: PCR/IgM ser/CSF (10)	-	-	Unknown	Meningoencephalitis	C	Unknown
2015, Brazil[200]	1	Ne: PCR CSF, ser, urine, saliva	6d	WCC N, prot↑	Seizures; abnormal MRI brain	Encephalitis	A	*Anticonvulsants.* Improved at 17d
2015, Honduras[142]	3	PCR ser	-	WCC ↑, prot ↑	Unknown	Meningoencephalitis	C	Unknown
2016*, India[201]	2	Ne&M: IgM ser	5d	-	Dizygotic twins; both had seizures, required ventilation, thrombocytopenia; abnormal MRI brain	Encephalopathy	A	Both improved and discharged at 24d
2016, Brazil[202]	1	Ne: PCR CSF; M: IgM ser	4	WCC ↑, prot ↑	Prostration, lethargy, seizures, required ventilation, thrombocytopenia; abnormal MRI brain & EEG	Encephalitis	A	Cerebral palsy, microcephaly, epilepsy at 1 year
Perinatal brain haemorrhage (n=7)								
2005-6, Réunion[61]	2	Ne&M: PCR/IgM CSF/ser	-	-	DIC, transient scattered parenchymal petechiae (1), cerebellar haematoma (1)	Haemorrhage	A	Unknown
2005-6, Réunion[126]	2	Ne&M: PCR/IgM CSF/ser	-	-	Unknown	Haemorrhage	C	Unknown
2005-6, Réunion[191]	1	Ne&M: PCR & IgM ser	3-7d	-	Severe thrombocytopenia, cerebral haemorrhage	Haemorrhage	A	Survived
2015, Brazil[203]	1	Nil	4d	NAD	Intraventricular bleed (cranial US), lethargy	Haemorrhage	A	Improved, discharged after 17d
2012, India[204]	1	M: IgM ser; Ne: NAD	3	NAD	Lethargic, severe thrombocytopenia, focal bleeds basal ganglia & subcortical areas	Haemorrhage	A	Fully recovered
Perinatal other (n=18)								
2005-6, Réunion[126]	17*	Ne&M: PCR/IgM CSF/ser	-	-	Seizures (6); hypotonia (17)	Seizures / hypotonia	N/A	Unknown
2005-6, Mayotte[128]	1	Ne&M: PCR/IgM CSF/ser	-	-	Hypotonia	Hypotonia	N/A	Unknown

Key:

PP = onset of neurological disease days post-partum; Ne = neonate; M = mother; PCR = polymerase chain reaction; IgM = immunoglobulin M; CSF = cerebrospinal fluid; Ser = serum; "-" = data unavailable; d = days; WCC = white cell count (↑ = >5cells/μl); Prot = protein (↑ = >0.4g adults, >1.5g neonates); N = normal; NAD = no abnormality detected; VEP = visual evoked potential; EEG = electroencephalogram; DIC = disseminated intravascular coagulation; Diagnostic certainty: A = probable, B = possible, N/A = not applicable; Dom Rep = Dominican Republic

Footnotes:

* At least 17 patients; unclear whether seizures and hypotonia were seen in the same or different patients; † Patient had a subarachnoid haemorrhage and optic atrophy in addition to encephalitis

3.3.11 Management

There are currently no specific antiviral agents or vaccines for chikungunya virus.[205] Various *in vitro* compounds active against chikungunya virus have been reported, including both direct-acting and host-targeting antivirals; however, most of these compounds are yet to find their way into *in vivo* models and clinical trials.[206] Two of the few that have been tested clinically, chloroquine and ribavirin, are already widely used in the treatment of other diseases and have a known safety profile. However, chloroquine was found not to have any benefit for arthritic chikungunya when compared to a non-steroidal anti-inflammatory drug in a randomised clinical trial.[207] Ribavirin, on the other hand, had promising results in a small case series of ten patients with severe arthritis post-chikungunya infection.[208] To the best of my knowledge, no antiviral has been evaluated in the management of chikungunya-associated neurological disease, which therefore remains the same as that of neurological disease without associated chikungunya infection: for patients with encephalitis, those with a reduced Glasgow coma score require assessment by intensive care specialists and may need intubation, ventilatory support, correction of electrolyte abnormalities, management of raised intracranial pressure and enhancement of cerebral perfusion pressure.[209] In patients with myelitis, corticosteroids are the standard first-line treatment, despite the lack of trial evidence for their use in this scenario.[210] The management of GBS focuses on immunotherapy with intravenous immunoglobulin or plasma exchange, and ventilatory support if the innervation of respiratory muscles is affected.[34]

Although not yet commercially available, it is hoped that a vaccine for chikungunya is on the horizon. Two phase 1 clinical trials have shown a good safety and immunogenicity profile to date.[211,212] A recent study that tested an insect-specific alphavirus as the vaccine platform found promising results in mice and macaques, including immunogenicity after a single-dose.[213]

Table 10: For the clinician – a summary.

Adults and children (transmission directly via mosquito bite)
Patients in areas endemic for chikungunya, Zika or dengue presenting with an acute neurological disorder should be investigated for all three arboviruses
Encephalitis is the most commonly reported neurological complication associated with chikungunya; encephalitis has a worse prognosis than encephalopathy alone; a CSF pleocytosis is not always seen
In myelitis associated with chikungunya, CSF pleocytosis and MRI changes are not always seen
Guillain-Barré syndrome associated with chikungunya follows a similar course compared to other infections such as *C. jejuni*; most patients recover after immunomodulatory treatment
Disease of both the central and peripheral nervous system in the same patient can be seen in association with chikungunya infection
Ophthalmological complications associated with chikungunya have been reported both at the time of infection and up to 12 weeks after; some reports describe treating with steroids, recovery is variable
Following chikungunya infection, complications of other organs can also occur at the same time as disease of the nervous system; such cases should be managed using a multidisciplinary approach
There is currently no available antiviral treatment or vaccine for chikungunya
Neonates (vertical transmission)
Neonates born to mothers experiencing symptoms of chikungunya infection near the time of delivery require admission and observation for signs of vertical transmission for at least 7 days post-partum, as they may be asymptomatic for the first few days of life
Neonates born to mothers infected outside of the peri-partum period are usually unaffected by chikungunya virus
Caesarean section does not appear to be protective in vertical transmission of chikungunya
Neonates infected with chikungunya should be followed up for at least 2 years, regardless of symptoms in the first week of life; the neurodevelopment of those without clinical encephalopathy at birth can still be affected

3.4 Conclusion

Neurological disease associated with chikungunya virus is being reported increasingly, in part due to the recent introduction of the virus to the South American population and associated large outbreaks. Clinicians and public health officials globally face challenges from the wide range of associated neurological disease and the complicating factor that dengue and Zika viruses are transmitted by the same mosquito vectors and have broadly similar epidemiology. In endemic areas, chikungunya virus should be tested for in all patients presenting with acute neurological disease and all mothers presenting with fever, arthralgia or rash; neonates with suspected symptomatic infection should be followed up for at least two years for evidence of neurodevelopmental delay. Future challenges include understanding the full scope of chikungunya neurological disease, both in neonatal and adult infection, and their underlying pathophysiological mechanisms. It is hoped that new direct therapeutic and vaccine candidates, some of which have shown promise in early studies, will augment the current supportive management strategies.

Chapter 4: General Discussion

4.1 Summary

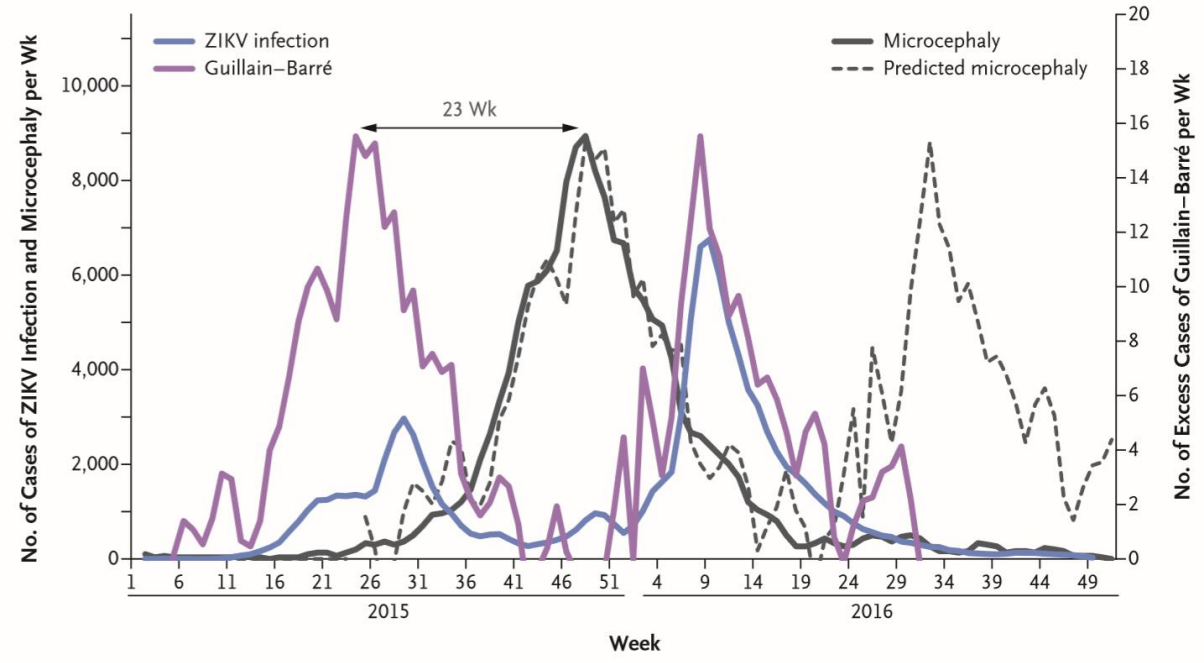
The Zika public health emergency was recently declared over, recognising that the virus is here to stay and a sustained technical and research response is needed.[21] Chikungunya virus continues to be a major concern in the tropics, with over 170,000 confirmed cases in Brazil in 2017 so far.[104] The retrospective case series documented a wide spectrum of neurological complications associated with Zika virus, including central nervous system disease, as well as highlighting the need to be vigilant for co-infection of more than one arbovirus in the setting of neurological disease. Furthermore, the study showed an unexpected role of chikungunya virus in disease originally thought to be due to Zika. Following on from this, the systematic review of the neurological complications associated with chikungunya provides a much-needed summary of the topic. Importantly, it emphasises the wide range of disease, with encephalitis as the most common presentation in adults. It highlights that neurological complications are also sometimes seen in conjunction with complications of other body systems; clinicians should be aware of this, and manage such cases using a multidisciplinary approach. Regarding vertical transmission, the review underlines the need for vigilance in pregnant women infected during the peri-partum period, and follow-up of at least two years required for the infant, regardless of symptoms at birth.

4.2 Further work done since this research

Since the retrospective case series, further work has been done that bears significance to the data above. Da Silva *et al.* performed a prospective case series in another hospital in Rio de Janeiro, over a similar time period (December 2015 – May 2016) to my study.[214] They described 35 patients with an acute neurological disorder and evidence of recent Zika infection. 27 patients had GBS, five had encephalitis, two had transverse myelitis and one had chronic inflammatory demyelinating polyneuropathy, a chronic form of GBS. The major limitation of this study was that chikungunya virus (circulating at the time) was not tested for, thus chikungunya and coinfection as the aetiology of disease in their cases could not be ruled out. Nonetheless, the study still adds to the evidence in favour of a wide spectrum of neurological disease associated with Zika virus.

A simple yet intriguing epidemiological study by Oliveira *et al.* argues further for the potential role of chikungunya virus in the midst of the Zika epidemic in Brazil.[215] The authors used routinely collected surveillance data from 2015 and 2016 to plot the number of suspected cases of Zika, GBS and microcephaly in the Northeast region of Brazil (see Figure 13).

Figure 13: Suspected cases of Zika virus infection, GBS and microcephaly in the Northeast region of Brazil (from de Oliveira *et al.*, Zika Virus Infection and Associated Neurologic Disorders in Brazil).[215]

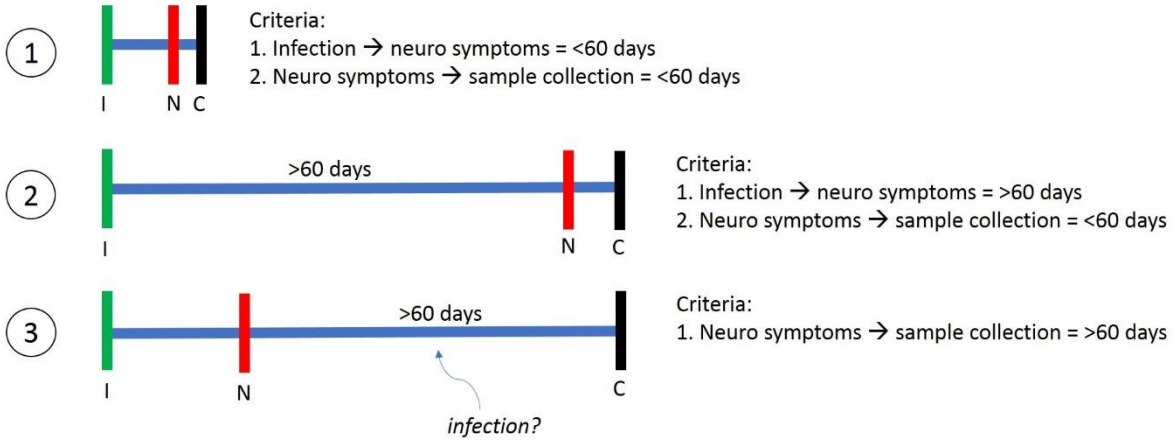


As seen on the graph, both year's increases in suspected Zika infections are associated with an increase in cases of GBS. However, only the 2015 suspected Zika outbreak is associated with ensuing microcephaly, whereas in 2016 the same pattern is not seen. The authors suggest three possibilities to explain this, which are not mutually exclusive: firstly, in 2016, the wave of suspected Zika may have in fact been due to chikungunya (which would have been difficult to distinguish clinically). This would explain a concomitant increased incidence of GBS but not microcephaly, which chikungunya does not cause; this explanation is in line with my retrospective work. Other possibilities raised include the potential need for an additional cofactor to cause microcephaly, which was present in 2015 but not in 2016; and that public fear of the effects of Zika in 2016 led to fewer conceptions and/or a greater number of terminations of pregnancy.

A limitation of many of the studies investigating arbovirus-mediated neurological disease, and indeed in my retrospective series, is the small number of cases usually described; this is due to the rarity of the investigated complications and also because many affected areas have variable referral pathways that can lead to under-diagnosis and under-reporting. However, one study currently underway (led by colleagues in Recife, Brazil) that I am involved in the analysis of has data for over 150 arbovirus-positive cases. The study has retrospectively investigated patients from a single, large-volume tertiary referral centre,

which accepts patients from the entire state of Pernambuco, Northeast Brazil. Preliminary analysis of this study is in accordance with the data from Chapter 2, with a large range of neurological disease described secondary to Zika, as well as many patients with chikungunya and arbovirus co-infection. With a greater number of patients and more complex variability surrounding diagnostics, I looked at classifying the strength of evidence of the link between arbovirus infection and neurological disease for each patient into different groups; this was lacking in my own series. I produced a simple classification into three groups (for cases with a positive detection of an arbovirus), which I felt was a learning point as a useful tool to consider such data in a more structured fashion:

Figure 14: Classification of arbovirus-positive cases depending on prodrome length and timing of sample collection.



Key:

I = initial symptoms of acute arbovirus **infection** (fever, rash, arthralgia)

N = initial symptoms of **neurological** disease

C = **collection** of sample for arbovirus testing

In group 1, the time between initial symptoms of arbovirus infection and onset of neurological disease, and between the onset of neurological disease and sample collection are short. This gives the strongest evidence for a causal association between arbovirus and neurological disease. In group 2, the time between arbovirus infection and onset of neurological disease is longer (the 60-day cut off is arbitrary); this group of patients should be viewed differently. Here, might the arbovirus infection be an incidental finding and unrelated to the neurological disease? If the evidence for arboviral infection is a positive PCR test, this may indicate prolonged viral persistence, making a causal association more plausible. However, if it is based purely on a positive IgM antibody, which, for example, can persist for more than five months in chikungunya infection,[216] the evidence for a causal association is not as strong. A caveat to this is when IgM is detected in the CSF; given IgM

antibodies do not cross the blood brain barrier, CSF detection of IgM implies CNS infection and thus causality in disorders of the nervous system. In group 3, there is an extended time between onset of neurological disease and sample collection. There are multiple potential reasons for this scenario, such as delayed presentation to hospital or samples only being collected at follow-up. Here, there is a risk that the patient may have had an arbovirus infection during this extended time, and thus a risk of attributing a causal role to an innocuous detected infection. Although classifying cases into the above group helps one assess the strength of evidence regarding a causal association between arbovirus and neurological disease, how can one try to mitigate such bias?

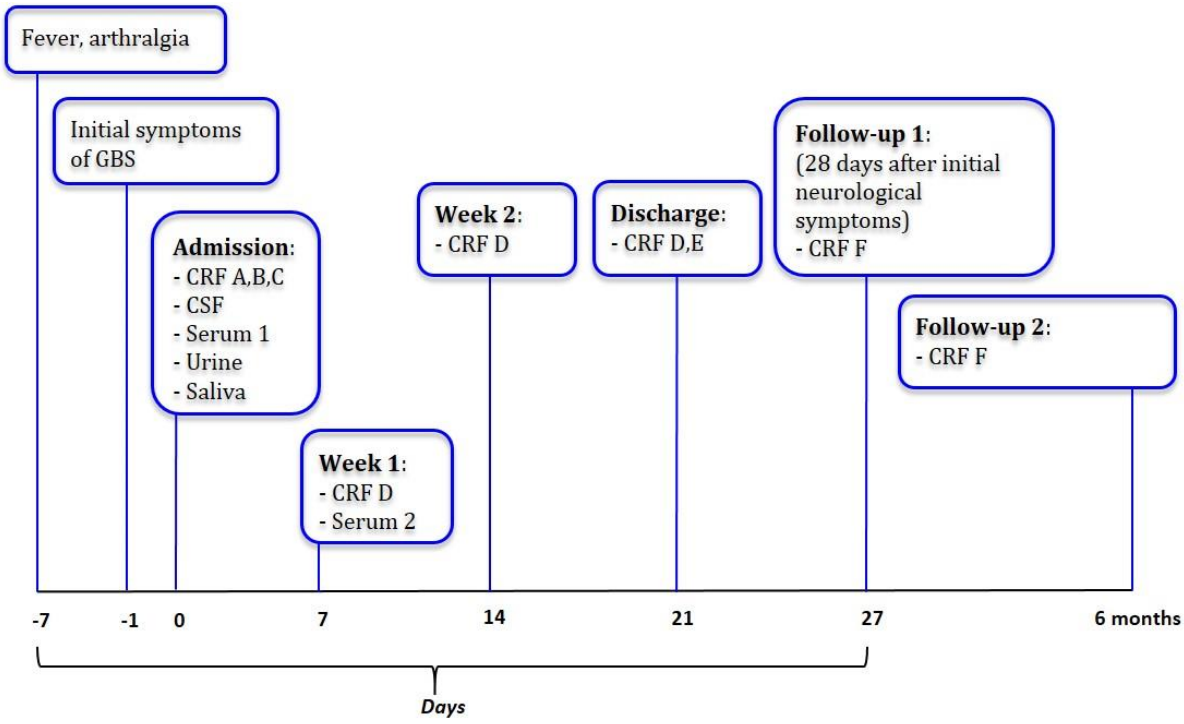
4.3 Future work

A prospective case-control study, which I have helped set up and is currently running in Rio de Janeiro and Recife, is designed to address some of these issues and also address other limitations faced in the retrospective setting. Firstly, analysing whether the proportion of recent arbovirus infection is statistically higher in those presenting with neurological disease compared with area-matched controls (without neurological disease) will clarify whether or not we are simply seeing an incidental finding of innocuous arbovirus infection; this will provide evidence for or against a causal relationship. Secondly, samples would always be collected at the point of admission, reducing the number of patients falling into group 3 above. Furthermore, this would reduce the number of missing samples (of urine, serum or CSF) faced in my retrospective work – this would likely allow for a higher detection rate of arboviruses, and more complete data to differentiate between CNS and systemic infection. The prospective nature of such a study would also improve follow-up data, as following up patients in a retrospective series is more difficult logistically. Lastly, a prospective study recruiting all cases of acute neurological disease would help answer an important question that I could not answer in the retrospective series, specifically whether asymptomatic arbovirus infection can also cause neurological complications. This question needs to be addressed in order to understand the full disease burden of both Zika and chikungunya viruses. In addition, I recently received communication from another research team, who report that they have seeing a wider spectrum of neurological disease in the outpatient setting, such as atypical Parkinsonian syndromes and multiple sclerosis associated with chikungunya infection. This has not been investigated for in the retrospective study, as these patients do not necessarily present acutely to a hospital; future studies will need to incorporate this outpatient cohort.

From an educational perspective, carrying out both the retrospective series and systematic review taught me valuable lessons for planning the prospective study, which is currently

underway. For example, when faced with the wide range of neurological disease associated with chikungunya identified in the review, I ensured that the study entry criteria allowed flexibility for such a range. I used strict criteria for suspected GBS, encephalopathy and myelopathy, but included a final criterion of "other: any other neurological syndrome (e.g. stroke, cranial nerve palsy, movement disorder) suspected to be linked to arbovirus infection." The wording allows, for example, patients with strokes suspected to be secondary to arbovirus infection to be recruited, without recruiting all cases of stroke (which would far exceed the study's capacity). Of note, the former criteria do not require a suspected arbovirus infection, in order to be able to recruit any cases that may have had a recent asymptomatic infection. Other lessons learnt relate to data generation, storage and analysis. In the retrospective study, manually entering all the retrospective data into an excel spreadsheet for analysis was time-consuming and inefficient; I therefore designed an online version of the case report form using the Research Electronic Data Capture (REDCap) tool.[217] This has proven to be easy to use, and will greatly assist analysis of the larger volumes of data generated. Additionally, given the limitation of not having had all three of CSF, serum and urine samples for all patients experienced in the retrospective series, I was able to design a protocol for the prospective series that ensured all samples (and clinical information) needed would be acquired and clearly organised.

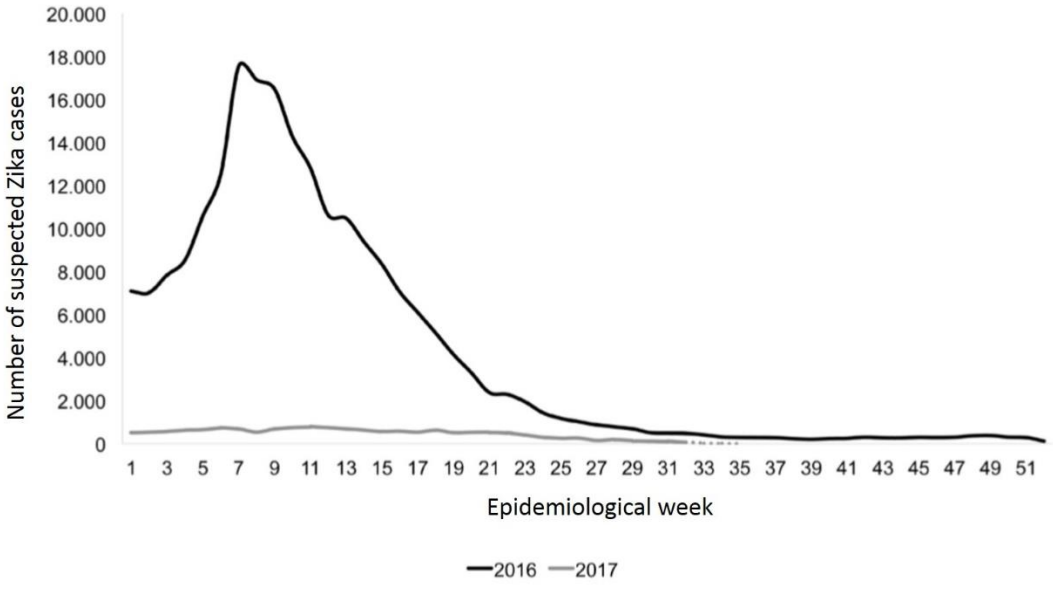
Figure 15: Prospective study case recruitment – example protocol for sample collection and case-report form (CRF) section completion.



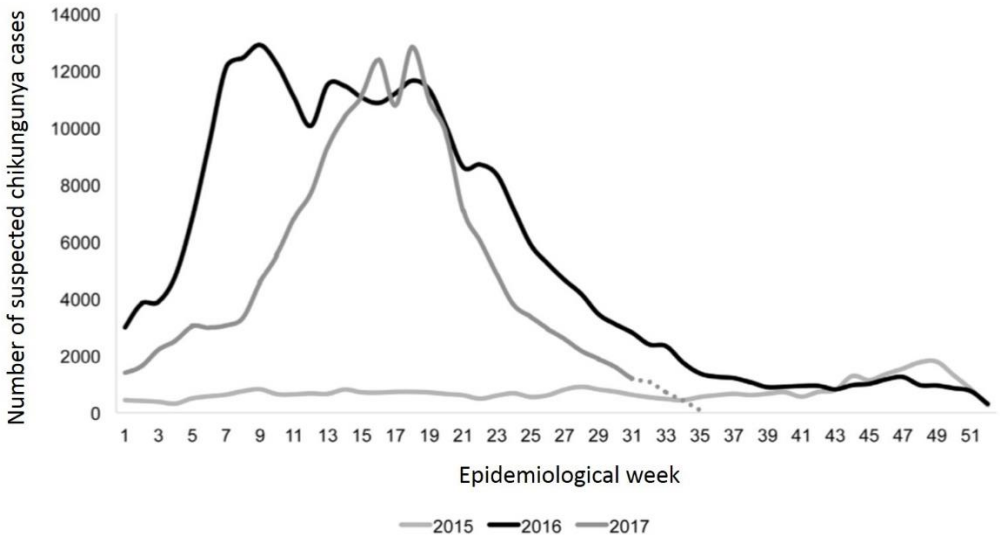
However, this prospective study, which started at the end of 2016, may only have limited relevance if Brazil does not continue to see arbovirus epidemics. Although chikungunya is still circulating (171,930 suspected cases this year as of September 2017), after the Zika epidemic of the 2015-16 summer (December-March), the country did not experience a similar outbreak during the 2016-17 summer, and has seen far fewer cases overall so far in 2017 than in 2016 (15,586 suspected cases this year as of September 2017, versus 216,207 suspected cases in 2016).[104] For the prospective study, the sample size needed for an odds ratio of 2:1 with a low incidence of arboviral disease increases significantly, and would not be feasible with the current protocol.

Figure 16: Suspected cases of (A) Zika and (B) chikungunya in Brazil in 2016 and 2017 (adapted from an epidemiological bulletin from the Brazilian Ministry of Health).[104]

A.



B.



There are multiple reasons why dramatic outbreaks might have been seen in 2015-16 but not currently. One study postulated that the 2015 El Niño event may have been partially responsible for the scale of the outbreaks.[218] The El Niño is a climatic phenomenon that occurs every few years, where a change in the Pacific Ocean surface temperature can lead to increases in rainfall and temperatures in South America; this in turn is conducive to greater mosquito populations and transmission of arbovirus disease. Another potential reason may be that a great enough proportion of the Brazilian population is now immune to the virus(es) after exposure in 2015-16; in effect a form of herd immunity. We do not yet know the seroprevalence of either chikungunya or Zika in Brazil, for differing reasons. For chikungunya, only two small-scale studies have investigated this, both in the state of Bahia. One study from a rural district sampling 120 people found a seroprevalence of 20%,[219] a second study from the city of Salvador sampling 633 people found a seroprevalence of 7.4%.[219] More data is needed from throughout the country to further characterise this. Regarding Zika, although the latter study estimated its seroprevalence at 63.3%, there is significant cross-reactivity with dengue virus in the commercial detection kits used for detecting IgG-specific antibodies (Medialdea-Carrera *et al.*, unpublished work). Given that the majority of the Brazilian population has been exposed to dengue, this is unreliable as a marker for previous Zika infection. However, I am currently involved in work using a new, in-house inhibition assay with a Zika-specific monoclonal antibody that will hopefully overcome this challenge. Regardless, if the tropics do not experience further epidemics of Zika or chikungunya, we will need to rely on only retrospective data to further characterise their neurological effects.

Given that diagnostics proved to be challenging in the retrospective studies, with issues such as the possibility of serological cross-reactivity and potentially false positive PCR results, upgrades in existing diagnostic technologies are welcome in the field. A recent interesting study looked at using the CRISPR-Cas (clustered regularly interspaced short palindromic repeat) system in diagnostics.[220,221] This is another example of optimising a cleverly adapted system seen in nature to scientific benefit. The researchers used Cas13a, an enzyme that is activated on contact with a pre-set, specific RNA sequence – on activation, the enzyme cleaves reporter RNA species, which fluoresce in solution, thus being able to specifically detect tiny concentrations of target RNA. The authors were able to use this system to separately identify Zika and dengue virus RNA. This technology remains to undergo clinical evaluation, but may hold promise with regard to improving sensitivity and specificity in the diagnostic workup of these patients.

Further important questions will need to be addressed in future research. We currently do not have an accurate estimate of the rate of neurological complications as a percentage of all Zika infections. This may be due to firstly, the aforementioned paucity of reliable seroprevalence data; secondly, the need for data regarding initially asymptomatic infections; and thirdly, a lack of awareness amongst clinicians of the full spectrum of neurological disease, potentially leading to under-reporting of Zika as the cause. In a pregnant cohort of mothers with symptomatic Zika infection from Rio de Janeiro, Brazil, 42% (49/117) of live births of foetuses exposed to Zika virus *in utero* were found to have grossly abnormal clinical or radiological findings; “almost all” abnormalities affected the CNS – however, only 31 of the 49 infants had abnormal results on clinical examination, the rest had abnormal radiology alone – those interpreting this imaging had knowledge of the Zika PCR status, which increases the risk of confirmation bias; therefore, this high result must be interpreted with caution.[23] Another study from the USA reported a rate of birth defects of 6% (26/442) from pregnancies with laboratory-confirmed evidence of Zika infection; all cases had reported neurological disease (22 had brain abnormalities, four had encephalocele, eye or hearing abnormalities).[222] A more recent prospective cohort study from the French territories in the Americas aimed to narrow down this range of 6-42% and found that of 555 fetuses and infants born to mothers who had preterm PCR-proven Zika infection, 39 (7.0%) had neurological and ocular defects possibly associated.[223] Regarding adult neurological disease secondary to Zika, one study estimated the prevalence of Zika-associated GBS at 1.23% by performing a meta-analysis of three studies.[224] However, the ‘denominators’ in the three studies, i.e. the population of Zika-infected individuals with or without neurological complications, were defined differently. For example, one study used PCR-confirmed Zika infection, whereas another included cases with clinical suspicion only. Without a

standardised denominator, it is difficult to draw a reliable conclusion. Regarding neurological complications other than GBS in Zika infection, to the best of my knowledge there is not yet any epidemiological evidence providing an association; this may be due to a very low incidence of disease or under-recognition of the potential manifestations of Zika infection.

Another important area of research for both viruses is in vaccines. As mentioned in the systematic review, trials of chikungunya vaccines are already beginning to show promise.[211,212] Zika, on the other hand, is more complicated. A number of studies trialling vaccine candidates on animal models have shown a good safety profile, induced humoral and cellular responses and protection from challenge with live virus.[225-227] A recent phase 1 human trial with 40 participants testing a DNA vaccine showed a good safety profile, induction of vaccine-specific antibodies in all cases, and neutralising antibodies in 62-95% of participants, depending on the cell line used.[228] However, a number of issues will need addressing as this field develops. Firstly, *in vitro* studies have already demonstrated the ability for prior dengue infection to enhance Zika infection via the process of antibody-dependent enhancement.[106,229] All patients in our retrospective series were positive for dengue IgG – whether this contributed to the progression to severe neurological disease resulting from Zika infection is unknown. Given that a large proportion of those at risk of infection with Zika live in dengue-endemic areas, there is concern whether antibody dependent enhancement might affect the safety or immunogenicity of a vaccine candidate.[230] Indeed very recently (December 2017), the distribution of the leading dengue vaccine, Dengvaxia (Sanofi), was halted in the Philippines amidst safety concerns of administering the vaccine to seronegative children, who may be at risk of developing more severe dengue disease (via antibody-dependent enhancement) should they become infected at a later stage.[231,232] In addition, the effect on the safety of commercial vaccines for (or previous infections with) other flaviviruses, namely yellow fever and Japanese encephalitis, is unknown. Secondly, there is a theoretical possibility that a vaccine itself may trigger neurological disease, either via molecular mimicry or, in the case of a live attenuated vaccine, via direct neurotropic effects (this applies to chikungunya as well). If this were to occur even rarely (as reported with the yellow fever vaccine),[233] it would further stress the importance of needing accurate data on the incidence of neurological complications seen in native Zika and chikungunya infection, in order to assess the value of the vaccine.

4.4 Pathophysiology

The mechanisms by which Zika and chikungunya viruses affect the nervous system have not been fully elucidated. Important questions include: whether the viruses act directly or indirectly towards neurons and if the process differs in the central and peripheral nervous

systems, how certain patients develop neurological disease after infection and others do not, the significance of the phylogenetic strain and factors driving placental transmission. Zika virus is a single-stranded, positive-sense RNA virus with a genome of 10,794 nucleotides, whose open reading frame (triplets of nucleic acid that have the potential to be translated to protein) codes for three structural proteins (capsid, premembrane/membrane and envelope) and seven non-structural proteins (NS: 1, 2A, 2B, 3, 4A, 4B and 5).[7] Chikungunya virus is also a single-stranded, positive-sense RNA virus; its genome is approximately 12kb in length and has two open reading frames encoding five structural proteins (capsid and glycoproteins E1, E2, E3 and 6K) and four non-structural proteins (NSP: 1, 2, 3 and 4).[234,235]

It has been shown in vitro that Zika virus can infect and affect various types of cells of the nervous system, including neural stem cells, oligodendrocytes, astrocytes and microglia; neuronal infection has been reported but appears limited.[236,237] As discussed in Chapter 2, the para-infectious temporal profile of certain neurological presentations associated with Zika infection are consistent with direct neuroinvasion by the virus, leading to disease. However, other possibilities also exist to explain this profile, including an autoimmune process starting before fever-arthralgia-rash symptoms of Zika start, or a hyperacute immune response. Direct Zika neuroinvasion was also supported by data from a mouse model looking at congenital infection, where after injection of the virus into embryonic brains, it was shown to infect, proliferate and cause apoptosis in neural progenitor cells, causing a clinical syndrome of microcephaly.[238] Regarding chikungunya, neurons, astrocytes and oligodendrocytes (but not microglia) have been shown to be susceptible to chikungunya infection in vitro; the former two cell types were shown to undergo apoptosis post-infection.[239,240] In vivo, subcutaneous inoculation in macaques resulted in morphological changes in astrocytes, including cell body hypertrophy and alteration in the pattern of branching of their primary processes.[241] Clearly, the in vitro and animal models have limitations with regard to extrapolating their data to human infection, such as route of infection and non-human cell types. However, they provide preliminary evidence that both viruses can potentially directly affect cells of the nervous system.

Aside from directly affecting cells of the nervous system, much attention has been focussed on the role of autoimmunity in Zika and chikungunya-associated neurological disease, both with regard to the humoral and adaptive immune systems. Regarding the former, a study showed a vast range of peptide sequences on proteins expressed by both Zika virus and humans – many of which are associated with both congenital neurological syndromes (53 sequences) and GBS (both demyelinating and axonal subtypes, 216 sequences).[242] Thus, this allows for disease via a process of molecular mimicry, whereby antibodies generated

against a Zika peptide sequence might inadvertently affect the host's own cells of the nervous system. Regarding chikungunya, the study on macaques above also found upregulation of toll-like receptor-2 (TLR2) in gray matter astrocytes, a gene coding for a sentinel cell surface receptor that plays an important role in identifying pathogens and the overall innate immune response.[241] However, the clinical neurological state of the macaques was not reported, which adds to the uncertainty of whether the immune response was protective or pathogenic in these cases. Another study that subcutaneously inoculated mice with chikungunya virus detected upregulation of TLR3 in the brain, a gene that is also associated with the innate immune response.[243] Amongst other clinical signs, these mice developed hind-limb paralysis, dehydration and weight loss, and 25% of them died after one week. However, pre-treatment with Polyinosinic: polycytidylic acid (a TLR3 agonist and interferon inducer), was protective clinically and promoted viral clearance from the brain, arguing for a *protective* innate immune response, at least in CNS disease. In concordance, faster viral clearance after chikungunya infection was seen in wild-type mice compared with a TLR3 knockout model; this was thought to be secondary to increased antibody neutralizing activity in the wild-type mice.[244] Chikungunya-infected TLR3 knockout mice had increased viral dissemination throughout the viscera, including the brain. Along with a U-shaped pattern of age-specific incidence, this critical role of TLR3 is reminiscent of susceptibility to HSV encephalitis. Thus overall, there is evidence for both a pathogenic and protective role of the innate immune system in such disease, and further work is required to help tease out which role is more pertinent and what other viral or host factors affect them.

A study from India compared the cytokine profile for patients with and without neurological complications following chikungunya infection.[245] Of those with neurological disease, four had encephalitis and one had "neuropathy". Concentrations of four cytokines (TNF-α, IFN-α, IL-6 and MIG) were found to be significantly higher in patients with neurological disease secondary to chikungunya, as opposed to uncomplicated chikungunya infection. These cytokines have a broad range of actions relating to the immune response. For example, MIG acts as a chemoattractant and potential stimulant of T cells.[246] Again, as with the examples cited above for the innate immune response, it is difficult to know whether in these cases, such an adaptive immune response is beneficial or the actual cause of the disease. Indeed, in a knockout mouse model lacking mature T and B lymphocytes, the levels of chikungunya RNA in ankles, quadriceps muscles and spleens were elevated compared to wild type mice, suggesting a protective role of the adaptive immune system – however, histological interrogation of these organs in the acute phase showed more inflammation in the wild type mice, suggesting a pathological role.[247] Regarding Zika, another knockout mouse study suggested a pathogenic role for the adaptive immune system in Zika-associated paralysis.[248]

The authors used type 1 interferon receptor knockout mice, in whom Zika virus was shown to heavily infect astrocytes, resulting in the breakdown of the blood brain barrier (shown by bright staining for mouse IgG, which is normally blocked by the barrier in mice). This, in turn, was accompanied by a vast infiltrate of CD8+ effector T cells associated with hindlimb paralysis; furthermore, CD8+ depleted mice had higher survival and only 1/9 developed paralysis – taken together, the results suggest a pathogenic role for these cells. Further such studies will need to take place in the human setting, however obvious ethical restrictions apply; peripheral blood mononuclear cell (PBMC) isolation will help in this scenario and indeed such studies are underway in the Recife cohort of patients presenting with arbovirus-associated neurological disease.

Patients diagnosed with myeloneuropathy or encephalomyeloneuropathy exhibit disease of both the central and peripheral nervous systems. Given the association between chikungunya and GBS, it is not clear whether in these cases, there is one underlying pathological process involving both the CNS and peripheral nerves, or dual pathology, with a myelopathy +/- encephalopathy centrally, and GBS peripherally. For example, a case report from India described a 73-year-old man who, a week after chikungunya infection, was admitted with drowsiness, weakness and absent reflexes, and eventually died.[120] His CSF and MRI showed evidence of CNS involvement, and an electromyogram showed a sensorimotor neuropathy. A brain autopsy showed subarachnoid haemorrhage, ischaemic changes and small foci of demyelination without identification of viral inclusion bodies. Clearly, this patient had involvement of both central and peripheral nervous systems at the same time, but it is not clear whether the same pathological process was responsible for both. Elucidation of these mechanisms may help to better guide management strategies.

Neurological disease secondary to chikungunya has been reported in areas with both ECSA (or ECSA-diverged IOL) and Asian strains, but whether these strains have differing neurovirulence is unknown. One study compared the effect of intracerebral inoculation of Asian and ECSA-diverged strains in mice.[249] Both spread within the brain to a similar extent, but the Asian strain was associated with higher mortality than the ECSA-diverged strain. Upregulation of a gene associated with apoptosis was seen in the former, whilst anti-apoptosis, antiviral and CNS protective gene upregulation were seen in the latter. This potentially suggests a higher neurovirulence of the Asian strain, and comparative clinical data from countries such as Brazil, where both strains are circulating, will be useful.

On neonatal neurological disease, given that caesarean section is not protective, vertical transmission is unlikely to occur via the birth canal, as is the case in other neonatal

infections such as herpes simplex.[250] Furthermore, the placenta seems to act as a barrier to transmission, as one study reported (as unpublished data) that placental cells from infected neonates were negative when labelled with anti-chikungunya antibody.[61] One hypothesis raised by the authors is that uterine contractions result in breaches of this placental barrier, allowing passive passage of the virus.

4.5 Conclusion

Zika and chikungunya viruses are likely to continue to be pathogens of global concern, with increasing rates of urbanisation, more accessible global travel and a warming climate all suiting ongoing transmission via their mosquito vector. The work in this thesis helps document in detail the clinical range of associated neurological disease of both viruses, as well as highlighting other important facets, including the utility of testing multiple body fluid samples, issues surrounding cross-reactivity in flavivirus serology, and the phenomenon of arbovirus co-infection. Future work in this area should aim to provide the data required to answer important questions, including the rate of neurological complications in both arbovirus infections and the effect of prior flavivirus exposure on disease progression and vaccine efficacy and safety. These answers will better equip researchers, clinicians and public health officials to reduce the disease burden of these two arboviruses.

Appendix

Hospitals

Hospital Federal dos Servidores do Estado

Hospital Universitário Pedro Ernesto

Instituto de Pesquisa Clínica Evandro Chagas (IPEC)

Hospital do Andaraí

Hospital Geral de Bonsucesso

Hospital Barra D'or

Hospital de Clínicas de Niterói

Hospital Bangu D'Or

Hospital Icaraí

Hospital Badim

Hospital São Vicente de Paulo

Diagnostic criteria

1. Guillain-Barré syndrome (adapted from Sejvar et al. 2011, Hadden et al. 1998)[251,252]

<table>
<tr><th>Level 1</th><th>Level 2</th><th>Level 3</th><th>Level 4</th></tr>
<tr><td colspan="3">[ ] Bilateral and flaccid weakness of the limbs
AND
[ ] Absence of an alternative diagnosis for weakness
AND
[ ] Decreased or absent deep tendon reflexes in affected limbs
AND
[ ] Monophasic illness pattern with weakness nadir between 12 hours and 28 days, followed by clinical plateau</td><td rowspan="4">Suspected GBS with no other diagnosis apparent, but does not fulfil level 3 criteria</td></tr>
<tr><td colspan="2">[ ] CSF total white cell count < 50 cells/mm^3
OR
[ ] If CSF results unavailable, electrophysiological findings consistent with GBS</td><td rowspan="2"></td></tr>
<tr><td>[ ] CSF protein level above laboratory normal value AND CSF total white cell count < 50 cells/mm^3
AND
[ ] Electrophysiological findings consistent with GBS</td><td></td></tr>
<tr><td>Classify → [ ] Level 1</td><td>[ ] Level 2</td><td>[ ] Level 3</td><td>[ ] Level 4</td></tr>
</table>

2. Encephalitis (adapted from Granerod et al. 2010, Venkatesan et al. 2013)[141,253]

Level 1	**Level 2**	**Level 3**
[ ] Acute or sub acute (<4 weeks) alteration in consciousness, cognition, personality or behaviour persisting for more than 24 hours [ ] Absence of an alternative diagnosis for symptoms		
[ ] New onset seizure **OR** [ ] New focal neurological signs **OR** [ ] Fever (≥ 38ºC) **OR** [ ] Movement disorder (includes: Parkinsonism, oromotor dysfunction etc.) **OR** [ ] EEG consistent with encephalopathy +/- epileptiform features		
[ ] CSF total white cell count > 5 cells/mm^3 **OR** [ ] Neuroimaging compatible with encephalitis		
Classify → [ ] Level 1	**[ ] Level 2**	**[ ] Level 3**

3. Myelitis (adapted from Transverse Myelitis Consortium Working Group Criteria, 2002.)[184]

<table>
<tr><td>Level 1</td><td>Level 2</td><td>Level 3</td><td>Diagnosis unclear</td></tr>
<tr><td colspan="4">[ ] Acute onset of weakness or sensory disturbance of upper and/or lower limbs
[ ] Absence of an alternative diagnosis for symptoms</td></tr>
<tr><td colspan="3">[ ] Brisk reflexes or extensor plantar response
OR
[ ] Bladder or bowel dysfunction
OR
[ ] Clearly defined sensory level</td><td></td></tr>
<tr><td colspan="2">[ ] Absence of extra-axial compressive aetiology by neuroimaging (MRI or CT myelography)
AND
[ ] Absence of flow voids on the surface of the spinal cord suggestive of arteriovenous malformation (MRI)</td><td colspan="2"></td></tr>
<tr><td>[ ] CSF total white cell count > 5 cells/mm^3
OR
[ ] MRI changes consist with myelitis</td><td colspan="3"></td></tr>
<tr><td>Classify →[ ]Level 1</td><td>[ ] Level 2</td><td>[ ] Level 3</td><td>[ ] Diagnosis unclear</td></tr>
</table>

4. Meningitis (adapted from McGill F, Heyderman RS, Michael BD, *et al.* 2016)[254]

<table>
<tr><th>Level 1</th><th>Level 2</th><th>Level 3</th></tr>
<tr><td colspan="3">[ ] Absence of an alternative diagnosis for symptoms
AND
[ ] Neck stiffness
OR
[ ] Kernig’s sign positive (with the hip and knee at 90° angles, subsequent extension of the knee causes pain)
OR
[ ] Brudzinsky’s sign positive (involuntary lifting of the legs on lifting the patient's head from the examining couch)</td></tr>
<tr><td colspan="2">[ ] Fever (≥ 38ºC)</td><td></td></tr>
<tr><td>[ ] CSF total white cell count > 5 cells/mm^3
OR
[ ] Meningeal enhancement seen on contrast enhanced CT or MRI</td><td colspan="2"></td></tr>
<tr><td>Classify → [ ] Level 1</td><td>[ ] Level 2</td><td>[ ] Level 3</td></tr>
</table>

5. Acute Disseminated Encephalomyelitis (ADEM) - adapted from the clinical definition proposed by Krupp, Banwell, *et al.,* 2007[255]

6. Radiculitis - based on clinical features and electrophysiological studies[256]

7. Myositis - based on clinical features and electrophysiological studies

8. Facial diplegia with paraesthesia (GBS variant) - modified from Dimachkie & Barohn, 2013[257]

Table 1: Diagnoses of 13 patients without evidence for a recent Zika, chikungunya or dengue virus infection.

Diagnosis	Number of patients
Guillain-Barré Syndrome	5
Miller Fisher Syndrome	1
Neuromyelitis optica	1
Encephalitis	1
Facial nerve palsy	1
Myeloradiculitis	1
Myelitis	1
Encephalomyeloradiculitis	1
Radicular pain	1

Table 2: Statistical analyses

A: Wilcoxon-Mann-Whitney U-test comparing median time from infection to neurology between CNS and peripheral nervous system disease

	Median time from infection to neurology (days)	P-value
CNS disease +/- peripheral disease (n=14)	4	0.009
Peripheral nervous system disease only (n=6)	11	

B: Wilcoxon-Mann-Whitney U-test comparing median time from infection to neurology between CNS disease (+/- peripheral disease) associated with and without CNS Zika

	Median time from infection to neurology (days)	P-value
CNS disease associated with CNS Zika (n=5)	1	0.216
CNS disease not associated with CNS Zika (n=9)	4	

C: Wilcoxon-Mann-Whitney U-test comparing median time from infection to neurology between peripheral nervous system disease only associated with and without CNS Zika

	Median time from infection to neurology (days)	P-value
Peripheral nervous sytstem disease only associated with CNS Zika (n=3)	10	0.400
Peripheral nervous system disease only not associated with CNS Zika (n=3)	29	

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