

# THE MULTIFACTORIAL BACKGROUND OF EMERGING VIRAL INFECTIONS WITH NEUROLOGICAL MANIFESTATIONS

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## ABSTRACT

The events of the past year have highlighted the continuing importance of emerging virus infections on the diagnosis and treatment of neurological disease. This review focusses on clarifying the effects of the multiple overlapping factors that impact emergence, including viral richness, transmission opportunity, and establishment. Case studies of the West Nile, chikungunya, and Zika viruses are utilised to illustrate the dramatic effects of expansion in the range and geographical distribution of emerging infectious disease, the acquisition of new virus vectors, and of increasing human anthropogenic factors such as global transport, climate change, and mosquito abatement programmes on the regional spread and clinical consequences of emerging infectious disease.

**Keywords:** Emerging infectious disease (EID), neurological disease, emerging viruses, Zika virus (ZIKV), chikungunya virus (CHIKV), West Nile virus (WNV), microcephaly, Guillain-Barré syndrome.

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## INTRODUCTION

The increasing occurrence of virus-induced emerging infectious disease (EID) is a significant global health concern.<sup>1,2</sup> EID is characterised by the presence of viruses that display either new transmission or increasing disease incidence, geographic range, or pathogenicity.<sup>3,4</sup> Approximately 60–75% of human EIDs are zoonotic,<sup>4,5</sup> thus the epidemiology of vectors and hosts represents a critical component of emergence. At one level emergence can be attributed to virus adaptation, but EID is increasingly impacted by anthropogenic effects as varied as changes in land use (e.g. deforestation), climate change, immigration, and the ease of human and product global transportation.

Infections with emerging viruses often profoundly impact the nervous system. Over the past decade, approximately 50% of EID in humans have exhibited some level of neurological involvement, often with life-threatening consequences.<sup>6</sup> We need look no further than the recent outbreak,

rapid spread, and neurological impact of Zika virus (ZIKV) in the Americas to appreciate the magnitude of this concern.<sup>7</sup>

Comprehensive overviews of viruses that cause neurological disease have been published in recent years.<sup>8-10</sup> Nonetheless, the direct consequences of viral infection within the nervous system are often difficult to diagnose clinically as symptoms may overlap with more common, non-infectious causes. Clinical diagnosis is also frequently hampered by the diversity of emerging viruses, their mode of transmission and latency, a knowledge gap in the biology of emerging viruses, and the corresponding lack of high-level clinical facilities and diagnostic tests that enable precise laboratory-based distinction of viruses and/or virus subtypes.

Viruses that were once geographically widespread and thought to be controlled, such as poliomyelitis, may re-emerge, and viruses that are endemic to one region may spread episodically to another, as has been the case with the Ebola virus. The regional origins of emerging infections contribute

to this dilemma, as it is common to assume that such infections only occur in exotic locations. However, it has been shown that the highest concentration per area of EID occurs between latitudes of 30–60° north and 30–40° south,<sup>5</sup> with predicted hotspots in tropical countries of Asia, Latin America, and Africa due to the abundance of vector-borne pathogens, increased animal zoonosis, and human population density. This constellation of effects contributes to a general lack of awareness and preparedness for the potential spread and corresponding neurological effects of emerging viruses.

The earliest phase of emergence is dependent on virus transmission, which is governed by the essential drivers of viral richness (genetic diversity, saturation frequency of host infection, and climate adaptability), transmission opportunity (population density, alternate host availability, geographic vector restriction, host mobility, and other unique exposure frequency), and establishment (ease of human transmission and herd immunity).<sup>11</sup> Perhaps the best example of richness as a driver of EID is the high mutation and recombination rates inherent to retroviruses (e.g. human immunodeficiency virus) and orthomyxoviruses (influenza viruses), respectively. However, many other viruses also display relatively high rates of mutation and adapt rapidly to new environmental conditions, often in spatially disparate locations.<sup>12,13</sup> EID is also linked to changes in transmission opportunity through either expansion of virus host range and/or geographical distribution. This is particularly evident in that many viruses do not survive for long periods of time outside of their hosts. As a result, transmission is often heavily influenced by the proximity of hosts and/or vectors. Several recent EID outbreaks of viruses transmitted by arthropod vectors (arboviruses) provide illustrative case studies of the interplay among these biological and anthropogenic factors in driving the rapid geographic spread of EID.

## THE IMPACT OF EXPANDED HOST RANGE ON EMERGING INFECTIOUS DISEASE

The spread of West Nile virus (WNV) in the USA provides a classic example of the impact of host range expansion on EID transmission. WNV is a mosquito-borne *Flavivirus*, first isolated in 1937 in the West Nile region of Uganda. Over the intervening years, WNV was detected in sporadic

EID outbreaks of encephalitis, meningitis, and more rarely, acute flaccid paralysis, throughout North Africa and Eastern Europe, with more recent discrete reports in Romania (1996), Morocco (1996), Tunisia (1997), Italy (1998), and Israel (1998). In contrast, WNV was not detected in the USA prior to 1999. Indeed, epidemic arbovirus infections in humans were rare in the USA over the past century, with the exception of a major epidemic of St Louis encephalitis virus in the 1970s.<sup>14,15</sup>

However, in 1999, a cluster of neurological disease involving 62 infected subjects, with 59 requiring hospitalisation, were reported in New York City. Epidemiological analysis coupled to antigenic and genetic sequence mapping definitively identified WNV as the causative agent.<sup>15</sup> Subsequently, WNV expanded geographically at an alarming rate within the USA, such that by 2003, over 2,500 cases of WNV neuroinvasive disease were reported. The estimated total number of WNV infections in the USA now exceeds 1 million, encompassing the 48 contiguous states.<sup>16</sup> The majority of WNV infections are asymptomatic; approximately 20% present as fever and myalgia, and the incidence of serious neurological disease from WNV infection remains relatively constant at approximately 0.7%.<sup>16–18</sup> Documentation of infection is not straightforward as antigenic cross-reactivity among *Flavivirus* proteins is the norm, and thus disease symptoms must be coupled to the presence of either antiviral IgM in acute-phase serum or cerebral spinal fluid (CSF), an IgG titre shift of 4-fold or higher between acute and convalescent phases, and/or the direct demonstration of virus antigen or RNA in serum, CSF, or tissue.<sup>15</sup>

Determining the origins of WNV in the USA presents a challenge that remains speculative to this date. The lifecycle of WNV includes birds as the principle host, with the spread to humans and other species driven primarily by mosquitoes of the *Culex* genus, which are found throughout the USA. Although several models have been proposed to explain the origins of the initial cases in the USA, the most plausible rests with expansion of the avian host range. Genetic analysis of the virus isolated from patients demonstrated that the 1999 WNV isolate in New York City closely resembled the virus isolated from two avian species in 1998, harvested at the time of a local WNV epidemic in Tel Aviv, Israel.<sup>19–21</sup> Although WNV is rarely fatal in avian species, both the outbreak in Israel and New York were characterised by significant avian deaths.<sup>15</sup> In addition, birds can support

exceptionally high levels of viraemia for several weeks with titres of up to  $10^{11}$  plaque forming units (pfu)/mL reported, whereas virus titre in humans is transitory and rarely exceeds  $10^{2-3}$  pfu/mL.<sup>22</sup> Lastly, the spread of WNV throughout the USA was attributed to high virus titre in a relatively small number of common avian species such as robins (*Turdus migratorius*), blue jays (*Cyanocitta cristata*), and house finches (*Haemorhous mexicanus*).

Collectively, these observations suggest that the most likely origin of WNV in New York City resulted from the introduction of infected birds to the USA, rather than mosquitoes or infected humans. This might have occurred either by transcontinental migratory patterns, or by the introduction of infected birds as components of commercial or other transport.<sup>24</sup> Once in the USA, it is likely that the virus percolated within common avian species such as crows, where multiple deaths were observed, reaching both high infection penetrance and titre, which in turn promoted efficient mosquito transmission to humans during the exceptionally hot summer of 1999.<sup>15,24</sup>

## VECTOR BIOLOGY AND HUMAN FACTORS ENHANCE THE ORIGINS OF EMERGING INFECTIOUS DISEASE

Chikungunya virus (CHIKV) provides an important example of the balance among virus genetics, vector diversity, and anthropogenic effects in driving the global spread of infection and disease incidence. CHIKV is a mosquito-borne *Alphavirus*, which, while not uniformly neuropathic, has been shown to induce neurological disease in up to 12% of infected individuals in isolated outbreaks.<sup>10,25</sup> The incubation period for the disease is 3–7 days and it typically manifests as encephalomyelitis, radiculitis, Guillain-Barré syndrome, or acute flaccid paralysis.<sup>9,10,26</sup> In children, symptoms may be more severe and include altered levels of consciousness, seizures, and paraplegia.<sup>27</sup> Rare maternal-fetal transmission may also occur in viraemic mothers, with severe fetal encephalopathy observed in 50% of these cases.<sup>27,28</sup>

CHIKV was discovered in 1952 in Tanzania and until the last decade only sporadic outbreaks were reported in Africa and Asia.<sup>29</sup> However, the geographic range of CHIKV has spread dramatically over the past 10 years, including outbreaks in East Africa (2004), islands of the Indian Ocean and Sri Lanka (2005–2007), and an index case for an

autochthonous CHIKV epidemic in Northeast Italy that was tracked to a visitor from Kerala, India, where CHIKV infection was widespread (2007).<sup>30</sup> Within the past 24 months, CHIKV has spread to other parts of Europe and rapidly throughout the Americas from Brazil to the USA with >750,000 cases reported in total.<sup>24,31</sup>

Phylogeographic analysis of CHIKV genomes indicates that the recent geographic expansion in the Caribbean and Americas is linked to the Asian genotype, whereas expansion to South Asia represents the Indian Ocean genotype of CHIKV.<sup>32</sup> The virus is primarily spread by *Aedes aegypti* and *Aedes albopictus* mosquitoes; importantly, as the geographic range of CHIKV expanded, the Asian lineage of the virus evolved by accumulation of a single amino acid change (A226V) in the viral E1 protein that enhanced transmission and infectivity in *Ae. albopictus*.<sup>33</sup>

While expansion of vector distribution and transmission were primary drivers of the spread of CHIKV, the globalisation of CHIKV can also be attributed to multiple anthropogenic factors. These include the failure of mosquito control efforts in the Americas, increased vector habitats from growing urbanisation in the tropics, increased tyre trade across regional boundaries in Asia, and greater human transport between Asia, Africa, and the Americas at times of peak vector activity.<sup>34–36</sup> Creating a precise linkage of CHIKV genetics and vector utilisation with the incidence of disease and, more specifically, the detailed characterisation of the neurological effects within each EID outbreak, has proven to be a significant challenge as many of the effected regions have suboptimal health care delivery and/or reporting systems.

## CONFOUNDING EFFECTS OF PUBLIC HEALTH AND EPIDEMIOLOGY PREPAREDNESS ON EMERGING INFECTIOUS DISEASE

The current spread of ZIKV in the Americas represents an ongoing pandemic, the causes and clinical outcomes of which remain hotly disputed. ZIKV is a mosquito-borne *Flavivirus*, first isolated in 1947 from a sentinel monkey in the Zika forest of Uganda, but it is likely that it originated between 1892 and 1943.<sup>37,38</sup> The virus distribution spread slowly over the course of 60 years, coupled to mutation and possibly genetic recombination that enabled adaptation to new vectors. This eventually

led to ZIKV spread to countries in Central, North, and West Africa, as well as Southeast Asia.<sup>38-40</sup> Serological evidence from these outbreaks indicated that approximately 80% of ZIKV infections were asymptomatic with penetrance in 5-10% of the population. Indeed, the relatively benign past nature of ZIKV infection is reinforced by the observation of only 14 confirmed human cases, and no significant morbidity or mortality, from discovery until 2007.<sup>38,40</sup>

This changed dramatically in 2007 with the detection of a ZIKV outbreak on Yap Island in the Federated States of Micronesia. Viral RNA analysis confirmed the presence of ZIKV in 49 individuals, with an additional 59 probable but unconfirmed cases.<sup>41</sup> This outbreak spread to multiple islands within French Polynesia between 2013 and 2014. In this area, there were 294 laboratory confirmed cases and in excess of 20,000 suspected cases of infection.<sup>42,43</sup> Disease symptoms were similar to those described previously including rash, fever, arthralgia, and conjunctivitis. However, a distinguishing clinical outcome was 72 cases of severe neurological symptoms including 40 suspected cases of Guillain-Barré syndrome.<sup>43</sup> Approximately half of these cases were in individuals who displayed illness compatible with ZIKV infection, although in none of these instances was ZIKV infection definitively confirmed by laboratory analysis.

Of increasing concern is the more recent spread of ZIKV to the Americas and Caribbean Islands. Beginning in April 2015, ZIKV was first reported in Brazil and subsequently, as of February 2016, confirmed infections were reported in 31 countries within the region.<sup>44,45</sup> Current estimates of the total number of infections in Brazil alone range from 0.4-1.3 million individuals. Consistent with past outbreaks, the vast majority of ZIKV infections remain asymptomatic and those who present with illness predominantly display symptoms that resolve within 2-6 days. The most troubling aspect of this pandemic lies with coincident data from six countries (French Polynesia, Brazil, El Salvador, Venezuela, Columbia, and Suriname) of an increasing incidence of Guillain-Barré syndrome and, more dramatically, of microcephaly in newborns.<sup>45</sup>

Reports of an increase in babies born with microcephaly first surfaced in 2015 from the state of Pernambuco, Northeast Brazil. Subsequently, similar data was reported nearby in Bahia

and Paraíba, with an increased incidence of microcephaly now reported in 23 of the 26 Brazilian states. Though the linkage of microcephaly to ZIKV infection remains speculative, several lines of evidence support this hypothesis. First is the increase in disease incidence: microcephaly was previously rare in Brazil, with an average of 163 cases per year from 2010-2014, but summative reports for 2015-2016 have now identified 5,640 cases of microcephaly and/or central nervous system malformations, 120 of which resulted in death.<sup>45,46</sup> Secondly, ZIKV can cross the placenta; ZIKV RNA has been detected in the amniotic fluid of mothers who birthed babies born with microcephaly.<sup>47</sup> Thirdly, placental and brain tissue from at least two newborns with microcephaly who died within 20 hours of birth tested positive for ZIKV by reverse transcriptase polymerase chain reaction.<sup>48</sup> Lastly, recent reports indicate that ZIKV can infect and attenuate the growth of human neural progenitor cells in an *in vitro* model system.<sup>49</sup>

Despite these observations, it is likely that reports of microcephaly in Brazil, both before and after the current ZIKV outbreak, are inaccurate. As noted above, prior to 2014, the incidence of microcephaly in Brazil was only 0.5 in 10,000 live births. In other countries where the criteria for diagnosing microcephaly are established and the analysis is conducted by well-trained health professionals, the incidence holds steady at 2-12 in 10,000 live births. Although the ZIKV outbreak is now widespread, >90% of cases in Brazil remain in the Northwest region, encompassing Pernambuco and neighbouring states. This relatively poor region is ill-equipped to deliver the necessary health care during this pandemic and to maintain consistent standards of clinical and laboratory epidemiological outcomes. A more detailed follow-up analysis by Brazilian health authorities on 1,533 cases reported in 2015 indicates that 950 of these should not be classified as microcephaly, and to date, only 30 of the 583 confirmed cases have been linked to congenital ZIKV infection.<sup>45</sup>

Taken collectively, these observations strongly suggest that the rate of microcephaly in Brazil has not increased as significantly as initially reported in the current pandemic. However, whether ZIKV infection induces microcephaly and perhaps Guillain-Barré syndrome remains an open question. It is certainly possible that the large number of ZIKV infections, including the unprecedented levels in pregnant women, may have uncovered a rare but extremely important clinical outcome.

As the majority of neurological disease cases are in medically underserved regions, associated factors such as malnutrition may also contribute to these effects. Lastly, in addition to potential direct pathogenic effects of ZIKV, it is important to highlight that ZIKV antigens share extensive cross-reactivity with other members of the *Flavivirus* genus, such as Dengue virus (DENV), which are also endemic to this region. This creates the potential for immune-mediated effects due to the preexistence of cross-reactive antibodies.

The ZIKV pandemic is likely to continue to spread in the Americas, and possibly to other regions during 2016. Virus transmission primarily occurs via *Ae. aegypti* and *Ae. albopictus* mosquitoes, though there is growing evidence that ZIKV may utilise a wide range of vectors, including eight species of *Aedes* and others within the genera *Anopheles*, *Mansonia*, and *Culex*.<sup>39,50</sup> Additionally, there are now several reports that suggest ZIKV can be sexually transmitted.<sup>51,52</sup> Given that the geographic distribution of *Ae. aegypti* alone now encompasses all continents, the breadth of vectors and transmission routes suggests that ZIKV has the potential to expand much on a global scale.

## CONCLUSIONS AND OUTLOOK

This review highlights several of the major factors that impact the global spread of EID. As outlined in the examples of WNV, CHIKV, and ZIKV, recent EID outbreaks and pandemics result from a combination of factors that simultaneously impact transmission frequency, establishment, and pathogenicity. EID can result from the origin of entirely new viruses, viruses new to a region, or the re-emergence of viruses once thought to be eradicated. Geographic spread can be mediated by utilisation of additional vectors or expansion in a vector's geographic range, addition of new host species, enhanced virus fitness within vectors or hosts, and/or by anthropogenic factors. Indeed, given global climatic changes, variable effectiveness of pesticides in longitudinal control of vector populations, and the ever-increasing reach, volume, and speed of human mobility and trade, the potential for global transmission of EID is greater now than ever before.

The historical challenges faced by Brazil in controlling the *Ae. aegypti* vector highlight the magnitude of this problem. Mosquito abatement efforts in Brazil date to 1946 amid concerns over

the spread of yellow fever.<sup>53</sup> Indeed, through the widespread use of dichlorodiphenyltrichloroethane, eradication of *Ae. aegypti* in Brazil was thought to be complete in the late 1950s. However, despite consistent awareness of the need to control mosquito populations, from the late 1960s through to the present, the vector serially reappeared due to the acquisition of drug resistance by new mosquito strains, social and environmental changes resulting from rapid urbanisation, failures in epidemiological surveillance, and inconsistent enactment of governmental policy. More recently, renewed abatement efforts were initiated by Brazil in response to the epidemic spread of DENV, which like ZIKA is transmitted by *Ae. aegypti* and *Ae. albopictus* mosquitoes. Despite this effort, since the detection of DENV in 1981, Brazilian infections have steadily risen with almost 1.5 million cases of DENV reported in 2013 alone. These observations highlight that mosquito vectors are extremely adaptable to human environments and display high reproductive capacity and genetic flexibility. In view of this, an integrated approach with well-coordinated governmental and regional action, and involving a combination of mechanical, biological, and chemical approaches that are linked to a public awareness campaign, is essential.

Despite the seemingly overwhelming variety of emerging viruses and the multiple confounding factors that affect their transmission, and contrary to present circumstances with regard to ZIKV, the future holds promise for greater awareness of, if not control over, the spread of EID. Efforts to block transmission by controlling mosquito populations using genetically modified strains has proven effective in field pilot studies.<sup>54</sup> Many regions of the world are now prepared for, or indeed already conduct routine sampling for emerging viruses. Portable low-cost diagnostic tests for viruses that couple to cellular phone technology are now in development.<sup>55</sup> Through in-country efforts and those of organisations such as the World Health Organization, there is now more rapid and accurate dissemination of the status of EID during outbreaks. Lastly, lower costs of sequencing and the growing abundance of existing virus genomes allows rapid statistical comparisons of emerging viruses across regions which, when coupled with cartography, accelerates the identification of causative viruses. Nonetheless, until each of these components are fully utilised on a global scale, we are likely to witness an acceleration of EIDs, and in that context, awareness of these events is essential when considering perplexing neurological cases.

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