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REVIEW ARTICLE

Zika virus infection—the next wave after dengue?

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Zika virus was initially discovered in east Africa about 70 years ago and remained a neglected arboviral disease in Africa and Southeast Asia. The virus first came into the limelight in 2007 when it caused an outbreak in Micronesia. In the ensuing decade, it spread widely in other Pacific islands, after which its incursion into Brazil in 2015 led to a widespread epidemic in Latin America. In most infected patients the disease is relatively benign. Serious complications include Guillain–Barré syndrome and congenital infection which may lead to microcephaly and maculopathy. *Aedes* mosquitoes are the main vectors, in particular, *Ae. aegypti*. *Ae. albopictus* is another potential vector. Since the competent mosquito vectors are highly prevalent in most tropical and subtropical countries, introduction of the virus to these areas could readily result in endemic transmission of the disease. The priorities of control include reinforcing education of travellers to and residents of endemic areas, preventing further local transmission by vectors, and an integrated vector management programme. The container habitats of *Ae. aegypti* and *Ae. albopictus* means engagement of the community and citizens is of utmost importance to the success of vector control.

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Introduction

The past decades have seen some hitherto exotic arboviruses and other arthropod-borne infections emerging from oblivion into epidemic diseases of global concern. The burden of dengue has been rising for five decades.[1] The 2004–2005 outbreak of chikungunya in East Africa and Indian Ocean was followed by worldwide spread in both the Old and New Worlds in the ensuing decade.[2] The expanding geographical distribution of these arboviral diseases is further fuelled by climate changes and importation of invasive arthropod species (most notably various *Aedes* mosquitoes such as *Ae. albopictus*, *Ae. japonicus*, *Ae. aegypti*, and *Ae. koreicus*) into temperate countries of Europe and North America.[3,4] The consequences of climate change and vector distribution can be seen in the autochthonous transmission of dengue and chikungunya in high-latitude areas such as Japan and European countries.[5,6], Zika virus is the latest culprit in a long list of arbovirus epidemics that emerged in the past two decades. As a largely neglected disease, little is known about the basic biology of the virus and the disease until the past decade when it made its mark outside Africa, not to mention vaccine development and antiviral studies. At the time of writing (early 2016), the epidemic in Latin America is still evolving. As in the case of other emerging epidemics such as chikungunya, new clinical and laboratory features may be recognized which may impact on future management of the disease. We herewith summarized what is currently known about the infection, highlighted the uncertainties, and examined the approaches to prevention and control of similar arthropod-borne infections which are pertinent to areas with risks of disease introduction and transmission.

Virology

The family *Flaviviridae* contains some of the most clinically important arboviruses (Table 1). The prototype agent, yellow fever virus, is indeed the first human virus discovered and found to be transmitted by an arthropod vector.[7,8] There are currently four genera in *Flaviviridae*, *Flavivirus* (53 species), *Hepacivirus* (one species, the hepatitis C virus), *Pegivirus* (two species), and *Pestivirus* (four species).[9] With the exception of the hepatitis C virus, most of the clinically relevant pathogens belong to the genus *Flavivirus*. Epidemiologically, the arthropod-borne flaviviruses can be divided into mosquito-borne and tick-borne viruses. Flaviviruses with no known vectors are also found in animals. Clinically, the most prominent syndromes caused by flaviviruses are undifferentiated febrile illnesses (often presenting as a fever with rash syndrome), central nervous system infection (especially encephalitis), visceral involvement, and haemorrhagic fever.

Flaviviruses are enveloped, single-stranded, positive sense RNA viruses measuring about 50 nm in size. The viral genome is about 10.5 to 11 kbp in size.[12,13] The viral genome produces a polyprotein with more than 3000 amino acids; this polyproptein is then cleaved into three structural and seven non-structural proteins.[10] The flaviviral genome encodes (from 5′ to 3′ end, i.e. from N- to C-terminal of the polyprotein) the structural C (capsid, ~11 kDa), prM (precursor M protein, ~26 kDa, which is further cleaved into the M protein), and E (envelope, ~53 kDa) proteins, and the non-structural NS1 (~46 kDa), NS2A (~22 kDa), NS2B (~14 kDa), NS3 (~70 kDa), NS4A (~16 kDa), NS4B (~27 kDa), and NS5 (~103 kDa) proteins.[10] Structurally, the E and M proteins are located at the surface of the viral particles, while the nucleocapsid is made up of the C protein and the genomic RNA molecule.

Table 2 summarizes the key functions and host effects of the flaviviral proteins. It must be remembered that the current knowledge on the effects of various viral proteins on host immune system and pathogenesis is based on previous studies on clinically important flaviviruses such as dengue virus, West Nile virus, yellow fever virus, Japanese encephalitis virus, and tick-borne encephalitis virus. Whether the findings can be generalized to other flaviviruses including Zika virus is unknown.

Initial isolation of Zika virus was made from a sentinel rhesus monkey in 1947 in the Zika Forest area of the Entebbe Peninsula in Uganda on the northwestern shore of Lake Victoria.[28] In 1948, the virus was also detected in a batch of *Aedes africanus* mosquitoes.[28] Isolation of the virus from humans were then reported in Uganda, Tanzania, and Nigeria in the 1950s.[29,30] The genomes of the three human isolates of Zika virus are 10,676 bp in size, which is comparable to other members of *Flaviviridae*.[31] Zika virus is phylogenetically closest to the Spondweni virus, which is

Table 1 Key clinical and epidemiological features of important flaviviruses causing human infections.[10,11]

Main clinical syndrome[a]	Vectors	
	Mosquito-borne	Tick-borne
Central nervous system infection	Japanese encephalitis virus, Murray Valley encephalitis virus, Ntaya virus, Rocio virus, St. Louis encephalitis virus, Usutu virus, West Nile virus	Louping ill virus, Powassan virus, tick-borne encephalitis virus
Viscerotropic infections ± haemorrhagic fever	Yellow fever virus, dengue virus	Alkhumra virus, Kyasanur Forest disease virus, Omsk haemorrhagic fever virus
Febrile illnesses	Dengue virus, Ilheus virus, Kokobera virus, Spondweni virus, Zika virus	

[a] Overlaps in the clinical syndromes do occur for individual viruses.

Table 2 Key proteins of flaviviruses and their functions.[10,14–27]

Proteins	Functions	Possible effects on hosts
C	RNA binding to form the nucleocapsid.	
prM, M	Stabilization, assisting the folding and secretion of E protein.	Antibodies towards prM enhances infectivity of immature virions, could be involved in pathogenesis of severe dengue in secondary infections.
E	Receptor binding, membrane fusion.	
NS1	RNA replication.	Localization to host cell surface and secreted extracellularly; modulates signalling of innate immune system, possible damages to platelets and endothelial cells through anti-NS1 antibodies, antagonizes C4 complement.
NS2A	RNA synthesis and viral assembly.	Interferon antagonist, induces host cell apoptosis.
NS2B	Complexes with NS3 to function as serine protease.	
NS3	Complexes with NS2B to function as serine protease; possess RNA helicase and triphosphatasae activities.	Induces apoptosis of host cells, modulates host microRNA, one of the targets of cytotoxic T cell response.
NS4A	RNA replication.	Blocks type I interferon signalling, induces autophagy and protects host cells from death during infection.
NS4B	RNA replication.	Blocks type I interferon signalling and RNA interference, modulator of stress granules in host cells.
NS5	Methytransferase and RNA guanylyltransferase activities; capping and synthesis of RNA; RNA-dependent RNA polymerase.	Blocks type I interferon signalling.

also a mosquito-borne flavivirus that has been found to cause a febrile illness in Africa.[32]

Several flaviviruses are noted for their propensity to cause central nervous system infections, in particular, encephalitis (Table 1). Globally, Japanese encephalitis virus, Murray Valley encephalitis virus, St. Louis encephalitis virus, West Nile virus, and tick-borne encephalitis (and to some extent, dengue virus, though neurological involvement is generally not a common feature of dengue) are the most commonly encountered pathogens in this regard.[33] In addition to the wild-type viruses, even apparently attenuated vaccine strains may still possess some degree of neurovirulence, as demonstrated by the yellow fever 17D vaccine.[34] The exact genetic determinants of flaviviral neurovirulence have not been completely elucidated, though it appears to be related to multiple genes such as E, NS1, NS3, and NS5.[33,35–41] This area is particularly relevant to Zika virus infection which was previously considered to be a relatively harmless febrile illness with low case-fatality ratio, but microcephaly has emerged as a unique potential sequel of the infection in pregnant women during the Latin American epidemic that began in 2015. Although the exact causality, risk, and mechanism between the infection and microcephaly remain to be investigated, a recent report described the presence of Zika virus in the brain tissues of an affected foetus as demonstrated by both reverse transcription polymerase chain reaction (RT-PCR) and electron microscopy.[42]

The phylogenetic relationships of flaviviruses have been well studied.[12,13,43] Phylogeny is most commonly studied by NS5 sequences, but the NS3 and E sequences or the entire coding region have also been utilized for studies. In general, clustering of flaviviruses can be seen in phylogenetic trees based on the type of transmission vectors (mosquitoes [*Aedes* versus *Culex*], ticks, or no known vectors), type of clinical syndromes (encephalitic versus non-encephalitic diseases), and geographical distribution of the viruses.[12] Figure 1 shows the phylogenetic relationship of the clinically relevant flaviviruses found in humans. As expected, phylogenetic trees based on NS5 and E sequences showed a clear clustering of the mosquito-borne versus tick-borne viruses, and viruses that cause central nervous system infections are also clustered together. Although Zika virus does possess neuropathogenic potentials, at least in the immature nervous system, it is not particularly closely related to other encephalitic viruses. It might be noted that the other potential serious complication of Zika virus infection, Guillain-Barré syndrome (*vide infra*), also affects the nervous system, albeit probably via a different pathogenic mechanism. Moreover, in infants with congenital infection, abnormalities of the retina (which is essentially a part of the central nervous system) were also detected (*vide infra*). Whether the phylogeny explains why Zika virus is not highly neurovirulent in adults but causes substantial damage to the immature neural tissues and possibly induces immunopathological damage in the adult nervous system remains to be studied. Another question begging for an answer is whether both lineages of Zika virus are equally neurovirulent for the foetal brain. This is relevant because the Yap 2007 strain and the Senegal 1984 strain of Zika virus differ from the prototype Uganda strain in that the latter has a four amino acid deletion in the E protein.[44] Microcephaly has not been reported previously in Africa where the disease is endemic (although the possibility of under-reporting cannot be excluded). Whether the difference in the four amino acids or other genetic changes is associated with virulence or transmissibility is currently unknown. Figure 1 also shows a clear separation of Zika viruses into the African and Asian lineages.[45] It has been suggested that Zika virus originated in east Africa near Uganda, which subsequently spread to west Africa and central Africa (the African lineage).[46] The Uganda strain also spread to

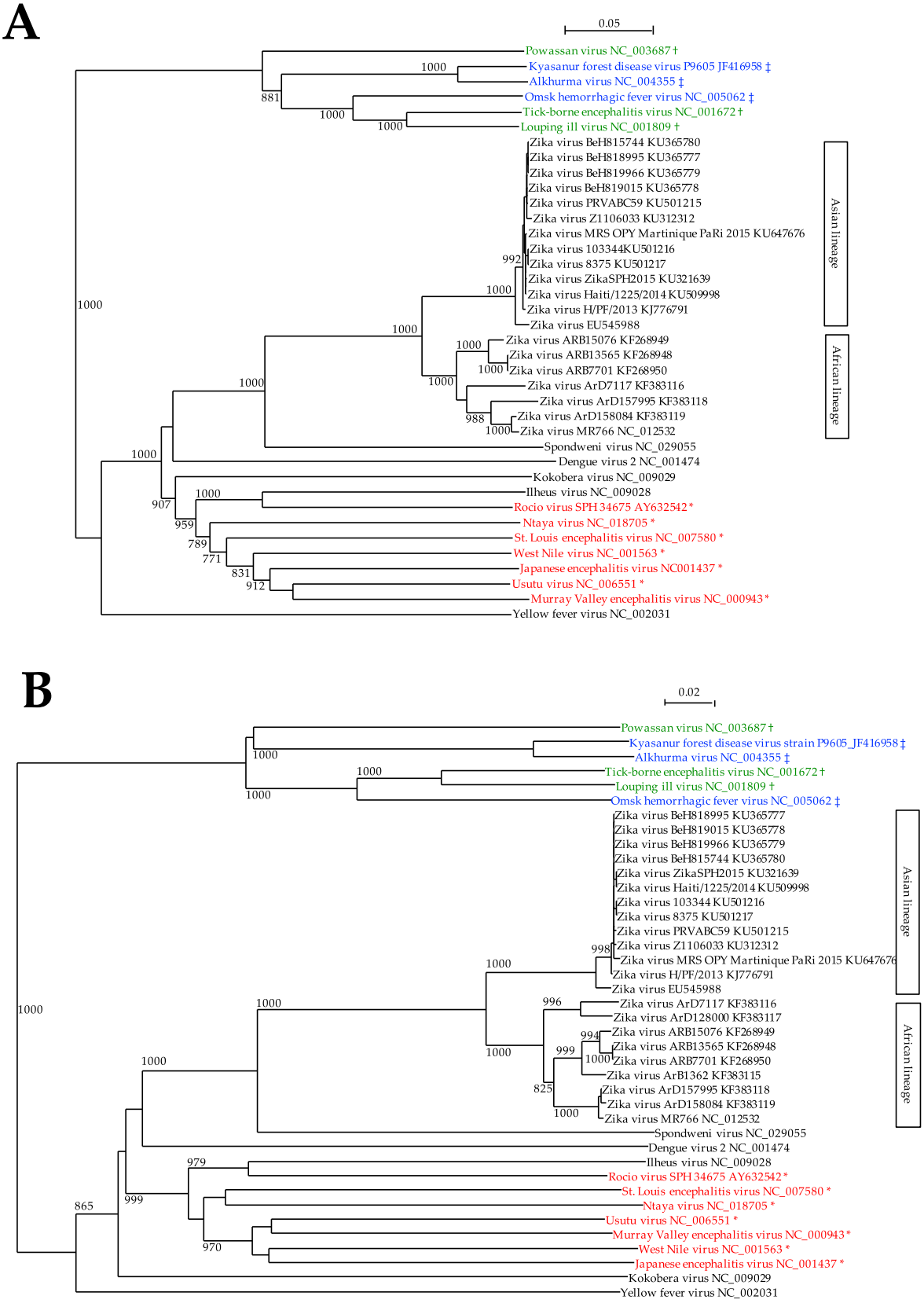

Figure 1 Phylogenetic trees showing the relationships between important flaviviruses causing human infection. A total of 1,536 nucleotide positions in envelope (*E*) gene (Figure 1A), 1,067 nucleotide positions in non-structural protein 1 (*NS1*) (Figure 1B), 1,925 nucleotide positions in non-structural protein 3 (*NS3*) (Figure 1C), 2,736 nucleotide positions in non-structural protein 5 (*NS5*) genes (Figure 1D) were included in the analysis. The trees were constructed using the neighbour-joining method. The bootstrap values calculated from 1,000 trees are shown when they are ≥70%. The scale bar indicates the estimated number of substitutions per 20 bases in *E* and *NS3* and per 50 bases in *NS1* and *NS5*. The names and accession numbers (in parentheses) are presented as cited in the GenBank database. * Mosquito-borne neurotropic viruses; † tick-borne neurotropic viruses; ‡ tick-borne haemorrhagic fever viruses.

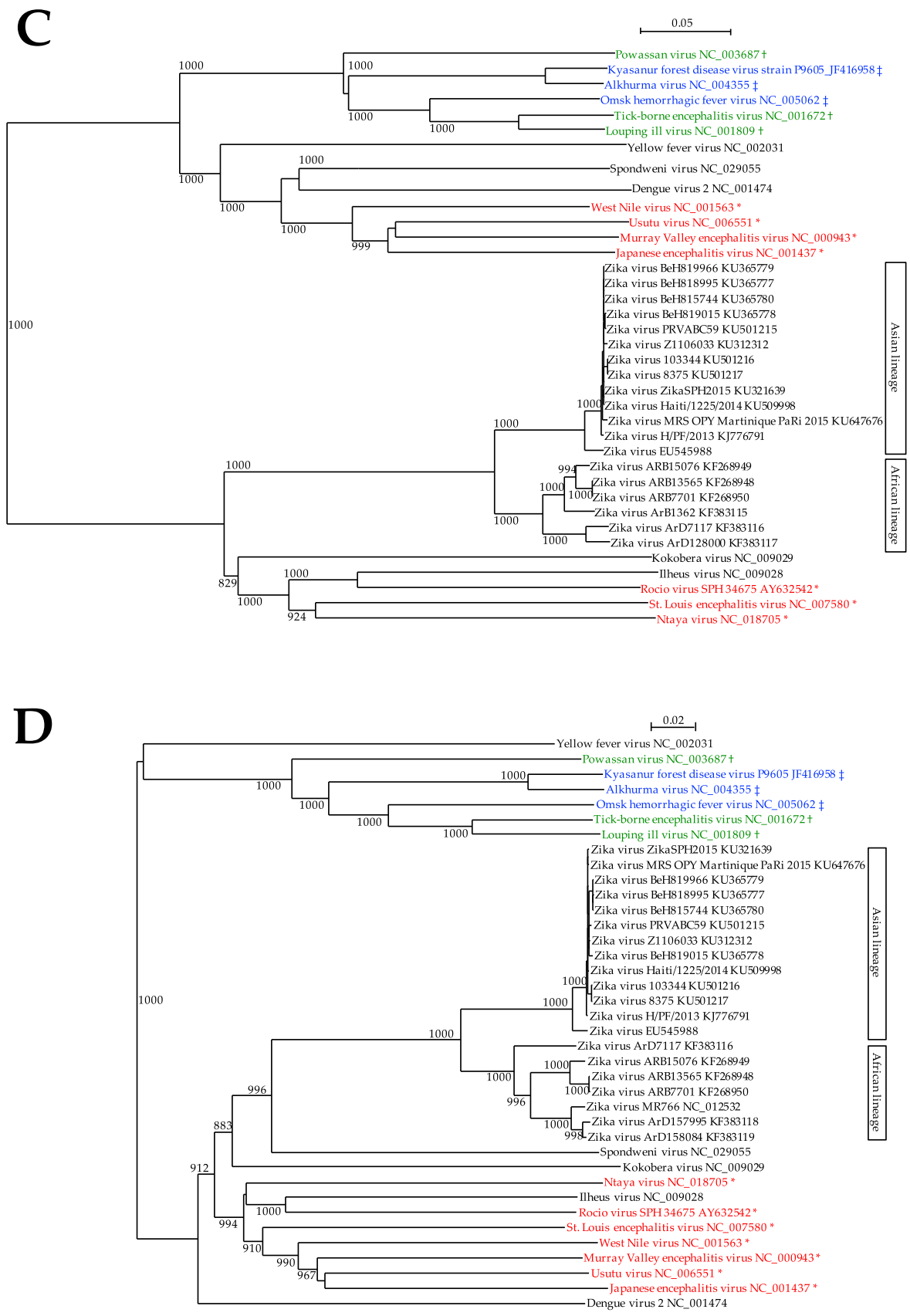

Figure 1 (*continued*).

Table 3 Outbreaks of human Zika virus infection since 2007.[59–63]

Year	Location	Estimated number of cases	Notable features
2007	Yap Island, Micronesia	49 confirmed, 59 probable, and 72 suspected cases in one study. Estimated over 900 clinical cases, 73% of population infected in 4 months.	*Aedes hensilli* implicated as the main vector.
2007	Gabon	Detected in 5 archived human samples; total number of cases unknown.	Retrospective study of a concurrent outbreak of dengue and chikungunya; detection of virus in patient sera and *Ae. albopictus* pools.
2013–2014	French Polynesia	8,723 suspected cases, over 30,000 sought medical care.	Derived from the Asian lineage, closely related to Cambodia 2010 and Yap state 2007 strains. Association with Guillain-Barré syndrome and other neurological complications suspected.
2014	The Cook Islands	932 suspected, 50 confirmed cases.	
2014	New Caledonia	1400 confirmed cases (35 imported).	
2014	Easter Island	51 confirmed out of 89 suspected cases from Jan – May 2014.	Infecting strain closely related to viral strain found in French Polynesia.
2015	Latin America[a]	Estimated 1.5 million cases in Brazil.	Association with microcephaly and maculopathy suspected.

[a] As of 10 February 2016. Includes Barbados, Bolivia, Colombia, Commonwealth of Puerto Rico, Costa Rica, Curacao, Dominican Republic, Ecuador, El Salvador, French Guiana, Guadeloupe, Guatemala, Guyana, Haiti, Honduras, Jamaica, Martinique, Mexico, Nicaragua, Panama, Paraguay, Saint Martin, Suriname, U.S. Virgin Islands, Venezuela.

Malaysia and the Micronesia, thereby establishing the Asian lineage. The Yap outbreak in 2007, French Polynesian outbreak in 2013–2014, and the Latin American epidemic since 2015 were due to viruses belonging to the Asian lineage, probably originating from a strain from Southeast Asia.[44,45,47]

The pathogenesis of Zika virus infection in humans is poorly understood, though studies are starting to unravel the disease process. In a study on the effects of Zika virus, human skin cells, the fibroblasts, keratinocytes, and dendritic cells are all permissive to infection with replication of the virus.[48] Molecules such as DC-SIGN, AXL, Tyro3, and TIM-1 are involved in cell entry, and both type I and type II interferons inhibit viral multiplication. The utilization of multiple cellular receptors for entry is similar to dengue virus. Another study described the cytokine profiles of six travellers who acquired Zika virus infection in Southeast Asia, French Polynesia, and Latin America.[49] Significant elevation of multiple cytokines (including interleukins 1b, 2, 4, 6, 9, 10, 13, 17, and IP-10) was observed during acute infection, but not interferon-gamma or tumour necrosis factor-alpha, suggesting a cytokine response towards Th2 reaction. Interleukins 1b, 6, 8, 10, 13, IP-10, RANTES, MIP-1a, MIP-1b, VEGF, FGF, and GM-CSF were elevated during the convalescent phase. The "cytokine storm" which occurs in some other viral infections was not demonstrated in the small cohort of subjects.

Epidemiology and transmission

Since its initial discovery, early virological and serological studies from the 1950s to 1980s showed that Zika virus infection is predominantly limited to African and Asian countries.[50] In Asia, endemic circulation of the virus (clinical disease and/or seroprevalence studies) has been reported in Indonesia, Cambodia, Thailand, The Philippines, Peninsular Malaysia, and Borneo, and among travellers returning from endemic areas.[51–58] The first major epidemic outside Africa occurred in Yap Island of the Federated States of Micronesia (in the western Pacific Ocean, north to Papua New Guinea) in 2007 (Table 3). Since then, Zika, dengue, and chikungunya viruses became rampant in the Pacific islands.[61] Another major epidemic occurred in the western Pacific islands of French Polynesia and New Caledonia in 2013–2014.[64]

The outbreak in Easter Island in 2014 heralded the incursion of the virus to mainland Latin America.[62] Since early 2015, Brazil reported the first autochthonous case in the city of Natal and this was quickly followed by another large epidemic in Brazil and neighbouring countries in Latin America.[63,65–68] As of 12 February 2016, there were over 2,000 confirmed and over 118,000 suspected cases in the Americas.[69] The exact time and route of spread of the virus to Brazil is unknown, but importation during the 2014 World Cup has been postulated.[65]

The virus is epizootic and enzootic in non-human primates in Africa (sylvatic cycle), and these mammals are the most important natural reservoir hosts.[70] However, as in the case of other arboviruses such as dengue and yellow fever, urban cycles involving human—mosquito—human transmission can readily occur when there are competent anthropophilic vectors. *Aedes* mosquitoes are the primary vectors for natural transmission of the Zika virus. The extrinsic incubation period of the virus in mosquitoes is about 10 days (similar to the 8–12 days required of dengue virus).[50,71] In *Ae. aegypti*, high levels of viruses could be found within the mosquitoes from days 20–60 after infection, though the average lifespan of female *Ae. aegypti* adults is shorter than this in the tropical field conditions.[71,72] Species from which the virus has been isolated or found to be capable of transmitting the virus include *Ae. africanus* (chiefly a forest-dwelling mosquito feeding on non-human primates), *Ae. apicoargenteus* (an African mosquito species), *Ae. luteocephalus* (an African mosquito species), *Ae. furcifer* (an African mosquito species), *Ae.*

vittatus (worldwide distribution), *Ae. unilineatus* (found in Africa and parts of Asia, including India, Pakistan, and Saudi Arabia), *Ae. opok* (an African mosquito species described in Uganda), *Ae. hensilli* (endemic species in Micronesia, implicated in the outbreaks of dengue, chikungunya, and Zika virus infections in Yap Island of Micronesia), *Ae. aegypti*, and *Ae. albopictus*.[59,60,73–79] To most countries of the world, the last two species, *Ae. aegypti* and *Ae. albopictus* (nowadays more properly known as *Stegomyia aegypti* and *Stegomyia albopicta* respectively),[80] may pose the greatest threats, given their almost ubiquitous presence in many tropical and subtropical countries, their adaptation to the urban and peri-domestic environments, their highly anthropophilic behaviours, invasion into some European and North American countries, and competence to act as vectors for numerous other arboviruses.[81] *Ae. aegypti*, in particular, is considered to be the prime vector for the transmission of Zika virus among humans. *Ae. aegypti* and *Ae. polynesiensis* are suspected to be the vectors involved in the French Polynesian outbreak.[82,83] In additon, Zika virus has also been isolated from other non-*Aedes* genera of mosquitoes including *Mansonia uniformis*, *Culex perfuscus*, and *Anopheles coustani*.[84] However, it must be remembered that the ability to isolate the virus from certain mosquito species and their *in vitro* competence to support viral replication does not mean that those species are necessarily important vectors epidemiologically. As in the case of other vectorborne infections, the vectorial capacity depends not only on vector competence, but also on the local density of the vectors, their host biting preference, feeding frequency, longevity (which is relevant to the extrinsic incubation period of the arbovirus), and level of viral replication in the vectors.[85–87]

In addition to mosquitoes, other routes of transmission of Zika virus are possible, although these are unlikely to be of major epidemiological significance under natural circumstances. Direct transmission from primates to human via animal bites has been suggested though not proven.[88] Coincidentally, Zika virus has also been detected in the saliva of 19.2% of infected individuals but the epidemiological significance of this remains to be determined.[89] Sexual transmission has been documented, and the virus has been detected in the semen up to 62 days after onset of febrile illness.[90–92] Likewise, perinatal and congenital infections can occur.[42,93] Another major clinical and public health concern is the potential for transmission through transfusion and transplantation. In endemic countries, the proportion of asymptomatic or subclincial flaviviral infections far exceeds the clinically overt cases. For example, the ratios between asymptomatic or inapparent to clinical cases of dengue, Japanese encephalitis, and yellow fever are 3–18:1, 250:1, and 7–12:1 respectively.[94–96] Detection of arboviruses in donated blood and their transmission through transfusion have been well documented for arboviruses such as dengue, West Nile, and tick-borne encephalitis viruses, with concerns over other viruses such as chikungunya and Ross River viruses.[97–103] During the French Polynesian Zika outbreak in 2013–2014, 3% of asymptomatic blood donors were found to be viraemic using RT-PCR screening, thereby underscoring the potential for transfusion transmission of Zika virus.[104]

Clinical and laboratory aspects

Clinically, Zika virus infection cannont be reliably differentiated from other arbovirus infections such as dengue and chikungunya as the symptoms and signs are not pathognomonic. The clinical and epidemiological features are also confounded by co-circulation of different arboviruses in the same geographical area.[60] Table 4 compares some of the features of dengue, Zika, and chikungunya. After an incubation period of 3 to 12 days, Zika virus infection presents initially with headache, a descending maculopapular rash involving palms and soles (which can be pruritic), fever, malaise, myalgia, anorexia, conjunctivitis, arthralgia, limb oedema and sometimes abdominal symptoms (abdominal pain, diarrhoea).[50,105] Limb oedema and conjunctivitis appeared to be commoner with Zika virus infections than dengue or chikungunya, while hepatomegaly, leukopenia, and thrombocytopenia were less common in Zika virus infections.[105] During the Yap outbreak, the main clinical manifestation (incidence in parentheses) were maculopapular rash (90%), fever (65%), arthritis/arthralgia (65%), conjunctivitis (55%), myalgia (48%), headache (45%), retro-orbital pain (39%), oedema (19%), and vomiting (10%).[59] Serious complications due to Zika virus infection were rarely reported in the past. However, during the French Polynesian outbreak, an increased incidence of Guillain-Barré syndrome was noted, with an incidence that is 20 times higher during the outbreak than non-outbreak periods.[83,115] An association with Guillain-Barré syndrome was also noted in the Latin American epidemic since 2015. In Brazil, 62% of the Guillain-Barré syndrome patients during the outbreak had preceding symptoms consistent with Zika virus infection.[63] Death from Zika virus infection in adults is rare but has been reported, although the exact contribution of the infection to mortality has not been detailed at the moment.[116] The duration of immunity after recovery from Zika virus infection is unknown. Co-infection with other arboviruses is possible, since the key *Aedes* vectors are capable of transmitting other arboviruses.[117]

The most striking and unexpected sequel of Zika virus infection is the possible association with congenital abnormalities, in particular, microcephaly. In some cases, intrauterine or neonatal death may ensue.[116] The epidemiological linkage was first observed in Brazil in 2015, where the number of infants born with microcephaly increased 20 times after the onset of the epidemic, with over 1200 cases being reported in 2015 (99·7 per 100,000 livebirths).[118] As of 30 January 2016, there were 4,783 cases of congenital central nervous system malformations recorded in Brazil (compared to an annual incidence of 163 microcephaly cases in 2001–2014) with 76 deaths.[63] In addition to microcephaly, affected foetuses and infants also have cerebral calcification seen in imaging.[119,120] In microcephalic infants, retinal abnormalities manifesting as macular neuroretinal atrophy, macular pigment mottling, foveal reflex loss, and chorioretinal atrophy, as well as optic nerve hypoplasia were also observed.[118,120] Ophthalmological examination of the infected mothers was uniformly normal.

The management of pregnant women with Zika virus infection remains problematic. The main difficulties

Table 4 Epidemiological and clinical features of dengue, Zika, and chikungunya virus infections.[59,61,105–114]

	Dengue virus	Zika virus	Chikungunya virus
Virology			
Family	*Flaviviridae*	*Flaviviridae*	*Togaviridae*
Nucleic acid	Single-strand, positive sense, RNA.	Single-strand, positive sense, RNA.	Single-strand, positive sense, RNA.
Main divisions	4 serotypes (1 to 4)	2 lineages (African and Asian)	4 major lineages (West African, East/Central/South African [ECSA], Indian Ocean, Asian)
Epidemiology			
Natural reservoir	Primates (sylvatic cycle).	Primates (sylvatic cycle).	Primates (sylvatic cycle).
Key vectors for natural transmission	*Aedes* mosquitoes. Sylvatic cycle: *Ae. furcifer, Ae. luteocephalus, Ae. vittatus, Ae. taylori, Ae. niveus.* Urban cycle: *Ae. aegypti* and *Ae. albopictus*, other locally predominant species implicated (e.g. *Ae. polynesiensis, Ae. pseudoscutellaris, Ae. malayensis, Ae. cooki*).	*Aedes* mosquitoes. Sylvatic cycle: *Ae. africanus, Ae. furcifer, Ae. luteocephalus, Ae. vittatus, Ae. unilineatus, Ae. opok.* Urban cycle: *Ae. aegypti, Ae. albopictus*; other locally predominant species implicated (e.g. *Ae. hensilli, Ae. polynesiensis*).	*Aedes* mosquitoes. Sylvatic cycle: *Ae. africanus, Ae. furcifer, Ae. luteocephalus, Ae. neoafricanus, Ae. taylori, Ae. dalzieli, Ae. vigilax, Ae. camptorhynchites, Ae. fulgens.* Possibly *Mansonia* spp. as well. Urban cycle: *Ae. aegypti, Ae. albopictus.*
Endemic areas	Tropics and subtropic areas. Widespread in Asia, Africa, Latin America, Pacific islands, northeast Australia. Increasing cases reported in southwestern and southeastern United States.[a]	*Asia*: Cambodia, Indonesia, Malaysia, Pakistan, The Philippines, Thailand. *Pacific islands*: Micronesia, French Polynesia, New Caledonia, The Cook Islands. *Africa*: Senegal, Uganda, Nigeria, Côte d'Ivoire, Gabon, Tanzania, Egypt, Central African Republic, Sierra Leone. *Latin America*: since 2015.[b]	Widespread in sub-Saharan Africa, Asia, Latin America, Pacific islands, Indian Ocean islands.[c] *Europe*: local transmission in northern Italy (2007) and southern France (2014) following importation of the virus.
Iatrogenic transmission	Transfusion-transsmission confirmed; possibly renal transplantation.	One case of transfusion-transmitted infection declared by Brazilian authorities.[d]	Transfusion-transmission potentially possible.
Vertical infections	Yes. No congenital abnormalities reported.	Yes. Possible association with microcephaly and maculopathy.	Yes. Possible centro-facial hyperpigmentation.
Sexual transmission	Not reported.	Yes.	Not reported.
Clinical features			
Incubation period	3–10 days (usually 5–7 days).	2–12 days (usually 2–7 days).	2–6 days.
Duration of viraemia	2–3 days before to 4–5 days (range: 2–12 days) after onset of symptoms.	Usually 3–5 days after onset of symptoms (possibly over 11 days in some cases). Duration of viraemia prior to disease onset unknown.	About 6 days after onset of symptoms (range: 3–10) days.
Asymptomatic infection	14% in adults, 53% in children. Over 75% in some series.	80%.	3–37%.
Common clinical manifestations	Fever, headache, retro-orbital pain, maculopapular rash or "white islands in a sea of red", arthralgia, myalgia.	Fever, headache, conjunctivitis, itchy maculopapular rash, arthralgia (small joints of hands and feet), oedema of extremities, oral ulcers.	Fever, rash, myalgia, polyarthralgia, polyarthritis, diarrhoea, vomiting, abdominal pain.

(*continued on next page*)

Table 4 (*continued*)

	Dengue virus	Zika virus	Chikungunya virus
Uncommon or severe manifestations	Severe dengue: vascular leakage, haemoconcentration, bleeding diathesis, shock, end organ involvement (previously referred to as dengue haemorrhagic fever and dengue shock syndrome)	Guillain-Barré syndrome, encephalitis, meningoencephalitis. Possible congenital infection leading to microcephaly and maculopathy.	Conjunctivitis, uveitis, iridocyclitis, retinitis, meningoencephalitis, myocarditis, hepatitis, multi-organ failure.
Case-fatality ratio	Less than 1% to 5% with dengue fever. Severe dengue without adequate treatment, up to 20% or above, but can be reduced to less than 1% with proper management.	Very low.	0.1%.
Key laboratory findings	Leukopenia, lymphopenia, thrombocytopenia, elevated transaminases. Haemoconcentration (increased haematocrit) and coagulation abnormalities in severe dengue.	Relatively normal blood tests. Occasional mild thrombocytopenia, leukopenia with monocytosis reported.	Leukopenia, lymphopenia, thrombocytopenia, hypocalcaemia, elevated transaminases.
Diagnostic tests of choice	NS1 antigen detection, RT-PCR, antibody detection.	RT-PCR, antibody detection (ELISA and neutralization assay).	RT-PCR, antibody detection.
Antiviral therapy	None.	None.	None.
Vaccine prevention	Vaccine first marketed in 2015.	None.	None.

[a] Updated map of countries with dengue transmission can be found at http://www.healthmap.org/dengue/en/.
[b] Updated map of American countries with autochthonous Zika virus transmission during the 2015 outbreak can be found at Regional Office for the Americas of the World Health Organization, http://www.paho.org/hq/index.php?option=com_content&view=article&id=11669&Itemid=41716&lang=en.
[c] Updated map of countries with chikungunya transmission can be found at http://www.cdc.gov/chikungunya/geo/.
[d] Quoted by the Center for Infectious Disease Research and Policy, The University of Minnesota, on 4 February 2016. No official scientific publications are available at the time of writing. Available at: http://www.cidrap.umn.edu/news-perspective/2016/02/brazil-confirms-blood-transfusion-zika-paho-calls-global-support. [accessed 18.02.16.].

include the uncertainty of the risk of developing congenital abnormalities after infection of the pregnant women, the risk associated with infection at different time of gestation, and the lack of alternative antenatal diagnostics other than imaging studies. Current guidelines generally recommend monitoring of pregnant women with a recent travel history to endemic areas, early diagnosis of infection, close antenatal and postnatal surveillance and monitoring of infected women, exclusion of other congenital infections (such as toxoplasmosis, cytomegalovirus infection, rubella), amniocentesis for virological investigations if radiological abnormalities are detected, and consideration of termination of pregnancy after thorough counselling of the pregnant women.[116,121,122] Zika virus can be detected by RT-PCR in the amniotic fluid of microcephalic foetuses, but the positive and negative predictive values of the RT-PCR finding (either in the amniotic fluid or even chorionic villus sampling) for the development of congenital abnormalities are unknown, and the test has not been well evaluated for specimens other than blood.[93,116,122]

Zika virus can be cultured in a number cell lines such as Vero and LLC-MK2, or by intracerebral inoculation of suckling mice.[123] While these are useful for virological studies and research, they are impractical for most clinical laboratories. Laboratory-acquired infections of Zika virus have also been reported.[50] Zika virus is classified as a biosafety level 2 organism according to the US CDC and human pathogen hazard group 3 according to the UK Advisory Committee on Dangerous Pathogens.[124,125] Antibody detection and nucleic acid amplification using RT-PCR are the usual diagnostic tests of choice. A commercial system (EUROIMMUN AG, Lübeck, Germany) detecting anti-Zika IgG and IgM using ELISA (with recombinant NS1 antigen) and indirect immunofluorescence assay (which also allows differentiation between Zika, chikungunya, and dengue viruses) was marketed in January 2016. An in-house ELISA antibody test was developed during the Yap outbreak by the Centers for Disease Control and Prevention, USA. As expected, IgG and IgM antibody testing using ELISA shows cross-reaction with other flaviviruses, especially in patients with prior flaviviral infections. IgM antibodies are detectable from days 3–8 after onset of illness. Antibody testing by the plaque reduction neutralization test is more specific.[44] In-house indirect immunofluorescent assays have

also been described for antibody detection.[126] Although antibody testing suffers from cross reactivities with other flaviviruses, it remains an essential diagnostic means, especially in patients who presented late in the course of disease where RT-PCR tests could be negative (about 5—7 days after onset of disease).[126] The usual laboratory test of choice for acute Zika virus infections is RT-PCR on clinical samples, most commonly the peripheral blood. RT-PCR allows accurate differentiation of Zika virus from other pathogens which may share similar clinical manifestations, and this is especially useful in areas where co-circulaion of different arboviruses is prevalent. Genotyping of the viral strains is also possible. In the Yap outbreak, one third of the sera collected within 10 days after disease onset were still positive by RT-PCR.[59] PCR protocols have been developed using primers directed towards various targets including E, NS5, and prM/E, and M.[44,51,127—129] Duration of viraemia in humans ranges from 1 to more than 11 days after the onset of disease.[50] The viral load in patient sera appears to be relatively low, ranging from 930 to 728,800 (median: 21,495) copies/mL in a series of 17 patients in the Yap outbreak in 2007 (sera were mostly collected within 3 days after onset of disease).[44] One patient in that study had a viral load of 338,797 copies/mL when the blood was collected on day 11 after disease onset. Zika virus RNA has also been detected in the saliva, urine, and semen of some patients; remarkably, the positive rate of RT-PCR in saliva is higher than that of blood (57.1% vs. 28.1%).[89,91,130] The additional value of performing RT-PCR on urine and saliva over blood, other than the ease of specimen collection, is unknown because the viral kinetics in these body fluids have not been determined.

The NS1 antigen of dengue virus has revolutionized the laboratory diagnosis of dengue, providing a simple and rapid point-of-care diagnostic means with high specificity and ability to diagnose the infection early in the course (especially in the first 3 days after disease onset).[131] Although commercially available NS1-based diagnostic technique is currently limited to dengue, this may potentially be applicable to other flaviviral infections because circulating NS1 antigens have also been detected in Japanese encephalitis and West Nile virus infections.[132,133] No information on the detection of circulating NS1 antigen in Zika virus infection is currently available. Caution should be excercised in the interpretation of dengue NS1 antigen testing results, for false positive result has been reported in a patient with acute Zika virus infection and other underlying diseases.[134,135]

Treatment of Zika virus infection is primarily supportive. Nonsteroidal anti-inflammatory drugs should be avoided unless dengue has been excluded.[116,122] Standard precautions in health care settings are adequate, with additional measures in mosquito-proofing of the health care facilities. Insect repellents and mosquito bite avoidance are recommended for health care workers looking after Zika patients.[116] It would be prudent to recommend patients in the first one to two weeks after the onset of illness to adhere to bite avoidance measures in order to reduce the risk of secondary transmission. No antivirals are currently licensed for specific therapy against flaviviral infections (except hepatitis C virus), although *in vitro* studies with therapeutic antibodies, small interfering RNA, and molecules against non-structural proteins (especially NS3 and NS5 proteins) are ongoing.[136—138] A few currently available drugs such as the tetracyclines, chloroquine, amodiaquine, and mefenamic acid have shown *in vitro* inhibitory acitivities against flavivirus (mostly with dengue virus), but it is still too early to comment on their potential clinical benefits.[139—144]

Prevention

Pregnant women are discouraged from travelling to Zika-endemic areas.[145] In addition to bite avoidance measures, non-pregnant, sexually active women of reproductive age residing in endemic areas should consider the issues of family planning and contraception, taking into account various social and religious precepts.[122] At present, the only flaviviral vaccines available for human use are yellow fever (live attenuated), Japanese encephalitis (inactivated, live attenuated, and chimeric), tick-borne encephalitis (inactivated) vaccines, and the newly marketed dengue vaccine (live attenuated, recombinant, tetravalent; marketed since 2015). Claims were made by an Indian biotechnology company that two Zika virus vaccine candidates (recombinant and inactivated) can be tested soon; however, no details on the vaccine preparations are currently available in the scientific literature.[146] In any case, a normal vaccine development cycle usually requires years of preclinical and clinical studies and a Zika virus vaccine for human use is unlikely to be available in the near future.

In the absence of vaccines or chemoprophylaxis, the prevention of Zika virus infection follows the general rules for other vectorborne infections. Broadly speaking, this involves two major areas, personal protection through bite avoidance and vector control. Bite avoidance is equally important to both residents in and travellers to endemic areas. Personal protection includes general measures such as protective clothings, proper choice and use of insect repellents, and mosquito-proofing of houses. The use of insecticide-impregnated bednets has been one of the core elements in the prevention and control vectorborne diseases such as malaria in endemic countries. However, its role against the *Aedes* vectors of Zika virus depends on the behaviours of the vectors in specific geographical areas. In general, *Ae. aegypti* mosquitoes are endophilic (resting indoors), endophagous (biting indoors), anthropophagic (preferentially biting humans), and diurnal and crepuscular in their activities. *Ae. albopictus* mosquitoes are generally exophilic (resting outdoors), exophagous (biting indoors), and anthropophilic, and are aggressive daytime biters.[147—149] However, it is known that the endophilic/exophilic and endophagous/exophagous behaviours are not absolute and these can be variable in different geographical areas.[150] A thorough knowledge of the local mosquitoes and their behaviours are therefore crucial to the control of vectorborne diseases, and this underlines the importance of long-term local vector surveillance.

The proper use of insect repellents is the keystone in personal protection against haematophagous arthropods. A number of insect repellents are widely available on the market, each of which has different repellent efficacies against different mosquito genera and other arthropods

such as mites, tickes, and flies. Despite negative publicity against it in recent years, N,N-diethyl-*m*-toluamide (DEET) remains the gold standard in insect repellents against which other newer compounds are benchmarked. DEET was initially developed and patented by the US Army in 1946 and commercialized since 1957. It is generally applied to skin in the form of liquids, aerosols, or lotions, and can be used to impregnate clothings if necessary (although permethrin is more commonly used for this purpose). Commercially available DEET formulations range from 4% to 100% in concentration. It is a common misconception that higher concentrations provide "more powerful" protection against arthropods. Higher concentrations merely prolong the duration of protection. At a concentration of 15%, DEET protects against *Ae. aegypti* and *Ae. albopictus* bites for about 7–8 hours.[151] The effect plateaus at a concentration of about 50%. Although percutaneous absorption of DEET does occur, the level is extremely low as compared to the lethal doses observed in animals.[152,153] DEET has an excellent record of safety and efficacy after 60 years of use. It is not oncogenic, teratogenic, or genotoxic at maximum tolerated doses in animals. Reports of serious human toxicity mainly relate to neurotoxicity, especially seizures in children. However, such reports remained rare (when compared to the total number of individuals exposed to DEET over the years) and in many of the reported cases, a definitive causal relationship between DEET and neurotoxicity cannot be established (other than the few cases of deliberate or accidental ingestion in huge quantities or improper use of the products).[154–158] Similarly, although detectable levels of DEET can be found in cord blood of infants born to mothers using DEET during second and third trimesters of pregnancy, no adverse outcomes of pregnancy have been found in a double-blind, randomized trial.[159] Hence, when used appropriately according to recommendations, DEET is still considered to be safe in children older than 2–6 months of age, as well as in pregnant and lactating women.[160–164] A DEET concentration of 20% to 30% is generally recommended for adult use.

Effective and safe alternatives to DEET are available. The most commonly used ones are ethyl butylacetylaminopropionate (IR3535, more effective against *Aedes* and *Culex* than *Anopheles* mosquitoes), picaridin (also known as icaridin; concentrations of $\geq$20% are needed), *p*-menthane-3,8-diol (PMD), and possibly 2-undecanone (BioUD).[151,165,166] For impregnation of clothings, shoes, and other equipment, permethrin is generally preferred.[158] Various botanical compounds have been advocated as natural and harmless insect repellents. These are often essential oils extracted from plants. While many of these oils do possess repellent activities, most of them are too volatile to offer lasting protections (usually lasting for less than 1 hour) and even these natural products may cause adverse reactions, especially skin irritation.[158,165,167–169] Insect repellent-treated wristbands, garlic, oral vitamin B, and electronic buzzers (which claim to produce ultrasound to repel insects) are completely ineffective as bite avoidance measures.[165,169] Whichever insect repellent is chosen, one must beware of potential limitations, such as repeating the application after heavy sweating, swimming, or raining, and applying the repellent about 20 minutes *after* the application of sunscreens. For international travellers, preventive measures against other vectorborne infections should not be forgotten. These include the use of antimalarial chemoprophylaxis and yellow fever vaccination (as dictated by the destination) as appropriate, as there are overlaps in the current endemic areas of these infections and the possibility of further spread of Zika virus infection in the future.[170] Vaccination against Japanese encephalitis and tick-borne encephalitis may be considered for travellers to endemic areas with high-risk exposures, and dengue vaccination may potentially be considered in the future when more data are available.

Vector control is the only long-term solution to the control of vectorborne diseases. During outbreak situations, emergency measures such as the use of space spray (fogging) may be deployed to rapidly bring down the number of biting adults and terminate disease transmission. However, this is not a sustainable measure in the long run. The details of mosquito control are beyond the scope of this article. In brief, this mainly involves source reduction by larval control. *Ae. aegypti* and *Ae. albopictus* are typical container-breeding species which thrive in urban and man-made environments. Unlike other mosquites which breed in open water areas, environmental management measures are less likely to be effective. Raising the awareness of the community and engaging citizens in source reduction in households and their vicinity is the key to success in controlling these mosquito species.[171,172] Other means of mosquito control, some of which are still experimental or in the early phases of field trials, include the use of biological measures such as entomopathogenic fungi and genetic measures including sterile insect techniques.[172,173] Obviously, all vector control strategies have to be continued as perennial exercises because various mosquito genera, including *Aedes* spp., are capable of overwintering, and that the eggs of *Aedes* mosquitoes are well known for their ability to withstand prolonged periods of desiccation.[174–176] Vector control measures must go hand in hand with vector surveillance, not only to monitor the density of mosquitoes (which may sometimes be correlated with the risk of transmission), but also to detect colonization by invasive species which may contribute to local spread of the infection.[177–179] The public health significance of vector control and surveillance cannot be overstated. *Aedes* mosquitoes, in particular, *Ae. aegypti* and *Ae. albopictus*, are notorious for their vectorial capacity in transmitting multiple vectorborne infections. Dengue, for example, has taken its toll in many continents. From 4 January 2015 to 14 February 2016, 44,196 cases of dengue fever have been recorded in Taiwan, making it one of the biggest outbreaks of the disease on the island.[180] Given the fact that the competent vectors *Ae. aegypti* and *Ae. albopictus* are highly prevalent in many tropical and subtropical countries, the potential for Zika virus to cause outbreaks, and worse, co-circulation with other arboviruses, in these areas is very high.[181]

The role of border screening in preventing the importation of infectious diseases has previously been discussed.[182] Although this is deployed in many countries (especially among Asian countries), border screening cannot reliably detect all infected individuals because of the asymptomatic incubation period. In the prevention of Zika virus infection, perhaps a more realistic approach is to

strengthen education of travellers prior to departure. This may involve travel medicine specialists in travel clinics as part of the pre-travel consultation, as well as reinforcing publicity and education in airports and other departure points. Of particular importance is a thorough pre-travel counselling of pregnant women intending to go to endemic areas for various arthropod-borne infections. This group of travellers may pose special difficulties in terms of vaccination, chemoprophylaxis, and choice of personal protection.[183] Exclusion of individuals with a recent history of travel to endemic areas from blood and organ donation would be a prudent precautionary measure. Given the risk of sexual transmission of Zika virus, abstinence or barrier contraceptives should be used by persons with recent visits to endemic areas and patients recovered from the illness. Nevertheless, because the longest duration of viraemia and viral shedding in other body fluids (such as semen, urine, and saliva) is presently unknown, the duration required for these precautions are at best tentative. For asymptomatic returned travellers, some authorities recommended that barrier contraception should be used for 28 days after returning, and for 6 months after recovery if symptomatic.[121,184]

Conclusion

The geographical distribution of Zika virus has expanded tremendously since 2007. The threat of further expansion is real because of the constant increase in the volume of international travel, difficulties in controlling *Aedes* populations, invasion of *Aedes* species to more temperate countries, and global climate change which may increase the geographical extents favourable to the breeding of mosquitoes. Nucleic acid amplification remains the main diagnostic test of choice, though the availability of commercial antibody detection assays should complement laboratory diagnosis of the infection. Control measures currently relies on standard bite avoidance measures by residents and travellers alike, as well as integrated vector management in the community. The unique challenge of Zika virus infection lies not only on disease control, but the potential sequelae of congenital infection and severe neurological complications. Further studies may provide insights to the pathogenic mechanisms, earlier and more sensitive predictors of congenital abnormalities, and the possibilities of vaccination.

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