Zika virus: history of a newly emerging arbovirus

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Zika virus was originally identified in a sentinel rhesus monkey in the Zika Forest of Uganda in 1947. The virus is a member of the family Flaviviridae, genus *Flavivirus,* and is transmitted to humans by *Aedes* species mosquitoes. The first report of Zika virus outside Africa and Asia was in 2007 when the virus was associated with a small outbreak in Yap State, part of the Federated States of Micronesia. Since then, Zika virus infections have been reported around the world, including in southeast Asia; French Polynesia and other islands in the Pacific Ocean; and parts of South, Central, and North America. Symptomatic infection in human beings normally results in a mild and self-limiting febrile disease, although recent reports have suggested a possible association with more serious sequelae such as Guillain-Barré syndrome, and microcephaly in newborn infants of mothers infected with Zika virus during pregnancy. In this Review, we summarise the history of Zika virus from its first detection to its current worldwide distribution.

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Introduction

The first formal description of Zika virus was published in 1952,[1] and for much of the following 60 years, interest in this virus was confined to a few specialised researchers. Nowadays, Zika virus is making headlines around the world, and WHO has recently declared a public health emergency of international concern for Zika virus.[2] The reason for this dramatic change has been the increased detection of Zika virus worldwide and its association with increasingly large outbreaks of disease.[3–6] Before 2007, virological and immunological evidence suggested that although Zika virus was distributed widely in Africa and Asia, Zika fever was not a disease of substantial concern to human beings because only 14 cases had been documented worldwide, consisting of 13 natural infections[7–10] and one laboratory-acquired infection.[11] The first substantial outbreak of Zika fever outside Africa and Asia occurred in 2007 in Yap State, which is part of the Federated States of Micronesia in the western Pacific Ocean.[12,13] In this outbreak, 49 confirmed and 59 probable cases of Zika virus infection were identified, whereas in the most recent outbreak in Brazil, an estimated 440 000–1 300 000 cases have been reported.[3] The alarming scale of the current outbreak and the potential for autochthonous transmission of this virus in North America and elsewhere[3,4,14] have heightened awareness of this emerging mosquito-transmitted disease.

Zika virus and transmission

Zika virus is an enveloped, spherical particle classified as a member of the family Flaviviridae, the genus *Flavivirus*. The virus belongs in the mosquito-borne cluster of the genus *Flavivirus*, and is grouped in the Spondweni virus serogroup. As with other flaviviruses, the viral genome is a positive-sense single-stranded RNA molecule of about 11 kb that encodes for an open reading frame, coding for three structural proteins and seven non-structural proteins.[15] Findings from studies of the aminoacid and nucleotide sequence of different isolates of Zika virus have shown that there are at least two major lineages, the African and Asian lineages,[16] although some studies further differentiate the African lineage into west and east African lineages.[13]

Zika virus is believed to be maintained primarily in nature in a sylvatic cycle of transmission between non-human primates and forest-dwelling mosquitoes,[17] although antibodies to Zika virus have been detected in several other non-primate mammals (as reported in[16]) and in rodents.[18] In this regard, many of the cases of Zika fever reported from Asia and Africa are likely to represent cases of spillover transmission from the sylvatic cycle, in which human beings became infected as an accidental host. The absence of monkeys in Yap State during the 2007 outbreak[12] and the scale of the more recent outbreaks[3] would suggest that an urban transmission cycle is possible, although early findings suggested that levels of viraemia in people infected with Zika virus were too low to support an urban transmission cycle.[13]

Zika virus identification and early epidemiology

Zika virus was originally isolated from a sentinel monkey that had been placed on a platform in the Zika Forest near Entebbe, Uganda.[1] The first sample from which Zika virus was isolated was collected in 1947, and a second isolation of the virus was achieved in 1948 when the virus was isolated from a pool of *Aedes africanus* mosquitoes collected in the same forest.[1] Subsequent studies of the pathogenicity of Zika virus in animals showed that the virus was neurotropic in mice,[19] but that subcutaneous injection of the virus extracted from mouse brain into monkeys resulted in an inapparent infection. Intracerebral inoculation of the virus to monkeys resulted in only mild fever in one of the five monkeys tested. In a concurrent serological survey,[19] high concentrations of neutralising antibodies were identified in about 6% of people tested, and antibodies were identified in one of 15 monkeys tested.[19]

Findings from studies subsequently showed, primarily through serological surveys, the widespread presence of Zika virus in several other parts of Africa in addition to Uganda, including Nigeria,[7,8,20] Senegal,[21] Sierra Leone,[22] Gabon,[23] Côte d'Ivoire,[24] and the Central African Republic.[25] Additionally, findings from serological surveys outside Africa suggested the presence of Zika virus in Egypt,[26] India,[27] Pakistan,[18] Malaysia,[28] Thailand and north Vietnam,[29] Philippines,[30] and Indonesia.[31] The presence of Zika virus in Asia was confirmed by its isolation from pools of *Aedes aegypti* mosquitoes in Malaysia in 1966.[32] Findings from these studies collectively suggest that Zika virus transmission was broadly distributed in Africa and Asia (figure 1).

As noted earlier, before 2007, only 13 cases of natural infection of human beings with Zika virus had been reported.[7–10] Although the first purported report of people infected with Zika virus was published in 1954,[33] subsequent investigation showed that the infectious pathogen was the closely related Spondweni virus,[10,34] a misidentification that also occurred in the case of the supposed report of experimental human infection with Zika virus.[35] The first bona-fide case of natural infection in people was therefore reported by Simpson,[10] who described his own course of disease acquired while isolating Zika virus from *A africanus* mosquitoes in Uganda between 1962 and 1963. Moore and colleagues[8] subsequently identified three cases of Zika fever through virus isolations from febrile children in 1968 in Nigeria, and Fabgami[7] identified two further cases of Zika fever in Nigeria through virus isolations between 1971 and 1975. Fabgami[7] also reported that 40% of Nigerians had neutralising antibodies to Zika virus, suggesting a high degree of population exposure. The remaining cases of Zika fever before 2007 were identified in patients with fever in hospitals in central Java, Indonesia, although identification was only based on serological investigation, and no confirmatory viral isolation was done.[9]

Several mosquito species belonging to the *Aedes* genus have been identified as potential transmission vectors for Zika virus. These include *A africanus*,[1,17,36] *Aedes luteocephalus* (reported in Fagbami[7]), *Aedes vittatus*,[24,37] *Aedes furcifer*,[24,37] *Aedes apicoargenteus*,[16,36,38] *Aedes hensilli*,[39] and, perhaps of the greatest concern because of their wide and increasing distribution,[40] *A aegypti*[9,24,32,41] and *Aedes albopictus*.[42]

Zika virus epidemiology in Yap State

In 2007, in the first identified transmission of Zika virus in people outside Africa and Asia, in Yap State 49 people with confirmed and 59 with probable Zika virus infection were identified by combined genetic and serological analysis.[12,13] A further 72 people were defined as suspected cases, and five did not have Zika virus infection.[12] Findings from initial laboratory testing with a commercially available dengue IgM assay suggested that dengue virus was the causative pathogen,[12] although local clinicians thought the disease was different clinically from dengue fever (as reported in[12,43]), a disease that had previously occurred in Yap State.[44,45] Therefore, final diagnosis was based on more detailed serological

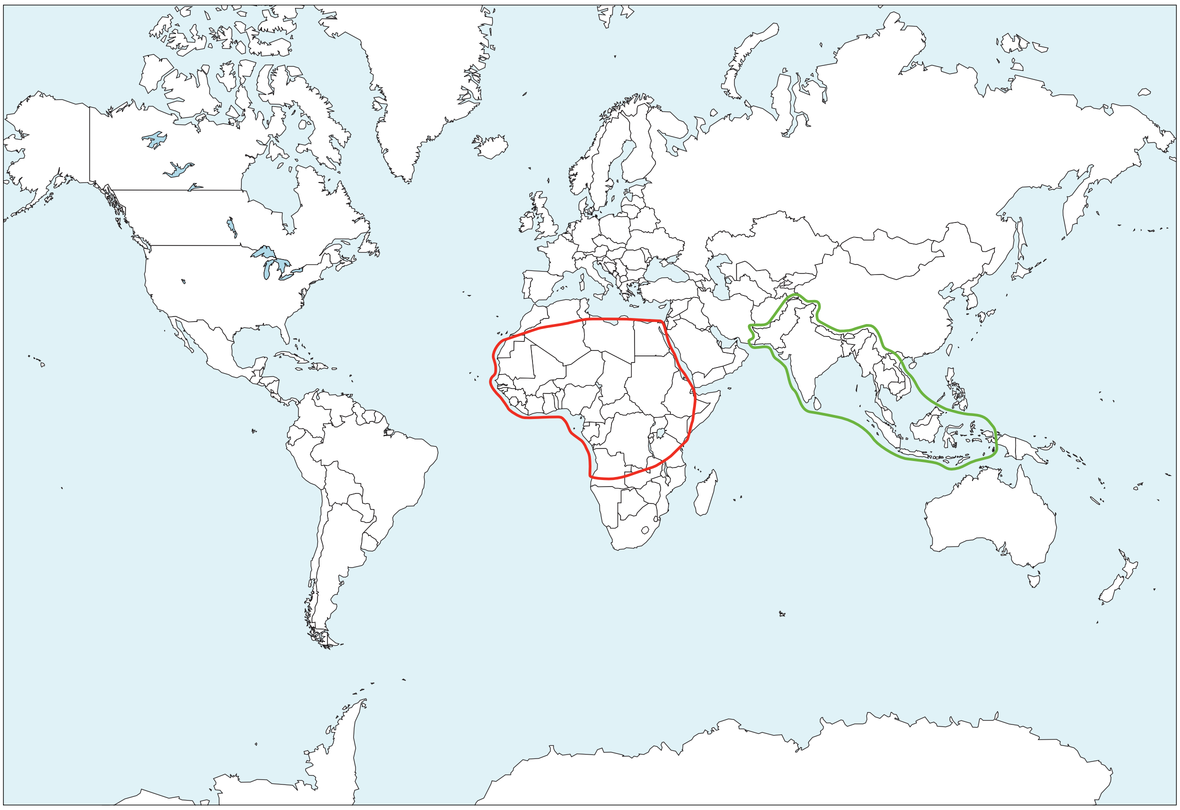

Figure 1: **Estimated distribution of Zika virus before 2007**
Based on findings from immunological and virological studies, the estimated extent of the distribution of Zika virus is shown for Africa (red outline) and Asia (green outline).

analysis, as well as specific detection of the viral genomic RNA. Sequencing of amplified RT-PCR products showed 90% homology with Zika virus, and, because the virus was not recovered from patient specimens during the outbreak, a consensus Zika genome was reconstructed from the sequencing data derived from four patients.[13] Phylogenetic analysis suggested the existence of two African subclades (west and east African lineages) and that the Yap State Zika virus was distantly related to the African clades and has probably resulted from a common ancestor that spread through southeast Asia and the Pacific.[13] Real-time PCR results suggested low levels of viraemia,[13] casting doubt on the ability of the virus to generate an urban epidemic cycle.

Zika virus and sexual transmission

Around the time of the outbreak in Yap State, two US scientists who had been working in southeast Senegal in August, 2008, returned to the USA where they became sick with a rash, headache, fatigue, and arthralgia, with one of them also reporting haematospermia (blood in the ejaculate).[46] Serological evidence suggested that Zika virus was the causative pathogen, and, the wife of one of the patients subsequently became sick (referred to as patient 3), with similar symptoms, and person-to-person transmission of Zika virus through sexual contact or saliva was inferred.[46] However, since the lifespan of a female *Aedes* mosquito is longer than 3 weeks,[47,48] the possibility of an infected mosquito being transported in clothing or personal baggage resulting in direct infection of patient 3 cannot be excluded. However, a second case of haematospermia has been reported,[49] and in this case Zika virus was positively identified in the semen, suggesting that sexual transmission of Zika virus through semen is a viable non-vector-borne route of infection. Other cases of possible sexual transmission[50] and high concentrations of Zika virus detected in semen[51] have been reported recently. In addition to semen, Zika virus RNA has been detected in salvia,[52] breast milk,[53] and urine,[54–57] and in at least one case the virus was recovered from a urine sample.[54]

Zika virus epidemiology: southeast Asia

In 2010, specimens were taken from a child attending a health clinic in Kampong Speu Province, Cambodia (figure 2), that subsequent investigations showed were positive for Zika virus.[58] The child had mild symptoms (fever, sore throat, cough, and headache, but no maculopapular rash) and did not need to be admitted to hospital. No other cases of Zika virus infection were reported around this time, and this was the only positive non-dengue, non-Japanese encephalitis *Flavivirus* infection detected, although around 10 000 samples of blood and throat swabs were screened as part of a US Naval Medical Research Unit 2 surveillance programme.[58]

In 2012, as part of a prospective longitudinal cohort study[59] with active surveillance for acute febrile illness undertaken in Cebu City, Philippines, blood samples were taken from a 15-year-old boy. His symptoms, which included subjective fever, muscle weakness, sore throat, and conjunctivitis, were resolved without medical care or need for a hospital admission. Zika virus was detected by real-time PCR and the virus recovered after intrathoracic inoculation of *Toxorhynchites splendens* mosquitoes and inoculation of serum onto monolayers of C6/36 cells. Sequence data and phylogenetic analysis confirmed the virus to be Zika virus of the Asian lineage.[59] So far, no further cases of Zika virus infection have been reported in either Cambodia or Philippines.

There has been one recent report of Zika virus infection in a resident in Indonesia, identified during an outbreak of dengue fever during December, 2014, to April, 2015, in Jambi Province, Sumatra.[60] Before this, two cases of Zika virus infection associated with travel to Indonesia had been reported.[61,62] In one report,[61] an Australian traveller to Jakarta, Indonesia, returned to Australia with fatigue followed by headache. Subsequently, a maculopapular rash developed as well as an occasional dry cough.[61] Although a dengue-specific RT-PCR test was negative, a *Flavivirus* pan-specific RT-PCR test was positive, and sequence analysis confirmed Zika virus as the causative pathogen.[61] In the other report,[62] a traveller to Indonesia was bitten by a monkey and was subsequently diagnosed with Zika virus infection on their return to Australia.

In early 2013, a traveller returning to Canada from a trip to Thailand via Hong Kong was diagnosed with Zika virus infection on the basis of identification of the Zika genomic RNA by RT-PCR and DNA sequencing.[54] In November, 2013, a traveller returned to Germany after a trip to Thailand, where they had experienced a period of weakness, fever, and chills as well as a

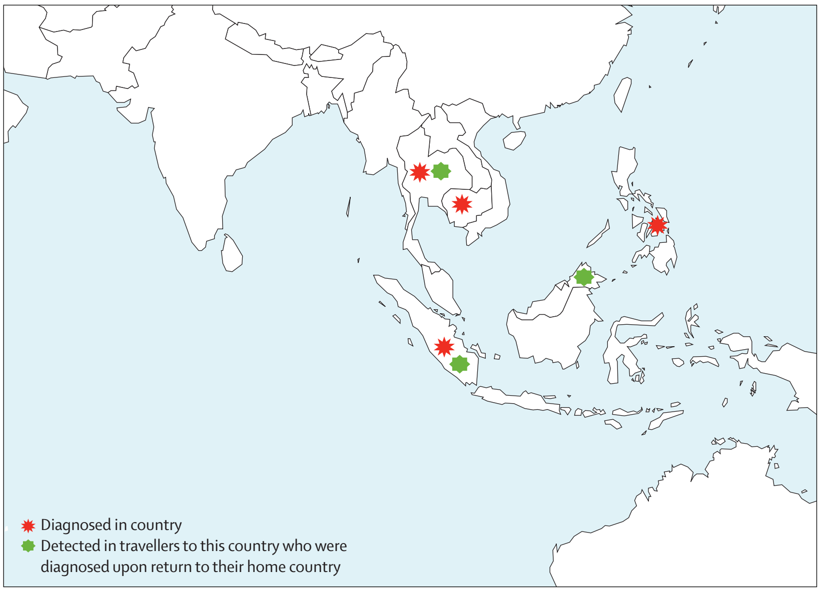

Figure 2: **Locations of Zika virus in southeast Asia from 2010 onwards**

maculopapular rash.[63] Although the patient was mostly asymptomatic upon return to Germany, detailed serological analysis implicated Zika virus infection as the causative pathogen.[63] Although findings from these studies[54,63] suggested that Zika virus was circulating in Thailand, definitive proof of Zika virus transmission in Thailand was not available until 2015, when Buathong and colleagues[64] provided evidence of Zika virus infection in the Thai population. In response to the reports of travellers to Thailand being diagnosed with Zika virus infection upon their return to their home countries[54,63] the Thai Ministry of Public Health started a review of outbreaks of between January, 2012, and December, 2014 (as reported in[64]). The criteria for further assessment of an outbreak were as follows: individuals in the outbreak had at least two of rash, conjunctivitis, or arthralgia; acute serum samples were negative for dengue virus and chikungunya virus; and rubella and measles IgM antibodies were not detected in convalescent serum.[64] These selection criteria identified four outbreaks for further investigation, and seven patients were identified as having had Zika virus infection on the basis of either detailed serological investigation or RT-PCR analysis.[64] Of these cases, three were identified as having occurred in March, 2012, confirming that Zika virus was circulating in Thailand before the visit of the travellers who returned to Canada[54] and Germany[63] after having become exposed to Zika virus. However, the Thai citizens who were identified as having had Zika virus infection were from central, northwest, and northeast Thailand,[64] whereas the travellers who were exposed to Zika virus stayed predominantly in southern Thailand.[54,63] Combined, these results suggest that Zika virus is widespread in Thailand, a result supported by findings from a recent serosurvey[65] of anti-Zika antibodies in serum samples from people in northeast Thailand. Further cases of travellers returning to Japan[57] and Italy[50] from Thailand with Zika fever have been reported recently. Analysis of the Thai Zika NS5 sequences identified by Buathong and colleagues[64] matched closely to the Cambodian 2010 sequences and to sequences from an outbreak of Zika virus in French Polynesia, and maximum likelihood phylogenetic trees clearly discriminated between the Asian and African sequences.[64]

A tourist returning home to Germany from a trip to Malaysia was diagnosed with Zika virus infection in September, 2014.[66] Although the tourist had visited both peninsular Malaysia and the Malaysian state of Sabah on the island of Borneo, the timing of the infection suggested that it occurred while the tourist was in Sabah. However, as noted earlier,[32] Zika virus was first formally identified in Asia in 1966, when the virus was isolated from *Aedes* mosquitoes in the peninsular Malaysian town of Bentong, and so infection having occurred in peninsular Malaysia should not be ruled out.

Zika virus epidemiology: French Polynesia and other islands in the Pacific Ocean

From October, 2013, onwards, reports started to appear of an outbreak of Zika virus infections in French Polynesia, with several islands being affected including Tahiti, Moorea, Raiatea, Taha'a, Bora Bora, Nuku Hiva, and Arutua.[67] Molecular and virological analysis of samples from patients showed the presence of the Zika virus genome by RT-PCR, and in some cases the virus was recovered through inoculation of Vero cells.[68] Within about 1 year after the first cases of Zika virus infection were confirmed, an estimated 19 000 suspected cases had occurred,[68] although other reports estimated the final numbers to be 8746 suspected cases and 30 000 medical consultations because of Zika virus (as reported in[69]). Zika virus infection was confirmed in 294 of 584 serum samples tested by RT-PCR.[68] Sequence analysis showed that the Zika virus circulating in French Polynesia was similar to the Cambodian 2010 and Yap State 2007 strains,[68] and was therefore of the Asian lineage of Zika virus. Findings from subsequent sequence analyses[5,59,64] suggested that the French Polynesian Zika virus was more closely related to southeast Asian strains than the Yap State strain, suggesting that importation of Zika virus to French Polynesia from southeast Asia was independent of the importation to Yap State. The time of introduction of Zika virus to French Polynesia has not been accurately established. However, findings from a retrospective survey[70] of seroprevalence of arboviruses among blood donors from French Polynesia in which serum samples were surveyed from 2011 to 2013 showed a low level of seropositivity towards Zika virus (0·8%), and seropositivity was confined solely to resident French Polynesians who had travelled abroad at least once. No case of Zika virus seropositivity was identified in residents of French Polynesia who had never travelled abroad,[70] suggesting Zika virus was introduced to French Polynesia around the time of the first detected case.

In December, 2013, a tourist from Norway spent 14 days in Tahiti and almost immediately after her return experienced fever and muscle and joint pain, coupled with a maculopapular rash and conjunctivitis.[71] A partial Zika virus genome was amplified using a commercial one-step RT-PCR kit and subsequently sequenced, and the identified virus belonged to the Asian Zika virus lineage.[71] Several other travellers to French Polynesia were diagnosed with Zika virus infection on their return to their home countries, which included France,[72] the USA,[73] and Italy.[74]

Several cases of importation of Zika virus were identified in the Pacific island of New Caledonia starting from late 2013, and by mid-January, 2014, autochthonous cases had been reported.[75] 1385 laboratory-confirmed cases of Zika virus infections were reported, which included 35 imported cases. Although most imported cases came from French Polynesia, importations were also noted from Vanuatu and the Cook Islands.[75] Two cases of dual infection with dengue virus and Zika virus were reported, one in a resident with no history of travel and one in a traveller who returned from French Polynesia.[75] Attempts were made to recover the viruses from these patients, but in both cases only dengue virus was recovered, possibly as a result of low levels of Zika viraemia.[75] At present, no information exists as to whether the patients were infected simultaneously or consecutively, and mosquitoes might carry both viruses simultaneously, although the low levels of Zika viraemia reported make this a less likely possibility. However, findings from an early study suggested that dengue virus immune serum might enhance Zika virus infection[76] and that yellow fever vaccination does not protect against Zika virus infection,[11] suggesting that complex immunological interactions are likely to occur where several flaviviruses are circulating at the same time.

As noted, one of the cases imported to New Caledonia was from the Cook Islands, on which an outbreak of Zika virus infections occurred, with 50 confirmed cases and 932 suspected cases (as reported in[69]). At least one case of importation of Zika virus to Australia from the Cook Islands has been reported,[77] and analysis of Zika E gene sequences showed the closest relation to the Asian Cambodia 2010 isolate.

Easter Island is located in the southeast Pacific Ocean and is the eastern marker for the Polynesian Triangle. An outbreak of Zika virus infection was reported to have started in January, 2014, and 89 serum samples were analysed by RT-PCR, of which 51 were positive for Zika virus.[78] Sequence analysis of the Zika NS5 coding sequence showed that the highest homology was to the French Polynesian Zika virus, and since none of the patients had a history of recent travel outside the island,[78] the mechanism of importation remains unclear, although importation through attendees at a festival held on Easter Island has been proposed.[79]

Zika virus epidemiology: South America

In March, 2015, serum samples were collected from 24 patients at Santa Helena Hospital in Camaçari, Bahia, Brazil, as a consequence of an ongoing outbreak of an illness characterised by fever, maculopapular rash, muscle and joint pain, and conjunctivitis.[80] RT-PCR analysis identified Zika virus in seven patients, and chikungunya virus in three, with no reported co-infection.[80] Sequence analysis of regions of the Zika virus envelope protein showed 99% identity with sequences from a French Polynesian isolate.[80] Findings from a second study,[81] done on serum samples obtained in the city of Natal, Rio Grande do Norte, Brazil, in March, 2015, also showed the presence of Zika virus at around the same time, and sequence data also showed high homology to the French Polynesian Zika virus sequences. The two collection sites are slightly over 1000 km apart (figure 3), and as such are possibly separate introductions of Zika virus to Brazil. There has been substantial speculation as to how Zika virus was introduced into Brazil. Although Zanluca and colleagues[81] suggested the introduction of Zika virus to Brazil was possibly associated with the hosting of the World Cup football tournament by Brazil, which was held between June 12, and July 13, 2014, no Zika-endemic Pacific countries took part in this competition.[79] However, spectators from Zika-endemic countries might have attended the competition and introduced the virus, and matches were held at both Natal and Salvador, which is only 37 km from Camaçari, Brazil (figure 3). A large outbreak of an acute exanthematous illness occurred in Salvador itself, starting from March, 2015, and Zika virus, together with dengue virus and chikungunya virus, were all separately implicated as causative pathogens.[82]

However, Zika virus might have been introduced during the IVF Va'a World Elite and Club Sprint Championship held in August, 2014.[79] This championship canoe race included competitors from four Pacific countries in which Zika virus was circulating: French Polynesia, New Caledonia, Cook Islands, and Easter Island (as reported in[79]). However, it was held in Rio de Janeiro (as reported in[79]), which is located about 1600 km from Camaçari and 2600 km from Natal (figure 3).

Since the first reported cases,[80,81] an estimated 440 000 to 1 300 000 Zika virus infections have occurred in Brazil,[3] and the virus has spread to several neighbouring countries. In a rapid risk assessment produced by the European Centre for Disease Prevention and Control,[83] and drawing on several non-peer-reviewed sources, ongoing Zika virus outbreaks were identified in Colombia, Paraguay, Venezuela, Suriname, French Guiana, Ecuador, Guyana, and Bolivia in South America;

Figure 3: **Introduction of Zika virus to Brazil**

Search strategy and selection criteria

We identified references for this Review through searches of PubMed and the Elsevier Scopus database using the Boolean search terms: "Zika virus" OR "Zika fever" for all articles published up to and including Jan, 29, 2016. Articles published in English and French were included. Articles identified from these searches and relevant references cited in those articles were reviewed. Additional references were sourced as needed on March 16, 2016.

Mexico in North America; El Salvador, Guatemala, Panama, and Honduras in Central America; and the Caribbean countries and territories of Martinique, Saint Martin, Puerto Rico, Haiti, Barbados, and Guadeloupe. Other countries with known or suspected Zika virus transmission include Cape Verde, Samoa, the Solomon Islands, Fiji, Vanuatu, and the Maldives,[83] although in this last case Zika virus infection was identified in a traveller to the Maldives upon their return to Finland.[56]

Zika virus infection: a changing clinical presentation?

The early clinical picture of natural human Zika virus infection was of a short duration, self-limiting, mild febrile illness that was accompanied by a maculopapular rash.[10] In the first reported substantial outbreak of Zika virus infections, in Yap State in 2007, the disease was associated with rash, fever, arthralgia, and conjunctivitis, but no hospital admissions or deaths were reported.[12] Similarly, the cases in Cambodia in 2010[58] and Philippines in 2012[59] were resolved without any hospital admissions. The cases in Thailand between 2012 and 2014 for which full clinical details were available were all classed as mild, with fever and rash as the main symptoms, and sore throat, muscle and joint pain, and headache as other reported symptoms.[64] Conjunctivitis, one of the screening review criteria for the Thai study, was only reported in two of the cases.[64]

The outbreak in French Polynesia was associated with about 70 cases of severe presentation including Guillain-Barré syndrome,[84] and other more severe pathological abnormalities have been associated with Zika virus infection, including meningoencephalitis in the Pacific Islands,[85] and myelitis in Guadeloupe.[86] A second cluster of Guillain-Barré cases occurred in French Polynesia less than 2 years after the cases associated with the Zika virus outbreak, after an outbreak of chikungunya virus infection between October, 2014, and March, 2015.[87] Most strikingly, the outbreak in Brazil has prompted concerns as to the dramatic increase in cases of microcephaly, with a 20 times increase in incidence and a prevalence of nearly 100 per 100 000 livebirths (as reported in[88]), prompting several agencies to issue advisories to pregnant women and those considering pregnancy.[89–92] A more recent analysis[93] in Brazil using a more precise definition of microcephaly than used previously[88] has supported a temporal relation between Zika virus infection in the mother during the first trimester and microcephaly, albeit with a significantly reduced overall prevalence of 2·80 per 100 000 livebirths (95% CI 1·86–4·05), and in a retrospective analysis of the outbreak in French Polynesia,[94] an increased risk of microcephaly associated with Zika virus infection in the first trimester was also found. In support of an association between Zika virus infection of the mother and microcephaly in the fetus, Zika virus has been found in the amniotic fluid of women whose fetuses had been diagnosed with microcephaly,[95] and in the brain but not in other tissues of fetuses aborted because of microcephaly.[96] A strong tropism of Zika virus for neuronal progenitor cells has also been noted, with the concomitant induction of cell death.[97] However, whether these findings imply an amount of neurotropism with Zika virus, as was implicated in the first animal studies,[19] or whether the more severe consequences (ie, microcephaly and Guillain-Barré syndrome) result from a complex immune interplay between successive *Flavivirus* infections, or between Zika virus and the placenta,[98] remains unclear.

Conclusions

Marked regional differences exist in the transmission of Zika virus in different parts of the world. Evidence suggests that Zika virus has been circulating in Thailand for at least 3–4 years,[64] and yet circulation of the virus has not been associated with outbreaks of Zika fever on the same scale as those in South America,[3] or with an increase in neurological complications. Whether this difference results from an as yet unidentified change in viral transmissibility or pathogenicity of Zika virus remains to be established.

Contributors
NW prepared the first draft, which was revised and expanded by DRS. NW prepared the figures with input from DRS. Both authors approved the final version of the manuscript and contributed equally.

Declaration of interests
We declare no competing interests.

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