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Zika virus: a previously slow pandemic spreads rapidly through the Americas

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Zika virus: a previously slow pandemic spreads rapidly through the Americas

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Abstract

Zika virus (*Flaviviridae*) is an emerging arbovirus. Spread by *Aedes* mosquitoes, it was first discovered in Uganda in 1947, and later in humans elsewhere in sub-Saharan Africa, arriving in south-east Asia at latest by mid-20th-century. In the 21st century, it spread across the Pacific Islands reaching South America around 2014. Since then it has spread rapidly northwards reaching Mexico in November 2015. Its clinical profile is that of a dengue-like febrile illness, but recently associations with Guillain-Barré syndrome and microcephaly have appeared. The final geographical range and ultimate clinical impact of Zika virus are still a matter for speculation.

Introduction

Zika virus (family *Flaviviridae*; genus *Flavivirus*) is a positive-sense single-stranded RNA arbovirus within a family that includes several other arboviruses of major clinical importance, such as yellow fever virus, West Nile virus, tick-borne encephalitis virus and dengue virus. First isolated in 1947 in the Zika forest region of Uganda from a *Macaca* monkey (Dick et al., 1952), the first human case was detected in Nigeria in 1954 (Macnamara, 1954). The arthropod vector is several mosquitos of the genus *Aedes* (Diagne et al., 2015). Both urban (Grard et al., 2014) and sylvatic (Berthet et al., 2014) transmission have been demonstrated. Epizootics occur in monkeys (McCrae and Kirya, 1982) but it is unclear as yet if primates are an obligatory reservoir in the transmission cycle in humans.

The classic clinical presentation resembles dengue fever but also chikungunya: a fever accompanied by polyarthralgia, myalgia, maculopapular rash and headache. This

complicates differential diagnosis. Serological testing, however, can distinguish Zika virus infection from that of dengue and chikungunya (Aubry et al., 2015). The virus remained one of the many neglected curiosities of tropical medicine and no efforts were made to develop a vaccine or treatment in view of its low case numbers, and low clinical impact relative to other arboviruses. This situation changed in the 21st century, first with the large-scale outbreaks in the Pacific islands, beginning on Yap in Micronesia in 2007 (Lanciotti et al., 2008), and then with the emergence of the first Zika virus disease cases in Brazil in early 2015 (Zanluca et al., 2015). Zika virus also began to spread northwards at a rapid rate across South and Central America, reaching Mexico by late November 2015 (ECDC, 2015).

The Zika virus genome

The positive strand RNA genome organisation of the virus follows that of related flaviviruses: 5'-C-prM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-3' (Kuno and Chang, 2007), with one single open reading frame encoding the structural proteins C, M and E and the nonstructural proteins which carry out functions in replication and assembly. In all likelihood, antagonism of host responses will be mediated by one or several of these non-structural proteins. 5' and 3' untranslated regions are important in flavivirus genome cyclisation and replication with conserved sequences (CS1-3) found in related flaviviruses. Kuno and Chang (Kuno and Chang, 2007) identified variation in CS1 and CS3 of Zika virus strain MR 766 (order CS3-CS2-CS1) and this should be further investigated when more sequencing data becomes available, as it may influence replication and possibly virus-host interactions and pathogenicity.

Phylogenetics, evolution and epidemiology

Analysis of the origins of what is now apparent as a pandemic of Zika virus has been largely retrospective, based on sequencing of isolates collected across Africa and south-east Asia during the course of the 20th century (Faye et al., 2014). Only with the arrival of Zika virus in the Pacific Islands (Lanciotti et al., 2008) did more systematic sequencing efforts commence and the first full-length genome was obtained (Kuno and Chang, 2007). 21 full-length Zika virus genomes are currently available in GenBank and 9 of those have collection date information in their GenBank record. Figure 1A shows a phylogenetic tree constructed using these, illustrating the emergence of the south-east Asian strain from Africa, and the subsequent seeding of the Pacific islands epidemic from south-east Asia as shown elsewhere (Buathong et al., 2015), and Figure 1B shows the wider relationship of Zika virus to other flaviviruses. Active tracking of the spread of Zika virus across the Pacific and into the Americas, and sequencing of older clinical isolates, have produced a total of 215 Zika virus sequences in GenBank, though many are short fragments.

Phylogenetic studies using these sequences (Faye et al., 2014) have nevertheless enabled the date of emergence of Zika virus in east Africa to be estimated at 1920 with a confidence range on this date of 1892-1947. Serological surveys carried out in Uganda in the wake of the initial discovery of the virus in the late 1940s showed seropositivity of 6.1% in humans (Dick et al., 1952). However, by the late 1960s, Kenya demonstrated seropositivity to Zika virus at 52% overall, but with wide variation between areas (Geser et al., 1970). Levels of seropositivity were lower in Nigeria during the late 1960s (Moore et al., 1975) but had risen to 56% by 1980 (Adekolu-John and Fagbami, 1983). Zika virus has subsequently been

reported across a wide range in central and west Africa, with some examples referenced here (Berthet et al., 2014, Grard et al., 2014).

The same phylogenetic study (Faye et al., 2014) also dated the transmission of east African Zika virus to south-east Asia around 1945 (confidence range 1920-1960), where the virus was first detected in the late 1960s in Malaysia (Marchette et al., 1969) and subsequently across south-east Asia. Various phylogenetic analyses have confirmed that Pacific Island Zika virus is related to the Asian lineages (for instance: Buathong et al., 2015, Alera et al., 2015) (see Fig. 1). Zika virus's first appearance in this new eastward movement was on the Micronesian island of Yap in 2007 (Duffy et al., 2009, Lanciotti et al., 2008). Confounding factors in establishing the exact dates of dispersion of Zika virus are the ease with which Zika virus disease can be confused with dengue fever and chikungunya fever.

The next Pacific outbreak occurred in French Polynesia in 2013 (Cao-Lormeau et al., 2014) and were associated with 42 cases of Guillain-Barre syndrome (Roth et al., 2014). The observation that blood samples collected from 2011 to 2013, had only 0.8% seropositivity to Zika virus, suggests that the introduction to Polynesia was not long before the identification of the index case (Aubry et al., 2015). The scale of the Polynesian outbreak was unprecedented with 28,000 infections recorded in the first four months. Further phylogenetic analyses (for instance: Buathong et al., 2015, Alera et al., 2015) showed Polynesian Zika virus to be more closely related to the south-east Asian strains than to the Yap Island outbreak sequences, suggesting an independent introduction to Polynesia from south-east Asia. Subsequent spread in the Pacific occurred in 2014 to New Caledonia (Dupont-Rouzeyrol et al., 2015), the Cook Islands (Pyke et al., 2014) and Easter Island (Tognarelli et al., 2015).

Transmission to the Americas appears to have originated in the Pacific Islands, a conclusion again based on phylogenetic analysis (Zanluca et al., 2015). The Brazilian state of Bahia was the first to identify cases (Campos et al., 2015). An official announcement by the Brazilian Ministry of Health was made on May 14th 2015, but patients with Zika symptoms had been reported in the city of Salvador from February 15th onwards. By December 10th 2015, Zika virus had spread to 18 other Brazilian states (ECDC, 2015). Two events that may have led to Zika virus's introduction to Brazil are the 2014 FIFA World Cup tournament and an international canoe racing event (Musso, 2015). Since Pacific nations were only represented among the canoe racers, the latter may be the likeliest introduction point.

The World Health Organization subsequently issued alerts to the presence of Zika virus in several Latin American countries: Colombia, Surinam, Guatemala, El Salvador, Mexico, Paraguay, Venezuela and Panama. Figure 2 illustrates the pandemic of Zika virus, drawing on empirical reports of seropositivity, genome sequences with collection information, phylogenetic analyses, and WHO reports for the American stages.

Clinical presentation of American Zika virus

The African form of Zika virus replicated many of the symptoms often associated with arboviruses. The 2007 outbreak in Micronesia presented with rash, fever, arthralgia and conjunctivitis as the most common symptoms and headache, vomiting and oedema in a minority. The disease is acute but self-limiting. Symptoms across six case clusters from 1962-2010 are reviewed by Heang et al (2012). The observation of Guillain-Barré syndrome among Zika cases in Polynesia represented an increase in the potential clinical severity of the disease (Roth et al., 2014).

On 21st November 2015, the WHO notified the presence of 739 cases of microcephaly in 9 states of north-eastern Brazil (http://www.who.int/csr/don/27-november-2015-microcephaly/en/), the same region as the Zika virus outbreak in that country. The association is not yet directly demonstrated but has been integrated into risk assessments by the European Centre for Disease Prevention and Control; additionally three deaths from Zika virus disease (one newborn, one 16 year old, one adult) have been reported, the first known occurrences (ECDC, 2015). The strong possibility exists of sexual transmission in two cases (Musso et al., 2015, Foy et al., 2011), perinatal transmission in two cases (Besnard et al., 2014) and a theoretical possibility of transmission by transfusion based on the presence of virus in 3% of asymptomatic Polynesian blood-donors (Musso et al., 2014). Such observations suggest that Zika virus, once introduced from an area of arboviral transmission, could lead in some cases to disease even in absence of vector-based transmission.

Conclusions and Future Prospects

Any country in which mosquitoes of the genus *Aedes* are present could be potential sites for future Zika virus disease outbreaks. This might include southern Europe and the USA where *Aedes albopictus* has been spreading invasively, but other competent species may also be present. Introductions by tourists have already occurred on several occasions, for example into Europe (Tappe et al., 2014). Competence studies are required in vulnerable regions in order to inform local risk assessments and efforts towards a vaccine and therapeutics need to be accelerated. Moreover, precautions need to be taken to avoid the pathogen entering public blood banks.

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References

ADEKOLU-JOHN, E. O. & FAGBAMI, A. H. 1983. Arthropod-borne virus antibodies in sera of residents of Kainji Lake Basin, Nigeria 1980. *Trans R Soc Trop Med Hyg,* 77**,** 149-51.

ALERA, M. T., HERMANN, L., TAC-AN, I. A., KLUNGTHONG, C., RUTVISUTTINUNT, W., MANASATIENKIJ, W., VILLA, D., THAISOMBOONSUK, B., VELASCO, J. M., CHINNAWIROTPISAN, P., LAGO, C. B., ROQUE, V. G., JR., MACAREO, L. R., SRIKIATKHACHORN, A., FERNANDEZ, S. & YOON, I. K. 2015. Zika virus infection, Philippines, 2012. *Emerg Infect Dis,* 21**,** 722-4.

AUBRY, M., FINKE, J., TEISSIER, A., ROCHE, C., BROULT, J., PAULOUS, S., DESPRES, P., CAO-LORMEAU, V. M. & MUSSO, D. 2015. Seroprevalence of arboviruses among blood donors in French Polynesia, 2011-2013. *Int J Infect Dis,* 41**,** 11-12.

BERTHET, N., NAKOUNE, E., KAMGANG, B., SELEKON, B., DESCORPS-DECLERE, S., GESSAIN, A., MANUGUERRA, J. C. & KAZANJI, M. 2014. Molecular characterization of three Zika flaviviruses obtained from sylvatic mosquitoes in the Central African Republic. *Vector Borne Zoonotic Dis,* 14**,** 862-5.

BESNARD, M., LASTERE, S., TEISSIER, A., CAO-LORMEAU, V. & MUSSO, D. 2014. Evidence of perinatal transmission of Zika virus, French Polynesia, December 2013 and February 2014. *Euro Surveill,* 19.

BUATHONG, R., HERMANN, L., THAISOMBOONSUK, B., RUTVISUTTINUNT, W., KLUNGTHONG, C., CHINNAWIROTPISAN, P., MANASATIENKIJ, W., NISALAK, A., FERNANDEZ, S., YOON, I. K., AKRASEWI, P. & PLIPAT, T. 2015. Detection of Zika Virus Infection in Thailand, 2012-2014. *Am J Trop Med Hyg,* 93**,** 380-3.

200 CAMPOS, G. S., BANDEIRA, A. C. & SARDI, S. I. 2015. Zika Virus Outbreak, Bahia, Brazil. *Emerg Infect*
201 *Dis,* 21**,** 1885-6.
202 CAO-LORMEAU, V. M., ROCHE, C., TEISSIER, A., ROBIN, E., BERRY, A. L., MALLET, H. P., SALL, A. A. &
203 MUSSO, D. 2014. Zika virus, French polynesia, South pacific, 2013. *Emerg Infect Dis,* 20**,**
204 1085-6.
205 DIAGNE, C. T., DIALLO, D., FAYE, O., BA, Y., GAYE, A., DIA, I., WEAVER, S. C., SALL, A. A. & DIALLO, M.
206 2015. Potential of selected Senegalese Aedes spp. mosquitoes (Diptera: Culicidae) to
207 transmit Zika virus. *BMC Infect Dis,* 15**,** 492.
208 DICK, G. W., KITCHEN, S. F. & HADDOW, A. J. 1952. Zika virus. I. Isolations and serological specificity.
209 *Trans R Soc Trop Med Hyg,* 46**,** 509-20.
210 DUFFY, M. R., CHEN, T. H., HANCOCK, W. T., POWERS, A. M., KOOL, J. L., LANCIOTTI, R. S., PRETRICK,
211 M., MARFEL, M., HOLZBAUER, S., DUBRAY, C., GUILLAUMOT, L., GRIGGS, A., BEL, M.,
212 LAMBERT, A. J., LAVEN, J., KOSOY, O., PANELLA, A., BIGGERSTAFF, B. J., FISCHER, M. &
213 HAYES, E. B. 2009. Zika virus outbreak on Yap Island, Federated States of Micronesia. *N Engl J*
214 *Med,* 360**,** 2536-43.
215 DUPONT-ROUZEYROL, M., O'CONNOR, O., CALVEZ, E., DAURES, M., JOHN, M., GRANGEON, J. P. &
216 GOURINAT, A. C. 2015. Co-infection with Zika and dengue viruses in 2 patients, New
217 Caledonia, 2014. *Emerg Infect Dis,* 21**,** 381-2.
218 ECDC. 2015. *Zika virus epidemic in the Americas: potential association with microcephaly and*
219 *Guillain-Barré syndrome* [Online]. Stockholm: European Centre for Disease Prevention and
220 Control

221 Available: http://ecdc.europa.eu/en/publications/Publications/zika-virus-americas-association-with-
222 microcephaly-rapid-risk-assessment.pdf [Accessed 16/12/2015 2015].
223 FAYE, O., FREIRE, C. C., IAMARINO, A., DE OLIVEIRA, J. V., DIALLO, M., ZANOTTO, P. M. & SALL, A. A.
224 2014. Molecular evolution of Zika virus during its emergence in the 20(th) century. *PLoS Negl*
225 *Trop Dis,* 8**,** e2636.
226 FOY, B. D., KOBYLINSKI, K. C., CHILSON FOY, J. L., BLITVICH, B. J., TRAVASSOS DA ROSA, A., HADDOW,
227 A. D., LANCIOTTI, R. S. & TESH, R. B. 2011. Probable non-vector-borne transmission of Zika
228 virus, Colorado, USA. *Emerg Infect Dis,* 17**,** 880-2.
229 GESER, A., HENDERSON, B. E. & CHRISTENSEN, S. 1970. A multipurpose serological survey in Kenya.
230 2. Results of arbovirus serological tests. *Bull World Health Organ,* 43**,** 539-52.
231 GRARD, G., CARON, M., MOMBO, I. M., NKOGHE, D., MBOUI ONDO, S., JIOLLE, D., FONTENILLE, D.,
232 PAUPY, C. & LEROY, E. M. 2014. Zika virus in Gabon (Central Africa)--2007: a new threat from
233 Aedes albopictus? *PLoS Negl Trop Dis,* 8**,** e2681.
234 HEANG, V., YASUDA, C. Y., SOVANN, L., HADDOW, A. D., TRAVASSOS DA ROSA, A. P., TESH, R. B. &
235 KASPER, M. R. 2012. Zika virus infection, Cambodia, 2010. *Emerg Infect Dis,* 18**,** 349-51.
236 KUNO, G. & CHANG, G. J. 2007. Full-length sequencing and genomic characterization of Bagaza,
237 Kedougou, and Zika viruses. *Arch Virol,* 152**,** 687-96.
238 LANCIOTTI, R. S., KOSOY, O. L., LAVEN, J. J., VELEZ, J. O., LAMBERT, A. J., JOHNSON, A. J., STANFIELD,
239 S. M. & DUFFY, M. R. 2008. Genetic and serologic properties of Zika virus associated with an
240 epidemic, Yap State, Micronesia, 2007. *Emerg Infect Dis,* 14**,** 1232-9.
241 MACNAMARA, F. N. 1954. Zika virus: a report on three cases of human infection during an epidemic
242 of jaundice in Nigeria. *Trans R Soc Trop Med Hyg,* 48**,** 139-45.
243 MARCHETTE, N. J., GARCIA, R. & RUDNICK, A. 1969. Isolation of Zika virus from Aedes aegypti
244 mosquitoes in Malaysia. *Am J Trop Med Hyg,* 18**,** 411-5.
245 MCCRAE, A. W. & KIRYA, B. G. 1982. Yellow fever and Zika virus epizootics and enzootics in Uganda.
246 *Trans R Soc Trop Med Hyg,* 76**,** 552-62.
247 MOORE, D. L., CAUSEY, O. R., CAREY, D. E., REDDY, S., COOKE, A. R., AKINKUGBE, F. M., DAVID-WEST,
248 T. S. & KEMP, G. E. 1975. Arthropod-borne viral infections of man in Nigeria, 1964-1970. *Ann*
249 *Trop Med Parasitol,* 69**,** 49-64.
250 MUSSO, D. 2015. Zika Virus Transmission from French Polynesia to Brazil. *Emerg Infect Dis,* 21**,** 1887.

MUSSO, D., NHAN, T., ROBIN, E., ROCHE, C., BIERLAIRE, D., ZISOU, K., SHAN YAN, A., CAO-LORMEAU, V. M. & BROULT, J. 2014. Potential for Zika virus transmission through blood transfusion demonstrated during an outbreak in French Polynesia, November 2013 to February 2014. *Euro Surveill,* 19.

MUSSO, D., ROCHE, C., ROBIN, E., NHAN, T., TEISSIER, A. & CAO-LORMEAU, V. M. 2015. Potential sexual transmission of Zika virus. *Emerg Infect Dis,* 21**,** 359-61.

PYKE, A. T., DALY, M. T., CAMERON, J. N., MOORE, P. R., TAYLOR, C. T., HEWITSON, G. R., HUMPHREYS, J. L. & GAIR, R. 2014. Imported zika virus infection from the cook islands into australia, 2014. *PLoS Curr,* 6.

ROTH, A., MERCIER, A., LEPERS, C., HOY, D., DUITUTURAGA, S., BENYON, E., GUILLAUMOT, L. & SOUARES, Y. 2014. Concurrent outbreaks of dengue, chikungunya and Zika virus infections - an unprecedented epidemic wave of mosquito-borne viruses in the Pacific 2012-2014. *Euro Surveill,* 19.

TAMURA, K., STECHER, G., PETERSON, D., FILIPSKI, A. & KUMAR, S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol,* 30**,** 2725-9.

TAPPE, D., RISSLAND, J., GABRIEL, M., EMMERICH, P., GUNTHER, S., HELD, G., SMOLA, S. & SCHMIDT-CHANASIT, J. 2014. First case of laboratory-confirmed Zika virus infection imported into Europe, November 2013. *Euro Surveill,* 19.

TOGNARELLI, J., ULLOA, S., VILLAGRA, E., LAGOS, J., AGUAYO, C., FASCE, R., PARRA, B., MORA, J., BECERRA, N., LAGOS, N., VERA, L., OLIVARES, B., VILCHES, M. & FERNANDEZ, J. 2015. A report on the outbreak of Zika virus on Easter Island, South Pacific, 2014. *Arch Virol*.

ZANLUCA, C., DE MELO, V. C., MOSIMANN, A. L., DOS SANTOS, G. I., DOS SANTOS, C. N. & LUZ, K. 2015. First report of autochthonous transmission of Zika virus in Brazil. *Mem Inst Oswaldo Cruz,* 110**,** 569-72.

Figure legends

Figure 1. Molecular phylogenetic analysis of dated Zika virus genomes. A: Zika virus genomes **B**: selected Flavivirus genomes. For both trees, genomes at, or near, full length were used to construct a maximum likelihood tree in MEGA (Tamura et al., 2013), under the GTR+G substitution model. Bootstrap confidence levels are given on nodes where >70%. Scale: substitutions per site. Sequence KF993678/Canada originated in Thailand.

Figure 2. Spread of Zika virus. This is inferred from phylogenetic analysis where available in the literature, otherwise reconstructed from patterns of case report clusters or seropositivity in populations. Map background: Wikimedia commons public domain.

Figure 1A

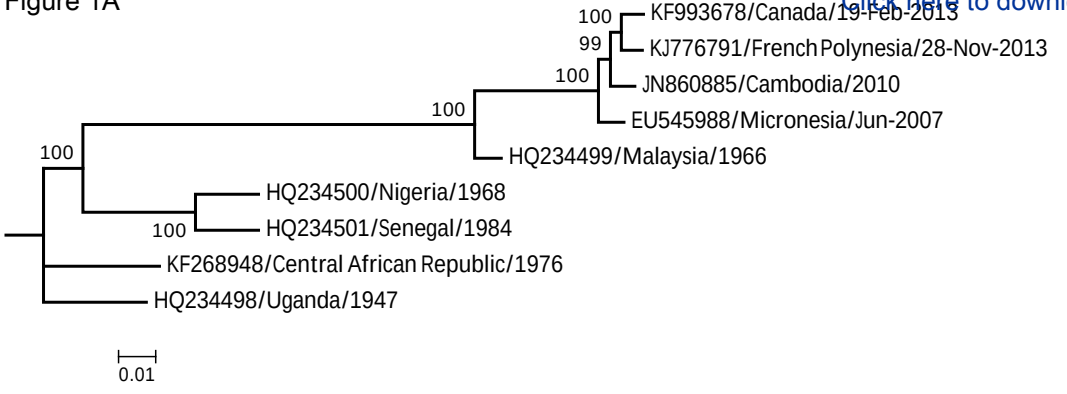

Figure 1B

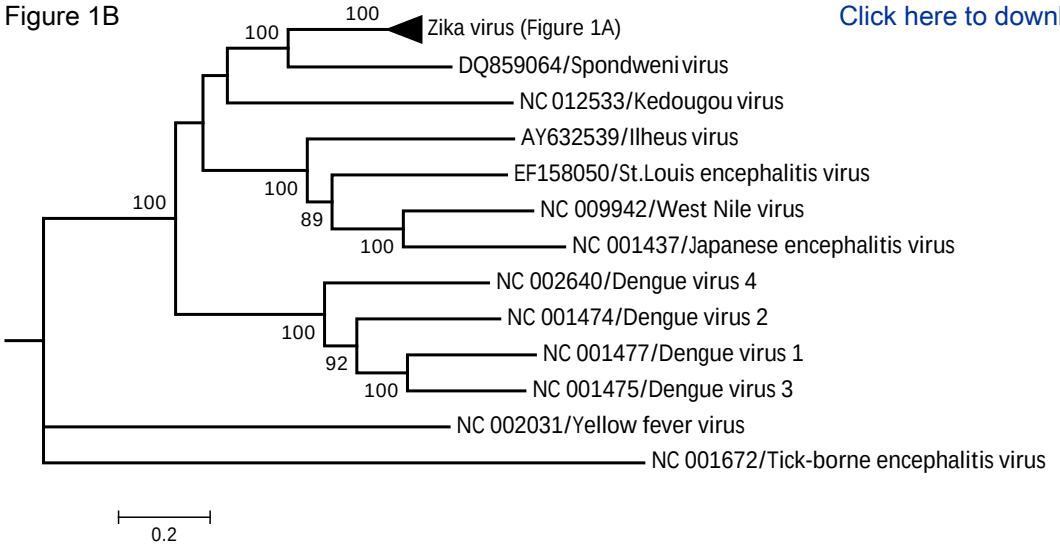

Figure 2

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