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# Complete Genome Sequences of Three Historically Important, Spatiotemporally Distinct, and Genetically Divergent Strains of Zika Virus: MR-766, P6-740, and PRVABC-59

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# Complete Genome Sequences of Three Historically Important, Spatiotemporally Distinct, and Genetically Divergent Strains of Zika Virus: MR-766, P6-740, and PRVABC-59

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Here, we report the 10,807-nucleotide-long consensus RNA genome sequences of three spatiotemporally distinct and genetically divergent Zika virus strains, with the functionality of their genomic sequences substantiated by reverse genetics: MR-766 (African lineage, Uganda, 1947), P6-740 (Asian lineage, Malaysia, 1966), and PRVABC-59 (Asian lineage-derived American strain, Puerto Rico, 2015).

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Zika virus (ZIKV) is a positive-stranded RNA virus of the family *Flaviviridae*, genus *Flavivirus* (1). ZIKV is spread among humans primarily through the bite of infected *Aedes* species mosquitoes (2), but it can also be transmitted from an infected mother to her child during pregnancy (3–7) or by sexual contact (8, 9) or blood transfusion (10). In nature, ZIKV is probably maintained in a sylvatic cycle involving wild primates and arboreal mosquitoes (11–16). Although ZIKV infection generally causes a mild and self-limiting illness in infected individuals (17, 18), it has recently been associated with a growing number of severe neurological disorders, including Guillain-Barré syndrome in adults and microcephaly in newborns (3–5, 19–21). Historically, ZIKV spread from equatorial Africa and Asia (22–33) to the Pacific Islands (34–40), and most recently, to Latin America (41–46); it is now a pandemic in progress (47–50).

Over the past half century, a significant number of ZIKV isolates have been obtained sporadically from three continents (Africa, Asia, and America); however, little is known about the genetic variation among these geographically and temporally

distinct ZIKVs because of the limited number of fully sequenced ZIKV genomes (27, 51–61). Here, we have determined the complete genome sequences of three historically important spatiotemporally distinct ZIKVs (Table 1): (i) MR-766, the first ZIKV isolated in Uganda from a sentinel rhesus monkey in 1947 (33); (ii) P6-740, the first non-African strain isolated in Malaysia from a pool of *Aedes aegypti* mosquitoes in 1966 (23); and (iii) PRVABC-59, the current American epidemic strain isolated in Puerto Rico from a human patient in 2015 (62). The consensus nucleotide sequence for each of the three genomic RNAs was determined by direct sequencing of three overlapping cDNA amplicons (nucleotides [nt] 1 to 4530, nt 2339 to 7386, and nt 5626 to 10611 [numbered based on LC002520]) that cover the entire viral genome except the 5' and 3' termini; 5'- and 3'-rapid amplification of cDNA ends was then performed to obtain the missing terminal sequences (nt 1 to 163 and nt 10053 to 10807), according to our established protocols (63, 64).

In all three ZIKV strains, the genomic RNA is 10,807 nt in length, with a single 10,272-nt open reading frame (ORF) flanked

TABLE 1 Summary of the complete genome sequences of three spatiotemporally distinct genetically divergent ZIKVs with the accession numbers deposited at the GenBank sequence database

Strain	Country of isolation	Collection date	Host	Complete genome size (nt)	5' NCR <sup>a</sup> (nt)	ORF <sup>b</sup> (nt)	3' NCR (nt)	Genetic lineage	GenBank accession no.
MR-766 <sup>c</sup>	Uganda	April 1947	Monkey ( <i>Macaca mulatta</i> )	10,807	106	10,272	429	African	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KX377335">KX377335</a>
P6-740 <sup>d</sup>	Malaysia	July 1966	Mosquito ( <i>Aedes aegypti</i> )	10,807	107	10,272	428	Asian	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KX377336">KX377336</a>
PRVABC-59 <sup>e</sup>	Puerto Rico	December 2015	Human ( <i>Homo sapiens</i> )	10,807	107	10,272	428	Asian	<a href="https://www.ncbi.nlm.nih.gov/nuccore/KX377337">KX377337</a>

<sup>a</sup> NCR, noncoding region.

<sup>b</sup> ORF, open reading frame.

<sup>c</sup> The genome of MR-766 has been fully sequenced in this study and by three other independent groups (accession numbers AY632535, LC002520, and KU955594), and their nucleotide sequences are not identical, most likely because of variations in the cultivation history of the virus.

<sup>d</sup> The partial coding sequence of P6-740 has been sequenced previously (accession no. HQ234499).

<sup>e</sup> The genome of PRVABC-59 has been sequenced previously by two research groups (accession numbers KU501215 and KX087101), but both lack the 5'- and 3'-terminal sequences.

by a 106- or 107-nt 5' noncoding region (NCR) and a 428- or 429-nt 3' NCR (Table 1). The ORF encodes a 3,423-amino acid (aa) polyprotein predicted to be cleaved into 10 proteins: 122-aa C, 168-aa prM, 504-aa E, 352-aa NS1, 226-aa NS2A, 130-aa NS2B, 617-aa NS3, 150-aa NS4A, 251-aa NS4B, and 903-aa NS5. The functionality of all three genomic sequences was validated by generating their full-length infectious cDNA clones (our unpublished data). A phylogenetic analysis using the nucleotide sequences of the 29 available ZIKV genomes (15 complete and 14 near-complete) revealed two major genetic lineages: African, including MR-766; and Asian, including both P6-740 and PRVABC-59, with PRVABC-59 derived from an ancestor of the Asian lineage, in agreement with recent reports (6, 7, 38, 43, 62, 65, 66). Our findings provide a foundation for comparative functional genomics studies of ZIKV biology.

**Accession number(s).** The accession numbers deposited at the GenBank sequence database are listed in Table 1.

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## REFERENCES

- Thiel HJ, Collett MS, Gould EA, Heinz FX, Houghton M, Meyers G, Purcell RH, Rice CM. 2005. Family *Flaviviridae*, p 981–998. In Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA (ed), *Virus taxonomy: eighth report of the International Committee on Taxonomy of Viruses*. Elsevier Academic, San Diego, CA.
- Weaver SC, Costa F, Garcia-Blanco MA, Ko AI, Ribeiro GS, Saade G, Shi PY, Vasilakis N. 2016. Zika virus: history, emergence, biology, and prospects for control. *Antiviral Res* 130:69–80. <http://dx.doi.org/10.1016/j.antiviral.2016.03.010>.
- Besnard M, Lastère 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:pii=20751. <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20751>.
- European Centre for Disease Prevention and Control. 2016. Rapid risk assessment. Zika virus disease epidemic: potential association with microcephaly and Guillain-Barré syndrome (first update). European Centre for Disease Prevention and Control, Stockholm, Sweden. <http://ecdc.europa.eu/en/publications/Publications/rapid-risk-assessment-zika-virus-first-update-jan-2016.pdf>.
- Pan American Health Organization. 2015. Epidemiological alert. Neurological syndrome, congenital malformations, and Zika virus infection. Implications for public health in the Americas. Pan American Health Organization, Washington, DC.
- Calvet G, Aguiar RS, Melo AS, Sampaio SA, de Filippis I, Fabri A, Araujo ES, de Sequeira PC, de Mendonça MC, de Oliveira L, Tschoeke DA, Schrago CG, Thompson FL, Brasil P, Dos Santos FB, Nogueira RM, Tanuri A, de Filippis AM. 2016. Detection and sequencing of Zika virus from amniotic fluid of fetuses with microcephaly in Brazil: a case study. *Lancet Infect Dis* 16:653–660. [http://dx.doi.org/10.1016/S1473-3099\(16\)00095-5](http://dx.doi.org/10.1016/S1473-3099(16)00095-5).
- Mlakar J, Korva M, Tul N, Popović M, Poljšak-Prijatelj M, Mraz J, Kolenc M, Resman Rus K, Vesnaver Vipotnik T, Fabjan Vodusek V, Vizjak A, Pizem J, Petrovec M, Zupanc AT. 2016. Zika virus associated with microcephaly. *N Engl J Med* 374:951–958. <http://dx.doi.org/10.1056/NEJMoa1600651>.
- Foy BD, Kobylinski KC, Chilson Foy JL, Blitvich BJ, Travassos da Rosa A, Haddow AD, Lanciotti RS, Tesh RB. 2011. Probable non-vector-borne transmission of Zika virus, Colorado, USA. *Emerg Infect Dis* 17:880–882. <http://dx.doi.org/10.3201/eid1705.101939>.
- Musso D, Roche C, Robin E, Nhan T, Teissier A, Cao-Lormeau VM. 2015. Potential sexual transmission of Zika virus. *Emerg Infect Dis* 21:359–361. <http://dx.doi.org/10.3201/eid2102.141363>.
- Musso D, Nhan T, Robin E, Roche C, Bierlaire D, Zisou K, Shan Yan A, Cao-Lormeau VM, Brout 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:p=20761. <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20761>.
- Haddow AJ, Williams MC, Woodall JP, Simpson DI, Goma LK. 1964. Twelve isolations of Zika virus from *Aedes (Stegomyia) africanus* (Theobald) taken in and above a Uganda Forest. *Bull World Health Organ* 31:57–69.
- Boorman JPT, Porterfield JS. 1956. A simple technique for infection of mosquitoes with viruses; transmission of Zika virus. *Trans R Soc Trop Med Hyg* 50:238–242. [http://dx.doi.org/10.1016/0035-9203\(56\)90029-3](http://dx.doi.org/10.1016/0035-9203(56)90029-3).
- Henderson BE, Hewitt LE, Lule M. 1968. Serology of wild mammals, p 48–51. *Virus Research Institute annual report*. East African Printer, Nairobi, Kenya.
- McCrae AW, Kirya BG. 1982. Yellow fever and Zika virus epizootics and enzootics in Uganda. *Trans R Soc Trop Med Hyg* 76:552–562. [http://dx.doi.org/10.1016/0035-9203\(82\)90161-4](http://dx.doi.org/10.1016/0035-9203(82)90161-4).
- McCrae AW, Kirya BG, Tukei PM. 1970. Summary of an apparent epizootic of Zika virus: pattern of incidence from *Aedes africanus* collected from the Zika Forest, 1969–1970, p 20–21. *Virus Research Institute annual report*. East African Printer, Nairobi, Kenya.
- Kirya BG, Okia NO. 1977. A yellow fever epizootic in Zika forest, Uganda, during 1972. Part 2: Monkey serology. *Trans R Soc Trop Med Hyg* 71:300–303.
- Petersen LR, Jamieson DJ, Powers AM, Honein AM, Honein MA. 2016. Zika virus. *N Engl J Med* 374:1552–1563. <http://dx.doi.org/10.1056/NEJMra1602113>.
- Lazear HM, Diamond MS. 2016. Zika virus: new clinical syndromes and its emergence in the Western Hemisphere. *J Virol* 90:4864–4875. <http://dx.doi.org/10.1128/JVI.00252-16>.
- Oehler E, Watrin L, Larre P, Leparc-Goffart I, Lastere S, Valour F, Baudouin L, Mallet H, Musso D, Ghanache F. 2014. Zika virus infection complicated by Guillain-Barré syndrome—case report, French Polynesia, December 2013. *Euro Surveill* 19:pii=20720. <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20720>.
- Schuler-Faccini L, Ribeiro EM, Feitosa IM, Horovitz DD, Cavalcanti DP, Pessoa A, Doriqui MJ, Neri JI, Neto JM, Wanderley HY, Cernach M, El-Husny AS, Pone MV, Seroo CL, Sanseverino MT, Brazilian Medical Genetics Society, Zika Embryopathy Task Force. 2016. Possible association between Zika virus infection and microcephaly—Brazil, 2015. *MMWR Morb Mortal Wkly Rep* 65:59–62. <http://dx.doi.org/10.15585/mmwr.mm6503e2>.
- Centers for Disease Control and Prevention. 2016. Health advisory. Recognizing, managing, and reporting Zika virus infections in travelers returning from Central America, South America, the Caribbean, and Mexico. Centers for Disease Control and Prevention, Atlanta, GA. <http://emergency.cdc.gov/han/han00385.asp>.
- Macnamara FN. 1954. Zika virus: a report on three cases of human infection during an epidemic of jaundice in Nigeria. *Trans R Soc Trop Med Hyg* 48:139–145. [http://dx.doi.org/10.1016/0035-9203\(54\)90006-1](http://dx.doi.org/10.1016/0035-9203(54)90006-1).
- Marchette NJ, Garcia R, Rudnick A. 1969. Isolation of Zika virus from *Aedes aegypti* mosquitoes in Malaysia. *Am J Trop Med Hyg* 18:411–415.
- Diallo D, Sall AA, Diagne CT, Faye O, Faye O, Ba Y, Hanley KA, Buememann M, Weaver SC, Diallo M. 2014. Zika virus emergence in mosquitoes in southeastern Senegal, 2011. *PLoS One* 9:e109442. <http://dx.doi.org/10.1371/journal.pone.0109442>.
- Faye O, Faye O, Diallo D, Diallo M, Weidmann M, Sall AA. 2013. Quantitative real-time PCR detection of Zika virus and evaluation with field-caught mosquitoes. *Virol J* 10:311. <http://dx.doi.org/10.1186/1743-422X-10-311>.
- Weinbren MP, Williams MC. 1958. Zika virus: further isolations in the

- Zika area, and some studies on the strains isolated. *Trans R Soc Trop Med Hyg* 52:263–268. [http://dx.doi.org/10.1016/0035-9203\(58\)90085-3](http://dx.doi.org/10.1016/0035-9203(58)90085-3).
27. Berthet N, Nakouné E, Kamgang B, Selekon B, Descorps-Declère S, Gessain A, Manuguerra JC, Kazanji M. 2014. Molecular characterization of three Zika flaviviruses obtained from sylvatic mosquitoes in Central African Republic. *Vector Borne Zoonotic Dis* 14:862–865. <http://dx.doi.org/10.1089/vbz.2014.1607>.
  28. Grard G, Caron M, Mombo IM, Nkoghe D, Mbouï Ondo S, Jiolle D, Fontenille D, Paupy C, Leroy EM. 2014. Zika virus in Gabon (Central Africa)—2007: a new threat from *Aedes albopictus*? *PLoS Negl Trop Dis* 8:e2681. <http://dx.doi.org/10.1371/journal.pntd.0002681>.
  29. Olson JG, Ksiazek TG, Suhandiman T, Triwibowo . 1981. Zika virus, a cause of fever in Central Java, Indonesia. *Trans R Soc Trop Med Hyg* 75:389–393. [http://dx.doi.org/10.1016/0035-9203\(81\)90100-0](http://dx.doi.org/10.1016/0035-9203(81)90100-0).
  30. Heang V, Yasuda CY, Sovann L, Haddow AD, Travassos da Rosa AP, Tesh RB, Kasper MR. 2012. Zika virus infection, Cambodia, 2010. *Emerg Infect Dis* 18:349–351. <http://dx.doi.org/10.3201/eid1802.111224>.
  31. Buathong R, Hermann L, Thaisomboonsuk B, Rutvisuttinunt W, Klungthong C, Chinnawirotpisan P, Manasatienkij W, Nisalak A, Fernandez S, Yoon IK, Akrasewi P, Plipat T. 2015. Detection of Zika virus infection in Thailand, 2012–2014. *Am J Trop Med Hyg* 93:380–383. <http://dx.doi.org/10.4269/ajtmh.15-0022>.
  32. Alera MT, Hermann L, Tac-An IA, Klungthong C, Rutvisuttinunt W, Manasatienkij W, Villa D, Thaisomboonsuk B, Velasco JM, Chinnawirotpisan P, Lago CB, Roque VG, Jr, Macareo LR, Srikiatkachorn A, Fernandez S, Yoon I. 2015. Zika virus infection, Philippines, 2012. *Emerg Infect Dis* 21:722–724. <http://dx.doi.org/10.3201/eid2104.141707>.
  33. Dick GWA, Kitchen SF, Haddow AJ. 1952. Zika virus. I. Isolations and serological specificity. *Trans R Soc Trop Med Hyg* 46:509–520. [http://dx.doi.org/10.1016/0035-9203\(52\)90042-4](http://dx.doi.org/10.1016/0035-9203(52)90042-4).
  34. Duffy MR, Chen TH, Hancock WT, Powers AM, Kool JL, Lanciotti RS, Pretrick M, Marfel M, Holzbauer S, Dubray C, Guillaumot L, Griggs A, Bel M, Lambert AJ, Laven J, Kosoy O, Panella A, Biggerstaff BJ, Fischer M, Hayes EB. 2009. Zika virus outbreak on Yap Island, Federated States of Micronesia. *N Engl J Med* 360:2536–2543. <http://dx.doi.org/10.1056/NEJMoa0805715>.
  35. Cao-Lormeau VM, Roche C, Teissier A, Robin E, Berry AL, Mallet HP, Sall AA, Musso D. 2014. Zika virus, French Polynesia, South Pacific, 2013. *Emerg Infect Dis* 20:1085–1086.
  36. Dupont-Rouzeyrol M, O'Connor O, Calvez E, Daurès M, John M, Grangeon J, Gourinat A. 2015. Co-infection with Zika and dengue viruses in 2 patients, New Caledonia, 2014. *Emerg Infect Dis* 21:381–382. <http://dx.doi.org/10.3201/eid2102.141553>.
  37. 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, Fernández J. 2016. A report on the outbreak of Zika virus on Easter Island, South Pacific, 2014. *Arch Virol* 161:665–668. <http://dx.doi.org/10.1007/s00705-015-2695-5>.
  38. Haddow AD, Schuh AJ, Yasuda CY, Kasper MR, Heang V, Huy R, Guzman H, Tesh RB, Weaver SC. 2012. Genetic characterization of Zika virus strains: geographic expansion of the Asian lineage. *PLoS Negl Trop Dis* 6:e1477. <http://dx.doi.org/10.1371/journal.pntd.0001477>.
  39. Pyke AT, Daly MT, Cameron JN, Moore PR, Taylor CT, Hewitson GR, Humphreys JL, Gair R. 2014. Imported Zika virus infection from the Cook Islands into Australia, 2014. *PLoS Curr* 6. <http://dx.doi.org/10.1371/currents.outbreaks.4635a54dbfba2155fb2fd76dc49f65e>.
  40. Waehre T, Maagard A, Tappe D, Cadar D, Schmidt-Chanasit J. 2014. Zika virus infection after travel to Tahiti, December 2013. *Emerg Infect Dis* 20:1412–1414. <http://dx.doi.org/10.3201/eid2008.140302>.
  41. Campos GS, Bandeira AC, Sardi SI. 2015. Zika virus outbreak, Bahia, Brazil. *Emerg Infect Dis* 21:1885–1886. <http://dx.doi.org/10.3201/eid2110.150847>.
  42. Zanluca C, Melo VC, Mosimann AL, Santos GI, Santos CN, Luz K. 2015. First report of autochthonous transmission of Zika virus in Brazil. *Mem Inst Oswaldo Cruz* 110:569–572. <http://dx.doi.org/10.1590/0074-02760150192>.
  43. Enfissi A, Codrington J, Roosblad J, Kazanji M, Rousset D. 2016. Zika virus genome from the Americas. *Lancet* 387:227–228. [http://dx.doi.org/10.1016/S0140-6736\(16\)00003-9](http://dx.doi.org/10.1016/S0140-6736(16)00003-9).
  44. World Health Organization. 2015. Zika virus outbreaks in the Americas. *Wkly Epidemiol Rec* 90:609–610.
  45. Hennessey M, Fischer M, Staples JE. 2016. Zika virus spreads to new areas—region of the Americas, May 2015–January 2016. *MMWR Morb Mortal Wkly Rep* 65:55–58. <http://dx.doi.org/10.15585/mmwr.mm6503e1>.
  46. Musso D. 2015. Zika virus transmission from French Polynesia to Brazil. *Emerg Infect Dis* 21:1887. <http://dx.doi.org/10.3201/eid2110.151125>.
  47. Musso D, Gubler DJ. 2016. Zika virus. *Clin Microbiol Rev* 29:487–524. <http://dx.doi.org/10.1128/CMR.00072-15>.
  48. Fauci AS, Morens DM. 2016. Zika virus in the Americas—yet another arbovirus threat. *N Engl J Med* 374:601–604. <http://dx.doi.org/10.1056/NEJMp1600297>.
  49. Gatherer D, Kohl A. 2016. Zika virus: a previously slow pandemic spreads rapidly through the Americas. *J Gen Virol* 97:269–273. <http://dx.doi.org/10.1099/jgv.0.000381>.
  50. Bogoch II, Brady OJ, Kraemer MUG, German M, Creatore MI, Kulkarni MA, Brownstein JS, Mekaru SR, Hay SI, Groot E, Watts A, Khan K. 2016. Anticipating the international spread of Zika virus from Brazil. *Lancet* 387:335–336. [http://dx.doi.org/10.1016/S0140-6736\(16\)00080-5](http://dx.doi.org/10.1016/S0140-6736(16)00080-5).
  51. Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lambert AJ, Johnson AJ, Stanfield SM, Duffy MR. 2008. Genetic and serologic properties of Zika virus associated with an epidemic, Yap state, Micronesia, 2007. *Emerg Infect Dis* 14:1232–1239. <http://dx.doi.org/10.3201/eid1408.080287>.
  52. Ladner JT, Wiley MR, Prieto K, Yasuda CY, Nagle E, Kasper MR, Reyes L, Vasilakis N, Heang V, Weaver SC, Haddow A, Tesh RB, Sovann L, Palacios G. 2016. Complete genome sequences of five Zika virus isolates. *Genome Announc* 4(3):e00377–16. <http://dx.doi.org/10.1128/genomeA.00377-16>.
  53. Ellison DW, Ladner JT, Buathong R, Alera MT, Wiley MR, Hermann L, Rutvisuttinunt W, Klungthong C, Chinnawirotpisan P, Manasatienkij W, Melendrez MC, Maljkovic Berry I, Thaisomboonsuk B, Ong-Ajchaowlerd P, Kaneechit W, Velasco JM, Tac-An IA, Villa D, Lago CB, Roque VG, Jr, Plipat T, Nisalak A, Srikiatkachorn A, Fernandez S, Yoon IK, Haddow AD, Palacios GF, Jarman RG, Macareo LR. 2016. Complete genome sequences of Zika virus strains isolated from the blood of patients in Thailand in 2014 and the Philippines in 2012. *Genome Announc* 4(3):e00359–16. <http://dx.doi.org/10.1128/genomeA.00359-16>.
  54. Liu L, Wu W, Zhao X, Xiong Y, Zhang S, Liu X, Qu J, Li J, Nei K, Liang M, Shu Y, Hu G, Ma X, Li D. 2016. Complete genome sequence of Zika virus from the first imported case in mainland China. *Genome Announc* 4(2):e00291–16. <http://dx.doi.org/10.1128/genomeA.00291-16>.
  55. Giovanetti M, Faria NR, Nunes MRT, de Vasconcelos JM, Lourenço J, Rodrigues SG, Vianez JL, Jr, da Silva SP, Lemos PS, Tavares FN, Martin DP, do Rosário MS, Siqueira IC, Ciccozzi M, Pybus OG, de Oliveira T, Alcantara LC. 2016. Zika virus complete genome from Salvador, Bahia, Brazil. *Infect Genet Evol* 41:142–145. <http://dx.doi.org/10.1016/j.meegid.2016.03.030>.
  56. Zhu Z, Chan JF, Tee KM, Choi GK, Lau SK, Woo PC, Tse H, Yuen KY. 2016. Comparative genomic analysis of pre-epidemic and epidemic Zika virus strains for virological factors potentially associated with the rapidly expanding epidemic. *Emerg Microbes Infect* 5:e22. <http://dx.doi.org/10.1038/emi.2016.48>.
  57. Cunha MS, Esposito DL, Rocco IM, Maeda AY, Vasami FG, Nogueira JS, de Souza RP, Suzuki A, Addas-Carvalho M, Barjas-Castro Mde L, Resende MR, Stucchi RS, Boin Ide F, Katz G, Angerami RN, da Fonseca BA. 2016. First complete genome sequence of Zika virus (*Flaviviridae*, *Flavivirus*) from an autochthonous transmission in Brazil. *Genome Announc* 4(2):e00032–16. <http://dx.doi.org/10.1128/genomeA.00032-16>.
  58. Baronti C, Piorkowski G, Charrel RN, Boubis L, Leparc-Goffart I, de Lamballerie X. 2014. Complete coding sequence of Zika virus from a French Polynesia outbreak in 2013. *Genome Announc* 2(3):e00500–14. <http://dx.doi.org/10.1128/genomeA.00500-14>.
  59. Kuno G, Chang GJ. 2007. Full-length sequencing and genomic characterization of Bagaza, Kedougou, and Zika viruses. *Arch Virol* 152:687–696. <http://dx.doi.org/10.1007/s00705-006-0903-z>.
  60. Lednicky J, Beau De Rochars VM, El Badry M, Loeb J, Telisma T, Chavannes S, Anilis G, Cella E, Ciccozzi M, Rashid M, Okech B, Salemi M, Morris JG, Jr. 2016. Zika virus outbreak in Haiti in 2014: molecular and clinical data. *PLoS Negl Trop Dis* 10:e0004687. <http://dx.doi.org/10.1371/journal.pntd.0004687>.
  61. Barzon L, Pacenti M, Berto A, Sinigaglia A, Franchin E, Lavezzo E, Brugnaro P, Palù G. 2016. Isolation of infectious Zika virus from saliva and prolonged viral RNA shedding in a traveller returning from the Do-

- minican Republic to Italy, January 2016. *Euro Surveill* 21:pii=21409. <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=21409>.
62. Lanciotti RS, Lambert AJ, Holodniy M, Saavedra S, Signor LdelC. 2016. Phylogeny of Zika virus in western hemisphere, 2015. *Emerg Infect Dis* 22:933–935. <http://dx.doi.org/10.3201/eid2205.160065>.
63. Yun SI, Kim SY, Rice CM, Lee YM. 2003. Development and application of a reverse genetics system for Japanese encephalitis virus. *J Virol* 77: 6450–6465. <http://dx.doi.org/10.1128/JVI.77.11.6450-6465.2003>.
64. Song BH, Yun GN, Kim JK, Yun SI, Lee YM. 2012. Biological and genetic properties of SA<sub>14</sub>-14-2, a live-attenuated Japanese encephalitis vaccine that is currently available for humans. *J Microbiol* 50:698–706. <http://dx.doi.org/10.1007/s12275-012-2336-6>.
65. Faye O, Freire CC, Iamarino A, Faye O, de Oliveira JV, Diallo M, Zanotto PM, Sall AA. 2014. Molecular evolution of Zika virus during its emergence in the 20th century. *PLoS Negl Trop Dis* 8:e2636.
66. Faria NR, Azevedo Rdo S, Kraemer MU, Souza R, Cunha MS, Hill SC, Thézé J, Bonsall MB, Bowden TA, Rissanen I, Rocco IM, Nogueira JS, Maeda AY, Vasami FG, Macedo FL, Suzuki A, Rodrigues SG, Cruz AC, Nunes BT, Medeiros DB. 2016. Zika virus in the Americas: early epidemiological and genetic findings. *Science* 352:345–349.