Review

Zika virus infection: a review of available techniques towards early detection

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Abstract

Zika virus belongs to the family *Flaviviridae* as do other viruses like Dengue, West Nile and Yellow Fever. They are arboviruses transmitted by the *Aedes* species of mosquito. Zika virus was first isolated in rhesus monkeys in Uganda in 1947. Human infections of the virus were found between the 1960s and 1980s in Africa, the Americas, Asia and the Pacific. The similarity in clinical presentation in Zika-infected patients compared with Dengue caused infections to be previously misdiagnosed as Dengue infection. The Zika virus pandemic in 2015 created a lot of concern globally because of little information about available techniques, samples as well as no available antiviral and vaccines for treatment and vaccination against infection. In addition, the vectors identified for transmission, *Aedes aegypti* and *Aedes albopictus,* were of great concern due to their ability to survive both temperate and tropical climatic conditions, hence indicating the possible global spread of Zika virus infection. Almost two years after the report of infection in pregnant women in Brazil resulting in microcephalic babies, Zika virus was identified as a public health problem. Thus, a lot of research into early detection and prevention has been conducted to control the spread of the virus. This review paper highlights available information on techniques currently available for diagnosis of infection caused by Zika virus.

Introduction

Zika virus, a once neglected tropical microorganism, has been in existence since 1947 when it was first isolated from the blood of a sentinel rhesus monkey (Macacamulatta) in the Zika forest near Entebbe, Uganda (Dick *et al.* 1952). Human infections caused by this virus were found between the 1960s and 1980s in Africa, the Americas, Asia and the Pacific. The first major outbreak of Zika infection outside Africa was reported in the Yap Islands of Micronesia in 2007 (Lanciotti *et al*. 2008). Fagbami in 1979 published a report of detection of Zika virus infection in 30% of the patient sera in four communities in Oyo state, Nigeria, along with three other Flavivirus, namely Yellow Fever (50%), West Nile (46%), and Wesselsbron (59%) (Fagbami 1979).

In French Polynesia in 2013, a woman with Guillain-Barr syndrome who exhibited influenza-like symptoms was negative for all 4 serotypes of dengue by plaque reduction neutralization test but positive for Zika virus (Oehler *et al*. 2014).

In Yap state, patients were presenting dengue like symptoms with conjunctivitis. Thus, they were initially diagnosed serologically as being infected with dengue virus (Zika virus outbreak, 2007). However, molecular diagnoses by reverse transcription–PCR (RT-PCR) and sequencing confirmed Zika virus infection, based on approximately 90% nucleotide identity with the Zika virus genome.

In Brazil, the first confirmed case of Zika virus was reported in a patient who was initially exhibiting dengue-like symptoms (Zanluca *et al.* 2015). Serological and molecular analysis of patient samples were negative for Dengue and Chikungunya, pathogenic re-emerging arboviruses (Lanciotti *et al.* 2008), but were positive for Zika by RT-PCR after detecting a 364bp amplicon expected only for Zika (Zanluca *et al.* 2015). However, in 2015 the world's attention was drawn to Brazil, when there was an increase in the number of microcephaic babies being born by pregnant women (Victora 2016). The number was initially below 200 prior to 2015 (Brazil Ministry of Health 2016). However, this figure increased to 4783 towards the later part of 2015 and the earlier part of 2016 (Brazil Ministry of Health, 2016).

Classification

Zika virus belongs to the family *Flaviviridae* which also includes other viruses such as dengue (DENV), Yellow Fever and West Nile viruses. All of these are of public health importance (Pierson & Diamond 2013, Faye *et al*. 2014). This virus has a single-stranded RNA genome with a positive sense polarity (Faye *et al*. 2014) and falls in group four according to the Baltimore system of classifying viruses.

The Zika viral genome is composed of 10,794 nucleotides which code for 3,419 amino acids (Kuno *et al.* 2007). The Zika virus genome, which acts as mRNA upon reaching the cytoplasm, is immediately translated into a polyprotein which is cleaved into 3 structural proteins; capsid (C), precursor membrane (prM), envelope (E) and 7 non-structural proteins (Kuno *et al.* 2007).

Flavivirus are known to replicate near the endoplasmic reticulum of the infected cell. However, some studies have reported Zika viral antigens in the nucleus of an infected cell, which requires further investigation (Buckley *et al*. 1988). Zika virus was classified into African and Asian lineages by phylogenetic analysis (Gatherer 2016). The Asian lineage originated during the virus's migration from Africa to Southeast Asia, where it was first detected in Malaysia. From there, Zika virus spread to the Pacific Islands, separately to Yap and French Polynesia, and then to New Caledonia, Cook Islands, Easter Island, and the Americas (Gatherer 2016).

Transmission

The vector that has been globally identified to transmit Zika virus is the *Aedes* species of mosquito. They are able to survive both tropical and temperate climatic conditions (Ledermann *et al.* 2014, Kraemer *et al.* 2015, Gaffigan *et al.* 2016). *Aedes aegypti* and *Aedes albopictus* have a wider global coverage as they are able to survive both climatic conditions. However, some species, namely *Aedes luteocephalus* in Africa and *Aedes hensilli* in the Pacific Islands, have limited global distribution. The *Aedes aegypti* is the main species of mosquito for transmission of Zika virus as its feeding habit of biting multiple hosts to complete its blood meals tends to spread the virus in the process. The mosquito is infected during a blood meal from an infected host which could be humans or animals after which the virus replicates within the vector and is later transmitted to another host which could be man during its next blood meal (Ioos *et al.* 2014). This species of mosquito is also known to transmit Dengue virus (Thomas *et al*. 2016). *Aedes albopictus* has not been implicated in most cases of Zika infections. However, in 2007, it was implicated in an outbreak of Zika infection in Gabon. Also, *Aedes albopictus* is endemic in the U.S.A. and the ability of the virus to survive in any *Aedes* species of mosquito indicates the *Aedes albopictus* vectoral role of Zika virus in the United States (Fauci *et al.* 2016).

Beside vector transmission, various research groups have shown evidence of other modes of transmission of the Zika virus. Transplacental transmission has been confirmed and is currently a global concern as infected babies are born with microcephaly (Schuler-Faccini *et al*. 2016). Sexual transmission of Zika virus has also been reported (Musso *et al.* 2015). Thus, some countries, like the U.S.A, have recommended couples who have visited endemic areas to delay child bearing, and advised couples to use condoms to reduce the spread of the virus. In an outbreak in French Polynesia, Zika virus was isolated from the semen of a patient diagnosed with hematospermia (Musso *et al.* 2015). In addition, a case of sexual transmission of Zika virus was reported in France in a woman who acquired Zika sexually from her partner who had recently visited Brazil (Elgot *et al*. 2016). CDC also is in the process of investigating 14 potential sexually acquired Zika virus cases (Duhaime-Ross 2016).

Zika virus has also been identified as one of the possible transfusion transmissible infections in addition to others like HIV and hepatitis B (Musso *et al*. 2014). The virus was detected by PCR in 42 (3%) of the 1505 blood samples donated from asymptomatic donors in French Polynesia (Musso *et al*. 2014). This finding suggested the Zika virus being a transfusion transmissible virus, which should be screened for before transfusing patients (Musso *et al*. 2014). Another report detected Zika virus serologically in blood donors in a Zika endemic area (Aubry *et al*. 2015).

Clinical manifestation

Concerning symptoms, Zika infection mimics that of Dengue (Ioos *et al.* 2014). Thus, Zika was previously misdiagnosed in patients as Dengue infection (Oehler *et al*. 2014). The period between infection and onset of clinical symptoms is approximately 3 to 12 days (Anna *et al.* 2016). However, infection is asymptomatic in approximately 80% of cases (Duffy *et al.* 2009*,* Ioos *et al.* 2014). The clinical symptoms manifest as a self-limited febrile syndrome associated with rash, conjunctivitis and arthralgias (Simpson 1964, Musso *et al*. 2016, Petersen *et al*. 2016). Rash, a prominent feature, is maculopapular and pruritic in most cases; it begins proximally and spreads to the extremities with spontaneous resolution within 1–4 days of onset (Simpson, 1964) while fever is typically low grade (37.4°C – 38.0°C) (Dupont-Rouzeyrol *et al*. 2014, Musso *et al*. 2015, Simpson 1964).

Pathogenesis

Currently, two diseases that have been reported to be associated with Zika virus infection are microcephaly

and Guillain–Barré syndrome (Cao-Lormeau *et al*. 2016, Oliveira *et al*. 2016). Microcephaly is a condition that results from the virus attacking the neural progenitor cells, disrupting their development and thereby affecting the formation of the fetal brain which affects the baby's development (Brasil *et al*. 2016, Marrs *et al*. 2016, Dang *et al*. 2016, Garcez *et al.* 2016, Qian *et al*. 2016, Tang *et al*. 2016). Neural progenitor cells (NPC) are self-renewing neural stem cells in the brain which differentiate into neurons, astrocytes, and oligodendrocytes (Delvecchio *et al*. 2016). Several research findings have established the link between Zika virus and microcephaly such as detection of viral RNA in the amniotic fluid of Zika infected pregnant women as well as in brain tissues from microcephalic babies (Brasil *et al*. 2016, Calvet *et al*. 2016, Driggers *et al*. 2016, Mlakar *et al*. 2016, Melo *et al*. 2016). *In vivo* experiments where pregnant mice were infected with Zika led to neuronal death, cell cycle arrest and apoptosis of NPCs leading to embryonic microcephaly and growth restriction (Cugola *et al*. 2016, Li *et al*. 2016, Miner *et al*. 2016, Shao *et al*. 2016).

Guillain-Barre syndrome is a rare but serious autoimmune disorder in which the immune system attacks healthy nerve cells in the peripheral nervous system. This leads to weakness, numbness, and tingling. It can eventually cause paralysis. Aetologic agents that were previous associated with its incidence were *Campylobacter jejuni*, influenza, *Epstein-Barr virus, Cytomegalovirus* and *HIV*. Recently, clusters of the Guillain–Barré syndrome and microcephaly have been spatially and temporally related to the current outbreak of Zika infection in the Americas (Beatriz *et al.* 2016, World Health Organization 2016). The World Health Organization on Feb 1 2016, declared Zika associated microcephaly and Guillain-Barre syndrome as a Public Health Emergency of International Concern (PHEIC), not based on the current information of Zika associated cases but on what is not known of clusters of microcephaly, Guillain-Barré syndrome, and possibly other neurological defects reported by country representatives from Brazil and French Polynesia (Heymann *et al*. 2016, Oehler *et al*. 2014, Pan American Health Organization 2016).

Laboratory diagnosis

Clinical presentation of patients infected with Zika is not reliable for preliminary diagnoses due to its similarity with other flaviviruses like Dengue. Thus, laboratory diagnosis is used to confirm Zika infection. Detection is based on the type of patient sample being analyzed. Samples that are currently used for detection of Zika infection in patients are blood or serum. Viremia is detectable between 3 to 5 days post infection. Other samples with Zika diagnostic potential are cerebrospinal fluid (CSF), urine, saliva, amniotic fluid, semen and fetal brain tissue (Plourde 2016). Urine and saliva which are noninvasive samples offer a better alternative for diagnosis as compared to blood and cerebrospinal fluid. Also, in most cases, collection of urine and saliva does not need any technical expertise as compared to blood and CSF which is collected by trained health professional in a designated health facility. Urine samples are currently recommended for diagnosis of Zika virus infection as viral RNA persists longer in urine as compared to blood (Plourde 2016, Gourinat *et al*. 2014, Besnard *et al*. 2014). One study reported that Zika virus RNA was detected in urine up to 20 days post infection after viral RNA was undetectable in the blood (Plourde 2016). Thus a patient urine sample must also be tested to confirm diagnosis when a negative blood test is reported for a Zika suspected patient. Detection of Zika viral RNA in saliva is best during the acute phase of the infection but is not ideal during the late stage. Musso *et al*. (2015) screened 182 Zika suspected patient's saliva and urine samples. Zika viral RNA was positive for 35 (19.2%) of their saliva while negative in their blood. On the other hand, 16 (8.8%) of the patients tested positive for Zika viral RNA in their blood but were negative in their saliva. This indicates that blood samples should also be screened in cases where saliva is used for Zika diagnosis.

Techniques for diagnosis

Serology and molecular diagnoses are currently the techniques being used for confirming Zika infection in patients. Detection of Zika virus in the blood or serum by molecular techniques, namely RT-PCR, is best during the acute stage of the infection when the patient is viremic (Lanciotti *et al*. 2008). However, serology cannot be used as Zika virus IgM may be undetectable during the acute phase of the infection (Hayes 2009). Serologic testing has a major limitation. Cross-reactivity with other flaviviruses (particularly Dengue), limits specificity (Beatriz *et al.* 2016). Therefore, positive serologic test results should be confirmed with molecular testing. The plaque reduction neutralization assay, a seroneutralization assay, generally has improved specificity over serology, but may still yield cross-reactive results in secondary Flavivirus infections (Hayes 2009, Anna *et al*. 2016).

Currently according to the CDC, symptomatic Zika suspected infected patients are diagnosed by RNA NAT (nucleic acid testing). Serum samples are tested two weeks after the onset of dengue-like symptoms. Urine samples are also tested before the 14^{th} day after the onset of symptoms. A positive result is confirmatory of Zika infection. However, a negative result does not exclude infection. Additional serum IgM testing must be done to rule out infection (Diagnostic Tests for Zika Virus, 2017).

In the case of pregnant women who fall under the high risk group, RNA nucleic acid testing must be

done on both serum and urine two weeks after any visit to a Zika endemic region. In addition, RNA testing must be done for those who are IgM positive for Zika after exposure. Pregnant women are routinely screened for Zika as part of their antenatal care in areas where Zika is endemic, especially during the 1st and 2nd trimester of pregnancy as most of the developmental processes of the growing fetus takes place at this stage (Diagnostic Tests for Zika Virus 2017).

The Trioplex Real-time RT-PCR Assay (Trioplex rRT-PCR) is a laboratory test designed to detect Zika virus, Dengue virus, and Chikungunya virus RNA (Centers for Disease Control and Prevention, 2016). This method has not yet received approval for Zika diagnosis by the Food and Drug Administration (FDA) except on Emergency Use Authorization (EUA) according to the CDC. The Trioplex rRT-PCR qualitatively detects and differentiates Zika from other Flavivirus in human sera, whole blood (EDTA), cerebrospinal fluid, urine and amniotic fluid. According to the CDC, patient samples analyzed by this assay must meet certain criteria; namely clinical signs and symptoms associated with Zika virus infection, history of residence in or travel to a geographic region with active Zika transmission at the time of travel, or other epidemiologic criteria for which Zika virus testing may be indicated as part of a public health investigation. A negative result must be combined with clinical observations, patient history, and epidemiological information (Centers for Disease Control and Prevention, 2016).

However, this assay can give both false positive and false negative results. False positive results can arise from contamination from previous positive samples. However, inclusion of negative controls helps detect any possible contamination of patient samples, thereby reducing their incidence. False negative results occur as a result of the method of sample collection which affects the quality of the sample analyzed, degradation of the viral RNA during sample transportation, the time of sample collection and analysis when viral RNA might no longer be detectable in some samples after the onset of symptoms (approximately 14 days post-onset of symptoms for serum, whole blood, and/or urine), failure to follow the authorized assay procedures and failure to use authorized extraction kits and platforms (Centers for Disease Control and Prevention CDC 2016, Plourde 2016, Musso *et al*. 2015). Addition of controls helps to assess the quality of samples as well as adhering to CDC guidelines on the use of assays helps reduce false negative and false positive results.

Proteomics, a tool that utilizes genetic signatures, is currently also being explored by many research groups for early diagnosis of diseases, vaccine and antiviral production. This molecular based technique has led to early identification and quantification of functionally active biomarkers at specific time points during disease progression. The technique can be used to detect the disease process caused by the virus early after infection and to prevent the pathology observed after infection. Also, the high sensitivity and specificity of this technique reduces false positive and negative results. Garcez *et al*. (2016) used combined proteomics to analyze human neurospheres derived from neural stem cells exposed to Zika virus (ZIKV) isolated in Brazil and identified 500 genes and proteins altered after viral infection, which contribute to the development of microcephaly in infected babies. Amongst the proteins detected, 199 were downregulated and 259 were upregulated when mock and Zika infected neurosphere protein expression profiles were compared (Garcez *et al*. 2016). Although biomarkers can help for the early diagnosis of a disease, they must be adequately validated over a period of time before being used for confirmation of a disease process.

The Zika outbreak in Brazil raised a lot of concern globally as there was little information about methods for diagnosing Zika as well as vaccine and antivirals for prevention. Currently, however, samples for detection of Zika infection as well as methods for diagnosing infection have been identified, thereby improving management of infected patients. In addition, two DNA ZIKV vaccine candidates have entered phase 1 human safety testing (ClinicalTrials.gov numbers, NCT01099852 and NCT0284048) (Marston *et al.* 2016). Thus, getting an effective vaccine for immunological protection is closer than expected. An effective antiviral is also being researched currently at the National Institutes of Health (NIH); compounds having antiviral properties are being screened to determine their antiviral effect on Zika (Awasthi 2016).

Conflict of interest

Authors declare that there is no conflict of interest in relation to this review paper.

Authors Contribution

Authors made equal contributions in writing up this review paper.

References

Aubry M, Finke J, Teissier A, Roche C, Broult J, Paulous S, Desprès P, Cao-Lormeau VM & Musso D 2015 Seroprevalence of arboviruses among blood donors in French Polynesia, 2011–2013. *Int J Infect Dis* **41** 11-12

Awasthi S 2016 Zika Virus: Prospects for the Development of Vaccine and Antiviral Agents. *Journal of Antivirals and Antiretrovirals* **8** 8–10

Parra B, Lizarazo J, Jiménez-Arango JA, Zea-Vera AF, González-Manrique G, Vargas J, Angarita JA,

Zuñiga G, Lopez-Gonzalez R, Beltran CL, Rizcala KH, Morales MT, Pacheco O, Ospina ML, Kumar A, Cornblath DR, Muñoz LS, Osorio L, Barreras P, Pardo CA 2016 Guillain-Barré Syndrome Associated with Zika Virus Infection in Colombia. *N Engl J Med* **375** 1513-1523

Besnard M., Lastère S, Teissier A, Cao-Lormeau VM, Musso D, 2014. Evidence of perinatal transmission of Zika virus, French Polynesia December 2013 and January 2014, Euro Surveill. 19, pii:20751

Brasil P, Pereira JP Jr, Moreira ME, Ribeiro Nogueira RM, Damasceno L, Wakimoto M, Rabello RS, Valderramos SG, Halai UA, Salles TS, Zin AA, Horovitz D, Daltro P, Boechat M, Raja Gabaglia C, Carvalho de Sequeira P, Pilotto JH, Medialdea-Carrera R, Cotrim da Cunha D, Abreu de Carvalho LM, Pone M, Machado Siqueira A, Calvet GA, Rodrigues Baião AE, Neves ES, Nassar de Carvalho PR, Hasue RH, Marschik PB, Einspieler C, Janzen C, Cherry JD, Bispo de Filippis AM, Nielsen-Saines K 2016 Zika Virus Infection in Pregnant Women in Rio de Janeiro. *N Engl J Med* **375** 2321-2334

Brazil Ministry of Health. Emergency Health Operations Center On Microcephaly. Monitoring of Microcephaly Cases in the Brazil. Epidemiological report 11-week epidemiological study 04/2016 (24 a1/30/2016). Brasília: Ministry of Health, 2016.

Brazil Ministry of Health. DATASUS. Anomaly or fault tab Congenital in live births. http:// www2.aids.gov.br/cgi/tabcgi.exe?caumul/anoma.def (accessed Feb 4, 2016).

Buckley A, Gould EA 1988 Detection of virus-specific antigen in the nuclei or nucleoli of cells infected with Zika or Langat virus. *J Gen Virol* **69** 1913-1920

Cao-Lormeau VM, Blake A, Mons S, Lastere S, Roche C, Vanhomwegen J, Dub T, Baudouin L, Teissier A, Larre P, Vial AL, Decam C, Choumet V, Halstead SK, Willison HJ, Musset L, Manuguerra JC, Despres P, Fournier E, Mallet HP, Musso D, Fontanet A, Neil J, Ghawché F 2016 Guillain-Barr Syndrome outbreak associated with Zika virus infection in French Polynesia: a case control study. *Lancet* **387** 1531-1539

Centers for Disease Control and Prevention (CDC), 2016. Trioplex Real-time RT-PCR Assay. Retrieved from https://www.cdc.gov/zika/pdfs/trioplex-real-time-rt-pcr-assay-instructions-for-use.pdf

Cugola FR, Fernandes IR, Russo FB, Freitas BC, Dias JL, Guimarães KP, Benazzato C, Almeida N, Pignatari GC, Romero S, Polonio CM, Cunha I, Freitas CL, Brandão WN, Rossato C, Andrade DG, Faria Dde P, Garcez AT, Buchpigel CA, Braconi CT, Mendes E, Sall AA, Zanotto PM, Peron JP, Muotri AR, Beltrão-Braga PC 2016 The Brazilian Zika virus strain causes birth defects in experimental models. *Nature* **534** 267-271

Elgot J 2016. France records first sexually transmitted case of Zika in Europe. The guardian 2016. [Online] Available from: http://www.theguardian.com/ world/2016/feb/27/zika-france-records-firstsexually-transmitted-case-europe [Accessed on 28 February 2016].

Fauci AS, Morens DM 2016. Zika virus in the Americas - yet another arbovirus threat. *N Engl J Med* **374** 601-604

Delvecchio R, Higa LM, Pezzuto P, Valadão AL, Garcez PP, Monteiro FL, Loiola EC, Dias AA, Silva FJ, Aliota MT, Caine EA, Osorio JE, Bellio M, O'Connor DH, Rehen S, de Aguiar RS, Savarino A, Campanati L, Tanuri A 2016 Chloroquine, an Endocytosis Blocking Agent, Inhibits Zika Virus Infection in Different Cell Models. *Viruses* **8** pii: E322

Diagnostic Tests for Zika Virus. Centre for Disease Control and Prevention. Accessed February 21, 2017.

Dick GW, Kitchen S, Haddow A 1952 Zika virus. Isolations and serological specificity. *Trans R Soc Trop Med Hyg* **46** 509–520

Driggers RW, Ho CY, Korhonen EM, Kuivanen S, Jääskeläinen AJ, Smura T, Rosenberg A, Hill DA, DeBiasi RL, Vezina G, Timofeev J, Rodriguez FJ, Levanov L, Razak J, Iyengar P, Hennenfent A, Kennedy R, Lanciotti R, du Plessis A, Vapalahti O 2016 Zika Virus Infection with Prolonged Maternal Viremia and Fetal Brain Abnormalities. *N Engl J Med* **374** 2142-2151

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

Duhaime-Ross A 2016. Zika linked to more birth defects than just microcephaly. The verge, 2016. [Online] Available from: http:// www.theverge.com/2016/3/8/11181088/zika-birth-defects-fetaldeath-growth-retardation-who [Accessed on 9 March 2016].

Dupont-Rouzeyrol M, O'Connor O, Calvez E, Daurès M, John M, Grangeon JP, Gourinat AC 2015 Co-infection with Zika and dengue viruses in 2 patients, New Caledonia, 2014. *Emerg Infect Dis* **21** 381-382

Fagbami AH 1979 Zika virus infections in Nigeria: virological and seroepidemiological investigations in Oyo State. *J Hyg (Lond)* **83** 213-219

Faye O, Freire CC, Iamarino A, Faye O, Oliveira JVC, Diallo M, Zanotto PMA, Sall AA 2014 Molecular evolution of Zika virus during its emergence in the 20th century. *PLOS Negl Trop Dis* **8** e2636

Gaffigan TVWR, Pecor JE, Stoffer JA, Anderson T 2016 Systematic catalog of culicidae. Suitland (MD): Smithsonian Institution; Silver Spring (MD): Walter Reed Army Institute of Research;. [cited 2016 Feb 10]. http://mosquitocatalog.org.

Garcez PP, Nascimento JM, de Vasconcelos JM, da Costa RM, Delvecchio R, Trindade P, Loiola EC, Higa LM, Cassoli JS, Vitória G, Sequeira P, Sochacki J, Aguiar RS, Fuzii HT, Bispo de Filippis AM, JLS Gonçalves Vianez Júnior, Martins-de-Souza D, Tanuri

A, Rehen SK 2016 Combined proteome and transcriptome analyses reveal that Zika virus circulating in Brazil alters cell cycle and neurogenic programmes in human neurospheres. *PeerJ Preprints* **4** e2033v1

Garcez PP, Loiola EC, Madeiro da Costa R, Higa LM, Trindade P, Delvecchio R, Nascimento JM, Brindeiro R, Tanuri A, Rehen SK 2016 Zika virus impairs growth in human neurospheres and brain organoids. *Science* **352** 816-818

Gatherer D, Kohl A 2016 Zika virus: a previously slow pandemic spreads rapidly through the Americas. *J Gen Virol* **97** 269–273

Gourinat AC, O'Connor O, Calvez E, Goarant C, Dupont-Rouzeyrol M 2015 Detection of Zika virus in urine. *Emerg Infect Dis* **21** 84-86

Hayes EB 2009 Zika virus outside Africa. *Emerg Infect Dis* **15** 1347-1350

Heymann DL, Hodgson A, Sall AA, Freedman DO, Staples JE, Althabe F, Baruah K, Mahmud G, Kandun N, Vasconcelos PF, Bino S, Menon KU 2016 Zika virus and microcephaly: Why is this situation a PHEIC? *Lancet* **387** 719-721

Ioos S, Mallet HP, Leparc Goffart I, Gauthier V, Cardoso T, Herida M 2014 Current Zika virus epidemiology and recent epidemics. *Med Mal Infect* **44** 302-307

Kraemer MU, Sinka ME, Duda KA, Mylne A, Shearer FM, Brady OJ, Messina JP, Barker CM, Moore CG, Carvalho RG, Coelho GE, Van Bortel W, Hendrickx G, Schaffner F, Wint GR, Elyazar IR, Teng HJ, Hay SI 2015 The global compendium of Aedes aegypti and Ae. albopictus occurrence. *Sci Data* **2** 150035

Kuno G, Chang GJ 2007 Full-length sequencing and genomic characterization of Bagaza, Kedougou, and Zika viruses. *Arch Virol* **152** 687-696

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

Ledermann JP, Guillaumot L, Yug L, Saweyog SC, Tided M, Machieng P, Pretrick M, Marfel M, Griggs A, Bel M, Duffy MR, Hancock WT, Ho-Chen T, Powers AM 2014 Aedes hensilli as a potential vector of Chikungunya and Zika viruses. *PLoS Negl Trop Dis* **8** e3188

Li C, Xu D, Ye Q, Hong S, Jiang Y, Liu X, Zhang N, Shi L, Qin CF, Xu Z 2016 Zika Virus Disrupts Neural Progenitor Development and Leads to Microcephaly in Mice. *Cell Stem Cell* **19** 120-126

Marrs C, Olson G, Saade G, Hankins G, Wen T, Patel J, Weaver S 2016 Zika Virus and Pregnancy: A Review of the Literature and Clinical Considerations. *Am J Perinatol* **33** 625-639

Marston HD, Lurie N, Borio LL, Fauci AS 2016 Considerations for Developing a Zika Virus Vaccine. *N Engl J Med* **375** 1209-1212

Victora CG, Schuler-Faccini L, Matijasevich A, Ribeiro E, Pessoa A, Barros FC 2016 Microcephaly in Brazil: how to interpret reported numbers? *Lancet* **387** 621-624

Miner JJ, Cao B, Govero J, Smith AM, Fernandez E, Cabrera OH, Garber C, Noll M, Klein RS, Noguchi KK, Mysorekar IU, Diamond MS 2016 Zika Virus Infection during Pregnancy in Mice Causes Placental Damage and Fetal Demise. *Cell* **165** 1081-1091

Mlakar J, Korva M, Tul N, Popović M, Poljšak-Prijatelj M, Mraz J, Kolenc M, Resman Rus K, Vesnaver Vipotnik T, Fabjan Vodušek V, Vizjak A, Pižem J, Petrovec M, Avšič Županc T 2016 Zika Virus Associated with Microcephaly. *N Engl J Med* **374** 951-958

Musso D, Gubler DJ 2016. Zika virus. *Clin Microbiol Rev* **29** 487-524

Musso D, Roche C, Nhan TX, Robin E, Teissier A, Cao-Lormeau VM 2015 Detection of Zika virus in saliva. *J Clin Virol* **68** 53-55

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

Musso D, Nhan T, Robin E, Roche C, Bierlaire D, Zisou K, Shan Yan A, Cao-Lormeau VM, 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** pii: 20761

Oehler E, Watrin L, Larre P, Leparc-Goffart I, Lastere S, Valour F, Baudouin L, Mallet H, Musso D, Ghawche F 2014 Zika virus infection complicated by Guillain-Barre syndrome--case report, French Polynesia, December 2013. *Euro Surveill* **19** pii: 20720

Oliveira Melo AS, Malinger G, Ximenes R, Szejnfeld PO, Alves Sampaio S, Bispo de Filippis AM 2016 Zika virus intrauterine infection causes fetal brain abnormality and microcephaly: tip of the iceberg? *Ultrasound Obstet Gynecol* **47** 6e7

Pan American Health Organization. Epidemiological update: neurological syndrome, congenital anomalies, and Zika virus infection. Jan 17, 2016. http:// www.paho.org/hq/index.php? option=com_content&view=article&id=11599&Itemid =41691 (accessed Feb 9, 2016).

Petersen LR, Jamieson DJ, Powers AM, Honein MA 2016 Zika virus *N Engl J Med* **374** 1552-1563

Pierson TC, Diamond MS 2013 Flaviviruses. In DM Knipe, PM Howley (eds.), *Fields virology*, 6th ed., Lippincott Williams and Wilkins, Philadelphia, p. 747-794

Plourde AR, Bloch EM 2016 A Literature Review of Zika Virus. *Emerg Infect Dis* **22** 1185-1192

Shao Q, Herrlinger S, Yang SL, Lai F, Moore JM, Brindley MA, Chen JF 2016 Zika virus infection disrupts neurovascular development and results in postnatal microcephaly with brain damage. *Development* **143** 4127-4136

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, Serao 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

Simpson DI 1964 Zika virus infection in man. *Trans R Soc Trop Med Hyg* **58** 335-338

Thomas DL, Sharp TM, Torres J, Armstrong PA, Munoz-Jordan J, Ryff KR, Martinez-Quiñones A, Arias-Berríos J, Mayshack M, Garayalde GJ, Saavedra S, Luciano CA, Valencia-Prado M, Waterman S, Rivera-García B 2016 Local transmission of Zika virus – Puerto Rico, November 23, 2015–January 28, 2016. *MMWR Morb Mortal Wkly Rep* **65** 154-158

World Health Organization, 2016 (http://apps .who.int/ iris/bitstream/ 10665/204526/ 2/ zikasitrep_4Mar2016_eng.pdf) http://doi.org/10.1056/ NEJMp1607762

Zanluca C, De Melo VCA, Mosimann ALP, Dos Santos GIV, dos Santos CND, Luz K 2015 First report of autochthonous transmission of Zika virus in Brazil. *Memorias Do Instituto Oswaldo Cruz* **110** 569–572

Zika virus outbreak. Micronesia (Yap). Suspected. ProMed June 27, 2007 [cited 2008 May 8]. Available from http://www.promed.mail.org, archive no.: 20070627.2065.