Title
Zika Virus: Critical Information for Emergency Providers

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Zika Virus: Critical Information for Emergency Providers

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KEY POINTS:

- Zika virus is a mosquito borne arbovirus
- The vast majority of individuals infected with Zika virus have minimal or no symptoms
- If present, typical symptoms include rash, conjunctivitis and fever
- Pregnant women infected with Zika, particularly during the first trimester, appear to be at increased risk of having infants with congenital abnormalities such as microcephaly
- There is currently no vaccine or treatment for Zika virus. Prevention through minimizing mosquito bites is the best means of decreasing risk of infection.

Synopsis

Zika virus is an arbovirus of the *Flaviviridae* family. It is primarily a minimally symptomatic mosquito-borne infection. However, with Zika's 2015-2016 introduction into the Western Hemisphere and its dramatic and rapid spread, it has become a significant public health concern, in large part due to congenital abnormalities associated with infection in pregnant women. In early 2016, the World Health Organization declared the microcephaly and other neurological conditions temporally associated with Zika virus infection a public health emergency of international concern. This paper discusses the current epidemiological and clinical understanding of Zika virus, focusing on critical information needed by emergency providers.

Introduction

Zika virus' recent introduction into the Western Hemisphere and its dramatic and rapid spread during 2015-2016 represents a global public health challenge. Associations between Zika virus with congenital anomalies and Guillain Barre Syndrome (GBS) underscore the importance of understanding strategies for management and control of the virus. There is significant concern for risk to pregnant women/women of reproductive age and the risks associated with the spreading disease and with travel to endemic areas. Preparations for the 2016 Olympic Games in Brazil cast an increased sense of urgency on the need for improved assessment/identification, management and containment of Zika virus. Given the potential for increased numbers of infected individuals, it is essential that emergency providers equip themselves with the knowledge and background necessary to effectively assess, manage and counsel patients. This article provides background and epidemiologic information on Zika virus followed by a discussion of diagnostic, treatment and management strategies.

History

Origins

The Zika Virus was first identified in 1947 at the Yellow Fever Research Laboratory in the Zika Forest area of Uganda.[1,2] During this time period, British scientists placed Asian rhesus monkeys in Africa. Blood samples from one sentinel rhesus monkey were injected into mice. These mice subsequently became ill. Several viruses were identified in the brain

tissue of the ill mice; one of the isolated viruses was the Zika virus. Zika virus was also isolated from mosquitos in the region.[2]

Epidemiology

From 1947-2007, there were limited and infrequent documented human cases of Zika virus infection.[3, 4] In 2007, scientists reported the first outbreak of Zika virus on the island of Yap within the Federated States of Micronesia. There were 49 confirmed and 59 probable cases documented over a 4-month period.[5, 6] Public health officials recorded a subsequent outbreak in 2013 in French Polynesia. There were 294 cases, confirmed by RNA assay, recorded over a 10-week period.[7, 8]

The first documented case of the Zika virus in the Americas was in 2014 with locally acquired cases in Easter Island.[9] Subsequently, in May 2015, initial cases were confirmed in Northeast Brazil.[10, 11, 12] From May 2015 until early 2016, Brazilian officials estimated that there were 1.5 million cases of Zika virus infections throughout the country.[11] Colombia also reported more than 25,000 cases from October 2015 through the middle of February 2016.[13] Given that infection is often subclinical with many affected patients not seeking medical care, officials advise that cases are likely underreported.

As of March 2016, there have been no documented cases of locally acquired Zika virus infection in the continental United States. However, there have been 153 travel-associated

cases within 28 states and the District of Columbia. Within the U.S. territories, to that date, there was 1 documented travel associated case and 107 locally acquired cases.[14]

Virology

Classification and Structure

Zika virus is an arbovirus within the genus Flaviviridae and family Flavivirus.[1] It is a single stranded RNA virus with Asian and African lineage.[6, 15, 16] Other flaviviruses include Yellow Fever, Dengue Fever and West Nile virus.[1] Within the African lineage, the life cycle of the virus is between non-human primates and mosquitoes. Humans are occasional, unintentional hosts within the African life cycle.[17, 18] Outside of Africa, humans are the primary host for infection.[5]

Transmission

The primary transmission vector is the *Aedas aegypti* mosquito. Laboratory testing has shown the possibility of transmission via the *Aedes albopticus* mosquito as well.[19, 20, 21, 22, 23]

Sexual transmission of the Zika virus has been reported.[24, 25, 26, 27] There has been at least one documented case of male to female sexual transmission of the virus as well as one probable case of male to female sexual transmission.[25, 26] In a third case, semen isolated from an infected male was shown to test positive for Zika virus serologic testing for 2-10 weeks

after initial symptom onset.[27] In all of these cases, the male patient was symptomatic. It is unclear whether sexual transmission can occur from an asymptomatic male. As of March 2016, there have been no documented cases of female to male transmission.[28] In April 2016, the CDC published a report of male-to-male sexual transmission of Zika virus. [29]

Concern has also emerged for Zika virus transmission via infected blood products.[30, 31] One 2014 study showed that 42 out of 1505 blood donors in the French Polynesian outbreak of 2013-2014 tested positive for Zika virus via nucleic acid testing. All blood donors were asymptomatic at the time of blood donation.[30] This has prompted new U.S. Food and Drug Administration (FDA) recommendations for blood and organ donation.[31]

As of March 2016, for blood donors in areas without active Zika virus transmission, the FDA suggests that donation should be deferred for 4 weeks in donors who are at risk for Zika virus infection. Donors are considered to be at risk if they either have had symptoms of Zika virus infection in the past 4 weeks, have had sexual contact with persons who either traveled to or resided in an area with active Zika virus transmission within the past 3 months, or if they have travelled to areas with active transmission of the Zika virus during the past 4 weeks. For areas with active Zika virus transmission, the FDA recommends that blood/blood products should be obtained from areas of the U.S. without active transmission of the Zika virus. The FDA is developing screening tests for blood.[31]

Evolving FDA recommendations for organ donation state that living donors should be considered ineligible if they were diagnosed with Zika virus infection, were in an area with

active Zika virus transmission, or had sex with a male with either of these two risk factors (travel to areas with active transmission or symptoms of Zika virus infection) within the past six months. Further, donors of umbilical cord blood, placenta or other gestational tissues should be considered ineligible if they had any of these risk factors (travel to area of active transmission, sexual contact with an at risk male or symptoms of Zika virus infection) during their pregnancy. Finally, deceased donors should be considered ineligible if they were diagnosed with Zika infection within 6 months prior to time of donation.[32]

Early studies noted a temporal association between incidence of Zika virus infection and incidence of congenital microcephaly/congenital defects. This led to concerns of possible transplacental/perinatal transmission of the Zika virus. Subsequent studies have identified Zika virus genetic material in amniotic fluid samples of fetuses with microcephaly whose mothers had symptoms suggestive of Zika virus infection. Other studies have shown RT-PCR confirmed Zika virus infection in both mothers and fetuses within four days of delivery. These data raised the possibility of transplacental transmission of the virus.[13, 32, 34, 35] A subsequent March 2016 study examined the proposed molecular basis for the link between congenital microcephaly and Zika virus. The study group identified that *in vitro,* Zika virus infects human neural progenitor cells, which subsequently leads to attenuation of cell growth.[36]

Clinical Presentation

Initial Signs and Symptoms

The virus has an incubation period of 3-12 days. This is followed by a subclinical or mild illness.[5, 37, 38, 39] Symptoms, when present, typically last for 2-7 days. Initial symptoms are generally mild and typically self-limited.[13] Typical symptoms of Zika infection are listed in Box 1.

Box 1: Typical Zika Symptoms

- Fever
- Conjunctivitis
- Arthralgia/myalgia
- diffuse rash
- Headache
- Retro-orbital pain
- Peripheral edema
- Gastrointestinal upset

Acute Sequela

Severe acute infections with Zika virus are rare. There have been fewer than 10 adult deaths caused by the Zika virus reported as of early 2016.[13, 40] However, investigators have detected an association with the serious sequelae of GBS.[41] A February 2016 *Lancet* case-control study reported that 41 patients in the case group (98%) had anti-Zika virus IgM or IgG compared with 54 patients (56%) in the control group ($p < 0.0001$). This study supported an association between Zika virus and GBS. In total, there was an estimated incidence of 0.24 per 1000 cases of GBS in the French Polynesian outbreak (compared with incidence of 0.25-0.65 per 1000 of GBS with C jejuni infection). [41] Authorities have attributed 3 deaths to GBS associated with Zika virus infection.[13]

Congenital Complications

As the incidence of Zika virus infection rose in Northeast Brazil, there was a simultaneous increase in the incidence of congenital microcephaly and other fetal complications. Subsequent studies established a possible link between Zika virus infection and congenital complications.[42, 43, 44] Up until February 2016, there had been no documented cases of microcephaly in Colombia despite over 30,000 cases of Zika virus infection in the country.[13] However, as of March 4, 2016, 3 infants who tested positive for Zika virus were diagnosed with microcephaly and other congenital anomalies. Colombian public health experts expect to see a further rise in congenital abnormalities related to Zika virus in Colombia.[45] A *New England Journal of Medicine* study published in February 2016 studied 88 women in Brazil from September 2015 to February 2016. This study identified fetal abnormalities in 29% of women who tested positive for Zika virus and in 0% of women who tested negative for Zika virus. Fetal abnormalities that were identified included: 2 cases of fetal death, 5 cases of in utero growth restrictions with or without microcephaly, 7 cases of ventricular calcifications or other central nervous system lesions and 7 cases of abnormal amniotic fluid volume or cerebral/umbilical artery flow.[44] In April 2016, the CDC concluded that Zika virus is a cause of microcephaly and other fetal anomalies.[46]

Testing and Diagnosis

Diagnostic Criteria

As of June 2016, the Pan American Health Organization (PAHO) provisional case definition of suspected acute Zika virus infection is[47] a rash that is typically pruritic and

maculopapular (Fig. 1) with two or more of the following accompanying signs/ symptoms:

- Arthralgia/myalgia
- Nonpurulent conjunctivitis
- Conjunctival hyperemia
- Fever (usually <38.5_C)
- Peri-articular edema

The signs and symptoms of Zika virus infection are non-specific, thus there is a broad differential diagnosis. The differential diagnosis includes Dengue fever, chikungunya virus, initial HIV seroconversion, measles, scarlet fever, rickettsial infection, leptospirosis, parvovirus, enterovirus, rubella and secondary syphilis. Given the non-specific nature of the signs/symptoms and the broad differential diagnosis, clinical presentation alone is insufficient to make the diagnosis without confirmatory laboratory testing.[13]

Laboratory testing

As of March 2016, definitive diagnosis of Zika virus is made by reverse transcriptase-polymerase chain reaction (RT-PCR) testing of blood and saliva, which can only be accomplished with assistance from public health authorities and is not yet available at individual hospitals.[13] Results are generally not accessible in a timely fashion. IgM antibody against the Zika virus can also be detected in serum. Data on the utility of urine and semen RT-PCR are limited. Delayed antibody testing (IgG) is less useful due to the cross reactivity of IgG antibodies to other flaviviruses, such as Dengue. Given that other flaviviruses can be endemic to the same region where there is active Zika virus transmission, false positive results are common.[13, 48, 49, 50, 51]

Infection Control and Public Health

Travel Recommendations/Restrictions

As of March 2016, the U.S. has no documented cases of locally acquired Zika virus infection, but there have been numerous travel-associated cases.[14] The first travel associated case of Zika virus among U.S. travelers was reported in 2007. From 2007-2014, 14 returning U.S. travelers tested positive for Zika virus at the U.S. Centers for Disease Control and Prevention (CDC). From 2015-2016, at least 8 returning U.S. travelers have tested positive for Zika virus per CDC reports.[52] The spread of Zika virus via travel prompted the release of interim CDC travel guidance in January 2016. CDC issued a Level 2 travel alert (practice enhanced precautions) for people traveling to regions where there is ongoing active transmission of Zika virus. This includes travel to: Brazil, El Salvador, French Guiana, Guatemala, Haiti, Honduras, Martinique, Mexico, Panama, Paraguay, Suriname, Venezuela and the Commonwealth of Puerto Rico. See Box 2 for specific recommendations.

Box 2: Recommendations for travelers to endemic areas include: Error: Reference source not found

- Wear long sleeved shirts and pants
- Use approved mosquito repellants
- Stay in lodgings that are screened or air-conditioned
- Safe eating habits
- Sexual education with condom use
    - for 6 months for men with infection
    - for 28 days in asymptomatic male travelers

Vector Control/Health Care Precautions

Vector control strategies include use of insecticides and removal of small stagnant pools of water, which tend to harbor the mosquito vector. In addition to these local control measures, research is ongoing for strategies to achieve mosquito eradication. For example, the World Health Organization (WHO) is exploring the release of sterile irradiated mosquitoes in order to curtail the breeding/propagation of the vector species.[13] Recommendations for healthcare providers encountering a patient potentially infection with Zika include vigorous hand hygiene, standard precautions and safe disposal of sharps/medical waste.[53] There is no indication for contact/droplet/airborne precautions in the healthcare setting. Standard measures to prevent sexual transmission are also advised for at risk populations.

Implications for pregnant women

As of early 2016, The CDC and the European Centre for Disease Prevention recommend that pregnant women and those who plan on becoming pregnant avoid travel to areas with active Zika virus transmission.[54, 55] If travel cannot be avoided, women should consult with their healthcare providers prior to traveling. These women should also take special precautions to avoid mosquito bites including wearing long-sleeved shirts and long pants, use of Environmental Protection Agency registered mosquito repellants, use of permethrin-treated clothing and gear, and staying in lodgings with air conditioning/screening.[56, 57] Women who are not pregnant at the time of travel should be counseled to avoid becoming pregnant within 28 days after return from travel.[58]

Mass Gatherings and International Implications

The 2016 Olympic Games in Rio De Janeiro, Brazil, poses a unique challenge for safety/infection control.[53, 59] In preparation for this mass gathering, WHO recommended that all participating member states maintain awareness of the possibility of Zika virus transmission. Member states were also advised to maintain adequate care facilities/resources to meet potential increased demand for care. States were advised to strengthen antenatal care/resources and improve infection control procedures.[57, 58] Vector control activities were accelerated in Brazil prior to start of the Olympic Games.[53, 59, 60, 61]

WHO drew international attention to the gravity and scope of the Zika virus outbreak on February 1st, 2016 when it declared the microcephaly and other neurological conditions temporally associated with Zika virus a Public Health Emergency of International Concern (PHEIC).[61] A PHEIC is defined as an "…extraordinary event which is determined…to constitute a public health risk to other states through the international spread of disease… and to potentially require a coordinated international response."[62]

Evaluating a Suspected Case

Initial Evaluation/Consideration

Initial evaluation should be guided by the defined criteria for case definition in conjunction with a thorough travel history. A history of sexual contact with at-risk travelers should also be elicited.[13] If Zika virus is suspected, the case should be reported to local and national public health authorities. In the United States and several other countries, Zika virus is a nationally reportable condition. This requirement facilitates appropriate epidemiologic follow-up, case tracking and management.[58, 63]

Serologic testing

Molecular serologic testing should be offered to pregnant women who present with signs and symptoms consistent with Zika virus infection within 2 weeks of travel to an endemic area. Laboratory testing should also be offered to asymptomatic pregnant women with a history of travel to areas with active Zika transmission. Initial test result interpretation can be complex due to possible cross reactivity with other flaviviruses, which can lead to false positive results. However, a negative IgM result at 2-12 weeks after initial travel can more definitively rule out acute infection with Zika virus. Serologic/molecular testing for asymptomatic women accordingly should be performed at 2-12 weeks after initial travel.[64]

In symptomatic pregnant women who reside in a region where there is active Zika virus transmission, testing should be performed within 7 days of symptom onset. For asymptomatic pregnant women who reside in an endemic area, testing should be performed at the initiation of prenatal care with follow up testing midway through the second trimester. Additionally, healthcare providers should provide sexual education and

counseling on reproductive options and risks/benefits of conception to women of reproductive age who live in endemic regions.[64]

Serologic/molecular testing is recommended in infants with microcephaly and/or intracranial calcifications whose mothers traveled to or resided in a region endemic for Zika virus while pregnant. Testing is also recommended in infants who are born to mothers with positive or inconclusive testing for Zika virus infection. Specimens for testing should be obtained from either the umbilical cord or directly via collection of serum. The placenta, umbilical cord tissues and cerebral spinal fluid may also be tested. Amniocentesis for *in utero* diagnosis is an emerging intervention; however, the clinical significance of *in utero* infection is unclear.[64, 65]

Treatment/Management Strategies

Management of Initial Infection/Sequela

Primary management of Zika virus infection is supportive and is directed toward management of associated symptoms. As of March 2016, no antiviral therapy exists for Zika virus infection. Symptomatic management includes rest, fluids/hydration and analgesics/antipyretics. Given the overlap of signs and symptoms with other arboviruses such as Dengue virus, aspirin and other non-steroidal anti-inflammatory drugs should be avoided until other infections have been ruled out. This is necessary in order to decrease the risk of Reye syndrome in children and hemorrhagic complications in the general

population.[13, 66] The symptoms of Zika virus are typically self-limited and mild. The vast majority of patients (approximately 80%) are asymptomatic so will not know they have been infected if never tested.[28]

Clinicians should pay close attention to early identification of neurologic symptoms that may herald an associated GBS. Typically, signs and symptoms of Zika virus infection occur 6 days prior to the neurologic symptoms of GBS. Patients with Zika virus associated GBS were not viremic at the time of hospital admission. Supportive management and ventilator support may be required in cases of GBS. IVIG and plasmapheresis may also be indicated for GBS treatment. With appropriate supportive care, clinical outcomes in patients with Zika virus and GBS have generally been favorable.[41]

Management of Pregnant Patients with Possible Zika Virus Infection

If initial serologic/molecular testing of a pregnant woman is positive or inconclusive, the pregnancy should be subsequently managed with serial fetal ultrasounds every 3-4 weeks to assess for microcephaly/intracranial calcifications and other fetal abnormalities. If initial serologic/molecular testing of a pregnant woman is negative, then initial fetal ultrasound should be performed for microcephaly/intracranial calcifications. If fetal abnormalities are identified on this ultrasound, then the pregnant patient should be re-tested. If ultrasound is negative, then it is appropriate to proceed with routine prenatal care.[64] Amniocentesis can also be considered for fetal testing, although the clinical implications of in utero infection are unclear.

Management of Neonates with Potential Zika Virus

In an infant with potential Zika virus (positive infant test, positive or inconclusive test in mother, mother who was untested with clinical symptoms and travel to endemic area while pregnant), clinicians should perform a comprehensive physical examination and neonatal testing for Zika virus. Rashes/skin lesions and other dysmorphic features should be documented with photographs. Ophthalmologic evaluation is necessary due to the possibility of abnormal eye findings in neonates with Zika virus. A cranial ultrasound should be performed to assess for intracranial abnormalities. Hearing should be assessed either prior to discharge or within 1 month of birth. If cranial ultrasound identifies microcephaly and/or intracranial calcifications, a clinical geneticist/pediatric neurologist should be consulted and testing/evaluation for other congenital infections should be undertaken. Patients should also undergo laboratory evaluation and comprehensive genetic testing as indicated.[65]

Patient Disposition

Initial Disposition

Given that acute Zika virus infection is typically mild and self-limited, admission is frequently unnecessary. Standard hospitalization criteria are indicated for symptomatic management of Zika virus infection.[28] As previously discussed, efforts should be made to

elicit neurologic symptoms/findings that may suggest associated GBS. In cases of associated GBS, hospital admission is warranted and intensive care unit admission with ventilator support may be necessary.[41]

Long-term Follow-up

As noted above, Zika virus is a nationally reportable disease in the United States. Suspected and documented cases should be reported to CDC for appropriate epidemiologic tracking and follow-up. For pregnant women with positive/inconclusive Zika virus test results, serial fetal ultrasound should be performed every 3-4 weeks and amniocentesis should be considered.[64]

For neonates with possible Zika virus infection, repeat vision and hearing screening should be performed at 6 months after birth. This follow up should be performed even if initial testing was normal. Close ongoing monitoring of head circumference and developmental milestones should also be performed in the first year of life.[65]

Future Directions

Zika Vaccine

Evolving approaches to vaccine development include the use of a live or killed, chimeric, DNA, or recombinant protein vaccine. The role of passive immunity after exposure to Zika

virus disease is unclear. The development of a vaccine against the Zika virus remains an active area for future research.[66]

Additional areas of future research include the development of antiviral therapies and the determination of whether Zika virus causes microcephaly and GBS or there is merely an association with these conditions. While some politicians and authors have advocated for quarantine or persons exposed to Zika virus, there is unlikely to be any future science that would support this point of view.[66]

Summary

Zika virus is a single stranded RNA filovirus that was originally isolated in 1947. Public health officials have documented outbreaks since 2007. A large outbreak originating in Brazil in 2015-2016 rapidly became a PHEIC involving multiple countries. As of March 2016, Zika virus in the United States is limited to travel associated cases. Transmission of Zika virus is primarily through the *A. aegeypti* mosquito, but may also occur via sexual, blood transfusion, and perinatal/transplacental transmission. While acute Zika virus infection is typically mild and self-limited, researchers have reported an association with GBS. In addition, there is concern about the temporal association between Zika virus infection and congenital microcephaly/intracranial calcifications and other congenital anomalies. Strategies to contain Zika virus infection include travel restrictions for women who are pregnant or trying to become pregnant and avoidance of mosquito bites in endemic regions of the world, as well as vector control. CDC has developed specific

molecular/serologic testing protocols and algorithms for follow-up care for suspected cases of infection. Suspected cases are reportable to CDC (directly or via local or county health departments). Until a vaccine and antiviral medications are developed, management of acute Zika virus infection will remain supportive. Improved infection control practices/vector control strategies were accelerated prior to the 2016 Olympics. Emergency physicians in member states should be prepared for assessment and management of increased numbers of potentially infected individuals, particularly women who are pregnant and considering becoming pregnant and their partners. Clinicians should consider the broad differential when encountering a patient with potential Zika virus so as not to miss other diseases or even novel emerging infections.

References

1. Dick GW, Kitchen SF, Haddow AJ. Zika virus. I. Isolations and serological specificity. *Trans R Soc Trop Med Hyg* 1952;46:509-20.
2. Chang C, Ortiz K, Ansari A, Gershwin E. The Zika Outbreak of the 21st Century. J Autoimmun. 2016 Feb 26. pii: S0896-8411(16)30008-7.
3. Simpson DI. Zika virus infection in man. *Trans R Soc Trop Med Hyg* 1964;58:335-8.
4. MacNamara FN. Zika virus: a report on three cases of human infection during an epidemic of jaundice in Nigeria. *Trans R Soc Trop Med Hyg* 1954;48:139-45.
5. Duffy MR, Chen TH, Hancock WT, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. *N Engl J Med* 2009;360:2536-43.
6. Haddow AD, Schuh AJ, Yasuda CY, et al. Genetic characterization of Zika virus strains: geographic expansion of the Asian lineage. *PLoS Negl Trop Dis* 2012;6:e1477.
7. Mallet HP, Vial AL, Musso D. BISES: Bulletin d'information sanitaire, epidemiologiques et statistiques. Papeete: Bureau de veille sanitaire (BVS) Polynesie francaise, May 2015.
8. Cao-Lormeau VM, Roche C, Teissier A, et al. Zika virus, French polynesia, South pacific, 2013. *Emerg Infect Dis* 2014;20:1085-6. doi:10.3201/eid2011.141380. 24856001.
9. Tognarelli J, Ulloa S, Villagra E, et al. A report on the outbreak of zika virus on Easter Island, South Pacific, 2014. *Arch Virol* 2015.26611910.
10. Da Saude M. (Brazil). Situacao Confirmacao do Zika virus no Brasil. Brasilia: Monisterion da Saude Brazil); May 2015. Accessed 10 January 2016. http://portalsaude.saude.gov.br/index.php/cidado/principal/agencia-saude/17701-confirmacao-do-zika-virus-no-brasil.
11. World Health Organization. Zika situation report 19 February 2016. Accessed 20 February 2016. http://apps.who.int/iris/bitstream/10665/204454/1/zikasitrep_19Feb2016_eng.pdf?ua=1.
12. Campos GS, Bandeira AC, Sardi SI. Zika virus outbreak, Bahia, Brazil. *Emerg Infect Dis* 2015;21:1885-6.doi:10.3201/eid2110.150847. 26401719.
13. Basarab M Bowman C, Aarons EJ, Cropley I. Zika virus. *BMJ* 2016; 352 :i1049

14. CDC. Zika virus. Atlanta, GA: US Department of Health and Human Services, CDC; 2016. http://www.cdc.gov/zika/index.html.
15. Kuno G, Chang GJ. Full-length sequencing and genomic characterization of Bagaza, Kedougou, and Zika viruses. *Arch Virol* 2007;152:687-96. doi:10.1007/s00705-006-0903-z. 17195954.
16. Faye O, Freire CC, Iamarino A, et al. Molecular evolution of Zika virus during its emergence in the 20(th) century. *PLoS Negl Trop Dis* 2014;8:e2636. doi:10.1371/journal.pntd.0002636. 24421913.
17. Haddow AJ, Williams MC, Woodall JP, Simpson DI, Goma LK. Twelve isolations of Zika virus from *Aedes* (Stegomyia) *Africanus* (Theobald) taken in and above a Uganda Forest. *Bull World Health Organ* 1964;31:57-69.14230895.
18. McCrae AW, Kirya BG. Yellow fever and Zika virus epizootics and enzootics in Uganda. *Trans R Soc Trop Med Hyg* 1982;76:552-62. doi:10.1016/0035-9203(82)90161-4. 6304948.
19. Marchette NJ, Garcia R, Rudnick A. Isolation of Zika virus from *Aedes aegypti* mosquitoes in Malaysia. *Am J Trop Med Hyg* 1969;18:411-5.4976739.
20. Li MI, Wong PSJ, Ng LC, Tan CH. Oral susceptibility of Singapore Aedes (Stegomyia) aegypti (Linnaeus) to Zika virus. *PLoS Negl Trop Dis* 2012;6:e1792. doi:10.1371/journal.pntd.0001792. 22953014.
21. Boorman JPT, Porterfield JS. A simple technique for infection of mosquitoes with viruses; transmission of Zika virus. *Trans R Soc Trop Med Hyg* 1956;50:238-42. doi:10.1016/0035-9203(56)90029-3. 13337908.
22. Grard G, Caron M, Mombo IM, et al. Zika virus in Gabon (Central Africa)—2007: a new threat from Aedes albopictus? *PLoS Negl Trop Dis* 2014;8:e2681. doi:10.1371/journal.pntd.0002681. 24516683.
23. Wong PS, Li MZ, Chong CS, Ng LC, Tan CH. Aedes (Stegomyia) albopictus (Skuse): a potential vector of Zika virus in Singapore. *PLoS Negl Trop Dis* 2013;7:e2348. doi:10.1371/journal.pntd.0002348. 23936579.

24. World Health Organization. Disease outbreak news: Zika virus infection—United States of America. Accessed 12 February 2016. www.who.int/csr/don/12-february-2016-zika-usa/en/
25. Foy BD, Kobylinski KC, Chilson Foy JL, et al. Probable non-vector-borne transmission of Zika virus, Colorado, USA. *Emerg Infect Dis* 2011;17:880-2. doi:10.3201/eid1705.101939. 21529401.
26. Musso D, Roche C, Robin E, Nhan T, Teissier A, Cao-Lormeau VM. Potential sexual transmission of Zika virus. *Emerg Infect Dis* 2015;21:359-61. doi:10.3201/eid2102.141363. 25625872.
27. Atkinson B, Hearn P, Afrough B, et al. Detection of Zika virus in semen[letter]. *Emerg Infect Dis* 2016. Accessed 20 February 2016. doi:10.3201/eid2205.160107. .
28. Oster AM, Brooks JT, Stryker JE, et al. Interim Guidelines for Prevention of Sexual Transmission of Zika Virus — United States, 2016. MMWR Morb Mortal Wkly Rep 2016;65:120–121. DOI: http://dx.doi.org/10.15585/mmwr.mm6505e1.
29. Deckard DT, Chung WM, Brooks JT, et al. Male-to-Male Sexual Transmission of Zika Virus— Texas, January 2016. MMWR Morb Mortal Wkly Rep 2016;65:372–4.
30. Musso D, Nhan T, Robin E, et al. Potential for Zika virus transmission through blood transfusion demonstrated during an outbreak in French Polynesia, November 2013 to February 2014. *Euro Surveill* 2014;19:20761. doi:10.2807/1560-7917.ES2014.19.14.20761. 24739982.
31. Avajlable at: http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm486359.htm. Accessed March 5, 2016.
32. Available at: http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm488612.htm. Accessed March 5, 2016.
33. Calvet G, Aguiar RS, Melo ASO et al. Detection and sequencing of Zika virus from amniotic fluid of fetuses with microcephaly in Brazil: a case study. *Lancet Infect Dis* 2016. Published online 17 February 2016. 10.1016/S1473-3099(16)00095-5. .

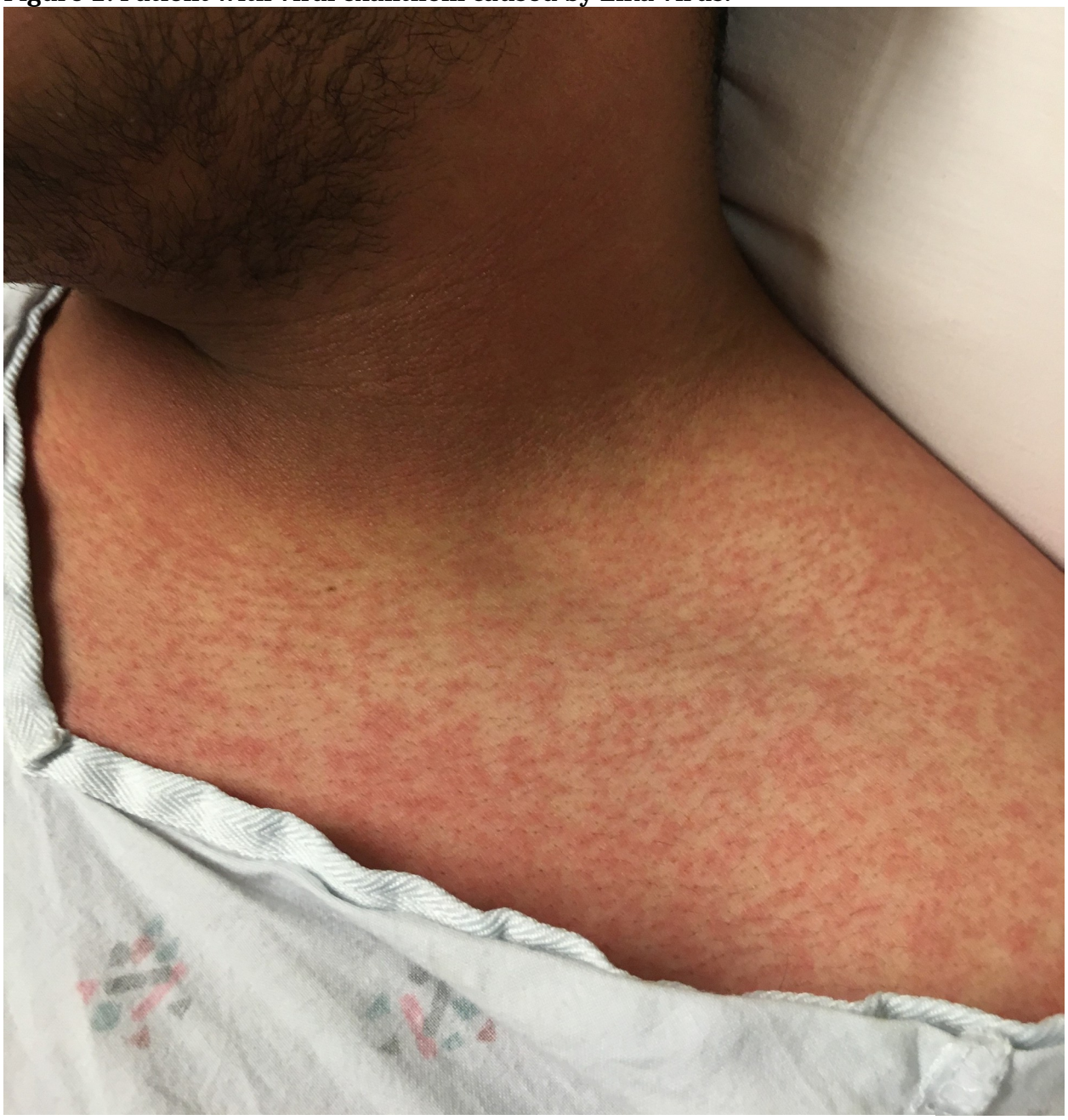
34. Besnard M, Lastère S, Teissier A, Cao-Lormeau V, Musso D. Evidence of perinatal transmission of Zika virus, French Polynesia, December 2013 and February 2014. *Euro Surveill* 2014;19:20751. doi:10.2807/1560-7917.ES2014.19.13.20751. 24721538.
35. Mlakar J, Korva M, Tul N, Popović M, Poljšak-Prijatelj M, Mraz J, Kolenc M, Resman Rus K, Vesnaver Vipotnik T, Fabjan Vodušek V, Vizjak A, Pižem J, Petrovec M, Avšič Županc T. Zika Virus Associated with Microcephaly. N Engl J Med. 2016 Feb 10. [Epub ahead of print]
36. Tang H, Hammack C, Ogden SC, et al. Zika virus infects human cortical neural progenitors and attenuates their growth. *Cell Stem Cell* 2016 (in press). DOI: http://dx.doi.org/10.1016/j.stem.2016.02.016
37. Ioos S, Mallet HP, Leparc Goffart I, Gauthier V, Cardoso T, Herida M. Current Zika virus epidemiology and recent epidemics. *Med Mal Infect* 2014;44:302-7. doi:10.1016/j.medmal.2014.04.008. 25001879.
38. Thomas DL, Sharp TM, Torres J, et al. Local transmission of Zika virus—Puerto Rico, November 23, 2015-January 28, 2016. *MMWR Morb Mortal Wkly Rep* 2016;65:154-8. doi:10.15585/mmwr.mm6506e2. 26890470.
39. Musso D, Nhan TX. Emergence of Zika virus. *Clin Microbiol* 2015;4:222.doi:10.4172/2327-5073.1000222.
40. World Health Organization. Zika virus: news and updates. Accessed 19 February 2016. www.who.int/emergencies/zika-virus/timeline-update/en/.
41. Cao-Lormeau, Van-Mai et al. Guillain-Barré Syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study. *The Lancet.* Published online February 29, 2016.
42. Soares de Araújo JS, Regis CT, Gomes RGS, et al. Microcephaly in northeast Brazil: a review of 16 208 births between 2012 and 2015. *Bull World Health Org* 2016;4. doi:10.2471/BLT.16.170639. .

43. Symmes Cobb J, Jaime Acosta L. Colombia's forecast on Zika-linked birth defect may be too high: minister. Reuters.com. Accessed 19 February 2016. www.reuters.com/article/us-health-zika-colombia-idUSKCN0VQ2AB.
44. Brasil P, Pereira JP Jr, Raja JC, et al. Zika Virus Infection in Pregnant Women in Rio de Janeiro - Preliminary Report. N Engl J Med. 2016 Mar 4. [Epub ahead of print]
45. Butler, D. First Zika-linked birth defects detected in Colombia. Nature 531, 153 (10 March 2016)
46. Available at: http://www.cdc.gov/media/releases/2016/s0413-zika-microcephaly.html. Accessed June 11, 2016.
47. Pan American Health Organization. Case definitions. Available at: http://www.paho.org/hq/index.php?option=com_content&view=article&id=11117&Itemid=41532&lang=en. Accessed June 11, 2016.
48. Musso D, Roche C, Nhan TX, Robin E, Teissier A, Cao-Lormeau VM. Detection of Zika virus in saliva. *J Clin Virol* 2015;68:53-5.doi:10.1016/j.jcv.2015.04.021. 26071336.
49. Gourinat AC, O'Connor O, Calvez E, Goarant C, Dupont-Rouzeyrol M. Detection of Zika virus in urine. *Emerg Infect Dis* 2015;21:84-6. doi:10.3201/eid2101.140894. 25530324.
50. Charrel RN, Leparc-Goffart I, Pas S, de Lamballerie X, Koopmans M, Reusken C. State of knowledge on Zika virus for an adequate laboratory response. *Bull World Health Organ* 2016; doi:10.2471/BLT.16.171207. .
51. Centers for Disease Control and Prevention. Revised diagnostic testing for Zika, chikungunya, and dengue viruses in US Public Health Laboratories. Released 7 February 2016. Accessed 12 February 2016. www.cdc.gov/zika/pdfs/denvchikvzikv-testing-algorithm.pdf
52. US-CDC 2016: Transcript for CDC Telebriefing: Zika Virus Travel Alert **http://www.cdc.gov/media/releases/2016/t0116-zika-virus-travel.html** - accessed 29th January, 2016.
53. Petersen, Eskild et al. Rapid Spread of Zika Virus in The Americas - Implications for Public Health Preparedness for Mass Gatherings at the 2016 Brazil Olympic Games. International Journal of Infectious Diseases, Volume 44 , 11 – 15

54. NaTHNaC. Zika virus: update and advice for pregnant women (updated 19 January 2016).http://travelhealthpro.org.uk/zika-virus-update-and-advice-for-pregnant-women/.
55. European Centre for Disease Prevention and Control. Microcephaly in Brazil potentially linked to the Zika virus epidemic [Internet]. Stockholm: ECDC; 2015. Available from:**http://ecdc.europa.eu/en/publications/_layouts/forms/Publication_DispForm.aspx?List=4f55ad51-4aed-4d32-b960-af70113dbb90&ID=1407** –accessed January 26th, 2016.
56. Petersen EE, Staples JE, Meaney-Delman D, Fischer M, Ellington SR, Callaghan WM, Jamieson DJ. Interim Guidelines for Pregnant Women During a Zika Virus Outbreak - United States, 2016. Jan 22;65(2):30-3 MMWR Morb Mortal Wkly Rep 2016;65:30-3.
57. Centers for Disease Control and Prevention (CDC). Interim Guidelines for Pregnant Women During a Zika Virus Outbreak — United States, 2016.**http://www.cdc.gov/mmwr/volumes/65/wr/mm6502e1.htm** –accessed January 26th, 2016.
58. Ahmad SSY, Amin TN, Ustianowski A. Zika virus: management of infection and risk. *BMJ* 2016;352:i1062. doi:10.1136/bmj.i1062. .
59. Elachola H[1], Gozzer E[2], Zhuo J[3], Memish ZA[4]. A crucial time for public health preparedness: Zika virus and the 2016 Olympics, Umrah, and Hajj. Lancet. 2016 Feb 13;387(10019):630-2. doi: 10.1016/S0140-6736(16)00274-9. Epub 2016 Feb 7.
60. World Health Organization 2016. Zika virus.**http://www.who.int/mediacentre/factsheets/zika/en/-** accessed January 23rd 2016.
61. WHO 2016. WHO statement on the first meeting of the International Health Regulations (2005) (IHR 2005) Emergency Committee on Zika virus and observed increase in neurological disorders and neonatal

malformations**http://www.who.int/mediacentre/news/statements/2016/1st-emergency-committee-zika/en/** -accessed February 1st 2016.
62. Available at: http://www.who.int/ihr/procedures/pheic/en/. Accessed March 7, 2016.
63. Koenig KL, Abdulmajeed A, Burns MJ. Identify-Isolate-Inform: A tool for initial detection and management of zika virus patients in the emergency department. WestJEM 2016;17(3):238–44.
64. Oduyebo T, Petersen EE, Rasmussen SA, et al. Update: Interim Guidelines for Health Care Providers Caring for Pregnant Women and Women of Reproductive Age with Possible Zika Virus Exposure — United States, 2016. MMWR Morb Mortal Wkly Rep 2016;65:122–127. DOI: http://dx.doi.org/10.15585/mmwr.mm6505e2.
65. Staples JE, Dziuban EJ, Fischer M, et al. Interim Guidelines for the Evaluation and Testing of Infants with Possible Congenital Zika Virus Infection — United States, 2016. MMWR Morb Mortal Wkly Rep 2016;65:63–67. DOI: http://dx.doi.org/10.15585/mmwr.mm6503e3.
66. Chan JF, Choi GK, Yip CC, Cheng VC, Yuen KY. Zika fever and congenital Zika syndrome: An unexpected emerging arboviral disease? J Infect. 2016 Mar 2. pii: S0163-4453(16)00061-X. doi: 10.1016/j.jinf.2016.02.011.
67. Koenig KL. Quarantine for Zika virus: Where's the science? Disaster Medicine and Public Health Preparedness 2016.

Figure 1: Patient with viral exanthem caused by Zika virus.



Photograph courtesy of Dr. David C. Pigott, University of Alabama at Birmingham