

Prolonged Detection of Zika Virus RNA in Pregnant Women

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OBJECTIVE: Zika virus infection during pregnancy is a cause of microcephaly and other fetal brain abnormalities. Reports indicate that the duration of detectable viral RNA in serum after symptom onset is brief. In a recent case report involving a severely affected fetus, Zika virus RNA was detected in maternal serum 10 weeks after symptom

onset, longer than the duration of RNA detection in serum previously reported. This report summarizes the clinical and laboratory characteristics of pregnant women with prolonged detection of Zika virus RNA in serum that were reported to the U.S. Zika Pregnancy Registry.

METHODS: Data were obtained from the U.S. Zika Pregnancy Registry, an enhanced surveillance system of pregnant women with laboratory evidence of confirmed or possible Zika virus infection. For this case series, we defined prolonged detection of Zika virus RNA as Zika virus RNA detection in serum by real-time reverse transcription-polymerase chain reaction (RT-PCR) 14 or more days after symptom onset or, for women not reporting signs or symptoms consistent with Zika virus disease (asymptomatic), 21 or more days after last possible exposure to Zika virus.

RESULTS: Prolonged Zika virus RNA detection in serum was identified in four symptomatic pregnant women up to 46 days after symptom onset and in one asymptomatic pregnant woman 53 days postexposure. Among the five pregnancies, one pregnancy had evidence of fetal Zika virus infection confirmed by histopathologic examination of fetal tissue, three pregnancies resulted in live births of apparently healthy neonates with no reported abnormalities, and one pregnancy is ongoing.

CONCLUSION: Zika virus RNA was detected in the serum of five pregnant women beyond the previously estimated timeframe. Additional real-time RT-PCR testing of pregnant women might provide more data about prolonged detection of Zika virus RNA and the possible diagnostic, epidemiologic, and clinical implications for pregnant women.

(*Obstet Gynecol* 2016;128:724–30)

DOI: 10.1097/AOG.0000000000001625

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The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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Financial Disclosure

The authors did not report any potential conflicts of interest.

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ISSN: 0029-7844/16



A causal relationship has been established between Zika virus infection during pregnancy and severe fetal brain abnormalities, including microcephaly.¹ Zika virus infection also has been linked to other adverse outcomes such as fetal loss.¹⁻³ Most Zika virus infections are asymptomatic or cause mild clinical disease.⁴ The duration of Zika virus RNA in serum is considered brief; the mean time from infection to viral clearance is reported as approximately 10 days.⁵⁻⁷ However, data on Zika virus RNA detection in individuals through repeated sampling are limited, and the duration of viral detection has not been studied systematically in pregnant women. A recent case report demonstrated Zika virus RNA in the serum of a pregnant woman 10 weeks after symptom onset, despite mild, short-lived maternal symptoms and an uneventful recovery.⁸ In that case, Zika virus infection contracted in the first trimester of pregnancy resulted in a severely affected fetus, and Zika virus was cultured from the fetal brain.⁸ Another pregnant woman infected with Zika virus in the first trimester, was reported to have ZIKV RNA detected in her serum 21 days after symptom onset.³ She experienced an early pregnancy loss at approximately 11 weeks of gestation and underwent dilation and curettage; Zika virus was cultured from amniotic fluid, placental tissue, and the fetus.³

The Centers for Disease Control and Prevention (CDC) recommends laboratory testing for all pregnant women with possible exposure to Zika virus.^{9,10} The CDC previously recommended testing symptomatic pregnant women by real-time reverse transcription-polymerase chain reaction (RT-PCR) on serum collected less than 7 days after symptom onset and urine collected less than 14 days after symptom onset.^{9,11} Serologic testing for Zika virus immunoglobulin M (IgM) antibodies was recommended for symptomatic pregnant women in whom Zika virus RNA was not detected by real-time PCR or for pregnant women who did not report signs or symptoms consistent with Zika virus disease but who had a known possible exposure to Zika virus.^{9,10} However, because information about the duration of RNA detection is limited, some health departments extended the interval between symptom onset and sample collection for real-time RT-PCR testing and included real-time RT-PCR testing as a component of their laboratory testing for asymptomatic pregnant women.

This report summarizes the clinical and laboratory characteristics of five pregnant women with prolonged detection of Zika virus RNA in serum that were reported to the U.S. Zika Pregnancy Registry.

MATERIALS AND METHODS

The U.S. Zika Pregnancy Registry was established as an enhanced surveillance initiative by the CDC in

collaboration with state, tribal, territorial, and local health departments to collect and aggregate information about pregnant women with any laboratory evidence compatible with Zika virus infection or a recent flavivirus infection possibly due to Zika virus (<http://www.cdc.gov/zika/hc-providers/registry.html>). This prospective surveillance was determined to be a nonresearch activity by the CDC and was therefore exempt from institutional review board approval. Laboratory evidence of Zika virus infection is defined as the presence of Zika virus RNA by real-time RT-PCR in serum, urine, or amniotic fluid or the presence of anti-Zika virus IgM and Zika virus-neutralizing antibodies in serum by enzyme-linked immunosorbent assay and plaque reduction neutralization test, respectively.¹² The U.S. Zika Pregnancy Registry also collects information on maternal exposure history, clinical presentation, laboratory testing, prenatal imaging, pregnancy screening and complications, fetal and neonatal outcomes, and infant development through the first year of life.

Real-time RT-PCR testing was performed in accordance with established laboratory testing protocols.¹³ For this case series, prolonged detection was defined as presence of Zika virus RNA detected in serum by real-time RT-PCR 14 or more days after symptom onset for symptomatic pregnant women or the presence of Zika virus RNA in serum 21 or more days after last possible exposure to Zika virus for asymptomatic pregnant women. Fourteen days after symptom onset was chosen as the timeframe for defining prolonged detection of Zika virus RNA because it accounts for the longest duration of Zika virus RNA detection in a nonpregnant person and is longer than the mean duration for detection of Zika virus RNA reported in a recent literature review by Lessler et al.⁷ In that study, the mean duration viral RNA was detectable in blood was 9.9 days (95% confidence interval 6.8–21.4). Twenty-one days after last potential exposure was chosen for asymptomatic pregnant women because 95% of nonpregnant persons infected with Zika virus will have no detectable RNA in serum by 19 days after infection.⁷

RESULTS

Five pregnant women with prolonged detection of Zika virus RNA in serum were reported to the U.S. Zika Pregnancy Registry in this case series. The five pregnant women with prolonged detection of Zika virus RNA included four symptomatic and one asymptomatic, and none was tested as a result of suspected or identified fetal abnormalities. These five pregnant women had travelled to or lived in one or more of



the following countries with active Zika virus transmission: Colombia, the Dominican Republic, El Salvador, Guyana, Honduras, and Mexico. Of the five pregnant women, the duration of travel to an area with active Zika virus transmission for three ranged from 13 to 87 days; the other two travelled to the United States at approximately 21 weeks of gestation from an area with active Zika virus transmission.

Among the four symptomatic women in whom Zika virus RNA was detected by real-time RT-PCR tests 14 or more days after symptom onset, a range of 17–46 days from symptom onset to positive real-time RT-PCR test was observed (Fig. 1). All symptomatic women reported a rash; some also reported fever, arthralgia, myalgia, headache, or chills. In one pregnancy that was electively terminated (Case A), there was evidence of fetal infection: Zika virus RNA was detected in amniotic fluid and fetal tissues by real-time RT-PCR. As of July 21, 2016, one pregnancy is ongoing and the other three pregnancies resulted in live births of apparently healthy neonates with no reported abnormalities. We summarize the clinical and laboratory characteristics of the five pregnancies with prolonged detection of Zika virus RNA below (Fig. 1).

Case A

A pregnant woman in her 30s reported symptoms of fever, maculopapular rash, arthralgia, myalgia, and headache at approximately 13 weeks of gestation, 3 days after returning from travel to an area with active Zika virus transmission. Zika virus RNA was detected by real-time RT-PCR in serum, along with Zika IgM and Zika virus–neutralizing antibodies 17 days after symptom onset. At approximately 16 weeks of gestation, 25 days after symptom onset, a fetal ultrasound scan was performed and was reported to be normal. Amniocentesis was performed, and Zika virus RNA was detected by real-time RT-PCR. After discussion with her health care providers, the patient elected to terminate her pregnancy at approximately 18 weeks of gestation. Based on molecular testing performed at local and state laboratories and at the CDC, conclusive evidence of Zika virus RNA was found in skeletal muscle, bone, and placenta, but not in umbilical cord, liver, or lung tissue. Real-time RT-PCR testing of the fetal brain did not detect Zika virus RNA; however, limited brain tissue was available for testing. Immunohistochemical staining to detect Zika viral RNA of the placenta, umbilical cord, and fetal heart, spleen, liver, lung, and brain was negative at the CDC; the immunohistochemical staining method used has been described previously.¹³ Limited information about this case has been reported previously.¹⁴

Case B

A pregnant woman in her 20s traveled to an area with active Zika virus transmission from approximately 19 to 21 weeks of gestation. She developed fever, rash, arthralgia, and chills 5 days before her return at approximately 20 weeks of gestation. She underwent Zika virus real-time RT-PCR and serologic testing 23 days after symptom onset. Zika virus RNA was detected by real-time RT-PCR in serum, and Zika virus IgM testing was positive with Zika virus–neutralizing antibodies present. Serum real-time RT-PCR testing was not repeated. Multiple prenatal ultrasound scans during pregnancy demonstrated a fetus without abnormalities. A full-term, apparently healthy neonate with no reported abnormalities was born at approximately 39 weeks of gestation. The newborn tested negative for Zika virus by real time RT-PCR and Zika IgM antibody testing.

Case C

A pregnant woman in her 20s traveled to an area with active Zika virus transmission from approximately 14 to 17 weeks of gestation. She reported a facial rash 5 days after return from travel; Zika virus real-time RT-PCR and serologic testing was performed 7 days after symptom onset at approximately 19 weeks of gestation. Zika virus RNA was detected in serum and urine, and Zika IgM was positive with Zika virus–neutralizing antibodies present. Additional maternal serum and urine were collected for testing at the state laboratory 9, 20, 34, and 44 days after symptom onset, and Zika virus RNA was detected only in serum at each time point. Real-time RT-PCR testing of a serum sample collected 51 and 62 days after symptom onset did not detect Zika virus RNA. Real-time RT-PCR testing at the CDC Division of Vector-Borne Diseases Arbovirus Diagnostic Laboratory of serum collected 7 and 9 days after symptom onset was equivocal, the result for the serum collected 20 days after symptom onset was positive, and at 34 days after symptom onset was negative. This discrepancy in findings between the two laboratories may be a consequence of the low level of Zika virus RNA in the original sample, which might have degraded to levels lower than the threshold level for detection during sample handling and transport to the CDC.¹⁵ Prenatal ultrasound scans at approximately 22 and 26 weeks of gestation revealed a head circumference smaller than expected for gestational age at 19 cm (3rd percentile) and 23 cm (4th percentile), respectively. The most recent ultrasound scan, at approximately 38 weeks of gestation, demonstrated a fetus with a head circumference smaller than expected for gestational age (32 cm; 6th percentile). The pregnancy is ongoing.



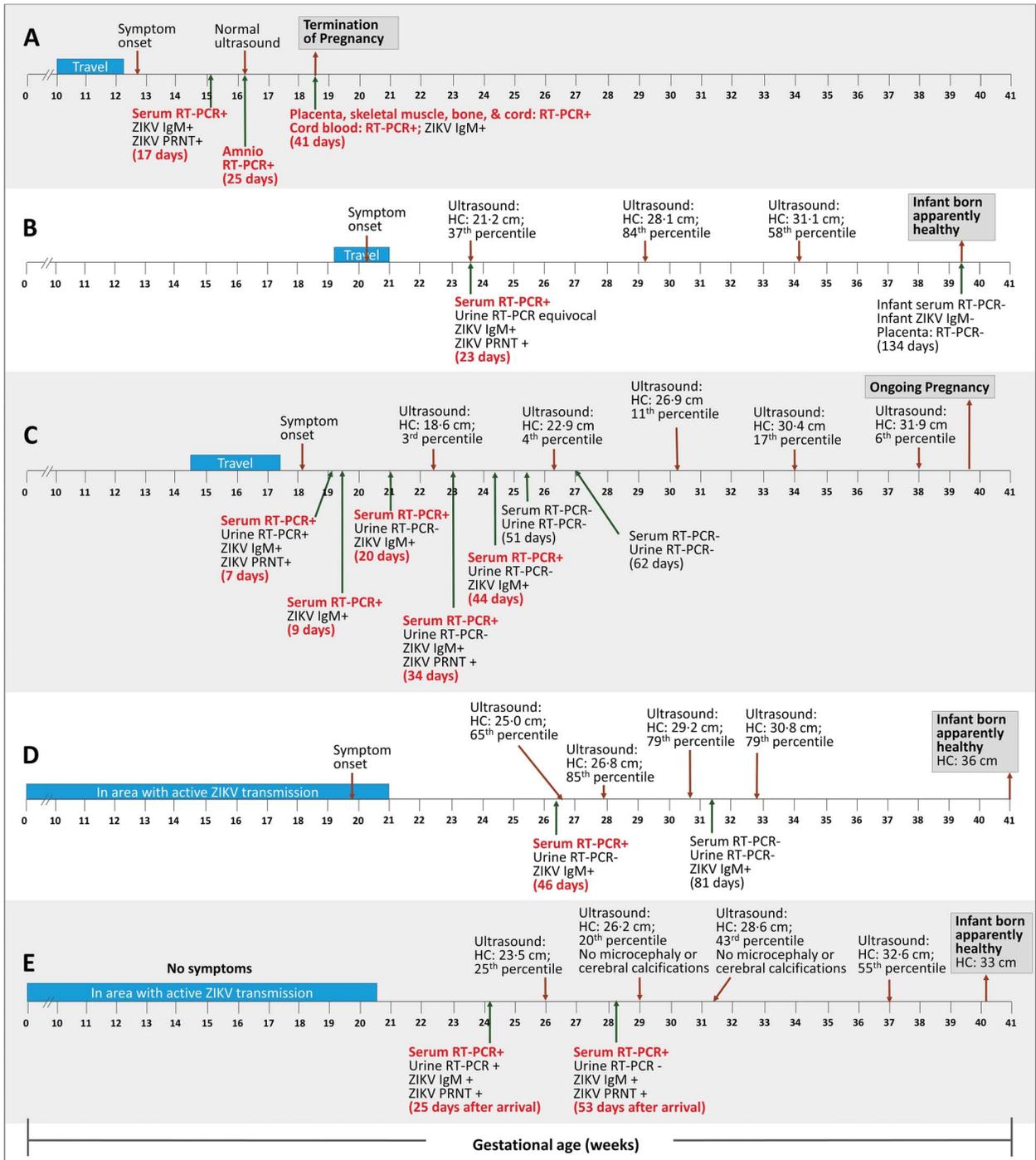


Fig. 1. Timelines of exposure, laboratory testing, and diagnostic imaging for five pregnant women (Cases A-E) with prolonged detection of Zika virus RNA, U.S. Zika Pregnancy Registry, 2016. The cycle threshold (Ct) values for each sample ranged from 32.2 to 42.2 (positive cutoff <38.0). All samples were tested twice; therefore the mean Ct values for each sample were calculated; the average Ct values for the samples ranged from 33.5 to 38.8. RT-PCR, real-time reverse-transcription polymerase chain reaction; IgM, immunoglobulin M; HC, head circumference; PRNT, plaque reduction neutralization test.

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Case D

A pregnant woman in her late teens traveled to the United States from an area with active Zika virus transmission at approximately 21 weeks of gestation. She reported having symptoms of fever and rash at approximately 20 weeks of gestation, and Zika real-time RT-PCR and serology was performed 46 days after symptom onset (26 weeks of gestation). Zika virus RNA was detected in serum; urine real-time RT-PCR was negative, and Zika IgM testing was positive. Repeat real-time RT-PCR testing 81 days after symptom onset did not detect Zika RNA in serum or urine. Multiple prenatal ultrasound scans during pregnancy demonstrated a fetus without abnormalities. A full-term, apparently healthy neonate with no reported abnormalities was born at approximately 41 weeks of gestation; the head circumference measured 36 cm.

Case E

A pregnant woman in her 30s traveled to the United States from an area with active Zika virus transmission at approximately 21 weeks of gestation. She was tested for Zika virus infection 25 days later. Zika virus RNA was detected by real-time RT-PCR in serum and urine specimens; Zika IgM testing was also positive with Zika virus–neutralizing antibodies present. She denied symptoms of Zika virus disease, and it is not known whether she had sexual contact with a partner who had traveled to or lived in an area with active Zika virus transmission after her travel to the United States; hence, the exact date range during which she may have been infected is unknown. Repeat Zika virus real-time RT-PCR on serum collected 53 days after travel from an area with active Zika virus transmission detected Zika virus RNA, and Zika IgM testing was positive with Zika virus neutralizing–antibodies present. Ultrasound scans performed at 26 and 29 weeks of gestation demonstrated no fetal abnormalities; head circumference was normal at the 25th and 20th percentiles, respectively. A full-term, apparently healthy neonate with no reported abnormalities was born at approximately 40 weeks of gestation; the head circumference measured 33 cm.

DISCUSSION

We describe five pregnant women with Zika virus infection who had prolonged detection of Zika virus RNA in serum. Four women reported symptoms consistent with Zika virus infections, and one woman was asymptomatic. This report adds to the existing evidence that Zika virus RNA in serum may be detected longer than previously expected, an observation now reported among at least eight pregnant women.^{3,8,16,17}

These data also suggest that Zika virus RNA might be detectable for prolonged periods in some asymptomatic pregnant women.

Animal data suggest that prolonged detection of Zika virus RNA may be associated with pregnancy.^{18,19} In an ongoing experimental trial comparing pregnant and nonpregnant Rhesus macaques, two pregnant Zika virus–infected Rhesus macaques had detectable Zika virus RNA in serum 29 and 57 days after Zika virus infection.^{18,19} Three nonpregnant Rhesus macaques did not have detectable Zika virus RNA by 17 days after infection.^{18,19}

Prolonged detection of viral RNA during pregnancy has been observed with hepatitis E, another RNA virus that has been linked to adverse pregnancy outcomes.²⁰ Delayed immune clearance of viruses from the maternal circulation may reflect altered immunity during pregnancy.^{21,22} Alternately, fetal infection and ongoing viral replication in the fetus or placenta might result in the transfer of viral genetic material into the maternal circulation. Although this has not yet been demonstrated for Zika virus, it has been well established that small amounts of fetal-derived genetic material can be detected in the maternal circulation.^{23,24}

Several limitations of this case series should be acknowledged. These cases reflect a small subset of women who were tested outside of the window of the previous testing recommendations. Because the symptoms of Zika virus disease are mild and nonspecific, symptom-onset dates might not be recorded precisely and the asymptomatic woman might have had symptoms that were unrecognized. Thus, the timing of exposure and infection for these cases was difficult to ascertain. In addition, sexual contact with a Zika virus–infected partner cannot be excluded and, thus, exposures might have been later than assumed based on travel history alone. Finally, variation in some real-time RT-PCR results were observed. The sensitivity of Zika virus RNA detection could be affected by several factors including storage time, specimen handling, processing, and temperature changes such as repeated freeze–thaw cycles.¹⁵ This is particularly challenging for viral infections such as Zika virus, in which the level of viremia is generally low, as observed in these samples.¹³

Several questions remain regarding the findings of prolonged detection of Zika virus RNA. Most notably, the duration of Zika virus RNA in serum requires further investigation to determine whether there is a correlation between prolonged viral RNA detection and the presence of infectious virus. It is also not known how frequently prolonged detection of





Fig. 2. Map of areas that have received or requested the Centers for Disease Control and Prevention Triplex Real Time Reverse Transcriptase Polymerase Chain Reaction Kits.

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Zika virus RNA occurs in nonpregnant persons and whether this differs by pregnancy status. Detection of West Nile viral RNA has been reported in blood donors for as long as 101 days after blood donation,²⁵ which could signify that prolonged detection of Zika virus RNA following a flavivirus infection may not be unique to Zika or to pregnant women. Finally, the clinical implications of prolonged detection of Zika virus RNA are not known; more data are needed to determine whether an association exists between this finding and fetal infection.

The reported results support previous data that demonstrate prolonged detection of Zika virus RNA in serum of pregnant women.^{3,8,16,17} Additional real-time RT-PCR testing of pregnant women with laboratory evidence of Zika virus infection will be valuable to further explore the prevalence and clinical implications of prolonged detection of Zika virus RNA. The CDC developed the Triplex test, a real-time (TaqMan) RT-PCR assay for the qualitative detection of Zika virus RNA and differentiation from dengue and chikungunya virus RNA in serum and cerebrospinal fluid. The test also detects Zika virus RNA in urine and amniotic fluid. Triplex tests can be performed on equipment present in most public health laboratories, and the CDC has

provided Triplex tests to more than 79 countries across the world (Fig. 2) and can provide additional tests to laboratories globally on request.

The CDC has updated the testing recommendations for pregnant women with possible Zika virus exposure. These testing recommendations extend real-time RT-PCR testing of serum from less than 1 week after symptom onset to less than 2 weeks and also include real-time RT-PCR testing of some asymptomatic pregnant women.²⁶

Expanding real time RT-PCR testing may provide additional diagnostic information and increase the proportion of pregnant women with Zika virus infection who receive a definitive diagnosis. Importantly, a positive real-time RT-PCR test would be useful for pregnant women in whom serologic test results do not readily distinguish between Zika virus and other flaviviruses. Second, real-time RT-PCR testing of asymptomatic pregnant women could allow for a more rapid diagnosis of Zika virus infection when compared with the processing time of IgM antibody testing paired with neutralizing antibody testing. Expanded real-time RT-PCR testing in pregnant women may improve our understanding of the diagnostic, epidemiologic, and clinical implications of prolonged detection of Zika virus RNA during pregnancy.



REFERENCES

- Rasmussen SA, Jamieson DJ, Honein MA, Petersen LR. Zika virus and birth defects—reviewing the evidence for causality. *N Engl J Med* 2016;374:1981–7.
- Martines RB, Bhatnagar J, de O Ramos AM, Davi HPF, Iglezias SD, Kanamura CT, et al. Pathology of congenital Zika syndrome in Brazil: a cohort study. *Lancet* 2016 June 29 [Epub ahead of print].
- van der Eijk AA, van Genderen PJ, Verdijk RM, Reusken CB, Mögling R, van Kampen JJA, et al. Miscarriage associated with Zika virus infection. *N Engl J Med* 2016 July 27 [Epub ahead of print].
- Duffy MR, Chen TH, Hancock WT, Powers AM, Kool JL, Lanciotti RS, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. *N Engl J Med* 2009;360:2536–43.
- Musso D, Gubler DJ. Zika virus. *Clin Microbiol Rev* 2016;29:487–524.
- 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.
- Lessler JT, Ott CT, Carcelen AC, Konikoff JM, Williamson J, Bi Q, et al. Times to key events in the course of Zika infection and their implications: a systematic review and pooled analysis. *Bull World Health Organ* 2016. Available at http://www.who.int/bulletin/online_first/16-174540.pdf. Retrieved July 21, 2016.
- Driggers RW, Ho CY, Korhonen EM, Kuivanen S, Jaaskelainen AJ, Smura T, et al. Zika virus infection with prolonged maternal viremia and fetal brain abnormalities. *N Engl J Med* 2016;374:2142–51.
- Centers for Disease Control and Prevention. Memorandum: revised diagnostic testing for Zika, chikungunya, and dengue viruses in US public health laboratories. 2016. Available at: <http://www.cdc.gov/zika/pdfs/denvchikvzikv-testing-algorithm.pdf>. Retrieved May 10, 2016.
- Oduyebo T, Petersen EE, Rasmussen SA, Mead PS, Meaney-Delman D, Renquist CM, 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;65:122–7.
- Interim guidance for Zika virus testing of urine—United States, 2016. *MMWR Morb Mortal Wkly Rep* 2016;65:474.
- Simeone RM, Shapiro-Mendoza CK, Meaney-Delman DE, Petersen E, Galang RR, Oduyebo T, et al. Possible Zika virus infection among pregnant women—United States and Territories, May 2016. *MMWR Morb Mortal Wkly Rep* 2016;65:514–9.
- Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lambert AJ, Johnson AJ, et al. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. *Emerg Infect Dis* 2008;14:1232–9.
- Meaney-Delman D, Hills SL, Williams C, Galang RR, Iyengar P, Hennenfent AK, et al. Zika virus infection among U.S. Pregnant travelers—August 2015–February 2016. *MMWR Morb Mortal Wkly Rep* 2016;65:211–4.
- Dauner AL, Gilliland TC Jr, Mitra I, Pal S, Morrison AC, Hontz RD, et al. Evaluation of nucleic acid stabilization products for ambient temperature shipping and storage of viral RNA and antibody in a dried whole blood format. *Am J Trop Med Hyg* 2015;93:46–53.
- Bocanegra C. Zika virus infection in pregnant women in Barcelona, Spain. *Clin Microbiol Infect* 2016 April 6 [Epub ahead of print].
- Pacheco O, Beltran M, Nelson CA, Valencia D, Tolosa N, Farr SL, et al. Zika virus disease in Colombia—preliminary report. *N Engl J Med* 2016 June 15 [Epub ahead of print].
- Project Zika Experimental Science Team (ZEST), Zika Open-Research Portal. Project Zika experimental science team (ZEST). 2016. Available at: <https://zika.labkey.com/project/OConnor/begin.view>. Retrieved April 24, 2016.
- Dudley DM, Aliota MT, Mohr EL, Weiler AM, Lehrer-Brey G, Weisgrau KL, et al. A Rhesus Macaque Model of Asian-lineage Zika virus infection. *Nat Commun* 2016;7:12204.
- Krain LJ, Atwell JE, Nelson KE, Labrique AB. Fetal and neonatal health consequences of vertically transmitted hepatitis E virus infection. *Am J Trop Med Hyg* 2014;90:365–70.
- Granovsky MO, Minkoff HL, Tess BH, Waters D, Hatzakis A, Devoid DE, et al. Hepatitis C virus infection in the mothers and infants cohort study. *Pediatrics* 1998;102(2 pt 1):355–9.
- Kourtis AP, Read JS, Jamieson DJ. Pregnancy and infection. *N Engl J Med* 2014;370:2211–8.
- Cell-free DNA screening for fetal aneuploidy. Committee Opinion No. 640. American College of Obstetricians and Gynecologists. *Obstet Gynecol* 2015;126:e31–7.
- Dawe GS, Tan XW, Xiao ZC. Cell migration from baby to mother. *Cell Adh Migr* 2007;1:19–27.
- Busch MP, Kleinman SH, Tobler LH, Kamel HT, Norris PJ, Walsh I, et al. Virus and antibody dynamics in acute West Nile virus infection. *J Infect Dis* 2008;198:984–93.
- Oduyebo T, Igbinsola I, Petersen EE, Polen KN, Pillai SK, Ailes EC, et al. Update: interim guidance for health care providers caring for pregnant women with possible Zika virus exposure—United States, July 2016. *MMWR Morb Mortal Wkly Rep* 2016. Available at: http://www.cdc.gov/mmwr/volumes/65/wr/mm6529e1.htm?s_cid=mm6529e1_w. Retrieved July 25, 2016.

