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THE ZIKA VIRUS AND PREGNANCY: EVIDENCE, MANAGEMENT, AND PREVENTION

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Abstract

Objective: To comprehensively review the available evidence and existing consensus reports and guidelines regarding the pregnancy and reproductive implications of the mosquito transmitted Zika virus infection. A primary focus was to provide pertinent information to aid clinicians in the management of pregnancies at risk for, exposed to or with confirmed Zika virus infection (ZIV).

Method: An extensive literature review was performed using Pubmed. Practice guidelines, and consensus reports were accessed from international, national and professional organizations' websites. The clinical articles for ZIKV infection testing varied from case reports to small epidemiologic studies.

Results: A ZIKV epidemic has been declared in several countries in the Americas. Fifty-two travel associated ZIKV infection cases have been reported throughout the United States (as of February 10, 2016). The consequences of congenital fetal/ newborn ZIKV infection could potentially have devastating consequences including miscarriage, fetal death, and major anomalies such as microcephaly, brain and brain-stem defects and long-term neurologic sequelae. While not definitive, current evidence suggest the existence of non-vector-borne transmission through sexual activity with an infected male partner. For women at risk for sexual transmission condom use is advised, especially during pregnancy.

Conclusion: While ZIKV infection appears to be a mild disease in the general population the potential consequences to the fetus and newborn could be profound. Management guidelines are currently evolving and will be significantly impacted as new evidence develops. It is therefore imperative that obstetric health care providers keep abreast of this rapidly evolving information landscape that has so far characterized this outbreak.

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Introduction

An epidemiological alert was issued by the Pan American Health Organization/World Health Organization (PAHO/WHO) on November 2015 due to an unusually large increase in the reported cases of microcephaly [1]. As of November (2015) the frequency of microcephaly reported in Brazil increased to 99.7 cases/100,000 live births compared to a rate of 5.7/100,000 live births in 2010 [2]. This increase coincided with the Zika virus (ZIKV) infection outbreak in Pernambuco, a northeastern State of Brazil [1]. As of November (2015) the number of reported microcephaly cases in Pernambuco had increased from an average of 10 per year to 141 cases from January to mid-November of 2015 [1]. Between October 2015 and January 2016 approximately 4,180 suspected cases of microcephaly have been reported in Brazil of which 270 have currently been confirmed [2,3]. Six of the 270 cases were found to be associated with the ZIKV [2,3]. Grave concerns have thus been raised regarding a potential association between ZIKV and microcephaly. Autochthonous (arising from the area) transmission has now been reported in 19 countries in the Americas in addition to Brazil [4]. Pregnancy data are urgently needed to answer questions such as the fetal risks, the rates of newborn transmission, and the risk associated with breastfeeding.

The aim of our review is to inform obstetric care providers about the potential implications of ZIKV exposure in pregnancy and present management strategies based on the current literature and guidelines, including algorithms from the Center for Disease Control and Prevention (CDC), the Society of Maternal Fetal Medicine (SMFM) and the American College of Obstetrics and Gynecology (ACOG). This reviewprovides: 1) a synopsis of ZIKV epidemiology, 2) information on symptomology and laboratory diagnosis of ZIKV infection, 3)

reported associations with microcephaly, 4) recommendations for evaluation in pregnancy, 5) prevention measures and finally 6) the potential for non-vector borne, including sexual transmission. Comprehensive analyses of the virology, serology and genetics of ZIKV have been published elsewhere and have been given only limited attention in this review.

Search Method

The search focused on pregnancy and microcephaly in geographic areas where ZIKV epidemics have occurred, modes of transmission of ZIKV, and recommendations for screening and management in pregnancy as well as information on newborn transmission through breastfeeding. Literature was retrieved through PubMed searches in January to March 2016. Bibliographies of published articles were searched. Specifically, website's of international, national and professional organizations namely. CDC, PAHO/WHO (Pan-American Health Organization and World Health Organization), European Centre for Disease Prevention and Control (ECDC), including Portal da Saude (Brazilian Health Ministry) were searched to identify original reports on the Zika virus in pregnancy and the neonatal period, practice guidelines and consensus statements.

ZIKV: Epidemiology - Incidence and Geographic Trail

A member of the family Flaviviridae, genus *Flavivirus*, ZIKV is a single stranded RNA arbovirus that uses a mosquito as a vector to transmit the disease [5,6,7]. In 1947-48 the virus was detected in the arboreal mosquito of the *Aedes* genus within the Zika forest in Uganda [6,7,8]. Once thought to be sylvatic (affecting only wild or peri-domestic animals), ZIKV was first isolated in rhesus monkeys in 1947 by a yellow fever surveillance network in the Zika forest [6,7,8,9]. Anti-Zika antibodies have been detected in non-human primates and large mammals (orangutan, zebras, elephants, etc.) including rodents in Pakistan. However, the definitive reservoir i.e. the long term host, remains unknown [10]. Antibodies were first identified in humans in Nigeria in 1954 [11]. Since that time the ZIKV has spread from Uganda to Nigeria and to countries in West Africa, and in addition has spread from Uganda to Malaysia, Micronesia, French Polynesia and the Cape Verde islands [8]. The ZIKV has now been isolated in mosquitoes and humans in Indonesia, Philippines, India, Thailand and Europe and from different mosquito species within the *Aedes* genus [8]. Prior to 2007, primates and large mammals served as the main hosts of ZIKV [7]. Documented human ZIKV exposures occurred sporadically in central and western Africa, Pakistan and South Asia, with only 14 human cases reported prior to 2007 [8].

The ZIKV is related to other flaviviruses namely: dengue, West Nile virus, yellow fever, and Japanese encephalitis virus [9]. The clinical symptoms of ZIKV can be similar yet milder than dengue infection and may have been confused for or occurred concurrently with dengue outbreaks in the past [5,8,12]. N-linked glycosylation on the E protein of the ZIKV enhances infectivity. It has been hypothesized that changes in N-linked glycosylation site could potentially affect ZIKV transmission and the development of complications such as Gillian-Barre syndrome (GBS) and fetal brain anomalies [8,9,12]. Further studies are required to resolve this question.

The first documented ZIKV epidemic among humans occurred in the Yap State of Micronesia in 2007 [5,9,12,13,14]. Clinical symptoms included rash, arthralgia and conjunctivitis and cases were initially misidentified as dengue based on rapid laboratory assay [13]. Further testing using reverse transcriptase-polymerase chain reaction (RT-PCR) technique ultimately identified ZIKV ribonucleic acid (RNA) [5,13]. In 2014, another ZIKV outbreak occurred in the Pacific Islands. Cook Island had 50 confirmed cases, New Caledonia had 35 imported confirmed cases, and 1,365 confirmed autochthonous cases. French Polynesia had 383 confirmed cases, and an autochthonous case was reported on Easter Island, in Chile [12,14].

In May 2015, the first autochthonous case was confirmed in Brazil and spread to 15 states by November 2015 [15,16]. From October-November 2015 additional autochthonous cases were reported in Columbia, Paraguay, Suriname, Venezuela, El Salvador, Guatemala and Mexico [16,17]. The first case of ZIKV infection acquired in the United States was identified in Texas, January 2016, and is currently undergoing investigation [18]. Texas Health Department officials reported that the infection was likely sexually transmitted from a partner who had recently traveled to Venezuela where he became ill and tested positive for ZIKV. [18,19]. Currently there are important unanswered questions regarding the pathogenesis of ZIKV: reservoirs, modes of transmission, the frequency of and the full spectrum of clinical presentation in human infection, and possible complications. Further, uncertainties remain as to the outcome of co-infection with other arboviruses such as the dengue virus.

ZIKV Transmission

The *Aedes* mosquito ingests the virus while feeding on an infected animal or human host. The mosquito harbors the virus without harm to itself and subsequently transmits it during the act of feeding on another victim [14]. *Aedes* mosquitoes actively feed from dawn until dusk but may do so at any time during the day and night [14]. It is a sequential feeder, ingesting a small amount of blood at a time and thus increasing the number of potential victims.

ZIKV has been detected in semen, and sexual transmission now appears to have been potentially responsible for two cases of non-vector borne infection of sexual partners [18,19,20]. In Tahiti in 2013, a male patient reported two illnesses consistent with ZIKV infection, with the first having occurred 8 weeks prior to the second infection [21]. Two weeks post resolution of clinical symptoms from the second reported infection he experienced hematospermia. A medical exam confirmed the diagnosis of hematospermia, and a questionnaire ruled out other potential causes. His semen, urine and blood were tested using RT-PCR - the blood and urine specimens were negative for ZIKV RNA while the semen specimen was positive for ZIKV RNA [21]. The duration after clinical infection in which the ZIKV can be isolated from the semen is currently unknown [21] Please refer to the section within this article labeled "Prevention of Sexual Transmission" for information on recommendations to reduce the risk of transmitting ZIKV during sexual activity.

During the New Caledonian ZIKV epidemic (2014), urine from six patients (gender not recorded) was tested for ZIKV by RT-PCR. Urine from two of the six patients tested positive for ZIKV RNA, with one test remaining positive for >20 days after serum ZIKV RNA became undetectable [22]. Further, transmission via a blood transfusion has not been documented [23]. However three percent of asymptomatic blood donors in French Polynesia were found to be

positive for ZIKV based on RT-PCR testing, thus transmission via this route appears plausible [23].

Perinatal Transmission

Concerns regarding transplacental transmission of ZIKV from mother to fetus have increased as reports of the potential association between maternal ZIKV exposure and fetal/newborn congenital microcephaly and other brain anomalies have surfaced [1,2,3,16]. Vertical transmission of ZIKV can potentially occur transplacentally or during labor and delivery [24]. Data on ZIKV vertical transmission rate and the probable period of transmission have yet to be generated.

Between December 2013 and February 2014, two cases of perinatal ZIKV transmission were reported in French Polynesia [24]. Transmission of ZIKV probably occurred it is thought during delivery. RT-PCR ZIKV RNA testing of maternal serum, saliva and breast milk, along with newborn serum, saliva and urine were reported. However, the testing was not performed consistently for each case thus confounding interpretation of the data. In Case 1, the mother experienced a rash two days prior to delivery, and her serum RT-PCR ZIKA RNA was positive on postpartum day 2. The newborn breastfed upon delivery and breast milk RT-PCR ZIKV RNA was positive on postpartum day 3. Her infant's RT-PCR ZIKV RNA serum and saliva test were found to be positive on postpartum day 3. In Case 2, the mother developed a pruritic rash, mild fever, and myalgia on postpartum day 3. She had positive serum RT-PCR ZIKV RNA on both postpartum days 1 and 5 followed by a negative test on postpartum day 8. Her infant was breastfed on postpartum day 3 and breast milk RT-PCR ZIKV RNA tested positive on postpartum day 8. Her infant's RT-PCR ZIKV RNA serum test was negative at delivery and on postpartum day 3 and subsequently converted to a positive test on postpartum days 4 and 7. The infant's RT-PCR ZIKV

RNA urine test was positive on day 8 and reverted to negative on postpartum day 9. The pregnancy of Case 2 was in addition complicated by gestational diabetes. Her infant suffered from hypoglycemia and neonatal jaundice, which could have resulted from the gestational diabetes. The important question as to whether ZIKV can be transmitted through breastfeeding cannot be resolved from the above data however and remains undetermined [24]. The saliva from both of these mothers tested positive for ZIKV, but these results reportedly may have been due to contamination [24].

Clinical Symptoms

The ZIKV incubation period ranges from 3-12 days after the mosquito bite [14,16]. The illness is usually mild and resolves within two to seven days [14, 16]. The clinical manifestations of an infection , should they develop, may include acute onset mild fever (<38.5℃) , mild-moderate arthralgia (notably of the small joints of hands and feet), non-purulent conjunctivitis or conjunctival hyperemia, maculopapular rashes usually spreading downward from the face to the limbs and frequently pruritic, and nonspecific symptoms such as headaches, myalgia, and asthenia [14,16]. It is estimated that only 18% of individuals infected with ZIKV become symptomatic [17].

In December 2013, during the French Polynesian ZIKV epidemic the first case of Guillain - Barre syndrome (GBS) associated with a ZIKV infection was reported [25]. The patient experienced influenza-like symptoms including fever and myalgia, accompanied by cutaneous rash and conjunctivitis seven days prior to hospitalization for GBS symptoms [25]. The diagnosis of ZIKV infection was made based on serum ZIKV IgM, IgG and plaque-reduction neutralization testing (PRNT) performed 28 days post onset of clinical symptoms. [25]. Ultimately, 40 GBS cases were diagnosed in 3 months compared to the usual average of 5 usually diagnosed during that period [14, 25]. The number of GBS cases previously reported in French Polynesia were 5 in 2009, 10 in 2010, 3 in 2011, and 3 in 2012 [26]. An additional 25 ZIKV cases experienced other neurological

complications such as paraesthesia, facial paralysis, encephalitis, meningo-encephalitis and myelitis [26]. French Polynesia had experienced a concurrent dengue and ZIKV epidemic potentially confounding assessment of the link between ZIKV and GBS [25]. It has been hypothesized that the increase in GBS cases may be due to an evolution of the virus to a more virulent form or to an immunologic deficiency, and /or the result of simultaneous dengue and ZIKV infection [14, 25].

Pregnant women who have a ZIKV infection experience symptoms concordant with the rate observed in the general population [17]. ZIKV is similar to other flaviviruses such as yellow fever and dengue and the arbovirus chikungunya and contribute to laboratory diagnostic difficulties due to the cross reactivity of these antibodies [17,27]. The clinical symptoms of ZIKV, dengue, and chikungunya vary in several ways: 1) joint pain is more severe in chikungunya and may last up to five years, 2) chikungunya and dengue induce immediate severe pyretic symptoms with temperatures above 39℃, 3) dengue causes eye pain, while ZIKV and chikungunya may cause conjunctivitis without secretions and 4) neurological complications have only been reported with ZIKV [28]. Knowledge of the distinguishing clinical features of ZIKV infection and related viruses is imperative. A detailed patient history and examination should be performed in an effort to try to distinguish ZIKV, dengue, and chikungunya virus infections. Clinical symptoms alone however are not diagnostic for ZIKV infection and laboratory testing is required to distinguish it from the other infections.

Zika Virus and Fetal Brain Anomalies

In November 2015, the Brazilian health ministry began reporting an increased incidence of brain and brain related abnormalities among newborns born in ZIKV infected areas [1,2,3,16]. These abnormalities include microcephaly, intracranial malformations, cerebral malformations or polyformative (severe congenital malformations with varying degrees of severity) syndromes, brainstem dysfunction and difficulty swallowing, ocular findings and probable fetal losses [16,29,30,31,32]. Fetal brain anomalies appear to be associated with maternal ZIKV infection occurring in the first or second trimesters of pregnancy [29,30,32]. Among 35 cases of microcephaly in which the mothers reported a history of ZIKV infection, 74% of these mothers (n=26 mothers) reported experiencing a macular rash. Among these 26 mothers, 21 reported experiencing a rash during the first trimester, and 5 reported that the rash first occurred in the second trimester.Additionally 4 mothers reported clinical symptoms (including fever and rash) associated with ZIKV infection in the first trimester - two of whose infants were diagnosed with microcephaly (delivered at 36 and 38 weeks gestation respectively), and the other 2 pregnancies experienced miscarriages at 11 and 13 weeks gestation [29]. Currently, there are no large scale studies that definitively prove that ZIKV infection causes microcephaly in the fetus. There are early suggestive data however. Recently, postmortem analysis after pregnancy termination retrieved the ZIKV from the brain of an infected fetus with microcephaly [30]. Prenatal ultrasound (US) in fetuses where maternal symptoms were consistent with ZIKV infection with negative serum testing and positive RT-PCR testing of amniotic fluid at the time was positive, have revealed a range of cranial abnormalities including microcephaly, brain atrophy, calcification, ventriculomegaly and mid-brain, cerebellar and brain-stem atrophy [31].

It should be borne in mind that microcephaly is a complex disorder and may be acquired or congenital [33]. Congenital microcephaly may be genetic or associated with teratogens, brain injury, hypoxic-ischemic lesions, and trans-placental infections such as syphilis, toxoplasmosis,

rubella, cytomegalovirus and herpes simplex virus (STORCH infections) [17]. The WHO defines microcephaly as a head circumference ≤ 2 standard deviations below the mean for age and sex [17]. If the head circumference is >3 standard deviations (SD) below the mean on prenatal US biometry there is a 70% chance of the newborn having an intellectual disability [34]. Microcephaly is difficult to detect prior to 28 weeks gestation [35]. This should be borne in mind in order to avoid giving premature assurance to the pregnant woman after an early apparently normal ultrasound is performed. Similarly, negative maternal symptomatology and history does not preclude the possibility of a congenital ZIKV infection. Viral testing, while imperative, is also not definitive. Further, given the complex etiology of microcephaly, evaluation for other potential causes including other infectious agents may be appropriate as dictated by clinical judgment. All of the above greatly complicates prenatal screening, counseling and management during pregnancy particularly in ZIKV epidemic regions. Important unanswered questions include the relationship of the trimester of onset of maternal infection with the frequency, severity and range of abnormalities that develop in the fetus and newborn .

Diagnosis & Management in Pregnancy

RT-PCR testing is recommended for ZIKV during the first week after the onset of maternal symptoms. Particularly in endemic regions, Plaque-reduction neutralization testing (PRNT) can be performed to measure virus specific neutralizing antibodies in order to differentiate between cross reacting antibodies in primary flavivirus infections, e.g., concomitant dengue virus and ZIKV infections, or a secondary flavivirus infection [36]. Neutralizing antibodies may still yield cross-reactive results in persons who were previously infected with another flavivirus, or have been vaccinated against yellow fever or Japanese encephalitis [4].

Infection with ZIKV may occur in any trimester. As previously stated the incubation period for a Zika virus infection ranges from 3-12 days after the bite of an infected mosquito. Zika virus IgM can be identified in serum as early as four days after the onset of clinical symptoms and may persist for up to 12 weeks [35]. A negative ZIKV screen may result if the RT-PCR was performed beyond the acute phase of the infection i.e. the first seven days of illness [35, 36]. Further, a negative RT-PCR can still result even if testing is performed 5-7 days after symptom onset and serologic testing along with PRNT testing is advised [35,36]. All positive Zika, dengue and chikungunya viral testing results must be reported to the CDC [36]. It is worth reemphasizing that the fact that most individuals who contract a ZIKV infection remain asymptomatic, coupled with the need for complex viral testing procedures and interpretation, this renders diagnosis of ZIKV infection difficult and at times inconclusive.

The algorithm in Table 1 serves as a convenient tool and guide for the management of pregnant women with possible ZIKV exposure. Detailed information to assist the obstetric health care provider in interpreting laboratory findings is included in Table 1 footnotes. The rationale for the screening recommendations is included. The table is based on the current literature presented in this article and in addition on the guidelines, algorithms and recommendations from the CDC, Society of Maternal Fetal Medicine (SMFM), and the American Congress of Obstetrics and Gynecology (ACOG) [35,37]. All pregnant women should be queried about recent travel, including recent travel of a male sexual partner during the current pregnancy, in order to ascertain potential exposure to ZIKV. There is currently no evidence that a previous ZIKV infection increases the risks for congenital birth defects in subsequent pregnancies [37].

(Insert Table 1)

Ultrasound Evaluation in Suspected or Confirmed Maternal Infection: A Suggested Approach

The CDC, Society for Maternal Fetal Medicine, and the American Congress of Obstetrics and Gynecology have all recommended ultrasounds in order to carefully evaluate the fetal brain and screen for other fetal anomalies in pregnant women who have been exposed to ZIKV, regardless of clinical symptoms or laboratory testing results [35, 37]. Serial US is recommended to detect microcephaly, intracranial calcifications or other fetal anomalies [35, 37]. It bears repeating that reduced brain growth can be associated with or occur in conditions with: 1) other fetal anomalies such as congenital heart disease (CHD), 2) toxoplasmosis, cytomegalovirus, herpes, syphilis, and rubella, 3) West Nile encephalitis virus - a flavivirus, 4) exposure to toxins, 5) consumption of teratogens, 6) malnutrition or metabolic disorders, or 7) genetic risk factors [31,38]. These conditions should therefore be considered particularly when microcephaly is suspected in non-epidemic areas and as dictated by clinical judgment may be considered in the work up of suspected ZIKV-related microcephaly.

While a targeted fetal US exam has been recommended, at this time there are no detailed guidelines outlining the full content of such an exam in fetuses at risk for congenital ZIKV infection. The US evaluation should however address the type of anomalies that have so far been reported in existing case reports. We are suggesting an approach based on the findings in these reports. Most importantly, it is hoped that at this stage a prospective, uniform and systematic approach to ultrasound evaluation will generate data to establish the diagnostic accuracy of ultrasound assessment detecting fetal abnormalities and to guide future recommendations. Biometry of the head, including head circumference measurements, should be performed to assess for microcephaly. Brain atrophy also involving the frontal lobes has been reported in congenital ZIKV [31]. The measurement of frontal-thalamic distance in the fetus has been used for the detection of frontal lobe hypoplasia in the mid-trimester fetus and could be considered

[39]. Lateral ventricular measurement of both ventricles will be useful in the assessment for unilateral ventriculomegaly [38]. Trans-cerebellar diameter measurement should also be performed at each assessment for the detection of cerebellar hypoplasia, a reported finding in congenital ZIKV. Routine measurement of the cisterna magna will also provide information on cerebellar hypoplasia and enlarged cisterna magna both of which are reported to occur in these cases. Finally, increased scalp redundancy as a result of reduced head growth has also been noted in these newborns. Mid-trimester nuchal thickness measurement frequently used for Down syndrome risk assessment may be of value in identifying this scalp redundancy. In addition, qualitative evaluation should in addition be performed to detect brain calcifications (particularly around the lateral and fourth ventricles) but also in the frontal lobes and cerebellum and indeed the entire brain. Abnormal cerebral gyral patterns, hypoplasia of the thalami and brain stem, cataract, micro-ophthalmia and calcifications in the eye have been reported. Currently the timing of manifestation of these abnormalities on prenatal ultrasound is not known. The question as to how frequently prenatal US should be performed and more problematically when US surveillance should cease in the presence of apparently normal US scans are important but cannot currently be definitively answered given the lack of data. The authors are of the opinion however that for confirmed or strongly suspected maternal infection, given the availability and relatively low cost of ultrasound, follow up US exams for head growth and detection of intracranial anomalies may be justifiable for the duration of the pregnancy.

Ultrasound is useful for assessing brain calcifications which appear at this point to be an important feature of congenital ZIKV infection. Placental calcification has also been reported so targeted ultrasound exams should justifiably involve a careful survey in the prenatal period. The presence of other findings in affected neonates should also guide the content of prenatal US assessment. As a consequence, overall growth should be assessed. Limb posture and movement

along with assessment for clubbing of the foot should be assessed given a report of arthrogryposis, hypertonia and spasticity in newborns [32]. Finally, brainstem abnormality appears to be a prominent consequence of congenital ZIKV infection. Fetal difficulty swallowing could plausibly manifest as polyhydramnios and or reduced stomach bubble. Both of these should therefore be assessed on prenatal US. Currently the frequency and timing of manifestation of these abnormalities on prenatal ultrasound has not yet been documented. The question as to how frequently prenatal US should be performed and more problematically when US surveillance should cease in the presence of apparently normal US scan are important but cannot currently be answered given the lack of data. We suggest US exams starting at 18-22 weeks and repeated every 3-4 weeks depending on risk status or diagnosis of infection. Several caveats should be borne in mind- an infected patient may in fact have a negative ZIKV test. Thus high-risk status and the need for US evaluation must be based on a combination of test results, symptomatology, travel history, partner travel history and of course residence in an epidemic area. Specifically, evaluation of the fetus for anomaly should be undertaken regardless of whether or not there is evidence of actual maternal infection but rather based on risk of maternal exposure [37]. A second caveat is that current interim CDC guidelines do not recommend continuation of serial US surveillance in women with a negative test in which the fetus does not have microcephaly or brain calcifications [35]. However, the best currently available data indicate that over 25% of congenital infection cases do not have microcephaly, likewise over 25% of congenital cases do not have brain calcifications [32]- thus negative findings on US screening do not guarantee an unaffected fetus. Similarly, prenatal US exam, particularly that performed earlier in pregnancy, is less sensitive for microcephaly than newborn measurements used to assess the cases referenced above. Moreover, positive US findings may only manifest or be appreciated after serial examinations and later in the pregnancy. These observations argue in

favor of serial US evaluations in those determined to be at high enough risk based on laboratory, history or epidemiologic factors. The authors are therefore of the opinion that particularly for confirmed or strongly suspected maternal infection, given the availability and relatively low cost of ultrasound, follow up assessment for head growth and detection of intracranial anomalies may be justifiable for the duration of the pregnancy.

 (Insert Table 2)

Magnetic Resonance Imaging (MRI): Assessing Fetal Brain Malformations

Fetal magnetic resonance imaging (MRI) is a complementary modality to US for suspected or diagnosed CNS abnormalities. The diagnostic accuracy of US for the subtler brain findings is likely to be somewhat limited for the average US examiner. Fetal MRI has good diagnostic accuracy for subtle cerebral and mid-brain anomalies. MRI has been shown to increase the sensitivity for the detection of multiple CNS disorders including isolated ventriculomegaly, along with improving identification of cortical, mid-brain and hind-brain anomalies [40]. A recent systematic review demonstrated the combination of ultrasound and fetal MRI were in agreement with the prenatal diagnosis of CNS anomalies in 65.4% of cases [41]. In 18.4% of cases however, MRI was positive with a negative ultrasound. Overall in 30% of cases, MRI was so different that clinical management was significantly changed. The additional contribution of fetal MRI is even more significant for mid-brain abnormalities, , ventriculomegaly, neuronal migration disorders, and abnormalities of gyration and sulcation –all of which are emerging as significant concerns in congenital ZIKV infection. Similarly MRI would be helpful for the evaluation of posterior fossa abnormalities involving the brain stem and pontine hypoplasia. MRI can be performed as early as the mid-trimester; however, in contrast to US the diagnostic accuracy improves with advancing gestation, highlighting the complementarity of the tools. We believe that the adjunctive use of fetal MRI should

therefore be strongly considered in appropriate circumstances such as confirmed or strongly suspicious cases of ZIKV or in fetuses with positive or suspected cranial abnormalities on prenatal ultrasound. There are no known adverse effects of MRI to the fetus, and there does not appear to be significant negative long-term effects [40]. Cost and availability are very relevant considerations in determining the frequency of use of both US and MRI, even in well-resourced countries such as the USA. This is particularly the case for MRI. Availability and economic considerations should be factored in and will affect the feasibility of MRI use in different localities.

Symptom Management in Pregnancy

Recommendations for clinical symptom management are presented in Table 3 as a practical tool to be used in the clinical setting. Treatment of the clinical symptoms associated with ZIKV infection is largely supportive. Included are recommendations on symptom relief measures that are safe in pregnancy. While aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) are not generally used in pregnancy, these medications should specifically be avoided until dengue infection is ruled out to reduce the risk of hemorrhage [17, 35].

(Insert Table 3)

Management of Postpartum Issues: Breastfeeding and Newborn Congenital ZIKV Infection Screening

Even though small amounts of ZIKV have been isolated in breast milk, the benefits of breastfeeding outweigh any short term neonatal risks [17, 24, 37, 41]. No definitive data exists in the published literature on oral transmission of ZIKV virus through breastfeeding. Thus ZIKV infection is not considered a deterrent to breastfeeding.

A comprehensive discussion of the guidelines for screening for ZIKV infection in the

newborn are beyond the scope of this article. Briefly, the CDC guidelines recommended newborn screening for ZIKV infection screening in two groups: 1) infants born to mothers with positive or inconclusive ZIKV test results during pregnancy, and 2) infants with microcephaly or intracranial calcifications born to mothers who traveled to or resided in an area with ZIKV transmission during pregnancy [41]. Newborn screening for congenital ZIKV infection requires the following specimens be sent to the CDC or the health department for molecular RT-PCR ZIKV RNA testing: 1) serum collected from the umbilical cord or directly from the infant (within two days of birth), 2) frozen and fixed placenta obtained at delivery, and 3) may include cerebrospinal fluid (CSF) if it is being drawn for other studies, and 4) amniotic fluid [42]. Additionally serologic testing for ZIKV IgM ELISA and dengue IgM ELISA on infant and maternal serum, and if available infant CSF, is required. Serologic testing should be accompanied by PRNT for evaluation of ZIKV and dengue virus neutralizing antibodies. The newborn is determined to have congenital ZIKV infection if the RT-PCR test for ZIKA RNA on any of the submitted specimens, including amniotic fluid is positive, or infant serum or infant CSF is positive for ZIKV IgM with confirmatory PRNT per guidelines [42]. It is important to note that the specificity and sensitivity for RT-PCR in amniotic fluid is unknown [35]. All positive postpartum newborn and maternal Zika and dengue virus results are to be reported to the CDC [36]. All newborns diagnosed with microcephaly or intracranial calcifications should have periodic evaluations by specialists for long term sequelae associated with this diagnosis. Parents should be informed of appropriate agencies offering social service assistance and counseling and other support services for children with microcephaly and other brain anomalies,.

Prevention: Environmental and Personal Measures, Avoiding Travel to Active ZIKV Transmission Areas

In the absence of an available vaccine, developing other prevention strategies is key [14]. It is estimated that it could take approximately 18 months to develop a ZIKV vaccine. Prevention of ZIKV infection encompasses environmental and personal measures [17,43,44]. Shown in Table 4 are outlines environmental and personal prevention measures to reduce ZIKV infection exposure. Up to date travel notices posted by the CDC can be found at http://wwwnc.cdc.gov/travel/notices. [45]. This includes travel advisories for countries where active transmission of the ZIKV is occurring. This advisory is labeled with an "Alert Level 2, Practice Enhanced Precautions" [45]. The website lists countries with active ZIKV transmission, along with specific information on prevention measures, and advises postponing travel for pregnant women, along with measures to reduce sexual transmission.

(Insert Table 4)

Prevention of Sexual Transmission

Sexual transmission of the ZIKV is a non-vector-borne method of transmission [20,21]. Sexual transmission does appear to be a plausible risk in men harboring the ZIKV in their semen after infection due to a mosquito bite [18,19,20,21]. As mentioned previously in the **ZIKV Transmission** section there were two cases of sexually transmitted infection originally reported in the literature. By February 23, 2016, 14 additional cases have been reported to CDC for evaluation for possible sexually transmission. Six of them are still under investigation. Two cases were excluded after obtaining additional information and two of them are confirmed to have been sexually transmitted ZIKV infections. Four of the cases were probably sexually

transmitted. These women did not have any other recognized risk factors other than sexual intercourse with symptomatic male partners who had recently traveled to areas with ongoing ZIKV infections [46]. Despite being no longer detectable in blood, ZIKV was detected in semen at 62 days after onset of febrile disease . The virus however could not be cultured from semen [47]. One study reported that at greater than two weeks from onset of symptoms, the viral load in semen may be higher than that in blood or urine by a factor of 100,000 times [48]. This finding suggests that the ZIKV can continue to replicate in semen regardless of viremic status. Neither transmission of virus from asymptomatic men to their partner or transmissions of virus from infected women to their partners have yet been shown to occur. It is not known if ZIKV can be spread from other body fluids that may be exchanged during oral sex or anal intercourse or whether sexual transmission of ZIKV poses a different risk for congenital infection than that of mosquito-borne transmission. The theoretical risk of sexual transmission of ZIKV merits particular attention in pregnancy given the association between ZIKV infection and fetal brain anomalies [2,3,29,30,31,32]. The CDC, SMFM and ACOG have posted guidelines for prevention of sexual transmission [37,49]. Prevention of sexual transmission guidelines are particularly relevant to men living within or traveling to and from active ZIKV transmission areas who have a pregnant partner. The duration that ZIKV remains in semen is unknown, thus the period of shedding of the virus remains unclear. Infected males are therefore advised to abstain from sexual activity, or to consistently and correctly use a condom FOR THE DURATION OF THE PREGNANCY in order to reduce sexual transmission of ZIKV . Testing in men for the purpose of assessing transmission risk is unpredictable and of questionable value at this time and so has not been recommended [21,49]. This information is also provided in Table 4 for ease of use in a clinical setting.

Family Planning

Pregnancy planning remains a family decision. However a high percentage of pregnancies are unplanned. It is strongly recommended that heterosexual couples living in or traveling to an area of active ZIKV transmission be advised about the possible risk of infection overall and during pregnancy in particular, and the potential impact on fetal development. The risks of sexual transmission of the ZIKV should also be discussed. If postponement of pregnancy is elected, concomitant use of condoms along with additional means of birth control are recommended for women and their male sexual partners who live in or travel to areas with active ZIKV transmission (refer to Table 4). Several South American and Caribbean governments have advised women to postpone pregnancy altogether during ZIKV epidemic [17].

Biosafety

The CDC has established biosafety levels (BSL) as a means of identifying hazards of biological agents and appropriate environmental protection and personnel protection equipment required upon handling of such agents [50]. Levels range from 1 to 4, with BSL 4 involving the greatest risk and requiring the highest level of safety precautions. Zika and dengue viruses are classified as BSL 2 and are considered a moderate hazard to the environment and to personnel working with these pathogens. Chikungunya is classified as BSL 3 which includes pathogens or agents that may cause a lethal disease if inhaled [36,50]. Handling of these infectious pathogens must follow specific guidelines that include standard and specialized microbiological procedures, safety equipment, and other laboratory procedures. Precautions and safety measures of increasing stringency are required with each increasing BSL classification. The CDC recommends that until the associated risk of ZIKV infection and fetal brain anomalies is better

understood, pregnancy may be regarded as imposing a significant risk in women working in laboratories where such specimens are tested.

Recent Update

In mid February 2016 the CDC received reports on 9 pregnant women in the United States who had traveled to ZIKV infected areas during their pregnancy. The ZIKV disease was confirmed via laboratory evaluation in all 9 of the pregnant women [51]. The outcomes among the 9 pregnancies are as follows: 2 early pregnancy losses, 2 elective terminations (one reportedly had a fetal ultrasound evaluation noting absence of corpus callosum, ventrigulomegaly and the MRI noted severe brain atrophy), 2 pregnancies are continuing without known complications, 2 delivered healthy infants, and 1 delivered at 39 weeks with severe microcephly (case in Hawaii) [51,52]. Ten additional pregnancy cases are currently under investigation by the CDC [51].

On March 22, 2016 the Miniserio da Saude (Ministry of Health) of Brazil released updated information on reported cases of microcephaly and/or other CNS anomalies throughout the country [53]. A total of 6,671 cases of microcephaly and/or CNS anomalies have been reported since 2015.. As reported by the Ministry: there are 907 confirmed cases of microcephaly and/or CNS anomalies suggestive of congenital infection, and of those confirmed cases 122 test positive for the ZIKV Subsequently 1,471cases were determined to have noninfectious causes of microcephaly and/or CNS anomalies, or the condition did not meet the criteria for microcephaly and/or CNS anomalies. The remaining 4,293 cases are currently under investigation. There were 198 reports of stillbirth and or abortion with microcephaly and or CNS anomalies, and among these cases46 were confirmed to have the diagnosis of microcephaly and/or CNS anomalies, 130are under investigation and 22 were withdrawn from this list for unspecified reasons.

Recent data suggest a relationship between timing of infection and fetal effects. Two recent studies (early March, 2016) provided information about the relationship between the onset and of infection using statistical coincidence models. According to their model, microcephaly is likely more strongly related to first trimester or at least early second trimester ZIKV transmission. There is however no information about other associated congenital anomalies [54,55].

Conclusion

Any country in which mosquitoes of the genus *Aedes* are present could be a potential site for future Zika virus outbreaks. This includes southern Europe and the USA where *A. aegyptii and A. albopictus* have been spreading and where other competent mosquito species may already be present. Moreover, precautions need to be taken to avoid the pathogen entering public blood banks. Overall, ZIKV outbreak represents a serious potential threat to the fetus. While there is significant variation between different countries and regions within countries, disease trends put large numbers of women in the Americas and the Caribbean at risk. Mobilization of governmental and public health resources with the focus on prevention is urgently needed. Accelerated work on vaccine development is a clear priority. While the burden should not be placed exclusively on women of childbearing age, the risks and prevention strategies need to be communicated to them in an effective manner. Family planning is a critical tool that should be discussed with at risk individuals and families. Health care providers and agencies must work vigorously to inform the most vulnerable population - pregnant women and women of childbearing age - of the risks ZIKV poses to the fetus/newborn while minimizing alarm and panic.

Declaration of Interest
The authors report no conflict of interest.

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Table 1. Guide for Management of Pregnant Women with Possible ZIKV Exposure

Clinical symptoms listed below ⊙; Zika active area = region of active transmission of Zika virus

Noteworthy Detail: 74% (26/35) of mothers reported having a rash who subsequently had ZIKV (+) infants with microcephaly [32].

- **Patient has ≥ 2 symptoms consistent with ZIKV during or within 2 weeks of TRAVEL to ZIKV active area, & or sexual partner(s) have traveled to ZIKV active area.** Test serum for all 3 viruses - ZIKV, dengue, chikungunya by RT-PCR with onset of symptoms during previous week AND IgM & PRNT ≥ 4 days after onset of symptoms*

- **Patient is asymptomatic with history of TRAVEL to a ZIKV active area, & or sexual partner(s) have traveled to a ZIKV active area. Offer testing.** Test serum IgM, PRNT 2 weeks after travel if possible, but may be performed up to 12 weeks after travel.**

- **Patient has ≥ 2 symptoms consistent with ZIKV and resides in active ZIKV area.** Test serum for all 3 viruses - ZIKV, dengue, chikungunya by RT-PCR within 7 days of symptom onset AND IgM & PRNT ≥ 4 days after onset of symptoms***

- **Patient is aysmptomatic and resides in active ZIKV area.** Test IgM at initial prenatal appointment. If initial IgM (-) retest IgM in mid second trimester as ongoing risk of exposure/infection persists throughout pregnancy

- Advise men who live or travel to ZIKV, dengue, chikungunya active areas who have a pregnant partner: to abstain from sexual activity or consistently & correctly wear condoms for the duration of the pregnancy; obtain history on potential ZIKV exposure & symptoms; ZIKV testing in men for the purpose of assessing transmission risk is of uncertain value. §

- Fetal risk for exposure to ZIKV infection persists regardless of maternal (+) or (-) or inconclusive ZIKV lab results
  ◦ Refer to a Maternal-Fetal Medicine Specialist
  ◦ Targeted ultrasound @ 18-20 weeks GA†

- If maternal lab result (+) ZIKV or Inconclusive test*, or microcephaly or intracranial calcifications, or other brain anomalies present
  ◦ Consider amniocentesis for ZIKV testing by RT-PCR****
  ◦ Serial US q 3-4 weeks †
  ◦ Consider targeted fetal brain MRI screening ‡
  ◦ Retest patient for ZIKV infection whose previous ZIKV was (-)

- Optional serial US q 3-4 weeks if maternal ZIKV screen (-) & normal targeted US @ 18-20

- Repeat ZIKV testing if clinical symptoms of ZIKV infection develop later in pregnancy

Table 1 Footnotes

⦿Symptoms: acute onset of mild fever (<38.5℃), mild-moderate arthralgia (small joint of hands & feet), nonpurulant conjunctivitis or conjunctival hyperemia, maculopapular rash usually spreading downward from the face to the limbs & may be pruritic, & nonspecific symptoms i.e. headaches, myalgia, asthenia. Symptoms usually resolve in 7 days. [14,216]

*Evidence of ZIKV infection: a positive RT-PCR test detecting ZIKV in any type of clinical specimen, OR 2) positive ZIKV IgM with PRNT evaluation with IgM neutralizing antibody titers ≥ 4-fold higher than dengue virus neutralizing antibody titers in serum. IgM neutralizing antibody titers that are < 4-fold higher than dengue virus neutralizing antibody titers in serum are considered inconclusive [36]. Zika, dengue or chikungunya viruses are frequently active in similar geographic locations, and the can complicate risk assessment. [35,36]

**A positive IgM test may be difficult to interpret. IgM results can persist up to 12 weeks after viral exposure, & may cross-react with other flavirus IgM. PRNT tests may be difficult to interpret due to previous exposure to flavivrius in a patient whose had previous flavivirus infection or was vaccinated against flavivirus. If IgM is NEGATIVE it would suggest that a ZIKV infection did not occur BUT IT IS NOT DEFINITIVE. [35,36].

***A negative RT-PCR result that was drawn within 5-7 days of symptoms doesn't R/O ZIKV infection as viremia decreases over time. A false positive IgM may occur due to previous exposure to a flavivirus. [36].

****Amniocentesis is not recommended until after 15 weeks GA. Risk for microcephaly or other fetal anomalies when amniotic fluid RT-PCR is (+) for ZIKV RNA is unknown. [35]

†Initial targeted US at 18-20 weeks permits evaluation of fetal growth consistent with pregnancy dating, careful evaluation of fetal anatomy, and to R/O fetal brain anomalies. Follow-up with targeted serial US q 3-4 weeks [26, 31,32,35,37,38,39].

‡For MRI considerations refer to the MRI section in the text of this article [40,41]].

§ Sexual activity is defined as inclusive of vaginal & anal intercourse & fellatio. The incidence of ZIKV in semen in infected men is based on 1 case report with laboratory confirmation, and possibly 2 other case reports at this time[18,20,21,37,46].

Updated 022116

Table 2. Targeted Ultrasound Exam in Pregnant Women with Possible ZIKV Exposure*
Using a Uniform & Systematic Approach.
Evaluate for Anomalies Reported in Existing Case Reports**‡

Assess for microcephaly†
• Biometry of the head, including head circumference measurements

Assessment for brain atrophy involving frontal lobes
• Frontal-thalamic distance in the fetus has been used for the detection of frontal lobe hypoplasia in the mid-trimester fetus and could be considered.
• Lateral ventricular measurement of both ventricles will be useful in the assessment for unilateral ventriculomegaly [38].
• Trans-cerebellar diameter measurement should also be performed at each assessment for the detection of cerebellar hypoplasia, a reported finding in congenital ZIKV.
• Routine measurement of the cisterna magna will also provide information on cerebral hypoplasia and enlarged cisterna magna in these cases.
• Increased scalp redundancy as a result of reduced head growth has been reported. Mid-trimester nuchal thickness measurement frequently used for Down syndrome risk assessment may be of value in identifying this scalp redundancy in the mid-trimester.

Evaluation to detect brain calcifications. Evaluate the entire brain.
• Conduct qualitative evaluation particularly around the lateral and forth ventricles
• Frontal lobes
• Cerebellum

Subtler findings (likely limited accuracy for the average examiner)
• Abnormal cerebral gyral patterns
• Hypoplasia of the thalami and brain stem
• Evaluate for evidence of cataract
• Evaluate for evidence of microophthalmia
• Evaluate for calcifications of the eye (has been reported)

Evaluate to detect placental calcifications

Overall fetal growth should be assessed

• Assess limb posture and movement
• Clubbing of the foot should be assessed   (given a report of arthrogryposis, hypertonia and spasticity in newborns [32]

• Assess brainstem for abnormalities

• Assess for potential fetal consequences of difficulty swallowing: polyhydramnios and absent or reduced stomach bubble

Table 2 Footnotes

* We suggest serial targeted anatomy US exams starting at 18-22 weeks & repeated every 3-4 weeks depending on risk status or diagnosis of infection. Several caveats should be borne in mind - an infected patient may in fact have a negative ZIKV test. Thus high-risk status and the need for US evaluation must be based on a combination of testing results, symptomatology, travel history, partner travel history and of course residence in an epidemic area. Specifically, evaluation of the fetus for anomaly should be undertaken regardless of whether or not there is evidence of actual maternal infection but rather based on risk of maternal exposure. [31,32,35,37,38,39]

**Current interim CDC guidelines do not recommend continuation of serial US surveillance in women with a negative test in which the fetus does not have microcephaly or brain calcifications [35]. However, the best currently available data indicate that over 25% of congenital infection cases do not have microcephaly, likewise over 25% of congenital cases do not have brain calcifications- thus negative findings on US screening DO NOT guarantee an unaffected fetus [32].

†Prenatal US exam, particularly that performed earlier in pregnancy, is less sensitive for microcephaly than newborn measurements used to assess the cases referenced above

‡Positive US findings may only manifest or be appreciated after **serial examinations** and later in the pregnancy. These observations argue in favor of serial US evaluations in those determined to be at high enough risk based on laboratory, history or epidemiologic factors.

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Table 3. Guide for Symptom Relief in Confirmed ZIKV Infection in Pregnancy

ZIKV incubation period: 3-12 days after bite. Illness usually mild - resolves in 2-7 days.
Primary Treatment Focus: Symptom Relief

Alert: Diagnosis of a ZIKV infection CANNOT be based on symptomatology alone. Laboratory assessment is required. Refer to Table 1 for laboratory screening and evaluation.

Prevent further transmission. Avoid exposure to Aedes mosquitoes during viremic phase (usually during 1st week of the disease). Refer to Table 4 ZIKV & Personal Protection

- **Fever with acute onset (< 38.5℃, 101.3°F). Primary Intervention:** light clothing, damp cloths, lukewarm shower or bath. **Secondary Intervention:** Acetaminophen (325 mg tabs) 650 mg every 4-6 hours, not to exceed 4000 mg/day. Avoid use of - ASA (acetlysalicylic acid), nonsteroidal anti-inflamatory (NSAIDs)†

- **Insensible fluid volume depletion (may occcur from vomiting, sweating, reduced intack due to nausea).** Maintain hydration with clear broth & juices.

- **Pain:Mild to moderate arthralgia (small joints of hands and feet), myalgia (muscle pain):** Acetaminophen (325 mg tabs) 650 mg every 4-6 hours, not to exceed 4000 mg/day. Avoid use of - ASA, NSAIDs†

- **Asthenia (abnormal weakness, lack of energy).** Promote rest.

- **Maculopapular rash (flat , raised lesion) usually spreading downward from the face to the limbs & may be pruritic (cause itching).** Calamine lotion, or menthol based aqueous cream. May advise patient to wear gloves while asleep to prevent *

- **Nonpurulant conjunctivitis or conjunctival hyperemia.** Loratadine 5 mg every 12 hours or 10 mg every 24 hours may reduce symptoms of "red, watery, itchy eyes" **

†ASA (aspirin) & NSAIDS are contraindicated in pregnancy as they increase the risk for bleeding, & are associated with hemorrhage & death in dengue viral infections. A dengue infection may occur concomitantly with Zika - Refer to Table 1 for information on screening for arbovirus (Zik, dengue, chikungunya) infections in pregnancy. [17, 35]
* There is no published data to support or refute use. Safety in pregnancy is based on clinical experience. [17]
** Loratadine is a FDA Catagory B medication and is used in pregnancy.

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Table 4. Reducing Risk of ZIKV Infection

MOSQUITO BITE PREVENTION
Environmental Measures [17,44]
• chemical mosquito control, & the elimination of conditions such as pooling/standing water (inside or outside of the home) that provide breeding grounds for the *Aedes* mosquitoes [17,42].

Personal Measures [17,43,44]
• covering exposed skin with clothing
• use of screens on windows and doors
• use of repellants which are deemed safe & OFFER THE HIGHEST SAFETY LEVEL IN PREGNANCY such as DEET, Picaridin (Icaridin, Bayrepel), IR3535 (ethyl butylacetylaminopropionate).
  -These repellants should be applied to exposed skin and over clothing (not under clothing), if using sunscreen apply sunscreen first then repellent.
  -Oil of lemon eucalyptus is often listed as an optional insect repellent but should be AVOIDED in pregnancy and breastfeeding [43]. The Environmental Protection Agency (EPA) has not evaluated natural insect repellents [44].
• Avoidance of subsequent mosquito bites by following the above recommendations prevents transmission of the disease to others [17].

PREVENTION OF SEXUAL TRANSMISSION [37,46]
• Men living within or traveling to & from active ZIKV transmission areas who have a pregnant partner are advised to abstain from sexual activity, or consistently and correctly use a condom FOR THE DURATION OF THE PREGNANCY to reduce ZIKV transmission during sexual activity
• Men living within or traveling to and from active ZIKV transmission areas who are concerned about sexual transmission may consider using condoms consistently and correctly to reduce transmission risk
• No guidelines exist for the recommended duration of condom use with a non-pregnant partner once outside of the ZIKV active area
• Testing in men for the purpose of assessing transmission risk is unpredictable and of questionable value.
• If postponement of pregnancy is elected, concomitant use of condoms & an additional means of birth control are recommended for women & their male sexual partners who live in or travel to & from an area with active ZIKV transmission

TRAVEL PRECAUTIONS
• Up to date travel notices posted by the CDC can be found at http://wwwnc.cdc.gov/travel/notices. Enhanced precautions are in effect. Pregnant women should consider postponing travel [45].

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Key Words/Associated Search Terms

Pairing 'ZIKV' or Zika virus with the following terms: 'epidemic', 'infection', 'pregnancy', 'fetal/newborn congenital anomalies', 'vector/non-vector-borne transmission', 'infection prevention', 'screening in pregnancy', "management in pregnancy', 'pregnancy and ultrasound', 'pregnancy and MRI', 'screening fetal brain anomalies', 'screening fetal microcephaly', 'sexual transmission risk and prevention', 'breastfeeding', 'symptom management in pregnancy'.