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TERATOGEN UPDATE

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Teratogen update: Zika virus and pregnancy

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Abstract

Zika virus was first identified in Uganda in 1947 but received little attention until 2015 when a large outbreak of Zika virus illness followed by an increased number of babies born with microcephaly occurred in Brazil. Zika virus spread rapidly throughout the Americas, and in 2016 was identified as a cause of microcephaly and other serious birth defects. Since that time, much has been learned about the Zika virus. The virus is primarily spread by the bite of *Aedes* species mosquitoes; however, other forms of transmission (e.g., sexual and intrauterine) have been recognized. Although postnatal Zika virus infection typically causes mild or no symptoms, effects on infants born to prenatally infected mothers can be severe and include structural birth defects and neurodevelopmental effects. The risk of a structural birth defect among infants born to mothers with confirmed or suspected Zika virus infection during pregnancy has ranged from 5 to 10%. The timing of Zika infection during pregnancy affects risk, with higher risks with the first-trimester infection. Neurodevelopmental effects are seen even in infants who appear normal in the newborn period. Although cases of Zika virus infection have fallen in the Americas, the Zika virus remains an active threat in some regions of the world. The development of a Zika vaccine will require continued focus and investment. Until a Zika vaccine is available, prevention efforts for pregnant women include avoidance of travel to areas with active Zika transmission, avoidance of mosquito bites for those living in or traveling to areas with Zika transmission, and protection against sexual transmission.

KEYWORDS

microcephaly, pregnancy, teratogen, Zika, zika virus

1 | INTRODUCTION

Although Zika virus was first identified in Uganda in 1947, it was not recognized as a cause of birth defects until 2016, after a large outbreak in Brazil led to a marked increase in the number of cases of microcephaly and other defects (Rasmussen, Jamieson, Honein, & Petersen, 2016). In recent years, much has been learned about the Zika virus, its transmission, adverse outcomes caused by maternal Zika virus infection during pregnancy, and ways to prevent Zika virus infection. However, many questions remain. In this Teratogen Update, we will review what is known about the Zika virus and its effects during pregnancy and will highlight gaps in knowledge that require future study.

2 | ZIKA VIRUS: HISTORY, CLINICAL FINDINGS, TRANSMISSION, AND EPIDEMIOLOGY

Zika virus is an RNA virus in the family *Flaviviridae*, closely related to several other arboviruses, including dengue, yellow fever, Japanese encephalitis, and West Nile viruses. The virus was first identified in 1947 as part of a sentinel monkey study of yellow fever in the Zika forest in Uganda (Dick, Kitchen, & Haddow, 1952), and was later documented to cause human illness in 1953 in Nigeria (Macnamara, 1954). Although subsequent serologic evidence suggests that human Zika virus infection had spread widely in Africa and Asia, the condition attracted little attention for more than 60 years. In 2007, the US Centers for Disease Control and Prevention (CDC) conducted an investigation of an outbreak of rash, conjunctivitis, fever, arthralgia, and arthritis on Yap Island in the Federated States of Micronesia, which was identified to be caused by Zika virus (Duffy et al., 2009). Based on serologic analysis, 73% of the island's population of nearly 7,000 inhabitants were estimated to have been infected during the outbreak. *Aedes hensilli* was identified as the most common type of mosquito responsible for transmission during this outbreak.

Based on the epidemiologic investigation on Yap Island (Duffy et al., 2009), clinical findings of Zika virus infection were found to be generally mild and included a macular or papular rash (in 90% of patients), fever (65%), arthritis or arthralgia (65%), nonpurulent conjunctivitis (55%), myalgia (48%), headache (45%), edema (19%), and vomiting (10%). Among those who tested positive for Zika serology (immunoglobulin M [IgM]), only 18% reported a clinical illness likely to be caused by the Zika virus, suggesting that as many as ~80% of patients had an asymptomatic disease. Later data from Brazil confirmed the mild symptomatology (Brasil et al., 2016); deaths due to postnatal Zika virus infection are rare (Ximenes et al., 2019).

The final paragraph of the paper describing the Yap Island experience foreshadowed the future: the authors noted that the ease of air travel and the abundance of mosquito vectors raised concern for Zika virus transmission to other Oceania islands and "even to the Americas" (Duffy et al., 2009). The authors emphasized the need for vigilance and surveillance systems to detect the transmission of infectious diseases.

The next major outbreak of the Zika virus illness occurred in French Polynesia in 2013–2014 with an estimated 32,000 persons infected (Petersen, Jamieson, et al., 2016). Although the clinical findings in this outbreak were similar to those seen on Yap Island, an increased number of cases of Guillain–Barre syndrome was seen following Zika virus infection (L. R. Petersen, Jamieson, et al., 2016). A later case-control study examining the association between Guillain–Barre syndrome and recent Zika virus infection (documented by the presence of neutralizing antibodies against Zika) conducted in French Polynesia demonstrated an odds ratio of >34 (odds ratio 34.1, 95% confidence intervals [CI] 5.8-infinity) (Cao-Lormeau et al., 2016). Outbreaks on several other Pacific islands were later seen (L. R. Petersen, Jamieson, et al., 2016).

Zika virus was first identified in the Americas in Bahia, Brazil in March of 2015 (Campos, Bandeira, & Sardi, 2015), although phylogenetic data suggest that the Zika virus had been introduced into the Americas more than 12 months earlier (Faria et al., 2016). The virus rapidly spread throughout Brazil. In September of 2015, an increased number of infants born with microcephaly was noted in Brazil in areas affected by the outbreak (Schuler-Faccini et al., 2016). A similar increase was retrospectively identified in French Polynesia following the 2013–2014 outbreak there (Cauchemez et al., 2016). This evidence led to the declaration of a Public Health Emergency of International Concern by the director-general of the World Health Organization on February 1, 2016 (World Health Organization, 2016). Additional cases continued to be reported, with evidence that many affected infants had a distinct phenotype consistent with the fetal brain disruption sequence, rarely seen prior to the Zika outbreak (Corona-Rivera et al., 2001). In early 2016, a review of available evidence was conducted using Shepard's criteria (a framework to assess teratogenicity; Shepard, 1994) and Bradford Hill criteria (a framework to assess causation) (Hill, 1965) and concluded that a causal relationship existed between Zika virus infection during pregnancy and microcephaly and other serious brain defects (Rasmussen et al., 2016). Since the publication of this paper, additional evidence has become available supporting this finding, including epidemiologic data (de Araujo et al., 2018), data from pregnancy registries in the United States and its territories (Reynolds et al., 2017; Rice et al., 2018), and animal models (Dong & Liang, 2018).

The primary mode of transmission of Zika virus is through the bite of *Aedes* mosquitoes (Hills, Fischer, & Petersen, 2017), most commonly *Aedes aegypti* and *Aedes albopictus*. Other modes of transmission include intrauterine (Calvet et al., 2016), sexual (Russell et al., 2017), perinatal (Besnard, Lastere, Teissier, Cao-Lormeau, & Musso, 2014), laboratory (Filipe, Martins, & Rocha, 1973), probably through transfusions (Barjas-Castro et al., 2016), and possibly through breast milk (Colt et al., 2017).

Zika virus spread from Brazil to other countries in South America, eventually with mosquito-borne

transmission to 87 countries and territories throughout the world (Musso, Ko, & Baud, 2019), including in the United States (US) with limited transmission in states (Florida and Texas) and widespread transmission in territories (Puerto Rico, US Virgin Islands, and American Samoa). Transmission in the Americas dropped significantly in late 2016, presumably related to sufficient herd immunity in areas of widespread transmission (Ribeiro et al., 2020). Modeling studies from work on the chikungunya virus suggest that sufficient herd immunity could suppress the widespread circulation of the Zika virus for at least 10 years (Ferguson et al., 2016). However, cases continue to be reported to the Pan American Health Organization in 2019 from areas of Central and South America (Ribeiro et al., 2020). Other areas of the world (e.g., southeast and south Asia) have reported more recent outbreaks, suggesting that Zika virus remains an active threat to pregnant women and their infants in some regions of the world (Duong, Dussart, & Buchy, 2017; Grubaugh, Ishtiaq, Setoh, & Ko, 2019; Wongsurawat et al., 2018).

Laboratory testing for maternal Zika virus infection has been complex (Rabe et al., 2016). Testing for Zika virus RNA (nucleic acid testing) is reliable, but RNA is only transiently present in body fluids (for a few days before and after the onset of symptoms in patients that are symptomatic). Thus the timing of testing is critical, and negative nucleic acid testing cannot rule out infection. Cross-reactivity with other flavivirus infections is a limitation of serologic testing, raising the possibility of false-positive results because of a previous infection with or vaccination for other flavivirus infections. As with nucleic acid testing, the timing of serologic testing is important. A negative test for IgM might have occurred because testing was too early (before the development of IgM antibodies) or too late (after waning of IgM levels), although the length of IgM persistence is unclear. In addition, it is difficult to determine if a positive IgM test during pregnancy is due to recent infection or because antibodies can remain from an infection that occurred before pregnancy. The issues of testing become even more complicated when the prevalence of Zika virus infection is low because lower disease prevalence results in a lower positive predictive value (and a higher probability of false-positive test results; Adebanjo et al., 2017).

The testing of infants born to mothers with Zika virus infection during pregnancy is also challenging (Adebanjo et al., 2017). Nucleic acid testing for Zika virus RNA in infant serum and urine and antibody testing for Zika virus IgM antibodies in serum are recommended, but results can be difficult to interpret. If nucleic acid testing is positive, congenital Zika virus infection is confirmed; however, a negative result does not exclude infection since the length of viral shedding in an infant with congenital Zika virus infection is not well understood. Positive IgM results can be helpful, although false-positive results can occur from cross-reacting IgM antibodies or nonspecific reactivity. If the cerebrospinal fluid is obtained for other testing, it can be tested for nucleic acid and IgM antibody since the cerebrospinal fluid has been the only specimen that has tested positive in some infants. Cord blood specimens should not be used for testing because both false-positive and false-negative tests have been seen. Testing of infants suspected to have congenital Zika virus infection should be performed as early as possible because both Zika virus RNA and IgM antibodies wane over time.

Whether Zika virus-associated birth defects were a new phenomenon related to genetic changes in the virus or whether the connection between the Zika virus and birth defects had previously gone undetected is unknown. Genetic and in vitro studies have suggested that mutations in the Zika virus might be responsible for increased virulence of Zika virus to human neural progenitor cells or increased transmission (Y. Liu et al., 2017; Z. Y. Liu, Shi, & Qin, 2019; Rossi, Ebel, Shan, Shi, & Vasilakis, 2018; Yuan et al., 2017). However, it should be noted that strains without the mutation can also lead to microcephaly in mice and humans (Grubaugh et al., 2019; Moi et al., 2017; Wongsurawat et al., 2018; Yuan et al., 2017). The possibility that the connection between previous cases of microcephaly and other birth defects and the Zika virus went undetected is also possible. A recent report (Chu et al., 2018) described two children with clinical and radiologic features of congenital Zika syndrome born in Cambodia several years before the outbreaks in French Polynesia and Brazil. The mothers of these children had symptoms of Zika virus infection during early pregnancy and serologic testing consistent with previous Zika infection, suggesting that the connection between the Zika virus and birth defects might have been missed in the past. The fact that the increased cases of microcephaly and other birth defects during the French Polynesia outbreak were not recognized until after concern had been raised in Brazil (Cauchemez et al., 2016) provides support for the hypothesis that the relationship had previously been undetected. One possibility is that surveillance systems in place in countries previously affected by Zika outbreaks might not have been sufficiently developed to identify an increase in a rare defect such as microcephaly. It is also possible that in areas with the endemic spread of Zika virus, most women are immune by the time they reach childbearing age, decreasing the occurrence of Zika-associated microcephaly, and other birth defects in these populations.

3 | ZIKA VIRUS AND BIRTH DEFECTS

While early on following the outbreak in Brazil, the focus regarding an increased risk of birth defects was on microcephaly (Pan American Health Organization, 2015), it was rapidly recognized that other findings, including brain calcifications, evidence of cell migration abnormalities, ventricular enlargement, redundant scalp skin, and arthrogryposis, were often seen (Schuler-Faccini et al., 2016). The recognition that some infants with congenital Zika virus infection had features of the fetal brain disruption sequence, a phenotype characterized by severe microcephaly, overlapping cranial sutures, redundant scalp skin, and neurological impairment that had been rarely reported before the Zika outbreak (Corona-Rivera et al., 2001), was essential to confirmation of Zika virus as a cause of birth defects (Rasmussen et al., 2016). A characteristic phenotype called the congenital Zika syndrome was later described that includes five features: (1) severe microcephaly with the partially collapsed skull; (2) thin cerebral cortices with subcortical calcifications; (3) ophthalmologic findings, including macular scarring and focal pigmentary retinal mottling; (4) congenital contractures; and (5) early hypertonia and manifestations of extrapyramidal involvement (Table 1; Moore et al., 2017). These features help to distinguish congenital Zika syndrome from other congenital infections; however, it was recognized that this phenotype likely represented only a portion of a broader spectrum of defects due to prenatal Zika virus exposure.

TABLE 1 Characteristic findings of congenital Zika syndrome

Location	Findings
Skull	Severe microcephaly with a partially collapsed skull with overlapping sutures, small or absent fontanel, occipital bone prominence, scalp rugae
Brain	Thin cerebral cortex, intracranial calcifications, primarily subcortical in location, hydrocephalus, hydranencephaly, gyral abnormalities (most consistent with polymicrogyria), absent or hypoplastic corpus callosum, hypoplasia of the cerebellum or cerebellar vermis
Eye	Microphthalmia, coloboma, cataracts, intraocular calcifications, optic nerve hypoplasia and atrophy, chorioretinal atrophy and scarring, macular pallor, gross pigmentary anomalies generally in the macular area
Joints	Congenital contractures—Isolated or multiple (arthrogryposis) joints—can be proximal or distal, upper or lower limb
Neurologic	Motor and cognitive impairment, significant early hypertonia and extrapyramidal symptoms, seizures, swallowing difficulties, cortical visual impairment, hearing impairment

Note: Adapted from Moore, C. A., Staples, J. E., Dobyns, W. B., Pessoa, A., Ventura, C. V., Fonseca, E. B.,..., Rasmussen, S. A. (2017). Characterizing the pattern of anomalies in congenital Zika syndrome for pediatric clinicians. *JAMA Pediatrics*, *171*(3), 288–295. doi:10.1001/jamapediatrics.2016.3982.

As additional information became available, the phenotype associated with the Zika virus has been modified. In most cases, the new information added other structural findings that could be seen in children whose mothers were infected with Zika virus infection during pregnancy, and include the finding that hydrocephalus and microcephaly, both of postnatal onset, could be seen in infants without these findings at birth (Juca et al., 2018; V. van der Linden et al., 2016). Another defect more recently identified as associated with congenital Zika virus infection is diaphragmatic paralysis due to phrenic nerve palsy (Rajapakse et al., 2018; V. van der Linden et al., 2019). This finding was important not only for the care of infants since rapid identification of this complication can lead to better medical care, but it also provided evidence of involvement of the peripheral nervous system in some infants with congenital Zika virus infection. At least two studies have reported an increased frequency of congenital heart defects among children with prenatal Zika virus exposure (Cavalcanti et al., 2017; Orofino et al., 2018). However, given that these defects have been mild in nature (e.g., secundum atrial and muscular ventricular septal defects), screening for these defects among Zika-exposed infants is not recommended. Information suggesting that certain defects are not caused by prenatal Zika virus infection is also emerging: neural tube defects and other early brain malformations were initially thought to be part of the spectrum of defects caused by Zika virus infection during pregnancy; however, recent data suggest that these defects are unlikely to be part of this spectrum (Delaney et al., 2018; Smoots et al., 2020).

The frequency of Zika virus-associated structural birth defects among infants following maternal infection with Zika virus during pregnancy has been estimated to be ~5–10% in most studies (Hoen et al., 2018; Reynolds et al., 2017; Rice et al., 2018), although a study from Brazil found a much higher proportion of adverse outcomes (Brasil et al., 2016). The study from Brazil enrolled pregnant women with an acute febrile illness with a rash, of which 182 women tested positive by nucleic acid testing. Among the offspring of women testing positive for Zika virus, 46% had adverse outcomes compared to 11.5%

among offspring of women testing negative ($p < .001$). Grossly abnormal clinical or brain imaging findings were seen in 42% of live infants, including four (3.4%) infants with microcephaly. The higher percentage of abnormalities, compared to other studies, is hypothesized to be related to the broad case definition used, which included findings on neuroimaging for which the clinical significance is unclear (Honein & Jamieson, 2016).

In contrast, analysis of data from the US Zika Pregnancy Registry from January 15 to December 27, 2016, showed Zika virus-associated birth defects in 51 of 972 (5%) of pregnancies with possible recent Zika virus infection in the 50 US States and District of Columbia. When the analysis was limited to completed pregnancies with laboratory-confirmed Zika virus infection, the proportion was higher, with birth defects reported in 10% (24 of 250 pregnancies). For this analysis, Zika virus-associated birth defects included brain abnormalities and/or microcephaly, neural tube defects, and other early brain malformations, eye abnormalities, or other consequences of central nervous system dysfunction (Reynolds et al., 2017). (As noted earlier, neural tube defects and other early brain malformations were later determined to not be part of the spectrum of Zika-associated birth defects.) A similar proportion was seen in data on children born in the US territories and freely associated states; in this analysis, 6% had at least one Zika-associated birth defect (Rice et al., 2018).

Results from a study from the French territories of the Americas were consistent with these findings (Hoen et al., 2018); among 546 pregnant women with symptomatic Zika virus infection, neurologic, and ocular defects presumed to be associated with Zika virus infections were seen in 39 fetuses and infants (7%). Thirty-two fetuses or infants (5.8%) had microcephaly, defined as a head circumference more than two standard deviations below the mean; of these, nine (1.6%) had severe microcephaly (>3 standard deviations below the mean). Seventeen fetuses or infants (3.1%) met the definition of congenital Zika syndrome.

The factors that affect the risk of birth defects are not fully understood. As is typical with teratogenic exposures, timing during pregnancy is critical. In the US Zika Pregnancy Registry of births occurring in the 50 US states and the District of Columbia, a higher proportion of fetuses or infants born to mothers infected during the first trimester of pregnancy had birth defects, compared to other pregnancy trimesters. Among mothers with possible Zika virus infection in the first trimester, 9% had reported birth defects; when limited to women with laboratory-confirmed Zika virus infection, 15% whose mothers were exposed in the first trimester were affected. In the study from the French territories in the Americas, maternal infections during the first trimester were shown to result in higher risk (12.7%) of neurologic and ocular abnormalities than those during the second or third trimesters (3.6% and 5.3%, respectively) (Hoen et al., 2018). An analogous relationship with pregnancy trimester was seen for severe microcephaly (3.7, 0.8, and 0.0%, respectively; $p = .02$) and for meeting the definition of congenital Zika syndrome (6.9, 1.2, and 0.9%, respectively; $p = .002$).

A recent systematic review identified three factors that affected the risk of microcephaly in an infant: infant sex (males were more likely to be affected than females, with a relative risk of 1.30, 95% CI 1.14–1.49), trimester of pregnancy (infection in the first trimester, compared to other trimesters, with a relative risk of 1.41, 95% CI 1.09–1.82), and presence of symptoms (asymptomatic compared to symptomatic infection during pregnancy, with a relative risk of 0.68, 95% CI 0.60–0.77; Gallo et al., 2020). Several other possible factors have been proposed, including maternal age, ethnicity, and nutritional status, but insufficient evidence is available to determine if these factors have an effect.

4 | ZIKA VIRUS AND OTHER ADVERSE OUTCOMES

Although the relationship between Zika virus infection during pregnancy and microcephaly and other birth defects is clear, the association with other adverse outcomes is sometimes less clear. Given the high proportion of normal pregnancies that end in spontaneous abortions and the challenges of their ascertainment (Lidegaard et al., 2020), documenting an association between a potential teratogen and pregnancy loss is difficult. Data are conflicting with regard to the Zika virus during pregnancy and pregnancy loss. Cases of women with spontaneous abortion following Zika virus infection have been reported (Gonce et al., 2018; van der Eijk et al., 2016); however, other studies do not support an association. For example, in the study by Brasil et al., 7% of both the Zika-affected and the Zika-unaffected pregnancies ended in spontaneous abortion or fetal death (Brasil et al., 2016). In the study from Hoen and colleagues, 28 of 546 pregnancies (5%) were not carried to term or were stillborn, including 11 miscarriages, 10 pregnancy terminations for medical reasons, six stillbirths, and one pregnancy termination for nonmedical reasons; however, no comparison group was included in this study (Hoen et al., 2018).

In addition to the structural birth defects seen among offspring born to mothers with Zika virus infection during pregnancy, nonstructural abnormalities are also a consequence of prenatal Zika virus exposure. Among children 1 year or older born in the US territories and

freely associated states to mothers with possible or laboratory-confirmed Zika virus infection during pregnancy, 9% had one or more Zika-associated neurodevelopmental abnormality (Rice et al., 2018). These abnormalities were detected from birth until age 2 years and included hearing abnormalities, congenital contractures, seizures, hypertonia or hypotonia, movement or swallowing abnormalities, possible developmental delay or a visual impairment, and postnatal onset microcephaly. When the analysis was limited to babies born to mothers with laboratory-confirmed Zika virus infection, 10% (99/943) had a Zika-associated neurodevelopmental abnormality.

Nielsen-Saines and colleagues recently published data on neurodevelopmental outcomes in 216 infants born to pregnant women who presented with a rash-like illness and tested positive for Zika virus RNA during the 2015–2016 Zika virus epidemic in Rio de Janeiro, Brazil (Nielsen-Saines et al., 2019). Among these infants, about a third (31.5%) between 7 and 32 months of age had below-average neurodevelopment based on Bayley Scales of Infant and Toddler Development, third edition (Bayley-III) and/or abnormal eye or hearing assessments. The language domain was most affected with 35% of children scoring below average. Among the 18 children (12% of those tested with Bayley-III) who scored more than two standard deviations below the median, six had microcephaly, and three developed autism spectrum disorder. Two children had microcephaly that resolved; both had normal neurodevelopment on testing. Factors that appeared to predict abnormal neurodevelopment were abnormal eye exams, preterm birth, male sex, and gestational age at infection, with better outcomes among mothers infected later in pregnancy.

In a recent study (Mulkey et al., 2020), 70 infants without evidence of congenital Zika syndrome born to Colombian mothers with Zika virus infection during pregnancy underwent neurodevelopmental assessments between 4 and 8 months of age (57%) and between 9 and 19 months of age (86%). These assessments showed scores decreased from normative scores as the children grew older, suggesting that long-term developmental monitoring is needed for infants born to mothers with prenatal Zika infection, even among those babies that appear normal at birth. The study also showed that nonspecific findings on brain imaging (e.g., lenticulostriate vasculopathy, germinolytic or subependymal cysts, and choroid plexus cysts) might be predictive of worse neurodevelopmental outcomes.

To evaluate the frequency of epilepsy among infants with congenital Zika virus infection, van der Linden evaluated 141 infants with congenital or acquired microcephaly, calcifications on neuroimaging, or unexplained developmental delay who also had congenital Zika virus infection confirmed by laboratory analysis. Among the infants examined at a mean age of 9 months (range 1–14 months), 67% were diagnosed with epilepsy, with a mean age of onset of 4.9 months. The main seizure types were epileptic spasms (72%), focal motor seizures (21%), and tonic seizures (4%). All required antiepileptic drugs with 56% requiring more than one medication, and seizure control was achieved in 65% (H. van der Linden Jr. et al., 2018).

To better understand the effects of congenital Zika virus infection beyond infancy, CDC collected data on children included in a microcephaly registry in Paraiba, Brazil, including 19 children who were 19–24 months of age who had microcephaly and confirmed or probably Zika virus infection (Satterfield-Nash et al., 2017). Among these children, 11 (58%) had seizures; 4 (21%) had retinal abnormalities; 8 (42%) had required hospitalization for pneumonia, bronchitis or other issues; 10 (53%) had sleeping difficulties, 9 (47%) had feeding difficulties, 13 (68%) had impaired response to auditory stimuli, and 11 (58%) had impaired response to visual stimuli. Fifteen (79%) had a severe motor impairment, with 14 (74%) diagnosed as having cerebral palsy. These findings emphasize the longer-term consequences of Zika virus infection among infants with congenital microcephaly.

5 | PREVENTION OF THE ADVERSE OUTCOMES CAUSED BY PRENATAL ZIKA VIRUS INFECTION

Prevention of the prenatal effects of the Zika virus infection has been a priority since early in 2016, even before the Zika virus was confirmed as a cause of birth defects. On January 15, 2016, based on evidence linking the Zika virus to the marked increase in the number of babies born with microcephaly in Brazil, CDC recommended that pregnant women consider postponing travel to areas with ongoing Zika virus transmission (Hennessey, Fischer, & Staples, 2016). As the association became more clear, pregnant women were advised not to travel to areas with active Zika virus transmission (CDC Zika Response, 2016). Women who lived in areas of active Zika virus transmission or who must travel to an area with Zika transmission were advised to avoid mosquito bites by using mosquito repellents, wearing long-sleeved shirts and pants, and preventing mosquitoes from entry into homes through screening of windows and doors, closing windows, and using air conditioning (Dirlikov et al., 2016). Although the numbers of cases of Zika virus infection have decreased, these recommendations

regarding travel and protection in areas with active transmission remain in place (American College of Obstetricians and Gynecologists Committee, 2019; Centers for Disease Control and Prevention, 2020). Information on areas with active Zika virus transmission is maintained on the CDC travel website (Centers for Disease Control and Prevention, 2020).

Mosquito control methodologies might be helpful in protecting communities. These include preventing breeding by reducing the larval habitat (e.g., by limiting standing water) and aerial spraying using insecticides or larvicides. Other methods include the release of mosquitoes infected with the bacteria Wolbachia (which make it more difficult for viruses to reproduce inside mosquitoes) and strategies to genetically modify mosquitoes (e.g., the release of genetically modified male mosquitoes that produce nonviable offspring when they mate with female mosquitoes) (Flores & O'Neill, 2018).

After the recognition of sexual transmission of Zika virus, CDC expanded its recommendations to advise that pregnant women with a sex partner who had traveled to or lives in an area with active Zika virus transmission either abstain from sex or use condoms for the remainder of the pregnancy (Brooks et al., 2016). For couples planning a pregnancy, recommendations to prevent infection around the time of conception were developed, given theoretical concerns for periconceptional transmission. If a female partner had possible Zika virus exposure, the use of condoms or abstaining from sex was recommended for at least eight weeks after the female partner's symptom onset or last possible Zika virus exposure. If a male partner or both partners had possible Zika virus exposure, condom usage or abstinence from sex was recommended for at least 6 months after the male partner's symptom onset or last possible Zika virus exposure (if asymptomatic; E. E. Petersen, Meaney-Delman, et al., 2016). After additional data became available about the length of time of infectious virus shedding in semen and of time from symptom onset to sexual transmission in a partner, the time of condom usage or abstinence following possible Zika virus exposure to male partners was decreased from 6 to 3 months (Polen et al., 2018).

In addition to guidance regarding the avoidance of mosquito bites and sexual transmission, recommendations for the care of pregnant women with Zika virus infection and their infants were developed to provide health care providers with information to guide with counseling and management. These recommendations, developed by CDC and professional organizations (Adebanjo et al., 2017; American College of Obstetricians and Gynecologists Committee, 2019; Oduyebo et al., 2017), were updated several times during the outbreak to ensure that they incorporated the latest available data. These recommendations provide information on how to identify infants with possible congenital Zika virus infection so that its associated complications can be recognized early (e.g., by performing an ophthalmologic exam, hearing testing, or cranial imaging) and optimally addressed.

Another approach to the prevention of Zika virus-associated birth defects is the development of a vaccine to prevent Zika virus infection. The World Health Organization has developed a Zika Vaccine Development Technology Roadmap that focuses on two scenarios for vaccine use: use in future outbreaks, focusing on vaccination of women who are pregnant or of childbearing age during an ongoing epidemic, and endemic use, focusing on vaccination of the general population of at-risk countries in between epidemics (World Health Organization, 2019). Several different vaccine candidates, including inactivated vaccines, live attenuated vaccines, and subunit vaccines have been developed with some candidates now in different stages of preclinical studies or in human clinical trials (Pattnaik, Sahoo, & Pattnaik, 2020). Several issues need to be taken into account during the development of Zika vaccines, including the phenomenon of antibody-dependent enhancement, in which binding of a virus to antibodies can enhance its entry into host cells, possibly increasing, rather than decreasing, the risk of disease following vaccination; and the concern for safety during pregnancy, in particular for live attenuated vaccines if they can cross the placenta and potentially cause fetal damage (Schwartz, 2018). In addition, testing a vaccine for efficacy is challenging as the numbers of cases of Zika virus infection have decreased substantially; in the absence of outbreaks, identification of immune markers that are predictive of protection is needed. Finally, interest and funding for vaccine development wane as numbers of cases have fallen; identifying ways to incentivize vaccine developers is needed to ensure that a safe and effective vaccine is available before the next Zika virus epidemic occurs (Pattnaik et al., 2020).

Despite the best efforts of avoidance of mosquito bites, mosquito control efforts in communities, and future development and distribution of a safe and effective vaccine, some pregnant women might still become infected with the Zika virus. Several drug candidates have been identified, although only a small number have advanced to clinical trials. Development of therapeutic interventions, either to be given prophylactically during an outbreak or therapeutically after identification of early infection, which could prevent the adverse outcomes associated with prenatal Zika virus infection, continue to be a focus of research (Bernatchez et al., 2020).

6 | CONCLUSIONS

While much has been learned in the nearly 5 years since Zika was identified as a cause of microcephaly and other serious brain defects, many gaps remain. Improved information on factors that increase the risk of adverse outcomes among women infected with the Zika virus during pregnancy is needed. The long-term effects of prenatal Zika virus infection on infants and children need to be better understood. The development of accurate diagnostic tests would allow for better counseling and management of pregnant women and their partners and optimal evaluation and management of their infants. Finally, efforts at developing a safe and effective vaccine need to continue so that future generations can be protected from the devastating effects of the Zika virus.

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CONFLICT OF INTEREST

The authors declare no conflict of interest relevant to this work.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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