Zika virus and the nonmicrocephalic fetus: why we should still worry

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Zika virus is a mosquito-transmitted flavivirus and was first linked to congenital microcephaly caused by a large outbreak in northeastern Brazil. Although the Zika virus epidemic is now in decline, pregnancies in large parts of the Americas remain at risk because of ongoing transmission and the potential for new outbreaks. This review presents why Zika virus is still a complex and worrisome public health problem with an expanding spectrum of birth defects and how Zika virus and related viruses evade the immune response to injure the fetus. Recent reports indicate that the spectrum of fetal brain and other anomalies associated with Zika virus exposure is broader and more complex than microcephaly alone and includes subtle fetal brain and ocular injuries; thus, the ability to prenatally diagnose fetal injury associated with Zika virus infection remains limited. New studies indicate that Zika virus imparts disproportionate effects on fetal growth with an unusual femur-sparing profile, potentially providing a new approach to identify viral injury to the fetus. Studies to determine the limitations of prenatal and postnatal testing for detection of Zika virus—associated birth defects and long-term neurocognitive deficits are needed to better guide women with a possible infectious exposure. It is also imperative that we investigate why the Zika virus is so adept at infecting the placenta and the fetal brain to better predict other viruses with similar capabilities that may give rise to new epidemics. The efficiency with which the Zika virus evades the early immune response to enable infection of the mother, placenta, and fetus is likely critical for understanding why the infection may either be fulminant or limited. Furthermore, studies suggest that several emerging and related viruses may also cause birth defects, including West Nile virus, which is endemic in many parts of the United States. With mosquito-borne diseases increasing worldwide, there remains an urgent need to better understand the pathogenesis of the Zika virus and related viruses to protect pregnancies and child health.

Key words: birth defect, Congenital Zika Syndrome, microcephaly, pregnancy, Zika virus

Zika virus (ZIKV) is a mosquito-transmitted flavivirus, which was recognized to cause fetal brain injury when an outbreak in Brazil became associated with a surge in congenital microcephaly. The World Health Organization rapidly declared the ensuing ZIKV epidemic a global public health emergency in 2016. The term Congenital Zika Syndrome (CZS) was coined to describe an array of birth defects associated with ZIKV infection including microcephaly, complex brain malformations, and ocular injury.[1–3] Although microcephaly is recognized as a classic finding for diagnosis of the CZS, evidence is now accumulating that subtle, but destructive brain and ocular injuries can also occur in infants with a normal head size at birth.[4–8] The expanding range of anomalies may be difficult or impossible to diagnose prenatally, and we remain uncertain as to the long-term neurocognitive effects of ZIKV exposure.

Although the initial emergence of ZIKV in the Americas was geographically limited, mosquito-borne infections are increasing globally and in the United

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States wherein cyclical epidemics may be expected. In addition to ZIKV, several related viruses can also infect the placenta and may pose a threat to pregnancies if a large outbreak or epidemic occurs.

It is critical to understand why ZIKV is so adept as a teratogen to predict which emerging viruses may have a similar profile of fetal injury and also to develop antiviral therapeutics that are safe in pregnancy. One major factor in enabling teratogenesis is the ability of the virus to evade the early (innate) immune response, which allows the virus to replicate and infect multiple cells in the human placenta and fetal brain including immature neural stem and progenitor cells.[9–13] Herein we summarize recent observations on the broadening clinical spectrum of ZIKV-associated injury and how the virus escapes the immune response to silently injure the fetus.

Congenital Zika Syndrome

The initial descriptions of radiological findings associated with the CZS were restricted to case series of infants with extensive fetal brain injury and severe microcephaly.[3,14–17] Classic features include a massive reduction in the parenchymal volume of the brain with ventriculomegaly and abnormalities of the corpus callosum and cortical migration. Ventriculomegaly may be symmetric or asymmetric; if asymmetric, the occipital horns are typically dilated out of proportion to the frontal horns with an associated loss of the parieto-occipital gray and white matter.

Intracranial calcifications are most common at the gray-white matter junction but can also involve periventricular white matter, the basal ganglia and/or thalami. Asymmetry and abnormalities of the gyral patterns may meet diagnostic criteria for polymicrogyria, lissencephaly, or pachygyria. The cerebellum is often abnormal and may be absent, atrophic, or underdeveloped. Craniofacial disproportion with a sloping forehead, due to frontal lobe hypoplasia, is typical in severe cases and may occur in the absence of microcephaly.[7] In severe cases, skull collapse may be evident with overlapping sutures and redundancy of skin folds. Arthrogryposis, or congenital contractures, may also occur in association with brain stem hypoplasia and thinning of the entire spinal cord.[18]

In summary, the extreme phenotype of the CZS is well described and clinically straightforward to diagnose using antenatal ultrasound and/or magnetic resonance imaging (MRI) and clinical examination.

Diagnostic challenges for congenital microcephaly and Zika virus infection in pregnancy

Although microcephaly is a central feature of the CZS, no single definition for microcephaly captures all infants with ZIKV-associated anomalies or injuries.[19,20] The Society for Maternal-Fetal Medicine recommended defining microcephaly by a fetal head circumference (HC) less than 3 SD below the mean when no other anomalies associated with ZIKV are seen.[21] This definition was consistent with standards for diagnosing microcephaly outside ZIKV exposure and designed to minimize the chance that a fetus with a constitutionally small head would be labeled as microcephalic.[22]

A less conservative definition was adopted by the World Health Organization and the International Society for Ultrasound in Obstetrics and Gynecology, which chose to define ZIKV-associated microcephaly as an HC less than 2 SD below the mean (Z-score ≤ -2 SD).[23] The Centers for Disease Control and Prevention (CDC; United States) similarly defined ZIKV-associated microcephaly as a fetal or neonatal HC less than 2 SD below the mean or the third centile for gestational age.[19]

Although these definitions are less conservative, use of a threshold at the third centile may still fail to capture fetuses or neonates with less dramatic reductions in brain parenchyma or excessive intracranial fluid (eg, ventriculomegaly, evolving hydrocephalus) that compensates for volume loss of the brain.[7,24] Although one study reported an 83% sensitivity to identify definite or probable CZS[20] using an HC threshold less than 2 SD below the mean (Intergrowth 21st sonographic standards),[25] this cohort was selected based on a clinical suspicion for microcephaly and thus this may be an overestimate.[26]

Others reported a 10% rate of a normal HC in the context of significant brain anomalies.[22,27] Note that a sex-independent threshold for microcephaly (single cutoff for neonatal HC by the Intergrowth 21st sonographic standard) is more likely to label female infants as microcephalic because girls tend to have smaller heads than boys.[28]

Diagnosis of CZS or a possible ZIKV-associated birth defect is best made in the context of definitive laboratory evidence of infection, but in many cases this may not be possible.[29] Laboratory evidence of ZIKV infection is defined by a combination of positive results using nucleic acid testing, serology, and plaque reduction neutralization tests.[30] However, ZIKV infections are often asymptomatic and when symptoms occur, they may be vague (headache, conjunctivitis) and insufficient to trigger contact with a health care provider.

The limited time window for detecting ZIKV nucleic acid can make it difficult to detect ZIKV RNA if maternal testing did not occur at the right time. Other viruses co-circulating with ZIKV (eg, dengue and Chikungunya viruses) may present similarly and serological tests for ZIKV may cross-react with antibodies from related flaviviruses.[31,32]

Finally, ZIKV RNA may be undetectable in the neonate at delivery if exposure occurred early in the pregnancy.[33] Limitations related to diagnostic testing are important factors driving the uncertainty with which birth defects may be attributed to ZIKV infection.

Broadening spectrum of ZIKV-associated fetal injury

Recent studies have begun to describe a range of more subtle anomalies that are not captured by the original diagnosis of CZS, which poses a clinical challenge to obstetrical providers for women with ZIKV exposure.[5] The CDC has defined a spectrum of birth defects that include typical anomalies in the CZS for the purposes of birth defects surveillance (Box).[19]

In many cases, the findings are challenging to detect without access to

BOX

Birth defects potentially related to Zika virus infection

Brain abnormalities with and without microcephaly

- Congenital microcephaly: HC less than the third centile for gestational age and sex
- Intracranial calcifications
- Cerebral atrophy
- Abnormal cortical formation (lissencephaly, polymicrogyria, pachygyria, schizencephaly, and gray matter heterotopia)
- Corpus callosum abnormalities
- Cerebellar abnormalities
- Porencephaly
- Hydranencephaly
- Ventriculomegaly[a] or hydrocephaly[a]
- Fetal brain disruption sequence (severe microcephaly, collapsed skull, overlapping sutures, scalp redundancy)
- Other major brain abnormalities (thalamus, hypothalamus, pituitary, basal ganglia or brainstem)

Neural tube defects and other early brain malformations[b]

- Anencephaly or acrania
- Encephalocele
- Spina bifida without anencephaly
- Holoprosencephaly or arhinencephaly

Eye abnormalities

- Microphthalmia or anopthalmia
- Coloboma
- Congenital cataract
- Intraocular calcifications
- Chorioretinal anomalies (eg, atrophy, scarring, macular pallor, retinal hemorrhage, and gross pigmentary changes excluding retinopathy of prematurity)
- Optic nerve atrophy, pallor, and other optic nerve abnormalities

Consequences of central nervous system dysfunction

- Congenital contractures (eg, arthrogryposis, club foot, congenital hip dysplasia) with associated brain abnormalities
- Congenital sensorineural hearing loss documented by postnatal testing

Box adapted from the US Centers for Disease Control and Prevention standard case definition for birth defects potentially associated with ZIKV infection.[19]

HC, head circumference; *ZIKV*, Zika virus.

[a] Excludes isolated mild ventriculomegaly without other brain abnormalities or hydrocephalus due to hemorrhage; [b] Evidence for a link between ZIKV infections and neural tube defects is weaker than for other listed anomalies.

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prenatal or postnatal MRI imaging[5]; the sensitivity of antenatal ultrasound may simply be insufficient to detect some fetal injuries. Sight-threatening ocular injuries have been described in infants without microcephaly (ie, optic nerve hypoplasia) that cannot be detected by ultrasound.[7,34] Even though intracranial calcifications should be easily detected by antenatal ultrasound, there are reports of calcifications first being detected using postnatal cranial ultrasound.[35]

It is also possible that neurological injury, which began with fetal exposure to ZIKV, may continue to evolve in the first year of life and become more obvious over time. Postnatal microcephaly and hydrocephalus are examples of this type of slowly evolving injury that may not be detected at the time of a pregnancy ultrasound or at birth.[7,24,36,37] Although ultrasound is an important tool to detect fetal structural brain anomalies, a normal scan cannot be taken as evidence that the fetus has escaped viral injury. Postnatal testing, as recommended by the CDC, is more likely to detect lesser viral injuries through cranial ultrasound imaging (and/or MRI, if feasible), eye examination, auditory screening (ie, automated auditory brainstem response), longitudinal developmental assessments, and close monitoring of growth.[38]

Fetal injuries detected in nonmicrocephalic infants exposed to ZIKV in utero

A maternal ZIKV infection is estimated to result in birth defects in 5–13% of cases, with higher rates of anomalies when infection occurs earlier in pregnancy;[22,35,39–43] however, these rates should be taken only as an estimate, given the clinical difficulty in detection of lesser injuries during pregnancy and in the neonatal period. Although the long-term risk for developing ocular and subtle brain injuries after ZIKV exposure in utero is unknown,[44] a few case series have reported rates of abnormalities detected in the prenatal and neonatal periods (Table). In a cohort of 1450 children exposed to ZIKV in utero and followed up to at least 1 year of age in the US Territories, neurodevelopmental abnormalities or birth defects were reported in 17% of children.[4]

In 116 pregnant Brazilian women with laboratory evidence of ZIKV infection and a rash, 42% of fetuses had abnormal clinical or brain imaging findings.[45] The variety of neurological findings included epilepsy, dysphagia with feeding

TABLE
Comparison of rates of microcephaly and other ZIKV-associated adverse outcomes or abnormalities in women with a diagnosis of ZIKV infection in pregnancy

ZIKV-associated birth defects or abnormalities	Rate	Reference
Rio de Janeiro, Brazil		
Congenital microcephaly	3% (4/125)	45
Small for gestational age	9% (11/125)	
Stillbirth	2% (3/125)	
Grossly abnormal results on neonatal neurologic exam (eg, hypertonicity, spasticity, contractures, seizures)	26% (31/117)	
Abnormalities affecting the central nervous system on examination, imaging, or both	42% (49/117)	
Barranquilla, Colombia		
Congenital microcephaly	4% (8/211)	22
Congenital microcephaly and/or fetal brain malformations	6% (12/214)	
Cerebral atrophy	5% (11/214)	
Intracranial calcifications (subcortical-cortical junction, basal ganglia, periventricular zones)	5% (11/214)	
Corpus callosum abnormalities	6% (12/214)	
Ventriculomegaly	5% (11/214)	
Club feet and arthrogryposis	1% (3/214)	
French Territories in the Americas		
Congenital microcephaly	6% (32/555)	40
Neurologic birth defects	7% (39/555)	
Intracranial calcifications	1% (8/555)	
Ventriculomegaly	1% (8/555)	
Stillbirth	1% (6/555)	
United States Territories		
Congenital microcephaly and/or brain abnormalities	4% (108/2549)	39
Total birth defects	5% (122/2549)	
Continental United States and Hawaii		
Congenital microcephaly	4% (18/442)	43
Brain abnormalities without microcephaly	1% (4/442)	
Total birth defects	6% (26/442)	
Miami, FL		
Congenital microcephaly	9% (5/53)	35
Abnormal neonatal head MRI	4% (2/53)	
Abnormal neonatal head ultrasound	24% (9/38)	
Ocular abnormalities	13% (7/53)	
Abnormal fundoscopic examination	47% (8/17)	
Audiology testing abnormalities	2% (1/44)	

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difficulties, visual and hearing deficits, spasticity, and hypertonicity.[45] In addition, 21% of infants had eye abnormalities; nearly half of these infants were not microcephalic and one third had no abnormalities identified within the central nervous system at all.[34] In a US cohort of 86 pregnancies from Miami-Dade County with laboratory evidence of ZIKV infection, abnormalities detected on postnatal cranial ultrasound (17%) and eye examination (13%) were common and exceeded the rate of congenital microcephaly (9%).[35] In both case series, ocular injuries and abnormal postnatal imaging were approximately twice as common as the outcome of microcephaly, which underscores the complexity of congenital ZIKV infections.

A few case reports of complex central nervous system defects in the context of a normal HC and ZIKV exposure are important to highlight that even significant injuries may not be captured by the diagnosis of microcephaly.[35] In the first case, ZIKV infection occurred in the first trimester, resulting in maternal fever, rash, myalgias, and conjunctivitis. Laboratory testing was positive for a maternal serum ZIKV immunoglobulin M and plaque reduction neutralization test. The HC of the fetus and neonate was consistent with a normal head size prenatally and at birth; the fetal HC Z-score was 0.2 at 33 weeks, −0.5 at 37 weeks, and 0.1 at birth, respectively, according to the Intergrowth 21st standard.[25] Postnatal testing revealed brain volume loss, abnormally smooth frontal and temporal lobes, atrophy of 1 cerebellar peduncle, and a hypopigmented retinal lesion (Figure 1). Postnatal testing in a second neonate revealed abnormally smooth frontal and temporal lobes with intracranial calcifications (Figure 2, A–E).

These cases illustrate specific injuries that may be sustained by neonates with a normal head size consistent with significant fetal brain injuries not captured by the current diagnostic criteria for congenital microcephaly.

The spectrum of organ injury associated with ZIKV is likely to be even greater than currently known because of broad viral tropism for most body organs, particularly in immunosuppressed

TABLE
Comparison of rates of microcephaly and other ZIKV-associated adverse outcomes or abnormalities in women with a diagnosis of ZIKV infection in pregnancy *(continued)*

ZIKV-associated birth defects or abnormalities	Rate	Reference
State of Texas		
Congenital microcephaly	3% (10/185)	42
Total birth defects	8% (15/185)	
New York City, NY		
Congenital microcephaly	5% (3/56)	41
Intrauterine growth restriction (AC <10th centile, Z-score <−1.3)	18% (10/56)	
Intrauterine growth restriction (AC less than second centile, Z-score <−2)	9% (5/56)	
Femur-sparing profile of intrauterine growth restriction (either an HC:FL or AC:FL ratio <10th centile, Z-score <−1.3)	52% (29/56)	
Stillbirth	2% (1/52)	

This table reports the rates for congenital microcephaly, ZIKV-associated birth defects, and other perinatal and neonatal outcomes in cohorts of pregnant women with a laboratory diagnosis of confirmed or presumptive ZIKV infection. Comparison of rates for microcephaly with other birth defects or abnormalities is best within each cohort because there are important differences in study design and outcome reporting. Stillbirth rates were included in the table only if it was clear that the authors referred to fetal death after 20 weeks' gestation.

AC, abdominal circumference; *HC*, head circumference; *FL*, femur length; *ZIKV*, Zika virus.

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individuals and mouse models.[46,47] Across a number of experimental mouse and nonhuman primate models of ZIKV infection, ZIKV RNA can be detected in the brain, spinal cord, placenta, testis/epididymis, ovary, uterus, kidney, spleen, heart, and lung.[8,46,48–51] Congenital heart disease has been reported in 14% of infants with CZS[52] and 40% of infants with presumptive ZIKV exposure during pregnancy;[53] anomalies included atrial and ventricular septal defects and, rarely, more complex anomalies.[52–54] Furthermore, ZIKV infection of germ cells within male testicular tissue and prolonged detection of ZIKV RNA in semen is very concerning for development of infertility.[55–59] This broad viral tropism may occur through ZIKV infection of microglia or vascular epithelium.[6,60–63] Whether viral injury to the placenta is necessary to induce fetal injury is unclear, but placental pathology has been associated with birth defects in some experimental models of ZIKV pathogenesis[48,64–66] but not others[8,49] and may be present only with first-trimester infection.[67,68]

Potential for long-term neurocognitive deficits because of ZIKV injury of the hippocampus

Even in the absence of abnormalities identified by prenatal and postnatal testing, the potential for long-term neurocognitive deficits remains. Recent studies in nonhuman primate models revealed that ZIKV injures neuroprogenitor cells in the hippocampus,[8,69] a specialized brain region important for learning, memory, cognition, and emotion/stress response, which begins to develop during mid- to late gestation and continues developing postnatally at least through childhood to young adulthood.[70–73]

Maternal-fetal ZIKV exposure led to loss of neural stem and progenitor cells (neurogenic arrest) and disruption of neural circuitry in the dentate gyrus (a key hippocampal subregion), while postnatal ZIKV exposure was associated wtih an overall hippocampal growth arrest and dysfunctional connectivity with other brain regions, leading to abnormal socioemotional behavior in older animals.[8,69] Hippocampal injuries would be expected to correlate with the reported early onset of seizures/epilepsy in human infants, perturbations to later learning and memory development, and potentially even later-onset depression and premature age-related cognitive decline.[73–75]

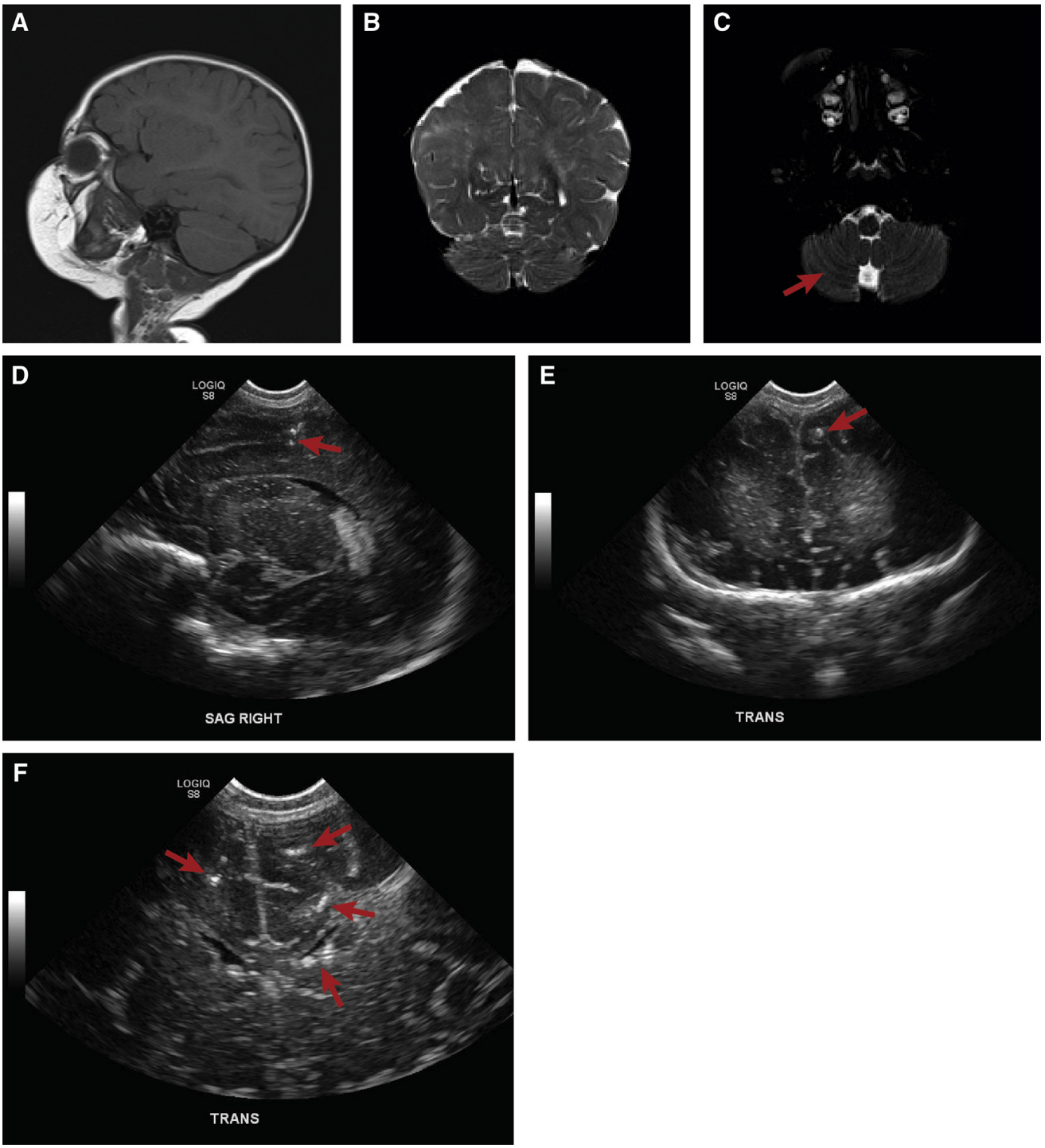
Unfortunately, the particular types of hippocampal injuries observed in experimental nonhuman primate models would not be easily detected in humans by standard prenatal MRI, rather requiring specific and repeated postnatal functional MRI scans to monitor hippocampal developmental progression in ZIKV-exposed non-microcephalic infants. Thus, further longitudinal studies are needed to understand the implications of ZIKV-associated hippocampal injury in the fetus or young children and whether this injury might result in a predisposition to learning disorders, developmental delay, and/or mental illnesses arising during later childhood and adolescence.[44]

Intrauterine growth restriction

Emerging evidence suggests that ZIKV infection in pregnancy has an impact on fetal growth and that intrauterine growth restriction (IUGR) may occur in approximately 9% of pregnancies.[41,45] Two large Brazilian case series of pregnant women with ZIKV infections have reported a profile of disproportionate fetal growth, which described a small head in relation to a longer body.[45,76] Other case series in Brazil[77] and the Caribbean[36] have also reported abnormal growth of the fetal head in the context of a normally growing femur.

A similar pattern of growth in early childhood among infants exposed to ZIKV during fetal life revealed a disproportionately small head (65% with HC ≤−3 SD) compared with the growth of the skeleton (14% with birth length ≤−3 SD).[16] This profile of femur-sparing growth restriction was also observed in nonhuman primate fetuses experimentally infected with ZIKV and was most dramatic when growth of the fetal head arrested (because of brain injury), but femur growth continued normally.[8,49] Notably, IUGR is also a hallmark feature of ZIKV infection in rodent models.[48,78,79]

FIGURE 1
Multiple brain abnormalities in a nonmicrocephalic neonate exposed to ZIKV



This figure depicts multiple brain anomalies in a neonate exposed to ZIKV in the first trimester with a normal head size at birth. A postnatal MRI demonstrated the following: global decrease in cerebral volume, more pronounced on the right **(A)**; a smooth appearance of the right frontal and anterior temporal lobes suggested a neuronal migration anomaly **(B)**; and right cerebellar peduncle atrophy **(C)**. Diffuse, coarse calcifications throughout the white matter were also observed on postnatal cranial ultrasound **(D, E, and F)**. At birth, the infant was found to have a left eye hypopigmented retinal lesion (not shown).

MRI, magnetic resonance imaging; *ZIKV*, Zika virus.

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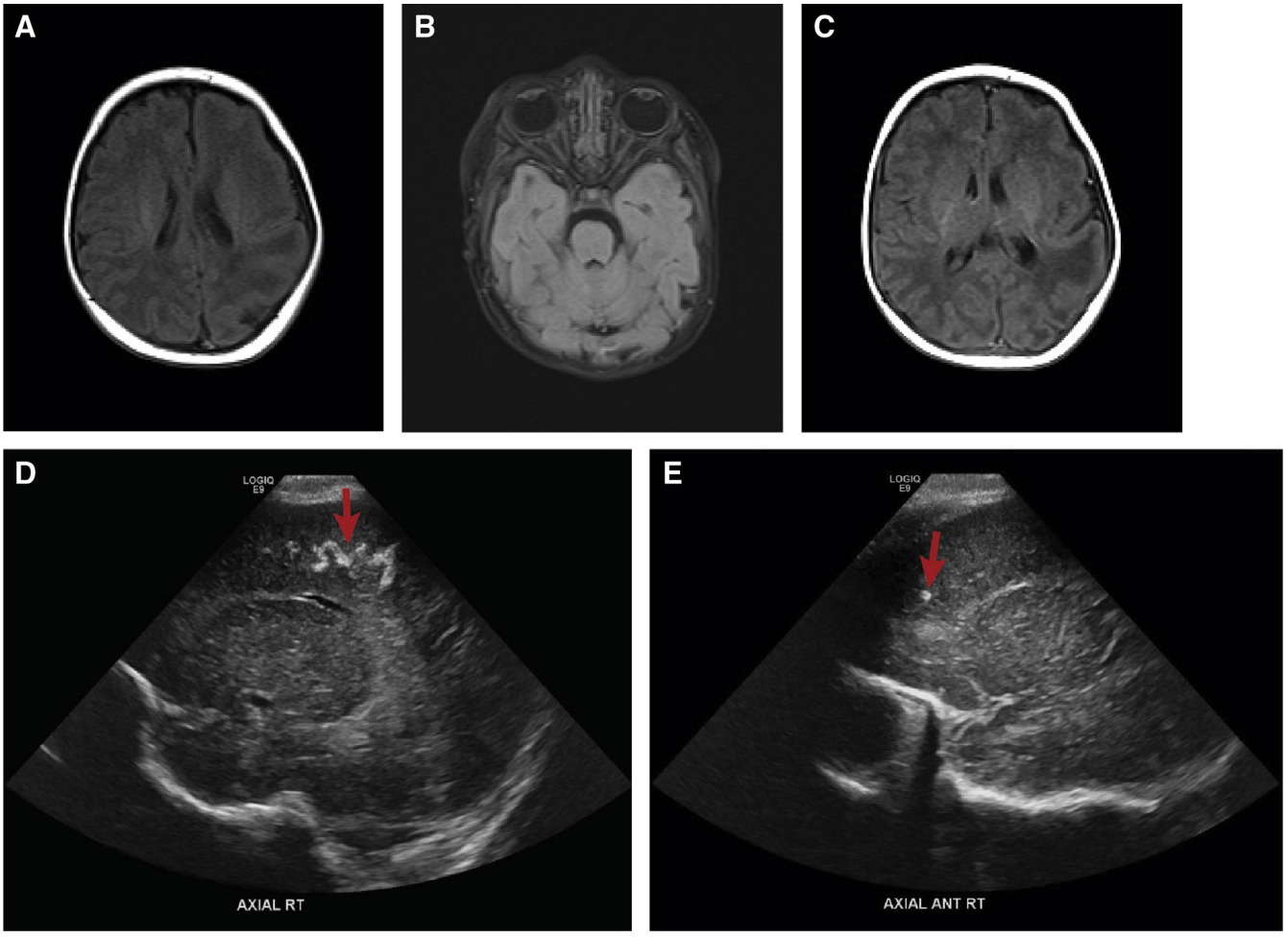
FIGURE 2
Spectrum of ZIKV-associated birth defects beyond diagnosis of congenital microcephaly



This figure demonstrates the spectrum of ZIKV-associated birth defects beyond the diagnosis of congenital microcephaly. A postnatal MRI revealed abnormalities of the gyri (**A**–**C**; polymicrogyria and loss of gyri) and intracranial serpiginous calcifications (**D and E**).

MRI, magnetic resonance imaging; *ZIKV*, Zika virus.

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The pathogenesis underlying development of a femur-sparing profile of IUGR is unknown but may occur if ZIKV selectively injures the brain and not the skeleton (Figure 3). An initial study to determine whether a femur-sparing profile of IUGR could be detected in association with ZIKV infection was recently performed in a cohort of 56 pregnant women in New York City, who acquired ZIKV infections through travel.[41] For this study, fetal body ratios with respect to femur length (FL) were calculated (HC:FL or abdominal circumference:FL) based on Intergrowth 21st standards.[41] A femur-sparing pattern of IUGR was detected in 52% of pregnancies based on either a HC:FL or abdominal circumference:FL less than the 10th centile.

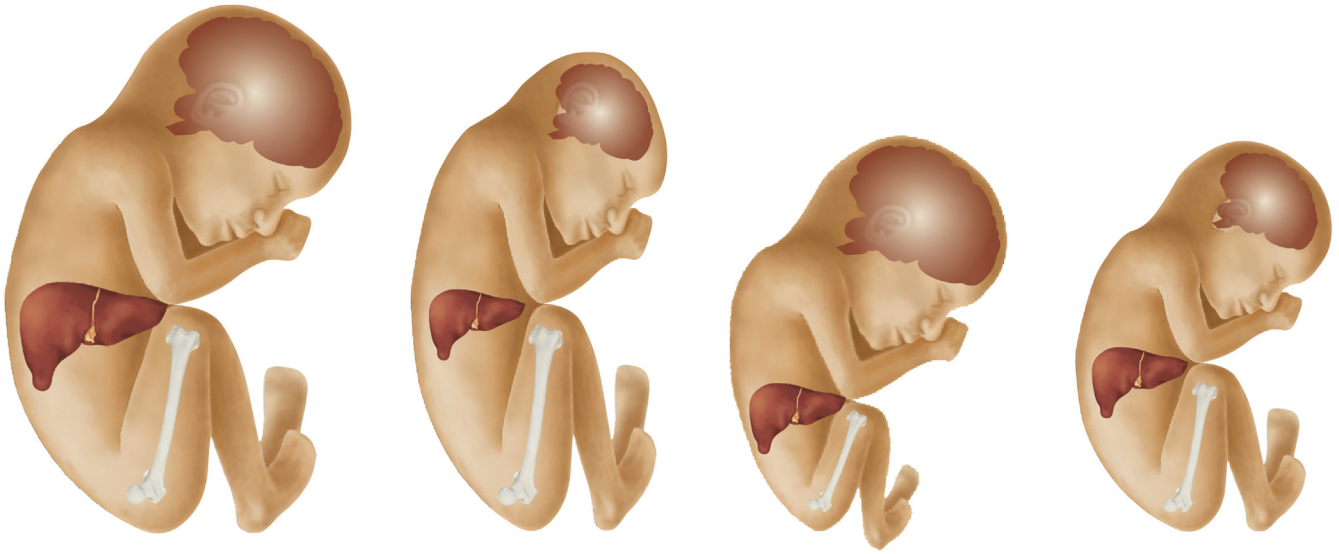
Whether aberrant fetal growth or a femur-sparing profile of IUGR is associated with neurological or ocular injuries is unknown, but future studies should evaluate whether fetal body ratios with respect to FL might provide a biomarker for a broader spectrum of ZIKV-associated fetal injury.

Stillbirth

Recently several human and animal studies indicated that ZIKV infections can cause spontaneous abortion and stillbirth, which also occur in association with other teratogenic infections.[40,45,80–83] Rates of ZIKV-associated pregnancy loss after 20 weeks have been reported to range between 1% and 2%,[40,41,45] which represents a 10- to 20-fold greater rate than in healthy pregnant women (~0.1%).[84] In nonhuman primates infected with ZIKV in early gestation, the rate of miscarriage and stillbirth was 26%, which represents a 3- to 7-fold higher rate than in comparably housed healthy, ZIKV-unexposed pregnant macaques.[85]

The mechanism for stillbirth is unknown, but evidence from a nonhuman

FIGURE 3
ZIKV-associated femur-sparing profile of fetal growth restriction



This figure illustrates how a femur-sparing profile of growth restriction, thought to be associated with ZIKV infection, compares with normal fetal growth and other aberrant patterns of growth restriction. A few studies indicate that growth of the femur is often spared in ZIKV infections, which may represent an internal standard to compare growth of the head or abdomen for possible ZIKV-associated viral injury of the fetus.

ZIKV, Zika virus.

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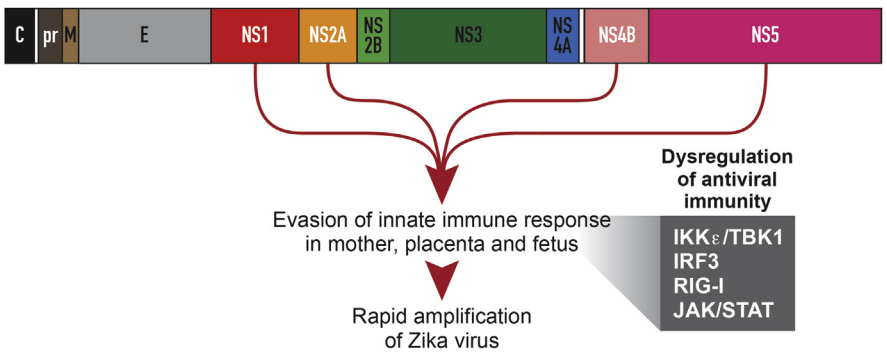
primate study suggests that placental injury and infarctions may compromise fetal oxygenation.[66] Furthermore, a new study suggests that type I interferon, a key antiviral defensive modulator, can alter placental development and trigger fetal death in the context of ZIKV infection in a mouse model.[86] The overall contribution of ZIKV infection to spontaneous abortion and stillbirth is difficult to estimate because of inconsistent reporting and data collection, but the rate appears to be significantly higher than baseline.[87]

FIGURE 4
Zika virus proteins associated with evasion of early immune response



This schematic illustrates the protein-coding regions of the ZIKV genome and specific NS proteins that have been implicated in inhibition of the innate immune response.

C, capsid; *E*, envelope; *IKKε*, inhibitor of kappa-B kinase subunit epsilon; *IRF3*, interferon regulatory factor 3; *JAK*, Janus kinase; *M*, membrane; *NS*, nonstructural; *pr*, precursor; *RIG-I*, retinoic acid inducible gene I; *STAT*, signal transducer and activator of transcription; *TBK1*, TANK-binding kinase 1.

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How ZIKV escapes the immune response

Molecular mechanisms of ZIKV pathogenesis and microcephaly remain poorly understood and, particularly, how the virus evades innate immunity to mediate vertical transmission and fetal brain injury.[88] ZIKV, like many flaviviruses, is transmitted to humans through the bite of an infected mosquito. In most human infections, there is an acute course with rapid amplification of virus in specific tissues, followed by clearance through the host immune response. ZIKV is unusual among flaviviruses because it can mediate a persistent infection and is detected in genital-mucosal tissues and other sites in the body for weeks to months after the initial exposure.[89–93]

To facilitate viral replication and spread within the infected host, ZIKV and other flaviviruses antagonize and/or evade the innate immune response, thus bypassing host defenses within infected cells to promote unchecked viral replication (Figure 4). The innate immune response plays an essential role in programming a robust adaptive immune response that clears the flavivirus infection.[94,95] Thus, it is important to understand the mechanisms that flaviviruses use to dysregulate host innate immunity because these directly contribute to human disease outcome.[96,97]

In the infected cell, flaviviruses sequester their genomic RNA within membranous intracellular compartments[98,99] as well as modify their RNA to mimic endogenous 5′-cap methylation.[100] These evasion strategies prevent host cell recognition of the viral pathogen-associated molecular patterns, allowing viral replication to continue undetected.

Flavivirus nonstructural (NS) proteins and subgenomic flavivirus RNA can also directly interfere with host antiviral signaling pathways to suppress immunity.[101–107] For ZIKV, the viral proteins NS1, NS2A, and NS4B have been demonstrated to antagonize the innate immune response at the level of the inhibitor of kappa B kinase epsilon/TANK-binding kinase 1 activation,[106,107] whereas NS5 has been shown to act downstream by binding interferon regulatory factor 3; Figure 4).[106] ZIKV subgenomic flavivirus RNA also prevents interferon induction in response to retinoic acid inducible gene I–like receptor stimulation by an unresolved mechanism.[101]

ZIKV further inhibits innate immunity via disruption of the Janus kinase/signal transducer and activator of transcription signaling cascade that is required to direct the expression of any antiviral genes induced by interferon.[103,105] Other mechanisms of flavivirus innate immune antagonism include evasion of the natural killer cell–mediated lysis and dysregulation of the humoral complement system.[108–111]

In support of these in vitro findings, an attenuated (or absent) inflammatory response associated with ZIKV infection in the fetal brain has been reported in pathological series from both human infants and fetuses[67] and nonhuman primates.[8,49] This pathological profile contrasts sharply with the cytopathic viral effects and robust inflammatory response, which is more typical for cytomegalovirus, herpes, and rubella viruses.[112,113] The absence of an inflammatory signature may have obscured the viral etiology of fatal cases associated with ZIKV infection before the epidemic in the Americas brought the congenital disease manifestations to light.

Emerging viruses with similar potential to injure the fetal brain

It is possible that other viruses and related flaviviruses may similarly exert a silent profile of fetal injury, which would require a high index of clinical suspicion for detection. Indeed, the pathogenic flavivirus, Japanese encephalitis virus, has been documented to transplacentally infect developing human fetuses, leading to miscarriage,[114,115] and other related members of the family *Flaviviridae* are well characterized to vertically transmit in vertebrates.[116,117]

A recent study suggests that several other emerging and ZIKV-related viruses may also infect the placenta and cause fetal demise.[118] Human maternal and fetal tissue explants were highly susceptible to West Nile virus (WNV) and Powassan virus infections, with less efficient infection by the alphaviruses, Mayaro virus, and Chikungunya virus; WNV could also infect the fetal brain and cause stillbirth or abortion in an immunocompetent mouse model.[118] Furthermore, a case series of WNV infections in human pregnancies reported neurological injuries similar to the CZS including meningitis, lissencephaly, and microcephaly.[119] Vertical transmission of Chikungunya virus has been reported with documented neonatal encephalitis and long-term adverse outcomes on child neurocognitive function.[120,121]

These studies suggest that emerging flaviviruses may share ZIKV's teratogenic capacity and that pregnancy outcomes in regions at risk for viral infections with unknown risk to the fetus should be monitored for birth defects.[118]

Conclusion

More than 50 years has passed since the rubella epidemic in the United States, which resulted in an estimated 20,000 births with the congenital rubella syndrome. ZIKV is an equivalent and contemporary threat to pregnancies with a widening array of associated anomalies that is not restricted to microcephaly. Sight-threatening ocular injuries and subtle fetal brain injuries may be impossible to detect prenatally. ZIKV exposure in utero is associated with a higher risk of IUGR and stillbirth. An unusual femur-sparing profile of growth restriction has been associated with ZIKV infection and may serve as a biomarker for fetal injury in the absence of microcephaly and structural brain anomalies.

Molecular mechanisms of how ZIKV induces birth defects are poorly understood but are likely related to dysregulation of the innate immune response; antagonism of host antiviral signaling allows ZIKV to evade destruction and continue to replicate. The recent demonstration that other viruses, some related to ZIKV, can infect the placenta and invade the brain suggest that we need to study the ability of other neuroinvasive viruses for their effects on the fetus. Although mosquito-borne diseases continue to increase globally, we remain unprepared to protect pregnancies from ZIKV and other teratogenic viral threats. ■

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REFERENCES

1. Kleber de Oliveira W, Cortez-Escalante J, et al. Increase in reported prevalence of microcephaly in infants born to women living in areas with confirmed Zika Virus transmission during the first trimester of pregnancy—Brazil, 2015. MMWR Morb Mortal Wkly Rep 2016;65:242–7.
2. Gulland A. Zika virus is a global public health emergency, declares WHO. BMJ 2016;352:i657.
3. Melo AS, Aguiar RS, Amorim MM, et al. Congenital Zika virus infection: beyond neonatal microcephaly. JAMA Neurol 2016;73:1407–16.

4. Rice ME, Galang RR, Roth NM, et al. Vital signs: Zika-associated birth defects and neurodevelopmental abnormalities possibly associated with congenital Zika virus infection—US territories and freely associated states, 2018. MMWR Morb Mortal Wkly Rep 2018;67:858–67.

5. Aragao M, Holanda AC, Brainer-Lima AM, et al. Nonmicrocephalic infants with congenital Zika syndrome suspected only after neuroimaging evaluation compared with those with microcephaly at birth and postnatally: how large is the Zika virus "iceberg"? AJNR Am J Neuroradiol 2017;38:1427–34.

6. Shao Q, Herrlinger S, Yang SL, et al. Zika virus infection disrupts neurovascular development and results in postnatal microcephaly with brain damage. Development 2016;143:4127–36.

7. van der Linden V, Pessoa A, Dobyns W, et al. Description of 13 infants born during October 2015–January 2016 with congenital Zika virus infection without microcephaly at birth—Brazil. MMWR Morb Mortal Wkly Rep 2016;65:1343–8.

8. Adams Waldorf KM, Nelson BR, et al. Congenital Zika virus infection as a silent pathology with loss of neurogenic output in the fetal brain. Nat Med 2018;24:368–74.

9. Qian X, Nguyen HN, Song MM, et al. Brain-region-specific organoids using mini-bioreactors for modeling ZIKV exposure. Cell 2016;165:1238–54.

10. Tang H, Hammack C, Ogden SC, et al. Zika virus infects human cortical neural progenitors and attenuates their growth. Cell Stem Cell 2016;18:587–90.

11. Tabata T, Petitt M, Puerta-Guardo H, et al. Zika virus targets different primary human placental cells, suggesting two routes for vertical transmission. Cell Host Microbe 2016;20:155–66.

12. El Costa H, Gouilly J, Mansuy JM, et al. ZIKA virus reveals broad tissue and cell tropism during the first trimester of pregnancy. Sci Rep 2016;6:35296.

13. Jurado KA, Simoni MK, Tang Z, et al. Zika virus productively infects primary human placenta-specific macrophages. JCI Insight 2016;1.

14. Soares de Oliveira-Szejnfeld P, Levine D, Melo AS, et al. Congenital brain abnormalities and Zika virus: what the radiologist can expect to see prenatally and postnatally. Radiology 2016;281:203–18.

15. Schuler-Faccini L, Ribeiro EM, Feitosa IM, et al. Possible association between Zika virus infection and microcephaly—Brazil, 2015. MMWR Morb Mortal Wkly Rep 2016;65:59–62.

16. Moura da Silva AA, Ganz JS, Sousa PD, et al. Early growth and neurologic outcomes of infants with probable congenital Zika virus syndrome. Emerg Infect Dis 2016;22:1953–6.

17. de Fatima Vasco Aragao M, van der Linden V, Brainer-Lima AM, et al. Clinical features and neuroimaging (CT and MRI) findings in presumed Zika virus related congenital infection and microcephaly: retrospective case series study. BMJ 2016;353:i1901.

18. Aragao M, Brainer-Lima AM, Holanda AC, et al. Spectrum of spinal cord, spinal root, and brain MRI abnormalities in congenital Zika syndrome with and without arthrogryposis. AJNR Am J Neuroradiol 2017;38:1045–53.

19. Centers for Disease Control and Prevention. Zika virus, 2018. Available at: www.cdc.gov/zika. Accessed September 11, 2018.

20. Franca GVA, Schuler-Faccini L, Oliveira WK, et al. Congenital Zika virus syndrome in Brazil: a case series of the first 1501 livebirths with complete investigation. Lancet 2016;388:891–7.

21. Society for Maternal-Fetal Medicine Publications Committee. Ultrasound screening for fetal microcephaly following Zika virus exposure. Am J Obstet Gynecol 2016;214:B2–4.

22. Sanz Cortes M, Rivera AM, Yepez M, et al. Clinical assessment and brain findings in a cohort of mothers, fetuses and infants infected with Zika virus. Am J Obstet Gynecol 2018;218:440.e1–3.

23. Papageorghiou AT, Thilaganathan B, Bilardo CM, et al. ISUOG interim guidance on ultrasound for Zika virus infection in pregnancy: information for healthcare professionals. Ultrasound Obstet Gynecol 2016;47:530–2.

24. Juca E, Pessoa A, Ribeiro E, et al. Hydrocephalus associated to congenital Zika syndrome: does shunting improve clinical features? Childs Nerv Syst 2018;34:101–6.

25. Papageorghiou AT, Ohuma EO, Altman DG, et al. International standards for fetal growth based on serial ultrasound measurements: the Fetal Growth Longitudinal Study of the INTERGROWTH-21st Project. Lancet 2014;384:869–79.

26. Heukelbach J, Werneck GL. Surveillance of Zika virus infection and microcephaly in Brazil. Lancet 2016;388:846–7.

27. Hazin AN, Poretti A, Turchi Martelli CM, et al. Computed tomographic findings in microcephaly associated with Zika virus. N Engl J Med 2016;374:2193–5.

28. Victora CG, Schuler-Faccini L, Matijasevich A, Ribeiro E, Pessoa A, Barros FC. Microcephaly in Brazil: how to interpret reported numbers? Lancet 2016;387:621–4.

29. Eppes C, Rac M, Dunn J, et al. Testing for Zika virus infection in pregnancy: key concepts to deal with an emerging epidemic. Am J Obstet Gynecol 2017;216:209–25.

30. Centers for Disease Control and Prevention. Guidance for laboratories testing for Zika virus infection. 2017. Available at: https://www.cdc.gov/zika/laboratories/lab-guidance.html. Accessed March 19, 2018.

31. Granger D, Hilgart H, Misner L, et al. Serologic testing for Zika virus: comparison of three Zika virus IgM-screening enzyme-linked immunosorbent assays and initial laboratory experiences. J Clin Microbiol 2017;55:2127–36.

32. Felix AC, Souza NCS, Figueiredo WM, et al. Cross reactivity of commercial anti-dengue immunoassays in patients with acute Zika virus infection. J Med Virol 2017;89:1477–9.

33. Reynolds MR, Jones AM, Petersen EE, et al. Vital signs: update on Zika virus–associated birth defects and evaluation of all US infants with congenital Zika virus exposure—US Zika Pregnancy Registry, 2016. MMWR Morb Mortal Wkly Rep 2017;66:366–73.

34. Zin AA, Tsui I, Rossetto J, et al. Screening criteria for ophthalmic manifestations of congenital Zika virus infection. JAMA Pediatr 2017;171:847–54.

35. Shiu C, Starker R, Kwal J, et al. Zika virus testing and outcomes during pregnancy, Florida, USA, 2016. Emerg Infect Dis 2018;24:1–8.

36. Sohan K, Cyrus CA. Ultrasonographic observations of the fetal brain in the first 100 pregnant women with Zika virus infection in Trinidad and Tobago. Int J Gynaecol Obstet 2017;139:278–83.

37. Petribu NCL, Aragao MFV, van der Linden V, et al. Follow-up brain imaging of 37 children with congenital Zika syndrome: case series study. BMJ 2017;359:j4188.

38. Adebanjo T, Godfred-Cato S, Viens L, et al. Update: interim guidance for the diagnosis, evaluation, and management of infants with possible congenital Zika virus infection—United States, October 2017. MMWR Morb Mortal Wkly Rep 2017;66:1089–99.

39. Shapiro-Mendoza CK, Rice ME, Galang RR, et al. Pregnancy outcomes after maternal Zika virus infection during pregnancy—US Territories, January 1, 2016–April 25, 2017. MMWR Morb Mortal Wkly Rep 2017;66:615–21.

40. Hoen B, Schaub B, Funk AL, et al. Pregnancy outcomes after ZIKV infection in French Territories in the Americas. N Engl J Med 2018;378:985–94.

41. Walker CL, Merriam AA, Ohuma EO, et al. Femur-sparing pattern of abnormal fetal growth in pregnant women from New York City after maternal Zika virus infection. Am J Obstet Gynecol 2018;219:187.e1–20.

42. Hall NB, Broussard K, Evert N, Canfield M. Notes from the field: Zika virus-associated neonatal birth defects surveillance—Texas, January 2016–July 2017. MMWR Morb Mortal Wkly Rep 2017;66:835–6.

43. Honein MA, Dawson AL, Petersen EE, et al. Birth defects among fetuses and infants of US women with evidence of possible Zika virus infection during pregnancy. JAMA 2017;317:59–68.

44. Adams Waldorf KM, Olson EM, Nelson BR, Little ME, Rajagopal L. The aftermath of Zika: need for long-term monitoring of exposed children. Trends Microbiol 2018;26:729–32.

45. Brasil P, Pereira JP Jr, Moreira ME, et al. Zika virus infection in pregnant women in Rio de Janeiro. N Engl J Med 2016;375:2321–34.

46. Chan JF, Zhang AJ, Chan CC, et al. Zika virus infection in dexamethasone-immunosuppressed mice demonstrating disseminated infection with multi-organ involvement including orchitis effectively treated by recombinant type I interferons. EBio Med 2016;14:112–22.

47. Miner JJ, Diamond MS. Zika virus pathogenesis and tissue tropism. Cell Host Microbe 2017;21:134–42.
48. Miner JJ, Cao B, Govero J, et al. Zika virus infection during pregnancy in mice causes placental damage and fetal demise. Cell 2016;165:1081–91.
49. Adams Waldorf KM, Stencel-Baerenwald JE, Kapur RP, et al. Fetal brain lesions after subcutaneous inoculation of Zika virus in a pregnant nonhuman primate. Nat Med 2016;22:1256–9.
50. Lazear HM, Govero J, Smith AM, et al. A mouse model of Zika virus pathogenesis. Cell Host Microbe 2016;9:720–30.
51. Rossi SL, Tesh RB, Azar SR, et al. Characterization of a novel murine model to study Zika virus. Am J Trop Med Hyg 2016;94:1362–9.
52. Cavalcanti DD, Alves LV, Furtado GJ, et al. Echocardiographic findings in infants with presumed congenital Zika syndrome: Retrospective case series study. PloS One 2017;12: e0175065.
53. Orofino DHG, Passos SRL, de Oliveira RVC, et al. Cardiac findings in infants with in utero exposure to Zika virus—a cross sectional study. PLoS Negl Trop Dis 2018;12:e0006362.
54. Angelidou A, Michael Z, Hotz A, et al. Is there more to Zika? Complex cardiac disease in a case of congenital Zika syndrome. Neonatology 2018;113:177–82.
55. Matusali G, Houzet L, Satie AP, et al. Zika virus infects human testicular tissue and germ cells. J Clin Invest 2018 Sep 10. pii: 121735. https://doi.org/10.1172/JCI121735. [Epub ahead of print] PMID: 30063220.
56. Medina FA, Torres G, Acevedo J, et al. Duration of infectious Zika virus in semen and serum. J Infect Dis 2018 Jul 27. https://doi.org/10.1093/infdis/jiy462. [Epub ahead of print].
57. Garcia-Bujalance S, Gutierrez-Arroyo A, De la Calle F, et al. Persistence and infectivity of Zika virus in semen after returning from endemic areas: report of 5 cases. J Clin Virol 2017;96: 110–5.
58. McDonald EM, Duggal NK, Ritter JM, Brault AC. Infection of epididymal epithelial cells and leukocytes drives seminal shedding of Zika virus in a mouse model. PLoS Negl Trop Dis 2018;12:e0006691.
59. Robinson CL, Chong ACN, Ashbrook AW, et al. Male germ cells support long-term propagation of Zika virus. Nat Commun 2018;9:2090.
60. Aleman TS, Ventura CV, Cavalcanti MM, et al. Quantitative assessment of microstructural changes of the retina in infants with congenital Zika syndrome. JAMA Ophthalmol 2017;135: 1069–76.
61. Singh PK, Guest JM, Kanwar M, et al. Zika virus infects cells lining the blood-retinal barrier and causes chorioretinal atrophy in mouse eyes. JCI Insight 2017;2:e92340.
62. Britt WJ. Adverse outcomes of pregnancy-associated Zika virus infection. Semin Perinatol 2018;42:155–67.
63. Muffat J, Li Y, Omer A, et al. Human induced pluripotent stem cell-derived glial cells and neural progenitors display divergent responses to Zika and dengue infections. Proc Natl Acad Sci USA 2018;115:7117–22.
64. Szaba FM, Tighe M, Kummer LW, et al. Zika virus infection in immunocompetent pregnant mice causes fetal damage and placental pathology in the absence of fetal infection. PLoS Pathogens 2018;14: e1006994.
65. Nguyen SM, Antony KM, Dudley DM, et al. Highly efficient maternal-fetal Zika virus transmission in pregnant rhesus macaques. PLoS Pathogens 2017;13:e1006378.
66. Hirsch AJ, Roberts VHJ, Grigsby PL, et al. Zika virus infection in pregnant rhesus macaques causes placental dysfunction and immunopathology. Nat Commun 2018;9:263.
67. Martines RB, Bhatnagar J, de Oliveira Ramos AM, et al. Pathology of congenital Zika syndrome in Brazil: a case series. Lancet 2016;388:898–904.
68. Ritter JM, Martines RB, Zaki SR. Zika virus: pathology from the pandemic. Arch Pathol Lab Med 2017;141:49–59.
69. Mavigner M, Raper J, Kovacs-Balint Z, et al. Postnatal Zika virus infection is associated with persistent abnormalities in brain structure, function, and behavior in infant macaques. Sci Transl Med 2018;10.
70. Spalding KL, Bergmann O, Alkass K, et al. Dynamics of hippocampal neurogenesis in adult humans. Cell 2013;153:1219–27.
71. Sorrells SF, Paredes MF, Cebrian-Silla A, et al. Human hippocampal neurogenesis drops sharply in children to undetectable levels in adults. Nature 2018;555:377–81.
72. Boldrini M, Fulmore CA, Tartt AN, et al. Human hippocampal neurogenesis persists throughout aging. Cell Stem Cell 2018;22: 589–99.e5.
73. Kempermann G, Gage FH, Aigner L, et al. Human adult neurogenesis: evidence and remaining questions. Cell Stem Cell 2018.
74. Goncalves JT, Schafer ST, Gage FH. Adult neurogenesis in the hippocampus: from stem cells to behavior. Cel 2016;167:897–914.
75. Toda T, Parylak SL, Linker SB, Gage FH. The role of adult hippocampal neurogenesis in brain health and disease. Mol Psychiatry 2018 Apr 20. https://doi.org/10.1038/s41380-018-0036-2. [Epub ahead of print].
76. Silva AA, Barbieri MA, Alves MT, et al. Prevalence and risk factors for microcephaly at birth in Brazil in 2010. Pediatrics 2018;141.
77. Oliveira Melo AS, Malinger G, Ximenes R, Szejnfeld PO, Alves Sampaio S, Bispo de Filippis AM. Zika virus intrauterine infection causes fetal brain abnormality and microcephaly: tip of the iceberg? Ultrasound Obstet Gynecol 2016;47:6–7.
78. Cugola FR, Fernandes IR, Russo FB, et al. The Brazilian Zika virus strain causes birth defects in experimental models. Nature 2016;534: 267–71.
79. Uraki R, Jurado KA, Hwang J, et al. Fetal growth restriction caused by sexual transmission of Zika virus in mice. J Infect Dis 2017;215:1720–4.
80. van der Eijk AA, van Genderen PJ, Verdijk RM, et al. Miscarriage associated with Zika virus infection. N Engl J Med 2016;375: 1002–4.
81. Schaub B, Monthieux A, Najihoullah F, et al. Late miscarriage: another Zika concern? Eur J Obstet Gynecol Reprod Biol 2016;207:240–1.
82. Magnani DM, Rogers TF, Maness NJ, et al. Fetal demise and failed antibody therapy during Zika virus infection of pregnant macaques. Nat Commun 2018;9:1624.
83. Seferovic M, Martin CS, Tardif SD, et al. Experimental Zika virus infection in the pregnant common marmoset induces spontaneous fetal loss and neurodevelopmental abnormalities. Sci Rep 2018;8:6851.
84. Wilcox AJ, Weinberg CR, O'Connor JF, et al. Incidence of early loss of pregnancy. N Engl J Med 1988;319:189–94.
85. Dudley DM, Van Rompay KK, Coffey LL, et al. Miscarriage and stillbirth following maternal Zika virus infection in nonhuman primates. Nat Med 2018;24:1104–7.
86. Yockey LJ, Jurado KA, Arora N, et al. Type I interferons instigate fetal demise after Zika virus infection. Sci Immunol 2018;3.
87. Blencowe H, Cousens S, Jassir FB, et al. National, regional, and worldwide estimates of stillbirth rates in 2015, with trends from 2000: a systematic analysis. Lancet Glob Health 2016;4: e98–108.
88. Lucchese G, Kanduc D. Zika virus and autoimmunity: from microcephaly to Guillain-Barre syndrome, and beyond. Autoimmun Rev 2016;15:801–8.
89. Barzon L, Pacenti M, Franchin E, et al. Infection dynamics in a traveller with persistent shedding of Zika virus RNA in semen for six months after returning from Haiti to Italy, January 2016. Euro Surveill 2016;21.
90. Aid M, Abbink P, Larocca RA, et al. Zika virus persistence in the central nervous system and lymph nodes of rhesus monkeys. Cell 2017;169:610–20.e14.
91. Gaskell KM, Houlihan C, Nastouli E, Checkley AM. Persistent Zika virus detection in semen in a traveler returning to the United Kingdom from Brazil, 2016. Emerg Infect Dis 2017;23:137–9.
92. Schaub B, Monthieux A, Najioullah F, Adenet C, Muller F, Cesaire R. Persistent maternal Zika viremia: a marker of fetal infection. Ultrasound Obstet Gynecol 2017;49:658–60.
93. Gonce A, Martinez MJ, Marban-Castro E, et al. Spontaneous abortion associated with Zika virus infection and persistent viremia. Emerg Infect Dis 2018;24:933–5.
94. Lindenbach BD, Thiel H-J, Rice CM. *Flaviviridae*: the viruses and their replication. In: Knipe DM, Howley PM, eds. Fields virology, 5th ed. Philidelphia: Lippincott-Raven Publishers; 2007. p. 1101–52.

95. Suthar MS, Diamond MS, Gale M. West Nile virus infection and immunity. Nat Rev Microbiol 2013;11:115–28.
96. Keller BC, Fredericksen BL, Samuel MA, et al. Resistance to alpha/beta interferon is a determinant of West Nile virus replication fitness and virulence. J Virol 2006;80:9424–34.
97. Liu WJ, Wang XJ, Mokhonov VV, Shi PY, Randall R, Khromykh AA. Inhibition of interferon signaling by the New York 99 strain and Kunjin subtype of West Nile virus involves blockage of STAT1 and STAT2 activation by nonstructural proteins. J Virol 2005;79:1934–42.
98. Fredericksen BL, Gale M Jr. West Nile virus evades activation of interferon regulatory factor 3 through RIG-I-dependent and -independent pathways without antagonizing host defense signaling. J Virol 2006;80:2913–23.
99. Miorin L, Albornoz A, Baba MM, D'Agaro P, Marcello A. Formation of membrane-defined compartments by tick-borne encephalitis virus contributes to the early delay in interferon signaling. Virus Res 2012;163:660–6.
100. Daffis S, Szretter KJ, Schriewer J, et al. 2′-O methylation of the viral mRNA cap evades host restriction by IFIT family members. Nature 2010;468:452–6.
101. Donald CL, Brennan B, Cumberworth SL, et al. Full genome sequence and sfRNA interferon antagonist activity of Zika virus from Recife, Brazil. PLoS Negl Trop Dis 2016;10:e0005048.
102. Best SM. The many faces of the flavivirus NS5 protein in antagonism of type I interferon signaling. J Virol 2017;91.
103. Grant A, Ponia SS, Tripathi S, et al. Zika virus targets human STAT2 to inhibit type I interferon signaling. Cell Host Microbe 2016;19:882–90.
104. Hertzog J, Dias Junior AG, et al. Infection with a Brazilian isolate of Zika virus generates RIG-I stimulatory RNA and the viral NS5 protein blocks type I IFN induction and signalling. Eur J Immunol 2018;48:1120–36.
105. Kumar A, Hou S, Airo AM, et al. Zika virus inhibits type-I interferon production and downstream signaling. EMBO Rep 2016;17:1766–75.
106. Xia H, Luo H, Shan C, et al. An evolutionary NS1 mutation enhances Zika virus evasion of host interferon induction. Nat Commun 2018;9:414.
107. Wu Y, Liu Q, Zhou J, et al. Zika virus evades interferon-mediated antiviral response through the co-operation of multiple nonstructural proteins in vitro. Cell Discov 2017;3:17006.
108. Glasner A, Oiknine-Djian E, Weisblum Y, et al. Zika virus escapes NK cell detection by upregulating major histocompatibility complex class I molecules. J Virol 2017;91.
109. Lobigs M, Mullbacher A, Regner M. MHC class I up-regulation by flaviviruses: immune interaction with unknown advantage to host or pathogen. Immunol Cell Biol 2003;81:217–23.
110. Avirutnan P, Mehlhop E, Diamond MS. Complement and its role in protection and pathogenesis of flavivirus infections. Vaccine 2008;26:I100–7.
111. Muller DA, Young PR. The flavivirus NS1 protein: molecular and structural biology, immunology, role in pathogenesis and application as a diagnostic biomarker. Antiviral Res 2013;98:192–208.
112. Silasi M, Cardenas I, Kwon JY, Racicot K, Aldo P, Mor G. Viral infections during pregnancy. Am J Reprod Immunol (New York, NY: 1989) 2015;73:199–213.
113. Singer DB, Rudolph AJ, Rosenberg HS, Rawls WE, Boniuk M. Pathology of the congenital rubella syndrome. J Pediatr 1967;71:665–75.
114. Mathur A, Tandon HO, Mathur KR, Sarkari NB, Singh UK, Chaturvedi UC. Japanese encephalitis virus infection during pregnancy. Indian J Med Res 1985;81:9–12.
115. Chaturvedi UC, Mathur A, Chandra A, Das SK, Tandon HO, Singh UK. Transplacental infection with Japanese encephalitis virus. J Infect Dis 1980;141:712–5.
116. Zhang Y, Li X, Chen H, et al. Evidence of possible vertical transmission of Tembusu virus in ducks. Vet Microbiol 2015;179:149–54.
117. Kim K, Shresta S. Neuroteratogenic Viruses and lessons for Zika virus models. Trends Microbiol 2016;24:622–36.
118. Platt DJ, Smith AM, Arora N, Diamond MS, Coyne CB, Miner JJ. Zika virus-related neurotropic flaviviruses infect human placental explants and cause fetal demise in mice. Sci Transl Med 2018;10.
119. O'Leary DR, Kuhn S, Kniss KL, et al. Birth outcomes following West Nile virus infection of pregnant women in the United States: 2003—2004. Pediatrics 2006;117:e537–45.
120. Bandeira AC, Campos GS, Sardi SI, Rocha VF, Rocha GC. Neonatal encephalitis due to Chikungunya vertical transmission: first report in Brazil. IDCases 2016;5:57–9.
121. Gerardin P, Samperiz S, Ramful D, et al. Neurocognitive outcome of children exposed to perinatal mother-to-child Chikungunya virus infection: the CHIMERE cohort study on Reunion Island. PLoS Negl Trop Dis 2014;8:e2996.