Studying the Effects of Emerging Infections on the Fetus: Experience with West Nile and Zika Viruses

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Emerging infections have the potential to produce adverse effects on the pregnant woman or her fetus; however, studying these effects is often challenging. We review our experiences with investigating the prenatal effects of two mosquito-borne infections that emerged in the past 2 decades, West Nile virus (WNV) and Zika virus. Concerns regarding teratogenicity were raised about both viruses; Zika virus has been confirmed to be teratogenic, while WNV appears not to increase the risk for adverse outcomes, although teratogenicity has not been excluded. Study designs used to examine the effects of both viruses include case reports and series, pregnancy registries, and cohort studies. Case–control studies and birth defects surveillance systems are being used to study the effects during pregnancy of Zika virus, but not the effects of WNV, because a specific phenotype was observed among infants with congenital Zika infection, but not among infants with congenital WNV infection. Experimental data that demonstrated that Zika virus was neurotropic have also been useful because they provided biologic plausibility for Zika virus's teratogenic effects: these findings were consistent with observations in congenitally infected infants. Challenges encountered with studies to evaluate the effects of these infections include the broad range of possible adverse outcomes, the inability to include all infected pregnant women in studies because many infections are asymptomatic, and the difficulty with interpretation of diagnostic testing of infants (WNV and Zika) and pregnant women (Zika). This review might be helpful to guide future studies of the effects of emerging infections during pregnancy.

Introduction

Emerging infections are defined as infections whose incidence has increased in the past two decades or is at risk for increasing in the near future (Centers for Disease Control and Prevention, 1998). In recent years, several infections meeting this definition have threatened human health. In some cases, new infections resulted from genetic changes in previous pathogens (e.g., pandemic H1N1 influenza virus [Novel Swine-Origin Influenza et al., 2009], H7N9 influenza virus [Uyeki and Cox, 2013], and Middle East Respiratory Syndrome Coronavirus [Rasmussen et al., 2016b]). In other cases, infections have emerged because of shifts in the geographic distribution of infectious pathogens (e.g., West Nile [Petersen and Roehrig, 2001] and Zika viruses [Petersen et al., 2016]). Infections can also emerge because of breakdowns in public health control measures (e.g., pertussis [Skoff et al., 2015]) or because of inappropriate antimicrobial use (e.g., *Clostridium difficile* [Lessa et al., 2015]).

Emerging infections have the potential to produce adverse effects on the pregnant woman or her fetus (Rasmussen and Hayes, 2005). Studying the potential adverse effects on the fetus can be challenging for several reasons, including the (1) broad range of adverse pregnancy outcomes that can be associated with congenital infections, ranging from pregnancy loss and preterm birth to birth defects to cognitive impairment and hearing loss of postnatal onset; (2) differing effects of a specific infection on the fetus depending on timing of maternal infection during pregnancy; (3) difficulty with detecting infections, either because infections are asymptomatic or because appropriate diagnostic testing is not performed; (4) problems diagnosing congenital infection because the sensitivity of diagnostic assays for a newly recognized congenital infection is often unknown; (5) possibility that the effects on the fetus might differ depending on the severity and nature of mother's illness, even in the absence of congenital infection; and (6) difficulty separating the effects of the infection itself from those of treatments for the infection (Rasmussen et al., 2007).

We previously reviewed these challenges and proposed study designs to assess the effects of emerging infections on the fetus (Rasmussen et al., 2007). In the past 2 decades, two mosquito-borne viruses have emerged in the Western Hemisphere, West Nile virus (WNV) and Zika virus. Concerns about teratogenicity have been raised about both viruses; Zika has been confirmed to be teratogenic, while WNV appears not to produce adverse fetal outcomes, although teratogenicity has not been excluded. Here, we review our experiences with the investigation of the effects on the fetus of these two emerging infections.

West Nile Virus

The emergence of WNV in the Western Hemisphere was first recognized following an encephalitis and meningitis outbreak in New York City in 1999. WNV is a flavivirus first isolated in 1937 from a febrile patient in the West

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Nile region of Uganda. Until its appearance in the United States, WNV was a cause of fever and sporadic encephalitis in parts of Africa, Europe, Asia, and the Middle East, and had attracted little attention in the medical literature (Petersen and Hayes, 2004). Following its emergence in New York City, the virus spread rapidly across the country to reach the West Coast and extended north and south to Canada and Mexico, respectively. As of 2015, nearly 44,000 U.S. cases of WNV had been reported to Centers for Disease Control and Prevention (CDC) from residents of all states except Alaska http://www.cdc.gov/westnile/resources/pdfs/data/2-west-nile-virus-disease-cases-reported-to-cdc-by-state_1999-2015_07072016.pdf.

Most persons with WNV are asymptomatic, with approximately 20% of those infected developing West Nile fever and approximately one in 140 developing West Nile neuroinvasive disease (i.e., encephalitis, meningitis, acute flaccid paralysis) (Petersen and Hayes, 2008). The primary mode of transmission of WNV is through the bite of an infected *Culex* species mosquito after the mosquito becomes infected by feeding on an amplifying host (usually a bird) (Petersen and Hayes, 2008). However, other modes of transmission have been recognized, including transmission through blood transfusions and transplanted organs (Iwamoto et al., 2003; Pealer et al., 2003), through percutaneous exposure (Centers for Disease Control and Prevention, 2002b), through breastfeeding (Hinckley et al., 2007), and from mother to fetus during pregnancy (Centers for Disease Control and Prevention, 2002a).

WEST NILE VIRUS AND PREGNANCY

Concerns about the effects of WNV during pregnancy were first raised by a report published in CDC's *Morbidity and Mortality Weekly Report* in 2002, which described an infant with birth defects born to a woman with West Nile encephalitis presenting at 27 weeks of pregnancy (Centers for Disease Control and Prevention, 2002a). The infant was born without obvious clinical abnormalities and with a normal head circumference. Ophthalmologic examination showed bilateral chorioretinitis, and brain imaging showed severe bilateral white-matter loss and cystic changes consistent with focal cerebral destruction (Alpert et al., 2003). Testing of infant blood and cerebrospinal fluid was positive for WNV-specific immunoglobulin M (IgM), demonstrating WNV transmission from the mother to the fetus (IgM typically does not cross the placenta; thus, a finding of IgM in a newborn provides evidence of intrauterine transmission). However, whether WNV was responsible for the abnormal findings was unknown.

Based on this report and the rapidly spreading WNV epidemic in the United States, the CDC developed interim guidelines for the evaluation of infants born to mothers with WNV during pregnancy (Centers for Disease Control and Prevention, 2004). Reports of four additional WNV-infected women and their infants were published; IgM

testing of the three infants who were tested at birth was negative, and all four infants were without abnormalities (Chapa et al., 2003; Hayes and O'Leary, 2004). In 2003, CDC initiated a registry for WNV-infected pregnant women in collaboration with state and local health departments; infant outcomes were assessed at birth through age 12 months. Among 77 women with WNV during pregnancy enrolled in the registry, four pregnancies ended in spontaneous abortions and two in elective abortions.

Among the 55 liveborn infants on whom cord serum was tested, 54 were WNV IgM negative; the one infant whose IgM test was positive for WNV on cord serum tested negative on peripheral serum at age 1 month. Among the 66 infants with information from a physical examination at birth, seven (10.6%, 95% confidence interval [CI], 5.2–20.3) had major birth defects, including one infant each with aortic coarctation, cleft palate, Down syndrome, lissencephaly, and polydactyly, and two infants with microcephaly (defined as head circumference >2 standard deviations below the mean). This frequency of congenital defects was higher than, but not statistically different from, the frequency in the reference group. However, only three infants had defects that could have been caused by WNV during pregnancy based on timing of the infection during pregnancy. In addition, none of the seven infants had conclusive evidence of congenital WNV infection; however, the sensitivity of IgM testing for congenital WNV infection in infants was unknown (O'Leary et al., 2006).

As part of a cooperative agreement between Tulane University and the CDC, in-depth follow-up of eleven infants identified through the West Nile Virus Pregnancy Registry was performed (Sirois et al., 2014). Infant medical records were reviewed, and ophthalmologic examinations and evaluations of child development at age 3 years using the Bayley Scales of Infant and Toddler Development®, Third Edition (Bayley-III®) were performed. None of the infants were of low birth weight or small for gestational age, and all had head circumferences that were appropriate for gestational age, except for one that was large for gestational age. Ophthalmologic examinations were normal on all infants. On developmental evaluation, the group's mean performance was at or above age level on all domains; one child had a mild delay in one domain, but this was consistent with the expected distribution of Bayley-III scores in the general population. Although these results were reassuring, the need for further research was emphasized, given the small number of children included in this study.

Results of a prospective study of WNV infection during pregnancy, also conducted as part of the Tulane-CDC collaboration, were recently published (Pridjian et al., 2016). In this analysis, outcomes from 28 mothers infected with WNV during pregnancy who were reported to the CDC and prospectively enrolled between 2005 and 2008 and

their infants were compared with those from 25 mothers without WNV infection and their infants. All infants in both groups delivered at or near term, except for one infant born to a WNV-infected mother delivered by emergency Cesarean delivery at 30 weeks gestation following a placental abruption. None of the infants born to mothers with WNV infection during pregnancy had positive WNV IgM on cord blood. Birth weight, length, and head circumference, and frequencies of major and minor birth defects were similar among infants born to mothers who were infected and those born to mothers who were not infected with WNV. Developmental evaluation (Bayley-III assessment) showed performance at or above age level domains for the 17 children born to women with WNV infection during pregnancy. While these results do not support an association between WNV and adverse outcomes, the study had insufficient power to rule out a small increased risk.

STUDY DESIGN AND CHALLENGES TO THE STUDY OF PRENATAL EFFECTS OF WNV

Several different study designs were used to study WNV's effects during pregnancy on the fetus (Table 1). These included case reports and case series (Centers for Disease Control and Prevention, 2002a; Alpert et al., 2003; Chapa et al., 2003; Hayes and O'Leary, 2004), a national pregnancy registry (O'Leary et al., 2006) with follow-up until 3 years of age (Sirois et al., 2014), and a prospective cohort study (Pridjian et al., 2016). Case–control studies and birth defects surveillance data have not been used to examine the prenatal effects of WNV because a specific phenotype has not been recognized among infants born to WNV-infected women.

Many challenges to the study of the prenatal effects of WNV have been encountered. First, as noted previously, a specific and consistent phenotype has not been observed among infants born to WNV-infected mothers, precluding the use of some study designs (case–control studies and birth defects surveillance systems) to investigate the issue. Studies performed thus far have not demonstrated an increased risk for adverse outcomes among infants born to WNV-infected mothers; however, proving that WNV is not a teratogen is difficult. Excluding a small increase in risk requires a very large epidemiologic study, which has not been performed because the number of pregnant women infected with WNV has been relatively small. Only 77 pregnant women were reported to a national West Nile Virus Pregnancy Registry during a 2-year period. Whether this is because few pregnant women were infected or because some WNV infections were missed is unknown; however, a significant proportion of WNV infections are asymptomatic (Pealer et al., 2003), and other WNV infections might not have been recognized and appropriately tested. In addition, limited data from the pregnancy registry suggest that the likelihood of intrauterine transmission of WNV is low because only a few infants have had positive IgM testing; however, the sensitivity of WNV IgM testing to detect congenital infection is unknown (O'Leary et al., 2006).

Another challenge to proving that an infection is not a teratogen is the broad range of adverse outcomes that can be associated with a prenatal exposure. Studies of children born to WNV-infected mothers to date have focused on birth, ophthalmologic, and developmental outcomes and have followed children only up until 2 to 3 years of age (Sirois et al., 2014; Pridjian et al., 2016). However, some teratogens have produced other types of adverse outcomes or outcomes not apparent until later in life (e.g., autism among children and diabetes among adults who were congenitally infected with rubella [Chess, 1977; Sever et al., 1985]). Thus, studies with longer follow-up and that include other adverse outcomes would be needed to state more definitively that WNV is not teratogenic.

Zika Virus

Zika virus is a flavivirus closely related to yellow fever, dengue, and West Nile viruses, most commonly transmitted through the bite of infected *Aedes aegypti* mosquitoes. Zika virus was first isolated from a sentinel monkey in the Zika forest of Uganda in 1947 (Petersen et al., 2016). For decades, Zika virus was associated with rare reports of mild illness until an outbreak occurred on the island of Yap, Federated States of Micronesia, in 2007 (Duffy et al., 2009). Like WNV, Zika virus garnered little attention in the medical literature until its recent emergence in the Western Hemisphere (Petersen et al., 2016). Zika virus was first recognized in Brazil in early 2015 (Zanluca et al., 2015); since then, Zika virus has spread rapidly, with 50 countries and territories in the Americas, including the United States, with reports of active mosquito-borne transmission. http://www.cdc.gov/zika/geo/active-countries.html.

ZIKA VIRUS AND PREGNANCY

In the fall of 2015, several months after the recognition of mosquito-borne transmission of Zika virus in Brazil, investigators noted a sharp increase in the number of infants born with microcephaly (Pan American Health Organization, 2015). Because of the rapid spread of Zika virus and concern about the increased number of babies with microcephaly born to mothers with prenatal Zika virus infection, the World Health Organization declared a Public Health Emergency of International Concern on February 1, 2016 (Heymann et al., 2016). Subsequently, an increase in cases of microcephaly was retrospectively recognized following an outbreak of Zika virus infection in French Polynesia in 2013 to 2014.

Early in the Brazil outbreak, much of the evidence in support of Zika as a cause of microcephaly was ecologic. Increases in the occurrence of febrile rash illnesses

TABLE 1. *Examples of Studies Used to Examine Effects of West Nile and Zika Viruses during Pregnancy on the Fetus by Types of Study Designs*

Types of study designs	West Nile virus	Zika virus
Case reports/case series	• Initial case report of infant with abnormal brain and eye findings born to mother infected with West Nile virus at 27 weeks gestation (Alpert et al., 2003; Centers for Disease Control and Prevention, 2002a). • Additional reports of four infants born to West Nile virus-infected mothers without abnormalities (Chapa et al., 2003; O'Leary et al., 2006).	• Case series of infants in Brazil assessed because of microcephaly demonstrated a consistent phenotype, now known as congenital Zika syndrome (Franca et al., 2016; Schuler-Faccini et al., 2016).
Birth defects surveillance systems	• Not used	• CDC has funded 45 jurisdictions to conduct birth defects surveillance defects believed to be associated with congenital Zika infection – in progress (see Gilboa et al., this issue).
Pregnancy registries	• West Nile Virus Pregnancy Registry, developed by CDC in collaboration with state and local health departments showed that most infants born to West Nile-infected mothers had no abnormalities evident at birth or during the first year of life (O'Leary et al., 2006). • Follow-up of eleven of these infants age focused on growth, ophthalmologic, and developmental outcomes up until 3 years of age show no evidence of adverse outcomes (Sirois et al., 2014).	• United States Zika Pregnancy Registry (in the 50 US states and District of Columbia, American Samoa, and US Virgin Islands) and the ZAPSS (Puerto Rico) (Simeone et al., 2016) were set up to better understand the effects of Zika virus during pregnancy. • United States Zika Pregnancy Registry data have been used to study the phenomenon of prolonged Zika viremia among pregnant women (Meaney-Delman et al., 2016) and to estimate the risk of Zika-associated defects among fetuses and infants born to women possibly infected with Zika during pregnancy (Honein et al., 2016).
Cohort studies	• A prospective cohort study was used to study the effects of West Nile virus during pregnancy, including the potential effects on developmental outcomes at age 24 months (Pridjian et al., 2016).	• A prospective cohort study was conducted of women with a rash illness during pregnancy who tested positive (Zika-affected) and negative (Zika unaffected) for Zika virus infection during pregnancy. No differences were noted in rates of fetal deaths, but adverse outcomes were noted in 46% of Zika-affected and 11% of Zika-unaffected pregnancies ($p < 0.001$). Adverse outcomes were noted in all three trimesters (Brasil et al., 2016a, 2016b).
Case–control studies	• Not used	• A case–control study conducted in Brazil demonstrated a substantial association between congenital Zika infection and microcephaly (crude odds ratio 55·5; 95% CI 8.6–∞) (de Araujo et al., 2016).

consistent with Zika virus disease were temporally and geographically associated with later appearance of an increased number of infants born with microcephaly (Kleber de Oliveira et al., 2016). Early cases were rarely laboratory-confirmed (Schuler-Faccini et al., 2016) and no consistent definition of microcephaly was used early on (Victora et al., 2016); these issues and the fact that a mosquito-borne virus had never before been proven to cause birth defects led many to be skeptical of the association (Butler, 2016; Triunfol, 2016).

Case reports and case series provided critical information in support of Zika virus as a cause of microcephaly. In several reports of fetuses or newborns with microcephaly, evidence of Zika virus infection was present. For example, in two pregnant women from Brazil whose fetuses were diagnosed with microcephaly and brain abnormalities on

prenatal ultrasonography, Zika virus RNA was identified in amniotic fluid by RT-PCR and genomic sequencing (Calvet et al., 2016). Pathological analysis of two newborns with microcephaly and brain abnormalities who died within 24 hr of birth and of two pregnancy losses at 11 and 13 weeks gestation (Martines et al., 2016) demonstrated Zika virus RNA by RT-PCR and immunohistochemistry. All four mothers resided in Brazil and had clinical symptoms compatible with Zika virus illness during their first pregnancy trimester.

Further evidence supporting a causal link between Zika virus and microcephaly was reported by Mlakar et al. who described a pregnant woman from Brazil with a rash and fever in her late first trimester of pregnancy (Mlakar et al., 2016). Prenatal ultrasonography at 29 weeks gestation revealed microcephaly with calcifications in the fetal brain and placenta, and autopsy performed after pregnancy termination showed cortical and subcortical calcifications, nearly complete agyria, and hydrocephalus. Zika virus was identified in fetal brain tissue by RT-PCR and the complete Zika virus genome was recovered.

Strong evidence for Zika virus as a teratogen came from a case reported by Driggers et al. in which a resident of the United States traveled to three countries with active Zika virus transmission (Mexico, Guatemala, and Belize) during her 11th week of gestation and developed symptoms of Zika virus illness shortly after her return home (Driggers et al., 2016). Prenatal ultrasonography showed decreasing head circumference, and abnormal intracranial anatomy was noted at 19 to 20 weeks of gestation by ultrasonography and fetal MRI. Following pregnancy termination, Zika virus was identified by RT-PCR in fetal tissues, with the highest viral loads found in fetal brain, compared with other fetal tissues. In addition, replicative Zika virus was isolated from the fetal brain.

While these case reports provided strong evidence for Zika virus as a cause of microcephaly and other serious brain anomalies, these alone were not considered sufficient to make the causal link. However, as has been seen with other teratogens (e.g., rubella, thalidomide, and isotretinoin) (Friedman, 1992), data from case series demonstrated a distinctive phenotype associated with congenital Zika infection. In the case series of 35 infants with microcephaly collected through a microcephaly registry and reported by Schuler-Faccini et al., a specific phenotype among infants began to emerge. This phenotype consisted of severe microcephaly (>3 SDs below the mean), intracranial calcifications, redundant scalp skin, hypertonia/spasticity, clubfoot, and arthrogryposis (Schuler-Faccini et al., 2016). These cases, selected based on the presence of microcephaly, were born to women who had resided in or traveled to an area with Zika virus circulation; most of these mothers reported a rash-like illness in the first or second trimester of pregnancy.

In addition, experimental data supported Zika virus as a cause of severe central nervous system damage. Zika virus had long been recognized as being neurotropic, with studies in mice as far back as 1952 demonstrating Zika virus's predilection for brain tissue (Dick, 1952). Recent studies confirmed these early findings; Zika virus was shown to infect, produce cell death, and attenuate future growth of human neural progenitor cells (Tang et al., 2016).

In April of 2016, a review by CDC authors was conducted to determine if available data were sufficient to implicate Zika as a cause of microcephaly and other serious brain defects (Rasmussen et al., 2016a). Data were reviewed using two sets of criteria: Shepard's criteria for teratogenicity (Shepard, 1994; Teratology Society Public Affairs Committee, 2005) and Bradford Hill criteria (Hill, 1965). Based on this review, CDC concluded that Zika virus caused microcephaly and other serious brain defects (Frieden et al., 2016; Rasmussen et al., 2016a). The authors recognized that many questions remained, including the level of risk, how timing of infection during pregnancy affects the defects observed, and the co-factors that might modify the risk. In addition, it was noted that microcephaly and other serious brain defects were likely to be only part of the Zika-associated phenotype and that further studies would be needed to identify the full spectrum of Zika-associated defects.

Since that time, additional data have accumulated in support of Zika virus as a cause of birth defects. These data include animal models that demonstrate that Zika virus is a teratogen in other species, including mice (Cugola et al., 2016; Li et al., 2016; Miner et al., 2016) and chick (Goodfellow et al., 2016) models. Additional epidemiologic data have also become available. In a case–control study with 32 case infants with microcephaly and 62 control infants, 13 (41%) of 32 case infants and none of the 62 controls had laboratory-confirmed Zika virus infection (de Araujo et al., 2016). This resulted in a crude overall odds ratio of 55·5 (95% CI, 8.6–∞), providing further strong evidence in support of Zika virus as a cause of microcephaly.

Additional case reports and case series have further defined the phenotype of congenital Zika syndrome (Moore et al., 2016). These data suggest that Zika virus infection during pregnancy produces a distinctive phenotype that includes severe microcephaly with partially collapsed skull, consistent with the fetal brain disruption sequence (Corona-Rivera et al., 2001); thin cerebral cortices with intracranial calcifications, primarily subcortical in location; retinal abnormalities including scarring of the macula and focal pigmentary retinal mottling (Ventura et al., 2016); contractures of single or multiple joints (van der Linden et al., 2016a); and significant hypertonia and extrapyramidal symptoms (Moura da Silva et al., 2016). In addition, recent data have shown that infants whose

mothers were infected with Zika during pregnancy who had normal head size at birth can develop microcephaly at a later age (van der Linden et al., 2016b).

To better understand the effects of Zika infection during pregnancy, CDC has launched several studies. CDC investigators have developed the US Zika Pregnancy Registry, a national registry for women with laboratory evidence of possible Zika infection during pregnancy and their infants, in collaboration with health care providers and state and local health departments (Simeone et al., 2016). This registry collects information on pregnant women and infants born to women with possible laboratory evidence of Zika infection in the United States and its territories, excluding Puerto Rico, including information on infant growth and development through the first year of life. This registry has already answered several questions; for example, after a report of prolonged Zika viremia in a pregnant woman with an infected fetus (Driggers et al., 2016), early data from the U.S. Zika Pregnancy Registry examined this phenomenon among a larger group of pregnant women (Meaney-Delman et al., 2016). In addition, data from the registry estimated the risk of microcephaly and other defects among women with possible Zika infection during pregnancy in the United States. Approximately 6% of fetuses or infants born to women with laboratory evidence of Zika infection during pregnancy had Zika-associated defects; among those with first trimester infection, the frequency was 11%. Presence or absence of maternal symptoms did not appear to affect the risk of abnormalities (Honein et al., 2016).

The Puerto Rico Department of Health and CDC have also developed a surveillance system in Puerto Rico to collect information on pregnant women and their infants called the Zika Active Pregnancy Surveillance System (ZAPSS). Pregnant women with laboratory evidence of Zika infection and their infants or fetuses are monitored. Monitoring is active (surveillance system staff visit hospitals and clinics to collect information from medical records of mothers and their infants); in this system, children will be followed up until age 3 years (Simeone et al., 2016).

As part of the emergency response to the Zika virus epidemic, CDC has funded 45 jurisdictions to develop systems to conduct birth defects surveillance for infants with birth defects associated with Zika infection (Gilboa et al. - in this issue). Several challenges to the monitoring of these defects are being addressed. For example, monitoring the prevalence of congenital microcephaly has previously been difficult because of varying methods of case ascertainment and differing case definitions, and birth prevalence estimates from birth defects surveillance systems have varied widely (Cragan et al., 2016). Use of consistent methods of ascertainment and specific case definitions for microcephaly are necessary to improve surveillance quality (Cragan et al., 2016). In addition, birth defects surveillance systems will need to have the ability to ascertain brain findings and other abnormalities beyond microcephaly to improve ascertainment of the spectrum of Zika-associated defects (Trevathan, 2016). Thus, these systems will use rapid, active methods of ascertainment and consistent case definitions for defects believed to be associated with Zika virus infection to enhance the identification of Zika-associated defects. These systems are planned to be complementary to the U.S. Zika Pregnancy Registry and ZAPSS; some infants will be identified by both systems, but infants who were not included in these systems, either because their mothers were not tested or for whom testing was misleading, will be identified by these birth defects surveillance systems if the infant has birth defects believed to be associated with congenital Zika infection (Gilboa et al. – this issue).

STUDY DESIGN AND CHALLENGES TO THE STUDY OF PRENATAL EFFECTS OF ZIKA VIRUS

Study designs that have been or are being used to examine Zika virus during pregnancy include case reports and case series, pregnancy registries, birth defects surveillance system data, case–control studies, and cohort analyses (Table 1). Other data useful to understand the prenatal effects of Zika virus include ecologic data of increases in infants with microcephaly following outbreaks of Zika (Kleber de Oliveira et al., 2016), mathematical modeling based on data following outbreaks (Cauchemez et al., 2016; Johansson et al., 2016), in vitro data on stem cells (Tang et al., 2016), and animal models (Cugola et al., 2016; Goodfellow et al., 2016; Li et al., 2016; Miner et al., 2016).

Investigators studying the prenatal effects of Zika virus have faced many challenges. The wide range of adverse pregnancy outcomes potentially associated with congenital infections makes determining whether a particular adverse outcome was associated with congenital Zika infection or occurred by chance difficult. For example, spontaneous abortions have occurred following prenatal Zika infection; the finding of Zika virus in the products of conception (Martines et al., 2016) suggests that Zika might be a cause of pregnancy loss, but given that pregnancy losses are relatively common, it is difficult to prove that Zika causes spontaneous abortions without collection of epidemiologic data. Although data are sufficient to conclude that Zika infection is a cause of microcephaly and other serious brain anomalies, further studies will be needed to determine the full spectrum of Zika-associated defects. The possibility that more subtle findings, such as cognitive impairment or autism that might not be recognized in infancy, can occur means that even infants who appeared normal at birth might later be recognized as having effects of congenital Zika infection; thus, studies at older ages will be needed.

Another challenge recognized in the study of congenital infections and other potential teratogens is that the effects of exposures during pregnancy differ depending on the

timing of the exposure (Teratology Society Public Affairs Committee, 2005). This central principle of teratology was well demonstrated by the effects of congenital rubella infection (Webster, 1998). Data suggest that microcephaly and other serious brain anomalies are related to maternal Zika infection during the first or early second trimester; however, data suggest that infections even late in pregnancy might be associated with effects on the infant (Brasil et al., 2016a, 2016b; Soares de Souza et al., 2016).

The possibility that the infection might not have been diagnosed in the pregnant woman, either because the woman was asymptomatic or because appropriate diagnostic testing was not performed, has been a significant challenge in the study of the effects of congenital Zika infection. Estimates suggest that most persons infected with Zika virus are asymptomatic (Duffy et al., 2009). Early studies in Brazil depended on the presence of a rash to identify a woman as potentially infected with Zika infection (Brasil et al., 2016b; Schuler-Faccini et al., 2016), leaving a significant proportion of infected mothers not identified as having Zika infection. In addition, serologic testing for Zika infection has been complicated: cross-reactivity with other flaviviruses (e.g., dengue, yellow fever) has challenged definitive diagnosis (Rabe et al., 2016). The US Zika Pregnancy Registry and ZAPSS have dealt with this issue by including pregnant women with any laboratory evidence of possible Zika infection; while this approach will decrease the likelihood of women with Zika infection being excluded from the registry, it will likely affect risk estimates for adverse outcomes.

Another significant issue complicating the study of Zika infections during pregnancy is related to problems with making a diagnosis of congenital infection in an infant because the sensitivity of diagnostic assays for a newly recognized congenital infection is unknown. Data from studies conducted in Brazil suggest that not all congenitally infected infants test positive for IgM (de Araujo et al., 2016; Melo et al., 2016). Additional data will be needed to assess the performance characteristics of testing for congenital Zika infection.

Conclusions

The study of the prenatal effects of emerging infections during pregnancy is challenging, as evidenced by the investigations conducted to understand the effects of WNV and Zika virus on the fetus. Many study designs were or are being used to examine the effects of WNV and Zika virus infections during pregnancy on the fetus. To examine the effects of WNV during pregnancy, case reports and series, a pregnancy registry, and a prospective cohort analysis were all used. Case–control studies and birth defects surveillance systems were not used to study prenatal effects of WNV, due to the lack of an established phenotype with WNV. In contrast, case–control studies and birth defects surveillance systems, as well as case reports and series, pregnancy registries, and cohort analyses all have been or will be used to study the prenatal effects of Zika infection. Case reports and series were of particular importance in identifying Zika as a teratogen, given the emergence of a recognizable pattern of defects observed in infants with congenital Zika infection (Moore et al., 2016).

Several other pieces of evidence have been helpful in understanding the teratogenicity of Zika virus (Rasmussen et al., 2016a), including ecologic data of increases in infants with microcephaly following outbreaks of Zika (Kleber de Oliveira et al., 2016), mathematical modeling (Cauchemez et al., 2016; Johansson et al., 2016), and experimental data (Tang et al., 2016). Animal models (Cugola et al., 2016; Goodfellow et al., 2016; Li et al., 2016; Miner et al., 2016) have provided further confirmatory evidence. Many challenges to the study of these infections remain. Studies to understand the prenatal effects of Zika infection, including studies discussed here and other studies, such as the large, multi-country prospective study funded by the National Institutes of Health (the Zika in Infants and Pregnancy study) https://www.nichd.nih.gov/news/releases/Pages/zika_zip_06202016.aspx, will continue for several years. These experiences with WNV and Zika virus might be helpful in the future to guide studies of emerging infections and their effects during pregnancy.

Acknowledgments

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