

Birth Outcomes Following West Nile Virus Infection of Pregnant Women in the United States: 2003-2004

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ABSTRACT

BACKGROUND. Congenital West Nile virus (WNV) infection was first described in a single case in 2002. The proportion of maternal WNV infections resulting in congenital infection and clinical consequences of such infections are unknown.

METHODS. In 2003 and 2004, women in the United States who acquired WNV infection during pregnancy were reported to the Centers for Disease Control and Prevention by state health departments. Data on pregnancy outcomes were collected. One of the maternal WNV infections was identified retrospectively after the infant was born. Maternal sera, placenta, umbilical cord tissue, and cord serum were tested for WNV infection by using serologic assays and reverse-transcription polymerase chain reaction. Infant health was assessed at delivery and through 12 months of age.

RESULTS. Seventy-seven women infected with WNV during pregnancy were clinically followed in 16 states. A total of 71 women delivered 72 live infants; 4 women had miscarriages, and 2 had elective abortions. Of the 72 live infants, 67 were born at term, and 4 were preterm; gestational age was unknown for 1. Of 55 live infants from whom cord serum was available, 54 tested negative for anti-WNV IgM. One infant born with umbilical hernia and skin tags had anti-WNV IgM in cord serum but not in peripheral serum at age 1 month. An infant who had no anti-WNV IgM in cord blood, but whose mother had WNV illness 6 days prepartum, developed WNV meningitis at age 10 days. Another infant, whose mother had acute WNV illness at delivery, was born with a rash and coarctation of the aorta and had anti-WNV IgM in serum at 1 month of age; cord serum was not available. A fourth infant, whose mother had onset of WNV illness 3 weeks prepartum that was not diagnosed until after delivery, had WNV encephalitis and underlying lissencephaly detected at age 17 days and subsequently died; cord serum was not available. The following major malformations were noted among live-born infants: aortic coarctation ($n = 1$); cleft palate ($n = 1$); Down syndrome ($n = 1$); lissencephaly ($n = 1$);

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Key Words

West Nile virus, congenital infection, birth outcomes, pregnancy, development, growth, pediatrics, birth defects, microcephaly

Abbreviations

WN—West Nile
WNV—West Nile virus
JEV—Japanese encephalitis virus
SLEV—St Louis encephalitis virus
CSF—cerebrospinal fluid
CDC—Centers for Disease Control and Prevention
ELISA—enzyme-linked immunosorbent assay
RT-PCR—reverse-transcription polymerase chain reaction

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microcephaly ($n = 2$); and polydactyly ($n = 1$). One infant had glycogen storage disease type 1. Abnormal growth was noted in 8 infants.

CONCLUSIONS. Of 72 infants followed to date in 2003 and 2004, almost all seemed normal, and none had conclusive laboratory evidence of congenital WNV infection. Three infants had WNV infection that could have been congenitally acquired. Seven infants had major malformations, but only 3 of these had defects that could have been caused by maternal WNV infection based on the timing of the infections and the sensitive developmental period for the specific malformations, and none had any conclusive evidence of WNV etiology. However, the sensitivity and specificity of IgM testing of cord blood to detect congenital WNV infection are currently unknown, and congenital WNV infection among newborns with IgM-negative serology cannot be ruled out. Prospective studies comparing pregnancy outcomes of WNV-infected and -uninfected women are needed to better define the outcomes of WNV infection during pregnancy.

WEST NILE VIRUS (WNV) is a neurotropic flavivirus that is antigenically related to Japanese encephalitis (JEV) and St Louis encephalitis (SLEV) viruses and is transmitted to humans primarily through the bite of infected mosquitoes. In 1999, WNV was discovered in the United States during an encephalitis epidemic in New York City and since has spread across North America causing >16 000 reported human WNV illness cases and >600 deaths.^{1,2} In 2002, the first case of human congenital WNV infection was reported in a normal-appearing neonate with bilateral chorioretinitis, severe bilateral cerebral destruction, and anti-WNV IgM antibodies in cord blood and cerebrospinal fluid (CSF).³ Four additional infants born to women with WNV illness during pregnancy were identified in 2002; 3 were born healthy at term with no evidence of congenital WNV infection, and 1 was born preterm but otherwise seemed normal and was not tested for WNV infection.⁴

Several reports have described adverse outcomes of pregnancy after mosquito-borne flaviviral infections in humans and nonhuman mammals. Spontaneous abortion with virus isolated from fetal tissue has been reported after JEV infection.^{5,6} Neonatal dengue, including hemorrhagic manifestations and neonatal death, has followed congenital dengue infection.⁷⁻⁹ SLEV, JEV, and WNV infection of pregnant mice has caused abortion and stillbirth, as well as neonatal encephalocele, hydrocephalus, and learning deficits.¹⁰⁻¹³ Hydranencephaly, porencephaly, abortion, stillbirth, and neonatal death were reported in lambs of pregnant sheep infected with WNV and the African flaviviruses Banzi and AR5189,

although the virus was not isolated from congenitally malformed fetuses.¹⁴

To further evaluate the frequency and consequences of congenital WNV infection, the Centers for Disease Control and Prevention (CDC) collaborated with state health departments to establish a surveillance registry for women who developed WNV illness during pregnancy. We describe here the birth outcomes, infant growth, and WNV laboratory test results assessing intrauterine WNV transmission in women identified through the surveillance registry in 2003 and 2004.

METHODS

Surveillance Methods

Health care providers in the United States reported WNV infection in pregnant women to state and local health departments. The health departments then reported to the CDC those infections documented by presence of IgM antibodies to WNV in serum by enzyme-linked immunosorbent assay (ELISA), a fourfold rise in WNV-specific neutralizing antibodies in serum by plaque-reduction neutralization testing, IgM antibody to WNV in CSF, or presence of WNV RNA in serum by reverse-transcription polymerase chain reaction (RT-PCR).¹⁵⁻¹⁷ Health department or CDC staff contacted the women's health care providers who asked their patients to voluntarily participate in the national surveillance registry for WNV illness during pregnancy. Health care providers were asked to report the outcomes of pregnancy, as well as the health and development of live-born infants, using standardized data collection forms. One of the maternal WNV infections was identified retrospectively after the infant was born. The collection of surveillance data on all of the pregnancies and their outcomes complied with CDC human subjects requirements.

Laboratory Methods

At the time of delivery, specimens of maternal serum, cord blood, placental tissue, umbilical cord tissue, and breast milk were collected for WNV testing. When clinically indicated, follow-up specimens of infant serum, infant CSF, or fetal and neonatal autopsy tissues were also collected. Maternal serum, infant serum, CSF, and breast milk were tested at state public health laboratories or the CDC for WNV- and SLEV-specific antibodies by IgM and IgG ELISA and plaque-reduction neutralization testing. All of the specimens except the maternal serum were tested for the presence of WNV RNA by RT-PCR at the CDC. Formalin-fixed specimens were tested for flavivirus antigen by immunohistochemical staining at the CDC.¹⁸

Data Collection and Analysis

Information regarding the mother's medical history, including estimated trimester of WNV infection, complica-

tions of pregnancy, date and route of delivery, and results of laboratory testing for other congenital infections was collected from each mother's health care provider. The mother's trimester of WNV infection was estimated based on the dates of illness onset and last menstrual period. Information collected on newborn infants included date of birth, estimated gestational age at delivery, physical examination findings at time of delivery, height, weight, head circumference, and Apgar scores. Gestational age at delivery was reported by the attending health care provider or, if not reported, was estimated at the CDC based on the date of the mother's last menstrual period. Observed frequencies of major birth defects, spontaneous abortions, preterm births, and low birth weights were compared with population-based estimates.^{19–28} Major birth defects were defined as structural or chromosomal abnormalities that can adversely affect health or development.^{19,29} Results of routine infant checkups at 2, 6, and 12 months of age were collected from the child's health care provider. Reported height, weight, and head circumference measurements were charted using Epi Info 2000 3.2 and reviewed for growth abnormalities.^{30,31}

RESULTS

Pregnancy Outcomes

In 2003 and 2004, 83 pregnant women with WNV illness were identified through national surveillance. Six women declined to participate in the registry, and their physicians provided no information regarding their pregnancy. Physicians of 2 other women declined to ask their patients to participate in the registry but reported that 1 woman had elected to terminate her pregnancy, and the other had offered her apparently healthy infant for adoption. Of 77 women from 16 states (including the 2 women with only basic information on WNV illness and pregnancy outcome), 52 (68%) had West Nile (WN) fever; 18 (23%) had WN neuroinvasive illness; 6 (8%) had unspecified clinical illness; and 1 (1%) had asymptomatic viremia identified through blood donor screening. None of the women died. The median age of the 77 women was 29 years (range: 15–45 years); 59 (77%) were white; 2 (3%) were American Indian; 1 (1%) was black; 1 (1%) was Asian; and for 14 (18%) race was unknown. WNV infection was documented at state or public health reference laboratories in 61 cases, at commercial or private laboratories without reference laboratory confirmation in 15 cases, and through blood donor screening shortly before pregnancy recognition in 1 case. Based on the dates of illness onset, 25 women (33%) were believed to have been infected during the first trimester of pregnancy, 27 (35%) in the second trimester, 24 (31%) in the third trimester, and the trimester of WNV infection could not be estimated for 1 (1%). Four women had spontaneous abortions. Two women had

elective abortions, and the remaining 71 delivered 72 live infants including 1 set of twins. Clinical information on 1 elective abortion and 5 of these infants has been reported in part in separate publications.^{32,33} Among women who spontaneously aborted, WNV illness onset preceded abortion by a mean of 47 days (range: 17–94 days).

Of 72 live-born infants, 42 were delivered vaginally, 23 by cesarean section, and for 7 the delivery method was not reported. There were 67 infants born at term (defined as gestational age of 37–41 weeks), 4 were born preterm, and 1 was born at unknown gestational age (Table 1). Three (4.8%) of 63 infants with reported birth weight had low birth weight (<2500 g). Of 66 live infants with information regarding physical examination at birth, 7 (10.6%) had major birth defects (aortic coarctation, 1; cleft palate, 1; Down syndrome, 1; lissencephaly, 1; microcephaly, 2; and polydactyly, 1); 4 infants had 5 minor birth defects (skin tag, 1; umbilical hernia, 4); and 1 infant had type 1 glycogen storage disease (Tables 1 and 2). The mother of the infant with lissencephaly had a WNV-like illness in the third trimester, which was not identified as WNV infection until after delivery. If this case was excluded, then 6 (9.2%) of

TABLE 1 Characteristics of Live-Born Infants of 71 WNV-Infected Pregnant Women

| Measurement | N ^a | Mean (Range) or n (%) With Condition |
|---|----------------|--------------------------------------|
| Gestational age, wk | 71 | 39 (26–43) |
| APGAR score 1 min | 59 | 8 (1–9) |
| Apgar score at 5 min | 59 | 9 (6–10) |
| Length, cm | 61 | 50.2 (33.5–55.8) |
| Weight, kg | 63 | 3.3 (0.8–4.8) |
| Head circumference, cm | 58 | 34.2 (23.5–37) |
| Adverse outcome | | |
| Low birth weight ^b | 63 | 3 (4.8) |
| Preterm birth ^c | 71 | 4 (5.6) |
| Major birth defect ^d | 66 | 7 (10.6) |
| Aortic coarctation with bicuspid aortic valve | 66 | 1 (1.5) |
| Cleft palate | 66 | 1 (1.5) |
| Down syndrome | 66 | 1 (1.5) |
| Lissencephaly | 66 | 1 (1.5) |
| Microcephaly ^e | 58 | 2 (3.4) |
| Polydactyly | 66 | 1 (1.5) |
| Other ^f | 66 | 7 (10.6) |
| Glycogen storage disease type 1 | 66 | 1 (1.5) |
| Neonatal death | 66 | 2 (3.0) |
| Skin tags | 66 | 1 (1.5) |
| Umbilical hernia | 66 | 4 (6.1) |

The number of live-born infants includes 1 set of twins.

^a Indicates the number of infants for whom information was available.

^b Defined as <2500 g.

^c Defined as delivery at <37 weeks' gestational age.

^d The mother of the infant with lissencephaly had a WNV-like illness in the third trimester, which was not identified as WNV infection until after delivery. If this case were excluded, then 6 (9.2%) of 65 infants had major birth defects.

^e Defined as head circumference >2 SD below mean; 1 of 2 microcephalic infants had several congenital abnormalities including multiple cerebral abnormalities, micrognathia, epicanthic folds, clinodactyly, splenomegaly, and cardiomegaly.

^f One infant had both skin tags and an umbilical hernia.

TABLE 2 Frequencies of Birth Abnormalities Among Live-Born Infants of 71 WNV-Infected Pregnant

| Women | | | |
|---------------------------------|-------------------------------------|--------------------------|--|
| Condition | Among 72 Live Births, % (95% CI) | General Population, % | Source |
| Major birth defects | | | |
| All major defects ^a | 10.6 (5.2–20.3) | 5.5 ^d | CDC, unpublished data, 2005; ref 24 ^d |
| Aortic coarctation | 1.5 (0.3–8.1) | 0.03 | Ref 20 |
| Cleft palate | 1.5 (0.3–8.1) | 0.04 | Ref 21 |
| Down syndrome | 1.5 (0.3–8.1) | 0.13 | Ref 22 |
| Lissencephaly | 1.5 (0.3–8.1) | 0.001 | Ref 23 |
| Microcephaly ^b | 3.5 (1.0–11.7) | 2.28 | Ref 24 |
| Polydactyly | 1.5 (0.3–8.1) | 0.2 | Ref 25 |
| Other adverse outcomes | | | |
| Glycogen storage disease type 1 | 1.5 (0.3–8.1) | 0.0005 | Ref 26 |
| Spontaneous abortion | 9.1 (3.4–21.2) ^c | 15.0 | Ref 27 |
| Low birth weight | 4.8 (1.6–13.1) | 7.9 | Ref 28 |
| Preterm | 5.6 (2.2–13.6) | 12.3 | Ref 28 |

CI indicates confidence interval.

^a The mother of the infant with lissencephaly had a WNV-like illness in the third trimester, which was not identified as WNV infection until after delivery. If this case was excluded, then 6 of 65 infants (9.2%) had major birth defects.

^b Defined as head circumference >2 SD below mean for term infants.

^c Calculated among 44 women with WNV illness onset estimated at <20 weeks' gestation.

^d Estimated as 3.29% from the Metropolitan Atlanta Congenital Defects Program (CDC, unpublished data, 2005), which includes only physician-diagnosed microcephaly (0.06% of births), plus 2.28% to include infants with microcephaly defined as head circumference <2 SD below mean²⁴; 3.25–0.06% + 2.28% = 5.47% ~ 5.5%.

65 infants had major birth defects, which is higher than expected. Major birth defects followed first-trimester maternal WNV infection in 1 case (polydactyly in a white infant), second-trimester infection in 3 cases (microcephaly, 2; and Down syndrome, 1), and third-trimester infection in 3 cases (aortic coarctation, 1; cleft palate, 1; and lissencephaly, 1). The mother of the infant with glycogen storage disease type 1 was probably infected in her third trimester.

Laboratory Testing for WNV Infection

Of 55 live infants from whom cord serum was collected, only 1 had anti-WNV IgM detected in cord blood. This was a term infant with umbilical hernia and facial skin tags delivered by emergency cesarean section. Infant serum collected at 1 month of age was negative for anti-WNV IgM, and serum collected at 8 months of age was negative for anti-WNV IgM and had no detectable WNV-neutralizing antibody, suggesting that the IgM detected in cord blood of this infant was of maternal origin (an unusual occurrence) or that the test was falsely positive.³⁴ At 6 months of age, the infant seemed healthy and had normal growth and development.

WNV RT-PCR was performed on 46 cord blood specimens and 50 cord tissue specimens tested; all were negative. Forty-nine of 50 placentae tested were negative by WNV RT-PCR. One was WNV PCR-positive on the fetal side and negative on the maternal side. This mother had onset of WNV illness 4 days before delivery. Both mother and infant had a transient rash at delivery; the infant was otherwise healthy. Cord blood and an infant serum specimen collected at 1 month of age tested negative for anti-WNV IgM. Neutralizing antibody to

WNV was present in infant serum at 1 month of age but absent at 6 months. At 12 months of age, the child had normal growth and development and seemed healthy.

Three infants had possible intrauterine WNV infections despite lacking evidence from cord blood or infant serum at delivery. One was a breastfed, term infant whose cord blood was negative for anti-WNV IgM and WNV RNA by RT-PCR but who developed WN meningitis at home at age 10 days with positive anti-WNV IgM in CSF and whose mother had onset of WN neuroinvasive disease 6 days before delivery. At 12 months of age, the infant's growth and development were normal. Another breastfed infant, whose mother had acute WN fever at delivery, was born with a transient rash and had bicuspid aortic valve and aortic coarctation. Neither cord blood nor neonatal serum was available for testing. Serum collected at 1 month of age was positive for anti-WNV IgM. At 14 months of age, the child had normal development and was growing close to the 25th percentile for weight, with height above the 90th percentile and head circumference at the 50th percentile. The third infant was born to a mother who had onset of a febrile illness 3 weeks before delivery. The mother was not tested for WNV infection at that time. The infant seemed healthy at delivery and was formula-fed and discharged at 3 days of age but developed seizures at 7 days of age; the seizures were not clinically evaluated until 17 days of age when the infant was found to have both lissencephaly and WNV encephalitis with anti-WNV IgM in CSF. A serum sample from the mother taken 1 month after delivery was positive for anti-WNV IgM. The infant's karyotype was normal; tests for anti-cytomegalovirus IgM, anti-rubella IgM, anti-SLEV IgM, and herpesvirus

nucleic acid were all negative. The infant died at 7 weeks of age.

In 1 of 2 elective abortion cases where fetal tissues were available, fetal cord serum, serum, CSF, liver, spleen, heart, and brain were negative by WNV RT-PCR. Two of 42 breast milk specimens were positive by WNV RT-PCR; 1 of the 2 breastfeeding infants was not subsequently tested for evidence of WNV infection; a follow-up serum specimen collected from the other infant at 7 months of age was negative for both anti-WNV IgM and WNV-neutralizing antibody.

Evaluation of Infants With Microcephaly

One infant with microcephaly was born at term after maternal WNV infection that probably occurred during the second trimester. The infant had a birth weight of 2.1 kg (<3rd percentile); head circumference of 29 cm (well below the 3rd percentile and >3 SD below the mean for term infants); and hydrocephalus, diencephalic hypoplasia, agenesis of the corpus callosum, micrognathia, epicanthic folds, clinodactyly, splenomegaly and cardiomegaly. The infant died at 3 days of age. Serum and CSF were negative for anti-WNV IgM by ELISA; brain, cord blood, cord tissue, CSF, liver, placenta, and serum were negative for WNV RNA by RT-PCR. Central nervous system tissue, heart, kidney, liver, lung, spleen, and placenta showed no evidence of flavivirus antigens by immunohistochemical staining. Additional serologic and nucleic acid tests were negative for cytomegalovirus, herpes simplex 1 and 2, rubella, syphilis, toxoplasmosis, and varicella zoster virus. The infant's karyotype was normal.

The second infant with microcephaly was born at term to a mother who had WNV illness during the second trimester. The infant had a birth weight of 3.0 kg (25th percentile) and a head circumference of 31 cm (below the 3rd percentile for term infants and >2 SD below the mean). Brain magnetic resonance imaging, a funduscopic examination, and a newborn hearing examination were normal. The infant's serum and CSF were negative for anti-WNV IgM. Cord blood, cord tissue, and placenta were negative for WNV RNA by RT-PCR. Tests for cytomegalovirus, herpes simplex virus 1 and 2, rubella, and toxoplasmosis were all negative. Karyotyping revealed duplication of part of the long arm of chromosome 15 (q11.2q13), which was determined by genetics consultation to be a normal variant of no clinical significance. At 12 months of age, her head circumference had increased to the 27th percentile, weight was at the 82nd percentile, height was at the 89th percentile, and she was reported to have normal development.

One other infant was born with small head size but was felt to have nearly symmetrical growth abnormalities. She was born at 29 weeks' gestation after maternal preeclampsia with a birth weight of 0.8 kg (<10th per-

centile for gestational age, the lowest reference percentile curve on available charts for intrauterine growth); a height of 33.5 cm (<10th percentile); and a head circumference of 24 cm (also below the 10th percentile).³⁵ An ultrasound of the head was normal, and the newborn physical examination was otherwise unremarkable. Serologic and PCR tests for WNV on cord blood, cord tissue, and placenta were all negative; test results for other infectious agents were not reported. By 12 months of age, her head circumference adjusted for gestational age was at the 54th percentile, weight was at the 4th percentile, height was at the 15th percentile, and her development seemed normal.

Infant Growth

Of 45 infants with weight measurements through 12 months of age, 41 seemed to have appropriate weight gain and 4 had marked flattening of the weight growth curve suggesting growth failure. All 12 of the infants with weight measurements through 6 months old seemed to have appropriate weight gain. Of 4 infants with weight measurements through 2 months of age, 3 seemed to have appropriate weight gain, and 1 had a marked decrease in weight for age. All 44 infants with height measurements through 12 months old seemed to have appropriate height gain. Ten of 11 infants with height measurements through 6 months of age seemed to have appropriate height gain and 1 had a decrease in height for age. All 4 infants who had height measurements through 2 months of age had appropriate height gain.

Of 40 infants with head circumference measurements through 12 months old, 37 seemed to have normal head growth and 3 seemed to have slightly increasing head circumference for age. Eleven of 12 infants with head circumference measurements through 6 months old seemed to have normal head growth, and 1 had a marked decrease in head circumference for age from the 90th percentile at birth to <5th percentile. Of 8 infants with head circumference measurements through 2 months of age, 6 seemed to have normal growth; 1 had a slight decrease in head circumference for age; and 1 had an apparent measurement error that precluded determination of head growth.

Forty-two of 43 infants for whom developmental status was reported through 12 months of age were reported to have normal development. One infant born preterm at 26 weeks of gestation was reported to have delayed development at 12 months of age with development appropriate for a 9-month-old. Eleven of 13 infants evaluated through 6 months of age were reported to have normal development. The infant with glycogen storage disease type 1 was reported to have abnormal development, and another infant was reported to have abnormal neck tone at 6 months of age. All 4 of the infants evaluated through 2 months of age

were reported to have normal development. No developmental follow-up was reported on the infant with Down syndrome.

DISCUSSION

Most of this cohort of 77 women who developed WNV illness during pregnancy delivered healthy infants without evidence of congenital WNV infection. The frequency of spontaneous abortion, preterm delivery, and delivery of low birth weight infants was no higher in this cohort than expected in the general population. Because of the surveillance methods, our study could not detect either asymptomatic or symptomatic WNV infections during pregnancy that were not reported by health care providers. Three infants who became infected with WNV may have had congenital WNV infection, but we were unable to document this either because umbilical cord, cord sera, and placental specimens were no longer available by the time the case was reported or maternal infection occurred so close to delivery that we would not expect the infant to have developed the IgM antibody to WNV at birth. In postnatal WNV infection, IgM may not develop for ≥ 8 days after illness onset.³⁶ In none of these cases can we rule out the possibility that the infant might have acquired WNV infection from a mosquito bite, and in the 2 breastfed infants we cannot rule out transmission through breast milk, given a published report of possible WNV transmission to an infant through breastfeeding.³⁷ Nevertheless, the detection of WNV infection in the 3 infants within 1 month of delivery and the documentation of maternal WNV infection suggest that congenital infection might have occurred.

One of the infants with possible congenital infection had lissencephaly and neonatal WNV encephalitis diagnosed at 17 days of age. Lissencephaly is believed to develop early in gestation, and onset of the mother's illness compatible with WN fever did not develop until the third trimester.³⁸ Unless the mother had an asymptomatic WNV infection earlier in pregnancy, this infant's lissencephaly is unlikely to have been caused by WNV infection. Rather, we surmise that the infant had underlying lissencephaly of unknown etiology and developed superimposed WNV encephalitis. Because the mother was not tested for WNV infection until after delivery, it is possible that both mother and infant were infected with WNV after delivery. However, the mother's febrile illness 3 weeks before delivery, the onset of seizures in the infant at 7 days of age, and the negative results for other common congenital infections suggests that congenital WNV infection might have occurred.

The 1 infant who had anti-WNV IgM detected in cord blood probably did not have WNV infection, because anti-WNV IgM was not detected at 1 and 8 months, and neutralizing antibody was not detected at 8 months. The lack of persistent IgM and loss of neutralizing antibody

within the first 8 months of age is most consistent with either a false-positive anti-WNV IgM test at birth or unusual leakage of maternal IgM into fetal circulation and loss of normally transferred maternal (IgG) neutralizing antibody.³⁴ Although this infant was born by emergency cesarean section, the delivering physician reported clean puncture of the umbilical cord to obtain the cord blood sample.

Given that congenital WNV infection has been described only recently and only 1 documented case is known, it remains uncertain whether occult congenital WNV infection (without serologic evidence of infection) might be responsible for adverse outcomes. The sensitivity of testing for anti-WNV IgM in cord blood to diagnose congenital WNV infection is not known. Detection of a specific IgM antibody has limited the sensitivity in diagnosing other congenital infections, and it is possible that in utero production of an antibody is impaired such that congenitally infected infants might fail to produce a sufficient WNV-specific antibody to be detected with standard assays, particularly if they were infected early in pregnancy.³⁹⁻⁴² In such cases, and without the detection of viral RNA or antigen, the diagnosis of congenital WNV infection would need to be made based on clinical abnormalities, but without knowledge of the clinical spectrum of congenital WNV infection this is problematic.

One way to evaluate the possibility of occult effects of maternal WNV infection on infant outcomes is to consider the frequency of malformations among the infants born after maternal WNV infection. Because these women were reported through passive surveillance, and 1 (the mother of the child with lissencephaly and WNV encephalitis) was reported after the outcome in her infant was discovered, comparisons to frequencies of birth defects in the general population may suffer from reporting and diagnostic bias. An additional difficulty arises from the method for classifying microcephaly as a major malformation.⁴³ The definition of microcephaly in existing literature varies from physician-diagnosed microcephaly, which has a reported incidence of 0.06% of births in the Metropolitan Atlanta Congenital Defects Program (CDC, unpublished data, 2005), to a head circumference < 2 SD below the mean, which, by definition, represents 2.28% of all births.^{24,43} We chose the latter definition to increase sensitivity for detecting possible effects of WNV on birth outcomes and included infants thus classified as microcephalic in the total of major malformations observed in this cohort. We did not classify as microcephalic the infant born at 29 weeks' gestation, because the infant had evidence of symmetrical growth retardation. Even if the infant with lissencephaly is excluded, but including the 2 infants with microcephaly, the frequency of major birth defects and glycogen storage disease in this cohort was higher than population-based estimates (Table 2). However, Down syndrome (a chromosome abnormality usually attrib-

uted to a nondisjunctional event during meiosis) and glycogen storage disease type 1 (an autosomal recessive disorder) are both present from conception and, therefore, could not be caused by intrauterine WNV infection. Similarly, cleft palate, aortic coarctation, and lissencephaly are generally thought to originate during the first trimester and, therefore, could not be caused by third-trimester WNV infection. The infant with polydactyly was born to a woman who was thought to be infected during the first trimester, but without laboratory evidence of congenital WNV infection, we cannot conclude that this or any of the other malformations seen in this cohort are because of WNV. Microcephaly is a common manifestation of congenital viral infection, but the 2 infants with microcephaly were examined in detail without finding any evidence of WNV infection. Both mothers had other possible risk factors for delivering an infant with microcephaly, but the contributing role of these other factors, if any, remains unclear.^{44,45} Thus, of the 7 infants with major malformations, only 3 had defects that could have been caused by maternal WNV infection based on the timing of the infections and the sensitive developmental period for the specific malformations, and none had any conclusive evidence of WNV etiology. However, given that available laboratory tests might be insensitive in diagnosing congenital WNV infection as discussed above, we cannot rule out the possibility that occult congenital WNV infection might have contributed to abnormalities that are temporally plausible with the timing of maternal WNV infection.

Similarly, it is unclear whether congenital WNV infection could account for some of the growth abnormalities seen in this cohort. The majority of infants, including those with low birth weight and major birth defects, subsequently had age-appropriate growth. Five infants had severe flattening of their weight growth curves, and 1 infant had a severe flattening of the head circumference curve. Although none of these infants had laboratory evidence of WNV infection, it remains unclear whether maternal WNV infection or occult congenital WNV infection could have contributed to this abnormal growth.

Nearly all of the infants have had reportedly normal development to date. In most cases, developmental status was determined by the health care provider during routine physical examination. A standardized assessment of development using a validated test has not been performed on this cohort. It is possible that maternal WNV infection might cause subtle developmental effects that would not be detected during routine health evaluations.

This article reports results for women with clinical illness who were reported to a national surveillance registry by health care providers and health departments, but there are likely many more pregnant women

who had mild clinical or asymptomatic WNV infections and for whom information on pregnancy outcomes is not available. Interim guidelines have been published for the clinical evaluation of infants born to mothers infected with WNV during pregnancy.⁴⁶ The identification of children with subtle developmental delays or those with previously undetected birth defects that might be caused by congenital WNV infection will require prospective studies using standardized assessments to compare infants of WNV-infected and noninfected women. A better understanding of the sensitivity and specificity of laboratory tests for diagnosing congenital WNV infection is also needed. In the United States, the CDC and state health departments continue to gather clinical and laboratory data on outcomes of pregnancies of WNV-infected women, and clinicians are encouraged to report known or suspected cases to their state health department. Investigators conducting a collaborative study of WNV during pregnancy are seeking enrollment of WNV-infected pregnant women through state health departments.

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