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SPECIAL REPORT

Zika Virus and Birth Defects — Reviewing the Evidence for Causality

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SUMMARY

The Zika virus has spread rapidly in the Americas since its first identification in Brazil in early 2015. Prenatal Zika virus infection has been linked to adverse pregnancy and birth outcomes, most notably microcephaly and other serious brain anomalies. To determine whether Zika virus infection during pregnancy causes these adverse outcomes, we evaluated available data using criteria that have been proposed for the assessment of potential teratogens. On the basis of this review, we conclude that a causal relationship exists between prenatal Zika virus infection and microcephaly and other serious brain anomalies. Evidence that was used to support this causal relationship included Zika virus infection at times during prenatal development that were consistent with the defects observed; a specific, rare phenotype involving microcephaly and associated brain anomalies in fetuses or infants with presumed or confirmed congenital Zika virus infection; and data that strongly support biologic plausibility, including the identification of Zika virus in the brain tissue of affected fetuses and infants. Given the recognition of this causal relationship, we need to intensify our efforts toward the prevention of adverse outcomes caused by congenital Zika virus infection. However, many questions that are critical to our prevention efforts remain, including the spectrum of defects caused by prenatal Zika virus infection, the degree of relative and absolute risks of adverse outcomes among fetuses whose mothers were infected at different times during pregnancy, and factors that might affect a woman's risk of adverse pregnancy or birth outcomes. Addressing these questions will improve our ability to reduce the burden of the effects of Zika virus infection during pregnancy.

POTENTIAL RELATIONSHIP BETWEEN ZIKA VIRUS INFECTION AND BIRTH DEFECTS

Since the identification of the Zika virus in Brazil in early 2015, the virus has spread rapidly throughout the Americas (www.cdc.gov/zika/geo/active-countries.html). An increase in the number of infants with microcephaly in Brazil was first noted in September 2015, after the recognition of Zika virus transmission in the country earlier in the year[1]; this was followed by the recognition of a similar increase in French Polynesia after an outbreak there in 2013 and 2014.[2] Despite accumulating evidence that supports the link between Zika virus infection and microcephaly, most experts have taken care not to state that Zika virus infection is causally related to these adverse outcomes.[3] This cautious approach toward ascribing Zika virus as a cause of birth defects is not surprising, given that the last time an infectious pathogen (rubella virus) caused an epidemic of congenital defects was more than 50 years ago, no flavivirus has ever been shown definitively to cause birth defects in humans,[4] and no reports of adverse pregnancy or birth outcomes were noted during previous outbreaks of Zika virus disease in the Pacific Islands.[5,6]

On the basis of the available evidence, the public health response to the outbreak of Zika virus disease has moved forward, with the distribution of health messages about the importance of mosquito-bite prevention, recommendations by public health authorities in some of the most severely affected countries to delay pregnancy, and advisories that pregnant women avoid travel to areas with active Zika virus transmission.[7] However, communications regarding Zika virus have been challenging: a recent survey showed low levels of knowledge and concern about Zika virus in the United States.[8] The recognition of Zika virus as a cause of microcephaly and other serious brain anomalies would allow for more direct communication, which might lead to improved understanding of and adherence to public health recommendations. Therefore, a review of the evidence linking Zika virus infection and adverse pregnancy and birth outcomes is needed.

As is typically the case in epidemiology and medicine, no “smoking gun” (a single definitive piece of evidence that confirms Zika virus as a cause of congenital defects) should have been anticipated. Instead, the determination of a causal relationship would be expected to emerge from various lines of evidence, each of which suggests, but does not on its own prove, that prenatal Zika virus infection can cause adverse outcomes. Two approaches have been used to identify potential teratogens (exposures to a mother during pregnancy that have a harmful effect on her embryo or fetus)[9]: first, the identification of a combination of a rare exposure and a rare defect (sometimes referred to as the astute clinician approach),[10] and second, the use of epidemiologic data to confirm an association. Many teratogens were first identified by means of the rare exposure–rare defect approach, including rubella virus, which was identified after an ophthalmologist noted a characteristic form of cataracts in infants whose mothers had rubella during pregnancy,[11] and heavy alcohol use, which was identified as a teratogen after the recognition of a characteristic pattern of malformations that became known as the fetal alcohol syndrome.[12] In contrast, some teratogens have been identified on the basis of epidemiologic studies (e.g., valproic acid was identified as a teratogen after a case–control study showed an odds ratio of 20 for the association of spina bifida with use of this drug during the first trimester of pregnancy).[13]

SHEPARD’S CRITERIA

In 1994, Thomas Shepard, a pioneer in the field of teratology, proposed a set of seven criteria for “proof” of human teratogenicity (Table 1) that incorporated both approaches.[9] These criteria were an amalgamation of criteria developed by other teratologists and guided by methods that were used to identify previous teratogens. These criteria have been used to guide discussions about causation in teratology-related litigation[30] and to assess other potential teratogens.[10] We used Shepard’s criteria[9] as a framework to evaluate whether the currently available evidence supports the hypothesis that prenatal Zika virus infection is a cause of microcephaly and other brain anomalies (Table 1).

According to these criteria, causality is established when either criteria 1, 3, and 4 (rare exposure–rare defect approach) or criteria 1, 2, and 3 (epidemiologic approach) are fulfilled. The first criterion states that a proven exposure to an agent must occur at a critical time during prenatal development. The severe microcephaly and other brain anomalies that have been observed in many infants are consistent with an infection occurring in the first or early second trimester of pregnancy. Several case reports and studies have shown that women who had fetuses or infants with congenital brain anomalies that were believed, on the basis of the mother’s symptoms or laboratory confirmation, to be due to Zika virus infection were infected in the first or early second trimester of pregnancy, as determined either according to the timing of the symptoms or according to the timing of travel to an area where Zika virus is endemic.[14-20] An analysis of the timing of laboratory-confirmed Zika virus transmission in certain states in Brazil and of the increase in the cases of microcephaly identified the first trimester as the critical time period for infection.[1] Zika virus infections that occur later in pregnancy have been associated with poor intrauterine growth, fetal death, or in some pregnancies, defects on prenatal imaging that have not yet been confirmed postnatally because the pregnancies are ongoing.[14] We conclude that Shepard’s first criterion has been met.

Shepard’s second criterion requires that two epidemiologic studies of high quality support the association. Although ecologic data do not necessarily qualify as an epidemiologic study, data from Brazil regarding the temporal and geographic association between Zika virus infection and the later appearance of infants with congenital microcephaly are compelling.[1,31,32] Two epidemiologic studies also provide support.[2,14] In a study conducted during the outbreak in Brazil, 88 pregnant women who had had an onset of rash in the previous 5 days were tested for Zika virus RNA. Among the 72 women who had positive tests, 42 underwent prenatal ultrasonography, and fe-

Table 1. **Shepard's Criteria for Proof of Teratogenicity in Humans as Applied to the Relationship between Zika Virus Infection and Microcephaly and Other Brain Anomalies.***

Criterion No.	Criterion	Evidence	Criterion Met?
1	Proven exposure to the agent at one or more critical times during prenatal development	On the basis of case reports, case series, and epidemiologic studies of microcephaly that are associated with laboratory-confirmed or presumed Zika virus infection, the timing of Zika virus infection associated with severe microcephaly and intracranial calcifications appears to be in the late first or early second trimester.[14-20]	Yes
2	Consistent findings by ≥2 high-quality epidemiologic studies, with control of confounding factors, sufficient numbers, exclusion of positive and negative bias factors, prospective studies if possible, and relative risk ≥6	On the basis of data from Brazil, the temporal and geographic association between Zika virus illness and cases of microcephaly is strong.[1] Two epidemiologic studies have been published. In a study in Brazil[14] that used a prospective cohort design, 29% of women with Zika virus infection at any time during pregnancy had abnormalities on prenatal ultrasonography, some of which have not been confirmed postnatally, In a study in French Polynesia,[2] retrospective identification of eight cases of microcephaly and the use of serologic and statistical data and mathematical modeling suggested that 1% of fetuses and infants born to women with Zika virus infection during the first trimester had microcephaly; the risk ratio in this analysis was approximately 50, as compared with the baseline prevalence of microcephaly. No other epidemiologic studies have examined this association to date.	Partially
3	Careful delineation of clinical cases; a specific defect or syndrome, if present, is very helpful	The phenotype has been well characterized in fetuses and infants with presumed congenital Zika virus infection, including microcephaly and other serious brain anomalies, redundant scalp skin, eye findings, arthrogryposis, and clubfoot.[15,20-23] The phenotype in some infants appears to be consistent with the fetal brain disruption sequence,[20,22] which has been observed after infection with other viral teratogens.[24]	Yes
4	Rare environmental exposure that is associated with rare defect	Reports of fetuses and infants with microcephaly who are born to women with brief periods of travel to countries with active Zika virus transmission are consistent with Zika virus being a rare exposure.[16,18,19] The defect, congenital microcephaly, is rare, with a birth prevalence of approximately 6 cases per 10,000 liveborn infants, according to data from birth-defects surveillance systems in the United States.[25]	Yes
5	Teratogenicity in experimental animals important but not essential	No results of an animal model with Zika virus infection during pregnancy and fetal effects have yet been published.	No
6	Association should make biologic sense	Findings are similar to those seen after prenatal infection with some other viral teratogens (e.g., cytomegalovirus, rubella virus).[26] Animal models have shown that Zika virus is neurotropic,[27,28] which supports biologic plausibility. Evidence that Zika virus infects neural progenitor cells and produces cell death and abnormal growth,[29] along with evidence of Zika virus in brains of fetuses and infants with microcephaly, on the basis of immunohistochemical staining and identification of Zika virus RNA and live virus,[16,17,19] provides strong biologic plausibility.	Yes
7	Proof in an experimental system that the agent acts in an unaltered state	This criterion applies to a medication or chemical exposure, not to infectious agents.	NA

* The criteria listed here were proposed by Shepard.[9] Criteria 1, 2, and 3 or criteria 1, 3, and 4 are considered to be essential, whereas criteria 5, 6, and 7 are helpful but not essential. Partial evidence is insufficient to meet a criterion. NA denotes not applicable.

tal abnormalities were observed in 12 (29%); none of the 16 women with negative tests had fetal abnormalities. The abnormalities that were observed on ultrasonography varied widely, and some findings lacked postnatal confirmation because the pregnancies were ongoing.[14]

A retrospective analysis after the 2013–2014 outbreak of Zika virus disease in French Polynesia identified eight cases of microcephaly; the authors used serologic and statistical data and mathematical modeling to estimate that 1% of the fetuses and neonates who were born to mothers who had been infected with Zika virus in the first trimester had microcephaly[2] — a prevalence that was approximately 50 times as high as the estimated baseline prevalence. However, this estimate was based on small numbers, confidence intervals were wide, and the risk of other adverse outcomes (e.g., other brain anomalies) was not assessed.[2] Although these studies provide important evidence in support of a causal relationship between Zika virus and microcephaly and other brain anomalies, both have limitations as noted by their authors, such as a lack of control for confounding factors and relatively small numbers of cases, and therefore they do not meet the stringent criteria set by Shepard. Thus, we conclude that Shepard's second criterion has not yet been satisfied.

The third criterion, careful delineation of clinical cases with the finding of a specific defect or syndrome, appears to be met. Previous teratogens have caused specific birth defects or syndromes rather than a broad range of birth defects.[33] Many fetuses and infants with presumed congenital Zika virus infection have had a typical pattern, including severe microcephaly, intracranial calcifications, and other brain anomalies, sometimes accompanied by eye findings, redundant scalp skin, arthrogryposis, and clubfoot[15,20-23]; such findings have led authors to use the term "congenital Zika syndrome."[22,34,35] On the basis of clinical details from a limited number of cases, some infants with presumed congenital Zika virus infection have had features that were consistent with fetal brain disruption sequence,[24] a phenotype involving the brain that is characterized by severe microcephaly, overlapping cranial sutures, prominent occipital bone, redundant scalp skin, and considerable neurologic impairment.[20,22] For example, 11 of 35 infants (31%) with microcephaly whose cases were reported to a Brazil Ministry of Health registry had excessive and redundant scalp skin,[20] a finding that is not typically seen in other forms of microcephaly.[36] These findings suggest an interruption of cerebral growth, but not in that of the scalp skin, after an injury (e.g., viral infection, hyperthermia, or vascular disruption) that occurred after the initial formation of brain structures, followed by partial collapse of the skull. The fetal brain disruption sequence is rare; only 20 cases were identified in a literature review in 2001.[24]

Shepard's fourth criterion refers to the association between a rare exposure and a rare defect; we conclude that this criterion also has been met. The concept behind this criterion is that a rare defect occurring after a rare exposure during pregnancy implies causation because of the unlikelihood of the two rare events occurring together.[10] Microcephaly is a rare defect that is estimated to occur in 6 infants per 10,000 liveborn infants in the United States.[25] Zika virus would not be a rare exposure among women living in Brazil during the Zika virus outbreak. However, reports of adverse birth outcomes among travelers who spent only a limited time period in an area where there is active Zika virus transmission are consistent with Zika virus being a rare exposure.[16,18,19]

A recent report is illustrative: a pregnant woman traveled for 7 days to Mexico, Guatemala, and Belize during her 11th week of gestation and had a positive test for Zika virus immunoglobulin M (IgM) antibodies 4 weeks later. On fetal ultrasonography and magnetic resonance imaging performed at 19 to 20 weeks of gestation, severe brain anomalies were diagnosed in the fetus, and the pregnancy was terminated at 21 weeks of gestation. Microcephaly was not present at the time of pregnancy termination, but the head circumference had decreased from the 47th percentile at 16 weeks of gestation to the 24th percentile at 20 weeks of gestation (a finding that is consistent with the timing of diminishing head sizes in previous cases),[14] which suggests that microcephaly would have developed in the fetus had the pregnancy continued.[16] In this woman, Zika virus would be considered a rare exposure, and her fetus had a rare outcome.

The last three criteria are helpful if they are present, but they are not considered to be essential. The fifth criterion, the need for an animal model that shows teratogenicity, has not been met. Although animal models have shown that Zika virus is neurotropic,[27,28] no studies that tested for teratogenicity in an animal model have been published, although studies are under way. The sixth criterion, that the association should make biologic sense, is clearly met here. Other viral infections have had similar effects (microcephaly and eye problems).[24,26] In addition, pathologic evidence supports this association: Zika virus RNA has been seen in damaged mononuclear cells (presumably glial cells and neurons) in the brains of newborns with microcephaly,[17] and the virus appears to be neurotropic.[17,19] Live Zika virus has been cultured from the brain of a fetus

Table 2. Bradford Hill Criteria for Evidence of Causation as Applied to the Relationship between Zika Virus Infection and Microcephaly and Other Brain Anomalies*

Criterion	Evidence	Criterion Met?
Strength of association	A recent epidemiologic study from French Polynesia suggests a strong association between prenatal Zika virus infection and microcephaly (estimated risk ratio, approximately 50).[2] The substantial increase in the number of cases of microcephaly and other brain anomalies that have been associated with the Zika virus outbreak in Brazil suggests a strong association.[1,2]	Yes
Consistency	Two epidemiologic studies, one from Brazil and one from French Polynesia,[2,14] support the association between prenatal Zika virus infection and microcephaly and other serious brain anomalies. The observed increase in the number of cases of microcephaly after outbreaks of Zika virus infection in Brazil and French Polynesia, as well as preliminary reports of cases in Colombia, support consistency.[1,2,42] Case reports of Zika virus infection in fetuses or infants with microcephaly or other brain anomalies who were born to mothers who traveled to areas of active Zika virus transmission support consistency.[16,18,19]	Yes
Specificity	Other causes of microcephaly exist; however, on the basis of clinical descriptions that are available for a small number of infants with presumed congenital Zika virus infection,[20] the clinical phenotype linked to the Zika virus appears to be an unusual form of microcephaly that is consistent with the fetal brain disruption sequence.	Yes
Temporality	Zika virus infection in mothers during pregnancy precedes the finding of microcephaly or other brain anomalies in fetuses or infants.[14-20] Zika virus outbreaks in Brazil and French Polynesia preceded the increase in the number of cases of microcephaly.[1,2]	Yes
Biologic gradient	Infection is a phenomenon that is either present or absent; there is no dose–response relationship. No data are available regarding whether women with an increased viral load have a higher risk of adverse pregnancy or birth outcomes.	NA
Plausibility	Findings are similar to those seen after prenatal infection with some other viral teratogens (e.g., cytomegalovirus and rubella virus).[26] Evidence that Zika virus infects neural progenitor cells and produces cell death and abnormal growth,[29] along with evidence of Zika virus in brains of fetuses and infants with microcephaly, on the basis of on immunohistochemical staining and identification of Zika virus RNA and live virus,[16,17,19] provides strong biologic plausibility.	Yes
Coherence	No results in an animal model of effects of Zika virus on pregnancy have yet been published, but animal models have shown that Zika virus is neurotropic,[27,28] a finding that is consistent with prenatal Zika virus infection causing microcephaly and other brain anomalies. Zika virus infects neural progenitor cells and produces cell death and abnormal growth,[29] a finding that is consistent with a causal relationship between Zika virus infection and microcephaly.	Yes
Experiment	No experimental animal model of Zika virus teratogenicity is available.	No
Analogy	No other flavivirus has been shown to definitively cause birth defects in humans,[4] but flaviviruses, Wesselsbron and Japanese encephalitis viruses, have been shown to cause stillbirth and brain anomalies in animals.[43] Findings are similar to those seen after prenatal infection with other viral teratogens (e.g., cytomegalovirus, rubella virus).[26]	Yes

* The criteria listed here were proposed by Hill.[40] We have updated a recent analysis by Frank et al.[41]

with severe brain anomalies after maternal infection at 11 weeks of gestation.[16] Furthermore, Zika virus efficiently infects neural progenitor cells and produces cell death and abnormal growth, thus providing a possible mechanism for microcephaly.[29] The seventh criterion, proof in an experimental system that the agent acts in an unaltered state, is aimed at medications or chemical exposures and does not apply to infectious agents. Thus, given Shepard's criteria as a framework, criteria 1, 3, and 4 have been satisfied — evidence that is considered sufficient to identify an agent as a teratogen.

OTHER CRITERIA

Other criteria can also be used to assess this relationship. Koch's postulates, developed in the late 19th century, are often cited as necessary to show causation in infectious disease; however, many authors have noted the need for Koch's postulates to be updated to accommodate modern technologies.[37-39] The Bradford Hill criteria[40] provide another framework to assess causation; Frank et al. recently used these criteria to assess the relationship between prenatal Zika virus infection and microcephaly and concluded that additional information was needed to assume that the relationship was causal.[41] However, several key pieces of evidence have become available since they performed their analysis, including two epidemiologic studies,[2,14] a study of the effects of Zika virus on neural progenitor cells,[29] and a case report of a fetus with brain anomalies and decreasing head size from whose brain live Zika virus was isolated.[16] On the basis of our update of their analysis, which incorporates newly available evidence (Table 2), nearly all the relevant criteria have been met, with the exception of the presence of experimental evidence. However, Hill emphasizes that meeting all nine criteria is not necessary[40]; instead, the criteria should serve as a framework to assess when the most likely interpretation of a relationship is causation.

ASSESSMENT OF CRITERIA

Thus, on the basis of a review of the available evidence, using both criteria that are specific for the evaluation of potential teratogens[9] and the Bradford Hill criteria[40] as frameworks, we suggest that sufficient evidence has accumulated to infer a causal relationship between prenatal Zika virus infection and microcephaly and other severe brain anomalies. Also supportive of a causal relationship is the absence of an alternative explanation; despite the extensive consideration of possible causes, researchers have been unable to identify alternative hypotheses that could explain the increase in cases of microcephaly that were observed first in Brazil and then retrospectively in French Polynesia, and now in preliminary reports that are being investigated in Colombia.[1,2,42]

Moving from a hypothesis that Zika virus is linked to certain adverse outcomes to a statement that Zika virus is a cause of certain adverse outcomes allows for direct communications regarding risk, both in clinical care settings and in public health guidance, and an intensified focus on prevention efforts, such as the implementation of vector control, the identification of improved diagnostic methods, and the development of a Zika virus vaccine.[44] In addition, after recognizing a causal relationship between Zika virus infection and adverse pregnancy and birth outcomes, we can focus research efforts on other critical issues: First, understanding the full spectrum of defects caused by congenital Zika virus infection; if Zika virus is similar to other teratogens, an expansion of the phenotype would be expected (e.g., with the congenital rubella syndrome, the phenotype was expanded from cataracts to include other findings such as hearing loss, congenital heart defects, and microcephaly).[11] Second, quantifying the relative and absolute risks among infants who are born to women who were infected at different times during pregnancy. Third, identifying factors that modify the risk of an adverse pregnancy or birth outcome (e.g., coinfection with another virus, preexisting immune response to another flavivirus, genetic background of the mother or fetus, and severity of infection). Addressing these issues will improve our efforts to minimize the burden of the effects of Zika virus infection during pregnancy.

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