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Commentary

The Difficult Design of Epidemiologic Studies on Zika Virus and Pregnancy

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While some evidence indicates a link between Zika virus and microcephaly and other congenital brain abnormalities (CBA), a firm causal relation is yet to be established.[1] Even in the recent CDC report of a causal connection with microcephaly, the 'consistent findings by ≥2 high-quality epidemiologic studies' criterion was only partially met.[2]

Measuring both exposure and outcome is problematic. Approximately 80% of Zika cases are asymptomatic.[3] Symptomatic Zika is characterized by rash, fever, joint pain, and conjunctivitis – fairly nonspecific symptoms.[4] The virus can be identified by RT-PCR only during a few days, and by IgM for a few weeks, after infection.[5] Moreover, Zika virus has serological cross-reactivity with other arboviruses, such as dengue,[5] although plaque reduction neutralisation tests can be performed for confirmation. Diagnosis of microcephaly is percentile-based, usually two or three standard deviations (SDs) below the mean for gestational age.[6] By definition, such an outcome will occur a certain percentage of the time, even with no underlying increase in prevalence. Both antenatal and postnatal measurement of head circumference are prone to error.[7,8] As gestational age at ultrasound is partly determined by biometry, including measurement of the head, antenatal diagnosis is particularly difficult to interpret. Other CBAs (such as cerebral calcifications and ventriculomegaly) are likely to be systematically over- or underdiagnosed, depending on the availability of ultrasound.

Experimental: Experimental designs will be useful for trials of vaccines, treatments, or vector control; and in animal studies, particularly of non-human primates.[9] A natural experiment (when study participants are assigned the exposure in a way that simulates randomisation) requires the strong assumption than exposure assignment is unrelated to other variables. Two populations that seem very similar could vary by genetics or nutrition, and diagnosis of congenital anomalies is likely to be more thorough in Zika-affected areas.

Ecological: Ecological studies can provide good negative evidence for causality: if microcephaly is measured consistently and population rates are constant before and after the introduction of Zika, this is evidence against a causal association, especially if results are consistent across areas. However, routinely collected data are often delayed years before being finalised, and as Zika enters a geographic area, screening and diagnosis are likely to be intensified. The effect of abortions on population rates also needs to be considered.

Cohort: A prospective cohort design allows for establishing temporality and limits diagnostic biases through standardised data collection on outcomes (staff ascertaining outcomes should be blinded to the woman's Zika infection status). Practical issues include enrolling women sufficiently early if first-trimester prenatal care is not standard; and follow-up, especially if women attend small clinics or providers rather than delivery hospitals for prenatal care, or if they deliver at home.

Prospective cohort studies present ethical issues. The study protocol is likely to involve testing for Zika or providing ultrasounds in conditions when it would not otherwise be done. Women with a positive Zika test will, at best, be subjected to unnecessary worry. While some women would appreciate this knowledge, and potentially, the opportunity to terminate the pregnancy, an ethical and scientific bind is created for the researchers, who (at least if US federally

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funded) are required to explicitly not have any part in a woman's decision to terminate a pregnancy. Comparable to other situations where a diagnosis occurs during the course of a study, research ethics might require referral for a safe abortion, which is not possible in many Zika-affected areas where abortion is illegal in almost all circumstances.[10] If such terminations are sufficiently common, affected cases may preferentially remove themselves from the study population and thus create a null or even inverse association between exposure and outcome. If known, terminations due to the study outcome should be included in the case group.

Case–control: In case–control studies, the exposure (Zika infection) is ascertained retrospectively, which is problematic: the earliest possible diagnosis of microcephaly or other brain anomalies is during the second or third trimester, meaning that infections that occurred in the first trimester cannot be reliably identified. In addition, defining the underlying population – those who, if they had the disease, would be cases in the study– can be difficult. As antenatal diagnosis requires an ultrasound, women who do not have an ultrasound will not become cases. However, if women are only offered ultrasounds when they are suffering a complication or when Zika infection is suspected, the study may be biased. In postnatal case–control studies, with cases identified at birth or beyond, identifying the source population is easier, but determining infection status is even more difficult.

Case-cohort: A case–control study nested in a prospective cohort. Blood is drawn early in pregnancy for all participants, but Zika status measured only on the cases and a small set of controls. Such studies are efficient and less vulnerable to diagnostic and recall bias, and can accommodate multiple case groups, such as other CBAs. Since exposure is not determined until after delivery, they avoid concerns about encouraging women to abort; however, many women would prefer to have that information and make their own decision. Similarly, participants may not want to sign up for a study where they may or may not be tested for a disease. If laboratory rapid diagnosis and abortion services are available, a case-cohort design may be unethical. However, in many of the affected areas, these resources are unavailable and finances do not allow for testing a large study population. If validity of the available tests is not established and if there is no accepted policy for care, it is generally acceptable to not provide test results to study participants. If a biobank is available and outcome data are routinely linked with biosamples, a case-cohort may be possible under a waiver of informed consent.

While congenital anomalies are garnering much attention, other Zika-related perinatal topics, including congenital transmission, pathogenesis in pregnancy, and neurodevelopmental sequelae in infants, should not be neglected. Rigorous study design will be required to truly understand the effects of Zika virus on women and children. Each design has specific strengths and weaknesses, and multiple studies with different approaches will be needed. Epidemiologists need to be sure that their voices are being heard as Zika studies are being conducted, interpreted, and incorporated into policy.

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