



Perspective

Zika Virus as a Cause of Neurologic Disorders

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Zika virus infections have been known in Africa and Asia since the 1940s, but the virus's geographic range has expanded dramatically since 2007. Between January 1, 2007, and March 1, 2016,

local transmission was reported in an additional 52 countries and territories, mainly in the Americas and the western Pacific, but also in Africa and southeast Asia. Zika virus infections acquired by travelers visiting those countries have been discovered at sites worldwide. *Aedes aegypti* mosquitoes are the principal vectors, though other mosquito species may contribute to transmission. The virus was found to be neurotropic in animals in experiments conducted in the 1950s, and recent experiments have shown how it can cause neural-cell death. A rise in the incidence of Guillain-Barré syndrome, an immune-mediated flaccid paralysis often triggered by infection, was first reported in 2013 during a Zika outbreak in French Polynesia. An increase in the incidence of mi-

crocephaly, a clinical sign that can be caused by underdevelopment of the fetal brain, was first reported in northeastern Brazil in 2015, after Zika virus transmission had been confirmed there. These reports of excess cases of Guillain-Barré syndrome and microcephaly led the World Health Organization (WHO) to declare a Public Health Emergency of International Concern on February 1, 2016, and to recommend accelerated research into possible causal links between Zika virus and neurologic disorders.¹

As researchers investigate whether and by what mechanisms Zika virus infections could affect the nervous system, there is a key question for public health: How can currently available evidence about causality guide the choice

and implementation of interventions? For this purpose, the WHO is developing a framework for the systematic appraisal of evidence about these causal relationships. How does the available evidence inform current WHO recommendations, and what are the priorities for research going forward?

Besides advancing scientific understanding, the main practical purpose of investigating causality is to evaluate, as accurately as possible, what reduction in the incidence of illness (here, especially neurologic disorders) can be expected from reducing human exposure to the putative cause (Zika virus infection). The conceptual framework is based on factors first proposed by Bradford Hill and commonly used to assess causality: temporality (cause precedes effect), biologic plausibility of causal mechanisms, consistency (same association found in different studies and populations), strength of association (as measured by risk ratio, rate ratio, or

odds ratio in cohort or case–control studies), exclusion of alternative explanations, dose–response relationship, cessation (removing the supposed cause reverses the effect), and analogy to cause-and-effect relationships in other diseases.² Temporality is the single necessary condition; none of the factors on its own is sufficient.

Causal relationships cannot be proven with absolute certainty in epidemiologic studies, but these factors help analysts judge the existence and strength of possible causal links. Their assessment should be complemented by controlled experiments, the most robust approach to drawing inferences about cause and effect.

A systematic strategy for identifying relevant evidence will enable a transparent and replicable approach that can be updated to capture new information. Study methods can be assessed for risks of selection and measurement bias, confounding, and the effect of chance. To illustrate the approach, we conducted a search of PubMed and selected journal and public health websites for information posted through March 4, 2016. The table and the Supplementary Appendix (available with the full text of this article at NEJM.org) provide a preliminary summary of population- and individual-level studies on possible associations between Zika virus infection and Guillain–Barré syndrome or microcephaly.

We found three published reports on Guillain–Barré syndrome studied at the population level. During the 2013–2014 outbreak in French Polynesia, the rise and fall of Zika virus infections was followed by a similar rise and fall in the incidence of Guillain–Barré syndrome, with a delay of

about 3 weeks.³ In the Americas, Guillain–Barré syndrome in the presence of Zika virus has now been reported to the WHO from Brazil, Colombia, El Salvador, Martinique, Panama, Puerto Rico, Suriname, and Venezuela, but we found no reports from these countries linking the syndrome with trends in Zika virus infections.

One study of microcephaly showed a higher-than-expected incidence in the state of Paraíba, Brazil, during the period when Zika transmission began, but data on the timing of Zika infections were not available (see table). In the state of Bahia, Brazil, an outbreak of cases of acute rash, suspected to be Zika virus disease, was followed by an increase in cases of microcephaly. Additional surveillance data describing the temporal relationship between Zika infections and neurologic disorders are likely to be published soon. Some recent epidemiologic observations invite further investigation: microcephaly has been reported in association with Zika infection in Brazil, but not yet in neighboring countries, perhaps because it is still too soon after the introduction of the virus. An outbreak of Zika virus infection in Cape Verde during 2015–2016 involving thousands of cases and possibly caused by an African strain of the virus has not been linked to any neurologic disorders.⁴

At the level of individual patients, we found 3 studies on Guillain–Barré syndrome and 14 on microcephaly. The only published case–control study showed, among other results, that 41 of 42 patients with Guillain–Barré syndrome diagnosed during the 2013–2014 outbreak in French Polynesia (98%) were carrying

Zika virus antibodies, as compared with 35 of 98 hospitalized controls (odds ratio, 59.7; 95% confidence interval, 10.4 to ∞).³ Serologic tests excluded some other infectious triggers for Guillain–Barré syndrome, and there was no association between the syndrome and exposure to dengue, which has the same mosquito vector and was circulating at the same time as the Zika virus outbreak.

One prospective study has compared ultrasound findings in pregnant women with confirmed Zika virus disease and in women with rash apparently from other causes (see table). Ultrasound findings were abnormal, including indications of microcephaly, in 12 of 42 women with Zika virus disease and were normal in all 16 women with no Zika virus disease. Eleven other reports, published between November 2015 and February 2016, involved a total of 93 neonates or fetuses with microcephaly, all in, or linked to, Brazil. In 9 of the 93 cases, Zika infection was detected in fetal or neonatal brain tissue or in amniotic fluid. In 4 cases, Zika virus was found in the brain but not in other organs on postmortem examination. In most case reports or case series, other infections and toxic exposures known to cause microcephaly were not completely excluded. One additional report compiled after the Zika virus outbreak in French Polynesia identified 17 cases of fetal or neonatal brain malformations, which included a number of cases of microcephaly.

The prevailing uncertainty about Zika virus infection and its consequences is now driving a vigorous program of research. One case–control study of Guillain–

Studies of Guillain-Barré Syndrome or Microcephaly in Association with Zika Virus Infection, According to Study Design and Date of Publication.*					
Study Type	Countries (Yr of Publication)	No. of Studies	Main Findings	Strengths	Weaknesses
Guillain-Barré syndrome					
Ecologic	French Polynesia (2015), Pacific Islands (2016)	2	French Polynesia: 8750 suspected Zika cases; 52% of 885 tested Zika-positive in saliva or blood; 73 with neurologic or autoimmune complications, including GBS; Pacific islands: AFP surveillance in children not associated with Zika emergence	French Polynesia: temporal ecologic association; studies cover several Pacific island countries	Mostly clinical diagnoses; AFP surveillance in children only and aggregated to yearly level
Surveillance	Brazil, Colombia, El Salvador, Venezuela (2016)	1	GBS cases in countries with reported Zika virus circulation: Brazil, 1708; Colombia, 201; El Salvador, 118 in 6 wk (vs. monthly average of 15); Venezuela, 252 in 1 mo	Covers multiple countries in the Americas	Ecologic association; no report of numbers of Zika cases; inconsistent or no GBS comparison data
Case reports	French Polynesia (2014), Puerto Rico (2016)	2	Patients with rash followed by GBS diagnosis; serum positive for ZIKV IgM; some other infections excluded in one case	Laboratory-confirmed ZIKV infections	Other infections not completely excluded
Case-control	French Polynesia (2016)	1	Cases: 42 people hospitalized with GBS during Zika outbreak; control group 1: 98 hospital inpatients with nonfebrile illness in same period; control group 2: 70 patients with RT-PCR-confirmed ZIKV infection; comparison of ZIKV antibodies, GBS vs. control group 1: OR, 59.7 (95% CI, 10.4-∞).	Two control groups; strong association	Cross-reactivity between DENV and ZIKV; few other infections investigated
Microcephaly					
Ecologic	Brazil: Paraíba, Bahia (2016)	2	Paraíba: retrospective review 2012-2015; 16,208 births; higher-than-expected (2-8%) incidence of microcephaly; Bahia: ecologic association between reports of acute rash, March-June 2015 and microcephaly cases October 2015-January 2016	Temporal associations; large number of births; Bahia data suggest association with late first and early second trimester	Ecologic associations only; no confirmed Zika cases; alternative explanations not excluded
Case reports or case series	French Polynesia (2015)	1	Retrospective review of 2013-2014 Zika outbreak period: 17 cases of congenital brain malformations, including microcephaly	Temporal association with Zika outbreak; other congenital brain abnormalities observed	No documented maternal infection; retrospective; most not tested for ZIKV; no control group
Case reports or case series	Brazil: several states (2015-2016)	11	93 Cases of microcephaly, 70 with history of maternal symptoms (laboratory-confirmed in 1 of 3 tested); 9 with ZIKV in amniotic fluid, fetal or neonatal brain; 4 with ZIKV in brain but not other organs	Biologic evidence of ZIKV in fetal or neonatal brain tissues and neurotropism	Most maternal ZIKV exposures were self-reported; other congenital infections not always excluded; no control group
Case reports or case series	Various countries (2016)	1	9 Women returning to United States from Zika-affected countries, August 2015-February 2016; all reported symptoms, all laboratory-confirmed recent ZIKV infection; 2 early pregnancy losses, 2 terminations, 1 microcephaly, 2 healthy newborns, 2 still pregnant	Temporal association; biologic evidence of maternal ZIKV infections	Alternative explanations not excluded; no control group
Cohort study	Brazil: Rio de Janeiro (2016)	1	88 Women with rash during pregnancy, 72 ZIKV-positive on RT-PCR; ultrasound normal in all 16 ZIKV-negative women; ultrasound abnormal in 12 of 42 ZIKV-positive women at all stages of pregnancy (29%); microcephaly mostly in association with intrauterine growth restriction	Temporal association; biologic evidence; strong association with abnormal ultrasound or neonatal outcome	Small study; control group presumed to have other causes for rash; some congenital infections not excluded

* References and a more detailed version of this table are provided in the Supplementary Appendix, available with the full text of this article at NEJM.org. AFP denotes acute flaccid paralysis, CI confidence interval, DENV dengue virus, GBS Guillain-Barré syndrome, OR odds ratio, RT-PCR reverse transcription-polymerase chain reaction, and ZIKV Zika virus.

Barré syndrome and one cohort study of pregnant women described above provide evidence for a causal link. However, most of the data summarized here derive from studies whose designs are typically classified as weak, and the data are not entirely consistent. The available data are mainly observations regarding temporal associations between infection and disease from routine population surveillance and clinical and pathological studies of single cases or groups of cases. Such data are essential for the discovery of new phenomena and as a source of testable hypotheses about cause and effect,⁵ but a more comprehensive approach to causality is needed. The WHO framework will set out research questions to address the various dimensions of causality as they apply to Zika virus and neurologic disorders and will ensure a systematic review of the literature to synthesize the evidence. Further case-control and cohort studies

are already under way to fill critical knowledge gaps.

Even with limited evidence linking Zika virus to neurologic disorders, the severe potential risks demand decisive, immediate action to protect public health. The WHO recommends applying key interventions such as intensive mosquito control; personal protection against mosquito bites; provision of appropriate clinical care for all patients with Guillain-Barré syndrome and for women before, during, and after pregnancy; and prevention of Zika virus transmission through sexual contact or blood transfusion.⁴ Most of these are not new interventions, but they do need strengthening. Populations must be informed of the potential current and future risks of neurologic disorders, wherever the virus is being or could be locally transmitted and in other regions inhabited by the mosquito vectors. As the putative link between Zika virus and neurologic

disorders is reinforced, refined, or even refuted, public health measures will be adjusted accordingly.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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This article was published on March 9, 2016, at NEJM.org.

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DOI: 10.1056/NEJMp1602708

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