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REVIEW

The Emergence of Zika Virus

A Narrative Review

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Zika virus (ZIKV) is yet another arbovirus that is rapidly emerging on a global scale, on the heels of a chikungunya epidemic in the Americas that began in 2013. A ZIKV epidemic that began in Brazil in 2015 has now spread rapidly to more than 30 countries in the Americas and the Caribbean, infecting more than 2 million inhabitants. This epidemic currently continues unabated. The explosive nature of recent outbreaks and concerning links to Guillain–Barré syndrome and microcephaly are incompletely understood. Also unknown is the relative importance of sexual transmission of ZIKV and asymptomatic ZIKV infections to the overall burden of transmission. The limited understanding of ZIKV presents an enormous challenge for responses to this rapidly emerging threat to human health. This article reviews the existing literature on ZIKV and proposes critical questions for vaccine development and other areas of needed research.

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Zika virus (ZIKV) was first isolated in 1947 from a sentinel rhesus monkey in the Zika forest near Entebbe, Uganda (1). It was one of many viruses discovered via the Rockefeller Foundation's program on yellow fever in Africa and South America (2). In 1948, ZIKV was again isolated from the Zika forest, from *Aedes africanus* mosquitos (1). Serologic studies in people living near the Zika forest showed an estimated ZIKV seroprevalence of 6% (3).

The first isolation of ZIKV from humans arose during investigation of an epidemic of jaundice in eastern Nigeria in 1952, when virus was isolated from a (nonjaundiced) child presenting with fever and headache (4). These symptoms were reproduced during experimental inoculation of a human volunteer in 1956, which resulted in a mild, self-limited febrile illness (5). The virus was next isolated in Uganda in 1962, from an individual with maculopapular rash, fever, and mild body pain (6). In the decade following initial discovery of the virus, sporadic febrile illnesses with seroconversion patterns suggestive of ZIKV infection were identified throughout Africa and Asia (7–11). Estimated seroprevalence was highest in Nigeria, at 48% to 56% (12, 13).

Recent Epidemics

The first recorded ZIKV epidemic occurred in 2007 on Yap Island, a small Micronesian island with approximately 10 000 inhabitants. Physicians noted an outbreak of a mild dengue-like illness characterized by rash, fever, arthralgia, arthritis, and conjunctivitis (14). ZIKV was identified in 14% of serum samples from patients with acute illness. A limited serosurvey performed on the island found that 73% of Yap inhabitants older than 3 years of age had demonstrable IgM antibodies to ZIKV, indicating recent infection. Only 19% of these individuals reported a history of clinical illness consistent with suspected ZIKV infection, suggesting an asymptomatic-to-symptomatic ratio of 4 to 1.

A second major ZIKV epidemic occurred in 2013 in French Polynesia, a chain of 67 islands with approximately 270 000 inhabitants (15). Expanded surveillance led to an estimate of 28 000 suspected cases during the epidemic, the largest on record at that time. This epidemic was also associated with the novel report of a case of Guillain–Barré syndrome (GBS) that developed 1 week after a ZIKV-like illness (16). At the time, there were concurrent epidemics of dengue virus serotypes 1 and 3 and chikungunya in the Pacific Islands (17). Scientists postulated that a reported 20-fold increase in GBS cases in French Polynesia may have been linked to dengue virus infection, as had been reported previously (18); ZIKV infection; or some pathologic mechanism related to sequential infection with the 2 viruses (16). After the outbreak in French Polynesia, outbreaks of ZIKV occurred in New Caledonia, the Cook Islands, and Easter Island (19, 20).

The largest ZIKV outbreak to date, by sheer numbers and geographic range, began in northeastern Brazil in May 2015 and is ongoing (21, 22). By October 2015, 14 states in Brazil and the department of Bolivar, Colombia, had confirmed autochthonous ZIKV transmission (23). By January 2016, the virus had spread to 20 countries or territories in the Americas and the Caribbean; regions with the highest burden of ZIKV transmission in Brazil, most notably Pernambuco State, were also reporting an increase in the number of infants born with microcephaly (24, 25). On 1 February 2016, the World Health Organization declared the reported clusters of microcephaly and other neurologic disorders a public health emergency of international concern (26), the fourth of its kind following swine flu (2009), the threatened resurgence of polio (2014), and Ebola (2014). As of March 2016, the epidemic had spread to 33 countries or territories, with transmission increasing in most countries (27).

Early estimates from Brazil suggested a 20-fold increase in the number of microcephaly cases compared with previous years (25). Estimation of true differences

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Key Summary Points

Zika virus (ZIKV) was identified in 1947 and for decades caused only sporadic cases of mild human disease. The explosive nature of recent epidemics and links to Guillain–Barré syndrome and microcephaly are concerning and remain poorly understood.

There is evidence that maternal ZIKV infection at any stage of pregnancy may result in an increased risk for microcephaly, intrauterine growth restriction, and fetal death. The contribution of ZIKV infection to the total increase in microcephaly cases being observed, relative to other potential (unidentified) causes, remains unknown.

There is evidence of sexual transmission of ZIKV by men, with virus detectable in semen by reverse transcriptase polymerase chain reaction for at least 2 months after infection. The relative importance of sexual transmission with regard to the overall burden of ZIKV transmission and risk for microcephaly is unknown.

At present, no specific antiviral or vaccine is available for ZIKV, although vaccines are in development. Treatment is supportive. Pregnant women in unaffected areas are currently advised to postpone travel to ZIKV-endemic regions if possible and to avoid sex with male partners who have traveled to endemic regions.

Providers should maintain a high level of suspicion for ZIKV infection in any patient presenting with rash and either a personal history of recent travel to an area with active ZIKV transmission or a history of travel in a sexual partner.

in incidence by affected region and by year has been complicated by increased sensitivity of the current surveillance system at the cost of decreased specificity (28) and likely increased awareness and reporting; however, there appears to be a true increase in the occurrence of microcephaly in several countries in the Americas.

Clear evidence now indicates that maternal ZIKV infection can cause fetal microcephaly. The virus has been isolated from the amniotic fluid of 2 pregnant women who had infants with microcephaly (29) and has been identified in brain tissue from 5 fetuses with microcephaly during autopsy (30, 31). All mothers reported a history of febrile illness with rash during their first trimester. It remains unclear whether other unidentified factors underlie the epidemic of microcephaly, including other known causes of microcephaly and possible surveillance artifacts.

Seven countries in the Americas and the Caribbean have reported increases in the incidence of GBS concurrent with a rise in the number of ZIKV infections (27). In Brazil, a 19% increase in the incidence of GBS was reported for 2015 compared with previous years (32). Sixty-two percent of patients with GBS in Brazil reported a history of symptoms consistent with suspected ZIKV infection, and 7 patients with neurologic disease (including GBS) had laboratory-confirmed ZIKV infection. A retrospective study of GBS cases during the French Polynesia outbreak demonstrated that 100% of patients with GBS had antibodies to ZIKV compared with 56% of controls (33).

To date, Europe, continental North America, and Australia have not reported mosquito-borne transmission of ZIKV (34). Transmission has been documented in Puerto Rico and the U.S. Virgin Islands and is expected to increase (35). Sexual transmission has been reported in the United States, Argentina, and Chile (27, 36, 37). Numerous imported cases of ZIKV infection in travelers returning from tropical countries to more temperate regions have also been reported, including 116 cases in the United States (38). Importation of ZIKV to susceptible countries across the globe raises concern for virus establishment in areas endemic for suitable mosquito vectors. A recent model that incorporated ecologic niche data for *A aegypti* and *A albopictus,* as well as regional travel patterns throughout the Americas, indicated that 60% of the U.S. population lives in areas conducive to at least seasonal, possibly year-round, ZIKV transmission (**Figure 1**) (39).

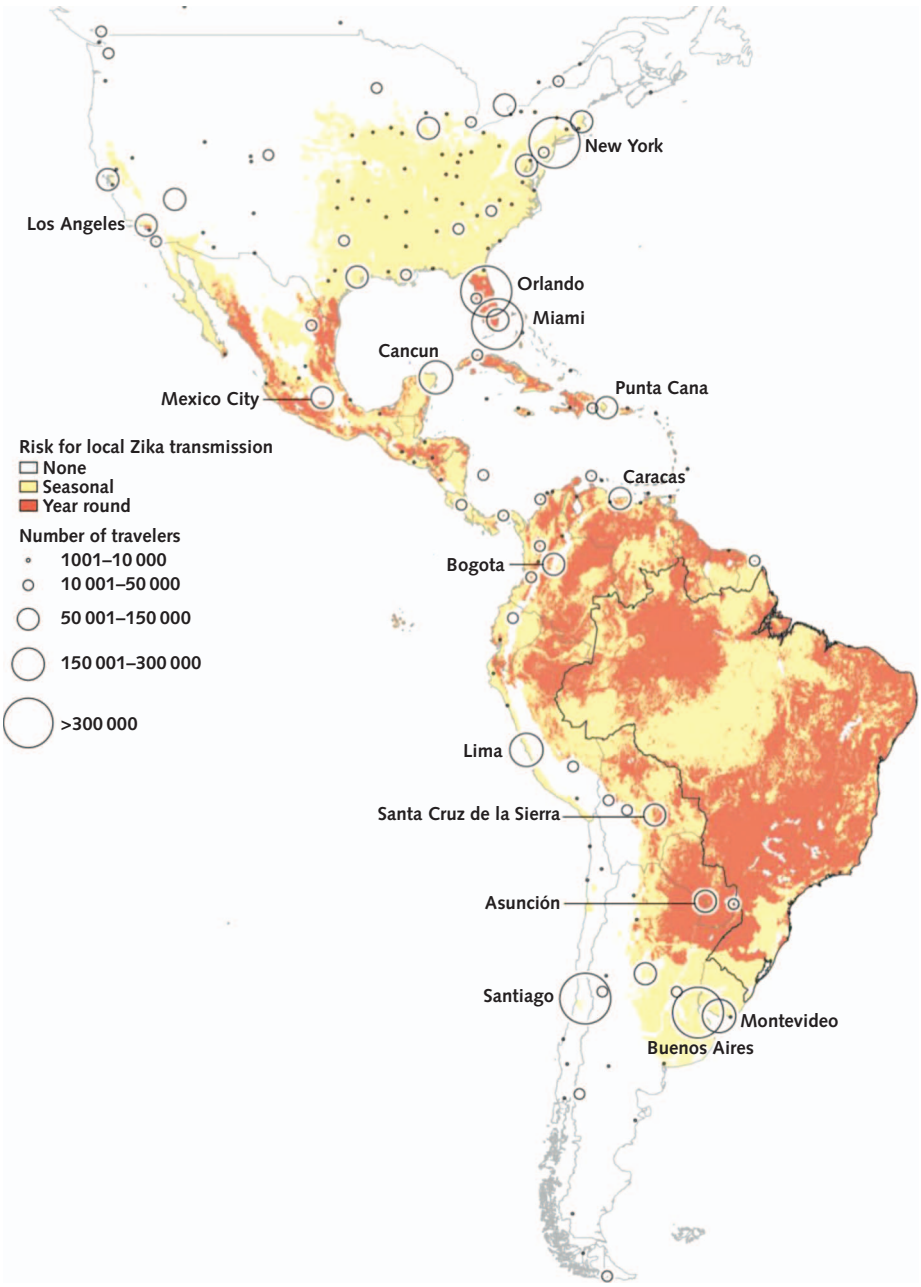
The Zika virus is closely related to the Spondweni virus. There is a single serotype of ZIKV, and infection is thought to lead to lifelong immunity. The 2 lineages, Asian and African, are derived from a common ancestor in Uganda (40, 41). All 3 recent epidemics have been linked to expansion of the Asian lineage of ZIKV (**Figure 2**) (22, 41). It is not known whether strains of ZIKV are associated with an increased risk for neurologic disease or whether mutations have facilitated the explosive spread of ZIKV in recent years.

TRANSMISSION

The Zika virus is spread through the bite of mosquitoes, and multiple mosquito species are capable vectors. The virus was first isolated from *A africanus* in Uganda (1, 42). *Aedes hensilli* was the primary vector in the Yap epidemic (43), and *A aegypti* is likely a dominant vector in Asia and the Americas (44–46). Multiple species are suggested as possible vectors, including a long list of *Aedes* species, *Mansonia uniformis*, *Culex perfuscis*, and *Anopheles coustani* (47, 48).

Besides nonhuman primates and humans, ZIKV has been isolated from small mammals, reptiles, birds, and livestock (49). The previously sporadic occurrence of human infections suggests that humans were once accidental hosts in a sylvatic transmission cycle involving primates, possibly other animals, and forest mosquitos. Recent outbreaks indicate that the evolving epidemiology of ZIKV may now more closely mirror that of yellow fever virus, involving a sylvatic cycle as well as an efficient urban cycle involving human hosts and *A aegypti* mosquitoes.

Figure 1. Predicted risk for local Zika transmission, based on final destinations for travelers departing Brazil and incorporating ecological niche information for *Aedes aegypti* and *A albopictus*.



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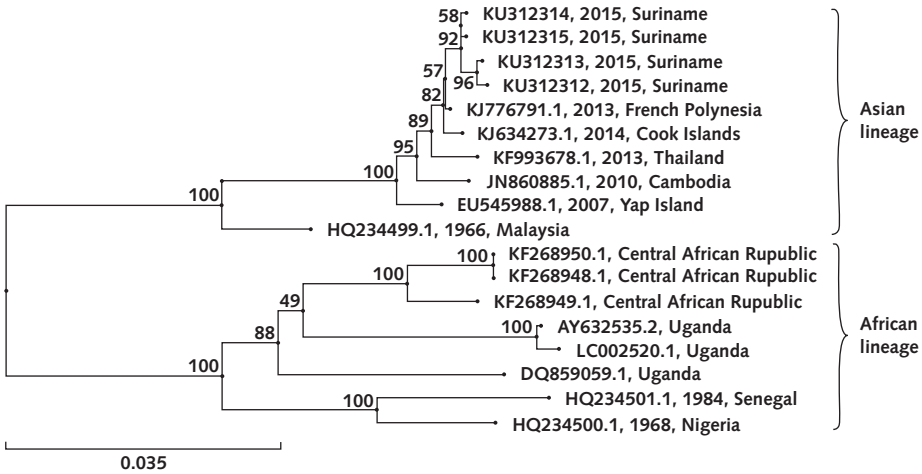
There is evidence of sexual transmission of ZIKV. In the first report in 2008, a U.S. scientist working in Senegal became ill after returning to the United States, and his wife developed symptomatic ZIKV infection 9 days later (36). Given her lack of travel, the inconsistency of the time interval with the suggested external incubation period of ZIKV (>15 days), and reported hematospermia, sexual transmission of the virus was suspected. In 2013, a patient in French Polynesia had 2 episodes of ZIKV-like illness separated by 8 weeks, followed by onset of hematospermia 2 weeks after the second episode. His semen was positive for ZIKV by reverse transcriptase polymerase chain reaction (RT-PCR), and the virus was found to be replicative (50). An additional patient from the Cook Islands had detectable ZIKV in his semen at 27 and 62 days after onset of illness; however, the replicative potential of these late samples was not investigated (51). Multiple cases of suspected sexual transmission are under investigation in the United States (37). Little is known regarding the poten-

Figure 2. Lineages of Zika virus isolates based on envelope gene sequences.



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tial for vaginal secretions or cervical mucous to transmit ZIKV.

Perinatal transmission was demonstrated during the epidemic in French Polynesia, when 2 mothers and their infants tested positive for ZIKV by RT-PCR on serum collected within 4 days of delivery (52). It is unclear whether infection occurred by transplacental transmission or during delivery. The association of ZIKV with birth defects suggests that transplacental transmission may occur. The risk window for fetal exposure to ZIKV, the rate of vertical transmission, and the incidence of birth defects with maternal infection are unknown. As a result, some countries have advised women to avoid pregnancy to decrease the risk for microcephaly.

Risk for transmission via blood transfusion was documented in the French Polynesia outbreak, during which 3% of blood donors tested were asymptomatically infected with ZIKV (53). There are 2 unpublished reports of ZIKV transmission by blood transfusion in Brazil in 2015 (54) and additional cases under investigation (55). The U.S. Red Cross is asking potential blood donors who have traveled to areas with ZIKV transmission to wait 28 days before donating blood, and Brazil's Health Ministry is requesting that those infected with ZIKV or dengue avoid donating blood for 30 days, a measure that will not exclude the estimated 80% of asymptomatic ZIKV infections from donation. Screening of the blood supply using nucleic acid amplification testing of pooled specimens has been performed in the United States for West Nile virus infection since 2003 and should be considered for ZIKV as well (56).

Clinical Manifestations

Up to 80% of Zika virus infections may be subclinical (14); the remainder are typically mild, self-limited illnesses lasting 5 to 7 days. A pruritic, maculopapular rash, headache, and fever are dominant features, although none are universally present. Arthralgia, particularly of the hands, wrists, and ankles, is common and is frequently associated with periarticular swelling. Such arthralgia may persist for weeks to months (36). In the Yap study, the most common symptom reported was rash (90%), followed by fever (65%), arthralgia (65%), and nonpurulent conjunctivitis (55%) (14). Other reported symptoms include aphthous ulcers, prostatitis-like symptoms (perianal pain and dysuria), and hematospermia. Jaundice, plasma leakage, and hemorrhage have not been noted during previous ZIKV epidemics.

ZIKV infection has been associated with development of GBS, typically a self-limited neurologic disease characterized by a relatively favorable prognosis and cerebrospinal fluid studies showing increased protein (57). Guillain–Barré syndrome may be severe if associated with respiratory failure, profound or persistent paralysis, or autonomic instability. The syndrome classically involves autoimmune-mediated myelin sheath destruction along peripheral nerves, manifesting as an ascending progressive polyradiculoneuritis (58). It can result from many bacterial or viral infections and, in some cases, vaccination. Classically associated pathogens are *Campylobacter jejuni* and the influenza viruses (58). It has also been associated with infections from other members of the flavivirus family, including Japanese encephalitis, West Nile virus infection, and dengue (59–61). Higher incidence rates have been reported for men and the elderly (62). A retrospective study of ZIKV-associated GBS in French Polynesia reported electrophysiologic findings compatible with the acute motor axonal neuropathy type (33).

Deaths due to ZIKV have been rare. A small number have occurred in the setting of GBS. Other deaths have occurred in individuals with underlying comorbid conditions, in whom the precise cause of death cannot be discerned. Few deaths have been attributed to ZIKV infection in previously healthy individuals. ZIKV PCR of

tissue specimens from a previously healthy patient who died showed widely disseminated disease throughout multiple organ systems (63).

Maternal infection with ZIKV at any stage of pregnancy may be associated with a range of serious and adverse fetal outcomes, including fetal death, placental insufficiency, intrauterine growth restriction, and central nervous system abnormalities, including microcephaly (64). Microcephaly is manifested as incomplete brain development in the setting of an abnormally small head circumference. Severe microcephaly can be associated with fetal death, seizures, significant developmental delay, and intellectual disability. Infants with microcephaly commonly require intensive developmental services to maximize their physical and intellectual abilities from an early age, and individuals with severe microcephaly typically require life-long care and advanced medical support, an especially significant challenge in resource-limited countries.

Historically, causes of microcephaly have included malnutrition, genetic abnormalities, and exposure to toxic substances, including alcohol, drugs, and chemicals. *Toxoplasma gondii*, rubella virus, cytomegalovirus, and herpes simplex virus (ToRCH complex) are common infectious causes (65). Although previous studies of the ToRCH complex of pathogens indicate that the first trimester is the period of greatest risk for transmission, evidence suggests that maternal infection with ZIKV at any stage of pregnancy may result in microcephaly (64).

Diagnostics

Several diagnostic RT-PCR–based assays have been used to identify ZIKV from the body fluids and tissues of infected individuals (66, 67). The virus appears to generate relatively low viremia (40), and virus in serum may be detectable for only 2 to 3 days after the onset of illness (68). One study found that viremia waned quickly with the onset of rash, which would challenge the identification of suspected cases and confirmation of infection by serum PCR (68). Virus has been detected by PCR in urine longer than in serum, with urine excretion continuing for more than 20 days in 1 case (68, 69). In a study of 182 ZIKV-positive individuals with both serum and saliva specimens, 9% were PCR-positive by serum but negative by saliva, and 19% were positive by saliva but negative by serum (70). The potential to identify ZIKV infection in saliva and urine, possibly for an extended period, offers unique opportunities for the development of noninvasive diagnostic tests and may facilitate epidemiologic and clinical studies.

Previous studies in Africa indicated little to no serologic cross-reactivity between ZIKV and other flaviviruses (2, 4); the Yap epidemic revealed that neutralizing and IgM antibodies were specific when ZIKV was a primary flavivirus infection but that antibody profiles on secondary ZIKV infection were highly and unpredictably cross-reactive with dengue, yellow fever, and Japanese encephalitis (40). "Original antigenic sin" has been demonstrated, wherein acute and convalescent sera from ZIKV cases may demonstrate a dramatic rise in antibodies to another flavivirus, presumably reflecting an anamnestic response to a previous infection or vaccination (36). These observations suggest that ZIKV serologic testing will be most informative when ZIKV occurs as a primary flavivirus infection and less helpful, even misleading, in the common setting of prior flavivirus exposure or vaccination. This cross-reactivity also complicates interpretation of seroprevalence data in most of the flavivirus-exposed world. More specific serologic assays to discern infections from ZIKV, dengue, yellow fever, and Japanese encephalitis are desperately needed.

Clinical Management

Zika virus infection is a nationally notifiable condition in the United States. In mild cases, supportive care, close follow-up, and reassurance are usually sufficient to achieve complete resolution of symptoms. Rest, oral hydration, and treatment of symptoms with acetaminophen-based products are appropriate. In the absence of confirmed ZIKV infection, treatment regimens should avoid aspirin-based products and nonsteroidal anti-inflammatory drugs because dengue may present similarly to ZIKV early in the disease course and platelet dysfunction induced by these drugs may predispose to bleeding complications.

Patients with GBS require comprehensive monitoring to identify potential respiratory failure, autonomic dysfunction, and arrhythmias. Essential to survival and recovery is intensive supportive care, including ventilator support, which may be problematic in resource-poor settings where ZIKV is being transmitted. Bowel and bladder care and treatment of neuropathic pain may be necessary (71). Plasma exchange and administration of intravenous immune globulin are the mainstays of GBS treatment (72); typically, these are effective in hastening recovery, and most cases recover almost completely after 2 to 4 weeks of disease.

Prevention of mosquito feeding on patients early in their disease course is encouraged on the basis of the hypothetical risk that a viremic patient could transmit ZIKV to mosquitoes. To prevent sexual transmission of ZIKV, the Centers for Disease Control and Prevention has developed guidance for men who reside in or have traveled to an area of active ZIKV transmission concerning sexual activity with pregnant or nonpregnant partners (73). Pregnant women are advised to postpone travel to areas with ZIKV transmission or to practice strict measures for mosquito bite avoidance if travel cannot be postponed (74).

There is no licensed antiviral for the treatment of ZIKV infections. Despite the mild nature of most cases, preventing potential complications of infection, such as GBS, adverse fetal outcomes, or sexual transmission, may provide sufficient rationale for development. Available human data on ZIKV kinetics suggest that duration of viremia may be extremely short and the opportunity to affect the disease process narrow. Passive immunotherapy with monoclonal or polyclonal antibodies is a

possible approach. Permutations of various scenarios (postexposure prophylaxis, outbreak disruption, passive protection during pregnancy) need to be explored.

VACCINE DEVELOPMENT

Numerous licensed flavivirus vaccines exist, so it is reasonable to assume that a safe and efficacious ZIKV vaccine is possible. All candidate vaccines thus far are in preclinical development, with optimism for human testing before the end of 2016.

Numerous uncertainties exist regarding the target population for vaccination and the desired outcomes of vaccination: to prevent transmission/epidemic interruption, to prevent complications with pregnancy-associated infection, to prevent GBS, and to generate sustained herd immunity. The ZIKV vaccine development pathway is complicated by an incomplete understanding of ZIKV pathogenesis, most notably the associations with GBS and microcephaly. In addition, complicated bioethical and development pathway issues are associated with inclusion of pregnant women in research and vaccination strategies (75–77).

Both live and replicating (for yellow fever, Japanese encephalitis, and dengue) vaccine constructs have been successfully developed for other flaviviruses as well as killed platforms. Live vaccines are generally believed to induce durable humoral and cellular immunity requiring fewer doses and boosters. Killed (nonreplicating) vaccines may be used in special populations (pregnancy, immunosuppressed) but may be less reactogenic, and they often require multiple doses, boosters, and possibly adjuvant to improve immunogenicity. Questions surrounding vaccine viral strain selection also exist. Although genetic homology between ZIKV strains circulating in Asia and the Americas appears highly similar (>99% identical), there is the potential that their clinical features may differ (for example, the incidence of GBS and complications of infection during pregnancy), and the basis for this difference may affect vaccine safety and performance.

The ZIKV vaccine development pathway is also complicated by the absence of a validated animal model. The possibility of developing a human infection (challenge) model exists, but without clarity on the cause and incidence of GBS after infection and the potential for sexual transmission remote from infection, the risk may not be acceptable. In light of these constraints, how will performance of a ZIKV vaccine be measured? Early animal experiments and study of human infection suggests that the antibodies generated in response to infection are neutralizing in vitro and demonstrate efficacy in passive protection studies in small animals (3, 5, 6). Therefore, focusing on the measurement of neutralizing antibodies for immunogenicity studies is reasonable. In contrast, for an infection that results in mild disease with a brief period of viremia, phase 2b or 3 vaccine trials measuring clinical end points (disease) would need extremely large sample sizes and incredibly robust disease surveillance systems. Given links between ZIKV, GBS, and microcephaly, is it possible to conduct a study large enough to assess vaccine effect on either of these potentially related end points? Finally, how can pregnant populations be ethically included?

OTHER CRITICAL RESEARCH QUESTIONS

There are additional key questions for Zika research. ZIKV has likely been endemic in Asia for many years (78–80). Why are the same neurologic complications not noted? Are differences in previous flavivirus exposure, particularly flavivirus vaccinations or exposure intensity to dengue viruses, playing a role in protection from, or predisposition toward, more severe ZIKV disease? What is the capacity for *A albopictus* to spread ZIKV? What is the relative importance of sexual versus mosquito-borne transmission in the overall burden of ZIKV transmission? What is the importance of asymptomatic infections in transmission of ZIKV, particularly given the apparent long-term persistence of ZIKV in semen?

The poorly understood association of ZIKV with adverse neurologic outcomes also raises important questions for research, including the incidence of vertical transmission, the risk for microcephaly at different gestational ages, and whether any other factors may contribute to the rise in reported microcephaly cases, such as toxin exposure or other infections. Does preexisting flavivirus antibody, particularly dengue antibody, from previous exposure, or maternally transferred antibody predispose toward, or protect against, GBS and microcephaly? If preexisting flavivirus immunity is found to be protective, could existing flavivirus vaccines be used to mitigate severe consequences of ZIKV infection?

Other ZIKV vaccine development questions include potential vaccine associations with GBS and fetal abnormalities, whether live virus or replicating ZIKV vaccine platforms are viable options, and possible safety and performance issues in flavivirus-primed (dengue, Japanese encephalitis, yellow fever) populations.

SUMMARY

The current, explosive epidemic of Zika virus in the Americas marks a shift in the epidemiology of this virus and its clinical features. Associations with GBS and birth defects, most notably microcephaly, are concerning and poorly understood. The scientific community has much to learn about the transmission and pathogenesis of this virus, which complicates the assessment of risk to travelers and residents of epidemic areas as well as the development of accurate diagnostic tests, antiviral agents, and vaccines. This review echoes a call to action by the scientific community and highlights important areas for future research.

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Note: Dr. Anderson accepts the final responsibility for the decision to submit the manuscript.

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Disclosures: Dr. Anderson has disclosed no conflicts of interest. Dr. Thomas reports that he is an inventor on a provisional invention disclosure for an inactivated ZIKV vaccine and for a combined flavivirus vaccine with a ZIKV component pending, with all rights assigned to the U.S. government. Dr. Endy reports grants from the National Institutes of Health outside the submitted work; personal fees from Sanofi Pasteur, Merck, and Veristat outside the submitted work; and a pending patent on a ZIKV vaccine. Disclosures can also be viewed at www.acponline.org/authors/icmje/ConflictOfInterestForms.do?msNum=M16-0617.

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