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Assessing the Global Threat from Zika Virus

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Abstract

First discovered in 1947, Zika virus (ZIKV) infection remained a little known tropical disease until 2015, when its apparent association with a significant increase in the incidence of microcephaly in Brazil raised alarms worldwide. There is limited information on the key factors that determine the extent of the global threat from ZIKV infection and resulting complications. Here, we review what is known about the epidemiology, natural history and public health impact of ZIKV infection; the empirical basis for this knowledge; and the critical knowledge gaps that need to be filled.

Introduction

Originally discovered in 1947, Zika virus (ZIKV) received little attention until a surge in microcephaly cases was reported following a 2015 outbreak in Brazil (1, 2). Prompted by the size of the outbreak and the severity of associated birth defects, the World Health Organization (WHO) declared ZIKV to be a Public Health Emergency of International Concern on February 1, 2016 (3). In response, there has been an explosion in research and planning as the global health community has turned its attention to understanding and

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controlling ZIKV. Still, much of the information needed to evaluate the global health threat from ZIKV remains unknown.

The global threat posed by any emerging pathogen depends on its epidemiology, clinical features and our ability to implement effective control measures (Figure 1). In an interconnected world, introductions of ZIKV to areas free of the virus may be inevitable. Whether these introductions result in only a few subsequent cases or a significant epidemic depends on the local ecology, population immunity, the demographics of the region, and random chance. The ability of the virus to transmit in any area can be characterized by its reproductive number, R: the number of people we expect to become infected from each case in that area (4). When R is greater than one, an epidemic can occur, and when it is less than one, onward transmission will be limited. When ZIKV successfully invades, the threat may be transient and the virus might become locally extinct, as appears to have been the case in Yap Island and French Polynesia (5, 6) or it may persist endemically, as seems to be the case in parts of Africa (7). There are two ways in which ZIKV can persist in a region: through ongoing transmission in animals (i.e., a sylvatic cycle) with occasional spillover into the human population, or through sustained transmission in humans (8, 9). Whichever scenario emerges, the natural history and pathogenesis of ZIKV will determine its impact on human health, with infection in pregnant women being particularly important (10). Finally, the extent of the global threat from ZIKV is mediated by our ability to control the virus and treat those cases that do occur.

In this review, we examine the empirical evidence for a global threat from ZIKV through the lens of these processes. We review what is known about the natural history and pathogenesis of ZIKV in humans, outline what we know about the ability of ZIKV and similar viruses to invade and persist in diverse settings, and summarize the challenges we face in studying and controlling ZIKV. Finally, we examine what we know about why ZIKV has emerged as a public health threat in the Americas after being known for decades as a rare and mild tropical disease.

A brief history of ZIKV

ZIKV was discovered in the blood of a rhesus monkey in 1947 at the Yellow Fever Research Institute in Entebbe, Uganda (1), and was isolated from *Aedes africanus* mosquitoes the following year (1). Soon after, multiple serosurveys found evidence of anti-ZIKV antibodies in human populations throughout Africa (11–14), India (15) and Southeast Asia (16, 17) (Figure 2). It was not initially clear that ZIKV caused clinical disease (13), though early evidence suggested it was neurotropic in mice (18). Human infection was first confirmed in 1953 in Nigeria (13), and ZIKV was definitively established as pathogenic in humans after later experimental (19) and natural (20) infections led to symptoms of fever and rash.

The globally distributed mosquito *Aedes aegypti* was identified as a likely vector for ZIKV transmission in the 1950s, after successful transmission of the virus to a mosquito from an infected human volunteer (19). Later experiments confirmed *Aedes aegypti*'s ability to transmit ZIKV to mice (21), and ZIKV has since been isolated from several *Aedes* species (and in a few cases other genera) (22), including *Aedes albopictus* (23–26).

In the decades following its discovery, intermittent serosurveys continued to find evidence of ZIKV infection in humans in Africa (27–29), the Indian subcontinent (30) and Southeast Asia (16, 31, 32). Evidence for ZIKV's continued presence was further bolstered by limited viral isolations from mosquitoes (33–38), humans (7, 20, 29, 39, 40) and non-human primates (9). However, few clinical cases had been reported in humans prior to 2007 (20, 29, 31, 40), and ZIKV was considered to be of limited public health importance.

In 2007, the first known significant outbreak of ZIKV occurred on Yap Island in the Federated States of Micronesia (6). Though several patients initially tested positive for dengue, the unusual clinical presentation prompted physicians to send serum to the Centers for Disease Control and Prevention (CDC) Arbovirus Diagnostic and Reference Laboratory, where it tested positive for ZIKV (6, 41). During the outbreak, approximately 73% of the island's residents were infected with ZIKV, and symptoms were generally mild and short-lived (6).

Following the Yap Island outbreak, there were sporadic isolations of ZIKV in residents of and travelers to Southeast Asia (42–44), but no other significant ZIKV outbreaks were observed until late 2013. From October 2013 to April 2014, French Polynesia experienced a large outbreak of ZIKV, estimated to have infected 66% of the general population (5, 45). A contemporaneous surge in the number of cases of Guillain-Barré syndrome raised concerns of an association with ZIKV (5, 45): 42 cases of Guillain-Barré syndrome were reported from November 2013 to February 2014, compared with three cases in all of 2012. These are the first known instances of neurologic sequelae associated with ZIKV infection. Though not noted at the time, retrospective analyses suggest that there may also have been an increase in microcephaly cases (46). Following the French Polynesia outbreak, ZIKV spread throughout the South Pacific, including outbreaks in New Caledonia, the Cook Islands and Easter Island in 2014 (47).

The earliest confirmed cases of ZIKV infection in the Americas occurred in late 2014 in northeastern Brazil (48). Recent work suggests the virus may also have been present simultaneously in Haiti (49). Over the following months, the virus spread rapidly throughout Brazil (50), followed by a significant rise in cases of Guillain-Barré syndrome and microcephaly in affected regions (51), prompting the WHO to declare a Public Health Emergency of International Concern on February 1, 2016 (3). Phylogenetic evidence suggests that the strains that seeded this outbreak are descendants of those that caused outbreaks in the South Pacific, which in turn descended from the Asian lineage of the virus (52).

Since late 2014, ZIKV has spread widely throughout South and Central America and the Caribbean (2). As of June 2016, over 35 countries throughout the Americas have reported locally circulating ZIKV (53). This includes a large outbreak in Colombia, with over 65,000 reported cases, numerous reports of potentially associated neurological syndromes, and ZIKV-associated microcephaly cases (54–56). As of June 2016, the ZIKV situation continues to evolve, and the global threat ultimately posed by ZIKV remains uncertain.

The natural history and pathogenesis of ZIKV

Transmission and Natural History of ZIKV

The primary source of ZIKV infection in humans is from bites of infected mosquitoes (57), although there have also been cases of sexual (58–60), perinatal (61) and suspected blood transfusion transmission (62). Evidence from outbreaks in the South Pacific indicates that a minority of those infected with ZIKV develop clinical illness: during the Yap Island outbreak 19% of people with serological evidence of recent infection (IgM-positive) reported ZIKV symptoms (6), and in French Polynesia 26% of ZIKV-positive blood donors who were asymptomatic at the time of donation later reported symptoms (63).

On average, those who do develop ZIKV symptoms will do so 6 days after infection (64), and 95% will do so within 11 days (Figure 3). Virus has been isolated from blood (13), urine (65, 66), saliva (67), semen (68), amniotic fluid (69) and neurologic tissue (70). Virus can be isolated in blood for an average of 10 days after infection (99% will clear virus by 24 days) (64), case reports indicate virus may remain in urine for 12 or more days after infection (65), and in semen for over 60 days (59). Pregnancy may affect the length of viral shedding: in one case a woman remained viremic for at least 10 weeks during pregnancy, but cleared virus within 11 days of termination (71). Antibodies to ZIKV become detectable on average 9 days after infection (64). While the duration of immunity against ZIKV remains unknown, evidence from other flaviviruses suggests it should be lifelong (72). Mosquitoes become infectious about 10 days after biting an infectious human, and likely remain so until death (19).

Unfortunately, many of these distributions are estimated based on fewer than 30 cases. Expansion of this pool of evidence is critical for accurate assessment of surveillance activities and modeling of ZIKV risk.

Clinical Illness

ZIKV symptoms are typically nonspecific and mild. Consistent with other reports (73), symptoms reported from 31 confirmed cases on Yap Island included maculopapular rash (90%), subjective fever (65%), arthralgia or arthritis (65%), nonpurulent conjunctivitis (55%), myalgia (48%), headache (45%), retro-orbital pain (39%), edema (19%) and vomiting (10%) (6, 70). Case reports suggest that acute symptoms of ZIKV will typically fully resolve within 1–2 weeks of onset (44, 60, 74–80). Deaths are rare and have primarily occurred in patients with pre-existing comorbidities or who are immunocompromised (81, 82).

Persons infected with ZIKV may be at increased risk for severe neurologic sequelae, notably Guillain-Barré syndrome. Data from French Polynesia suggest a risk of Guillain-Barré of 24 per 100,000 ZIKV infections (5, 45), over 10-fold the annual rate in the USA (1.8 per 100,000) (83). Regardless of cause, Guillain-Barré is associated with significant morbidity and 3–10% mortality (84). Guillain-Barré may be more common in symptomatic ZIKV cases; during the French Polynesia outbreak, 88% of Guillain-Barré cases reported symptoms a median of 6 days prior to Guillain-Barré onset (5, 45). There have been reports

of other neurological sequelae, including meningoencephalitis (85) and acute myelitis (86), though no causal link has been established.

ZIKV in Pregnancy

Much of the concern surrounding ZIKV has focused on the link between infection in pregnancy and fetal microcephaly. As of May 7th, 2016, 7,438 suspected microcephaly cases have been reported in Brazil since ZIKV's emergence (1,326 confirmed/4,005 investigated), versus fewer than 200 per year prior to the outbreak (87, 88). Quantifying the risk of microcephaly has been complicated by uncertainty in the number of ZIKV affected pregnancies, owing to the large fraction of cases that are asymptomatic, a lack of consensus on the definition of microcephaly, and other infectious causes of microcephaly, such as cytomegalovirus and rubella (89). However, in light of multiple epidemiologic studies and the isolation of ZIKV in amniotic fluid and fetal brain tissue, the CDC confirmed a causal link between ZIKV infection during pregnancy and severe birth defects, including microcephaly in April 2016 (90). This conclusion is further supported by the presence of microcephaly and other brain abnormalities in the pups of mice experimentally infected with ZIKV (91).

ZIKV symptoms in pregnant women are similar to the general population (92), but it is unknown if immunosuppression during pregnancy changes the rate at which they occur. Among those who are symptomatic, adverse fetal outcomes appear to be frequent, occurring in 29% (12/42) of symptomatic ZIKV infected pregnant women in a prospective study in Brazil (92). A second Brazilian study found that 74% (26/35) of mothers of infants with microcephaly reported a rash in the first or second trimester (51). The rate of birth defects in asymptomatic pregnant women is likely lower but not zero. For example, a Colombian study identified four microcephaly cases with virologic evidence of ZIKV infection, all of which were born to women who did not report symptoms of ZIKV (54). Modeling studies suggest the overall risk of ZIKV-associated microcephaly in the first trimester is around 1 per 100, regardless of symptoms, and low to negligible thereafter (46, 93).

While microcephaly was the first fetal abnormality recognized, there is increasing evidence that ZIKV may be responsible for other fetal sequelae such as intracranial calcifications, ventriculomegaly, ocular impairment, brainstem hypoplasia, intrauterine growth restriction (IUGR) and fetal demise (92, 94). Placental pathology has also been reported. While microcephaly is detectable at birth, other findings may require additional, less routine procedures such as imaging or autopsy, and thus may be underreported. Brasil *et al.* found only 1 in 4 fetuses with abnormalities in ZIKV-infected women met the criteria for microcephaly (92), indicating the total number of ZIKV impacted pregnancies may be four-fold the number of reported microcephaly cases.

Beyond an association with symptoms, it is unclear what factors increase the risk of adverse pregnancy outcomes after maternal ZIKV infection. For other infections that cause fetal abnormalities, risk is often associated with gestational age at infection. For instance, the risk of birth defects from cytomegalovirus and rubella is highest if infection occurs in the first or early second trimester (89). Epidemiologic evidence suggests a similar association with first trimester ZIKV infection (46, 95). In a prospective study of 88 women, microcephaly and

brain abnormalities occurred only in first and second trimester infections (92). However, 8 of 12 cases of fetal abnormalities overall occurred in second and third trimester infections, and women infected as late as 35 weeks experienced fetal death, intrauterine growth restriction (IUGR), or anhydramnios (although these outcomes commonly occur in the absence of ZIKV; e.g., in Brazil 11 fetal deaths occur per 1,000 births (*96*), IUGR rates range from 5–7% in developed countries (97). A recent Colombian study suggests little to no risk from infection in the third trimester; among 616 Colombian women with clinical symptoms of ZIKV during the third trimester, none gave birth to infants with microcephaly or other brain abnormalities (7% were still pregnant at the time of reporting) (54).

Adverse outcomes in pregnancy are the most concerning side effects of ZIKV infection, and research into this association is progressing rapidly. Still, much remains to be learned, particularly about the frequency and spectrum of ZIKV sequelae in pregnancy, and how we can assess and reduce risk. ZIKV related birth defects can have long standing financial, social and health effects on affected families and communities (98). Hence the threat from ZIKV cannot purely be assessed based on immediate clinical outcomes, but also must account for its lifelong effects.

The potential range and impact of ZIKV

Transmissibility and Potential Range of ZIKV

Transmission of ZIKV in a population is a function of local ecology, the natural history of ZIKV and the population's susceptibility to infection. The suitability of the local environment for ZIKV transmission and the impact of ZIKV's natural history are captured by the basic reproductive number, R_0, the number of secondary infections expected from a single case in a population with no preexisting immunity (e.g., French Polynesia before 2013). R_0 is a function of both disease and setting, and will vary between locales based on the local environment, human behavior, vector abundance and, potentially, interactions with other viruses. The combined impact of these factors and susceptibility will be captured by the reproductive number, R, which is related to R_0 by the equation $R=R_0 \times S$, where S is proportion of the population susceptible to ZIKV. This value, combined with the generation time (the time separating two consecutive infections in a chain of transmission) tells us the speed at which ZIKV will spread in a population. As we consider how to assess the range and impact of ZIKV, we rely both on previous experience with ZIKV and related viruses, and an assessment of factors likely to influence R and R_0.

The size of an outbreak after an introduction will depend on R (R_0 in a ZIKV naive population) (99), with small, self-limiting outbreaks becoming more likely as R approaches one, and increasing epidemics with larger Rs. Hence, ZIKV can successfully spread to a new region if $R>1$, which requires, among other factors, sufficient density of the vector population. ZIKV has been isolated from multiple *Aedes* genus mosquitoes (23–26, 38), including *Aedes albopictus* and *Aedes aegypti*, which have a large global range (Figure 2B) (100). While ZIKV has been occasionally isolated from or experimentally passed to other genera, including *Culex* species, there is no current evidence they contribute substantially to its spread (22, 23, 101). It is unclear if all areas across the range of these mosquitoes are at risk for ZIKV epidemics. Dengue, a virus that is also transmitted by *Aedes* mosquitoes, has

caused epidemics throughout the Americas (Figure 2C), but has not achieved sustained transmission in the continental USA, despite widespread vector presence (100, 102, 103). The reasons for this may include not only climate but also differences in built environments and social factors (104), all of which are likely to affect ZIKV transmission.

Several groups have attempted to map ZIKV's potential global range based on currently available data. These maps have been constructed around combinations of environment, vector abundance and socio-economic factors (105–109). There is wide agreement that much of the world's tropical and sub-tropical regions are at risk for ZIKV spread, including significant portions of the Americas, Africa, Southeast Asia and the Indian sub-continent, as well as many Pacific islands and Northern Australia. These maps differ notably in the extent of risk projected in the Southeastern USA and inland areas of South America and Africa, with Carlson and colleagues suggesting a more limited range (107), particularly in the continental USA, than Messina *et al.* and Samy *et al.* (108, 109). These maps are important attempts to refine estimates of ZIKV's global range beyond those based solely on the distribution of dengue or *Aedes* mosquitoes; but, as noted by the authors, are based on limited evidence, and should be refined as we learn more about ZIKV. These analyses are, arguably, best interpreted as an assessment of the risk of initial post-invasion ZIKV epidemics, not its long term persistence. Whether ZIKV will in fact spread throughout these areas is uncertain; similar viruses have failed to spread to or take hold in areas theoretically at risk (e.g. Yellow Fever in Southeast Asia) (110).

R_0 in ZIKV outbreaks in Yap Island and French Polynesia was estimated to be between 1.8–5.8 (111–113), corresponding to 73.2–99.9% of the at-risk population becoming infected in an uncontrolled outbreak, based on classic epidemic theory (4) (although the true relationship between *R0* and final attack rates for ZIKV will be somewhat more complex (*99*)). Serosurveys in French Polynesia suggest 66% of the population was infected (46), which is somewhat lower but not inconsistent with these projections. Preliminary estimates of R_0 from Colombia vary by location, and range from 1.4–6.6 (114, 115). These are similar to R_0 estimates presented by Ferguson *et al.* for 13 countries in the Americas (116), and recent estimates of R_0 for Rio de Janeiro (117). These values are consistent with R_0 estimates for dengue in similar settings. Of note, all of these are from settings with recently observed endogenous transmission of ZIKV, and R_0 will vary widely across settings and is likely to be far lower near the limits and outside of ZIKV's range.

ZIKV's potential for endemic circulation

After the initial, post-invasion epidemic of ZIKV, the virus may either go extinct locally, or be maintained through endemic human spread or sylvatic transmission (Figure 1). Early age stratified serosurveys in Africa and Asia offer some insight into past transmission patterns of ZIKV in these regions and ZIKV's past dynamics (Figure 4). Serosurveys in Nigeria, the Central African Republic and Malaysia are consistent with ongoing ZIKV transmission, common spillover infections from a sylvatic reservoir, or frequent reintroductions from other regions over multiple decades (13, 16, 118).

However, these results must be interpreted with caution owing to cross reactivity with other flaviviruses in serologic tests (22). Up-to-date, age-stratified serosurveys, broadly covering

regions where ZIKV has previously been detected, would tell us much about the virus's ability to persist.

More recent evidence of sustained transmission comes from Thailand, where seven samples collected in independent outbreak investigations tested positive for ZIKV infection (43). The broad geographic spread of these cases is consistent with endemic transmission throughout Thailand. Furthermore, occasional but consistent serologic and virologic evidence of ZIKV transmission in humans and mosquitoes from across Africa, India and southeast Asia spanning more than 60 years suggests ZIKV has been persistently present throughout these regions (22) (Figure 1A). Phylogenetic evidence further supports this supposition, as the African and Asian lineages divided in the 1940s and remain distinct up unto the present day (22, 26) (Figure 5).

The evidence supports ZIKV's ability to persist regionally, but it is unclear if the human population alone can maintain ZIKV endemically. After an initial post invasion epidemic, the time until there is a risk of additional epidemics will be driven by the replenishment of susceptibles through births and waning immunity (the latter seems unlikely based on evidence that other flaviviruses provide lifelong immunity to the infecting strain (22)). For ZIKV to persist in the human population over this period, the population must be large enough to support low levels of transmission between epidemics (4).

However, all countries with evidence of persistent ZIKV transmission have a plausible sylvatic cycle. Patterns of ZIKV isolations in a study of samples from multiple hosts in Senegal spanning 50 years support episodic transmission across species (9); phylogenetic evidence indicates ZIKV passes frequently between non-human primates and humans in Africa (26); and numerous studies in Africa and Asia show serologic evidence for ZIKV infection in non-human primates (1, 18, 22, 33, 119). Some areas, where there has been serological evidence of long periods of consistent risk of ZIKV infection, are near to areas where serological evidence suggest that human populations are largely ZIKV free (e.g., Nigeria versus Kenya) (120, 121): a pattern more consistent with spillover infections from a sylvatic reservoir than of endemic transmission in humans.

In light of this evidence, it is plausible that the persistence of ZIKV in Africa and Asia may depend on the presence of a sustainable sylvatic cycle. However, it is unclear if the primate population in the Americas could support sylvatic transmission (122), or if such a cycle is necessary for ZIKV to remain endemic. Non-human primates are present throughout South and Central America, and ZIKV has recently been isolated from two species in the Ceará State of Brazil (123), suggesting at least the possibility for sustained sylvatic transmission in the region. Further characterization of ZIKV ecology in Asia and Africa and monitoring of the developing situation in the Americas is needed to assess the long term risk from ZIKV in newly affected regions.

Because the most severe outcomes of ZIKV infection are associated with pregnancy, the risk from endemic ZIKV will depend on the age distribution of those infected. Serosurveys indicating ongoing ZIKV circulation (Figure 4A–C) support average ages of infection of 17 (Nigeria 1952), 29 (Central African Republic 1979) and 30 years (Malaysia 1953–1954) (13,

16, 118). Likewise, R_0 estimates from the literature are consistent with average ages of infection ranging between 10 and 38 years in the setting of endemic human-to-human transmission (although human-to-human transmission should not be necessarily assumed in the settings covered in Fig. 4A–C). These ages suggest that in endemic settings, risk of ZIKV infection may be significant during childbearing years. Importantly, this information could potentially be used to estimate the expected rate of microcephaly and other birth defects in regions where ZIKV becomes endemic.

Why has ZIKV invaded the Americas now

Little is known about ZIKV's introduction into the Americas. Phylogenetic analyses indicate that a virus descended from the French Polynesian ZIKV strain entered Brazil between May and December 2013 (52). Although there has been speculation about introduction during specific sporting events (52, 124), Brazil has over 6 million visitors per year, providing numerous opportunities for ZIKV introduction. Regardless of how and when ZIKV entered the Americas, the reasons for the size and severity of this outbreak are unclear.

The unprecedented size and impact of the ZIKV epidemic in the Americas may be the natural result of a random introduction into a large population without preexisting immunity. Like the Americas, the populations of Yap Island and French Polynesia were fully susceptible when ZIKV was introduced, and both had large outbreaks infecting over 65% of their populations (6, 45). However, on these small islands the absolute number of adverse outcomes may have been too low to be noticed initially. Likewise, it is possible that small ZIKV epidemics, and even invasion into Southeast Asia in the mid-1900s, resulted in effects that were unnoticed against the backdrop of other infectious diseases, particularly since small population sizes (compared to Brazil) mean that excess microcephaly cases would likely be in the hundreds (or less) in any given country. Endemic transmission would be even less likely to be noticed, as yearly attack rates would be a tenth again lower (Fig. 4) (116). Still, given the magnitude and severity of the outbreak in the Americas, it seems implausible that if such outbreaks were occurring, none was observed for over 60 years. Hypothesized changes in the biological and ecological drivers of ZIKV transmission must be carefully assessed, as they will influence how we quantify the risk from ZIKV globally.

Warmer temperatures and rainfall resulting from the 2015–2016 El Nino may have facilitated ZIKV transmission throughout the region (125) and increased the geographic range of *Aedes* mosquitoes. Warmer temperatures have been associated with more efficient transmission of related flaviviruses (126) and greater production of adult mosquitoes (127, 128). El Nino-associated periods of flooding (which increases mosquito breeding sites) and of droughts (which can increase human-mosquito interactions) may facilitate ZIKV transmission (129, 130). However, it should not be assumed that increased temperature or rainfall will universally promote ZIKV transmission, as climatic changes have complex repercussions across food webs (from plant growth to bird behavior) and the thermal effects on the virus itself are likely to be non-linear (131). Over a longer time scale, development and urbanization has led to a proliferation of *Aedes aegypti* and *Aedes albopictus* in densely populated areas, which may have facilitated the rise of dengue in the region and may also have provided conditions that favoured ZIKV spread (132).

There is some possibility that immunological interactions with other flaviviruses may be facilitating the spread or pathogenesis of ZIKV in the Americas. In dengue, pre-existing antibodies to one serotype are hypothesized to enhance subsequent infections with another serotype through a mechanism known as antibody dependent enhancement (ADE)(133). ADE may result in increased susceptibility to infection, the likelihood of developing severe disease and the chances of transmission (134, 135). Evidence from some *in vitro* experiments and epidemiological studies show both protective and enhancing effects between immunity to Japanese encephalitis and dengue (136, 137), and several *in vitro* studies have shown enhancement of ZIKV replication in the the presence of antibodies to other flaviviruses (135, 139). Dengue has circulated throughout much of Central and South America since it re-emerged 30 years ago, hence it is possible that such interactions are contributing to the current outbreak of severe disease. However, this would raise questions as to why similar interactions have not been seen in the dengue endemic regions of Southeast Asia that also show evidence of ZIKV circulation. Studies that measure preexisting dengue and ZIKV antibodies and track clinical outcomes may help illuminate the issue.

The severity of outcomes in recent outbreaks, compared with past observations of mild disease, has led some to hypothesize the virus has mutated to be more pathogenic (140). Recent evidence suggests distinct codon preferences between African and Asian ZIKV lineages, although adaptive genetic changes that may have an impact on viral replication and titers (141), while the genetic diversity of viruses isolated in ZIKV associated microcephaly cases suggest recent mutations may not be involved (142). Epidemiologic and laboratory studies are needed to determine if these changes have had a substantive effect on viral pathogenesis. Until the impact of ZIKV evolution is better understood, we should be careful to balance the need to learn from previous research with the possibility that the virus has fundamentally changed.

Human genetics is known to have a profound effect on the pathogenesis of many infectious diseases (143), and there is some indication the same could be true for flaviviruses (144, 145). There is evidence of ancient intermixing between Polynesian and American populations (146), there are no indications of a link between ancestry and severe outcomes from ZIKV at this point. Likewise, genetic variation in *Aedes aegypti* is known to affect vector competence to transmit flaviviruses (147), hence it is possible that changes in the makeup of the vector population also influence ZIKV transmission and account for regional differences in ZIKV impact.

Challenges and research priorities for responding to the ZIKV threat

Surveillance and Clinical Outcomes

The key challenge in ZIKV surveillance is the proportion of cases that remain asymptomatic and the nonspecificity of ZIKV symptoms (148). Dengue and chikungunya are also transmitted by *Aedes* mosquitoes, co-circulate with ZIKV, and can have a similar presentation, further complicating surveillance efforts.

Laboratory testing is needed to confirm ZIKV infection. Molecular (RT-PCR) techniques can be used to detect ZIKV in serum, saliva and urine (67, 149). However, there are frequent

cases where testing of different fluids gives discrepant results, and additional studies are needed to assess diagnostic accuracy (67). The timing of sample collection is crucial; viral RNA is only detectable in serum for 3–5 days after symptom onset (~10 days after infection), but may persist longer in other fluids (59, 64, 66).

A highly specific, easily administered antibody test would be a boon to surveillance and patient care. Such a test could be used to estimate underlying ZIKV incidence and thus rates of severe outcomes, confirm infection in studies of ZIKV pathogenesis, and to test for immunity to ZIKV early in pregnancy so women can know if they are at risk. However, serological testing is complicated by potential cross-reactivity with other flaviviruses (22). Newer ELISA tests show promise, such as an IgG-ELISA test used in French Polynesia that, despite endemic dengue circulation, found <1% ZIKV seropositivity in blood donors prior to the outbreak (150).

To assess the risk and determinants of ZIKV related clinical outcomes, we need studies aimed at measuring the underlying incidence of ZIKV infection, regardless of clinical presentation (e.g., serosurveys), the spectrum of illness and risk factors for severe outcomes (e.g., cohort and case-control studies), and the impact of ZIKV over longer time scales, including the length of immunity.

Ecology and Evolution

There has been a high level of global concern surrounding the threat from ZIKV. One reason the concern is so great is that we are unable to accurately assess the global threat from the virus, and differing lines of evidence point to conflicting conclusions. For instance, the range of *Aedes* mosquitos and ecological analyses would suggest that much of the continental U.S. is at risk from ZIKV, while recent experience with dengue and chikungunya would suggest that ZIKV is unlikely to persist in this region. To assess the epidemiologic and ecologic factors that drive global risk, there is a need for studies that more accurately assess where ZIKV circulation persists over long periods (e.g., global age stratified serosurveys), and the ecological determinants of persistence (e.g., reservoirs, critical population size, vector competence); as well as studies characterizing interactions between ZIKV and other flaviviruses. Across both clinical and ecological studies, it is important to evaluate the impact of host, viral and mosquito genetics.

Interventions and Control

A ZIKV vaccine may be the best way to protect at-risk populations over the long term. Vaccine development has been prioritized by the WHO and other public health agencies, and there are at least 18 active manufacturers and research institutions pursuing early stages of ZIKV vaccine development (151). However, phase 1 clinical studies are not expected to begin until the end of 2016 (151), hence a vaccine is unlikely to become available in time to change the course of the current outbreak in the Americas.

Without a vaccine or antiviral drugs, the tools at our disposal for reducing ZIKV incidence are based on vector control and limiting ZIKV exposure. We have little direct evidence of the effectiveness of these approaches in controlling ZIKV transmission, but there are decades of experience in controlling dengue and other flaviviruses (152–154). Effective

vector control is possible: Gorgas virtually eliminated Yellow Fever from Havana and the Panama Canal region in the early 1900 using crude and draconian, methods of vector control (155). Intensive vector control in the 1950s and 1960s, including mass DDT spraying, successfully eliminated *Aedes aegypti* from 18 countries in the Americas, substantially reducing dengue incidence (154, 156, 157). Later, Singapore and Cuba implemented successful vector control programs lasting decades (154, 158, 159). However, all of these efforts ultimately proved to be unsustainable, and *Aedes aegypti* and dengue re-emerged following their discontinuation (154, 158, 159). Nevertheless, there could be benefits from even short term elimination, but research is needed to identify sustainable policies that can protect areas from ZIKV and or other *Aedes* borne diseases in the long term.

There is limited evidence for the effectiveness of measures aimed at reducing individual exposure to mosquitoes for dengue control. A meta-analysis suggests that use of screens in houses reduces the odds of dengue incidence by 78%, as does combined community environmental management and use of water container covers (152). Other interventions, such as indoor residual spraying, repellents, bednets and traps, showed no significant effect or a negative effect (insecticide aerosols) (152). However, these results are predominantly based on observational studies, limiting the strength of the evidence they provide. Topical insect repellents and other personal protective measures do reduce mosquito biting (160), and should decrease the risk of ZIKV infection. Some randomized trials have assessed the effect of interventions on mosquito populations with inconsistent results (152, 161), and there have been no well-designed trials assessing the impact of the common, WHO recommended practice of space spraying or fogging to control dengue transmission (152). Well-designed experimental studies with endpoints of transmission and disease in humans are needed to better evaluate the effectiveness of interventions aimed at vector control and personal risk reduction.

Conclusion

The rise of ZIKV after its long persistence as a disease of apparently little importance highlights how little we truly understand about the global spread of flaviviruses and other vector borne diseases. Over the past decades, dengue, chikungunya, West Nile virus, and now ZIKV have emerged or re-emerged throughout the globe (2, 145). However, why these viruses have expanded their range, while others (e.g., Yellow Fever) have failed to invade areas potentially ripe for their spread remains a mystery. New analytic and molecular tools have greatly expanded our ability to forecast risk and track the spread of these viruses, but a deep understanding of what makes one virus a global threat while another is not remains elusive. While the important role of random chance and the continuing evolution of viral species may make precise forecasting of emerging pandemics impossible, we can continue to improve the speed with which we assess and respond to emerging threats.

The evidence highlighted in this review is both encouraging and disheartening. On the one hand, the speed with which the global community has collected and disseminated clinical, epidemiologic and laboratory information on ZIKV after identification of the threat is impressive. But the development of therapeutics and diagnostics is hampered by our ignorance, despite knowing of ZIKV's existence for over half a century. Consequently, we

have been able to do little to contain the virus's rapid spread across the Americas. New threats from infectious diseases may emerge from unexpected places, and we need strategies in place we can roll out to rapidly gain an understanding of the transmission, pathogenesis and control of previously little known pathogens to protect global public health.

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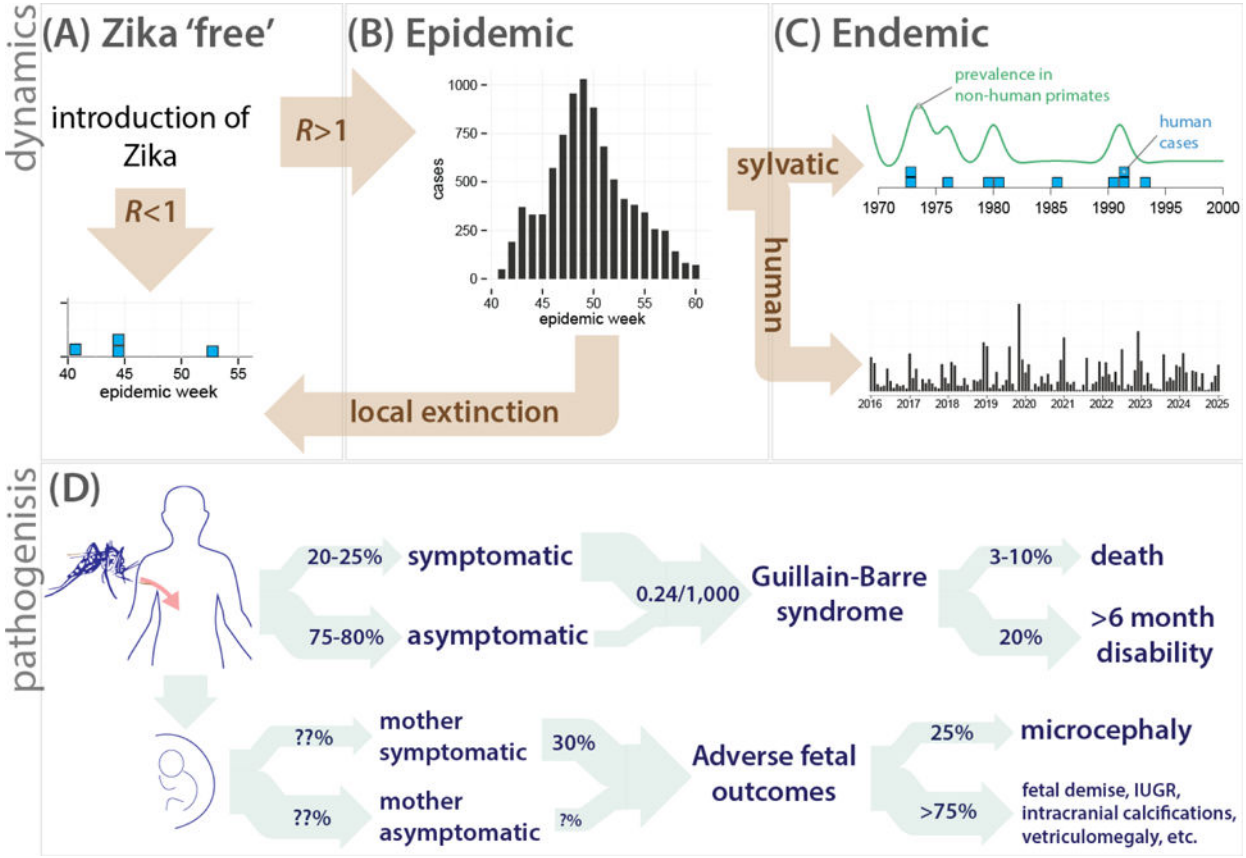

Fig. 1. Factors determining the global risk from ZIKV

(A) As long as ZIKV circulates anywhere, periodic introductions into ZIKV free regions will occur. Whether these lead to an epidemic depends on the reproductive number, *R*, a measure of transmission efficiency determined by local ecology and population susceptibility to ZIKV. **(B)** When *R*>1, introductions can result in significant epidemics, after which the virus may go locally extinct or become endemic. **(C)** ZIKV could be maintained endemically either in local non-human primates (the sylvatic cycle) or through ongoing human transmission. **(D)** Most ZIKV infections (75–80%) are asymptomatic, and those with symptoms are likely at highest risk for rare neurological complications (6, 63, 92), particularly Guillain-Barré (45). Adverse fetal outcomes, notably microcephaly, may also be more common when the mother is symptomatic. Owing to its association with pregnancy, ZIKV's health impact depends on the fertility rate and the age distribution of infections. The age distribution mirrors the general population in ZIKV free (A) and epidemic (B) settings, but is a function of the force of infection in endemic settings (C) (4, 45). Appropriate control measures can reduce R, decreasing the probability of successful ZIKV invasion (A) and its subsequent impact (B–C) (see *116*).

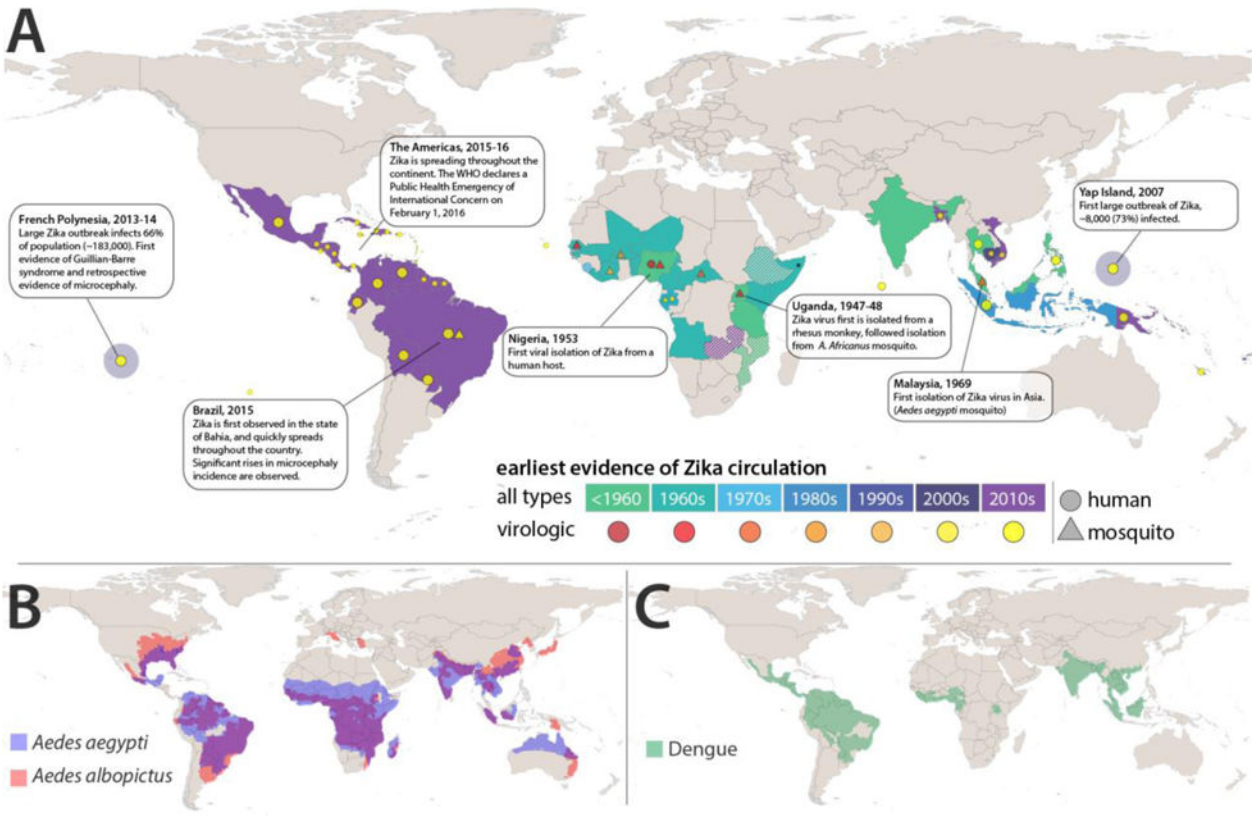

Fig. 2. Current and potential distribution of ZIKV

(A) Spread of ZIKV across the globe to date. Countries are colored by the timing of the first indication of local ZIKV transmission by serologic evidence or confirmation of human cases. Solid shading indicates clusters of confirmed cases or seropositivity to ZIKV of >10% in some sub-population, while hatched colors indicate 5–10% seropositivity (serosurveys showing <5% seropositivity are not shown). Symbols indicate locations and timings of viral isolations from mosquitoes (triangles) and humans (circles). **(B)**. Map of the global occurrence of the widely distributed ZIKV vectors *Aedes aegypti* and *Aedes albopictus*. Adapted from (100, 116). **(C)** Map of the occurrence of dengue, a closely related *Aedes* transmitted flavivirus. Adapted from (103). Shaded regions correspond to areas with predicted probability of vector or dengue occurrence of >30%. * - Somalia did not report the total percent ZIKV seropositive, but there were a small percentage of subjects seropositive to ZIKV and no other flavivirus, and a large percentage seropositive to two or more flaviviruses, so is included.

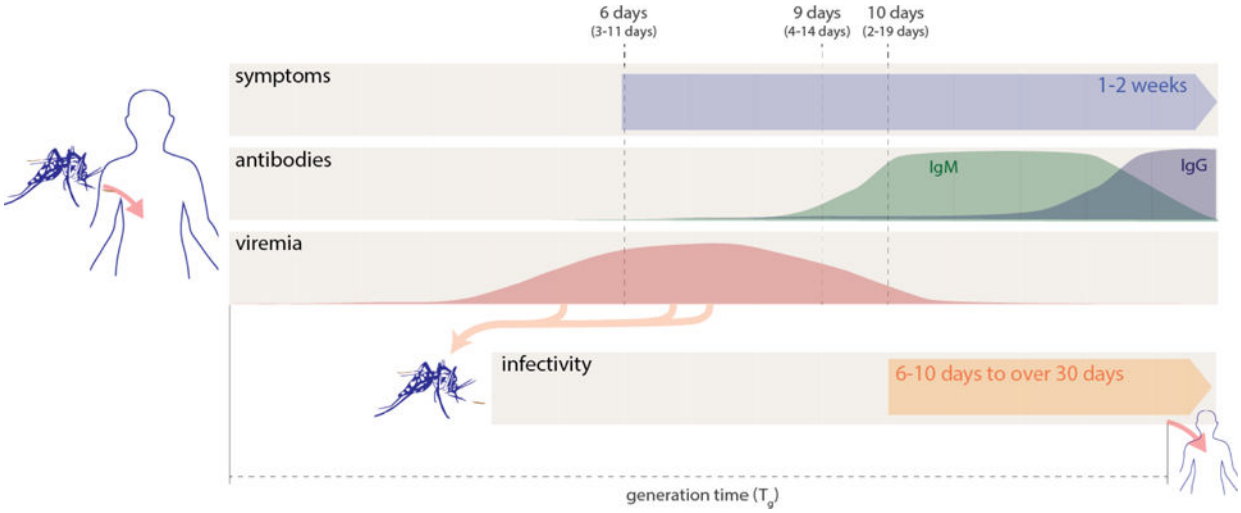

Fig. 3. Schematic of the course of human and mosquito infection

Symptoms develop on average 6 days (95% range 3–11 days) after ZIKV infection (64). Approximately 9 days (95% range 4–14 days) after infection, antibodies start increasing: the first antibodies detectable will be IgM, which will later decline as IgG antibodies increase then persist indefinitely (note the timing of IgM/IgG switch is for illustrative purposes only and not meant to indicate actual length of IgM persistence). Viremia likely starts to increase before symptoms appear, and the magnitude and length of viremia will shape the risk of infection of susceptible mosquitoes that bite this host. After an incubation period, this infected mosquito will be able to transmit infection to susceptible humans (19). The interval from the initial to the subsequent human infection is the generation time of ZIKV, T_g (for estimates, see *116*)).

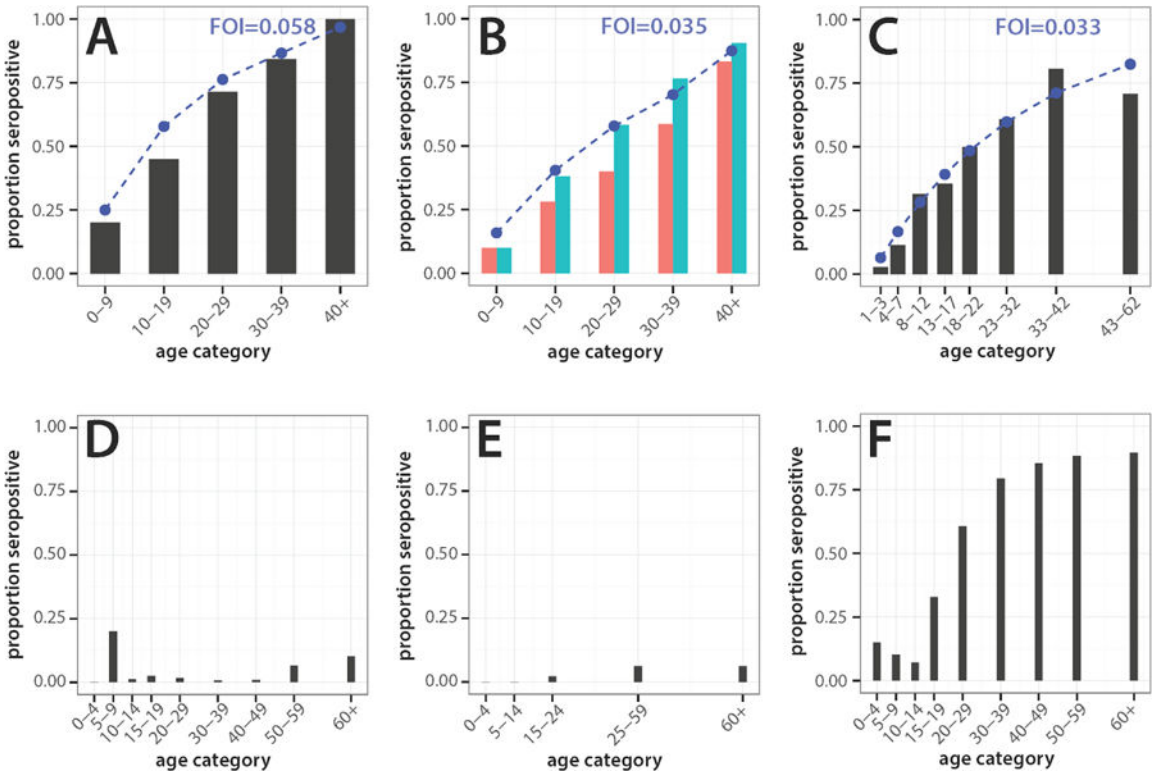

Fig. 4. Age stratified serosurveys provide important clues to local ZIKV epidemiology

Results must be interpreted with caution bacuse of the possibility of cross-reactivity with other flavivirus antibodies. **(A–C)** Ongoing ZIKV transmission, whether from endemic human transmission or a constant risk of zoonotic infection, manifests as a smooth increase in the proportion of the population seropositive with increasing age. This pattern is also consistent with frequent reintroductions leading to periodic outbreaks. If we assume that the risk of ZIKV infection is constant over a lifetime, we can estimate the force of infection (FOI): the proportion of the susceptible population infected each year. Serosurvey results consistent with ongoing transmission include: **(A)** Uburu, Nigeria, 1952 (13), **(B)** Central African Republic, 1979 (pink=female, red=male) (118), and **(C)** Malaysia, 1953–54 (16). Blue dashed lines and text represent the expected trajectory from the estimated FOI. **(D–E)** In areas without significant ZIKV transmission there will be very low levels of seropositivity across age groups, and no clear age pattern. Some individuals may still be seropositive due to cross-reactivity in serological assays, infection of travelers, and limited imported cases. Examples include **(D)** Central Nyanza, Kenya, 1966–1968 (121) and **(E)** Mid-Western Region, Nigeria, 1966–1967 (120). **(F)** Significant shifts in seropositivity between age groups inconsistent with ongoing transmission suggest past epidemics, e.g., results from a 1966–1968 serosurvey in the Malindi district of Kenya are consistent with one or more epidemics of ZIKV occurring 15–30 years prior (121). Similar patterns could also occur due to differences in infection risk by age or a sharp reduction in transmission intensity at some point in the past.

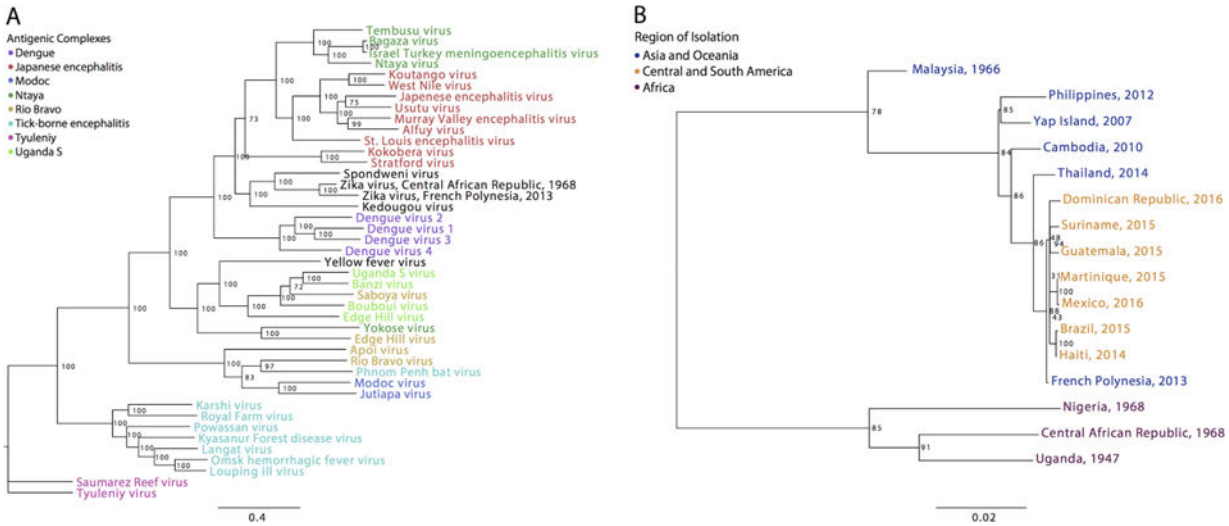

Fig. 5. ZIKV Phylogenetics

(A) Maximum likelihood tree of phylogenetic relationships between 43 flaviviruses (numbers indicate support from 1,000 ultrafast bootstrap replicates), with antigenic clusters from Calisher *et al.* indicated by color (162). **(B)** The phylogenetic relationship between ZIKV strains isolated from throughout the globe. Whole-genome nucleotide sequences were aligned using Clustal Omega (163) and trees were constructed using IQ-TREE (164) under a GTRM+G+I evolutionary model.