JVI Accepted Manuscript Posted Online 9 March 2016
J. Virol. doi:10.1128/JVI.00252-16

Zika Virus: New Clinical Syndromes and its Emergence in the Western Hemisphere

Helen M. Lazear[1] and Michael S. Diamond[2]

[1] Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599. [2] Departments of Medicine, Molecular Microbiology, Pathology & Immunology, and The Center for Human Immunology and Immunotherapy Programs, Washington University School of Medicine, St Louis, MO 63110.

*Address correspondence to:
Helen M. Lazear, Ph.D., Department of Microbiology and Immunology, University of North Carolina, Chapel Hill, NC 27599 USA. helen.lazear@med.unc.edu, (919) 966-3708
and/or
Michael S. Diamond, M.D. Ph.D., Departments of Medicine, Molecular Microbiology, and Pathology and Immunology, Washington University School of Medicine, 660 South Euclid Ave. Box 8051, Saint Louis, MO 63110, USA. diamond@borcim.wustl.edu, (314) 362-2842 or

Running title: Emergence of Zika virus

ABSTRACT

Zika virus (ZIKV) had remained a relatively obscure flavivirus until a recent series of outbreaks accompanied by unexpectedly severe clinical complications brought this virus into the spotlight as an infection of global public health concern. In this review, we discuss the history and epidemiology of ZIKV infection, recent outbreaks in Oceania and the emergence of ZIKV in the Western Hemisphere, newly ascribed complications of ZIKV infection including Guillain-Barré syndrome and microcephaly, potential interactions between ZIKV and dengue virus, and the prospects for the development of antiviral agents and vaccines.

Zika virus: history and epidemiology

Zika virus (ZIKV) is a member of the *Flavivirus* genus of the *Flaviviridae* family, which includes other globally relevant human pathogens such as dengue (DENV), yellow fever (YFV), West Nile (WNV), Japanese encephalitis (JEV) and tick-borne encephalitis (TBEV) viruses (1, 2). ZIKV is an enveloped virus with an approximately 10.7 kilobase positive-sense RNA genome. Similar to other flaviviruses, the ZIKV genome encodes a single polyprotein that is cleaved post-translationally by host and viral proteases into three structural proteins (capsid (C), pre-membrane (prM), and envelope (E)) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A NS4B, and NS5) (3, 4). C binds to the viral RNA to form a nucleocapsid, prM prevents premature fusion with host membranes, and E mediates cellular attachment, entry, and fusion (5). The viral nonstructural proteins regulate viral transcription and replication and also attenuate host antiviral responses (1, 6, 7). ZIKV is a member of the Spondweni group within the mosquito-borne clade of flaviviruses (**Figure 1**) and is closely related to the four serotypes of DENV with approximately 43% amino acid identity across the viral polyprotein as well as in the ectodomain of E.

ZIKV was first isolated in 1947 from a febrile sentinel rhesus monkey in the Zika forest, a research station of the East African Virus Research Institute (now the Uganda Virus Research

Institute) in Entebbe, Uganda (8, 9). The virus was isolated subsequently from *Aedes africanus* mosquitoes in the same forest (9-11), and multiple monkey species in the Zika forest were found to be seropositive for ZIKV (11). Small mammals in the Zika forest (including squirrels, tree rats, giant pouched rats, and civets) did not show serological evidence of ZIKV infection, consistent with a model where primates (both humans and monkeys) are the primary vertebrate hosts for ZIKV (10). Multiple species of *Aedes* mosquitoes contribute to enzootic maintenance of ZIKV, but likely only a subset of these transmit the virus to humans (12, 13). There is evidence of high rates of ZIKV seroprevalence in Africa and Asia (9, 14-17), although the specificity of such assays is uncertain, given the significant serological cross-reactivity between ZIKV and other flaviviruses (see below). In the decades following its discovery, ZIKV was isolated from human patients sporadically during outbreaks in Africa and Southeast Asia (15, 18), but remained obscure due to the fairly benign nature of the infection (generally a self-limiting febrile illness, see below).

ZIKV came to global attention in 2007, when it caused of an explosive outbreak in Micronesia (18-21). On the island of Yap, it is estimated that approximately 75% of the population became infected during a 4-month period (19). In the ensuing years, ZIKV spread throughout Oceania (22-25) and then was detected in Brazil in early 2015 (26, 27). Although the precise means by which ZIKV was introduced to the Western Hemisphere is unknown, the presumption is that the virus came to Brazil from Polynesia via a viremic traveler or an infected mosquito (2, 26, 28, 29). The *Aedes aegypti* mosquito, which can transmit ZIKV, is abundant in Brazil and autochthonous transmission was established. The outbreak initially was concentrated in Northeastern Brazil. However, the virus rapidly spread throughout Latin America and the Caribbean, such that within one year most countries in the region have reported local transmission (30-32). Further spread of the virus is anticipated and imported cases already have been reported in the United States, Europe, and elsewhere in travelers returning from Latin America and the Caribbean during the current outbreak (30, 33-35). The rate at which ZIKV has

spread through Latin America and the Caribbean since its introduction appears comparable to chikungunya virus (CHIKV) after its introduction to the Western Hemisphere in late 2013, suggesting that it reflects the abundance and competence of the *Aedes aegypti* mosquitoes that are used as vectors by both viruses, as well as the availability of a susceptible host population (36). ZIKV genome sequences from Polynesia and South America are highly similar (2, 26, 29) (approximately 99% nucleotide identity across the viral genome), but there are genetic differences, for example 6 amino acid changes between the H/PF/2013 strain from French Polynesia and the SPH2015 strain from Brazil (Genbank accession: KJ776791.1 and KU321639.1) (37). Future studies are needed to determine whether such changes impact disease pathogenesis, tropism, or vector competence. The ability of changes in viral sequence to impact the epidemic potential of arboviruses was seen previously with CHIKV, where a small number of mutations, including a A226V change in the E1 glycoprotein, enabled the virus to use *Aedes albopictus* mosquitoes as vectors, which have an expanded geographic range compared to *Aedes aegypti*, facilitating epidemic spread into new areas (37-39).

Modes of transmission

Vector-borne transmission. ZIKV is a mosquito-transmitted virus (**Figure 2**). ZIKV has been isolated from many species of *Aedes* mosquito, but only a subset of these are competent vectors for transmission (including *Ae. aegypti, Ae. albopictus, Ae. hensilii*, and *Ae. polynesiensis)* (9-13, 18, 21, 40-42). *Aedes aegypti* is thought to be the principal vector spreading ZIKV during the current outbreak in Latin America and the Caribbean, likely due to the urban abundance and anthropophillic nature of this mosquito (43). Monkeys are presumed to serve as reservoir hosts for ZIKV, although the primary species has not been identified (11, 18). It is unclear whether ZIKV will become endemic in New World monkeys and establish a sylvatic transmission cycle in Latin America analogous to YFV, or be maintained exclusively through urban transmission cycles with no New World sylvatic cycle, similar to DENV (44).

Humans are amplifying hosts for ZIKV and urban cycles of transmission between humans and mosquitoes sustain and cause epidemics. Indeed, the island of Yap in Micronesia experienced an extensive ZIKV outbreak yet there are no non-human primates on this island (19). There currently is no evidence that animals other than humans and non-human primates serve as amplifying hosts for ZIKV, suggesting a mode of transmission similar to DENV, YFV, and CHIKV. While mosquito-borne transmission clearly is the main cause of ZIKV outbreaks, other modes of transmission have been reported.

Blood-borne transmission. As is the case for other blood-borne infections, a ZIKV viremic donor could potentially contaminate the blood supply (45, 46) and cases of ZIKV transmission through transfusions of donated blood have been reported in Brazil, although not yet published. In many areas, including the United States, Canada, and Europe, the blood supply already is screened by nucleic acid amplification tests to detect WNV (47-50). The same approach, once a screening test becomes available, could be used to detect ZIKV, and plans exist in several countries to screen the blood supply for ZIKV or to defer blood donation from those who have travelled to countries where ZIKV is circulating. In the absence of an approved diagnostic assay to detect ZIKV contamination, strategies are available to inactivate infectious agents in the blood supply (46, 51).

Sexual transmission. There is evidence of sexual transmission of ZIKV (34, 52, 53), and ZIKV RNA has been detected in semen (54, 55). To date, all reported sexually transmitted cases of ZIKV infection have been from infected men to their female partners. Although some of these cases were accompanied by hematospermia, infectious ZIKV was detectable in semen even after viremia had cleared (undetectable ZIKV RNA in serum), arguing against blood-borne transmission (54). Moreover, while other sexually transmitted infections cause hematospermia (56), this has not been a common presentation of ZIKV infection, nor has it been evident in all cases of sexually transmitted ZIKV (34). Recent reports of infectious ZIKV in urine, along with the detection of ZIKV RNA in urine even after viremia has cleared (57), could be consistent with

ZIKV replication in urogenital tissues. ZIKV RNA has been detected in saliva (58) and infectious ZIKV in saliva recently was reported. Due to the highly correlated nature of behaviors, sexual and salivary transmission can be difficult to distinguish. Indeed, Kaposi's sarcoma-associated herpesvirus initially was thought to be sexually transmitted, but subsequent findings indicated that the primary mode of transmission was through saliva (59). Indeed, pigs can develop high viral loads in the tonsils and transmit JEV through oronasal secretions, which demonstrates this as a possible transmission route for flaviviruses (60). Although sexual transmission is unlikely to be a major cause of ZIKV outbreaks, the presence of virus in semen warrants investigation, especially given recent evidence that Ebola virus RNA can be detected in the semen of survivors for months after the acute infection has cleared. Similarly, ZIKV RNA was detected in semen 62 days after the onset of febrile symptoms (55). The immune privileged nature of the testes may allow ZIKV to persist in this tissue. Such reservoirs have the potential to initiate new transmission cycles from seemingly healthy individuals (61, 62). The growing number of imported ZIKV cases in areas of the United States and Europe where local mosquito transmission is less likely provides an opportunity to detect and determine the significance of alternative transmission mechanisms (34).

Maternal transmission. ZIKV RNA has been detected in breast milk (63). As this route of transmission has been documented for other flaviviruses (64-66), ZIKV-infected mothers may be able to pass the virus to nursing children. However, it is not known whether infectious ZIKV is present in breast milk nor its possible duration relative to acute infection, and ZIKV-infected mothers are still encouraged to breastfeed their infants (67). Perinatal transmission of ZIKV was documented in French Polynesia (63), but it is unknown whether this represented transmission in breast milk, blood-borne transmission during delivery, or *in utero* transmission.

The question of *in utero* transmission has gained urgency as the emergence of ZIKV in Brazil has coincided with an alarming increase in the number of cases of microcephaly, with the Northeastern states reporting >4,000 cases over approximately four months, a more than 20-

fold increase from prior years (68-71). Microcephaly is a congenital abnormality in which the fetal brain is underdeveloped (72, 73). There is not a standard definition of microcephaly, as definitions range from a newborn head circumference ≤32 or 33 cm, or ≥2 or 3 standard deviations below the mean for gestational age (69). Many factors during pregnancy can cause microcephaly, including other viral infections (*e.g.*, human cytomegalovirus, rubella virus, and varicella-zoster virus), exposure to toxins (*e.g.*, drugs or alcohol), and genetic mutations. Microcephaly can be asymmetric, meaning a small head on an otherwise normally proportioned body, or symmetric, meaning that the small head is proportional to a small overall body size; the type of microcephaly can be characteristic of its etiology. Microcephaly can be diagnosed by prenatal ultrasound, but generally not until the late second trimester and many cases are not evident until after birth. The long-term effects of microcephaly can vary widely, from virtually no defects to cognitive deficits and severe physical disability (73).

It is important to note that the majority of the microcephaly cases reported during the current outbreak have yet to be confirmed or linked directly to ZIKV; in ongoing follow-up studies, approximately one third of reported microcephaly cases had been corroborated, and presumably some of these will be attributable to causes other than ZIKV infection (68, 70, 71). Further complicating the analysis, the case definition for microcephaly has changed over the course of the current outbreak: in December 2015 the Brazilian Ministry of Health adopted a newborn head circumference ≤32 cm as the case definition, compared to the less stringent ≤33 cm cutoff used previously (69). Clearly, better data are required to assess the potential connection between ZIKV infection and microcephaly; epidemiological studies, including case-control and prospective cohort studies, are underway and should bring clarity to this question in time. Nonetheless, accumulating evidence strongly suggests a causal role for ZIKV in the development of microcephaly. In addition to the timing and geographic distribution of microcephaly cases relative to ZIKV infections, data supporting trans-placental infection includes the following: (i) detection of ZIKV RNA and sequencing of full-length viral genome

179 from the amniotic fluid of fetuses diagnosed with microcephaly by ultrasound in mothers who
180 reported previous ZIKV infection but were not viremic at the time of amniocentesis; (ii) detection
181 of ZIKV RNA and/or antigen in the tissues of three microcephalic infants who died shortly after
182 birth; (iii) detection of ZIKV RNA in the placenta from a microcephalic fetus after miscarriage;
183 (iv) Partial sequence of ZIKV genome and viral antigen detection in four fetal brain tissue
184 samples recovered from miscarriages and neonatal death; (v) sequencing of full-length ZIKV
185 RNA genome and visualization of ZIKV-like particles by electron microscopy in the fetal brain
186 from a terminated pregnancy (74-81). A recent report of anti-ZIKV IgM in the cerebral spinal
187 fluid of 12 infants with microcephaly also supports *in utero* infection with ZIKV.

188 Although other viruses can cross the placenta and cause microcephaly in humans
189 and/or animals, this presentation has never been associated previously with flaviviruses (82-86).
190 *In utero* infection with WNV has been studied, with no clear evidence of an association with
191 microcephaly (87-89). Furthermore, there are an estimated >390 million DENV infections
192 annually (including ~25 million estimated in Brazil (90)), so even a very low rate of DENV-
193 induced microcephaly would have been observed. While the mechanisms by which ZIKV may
194 cause microcephaly are unknown, the preliminary evidence and the severity of the disease has
195 prompted the United States Centers for Disease Control and Prevention (CDC), Public Health
196 Agency of Canada, Australian Department of Foreign Affairs and Trade, and Public Health
197 England, among others, to recommend that women who are pregnant or planning to become
198 pregnant avoid travel to areas where ZIKV is circulating (in effect, nearly all of Latin America
199 and the Caribbean, among other locations) (74, 75, 80, 91, 92). Such travel advisories have
200 significant economic impact on the affected countries, especially with the approach of the 2016
201 Olympic Games in Rio de Janeiro. Furthermore, in response to the potential for sexual
202 transmission of ZIKV, CDC has cautioned pregnant women against unprotected sex with
203 partners who have potential ZIKV exposure (34, 91, 93). Remarkably, health officials in several
204 Latin American and Caribbean countries have recommended that women postpone pregnancy

in response to the ZIKV outbreak. In the United States, pregnant women have become infected while traveling to areas with active ZIKV transmission, or by sexual contact with ZIKV-infected male partners. The outcomes of these ZIKV-exposed pregnancies have been variable, including early pregnancy loss, elective termination, delivery of an infant with severe microcephaly, and seemingly unaffected infants (80). Many unanswered questions remain about *in utero* transmission of ZIKV infection and the development of microcephaly, as discussed further below.

Clinical features of Zika virus infection

Historically, ZIKV infection caused a variable clinical syndrome in humans ranging from no signs or symptoms to an influenza-like viral illness that appeared similar in the early stages to those caused by other epidemic arboviruses including DENV and CHIKV. For ZIKV, approximately 20 percent of individuals who become infected progress to a clinically apparent febrile illness, although hospitalization is rare (18, 19). Signs and symptoms associated with ZIKV infection occur on average within 3 to 7 days of mosquito inoculation and include an abrupt onset of fever accompanied by headache, arthralgia, myalgia, conjunctivitis, vomiting, fatigue, and/or maculopapular rash (94) (**Figure 2**). For many years, ZIKV infection was considered self-limiting with no long-term sequelae, but more severe complications have become apparent during the more recent ZIKV outbreaks in the South Pacific and Latin America, possibly because the greater number of infections has facilitated detection and reporting of rare outcomes (though other factors may also contribute to increased ZIKV pathogenesis). Although ZIKV infection has not been reported to cause the plasma leakage and hemorrhage associated with severe DENV disease, ZIKV has caused thrombocytopenia and hematospermia (52, 54, 95). There are no reported fatal cases of ZIKV in otherwise healthy people. However, ZIKV-associated mortality has been described in patients with co-morbidities

including sickle cell disease (96), and congenital ZIKV infection and post-ZIKV Guillain-Barré syndrome (GBS) can be fatal.

During the 2013-2014 ZIKV outbreak in French Polynesia, neurological disorders were linked to ZIKV infection, as there was an increase in the incidence of GBS, a post-infection autoimmune neuropathy that can result in weakness, paralysis, and death (92, 97-99). A case-control study of the outbreak found that GBS patients were more likely to have evidence of past ZIKV infection compared to controls, with 0.24 cases of GBS per 1,000 ZIKV infections (98). Patients with post-ZIKV GBS had atypically low levels of anti-ganglioside antibodies compared to patients with GBS of other etiologies, suggesting that ZIKV may induce GBS by different mechanisms than other causes (98). Cases of a diffuse demyelinating disorder consistent with GBS that are temporally associated ZIKV infection also have been reported in Brazil, El Salvador, Colombia, and Venezuela (75, 92). More studies are needed to understand the linkage between ZIKV infection and GBS, particularly the pathophysiological mechanisms at play. Possible mechanisms include (i) immunopathology due to viral antigen mimicry with a host protein; (ii) virus sequence changes resulting in enhanced tropism for the peripheral nervous system; and (iii) an association with prior or concurrent immune responses to DENV (97-100).

Most concerning is the sharp increase in cases of microcephaly in newborns in the Northeastern region of Brazil that is associated with ZIKV infection of pregnant women (101). Several cases of presumed intrauterine ZIKV infection resulted in coarse cerebral calcifications in different brain regions of newborn infants or fetuses *in utero* (76). A recent study of a fetus with microcephaly recovered after elective termination at 32 weeks of gestation also revealed numerous calcifications in the cortical and subcortical regions of the frontal, parietal, and occipital lobes of the cerebral cortex (77). Hydrops fetalis and hydranencephaly were noted in a fetus with microcephaly, which was followed by fetal demise (81). The reported microcephaly cases may represent only the severe end of the spectrum, such that newborns with less severe infection could still have long-term cognitive or functional sequelae (76). Indeed, ocular findings

in infants with presumed ZIKV-associated microcephaly were described recently. Approximately 30% of children with suspected ZIKV infection *in utero* had evidence of significant retinal and optic nerve abnormalities (102).

Pathogenesis of ZIKV infection.

Although no recent ZIKV pathogenesis studies have been performed to explain the possible microcephaly observed in Brazil, experiments in mice that were performed 40 and 60 years ago suggest that under certain circumstances ZIKV has a tropism for cells in the brain. The original ZIKV strain (MR 766) was isolated by George Dick and colleagues in 1947 from the brain of a 5 to 6 week-old Swiss mouse after it was inoculated via an intracerebral route with the serum of a febrile sentinel rhesus macaque (9). The same group showed subsequently that passaged ZIKV strains caused signs of central nervous system (CNS) disease including motor weakness and paralysis after intracerebral inoculation in mice of different ages (8). Mice under seven days of age were susceptible to lethal ZIKV infection when inoculated by an intraperitoneal route whereas adult mice were less sensitive (103). In mice, the pathological manifestations of disease were restricted to CNS tissues. Neuronal degeneration and cellular infiltration were observed in regions of the spinal cord and brain with evidence of Cowdry type A inclusion bodies (8), which also are described after neuronal infection by herpesviruses. Evidence of neuronal injury also was observed in the pathological evaluation of a human fetus infected *in utero* with ZIKV. In this case, diffuse astrogliosis and activation of microglia were present, and damage extended to the brain stem and spinal cord with Wallerian degeneration of the descending corticospinal tracts noted (77). Beyond the CNS, no other tissue supported significant ZIKV infection including the kidney, lung, spleen, and liver. In comparison, other animals, including cotton rats, guinea pigs, rabbits, and rhesus monkeys did not develop CNS disease, even after intracerebral inoculation (8). More recent studies using a ZIKV isolate from French Polynesia demonstrated infection of human keratinocytes, dermal fibroblasts, and skin

biopsies, consistent with the skin being the initial site of ZIKV replication after mosquito inoculation, similar to WNV and DENV (104-107). Similar to DENV, ZIKV can use DC-SIGN and the TAM receptors Axl and Tyro3 as attachment factors (104). Also similar to other flaviviruses, ZIKV infected human dendritic cells in culture and was restricted by the antiviral effects of type I and type II interferon (104).

Some ZIKV strains have one N-linked glycosylation site in their envelope (E) protein (N154), whereas others lack predicted glycosylation sites (108). This pattern contrasts with DENV, which has two N-linked glycosylation sites (N67 and N154), and is similar to the E proteins of more distantly related flaviviruses including WNV and TBEV (N154) (109-111). Although N-linked glycosylation on E is associated with enhanced mosquito transmission and/or increased virulence in mammals for some flaviviruses including WNV, TBEV, and others (112-118), it remains unknown whether differential glycosylation between ZIKV strains determines or even correlates with pathogenicity.

Diagnosis of ZIKV infection

Because ZIKV causes a non-specific influenza-like illness without pathognomonic features, it is challenging clinically to distinguish it from other viral illnesses. This is especially true because ZIKV co-circulates and shares mosquito vectors with DENV and CHIKV which present similarly with fever, rash, arthralgia, and myalgia (25, 119). In addition to co-circulation, recent reports have described co-infection of multiple arboviruses including ZIKV and DENV (24).

Given the challenges in clinical diagnosis, a laboratory-based diagnosis of ZIKV is the gold standard (120). Beyond direct virus isolation, which can be difficult outside of highly specialized laboratories, the most definitive current diagnostic tool is a RT-PCR-based assay that detects ZIKV RNA and can distinguish it from DENV, CHIKV, and other viral infections (120). Because ZIKV viremia in humans lasts for a short duration of 3 to 5 days (20, 121),

serum RT-PCR assays, while highly specific, have low sensitivity rates. Urine and saliva samples may have greater utility for diagnosing ZIKV infection by RT-PCR, as viral RNA is detectable at a higher load and with a longer duration in these body fluids than in serum (57, 58). In one study in French Polynesia, 19.2% of tests were positive for ZIKV RNA in saliva while negative in blood. The use of saliva sample increased the rate of molecular detection of ZIKV and was of particular interest in groups (e.g., children and newborns) where blood was difficult to collect (58). Viral detection in urine and saliva is not unique to ZIKV, as DENV RNA has been detected in both fluids, whereas infectious WNV and WNV RNA have been detected in urine (122-124).

Serology-based diagnosis of ZIKV infection, which is critical to surveillance, epidemiologic analyses, and acute diagnoses, poses a challenge even to experienced laboratory personnel due to the extensive cross-reactivity of antibodies against related flaviviruses that are derived from natural infection or vaccination (e.g., YFV, DENV, or JEV) (19, 20, 120). As an example, ZIKV-infected patients can be positive in an IgM assay for DENV, particularly if ZIKV occurs as a secondary flavivirus infection. Cross-reactivity was observed more frequently with DENV than with YFV, JEV, or WNV, although further studies are needed as small numbers of samples were tested. In comparison, if ZIKV is the first flavivirus encountered, the extent of cross-reactivity is less (20). Anti-ZIKV IgM was detectable as early as 3 days after onset of illness with most having it present by day 8. Neutralizing antibody developed as early as 5 days after illness onset but again but may still yield substantial cross-reactivity in the setting of prior flavivirus infection or vaccination. The use of paired acute and convalescent sera and a greater than 4-fold rise in ZIKV antibody titers specifically may increase the accuracy of serological testing.

Thus, if ZIKV epidemics occur in populations with DENV or other flavivirus vaccine or natural immunity, extensive cross-reactivity in the IgM and neutralization assays can occur, which could lead to an incorrect diagnosis. This is particularly problematic as ZIKV epidemics

spread through Latin America and the Caribbean, where DENV prevalence is high. Ideally, a serological assay that minimizes cross-reactivity of other flaviviruses is needed to increase the specificity of IgM and IgG assays. Based on published studies with related flaviviruses (125-127), the development of diagnostic assays with ZIKV NS1 proteins or ZIKV E proteins and subviral particles encoding mutations in the highly cross-reactive fusion loop in domain II might enhance the specificity of serological tests substantially.

Unanswered questions

In utero transmission and teratogenic effects. While the introduction of a pathogen into a new environment often brings epidemiological and diagnostic challenges, at the outset of the ZIKV outbreak in Brazil, there was no reason to expect a unique presentation; indeed, Zika fever is typically milder than dengue fever. The association between ZIKV and microcephaly was unexpected, as this presentation has not been associated with flaviviruses, and congenital abnormalities are not characteristic of flavivirus infection. Accumulating evidence indicates a role for maternal ZIKV infection as an explanation for the increase in microcephaly cases in Brazil, although further assessment of reported and historical cases is necessary to determine the magnitude of the increase and the attack rate (68, 70, 71). Many questions remain regarding the mechanisms by which ZIKV might cause congenital defects, including microcephaly. The simplest mechanism would be an inherent ability of ZIKV to cross the placenta, followed by direct infection of nervous tissue in the developing fetus. This mechanism is supported by the detection of ZIKV RNA, complete genomes, antigen, and viral particles in fetal tissues, placenta, and amniotic fluid from pregnancies with microcephaly (74, 76-78, 80, 81, 92), and prior studies in mice suggesting a tropism for central nervous system tissues (8). If ZIKV is neurotropic and neurovirulent in the developing fetus, it seems unlikely to manifest only as microcephaly. While microcephaly may be the most apparent congenital abnormality from ZIKV infection, it remains possible that the virus can cause a spectrum of neurological effects, some of which may not be

evident for months or years. The association between ZIKV and microcephaly also could be because of its introduction into a ZIKV-naïve population, or alternatively into a population with unique patterns of flavivirus immunity, with prior immunity to DENV or other flaviviruses modulating ZIKV pathogenesis.

As the placenta generally is an effective barrier in preventing microorganisms in the maternal circulation from accessing the developing fetus, it will be important to determine what mechanisms ZIKV uses to circumvent this barrier. For example, can ZIKV infect placental trophoblast cells directly, or does it employ some other method to access the fetal compartment? For other congenital infections, the risk of fetal infection varies at different stages of pregnancy (82, 83), and the most extensively described cases of ZIKV-associated microcephaly have all involved infection during the first trimester (76-78, 80). It will be important to determine the temporal risk of congenital ZIKV infection, in order to make informed recommendations to pregnant women about the risks of exposure to ZIKV (74, 91).

A growing body of evidence indicates that ZIKV can cross the placenta, infect the fetus, and damage the developing brain (74, 76-80, 92). However, demonstrating a direct causal role for congenital ZIKV infection in the development of microcephaly will require more extensive clinical and epidemiological studies, many of which are now in progress. The existing data do not demonstrate that ZIKV is sufficient to cause microcephaly, and other factors may potentiate the teratogenic effects of ZIKV, including co-infections, environmental factors, viral strain differences, or host genetics. It is noteworthy that to date, ZIKV-associated microcephaly has been observed only in Brazil, and not in previous outbreaks or in other countries. This may reflect the large number of ZIKV infections in Brazil (>1.5 million estimated) and the timing of the outbreak, with Brazil experiencing the earliest effects. However, if microcephaly remains exclusive to women in Brazil or who were infected with the virus while travelling there, it will be important to consider co-factors that may impact *in utero* infection by ZIKV.

Interactions between ZIKV and DENV. One of the characteristic features of DENV pathogenesis is that while infection with one serotype provides durable immunity to that same serotype, antibodies to one DENV serotype can exacerbate infection with different serotypes via antibody-dependent enhancement (ADE) (128-130). ADE occurs when cross-reactive non-neutralizing antibodies bind to a heterologous DENV serotype. Antibody-opsonized but non-neutralized virus can infect myeloid cells (e.g., monocytes or macrophages) expressing Fc-gamma receptors at a higher rate, allowing for enhanced infection and yield. Because of this, secondary DENV infections (or primary infections in infants with circulating maternal antibodies) can produce severe disease manifestations, including plasma leakage, hemorrhage, and circulatory collapse. ADE can be demonstrated for many flaviviruses in cell culture, but the phenomenon appears to be biologically relevant only in the context of DENV, possibly due to the degree of antigenic relatedness between different DENV serotypes or because of the unique biology of the DENV NS1 protein (131, 132). Given the relatedness between DENV and ZIKV, and the high cross-reactivity demonstrated in serological assays, ADE between DENV and ZIKV and altered disease pathogenesis warrants further evaluation. Recent outbreaks of ZIKV have been associated with more severe disease than historical ones. While explanations for this include changes in the virus, and an enhanced ability to detect rare presentations in larger outbreaks, one feature that distinguishes the most recent ZIKV outbreaks is that they occurred in regions of DENV hyperendemicity, where multiple strains of DENV co-circulate and most people have been infected previously by one or more DENV serotypes. This raises the possibility that ZIKV infection in DENV immune individuals could result in more severe disease presentations. While the natural history of recent DENV outbreaks has been of ZIKV introduction into regions with high DENV prevalence, as ZIKV becomes endemic in the Western Hemisphere it also will be important to monitor reciprocally how ZIKV immunity impacts DENV pathogenesis. If prior DENV immunity impacts ZIKV pathogenesis, we might expect an even

Journal of Virology

410 greater burden of ZIKV disease if outbreaks emerge in areas of Southeast Asia where the
411 burden of DENV infection is even greater than in Latin America (90).

412 *Vaccine development.* Successful vaccination programs have reduced the global health
413 burden of many flavivirus infections. More than 500 million doses of vaccine to prevent YFV
414 infection have been administered since its development in 1937, and effective vaccines have
415 blunted the impact of JEV and TBEV. Recently, after decades of study, the first live-attenuated
416 tetravalent DENV vaccine (Dengvaxia®) completed phase III human trials and is being deployed
417 in Brazil, the Philippines, and Mexico.

418 As no ZIKV vaccines have been tested even at the pre-clinical stage, we are likely years
419 away from introduction of a ZIKV vaccine. It is expected that at least some groups with existing
420 flavivirus vaccine platforms (e.g., chimeric live attenuated strains, passaged or genetically
421 engineered live attenuated strains, E protein subunit, subviral particles, inactivated virions, or
422 DNA plasmid) will apply these strategies towards ZIKV vaccine development in an expedited
423 manner. A major question remains as to whether it will be easy or difficult to generate an
424 immunogenic and safe vaccine against ZIKV. The issues related to this question include the
425 following: ***(a) Strain diversity*.** Given the relatively low variation between ZIKV strains (2, 26,
426 29, 108) (approximately 94% amino acid identity across the viral genome), and lack of existence
427 of different genotypes or serotypes, it is plausible that an effective vaccine against one strain will
428 function broadly against all circulating ZIKV strains; ***(b) Effect of pre-existing flavivirus***
429 ***immunity on ZIKV vaccine responses.*** ZIKV outbreaks are occurring in areas with high
430 seroprevalence rates for DENV infection and vaccination with YFV. Thus, at least some fraction
431 of candidates for ZIKV vaccines will have pre-existing cross-reactive antibodies derived from
432 natural or vaccine-induced flavivirus immunity. This could impact ZIKV responses in one of
433 three ways: (i) boost cross-reactivity immunity, conferring protection against ZIKV; (ii) boost
434 cross-reactive immunity at the expense of generating protective type-specific ZIKV responses

("original antigenic sin"); (iii) neutralize live-attenuated ZIKV without appreciably affecting cross-reactive immunity (sterilizing immunity).

Development of therapeutics. Given that vaccines against ZIKV may be years away, the development of immediate measures to control or limit ZIKV disease should be a priority. To date, no drug screening studies have been published with ZIKV. Because DENV infections are so frequent worldwide, effort over the past decade has been made in evaluating inhibitors of specific steps in the DENV lifecycle. Such drugs, were they to advance through clinical trial, might have inhibitory activity against other flaviviruses, including ZIKV. Indeed, antiviral drug discovery screens have been performed to identify inhibitors of the fusogenic activity of E protein; the protease and helicase activity of NS3; and the RNA-dependent RNA polymerase and methyltransferase activities of NS5, with further pre-clinical development ongoing (133). Additional strategies being considered are repurposing drug screens including the testing of FDA-approved or well-studied "orphan" drugs against ZIKV infection. Because drugs against flavivirus proteins could select rapidly for resistant variants, the concept of targeting host molecules required for DENV infectivity (134) or viral proteins that require oligomerization (135) has emerged as a possible strategy. Drugs that target steps in flavivirus infection or cell-intrinsic immunity also could be considered. Finally, passive transfer or antibody-based therapeutics against ZIKV as prophylaxis or treatment may be possible, once strongly neutralizing human monoclonal antibodies are isolated, analogous to studies with other flaviviruses (136, 137). Regardless of the approach, one obstacle to developing ZIKV therapeutics is that a key target population would be pregnant women; the design and implementation of trials to test new drugs in pregnant women will be challenging.

Animal models of ZIKV pathogenesis. Development of vaccines and therapeutics would be expedited by the development of animal models of the different manifestations of ZIKV disease. There are few available data in non-human primates apart from the original isolation of ZIKV from the serum of a febrile rhesus monkey (9) and a recently initiated study to assess

ZIKV infection dynamics in three rhesus macaques (https://dholk.primate.wisc.edu/project/dho/public/Zika/public/ZIKV-001-public/begin.view?). There also is little available data in mice, as only three papers have reported on ZIKV infection in mice and nothing has been published in almost 40 years (8, 103, 138). Although these studies suggest that ZIKV can replicate and cause injury in cells of the central nervous system, whether this pathogenesis is related or not to the current linkages to GBS or microcephaly remains uncertain and requires further study. A systematic analysis of ZIKV infection and disease through multiple routes (e.g., intradermal, subcutaneous, or intravenous) in different strains of mice at different ages is needed. Such studies might include panels of genetically diverse mice, such as Collaborative Cross mice (139), to identify genetic susceptibility loci that could be related to human disease or to develop infection models for therapeutic and vaccine testing (140, 141). In addition to direct infection of newborn, juvenile, adult, and old mice, studies in which pregnant dams are inoculated with ZIKV and the effects on fecundity, neonatal infection, and brain development are evaluated could address the presumed linkage to microcephaly in humans.

Public Health Considerations. The association between ZIKV infection and neurological complications such as microcephaly and GBS prompted the World Health Organization on February 1, 2016 to declare a Public Health Emergency of International Concern surrounding the current ZIKV epidemic in Latin America and the Caribbean (142). The sudden surge of public health, clinical, and basic science interest in ZIKV will increase our understanding of this virus that had remained an obscure viral curiosity until quite recently.

Analogous to the introduction of WNV into the United States in 1999 and the arrival of CHIKV in the Caribbean in 2013, the emergence of ZIKV in Brazil represents another example of an arbovirus introduction to the Western Hemisphere with significant impacts on human health and ecology (143). The appearance of new, more severe clinical presentations in recent ZIKV outbreaks also highlights that familiar infections can produce new phenotypes when

introduced to new ecological and host systems. The abundance of *Aedes aegypti* mosquitoes in Latin America and the Caribbean suggests that ZIKV may become endemic in the region. Autochthonous transmission also is a possibility in the southern United States, where *Aedes aegypti* mosquitos are common, and perhaps farther north where *Aedes albopictus* may serve as a vector. However, the presence of cultural and economic factors such as air conditioning, window screens, indoor lifestyles, and vector control measures, as well as a temperate climate, may prevent widespread ZIKV outbreaks in the United States, much as DENV and CHIKV have not caused epidemics here. Nonetheless, imported cases from travelers are likely to increase in the United States, Europe, and elsewhere (30, 33-35). Indeed, ZIKV infection is now a nationally reportable disease in the United States.

The lack of specific antiviral measures to combat ZIKV emphasizes the importance of vector control strategies for combatting arbovirus disease. Such approaches (removing sources of standing water that serve as breeding sites, larvicide and insecticide application, behavioral modifications to avoid mosquito exposure, and possibly the controlled introduction of genetically modified or sterile mosquitoes into an epidemic site) also will protect against DENV, CHIKV, and other mosquito-transmitted diseases (144). The unexpected linkage between ZIKV and microcephaly, and the lack of specific measures to prevent or treat ZIKV in pregnant women, as well as a lack of information to assess the risks posed by ZIKV infection during pregnancy has prompted public health authorities in some countries to issue highly unusual recommendations regarding pregnancy including postponement. In the US, the CDC has recommended enhanced prenatal surveillance of pregnant women who have travelled to areas with ZIKV circulation (74, 80, 91). Such recommendations are framed as “abundance of caution” but must be considered in light of the reality of implementation. Access to contraceptives, prenatal care, and safe abortion services should be components of any public health response to ZIKV.

Conclusions

ZIKV emergence in the Western Hemisphere has followed what has become a familiar script, in which a previously obscure vector-borne disease is introduced into a new ecological system and host population and then spreads rapidly with significant implications for human health. In the case of ZIKV, this most recent outbreak has been associated with unexpected clinical presentations, and it has been difficult to evaluate the risks and severity of ZIKV infection due to an absence of specific diagnostic reagents and a basic understanding of the molecular virology and pathogenic mechanisms of this virus.

ACKNOWLEDGEMENTS

This work was supported by start-up funds from the University of North Carolina Department of Microbiology and Immunology and the Lineberger Comprehensive Cancer Center (H.M.L), as well as grants from the Burroughs Wellcome Fund (M.S.D) and NIH (R01 AI073755) (M.S.D).

526 **Figure Legends**

527 **Figure 1.** Schematic phylogeny illustrating the genetic relationships between selected
528 flaviviruses that are human pathogens. Dendrogram adapted from (145), based on the amino
529 acid sequence of the complete polyprotein.

530 **Figure 2. ZIKV pathogenesis.** The typical course of ZIKV infection is illustrated (green
531 background), with potential severe effects requiring further investigation indicated (blue
532 background). DENV, dengue virus; ADE, antibody-dependent enhancement.

533

REFERENCES

1. **Pierson TC, Diamond MS.** 2013. Flaviviruses, p 747-794. *In* Knipe DM, Howley PM (ed), Fields Virology, 6th ed, vol 2. Wolter Kluwer.
2. **Gatherer D, Kohl A.** 2016. Zika virus: a previously slow pandemic spreads rapidly through the Americas. J Gen Virol **97:**269-273.
3. **Chambers TJ, Hahn CS, Galler R, Rice CM.** 1990. Flavivirus genome organization, expression, and replication. Annu Rev Microbiol **44:**649-688.
4. **Kuno G, Chang GJ.** 2007. Full-length sequencing and genomic characterization of Bagaza, Kedougou, and Zika viruses. Arch Virol **152:**687-696.
5. **Mukhopadhyay S, Kuhn RJ, Rossmann MG.** 2005. A structural perspective of the flavivirus life cycle. Nat Rev Microbiol **3:**13-22.
6. **Diamond MS, Pierson TC.** 2015. Molecular Insight into Dengue Virus Pathogenesis and Its Implications for Disease Control. Cell **162:**488-492.
7. **Suthar MS, Diamond MS, Gale M, Jr.** 2013. West Nile virus infection and immunity. Nat Rev Microbiol **11:**115-128.
8. **Dick GW.** 1952. Zika virus. II. Pathogenicity and physical properties. Trans R Soc Trop Med Hyg **46:**521-534.
9. **Dick GW, Kitchen SF, Haddow AJ.** 1952. Zika virus. I. Isolations and serological specificity. Trans R Soc Trop Med Hyg **46:**509-520.
10. **Haddow AJ, Williams MC, Woodall JP, Simpson DI, Goma LK.** 1964. Twelve Isolations of Zika Virus from Aedes (Stegomyia) Africanus (Theobald) Taken in and above a Uganda Forest. Bull World Health Organ **31:**57-69.
11. **McCrae AW, Kirya BG.** 1982. Yellow fever and Zika virus epizootics and enzootics in Uganda. Trans R Soc Trop Med Hyg **76:**552-562.
12. **Diagne CT, Diallo D, Faye O, Ba Y, Faye O, Gaye A, Dia I, Faye O, Weaver SC, Sall AA, Diallo M.** 2015. Potential of selected Senegalese Aedes spp. mosquitoes (Diptera: Culicidae) to transmit Zika virus. BMC Infect Dis **15:**492.
13. **Diallo D, Sall AA, Diagne CT, Faye O, Faye O, Ba Y, Hanley KA, Buenemann M, Weaver SC, Diallo M.** 2014. Zika virus emergence in mosquitoes in southeastern Senegal, 2011. PLoS One **9:**e109442.
14. **Fagbami AH.** 1979. Zika virus infections in Nigeria: virological and seroepidemiological investigations in Oyo State. J Hyg (Lond) **83:**213-219.
15. **Haddow AD, Schuh AJ, Yasuda CY, Kasper MR, Heang V, Huy R, Guzman H, Tesh RB, Weaver SC.** 2012. Genetic characterization of Zika virus strains: geographic expansion of the Asian lineage. PLoS Negl Trop Dis **6:**e1477.
16. **Wolfe ND, Kilbourn AM, Karesh WB, Rahman HA, Bosi EJ, Cropp BC, Andau M, Spielman A, Gubler DJ.** 2001. Sylvatic transmission of arboviruses among Bornean orangutans. Am J Trop Med Hyg **64:**310-316.
17. **Smithburn KC, Kerr JA, Gatne PB.** 1954. Neutralizing antibodies against certain viruses in the sera of residents of India. J Immunol **72:**248-257.
18. **Hayes EB.** 2009. Zika virus outside Africa. Emerg Infect Dis **15:**1347-1350.
19. **Duffy MR, Chen TH, Hancock WT, Powers AM, Kool JL, Lanciotti RS, Pretrick M, Marfel M, Holzbauer S, Dubray C, Guillaumot L, Griggs A, Bel M, Lambert AJ, Laven J, Kosoy O, Panella A, Biggerstaff BJ, Fischer M, Hayes EB.** 2009. Zika virus outbreak on Yap Island, Federated States of Micronesia. N Engl J Med **360:**2536-2543.
20. **Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lambert AJ, Johnson AJ, Stanfield SM, Duffy MR.** 2008. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. Emerg Infect Dis **14:**1232-1239.
21. **Li MI, Wong PS, Ng LC, Tan CH.** 2012. Oral susceptibility of Singapore Aedes (Stegomyia) aegypti (Linnaeus) to Zika virus. PLoS Negl Trop Dis **6:**e1792.

22. **Cao-Lormeau VM, Roche C, Teissier A, Robin E, Berry AL, Mallet HP, Sall AA, Musso D.** 2014. Zika virus, French polynesia, South pacific, 2013. Emerg Infect Dis **20:**1085-1086.
23. **Musso D, Cao-Lormeau VM, Gubler DJ.** 2015. Zika virus: following the path of dengue and chikungunya? Lancet **386:**243-244.
24. **Dupont-Rouzeyrol M, O'Connor O, Calvez E, Daures M, John M, Grangeon JP, Gourinat AC.** 2015. Co-infection with Zika and dengue viruses in 2 patients, New Caledonia, 2014. Emerg Infect Dis **21:**381-382.
25. **Roth A, Mercier A, Lepers C, Hoy D, Duituturaga S, Benyon E, Guillaumot L, Souares Y.** 2014. Concurrent outbreaks of dengue, chikungunya and Zika virus infections - an unprecedented epidemic wave of mosquito-borne viruses in the Pacific 2012-2014. Euro Surveill **19**.
26. **Zanluca C, de Melo VC, Mosimann AL, Dos Santos GI, Dos Santos CN, Luz K.** 2015. First report of autochthonous transmission of Zika virus in Brazil. Mem Inst Oswaldo Cruz **110:**569-572.
27. **Campos GS, Bandeira AC, Sardi SI.** 2015. Zika Virus Outbreak, Bahia, Brazil. Emerg Infect Dis **21:**1885-1886.
28. **Musso D.** 2015. Zika Virus Transmission from French Polynesia to Brazil. Emerg Infect Dis **21:**1887.
29. **Lanciotti RS, Lambert AJ, Holodniy M, Saavedra S, del Carmen Castillo Signor L.** 2016. Phylogeny of Zika Virus in Western Hemisphere, 2015. Emerg Infect Dis.
30. **Hennessey M, Fischer M, Staples JE.** 2016. Zika Virus Spreads to New Areas - Region of the Americas, May 2015-January 2016. MMWR Morb Mortal Wkly Rep **65:**55-58.
31. **Thomas DL, Sharp TM, Torres J, Armstrong PA, Munoz-Jordan J, Ryff KR, Martinez-Quinones A, Arias-Berrios J, Mayshack M, Garayalde GJ, Saavedra S, Luciano CA, Valencia-Prado M, Waterman S, Rivera-Garcia B.** 2016. Local Transmission of Zika Virus - Puerto Rico, November 23, 2015-January 28, 2016. MMWR Morb Mortal Wkly Rep **65:**154-158.
32. **Camacho E, Gomez MP, Blanco PJ, Osorio JE, Aliota MT.** 2016. Detection of Autochthonous Zika Virus Transmission in Sincelejo, Colombia. Emerg Infect Dis.
33. **Zammarchi L, Tappe D, Fortuna C, Remoli ME, Gunther S, Venturi G, Bartoloni A, Schmidt-Chanasit J.** 2015. Zika virus infection in a traveller returning to Europe from Brazil, March 2015. Euro Surveill **20**.
34. **Hills SL, Russell K, Hennessey M, Williams C, Oster AM, Fischer M, Mead P.** 2016. Transmission of Zika Virus Through Sexual Contact with Travelers to Areas of Ongoing Transmission — Continental United States, 2016. MMWR Morb Mortal Wkly Rep **65**.
35. **Maria AT, Maquart M, Makinson A, Flusin O, Segondy M, Leparc-Goffart I, Le Moing V, Foulongne V.** 2016. Zika virus infections in three travellers returning from South America and the Caribbean respectively, to Montpellier, France, December 2015 to January 2016. Euro Surveill **21**.
36. **Morrison TE.** 2014. Reemergence of chikungunya virus. J Virol **88:**11644-11647.
37. **Baronti C, Piorkowski G, Charrel RN, Boubis L, Leparc-Goffart I, de Lamballerie X.** 2014. Complete coding sequence of zika virus from a French polynesia outbreak in 2013. Genome Announc **2**.
38. **Tsetsarkin KA, Chen R, Sherman MB, Weaver SC.** 2011. Chikungunya virus: evolution and genetic determinants of emergence. Curr Opin Virol **1:**310-317.
39. **Tsetsarkin KA, Vanlandingham DL, McGee CE, Higgs S.** 2007. A single mutation in chikungunya virus affects vector specificity and epidemic potential. PLoS Pathog **3:**e201.
40. **Ledermann JP, Guillaumot L, Yug L, Saweyog SC, Tided M, Machieng P, Pretrick M, Marfel M, Griggs A, Bel M, Duffy MR, Hancock WT, Ho-Chen T, Powers AM.**

2014. Aedes hensilli as a potential vector of Chikungunya and Zika viruses. PLoS Negl Trop Dis **8:**e3188.

41. **Grard G, Caron M, Mombo IM, Nkoghe D, Mboui Ondo S, Jiolle D, Fontenille D, Paupy C, Leroy EM.** 2014. Zika virus in Gabon (Central Africa)--2007: a new threat from Aedes albopictus? PLoS Negl Trop Dis **8:**e2681.
42. **Marchette NJ, Garcia R, Rudnick A.** 1969. Isolation of Zika virus from Aedes aegypti mosquitoes in Malaysia. Am J Trop Med Hyg **18:**411-415.
43. **Powell JR, Tabachnick WJ.** 2013. History of domestication and spread of Aedes aegypti--a review. Mem Inst Oswaldo Cruz **108 Suppl 1:**11-17.
44. **Hanley KA, Monath TP, Weaver SC, Rossi SL, Richman RL, Vasilakis N.** 2013. Fever versus fever: the role of host and vector susceptibility and interspecific competition in shaping the current and future distributions of the sylvatic cycles of dengue virus and yellow fever virus. Infect Genet Evol **19:**292-311.
45. **Musso D, Nhan T, Robin E, Roche C, Bierlaire D, Zisou K, Shan Yan A, Cao-Lormeau VM, Broult J.** 2014. Potential for Zika virus transmission through blood transfusion demonstrated during an outbreak in French Polynesia, November 2013 to February 2014. Euro Surveill **19**.
46. **Kleinman S.** 2015. Pathogen inactivation: emerging indications. Curr Opin Hematol **22:**547-553.
47. **Centers for Disease Control and Prevention.** 2013. Fatal West Nile virus infection after probable transfusion-associated transmission--Colorado, 2012. MMWR Morb Mortal Wkly Rep **62:**622-624.
48. **O'Brien SF, Scalia V, Zuber E, Hawes G, Alport EC, Goldman M, Fearon MA.** 2010. West Nile virus in 2006 and 2007: the Canadian Blood Services' experience. Transfusion **50:**1118-1125.
49. **European Union Directorate-General for Health and Food Safety.** 2012. WEST NILE VIRUS AND BLOOD SAFETY INTRODUCTION TO A PREPAREDNESS PLAN IN EUROPE
50. **Custer B, Kamel H, Kiely NE, Murphy EL, Busch MP.** 2009. Associations between West Nile virus infection and symptoms reported by blood donors identified through nucleic acid test screening. Transfusion **49:**278-288.
51. **Aubry M, Richard V, Green J, Broult J, Musso D.** 2016. Inactivation of Zika virus in plasma with amotosalen and ultraviolet A illumination. Transfusion **56:**33-40.
52. **Foy BD, Kobylinski KC, Chilson Foy JL, Blitvich BJ, Travassos da Rosa A, Haddow AD, Lanciotti RS, Tesh RB.** 2011. Probable non-vector-borne transmission of Zika virus, Colorado, USA. Emerg Infect Dis **17:**880-882.
53. **Dallas County Health and Human Services.** 2016. DCHHS Provides Update on Zika Virus Cases. http://www.dallascounty.org/department/hhs/press/documents/PR2-4-16DCHHSProvidesUpdateonZikaVirusCases.pdf.
54. **Musso D, Roche C, Robin E, Nhan T, Teissier A, Cao-Lormeau VM.** 2015. Potential sexual transmission of Zika virus. Emerg Infect Dis **21:**359-361.
55. **Atkinson B, Hearn P, Afrough B, Lumley S, Carter D, Aarons EJ, Simpson AJ, Brooks TJ, Hewson R.** 2016. Detection of Zika Virus in Semen. Emerg Infect Dis **22**.
56. **Stefanovic KB, Gregg PC, Soung M.** 2009. Evaluation and treatment of hematospermia. Am Fam Physician **80:**1421-1427.
57. **Gourinat AC, O'Connor O, Calvez E, Goarant C, Dupont-Rouzeyrol M.** 2015. Detection of Zika virus in urine. Emerg Infect Dis **21:**84-86.
58. **Musso D, Roche C, Nhan TX, Robin E, Teissier A, Cao-Lormeau VM.** 2015. Detection of Zika virus in saliva. J Clin Virol **68:**53-55.

59. **Martin JN.** 2007. The epidemiology of KSHV and its association with malignant disease. *In* Arvin A, Campadelli-Fiume G, Mocarski E (ed), Human Herpesviruses: Biology, Therapy, and Immunoprophylaxis. Cambridge University Press, Cambridge.
60. **Ricklin ME, Garcia-Nicolas O, Brechbuhl D, Python S, Zumkehr B, Nougairede A, Charrel RN, Posthaus H, Oevermann A, Summerfield A.** 2016. Vector-free transmission and persistence of Japanese encephalitis virus in pigs. Nat Commun **7:**10832.
61. **Mate SE, Kugelman JR, Nyenswah TG, Ladner JT, Wiley MR, Cordier-Lassalle T, Christie A, Schroth GP, Gross SM, Davies-Wayne GJ, Shinde SA, Murugan R, Sieh SB, Badio M, Fakoli L, Taweh F, de Wit E, van Doremalen N, Munster VJ, Pettitt J, Prieto K, Humrighouse BW, Stroher U, DiClaro JW, Hensley LE, Schoepp RJ, Safronetz D, Fair J, Kuhn JH, Blackley DJ, Laney AS, Williams DE, Lo T, Gasasira A, Nichol ST, Formenty P, Kateh FN, De Cock KM, Bolay F, Sanchez-Lockhart M, Palacios G.** 2015. Molecular Evidence of Sexual Transmission of Ebola Virus. N Engl J Med **373:**2448-2454.
62. **Deen GF, Knust B, Broutet N, Sesay FR, Formenty P, Ross C, Thorson AE, Massaquoi TA, Marrinan JE, Ervin E, Jambai A, McDonald SL, Bernstein K, Wurie AH, Dumbuya MS, Abad N, Idriss B, Wi T, Bennett SD, Davies T, Ebrahim FK, Meites E, Naidoo D, Smith S, Banerjee A, Erickson BR, Brault A, Durski KN, Winter J, Sealy T, Nichol ST, Lamunu M, Stroher U, Morgan O, Sahr F.** 2015. Ebola RNA Persistence in Semen of Ebola Virus Disease Survivors - Preliminary Report. N Engl J Med doi:10.1056/NEJMoa1511410.
63. **Besnard M, Lastere S, Teissier A, Cao-Lormeau V, Musso D.** 2014. Evidence of perinatal transmission of Zika virus, French Polynesia, December 2013 and February 2014. Euro Surveill **19**.
64. **Barthel A, Gourinat AC, Cazorla C, Joubert C, Dupont-Rouzeyrol M, Descloux E.** 2013. Breast milk as a possible route of vertical transmission of dengue virus? Clin Infect Dis **57:**415-417.
65. **Centers for Disease Control and Prevention.** 2002. Possible West Nile virus transmission to an infant through breast-feeding--Michigan, 2002. MMWR Morb Mortal Wkly Rep **51:**877-878.
66. **Kuhn S, Twele-Montecinos L, MacDonald J, Webster P, Law B.** 2011. Case report: probable transmission of vaccine strain of yellow fever virus to an infant via breast milk. CMAJ **183:**E243-245.
67. **Fleming-Dutra KE, Nelson JM, Fischer M, Staples JE, Karwowski MP, Mead P, Villanueva J, Renquist CM, Minta AA, Jamieson DJ, Honein MA, Moore CA, Rasmussen SA.** 2016. Update: Interim Guidelines for Health Care Providers Caring for Infants and Children with Possible Zika Virus Infection — United States, February 2016. MMWR Morb Mortal Wkly Rep **65**.
68. **Butler D.** 2016. Zika virus: Brazil's surge in small-headed babies questioned by report. Nature **530:**13-14.
69. **Victora CG, Schuler-Faccini L, Matijasevich A, Ribeiro E, Pessoa A, Celso Barros F.** 2016. Microcephaly in Brazil: how to interpret reported numbers? Lancet.
70. **Soares de Araújo JS, Regis CT, Gomes RGS, Tavares TR, Rocha dos Santos C, Assunção PM, Nóbrega RV, de Fátima Alves Pinto D, Vinícius B, Bezerrab D, da Silva Mattos S.** 2016. Microcephaly in northeast Brazil: a review of 16 208 births between 2012 and 2015. Bull World Health Organ.
71. **Schuler-Faccini L, Ribeiro EM, Feitosa IM, Horovitz DD, Cavalcanti DP, Pessoa A, Doriqui MJ, Neri JI, Neto JM, Wanderley HY, Cernach M, El-Husny AS, Pone MV, Serao CL, Sanseverino MT, Brazilian Medical Genetics Society-Zika Embryopathy**

Task F. 2016. Possible Association Between Zika Virus Infection and Microcephaly - Brazil, 2015. MMWR Morb Mortal Wkly Rep **65:**59-62.

72. **Faheem M, Naseer MI, Rasool M, Chaudhary AG, Kumosani TA, Ilyas AM, Pushparaj P, Ahmed F, Algahtani HA, Al-Qahtani MH, Saleh Jamal H.** 2015. Molecular genetics of human primary microcephaly: an overview. BMC Med Genomics **8 Suppl 1:**S4.
73. **Harris SR.** 2015. Measuring head circumference: Update on infant microcephaly. Can Fam Physician **61:**680-684.
74. **Petersen EE, Staples JE, Meaney-Delman D, Fischer M, Ellington SR, Callaghan WM, Jamieson DJ.** 2016. Interim Guidelines for Pregnant Women During a Zika Virus Outbreak - United States, 2016. MMWR Morb Mortal Wkly Rep **65:**30-33.
75. **European Centre for Disease Prevention and Control.** 2016. Zika virus disease epidemic: potential association with microcephaly and Guillain-Barré syndrome (first update). Stockholm: ECDC.
76. **Oliveira Melo AS, Malinger G, Ximenes R, Szejnfeld PO, Alves Sampaio S, Bispo de Filippis AM.** 2016. Zika virus intrauterine infection causes fetal brain abnormality and microcephaly: tip of the iceberg? Ultrasound Obstet Gynecol **47:**6-7.
77. **Mlakar J, Korva M, Tul N, Popovic M, Poljsak-Prijatelj M, Mraz J, Kolenc M, Resman Rus K, Vesnaver Vipotnik T, Fabjan Vodusek V, Vizjak A, Pizem J, Petrovec M, Avsic Zupanc T.** 2016. Zika Virus Associated with Microcephaly. N Engl J Med doi:10.1056/NEJMoa1600651.
78. **Martines RB, Bhatnagar J, Keating MK, Silva-Flannery L, Muehlenbachs A, Gary J, Goldsmith C, Hale G, Ritter J, Rollin D, Shieh WJ, Luz KG, Ramos AM, Davi HP, Kleber de Oliveria W, Lanciotti R, Lambert A, Zaki S.** 2016. Notes from the Field: Evidence of Zika Virus Infection in Brain and Placental Tissues from Two Congenitally Infected Newborns and Two Fetal Losses - Brazil, 2015. MMWR Morb Mortal Wkly Rep **65:**159-160.
79. **Calvet G, Aguiar RS, Melo AS, Sampaio SA, de Filippis I, Fabri A, Araujo ES, de Sequeira PC, de Mendonca MC, de Oliveira L, Tschoeke DA, Schrago CG, Thompson FL, Brasil P, Dos Santos FB, Nogueira RM, Tanuri A, de Filippis AM.** 2016. Detection and sequencing of Zika virus from amniotic fluid of fetuses with microcephaly in Brazil: a case study. Lancet Infect Dis doi:10.1016/S1473-3099(16)00095-5.
80. **Meaney-Delman D, Hills SL, Williams C, Galang RR, Iyengar P, Hennenfent AK, Rabe IB, Panella A, Oduyebo T, Honein MA, Zaki S, Lindsey N, Lehman JA, Kwit N, Bertolli J, Ellington S, Igbinosa I, Minta AA, Petersen EE, Mead P, Rasmussen SA, Jamieson DJ.** 2016. Zika Virus Infection Among U.S. Pregnant Travelers —August 2015–February 2016. MMWR Morb Mortal Wkly Rep **65**.
81. **Sarno M, Sacramento GA, Khouri R, do Rosario MS, Costa F, Archanjo G, Santos LA, Nery N, Jr., Vasilakis N, Ko AI, de Almeida AR.** 2016. Zika Virus Infection and Stillbirths: A Case of Hydrops Fetalis, Hydranencephaly and Fetal Demise. PLoS Negl Trop Dis **10:**e0004517.
82. **Banatvala JE, Brown DW.** 2004. Rubella. Lancet **363:**1127-1137.
83. **Naing ZW, Scott GM, Shand A, Hamilton ST, van Zuylen WJ, Basha J, Hall B, Craig ME, Rawlinson WD.** 2016. Congenital cytomegalovirus infection in pregnancy: a review of prevalence, clinical features, diagnosis and prevention. Aust N Z J Obstet Gynaecol **56:**9-18.
84. **Teissier N, Fallet-Bianco C, Delezoide AL, Laquerriere A, Marcorelles P, Khung-Savatovsky S, Nardelli J, Cipriani S, Csaba Z, Picone O, Golden JA, Van Den Abbeele T, Gressens P, Adle-Biassette H.** 2014. Cytomegalovirus-induced brain malformations in fetuses. J Neuropathol Exp Neurol **73:**143-158.

85. **Calisher CH, Sever JL.** 1995. Are North American Bunyamwera serogroup viruses etiologic agents of human congenital defects of the central nervous system? Emerg Infect Dis **1:**147-151.
86. **Agerholm JS, Hewicker-Trautwein M, Peperkamp K, Windsor PA.** 2015. Virus-induced congenital malformations in cattle. Acta Vet Scand **57:**54.
87. **Centers for Disease Control and Prevention.** 2002. Intrauterine West Nile virus infection--New York, 2002. MMWR Morb Mortal Wkly Rep **51:**1135-1136.
88. **O'Leary DR, Kuhn S, Kniss KL, Hinckley AF, Rasmussen SA, Pape WJ, Kightlinger LK, Beecham BD, Miller TK, Neitzel DF, Michaels SR, Campbell GL, Lanciotti RS, Hayes EB.** 2006. Birth outcomes following West Nile Virus infection of pregnant women in the United States: 2003-2004. Pediatrics **117:**e537-545.
89. **Paisley JE, Hinckley AF, O'Leary DR, Kramer WC, Lanciotti RS, Campbell GL, Hayes EB.** 2006. West Nile virus infection among pregnant women in a northern Colorado community, 2003 to 2004. Pediatrics **117:**814-820.
90. **Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, Drake JM, Brownstein JS, Hoen AG, Sankoh O, Myers MF, George DB, Jaenisch T, Wint GR, Simmons CP, Scott TW, Farrar JJ, Hay SI.** 2013. The global distribution and burden of dengue. Nature **496:**504-507.
91. **Oduyebo T, Petersen EE, Rasmussen SA, Mead PS, Meaney-Delman D, Renquist CM, Ellington SR, Fischer M, Staples JE, Powers AM, Villanueva J, Galang RR, Dieke A, Munoz JL, Honein MA, Jamieson DJ.** 2016. Update: Interim Guidelines for Health Care Providers Caring for Pregnant Women and Women of Reproductive Age with Possible Zika Virus Exposure - United States, 2016. MMWR Morb Mortal Wkly Rep **65:**122-127.
92. **European Centre for Disease Prevention and Control.** 2016. Rapid Risk Assessment. Zika virus disease eepidemic: potential association with microcephaly and Guillain–Barré syndrome. Second update, 8 February 2016. Stockholm: ECDC.
93. **Oster AM, Brooks JT, Stryker JE, Kachur RE, Mead P, Pesik NT, Petersen LR.** 2016. Interim Guidelines for Prevention of Sexual Transmission of Zika Virus - United States, 2016. MMWR Morb Mortal Wkly Rep **65:**120-121.
94. **Heang V, Yasuda CY, Sovann L, Haddow AD, Travassos da Rosa AP, Tesh RB, Kasper MR.** 2012. Zika virus infection, Cambodia, 2010. Emerg Infect Dis **18:**349-351.
95. **Karimi O, Goorhuis A, Schinkel J, Codrington J, Vreden SGS, Vermaat JS, Stijnis C, Grobusch MP.** 2016. Thrombocytopenia and subcutaneous bleedings in a patient with Zika virus infection. Lancet.
96. **Ortega LA, Polo A, Tatis GP, García HL, Lissethe EP, Herrera CP, Turca AMR, Gómez WV, Morales AJR.** 2016. Fatal Zika Virus Infection in Girl with Sickle Cell Disease, Colombia. Emerg Infect Dis.
97. **Oehler E, Watrin L, Larre P, Leparc-Goffart I, Lastere S, Valour F, Baudouin L, Mallet H, Musso D, Ghawche F.** 2014. Zika virus infection complicated by Guillain-Barre syndrome--case report, French Polynesia, December 2013. Euro Surveill **19**.
98. **Cao-Lormeau V, Blake A, Mons S, Lastère S, Roche C, Vanhomwegen J, Dub T, Baudouin L, Teissier A, Larre P, Vial A, Decam C, Choumet V, Halstead SK, Willison HJ, Musset L, Manuguerra J, Despres P, Fournier E, Mallet H, Musso D, Fontanet A, Neil J, Ghawché F.** 2016. Guillain-Barré Syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study. Lancet.
99. **Ansar V, Valadi N.** 2015. Guillain-Barre syndrome. Prim Care **42:**189-193.
100. **Ubogu EE.** 2015. Inflammatory neuropathies: pathology, molecular markers and targets for specific therapeutic intervention. Acta Neuropathol **130:**445-468.
101. **Ventura CV, Maia M, Bravo-Filho V, Gois AL, Belfort R, Jr.** 2016. Zika virus in Brazil and macular atrophy in a child with microcephaly. Lancet **387:**228.

102. **de Paula Freitas B, de Oliveira Dias JR, Prazeres J, Sacramento GA, Ko AI, Maia M, Belfort R, Jr.** 2016. Ocular Findings in Infants With Microcephaly Associated With Presumed Zika Virus Congenital Infection in Salvador, Brazil. JAMA Ophthalmol doi:10.1001/jamaophthalmol.2016.0267.
103. **Way JH, Bowen ET, Platt GS.** 1976. Comparative studies of some African arboviruses in cell culture and in mice. J Gen Virol **30:**123-130.
104. **Hamel R, Dejarnac O, Wichit S, Ekchariyawat P, Neyret A, Luplertlop N, Perera-Lecoin M, Surasombatpattana P, Talignani L, Thomas F, Cao-Lormeau VM, Choumet V, Briant L, Despres P, Amara A, Yssel H, Misse D.** 2015. Biology of Zika Virus Infection in Human Skin Cells. J Virol **89:**8880-8896.
105. **Lim PY, Behr MJ, Chadwick CM, Shi PY, Bernard KA.** 2011. Keratinocytes are cell targets of West Nile virus in vivo. J Virol **85:**5197-5201.
106. **Limon-Flores AY, Perez-Tapia M, Estrada-Garcia I, Vaughan G, Escobar-Gutierrez A, Calderon-Amador J, Herrera-Rodriguez SE, Brizuela-Garcia A, Heras-Chavarria M, Flores-Langarica A, Cedillo-Barron L, Flores-Romo L.** 2005. Dengue virus inoculation to human skin explants: an effective approach to assess in situ the early infection and the effects on cutaneous dendritic cells. Int J Exp Pathol **86:**323-334.
107. **Surasombatpattana P, Hamel R, Patramool S, Luplertlop N, Thomas F, Despres P, Briant L, Yssel H, Misse D.** 2011. Dengue virus replication in infected human keratinocytes leads to activation of antiviral innate immune responses. Infect Genet Evol **11:**1664-1673.
108. **Faye O, Freire CC, Iamarino A, Faye O, de Oliveira JV, Diallo M, Zanotto PM, Sall AA.** 2014. Molecular evolution of Zika virus during its emergence in the 20(th) century. PLoS Negl Trop Dis **8:**e2636.
109. **Hanna SL, Pierson TC, Sanchez MD, Ahmed AA, Murtadha MM, Doms RW.** 2005. N-linked glycosylation of west nile virus envelope proteins influences particle assembly and infectivity. J Virol **79:**13262-13274.
110. **Lee E, Leang SK, Davidson A, Lobigs M.** 2010. Both E protein glycans adversely affect dengue virus infectivity but are beneficial for virion release. J Virol **84:**5171-5180.
111. **Mondotte JA, Lozach PY, Amara A, Gamarnik AV.** 2007. Essential role of dengue virus envelope protein N glycosylation at asparagine-67 during viral propagation. J Virol **81:**7136-7148.
112. **Yoshii K, Yanagihara N, Ishizuka M, Sakai M, Kariwa H.** 2013. N-linked glycan in tick-borne encephalitis virus envelope protein affects viral secretion in mammalian cells, but not in tick cells. J Gen Virol **94:**2249-2258.
113. **Prow NA, May FJ, Westlake DJ, Hurrelbrink RJ, Biron RM, Leung JY, McMinn PC, Clark DC, Mackenzie JS, Lobigs M, Khromykh AA, Hall RA.** 2011. Determinants of attenuation in the envelope protein of the flavivirus Alfuy. J Gen Virol **92:**2286-2296.
114. **Moudy RM, Zhang B, Shi PY, Kramer LD.** 2009. West Nile virus envelope protein glycosylation is required for efficient viral transmission by Culex vectors. Virology **387:**222-228.
115. **Beasley DW, Whiteman MC, Zhang S, Huang CY, Schneider BS, Smith DR, Gromowski GD, Higgs S, Kinney RM, Barrett AD.** 2005. Envelope protein glycosylation status influences mouse neuroinvasion phenotype of genetic lineage 1 West Nile virus strains. J Virol **79:**8339-8347.
116. **Shirato K, Miyoshi H, Goto A, Ako Y, Ueki T, Kariwa H, Takashima I.** 2004. Viral envelope protein glycosylation is a molecular determinant of the neuroinvasiveness of the New York strain of West Nile virus. J Gen Virol **85:**3637-3645.
117. **Jiang WR, Lowe A, Higgs S, Reid H, Gould EA.** 1993. Single amino acid codon changes detected in louping ill virus antibody-resistant mutants with reduced neurovirulence. J Gen Virol **74 (Pt 5):**931-935.

118. **Fuchs A, Lin TY, Beasley DW, Stover CM, Schwaeble WJ, Pierson TC, Diamond MS.** 2010. Direct complement restriction of flavivirus infection requires glycan recognition by mannose-binding lectin. Cell Host Microbe **8:**186-195.
119. **Cardoso CW, Paploski IA, Kikuti M, Rodrigues MS, Silva MM, Campos GS, Sardi SI, Kitron U, Reis MG, Ribeiro GS.** 2015. Outbreak of Exanthematous Illness Associated with Zika, Chikungunya, and Dengue Viruses, Salvador, Brazil. Emerg Infect Dis **21:**2274-2276.
120. **Waggoner JJ, Pinsky BA.** 2016. Zika Virus: Diagnostics for an Emerging Pandemic Threat. J Clin Microbiol doi:10.1128/JCM.00279-16.
121. **Balm MN, Lee CK, Lee HK, Chiu L, Koay ES, Tang JW.** 2012. A diagnostic polymerase chain reaction assay for Zika virus. J Med Virol **84:**1501-1505.
122. **Andries AC, Duong V, Ly S, Cappelle J, Kim KS, Lorn Try P, Ros S, Ong S, Huy R, Horwood P, Flamand M, Sakuntabhai A, Tarantola A, Buchy P.** 2015. Value of Routine Dengue Diagnostic Tests in Urine and Saliva Specimens. PLoS Negl Trop Dis **9:**e0004100.
123. **Barzon L, Pacenti M, Franchin E, Pagni S, Martello T, Cattai M, Cusinato R, Palu G.** 2013. Excretion of West Nile virus in urine during acute infection. J Infect Dis **208:**1086-1092.
124. **Barzon L, Pacenti M, Franchin E, Squarzon L, Sinigaglia A, Ulbert S, Cusinato R, Palu G.** 2014. Isolation of West Nile virus from urine of patients with acute infection. J Clin Microbiol doi:10.1128/JCM.01328-14.
125. **Chiou SS, Crill WD, Chen LK, Chang GJ.** 2008. Enzyme-linked immunosorbent assays using novel Japanese encephalitis virus antigen improve the accuracy of clinical diagnosis of flavivirus infections. Clin Vaccine Immunol **15:**825-835.
126. **Chabierski S, Barzon L, Papa A, Niedrig M, Bramson JL, Richner JM, Palu G, Diamond MS, Ulbert S.** 2014. Distinguishing West Nile virus infection using a recombinant envelope protein with mutations in the conserved fusion-loop. BMC Infect Dis **14:**246.
127. **Rockstroh A, Barzon L, Pacenti M, Palu G, Niedrig M, Ulbert S.** 2015. Recombinant Envelope-Proteins with Mutations in the Conserved Fusion Loop Allow Specific Serological Diagnosis of Dengue-Infections. PLoS Negl Trop Dis **9:**e0004218.
128. **Acosta EG, Bartenschlager R.** 2015. Paradoxical role of antibodies in dengue virus infections: considerations for prophylactic vaccine development. Expert Rev Vaccines doi:10.1586/14760584.2016.1121814**:**1-16.
129. **de Alwis R, Williams KL, Schmid MA, Lai CY, Patel B, Smith SA, Crowe JE, Wang WK, Harris E, de Silva AM.** 2014. Dengue viruses are enhanced by distinct populations of serotype cross-reactive antibodies in human immune sera. PLoS Pathog **10:**e1004386.
130. **Guzman MG, Alvarez M, Halstead SB.** 2013. Secondary infection as a risk factor for dengue hemorrhagic fever/dengue shock syndrome: an historical perspective and role of antibody-dependent enhancement of infection. Arch Virol **158:**1445-1459.
131. **Beatty PR, Puerta-Guardo H, Killingbeck SS, Glasner DR, Hopkins K, Harris E.** 2015. Dengue virus NS1 triggers endothelial permeability and vascular leak that is prevented by NS1 vaccination. Sci Transl Med **7:**304ra141.
132. **Modhiran N, Watterson D, Muller DA, Panetta AK, Sester DP, Liu L, Hume DA, Stacey KJ, Young PR.** 2015. Dengue virus NS1 protein activates cells via Toll-like receptor 4 and disrupts endothelial cell monolayer integrity. Sci Transl Med **7:**304ra142.
133. **Whitehorn J, Yacoub S, Anders KL, Macareo LR, Cassetti MC, Nguyen Van VC, Shi PY, Wills B, Simmons CP.** 2014. Dengue therapeutics, chemoprophylaxis, and allied tools: state of the art and future directions. PLoS Negl Trop Dis **8:**e3025.

134. **Krishnan MN, Garcia-Blanco MA.** 2014. Targeting host factors to treat West Nile and dengue viral infections. Viruses **6:**683-708.

135. **Mateo R, Nagamine CM, Kirkegaard K.** 2015. Suppression of Drug Resistance in Dengue Virus. MBio **6:**e01960-01915.

136. **Dejnirattisai W, Wongwiwat W, Supasa S, Zhang X, Dai X, Rouvinski A, Jumnainsong A, Edwards C, Quyen NT, Duangchinda T, Grimes JM, Tsai WY, Lai CY, Wang WK, Malasit P, Farrar J, Simmons CP, Zhou ZH, Rey FA, Mongkolsapaya J, Screaton GR.** 2015. A new class of highly potent, broadly neutralizing antibodies isolated from viremic patients infected with dengue virus. Nat Immunol **16:**170-177.

137. **Oliphant T, Engle M, Nybakken GE, Doane C, Johnson S, Huang L, Gorlatov S, Mehlhop E, Marri A, Chung KM, Ebel GD, Kramer LD, Fremont DH, Diamond MS.** 2005. Development of a humanized monoclonal antibody with therapeutic potential against West Nile virus. Nat Med **11:**522-530.

138. **Bell TM, Field EJ, Narang HK.** 1971. Zika virus infection of the central nervous system of mice. Arch Gesamte Virusforsch **35:**183-193.

139. **Collaborative Cross Consortium.** 2012. The genome architecture of the Collaborative Cross mouse genetic reference population. Genetics **190:**389-401.

140. **Gralinski LE, Ferris MT, Aylor DL, Whitmore AC, Green R, Frieman MB, Deming D, Menachery VD, Miller DR, Buus RJ, Bell TA, Churchill GA, Threadgill DW, Katze MG, McMillan L, Valdar W, Heise MT, Pardo-Manuel de Villena F, Baric RS.** 2015. Genome Wide Identification of SARS-CoV Susceptibility Loci Using the Collaborative Cross. PLoS Genet **11:**e1005504.

141. **Rasmussen AL, Okumura A, Ferris MT, Green R, Feldmann F, Kelly SM, Scott DP, Safronetz D, Haddock E, LaCasse R, Thomas MJ, Sova P, Carter VS, Weiss JM, Miller DR, Shaw GD, Korth MJ, Heise MT, Baric RS, de Villena FP, Feldmann H, Katze MG.** 2014. Host genetic diversity enables Ebola hemorrhagic fever pathogenesis and resistance. Science **346:**987-991.

142. **Heymann DL, Hodgson A, Sall AA, Freedman DO, Staples JE, Althabe F, Baruah K, Mahmud G, Kandun N, Vasconcelos PF, Bino S, Menon KU.** 2016. Zika virus and microcephaly: why is this situation a PHEIC? Lancet **387:**719-721.

143. **Fauci AS, Morens DM.** 2016. Zika Virus in the Americas--Yet Another Arbovirus Threat. N Engl J Med **374:**601-604.

144. **Yakob L, Walker T.** 2016. Zika virus outbreak in the Americas: the need for novel mosquito control methods. Lancet Glob Health doi:10.1016/S2214-109X(16)00048-6.

145. **Alkan C, Zapata S, Bichaud L, Moureau G, Lemey P, Firth AE, Gritsun TS, Gould EA, de Lamballerie X, Depaquit J, Charrel RN.** 2015. Ecuador Paraiso Escondido Virus, a New Flavivirus Isolated from New World Sand Flies in Ecuador, Is the First Representative of a Novel Clade in the Genus Flavivirus. J Virol **89:**11773-11785.