An update on Zika virus infection

David Baud, Duane J Gubler, Bruno Schaub, Marion C Lanteri, Didier Musso

The epidemic history of Zika virus began in 2007, with its emergence in Yap Island in the western Pacific, followed in 2013–14 by a larger epidemic in French Polynesia, south Pacific, where the first severe complications and non-vector-borne transmission of the virus were reported. Zika virus emerged in Brazil in 2015 and was declared a national public health emergency after local researchers and physicians reported an increase in microcephaly cases. In 2016, WHO declared the recent cluster of microcephaly cases and other neurological disorders reported in Brazil a global public health emergency. Similar clusters of microcephaly cases were also observed retrospectively in French Polynesia in 2014. In 2015–16, Zika virus continued its spread to cause outbreaks in the Americas and the Pacific, and the first outbreaks were reported in continental USA, Africa, and southeast Asia. Non-vector-borne transmission was confirmed and Zika virus was established as a cause of severe neurological complications in fetuses, neonates, and adults. This Review focuses on important updates and gaps in the knowledge of Zika virus as of early 2017.

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Materno-fetal and Obstetrics Research Unit, Obstetric Service, Department "Femme-Mère-Enfant", University Hospital, Lausanne, Switzerland (Prof D Baud MD); **Program in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore** (Prof D J Gubler ScD); **Centre Pluridisciplinaire de Diagnostic Prénatal de Martinique, Service de Gynécologie Obstétrique, Maison de la Femme de la Mère et de l'Enfant, and Registre des Malformations des Antilles (REMALAN), Maison de la Femme de la Mère et de l'Enfant, Centre Hospitalier Universitaire de Martinique, Fort de France, Martinique, France** (B Schaub MD); **Blood Systems Research Institute, San Francisco, CA, USA** (M C Lanteri PhD); **Department of Laboratory Medicine, University of California San Francisco, San Francisco, CA, USA** (M C Lanteri); **Cerus Corporation, Concord, CA, USA** (M C Lanteri); **and Unit of Emerging Infectious Diseases, Institut Louis Malardé, Tahiti, French Polynesia** (D Musso MD)

Correspondence to:
Prof David Baud, Materno–fetal and Obstetrics Research Unit, Obstetric Service, Department "Femme-Mère-Enfant", University Hospital, Lausanne 1011, Switzerland
david.baud@chuv.ch

Introduction

Zika virus, a member of the family Flaviviridae and genus flavivirus, was first isolated in 1947 from a sentinel monkey in the Zika forest in Uganda, east Africa. Subsequent epidemiological studies suggested that Zika virus had a broad geographical distribution in sub-Saharan Africa and southeast Asia. The first human infection was reported in 1954 in Nigeria, but the identification of this virus was subsequently questioned and thought to be Spondweni. The first confirmed human infection was reported in Uganda in 1962–63.[1] Although known to infect people, Zika virus infection was rarely investigated and could have been misdiagnosed as dengue virus infection based on clinical presentation and serological cross-reactivity with closely related viruses. Thus, silent transmission in the absence of severe disease and large outbreaks allowed Zika virus infection to go undetected while spreading throughout Africa and Asia, with fewer than 20 human infections confirmed in 60 years.[2] The 2007 Yap Island outbreak marked the beginning of a new chapter in the history of Zika virus (figure 1).

Geographical distribution of Zika virus infections

In 2007, the first known Zika virus outbreak occurred on the isolated island of Yap, in the western Pacific.[3] 6 years later, a larger epidemic occurred in French Polynesia, in the south Pacific, followed by smaller outbreaks on other Pacific islands.[4] The virus was introduced into Brazil between 2013 and 2015, most probably from the Pacific, and caused a large epidemic that peaked in November, 2015, subsequently spreading rapidly throughout Brazil and the Americas, while continuing to circulate in the Pacific islands in 2016–17.[5] In Brazil, most Zika virus cases have been reported in the northern states and in the southeast region (Rio de Janeiro).[6,7] As of January, 2017, almost all Latin American and Caribbean countries have reported active Zika virus circulation.[2,5] Although circulation has been documented in Africa over the past 60 years and in Asia over the past 50 years, awareness of Zika virus circulation and the potential for severe disease outcomes was revealed only after the French Polynesian and Brazilian outbreaks. Enhanced diagnostic techniques and surveillance systems allowed investigation and reporting of autochthonous cases, and new circulation was reported in southeast Asia and Africa.[5] However, Zika virus infection incidence in these regions remains uncertain. In some areas, such as the Maldives or Vanuatu, Zika virus circulation was supported only by detection of infections in returning travellers.[8] In 2016, Zika virus emerged in the continental USA, causing a small outbreak in Florida and autochthonous cases in Texas.[9] Figures 2 and 3 show geographical distribution and emergence of Zika virus, and table 1 details key outbreaks.

The virus strains that emerged in the Pacific, Americas, Africa (Cape Verde), and southeast Asia (Singapore) were all from the Asian lineage. Phylogenetic studies suggest that Zika virus was probably imported to Brazil from the Pacific, and to Cape Verde from Brazil.[1,12] The outbreak in Singapore was caused by a strain closely related to the strain that had previously circulated in Asia before 2007, and was not the consequence of a new introduction from the Pacific or Americas.[12]

Favourable factors for Zika virus emergence

As Zika virus has circulated for several decades with sporadic or silent transmission to human beings and no reported epidemics, it was surprising when it suddenly emerged as a major public health problem. Although the epidemic potential of the virus has changed, allowing epidemic transmission, it is unclear whether the virulence has changed. Because of the close genetic and epidemiological relationship between

Search strategy and selection criteria

References for this Review were identified through searches of PubMed, Scopus, Web of Science, and Google Scholar, for articles published before April, 2017 by use of the terms "Zika virus", "ZIKV", "microcephaly", "Guillain-Barré syndromes", and "emerging viruses". Articles in English and French resulting from these searches and relevant references cited in those articles were reviewed.

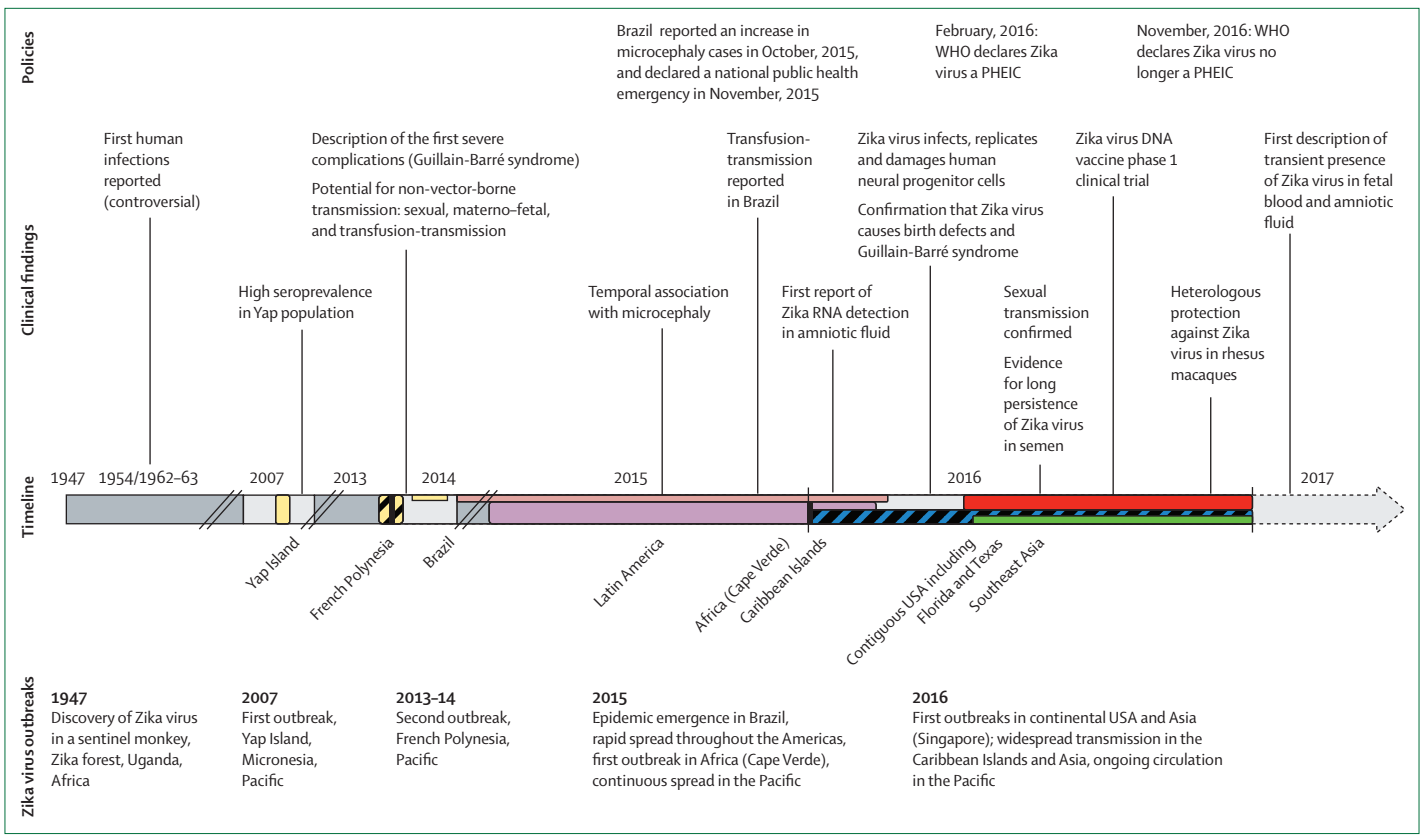


***Figure 1*: Timeline of Zika virus infection**
PHEIC=public health emergency of immediate concern.

dengue virus and Zika virus, the same demographic, societal, and technological factors that drove the emergence and spread of pandemic dengue virus probably also were a factor in the emergence and spread of Zika virus.[13]

Economic growth in tropical developing countries was a major driver of unprecedented and unplanned urban growth, which provided the ideal ecological conditions for increased aedes mosquito populations living in intimate contact with crowded human populations.[13] This, combined with ineffective mosquito control and modern transportation, provided the ideal mechanism to transport both the mosquitoes and viruses around the world. As with dengue virus, the resulting increased transmission expanded the probability of genetic change in Zika virus, and thus led to the emergence of viruses with greater epidemic potential and virulence. Therefore, globalisation facilitated the geographical spread of these new viral strains.

Two hypotheses can explain the emergence of Zika virus and the change in disease epidemiology. Genetic change might have resulted in emergence of a virus strain with greater epidemic potential and virulence, causing epidemics with more severe disease.[14,15] This theory should be confirmed by full-length sequencing of virus strains and reverse genetic studies. Another hypothesis might be the insufficiency of previous statistical power to detect rare events before 2013. Thus, the increased incidence of infections during the epidemics in French Polynesia and the Americas allowed rare clinical syndromes, including microcephaly and Guillain-Barré syndrome, to be recognised as associated with Zika infection.

See Online for appendix

Pathophysiology of Zika virus infection

In-vitro and in-vivo models

Zika virus infects human embryonic cortical neural progenitor cells, inducing cell death and providing evidence that human neurons are susceptible to the virus.[16] The virus seems to mainly target neuronal progenitors in the developing brain and, in rare instances, some areas of the adult brain.[17,18] Early infection is associated with proliferation arrest and an increase in neuronal progenitor death. Similar results were observed in cortical neurospheres.[19]

Mouse and rhesus macaque models supported a causal role for Zika virus in adverse pregnancy and neurological outcomes observed in people (appendix). In mice, Zika virus was recovered from fetal tissues after maternal peripheral infection, suggesting

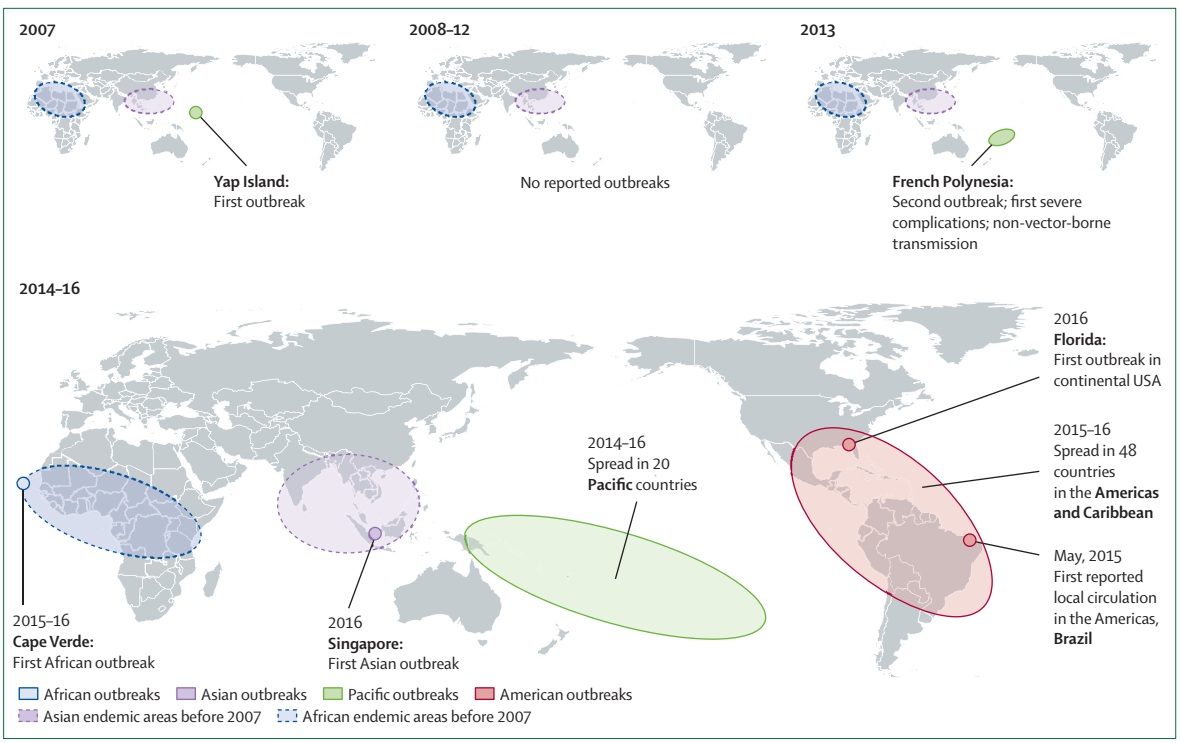


Figure 2: **Zika virus outbreaks from 2007–16**

materno–fetal transmission.[19,20] Fetuses of infected mice had abnormalities similar to those described in people.[17,19–22] Vaginal infection in mice was associated with fetal defects.[21]

After subcutaneous infection in primates, Zika virus RNA was recovered in blood, semen, vaginal fluids, urine, saliva, and cerebrospinal fluid;[23–25] and after intravenous inoculation in primates viral RNA was recovered in blood, saliva, urine, lymph nodes, heart, spleen, uterus, vagina, and gastrointestinal tissues.[26] Zika virus RNA has been found to persist for longer in whole blood than in plasma, and this phenomenon has been observed in people.[27] The virus is rapidly cleared from the plasma through development of an efficient adaptive immune response.[26] Prolonged maternal viraemia and severe fetal brain malformations were observed after maternal infection in primates.[23,25]

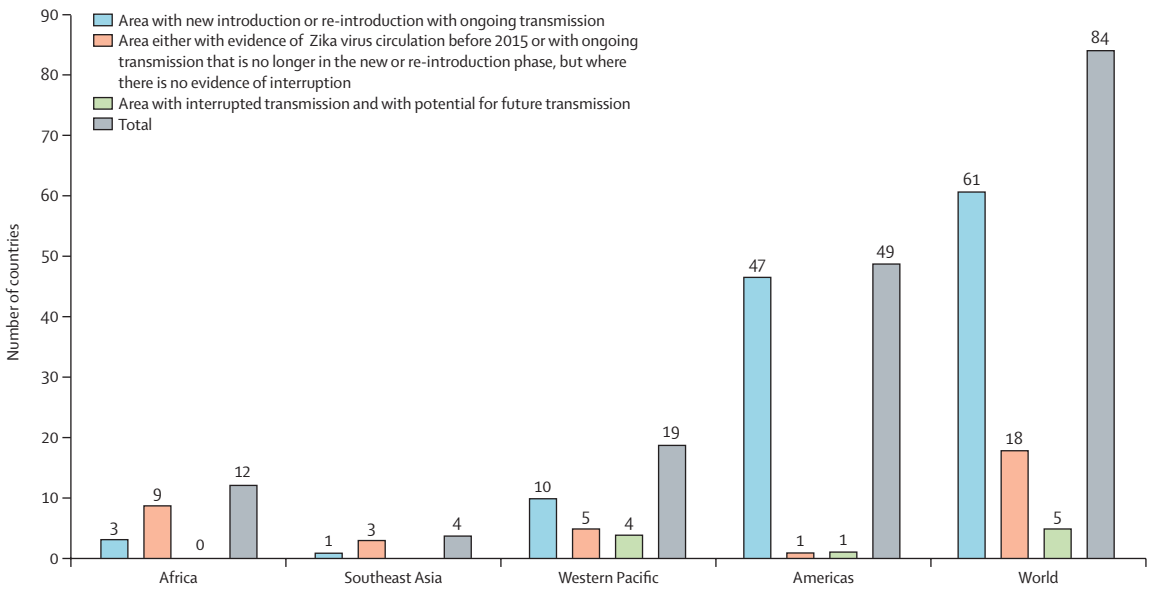
Immunity against Zika virus

In primary flavivirus infections, the antibody response is often monotypic, allowing serological identification of the infecting virus. In secondary flavivirus infections, which commonly occur in endemic Zika virus areas, the antibody response becomes broadly heterotypic, cross-reacting with multiple flavivirus antigens, making reliable identification of the infecting virus by serology-based assays challenging. There is one Zika virus serotype, despite two lineages (African and Asian) and three genotypes (west African, east African, and Asian).[2,28] Zika virus, like other flaviviruses, induces a humoural antibody response that is thought to provide lifelong protection against reinfection, although this assumption should be further confirmed across the multiple human cohorts that are being investigated. The amount of background immunity required in the population to prevent emergence or recirculation of the virus is unknown. In-vitro cell infection with Zika virus (like other flaviviruses) can be enhanced by heterologous cross-reacting anti-flavivirus antibodies through the Fc receptor, a phenomenon called antibody-dependent enhancement, which is postulated to play a part in dengue virus pathogenesis.[29] Although in-vitro studies have shown that pre-existing dengue virus antibodies might drive increased Zika virus replication, there is no evidence that this phenomenon plays a part in viral pathogenesis.[30,31] Studies in rhesus macaques suggest that immunity against the Zika virus African lineage confers immunity against the heterologous Asian lineage and vice versa.[32] Very little is known about the innate and cellular immune responses to Zika virus, but these responses are probably similar to those triggered by other flaviviruses.[33–35]

Zika virus transmission

Vector-borne

Zika virus is part of the mosquito-borne group of flaviviruses that are mainly transmitted in the urban environment by aedes (subgenus stegomyia) mosquitoes,



Africa	Southeast Asia	Western Pacific	Americas
Angola, Burkina Faso, Burundi, Cape Verde, Cameroon, Central African Republic, Gabon, Guinea-Bissau, Côte d'Ivoire, Nigeria, Senegal	Bangladesh, Indonesia, Maldives, Thailand	American Samoa, Cambodia, Cook Islands, Federated States of Micronesia, French Polynesia, Fiji, Laos, Malaysia, Marshall Islands, New Caledonia, Papua New Guinea, Palau, Philippines, Samoa, Singapore, Solomon Islands, Tonga, Vanuatu, Vietnam	Anguilla, Antigua and Barbuda, Argentina, Aruba, The Bahamas, Barbados, Belize, Bolivia, Caribbean Netherlands (Bonaire, Sint Eustatius, and Saba), Brazil, British Virgin Islands, Cayman Islands, Chile, Colombia, Costa Rica, Cuba, Curaçao, Dominica, Dominican Republic, Ecuador, El Salvador, French Guiana, Grenada, Guadeloupe, Guatemala, Guyana, Haiti, Honduras, Jamaica, Martinique, Mexico, Montserrat, Nicaragua, Panama, Paraguay, Peru, Puerto Rico, Saint Barthélemy, Saint Kitts and Nevis, Saint Lucia, Saint Martin, Saint Vincent and the Grenadines, Sint Maarten, Suriname, Trinidad and Tobago, Turks and Caicos Islands, USA, Virgin Islands, Venezuela
12	4	19	49

***Figure 3*: Countries and territories that reported Zika virus circulation up to March, 2017**
Categories according to WHO classification.[5]

which also transmit dengue virus, chikungunya, and yellow fever virus.[2]

Nearly all tropical and subtropical areas of the world, containing a total population of approximately 3·6 billion people, are infested with *Aedes aegypti*, *Aedes albopictus*, and a variety of other aedes mosquitoes, and are at risk for Zika virus, dengue virus, chikungunya, and yellow fever virus infection and epidemic transmission.[13] However, between April, 2007, and March, 2017, Zika virus transmission had only been reported in 84 countries worldwide.[5]

Conflicting studies suggest that mosquitoes belonging to other genera, mainly culex mosquitoes, which transmit West Nile and Japanese encephalitis viruses, might be involved in Zika virus transmission.[36,37] Additional investigations are needed concerning whether culex is a viable vector that might substantially affect operational prevention and control programmes. Vertical transmission of the virus has been shown in *A aegypti* mosquitoes.[38]

Non-vector-borne

Sexual

Sexual transmission of Zika virus was suspected in 2008, when a scientist infected in Senegal transmitted the virus to his wife upon returning to the USA, and in 2013 when infectious viral particles were detected in the semen of a French Polynesian patient.[2,39] Zika virus sexual transmission has since been documented when people living in non-endemic areas became infected after sexual intercourse with partners returning from endemic areas. Sexual transmission is possible from both asymptomatic and symptomatic infections through genital, oral, and anal intercourse, and male to male, male to female, and female to male contact.[40] Sexual transmission is reported to have occurred up to 44 days after symptom onset;

	Yap Island (Pacific)[2]	French Polynesia (Pacific)[1]	Brazil (Americas) as of May, 2017[4,5]
Population	7500	270 000	206 000 000
Confirmed cases	49	340[10]	697
Estimated infections (% of population)	5005 (75%)	30 000 (11·5%)	220 213 (0·1%)
New epidemiological findings	First reported outbreak; first detection of Zika virus outside Asia and Africa	Non-vector-borne transmission possible (materno–fetal, sexual, transfusion)	First detection in the Americas; microcephaly association
Clinical findings	Rash, fever, arthralgia, and conjunctivitis	GBS; CNS malformation	GBS; CNS malformation
CNS malformation cases*	0	17[11]	2653
Main challenges	Identification of Zika virus	High incidence of GBS	Laboratory capacities to confirm cases; high incidence of microcephaly

GBS=Guillain-Barré syndrome. *Number of microcephaly or CNS malformation cases suggestive of congenital Zika virus infection or potentially associated with viral infection.

Table 1: **Comparison of Zika virus outbreaks in Yap, French Polynesia, and Brazil**

infectious viral particles have been isolated up to 69 days after symptom onset in semen and up to 2 days after symptom onset in the female genital tract.[41,42] Zika virus RNA has been detected for up to 6 months after symptom onset in semen and up to 13 days after symptom onset in the female genital tract,[42] which does not necessarily associate with the presence of infectious virus.[43] Therefore, the exact duration of infectivity of genital fluids is unknown.[42]

The effect of Zika virus infection on male fertility is unknown. Sexual transmission of the virus can have an impact on assisted reproductive technology and gamete or embryo banking. A validated RT-PCR to detect Zika virus in semen should be implemented to screen donors.[43]

Although sexual transmission might play a part in non-endemic areas, the main mode of Zika virus transmission is through mosquito bites.[44] The effect of sexual transmission in endemic areas is impossible to assess because the entire population is exposed to mosquitoes. As of March, 2017, 13 countries have reported evidence of non-vector person to person transmission of the virus.[5]

Transfusion

The potential for Zika virus transfusion-transmitted infection was suspected in French Polynesia after viral RNA was detected in 2·8% of asymptomatic blood donors in 2014,[2] and further confirmed in Puerto Rico in 2016 with 1·1% of blood donors identified as viraemic.[45] In 2017, Zika virus RNA-positive asymptomatic blood donors were detected in Florida and Texas.[46–48] Transfusion-transmitted infection was confirmed in Brazil in 2016.[49] As with sexual transmission, transfusion-transmitted infection is difficult to prove in endemic areas.[50] With the poor availability of molecular biology-based assays and the challenges associated with interpretation of serology data, the number of documented cases of transfusion-transmitted infection is probably underestimated.

Zika virus is a new challenge for the blood supply.[51,52] WHO, the US Food and Drug Administration (FDA), and the American Association for Blood Banks have issued recommendations to prevent transfusion-transmitted infections (appendix). As most infections are asymptomatic, the most effective mitigation strategies to prevent transfusion-transmitted infection are nucleic acid testing of blood donations or pathogen inactivation.[46] A commercially available licensed pathogen-inactivation system has been shown to inactivate a wide range of pathogens, including Zika virus, after photochemical treatment of plasma and platelets.[53–55] Robust inactivation of Zika virus has also been shown with a pathogen-inactivation system under development for the chemical treatment of red blood cells.[56]

The FDA has also issued guidance for donors of other substances of human origin (appendix), even though Zika virus transmission has not been reported through organ transplantation.

Materno–fetal

Perinatal transmission of Zika virus was first reported during the French Polynesian outbreak in 2013.[57] Intrauterine transmission was subsequently confirmed during the Brazilian outbreak.[58,59] Viral RNA was detected in the amniotic fluid of pregnant women suffering from symptoms compatible with Zika virus infection, and later in fetal brains and products of miscarriages, supporting materno–fetal transmission of the virus.[58–62]

Chronic placentitis (TORCH type—Toxoplasmosis or *Toxoplasma gondii*; Other infections; Rubella; Cytomegalovirus; Herpes simplex virus-2 or neonatal herpes simplex) has been described and implicated in the destruction of the placental immunological barrier. However, unlike other TORCH pathogens, Zika virus does not cause a massive inflammatory response within the placenta, but still induces severe damage in the fetal brain.

Vertical transmission will not occur in all pregnant women infected with Zika virus and symptomatic congenital infection will not be observed in all exposed fetuses, similar to cytomegalovirus and toxoplasmosis.[63,64] The exact rate of vertical transmission and congenital infection remains to be identified. Infectious viral

particles have been detected in breastmilk, but neonatal transmission by breastfeeding has so far not been described.[65]

Prevention of materno–fetal transmission of the virus relies on prevention of mosquito bites and practising safe sex (appendix).[66,67] According to the US Centers for Disease Control and Prevention, all pregnant women returning with possible Zika virus exposure should be tested, regardless of symptom status.[68] Women living in areas of active virus circulation should avoid pregnancy, those not living in endemic areas should be advised to avoid travelling in endemic countries, and all pregnant women should avoid sexual contact with partners with the same exposure risks. Recommendations are empirical and based on the detection of Zika virus RNA up to 6 months after exposure in semen and 2 weeks after exposure in the female genital track.

Contact with infected body fluids

Non-sexual direct person to person transmission has been reported only once through contact with body fluids of a highly viraemic, severely ill patient, but this mode of transmission remains to be confirmed.[69]

Laboratory diagnosis of Zika virus infection

In the absence of an antigenic detection test, acute phase diagnosis relies on molecular detection of Zika virus RNA. Blood and urine are the samples of choice. The virus can be detected only briefly in plasma or serum during acute illness. Compared with serum, urine was reported to increase the detection rate of viral RNA within the first week after symptom onset and expand the window of detection, as Zika virus RNA was detectable up to 39 days after exposure.[70,71] However, discrepant results were found in Puerto Rico, with a lower sensitivity of urine than blood.[72] In blood, Zika virus RNA has been reported up to 107 days after symptom onset in pregnant women and up to 60 days after birth in a virus-infected neonate.[73,74] Zika virus RNA can be detected for an increased duration in whole blood compared to serum, therefore whole blood testing should be considered for both men and women, espcially for diagnosis of Zika infection in pregnant women living in or returning from endemic areas.[27,75,76] As the timing of infection is difficult to establish, a negative RT-PCR does not exclude infection.[77]

Serology relies on detection of specific IgM by ELISA, which detects IgM antibodies as early as 4–5 days and up to 12 weeks or more after symptom onset. For blood collected from 1–12 weeks after symptom onset, a negative IgM ELISA is a strong argument against Zika virus infection; however, IgM might not always be detectable.[77–79] Serological testing for viral IgM is recommended up to 12 weeks after exposure. All positive or inconclusive IgM ELISA results should be confirmed by a plaque reduction neutralisation test (PRNT), a technique only available in few laboratories. Cross-reactions have been reported in individuals with a previous history of flavivirus infection or vaccination against another flavivirus when using ELISA and PRNT,[77] and Zika virus infection might also be responsible for false positive dengue virus serology.[80] Serology is difficult to interpret in endemic areas and in returning travellers with a previous history of flavivirus infection, as most cases are secondary flavivirus infections.

For Zika virus infection diagnosis, RT-PCR should be performed, and serum should be tested for IgM.[81] In pregnant women infected with the virus, both RT-PCR, on serum and whole blood, and serology should be considered at any time;[82] however, laboratory confirmation of fetal infection during pregnancy is challenging. Detection of Zika virus RNA in blood, urine, and amniotic fluid can be negative or transient, despite proven fetal infection.[78] Conversely, the virus can be detected in pregnant mothers or amniotic fluid without fetal abnormalities. Sensitivity, specificity, and negative and positive predictive values of detection of Zika virus RNA in amniotic fluids are unknown, challenging maternal counselling.

For infants with possible congenital Zika virus infection, RT-PCR should be performed within the first 2 days of birth on both serum and urine, and IgM ELISA should be performed on serum. Molecular and serology diagnosis tests are commercially available.

Clinical features and complications of Zika virus infections

The clinical presentation of uncomplicated Zika virus infection has been extensively described.[2] Because of its non-specific nature, infection is often not detected, or is misdiagnosed. The percentage of asymptomatic infections has been reported to be around 80%; however, a retrospective serosurvey in French Polynesia showed that, among patients who were seropositive for the virus, the percentage of asymptomatic infections was about 30% in infants and 50% in adults.[83] These results underscore that the strain of virus can influence the asymptomatic to symptomatic ratio in flavivirus infections.

Complications in fetuses and neonates

A potential link between maternal Zika virus infection and a congenital syndrome was identified in October, 2015 in Brazil, when neurologists and physicians in the state of Pernambuco observed an increase in microcephaly cases. The Brazilian Ministry of Health declared a national health emergency in November, 2015.[6,84] In February, 2016, this microcephaly epidemic was declared a public health emergency of international concern by WHO.[85]

The temporal association between the Zika virus outbreak and severe congenital CNS malformations was also reported in a retrospective analysis conducted in French Polynesia. Compared with uninfected people, the

risk ratio of developing brain anomalies was approximately 50 in French Polynesia and Recife, Brazil.[86,87] Analysis of the Zika virus and microcephaly epidemics in these countries and in returning travellers in the USA suggested that the greatest risk of fetal brain anomalies was during the first trimester.[84,86,88]

Detection of viral RNA in the amniotic fluid, placenta, brain tissue of fetuses and infants with microcephaly, and the high rates of microcephaly among children born to mothers with proven acute Zika virus infection during pregnancy, provided strong evidence linking CNS anomalies to maternal infection.[59–61,89] By use of Shepard's criteria for the assessment of potential teratogens, it was concluded that a causal relationship existed between prenatal infection with Zika virus and serious brain anomalies.[90]

Unlike some congenital pathogens, Zika virus seemed to harbour a specific neurotropism.[91] Major anomalies observed in fetuses and neonates include microcephaly, ventriculomegaly, diffuse calcifications, cerebral atrophy, signs of abnormal gyration, cortical development, and ocular anomalies that might lead to severe mental retardation and substantial motor disabilities, and visual and auditory impairments.[91] Microcephaly might not always be observed, as a normal head circumference was observed in 20% of Zika virus congenital syndromes;[92] screening for microcephaly at birth is thus not sufficient to detect congenital syndromes.[93] Head growth deceleration has been reported in infants born with a normal head circumference, leading to development of microcephaly after birth.[94]

The clinical presentation of Zika virus infection is similar in pregnant and non-pregnant women, and congenital infection is possible even in asymptomatic women.[92] The prognosis and adequate management of virus-exposed fetuses remains to be established and should include close ultrasound monitoring, amniocentesis, and fetal blood analysis.[78,82,93] Persistent maternal viraemia might act as a prognostic factor, as viral RNA was detected in maternal blood up to 107 days after symptom onset, and might be the result of viral replication in the fetus or placenta.[60,73,74,82]

In Rio de Janeiro, Brazil, adverse fetal outcomes were significantly more likely among offspring of mothers infected with Zika virus than they were among uninfected mothers presenting with a rash (44% *vs* 12%).[89] By contrast with previous studies, these adverse outcomes were observed regardless of the trimester during which infection might have occurred.[84,86,88,89]

Among 442 completed pregnancies in women with laboratory evidence of possible Zika virus infection in the US Zika Pregnancy Registry, birth defects potentially related to the virus were identified in 26 cases (6%).[88] Similar rates of congenital anomalies were observed among pregnant women with and without symptoms. As of March, 2017, the WHO registered 2656 congenital syndromes associated with Zika virus infections in 31 countries, of which 2653 were in Brazil.[6,5] Data from an international registry, open to any health-care provider willing to participate, can provide country-specific estimates.[95] Because of the preliminary nature of the studies mentioned, long-term neurological outcomes are not yet available.

Some infected newborns will remain asymptomatic or only develop minor symptoms later in life. However, blindness, hearing loss, hypotonia, paresy, epilepsy, and severe neurodevelopmental delay are the long-term outlook for children severely infected in utero, similar to the 15% of fetuses infected by cytomegalovirus. Prospective follow-up of exposed and infected infants is crucial.

Complications in adults

Guillain-Barré syndrome was the first reported severe complication of Zika virus infection in adults. The link between Zika virus and Guillain-Barré syndrome was confirmed by a case-control study conducted in French Polynesia.[96] The increased incidence of Guillain-Barré syndrome during Zika virus outbreaks varies by country, and was up to 20-times baseline in French Polynesia and 10-times baseline in Venezuela.[2,97] In the largest series of Zika virus-associated Guillain-Barré syndrome (French Polynesia and Colombia), the main characteristics of Guillain-Barré syndrome were a rapid progression to nadir (about 1 week), a short plateau phase (median 4 days), and a high proportion of facial palsy. Electrophysiological findings showed that most of the French

	Gaps in knowledge
Lineages	
Severe complications of Zika virus infections in fetuses, neonates, and adults are associated with the Asian lineage	Can the Zika virus African lineage cause severe complications?
Vector-borne transmission	
Proven competent vectors for Zika virus are *Aedes aegypti*, *Aedes albopictus*, and *Aedes hensilli*	Is *A albopictus* alone able to support active circulation of Zika virus? Do culex mosquitoes have a role in transmission of the virus?
A aegypti can transmit Zika virus vertically	Does vertical Zika virus transmission occur in other species and what part does it play in virus maintenance?
New vector control strategies are in development	New vector control strategies are not yet available to treat large areas
Host	
Human and non-human primates are amplification hosts for Zika virus	Can non-primate species serve as reservoirs or amplification hosts?
Non-vector-borne transmission of Zika virus	
Transfusion-transmission confirmed	How many Zika virus transfusion transmissions occurred? Are blood donations with persistent RNA-positivity infectious? What is the minimum infectious dose for transmission by transfusion?
Sexual transmission confirmed	What is the epidemiological effect of sexual transmission of Zika virus in endemic and non-endemic areas? What is the maximum duration of infectivity of semen and female genital fluids? What is the effect of infection on male fertility?

(Table 2 continues on next page)

	Gaps in knowledge
(Continued from previous page)	
Materno–fetal transmission of Zika virus and link between the virus and severe congenital CNS malformations confirmed	What is the percentage of transmission from infected pregnant women to fetuses? What is the percentage of infected fetuses that will develop malformations? What are the long-term outcomes for infected neonates without detectable abnormalities at birth? Can markers of fetal infection or predictive markers of congenital syndrome be identified in peripheral maternal blood?
Immunity against Zika virus	
Only one Zika virus serotype exists	Does Zika virus infection confer lifelong immunity? What is the effect of previous flavivirus infection on severity of Zika virus infections? How much background immunity is required in the population to prevent emergence of further circulation of new Zika virus strains? What is the effect of previous vaccination against a heterologous flavivirus (eg, yellow fever virus or Japanese encephalitis virus)? Is there antibody-dependent enhancement between Zika virus, dengue virus, or other flaviviruses?
Laboratory diagnosis of Zika virus infections	
Commercial kits are available for molecular diagnosis and serology	No diagnostic tests have been adapted to low-resource countries and there are no rapid tests; the sensitivity, specificity, and positive and negative predictive values of RT-PCR in amniotic fluids is unknown
Complications of Zika virus infections	
Link between Zika virus and severe CNS malformation in neonates and neurological complications in adults has been shown	What is the real incidence of these complications in Zika virus infections?
Emergence of Zika virus	
Small Zika virus outbreaks have been reported in Africa and Asia	Will Zika virus cause large outbreaks in African and Asian countries? Are specific mutations in the genome of the virus associated with increased epidemic potential and virulence? What is the exact geographical distribution and incidence of infections in the Pacific, Africa, and Asia?
Recommendations and guidelines	
Available guidelines rely on gold standard procedures	What is the applicability and effectiveness of Zika virus recommendations in endemic areas, as most are located in low-resource countries?
Drugs and vaccine	
Some drugs have in-vitro activity against Zika virus	When will drugs be available against Zika virus?
Vaccines are in the pipeline	When will a vaccine be available against Zika virus?

Table 2: **Current knowledge and associated gaps in knowledge for key areas concerning Zika virus infections**

Polynesian cases were of the acute motor axonal neuropathy subtype of Guillain-Barré syndrome, whereas in Colombia the predominant subtype was acute inflammatory demyelinating polyradiculoneuropathy, which is the most common subtype of Guillain-Barré syndrome.[96,98]

The pathogenesis of Zika virus-associated Guillain-Barré syndrome is still unknown: direct neuropathogenic mechanisms, hyperacute immune response, immune disregulation, and molecular mimicry against nervous antigens are all hypotheses.[99] Treatment of Guillain-Barré syndrome that requires intensive care facilities, plasma exchange, and intravenous immunoglobulins, can be challenging in remote areas and low-resource countries.

As of March, 2017, 23 countries or territories have reported an increased incidence of Guillain-Barré syndrome or laboratory confirmation of a Zika virus infection among Guillain-Barré syndrome cases.[5] Other CNS and non-CNS complications of infection potentially related to Zika virus are reported in the appendix.

Vaccines and drugs against Zika virus

Vaccine development has been well supported by international funding agencies. A DNA vaccine has entered phase 1 clinical trials and there are more than 40 vaccine candidates in the pipeline, some of which are being fast tracked for licensure.[100] Nevertheless, a vaccine will probably not be available for at least 2 years. It is also not known if Zika virus infections lead to lifelong immunity.[101]

Using large screening strategies, several compounds have been found to have in-vitro activity against Zika virus, but there are no antiviral drugs that have shown activity against the virus in vivo.[102]

Knowledge gaps

Although important advances have been made since Zika virus emerged in the Pacific, gaps remain in the knowledge of the epidemiology, virology, and biology of infections (table 2). For example, in July, 2016, WHO told *The Lancet* that "we don't know what the full spectrum of the Zika-caused congenital defects will be. Will apparently unaffected children whose mothers had Zika in pregnancy develop normally? Will they be able to walk and talk normally? Will they be mentally impaired or have other problems that only become evident years later?"[103] Almost a year later, most of these questions remain unanswered.

Applicability and effectiveness of recommendations

The effectiveness and applicability of strict recommendations are debatable. Recommendations are based on an extreme interpretation of the precautionary principle, which is awaiting data that will be generated by ongoing studies to further inform guidelines. Is the response always proportionate to the risk? For example, guidelines recommending that all blood donations be tested for Zika virus RNA in the USA, even in states without competent vectors, were issued without consultation with the US blood community. This warranted consideration of the challenges around donor deferrals, which are complicated by the seasonality of arbovirus outbreaks throughout the world, increased exchanges and travel between non-active and active virus transmission areas, and sexual transmission.[104] Can couples of reproductive age living in and travelling to endemic areas be asked to have strict protected sexual intercourse for 6 months after potential exposure?[105] What is the effect of this recommendation on sexual transmission of Zika virus in endemic areas with year-round transmission, in which all people are exposed to

mosquito bites? By contrast with Zika virus, no recommendations regarding sexual behaviour have been elaborated for other teratogenic viruses, such as cytomegalovirus, which can also persist for months in genital secretions.[105] What is the effect of antenatal diagnosis of infections in countries where abortion is illegal or highly restricted?

Scientists sent an open letter to the WHO suggesting cancellation, delay, or relocation of the 2016 Rio de Janeiro Olympic Games, but local and international specialists correctly stated that the risk of Zika virus transmission would be very low during the event.[106]

Recommendations and guidelines on the virus rely on gold standard laboratory testing, imaging, obstetrical, neurological, and paediatric expertise; such materials and expertise are sometimes lacking in areas with active virus circulation.

Is our response to Zika an overreaction? If the response sometimes seems exaggerated, it is important to note that this is the first time since the emergence of HIV that such dramatic complications have been reported on a large scale after the emergence or re-emergence of a pathogen. Therefore, all possible mitigation strategies should be implemented, even if some of them are subsequently found to be unnecessary.

Conclusion

WHO declared Zika virus a public health emergency of international concern in February, 2016, subsequently declaring the virus an ongoing challenge requiring intense action, but no longer a public health emergency of international concern, in November, 2016.[107] As of May, 2017, more than 200 000 infections and 3000 congenital syndromes associated with Zika virus infections have been confirmed in the Americas in a susceptible population of 1 billion inhabitants.[5] The number of cases in other endemic areas (Pacific, southeast Asia, and Africa) is unknown. Virus transmission has been decreasing in Latin America and the Caribbean over the past months because of the seasonality of arbovirus outbreaks. However, as we enter a new arbovirus season in the northern hemisphere, the persistence of Zika virus as an ongoing major concern might be confirmed. Research on Zika virus will continue, as approximately 3·6 billion people are living in at-risk areas for transmission. Moreover, the long-term immunity against Zika virus and the interplay between dengue virus, yellow fever virus, west Nile virus, Japanese encephalitis virus, and Zika immunity should be further characterised to inform decisions around vaccine efficacy.[10,11] Zika is still challenging in several parts of the world and there is potential for new epidemic transmission, spread beyond the currently impacted geographical areas, and increased virulence as the virus adapts to wider human populations. The current knowledge of Zika virus infection is based only on a short period of 10 years, with most efforts deployed over the last year, and the consequences of long-term congenital Zika-associated syndrome are unknown and should become clearer over the next decade. It is not possible to accurately predict when the next pathogen will emerge, or what it will be, but the experience of Zika virus emphasises the need for preparedness and envisioning worst case scenarios.

Contributors

DB, DJG, and DM did the literature search and drafted the manuscript in their domain of expertise. MCL and BS contributed unpublished data, participated in the discussion of ideas, helped with the writing, and provided critical inputs to the paper. All authors critically reviewed and approved the final manuscript.

Declaration of interests

We declare no competing interests.

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References

1 Wikan N, Smith DR. Zika virus: a history of newly emerging arbovirus. *Lancet Infect Dis* 2016; **16:** e119–26.
2 Musso D, Gubler DJ. Zika virus. *Clin Microbiol Rev* 2016; **29:** 487–524.
3 Duffy MR, Chen TH, Hancock WT, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. *N Engl J Med* 2009; **360:** 2536–43.
4 Musso D, Nilles EJ, Cao-Lormeau VM. Rapid spread of emerging Zika virus in the Pacific area. *Clin Microbiol Infect* 2014; **20:** O595–96.
5 WHO. Situation report. Zika virus microcephaly Guillain-Barré syndrome. March 10, 2017. Data as of 9 March 2017. http://reliefweb.int/report/world/zika-virus-microcephaly-and-guillain-barr-syndrome-situation-report-10-march-2017 (accessed May 26, 2017).
6 Brito CAA, Cordeiro MT. One year after the Zika virus outbreak in Brazil: from hypotheses to evidence. *Rev Soc Bras Med Trop* 2016; **49:** 537–43.
7 Brasil P, Calvet GA, Siqueira AM, et al. Zika virus outbreak in Rio de Janeiro, Brazil: clinical characterization, epidemiological and virological aspects. *PLoS Negl Trop Dis* 2016; **10:** e0004636.
8 Musso D, Baud D, Fredman DO. Should testing of donors be resticted to active Zika virus areas? *Lancet Infect Dis* 2016; **16:** 1108–09.
9 McCarthy M. Four in Florida are infected with Zika from local mosquitoes. *BMJ* 2016; **354:** i4235.
10 Musso D, Roche C, Nhan TX, Robin E, Teissier A, Cao-Lormeau VM. Detection of Zika virus in saliva. *J Clin Virol* 2015; **68:** 53–55.
11 Besnard M, Eyrolle-Guignot D, Guillemette-Artur P, et al. Congenital cerebral malformations and dysfunction in fetuses and newborns following the 2013 to 2014 Zika virus epidemic in French Polynesia. *Euro Surveill* 2016; **21:** 1–9.
12 Musso D, Lanteri M. Emergence of Zika virus: where does it come from and where is it going to? *Lancet Infect Dis* 2017; **17:** 255.
13 Gubler DJ. Dengue, urbanization and globalization: the unholy trinity of the 21(st) century. *Trop Med Health* 2011; **39:** 3–11.
14 Pettersson JH, Eldholm V, Seligman SJ, et al. How did Zika virus emerge in the Pacific islands and Latin America. *MBio* 2016; **7:** e01239–16.
15 Yokoyama S, Starmer WT. Possible roles of new mutations shared by Asian and American Zika viruses. *Mol Biol Evol* 2017; **34:** 525–34.
16 Tang H, Hammack C, Ogden SC, et al. Zika virus infects human cortical neural progenitors and attenuates their growth. *Cell Stem Cell* 2016; **18:** 587–90.
17 Li C, Xu D, Ye Q, et al. Zika virus disrupts neural progenitor development and leads to microcephaly in mice. *Cell Stem Cell* 2016; **19:** 120–26.
18 Li H, Saucedo-Cuevas L, Regla-Nava JA, et al. Zika virus infects neural progenitors in the adult mouse brain and alters proliferation. *Cell Stem Cell* 2016; **19:** 593–98.

19 Cugola FR, Fernandes IR, Russo FB, et al. The Brazilian Zika virus strain causes birth defects in experimental models. *Nature* 2016; **534:** 267–71.

20 Miner JJ, Cao B, Govero J, et al. Zika virus infection during pregnancy in mice causes placental damage and fetal demise. *Cell* 2016; **165:** 1081–91.

21 Yockey LJ, Varela L, Rakib T, et al. Vaginal exposure to Zika virus during pregnancy leads to fetal brain infection. *Cell* 2016; **166:** 1247–56.

22 Wu KY, Zuo GL, Li XF, et al. Vertical transmission of Zika virus targeting the radial glial cells affects cortex development of offspring mice. *Cell Res* 2016; **26:** 645–54.

23 Dudley DM, Aliota MT, Mohr EL, et al. A rhesus macaque model of Asian-lineage Zika virus infection. *Nat Commun* 2016; **7:** 12204.

24 Osuna CE, Lim SY, Deleage C, et al. Zika viral dynamics and shedding in rhesus and cynomolgus macaques. *Nat Med* 2016; **22:** 1448–55.

25 Adams Waldorf KM, Stencel-Baerenwald JE, Kapur RP, et al. Fetal brain lesions after subcutaneous inoculation of Zika virus in a pregnant nonhuman primate. *Nat Med* 2016; **22:** 1256–59.

26 Coffey LL, Pesavento PA, Keesler RI, et al. Zika virus tissue and blood compartmentalization in acute infection of rhesus macaques. *PLoS One* 2017; **12:** e0171148.

27 Lustig Y, Mendelson E, Paran N, Melamed S, Schwartz E. Detection of Zika virus RNA in whole blood of imported Zika virus disease cases up to 2 months after symptom onset, Israel, December 2015 to April 2016. *Euro Surveill* 2016; **21: 1–4.**

28 Dowd KA, DeMaso CR, Pelc RS, et al. Broadly neutralizing activity of Zika virus-immune sera identifies a single viral serotype. *Cell Rep* 2016; **16:** 1485–91.

29 Gubler DJ. Dengue and dengue hemorrhagic fever. *Clin Microbiol Rev* 1998; **11:** 480–96.

30 Priyamvada L, Quicke KM, Hudson WH, et al. Human antibody responses after dengue virus infection are highly cross-reactive to Zika virus. *Proc Natl Acad Sci USA* 2016; **113:** 7852–57.

31 Dejnirattisai W, Supasa P, Wongwiwat W, et al. Dengue virus sero-cross-reactivity drives antibody-dependent enhancement of infection with Zika virus. *Nat Immunol* 2016; **17:** 1102–08.

32 Aliota MT, Dudley DM, Newman CM, et al. Heterologous protection against Asian Zika virus challenge in rhesus macaques. *PLoS Negl Trop Dis* 2016; **10:** e0005168.

33 Lanteri MC, O'Brien KM, Purtha WE, et al. Tregs control the development of symptomatic West Nile virus infection in humans and mice. *J Clin Invest* 2009; **119:** 3266–77.

34 Bowen JR, Quicke KM, Maddur MS, et al. Zika virus antagonizes type I interferon responses during infection of human dendritic cells. *PLoS Pathog* 2017; **13:** e1006164.

35 Miner JJ, Diamond MS. Zika virus pathogenesis and tissue tropism. *Cell Host Microbe* 2017; **21:** 134–42.

36 Amraoui F, Atyame-Nten C, Vega-Rúa A, Lourenço-de-Oliveira R, Vazeille M, Failloux AB. *Culex* mosquitoes are experimentally unable to transmit Zika virus. *Euro Surveill* 2016; **21:** 30333.

37 Guo XX, Li CX, Deng YQ, et al. Culex pipiens quinquefasciatus: a potential vector to transmit Zika virus. *Emerg Microbes Infect* 2016; **5:** e102.

38 Thangamani S, Huang J, Hart CE, Guzman H, Tesh RB. Vertical transmission of Zika virus in *Aedes aegypti* mosquitoes. *Am J Trop Med Hyg* 2016; **95**: 1169–73.

39 Musso D, Roche C, Robin E, Nhan T, Teissier A, Cao-Lormeau VM. Potential sexual transmission of Zika virus. *Emerg Infect Dis* 2015; **21**: 359–61.

40 Davidson A, Slavinski S, Komoto K, Rakeman J, Weiss D. Suspected female-to-male sexual transmission of Zika virus—New York City, 2016. *MMWR Morb Mortal Wkly Rep* 2016; **65:** 716–17.

41 Penot P, Brichler S, Guilleminot J, et al. Infectious Zika virus in vaginal secretions from an HIV-infected woman, France, August 2016. *Euro Surveill* 2017; **22:** 30444.

42 Moreira J, Peixoto TM, Siqueira AM, Lamas CC. Sexually acquired Zika virus: a systematic review. *Clin Microbiol Infect* 2017; published online Jan 3. DOI:10.1016/j.cmi.2016.12.027.

43 Baud D, Musso D, Vouga M, Alves MP, Vulliemoz N. Zika virus: a new threat to human reproduction. *Am J Reprod Immunol* 2017; **77:** e12614

44 Musso D, Lanteri MC. Thoughts around the Zika virus crisis. *Curr Infect Dis Rep* 2016; **18:** 46.

45 Kuehnert MJ, Basavaraju SV, Moseley RR, et al. Screening of blood donations for Zika virus infection—Puerto Rico, April 3–June 11, 2016. *MMWR Morb Mortal Wkly Rep* 2016; **65:** 627–28.

46 Musso D, Broult J, Bierlaire D, Lanteri M, Aubry M. Prevention of transfusion-transmitted Zika virus in French Polynesia, nucleic acid testing versus pathogen inactivation. *ISBT Sci Ser* 2016; **12:** 254–59.

47 Galel SA, Williamson PC, Busch MP, et al. First Zika-positive donations in the continental United States. *Transfusion* 2017; **57:** 762–69.

48 Williamson PC, Linnen JM, Kessler DA, et al. First cases of Zika virus-infected US blood donors outside states with areas of active transmission. *Transfusion* 2017; **57:** 770–78.

49 Motta IJF, Spencer BR, Cordeiro da Silva SG, et al. Evidence for transmission of Zika virus by platelet transfusion. *N Engl J Med* 2016; **375:** 1101–03.

50 Musso D, Gould E, Lanteri M. Documentation of transfusion-transmitted arbovirus infections in endemic areas. *Transfusion* 2016; **56:** 3143–44.

51 Musso D, Stramer SL, AABB Transfusion-Transmitted Diseases Committee, Bush MP, for the International Society of Blood Transfusion Working Party on Transfusion-Transmitted Infectious Diseases. Zika virus: a new challenge for blood transfusion. *Lancet* 2016; **387:** 1993–94.

52 Lanteri MC, Kleinman SH, Glynn SA, et al. Zika virus: a new threat to the safety of the blood supply with worldwide impact and implications. *Transfusion* 2016; **56:** 1907–14.

53 Aubry M, Richard V, Green J, Broult J, Musso D. Inactivation of Zika virus in plasma with amotosalen and ultraviolet A illumination. *Transfusion* 2016; **56:** 33–40.

54 Santa Maria F, Laughhunn A, Lanteri M, Aubry M, Musso D, Stassinopoulos A. Inactivation of Zika virus in platelet components using amotosalen and ultraviolet A illumination. *Transfusion* (in press).

55 Katz LM, Rossmann SN. Zika and the blood supply: a work in progress. *Arch Pathol Lab Med* 2017; **141:** 85–92.

56 Laughhunn A, Santa Maria F, Broult J, et al. Amustaline (S-303) treatment inactivates high levels of Zika virus in red blood cell components. *Transfusion* 2017; **57:** 779–89.

57 Besnard M, Lastere S, Teissier A, Cao-Lormeau V, Musso D. Evidence of perinatal transmission of Zika virus, French Polynesia, December 2013 and February 2014. *Euro Surveill* 2014; **19:** 20751.

58 Oliveira Melo AS, Malinger G, Ximenes R, Szejnfeld PO, Alves Sampaio S, Bispo de Filippis AM. Zika virus intrauterine infection causes fetal brain abnormality and microcephaly: tip of the iceberg? *Ultrasound Obstet Gynecol* 2016; **47:** 6–7.

59 Calvet G, Aguiar RS, Melo AS, et al. Detection and sequencing of Zika virus from amniotic fluid of fetuses with microcephaly in Brazil: a case study. *Lancet Infect Dis* 2016; **16:** 653–60.

60 Driggers RW, Ho CY, Korhonen EM, et al. Zika virus infection with prolonged maternal viremia and fetal brain abnormalities. *N Engl J Med* 2016; **374:** 2142–51.

61 Mlakar J, Korva M, Tul N, et al. Zika virus associated with microcephaly. *N Engl J Med* 2016; **374:** 951–58.

62 van der Eijk AA, van Genderen PJ, Verdijk RM, et al. Miscarriage associated with Zika virus infection. *N Engl J Med* 2016; **375:** 1002–04.

63 Vouga M, Musso D, Van Mieghem T, Baud D. CDC guidelines for pregnant women during the Zika outbreak. *Lancet* 2016; **287:** 843–44.

64 Vouga M, Musso D, Panchaud A, Baud D. Clinical management of pregnant women exposed to Zika virus. *Lancet Infect Dis* 2016; **16:** 773.

65 Dupont-Rouzeyrol M, Biron A, O'Connor O, Huguon E, Descloux E. Infectious Zika viral particles in breastmilk. *Lancet* 2016; **387:** 1051.

66 Panchaud A, Stojanov M, Ammerdorffer A, Vouga M, Baud D. Emerging role of Zika virus in adverse fetal and neonatal outcomes. *Clin Microbiol Rev* 2016; **29:** 659–94.

67 Petersen EE, Meaney-Delman D, Neblett-Fanfair R, et al. Update: interim guidance for preconception counseling and prevention of sexual transmission of Zika virus for persons with possible Zika virus exposure—United States, September 2016. *MMWR Morb Mortal Wkly Rep* 2016; **65:** 1077–81.

68 Oduyebo T, Igbinosa I, Petersen EE, et al. Update: Interim guidance for health care providers caring for women of reproductive age with possible Zika virus exposure—United States, 2016. *MMWR Morb Mortal Wkly Rep* 2016; **65:** 315–22.

69 Swaminathan S, Schlaberg R, Lewis J, Hanson KE, Couturier MR. Fatal Zika virus infection with secondary nonsexual transmission. *N Engl J Med* 2016; **375:** 1907–09.

70 Bingham AM, Cone M, Mock V, et al. Comparison of test results for Zika virus RNA in urine, serum, and saliva specimens from persons with travel-associated Zika virus disease—Florida, 2016. *MMWR Morb Mortal Wkly Rep* 2016; **65:** 475–78.

71 Fréour T, Mirallié S, Hubert B, et al. Sexual transmission of Zika virus in an entirely asymptomatic couple returning from a Zika epidemic area, France, April 2016. *Euro Surveill* 2016; **21:** 30254.

72 Paz-Bailey G, Rosenberg ES, Doyle K, et al. Persistence of Zika virus in body fluids—preliminary report. *N Engl J Med* 2017; published online Feb 14. DOI:10.1056/NEJMoa1613108.

73 Suy A, Sulleiro E, Rodó C, et al. Prolonged Zika virus viremia during pregnancy. *N Engl J Med* 2016; **375:** 2611–13.

74 Oliveira DBL, Almeida FJ, Durigon EL, et al. Prolonged shedding of Zika virus associated with congenital infection. *N Engl J Med* 2016; **375:** 1202–04.

75 Froeschl G, Huber K, von Sonnenburg F, et al. Long-term kinetics of Zika virus RNA and antibodies in body fluids of a vasectomized traveller returning from Martinique: a case report. *BMC Infect Dis* 2017; **17:** 55.

76 Murray KO, Gorchakov R, Carlson AR, et al. Prolonged detection of Zika virus in vaginal secretions and whole blood. *Emerg Infect Dis* 2017; **23:** 99–101.

77 Rabe IB, Staples JE, Villanueva J, et al. Interim guidance for interpretation of Zika virus antibody test results. *MMWR Morb Mortal Wkly Rep* 2016; **65:** 543–46.

78 Schaub B, Vouga M, Najioullah F, et al. Analysis of blood from Zika virus-infected fetuses: a prospective case series. *Lancet Infect Dis* 2017; **17:** 520–27.

79 Landry ML, St George KG. Laboratory diagnosis of Zika virus infection. *Arch Pathol Lab Med* 2017; **141:** 60–67.

80 Felix AC, Souza NC, Figueiredo WM, et al. Cross reactivity of commercial anti-dengue immunoassays in patients with acute Zika virus infection. *J Med Virol* 2017; published online Feb 23. DOI:10.1002/jmv.24789.

81 US Centers for Disease Control and Prevention. Guidance for U.S. laboratories testing for Zika virus infection. 2016. https://www.cdc.gov/zika/laboratories/lab-guidance.html (accessed May 26, 2017).

82 Baud D, Van Mieghem T, Musso D, Truttmann AC, Manchaud A, Vouga M. Clinical management of pregnant women exposed to Zika virus. *Lancet Infect Dis* 2016; **16:** 523.

83 Aubry M, Teissier A, Huart M, et al. Zika virus seroprevalence, French Polynesia, 2014–2015. *Emerg Infect Dis* 2017; **23:** 669–72.

84 Kleber de Oliveira W, Cortez-Escalante J, De Oliveira WTGH, et al. Increase in reported prevalence of microcephaly in infants born to women living in areas with confirmed Zika virus transmission during the first trimester of pregnancy—Brazil, 2015. *MMWR Morb Mortal Wkly Rep* 2016; **65:** 242–47.

85 Gulland A. Zika virus is a global public health emergency, declares WHO. *BMJ* 2016; **352:** i657.

86 Cauchemez S, Besnard M, Bompard P, et al. Association between Zika virus and microcephaly in French Polynesia, 2013–15: a retrospective study. *Lancet* 2016; **387:** 2125–32.

87 de Araújo TVB, Rodrigues LC, de Alencar Ximenes RA, et al. Association between Zika virus infection and microcephaly in Brazil, January to May, 2016: preliminary report of a case-control study. *Lancet Infect Dis* 2016; **16:** 1356–63.

88 Honein MA, Dawson AL, Petersen EE, et al. Birth defects among fetuses and infants of US women with evidence of possible Zika virus infection during pregnancy. *JAMA* 2011; **317:** 59–68.

89 Brasil P, Pereira JP Jr, Moreira ME, et al. Zika virus infection in pregnant women in Rio de Janeiro. *N Engl J Med* 2016; **375:** 2321–34.

90 Rasmussen SA, Jamieson DJ, Honein MA, Petersen LR. Zika virus and birth defects—reviewing the evidence for causality. *N Engl J Med* 2016; **374:** 1981–87.

91 Vouga M, Baud D. Imaging of congenital Zika virus infection: the route to identification of prognostic factors. *Prenat Diagn* 2016; **36:** 799–811.

92 França GVA, Schuler-Faccini L, Oliveira WK, et al. Congenital Zika virus syndrome in Brazil: a case series of the first 1501 livebirths with complete investigation. *Lancet* 2016; **388:** 891–97.

93 Eppes C, Rac M, Dunn J, et al. Testing for Zika virus infection in pregnancy: key concepts to deal with an emerging epidemic. *Am J Obstet Gynecol* 2017; **216:** 209–25.

94 van der Linden V, Pessoa A, Dobyns W, et al. Description of 13 infants born during October 2015–January 2016 with congenital Zika virus infection without microcephaly at birth—Brazil. *MMWR Morb Mortal Wkly Rep* 2016; **65:** 1343–48.

95 Panchaud A, Vouga M, Musso D, Baud D. An international registry for women exposed to Zika virus during pregnancy: time for answers. *Lancet Infect Dis* 2016; **16:** 9995–96.

96 Cao-Lormeau VM, Blake A, Mons S, et al. Guillain-Barré Syndrome outbreak caused by Zika virus infection in French Polynesia: a case control study. *Lancet* 2016; **387:** 1531–39.

97 Dos Santos T, Rodriguez A, Almiron M, et al. Zika virus and the Guillain-Barré Syndrome—case series from seven countries. *N Engl J Med* 2016; **375:** 1598–601.

98 Parra B, Lizarazo J, Jiménez-Arango JA, et al. Guillain-Barré syndrome associated with Zika virus infection in Colombia. *N Engl J Med* 2016; **375:** 1513–23.

99 Uncini A, Shahrizaila N, Kuwabara S. Zika virus infection and Guillain-Barré syndrome: a review focused on clinical and electrophysiological subtypes. *J Neurol Neurosurg Psychiatry* 2017; **88:** 266–271.

100 Thomas SJ, L'Azou M, Barrett ADT, Jackson NAC. Fast-track Zika vaccine development—is it possible? *N Engl J Med* 2016; **375:** 1212–16.

101 Cohen J. Infectious disease. The race for a Zika vaccine is on. *Science* 2016; **351:** 543–44.

102 Retallack H, Di Lullo E, Arias C, et al. Zika virus cell tropism in the developing human brain and inhibition by azithromycin. *Proc Natl Acad Sci USA* 2016; **113:** 14408–13.

103 Maurice J. The Zika virus public health emergency: 6 months on. *Lancet* 2016; **388:** 449–50.

104 American Association for Blood Banks. A joint statement to the Food and Drug Administration's blood product advisory commitee. Nov 18, 2016. https://www.aabb.org/advocacy/statements/Documents/statement161118.pdf (accessed May 25, 2017).

105 Vouga M, Musso D, Schaub B, Panchaud A, Baud D. Zika virus: are we going too far? *Lancet* 2017; **389:** 151.

106 Castro MC. Zika virus and the 2016 Olympic Games—evidence-based projections derived from dengue do not support cancellation. *Travel Med Infect Dis* 2016; **14:** 384–88.

107 WHO. Fifth meeting of the emergency committee under the international health regulations (2005) regarding microcephaly, other neurological disorders and Zika virus. November 18, 2016. http://www.who.int/mediacentre/news/statements/2016/zika-fifth-ec/en/ (accessed May 25, 2017).