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SUPPLEMENT ARTICLE

Modes of Transmission of Zika Virus

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For >60 years, Zika virus (ZIKV) has been recognized as an arthropod-borne virus with *Aedes* species mosquitoes as the primary vector. However in the past 10 years, multiple alternative routes of ZIKV transmission have been identified. We review the available data on vector and non-vector-borne modes of transmission and interventions undertaken, to date, to reduce the risk of human infection through these routes. Although much has been learned during the outbreak in the Americas on the underlying mechanisms and pathogenesis of non-vector-borne ZIKV infections, significant gaps remain in our understanding of the relative incidence of, and risk from, these modes compared to mosquito transmission. Additional research is urgently needed on the risk, pathogenesis, and effectiveness of measures to mitigate non-vector-borne ZIKV transmission.

Keywords. Zika virus; transmission modes; *Aedes* mosquitoes; pathogenesis.

Since the initial isolation of Zika virus (ZIKV) in 1947 as part of investigations by the Rockefeller Institute to characterize the yellow fever transmission cycle and its identification the following year in canopy-dwelling *Aedes africanus* mosquitoes [1], ZIKV has been recognized as an arthropod-borne virus (arbovirus). Since then, multiple confirmed, probable, and potential secondary modes of transmission have been identified. In this article, we review the available data on known and possible transmission modes of ZIKV and preventive measures to reduce the risk of human-to-human spread.

MOSQUITO TRANSMISSION

Epidemiology

In Africa, ZIKV circulates in a sylvatic transmission cycle involving nonhuman primates (NHPs) and forest-dwelling *Aedes* species mosquitoes. As with yellow fever, human exposure to ZIKV may occur from mosquitoes participating in this sylvatic transmission cycle. In Asia, no evidence exists for a sylvatic transmission cycle, but surveillance for sylvatic arboviruses is lacking in that region. In addition, ZIKV joins yellow fever, chikungunya, and dengue viruses as a select group of emerging arboviruses capable of sustained transmission in an urban human–mosquito–human transmission cycle.

The extent of viral transmission to humans that results from exposure to the sylvatic transmission cycle has not been well defined, in part because of lack of surveillance as well as serologic cross-reactivity with other circulating flaviviruses. In asymptomatic African residents, seroprevalences of anti-Zika antibodies as high as 26% have been observed in Angola [2] A recent study examining blood samples collected over 20 years from 3 distinct cohorts of clinic patients showed that 5%–8% had serologic evidence of past Zika virus infection by immunoglobulin M (IgM) enzyme-linked immunosorbent assay (ELISA) [3]. However, it cannot be definitely determined to what extent this exposure resulted from sylvatic vs urban transmission and to what extent serologic cross-reactivity influenced the results. In recent years, as the virus has spread to the Pacific Islands and the Americas, the urban transmission cycle has accounted for hundreds of thousands of recorded cases.

Biology and Pathophysiology

Several mosquito species primarily belonging to the *Aedes* (*Stegomyia*), *Aedes* (*Diceromyia*), *and Aedes* (*Fredwardsius*) subgenera, including *Aedes africanus*, *Aedes luteocephalus*, *Aedes furcifer*, and *Aedes vittatus*, are likely enzootic vectors in Africa [4–7]. Little is known about the epidemiology and potential animal reservoirs for ZIKV in Africa. Serological evidence of Zika virus exposure in NHPs has been observed in several sub-Saharan countries including the Central African Republic, Ethiopia, Gabon, Nigeria, Senegal, and Uganda [8]. A recent study indicated that up to 16% of some populations of wild African green monkeys and baboons have been exposed to ZIKV, even in areas where ZIKV infections in humans have not been observed [9]. While evidence indicates that NHPs play a critical role in viral transmission in Africa, serological investigations demonstrating antibodies against Zika virus in many animal species raise questions about the relative importance of other animal species [10]. Limited data exist as to the level of host competence present in NHPs during natural infection, but viremia levels in experimental NHP models of infection have been in a range compatible with the ability to infect a feeding

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mosquito for other flaviviruses [11]. Modeling work is supportive of the potential to establish sylvatic ZIKV transmission in a wide range of potential settings [12].

Aedes aegypti has been linked with nearly all known urban Zika virus outbreaks, although two other *Aedes* (*Stegomyia*) species, *Aedes hensilli* and *Aedes polynesiensis*, were thought to be vectors in outbreaks in Yap State, Federated States of Micronesia [13] and in French Polynesia [14], respectively. *Aedes aegypti* is widely distributed throughout the tropical and subtropical world. In the United States, *Aedes aegypti* is endemic throughout Puerto Rico and the US Virgin Islands and in parts of the contiguous United States and Hawaii [15, 16]. Despite the presence of *Ae. aegypti* in many areas of the contiguous United States, as of April 2017, autochthonous mosquito-borne transmission has only occurred in Florida and southern Texas, which suggests that other unknown factors greatly influence transmission. This finding is consistent with a similar restricted transmission range observed in the contiguous United States for dengue virus, another flavivirus also transmitted by *Ae. aegypti* [17, 18]. Investigations of dengue outbreaks in the continental United States have indicated that socioeconomic and infrastructure factors such as the use of air-conditioning is associated with lower levels of infection [17, 19].

Despite its association with outbreaks, studies examining competency of *Ae. aegypti* to transmit ZIKV, usually determined by a high proportion of infected mosquitoes with infectious saliva following ingestion of an infected blood meal, have yielded mixed results. Some studies have demonstrated high competency [20], others low competency [5, 21], and yet others variable competency depending on the geographic origin of the *Ae. aegypti* tested, Zika viral strain, or mode of mosquito infection [22–24]. Two studies suggested higher vector competence of *Ae. aegypti* infected with African compared to Asian genotype strains [22, 23, 25]. Regardless, *Ae. aegypti* is thought to have high vectorial capacity (the overall ability of a vector species to transmit a pathogen in a given location and at a specific time) as it primarily feeds on humans, often bites multiple humans in a single blood meal, and lives in close association with human habitation [26]. Vertical transmission of Zika virus in *Ae. aegypti* has been demonstrated, but its role in maintaining the virus is unknown [27, 28].

ZIKV has infrequently been identified from >25 mosquito species [4, 29, 30] and, in particular, the role of *Aedes albopictus* and *Culex* species mosquitoes in viral transmission has been debated. *Aedes aegypti* and *Ae. albopictus* are the only known *Aedes* (*Stegomyia*) species endemic in the Americas. The concern about the vector potential of *Ae. albopictus* stems from the fact that its range extends beyond that of *Ae. aegypti* to more temperate climates, thus potentially expanding the areas at risk for outbreaks. The authors of one study in Gabon speculated that *Ae. albopictus* may have been a vector in an area with scarce *Ae. aegypti* and abundant *Ae. albopictus* populations and where mosquito testing identified ZIKV infections only in *Ae. albopictus* [31]. However, mosquito-borne transmission has not been detected in areas of the United States and Europe endemic for *Ae. albopictus* but not *Ae. aegypti* despite the influx of thousands of infected travelers returning to those areas from areas of ongoing transmission in 2016. Vector competence studies for *Ae. albopictus* have yielded mixed results, demonstrating high [32], medium [33, 34], or low competence for ZIKV [21, 23]. Differing conditions used in these studies including the source and treatment of the ZIKV used, the mosquito populations employed, and route of infection (artificial blood meals vs feeding on viremic animals) likely explain at least some of the variability in these results. As *Ae. albopictus* does not live in such close proximity to humans as *Ae. aegypti* and does not preferentially feed on humans, it is thought to have a lower inherent vectorial capacity than *Ae. aegypti*. The potential role of *Culex* mosquitoes as ZIKV vectors has been debated [35]. One study suggested *Culex quinquefasciatus* as a competent vector [36]; however, many other studies have failed to demonstrate vector competence of *Culex pipiens* [25, 37–39], *Cx. quinquefasciatus* [25, 33, 39–41], and *Culex tarsalis* [25].

Knowledge Gaps and Future Research Directions

Little is known about the enzootic transmission cycles for ZIKV. Particular areas of future emphasis include further definition of the enzootic cycle in Africa and its impact on human infection incidence, determining whether an enzootic transmission cycle exists in Asia, and whether an enzootic transmission cycle will develop in the Americas. Although inoculation of a large number of North American animal species failed to find any that developed sufficient viremia to implicate them as a potential reservoir species [10], further work globally could help determine the importance, if any, of other animal species beyond NHPs in maintaining the virus. Finally, given the importance of *Ae. aegypti* as a transmission vector, further development of effective prevention and control measures are important priorities.

SEXUAL TRANSMISSION

Epidemiology

Possible sexual transmission of ZIKV was initially reported in 2008 [42]. Six days after returning home from Senegal (an area with known ZIKV presence), an American scientist developed hematospermia and other symptoms compatible with ZIKV disease. Four days later, his wife, who had not traveled internationally in the previous year and with whom he was sexually active shortly after his return, developed symptoms. In both patients, the results of serological analyses of acute- and convalescent-phase paired sera were consistent with ZIKV infection; however, efforts to detect ZIKV nucleic acid (RNA) and to isolate virus from sera were unsuccessful. Competent mosquito vectors for ZIKV have not been detected in the geographic region of residence of the scientist and his wife, and were absent

around their home upon specific investigation. Further evidence for the potential for sexual transmission was provided by the detection of a high ZIKV RNA load and isolation of cultured virus from semen samples of a patient also exhibiting hematospermia during an outbreak in French Polynesia in 2013 [43].

In 2016 early in the course of the outbreak in the Americas, experiences with travelers returning from areas of active transmission to North America and Europe confirmed sexual contact as a mode of transmission. As of 18 April 2017, 46 cases of sexually acquired Zika virus disease have been reported in the United States to the Centers for Disease Control and Prevention (CDC). While the vast majority of these transmissions occurred from symptomatic men to women, cases of male-to-male [44], female-to-male [45], and asymptomatic male-to-female transmission [46, 47] have also been documented. These findings spurred efforts to determine the frequency and duration (ie, persistence) of ZIKV shedding in genital fluids and especially semen, the risk of transmission through sexual contact, and the potential for ongoing Zika virus transmission sustained through sexual transmission.

Current CDC and World Health Organization (WHO) guidance recommends that men, regardless of presence or absence of ZIKV-compatible symptoms, wait at least 6 months following ZIKV exposure before cessation of barrier-protected sexual intercourse [48]. The CDC similarly recommends that women wait at least 8 weeks following ZIKV exposure. At this time, no cases of adverse outcome following periconceptual sexual transmission of ZIKV have been documented and all known cases of male-to-female sexual transmission would have been prevented by adherence to this guidance, but ongoing surveillance and further investigation are essential. Simple mathematical models suggest that sexual transmission of ZIKV is unlikely to be sustained in areas without ongoing mosquito-borne transmission [49].

Biology and Pathophysiology

The presence of potentially infectious ZIKV in human semen has been assessed in 2 ways: by the presence of ZIKV RNA detectable by reverse-transcription polymerase chain reaction (RT-PCR) and by the demonstration of virus by passage amplification in culture. Assessing persistence of virus by the presence of ZIKV RNA is unreliable as the presence of RNA may not necessarily represent the presence of infectious virus. The longest published time point after onset of illness that ZIKV RNA has been detected in semen has been reported to be 188 days [50]; unpublished data from individual case reports indicate even longer periods of detectability on very rare occasions. A study with serial specimen collection from 55 men from Puerto Rico who had laboratory-confirmed infection found that semen RNA concentrations in general declined steadily after illness onset. Among the 24 (44%) men with ZIKV RNA detected in any semen sample, the estimated median time until loss of RNA detection in semen was 34 days (95% confidence interval [CI], 28–41 days); by 81 days (95% CI, 64–98 days), the likelihood of detecting ZIKV RNA was only 5% [51]. The longest published time point after onset of illness that ZIKV was possibly culturable from semen was 69 days [52]; however, methodological issues make generalizing from this single report difficult. ZIKV RNA was detected in two cell culture samples 5–7 days after inoculation, but no data were reported on changes in ZIKV RNA copy number in the inocula vs in the supernatant of the cell culture passage such as by cytopathic effect, plaque formation, or serial transfer of infectious material. Other publications have reported culturing virus up to 24 days [43, 53–55] after illness onset.

It is unclear which semen component(s) contain infectious virus; however, ZIKV RNA has been detected in the semen of men without spermatocytes due to vasectomy [52] and azoospermia [47] and in semen from which spermatocytes have been removed by centrifugation [56]. However, ZIKV antigens have also been visualized within the head of spermatocytes by immunofluorescent staining [57] of a ZIKV-infected man, and ZIKV RNA has been detected directly by in situ hybridization in the head and flagella of spermatocytes from inoculated mice [58].

Data from experimental inoculation of adult type I and type II interferon receptor deficient (AG129) mice with an Asian ZIKV genotype isolated during the current Western hemisphere outbreak demonstrated a 75% (95% CI, 53%–89%; 15/20 males) seminal shedding rate of viable virus between 7 and 21 days postinoculation (dpi). Mean peak titers in semen from nonvasectomized male mice were 3.8 $\log_{10}$ plaque-forming units (PFU)/ejaculate (maximum: 5.6 $\log_{10}$) [59]. Infected male AG129 mice demonstrated a 50% sexual transmission rate to AG129 female mice at 7–19 days dpi as evidenced by isolation of virus from the brains of symptomatic mated females. Overall, 73% of male mice (8/11) were responsible for at least 1 sexual transmission event. Zika viral RNA was detected in semen through 56 dpi despite the last observation of infectious virus at 21 dpi, indicating prolonged RNA shedding in mouse seminal fluid following cessation of detection of infectious virus by plaque assay. Eight vasectomized AG129 mice were assessed for viral shedding and sexual transmission potential. In contrast to data generated from nonvasectomized mice, virus was only detected in 2 samples of seminal fluids. Titers of ZIKV in these 2 seminal samples were significantly lower (<2.2 $\log_{10}$ PFU/ejaculate); nevertheless, 2 of 7 (29%) matings of susceptible AG129 females that occurred within 24 hours of these 2 ZIKV-positive surrogate samplings resulted in symptomatic infections of mated female AG129 mice. Published data on semen from NHPs are limited to assessment of ZIKV RNA in seminal fluids of five rhesus macaques inoculated with a Thai ZIKV isolate [60]. These data demonstrated the presence of viral RNA at dpi 7 through 28 and observed increased ZIKV RNA copy numbers

in subsequent inoculated culture from semen obtained at dpi 7 and 14, providing an indication of the presence of infectious virus.

No human data exist on the infectious dose required for sexual transmission of ZIKV. Direct intravaginal inoculation of pregnant AG129 mice with 1000 PFU resulted in a 60% symptomatic infection rate. In contrast, 22% of nonpregnant females given the same intravaginal dose were infected [59]. In this mouse model of sexual transmission, levels of virus as low as 40–160 PFU per ejaculate (approximately 3–4 $\log_{10}$ RNA copies) resulted in sexual transmission [59]. Peak ZIKV titers in vasectomized male mice were significantly lower than titers observed in nonvasectomized males but did not translate to a statistically lower transmission rate in this study. Additional intravaginal mouse model development studies have demonstrated that intravaginal susceptibility is dependent upon the estrus status of the female mouse and that hormonal fluctuations can alter expression of putative ZIKV receptors on the surface of vaginal mucosal cells [61, 62]. Human data on the correlation between RNA copy and the presence of infectious virus in semen is limited, and this relationship likely varies between individuals and at different time periods after symptom onset. Absent this data and an understanding of the factors associated with female intravaginal susceptibility, it is not possible to make assessments of transmission risk for an individual person or couple.

Regarding women, data from case reports and from female participants in the Puerto Rican serial specimen study indicate that shedding of detectable ZIKV RNA in vaginal secretions is uncommon and limited in duration (usually <14 days) [51, 63–66]. Mouse models suggest that that vaginal shedding could be affected by the timing within the estrus/menstrual cycle [61] as well as pregnancy status [59]; however, inoculated female AG129 mice failed to demonstrate sexual transmission to male mice. Given that the one suspected case of female-to-male sexual transmission occurred in a period at the onset of the woman's menses, and mice do not shed their endometrial layer in a similar manner, this mouse model is likely not directly applicable to female-to-male human transmission potential.

Knowledge Gaps and Future Research Directions

In areas with widespread ongoing mosquito-borne transmission, assessing the contribution of sexual transmission to all ZIKV infections is exceptionally challenging. It is unknown how the per-act risk of sexual transmission from infected men compares to the per-bite risk of an infected mosquito or if infection through sexual contact confers any different risk for fetal injury than vector-borne infection. Studies investigating differential viral RNA signatures or vaginal secretory immune responses of women known to be infected by mosquito bite vs sexually could be useful in further characterizing the subsequent pathogenesis of alternative routes of infection.

INTRAUTERINE AND INTRAPARTUM TRANSMISSION

Epidemiology

Maternal ZIKV infection has been associated with adverse fetal and infant outcomes, including microcephaly, brain abnormalities, and visual and hearing deficits [67–72]; however, the potentially devastating effects following maternal infection during pregnancy have only been recently reported. Intrapartum transmission of ZIKV has been reported; however, the precise mechanism is unknown [73]. ZIKV has been demonstrated in vaginal secretions [74] and female-to-male sexual transmission has been reported [45]. Thus, it is conceivable that vertical transmission can occur through contact of infant mucosal membranes with ZIKV in blood and vaginal secretions during the birth process.

Biology and Pathophysiology

The precise pathogenesis of congenital transmission remains poorly understood. Human and animal studies indicate that the placenta and fetus are at risk for infection via transplacental transmission [59, 75–85]. Sexual transmission and ascending vaginal infection leading to congenital ZIKV infection have been demonstrated in a mouse study [85]. Most of the focus to date has been on the transplacental maternofetal transmission of Zika virus. The placenta plays a major role in preventing transmission of pathogens, acting as a physical barrier and through the innate and adaptive maternal immune response [86]. However, these protective features do not prevent all infections from reaching the fetus. As seen with other congenital infections such as *Toxoplasma gondii* and cytomegalovirus [87, 88], pathogens can bypass the physical and immune functions of the placenta and be transferred from maternal tissues to the fetus. Human and animal studies have explored mechanisms by which ZIKV may cause fetal infection, as well as the mechanisms responsible for adverse sequelae observed among infants with congenital ZIKV infection [59, 75–85].

Examination of the architecture of the placenta may yield important insight into possible mechanisms contributing to placental and fetal infection. A mature placenta weighs approximately 500 g and is comprised of the chorionic plate, the surface that faces the fetus, and the basal plate, which is in contact with the maternal endometrium or decidua [86]. Between these chorionic and basal plates is the intervillous space where thousands of fetally derived villi are bathed in maternal blood originating from maternally derived spiral arteries. Each fetal villus originates from the chorionic plate and is covered by a trophoblast cell layer consisting of syncytiotrophoblasts (STBs), subsyncytial cytotrophoblasts (CTBs), and a basement membrane. The villi are highly vascularized by fetal capillaries allowing for the exchange of oxygen and nutrients. Also, within the villi are Hofbauer cells (HBCs), highly vacuolated macrophages located in close proximity to fetal capillaries [80]. For transplacental transmission from the maternal to the fetal blood to

occur, ZIKV must somehow cross these multiple cellular layers or perhaps be transported. Some studies indicate that STBs are resistant to ZIKV replication [89]; however, other reports indicate that because ZIKV infection triggers vascular damage and apoptosis, the placenta may become more permeable [78, 82, 83], facilitating entry into CTBs [78, 81, 83]. Entry of ZIKV into the CTBs could result in propagation of infection into the villus stroma and infection of HBCs.

Once in the placenta, ZIKV has been shown to replicate in various cell types, including macrophages and fetal endothelial cells [76, 78–80]. ZIKV has been shown to infect HBCs in isolated cultures and placental explants. Given the location of HBCs within the villi, these cells can serve as a reservoir of ZIKV and disseminate ZIKV into fetal blood. Detection of ZIKV RNA in the chorionic plate, specifically within the HBCs and histiocytes in the intervillous spaces, has been observed in placental specimens from pregnancies with maternal ZIKV infections [75, 90].

Similar to other flaviviruses, studies indicate that Tyro3, Axl, Mert (TAM) receptors, members of the tyrosine kinase family, may play a role in promoting ZIKV entry into placental cells [79, 91]. Axl is expressed in several cell types present at the maternofetal interface that are known to be susceptible to ZIKV infection, including trophoblasts, endothelial cells, fibroblasts, and HBCs. In addition, TAM receptors, once activated can dampen the innate immune response, and inhibit type I interferon, which can block its antiviral effects [84]. TIM1, a member of the T cell immunoglobulin and mucin domain protein family that regulates innate and adaptive immune functions and cell survival, has also been suggested as an important entry co-factor, facilitating entry of ZIKV into placental cells.

Knowledge Gaps and Future Research Directions

While much has been learned about ZIKV over the past year, many questions remain about the pathophysiology of maternofetal transmission. Future research should include an emphasis on studying the basic science of maternofetal transmission, differences in frequency of transmission based on gestational timing of infection, and correlation of maternal serum ZIKV viral load with the risk of infection. Additional research could greatly inform the understanding of risk associated with ZIKV infection during pregnancy and could provide insight into the relationship between fetal infection and adverse outcomes. Given the absence of histopathological findings in placental tissues where ZIKV is detected by RT-PCR [92], further examination of and elucidation of placental pathology may improve understanding on the mechanism of placental infection and of maternofetal transmission.

BLOOD TRANSFUSION

Investigations in Brazil have detected 3 probable transmissions of ZIKV associated with platelet transfusion, although mosquito-borne transmission could not be definitively ruled out in these cases and none of the recipients experienced clinical disease [93, 94]. Transmission of flaviviruses closely related to ZIKV, such as dengue virus, yellow fever vaccine virus, and West Nile virus (WNV), via blood product transfusion has been well documented. Following confirmation of 23 WNV infections from transfusion of blood products in the United States in 2002 [95], universal screening of blood products in the United States by a sensitive nucleic acid test (NAT) has been routinely performed. In addition, ZIKV RNA has been detected in 2.8% (42/1505) of asymptomatic blood donors by reverse-transcription polymerase chain reaction (RT-PCR) during the 2013–2014 ZIKV outbreak in French Polynesia [96].

Prevention of transmission of ZIKV requires either pathogen reduction of blood products or identification of potentially infectious blood products through laboratory screening and removal of those products from distribution. Pathogen reduction technology (PRT) has been demonstrated to be effective in inactivating ZIKV for both platelet and plasma [97] and red blood cell components [98]; however, PRT is currently only US Food and Drug Administration (FDA)–approved for plasma and apheresis platelets. The FDA issued updated guidance for industry to reduce the risk of transfusion-related transmission of ZIKV in August 2016 [99]. The new recommendations call for blood collection establishments in all states and US territories to screen individual units of donated whole blood and blood components with a ZIKV screening test authorized for use by FDA under an investigational new drug (IND) application, or with a licensed test when available. Alternatively, PRT may be used for plasma and certain platelet products.

Following identification of local transmission of ZIKV in Puerto Rico in December 2015, initial efforts to protect the safety of the blood supply involved the importation of blood products from unaffected areas in the continental United States and treatment of plasma and apheresis platelets with PRT, facilitated by the Department of Health and Human Services. Starting in early April 2016, individual donor screening for ZIKV by NAT under an IND authorization was implemented. By June 2016, 2 blood screening NATs were available under IND applications in the United States. During the peak of the outbreak, 1.8% of donated blood products in Puerto Rico had detectable ZIKV nucleic acid [100, 101]. To date, 325 presumptive viremic donors in Puerto Rico have been identified [102].

Since the availability of NAT screening assays under IND, 219 blood presumptive viremic donors in the continental United States and Hawaii have been identified. The first 14 probable ZIKV infections identified in blood donors from the continental United States were described in detail by Galel and colleagues [103]. All probable infected donations were collected in Florida, and 10 of 14 donors reported travel to an area with active ZIKV transmission in the previous 90 days. Viral loads in these donations ranged from 1×10^3 to 8×10^6 copies/mL, 9 of

14 had anti-Zika IgM antibody present. The infectious dose of ZIKV necessary for transmission via transfusion and the effect of antibody on transmissibility are currently unknown.

LABORATORY EXPOSURE

One of the earliest documented human infections with ZIKV occurred in 1963 in an individual working in a Ugandan laboratory with a strain isolated from *Ae. africanus* mosquitoes [104]. However the location and route of transmission could not be definitively determined in this case as mosquito exposure was also present. In 1973, ZIKV was again isolated from a blood sample from a laboratorian working with arboviruses, this time in the absence of a potential vector-borne route of transmission [105]. In 1980, the American Committee on Arthropod-Borne Viruses reported, without details, 3 additional suspected laboratory-acquired ZIKV infections identified through global laboratory safety surveys conducted in 1976 and 1979 [106]. During the current outbreak, a laboratorian in Pennsylvania developed a symptomatic laboratory-confirmed ZIKV infection following a needle-stick injury in the absence of other apparent routes of transmission [107]. BSL-2 practices, containment equipment, and facilities are recommended for activities with human diagnostic specimens [108].

ORGAN AND TISSUE TRANSPLANTATION AND OTHER POTENTIAL ROUTES OF TRANSMISSION

Transmission of ZIKV by organ transplantation has not yet been documented, but concern exists based on previous documented transmission of WNV through solid organ transplantation and the often immunosuppressed status of transplant recipients. Testing of donor serum for WNV has had limited utility in excluding the risk for transplantation transmission with 50% of the 8 organ donors associated with virus transmission having negative NAT results prior to organ recovery [109]. The risk for ZIKV transmission through organ transplantation is unknown, but plausible. The Organ Procurement and Transplantation (OPTN) and United Network for Organ Sharing (UNOS) have put out guidance on considerations for evaluating potential organ donors given this potential risk [110, 111]. Data on outcomes of ZIKV infection in solid organ transplant recipients are currently limited to a small number of case reports from non-transplant-associated infections [112, 113].

Transmission via tissue transplantation has never been demonstrated for any flavivirus, but WNV RNA was identified in skin, fat, muscle, tendon, and bone marrow from a deceased donor who was associated with WNV transmission through solid organ transplantation [114]. However, WNV could not be cultured from the RNA-positive tissues. Concern exists for ZIKV due to prolonged RNA detection in reproductive and eye tissues and the lack of processing for certain tissue types, including semen. Donor deferral recommendations have therefore been issued by FDA for both living and cadaveric donors [115], but the use of donor screening tests have not been recommended to date, in part due to an absence of data on the performance of available assays for this purpose and specimen type.

OTHER POTENTIAL ROUTES

Nucleic acid from flaviviruses including dengue virus and WNV have been detected in breast milk, but cases of confirmed or probable transmission events from breastfeeding appear to be very rare [116, 117]. Recent case reports have identified at least 9 lactating mothers with ZIKV RNA detected in breast milk samples, 3 of which had infectious viral particles as evidenced by culture [73, 118–121]. Two of the mothers without cultured virus had infants with evidence of ZIKV infection. In neither of these cases were the data sufficient to establish the route of infection for the newborn. In an additional case, complete genome sequences that differed only by two synonymous nucleotide substitutions were obtained from maternal breast milk and the urine of her 5-month-old breastfeeding infant [121]. After careful review of the available evidence, WHO and CDC concluded that the benefits of breastfeeding outweigh any potential risk [122].

ZIKV RNA has been detected on multiple occasions in a variety of other bodily specimens including urine, saliva, amniotic fluid, female genital tract secretions, cerebrospinal fluid, aqueous humor, conjunctival fluid, and nasopharyngeal swabs [64, 123–128]. Infectious viral particles have been documented occasionally in some of these specimens, but have not been linked to transmission events to date, with the possible exception of 1 case of female-to-male sexual transmission [45]. A case of locally acquired ZIKV infection in an area without competent mosquito vectors was extensively investigated without identification of the probable source of infection or additional transmission events [129]. The index patient in this instance had a viral load that was approximately 100 000 times that of the average ZIKV infection. The case patient reported hugging and kissing the index patient in a similar fashion to other family contacts, and assisted hospital personnel in holding the index patient as he was being cleaned, but did not have direct contact with body fluids. No other infections in family contacts or healthcare workers could be identified, suggesting an unknown, but rare, person-to-person transmission event. These findings reinforce the continued need for healthcare workers to follow standard precautions when handling body fluids from patients.

CONCLUSIONS

While mosquito-borne transmission still appears to account for the vast majority of ZIKV infections globally, the recent large outbreaks of the disease have identified several additional routes of transmission for this virus. These multiple possible routes of transmission have considerably increased the complexity of responding to the ongoing outbreak. Public health prevention efforts have needed to expand beyond the

traditional cornerstone of vector control for arthropod-borne diseases to encompass strategies to prevent blood-borne, congenital, and sexual transmission. Additional research is urgently needed to develop interventions to mitigate non-vector borne ZIKV transmission. This includes information on what additional routes of transmission can occur, the risk of various fluids to transmit ZIKV, the relative risk for each mode of transmission, and the potential pathogenesis following each potential mode of transmission.

Notes

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References

1. Dick GW, Kitchen SF, Haddow AJ. Zika virus. I. Isolations and serological specificity. Trans R Soc Trop Med Hyg **1952**; 46:509–20.
2. Kokernot RH, Casaca VM, Weinbren MP, McIntosh BM. Survey for antibodies against arthropod-borne viruses in the sera of indigenous residents of Angola. Trans R Soc Trop Med Hyg **1965**; 59:563–70.
3. Herrera BB, Chang CA, Hamel DJ, et al. Continued transmission of Zika virus in humans in West Africa, 1992–2016. J Infect Dis **2017**; 215:1546–50.
4. Diallo D, Sall AA, Diagne CT, et al. Zika virus emergence in mosquitoes in southeastern Senegal, 2011. PLoS One **2014**; 9:e109442.
5. Diagne CT, Diallo D, Faye O, et al. Potential of selected Senegalese *Aedes* spp. mosquitoes (Diptera: Culicidae) to transmit Zika virus. BMC Infect Dis **2015**; 15:492.
6. Fagbami AH. Zika virus infections in Nigeria: virological and seroepidemiological investigations in Oyo State. J Hyg (Lond) **1979**; 83:213–9.
7. Akoua-Koffi C, Diarrassouba S, Benie VB, et al. Investigation surrounding a fatal case of yellow fever in Cote d'Ivoire in 1999. Bull Soc Pathol Exot **2001**; 94:227–30.
8. Musso D, Gubler DJ. Zika virus. Clin Microbiol Rev **2016**; 29:487–524.
9. Buechler CR, Bailey AL, Weiler AM, et al. Seroprevalence of Zika virus in Wild African green monkeys and baboons. mSphere **2017**; 2. doi:10.1128/mSphere.00392-16.
10. Ragan IK, Blizzard EL, Gordy P, Bowen RA. Investigating the potential role of North American animals as hosts for Zika virus. Vector Borne Zoonotic Dis **2017**; 17:161–4.
11. Haddow AD, Nalca A, Rossi FD, et al. High infection rates for adult macaques after intravaginal or intrarectal inoculation with Zika virus. Emerg Infect Dis **2017**; 23:1274–81.
12. Althouse BM, Vasilakis N, Sall AA, Diallo M, Weaver SC, Hanley KA. Potential for Zika virus to establish a sylvatic transmission cycle in the Americas. PLoS Negl Trop Dis **2016**; 10:e0005055.
13. Ledermann JP, Guillaumot L, Yug L, et al. *Aedes hensilli* as a potential vector of chikungunya and Zika viruses. PLoS Negl Trop Dis **2014**; 8:e3188.
14. Musso D, Nilles EJ, Cao-Lormeau VM. Rapid spread of emerging Zika virus in the Pacific area. Clin Microbiol Infect **2014**; 20:O595–6.
15. Petersen LR, Jamieson DJ, Honein MA. Zika virus. N Engl J Med **2016**; 375:294–5.
16. Hahn MB, Eisen RJ, Eisen L, et al. Reported distribution of *Aedes (Stegomyia) aegypti* and *Aedes (Stegomyia) albopictus* in the United States, 1995–2016 (Diptera: Culicidae). J Med Entomol **2016**; 53:1169–75.
17. Radke EG, Gregory CJ, Kintziger KW, et al. Dengue outbreak in Key West, Florida, USA, 2009. Emerg Infect Dis **2012**; 18:135–7.
18. Thomas DL, Santiago GA, Abeyta R, et al. Reemergence of dengue in southern Texas, 2013. Emerg Infect Dis **2016**; 22:1002–7.
19. Reiter P, Lathrop S, Bunning M, et al. Texas lifestyle limits transmission of dengue virus. Emerg Infect Dis **2003**; 9:86–9.
20. Li CX, Guo XX, Deng YQ, et al. Vector competence and transovarial transmission of two *Aedes aegypti* strains to Zika virus. Emerg Microbes Infect **2017**; 6:e23.
21. Chouin-Carneiro T, Vega-Rua A, Vazeille M, et al. Differential susceptibilities of *Aedes aegypti* and *Aedes albopictus* from the Americas to Zika virus. PLoS Negl Trop Dis **2016**; 10:e0004543.
22. Roundy CM, Azar SR, Rossi SL, et al. Variation in *Aedes aegypti* mosquito competence for Zika virus transmission. Emerg Infect Dis **2017**; 23:625–32.
23. Ciota AT, Bialosuknia SM, Zink SD, et al. Effects of Zika virus strain and *Aedes* mosquito species on vector competence. Emerg Infect Dis **2017**; 23:1110–7.
24. Pompon J, Morales-Vargas R, Manuel M, et al. A Zika virus from America is more efficiently transmitted than an Asian virus by *Aedes aegypti* mosquitoes from Asia. Sci Rep **2017**; 7:1215.
25. Weger-Lucarelli J, Rückert C, Chotiwan N, et al. Vector competence of American mosquitoes for three strains of Zika virus. PLoS Negl Trop Dis **2016**; 10:e0005101.
26. Gubler DJ. The global emergence/resurgence of arboviral diseases as public health problems. Arch Med Res **2002**; 33:330–42.
27. Ciota AT, Bialosuknia SM, Ehrbar DJ, Kramer LD. Vertical transmission of Zika virus by *Aedes aegypti* and *Ae. albopictus* mosquitoes. Emerg Infect Dis **2017**; 23:880–2.
28. 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.
29. Althouse BM, Hanley KA, Diallo M, et al. Impact of climate and mosquito vector abundance on sylvatic arbovirus circulation dynamics in Senegal. Am J Trop Med Hyg **2015**; 92:88–97.
30. Ayres CF. Identification of Zika virus vectors and implications for control. Lancet Infect Dis **2016**; 16:278–9.
31. Grard G, Caron M, Mombo IM, et al. Zika virus in Gabon (Central Africa)—2007: a new threat from *Aedes albopictus*? PLoS Negl Trop Dis **2014**; 8:e2681.
32. Wong PS, Li MZ, Chong CS, Ng LC, Tan CH. *Aedes (Stegomyia) albopictus* (Skuse): a potential vector of Zika virus in Singapore. PLoS Negl Trop Dis **2013**; 7:e2348.
33. Liu Z, Zhou T, Lai Z, et al. Competence of *Aedes aegypti*, *Ae. albopictus*, and *Culex quinquefasciatus* mosquitoes as Zika virus vectors, China. Emerg Infect Dis **2017**; 23:1085–91.
34. Di Luca M, Severini F, Toma L, et al. Experimental studies of susceptibility of Italian *Aedes albopictus* to Zika virus. Euro Surveill **2016**; 21. doi:10.2807/1560-7917.ES.2016.21.18.30223.
35. Leal WS. Zika mosquito vectors: the jury is still out. F1000Res **2016**; 5:2546.
36. Guo XX, Li CX, Deng YQ, et al. *Culex pipiens* quinquefasciatus: a potential vector to transmit Zika virus. Emerg Microbes Infect **2016**; 5:e102.
37. Heitmann A, Jansen S, Luhken R, et al. Experimental transmission of Zika virus by mosquitoes from central Europe. Euro Surveill **2017**; 22. pii:30437.
38. Boccolini D, Toma L, Di Luca M, et al. Experimental investigation of the susceptibility of Italian *Culex pipiens* mosquitoes to Zika virus infection. Euro Surveill **2016**; 21. doi:10.2807/1560-7917.ES.2016.21.35.30328.
39. Huang YJ, Ayers VB, Lyons AC, et al. *Culex* species mosquitoes and Zika virus. Vector Borne Zoonotic Dis **2016**; 16:673–6.
40. Dodson BL, Rasgon JL. Vector competence of *Anopheles* and *Culex* mosquitoes for Zika virus. Peer J **2017**; 5:e3096.
41. Fernandes RS, Campos SS, Ferreira-de-Brito A, et al. *Culex quinquefasciatus* from Rio de Janeiro is not competent to transmit the local Zika virus. PLoS Negl Trop Dis **2016**; 10:e0004993.
42. Foy BD, Kobylinski KC, Chilson Foy JL, et al. Probable non-vector-borne transmission of Zika virus, Colorado, USA. Emerg Infect Dis **2011**; 17:880–2.
43. 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.
44. Deckard DT, Chung WM, Brooks JT, et al. Male-to-male sexual transmission of Zika virus—Texas, January 2016. MMWR Morb Mortal Wkly Rep **2016**; 65:372–4.
45. 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**; 65:716–7.
46. Brooks RB, Carlos MP, Myers RA, et al. Likely sexual transmission of Zika virus from a man with no symptoms of infection—Maryland, 2016. MMWR Morb Mortal Wkly Rep **2016**; 65:915–6.
47. Freour T, Mirallie 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. doi:10.2807/1560-7917.ES.2016.21.23.30254.
48. 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.
49. Yakob L, Kucharski A, Hue S, Edmunds WJ. Low risk of a sexually-transmitted Zika virus outbreak. Lancet Infect Dis **2016**; 16:1100–2.

50. Nicastri E, Castilletti C, Liuzzi G, Iannetta M, Capobianchi MR, Ippolito G. Persistent detection of Zika virus RNA in semen for six months after symptom onset in a traveller returning from Haiti to Italy, February 2016. Euro Surveill **2016**; 21. doi:10.2807/1560-7917.ES.2016.21.32.30314.
51. Paz-Bailey G, Rosenberg ES, Doyle K, et al. Persistence of Zika virus in body fluids—preliminary report. N Engl J Med **2017**. doi:10.1056/NEJMoa1613108.
52. Arsuaga M, Bujalance SG, Díaz-Menéndez M, Vázquez A, Arribas JR. Probable sexual transmission of Zika virus from a vasectomised man. Lancet Infect Dis **2016**; 16:1107.
53. D'Ortenzio E, Matheron S, Yazdanpanah Y, et al. Evidence of sexual transmission of Zika virus. N Engl J Med **2016**; 374:2195–8.
54. Mansuy JM, Dutertre M, Mengelle C, et al. Zika virus: high infectious viral load in semen, a new sexually transmitted pathogen? Lancet Infect Dis **2016**; 16:405.
55. Jang HC, Park WB, Kim UJ, et al. First imported case of Zika virus infection into Korea. J Korean Med Sci **2016**; 31:1173–7.
56. Barzon L, Pacenti M, Franchin E, et al. Infection dynamics in a traveller with persistent shedding of Zika virus RNA in semen for six months after returning from Haiti to Italy, January 2016. Euro Surveill **2016**; 21. doi:10.2807/1560-7917.ES.2016.21.32.30316.
57. Mansuy JM, Suberbielle E, Chapuy-Regaud S, et al. Zika virus in semen and spermatozoa. Lancet Infect Dis **2016**; 16:1106–7.
58. Govero J, Esakky P, Scheaffer SM, et al. Zika virus infection damages the testes in mice. Nature **2016**; 540:438–42.
59. Duggal NK, Ritter JM, Pestorius SE, et al. Frequent Zika virus sexual transmission and prolonged viral RNA shedding in an immunodeficient mouse model. Cell Rep **2017**; 18:1751–60.
60. Osuna CE, Lim SY, Deleage C, et al. Zika viral dynamics and shedding in rhesus and cynomolgus macaques. Nat Med **2016**; 22:1448–55.
61. Tang WW, Young MP, Mamidi A, Regla-Nava JA, Kim K, Shresta S. A mouse model of Zika virus sexual transmission and vaginal viral replication. Cell Rep **2016**; 17:3091–8.
62. Pierce A, Bliesner B, Xu M, et al. Axl and Tyro3 modulate female reproduction by influencing gonadotropin-releasing hormone neuron survival and migration. Mol Endocrinol **2008**; 22:2481–95.
63. Prisant N, Breurec S, Moriniere C, Bujan L, Joguet G. Zika virus genital tract shedding in infected women of childbearing age. Clin Infect Dis **2017**; 64:107–9.
64. Prisant N, Bujan L, Benichou H, et al. Zika virus in the female genital tract. Lancet Infect Dis **2016**; 16:1000–1.
65. Nicastri E, Castilletti C, Balestra P, Galgani S, Ippolito G. Zika virus infection in the central nervous system and female genital tract. Emerg Infect Dis **2016**; 22:2228–30.
66. 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.
67. 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.
68. França GV, 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**; 388:891–7.
69. Reynolds MR, Jones AM, Petersen EE, et al. Vital signs: update on Zika virus-associated birth defects and evaluation of all U.S. infants with congenital Zika virus exposure—U.S. Zika Pregnancy Registry, 2016. MMWR Morb Mortal Wkly Rep **2017**; 66:366–73.
70. Moore CA, Staples JE, Dobyns WB, et al. Characterizing the pattern of anomalies in congenital Zika syndrome for pediatric clinicians. JAMA Pediatr **2017**; 171:288–95.
71. Ventura LO, Ventura CV, Lawrence L, et al. Visual impairment in children with congenital Zika syndrome. J AAPOS **2017**. doi:10.1016/j.jaapos.2017.04.003.
72. Martines RB, Bhatnagar J, Keating MK, et al. 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 **2016**; 65:159–60.
73. 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. pii:20751.
74. 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. doi:10.2807/1560-7917.ES.2017.22.3.30444.
75. Bhatnagar J, Rabeneck DB, Martines RB, et al. Zika virus RNA replication and persistence in brain and placental tissue. Emerg Infect Dis **2017**; 23:405–14.
76. Cao B, Diamond MS, Mysorekar IU. Maternal-fetal transmission of Zika virus: routes and signals for infection. J Interferon Cytokine Res **2017**; 37:287–94.
77. de Carvalho NS, de Carvalho BF, Doris B, Silverio Biscaia E, Arias Fugaca C, de Noronha L. Zika virus and pregnancy: an overview. Am J Reprod Immunol **2017**; 77. doi:10.1111/aji.12616.
78. 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.
79. Nowakowski TJ, Pollen AA, Di Lullo E, Sandoval-Espinosa C, Bershteyn M, Kriegstein AR. Expression analysis highlights AXL as a candidate Zika virus entry receptor in neural stem cells. Cell Stem Cell **2016**; 18:591–6.
80. Simoni MK, Jurado KA, Abrahams VM, Fikrig E, Guller S. Zika virus infection of Hofbauer cells. Am J Reprod Immunol **2017**; 77:e12613.
81. Aagaard KM, Lahon A, Suter MA, et al. Primary human placental trophoblasts are permissive for Zika virus (ZIKV) replication. Sci Rep **2017**; 7:41389.
82. Noronha Ld, Zanluca C, Azevedo ML, Luz KG, Santos CN. Zika virus damages the human placental barrier and presents marked fetal neurotropism. Mem Inst Oswaldo Cruz **2016**; 111:287–93.
83. Quicke KM, Bowen JR, Johnson EL, et al. Zika virus infects human placental macrophages. Cell Host Microbe **2016**; 20:83–90.
84. Vermillion MS, Lei J, Shabi Y, et al. Intrauterine Zika virus infection of pregnant immunocompetent mice models transplacental transmission and adverse perinatal outcomes. Nat Commun **2017**; 8:14575.
85. 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.e4.
86. Burton GJ, Fowden AL. The placenta: a multifaceted, transient organ. Philos Trans R Soc Lond B Biol Sci **2015**; 370:20140066.
87. Abbasi M, Kowalewska-Grochowska K, Bahar MA, Kilani RT, Winkler-Lowen B, Guilbert LJ. Infection of placental trophoblasts by *Toxoplasma gondii*. J Infect Dis **2003**; 188:608–16.
88. Cheeran MC, Lokensgard JR, Schleiss MR. Neuropathogenesis of congenital cytomegalovirus infection: disease mechanisms and prospects for intervention. Clin Microbiol Rev **2009**; 22:99–126.
89. Tabata T, Petitt M, Puerta-Guardo H, et al. Zika virus targets different primary human placental cells, suggesting two routes for vertical transmission. Cell Host Microbe **2016**; 20:155–66.
90. Rosenberg AZ, Yu W, Hill DA, Reyes CA, Schwartz DA. Placental pathology of Zika virus: viral infection of the placenta induces villous stromal macrophage (Hofbauer cell) proliferation and hyperplasia. Arch Pathol Lab Med **2017**; 141:43–8.
91. Hamel R, Dejarnac O, Wichit S, et al. Biology of Zika virus infection in human skin cells. J Virol **2015**; 89:8880–96.
92. Ritter JM, Martines RB, Zaki SR. Zika virus: pathology from the pandemic. Arch Pathol Lab Med **2017**; 141:49–59.
93. Motta IJ, Spencer BR, Cordeiro da Silva SG, et al. Evidence for transmission of Zika virus by platelet transfusion. N Engl J Med **2016**; 375:1101–3.
94. Barjas-Castro ML, Angerami RN, Cunha MS, et al. Probable transfusion-transmitted Zika virus in Brazil. Transfusion **2016**; 56:1684–8.
95. Pealer LN, Marfin AA, Petersen LR, et al. Transmission of West Nile virus through blood transfusion in the United States in 2002. N Engl J Med **2003**; 349:1236–45.
96. Musso D, Nhan T, Robin E, et al. Potential for Zika virus transmission through blood transfusion demonstrated during an outbreak in French Polynesia, November 2013 to February 2014. Euro Surveill **2014**; 19. pii:20761.
97. 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.
98. 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.
99. Food and Drug Administration. Revised recommendations for reducing the risk of Zika virus transmission by blood and blood components. https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/UCM518213.pdf. Accessed 3 May 2017.
100. Adams L, Bello-Pagan M, Lozier M, et al. Update: Ongoing Zika virus transmission—Puerto Rico, November 1, 2015–July 7, 2016. MMWR Morb Mortal Wkly Rep **2016**; 65:774–9.
101. 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**; 65:627–8.
102. Centers for Disease Control and Prevention. Case counts in the US. Atlanta, GA: CDC, **2017**.
103. Galel SA, Williamson PC, Busch MP, et al; Cobas Zika IND Study Group. First Zika-positive donations in the continental United States. Transfusion **2017**; 57:762–9.
104. Simpson DI. Zika virus infection in man. Trans R Soc Trop Med Hyg **1964**; 58:335–8.
105. Filipe AR, Martins CM, Rocha H. Laboratory infection with Zika virus after vaccination against yellow fever. Arch Gesamte Virusforsch **1973**; 43:315–9.
106. Subcommittee on Arbovirus Laboratory Safety of the American Committee on Arthropod-Borne Viruses. Laboratory safety for arboviruses and certain other viruses of vertebrates. Am J Trop Med Hyg **1980**; 29:1359–81.
107. Alleghany County Health Department. Health department reports fourth confirmed case of Zika in the county: virus contracted in an isolated lab incident. http://www.achd.net/pr/pubs/2016release/060916_zika.html. Accessed 3 May 2017.

108. Centers for Disease Control and Prevention. Laboratory safety when working with Zika virus. https://www.cdc.gov/zika/laboratories/lab-safety.html. Accessed 3 May 2017.
109. Winston DJ, Vikram HR, Rabe IB, et al; West Nile Virus Transplant-Associated Transmission Investigation Team. Donor-derived West Nile virus infection in solid organ transplant recipients: report of four additional cases and review of clinical, diagnostic, and therapeutic features. Transplantation **2014**; 97:881–9.
110. Organ Procurement and Transplantation Network. Guidance on Zika virus. https://optn.transplant.hrsa.gov/news/guidance-on-zika-virus/. Accessed 3 May 2017.
111. United Network for Organ Sharing. Guidance for organ donation and transplantation professionals regarding the Zika virus. https://www.unos.org/guidance-for-organ-donation-and-transplantation-professionals-regarding-the-zika-virus/. Accessed 3 May 2017.
112. Schwartzmann PV, Ramalho LN, Neder L, et al. Zika virus meningoencephalitis in an immunocompromised patient. Mayo Clin Proc **2017**; 92:460–6.
113. Nogueira ML, Estofolete CF, Terzian AC, et al. Zika virus infection and solid organ transplantation: a new challenge. Am J Transplant **2017**; 17:791–5.
114. Blau DM, Rabe IB, Bhatnagar J, et al; West Nile Virus Transplant-Associated Transmission Investigation Team. West Nile virus RNA in tissues from donor associated with transmission to organ transplant recipients. Emerg Infect Dis **2013**; 19:1518–20.
115. US Food and Drug Administration. Donor screening recommendations to reduce the risk of transmission of Zika virus by human cells, tissues, and cellular and tissue-based products. https://www.fda.gov/downloads/biologicsbloodvaccines/guidancecomplianceregulatoryinformation/guidances/tissue/ucm488582.pdf. Accessed 3 May 2017.
116. Centers for Disease Control and Prevention (CDC). Transmission of yellow fever vaccine virus through breast-feeding—Brazil, 2009. MMWR Morb Mortal Wkly Rep **2010**; 59:130–2.
117. Hinckley AF, O'Leary DR, Hayes EB. Transmission of West Nile virus through human breast milk seems to be rare. Pediatrics **2007**; 119:e666–71.
118. Dupont-Rouzeyrol M, Biron A, O'Connor O, Huguon E, Descloux E. Infectious Zika viral particles in breastmilk. Lancet **2016**; 387:1051.
119. Cavalcanti MG, Cabral-Castro MJ, Gonçalves JL, Santana LS, Pimenta ES, Peralta JM. Zika virus shedding in human milk during lactation: an unlikely source of infection? Int J Infect Dis **2017**; 57:70–2.
120. Sotelo JR, Sotelo AB, Sotelo FJB, et al. Persistence of Zika virus in breast milk after infection in late stage of pregnancy. Emerg Infect Dis **2017**; 23:856–7.
121. Blohm GM, Lednicky JA, Marquez M, et al. Complete genome sequences of identical Zika virus isolates in a nursing mother and her infant. Genome Announc **2017**; 5. pii:e00231-17.
122. WHO Guidelines Approved by the Guidelines Review Committee. Guideline: infant feeding in areas of Zika virus transmission. Geneva, Switzerland: WHO, **2016**.
123. Bonaldo MC, Ribeiro IP, Lima NS, et al. Isolation of infective Zika virus from urine and saliva of patients in Brazil. PLoS Negl Trop Dis **2016**; 10:e0004816.
124. 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.
125. Sun J, Wu, Zhong H, et al. Presence of Zika virus in conjunctival fluid. JAMA Ophthalmol **2016**; 134:1330–2.
126. Rozé B, Naijioullah F, Fergé J, et al. Zika virus detection in urine from patients with Guillain-Barré syndrome on Martinique, January 2016. Euro Surveill **2016**; 21:30154.
127. Furtado JM, Espósito DL, Klein TM, Teixeira-Pinto T, da Fonseca BA. Uveitis associated with Zika virus infection. N Engl J Med **2016**; 375:394–6.
128. Fonseca K, Meatherall B, Zarra D, et al. First case of Zika virus infection in a returning Canadian traveler. Am J Trop Med Hyg **2014**; 91:1035–8.
129. Brent C, Dunn A, Savage H, et al. Preliminary findings from an investigation of zika virus infection in a patient with no known risk factors—Utah, 2016. MMWR Morb Mortal Wkly Rep **2016**; 65:981–2.