RAPID RISK ASSESSMENT

Zika virus disease epidemic: potential association with microcephaly and Guillain–Barré syndrome

Fifth update, 11 April 2016

Main conclusions and options for response

Considering the continued spread of Zika virus in the Americas and Caribbean, the strong evidence of an association between Zika virus infection during pregnancy and congenital central nervous system malformations, the association between Zika virus infection and Guillain—Barré syndrome, and the risk of establishment of local vector-borne transmission in Europe during the 2016 summer season, EU/EEA Member States are recommended to consider a range of mitigation measures.

The following uncertainties have been taken into consideration in developing the proposed options for response:

- At the present time, there is a lack of evidence on which stage of the pregnancy the foetus is most vulnerable to Zika virus infection. Therefore the entire duration of pregnancy should be considered at risk.
- The presence of infectious Zika virus in semen has been detected up to three weeks after onset of disease; the longest interval reported between the onset of symptoms in a male and the subsequent onset of the disease thought to be due to sexual transmission in a female partner is 19 days.
- The role of asymptomatic males in the sexual transmission to women is unknown.
- The roles of different mosquito species as potential vectors of Zika virus should be clarified. If current assumptions prove inaccurate or incorrect, vector control strategies have to be adapted and revised.

Information to travellers and EU residents in affected areas

Over the past two months, as of 4 April 2016, autochthonous cases of Zika virus infection have been reported from 45 countries or territories worldwide. In the past nine months, 47 countries or territories have reported autochthonous cases of Zika virus infection.

A list of countries and territories with documented autochthonous transmission during the past two months is available on the ECDC website.

Information for travellers and EU citizens residing in areas with active transmission

- Travellers visiting countries where there is active transmission of Zika virus should be made aware of the ongoing outbreak of Zika virus infection. A list of countries and territories with documented autochthonous transmission during the past two months is available on the ECDC website.

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- Travellers visiting these countries, and EU citizens residing in these countries, should take measures to prevent mosquito bites indoors and outdoors, especially from sunrise to sunset when *Aedes* mosquito vectors are most active in biting. These measures include:
  - The use of mosquito repellent in accordance with the instructions indicated on the product label.
  - Wearing long-sleeved shirts and long trousers, especially during the hours when the type of mosquito that is known to transmit the Zika virus (*Aedes*) is most active.
  - Sleeping or resting in screened or air-conditioned rooms, otherwise use mosquito nets, even during the day.
- Pregnant women and women who are planning to become pregnant should consider postponing non-essential travel to affected areas until after delivery.
- Pregnant women who plan to travel to affected areas and pregnant women residing in affected areas should consult their healthcare providers for advice and follow strict measures to prevent mosquito bites.
- Travellers with immune disorders or severe chronic illnesses should consult their doctor or seek advice from a travel clinic before travelling, particularly on effective prevention measures.
- Travellers to Zika-affected areas and EU citizens residing in affected areas should be advised that using condoms could reduce the risk of sexual transmission through semen.

Information for travellers returning from areas with transmission of Zika virus

- Pregnant women who have travelled or resided in areas with Zika virus transmission should mention their travel during antenatal visits in order to be assessed and monitored appropriately.
- In order to protect the foetus, male travellers returning from affected areas should consider using a condom with a pregnant partner until the end of pregnancy.
- Travellers showing symptoms compatible with Zika virus disease within two weeks of return from an affected area are advised to contact their healthcare provider and mention their recent travel.
- On 11 April, WHO published an update of its travel health advice on Zika virus in which they advise travellers returning from areas with ongoing Zika virus transmission to practise safer sex for at least one month after returning, in order to reduce the potential risk of onward sexual transmission. This WHO guidance will be reviewed and the recommendations updated as new evidence emerges.

Information to healthcare providers in EU Member States

Ensure that Zika virus-infected patients in areas with *Aedes* mosquito vectors avoid getting bitten during the first week of illness (bed nets, screened doors and windows as recommended by PAHO/WHO).

Increase awareness among health professionals who provide prenatal care of the possible association between Zika virus and microcephaly and adapt prenatal monitoring in accordance with the exposure to the vector.

In addition, due to the unprecedented size of the Zika virus epidemic, health services and practitioners should be alerted to the possible occurrence of neurological syndromes (Guillain–Barré syndrome and other neurological syndromes such as meningitis, meningoencephalitis and myelitis according to WHO/PAHO) and potential disease complications not yet described in the scientific literature, and atypical clinical presentation among specific populations (i.e. children, the elderly, immunocompromised individuals and those with sickle cell disease).

Source and date of request

ECDC internal decision, 31 March 2016.

Public health issue

This document assesses the risks associated with the Zika virus epidemic in currently affected countries (notably South and Central America and in the Caribbean). It assesses the association between Zika virus infection and congenital central nervous system (CNS) malformations, including microcephaly, as well as the association between Zika virus infection and Guillain–Barré syndrome (GBS).

To date, ECDC has published seven risk assessments related to Zika virus epidemics:

- 'Zika virus infection outbreak, French Polynesia', 14 February 2014 [1];
- 'Zika virus infection outbreak, Brazil and the Pacific region', 25 May 2015 [2];
- 'Microcephaly in Brazil potentially linked to the Zika virus epidemic', 24 November 2015 [3];
- 'Zika virus epidemic in the Americas: potential association with microcephaly and Guillain–Barré syndrome', 10 December 2015 [4];
- 'Zika virus disease epidemic: potential association with microcephaly and Guillain–Barré syndrome (first update) ', 21 January 2016 [5].
- 'Zika virus disease epidemic: potential association with microcephaly and Guillain–Barré syndrome (second update) ', 8 February 2016 [6].
- 'Zika virus disease epidemic: potential association with microcephaly and Guillain–Barré syndrome (third update)', 23 February 2016 [7]
- 'Zika virus disease epidemic: potential association with microcephaly and Guillain–Barré syndrome (fourth update)', 9 March 2016 [8].

ECDC issues this risk assessment document according to Article 7(1) of Regulation (EC) No 851/2004 establishing a European centre for disease prevention and control. In the framework of ECDC's mandate, the specific purpose of an ECDC risk assessment is to present different options on a certain matter. The responsibility on the choice of which option to pursue and which actions to take, including the adoption of mandatory rules or guidelines, lies exclusively with the EU/EEA Member States. In its activities, ECDC strives to ensure its independence, high scientific quality, transparency and efficiency.

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Disease background information

Zika virus disease is caused by an RNA virus transmitted to humans by *Aedes* mosquitoes, especially by the *Aedes aegypti* species. Up to 80 per cent of infections are asymptomatic [9]. Symptomatic infections are characterised by a self-limiting febrile illness of 4 to 7 days' duration accompanied by rash, arthralgia, myalgia and non-purulent conjunctivitis. Based on systematic review and pooled analysis on 25 Zika cases, the estimated median incubation period of Zika virus infection is 5.9 days (95% CI: 4.4–7.6) [10]. A recent study on 56 confirmed symptomatic Zika virus cases identified in Rio de Janeiro between April and June 2015 found the frequency of symptoms to be: exanthema (98%), headache (67%), fever (67%), arthralgia (58%), myalgia (49%), retro-orbital eye pain (40%), conjunctivitis (39%) and joint swelling (23%) [11].

Zika virus infection was linked to GBS for the first time in 2014 when a possible association between Zika virus infection and GBS was reported during an outbreak in French Polynesia [12]. Zika virus infection during pregnancy was first linked to congenital microcephaly in October 2015 in Brazil. It has since become clear that transplacental infections occur and that these infections can cause severe damage to the development of the foetus' central nervous system. What has not yet been established is how often maternal infections result in foetal infection, and congenital brain damage, and when during pregnancy the risk of adverse outcomes is highest. There is also a paucity of information regarding the spectrum of CNS damage caused by intrauterine infections, partly because of the short observation time of children born to mothers who had Zika infections during pregnancy. There is no vaccine to prevent Zika virus infections nor is any specific antiviral treatment available.

Hazin, et al reported the first series of computed tomography in 23 infants from Pernambuco state, Brazil, with congenital microcephaly and clinical and epidemiologic data compatible with congenital Zika virus infection [13]. All

mothers had symptoms of Zika virus infection during the first or second trimester of pregnancy. Enzyme-linked immunosorbent assay (ELISA) test results for Zika virus IgM antibodies in samples of cerebrospinal fluid was available for seven of the infants and all were positive. Serological tests for the TORCH group of congenital infections were negative for all 23 infants. All infants presented severe brain anomalies associated with disruption in brain development such as brain calcifications mainly at the corticomedullary junction, general cortical hypogyration, white-matter abnormalities and significant ventriculomegaly.

A clinical series of 35 congenital syndromes presumably associated with infection by Zika virus in Brazil has been published. This study presents clinical findings in comparison with TORCH infections [14].

Zika virus infection can be confirmed by direct detection of Zika virus RNA or specific viral antigens in clinical samples. Based on a modelling study, seroconversion occurs on average at 9.0 days (95% CI, 7.0–11.6) after infection but serological results should be interpreted with caution due to cross-reactivity with other flaviviruses and according to the vaccination status against flaviviruses [10,15]. It is estimated that Zika virus clearance in the blood is on average 9.9 days (95% CI: 6.8–21.4) [10]. Zika virus isolation from saliva has been reported on day six after onset of a febrile illness in a patient returning from the Dominican Republic to Italy [16]. The Zika virus RNA was detected for up to 29 days in saliva and urine and for up to 10 days in blood after onset of symptoms [16]. A second study assessed the occurrence of Zika viral particles in saliva and urine among nine suspected patients (including six pregnant women) from Rio de Janeiro [17]. Zika virus was isolated only in one urine sample and one saliva sample for two different patients. Further investigation would be needed to evaluate the infectivity of Zika virus in saliva as non-vector-borne viral transmission mode of Zika disease.

Onward transmission from imported cases within the continental EU is possible because *Aedes albopictus* might be considered a competent vector for the transmission of Zika virus, although a recent study showed an unexpected low vector competence of this species [18]. These findings are in line with a previous vector competence study from Diagne, et al. based on several *Aedes* mosquito species from West Africa (namely *Ae. aegypti*, *Ae. unlineatus*, *Ae. vittatus* and *Ae. luteocephalus*) using six Zika virus strains from Senegal [19]. Despite being susceptible to oral infection, a low transmission rate was observed for all the *Aedes* mosquito populations tested. Both these latter studies found a lower transmission rate than a Zika virus vector competence laboratory study using *Ae. aegypti* and *Ae. albopictus* mosquito populations from Singapore and Ugandan MR776 ZIKV strain at 14 days after inoculation [20,21]. Chouin-Carneiro, et al suggest that other factors such as the high density of human-biting mosquitoes and a naïve human population for Zika virus might contribute to the observed outbreak dynamics and explain the rapid spread of the disease in the Americas.

More information on Zika virus disease can be found in the previous risk assessments [1-8] and in the ECDC factsheet for health professionals. Four literature reviews have been published since the last risk assessment [22-25].

Event background information

Towards the end of 2014, Brazil detected a cluster of cases of febrile rash in the northeast region of the country. The diagnosis of Zika virus infection was confirmed in May 2015 by reverse transcriptase polymerase chain reaction (RT-PCR) assay. The Brazilian Ministry of Health estimated that in 2015 between 0.4 and 1.3 million cases of Zika virus infection occurred in the country, significantly exceeding the number of reported cases [26]. On 18 February 2016, Zika virus infection became a notifiable disease in Brazil [27]. As of 29 March 2016, all 27 Brazilian states report ongoing transmission of Zika virus.

Situation worldwide

Colombia is the second most affected country in the Americas. Since October 2015 and as of 26 March 2016, 58 790 suspected and 2 603 confirmed cases have been reported nationally. The epidemic in Colombia seems to have reached its peak, indicated by a steady decline in the number of suspected and confirmed cases reported per week since week 5 of 2016 [28].

As of 5 April 2016, thirteen cases of non-vector-borne transmission of Zika virus, probably through sexual transmission, have been reported by seven countries: Argentina (1), Chile (1), France (1), Italy (1), New Zealand (1), Portugal (in the Autonomous Region of Madeira) (1) and the United States of America (7).

Over the past two months, and as of 4 April 2016, autochthonous cases of Zika virus infection have been reported from 45 countries or territories worldwide. In the past nine months, 47 countries or territories have reported autochthonous cases of Zika virus infection. The latest information on the spread of the Zika virus epidemic and an update on adverse pregnancy outcomes and post-infectious GBS is available through the ECDC Zika outbreak webpage [29]. Regular updates on the epidemiological situation are available on an ECDC webpage entitled Countries and territories with local Zika transmission [30].

Situation in the EU/EEA and EU outermost regions and overseas countries and territories

As of 5 April 2016, no autochthonous vector-borne Zika virus transmission has been reported in the continental EU. ECDC is collecting data regarding imported cases through the media and official government communication lines. As of 5 April 2016, ECDC has recorded 359 imported cases in 17 EU/EEA countries. Twenty-three of the imported cases are pregnant women. In addition, information about one confirmed case was published following the diagnosis in a Slovenian hospital [31].The number of imported cases reported is not based on systematic reporting through surveillance systems and hence cannot be considered exhaustive.

Several outermost regions (OMR) and overseas countries and territories (OCT) continue to report autochthonous transmission: French Guiana, Guadeloupe, Martinique, Saint Martin and Sint Maarten.

Microcephaly and congenital central nervous system malformations

Only French Polynesia and Brazil have reported an increasing number of congenital CNS malformations concomitant with the Zika virus infection outbreak [32]. Since the last ECDC Rapid Risk Assessment on 9 March 2016, Cape Verde, Martinique and Panama have reported cases of congenital CNS malformation concomitant with Zika virus circulation, although these are not of sufficient number to assess whether they represent an increase over background rates.

Brazil

Between 22 October 2015 and 2 April 2016, 6 906 cases of microcephaly and/or CNS malformations were reported by Brazil. This contrasts with the period from 2001 to 2014, when an average of 163 microcephaly cases was recorded nationally per year. Of the 6 906 cases, investigations have been concluded for 2 860 cases and 1 046 were suggestive of congenital infection. Microcephaly and/or CNS malformation cases have been detected in 21 out of 27 states in Brazil, but the reported increase is concentrated in the northeast region [33].

Among those 6 906 cases, 227 child deaths occurred after birth or during pregnancy (including miscarriage or stillbirth); 51 of these had microcephaly and/or CNS malformation suggestive of congenital infection, 148 remain under investigation and 28 were discarded.

Colombia

On 30 March, Colombia reported 50 live births with microcephaly between 4 January and 20 March 2016. This number represents a limited increase on the historical annual average expected (140 cases per year). Of the 50 cases registered, 18 were subsequently discounted.

So far, seven of the remaining 32 cases have been reported to have Zika virus positive results by PCR [33].

Molecular genetic analyses suggest that Zika virus introduction into the Americas is linked to a single introduction occurring between May and December 2013 [34]. Therefore, the introduction might have taken place more than 12 months prior to the first identification of Zika virus in Brazil in May 2015. No specific amino acid changes were found among the three currently available virus genomes coming from microcephaly cases.

Panama

On 18 March 2016, the Ministry of Health reported a case of Zika congenital syndrome in a newborn baby that died on 17 March [35,36].

Cape Verde

On 14 March 2016, a baby born with microcephaly was reported by Cape Verde. Samples taken from the mother and the infant detected anti-Zika virus IgG antibodies confirmed by seroneutralisation. In addition, a new report was received on 24 March 2016 from the US CDC of a resident from Cape Verde who gave birth in the USA to a full-term baby with microcephaly. Preliminary testing of the woman's serum yielded positive results for Zika virus IgM antibodies [32].

Martinique

As of 31 March 2016, two cases of microcephaly and one additional malformation case in a Zika-positive patient have been reported, according to Agence Régionale de Santé Martinique [37].

Recent scientific developments

The risk of microcephaly in foetuses and neonates in French Polynesia between September 2013 and July 2015 has been retrospectively estimated [38]. Based on sero-survey results, the authors estimated that 66% (95% CI 62–70) of the population was infected with Zika virus during the seven-month outbreak from October 2013 to April 2014. The likelihood that a resident woman who was pregnant during this period would have become infected with Zika virus was high. Using modelling, the researchers found that the incidence of microcephaly in French Polynesia

following the outbreak was best explained by Zika infection during the first trimester of pregnancy. They estimated the risk of microcephaly to be one per hundred women infected by Zika virus in the first trimester (point estimate: 95 microcephaly cases (95% CI 34–191) per 10 000 women infected).

In addition, Besnard, et al conducted a comprehensive review of the characteristics of the clinical spectrum, the prenatal monitoring findings and laboratory results for a cluster of 19 congenital cerebral malformations in foetuses and newborns reported during the same Zika virus outbreak [39]. Of the 19 cases, Zika virus infection was confirmed by RT-PCR in amniotic fluid in four microcephaly cases. This cluster represents a 14-fold increase in congenital microcephaly and 31-fold increase in brain stem dysfunction compared to the annual incidence baseline in French Polynesia.

On 30 March 2016, Driggers, et al. reported an in-depth case report investigation of one case with foetal brain abnormalities linked to Zika virus infection during pregnancy. Prolonged low-level maternal viraemia was reported (at week 16 and week 20) which contrasts with expected short viraemia described among non-pregnant cases. The mother's viraemia was found to have cleared 11 days after termination of pregnancy. According to the authors, prolonged viraemia in the mother might be explained by replication of the virus in the foetus or placenta as high Zika viral loads were found in foetal brain, placenta, and umbilical cord. The report also provided insights into histopathological lesions observed which are in favour of selective neuronal vulnerability of cortical neural progenitor cells [40].

Guillain–Barré syndrome and other neurological syndromes

Since October 2015, eight countries or territories (Brazil, Colombia, Dominican Republic, El Salvador, French Polynesia, Honduras, Suriname and Venezuela) have reported increased GBS incidence with at least one reported GBS case with laboratory confirmation of Zika virus infection.

Five countries or territories (French Guiana, Haiti, Martinique, Panama and Puerto Rico) did not report an increase in GBS incidence but reported at least one GBS case with confirmed Zika virus infection.

In mainland France, neurological complications have been reported in an imported Zika virus disease case. In Honduras, a pregnant woman with laboratory-confirmed Zika virus infection was reported to have an undefined neurological syndrome [32]. According to media reports, this neurological syndrome is a case of sensory neuropathy [41].

There have been additional new case reports indicating that other neurological conditions could be associated with Zika virus infection:

- In Guadeloupe, a 15-year-old previously healthy girl developed acute myelitis [42] and Zika virus RNA was detected in high concentration in her serum, urine and cerebrospinal fluid nine days after onset of the neurological symptoms. She was treated with corticosteroids and recovery was satisfactory one month after admission.
- A case of meningoencephalitis in an 81-year-old man attending a four-week cruise in the Pacific. Zika virus RNA was detected in cerebrospinal fluid (CSF) by RT-PCR and isolated on Vero cell line. The patient's cognitive function recovered at day 38 after admission and presented only a slight residual weakness of the left arm as sequelae [43].

WHO public health emergency of international concern

On 8 March 2016 WHO convened the second meeting of IHR Emergency Committee on Zika virus [44]. The Committee noted the new information from State Parties and academic institutions in terms of case reports, case series, one case–control study (GBS) and one cohort study (microcephaly) in the presence of Zika virus infection. The Committee advised that the clusters of microcephaly cases and other neurological disorders continue to constitute a Public Health Emergency of International Concern (PHEIC).

WHO and international, regional and national partners are responding to the Zika virus disease PHEIC under the Strategic Response Framework and the Joint Operational Response Plan [32]. Based on the outputs of the first global consultation on Zika virus research in early March 2016, key priority areas have been defined by WHO for public health research as follows: establish causality between Zika virus infection and neurological disorders, establish a cohort study of pregnant women to assess adverse outcomes of pregnancy, study sexual transmission of Zika virus, enhance research about vector control intervention and Public health system responding to Zika outbreak [32].

ECDC threat assessment for the EU

Since the Rapid Risk Assessment issued on 9 March 2016, six additional countries or territories have reported laboratory-confirmed autochthonous transmission: the Philippines, Fiji, Vietnam, Kosrae (Federated States of Micronesia), Papua New Guinea and Cuba [45-48]. The occurrence of sporadic cases of Zika virus in Asia is expected because of the historical records of Zika virus circulation based on case reports of travel-related cases or

previous sero-surveys. ECDC is closely monitoring the situation and there is no additional information about the genetic linkage with the Zika virus strain circulating in Americas and the Caribbean.

The Zika epidemic in the Americas continues to evolve and expand geographically among countries in the Americas and Caribbean. Vector-borne transmission of Zika virus is expected to have a similar seasonal pattern to that of dengue and chikungunya, two arboviruses transmitted by the same vectors *Ae. aegypti* and *Ae. albopictus.* The dynamic of the Zika virus outbreak evolution in Central American countries and Mexico will need to be monitored as the vector-borne transmission season is expected to start in April–May. In Colombia a decrease has been observed since week 5 of 2016 (peak of the outbreak) returning to an incidence comparable to December 2015.

Severe outcomes

Uncertainties persist about the frequency and clinical spectrum of intra-uterine Zika virus infections. On 31 March 2016, WHO reported that there is 'strong scientific consensus that Zika virus is a cause of GBS, microcephaly and other neurological disorders based on observational, cohort and case–control studies currently published' [32]. The consistency of the concomitant occurrence of Zika virus and severe outcomes as described below indicate an increased likelihood of an association with Zika virus infection.

Microcephaly and congenital central nervous system malformations

An increase in microcephaly cases and other neonatal malformations related to Zika virus infection is reported in Brazil and French Polynesia. The trend in Colombia remains under investigation. Sporadic cases of microcephaly and other birth defects in foetuses with suspected links to Zika virus infection have been reported from Cape Verde (two cases), Colombia (32 cases), Martinique (two cases) and Panama (one case). In addition, two cases linked to exposure in Brazil were detected in two other countries: one case in Hawaii, USA, and one case published following the diagnosis in a Slovenian hospital [31]. Another case was diagnosed in Finland, with possible exposure in Guatemala [40].

The evidence in favour of a causal link between transplacental infection and congenital CNS malformations is substantial and accumulating. The isolation of infectious Zika virus from human foetal brain fulfils Koch's second postulate regarding the isolation of pathogens from a diseased organism [40]. This supports the association between congenital Zika virus infection and foetal brain damage.

The magnitude and geographical range of the increase in congenital CNS malformations has not yet been well characterised. The currently available data are still insufficient to quantify accurately the risk of transplacental transmission of Zika virus during pregnancy. To date, only one study provides a first estimate of microcephaly risk, i.e. about one per 100 women infected by Zika virus in the first trimester of pregnancy (95 microcephaly cases (95% CI 34–191) per 10 000 women infected) [38]. Although this risk of microcephaly among infected mothers is below the transmission rate of other infectious diseases implicated in congenital malformations (i.e. Cytomegalovirus with a transmission rate to infants born to mothers who had a primary infection estimated at around 32%), it represents a significant public health concern in the area with the current outbreak with a high attack rate. Based on the estimated risk of microcephaly under a scenario of widespread transmission of Zika virus in the general population (as shown in the French Polynesia outbreak with two thirds of the population likely infected), it is plausible that an increase of congenital CNS malformations would be detectable in areas where Zika virus disease incidence is high.

Preliminary findings from a prospective cohort study in Rio de Janeiro of women who presented with rash during pregnancy indicate that the risk could be higher. The mothers were included in this study, which has been running since 2007, if the rash had developed within the five days prior to being seen at the study site. Blood and urine samples were tested for Zika virus by RT-PCR assays [49]. From September 2015 through February 2016, a total of 88 women with rash were enrolled in the study, of whom 72 (82%) tested positive for Zika virus in either blood, urine, or both. Ultrasonography results were available for 42 of the women with confirmed Zika virus infection and 12 (29%) of them had findings of foetal CNS pathology. None of the pregnant women with rash who tested negative for Zika virus by RT-PCR assays (16 of 88) had abnormal ultrasonography findings during follow-up.

The currently available data are insufficient to quantify the risk of transplacental transmission during pregnancy and the resulting risk of adverse pregnancy outcomes. However, for symptomatic pregnant women the likelihood of congenital malformations is substantial based on the results of the study of Brasil, et al. [49]. It is still uncertain how many of the pregnant women who become infected will transmit the virus to the foetus, and how many of the foetuses will develop brain damage or other malformations.

It is probable that the risk of transplacental infection as well as the risk of developing congenital CNS malformations depends on the gestational age at the time of infection. On 5 April 2016, the preliminary result of a case–control study in Paraíba state in Brazil supports the idea that mothers who had a Zika virus infection in the first trimester of pregnancy were more likely to have children with microcephaly as seen in the retrospective analysis of the French Polynesia outbreak [38,50].

It is also conceivable that other factors, such as the mother's age and her nutritional status, influence the risk of transplacental transmission. Yet there are currently no data available to support such a hypothesis. It is possible

that Zika virus infection is a necessary factor in the aetiology of congenital CNS malformations but that there are, as of yet unidentified, co-factors that influence the risk.

For surveillance purposes, the US CDC has developed a voluntary registry to collect information on pregnant women in the US with confirmed Zika virus infection and their infants [51]. ECDC is developing a framework for the surveillance of Zika virus infection in EU/EEA Member States and specific surveillance of Zika congenital syndrome in Europe. EU surveillance would aim to monitor the occurrence of autochthonous Zika virus infections and support assessment of the risk of local transmission in the EU/EEA in order to trigger appropriate control measures, especially in receptive areas where the vector is present. Surveillance objectives are: i) early detection of autochthonous Zika virus infections; ii) timely detection of imported Zika virus infections in regions where the vector is established; iii) support for the assessment of the risk of local transmission in the EU/EEA. The case definition for EU reporting is available on the website [52].

In conclusion, results from ongoing and further case–control and cohort studies are still required to confirm the estimated risk of microcephaly and other congenital CNS malformations potentially linked with Zika virus infection.

Guillain–Barré syndrome and other post-infectious neurological syndromes

Cases of GBS continue to be reported from the affected countries: according to WHO and as of 31 March 2016, 13 countries or territories have reported an increase in GBS and/or laboratory-confirmed cases related to Zika virus infection [32]. Observations support the role of Zika virus infection as a presumptive infection event preceding GBS. Countries reporting an increase of GBS are affected by Zika viral strains from the Asian lineage.

Risk of Zika virus transmission via substances of human origin

People with asymptomatic infections and those who are viraemic in the incubation period of Zika disease could potentially donate contaminated substances of human origin (SoHO) without their infections being recognised at the time of donation [53]. The virus can also be transmitted by SoHO from donors after clinical recovery from Zika virus disease due to possible prolonged viraemia or a persistence of the virus in semen after viraemia has cleared. Zika virus RNA has been detected in blood, urine, saliva, seminal fluid and breast milk [54-58] (Table 1). Data on the survival of Zika virus in processed and stored SoHO are lacking.

Assessing the risk of Zika virus transmission through contaminated SoHO is currently difficult because of the paucity of data on the prevalence of Zika virus in the donor population and the limited number of case reports of transmission via SoHO. According to Musso, et al, during the last Zika virus outbreak in French Polynesia, 42 of 1 505 (3%) blood donors, although asymptomatic at the time of donation, were found to be positive for the Zika virus genome by RT-PCR, supporting a potential risk of transfusion-derived transmission [54,59]. The Brazilian media reported possible cases of transfusion-transmitted Zika virus in March 2015 and February 2016 [60-62]. Reports of sexual transmission of Zika virus through contaminated male semen to a partner indicate the possible virus transmission through donated sperm [63-67]. There are no documented transmissions of the virus via saliva, urine or breastfeeding. Cases of Zika virus transmission through donated cells, tissues and organs have not been reported, but this possibility cannot be excluded due to the confirmed presence of the virus in human blood and bodily fluids.

Table 1. Time of detection and Zika virus RNA load in samples of infected individuals

Sample origin	Time of detection (days)		Viral RNA load	Isolation of replicative particles	References
	Before the onset of symptoms	After the onset of symptoms			
Blood	2–3	11	up to 8.1 x 10^6 copies/mL	+	[54]
Urine	-	10 to 22	0.7–220 x 10^6 copies/mL	+	[16,55,68]
Saliva	-	2 to 29	3 x 10^6 copies/mL	+	[16,56,69]
Seminal fluid	-	21 to 62	up to $10^{8.6}$ copies/mL	+	[57,70-72]
Breast milk	-	3 to 8 after delivery	up to 2.1 x 10^6 copies/mL	+	[58,73]

A recent case report of Zika congenital infection showed a prolonged detection at low level by quantitative RT-PCR of Zika virus RNA in serum from the mother at between week 16 and week 20 of pregnancy after termination of the pregnancy, RT-PCR returned to negative. The kinetics of Zika virus RNA in the sera of infected pregnant women are not yet well understood and would require assessment in larger studies [40].

The limited set of data indicates that there is a potential risk of Zika virus transmission through SoHO that may cause serious consequences to the health of recipients. However, a scarcity of reported cases of donor-derived Zika virus infection precludes a more accurate risk assessment. The evidence of association between Zika virus infection and congenital malformations and GBS justifies preventive measures to reduce the risk of transmission via SoHO supply [74].

Risk of sexual transmission

Live Zika virus particles have been detected on one occasion in semen more than three weeks after onset of Zika symptoms. Zika viral RNA has been reported in semen up to 62 days after clinical disease [57,71]. Zika virus genome has also been detected in saliva during and after the acute phase of the disease and viral isolation reported on day 6 after symptom onset [16]. A second viral isolation from saliva was recently reported but the date on sampling is not available [17]. Comprehensive data about the presence of viable virus, viral load or kinetics are lacking, and at this point in time the risk of transmission via saliva cannot be further assessed.

Several cases of sexual transmission from males to their partners have been reported or are under investigation in recent weeks (see 'Event background', above). In all cases, except one case where information is currently unavailable, males presented with a clinical illness compatible with Zika virus infection. The interval between onset of symptoms in the man and in his female partner varies at between 4 and 19 days. The shortest period reported corresponded to a male who might have had sexual contact several days before onset of disease. So far, no sexual transmission of Zika virus from infected women to their partners or from persons who are asymptomatically infected has been reported.

On 11 April, WHO published an update of its travel health advice on Zika virus in which they advise travellers returning from areas with ongoing Zika virus transmission to practise safer sex for at least one month after returning, in order to reduce the potential risk of onward sexual transmission [75]. This WHO guidance will be reviewed and the recommendations updated as new evidence emerges.

Travel-related risk for EU citizens

The spread of Zika virus infections in the Americas, in the Caribbean and Pacific Islands constitutes a significant development in the epidemiology of this emerging vector-borne disease. Travellers to countries where competent vectors are present and Zika virus circulation is documented are at risk of becoming infected through mosquito bites. Due to the strong evidence of a link between Zika virus infection and severe congenital anomalies, pregnant women and women who are trying to become pregnant constitute a high-risk group with regard to serious adverse outcomes of Zika virus infection. Residents in EU OCTs and OMRs with competent and active vectors are at increased risk of exposure to Zika virus.

Risk related to mass gatherings

The Rio de Janeiro 2016 Olympics (5–21 August 2016) and the Paralympic Games (7–18 September 2016) are the two most prominent mass gathering events that will take place in the Americas in the coming months. A large number of visitors are expected for these events. The Olympic Games will take place during Brazil's winter when the cooler and drier weather will reduce mosquito populations and significantly lower the risk of infection for visitors. ECDC is preparing a comprehensive risk assessment for communicable diseases ahead of the Games.

An analysis of the 2014 World Cup in Brazil indicated the following:

- The density of dengue cases in Brazil was very low in the southern hemisphere (mid-June to mid-September). Therefore, the risk of vector-borne transmission of Zika virus infection during the Olympic Games is expected to be low – by analogy with the transmission of dengue which involves the same vectors [76].
- Only three exported cases of dengue fever were reported among returning travellers who attended the event [76]. The estimated expected number of dengue cases among the 600 000 foreign tourists during the World Cup was 33 (range 3 to 59) according to a modelling exercise conducted before the event [78].

Although the probability of being bitten by an infected mosquito is expected to be very low during the events, it cannot be excluded that Zika-infected travellers will return to regions of the EU where competent vectors are active. This may create an opportunity for local vector-borne transmission.

People travelling to Brazil before the Olympic Games should follow the precautions and recommendations issued by their national health authorities. The US CDC have issued specific travel recommendations related to the Olympics and the Paralympic Games [79].

Risk of importation and transmission in EU OCTs and OMRs

Aedes aegypti mosquitoes are present in the EU OCTs and OMRs in the Americas and the Caribbean, and most of them have reported autochthonous transmission. The risk associated with spread to yet unaffected OCTs and OMRs in the area is significant because of the immunologically naïve populations, the presence of competent vectors, the permissive climate and the intense movement of people in and between countries and territories.

Other EU OMRs and OCTs outside of the Caribbean where mosquito vectors are present such as *Aedes aegypti* in Madeira or *Aedes albopictus* in La Réunion are at risk of establishment of local transmission should the virus be introduced. Madeira is of particular concern because of the close relationship with Brazil and Venezuela where Zika virus is currently circulating. The 2012 dengue epidemic demonstrated the favourable conditions for vector-borne outbreaks in Madeira [80].

Risk of importation and transmission in the continental EU

The continued rise in cases of Zika virus infection in the Americas and the Caribbean increases the risk of infection among travellers. ECDC is collecting reports of imported cases in the EU/EEA through the media and official government communication lines [81]. Cases of Zika virus infection coming from countries with autochthonous transmission continue to be reported in the EU.

There is no evidence to date of 'airport transmission' of mosquito-borne viral disease, similar to airport malaria [82]. The risk of importation of Zika-infected mosquitoes inside aircraft cabins is low, and there is no evidence that this plays a role in the transmission of arbovirus infections. WHO has issued specific guidance and recommendations for aircraft disinsection [83,84].

The risk of transmission of Zika virus infection in the EU is variable and depends on several co-factors, for example:

- The **presence of a potential mosquito vector**: *Aedes albopictus* is established in most places around the Mediterranean coast [85].
- The **competence** of *Aedes albopictus* to transmit Zika virus, which depends on characteristics of the pathogen (strain-specific vector competence) and of the mosquito species. Onward transmission from imported cases within the continental EU is possible because *Aedes albopictus* might be considered a competent vector for the transmission of Zika virus, although a recent study showed an unexpected low vector competence of this species [18]. The vector competence of this species has not yet been confirmed for European mosquito populations; experiments with European *Aedes albopictus* populations are ongoing.
- The **capacity of the vector** to transmit the infection is determined by a number of factors such as vector competence, the mosquito population density, feeding host preferences, biting rates and survival of the mosquito population. Spatial variation in vector capacity is expected in areas where *Aedes albopictus* is present, and further depends on environmental conditions and locations. In practice, the presence of a competent vector in a location is necessary, but is not sufficient to allow further transmission when an arbovirus is introduced in a mosquito's population.

The risk of transmission of Zika virus infection is extremely low in the EU during the early spring season as the climatic conditions are not suitable for the activity of the *Aedes albopictus* mosquitoes.

The suitable conditions for *Aedes albopictus* activity will increase progressively during the spring (April to June) especially in southern Europe. For the months April to June, the International Research Institute for Climate and Society of the Earth Institute (University of Colombia, US) predicts above-normal temperatures in western Europe coinciding with a normal precipitation pattern, which might result in an early start of the mosquito activity season in southern Europe [86].

During the summer season most likely from July by analogy with other mosquito-borne disease transmission in the EU, autochthonous transmission in the continental EU following the introduction of the virus by a viraemic traveller is possible in areas where *Aedes albopictus* is established [85].

Conclusions and options for response

Considering the continued spread of Zika virus in the Americas and Caribbean, the strong evidence of an association between Zika virus infection during pregnancy and congenital CNS malformations, the association between Zika virus infection and Guillain–Barré syndrome, and the risk of establishment of local vector-borne transmission in Europe during the 2016 summer season, EU/EEA Member States are recommended to consider a range of mitigation measures.

The following uncertainties have been taken into consideration in developing the proposed options for response:

- At the present time, there is a lack of evidence on which stage of the pregnancy the foetus is most vulnerable to Zika virus infection. Therefore the entire duration of pregnancy should be considered at risk.
- The presence of infectious Zika virus in semen has been detected up to three weeks after onset of disease; the longest interval reported between the onset of symptoms in a male and the subsequent onset of the disease thought to be due to sexual transmission in a female partner is 19 days.
- The role of asymptomatic males in the sexual transmission to women is unknown.
- The roles of different mosquito species as potential vectors of Zika virus should be clarified. If current assumptions prove inaccurate or incorrect, vector control strategies have to be adapted and revised.

Information to travellers and EU residents in affected areas

Over the past two months, as of 4 April 2016, autochthonous cases of Zika virus infection have been reported from 45 countries or territories worldwide. In the past nine months, 47 countries or territories have reported autochthonous cases of Zika virus infection.

A list of countries and territories with documented autochthonous transmission during the past two months is available on the ECDC website.

Information for travellers and EU citizens residing in areas with active transmission

- Travellers visiting countries where there is active transmission of Zika virus should be made aware of the ongoing outbreak of Zika virus infection. A list of countries and territories with documented autochthonous transmission during the past two months is available on the ECDC website.
- Travellers visiting these countries and EU citizens residing in these countries should take measures to prevent mosquito bites indoors and outdoors, especially from sunrise to sunset when *Aedes* mosquito vectors are most active in biting. These measures include:
  - The use of mosquito repellent in accordance with the instructions indicated on the product label.
  - Wearing long-sleeved shirts and long trousers, especially during the hours when the type of mosquito that is known to transmit the Zika virus (*Aedes*) is most active.
  - Sleeping or resting in screened or air-conditioned rooms, otherwise use mosquito nets, even during the day.
- Pregnant women and women who are planning to become pregnant should consider postponing non-essential travel to affected areas until after delivery.
- Pregnant women who plan to travel to affected areas and pregnant women residing in affected areas should consult their healthcare providers for advice and follow strict measures to prevent mosquito bites.
- Travellers with immune disorders or severe chronic illnesses should consult their doctor or seek advice from a travel clinic before travelling, particularly on effective prevention measures.
- Travellers to Zika-affected areas and EU citizens residing in affected areas should be advised that using condoms could reduce the risk of sexual transmission through semen.

Information for travellers returning from areas with transmission of Zika virus

- Pregnant women who have travelled or resided in areas with Zika virus transmission should mention their travel during antenatal visits in order to be assessed and monitored appropriately [87].
- In order to protect the foetus, male travellers returning from affected areas should consider using a condom with a pregnant partner until the end of pregnancy.
- Travellers showing symptoms compatible with Zika virus disease within two weeks of return from an affected area are advised to contact their healthcare provider and mention their recent travel.
- On 11 April, WHO published an update of its travel health advice on Zika virus in which they advise travellers returning from areas with ongoing Zika virus transmission to practise safer sex for at least one month after returning, in order to reduce the potential risk of onward sexual transmission [75]. This WHO guidance will be reviewed and the recommendations updated as new evidence emerges.

Surveillance of imported cases and monitoring of transmission in the continental EU

- Increase awareness among clinicians and travel health clinics about the evolution of the Zika virus outbreak and the affected areas so that they can include Zika virus infection in their differential diagnosis for travellers from those areas.
- Enhance vigilance towards the early detection of imported cases of Zika virus infection in EU Member States, EU OCTs and OMRs, in particular where vectors are present, in order to reduce the risk of autochthonous transmission.
- Clusters of unexplained illness with rash detected in receptive areas between 1 May and 31 October should be investigated, and Zika virus infection should be considered as an underlying cause.
- Ensure early warning reporting of autochthonous cases, in particular in receptive areas.
- Strengthen laboratory capacity to confirm suspected Zika virus infections in the European region in order to differentiate Zika virus infections from other arboviral infections (e.g. dengue, chikungunya).
- Increase awareness among obstetricians, paediatricians and neurologists in the EU/EEA that Zika virus infections should be investigated in patients presenting with congenital CNS malformations, microcephaly and GBS.

Safety of substances of human origin

Competent authorities, establishments and clinicians dealing with SoHO need to be vigilant and aware of the risk of donor-derived Zika virus transmission through transfusion and transplantation. Measures to prevent Zika virus transmission through SoHO should be taken in both affected and non-affected areas. Detailed SoHO safety measures were already described in Annex 1 of the previous risk assessment on 9 March 2016 [8]. The European Commission's Directorate General for health and food safety established a working group for the preparation a preparedness plan in Europe related to safety of substances of human origin in the event of a Zika virus outbreak.

Non-affected areas

The primary measure to prevent Zika virus transmission in non-affected areas is the temporary deferral of blood donors from donation. Living donors of cells and tissues who are at risk of having been infected should also be deferred. Criteria for deferral are:

- a medical diagnosis of Zika virus disease;
- return from affected areas; or
- sexual intercourse with males diagnosed with Zika virus disease or returned from affected areas.

Deceased donors of cells and tissues with a recent medical diagnosis of Zika virus infection should not be accepted for donation. Periods defined for donor deferral/acceptance should provide a sufficient safety margin for a virus-free donation. This includes taking into account viral persistence in the particular type of SoHO during and after the clinical course of Zika virus disease.

Affected areas

Blood and tissue establishments may temporarily interrupt donations and import blood components or cells and tissues from unaffected parts of the country and consider the use of pathogen inactivation for plasma, platelets and some tissues. The screening of all donated blood and all donors of cells and tissues for the presence of Zika virus RNA by nucleic acid testing (NAT) may be considered necessary to assure the safety and sustainability of supply in affected areas. A systematic review and pooled analysis to estimate the distribution of times from Zika infection to symptom onset, seroconversion, and viral clearance, showed that symptom-based screening reduces the risk of a positive Zika virus blood donation by 7% (RR 0.93, 95% CI 0.86–0.99), and antibody screening by 29% (RR 0.71, 95% CI: 0.28–0.88) [10]. This estimate confirms that in areas with a high incidence of Zika virus, blood establishments may consider NAT testing to identify lots safe for use in pregnant women.

At the end of March 2016, the US Food and Drug Administration approved the use of an investigational test to screen blood donations for Zika virus under an investigational new drug application in areas with an active mosquito-borne transmission of Zika virus [88]. European Member States, which will most likely be impacted by a spread of the Zika virus infection, can potentially use this test for screening blood donations.

Irrespective of the presence of ongoing local virus transmission in the area, the risk of Zika virus transmission through organs donated by living or deceased donors should be recognised and assessed during a pre-donation evaluation and balanced against the benefits of the transplantation for each potential recipient.

Information to healthcare providers in EU Member States

Ensure that Zika virus-infected patients in areas with *Aedes* mosquito vectors avoid getting bitten during the first week of illness (bed nets, screened doors and windows as recommended by PAHO/WHO).

Increase awareness among health professionals who provide prenatal care of the possible association between Zika virus and microcephaly and adapt prenatal monitoring in accordance with the exposure to the vector [88,89].

In addition, due to the unprecedented size of the Zika virus epidemic, health services and practitioners should be alerted to the possible occurrence of neurological syndromes (GBS and other neurological syndromes such as meningitis, meningoencephalitis and myelitis according to WHO/PAHO) and potential disease complications not yet described in the scientific literature, and atypical clinical presentation among specific populations (i.e. children, the elderly, immunocompromised individuals and those with sickle cell disease).

Preparedness in the EU

Preparedness for the prevention and control of Zika virus infection in the EU/EEA will require capacities and capabilities for early detection, response and communication. Consistent with the evidence presented in this document, the following components might be considered with regard to Zika virus preparedness [79,90-94]. ECDC published a preparedness planning guide for diseases transmitted by *Aedes aegypti* and *Aedes albopictus*.

Early detection mechanisms should ensure the following:

- Rapid notification of human cases (imported and/or autochthonous).
- Surveillance of those *Aedes* mosquito species that are vectors for Zika virus; this should include consideration of entomological and environmental indicators. ECDC Guidelines for the surveillance of invasive mosquitoes in Europe provides a useful overview of entomological surveillance at national and subnational levels [96].
- Laboratory diagnosis capacity.

Response mechanisms should cover the following:

- Organisational and planning mechanisms aimed at the prevention and control of mosquito-borne diseases.
- Intersectoral and cross-disciplinary collaboration with all relevant partners.
- Case management.
- Safety of substances of human origin.

- Gynaecological, obstetric and neonatal services to follow-up on infected pregnant women and to provide reproductive health guidance.
- Outbreak investigation capacity (including epidemiological, entomological and environmental aspects);
- Rapid vector control measures against imported cases in areas with those *Aedes* mosquito species that are vectors for Zika virus.

Communication mechanisms:

- Advice to travellers, with special focus on pregnant women.
- Training of healthcare professionals on health impacts of Zika virus.
- Community involvement in the control of mosquito populations through both individual and collective preventive measures.
- Involvement of mass media for communication purposes and to promote public awareness and protection.

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