RAPID RISK ASSESSMENT

Zika virus disease epidemic

Seventh update, 8 July 2016

Conclusions and options for response

Given the current circulation of Zika virus; the evidence of an association between Zika virus infection during pregnancy and congenital malformations of the central nervous system (CNS); the association between Zika virus infection and Guillain–Barré Syndrome (GBS) and the risk 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 when developing the proposed options for response:

- There is growing evidence that Zika virus infection during the first and second trimester is associated with increased risk for CNS malformations and microcephaly. The risk of CNS malformations when the infection occurs during the third trimester is unknown, hence Zika virus infection should be considered as a risk throughout the entire duration of pregnancy.
- Viable Zika virus have been detected in semen up to 24 days after onset of symptoms of Zika virus infection. The longest interval reported between the onset of symptoms in an infected man and the subsequent onset of disease in a female sexual partner is between 34 and 41 days.
- The majority of the reported sexual transmission events are linked to symptomatic index cases. There is just one probable transmission from an asymptomatic sexual partner reported in the literature to date.

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

A list of countries and territories with active transmission (i.e. sporadic and widespread transmission) during the past three months is available on the ECDC website.

- Travellers visiting countries where there is active transmission of Zika virus and EU citizens residing in these countries should:
  - be made aware of the ongoing outbreak of Zika virus infection and the fact that Zika virus is usually transmitted by mosquitoes, but can be also transmitted by sexual intercourse.
  - take measures to prevent mosquito bites indoors and outdoors, especially between sunrise and sunset when *Aedes* mosquito vectors are most active. These measures include:
    - using 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 using mosquito bed nets, at night and during the day.

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- Pregnant women and women who are planning to become pregnant and planning to travel to areas with widespread transmission should postpone non-essential travel.
- Pregnant women and women who are planning to become pregnant and planning to travel to areas with sporadic transmission should consult their physician or a travel clinic and consider postponing non-essential travel.
- Pregnant women residing in countries with active transmission (sporadic and widespread) 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 - particularly as regards effective prevention measures - before travelling to countries with active transmission.
- Travellers to countries with active Zika transmission and EU citizens residing there should be advised that using condoms is likely to reduce the risk of sexual transmission.

Information for travellers returning from areas with active transmission of Zika virus

- Pregnant women who have travelled or resided in areas with active 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 areas with active transmission should consider using a condom with a pregnant partner until the end of her pregnancy.
- Travellers returning from areas with ongoing Zika virus transmission should be advised to use a condom for at least eight weeks after returning, in order to reduce the potential risk of onward sexual transmission. If before or during that period Zika virus symptoms occur, men should use condoms or consider abstinence for at least six months.
- Travellers, including those with immune disorders or severe chronic illnesses, who develop symptoms compatible with Zika virus disease within two weeks of returning from an area with active transmission, should contact their healthcare provider and mention their recent travel.
- All travellers, irrespective of symptoms, who return from an area with Zika virus transmission to an area in the EU/EEA where *Aedes aegypti* or *Aedes albopictus* mosquitoes are active, should take measures to prevent mosquito bites for three weeks so that they do not pass Zika virus to uninfected mosquitoes.

Surveillance of imported cases and local transmission in the EU Member States of continental Europe

- Increase awareness among clinicians and travel health clinics of the evolution of the Zika virus outbreak and the areas around the world with active and past transmission (ECDC website) to allow them to consider Zika virus infection in their differential diagnosis for travellers from those areas. Clinicians should be aware that Zika virus infections can be paucisymptomatic.
- Enhance vigilance towards the early detection of imported cases of Zika virus infection into EU Member States, EU Overseas Countries and Territories (OCTs) and EU Outermost Regions (OMR), in particular where Zika vectors are present, in order to reduce the risk of onward autochthonous transmission.
- Clusters of unexplained illness with a rash, detected in receptive areas of continental Europe between 1 May and 31 October should be investigated, and Zika virus infection should be considered as a possible cause.
- Ensure timely reporting of autochthonous cases, in particular in the receptive areas of EU Member States in continental Europe.
- Strengthen laboratory capacity and capabilities to confirm Zika virus infections in the EU/EEA and to differentiate Zika virus infections from other arboviral infections (e.g. dengue, chikungunya).
- Increase awareness among obstetricians, paediatricians and neurologists that the possibility of Zika virus infection should be investigated in patients presenting with congenital CNS malformations, microcephaly and Guillain-Barré syndrome.

Information to healthcare providers in EU Member States

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

Efforts should be made to increase awareness among health professionals providing antenatal care of the risk of neurological congenital syndrome associated with maternal Zika virus infection, especially during the first two trimesters. Antenatal monitoring should be adapted in accordance with the possibility of exposure to the virus (through vector or sexual transmission) [1,2]. ECDC maps showing Zika transmission in the past nine months are provided to aid clinicians assessing returning travellers, especially pregnant women, –who have visited countries and territories with recent or current local active transmission of Zika virus.

Due to the unprecedented size of the Zika virus epidemic, health services and practitioners should be alerted to the association between Zika virus infections and GBS, the possible association with other neurological conditions (such as meningitis, meningoencephalitis and myelitis) and with as yet undocumented

complications of Zika virus infections, particularly among children, the elderly, immunocompromised individuals and those with sickle cell disease.

Safety of substances of human origin

Competent authorities, establishments and clinicians dealing with substances of human origin (SoHO) need to be vigilant about the risk of donor-derived Zika virus transmission through transfusion and transplantation. Measures should be taken to prevent Zika virus transmission through SoHO in both affected and non-affected areas. Implementation of safety measures related to SoHO should be informed by risk assessments performed at the national level. The European Commission's Directorate General for health and food safety has established a working group for the preparation of a European preparedness plan for the safety of substances of human origin in the event of a Zika virus outbreak. ECDC will synchronise detailed SoHO safety measures, already described in Annex 1 of the risk assessment dated 9 March 2016 [3], with those defined in the guide for SoHO safety preparedness activities at EU level.

Non-affected areas and areas with sporadic transmission

The primary measure to prevent Zika virus transmission via SoHO in non-affected areas and areas with sporadic transmission is the temporary deferral of donations from blood donors and living donors of cells and tissues who are at risk of having been infected. When identifying donors at risk of Zika virus infection, the following criteria should be considered:

- A medical diagnosis of Zika virus disease;
- Returning from areas with widespread transmission;
- Reporting sexual intercourse with a man who has been diagnosed with Zika virus infection or a man who have returned from areas with widespread transmission.

Based on the frequency of travel to currently affected areas, the Netherlands have assessed the risk of Zika virus transmission from blood donors who have had sexual contact with an asymptomatic man who has returned from an affected area as too small to warrant their deferral [4]. Similarly, based on risk assessments, Australia and France do not defer blood donors who have had sexual contact with asymptomatic men who have returned from affected areas [5,6]. Thus, current practice, as reported to date, is that the implementation of safety measures for this category of risk donor is being considered and re-assessed as required, as part of the risk assessment for national preparedness plans.

Cells and tissues from deceased donors with a recent medical diagnosis of Zika virus infection should not be accepted for donation. Defined periods should be set for living SoHO donor deferral/acceptance to provide a sufficient safety margin for virus-free donation. This includes taking into account viral persistence in particular tissues, fluids and anatomical sites during and after symptomatic and asymptomatic Zika virus infections.

Areas with widespread transmission

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 areas with widespread transmission. 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) [7]. 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.

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.

Laboratory screening of SoHO donors/donations

Test kits, registered/approved for screening tests, should be used for determining SoHO donor/donation suitability. Commercial Zika tests for screening are still under development. Based on scientific data, SoHO establishments and laboratories may develop in-house or adapt available commercial diagnostic tests for screening purposes. In the event of a Zika virus outbreak the use of such screening tests should be validated and approved by the responsible national authority. Some blood establishments are gaining experience with in-house testing or using adapted commercial tests. Semi-automated platforms for NAT screening using CE marked kits for diagnostics were implemented for NAT screening in the French West Indies during the 2014 outbreak of chikungunya [8] and are currently being implemented for NAT screening of blood donors for Zika virus in the French Antilles using the RealStar RT-PRC Zika kit 1.0, Altona. 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 a new drug application being investigated for areas with an active mosquito-borne transmission of Zika virus [9]. A number of commercial laboratory tests for in vitro diagnostics of Zika virus infection have also been submitted to WHO for an Emergency Use Assessment and Listing (EUAL) [10]. Products that are

reviewed favourably are then listed as eligible for WHO procurement and could be used for an emergency application until final registration/approval for commercial use is available.

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. ECDC has published a preparedness planning guide for diseases transmitted by *Aedes aegypti* and *Aedes albopictus*. The guide focuses on the main components that should be considered when developing preparedness plans. Consistent with the evidence presented in this document, the following components might be considered with regard to Zika virus preparedness [11-16].

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's publication 'Guidelines for the surveillance of invasive mosquitoes in Europe' provides a useful overview of entomological surveillance at national and subnational levels [14,15].
- Laboratory diagnosis capacity and capabilities.

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 *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.

Source and date of request

ECDC internal decision, 22 June 2016.

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 for the choice of which option to pursue and which actions to take lies exclusively with the EU/EEA Member States.

Public health issue

This document assesses the risks associated with the Zika virus epidemic in currently affected countries, in EU Overseas Countries and Territories (OCTs) and Outermost Regions (OMRs) and in the EU Member States within continental Europe.

Since February 2014, ECDC has published eight risk assessments related to Zika virus epidemics [3,17-25].

Consulted experts

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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. More information about Zika virus disease can be found in the previous risk assessments [3,17-25] and in the ECDC factsheet for health professionals (last update 23 June 2016). Three extensive literature reviews have been published since the previous ECDC risk assessment [26-28].

Highlights of recent scientific developments

Pathogen

Zika virus is able in vitro to infect various cell lines derived from human-induced pluripotent stem cells (iPSCs) or embryonic stem cells such as neural progenitors, neurospheres, and organoids [29-33]. Hanners et al. demonstrated that a 2015 Zika virus isolate (Puerto Rico) replicates in primary human foetal neural progenitor cells with a cytopathic effect and that viral replication may persist in these cell lines for many weeks, a finding that is consistent with histopathological findings in affected foetuses [34].

Several recent publications based on animal models support an in vivo deleterious effect on neural progenitor cells, leading to reduction of their proliferation and differentiation and to increased apoptosis. Using a murine model, Wu et al. showed that Zika virus is able to infect radial glial cells which are cortical neurone progenitors [35]. A Zika virus strain from Puerto Rico is able to replicate in Hofbauer cells, primary human placental macrophages, and to a lesser extend in cytotrophoblasts isolated from villous tissue of full-term placentas [36]. Despite the fact that both cell types are permissive to Zika virus, the cellular pathways of trans-placental transmission are not fully understood yet. Further investigations are now being undertaken to elucidate the mechanism(s) of intrauterine infection.

Histopathological investigations on samples of brain tissue from three new-borns with microcephaly who died after birth (two shortly after birth, one two months later) and two placentas from spontaneous abortions were performed. The pregnant women all reported rash and fever during the first trimester of pregnancy [37]. All samples were positive for Zika virus RNA by RT-PCR. Viral antigens were localised in the cytoplasm of degenerating and necrotic neurones and glial cells in the vicinity of the micro-calcifications in the brains of the microcephaly cases. Zika virus antigens were identified in the chorionic villi of one placenta from a spontaneous abortion in the first trimester.

Overall, these results support the view that Zika virus is neurotropic and cytotoxic for several foetal neural cells, and that it interferes with the neurogenesis during human brain development.

Animal models

The kinetics of Zika virus in rhesus macaques has been reported by Dudley et al [38]. Eight Indian-origin rhesus macaques (including two pregnant animals) were inoculated with Zika virus of Asian-lineage from French Polynesia (by sub-cutaneous injection of three different doses ranging from 10^4 to 10^6 plaque-forming units (PFU)). All animals were susceptible to the infection and Zika virus RNA was found in their blood (peak: 2–6 days post inoculation (d.p.i), longest duration: 11 d.p.i), urine (range: 2–17 d.p.i.), saliva (3–14 d.p.i), cerebrospinal fluid (at 4 d.p.i. only) and vaginal fluid (range 1–7 d.p.i). Zika virus neutralising antibodies were detected on day 21 post-inoculation. No viral replication was found following a challenge with the same virus strain, indicating that natural infection induces protective immunity against homologous strains. The two pregnant animals who were inoculated mid-first trimester of pregnancy had persistent viraemia for 29 and 57 days post inoculation. Foetal infection and pregnancy outcome has yet to be determined.

Cross-immunity between the two Zika lineages has been investigated in animal models. Rhesus macaques who were infected by the East African strain (MR 766 ZIKV) were protected from subsequent infection when challenged with the heterologous Asian Zika virus strain [39].

Microcephaly and congenital central nervous system malformations

In March 2016, WHO reported that there is 'strong scientific consensus that Zika virus is a cause of Guillain–Barré Syndrome (GBS), microcephaly and other neurological disorders based on observational, cohort and case–control studies currently published' [40]. Epidemiological observations are supported by cumulative evidence from in vitro and in vivo laboratory studies of the damaging effect of Zika virus on neuronal tissue, especially neural progenitor cells, therefore possibly impairing the development of the foetus' brain. These laboratory studies are strengthening the coherence of the evidence and its consistency with Hill's criteria for causation. Overall data from scientific investigations add to the evidence that the emerging Zika virus strain from the Asian lineage can cause transplacental infection and congenital central nervous system (CNS) malformations in the developing brain [41,42].

Modelled risk estimates for microcephaly cases have been retrospectively made for each state in Brazil between November 2015 and April 2016 [43]. In this study, microcephaly cases were defined on the basis of clinical criteria only. In Pernambuco state, the estimated risk was 20 to 200 per 10 000 live births (assuming a 50% exposure) with a background risk of around 2 per 10 000 live births in Brazil.

Additional new information about the gestational age at the time of Zika virus infection and the risk of congenital CNS malformations includes the following:

- Among 11 944 pregnant women with clinical Zika virus disease reported in Colombia through the Zika virus national surveillance system between August 2015 and early April 2016, 1 484 (12%) were confirmed positive with Zika virus using RT-PCR assay [44]. In a subgroup of pregnant women (n=1 850) with complete information on gestational age at the time of Zika virus symptom onset, 56% of the 582 serum samples available were positive for Zika virus. Among these 1 850 pregnant women, 27.8% reportedly contracted the infection in the first trimester, 37.9 % in the second trimester, and 33.2% in the third trimester. As the majority of the women who contracted Zika virus disease in the first or second trimester were still pregnant at the time of this report, information about pregnancy outcomes is not yet available. However, for the group of women who were reportedly infected during the third trimester (n=616), no cases of microcephaly or brain abnormalities have been reported to date. Among this cohort, 82% of the infants were born at term with a normal birth weight, 2% were born at term with a low birth weight, 8% were preterm, 1% died during the perinatal period while 7% are still being followed. Hence, Zika congenital syndrome with microcephaly does not appear to be associated with symptomatic infection during the third trimester of pregnancy in this study. These results do not, however, account for asymptomatic infection or unreported clinical illness. Among 50 infants with possible microcephaly who were reported through the national surveillance system for birth defects (and not the national Zika virus surveillance system) just four of those born to mothers who did not report symptoms of Zika virus disease during pregnancy had laboratory evidence of congenital Zika virus infection (positive RT-PCR for Zika and negative results for the most common group of congenital infections). The follow-up study on pregnancy outcomes for women with Zika virus infection during the first and the second trimester should provide a better estimate of the risk of congenital CNS malformations in the coming months.
- In Brazil, 1 501 cases of microcephaly cases reported to the national surveillance system between November 2015 and February 2016 were investigated [45]. The cases were classified in one of four groups according to the diagnostic certainty of the congenital Zika infection. Definite cases are defined as new-borns with laboratory evidence of Zika virus infection during pregnancy through serology or PCR, independently of other findings. The other classes are define as: 'i) highly probable cases, new-born babies with imaging reports mentioning specific findings that were highly suggestive of Zika virus infection, including brain calcifications, ventricular enlargement, or both, with negative laboratory results for syphilis, toxoplasmosis, and cytomegalovirus; ii) moderately probable cases, new-born babies with imaging findings as in category 2, but without results for one or more of the three infections (syphilis, toxoplasmosis, and cytomegalovirus); iii) somewhat probable cases, new-born babies with imaging reports lacking a detailed description of the findings, for which a state-level physician concluded that a congenital infection was likely to have been involved, for whom laboratory results for syphilis, toxoplasmosis, or cytomegalovirus were negative or unavailable'. The discarded cases are new-born babies that were not included in the above categories. In total 899 (59.9%) of the 1 501 cases were discarded as not fitting into any of the above categories.
- Definite cases consisted of 76 (5%) new-borns. Fifty four cases (3.6%) were classified as highly probable congenital Zika virus syndrome cases, 181 (12%) as moderately probable and 291 (19.4%) as somewhat probable. One in five children classified as definite or probable Zika virus syndrome had a head circumference within the normal range. Information about the presence of a rash during pregnancy was available for 664 women, of whom 266 (40%) reported a rash. Rash was reported in 71.4% of cases classified as definite and in 75% classified as highly probable. In the subset of mothers for whom the timing of the rash was known (n=183), it was more frequently reported in the first trimester (77%) than the second (18%) and third trimester (5%). Rashes in the third trimester of pregnancy were associated with brain abnormalities despite normal-sized heads and for one third of definite and probable cases there was no history of a rash during pregnancy. As the positive predictive value for microcephaly of a rash among suspected cases was only 71.1% and a significant proportion of the definite and or probable cases presented with a normal head circumference, the authors also suggest a need to revise the criteria for including new-borns in the medical follow-up in the aftermath of a Zika outbreak.
- In a population-based retrospective study in Brazil, the number of suspected severe microcephaly cases were best predicted by Zika virus incidence during week 14 of pregnancy (95% confidence interval of mean = +/−0.08 weeks) [46].
- Johansson et al. estimated the relationship between trimester-specific Zika virus infection risk and microcephaly in Brazil [47]. They report a strong association between the risk of microcephaly and infection risk during the first trimester of pregnancy; ranging from 0.88% (assuming an 80% overall Zika virus infection rate and 100% over-reporting of microcephaly) to 13.2% (assuming a 10% Zika virus infection rate and no over-reporting). No substantial association was found for the second and third trimesters.
- A recent case report of severe foetal malformations during early pregnancy provides additional evidence [48]. The mother presented with a two-day rash at week four of pregnancy gestation and was later confirmed positive by Zika virus serology. Throughout the routine follow up, ultrasound examinations revealed foetal malformations and Zika virus was detected in the amniotic fluid. The pregnancy was terminated at week 21 and foetal autopsy demonstrated severe neurological malformations. Zika virus RNA was detected in brain and umbilical cord samples.

It is conceivable that other co-factors, such as the mother's age, genetic pre-disposition, concomitant infections and nutritional status among others, influenced the probability of transplacental transmission. Severe congenital impairments, such as microcephaly, may represent the severe phenotype of a broader spectrum of conditions included in the Zika congenital syndrome, which might also include other congenital impairment and adverse pregnancy outcomes.

To date, the information available is still insufficient to provide a robust estimate of the absolute and relative risk of adverse pregnancy complications by gestational age and the role of potential co-factors. Results from ongoing and further case–control and cohort studies are still required to more accurately estimate the risk of microcephaly and other adverse foetal outcomes in relation to Zika virus infection during pregnancy. Further prospective epidemiological studies on Zika virus in population should provide a better understanding of the clinical spectrum of the Zika virus disease and the potential associated cognitive and functional sequelae.

Ocular manifestations

In Salvador (Brazil), a high frequency of ocular lesions (bilateral macular and peri-macular lesions as well as optic nerve abnormalities) were identified in a case series of 29 infants with microcephaly with a presumed diagnosis of congenital Zika virus infection [49]. Pigmentary maculopathy was also described among three male infants who were born with microcephaly from mothers who had a viral syndrome compatible with Zika infection during the first trimester of gestation [50]. It is unclear if the lesions are secondary to microcephaly or caused directly by Zika virus infection of the retina. Since the last Rapid Risk Assessment on Zika virus [25], additional case reports have been published of ophthalmological complications among cases of congenital Zika virus infections without microcephaly:

- In June 2016, ocular impairments were first described in one infant without microcephaly by Ventura et al. [51]. The infant was referred for ophthalmic examination and was suspected of congenital Zika virus infection, due to lower limb and upper limb spasms at birth and computed tomography scan anomalies without microcephaly, although the mother did not report Zika virus compatible symptoms during the pregnancy. Congenital Zika virus infection was supported by the detection of IgM antibodies in the infant's cerebral spinal fluid. Chorioretinal scarring was detected on the macular region similar to scars previously reported in congenital Zika virus infection among microcephalic cases. This case report supports the occurrence of ocular impairment among congenital Zika virus infection cases, despite the absence of microcephaly. Further systematic ophthalmic examination of such cases are necessary to assess the frequency of ophthalmological complications as part of the congenital Zika virus syndrome.
- Based on several case series, non-purulent conjunctivitis is described as a frequent symptom of Zika virus disease [26]. Recently, the first case of uveitis associated with Zika virus infection was reported in a 40-year-old man eight days after symptomatic Zika infection. Aqueous humour from anterior-chamber obtained through paracentesis was positive for Zika virus RNA by RT-PCR. Visual acuity returned to its baseline after seven days of topical glucocorticoids. As other immune and infectious causes of uveitis were excluded, the case report indicates that anterior uveitis may be a clinical manifestation of Zika virus infection [52].

Guillain–Barré syndrome and other post-infectious neurological syndromes

A case-control study in French Polynesia and recent observations support the link between a previous Zika virus infection and development of Guillain–Barré syndrome [53]. The countries that have so far reported increases in Guillain–Barré syndrome are all affected by outbreaks of the Asian lineage of Zika virus.

Epidemiology

The relative contributions of both vector-borne transmission and sexual transmission were modelled using the daily number of clinically suspected cases of Zika virus in Barranquilla, Colombia. Using a vector-borne disease deterministic model (SEIR/SEI), a reproduction number of 4.4 (95% CI, 3.0 to 6.2) was estimated. By comparing with earlier chikungunya virus outbreaks in naïve populations, the authors hypothesise that the reproduction number for sexual transmission alone is too low to achieve sustainable transmission but can contribute to the occurrence of a substantial number of cases. A second study investigating the impact of mosquito-borne and sexual transmission estimated a basic reproduction number of 2.05 (95% CI: 0.52–6.30). Incidence data from Brazil, Colombia, and El Salvador were used to calibrate the deterministic model. The percentage contribution of sexual transmission is estimated to 3.04% (95% CI: 0.13–45.7). The sensitivity analyses of R_0 showed that the biting rate and mortality rate of mosquitoes are key parameters influencing the reproductive number. Sexual transmission increased the risk of infection and epidemic size and duration but may not initiate or sustain an outbreak by itself. The result of both modelling studies supports recommending both mosquito-control and personal biting protection as well as safe sexual practices to reduce Zika virus incidence during outbreaks [54].

With regard to temperate areas, a modelling study has estimated the basic reproduction number of Zika virus in areas where *Aedes* mosquito populations exist in Europe (*Aedes aegypti* in Madeira, Portugal and *Aedes albopictus* in continental Europe) [55]. Using a temperature-driven vectorial capacity model and assuming that the European population of *Aedes albopictus* have competence for the transmission of Zika virus in natural settings, potential areas for autochthonous transmission were centered on Italy, south-eastern France, the southern and eastern

coasts of Spain, western regions of the Balkans and in southern Greece. Potential transmission rates were predicted to follow a marked seasonal pattern, with an increase in July (R_0 values mainly ranging from 2 to 3), reaching a peak in August (R_0 values of 3 to 4) and decreasing in September to fall below one in October.

Transmission

Since the last rapid risk assessment, several cases of sexual transmission have been reported. A case of male-to-female sexual transmission occurred between 34 and 41 days after onset of symptoms of the male partner [56]. Similarly, Freour et al. reported a probable event of male-to-female sexual transmission between 21 and 36 days after returning from an affected area. Both partners were potentially exposed to Zika in an affected area, but the woman was viraemic 39 days after her return which cannot be explained by an exposure in the affected area, given a viraemia clearance ranging between 2 to 19 days. In addition the detection of Zika virus RNA in the man's semen and in the urine may argue for male-to-female sexual transmission. Interestingly both partners experienced an asymptomatic Zika virus infection [57].

A study of a family cluster of four Zika cases returning from Venezuela to China showed that in one asymptomatic case Zika viraemia was at the same level as seen among symptomatic cases (10 000 copies/ml) between seven to nine days after the last possible exposure to mosquito bites [58]. This observation confirms that asymptomatic cases can present as high a viral load as symptomatic cases. The epidemiological significance of such findings with regard to transmission from asymptomatic human to vector is unknown but should be considered as reported for dengue [59].

Further investigations are required into the mode of transmission and viral kinetics in bodily fluids in order to adapt prevention and control measures.

No new data on the vector competence of *Aedes aegypti* and *Aedes albopictus* have been reported since the last rapid risk assessment.

Diagnostics

The kinetics of Zika virus RNA was studied in serum, whole blood and urine samples from six symptomatic Zika virus cases [60]. Serum samples were positive three days after onset of symptoms, urine samples were positive for 26 days and whole blood samples were positive at 58 days. Values in serum and urine samples were similar to earlier findings. Zika virus RNA was found positive for all cases in the first set of whole blood samples (day post onset ranging for 5−58 days). These findings indicate that Zika virus RNA can be detected in whole blood samples for up to two months after infection; representing a notably longer period than that for serum and urine samples. Further studies are required to assess and validate these findings, which were also reported for West Nile virus [60].

A retrospective analysis of samples from 65 patients with rRT-PCR–confirmed acute Zika virus infection showed no false-positive results using two dengue NS1 tests (Platelia Dengue NS1 test, n=36; SD Bioline Dengue Duo test, n=21, and both tests applied on 8 samples) [61]. Despite the fact that Zika virus is related to dengue virus, the non-structural (NS) protein from Zika virus seems not to cross react with NS1 dengue tests.

EUROIMMUN Anti-Zika Virus ELISA (IgG, IgM) showed reduced cross-reactivity to other flaviviruses [62]. However, a probable cross-reactivity with samples from malaria patients with a current infection has been described since Zika virus neutralisation tests could not demonstrate a Zika virus infection in 11 of the 14 samples with positive or borderline ELISA results. Therefore, serum panels for laboratory assessment of Zika serological test specificity should include samples from malaria patients [63].

Treatment and vaccine development

Azithromycin (macrolide) has been shown to significantly reduce the cytopathic effect of Zika virus infection in vitro on [64]. On U87 cells (glioblastoma line expressing high levels of astrocyte and radial glia marker genes) the molecule inhibited viral proliferation for a range of concentrations comparable to those measured in adult brain tissue or in placental tissues. These findings require further investigation.

Using a susceptible mouse model, Larocca et al. showed that a single immunisation of a plasmid DNA vaccine expressing full-length Zika virus pre-membrane (prM) and envelope (Env) or a purified inactivated virus vaccine provides complete protection against a Zika virus strain from north-east Brazil [65]. Plasmid DNA vaccinated mouse showed a protective efficacy correlated with Env-specific antibody titers and an absence of detectable viraemia following a challenge with Zika virus. Deletion mutants from full-length prM-Env DNA vaccine were not able to provide protection. These data demonstrate that protection against Zika virus challenge can be achieved by single-shot DNA vaccines and inactivated virus vaccines in susceptible mouse models.

Event background information

Current situation worldwide

Autochthonous transmission of Zika virus was confirmed in Brazil in April 2015. Since January 2016 and as of 30 June, 159 939 probable and 40 086 confirmed cases of Zika virus infection have been reported [66].

Colombia remains the second most affected country in the Americas. Since October 2015 and as of 25 June 2016, 87 844 suspected and 8 850 confirmed cases have been reported nationally [67]. However, since the epidemic reached its peak in week 5 of 2016, the number of suspected and confirmed cases has been steadily declining.

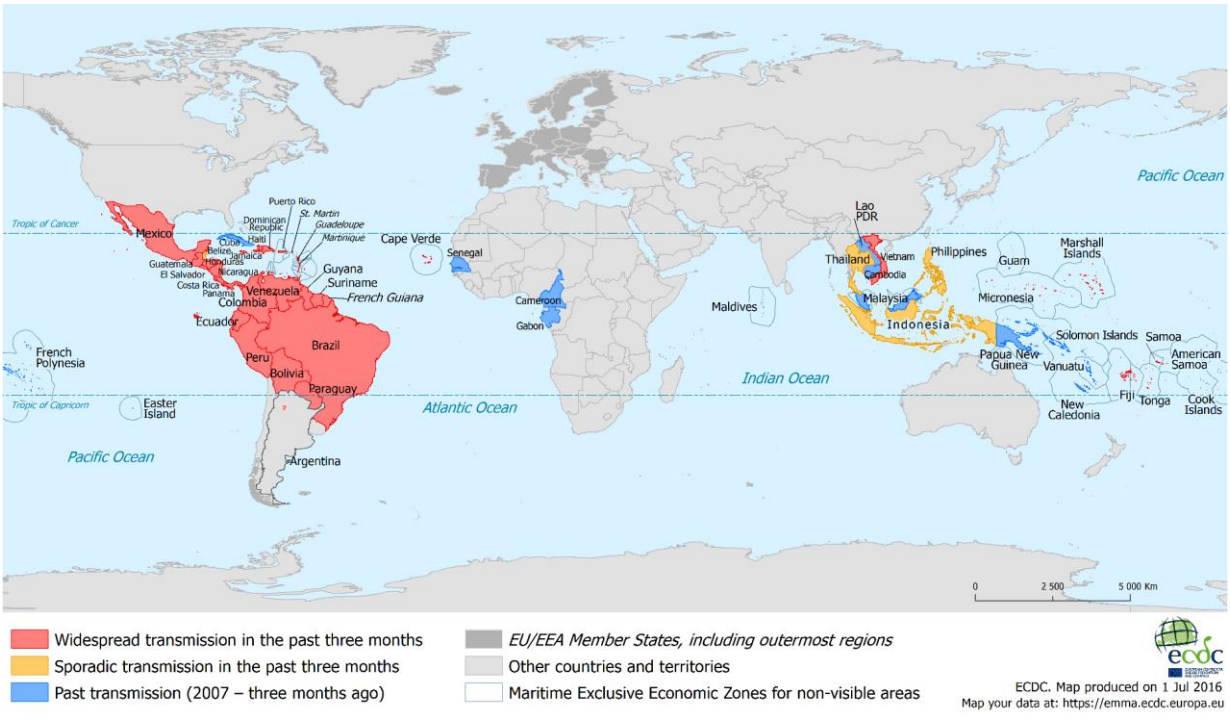
As of 4 July 2016, 11 countries reported non-vector-borne transmission of Zika virus, most likely through sexual transmission: Argentina, Canada, Chile, France, Italy, Germany, New Zealand, Peru, Portugal (in the Autonomous Region of Madeira), Spain and the United States of America [48,68].

Over the past three months and as of 1 July 2016, autochthonous cases of Zika virus infection have been reported from 49 countries or territories worldwide. In the past nine months, 53 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 Guillain–Barré syndrome is available through the ECDC Zika outbreak webpage [69]. Regular updates on the epidemiological situation are available on ECDC's webpage Countries and territories with local Zika transmission [70].

WHO AFRO reported that the strain currently circulating in Cape Verde is genetically related to the strain circulating in the Americas according to Institut Pasteur, Dakar [71]. Between week 41 in 2015 and week 17 in 2016, 7 750 suspected Zika cases were reported by the Ministry of Health in Cape Verde [72]. WHO and its partners are continuing to support preparedness in the WHO African Region.

On 1 July 2016, media reported three confirmed cases of Zika virus infection on the island of Bubaque (Bijagós) in Guinea Bissau. The cases were confirmed by Institut Pasteur in Dakar and the National Institute for Health Ricardo Jorge in Lisbon. Investigations are underway to determine the type of virus and its origin, since the three infected people claim to have had no contact with individuals from Cape Verde or Brazil [73].

Figure 1. Countries and territories with reported confirmed autochthonous vector-borne transmission of Zika virus infection in the past three months*, as of 1 July 2016



*As of week 17 in 2016, ECDC extended the period for classifying whether a country or territory has active local transmission from two to three months. This change is based on the observation that previous Zika virus outbreaks usually lasted more than two months. In addition, ECDC added a 'countries and territories with past vector-borne transmission' category for countries having experienced transmission since 2007 and up to three months ago. More information about country classification is available on the ECDC website.

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

As of 1 July 2016, no autochthonous vector-borne Zika virus transmission has been reported in the EU Member States of continental Europe. Since January 2016 and as of 1 July, ECDC has recorded 977 imported cases of Zika infection in 20 EU/EEA countries. The number is based on reporting through surveillance systems, TESSy and event-based surveillance. Two countries have reported the majority of the imported cases; France reported 534 cases of Zika virus and Spain reported 154 cases since late January 2016. Forty-six of the imported cases were among pregnant women.

Several EU Outermost Regions (OMR) and Overseas Countries and Territories (OCT) continue to report vector-borne autochthonous Zika transmission: French Guiana, Guadeloupe, Martinique, Saint Martin and Saint-Barthélemy. In addition, islands in the Dutch Antilles (Aruba, Bonaire, Curacao and Sint Maarten) continue to report autochthonous transmission. According to PAHO and WHO, the number of Zika cases is still increasing in the EU Outermost Regions and Overseas Countries and Territories in the Caribbean [74].

Microcephaly and congenital central nervous system malformations

As of 29 June 2016, congenital microcephaly, CNS malformations and other foetal malformations potentially associated with Zika virus infection during pregnancy have been reported in ten countries or territories: Brazil, Cape Verde, Colombia, El Salvador, French Guiana, French Polynesia, Martinique, Marshall Islands, Panama and Puerto Rico. Outside of the epidemic area, Slovenia reported one case exposed in Brazil, Spain reported two cases exposed in Colombia and Venezuela and US reported eight cases [68].

Brazil

Between October 2015 and 30 June 2016, Brazil reported 8 165 suspected cases of microcephaly and other nervous system disorders suggestive of congenital infection. Of these, 1 638 are confirmed cases of microcephaly, 270 of which are laboratory-confirmed for Zika virus infection.

Colombia

Between weeks 1 and 25 in 2016, Colombia reported 13 confirmed cases of microcephaly associated with Zika virus infection, 56 cases were investigated and discarded and 112 cases are still under investigation [67].

Guillain–Barré syndrome and other neurological syndromes

As of 29 June 2016, in the context of Zika virus circulation, 14 countries and territories worldwide have reported an increased incidence of Guillain-Barré syndrome (GBS) and/or laboratory confirmation of a Zika virus infection among GBS cases: Brazil, Colombia, Dominican Republic, El Salvador, French Guiana, French Polynesia, Honduras, Martinique, Suriname, Venezuela, Guadeloupe, Haiti, Panama and Puerto Rico [68].

International guidance and response plans

The World Health Organization has published:

- A revised Zika strategic response plan for July 2016–December 2017 [75].
- A summary of a rapid advice guidelines for infant feeding in areas of Zika virus transmission (updated on 29 June 2016), which concluded that the benefits of breastfeeding for the infant and mother outweigh any potential risk of Zika virus transmission through breastfeeding on the basis of the evidence currently available [76].
- A vector control operations framework for Zika virus [77].

US Centers for Disease Control and Prevention published a Draft Interim Zika Response Plan in June 2016 [78].

ECDC published a preparedness planning guide for diseases transmitted by *Aedes aegypti* and *Aedes albopictus* and a Policy briefing 'Preparing for Zika in the EU' on 27 June 2016 [79,80].

ECDC threat assessment for the EU

The Zika epidemic remains a significant concern for public health. During the third meeting of the International Health Regulations Emergency Committee on 14 June 2016, convened by the Director-General of WHO under the International Health Regulations (2005), the Committee 'concurred with the international scientific consensus, reached since the Committee last met, that Zika virus is a cause of microcephaly and GBS, and, consequently, that Zika virus infection and its associated congenital and other neurological disorders is a Public Health Emergency of International Concern (PHEIC)' [81].

The epidemic continues to evolve but the number of affected countries in the Americas and Caribbean appears not to have changed during June 2016 [68]. In some countries and territories in the Americas and the Caribbean, such as Colombia, Brazil, El Salvador and Haiti, there has even been a decrease in the number of reported cases during the past month [82]. Vector-borne transmission is expected to continue in the coming months, notably in Central American countries and the Caribbean, where vector activity is significant during the summer season.

The outbreak is unprecedented and constitutes a significant development in the epidemiology of this emerging vector-borne disease. The evolution of the Zika epidemic in the Americas demands close monitoring as it has a direct impact on the risk of importation and possible occurrence of local transmission in the European Union. The viral circulation in affected countries coincides with the summer holiday period in Europe and it is expected that Zika viraemic travellers will continue to return to the EU during the entire 2016 European mosquito season. This will create the possibility of onward transmission of Zika virus in receptive areas in Europe.

The occurrence of occasional cases of Zika virus in Asia is expected, in view of the historical records of Zika virus circulation, case reports of travel-related cases and previous sero-surveys.

Travel-related risk for EU citizens

Travellers to countries where competent vectors are present and Zika virus circulation is ongoing are at risk of becoming infected through mosquito bites. Due to the link between Zika virus infection and severe congenital anomalies, pregnant women and women who are trying to become pregnant constitute a high-risk group in terms of serious adverse outcomes of Zika virus infection.

Risk related to mass gatherings

The Rio de Janeiro 2016 Olympic Games (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. ECDC has published a specific risk assessment and an update on these events, including an assessment of Zika virus infection [83,84]. ECDC is continuing to follow the evolution of the Zika virus epidemic in order to assess and monitor the trends in Brazil.

On 12 May, WHO published a statement on Zika virus and the Olympic and Paralympic Games, Rio 2016, providing specific advice for those participating and visiting Brazil for the Games [85]. On 29 June 2016, WHO updated the health advice for travellers to the 2016 Summer Olympic and Paralympic Games providing specific recommendations for Zika virus disease [86].

Risk of importation and transmission in EU Outermost Regions and Overseas Countries and Territories

Residents in EU Outermost Regions (OMR) and Overseas Countries and Territories (OCT) with competent and active vectors are at increased risk of exposure to Zika virus. *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 (see Countries and territories with local Zika transmission) [70].

The risk associated with spread to as yet unaffected OCTs and OMRs in the area is significant because of the immunologically naïve populations, the presence of competent vectors, the occurrence of prior outbreaks of arboviruses transmitted by *Aedes* mosquitoes, the permissive climate and the movement of people in and between countries and territories.

Other EU OMRs and OCTs outside of the Caribbean where mosquito vectors are present, such Madeira and Mayotte with *Aedes aegypti* or La Réunion with *Aedes albopictus*, are at risk of local transmission should the virus be introduced.

Madeira is of particular concern because of the presence of *Aedes aegypti* and the probability of vector-borne pathogen transmission is considered high during the summer months. The 2012 dengue epidemic demonstrated the favourable conditions for mosquito-borne outbreaks during the summer season and the close relationship with countries (such as Brazil and Venezuela) where Zika virus is currently circulating increases the risk of the virus being imported [13].

According to the Interim Risk Assessment issued by WHO's European Region, the capacity to contain Zika virus transmission at an early stage is good for the countries of the WHO European Region overall [87].

Risk of importation and transmission within the continental EU

The outbreak in the Americas and the Caribbean has considerably increased the risk of Zika virus importation into the EU through infected returning travellers and visitors from the affected countries. Cases of Zika virus infection arriving from countries with autochthonous transmission continue to be reported in the EU and it is expected that this will continue during the EU/EEA summer.

There is no evidence to date of 'airport transmission' of mosquito-borne viral disease, unlike the evidence for 'airport malaria' [88]. 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 [89,90]. On 21–22 April 2016, WHO organised an ad-hoc advisory group to review the evidence on effectiveness of aircraft disinsection to prevent the international spread of mosquito-borne disease, including Zika [91].

On 13 April the SHIPSAN Act Interim guidance on maritime transport and Zika virus disease [92] was updated. The European Transport Workers' Federation (EFT) and European Community Shipowners' Association (ECSA) acknowledged in a common statement the need to draw shipping companies' and seafarer's attention to the risks of the Zika virus and to provide crew members on board ships calling at ports in affected countries with relevant guidance to protect themselves [93].

The likelihood of mosquito-borne transmission of Zika virus infection in the EU is considered plausible only for those areas where mosquitoes capable of carrying and transmitting the virus are present. The transmission depends on several factors related to the mosquito, the virus and the environment [94,95]:

- The introduction of the virus by a viraemic traveller during the summer season where *Aedes albopictus* is established can be expected (see situation in the EU/EEA and EU Outermost Regions and Overseas Countries and Territories). *Aedes albopictus* is established around the Mediterranean basin. As part of the VectorNet project, ECDC shows the current known distribution of invasive mosquito species in Europe at regional administrative level (NUTS 3) [96].
- The suitable conditions for *Aedes albopictus* activity increase progressively during the spring (April to June), especially in southern Europe. By analogy with other mosquito-borne disease transmission, the conditions for autochthonous Zika virus transmission will remain favourable in continental Europe during summer and autumn [55].
- Factors such as survival, density, and biting behaviour of the vector species will determine the final transmission potential of the vector species. Local vector-borne transmission in the EU therefore cannot be excluded.

Given the low vector competence of the studied European populations of *Aedes albopictus*, the likelihood of local vector-borne transmission in the EU is considered to be low to moderate.

Risk of Zika virus transmission via substances of human origin

Zika virus RNA has been detected in blood, urine, saliva, seminal fluid and breast milk [97-101] (see Annex 1).

People with asymptomatic infections and those who are viraemic during the incubation period of Zika virus disease could potentially donate contaminated substances of human origin (SoHO) without their infections being recognised at the time of donation. The virus could 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. There are no data available on the survival of Zika virus in processed and stored SoHO.

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 [97,102]. From 3 April to 11 June 2016, a total of 68 (0.5%) presumptive viraemic donors were identified from 12 777 donations tested in Puerto Ricco. The highest weekly incidence was 1.1% for the last week of reporting (5–11 June). The incidence has been increasing over time [103]. The Brazilian media reported possible cases of transfusion-transmitted Zika virus in March 2015 and February 2016 [104-106]. A probable case of transfusion-transmitted Zika virus infection in Brazil has recently been published [107].

Several reported cases of sexual transmission from males to their partners (see 'Risk of sexual transmission' below) strongly support a possibility of Zika transmission through donated sperm.

Zika virus RNA has been detected in brain, liver, spleen, kidney, lung and heart samples from a fatal case in an adult with underlying chronic health conditions [108]. Yet, it is unknown whether organs, infected with Zika virus, could transmit a disease.

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.

A recent case report of Zika congenital infection showed a prolonged detection of low viral level by quantitative RT-PCR of Zika virus RNA in serum from the mother in weeks 16 and 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 [30].

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 Guillain–Barré syndrome justifies preventive measures to reduce the risk of transmission via SoHO supply [109].

Risk of sexual transmission

Replicative Zika virus particles have been detected on two occasions in semen at 21 and 24 days after onset of Zika symptoms [100,110].

Zika viral RNA has been reported in semen at 14 days [111], 21 days [100], 24 days [110], 45 days [112], 47 days [113], and 62 days after clinical onset of disease [114], and as late as 92 days (Ct >35) from a symptomatic patient (personal communication: Emma Aarons and Daniel Bailey, Public Health England. Manuscript in preparation). In another study a gradual decrease of viral RNA load was reported in two patients until Zika virus was undetectable after 56 and 68 days post-onset of symptoms respectively [115]. In two other patients, tests for Zika virus RNA in semen were negative. Viral isolation from the semen of these patients was not successful.

Zika virus genome has also been detected in saliva during and after the acute phase of the disease and reported from the symptom onset and for up to 29 days afterwards [116]. Isolation of virus from saliva was reported on day 6, as well as a second isolation, but only stating the date when the sample was collected [117].

Time of detection Zika virus in biological samples is summarised in Annex 1 (Table 1).

Reports of sexual transmission of Zika virus from men with symptomatic infection to both female and male sexual partners via contaminated semen indicate the possibility of virus transmission through donated sperm [118-123]. With regard to sexual transmission, the interval between onset of symptoms in the man and in his female partner was 44 days [56]. So far, no sexual transmission of Zika virus from infected women to their partners has been reported.

Freour et al. reported a probable event of asymptomatic male-to-female sexual transmission between 21 to 36 days after returning from an affected area [57] (see above section 'Disease background' for more details).

Comprehensive data about the presence of viable virus, viral load or kinetics is not available, and at this point in time the risk of transmission via saliva cannot be further assessed. Further comprehensive information about the kinetics of the Zika virus in bodily fluids and the consequent clinical implication is required in order to adapt prevention and control measures accordingly.

Annex 1. Time of detection Zika virus in biological samples

Table 1. Time of detection of Zika virus in human samples among symptomatic cases

Sample origin	Methods	Range of detection in days from onset of symptoms			
		Minimum (days)	Ref.	Maximum (days)	Ref.
Blood	Molecular diagnostic	First day of symptoms	[58,98,124]	14 to 16	[111,125]
Whole blood	Molecular diagnostic	5	[126]	58	[126]
Urine	Molecular diagnostic	First day of symptoms	[98,124]	15 to 29	[98,116,124]
	Virus isolation			4	[127]
Saliva	Molecular diagnostic	First day of symptoms	[58,124]	29	[116]
	Virus isolation			6	[116]
Seminal fluid	Molecular diagnostic	10	[113]	From 58 and 62 up to 92	[114] (Personal communication. Emma Aarons & Daniel Bailey, Public Health England. Manuscript in preparation)
	Virus isolation	NA		21 to 24	[100,110]
Breast milk	Molecular diagnostic	3 (after delivery)	[101]	8 (after delivery)	[101,128].
	Virus isolation	NA		4 (after delivery)	[128,129].

NA = not available.

Zika virus RNA was detected in three asymptomatic individuals during a biological, clinical, serological and virological follow-up of a military community exposed to Zika virus in Suriname over a two-week period (two urine samples and one blood sample) [130] and in one case returning from Venezuela to China among a family cluster of symptomatic cases (positive sample serum, urine, saliva) [58].

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 between weeks 16 and 20 of pregnancy and 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 [30].

Based on a modelling study, seroconversion occurs on average nine 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 depending on the vaccination status against flaviviruses [7,131].

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