We report a case of Zika virus infection imported in Florence, Italy ex-Thailand, leading to a secondary autochthonous case, probably through sexual transmission. The two cases occurred in May 2014 but were retrospectively diagnosed in 2016 on the basis of serological tests (plaque reduction neutralisation) performed on stored serum samples. Our report provides further evidence that sexual transmission of Zika virus is possible.

Case reports

At the beginning of May 2014, an Italian man is his early 30s (patient 1) returned to Florence, Italy, after a 10-day holiday in Thailand. On the day after his arrival, he developed a confluent maculopapular rash, on the face, trunk, arms, and legs, with fever (maximum temperature 38°C), conjunctivitis, and frontal headache with retroocular pain.

Four days later, patient 1 was admitted to the Infectious and Tropical Diseases Unit of the Florence Careggi University Hospital. Blood tests revealed leucopenia (3,000 cells/µL; reference: 4,000–10,000/µL) while creatinine, platelet count and transaminases were normal. Serological investigation two days after (i.e. 6 days after symptoms onset), showed past exposure to measles and parvovirus, negative results for human immunodeficiency virus (HIV) 1–2 Ab/Ag and chikungunya IgM, a positive result for dengue virus (DENV) IgM, and negative results for DENV IgG, as well as DENV NS1 Ag (Table).

The symptoms subsequently rapidly resolved (total duration of fever and rash: 6 days) and he was discharged nine days after admission with a probable diagnosis of DENV infection.

Perifocal vector control activities (including spraying adult mosquitoes and destruction of larval breeding sites) were implemented the day after the availability of DENV IgM positive results, around the patient’s residence and workplace, even though the period of activity of Aedes albopictus in Italy is usually considered to start in June and end in October [1]. A second and third blood test using enzyme-linked immunosorbent assay (ELISA), performed 38 and 109 days after symptoms onset, showed DENV IgG seroconversion and IgM negativisation in the third sample.

Nineteen days after the onset of symptoms in patient 1, his girlfriend (patient 2), who was in her late 20s developed diffuse pain, associated to both wrists and oedema on fingers of each hand, maculopapular rash on the trunk, arms, and legs, without fever. Four days later she was evaluated at the outpatient facility of the same hospital. Patient 2 had not travelled to tropical areas during the previous year. Blood tests performed on the next day (5 days after her symptoms started) showed normal white blood cells and platelet count, normal C-reactive protein, creatinine, transaminases, and undetectable beta-human chorionic gonadotropin (HCG). The patient had IgG antibodies against cytomegalovirus, Epstein–Barr virus, parvovirus and rubella, while she was seronegative for coxsackie A, coxsackie B, echovirus and DENV (IgG, IgM and NS1 Ag). Serological tests were repeated 39 and 93 days after symptoms onset, respectively, showing a slight positivity for DENV IgG, with IgM and NS1Ag persistently negative (Table).

Retrospective testing of serum samples in 2015 and 2016

Serum samples of both patients were sent to the Istituto Superiore di Sanità (ISS), Rome, Italy, to perform confirmatory tests (Table) for DENV in June and September 2015, respectively. Plaque reduction neutralisation tests (PRNTs) for DENV gave inconclusive results for both patients: indeed, a 50% of plaque reduction was
Background

ZIKV is an *Aedes*-borne virus (Flaviviridae family), identified in 1947 in monkey rhesus in Uganda [2,3]. Sporadic human cases were reported in Asia and Africa until 2007, when a ZIKV outbreak occurred in Yap, Micronesia [4]. Subsequently, in October 2013, ZIKV reached French Polynesia, causing a large outbreak [5]. In early 2015, autochthonous cases of ZIKV were reported in Brazil [6], and the virus subsequently spread throughout South America, Central America, and the Caribbean [7-9]. An increasing number of imported cases has been observed in Europe and United States (US) [10-13]. The presumed association of ZIKV infection during pregnancy with increased number of babies born with microcephaly in Brazil [14] convinced the World Health Organization to declare ZIKV a ‘Global Emergency of Public Health Concern’ in February 2016 [15].

Discussion and conclusions

Even if ZIKV transmission is mostly vectorial, transplacental and perinatal transmission have been reported; transmission through blood transfusion may also occur [16-18].

Little evidence supports the possibility of ZIKV sexual transmission to date. In December 2013, ZIKV was isolated from the semen of a patient with haematospermia in Tahiti [19]. Further in 2014, ZIKV RNA was detected 62 days after onset of febrile illness in the semen of a person with ZIKV infection, imported into the United Kingdom from the Cook Islands [20]. Sexual transmission from a man who acquired ZIKV infection in Senegal, to his wife was reported in Colorado, US, in 2007 [21], and more recently from a person who had travelled to Latin America, to his partner in Texas [22].

Possible sexual transmission of ZIKV is of particular concern during pregnancy, and specific guidelines for prevention of ZIKV infection through this route have been published recently [23].

Because patient 2 had not travelled to tropical areas during the previous year and had unprotected sexual intercourse with patient 1 during a 20 day period between his return to Italy and her own onset of symptoms, transmission by semen was suggested. Even if ZIKV transmission is mostly vectorial, transplantation and perinatal transmission have been reported; transmission through blood transfusion may also occur [16-18].

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Possible sexual transmission of ZIKV is of particular concern during pregnancy, and specific guidelines for prevention of ZIKV infection through this route have been published recently [23].

Because patient 2 had not travelled to tropical areas during the previous year and had unprotected sexual intercourse with patient 1 during a 20 day period between his return to Italy and her own onset of symptoms, transmission by semen was suggested. Exact dates of sexual intercourse could not be recalled by the patients, who reported several sexual contact events before patient 2’s symptom onset. Other transmission modalities (i.e. direct contact with other bodily fluids)
are unlikely to play a role but may not be completely ruled out.

Transmission through local potentially competent vectors, *Ae. albopictus*, can likely be excluded considering that patient 1 came back to Italy outside the usual period of vector activity and vector control measures were implemented within eight days after his arrival to Italy, possibly before the estimated extrinsic incubation period could be completed [1, 24].

Failure to detect viral RNA even in samples collected few days after the onset of symptoms, and an early detection of ZIKV-specific neutralising antibodies, are consistent with previous reports [10, 19, 25]; however, limits in the sensitivity of the real-time PCR method used in this study cannot be definitively excluded. Serological test results confirm the broad cross-reactivity between DENV and ZIKV. With respect to PRNT results, borderline results for DENV are likely to be due to a low degree of residual cross-reactivity which may not be eliminated even using this test, which is considered highly specific. Another possible limit of our study consists in the fact that only serotype 2 DENV PRNT could be performed; however, this is not likely to affect the interpretation of the results, which clearly show a pattern consistent with ZIKV infection.

Current evidence supports the combined use of PCR and serological tests for the diagnosis of ZIKV infection. PCR can be positive in early serum and saliva samples (<8 days after symptoms onset), with saliva showing higher detection rates, while PCR on urine seems to enlarge the window of detection of ZIKV RNA up to ca 30 days after symptoms onset [26, 27]. Five days after disease onset, serological investigations can be conducted by detection of ZIKV-specific IgM antibodies and confirmation by neutralisation [28].

In conclusion, we provide additional evidence for sexual transmission of ZIKV. Further studies are needed to estimate the probability of sexual transmission and its role as a secondary route of transmission of ZIKV in epidemic and non-epidemic areas.

**Conflict of interest**

None declared.

**Authors’ contributions**

Wrote the manuscript: LZ, GR, GV, AB; performed laboratory investigations: AM, CF, MER, EB, CF, GV; revised the manuscript: GR, MT, CR; managed the patients: LZ, MT.

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