

CASE REPORT

An autochthonous sexually transmitted Zika virus infection in Italy 2016

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SUMMARY

We describe two cases of Zika virus infection involving an Italian patient returning from the Dominican Republic and his wife, who remained in Italy and had not travelled to Zika virus endemic areas in the previous months. The infection was transmitted through unprotected sexual intercourse after the man's return to Italy.

Received April 25, 2017

Accepted September 29, 2017

INTRODUCTION

Zika virus (ZIKV) is a mosquito-borne virus belonging to the *Flaviviridae* family (genus *Flavivirus*). It is endemic in Africa and Southeast Asia (Peterson *et al.*, 2016), but only few human cases had been reported before 2007. In 2007, a ZIKV outbreak occurred in the Micronesian Yap Islands (Duffy *et al.*, 2009). Subsequently, in 2013-2014 a large ZIKV outbreak was reported in French Polynesia (Cao-Lormeau *et al.*, 2013). In March 2015, the first local transmission of ZIKV in the Americas was detected in Bahia, Brazil (Campos *et al.*, 2015). Since then, local transmission has been documented in about 47 countries in the Americas (Pan American Health organization/World Health Organization, 2016). ZIKV infections in humans are frequently asymptomatic or are characterized by maculopapular rash, fever, myalgia/arthralgia, headache, retro-orbital pain and conjunctivitis (Peterson *et al.*, 2016). ZIKV infection appears to be associated with fetal malformations, as shown by the striking increase in microcephalia in endemic regions (Mlakar *et al.*, 2016). *Aedes* spp. mosquitoes are the vector of ZIKV infection in humans (Peterson *et al.*, 2016). Non-vector-borne transmission of Zika virus has also been documented by sexual transmission (D'Ortenzio *et al.*, 2016) and trans-placental virus transmission during pregnancy (Mlakar *et al.*, 2016).

CASE REPORTS

From 16th to 22nd March 2016, a 50-year-old Italian man visited Santo Domingo in the Dominican Republic. Five

days after his return to Italy (27th March) the man complained of arthralgia, myalgia, macular cutaneous rash and conjunctivitis. On 1st April, the patient was evaluated at the Department of Infectious Diseases of the University of Insubria, Varese, Italy.

Upon examination, a potential arbovirus infection was suspected and serum, plasma, urine and saliva samples were collected and referred to the regional reference laboratory (Molecular Virology Unit, Microbiology and Virology Department, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy). The patient was advised not to have unprotected sex but he reported that he had already had unprotected vaginal intercourse with his wife. Serum tests revealed ZIKV-specific IgM (Anti-Zika virus ELISA (IgM), Euroimmun, Lübeck, Germany) and ZIKV neutralizing antibodies (Timiryasova *et al.*, 2013) and an absence of ZIKV-specific IgG (Anti-Zika virus ELISA (IgG), Euroimmun, Lübeck, Germany), suggesting an acute ZIKV infection.

The diagnosis was confirmed by the presence of ZIKV RNA in his urine sample, while plasma and saliva specimens tested negative by two molecular methods (Scaramozzino *et al.*, 2001; Lanciotti *et al.*, 2008), as shown in *Table 1*. Sequencing of amplicon from the man's urine confirmed infection by ZIKV (GenBank accession number KY014328). Furthermore, DENV and CHIKV infections were ruled out since specific DENV and CHIKV IgM and IgG were negative (Dengue virus IgM Capture DXSelect™ and Dengue virus IgG DxSelect™ by Focus Diagnostics, Cypress, CA 90630, USA; Anti-Chikungunya virus IFA (IgG), Anti-Chikungunya virus IFA (IgM), Euroimmun, Lübeck, Germany), as were all the specific molecular assays performed on serum and urine samples. Twenty-four days after the onset of illness, ZIKV IgM were positive and ZIKV IgG seroconversion was observed. ZIKV RNA was positive in urine and semen with both molecular assays (Scaramozzino *et al.*, 2001; Lanciotti *et al.*, 2008). At day 27 post onset of symptoms, ZIKV RNA was still positive in urine with pan-Flavivirus

Key words:

Zika virus, Sexual transmission, Non-vector-borne transmission.

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RT-PCR (Scaramozzino *et al.* 2001), while after 41 days it was negative with both ZIKV molecular assays (Scaramozzino *et al.*, 2001; Lanciotti *et al.*, 2008) (Table 1).

On 10th April, the patient's wife (47 years old), developed macular cutaneous rash and retro-orbital pain. She was evaluated the next day at the Department of Infectious and Tropical Diseases of the University of Insubria. The woman had no history of travelling to ZIKV endemic areas in the previous months. Suspecting a potential sexual transmission of ZIKV infection between the partners, the woman's serum, plasma, urine and saliva samples were collected and referred to the regional reference laboratory. One day after symptoms onset, ZIKV IgM and IgG scored negative, while ZIKV RNA was detected in her plasma and saliva with both molecular assays performed (Scaramozzino *et al.*, 2001; Lanciotti *et al.*, 2008) and in urine only with the ZIKV Real-time RT-PCR (Lanciotti *et al.*, 2008) (Table 1).

Sequencing of amplicon from the woman's saliva confirmed infection by ZIKV (GenBank accession number KY014329). Ten days after the onset of illness, ZIKV IgM and ZIKV neutralizing antibodies (Timiryasova *et al.*, 2013) became positive while ZIKV IgG were still negative. ZIKV RNA in plasma and saliva was negative, while it was still positive in urine with both molecular assays (Scaramozzino *et al.*, 2001; Lanciotti *et al.*, 2008). Seventeen days after symptoms onset, ZIKV IgM and ZIKV neutralizing antibodies (Timiryasova *et al.*, 2013) were positive whereas ZIKV IgG began to increase, reaching borderline values. ZIKV RNA was still positive in urine with pan-Flavivirus RT-PCR (Scaramozzino *et al.*, 2001). Twenty-four days after the onset of illness, ZIKV IgM were positive and ZIKV IgG seroconversion was observed. ZIKV RNA was negative in urine with both ZIKV molecular assays (Scaramozzino *et al.* 2001; Lanciotti *et al.*, 2008) (Table 1).

Neutralizing antibodies increased more than fourfold from the first to the last serum sample in the man, and from the second to the last serum sample in the woman, as shown in Table 1. The comparison of the ZIKV sequenc-

es from the man's urine and the woman's saliva showed a complete homology (100%).

DISCUSSION AND CONCLUSIONS

The two cases of ZIKV infection described in this report support the observation that ZIKV could be transmitted by vaginal intercourse. This hypothesis is sustained by the following evidence:

- 1) the woman had not travelled to ZIKV endemic areas in the previous months;
- 2) she contracted ZIKV infection during a period characterized by the absence of vector activity in Italy;
- 3) she had unprotected vaginal intercourse with a ZIKV-symptomatic patient shedding virus in semen;
- 4) the comparison of the ZIKV sequences from the spouses showed a complete homology (100%).

The sexual transmission of ZIKV is of particular interest for its obvious implications in pregnancy outcomes. Indeed, the greatest impact of ZIKV infection on fetal damage is reported in the first trimester of pregnancy (Cauchemez *et al.*, 2016). As reported in a current review (Moreira *et al.*, 2017), ZIKV is potentially sexually transmitted either from symptomatic or asymptomatic infected individuals, with a prolonged period of shedding in the male genital tract after symptom onset. A recent study reported the detection of ZIKV-RNA in semen six months after onset of symptoms (Barzon *et al.*, 2016), showing a prolonged potential risk for virus sexual transmission. ZIKV sexual transmission is an important additional risk factor for pregnancy in both endemic and non-endemic areas. In view of the obvious implications of positive ZIKV genital secretion for autochthonous transmission, it is strongly advised to include semen analysis by RT-PCR as part of the routine diagnostic work-up in subjects with suspected ZIKV infection. In addition, advice on avoiding unprotected sex should be provided. Finally, sexual partners, in particular women of child-bearing age, should be mandatorily included in surveillance protocols.

Table 1 - Virological values of the couple infected with Zika virus (April 2016).

Patient	Days after symptom onset samples taken	ZIKV IgG	ZIKV IgM	ZIKV NT Ab	Zika virus-specific real-time RT-PCR (copies/ml)				Pan-flavivirus RT-PCR			
					plasma	urine	saliva	semen	plasma	urine	saliva	semen
Case 1	5	Neg	Pos	Pos (1:160)	Neg	Pos (2.775)	Neg	na	Neg	Pos	Neg	na
Case 1	24	Pos	Pos	Pos (1:640)	Neg	Pos (26)	Neg	Pos (45.804)	Neg	Pos	Neg	Pos
Case 1	27	ND	ND	Pos (1:1280)	Neg	Neg	Neg	na	Neg	Pos	Neg	na
Case 1	41	ND	ND	Pos (1:5000)	Neg	Neg	Neg	na	Neg	Neg	Neg	na
Case 2	1	Neg	Neg	ND	Pos (664)	Pos (556)	Pos (11.351)	NA	Pos	Neg	Pos	NA
Case 2	10	Neg	Pos	Pos (1:160)	Neg	Pos (51)	Neg	NA	Neg	Pos	Neg	NA
Case 2	17	borderline	Pos	Pos (1:320)	Neg	Neg	Neg	NA	Neg	Pos	Neg	NA
Case 2	24	Pos	Pos	Pos (1:1280)	Neg	Neg	Neg	NA	Neg	Neg	Neg	NA

ZIKV: Zika virus; ND: not done; NA: not applicable; na: not available; NT Ab: neutralizing antibody titre; Pos: positive; Neg: negative; RT-PCR: reverse transcription-polymerase chain reaction.

Acknowledgements

We thank all the technical staff for handling the specimens and performing the assays. We thank Mrs Daniela Sartori for manuscript editing and Mrs Sheila McVeigh for English revision. This work was funded by Ministero della Salute, Fondazione IRCCS Policlinico San Matteo, Ricerca Corrente (grant no. 80206).

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