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Case report

Confirmed Zika virus infection in a Belgian traveler returning from Guatemala, and the diagnostic challenges of imported cases into Europe

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ABSTRACT

We report the first laboratory-confirmed Zika virus (ZIKV) infection in a Belgian traveler after a three week holiday in Guatemala, December 2015. This case along with other imported cases into Europe emphases once again the need for accurate diagnostic tools for this rapidly emerging virus. The challenge is to diagnose patients in the acute phase, which appears short, as serological testing is complicated by cross-reactivity, vaccination status and scarce availability of specific ZIKV tests.

1. Why this case is important

Zika virus (ZIKV) is a flavivirus transmitted by *Aedes* mosquitos. Clinical symptoms include fever, rash, arthralgia, myalgia and conjunctivitis, but an estimated 80% of persons infected with ZIKV are asymptomatic [1]. In the acute phase, the clinical presentation is difficult to differentiate from other arbovirus infections such as dengue virus (DENV) and chikungunya virus (CHIKV), which are transmitted by the same vectors and commonly co-circulate in the same geographic area. The course of ZIKV disease is usually mild and self-limiting, but further in-depth studies are needed to investigate if there is a causal link between ZIKV infections and Guillain-Barré syndrome cases, newborns with microcephaly and other neurological manifestations which have been increasingly reported in countries hit by the current and past outbreaks [2]. In the meantime specific prevention recommendations for and follow-up of pregnant woman are warranted, both in endemic regions and for travelers [3], and a growing demand for asymptomatic ZIKV testing related to pregnancy is faced. To follow-up transmission of ZIKV towards new regions, rapid diagnosis of viremic patients is needed.

1.1. Case description

A Belgian 27-year old female traveled for three weeks in Guatemala and visited Antigua, Lake Atitlán, Panajachel, Chichicastenango, Flores, Tikal, Rio Dulce, Livingstone and Monterrico. Despite using a DEET containing insect repellent she recalled several mosquito bites, especially in Monterrico where she stayed from November 30th until December 4th 2015. On December 7th 2015, one day after her return to Belgium, she developed a headache and retro-orbital pain, followed by fever and arthralgia mainly of the knees, wrists and fingers one day later. On the 9th of December she had a rash on the thorax and upper limbs and nausea without vomiting. The next day she presented at the local Travel Clinic of Clinique Saint-Pierre, Ottignies, Belgium.

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Table 1
Laboratory data.

	serum sample, day after symptom onset	3d	17d
Direct virus detection tests	ZIKV RT-PCR[a]	**Ct 40.57/ Ct 40.63/Neg**	ND
	flavivirus RT-PCR[b]	**Ct 46.62**	ND
	DENV RT-PCR[c]	Neg	ND
	DENV NS1 Ag test[d]	Neg	ND
	ZIKV culture	Neg	ND
IIFT[e]	Anti-ZIKV-IgG	Neg	**1:5,120**
	Anti-ZIKV-IgM	Neg	**1:320**
IIFT[f]	Anti-ZIKV-IgG	Neg	**>1:10,000**
	Anti-ZIKV-IgM	Neg	**1:100**
	Anti-DENV-IgG	Neg	**1:10,000**
	Anti-DENV-IgM	Neg	**DEN23 1:10**
	Anti-CHIKV-IgG	Neg	Neg
	Anti-CHIKV-IgM	Neg	Neg
ELISA[g]	Anti-DENV-IgG	Neg (0.72)	**Pos (5.98)**
	Anti-DENV-IgM	Neg (0.18)	Neg (0.96)
IFFA[h]	Anti-DENV-IgG	Neg	**DEN2 1:10,000 DEN134 1:1,000**
	Anti-DENV-IgM	Neg	Neg
	Anti-JEV-IgG	Neg	**1:1,000**
	Anti-JEV-IgM	Neg	Neg
	Anti-WNV-IgG	Neg	**1:1,000**
	Anti-WNV-IgM	Neg	Neg
	Anti-YFV-IgG	**1:100**	**1:10,000**
	Anti-YFV-IgM	**1:10**	Neg
	Anti-TBEV-IgG	Neg	**1:1,000**
	Anti-TBEV-IgM	Neg	Neg
IIFA[i]	Anti-CHIKV-IgG	ND	Neg
	Anti-CHIKV −IgM	ND	Neg
ZIKV VNT[j]	ZIKV-NT90	ND	**>640**

Neg, negative test; ND, test not done; Positive results are highlighted in **bold**; ZIKV, Zika virus; DENV, Dengue virus; JEV, Japanese encephalitis virus; WNV, West Nile virus; YFV, Yellow Fever virus; TBEV, Tick Borne encephalitis virus; CHIKV, Chikungunya virus; IIFA, Indirect immunofluorescence assay; ELISA, enzyme-linked immunosorbent assay; VNT, Virus Neutralization Test.

[a] In-house/adapted from Faye *et al.*, 2013/Faye *et al.*, 2013; PCR positivity cut-off < 50 cycles, and if Ct > 38: to be confirmed by a second positive result.
[b] Patel *et al.*, 2013.
[c] In-house.
[d] SD Bioline Dengue NS1 Ag.
[e] IIFA WHO CC, Hamburg.
[f] IIFA Euroimmun Biochip Arboviral Fever Mosaic 2.
[g] ELISA Dengue Capture DxSelect™, Focus Diagnostics, cut-off 1.0.
[h] IIFA Euroimmun Biochip Mosaic Flavivirus Profile 2.
[i] IIFA Euroimmun Biochip Chikungunya.
[j] In-house VNT using Vero cells, 90% Neutralizing titer (NT90) calculated using the Reed-Muench method.

Serum taken on the 10th of December (three days after symptom onset), was sent to the Institute of Tropical Medicine (ITM) (Antwerp, Belgium) for DENV diagnosis but RT-PCR and NS1 antigen testing were negative. On December 24th (17 days after symptom onset) a convalescent serum sample was sent to ITM with the request for ZIKV RT-PCR and serology. A specific ZIKV and a generic flavivirus real-time RT-PCR were performed on the acute phase serum sample (stored at 4 °C) while IgG and IgM antibody detection for several flaviviruses was performed on the convalescent serum sample with an indirect immunofluorescence assay (IIFA). Additionally DENV serology was performed with an enzyme-linked immunosorbent assay (ELISA) (Table 1).

Both ZIKV specific and pan-flavivirus RT-PCRs resulted in a weak positive signal. Repeated RNA extraction and RT-PCRs were confirmative. Sequencing of both RT-PCR products revealed incomplete amplicon sequences, as expected with these high cycle threshold (Ct) values, but they were highly suggestive for ZIKV with BLAST analysis results of 96% and 100% identity to the Brazilian ZikaSPH2015 sequence (GenBank accession number KU321639, submitted on December 21th 2015). Viral culture was not successful in our case, possibly due to low viremia.

Further anamnesis revealed that the patient was vaccinated against yellow fever virus (YFV) in 2012 and was not aware of a previous DENV infection. Both the acute phase and convalescent serum samples were sent to a WHO Collaborating Centre for Arbovirus and Haemorrhagic Fever Reference and Research Institute (Hamburg, Germany) for specific ZIKV serology testing (IIFA), revealing a seroconversion for ZIKV IgM (titer 1:320) and IgG (titer 1:5,120). In a second stage both samples were also tested with the Euroimmun Arboviral Fever Mosaic 2 test at ITM. The presence of ZIKV-specific neutralizing antibodies was confirmed by an in-house virus neutralization assay (Table 1).

1.2. Other similar and contrasting cases in the literature

To our knowledge, twelve ZIKV cases imported to Europe were published (PubMed search up to 16/02/2016, Table 2). Six of the cases are imported from the current outbreak in South and Central America in 2015–2016.

2. Discussion

In the case described here, RT-PCR was positive on serum at day three post symptom onset. In comparison, out of the twelve previously published ZIKV cases imported into Europe [4–12] (Table 2) an acute phase serum sample (≤5 days), was available in seven cases, of which three cases were positive by ZIKV RT-PCR. Current knowledge on the viral load and variations in ZIKV viremia over time is scarce [2]. The viremic phase appears to be short, as Duffy *et al.* reported that 15/17 (88%) of RT-PCR positive samples were