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False positive dengue NS1 antigen test in a traveller with an acute Zika virus infection imported into Switzerland

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Summary

We report the first case of an acute Zika virus infection imported into Switzerland by a traveller returning from Canoa Quebrada, Ceará state, in the north-eastern part of Brazil. Due to a false positive dengue virus NS1 antigen test, IgG-antibody seroconversion and a suggestive clinical picture, an acute dengue fever was initially considered. However, because of lack of specific IgM-antibodies, stationary IgG-antibody titre and a negative dengue virus PCR test result, a dengue virus infection was excluded and a cross-reaction with other, causative flaviviruses was postulated. Based on recent reports of Zika fever cases in the north-eastern parts of Brazil, an acute Zika virus infection was suspected. Because of a lack of commercially available Zika virus diagnostic tests, the case was confirmed in the WHO reference laboratory. As the clinical presentation of Zika virus infection can be confused with dengue fever and chikungunya fever, and because of possible public health implications, all patients returning from affected areas should be additionally tested for Zika virus. This case illustrates the urgent medical need for a broadly available assay capable of differentiating Zika from Dengue infections.

Key words: *Zika virus; dengue virus; false positive NS1 antigen; traveller*

Introduction

As of May 15, 2015, the Brazilian Ministry of Health confirmed Zika virus to be circulating in the country [1]. A possible link between Zika virus infection in pregnancy and an unusual increase in microcephaly cases in Northeast Brazil is currently under investigation [2].

Zika virus is a positive-sense, single-stranded RNA virus of the genus *Flavivirus*, family *Flaviviridae*, and is closely related to other flaviviruses of public health relevance including dengue virus, tick-borne encephalitis virus, and West Nile virus [3]. At present, *Aedes aegypti* is considered to transmit the virus outside the African continent, though *Ae. albopictus* has long been a suspected vector as well [4].

Zika virus was first isolated from a rhesus monkey in Uganda in 1947 [5]. In 2007, Zika virus spread for the first time outside Africa and Asia and can be considered an emerging pathogen [6]. Recently, an imported Zika virus infection in a traveller returning from Brazil to Italy was described [7]. We report the first case of an acute Zika virus infection imported into Switzerland by a traveller in whom an acute dengue fever , based on false positive dengue virus NS1 antigen test, was initially suspected.

Case report

On June 8, 2015, a 44-year-old nonpregnant woman presented at our travel clinic in Zurich, Switzerland with a 3-day history of flu-like illness, chills, headache, fever up to 38.6°C and a painful swelling of the cervical, retro auricular, occipital and inguinal lymph nodes. No signs of conjunctivitis were observed. Symptoms appeared on June 5, 5 days after her return from a 12-day vacation at Canoa Quebrada, Ceará state, in the north-eastern part of Brazil, a neighbouring state to Rio Grande do Norte, where cases of Zika fever have recently been confirmed [1-2].

Three days later, on June 8 she presented a maculopapular rash (rubelliform exanthema) on the upper part of her chest except for the head, which lasted for 5 days. On day 5 of her illness, she developed a very painful swelling of joints in her hands and elbow, with an increasing arthralgia of the wrists, palms and fingers. The symptoms were less prominent on the soles. Blood pressure, pulse, general examination of heart, lung, abdomen, neurological examinations and haematology/blood chemistry did not show any abnormalities during the course of illness, and C-reactive protein, liver function tests and complete blood count results were within reference ranges. In our case, only acetaminophen (paracetamol) was administered, in doses of 4

grams per day over a period of two weeks. On day 13 after the onset of the symptoms**,** almost all complaints had disappeared. The remaining back pain and headache lasted for another two weeks. After that the patient recovered completely (*restitutio ad integrum*).

Because of the symptoms and the travel history of the patient to Brazil, dengue virus and chikungunya virus were initially suspected as possible causative agents. In the first serum sample obtained on June 8 neither dengue virus NS1 antigen nor specific antibodies to dengue virus or chikungunya virus (Anti-*Dengue Virus* IIFA *Mosaic* Types *1-4* and Anti-Chikungunya Virus IIFA, EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany) were detected. Three days later a positive NS1 antigen result was obtained at two different laboratory settings by the SD Dengue Duo NS1 antigen and IgG/IgM combo device (Standard Diagnostics, South Korea), which is our first line antigen test and is in common use in Switzerland, as well as world wide. No antibodies against dengue virus or chikungunya virus were detected at that time. In the follow-up on June 17, dengue virus NS1 antigen remained positive. An indirect immunofluorescence assay (IIFA) for dengue virus demonstrated a seroconversion of the dengue virus antibodies with an IgG titre of 1:80, but no dengue virus IgM antibodies were detected. In relation to the unusual dengue virus laboratory results, an additional dengue virus NS1 antigen test, the Bio-Rad NS1 antigen strip (Bio-Rad, France) and dengue virus specific real time reverse transcription-PCR were performed and neither NS1-antigen nor dengue virus RNA were detected. Because of atypical progression of dengue virus serology, a cross-reaction with other flaviviruses was suspected, and patient sera were tested for other flaviviruses using the Flavivirus Profile 2 IIFA (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany), whereby no specific flavivirus antibodies were detected (table 1).

Based on recent reports of the confirmed circulation of Zika virus in Brazil, a Zika virus infection was additionally taken into account. Because of lack of commercially available Zika virus diagnostic tests, all serum samples were sent for further analysis to the World Health Organization Collaborating Centre for Arbovirus and Haemorrhagic Fever Reference and Research, Hamburg, Germany.

In the first serum sample obtained on June 8, no specific Zika virus antibodies were detected. Three days later (in the serum of June 11) a seroconversion to Zika virus was observed and an IIFA for Zika virus demonstrated an IgM titre of 1:1,280 and an IgG titre of 1:320 (cut-off <1:20). Zika virus-specific real time reverse transcription-PCR was negative from the second serum sample, as well as generic flavivirus and alphavirus RT-PCR [7]. This can be explained with a short viraemic period allowing direct Zika virus detection only during the first three to five days after onset of symptoms [8]. Since the serum sample of June 8 was no longer available, the retrospective PCR analysis was not possible. Follow-up Zika virus serological testing on June 17 showed a fourfold increased IgG titre of 1:2,560

Table 1: Serological and virological data of a case of Zika virus imported from Brazil into Switzerland, June 2015.

Test Method	**Sample collection date**			
	June 8, 2015	**June 11,2015**	**June 17, 2015**	**July 1, 2015**
Anti-ZIKV-IgM[1]	<1:20	1:1280	1:1280	1:640
Anti-ZIKV-IgG[1]	<1:20	1:320	1:2560	1:2560
Pan-flavivirus RT-PCR	ND[4]	negative	ND[4]	ND4
ZIKV qRT-PCR	ND[4]	negative	ND[4]	ND[4]
ZIKV VNT[2]	ND[4]	ND[4]	ND[4]	1:2560
Anti-DENV-IgM[1]	<1:20	<1:20	<1:20	<1:20
Anti-DENV-IgG[1]	<1:20	<1:20	1:80	1:80
DENV NS1 antigen (SD Dengue Duo)[3]	negative	strong positive	strong positive	weak positive
DENV NS1 antigen (Bio-Rad antigen strip)[4]	negative	negative	negative	negative
DENV NS1 antigen (Bio-Rad Platelia EIA)[5]	negative	negative	negative	negative
DENV RT-PCR	negative	negative	negative	ND[6]
DENV VNT[2]	ND[6]	ND[6]	ND[6]	<1:20
Anti-TBEV-IgM[1]	ND[6]	<1:20	ND[6]	<1:20
Anti-TBEV-IgG[1]	ND[6]	<1:20	ND[6]	<1:20
TBEV VNT[2]	ND[6]	ND	ND[6]	<1:20
Anti-YFV-IgM[1]	ND[6]	<1:20	ND[6]	<1:20
Anti-YFV-IgG[1]	ND[6]	<1:20	ND[6]	<1:20
Anti-WNV-IgM[1]	ND[6]	<1:20	ND[6]	<1:20
Anti-WNV-IgG[1]	ND[6]	<1:20	ND[6]	<1:20
Anti-JEV-IgM[1]	ND[6]	<1:20	ND[6]	<1:20
Anti-JEV-IgG[1]	ND[6]	<1:20	ND[6]	<1:20
Anti-CHIKV-IgM[1]	<1:20	<1:20	<1:20	<1:20
Anti-CHIKV-IgG[1]	<1:20	<1:20	<1:20	<1:20

[1] ZIKV: Zika virus; DENV: dengue virus; TBEV: tick borne encephalitis virus; YFV: yellow fever virus; WNV: West Nile virus; JEV: Japanese encephalitis virus; CHIKV: chikungunya virus; Indirect immunofluorescence assay (IIFA) – titres <1:20 for serum were considered negative; [2] ZIKV and DENV virus neutralisation test (VNT) – titres <1:20 for serum were considered negative; [3] NS1: non-structural protein; SD Dengue Duo NS1 antigen and IgG/IgM combo device (Standard Diagnostics, South Korea); [4] Bio-Rad NS1 antigen strip (Bio-Rad, France); [5] Platelia Dengue NS1 EIA (Bio-Rad); [6] ND: not done

(table 1). This is underlined by the presence of Zika virus -specific neutralizing antibodies and the absence of dengue virus-specific neutralizing antibodies in the serum sample taken on July 1 (table 1). In the same serum, an IIFA for dengue virus demonstrated an IgG titre of 1:80, but neither IgM antibodies nor dengue virus RNA were detected in any serum sample (table 1). Further, in the serum of July 1 no IgG antibody titre increase to dengue virus was noted and the SD Bioline dengue virus NS1 antigen test became weakly reactive.

Discussion

The SD Dengue Duo test is in common use worldwide and proved in several studies to be highly sensitive and specific [9–12]. Although the dengue virus NS1 antigen in the SD Dengue Duo kit has high specificity, false positive and misleading dengue virus NS1 antigen results have been described in patients with chikungunya fever and haematological malignancies [11–13]. No dengue virus NS1 antigen false positivity in Zika virus infections has been reported so far. The true reason for the false reactivity in this particular case is not clear and further studies on the test specificity in Zika virus acute infections are necessary. Serological cross-reactivity between Zika virus and dengue virus, observed in our case, is known from earlier studies [8].

Thus far, Zika virus infection has been associated with a relatively mild illness, but its true potential to cause severe disease is currently unknown. Neurological complications of Zika virus infections had previously been reported as Guillain-Barré syndrome and hearing difficulties (sudden bilateral dull and metallic hearing) [14]. Further elaboration and monitoring of additional clinical symptoms in association with an acute Zika virus infection are needed [2]. The clinical presentation of Zika virus infection is usually not specific (mild fever, rash, arthralgia, and conjunctivitis) and can be confused with other diseases, especially dengue fever and chikungunya fever [15]. As no specific treatment of Zika virus infection is available and there is no vaccine or preventive drug, the treatment is symptomatic and mainly based on pain relief, fever reduction and antihistamines for pruritic rash. Travel-related cases of Zika virus infection in patients returning from affected areas are likely to occur in the future and clinical cases suspected of dengue virus or chikungunya virus infection should be additionally tested for Zika virus. This case illustrates the urgent medical need for a broadly available assay capable of differentiating Zika from Dengue infections.

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