TWENTY FOUR CASES OF IMPORTED ZIKA VIRUS INFECTIONS DIAGNOSED BY MOLECULAR METHODS

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Abstract

Zika virus is an emerging flavivirus widely spreading through Latin America. Molecular diagnosis of the infection can be performed using serum, urine and saliva samples, although a well-defined diagnostic algorithm is not yet stablished. We describe a series of 24 cases of imported zika virus infection into Catalonia (northeastern Spain). Based on our findings, testing of paired serum and urine samples is recommended.

Background

Zika virus (ZIKV) is an arthropod-borne virus from the genus *Flavivirus*, family *Flaviviridae*. It was first identified in 1947 after isolation from a rhesus monkey in Uganda[1]. Sporadic cases of ZIKV infection in humans had been reported until the 2007 outbreak in Yap islands[2]. The French Polinesia epidemic in 2013 preceded the current expansion of the virus in Latin America[3]. Most cases of ZIKV infection are considered to be either asymptomatic or inducing a mild disease presenting with symptoms such as fever, malaise, rash, arthralgia and conjunctivitis. However, the rapid dissemination of the virus and the possibility of severe complications such as Guillain-Barré syndrome or fetal abnormalities have placed ZIKV as the latest viral threat to public health systems[4] [5].

Since the clinical presentation is unspecific and may overlap with that of other arboviral diseases circulating in the same region, laboratory confirmation of suspected patients is essential for an accurate classification of cases. As for dengue and chikungunya, diagnosis of ZIKV infection is generally achieved by molecular detection of the viral genome and by detection of IgM and IgG ZIKV-induced antibodies. Serological diagnosis is challenging mainly due to cross reactivity with antibodies against other flaviviruses[6] and may require performing laborious seroneutralization assays. In this sense, more specific serological assays are needed and recently a ZIKV ELISA has shown promising results[7]. In general, molecular detection by reverse transcription polymerase chain reaction (RT-PCR) would be preferable since it offers rapid and specific diagnosis, although its use is limited by the presence of the virus in clinical samples during the acute phase of the infection. Determining the type of samples and the duration of the virus in those biological fluids is important for an optimal molecular diagnosis. A short viremia (3-5 days) has been reported [6], but the virus may be detectable for nearly 2 months in certain pregnant women with congenital infection[8]. The diagnostic utility of saliva and urine samples has also been reported. Saliva may increase the

rate of detection but it does not seem to expand the period of detection compared to serum [9]. The virus may be present in urine for prolonged periods up to 20 days after the onset of symptoms [10] [11]. However, there is a lack of published series of ZIKV in travelers addressing the optimal time for testing each sample. An accurate description of the optimal samples and collection time would be useful for improved detection of ZIKV infections, given its implications on pregnancy and the possibility of autochtonous vector borne or sexual transmission in non endemic countries. Recently, provisional guidelines for testing have been provided by the ECDC suggesting optimal periods for testing of serum (0-4 days after onset of symptoms) and urine (3-8 days) samples [12].

Results and Discussion

We report a series of 24 travelllers with ZIKV infection diagnosed by molecular methods in serum and urine samples at our laboratory from January 2016 onwards. Cases detected were either attended at the Tropical Medicine Department of Hospital Clinic de Barcelona or detected through a regional arbovirus surveillance program (http://canalsalut.gencat.cat/web/.content/home_canal_salut/professionals/temes_de_salut/vigilancia_epidemiologica/documents/arxius/protocol_arbovirosis_cat.pdf).

A commercial ZIKV specific real time RT-PCR (RealStar® Zika Virus RT-PCR kit, Altona Diagnostics) was used as a first line test and all positive samples were confirmed by a second molecular test: an in house generic flavivirus RT-PCR[13] followed by sequencing or another commercial real time RT-PCR (VIASURE Zika Virus Real Time PCR Detection kit, Certest Biotec). ZIKV-IgM and IgG antibodies were detected in serum diluted 1/10 by a commercial immunofluorescence test (Euroimmun, AG). When available, both urine and serum samples were analyzed. All serum samples collected within 5 days after the onset of symptoms tested negative for dengue and chikungunya by specific real time RT-PCR assays.
A total of 24 patients were diagnosed of ZIKV infection by real time RT-PCR. Seventeen patients (70,8%) were female and the median age was 38 years-old. All patients presented with symptoms. The main clinical and epidemiological characteristics of the patients and how the diagnosis of ZIKV infection was performed are summarized in Table 1. The type of sample and the time point of collection after the onset of symptoms are also described. In 12 cases, serum and urine samples were analyzed whereas in 11 patients only serum samples were collected and in one patient only urine was tested. A total of 36 samples were tested (23 serum and 13 urine samples). Among the patients with both serum and urine samples tested, four of them had a positive result in both samples, four patients had positive results only in urine and four were only positive for ZIKV in serum. Significantly lower cycle threshold (Ct) values were observed in urine samples obtained 5 or 6 days after the onset of symptoms compared to serum samples, indicating higher viral loads in urine, as previously described [11]. Interestingly, 20/26 (76,9%) of the samples collected within the suggested optimal time for testing [12] were positive, 6/7 (85,7%) of the samples collected within the suboptimal time tested positive and 2/3 (66,7%) of the samples collected out of the optimal or suboptimal times tested positive. Patient 24 showed a positive RT-PCR in serum on day 8 after the onset of the symptoms, with a negative RT-PCR in urine. Both results are unexpected as at this time a positive PCR would be more likely in urine and a negative result in serum. Other unexpected findings include a positive RT-PCR in urine at day 1 (patient 1) or negative RT-PCR in serum at day 3 (patients 8 and 10). Although saliva samples are not considered to expand the ZIKV period of detection and were not collected in our series, systematic testing of these samples may yield surprising results, as has been recently shown in a case with prolonged viral shedding in saliva for 29 days[14]. Taken together, it seems that the kinetics of ZIKV are not yet fully understood. Our results support the testing of paired serum and urine samples for an

improved ZIKV molecular diagnosis and may contribute to a better understanding of the diagnostic markers of ZIKV infection.

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Table

Table 1. Clinico-epidemiological characteristics and virological diagnosis of 24 cases of imported ZIKV infection.

Patient	Sex/Age	Country visited	Symptoms	Days after onset of symptoms	RT-PCR in serum (Ct)	RT-PCR in urine (Ct)	Zika serology[1]	
							IgM	IgG
1	F/in her 40s	Bolivia	Rash, arthralgia, conjunctivitis	1	Positive (32,67)	Positive (34,56)	NI	Positive*
2	F/under 15	Honduras	Fever, chill, headache, rash, conjunctivitis	2	Positive (35,93)	NA	Negative	Negative
3	F/in her 40s	Puerto Rico	Rash, low fever	2	Positive (38,06)	NA	Negative	Negative
4	F/under 15	Honduras	Fever, chill, headache, rash, conjunctivitis	2	Positive (36,63)	NA	Positive **	Negative
5	F/in her 40s	Brazil	Arthralgia, rash	2	Positive (37,59)	NA	Negative	Negative
6	M/in his 30s	Honduras	Fever, chill, headache, rash, conjunctivitis	2	Positive (38,14)	NA	Negative	Positive*
7	F/in her 40s	Honduras	Low fever, arthralgia, rash, headache	2	Positive (34,27)	NA	Negative	NI
8	M/in his 20s	Bolivia	Arthralgia, rash, dysthermia	3	Negative	Positive (35,04)	Negative	Positive*

9	M/in his 30s	Venezuela	Myalgia, cehalea, fever, adenopathies, rash	3	Positive (24,78)	NA	Negative	Positive **
10	M/in his 40s	Colombia	Low fever, arthralgia, myalgia, rash, diarrhea	3	Negative	Positive (33,6)	Negative	Negative
11	F/in her 40s	Colombia	Rash, arthralgia, low fever	3	Positive (35,38)	NA	Negative	Positive*
12	F/in her 50s	Colombia	Dysthermia, rash, facial pruritus	3	Positive (26,76)	Negative	NA	NA
13	F/in her 30s	Dominican Republic	Fever, arthralgia, headache, rash, asthenia	3	Positive (36,24)	NA	Positive**	Negative
14	F/in her 20s	Dominican Republic	Fever, arthralgia, rash	3	Positive (35,45)	Negative	NA	NA
15	F/in her 30s	Dominican Republic	Low fever, rash	3	Positive (30,73)	Negative	Negative	Positive*
16	F/in her 40s	Bolivia	Rash, fever, arthralgia, conjunctivitis, headache, asthenia	4	NA	Positive (26,66)	NA	NA
17	F/in his 20s	Dominican Republic	Rash, arthralgia	4	Negative	Positive (33,95)	Negative	Negative
18	M/in his 30s	Venezuela	Arthralgia, rash, dysthermia	5	Positive (34,22)	Positive (31,32)	Negative	Negative
19	M/in his 30s	México and El Salvador	Arthralgia, rash, conjunctivitis, adenopathy	6	Positive (37,25)	Positive (24,14)	Positive*	Positive*

20	M/in his 40s	Venezuela	Fever, rash, arthralgia, conjunctivitis	6	Negative	Positive (34,74)	Positive**	Positive*
21	F/in his 30s	Dominican Republic	Arthralgia, fever, conjunctivitis, rash	6	Positive (31,57)	NA	Negative	Positive*
22	F/in her 20s	Martinique	Fever, cephalea, rash, adenopaty	6	Positive (37,55)	Positive (33,16)	Positive **	Negative
23	F/in her 60s	Colombia	Asthenia, rthralgia, myalgia, rash, headache	8	Positive (37,87)	NA	Positive	Negative
24	F/in her 40s	Brazil	Rash, dysthermia, conjunctivitis	8	Positive (35,17)	Negative	Negative	Negative

*: samples were also positive for antibodies against dengue; **: weakly positive; NA: samples not available; NI: not interpretable due to homogeneous staining of both infected and non-infected cells of the immunofluorescence slide. Ct: cycle threshold values of the RealStar® Zika Virus RT-PCR kit (Altona Diagnostics)

Highlights

-A series of 24 confirmed cases of zika virus infection in travelers is described.

-Molecular detection of zika virus in serum and urine samples revealed positive samples outside the considered optimal time for testing.

-Testing of paired serum and urine samples is recommended for optimal molecular detection.