Journal of Clinical Virology 82 (2016) 1–4

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Case report

Viral load kinetics of Zika virus in plasma, urine and saliva in a couple returning from Martinique, French West Indies

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ARTICLE INFO

Article history:
Received 18 March 2016
Received in revised form 6 June 2016
Accepted 21 June 2016

Keywords:
Zika virus infection
Kinetics
Plasma
Urine
RNA
Antibodies

ABSTRACT

While the rapid spread of Zika virus (ZIKV) in South America has been declared a public health emergency few data are available on the kinetics of the virus load and the specific antibodies in individual patients. This report describes the kinetics of ZIKV decay in the body compartments and the kinetics of anti ZIKV IgG and IgM of two people returning from Martinique, French West Indies. ZIKV remained detectable in the plasma for roughly 2 weeks indicating that mosquito control measures should be prolonged accordingly. Remarkably, their urine samples consistently tested positive for even longer. The antibodies responses were different between the two patients but for both the rapid onset of IgM allowed a diagnosis from the end of the first week.

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1. Why this case is important?

The recent emergence of Zika virus (ZIKV) infections and the current outbreak in the Americas and Caribbean islands is of major concern. Links between ZIKV and neurological disorders and congenital malformations are now well established [1,2], although most cases of ZIKV infection are asymptomatic or mild. While interest in ZIKV within the scientific community has increased exponentially, data on the viral load and specific antibodies kinetics in individual patients are still lacking. This report describes the kinetics of ZIKV decay in the body compartments and the kinetics of anti-ZIKV IgG and IgM of two people returning from Martinique, French West Indies.

2. Case description

A French couple living in of southwest France with no unusual medical history spent eight days in Martinique during January 2016. They showed no symptoms during their stay and returned to France on January 20. The 66-year-old woman immediately developed symptoms of intense fatigue and three days later, on January 23, she began to shiver, developed myalgia, arthralgia and a cutaneous rash. Two days later, on January 25, the 69-year-old man suddenly developed a fever with a cutaneous rash, myalgia and arthralgia. Both patients had begun to recover spontaneously from the fever when they were examined on January 27th, but they still had the remains of a maculo-papular rash. Neurological examination was normal. The man's white blood cell count showed mild neutropenia (900 cells/μL, norm: >2000 cells/μL). Their blood hemoglobin, platelet counts, creatinine, C-reactive protein and hepatic enzyme activities were all normal. Their plasma and urine tested positive for ZIKV by real-time reverse transcriptase polymerase chain reaction (real-time RT-PCR) (Real Star Zika virus RT-PCR kit 1.0, Altona Diagnostic GmbH, Hamburg, Germany), while Chikungunya and Dengue virus RNAs were not detected by an in-house real-time RT-PCR system. Zika virus RNA was quantified using a synthetic RNA transcript (Altona Diagnostic GmbH, Hamburg, Germany). The range was 200–2.000.000 copies/mL. Serial samples of several body fluids were assayed for ZIKV by real-time

Abbreviations: ZIKV, Zika virus; RT-PCR, reverse transcriptase polymerase chain reaction.

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http://dx.doi.org/10.1016/j.jcv.2016.06.011
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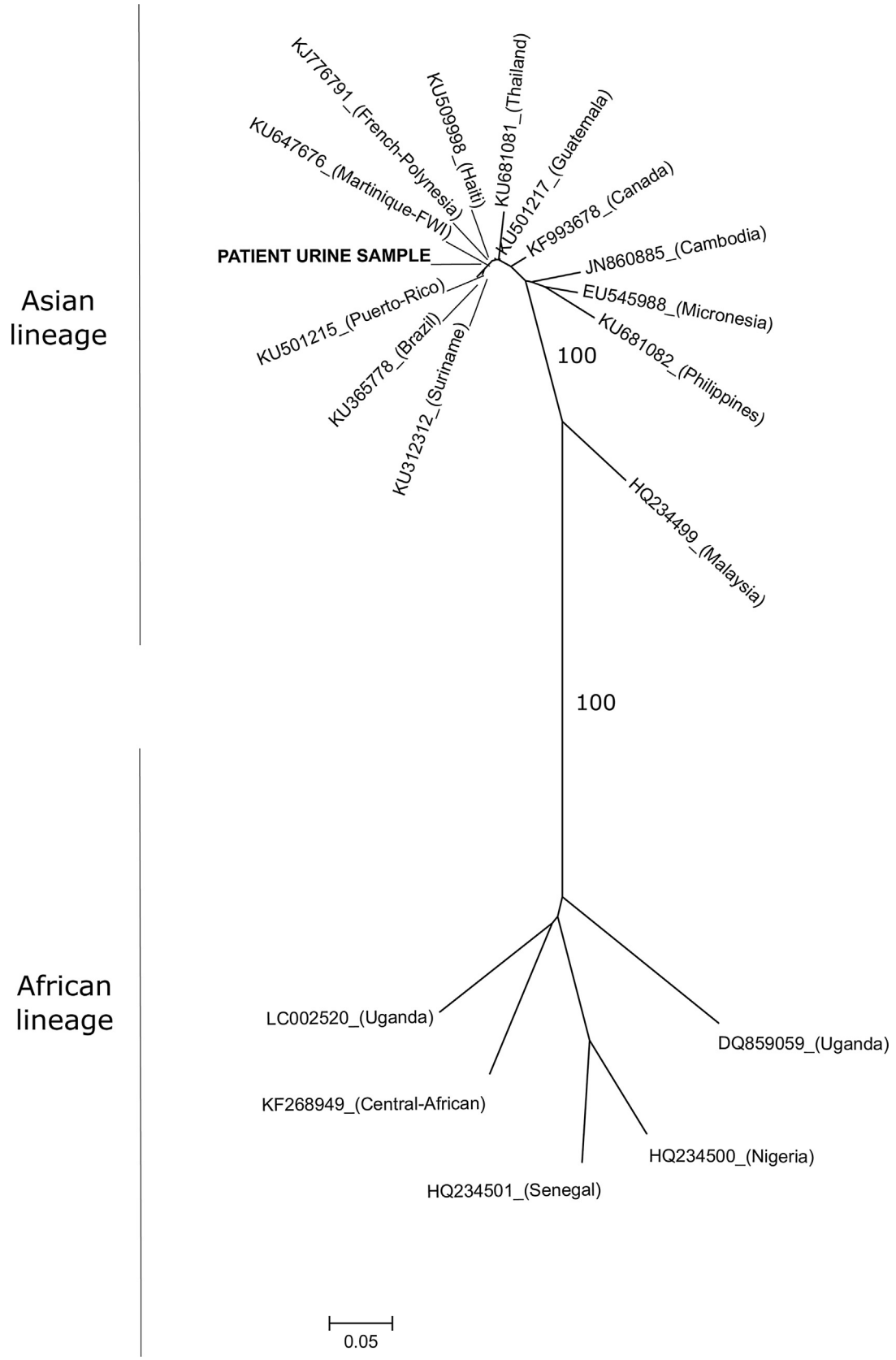

Fig. 1. Phylogenetic tree constructed using the neighbor-joining method and based on a partial (1079 nt) sequence of the ZIKA virus NS5 gene that was imported to metropolitan France from Martinique. Phylogenetic analysis includes reference sequences of ZIKA viruses from Asian and African lineages. Sequences are indicated as GenBank accession numbers and countries. The imported ZIKA strain isolated in this study is indicated in boldface. Bootstrap support values (100 replicates) are indicated at major nodes. Scale bar indicates nucleotide substitutions per site.

RT-PCR until February 17 (Table 1). ZIKV RNA was detected in plasma and urine samples from both patients, but the rates of disappearance from these compartments differed; ZIKV RNA was found in the saliva of only the woman (data not shown in Table 1). ZIKV remained detectable in their plasma for about 2 weeks, while the urine of the man was consistently positive until day 14 and that of the woman until day 25. The greater virus load in the urine was observed in the man. While both patients recovered within a week, the woman developed unilateral conjunctivitis few days after the first examination. Real-time RT-PCR on her conjunctiva detected no ZIKV; those of her husband tested positive (detected but not quantified) but were asymptomatic. We identified the virus in a urine sample from the woman by sequencing a 1079-nt fragment within the NS5 gene of ZIKV and performing phylogenetic analyses using reference sequences. As expected the virus was an Asian lineage strain (Fig. 1). On the other hand serial samples of plasma from the two patients were tested for anti-ZIKV IgG and IgM (ELISA Anti-virus ZIKA, Euroimmun, Lübeck, Germany [3]). These assays detect antibodies against NS1 antigen and were used according to the manufacturer's instructions. Anti-ZIKV IgM were positive for both patients and could be a diagnostic tool from nearly one week after symptom onset. Nevertheless the responses were different between the two patients; the man exhibited a doubtful reactivity from the 23rd day. Similarly the anti-ZIKV IgG responses varied but both patients were positive around three weeks after symptom onset (Table 1).

3. Other similar and contrasting published cases

Zika virus infection is an emerging arthropod-borne flavivirus that is transmitted by *Aedes* mosquito species. Sporadic cases were initially reported in Africa and Asia and the first outbreak occurred in 2007 on Yap Island (Micronesia) [4]. This was followed by a larger epidemic in 2013–2014 in French Polynesia [5]. The first evidence of a ZIKV infection in the Americas appeared in northeast Brazil in May 2015 [6]. An Asian lineage virus spread rapidly through South and Central America and the Caribbean islands a few months later. It eventually affected 42 countries worldwide [7]. Most recently, in the last week of 2015, 12 confirmed and 150 suspected cases of ZIKV infection were reported on the island of Martinique (French West Indies) [8].

Rash, fever, arthralgia and conjunctivitis are the most common symptoms and they usually last about a week [9]. The most severe risk posed by the disease is that of developing Guillain-Barré syndrome [1], and for pregnant women of fetal abnormalities and death [2]. Travel-related imported ZIKV infections were reported during the 2013 epidemic [10,11]. Anti- ZIKV antibodies were detected, but they cross-reacted with Dengue antibodies [10,12] and often required confirmation with neutralizing antibodies. A ZIKV infection is presently diagnosed within the first week after the symptom onset using RT-PCR to detect virus RNA in the blood and/or urine [11,13–15]. The 157 blood samples tested during the Yap Island outbreak included 17 that were positive for ZIKV by real-time RT-PCR; most of the positive samples were collected within three days of symptom onset, although one positive specimen was collected 11 days after symptom onset [4]. A recent report of three imported cases returning from the Americas to France indicated that viremia was short, less than five days, in all cases and virus was detected in the urine and saliva eight days after symptom onset in one case, but not after two weeks in another [12]. In this report the serological response was also studied; IgM antibodies appeared between five to nine days after symptom onset whereas the IgG rise was delayed for one patient.

4. Discussion

It is most important to know the duration of ZIKV viremia so as to be better prepared for disease control worldwide. ZIKV may be transmitted from viremic humans to vectors such as *Aedes* mosquito species under suitable climatic conditions, and some of these mosquitos are already established in Europe, where they could perpetuate local transmission cycles. Short-term low-level ZIKV viremia was considered to be common, lasting usually less than five days after the onset of symptoms [13]. The data from these 2 mildly symptomatic patients show that ZIKV RNA may remain in the plasma for about 2 weeks, although its concentration decreases, indicating that mosquito control measures should be continued for at least 2 weeks after symptom onset. On the other hand we found the anti-ZIKV IgM detection as a useful tool for an early biological diagnosis.

The route of ZIKV virus shedding may also be relevant. There may be short-term ZIKV shedding into the saliva [13]. Greater virus loads of ZIKV that persist for longer can also be detected in the urine [14,15]. But the finding that the man had a higher viral load in urine than the woman needs further investigations to evaluate if this is only casual or a tendency relative to man *vs* woman virus excretion in urine. Thus protection from in-situ vectors could include real-time RT-PCR screening for ZIKV of the urine of asymptomatic but "contagious" travelers returning from epidemic areas to regions where *Aedes* species are established. ZIKV shedding into the semen is a much greater problem [16]. Although almost all cases of ZIKV infection are transmitted by mosquito bites, rare cases of ZIKV shedding into the semen and its subsequent sexual transmission have been reported [17]. However, nothing is known about how long such shedding persists. Hence, women who are already pregnant or trying to conceive with men who have been exposed to ZIKV should be made aware of this risk.

The trends towards urbanization and globalization both point to the potential for major urban epidemics of ZIKV. It is imperative that we delineate as precisely as possible the life cycle of ZIKV and virus shedding in order to develop effective mosquito control [18].

Table 1
Kinetics of ZIKV RNA in plasma and urine, kinetics of ZIKV antibodies in plasma.

Case		27 Jan 2016	30 Jan 2016	01 Feb 2016	04 Feb 2016	08 Feb 2016	17 Feb 2016
Man	Days after symptom onset	2	5	7	10	14	23
	RT-PCR plasma (copies/mL)	100[a]	NS	75[a]	230	630	0
	RT-PCR urine (copies/mL)	51,000	74,000	1500	340	1560	0
	Anti-ZIKV IgG	NS	–	–	NS	±	+
	Anti-ZIKV IgM	NS	+	+	NS	+	±
Woman	Days after symptom onset	4	7	9	12	16	25
	RT-PCR plasma (copies/mL)	180[a]	270	120[a]	160[a]	560	0
	RT-PCR urine (copies/mL)	5550	1800	340	260	0	670
	Anti-ZIKV IgG	NS	+	+	+	+	+
	Anti-ZIKV IgM	NS	+	+	+	+	+

[a] Estimated values outside of the standard curve range, NS: not sampled, –: negative, ±: doubtful, +: positive.

Competing interests

None declared.

Funding

None.

Ethical approval

Not required.

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