Case Report

First dengue co-infection in a Belgian traveler returning from Thailand, July 2013

Lieselotte Cnops a,*, Cristina Domingo b,1, Dorien Van den Bossche a,2, Evilien Vekens c,3, Evelien Brigou d,4, Marjan Van Esbroeck a,5

a Institute of Tropical Medicine (ITM), Department of Clinical Sciences, Central Laboratory for Clinical Biology (CLKB), Kronenburgstraat 43/3, B-2000 Antwerp, Belgium
b Robert Koch Institute (RKI), Center for Biological Threats and Special Pathogens (ZBS-1), Nordufer 20, 13353 Berlin, Germany
c Medisch Labo Medina, Hoogveld 10, B-9200 Dendermonde, Belgium
d Huisartsenpraktijk De Mier, Luiaardweg 16, 1850 Grimbergen, Belgium

ARTICLE INFO

Article history:
Received 25 July 2014
Received in revised form 7 October 2014
Accepted 11 October 2014

Keywords:
Dengue virus
Mixed infection
RT-PCR
Imported case
Travel
Thailand

ABSTRACT

We report a dengue virus (DENV) co-infection in a Belgian traveler after a three–weeks holiday to Thailand. The patient recovered well without any complication. The infection was diagnosed by NS1 antigen testing and the concurrent presence of serotype DENV1 and DENV2 was demonstrated by reverse transcriptase polymerase chain reaction (RT-PCR) in acute phase serum sampled three days after symptoms onset. The predominant DENV1 serotype was identified as genotype I, lineage Asia-3 by sequencing. To our knowledge, this is the first time that a dengue co-infection is reported in a European traveler. The co-infection accounts for 1.0% of the total number of RT-PCR-positive samples (n = 105) diagnosed in the reference laboratory of Belgium between 2008 and 2013. We expect that the number of reports on acute co-infections will increase in the coming years considering the increasing number of regions that are progressively becoming hyperendemic, especially in Southeast Asia.

© 2014 Elsevier B.V. All rights reserved.

1. Why this case is important

Dengue virus (DENV) is the most genetically diverse member within the family of Flaviviridae. The DENV complex consists of four antigenically distinct serotypes (DENV1–DENV4). Within each serotype there is considerable genetic variation between phylogenetically defined genotypes [1].

Dengue is the most rapidly spreading viral disease worldwide and its global epidemiology has changed dramatically during the last decades. Due to globalization, international travel and transport, dengue transmission expanded into new geographic areas and re-appeared in regions that had been free of virus for many years. And, new dengue serotypes and strains were introduced in regions on top of the already existing serotypes. Co-circulation of different serotypes in one region exists with fluctuations of the dominant serotype or genotype over time and from place to place [2,3].

While co-circulation has been frequently described, reports on co-infections were very rare up to a decade ago. The first naturally acquired co-infection in humans, caused by DENV2 and DENV3, was reported in Puerto-Rico in 1982 [4]. Two other reports described six patients co-infected by DENV1 and DENV3 in New Caledonia [2] and an autochthonous case with a dual DENV1/DENV2 viremia in São Paulo in 2001 [5]. In the last few years, the number of reported autochthonous co-infections seems to increase, mainly in India [6–8].

We here described the rare event of a dengue co-infection in an European traveler with identification of the causing serotypes and one of the genotypes. It serves as sentinel as no other data on the circulating genotypes causing the major epidemic in Thailand 2013 are available.

2. Case description

In July 2013, a DENV1/DENV2 co-infection was detected by RT-PCR in a 25-year old Belgian woman returning from Thailand.
Table 1
Laboratory results of the DENV1-DENV2 mixed infection.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Date</th>
<th>NS1 Ag RDT</th>
<th>DENV IgM ELISA (ratio)*</th>
<th>DENV IgG ELISA (ratio)*</th>
<th>Real-time RT-PCR multiplex† (CT-value)</th>
<th>Real-time RT-PCR simplex DENV1 (CT-value)</th>
<th>Real-time RT-PCR simplex DENV2 (CT-value)</th>
<th>Real-time RT-PCR multiplex† (CT-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>08/07/2013</td>
<td>Positive</td>
<td>Positive 3.41</td>
<td>Positive 3.67</td>
<td>Positive (DENV1 25.32 + DENV2 31.16)</td>
<td>Positive (23.21)</td>
<td>Positive (27.62)</td>
<td>Positive (DENV1 + DENV2)</td>
</tr>
</tbody>
</table>

* ELISA is considered positive if ratio > 1.0.
† Performed at ITM.
‡ Performed at RKI.

She traveled to Bangkok, Kanchanaburi, Ayutthaya, Sukhothai, Chiang Mai and Koh Tao-Koh Samui between June 16th and July 6th. Upon return, the patient presented to her general practitioner with characteristic dengue symptoms: fever, headache, maculopapular rash, myalgia, nausea and anorexia. Fever lasted for three days and the patient fully recovered. Serum collected three days after fever onset, was submitted to the national reference center (Institute of Tropical Medicine (ITM), Belgium) where a dengue infection was diagnosed by antigen detection (SD Bioline Dengue NS1 Ag, Standard Diagnostics Korea), real-time RT-PCR adapted from [9], and serology (Dengue Virus IgM and IgG Capture DxSelectTM (Focus Diagnostics, Cypress, CA, USA)). Travel history and clinical data were retrieved by a questionnaire sent to the treating physician. Laboratory analysis on the acute phase serum demonstrated thrombocytopenia (145 × 10^9/L) and leucopenia (2.4 × 10^9/L). Malaria tests (microscopy, Binax NOW antigen test (Alere)) were negative. Rapid diagnostic testing detected the presence of NS1-antigen and ELISA demonstrated a positive IgG and IgM ratio (Table 1).

A first dengue multiplex RT-PCR revealed a Ct-value of 25.32 for DENV1 and 31.16 for DENV2. A simplex DENV1 and a simplex DENV2 RT-PCR with the same primers and probe confirmed the multiplex RT-PCR result. Comparable RT-PCR results were obtained after repeated extraction. The sample was sent to the Center for Biological Threats and Special Pathogens of the Robert Koch Institute (RKI, Berlin, Germany) where the co-infection was confirmed by a second multiplex real-time RT-PCR. A RT-nested-PCR [10] was performed and after TA cloning and E. coli transformation (TOPO TA cloning kit, Life Technologies, Carlsbad, CA, USA) following manufacturer’s instructions, plasmid DNA was isolated (Plasmid Mini kit, Qiagen) and sequenced. A phylogenetic analysis was performed as described [11] using MEGA6 software [12]. Genotyping (Figure) revealed the identification of DENV1 genotype I (Asia), lineage Asia-3. Due to the predominance of DENV1, it was not possible to determine the genotype of DENV2, even after repeated analysis. Viral culture was not successful due to cytotoxicity and would probably have given competitive suppression of the minor serotype by the dominant serotype [13].

No follow-up serum sample was requested as the patient was a confirmed case of non-severe dengue fever [14].

2.1. Other similar and contrasting cases in the literature

The only co-infection diagnosed in a traveler up to date concerned a Japanese girl with a DENV1/DENV4 infection acquired in Cambodia in August 2013 [15].

3. Discussion

We report a co-infection with DENV1 and DENV2 in a Belgian traveler returning from Thailand, where different dengue serotypes have been co-circulating since decades [16,17]. Most cases of travel-acquired dengue fever in European travelers are imported from Thailand [18,19], with DENV1 as most commonly detected serotype followed by DENV2 [10].

This is the first co-infection detected in our reference center during the six years of molecular diagnostics. This one case accounts for 1.0% of the total number of RT-PCR-positive single infections (n = 105) diagnosed in our laboratory between 2008 and 2013. It seems that co-infections are more often reported for other tropical diseases such as malaria; even given the fact that the percentage of Plasmodium mixed-infections detected by PCR (0.8%; 27/334) in our travel-related setting between 1995 and 2009 [20] is comparable to that of DENV. DENV co-infections are probably underdiagnosed in travelers because concurrent infections can only be demonstrated by the use of serotype-specific RT-PCRs and the short period of viremia after symptom onset is often passed when patients return home.

Due to this short viremic phase, which is known to last no longer than about one week, the patient acquired the two infections at (nearly) the same moment. Either both serotypes were transmitted by one mosquito or the patient was bitten by two different mosquitoes within a period of maximum one week. Thavara and colleagues demonstrated for the first time the presence of two serotypes in a field-caught mosquito. Consequently, it is possible to get a double infection from a single bite in endemic areas where more than one serotype is circulating [21]. Because the DENV2 signal by RT-PCR was weaker compared to DENV1 for the case described here, we hypothesize that the infection was caused by a single bite of a mosquito with a lower viral load of DENV2 than of DENV1, or that the patient contracted the DENV2 infection some days before the DENV1 infection. Another possible explanation is competition between both viruses within the patient or the mosquito with DENV1 being most successful.

Little is known about the clinical implications of DENV co-infections. The patient described here presented with typical symptoms without complications and recovered easily and completely. According to Ponson et al. [7], co-infections may not necessarily cause more severe disease. Other studies suggest that more severe forms of dengue, including dengue hemorrhagic fever (DHF), are more often seen in co-infections than in single infections [8,21]. The cause of severe dengue infections is multifactorial. Hyperendemicity (co-circulation of multiple serotypes in one region) with the subsequent risk of secondary infections is believed to be one of the most significant factors. Severity may also be related to the infecting serotype and strain with DENV1 being more severe than other serotypes and the Asian DENV2 genotype more severe than the American one [22,23]. More data is needed to demonstrate if DHF is indeed more frequent in co-infections than in single infections and if so, to gain a better understanding of the factors that influence disease severity in co-infections and whether they differ from those in subsequent single infections.

The detection of a co-infection in a person living in or visiting an endemic region can serve as sentinel for ongoing co-circulation in a certain region and at a certain time point. In Thailand the incidence of dengue increased significantly since the late 1980s [21] and an ‘invasion’ phase of new strains causing local outbreaks between
1985 and 1995, was followed by a stationary phase from 2000 onwards with genetically stable strains [17]. But, DENV serotypes still demonstrate changing patterns with shifts in the dominant serotype over time and with more virulent genotypes displacing those of lower epidemiological impact, resulting in outbreaks [2,11]. While DENV1 was predominant in 2004 and in 2008, DENV4 was prominent in 2007 and DENV3 in 2010 [7]. In 2013, the year in which the Belgian traveler contracted the co-infection, Thailand experienced its largest epidemic in more than two decades. No data are available on the circulating strain responsible for this outbreak. Our genotyping results demonstrated that at least DENV1 genotype I, lineage Asia-3 was circulating at that moment.

Together with the increasing evidence of DENV serotype co-circulation in endemic regions, there is an upward trend in the number of autochthonous co-infections. A study with RT-PCR data from Indonesia and Mexico from the period 1980 to 1996 revealed that 5.5% of the samples contained more than one serotype [24]. A more recent study of dengue outbreaks in India in 2006 demonstrated 19% of DENV co-infections [6] and in 2011–2012 even more than 40% of the samples contained two serotypes [8]. In contrast, few data exist on co-infections in Thailand where hyperendemic transmission is ongoing for decades. A retrospective study on samples collected between 2004 and 2010 in Thailand demonstrated that only 1/185 patients (0.5%) with a RT-PCR-positive sample was infected with two serotypes [7]. We expect that due to the increasing incidence of DENV infections, also the number of co-infections will rise in the upcoming years. More data will contribute to the understanding of the clinical features associated with co-infection. Traveling to and in endemic regions might increase the risk for further spread of DENV in new regions. Therefore, awareness among travelers is needed and ongoing surveillance remains very important.

Funding

The national reference center of WNV (and other arboviruses) in Belgium is partially supported by the Belgian Ministry of Social Affairs through a fund with the Health Insurance System.

Competing interests

We state that there is no potential conflict of interest of the authors.

Ethical approval

The diagnostic procedures described in this manuscript are part of the standard diagnostic work-up of patients suspected of dengue. All samples for routine diagnostic from patients presenting at the Institute of Tropical Medicine (ITM, Antwerp, Belgium) polyclinic are stored after completion of the routine tests. The ITM has the policy that sample left-overs of patients presenting at the ITM polyclinic can be used for research unless the patients explicitly state their objection. The Institutional Review Board of ITM approved the institutional policy of this presumed consent as long as patients’ identity is not disclosed to third parties. All data have been analyzed anonymously. We declare that informed consent has been obtained from the person whose details are described in article that this information may be published.

References