Three Cases of Imported Dengue Virus Infection From Madeira to Belgium, 2012

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We report three laboratory-confirmed dengue virus (DENV) infections imported to Belgium by travelers returning from Madeira (Portugal). Despite the use of a mosquito-repellent spray as reported by two patients, the infection could not be prevented. Diagnosis was made by antigen detection and real-time reverse transcriptase polymerase chain reaction (RT-PCR) in two cases and by serology 1 month after onset of symptoms in a third one. The responsible virus was identified as DENV serotype 1, American/African genotype (genotype V). The close relationship to isolates from Colombia supports the previous findings that a South American strain originated the outbreak in Madeira in 2012.
Three DENV Cases, Imported to Belgium From Madeira

Table 1

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Gender</th>
<th>Destination</th>
<th>Reason of visit (duration)</th>
<th>Reported prevention</th>
<th>Date of return</th>
<th>Onset of fever</th>
<th>Date of return</th>
<th>Blood values</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>29</td>
<td>Female</td>
<td>East Funchal</td>
<td>Holiday (7 days)</td>
<td>No prevention</td>
<td>03/11/2012</td>
<td>07/11/2012</td>
<td>Thrombocytopenia (123 × 10^9/L)</td>
<td>Leukopenia (2.1 × 10^9/L)</td>
<td>Fever (39°C, 5–6 days), maculopapular rash (+−), myalgia/arthralgia, mild head and swollen throat for 9 days</td>
</tr>
<tr>
<td>Case 2</td>
<td>31</td>
<td>Male</td>
<td>East Funchal</td>
<td>Holiday (7 days)</td>
<td>Insect repellent spray</td>
<td>03/11/2012</td>
<td>06/11/2012</td>
<td>No thrombocytopenia</td>
<td>No leukopenia</td>
<td>Fever (2 days), maculopapular rash (+−), myalgia/arthralgia, headache, swollen throat (difficult to eat and swallow) for 9 days</td>
</tr>
<tr>
<td>Case 3</td>
<td>53</td>
<td>Female</td>
<td>Funchal center</td>
<td>Visiting friends (7 days)</td>
<td>DEET spray</td>
<td>28/10/2012</td>
<td>26/10/2012</td>
<td>No thrombocytopenia</td>
<td>Leukopenia (3.2 × 10^9/L) Erythrocytopenia (3.89 × 10^12/L)</td>
<td>Fever (38°C, 11 days), maculopapular rash, myalgia/arthralgia, nausea, nose bleeding, itching</td>
</tr>
</tbody>
</table>

Flemish regional, Belgian national, and European health authorities were notified of all cases. Written informed consent was obtained from the three patients.

Sequencing of the E/NS1 region at the Instituto de Salud Carlos III (Madrid, Spain) revealed the genotype for one traveler (case 3). BLAST analysis (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) showed a similarity index of 98% with a South American strain of DENV-1 (accession number GQ868570, isolate CO/BID-VE3391) isolated from Santander (North East Colombia) in 2008. Phylogenetic analysis of 34 sequences of DENV-1 retrieved from GenBank (total alignment length of 225 nucleotides; December 9, 2013) was aligned with MEGA 5. The sequence of the Belgian case (BE56, Figure 1) fell into the DENV-1 American/African (V) genotype, within South American isolates from Colombia and Venezuela and was most closely related to the isolate from Colombia in 2008 (GQ868570). Interestingly, a sequence with 98% similarity to isolate BE56 was detected in another Belgian traveler (BE49) returning from Colombia at the same period as that of the Madeira outbreak. In addition, the E/NS1 sequence of the DENV-1 isolate detected during the dengue outbreak in Croatia in 2010 (FR847064) was also included in the phylogenetic analysis and belongs to the same American/African genotype but clustered within the Indian lineage while the Madeiran isolate grouped together with the American lineage.

Discussion

Among the imported arboviral infections diagnosed in Belgium, DENV infections are the most frequent, with Southeast Asia and tropical America accounting for the majority of travel destinations. In 2010, it became clear that even travelling to non-endemic regions, such as southern Europe, pose a risk to acquire dengue fever as illustrated by reports on autochthonous cases in France and Croatia.3–6 It is noteworthy that this phenomenon is of concern for other arboviruses as well as demonstrated for chikungunya virus in Italy12 and West Nile virus in Greece.13

The epidemic in Madeira7,8 illustrates the recent reemergence of DENV in Europe. The high mobility of travelers across European borders contributes to an increased invasion risk of DENV into new regions. Apart from the 2,164 cases diagnosed in Madeira island residents, 78 imported cases have been diagnosed in several European countries: UK, Germany, mainland Portugal, Finland, Sweden, France, Denmark, Austria, Norway, Croatia, Slovenia, Spain, and Switzerland.8

In contrast to malaria that is often imported by migrants, DENV-imported cases in Europe represent mostly tourists (84%).14 Accurate travel information

J Travel Med 2014; 21: 344–348
is therefore crucial, especially because DENV is transmitted by day-biting mosquitoes. Despite the use of a mosquito-repellent spray as reported by two patients, the infection could not be prevented. This might be because of the incorrect use of the spray (not covering the whole body, no reapplication after 6 hours, or not at the correct time of the day when dengue mosquitoes are mostly active, ie, from dusk till dawn) or because of the use of a repellent containing less than the effective 20% to 50% N,N-diethyl-3-methylbenzamide (DEET) concentration. As reported by Frank and colleagues,15 during the Madeiran outbreak, there was an overall increased infection risk after rainfall, which could also have played a role in acquiring the infection in the cases described here.

Clinical diagnosis of dengue fever might be difficult because it resembles other (arbo)viral diseases such as influenza, hepatitis, and malaria. Moreover, the disease might be overlooked if patients have not traveled to tropical destinations. Timely diagnosis is important to exclude other causes of disease, to avoid improper treatment with drugs having anticoagulant effects that might cause hemorrhagic complications, and to postpone surgical interventions. Dengue infection can become life-threatening in severe cases, which occur in less than 1% of the imported cases.16 Fortunately, none of the cases infected in Madeira were fatal.7,8

In Madeira, only DENV-1 was circulating during the epidemic. Sequencing of the E/NS1 junction genome fragment is useful for accurate determination

### Table 2 Laboratory results of the three imported dengue cases to Belgium returning from Madeira.

<table>
<thead>
<tr>
<th>Case</th>
<th>Sampling date</th>
<th>Days after onset of symptoms</th>
<th>DEN IgM/IgG rapid test*</th>
<th>DEN IgM ELISA†</th>
<th>DEN IgG ELISA†</th>
<th>DEN NS1 Ag‡</th>
<th>RT-PCR</th>
<th>Genotyping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>12/11/2012</td>
<td>5</td>
<td>IgM weakly positive</td>
<td>0.41</td>
<td>0.09</td>
<td>Positive</td>
<td>DENV-1 (Ct: 33.16)</td>
<td>Undetermined</td>
</tr>
<tr>
<td>Case 2</td>
<td>06/12/2012</td>
<td>30</td>
<td>Not done</td>
<td>8.43</td>
<td>2.79</td>
<td>Not done</td>
<td>Not done</td>
<td>Not done</td>
</tr>
<tr>
<td>Case 3</td>
<td>30/10/2012</td>
<td>4</td>
<td>IgM positive</td>
<td>0.83</td>
<td>0.07</td>
<td>Positive</td>
<td>DENV-1 (Ct: 27.7)</td>
<td>Genotype V</td>
</tr>
</tbody>
</table>

*The cutoff value of the IgM and IgG ELISA is 1.00. Genotyping was not possible for case 1, probably because of the low nucleic acid concentration.
†IgM=immunoglobulin M; IgG=immunoglobulin G; ELISA=enzyme-linked immunosorbent assay; RT-PCR=reverse transcriptase polymerase chain reaction; DENV=dengue virus.
‡11FK50, SD Bioline.
of the causing genotype as shown in cases in Croatia and imported to Europe and can contribute to the epidemiology of the disease. The phylogenetic analysis of this study was also based on this fragment and revealed that isolate BE56 of the Belgian traveler falls within the South American lineage of the American/African (V) genotype of DENV-1, being most closely related to the isolate detected in Colombia in 2008. This finding is in agreement with the previous reports related to the Madeira outbreak. In addition, the DENV sequence of case BE49 imported to Belgium from Colombia in September 2012 showed 98% identity with the sequence of BE56, and 97% identity with the Colombian strain. Our results support the suggestion of Alves and colleagues and Huhtamo and colleagues that this South American strain originated the outbreak in Madeira in 2012. Direct comparison of the BE56 sequence with those described in the previous reports was not possible because of the use of other sequencing targets. However, comparison was possible with the sequence of the autochthonous case from Croatia in 2010 and indicated that both strains belong to the same American/African genotype but that the Croatian genotype clustered together with Indian lineage. It is thus very unlikely that DENV1 was introduced from the same origin in both Southern European countries, which are about 5,000 km away from each other. The exact circumstances of DENV introduction to Madeira remain to be elucidated.

So far, autochthonous DENV infections have never been observed in Belgium, and there are no reports on the presence of Aedes aegypti in Belgium. This vector is however well established in Madeira since 2005 and has been sporadically discovered in the UK, France, Italy, Malta, Croatia, Ukraine, Russia, Turkey, and recently in the Netherlands, the northern neighboring country of Belgium. The presence of Aedes albopictus is described in 16 European countries, including Belgium and the Netherlands, and its establishment is at least seen in Italy, France, Spain, and Greece. Also other potential dengue vectors, such as Aedes japonicus, invade Europe, which is established in parts of Belgium, France, Switzerland, and Germany.

Besides the presence of the vector, additional factors, such as climatic conditions and optimal breeding sites, are needed for DENV introduction in a country; thus, the estimated risk of DENV introduction to Belgium is currently very low.

In conclusion, dengue fever should be considered in the differential diagnosis of travelers presenting with fever and myalgia and/or arthralgia shortly after a visit to DENV endemic or epidemic regions. As there is need for increased awareness, accurate prevention instructions, and timely diagnosis, health care workers have to be informed about the distribution of the virus that is currently expanding into previously unaffected regions.

Acknowledgments

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. This study was partially supported by The Spain Sanitary Research Funds, project no. 10/00069, P.I. MP Sanchez-Seco, PhD. We thank Arantxa Potente, Elke Gintelenberg, and Kathy Demeulemeester for technical assistance.

Declaration of Interests

The authors state that they have no conflicts of interest.

References


The city of Brussels in Belgium has a unique comic strip route. This route takes people along several walls in Brussels with big paintings of famous comic book heroes. This wall in the “Rue haute” features Quick and Flupke who were amongst the first comic book heroes drawn by Hergé who will become famous later as the renowned creator of Tintin. Photo Credit: Eric Caumes