Journal of
TRAVEL MEDICINE

BRIEF COMMUNICATION

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

Lieselotte Cnops, PhD,* Leticia Franco, PhD,† Britt Van Meensel, MD,‡ Jef Van den Ende, PhD,* Maria Paz Sanchez-Seco, PhD,† and Marjan Van Esbroeck, MD*

*Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium; †National Center for Microbiology, Instituto de Salud Carlos III, Madrid, Spain; ‡Laboratory Medicine, Medisch Centrum Huisartsen, Leuven, Belgium

DOI: 10.1111/jtm.12113

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.

Dengue is the most important mosquito-borne viral infection worldwide and affects about 390 million people yearly in endemic areas of Southeast Asia, tropical America, and Africa.[1,2] The infection is caused by any of the four dengue virus serotypes (DENV-1 to DENV-4). DENV is primarily transmitted by *Aedes aegypti*, a mosquito that lives in close proximity to humans, or secondarily transmitted by *Aedes albopictus*, known as the Asian tiger mosquito. The global prevalence of dengue has grown dramatically in recent decades[2,3] because of the spread of the vector to new areas and the increase in international transport and travel.

Recently, dengue fever reached the shores of Europe. Several southern European countries that have an established mosquito vector are now at risk of an outbreak. Locally acquired DENV infections occurred in France in 2010 and 2013[3,4] and in Croatia in 2010.[5,6] In 2012, the Madeira islands (Portugal) were confronted with the first sustained epidemic of dengue fever in Europe since 1927 to 1928 when dengue hit Greece.[7] Between October 3, 2012 and February, 3 2013, 2,164 cases were reported from Madeira islands.[8] Genetic characterization of the virus demonstrated DENV-1 genotype V in one autochthonous case from Madeira archipelago[7] and in two imported cases returning from Madeira to Finland.[9]

Corresponding author: Lieselotte Cnops, PhD, Central Laboratory for Clinical Biology (CLKB), Department of Clinical Sciences, Institute of Tropical Medicine, Kronenburgstraat 43/3, B-2000 Antwerp, Belgium. E-mail: lcnops@itg.be

Case Reports

Between October and November 2012, during the peak of the epidemic, three Belgian travelers contracted dengue on their return from Funchal (Madeira). They presented with classical symptoms of dengue fever, none developed severe disease and all recovered well. Two patients were aware of the dengue outbreak and reported the use of a mosquito-repellent spray during their stay (Table 1).

DENV infection was diagnosed on acute-phase serum samples of two patients by detection of IgM antibodies by rapid immunochromatography testing and confirmed in the national reference center [Institute of Tropical Medicine (ITM), Antwerp, Belgium] by detection of DENV NS1 antigen and by real-time reverse transcriptase polymerase chain reaction (RT-PCR) [adapted from Ref. (10)] revealing serotype DENV-1 (Table 2; case 1 and case 3). DENV diagnosis was delayed in one patient (case 2; travelling together with case 1), although he visited his general practitioner immediately upon return and mentioned the dengue

Table 1 Travel history and clinical presentation of the three Belgian travelers visiting Madeira

Case	Age	Gender	Destination	Reason of visit (duration)	Reported prevention	Onset of fever	Date of return	Blood values	Symptoms
Case 1	29	Female	East Funchal	Holiday (7 days)	No prevention	07/11/2012	03/11/2012	Thrombocytopenia (123×10^9/L) Leukopenia (2.1×10^9/L)	Fever (39°C, 5–6 days), maculopapular rash (+/−), myalgia/arthralgia, loss of appetite
Case 2	31	Male	East Funchal	Holiday (7 days)	Insect repellent spray	06/11/2012	03/11/2012	No thrombocytopenia (189×10^9/L) No leukopenia (5.07×10^9/L)	Fever (2 days), maculopapular rash (+/−), myalgia/arthralgia, headache, swollen throat (difficult to eat and swallow) for 9 days
Case 3	53	Female	Funchal center	Visiting friends (7 days)	DEET spray	26/10/2012	28/10/2012	No thrombocytopenia (197×10^9/L) Leukopenia (3.2×10^9/L) Erythrocytopenia (3.89×10^{12}/L)	Fever (38°C, 1 day), maculopapular rash, myalgia/arthralgia, nausea, nose bleeding, itching

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

outbreak in Madeira. He received a prescription for oral rehydration therapy, paracetamol, and advice to take rest. One month later, the patient consulted a physician at ITM because of the reappearance of rash with unknown etiology, and DENV diagnosis was made on a convalescent phase serum by enzyme-linked immunosorbent assay (Table 2).

Sequencing of the E/NS1 region[11] 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 Italy[12] and West Nile virus in Greece.[13]

The epidemic in Madeira[7,8] 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

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

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

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.
*IgM/IgG, Standard Diagnostic (SD), Korea.
†IgM Capture DxSelect™, Focus Diagnostics, Cypress, CA, USA; IgG capture ELISA test, Panbio Diagnostics, Queensland, Australia.
‡11FK50, SD Bioline.

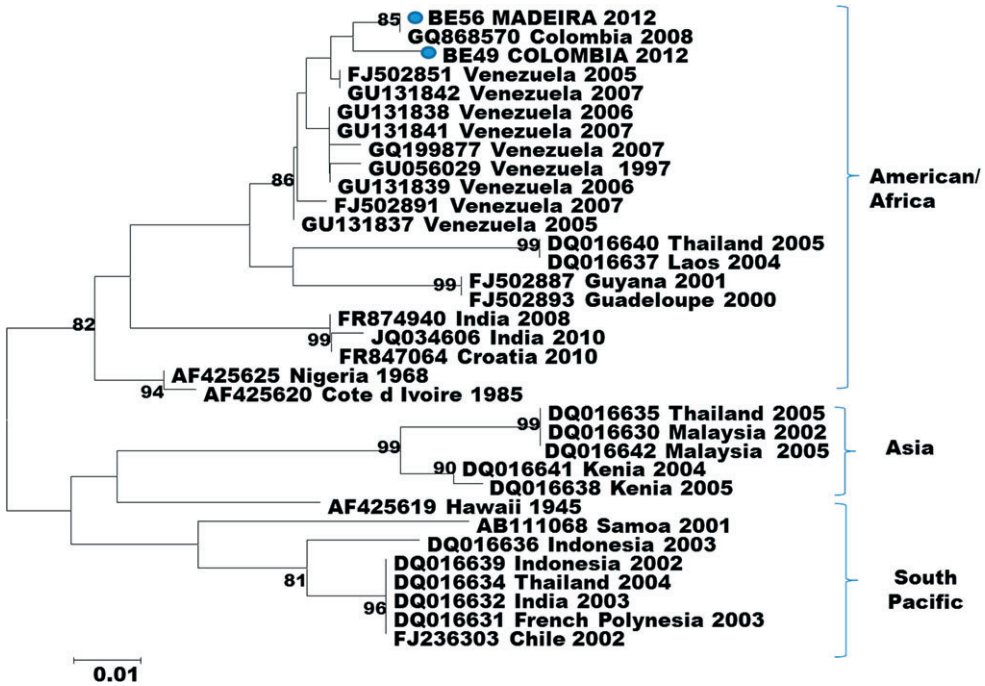

Figure 1 Phylogenetic tree based on a 225-bp fragment from the E-NS1 junction genome region of DENV-1 strains containing the sequences obtained from case 3 (BE56) and an additional case imported to Belgium from Colombia at the same period (BE49). This neighbor-joining unrooted tree was constructed by bootstrap analysis of 1,000 replicates using MEGA 5 software. The sequence obtained from the two Belgian patients is marked with circles.

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

of the causing genotype as shown in cases in Croatia[6] and imported to Europe[11] 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.[7,9] 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[7] and Huhtamo and colleagues[9] 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[7,9] 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[6] 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 *A. aegypti* in Belgium. This vector is however well established in Madeira since 2005[17] and has been sporadically discovered in the UK, France, Italy, Malta, Croatia, Ukraine, Russia, Turkey,[18] and recently in the Netherlands,[14] the northern neighboring country of Belgium. The presence of *A. albopictus* is described in 16 European countries, including Belgium[19] and the Netherlands,[20] 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.[19]

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

1. Bhatt S, Gething PW, Brady OJ, et al. The global distribution and burden of dengue. Nature 2013; 496:504–507.
2. Wilder-Smith A, Gubler DJ. Geographic expansion of dengue: the impact of international travel. Med Clin North Am 2008; 92:1377–1390.
3. La Ruche G, Souarès Y, Armengaud A, et al. First two autochthonous dengue virus infections in metropolitan France, September 2010. Euro Surveill 2010; 15:19676.
4. Marchand E, Prat C, Jeannin C, et al. Autochthonous case of dengue in France, October 2013. Euro Surveill 2013; 18:20661.
5. Gjenero-Margan I, Aleraj B, Krajcar D, et al. Autochthonous dengue fever in Croatia, August–September 2010. Euro Surveill 2011; 16:19805.
6. Kurolt IC, Betica-Radić L, Daković-Rode O, et al. Molecular characterization of dengue virus 1 from autochthonous dengue fever cases in Croatia. Clin Microbiol Infect 2013; 19:E163–E165.
7. Alves MJ, Fernandes PL, Amaro F, et al. Clinical presentation and laboratory findings for the first autochthonous cases of dengue fever in Madeira island, Portugal, October 2012. Euro Surveill 2013; 18:20398.
8. European Centre of Disease Prevention and Control. Dengue outbreak in Madeira, Portugal, October–November 2012. Stockholm: ECDC, 2013.
9. Huhtamo E, Korhonen E, Vapalahti O. Imported dengue virus serotype 1 from Madeira to Finland 2012. Euro Surveill 2013; 18:20405.
10. Johnson BW, Russell BJ, Lanciotti RS. Serotype-specific detection of dengue viruses in a fourplex real-time reverse transcriptase PCR assay. J Clin Microbiol 2005; 43:4977–4983.
11. Domingo C, Niedrig M, Gascón J, et al. Molecular surveillance of circulating dengue genotypes through European travelers. J Travel Med 2011; 18:183–190.
12. Rezza G, Nicoletti L, Angelini R, et al., CHIKV Study Group. Infection with chikungunya virus in Italy: an outbreak in a temperate region. Lancet 2007; 370:1840–1846.
13. Cnops L, Papa A, Lagra F, et al. West Nile virus infection in Belgian traveler returning from Greece. Emerg Infect Dis 2013; 19:684–685.
14. Marí RB, Peydró RJ. Chapter 27: Re-emergence of malaria and dengue in Europe. In: Rodriguez-Morales A, ed.

Current topics in tropical medicine. Vol. 2012. Rijeka, Croatia: InTech, 2012:483–512.
15. Frank C, Höhle M, Stark K, Lawrence J. More reasons to dread rain on vacation? Dengue fever in 42 German and United Kingdom Madeira tourists during autumn 2012. Euro Surveill 2013; 18:20446.
16. Wichmann O, Gascon J, Schunk M, et al. European network on surveillance of imported infectious diseases. Severe dengue virus infection in travellers: risk factors and laboratory indicators. J Infect Dis 2007; 195:1089–1096.
17. Margarita Y, Santos Gracio AJ, Lencastre I, et al. Mosquitos de Portugal: primeiro registo de *Aedes (Stegomia) aegypti* Linnaeus, 1762 (Diptera, Culicidae) na Ilha da Madeira. Acta Parasitol Port 2006; 1: 59–61.
18. Snow K, Ramsdale C. Distribution chart for European mosquitos. Eur Mosq Bull 1999; 3:14–31.
19. Schaffner F, Bellini R, Petrić D, et al. Development of guidelines for the surveillance of invasive mosquitoes in Europe. Parasit Vectors 2013; 6:209.
20. ProMED-mail. Aedes albopictus–Netherlands. Archive number 20130817.1885955. Available at: http://www.promedmail.org/direct.php?id=20130817.1885955. (Accessed 2013 Sep 9)

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*