

Molecular epidemiology suggests Venezuela as the origin of the dengue outbreak in Madeira, Portugal in 2012–2013

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Abstract

An explosive epidemic occurred in Madeira Island (Portugal) from October 2012 to February 2013. Published data showed that dengue virus type I introduced from South America was the incriminated virus. We aim to determine the origin of the strain introduced to Madeira by travellers returning to Europe. Using phylogeographic analysis and complete envelope sequences we have demonstrated that the most probable origin of the strain is Venezuela.

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After more than 80 years without dengue fever in Europe, in 2010 autochthonous transmission was reported in France and Croatia [1]. In October 2012, a cluster of autochthonous cases was reported in Madeira, Portugal [2]. The outbreak in Madeira evolved rapidly because of the abundance of the competent vector *Aedes aegypti* [3] and the totally naive population, resulting in over 2168 cases from 26 October 2012 to 3 March 2013. Until 3 February 2013, at least 78 cases in 13 other European countries were reported as introductions via travellers departing from Madeira [4]. The causative virus was identified as dengue virus type I (DENV-I) [5].

A recent study investigating Madeira air travel interconnectivity to track the possible origin of DENV-I introduction in Europe indicated that from 22 948 air travellers to Madeira in 2012 arriving from 29 dengue endemic countries, almost 90% of them came from Venezuela and Brazil [6]. In addition, in 2012 DENV-I partial capsid pre-membrane sequences were obtained from two Finnish travellers (399 and 396 nucleotides, respectively) returning from Madeira to Finland with dengue fever [7]. These sequences were similar to that of an autochthonous case from Madeira and grouped phylogenetically with South American lineages within the America/Africa genotype. Analysis of partial E sequences from Belgian travellers reported similar findings [7,8]. These findings suggest a unique introduction from a South American country to Madeira.

Here we report for the first time the full envelope dengue sequence data from travellers who had acquired dengue fever in Madeira and compared them with recent sequences obtained from travellers returning from South America to find the most probable source of the epidemic.

As part of a collaborative study to monitor dengue virus importation into Europe through travellers (Dengue tools project, www.denguetools.net), we collected acute positive samples (as detected by RT-PCR or Non-structural protein I antigen test (NSI-Ag)), from the beginning of 2012 from patients seen by each of the 17 Tropical Medicine centres enrolled in the study in 11 European countries [9] onwards. Positive samples were sent to the reference laboratory (Instituto de Salud Carlos III, Madrid, Spain) for strain isolation and characterization. Samples from travellers returning to Europe from Madeira or South America were selected for comparing virus sequences at the Envelope (E) gene and its junction with the NSI gene. The ethics review board of both hospitals approved this study.

Briefly, RNAs were extracted with a Qiamp viral RNA extraction kit (Qiagen, Hilden, Germany), and the complete E gene (1485 nucleotides) and the E/NSI junction (441 nucleotides) of DENV-I strains were RT-PCR amplified [10]. The



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amplification products were purified and sequenced using a set of 15 primers (11 internal and two external primers for *E*, plus two external primers for *E/NSI*). In total, we obtained the sequences from three new isolates: one from a German traveller returning from Madeira in 2012, and two from Spanish travellers returning from Venezuela in 2010 and 2012. These sequences were aligned with 140 other DENV-I sequences retrieved from GenBank, and chosen to represent the different virus genotypes and different geographical origins and time of detection for each genotype. Using this sequence data set, a Bayesian Markov Chain Monte Carlo (MCMC) phylogenetic tree was inferred with BEAST v1.7.5 [11], incorporating the best-fitted nucleotide substitution model as determined by jModelTest 2.1.3 [12].

The MCMC tree revealed that the DENV-I strains obtained in the present study belonged to genotype V (America/Africa), and clustered within one of the South American sub-lineages, forming a monophyletic group together with strains recently isolated in Colombia and Venezuela (Fig. 1). Nucleotide identities in the complete *E* gene among the sequences belonging to this monophyletic group (Venezuela and Colombia sub-lineages) displayed a 99.93% nucleotide identity within Madeira strain (MUC15-2012) and Venezuela strain (51019-2010) and 99.86% with the most recent Venezuelan strain (1052-2012). The MCMC tree was also used to reconstruct the ancestral state of the geographical origin of DENV-I sequences at each node of the phylogeny, and hence to trace the origin of the strain introduced in Madeira. The MCMC tree annotated with node state (i.e. country of origin) indicated that Venezuela was the most probable source of the strain introduced in Madeira (Posterior probability = 0.99) (see insert in Fig. 1). Our phylogenetic data directly comparing two Venezuelan strains against one from Madeira, obtained in 2010 and 2012, respectively, show a very high identity (99%). According to our phylogeographical analysis, the strain GQ868570, Colombia 2008, probably originated in Venezuela and circulated in North Colombia (North Santander state, border with Venezuela's Zulia and Táchira states) and was circulating in the region from 2006 until 2008, and meanwhile was detected Venezuela in 2010 and 2012. Hence, the genetic characterization of dengue virus strains from travellers recently returning from South America indicated that the DENV-I strain responsible for the outbreak in Madeira was most probably introduced from Venezuela. This finding is in accordance with recent investigations by Wilder-Smith et al. [6] on the interconnectivity via air travel between countries where dengue is endemic and Madeira. In this study, the importation index into Madeira—a measure of the risk of dengue introduction from a given country, based on both travel volume and dengue incidence in that country, was higher for Venezuela than for the other 29

dengue endemic countries with interconnectivity with Madeira. This abundant exchange of travellers is due to the large Portuguese community in Venezuela, mostly coming from Madeira, which is the second biggest in Latin America [13]. Indeed, Venezuela is the only Latin American country with direct flights from its capital (Caracas) to Madeira. This cultural connection may have contributed to dengue introduction in Madeira.

The recent explosive epidemic of chikungunya in the Americas showed how a single introduction of an Asian strain [14] spread from the initial outbreak in the Caribbean and circulated over the continent, reaching more than 1 million cases by January 2015. In this regard, Venezuela with more than 34 000 affected people [15] poses a risk of introduction of chikungunya into Madeira during the next months as occurred with dengue in 2012. In conclusion, our phylogenetic analyses provide compelling evidence that Venezuela is the source of DENV-I introduced into Madeira in 2012.

Contribution to authorship

LF and AT conceived and designed the study; LF, IP, NS, MS, FM, AP, AN, MPSS and CH contributed to acquisition, analysis and interpretation of data; LF, IP, AWS and AT drafted the article. All authors revised it critically and approval the final version.

Transparency declaration

The authors declare that they have no conflicts of interest.

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