International Journal of Infectious Diseases 92 (2020) 46–48

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

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

Case Report

Full-length genome sequence of a dengue serotype 1 virus isolate from a traveler returning from Democratic Republic of Congo to Italy, July 2019

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ARTICLE INFO

Article history:
Received 6 November 2019
Received in revised form 13 December 2019
Accepted 16 December 2019

Keywords:
Dengue virus
Full-genome sequence
Travel-associated infection
Democratic Republic of Congo

ABSTRACT

We report the full-genome sequence of a Dengue serotype-1 virus (DENV-1) isolated from a traveler returning in July 2019 to Italy from Democratic Republic of Congo (DRC), which is currently affected by Ebola and measles outbreaks. The sequence shows high similarity with two 2013 strains isolated in Angola and China.

Background

Dengue fever (DF) is a mosquito-borne disease caused by four phylogenetically and antigenically distinct dengue viruses (DENV 1-4) which belong to the *Flavivirus* genus (World Health Organization, 2019). The disease is endemic throughout the tropics and international travels contribute to spreading DENV infection and determine imported cases in non-endemic areas (Bhatt et al., 2013). However, circulation of DENV is underreported in sub-Saharan African countries due to the lack of diagnostic capacity, epidemiological surveillance and nonspecific clinical signs and symptoms, which overlap with those of other prevalent diseases (Amarasinghe et al., 2011; Jaenisch et al., 2014). DRC is one of those countries home to a variety of pathogens and plagued by several emergencies. Among these, an Ebola virus disease (EVD) outbreak was declared on the 1st of August, 2018 in the North Kivu province and on July 17, 2019, the WHO Emergency Committee declared it to be a Public Health Emergency of International Concern (PHEIC) (https://www.who.int/ihr/procedures/statement-emergency-committee-ebola-drc-july-2019.pdf).

International scientific agencies and public health institutions in non-endemic European Union and European Economic Area (EU/EEA) countries are again on alert to screen and monitor and eventually manage travelers returning from DRC with VHF symptoms. Appropriate differential diagnosis is key to identify the cause of infectious febrile syndromes in patients with travel histories. Although the *Aedes spp* mosquitoes are present in DRC, little information about DENV circulation is available so far (Mbanzulu et al., 2017). Moreover, travelers may serve as sentinels to local epidemic risks, and their role is especially important in areas with scarce public health reporting and resources.

Findings

A previously healthy Caucasian woman (18-years-old) returning from Kinshasa, DRC, to Italy was admitted to the Policlinico Umberto I, University of Rome "Sapienza" in Rome, Italy on the 20th of July. The patient reported the onset of asthenia and retro-orbital pain on the 17th of July followed by the onset of fever (>38.5 °C) and rash on the following day. The patient had been staying in Kinshasa working for a humanitarian organization for two weeks and returned to Italy on the 14th of July 2019.

Virological diagnosis was made at the National Institute for Infectious Diseases "Lazzaro Spallanzani" IRCCS (INMI) in Rome,

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https://doi.org/10.1016/j.ijid.2019.12.023

Regional Reference Laboratory for arboviral infections. DENV NS1 antigen (STANDARD F Dengue NS1 Ag FIA, SD Biosensor, Korea) and viral RNA (CDC DENV-1–4 Real-Time RT-PCR, Santiago et al., 2013) was detected in the acute-phase serum sample. DENV 1-4 multiplex Real-Time RT-PCR showed the presence of DENV type 1, which was confirmed also by pan-flavivirus nested RT-PCR (modified from Moureau et al., 2007) targeting the non-structural protein 5 (NS5) gene, followed by the amplicon sequencing (241 bp). The virus was isolated after a single passage in Vero E6 cell culture from an acute-phase serum sample. DENV-specific IgM and IgG resulted negative using both rapid test (STANDARD F Dengue IgM/IgG FIA, SD Biosensor, Korea) and indirect immune fluorescence assay (IFA, Arboviral Fever Mosaic-2, IgM and IgG, Euroimmun, Hamburg, Germany), consistent with the short time between clinical onset and sample collection. Chikungunya virus and Zika virus specific IgM and IgG were negative as well as specific RT-PCR in serum. Thin and thick smears for malaria were negative and the patient was under prophylactic treatment with atovaquone/proguanil. The patient recovered after 11 days since symptoms onset without any further complications and no sequelae.

A 10,568-nt-long sequence was amplified in 21 overlapping reverse transcriptase-PCR amplicons and Sanger sequenced from cell culture-derived isolate. Primers were designed using the 2013 Angola strain (GenBank acc no KF184975) as template, since the NS5 sequencing showed high similarity (99.56% on 241 bp) with 2013 Angola strain (primers sequences available on request). Thirty-four DENV full genome sequences from the most recent literature describing the circulation of DENV in DRC neighbouring countries (e.g. 2013 Angola outbreak) were used for phylogenetic analysis (Parreira et al., 2014). The results showed that the closest related sequences to the DENV-1 isolated from this patient (INMI/DRC-2019 – GenBank acc no MN577472) were from two 2013 strains isolated in China and Angola (99.3% and 98.5% similarity on 10141 bp, respectively).

The phylogenetic tree, shown in Figure 1, confirms this genetic correlation. Notably, the strain isolated in China is reported as an imported infection, but no information on the travel history of the patient is available (https://www.ncbi.nlm.nih.gov/nuccore/KF864667).

Discussion

Here, we report an imported case of acute primary DENV-1 infection in a patient who had travelled to Kinshasa in DRC. The strain identified in the patient's serum is most closely related to a strain isolated from a DENV infection imported to China in 2013, and to the strain responsible for the 2013 Angola outbreak (Centers for Disease Control and Prevention (CDC), 2013). It is possible that the case imported to China was actually imported from Angola or neighbour countries, but, to our knowledge, no information is available to this respect. The last published report regarding DENV

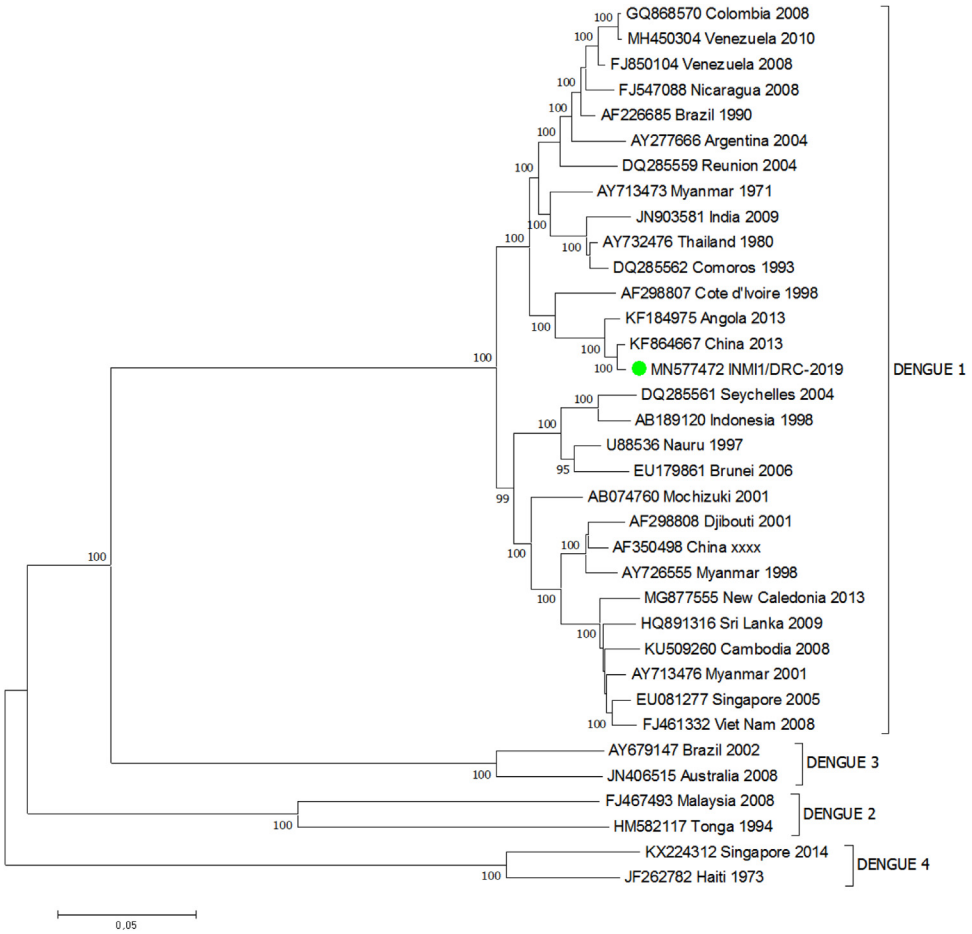

Figure 1. Phylogenetic tree based on complete genome (10141 nt), built using the Maximum Likelihood method. The evolutionary distances were computed using the General Timer Reversible model (GTR). The rate variation among sites was modelled with a gamma distribution (shape parameter = 0.31). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. Each record consists of accession number, place and year of detection/isolation, when available. The sequence analyzed in this work is highlighted with a green dot. Phylogenetic analysis was conducted in the MEGA7 software package. The complete genome sequence of DENV-1 isolate here described (INMI/DRC-2019) has been deposited in GenBank under the accession number MN577472.

circulation in the DRC dates back to 2015, when a man travelling from Kinshasa (DRC) to Japan was found positive for DENV-1. Also in this case, the strain identified showed >99% identity to the Angola strain (Yamamoto et al., 2019), indicating that the circulation of DENV1 in DRC goes back at least to 2015. Since then, to our knowledge, there has been no report of DENV circulation in DRC. Previously, DENV has also been detected in DRC during a retrospective cross-sectional study, in which samples collected between 2003 and 2012 from yellow fever suspects were tested for other arboviruses. Sixteen (3.5%) patients enrolled from 2010 to 2011 resulted positive for DENV RNA. The majority of cases were located in the north provinces of DRC and DENV-2 was the main involved serotype (n = 11); 3 patients had DENV-1 and 2 were co-infected. No phylogenetic analysis was performed on these virus strains (Makiala-Mandanda et al., 2018).

Given the similarity between the strain involved in the 2015 outbreak and the strain detected in the patient here described, a sustained unrecognized circulation in the country could be hypothesized in these years. However, a national serosurvey on samples collected in 2013–2014 from children ranging in age between 6 months to 5 years revealed a very low DENV seroprevalence (0.4%, 95% Confidence Interval (CI) 0.1–0.9%) (Willcox et al., 2018). This low value among children is expected in countries with a low transmission and sporadic spillover events from hidden ecology niches to raise urban cycles.

More studies on seroprevalence in the adult population should be carried out to determine seroprevalence of flaviviruses across a broader age range, and investigate how these viruses contribute to the burden of acute febrile illness in the DRC.

This report highlights the need to consider DENV in surveillance programs in DRC and the likelihood of travel-related DENV infections in patients returning from that country. The current EVD outbreak in North Kivu province has greatly increased the number of international health care workers operating in DRC. There are currently more than 100 international humanitarian agencies in DRC. Although the relative contribution of travel-associated DENV cases from DRC would be minor compared to overall importation of cases from other endemic countries (i.e. southern Asia and latin America), the increase of clinical awareness of DENV in DRC would actually help to guide the differential diagnosis in endemic and non-endemic areas, which may receive travelers returning from DRC with suspicion of EVD.

In addition, epidemiological information about DENV circulation is also useful for surveillance intervention in dengue non-endemic countries given that *Aedes albopictus*, one of the DENV vectors, is currently established throughout most of the Mediterranean basin, expanding the potential for DENV and other arboviruses to diffuse after introduction by imported cases. In fact, autochthonous arboviruses outbreaks as the consequence of spill over from large ongoing transmission in tropical areas occurred in continental Europe in the last years (Vairo et al., 2018; European Centre for Disease Prevention and Control, 2013; Calba et al., 2017).

Further epidemiological investigation would be important to elucidate the prevalence of DENV infection in the country and helpful in providing up to date geographic risk profiles.

Conflict of interest

The authors declare no financial or personal relationships or conflict of interest with other people or organizations that could inappropriately influence (bias) their work.

Funding source

This work was supported by grants from the Italian Ministry of Health[Ricerca Corrente “Linea 1” and Ricerca Finalizzata GR-2016-02362110]; and from theEDCTP-funded project “PANDORA-ID-NET” [Grant number RIA2016E-1609].

Ethical approval

The work has been approved and written informed consent was obtained from the patient for publication.

References

Amarasinghe A, Kuritsk JN, Letson GW, Margolis HS. Dengue virus infection in Africa. Emerg Infect Dis 2011;17(August (8)):1349–54, doi:http://dx.doi.org/10.3201/eid1708.101515.

Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. Nature 2013;496(7446):504–7, doi:http://dx.doi.org/10.1038/nature12060.

Calba C, Guerbois-Galla M, Franke F, Jeannin C, Auzet-Caillaud M, Grard G, et al. Preliminary report of an autochthonous chikungunya outbreak in France, July to September 2017. Euro Surveill 2017;22(39), doi:http://dx.doi.org/10.2807/1560-7917.ES.2017.22.39.17-00647.

Centers for Disease Control and Prevention (CDC). Ongoing dengue epidemic—Angola, June 2013. MMWR Morb Mortal Wkly Rep 2013;62:504–7.

European Centre for Disease Prevention and Control. Mission report-dengue outbreak in Madeira, Portugal, March. Stockholm: ECDC; 2013.

Jaenisch T, Junghanss T, Wills B, Brady OJ, Eckerle I, Farlow A, et al. Dengue expansion in Africa-not recognized or not happening?. Emerg Infect Dis 2014;20(10), doi:http://dx.doi.org/10.3201/eid2010.140487.

Makiala-Mandanda S, Ahuka-Mundeke S, Abbate JL, Pukuta-Simbu E, Nsio-Mbeta J, Berthet N, et al. Identification of dengue and chikungunya cases among suspected cases of yellow fever in the Democratic Republic of the Congo. Vector Borne Zoonotic Dis 2018;18(July (7)):364–70, doi:http://dx.doi.org/10.1089/vbz.2017.2176.

Mbanzulu KM, Wumba R, Mukendi JK, Zanga JK, Shija F, Bobanga TL, et al. Mosquito-borne viruses circulating in Kinshasa, Democratic Republic of the Congo. Int J Infect Dis 2017;57:32–7, doi:http://dx.doi.org/10.1016/j.ijid.2017.01.016.

Moureau G, Temmam S, Gonzalez JP, Charrel RN, Grard G, de Lamballerie X. A real-time RT-PCR method for the universal detection and identification of flaviviruses. Vector Borne Zoonotic Dis 2007;7(4):467–77, doi:http://dx.doi.org/10.1089/vbz.2007.0206.

Parreira R, Conceição C, Centeno-Lima S, Marques N, da Cunha JS, Abreu C, et al. Angola’s 2013 dengue outbreak: clinical, laboratory and molecular analyses of cases from four Portuguese institutions. J Infect Dev Ctries 2014;8(9):1210–5, doi:http://dx.doi.org/10.3855/jidc.4910.

Santiago GA, Vergne E, Quiles Y, Cosme J, Vazquez J, Medina JF, et al. Analytical and clinical performance of the CDC real time RT-PCR assay for detection and typing of dengue virus. PLoS Negl Trop Dis 2013;7(7):e2311, doi:http://dx.doi.org/10.1371/journal.pntd.0002311.

Vairo F, Mammone A, Lanini S, Nicastri E, Castilletti C, Carletti F, et al. Local transmission of chikungunya in Rome and the Lazio region, Italy. PLoS One 2018;13(12)e0208896, doi:http://dx.doi.org/10.1371/journal.pone.0208896.

Willcox AC, Collins MH, Jadi R, Keeler C, Parr JB, Mumba D, et al. Seroepidemiology of dengue, zika, and yellow fever viruses among children in the Democratic Republic of the Congo. Am J Trop Med Hyg. 2018;99(3):756–63, doi:http://dx.doi.org/10.4269/ajtmh.18-0156.

World Health Organization. Dengue and severe dengue. Fact sheet. April 15th, 2019. 2019. https://www.who.int/en/news-room/fact-sheets/detail/dengue-and-severe-dengue.

Yamamoto SP, Kasamatsu Y, Kanbayashi D, Kaida A, Shirano M, Kubo H, et al. Dengue virus in traveler returning to Japan from the Democratic Republic of the Congo, 2015. Jpn J Infect Dis 2019;, doi:http://dx.doi.org/10.7883/yoken.JJID.2018.554.