A secondary dengue 4 infection in a traveler returning from Haiti confirmed by virus isolation, complete genome sequencing and neutralisation assay: A brief report

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Summary Here we report the clinical and laboratory findings of a dengue 4 virus (DENV) secondary infection in a patient returning from Haiti to France. The diagnostic of acute DEN-4 virus infection was demonstrated by (i) the presence of DEN-4 RNA in two successive serum samples, (ii) the isolation of a DEN-4 virus in Vero cells and subsequent identification of subtype IIb through complete genome sequencing, (iii) the presence of dengue NS1 antigen, (iv) the seroconversion with detection of dengue IgM in the second serum while negative in the first serum. The diagnosis of secondary dengue episode was demonstrated by (i) the presence of dengue IgG in the early serum, and (ii) the demonstration that neutralising antibodies against DEN-3 were present at the acute stage of the disease. Next-generation sequencing has a primary role to play in phylogeographic studies including database sequences, sequences from imported cases, and sequences from autochthonous cases.

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1. Introduction

Dengue fever is endemic in most tropical parts of the world, of which many are popular tourist destinations [1]. Dengue virus infection has been reported in more than 100 countries, with 2.5 billion people living in areas where dengue is endemic [2,3]. Annual global incidence of dengue infection is 50–100 millions of dengue fever and 250,000 cases of dengue hemorrhagic fever and a mortality rate of 25,000 per year [4,5]. Dengue viruses are arthropod-borne viruses transmitted by Aedes sp. mosquitoes. There are four antigenically distinct serotypes (DEN-1 to DEN-4), which are genetically distinct although their epidemiology is nearly identical. Infection with one serotype confers long-term immunity only to that serotype, and therefore persons may be infected up to four times during their life [4,6]. The proportionate morbidity associated with dengue is especially high among travelers returning from Southeast Asia and the Caribbean [7]. Imported cases in returning travelers are important to report as they serve as sentinels for local outbreaks of dengue fever in tropical areas to which it is endemic [8,9]. Although there are many published cases of imported dengue infections, DEN-4 cases are rarely reported and even more rarely DEN-4 secondary infection with identification of the serotype of primary infection.

Here we report the clinical and laboratory findings of a case of secondary DEN-4 infection acquired in Haiti in late summer 2014 and imported into France.

2. Case report

A 64 year-old diabetic woman was hospitalized in September 2014 for a febrile syndrome after returning from Haiti, her native country where she stays each summer for visiting relatives and friends living in Port-au-Prince and Ganthier districts. The onset that occurred on the day of return to France was characterized by high grade fever (40 °C), nausea without vomiting, and non productive cough. Two days later, she was hospitalized for extreme fatigue, arthralgia and headache. A maculopapular rash was neither seen during physical examination, nor noticed by her. She reported massive mosquito bites in Ganthier. She could not recall previous dengue fever episode.

Laboratory tests performed on blood samples collected at the day of hospital admission showed microcytic normochromic anemia (hemoglobin 74 g/L, Hematocrite 24%) for which she received blood transfusion. Platelet and leukocyte counts were 307 G/L and 7.82 G/L, respectively. Biochemistry tests showed moderate hyponatremia (133 mmol/L) and hypokaliemia (2.4 mmol/L), C-reactive protein (194 mg/L), urea (3.94 mmol/L) and creatinine (54 μmol/L). Transaminases (SGOT at 55 UI/L and SGPT 60 UI/L) were slightly elevated. Upon admission, chikungunya virus-specific immunoglobulin (Ig) G and IgM were not detected by indirect immunofluorescence test [10]. Dengue NS1 antigen and dengue IgG were positive, whereas dengue IgM was negative using a lateral-flow diagnostic assay (SD BIOLINE Dengue Duo NS1 Ag + Ab Combo, ALERE, France). DEN-4 virus RNA was detected (Cycle threshold [Ct] at 32) using four serotype-specific in-house real-time reverse transcription polymerase chain reaction (rt-RT-PCR) while DEN-1, DEN-2 and DEN-3 tests were negative, and rt-RT-PCR for chikungunya was also negative [11]. The serum sample was inoculated onto Vero cells and despite cytopathic effect was not observed after 5 days, virus replication was demonstrated based on positive rt-RT-PCR for DEN-4 (Ct < 20). Supernatant was used for complete genome sequencing using next-generation sequencing on Ion-Torrent Personal genome machine (Life Technologies) and was attributed Genbank accession number KP140942. The complete sequence was aligned with homologous DEN-4 sequences retrieved from the Genbank database and used for phylogenetic analysis (Fig. 1). This patient was infected with a DEN-4 strain belonging to the genotype Ib, which is most closely related to human strains isolated in 1994 in Puerto Rico (98% identity at the nucleotide level) [12].

Five days later, a second serum was collected and showed (i) that Dengue IgM and IgG were positive whereas NS1 antigen was negative using the Dengue Duo NS1 test, (ii) and that DEN-4 virus RNA was still detected (Cycle threshold at 37) by rt-RT-PCR.

In the early serum, the concomitant finding of DEN-4 RNA and IgG against dengue virus suggests that this patient had contracted another dengue virus infection before this episode. To identify the serotype of the previous dengue infection, the affinity of IgG antibodies detected in the acute stage serum was assayed by PRNT50-neutralisation assay using independently each of the 4 dengue serotypes. The acute serum of this patient was capable to neutralize DEN-3 virus at 1:160 titer, thus demonstrating that the primary dengue infection has been cause by a DEN-3 virus.

The fever and cough resolved without complications and after one week in infectious disease unit care. The patient was transferred in the digestive surgery department for management of a primary malignant tumor of the small intestine that was discovered during the check-up done for anemia.

3. Discussion

Dengue is endemic in most tropical and subtropical countries, many of which are popular tourist destinations [13]. International travelers may both acquire and spread dengue virus infection. The 1980s and 1990s witnessed a dramatic geographic expansion of epidemic dengue fever and dengue hemorrhagic fever from Southeast Asia to the South Pacific Islands, the Caribbean, and Latin America, with regions changing from nonendemic (no serotypes present) to hyperendemic (multiple serotypes present) [14]. All four serotypes have now been reported in the Caribbean where most outbreaks have consisted of a mixture of DEN serotypes [15]. DEN-4 was introduced in the Americas in 1981 and has spread to most countries of the region within 3 years [5]. DEN-4 is known to be present in Haiti for at least 3 decades and accounted for a significant proportion of past infection, all caused by subtype Ib [12,16]. Two studies conducted in travelers respectively returning to Japan and to Europe [17,18] showed that DEN-4 imported cases are less frequent than those due to other serotypes. There are 8 dengue sequences from Haiti in Genbank (DEN-1 [n = 6], DEN-2 [n = 1], DEN-4 [n = 1]) isolated in 1994.
In this study, the diagnostic of acute DEN-4 virus infection was demonstrated by (i) the presence of DEN-4 RNA in two successive serum samples, (ii) the isolation of a DEN-4 virus in Vero cells and subsequent identification of subtype IIb through complete genome sequencing (strain MRS616942904/2014, acc no KP140942), (iii) the presence of dengue NS1 antigen, (iv) the seroconversion with detection of dengue IgM in the second serum while negative in the first serum.

The diagnosis of secondary dengue episode was demonstrated by (i) the presence of dengue IgG in the early serum, and (ii) the demonstration that neutralising antibodies against DEN-3 were present at the acute stage of the disease.

Since 2006, in response to Ae. albopictus establishment in southern France, the risk of dissemination of dengue and chikungunya viruses in mainland France is surveyed and prevented by the French authorities based on a plan that combines entomological, epidemiological and virological surveillance from May to November [19,20]. In 2014, a total of 1434 suspect cases have been investigated, with confirmation of 160 cases of dengue. In October 2014, four autochthonous DEN cases were identified (2 unrelated DEN-1 and 2 DEN-2), of which the two DEN-2 cases were epidemiologically related. Accordingly, NGS-based complete sequencing appears as an efficient tool to trace imported cases of dengue in returning travelers and to document phylogeographic links between local and imported strains, but also between imported strains and autochthonous strains [21]. During 2014, of the 160 confirmed dengue cases, 45 were due to DEN-4 virus; all of these DEN-4 were observed in patients returning from the Caribbean area [22], Leparc-Goffart personal data. The DEN-4 case presented in this study exemplifies that such sequence-based surveillance system is to be developed and implemented to provide the opportunity for tracing imported cases referred to reference centers and to help for the sourcing of autochthonous cases [21].

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Conflict of interest

None.

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