Short communication

Imported dengue from 2013 Angola outbreak: Not just serotype 1 was detected

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ABSTRACT

Background: All the reports from Angola’s 2013 dengue outbreak revealed serotype 1. However, previously dengue serotypes 1–4 have been reported in Africa and in 2014 serotype 4 was reported in Angola.

Objectives: To report dengue serotypes in patients returning from Angola during 2013 outbreak.

Study design: Retrospective, cross-sectional study. We serotyped the dengue by an in house Polymerase Chain Reaction technique in randomly selected cases.

Results: From the 2013 Angola’s dengue outbreak we treated 47 adult patients. None had history of past dengue. A combo kit test for dengue revealed positive NS1 antigen in 39 and IgM antibodies in 8. From 17 randomly patients tested by RNA Real Time-PCR, 11 were positive: 7 for DENV-1, 2 for DENV-2, 1 for DENV-3 (co-infected with DENV-1) and 1 for DENV-4. None had a complicated or fatal evolution.

Conclusion: Unlike previous reports the 4 serotypes were detected, and this resulted in a different epidemiological situation, raising the risk of future outbreaks of severe dengue.

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1. Background

Serosurveys in Africa document the presence of dengue since 1926 [1]. In Africa the surveillance data are poor and its knowledge is not accurate [2]. Despite this, there are reports from serotypes 1, 2, 3 and, more rarely, serotype 4 in several African countries [3–6].

Angola’s dengue cases have been described in 1986 and 1999–2002 [7], but no dengue outbreak was reported until 2013. All isolates from this outbreak have been consistently reported as serotype 1 [8–10]. However, in 2014 Parreira et al. published a case report of a co-infection of serotype 4 dengue and Chikungunya virus imported from Angola [11].

2. Objectives

To report clinical and laboratory data concerning imported dengue in 2013 in patients returning from Angola.

3. Methods

We made a hospital-based, retrospective and cross-sectional study, analysing all the clinical and laboratory records of adults returning from Angola and diagnosed with dengue. We used an in-vitro rapid immunochromatographic assay, SD BIOLINE Dengue Duo® (Dengue NS1 Ag+IgG/IgM) kit following the manufacture protocol. From some randomly selected patients the plasma was frozen (−20 °C). Later we tested for Dengue virus RNA and serotyped. We used an in-house one step Reverse Transcription Real Time Polymerase Chain Reaction (RT-PCR) test with primers and probes described by Laue et al. [12]. All samples were tested separately with each of the four primers/probe sets. One step RT-PCR Kit (Qiagen®) with Q-solution was used with primers and probes described with a final 50 μl reaction with 10 μl of RNA extracted plasma sample (EZ1 RNA virus mini-kit, Qiagen®) or water for negative control. We used a real-time thermocycler...
(Rotor gene 3000, Corbett Research®) and after a 30-min reverse transcription step at 50 °C and 15 min of Taq activation, cDNA was amplified using a two-step protocol with the initial 10 cycles with 56 °C for annealing temperature and 50 °C with fluorescence acquisition during the next 35 cycles. The test performance was verified with participation in Quality Control for Molecular Diagnostic (QCMD) 2014 Dengue virus RNA External Quality Assessment (EQA) program (all 4 serotypes were tested in the external control and the results were 100% concordant). We used negative controls for each sample. All patients were screened for Plasmodium spp. – blood smear and a rapid test (BinaxNOW® Malaria) – and for HIV infection – commercial fourth generation test (Architect® HIV Ag/Ab Combo, Abbott), hepatitis (A, B and C – commercial tests) and typhoid fever (blood cultures).

4. Results

From the 47 patients with dengue, 45 were non-Angolan and 20 were expatriate in Angola. No patient had previously reported clinical dengue fever. They all lived or stayed for some days in Luanda, before travelling to Portugal. At diagnosis fever was reported in all patients, myalgia in 40, headache in 37, asthenia in 31, retro-orbital pain in 17 and arthralgia in 15. Gum bleeding and metrorrhagia was elicited in 2 patients.

4.1. Laboratory data

Diagnosis was based on NS1 antigenaemia and/or Dengue IgM positivity (Table 1). A dengue virus RT-PCR in-house was performed in 17 patients (Table 2). All four dengue serotypes were detected: 7 for DENV-1, 2 for DENV-2, 1 for DENV-3 (co-infected with DENV-1) and 1 for DENV-4 (Table 2). Amongst the six patients with RT-PCR in-house negative test, four were NS1 antigen positive.

4.2. Co-infections

In two patients, malaria and dengue were simultaneously diagnosed. The blood smear was positive for Plasmodium (parasitaemia <1%) and antigen test revealed P. falciparum. One man expatriated in Luanda was under treatment for HIV infection, having an undetectable viral load and CD4 lymphocytes count of 600 cells/mm³. Evolution was unremarkable in these three patients.

4.3. Outcome

Clinical outcome was good for all the 47 patients.

Prolonged asthenia was reported in 12 patients, headache, insomnia and itching in 2 each, transitorily arthralgia and dysesthesia in one each.

5. Discussion

This study addresses for the first time the circulation of the four dengue serotypes in the 2013 Angola’s outbreak. The outbreak was linked to serotype 1 [8]. In Africa very few studies explore the serotypes identification, evolutionary history and dynamic of the disease and there is not any report of these serotypes co-circulating [13]. The detection of the four serotypes in this outbreak may translate a large dissemination of dengue virus.

We screened all the samples with the SD BIOLINE Dengue Duo® kit following the manufacture protocol and the diagnosis was based on NS1 antigenaemia and/or Dengue IgM positivity. We also performed a dengue virus RT-PCR in 17 patients. We tested only 17 samples because we did not have samples from all the patients, as this was a retrospective study. The RT-PCR was previously tested and verified by an external Quality Assessment Program (Quality Control for Molecular Diagnostic (QCMD) 2014 Dengue virus RNA). This program is accredited to ILAC-G13:08/2007/ISO/IEC Guide 43-96 01:1997 (ISO17043:2010) and is also ISO9001:2008 certified. For all of these reasons we believed that the results that we found in this cohort are real. Although we did not have the opportunity to appreciate the real dimension of these results because we did not have access to more samples from this outbreak. Four of the seventeen positive patient’s sample for NS1 antigen had negative serotype determination. This can be easily understood when we look at the kinetics of the antigenaemia and viremia [2]. Six
Portuguese patients were antigen NS1 and Ig M plus IgG positive; 3 of them were living in Angola for more than 2 years, although none of them has past clinical history of dengue.

At that time (2013) the Chikungunya and Zika virus tests were not available in our hospital, so none of our patients was tested for these viruses. Besides, co-infection with Chikungunya in Angola was reported only in the following year [11]. In 2013, neither we knew Zika virus widespread distribution [14] nor its clinical implications [15,16] and no case has yet been reported in Angola. Because of this facts, we were not so aware of possible co-infection with dengue or Zika virus infection at that time. Apart from this, clinically the symptoms suggested dengue fever, as patients had no conjunctivitis or relevant arthralgia.

The relevance of urban growth, travel, migration, and commercial trade in the spread of dengue and in the introduction of new dengue serotypes cannot be missed. Portugal may be a gate for dengue dissemination in Southern Europe, considering the close relationship with several sub-Saharan African countries and the rapid spreading and establishing of the vector *A. albopictus*, already presented in Spain [17]. Co-circulation of all dengue serotypes may predispose infected individuals to Dengue hemorrhagic fever in infection by a different serotype. Studies that address the issue of concomitant circulation of multiple dengue virus serotypes in this outbreak are welcome. Closely monitoring the ongoing Dengue cases and spread of the virus type in Africa is essential to understand the dynamic and the global burden of disease.

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References


