

Emerging arbovirolosis in Italy: report on 8 cases of imported Dengue virus infection in Sardinia

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ABSTRACT:

- **Introduction:** Dengue fever is the most common viral disease transmitted by arthropods. It is endemic in Puerto Rico, Latin America, Southeast Asia and the Pacific islands; several cases occurred also in Africa and the Eastern Mediterranean. The spread in many popular tourist destinations and the recent increase in migrants from these areas towards Italy may lead to an increase of cases. The aim of this work is to investigate the clinical presentation, laboratory characteristics and diagnostic procedures of Dengue fever.
- **Patients and Methods:** We consecutively enrolled 8 patients admitted to the Infectious Disease Unit of the University of Sassari. Clinical and laboratory results were collected from clinical records. Diagnostic test for Dengue infections included serology (ELISA) and PCR on blood samples.
- **Results:** The mean age of the patients, all males, was 28 ± 5 years. Six patients were from Nigeria, one from Mali and one from Italy. Seven out of 8 patients have transited from Libya before arriving in Italy whereas one was an Italian traveller returning from Thailand. The most frequent clinical presentations included fever, headache and arthromyalgia. No cases of rash were reported. Hemorrhagic manifestations were rare and mostly represented by gross haematuria. The most frequent laboratory test alterations were an increase of aminotransferase and creatine phosphokinase levels and leukopenia. Only one patient developed thrombocytopenia. The clinical suspicion was confirmed by serology in all cases. PCR on blood for Dengue virus was negative.
- **Conclusions:** The clinical suspicion of Dengue fever should be supported by epidemiological criteria and serology results in order to correctly diagnose the infection in its early stage. Given the recent increase in the incidence in the last decades, the geographical expansion of Dengue fever and the flow of migrants from Africa to Italy, surveillance systems based on laboratory tests are becoming crucial.
- **Keywords:** Arbovirolosis, Dengue virus, Italy, Sardinia.

INTRODUCTION

Dengue fever is the most important mosquito-borne viral infection¹, caused by *Dengue virus* (DENV). The infection could be caused by 4 different serotypes (DENV-1, DENV-2, DENV-3, DENV-4).

After being bitten by a mosquito carrying the dengue virus, the incubation period ranges from 3 to 14 (usually 4 to 7) days before the signs and symptoms of dengue appear. The different clinical forms may vary from a flu-like infection to a hemorrhagic syndrome.

An estimated 390 million cases occur annually, 96 million of whom are associated with clinical disease². Dengue fever is endemic in Puerto Rico, Latin America, Southeast Asia and the Pacific islands; several cases have occurred also in Africa and the Eastern Mediterranean. Over 50 million infections each year, the mortality rate is over 5% in some countries³⁻⁶.

The principal mosquito vectors of dengue virus are *Aedes aegypti* and *Aedes albopictus* (*A. albopictus*), insects closely associated with humans and their dwellings⁷. The second one is ubiquitously diffused in Italy.

Dengue virus is spread in many popular tourist destinations, and the recent increase in immigration from these areas towards Italy allowed the discovery of this imported disease also in our hospital wards⁸.

PATIENTS AND METHODS

Overall, 8 patients admitted with Dengue fever to our Infectious Diseases Unit were consecutively enrolled from June to August 2015. They were referred by Internal Medicine Departments, where they had been admitted for fever of unknown origin.

For each patient, serology with ELISA was performed at entry and, in some cases, during follow-up visits, at 15 days.

Specific antibodies for *Dengue virus* were determined using commercially available ELISA diagnostic tests (Dengue ELISA IgG/IgM, Vircell Microbiologist, Granada, Spain).

Furthermore, serological tests on the most frequent viral infections in our geographical area were carried out, in order to rule out the diseases contracted after the immigration, such as Influenza virus, rhinovirus and adenovirus.

ELISA test for *Mycoplasma* and *Chlamydia pneumoniae* were also performed in patients with more important respiratory tract symptoms.

RESULTS

We enrolled 8 patients, all males, with a median age of 28 ± 5 years. Six patients were from Nigeria, one from Mali, one from Italy. Seven out of 8 transited from Libya before arriving in Italy, whereas one was an Italian tourist returning from Thailand.

Fever, headache, and arthromyalgia were present in 7/8 (87.5%) of cases, rhabdomyolysis was present in 6/8 (75.0%) of the cases, weakness and bleeding were present in 4/8 (50.0%) and 3/8 (37.0%) of the cases respectively. All cases of bleeding occurred with haematuria. Abdominal pain and serositis were present with a percentage of 12.5% of the cases (1/8), while nobody developed a skin rash.

Laboratory data showed that transaminase increase was present in 7/8 (87.5%) and creatine phosphokinase (CPK) increase was present in 6/8 (75.0%) of the cases of Dengue fever. Leukopenia and thrombocytopenia were present in 5/8 (62.0%) and 3/8 (37.5%) of the cases, respectively, anemia and renal failure affected 1/8 (12.5%) patients. Clinical features and laboratory test results are showed in Table 1.

Overall, the duration of hospitalization was on average 9 ± 2.9 days, fever lasted on average for 2 ± 1.6 days, only considering the length of hospitalization in our unit and it was 5-8 days when considering the onset of fever before admission.

Diagnostic tests confirmed the clinical suspect of Dengue fever 100% of cases (8/8) with ELISA test. Three cases had IgM antibodies at the first test, and five showed IgG seroconversions. Serology tests of the 8 patients are summarized in Table 2.

Table 1. Clinical features and laboratories abnormalities in 8 patients with Dengue fever.

Variable	Number/total (%) - Median \pm SD
Fever	7/8 (87.5%)
Headache	7/8 (87.5%)
Arthromyalgia	7/8 (87.5%)
Fatigue	4/8 (50%)
Bleeding	3/8 (37%)
Abdominal pain	1/8 (12.5%)
Pleural effusion	1/8 (12.5%)
WBC (cells/mL)	4486 \pm 870
Hb (g/dL)	13.9 \pm 1.3
PLT (cells/mL)	156837 \pm 59413
AST (IU/L)	343.4 \pm 304
ALT (IU/L)	131.9 \pm 72.9
Mioglobine	6960 \pm 11515
CPK (IU/l)	7948 \pm 7707

SD: standard deviation; WBC: White Blood Cells; Hb: Hemoglobin; PLT: Platelets; AST: aspartate aminotransferase; ALT: alanine aminotransferase; CPK: creatine phosphokinase; IU: international units.

DISCUSSION

Fever, headache, arthromyalgia and rhabdomyolysis were the most frequent clinical features in patients with Dengue fever, whereas only three patients had bleeding, abdominal pain and pleural effusion were rarely observed. None of the reported cases developed a rash.

The increase of aminotransferase levels, leukopenia and CPK were the most represented laboratory abnormalities.

None of the patients showed a significant reduction of hemoglobin and hematocrit, also in the case of bleeding. Thrombocytopenia was a rare finding.

The ELISA method has been concordant with the clinical suspicion in all of the cases.

We did not perform entomologic studies. However, *A. albopictus*, which is considered the second most important vector of DENV, is widely distributed in Italy as well as in Sardinia.

A. albopictus has spread widely in Europe over the last twenty years, especially in the Mediterranean region^{9,10}, with potential risk of local circulation of many arboviruses such as DENV, chikungunya (CHIKV) and Zika virus¹¹⁻¹³. Furthermore, *A. albopictus* diffusion could represent a favouring factor for the potential spread of tropical diseases through the infection of international travellers.

A study conducted from 2008 to 2011 highlighted the diffusion of mosquito-borne infections, reporting a total of 130 cases of DENV/CHIKV in travellers returning to Italy. In addition, prediction models showed a risk for Chikungunya between 4.9% and 25% and Dengue between 4.2% and 10.8% for cases imported in Italy, as well as other temperate regions¹⁴.

Similar trends have been confirmed by various outbreaks described in Europe, such as in Croatia, Portugal, and France¹⁵⁻¹⁷.

This is the reason why in Italy infections by arboviruses are included in the mandatory medical surveil-

Table 2. ELISA serology test in 8 patients with Dengue fever.

Patient	DENV IgM T0 (AU)	DENV IgG T0 (AU)	DENV IgM T1 (AU)	DENV IgG T1 (AU)
Patient 1	Positive (44)	Indeterminate	–	–
Patient 2	Positive (26)	Indeterminate	–	–
Patient 3	Positive (26)	Negative	–	–
Patient 4	Indeterminate	Negative	Negative	Positive (46)
Patient 5	Negative	Negative	Negative	Positive (32)
Patient 6	Negative	Negative	Negative	Positive (31)
Patient 7	Negative	Negative	Negative	Positive (25)
Patient 8	Negative	Negative	Negative	Positive (26)

T0: serology performed at the admission; T1: serology performed after 15 days; AU: arbitrary units. Elisa cut-off: < 9 AU= negative; >11 AU= positive; Indeterminate = 11 AU.

lance system, which showed an increasing number of Dengue cases from 79 in 2012 to 142 in 2014¹⁸.

However, only a few data are available on the real circulation of DENV in Italy, probably due to the non-specificity of symptoms in most cases. Our study also confirms previous reports showing that clinical features could be frequently flu-like thus misleading the diagnosis.

The same applies for other viruses, such as the West Nile (WNV). In a study conducted in Veneto, Italy, between 2010 and 2012, the screening for WNV of autochthonous population showed a seroprevalence of 5.9%, further highlighting the importance of human and entomological surveillance¹⁹.

CONCLUSIONS

Given the geographic expansion of viruses previously detected only in tropical regions and the migratory flow from Africa to Italy, the surveillance systems based on laboratory tests are becoming increasingly important. It is also essential for a correct diagnosis to confirm the clinical suspicion of Dengue Fever with epidemiological and laboratory criteria.

In consideration of the presence in Sardinia of *A. albopictus*, a vector of Dengue virus, the need for a strict epidemiological surveillance to detect potential indigenous cases is a priority.

CONFLICT OF INTERESTS:

The Authors declare that they have no conflict of interests.

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