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## Laboratory and surveillance studies following a suspected Dengue case in Greece, 2012



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### ABSTRACT

**Objectives:** To describe the laboratory assays used to investigate a suspected dengue case in summer 2012 in western Greece and to report the public health response.

**Design of methods:** Samples from the patient were tested for detection of Dengue virus (DENV) antibodies, NS1 antigen and RNA. Public health professionals interviewed residents of the village, and blood samples taken from 132 persons were tested for antibodies for DENV and West Nile virus (WNV). Samples from 10 persons who reported symptoms in the prior 15 days (including 3 persons who had fever at the time of sampling) were tested for DENV, WNV and additional arboviruses. Entomological missions were organized in the area of potential exposure to investigate the presence of competent DENV vectors.

**Results:** Based on a combination of serological and molecular methods, DENV infection was excluded, and the problems in the serology, especially in the DENV NS1 antigen, were attributed to interfering factors. A 6.1% WNV seroprevalence was detected in the region, and phlebovirus IgM and IgG antibodies were detected in two of three persons who had fever at the time of sampling. *Aedes albopictus* adult mosquitoes were present in the region.

**Conclusions:** A multi-disciplinary field and laboratory investigation showed no evidence of DENV infection. There is a need for industries to improve the immunometric assays to avoid interference with rheumatoid or other factors, and increased awareness is needed for the evaluation of the diagnostic assays. The high WNV seroprevalence in the investigated region highlights the need for strengthening awareness on vector borne diseases. The presence of *Ae. albopictus* suggests that the possibility of introduction of DENV exists, and preparedness plans are needed.

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## 1. Introduction

Dengue virus (DENV) is a flavivirus (family *Flaviviridae*) transmitted to humans by bites from infected *Aedes* mosquitoes,

mainly *Aedes aegypti*, whereas *Aedes albopictus* is considered a competent although secondary DENV vector. An estimated 50 to 100 million DENV infections occur annually in 125 countries, and DENV infection is considered a major public health threat by the World Health Organization (WHO), as Dengue fever global incidence has increased 30-fold in the last 50 years.<sup>1</sup> The main symptoms of Dengue fever include fever, myalgia, arthralgia, and rash, whereas severe cases lead to Dengue hemorrhagic fever or even Dengue shock syndrome. The disease is endemic in tropical

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and subtropical regions, and several imported cases are being reported annually in travelers returning from these regions. The last large epidemic in Europe was observed during 1927–28 in Greece (>80% of the population in the Athens area was affected), with more than 1,000 reported deaths.<sup>2</sup> The possibility of re-introduction of DENV to Europe exists, in view of the increasing global travel rates and the wide distribution of its secondary vector, *Ae. albopictus*, in more than 16 European countries,<sup>3</sup> also present in the wider region of West Greece since 2003.<sup>3</sup> Autochthonous cases of Dengue fever have been reported in 2010 in South France<sup>4,5</sup> and Croatia,<sup>6</sup> while a large outbreak was detected in 2012 in Madeira, Portugal,<sup>7</sup> suggesting that DENV is an emerging public health threat for Europe.

Another flavivirus, West Nile virus (WNV), transmitted to humans mainly by bites of infected *Culex* spp. mosquitoes, is widely distributed in the world, causing sporadic cases and outbreaks. Since 2010, when the virus emerged in Greece,<sup>8</sup> up to 2013, 609 human cases (432 of them accompanied by neurological symptoms) have been observed.<sup>9</sup> Cross-reactivity is often seen among flaviviruses, especially among those belonging to the same serocomplex.<sup>10</sup> In the present study we report the laboratory and field investigation of a suspected autochthonous Dengue case observed in Greece in 2012.

## 2. Case report

In late August 2012, an 84-year old male resident of a village in the municipality of Agrinio, western Greece, presented to a local private clinic with fever, fatigue, neck pain and weakness of his lower limbs. Laboratory tests showed elevated bilirubin, thrombocytopenia, and leukocytosis. The patient was transferred to a tertiary care center. His condition deteriorated rapidly, and he died on the 10<sup>th</sup> day of hospitalization due to *Staphylococcus aureus* septicemia despite adequate antimicrobial treatment and supportive care. During his hospitalization he developed epistaxis (not thrombocytopenic at the time) that was attributed to a medical history of Rendu-Weber-Osler syndrome. The patient had not received blood transfusions or other blood products during the incubation period, and he did not report any travel beyond the prefecture of his residence. His past medical history included atrial fibrillation, for which he was treated with an oral anti-coagulant.

## 3. Laboratory testing

Since an outbreak of WNV infection was ongoing at the time in Greece in August 2012, blood and cerebrospinal fluid (CSF) samples drawn on the 8<sup>th</sup> day of illness were sent to the National Reference Laboratory for Arboviruses to be tested for probable WNV infection. Samples were tested for presence of WNV IgM and IgG antibodies using ELISA (WNV IgM capture DxSelect and WNV IgG DxSelect, Focus Diagnostics, Cypress, CA). No WNV IgM and IgG antibodies were detected in the CSF; a borderline WNV IgM antibody index (1.6 with cut-off 1.5) was seen in the patient's serum sample, which became negative after subtraction of the background).

Due to the presence of epistaxis and thrombocytopenia in the patient, samples were additionally tested for DENV IgM and IgG antibodies using ELISA (DENV IgM capture DxSelect and DENV IgG DxSelect, Focus Diagnostics, Cypress, CA). No DENV IgM and IgG antibodies were detected in the CSF; results also were negative for DENV IgG antibodies in the serum and CSF. A high positive DENV IgM index (out of range) was seen in the patient's serum, which resulted in a negative after subtracting the background.

Blood and CSF samples were also tested for DENV NS1 antigen (Platelia Dengue NS1 Ag-ELISA (Biorad Laboratories, Marnes-La-Coquette, France); CSF was negative, but a positive result was obtained in the serum sample (3.98, cut-off 1). An NS1 kit was kindly provided by Focus Diagnostics, and serum and CSF samples results were negative. The large difference between indices before and after subtraction of the background in the DENV IgM ELISA prompted us to repeat the Biorad NS1 antigen assay after treatment of the patient's serum with rheumatoid factor (Rf)-absorbent (although it is not written in the instructions for use) to investigate any interference with IgM- Rfs, and the result was negative.

Two RT-nested PCRs, one using generic flavivirus primers<sup>11</sup> and a second using DENV-specific primers,<sup>12</sup> as well as a DENV-specific real time RT-PCR (reagents kindly provided by Ana Vazquez and Antonio Tenorio, Spain), were applied on serum and CSF samples and had negative results. Based on all of the above results, the case was declared DENV-negative.

Serum samples from another WNV-suspected case tested in the Department of Microbiology in the University of Athens also gave borderline WNV IgM and a high DENV IgM antibody index; in that case the DENV infection was easily excluded, since the DENV IgM was negative after the background subtraction and the NS1 antigen was negative. Five serum samples from additional WNV-suspected cases with borderline WNV IgM antibodies were negative after subtraction of the background, and when tested for DENV IgM and IgG antibodies and NS1 antigen were all negative. [Table 1](#)

## 4. Public health response

Immediately after the first suspicion of DENV infection, and before finalization of the laboratory testing, an active case-finding mission was organized by the Hellenic Centre for Disease Control and Prevention (HCDCP) in the village in western Greece where the patient lived. Public health (PH) professionals from the HCDCP and the University of Thessaly, in cooperation with regional PH staff, visited the area and offered a physical exam for all residents of the village; the participants were interviewed through a structured questionnaire aiming to identify persons with possible DENV infection. A total of 132 persons [57 (43.18%) male] were examined, focusing on an area up to 200-m radius from the case's residence, also including the patient's relatives. Their median age was 57 years (range 7–85 years). Ten persons reported fever, headache, arthralgia, myalgia, nausea, vomiting or upper respiratory infection in the last 15 days (3 at the time of examination).

**Table 1**

Initial laboratory testing results of the suspected Dengue case, Greece, August 2012. CSF: cerebrospinal fluid; neg: negative.

Sample	WNV IgM before/after subtraction cut off: 1.5	WNV IgG	DENV IgM cut off: 1.0	DENV IgG	NS1 antigen cut off: 1.0	PCR for flaviviruses and DENV-specific
	Focus Diagnostics				BioRad	
Serum	1.6/neg	neg	Out of range/neg	neg	3.98	neg
CSF	neg	neg	neg	neg	neg	neg

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