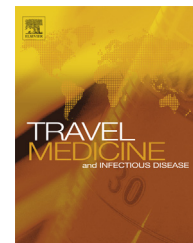


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Molecular epidemiology of dengue fever cases imported into Romania between 2008 and 2013

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Summary *Background:* Dengue fever is the commonest arthropod-borne infection worldwide. In recent years, rapid growth in global air travel has resulted in a considerable increase in the incidence of imported cases. In Romania it is now the second most frequent cause for hospitalization (after malaria) in patients arriving from tropical regions.

Methods: Serological and molecular diagnostics were applied to samples obtained between 2008 and 2013 from travelers with suspected dengue. Molecular typing was performed by RT-PCR followed by sequencing of the E-NS1 junction.

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Results: Twelve of 37 suspected cases were confirmed and three remained probable. The infections were acquired in endemic regions in Asia, Africa and in Europe (Madeira Island). Dengue virus nucleic acid was detected and sequenced in nine cases. Phylogenetic analysis indicated that the viruses were of genotypes I and V of serotype 1, cosmopolitan genotype of serotype 2 and genotypes I and III of serotype 3.

Conclusions: Romanian tourists traveling to dengue-endemic countries are at risk of acquiring dengue infection. Appropriate prevention measures prior to travel and upon return should be taken, particularly as the dengue secondary vector *Aedes albopictus* is now established in Bucharest.

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1. Introduction

Dengue fever is the most widely distributed arthropod-borne infection worldwide. According to World Health Organization 2.5 billion humans living in tropical and subtropical regions are at risk of acquiring dengue infection [1], with an estimated 390 million cases per year [2]. The dengue viruses (DENVs) comprise a distinct serocomplex of the family *Flaviviridae* in the genus *Flavivirus* [3,4]. There are four divergent serotypes (DENV-1 to DENV-4), based on genetic and antigenic properties. These display 63–68% amino acid sequence homology [5,6]. In addition, a new viral serotype (DENV-5) has been identified in a human outbreak in Malaysia in 2007; there is evidence that this circulates among macaques [7]. Serotypes 1–4 are further classified into genotypes according to nucleotide divergence of up to 6% in the envelope glycoprotein coding region. DENV-1 comprises five genotypes (I–V) with a broad distribution: Southeast Asia, China, East Africa (genotype I); Thailand (genotype II); Malaysia (genotype III); West Pacific islands and Australia (genotype IV); America, West Africa and Asia (genotype V) [8]. Six genotypes have been proposed for DENV-2: Asian I (Southeast Asia and East Asia); Asian II (Southeast Asia, East Asia, Oceania, Caribbean region and the Americas); Asian/American (Southeast Asia, Caribbean region and Americas); a cosmopolitan genotype (India, South-Central Asia, East Asia, East Africa, Middle East, Southeast Asia and Oceania); an American genotype (India, Caribbean region, South America, Central America, Oceania and Trinidad) and sylvatic genotype (West Africa and Malaysia) [9]. DENV-3 isolates are assigned to four genotypes: genotype I (Indonesia, Malaysia, Philippines and South Pacific islands), genotype II (Thailand, Vietnam and Bangladesh), genotype III (Sri Lanka, India, Africa and Samoa), and genotype IV (Puerto Rico, Latin and Central America and Tahiti) [10]. Phylogenetic analysis indicates the existence of four genotypes within the DENV-4 serotype: genotype I (Thailand, the Philippines, Sri Lanka and Japan), genotype II (Indonesia, Malaysia, Tahiti, the Caribbean and the Americas), genotype III (Thailand) and genotype IV (sylvatic strains from Malaysia) [10].

The virus is transmitted by mosquitoes of the genus *Aedes*, primarily *Aedes aegypti* and *Ae. albopictus*, and is maintained in urban cycles in humans and sylvatic cycles in non-human primates [1,11]. Spillover from the sylvatic cycle to the urban cycle has been documented [12,13]. The

infection can be asymptomatic or range from an undifferentiated acute febrile illness to “classic” dengue fever (DF) which, in some cases, can progress to life-threatening dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) [14]. At present there are no specific treatments or commercially available vaccines [15].

One of the largest epidemics of dengue ever recorded occurred world-wide was in refugee camps in Greece (1927–1928), with at least 650,000 cases and 1,000 deaths. The vector was *Ae. aegypti* [16]. In recent years there has been a sharp rise in the number of imported DF in Europe, attributable to a rapid rise in international air travel and high rates of transmission in endemic countries [17]. The introduction of *Ae. albopictus* followed by rapid colonization has given rise to autochthonous cases in mainland France (2010, 2013) and Croatia (2010) [18–20]. In addition, more than 2,000 cases were recorded in Madeira (2012), an autonomous region of Portugal and therefore technically a part of Europe. In this case the vector was *Ae. aegypti*, a recent arrival in the archipelago [21,23]. The rapid dissemination and establishment of the *Ae. albopictus* in Southern Europe, and the intense increase in traveling fuel the introduction of dengue in non-endemic areas [24]. This species was detected in 2012 in Bucharest, Romania, and has remained present in subsequent years (Prioteasa et al., personal communication). Dengue is now included in the list of notifiable communicable diseases; laboratory diagnosis by the Romanian National Reference Centre for Vector-Borne Infections was implemented starting 2008. In the present study we present the molecular epidemiology of imported DF cases in Romania between 2008 and 2013.

2. Material and methods

2.1. Patients and laboratory diagnosis

Sera from travelers with clinical suspicion of dengue fever after return from dengue-endemic regions were received by the National Reference Centre for Vector-Borne Infections (“Cantacuzino” N.I.R.D.M.I.) from Public Health Departments and from Clinical Hospital of Infectious and Tropical Diseases “Dr. Victor Babeş” in Bucharest. The samples were tested for DENVs antibodies with IgM and IgG indirect ELISA (Euroimmun, Lübeck, Germany). Paired sera were requested but convalescent serum was not available

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