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BRIEF COMMUNICATION

Imported Dengue Fever in Tuscany, Italy, in the Period 2006 to 2012

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This report focuses on epidemiological and clinical features of dengue fever (DF) in Tuscany (Italy) between 2006 and 2012. Sixty-one DF cases were diagnosed, 32 of which were in the period of *Aedes albopictus* activity. Some clinical (arthralgia/myalgia, nausea/vomiting, and skin rash), laboratory (leukopenia and thrombocytopenia), and epidemiological characteristics (travel in a continent other than Africa) significantly distinguished DF cases from other febrile illnesses. Our data stress the importance of increasing awareness on dengue in Italy among clinicians in order to reach an early diagnosis in returning travelers and to implement appropriate clinical and public health interventions.

Dengue fever (DF) is distributed widely in tropical and subtropical countries with an estimated range from 50 to over 200 million cases per year.[1,2] DF is caused by four serotypes of the dengue virus (DENV) and is transmitted by *Aedes* spp. mosquitoes. DF often presents itself as a short, self-limiting viral disease or may even be asymptomatic. However, in about 1% of cases DF can progress to a severe clinical form with hemorrhagic manifestations, plasma leakage, severe involvement of organs, shock, and death.[3] Imported cases of DF in travelers returning from endemic countries have been frequently reported in Europe in recent years.[4] Moreover, in 2010, autochthonous cases have been reported in France and Croatia. This was the first instance of locally acquired cases since the last European outbreak in Greece between 1927 and 1928.[5,6] In addition, a large epidemic of DF was recently observed in the Portuguese island of Madeira.[7]

The risk of emergence of autochthonous cases is related to the presence of competent vector mosquitoes such as *Aedes albopictus* and *Aedes aegypti* in European territories. *Aedes albopictus* is present in 20 European

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countries, among which Italy is the most heavily infested.[8] *Aedes aegypti*, the most effective DENV vector,[9] is present in Europe, but currently limited to Madeira, few countries around the Black Sea (southern Russia, Abkhazia, and Georgia), and the Netherlands.[8]

The diagnosis of fever in returned travelers may be challenging and, after excluding malaria, prompts the physician to consider a wide range of diseases and diagnostic tests. The main purpose of this study was to increase awareness of dengue in Italy among clinicians, who are advised to request diagnostic tests for suspected cases and report confirmed cases to public health authorities in order to implement vector control measures as required. We report the results of a retrospective study carried out with the aim of (1) describing epidemiological, clinical, and laboratory characteristics of DF cases observed in Tuscany region of Italy and (2) comparing data on laboratory-confirmed dengue (LCD) to suspected DF patients with negative serological test results for DF, who were classified as cases of other febrile illnesses (OFI).

Patients and Methods

The study was promoted by the Tuscan Reference Centre for Tropical Diseases (TRCTD) and proposed to include all infectious diseases units ($n = 11$) of Tuscany. Ten infectious diseases units from eight of nine provinces agreed to participate.

The TRCTD laboratory provides diagnostic support for some tropical diseases such as DF to other Tuscan health facilities. Serological tests for DF available at TRCTD are: (1) Dengue IgG Capture ELISA, Panbio Diagnostics (Brisbane, Australia); (2) Dengue IgM Capture ELISA, Panbio Diagnostics; and (3) Platelia Dengue NS1 Ag assay (EIA), Bio-Rad Laboratories (Marnes la Coquette, France). For this study, serological criteria for LCD cases were defined as presence of anti-DENV IgM antibodies and/or positivity for NS1 antigen. Epidemiological, clinical, and laboratory characteristics of patients with LCD diagnosed in the period 2006 to 2012 were collected retrospectively by reviewing medical records. Moreover, the LCD cases observed in the period 2006 to 2010 were then compared with patients classified with OFI. For the second aim, the exclusion criteria concerning both LCD and OFI patients were: (1) cases without available clinical data; (2) positivity for dengue IgG only; (3) single dengue IgG and dengue IgM negative result on tests performed during the first 4 days from onset of symptoms; (4) patients diagnosed with malaria; and (5) patients whose onset of fever was >15 days after return from travel. Malaria was excluded considering that any returned traveler with fever is assumed to have malaria unless proven otherwise; hence parasitological exams are performed before any other laboratory diagnostic test. The statistical analysis compared categorical variables between the two groups, LCD and OFI, using chi-square and Fisher's exact test when appropriate. Odds ratio (OR) was also evaluated for variables with $p < 0.05$ (Table 1).

Results

A total of 61 LCD cases were confirmed in Tuscany in the period 2006 to 2012. Stratification of the 61 cases by province revealed that 32 cases were observed in Florence, 10 in Arezzo, 6 in Leghorn, 3 in Lucca, 3 in Grosseto, 3 in Siena, 2 in Pistoia, and 2 in Massa-Carrara. According to the year of diagnosis, 2 cases were observed in 2006, 5 in 2007, 8 in 2008, 8 in 2009, 15 in 2010, 12 in 2011, and 11 in 2012.

Neither autochthonous nor severe cases of DF were diagnosed. Information concerning the origin of infection was available for 51 patients. A total of 30 LCD cases (58.8%) were acquired in Asia, 11 (21.5%) in Central America, 7 (13.7%) in South America, 2 (3.9%) in Oceania, and 1 (1.9%) in Africa. In regard to the date of diagnosis, 32 cases (52.4%) were diagnosed in the period of *A. albopictus* activity, which is between June 15 and November 30, according to the Italian Ministry of Health.[10] In the comparison between LCD and OFI cases in the period 2006 to 2010, samples from 193 patients were tested for suspected DF at the TRCTD. Among them, 90 were excluded (49 OFI and 2 LCD had no available clinical data, 23 had fever onset >15 days after return, 9 had only IgG positivity, 5 were IgG and IgM negative in the first 4 days after onset of symptoms and follow-up samples were not available, and 2 patients had malaria). A total of 36 LCD cases were observed, accounting for 19% of tested subjects. Of those, 17 were IgG positive/IgM positive, 15 IgG negative/IgM positive, 2 NS1Ag positive/IgG negative/IgM negative, 1 NS1Ag positive/IgG positive/IgM negative, and 1 NS1Ag positive/IgG positive/IgM positive. The comparison of epidemiological, clinical, and laboratory characteristics of LCD versus OFI is reported in Table 1. As far as epidemiological features are concerned, males ($p = 0.015$; OR: 3.1), traveling to visit family/friends ($p = 0.046$; OR: 3.8) and travel in a continent other than Africa ($p < 0.001$; OR: 20.8) were more common in LCD patients rather than OFI patients. For clinical features, arthralgia/myalgia ($p = 0.0006$; OR: 4.4), nausea/vomiting ($p = 0.0024$; OR: 3.6), and skin rash ($p < 0.0001$; OR: 15.2) was more common in patients with LCD. In terms of laboratory findings, LCD patients most commonly had leukopenia ($p < 0.001$; OR: 9.8) and thrombocytopenia ($p < 0.001$; OR: 16.7).

Discussion

After malaria, DF is the second-most common diagnosis made on febrile patients returning from tropical areas and a test for DF must always be included in the diagnostic work-up in such subjects.[4] Physicians who manage febrile, returning travelers must always place priority on the differential diagnosis of conditions

Table 1 Clinical, demographic, and epidemiological features of patients with laboratory-confirmed dengue (LCD) fever and other febrile illness (OFI) observed in Tuscany (Italy) in the period 2006 to 2010

	LCD ($N = 36$)	OFI† ($N = 67$)	p Value*
Demographic features			
Male gender	29 (80.5%)	38 (57%)	0.01 OR: 3.1 (95% CI: 1.215 to 8.228)
Female gender	7 (19.5%)	29 (43%)	0.01 OR: 0.3 (95% CI: 0.122 to 0.823)
Age in years: median (range)	39 (21–72)	28 (70–16)	
Age in years: mean	40	37.5	
Onset after return			
Days: median (range)	1 (−17 to 7)	1 (−18 to 14)	
Onset after return			
Days: mean	4.02	1.8	
Country of origin			
Italy	31 (86.1%)	58 (86.5%)	1.00
European countries other than Italy	0	3 (4.4%)	0.54
Central America	0	2 (2.9%)	0.54
Latin America	1 (2.8%)	1 (1.4%)	1.00
Oceania	1 (2.8%)	0	0.34
Africa	2 (5.5%)	0	0.11
Asia	1 (2.8%)	3 (4.4%)	1.00
Travel destinations			
Asia	18 (50%)	21 (31.3%)	0.06
Central America	10 (27.8%)	11 (16.4%)	0.17
South America	5 (13.9%)	10 (14.9%)	1.00
Oceania	2 (5.5%)	0	0.11
Africa	1 (2.8%)	25 (37.3%)	<0.01 OR: 0.6 (95% CI: 0.00 to 0.52)
Continent other than Africa	35 (97.2%)	42 (62.7%)	<0.01 OR: 20.8 (95% CI: 2.68 to161.58)
Reason of travel			
Business	5 (13.9%)	9 (13.4%)	1.00
Tourism	23 (63.9%)	52 (77.6%)	0.13
Family	7 (19.4%)	4 (5.9%)	0.04 OR: 3.8 (95% CI: 1.03 to14.01)
Others	1 (2.8%)	2 (2.9%)	1.00
Clinical presentation			
Headache	15 (41.7%)	25 (37.3%)	0.66
Arthralgia/myalgia	23 (63.9%)	19 (28.3%)	<0.01 OR: 4.4 (95% CI: 1.88 to 10.59)
Nausea/vomiting	23 (63.9%)	22 (32.8%)	<0.01 OR: 3.6 (95% CI: 1.54 to 8.46)
Mucosal bleeding/petechiae	2 (5.5%)	0	0.11
Rash	15 (41.7%)	3 (4.4%)	<0.01 OR: 15.2 (95% CI: 4.01 to 57.84)
Cough/pharyngitis	6 (16.7%)	13 (19.4%)	0.79
Urinary symptoms	1 (2.8%)	3 (4.4%)	1.00
YF, JE, TBE vaccinated	3 (8.3%)	7/45 (15.5%)	0.49
Laboratory findings			
Leukopenia (white blood cell <4,000/μL)	24 (66.7%)	10/59 (16.9%)	<0.01 OR: 9.8 (95% CI: 3.71 to 25.87)
Thrombocytopenia (platelet counts <150,000/μL)	26 (72.2%)	8/59 (13.5%)	<0.01 OR: 16.7 (95% CI: 5.84 to 47.02)

OR = odds ratio; YE = yellow fever; JE = Japanese encephalitis; TBE = tick-borne encephalitis.

*p Value has been determined by chi-square and Fisher's exact test when appropriate.

†OFI (42 undiagnosed fever in returning traveler, 5 pneumonia, 4 meningitis, 3 urinary tract infection, 3 bacterial sepsis, 2 Epstein–Barr virus (EBV) acute infection, 2 paratyphoid fever, 1 cytomegalovirus (CMV) acute infection, 1 rickettsiosis, 1 pulmonary tuberculosis, 1 amebic dysentery, 1 measles, 1 visceral leishmaniasis).

that are treatable, that may cause serious sequelae or death, and pose a risk to public health.[11] DF fulfills all the mentioned priority conditions. Severe DF has a fatality rate as high as 20% in absence of appropriate medical intervention.[12] Although a direct antiviral for DENV is currently unavailable, supportive therapy for the management of DF improves the outcome in the affected patients, if strictly implemented, by reducing the fatality to 1%.[12] Moreover, avoidance of nonsteroidal anti-inflammatory drugs and invasive procedures is recommended to prevent severe and even fatal hemorrhagic complications.[13] From the public health point of view, a subject returning from a DF-endemic area represents a risk for the emergence of autochthonous cases in areas where competent vectors are present. All patients included in this study were febrile from <15 days and were returning from a dengue-endemic area. As reported in other studies conducted in Europe, DF is more often diagnosed in adult male tourists returning from Asia, particularly Thailand and India, or Central and South America, mainly from Brazil.[12] In three cases, acute DF was diagnosed by NS1 antigen detection, providing supportive evidence that this test is a valid tool for early diagnosis of dengue in travelers returning from endemic areas.[14] In our series, three key clinical features (arthralgia/myalgia, nausea/vomiting, and skin rash), two laboratory results (leukopenia and thrombocytopenia), and one epidemiological feature (travel in a continent other than Africa) significantly distinguished LCD from OFI, as previously reported.[15] In addition, about 52% of dengue cases were diagnosed in the period of *A. albopictus* activity. As *A. albopictus* is present widely in Tuscany, the reported dengue cases led to implementation of the regional surveillance program for arthropod-borne diseases that is based on home isolation and pest control methods around the patient's house.[10,16]

This study however has a number of limitations. It was based on retrospectively collected data, included only febrile subjects evaluated at infectious diseases departments, and does not provide information on serotype as reverse-transcriptase polymerase chain reaction (RT-PCR) was not performed. Moreover, the presence of anti-DENV IgM antibodies in a single specimen as a criterion to define a confirmed DF case, although coupled with clinical and epidemiological information, does not completely rule out the possibility of a false-positive result.

In conclusion, awareness of the main epidemiological and clinical features of DF may help physicians to recognize DF early on and diagnose the same in febrile travelers from DF-endemic areas.[3,13] An appropriate diagnostic approach is crucial to limit the risk of introduction and transmission of dengue virus in areas where *A. albopictus* or *A. aegypti* are present and in their period of activity. A rapid identification of an imported DF case would allow for implementation of adequate control measures aimed at preventing local transmission. Furthermore, travelers may act as sentinels for providing information regarding the emergence or reemergence of DF in a region.

Declaration of Interests

The authors state they have no conflicts of interest to declare.

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