Le Infezioni in Medicina, n. 2, 243-252, 2020

Imported dengue fever: a 16-years retrospective analysis in Milan (Italy) and a brief review of the European literature

Gabriele Pagani[1,2], Nadia Zanchetta[3], Laura Galimberti[2], Letizia Oreni[1], Simone Passerini[4], Andrea Giacomelli[1], Laura Cordier[4], Maria Rita Gismondo[1,3], Giuliano Rizzardini[4], Massimo Galli[1,2], Spinello Antinori[1,2]

[1]Dipartimento di Scienze Biomediche e Cliniche "L. Sacco", Università degli Studi di Milano, Milano, Italy;
[2]III Divisione di Malattie Infettive, ASST Fatebenefratelli Sacco, Università degli Studi di Milano, Milano, Italy;
[3]Microbiologia, Virologia e Diagnostica Bioemergenze, ASST Fatebenefratelli Sacco, Milano, Italy;
[4]I Divisione di Malattie Infettive, ASST Fatebenefratelli Sacco, Milano, Italy

SUMMARY

Dengue Fever (DF), transmitted by *Aedes* mosquitoes, is the most common arthropod-borne infection. It is almost ubiquitous in tropical and subtropical areas with an estimate of 360 million infections per year. A competent vector (*A. albopictus*) is present in most of Southern Europe and is endemic in Italy. We conducted a 16-year retrospective study of probable/confirmed dengue fever observed at the Department of Infectious Diseases of Luigi Sacco Hospital in Milan, Italy. Overall 122 patients were included in the study, 106 with probable and 16 with proven diagnosis of dengue fever. Most patients (91%) were Italian, with a median age of 35 years (IQR 29-46 years) and similar gender distribution, travelling for tourism (80%). Asia (mainly South East Asia and Indian Subcontinent) was the most frequent travel destination (55%), followed by Central America and the Caribbeans (22%). August-September was the peak season of presentation (42.6%). The majority of our diagnoses were based on serology alone. The most common signs and symptoms were fever (99,2%), maculopapular rash (50,8%), headache (50,8%), arthralgias (50,8%) and myalgias (46,7%). Leukopenia (77%), thrombocytopenia (81%) and altered LDH, AST and ALT (respectively 60,6%, 54,1% and 45,9%) were the most common laboratory test's abnormalities. No cases of severe DF were recorded. Our epidemiological and clinical findings are largely in accordance with most recent studies about imported DF in Europe. Although very similar in presentation to other arthropod-borne illnesses, some clinical features may help in differentiating DF from other causes of fever in the returning traveler.

Keywords: Dengue, dengue fever, arboviral infections, imported infections, travel medicine.

INTRODUCTION

Dengue fever is caused by a single-stranded RNA virus belonging to Flaviviridae *(Dengue virus)*, subdivided in four closely related, but antigenically different, serotypes (DENV 1-4).

Dengue fever is the most common arthropod-borne viral infection worldwide and an increasingly recognized public health problem. It is endemic in more than one hundred tropical and subtropical countries and 2.5 billion people are at risk of transmission, the greatest disease burden being observed in Central and South America and Southern and South-Eastern Asia. According to recent estimates there are as much as 360 million infections per year (96 million of which clinically evident) [1-3].

Corresponding author
Gabriele Pagani
E-mail: gabriele.pagani@unimi.it

Infection with one serotype grants permanent immunity against the same serotype, but only temporary protection against the others. *Aedes* mosquitoes are the vectors of the infection, which is most efficiently transmitted by *A. aegypti*, an urban day-biting tropical mosquito, and less frequently by *A. albopictus* or other species [4].
Clinical manifestations range from a self-limiting febrile flu-like disease (non-severe dengue) to a severe and potentially fatal syndrome characterized by capillary leak syndrome (possibly leading to shock), end-organ failure and haemorragic manifestations (severe dengue), the latter more common in secondary infections [4].
Dengue is recognized as a frequent cause of morbidity in international travelers, being the most common cause of fever in travelers returning from South and Southeast Asia and Latin America [5]. Due to the presence of the virus in some of the most popular tourism destinations and to the increase in trans-continental travelling, a rising number of imported cases of Dengue has been reported in Europe [6-9].
At present there is concern regarding the possible reintroduction of dengue fever in Southern Europe, where several international airports are located in areas where a competent vector (*i.e.*, *A. albopictus*, commonly known as the "Asian Tiger Mosquito") is widespread [10, 11]. As a matter of fact, several cases of autochthonous transmission have already been reported in France, Spain and Croatia and a recent outbreak (sustained by *A. aegypti* transmission) occurred on the Portuguese island of Madeira in 2012-2013, with more than 2200 infections [12-16].
We aimed to analyze the epidemiological and clinical features of imported dengue fever (DF) observed at Luigi Sacco Hospital's Department of Infectious diseases in Milan (Italy) over a 16-years period.

PATIENTS AND METHODS

A retrospective study was carried out at the Luigi Sacco Hospital from January 2001 to December 2016. This university-affiliated hospital is a referral center for imported tropical infections in Northern Italy.
Patients were selected using two complementary methods: all the patients whose discharge diagnosis codec according to the International Classification of Diseases, ninth Revision [ICD-9], corresponded to "dengue fever" and cross-checked with Microbiology's electronic archive for patients with positive serologies for DENV antibodies and/or positive PCR for DENV, were included in the analysis.
Clinical data were subsequently retrieved from their clinical records and anonymized. The following parameters were recruited: personal data (age, gender, nationality), year and month of admission and discharge, diagnosis, country of origin, visited countries, reason of travel, travel period (dated of beginning and end of travel, travel duration), signs and symptoms at presentation and their onset date, comorbidities, haematologic and biochemical tests' results (complete blood count, creatinine, blood glucose, blood urea nitrogen (BUN), lactate dehydrogenase (LDH), transaminases, blood bilirubin, gamma glutamyl transpeptidase (gGT), prothrombin and activated tromboplastin time (as normalized ratios), serologies for DENV (IgM and IgG class antibodies).

Definitions
According to CDC definitions a case of "probable" DF was defined as a positive serology for DENV together with compatible clinical and epidemiological features; a case of "proven" DF was defined as a positive PCR for DENV RNA or at least a fourfold increase in IgG titer between serum samples withdrawn during the acute phase and the convalescent phase (17).
Travel destinations were grouped under five areas: A (Asia and Southeast Asia), B (Oceania), C (Africa and Middle East), D (Central America and Caribbean), E Area (South America).
Leukopenia was defined as a white cell count ≤4000 cells/μL (neutropenia as a neutrophil count ≤1500 cells/μL), thrombocytopenia as a platelet count ≤150.000 cells/μL.
Altered values were defined if >220 IU/L for LDH, >35 IU/L for AST, >40 IU/L for ALT.

Diagnostic Methods
Research of DENV specific antibodies was performed by commercially available Dengue virus specific IgM and IgG detection kit (NovaLisa Dengue IgG/IgM ELISA, Immunodiagnostica Gmbh, Dietzenbach, Germany) according to manufacturer's instructions. From 2015 on, real-time reverse transcription polymerase chain reaction (RT-PCR)

for DENV-RNA was performed by means of a commercially available kit (Dengue/CHICKV RT-PCR, Fast Track Diagnostics Ltd., Malta).

Review Methods

The PubMed and Google Scholar databases were searched for articles in English, published between 2004 and 2017 using the following combination of MESH terms: "dengue", "imported" and "Europe". We then selected epidemiological studies about imported DF set in European countries.

Statistical methods

Continuous variables were described as median and interquartile range (IQR).

Discrete variables were described as absolute number and percentage.

Patients were also divided in two groups based on the time elapsed between the onset of symptoms and hospital admission (equal or less than 4 days and more than 4 days): Wilcoxon-Mann-Whitney Test was used to compare continuous variables between two groups.

A p-value <0.05 was considered significant.

RESULTS

A total of 122 patients observed between 2001 and 2016 were included. We did not observe an increasing trend in the number of diagnosed cases over the period of study (Figure 1).

The majority of patients were young (median age: 35 years; IQR 29-46) and Italian (91%). Males and females were almost equally represented (M:F=1:1,07) (Figure 2). One hundred and six patients had "probable" DF and 16 fulfilled a diagnosis of "proven" DF. The median duration of travel was 18 days (RIQ 14-25).

Tourism was the main reason of travel (n=97; 80%), 12 patients (10%) were travelling for working reason, 9 (7,5%) were visiting friends or relatives (VFR). Four patients were travelling for other reasons (volunteering, religious missions, etc.).

More than half of the patients (n=67; 55%) were returning from Asia, 27 (22%) were returning from the Central America/Caribbean area, 16 (13%) from South America, 9 (7%) from Africa or the Middle East and two (2%) from Oceania. Travel destination was unknown for one patient. Absolute and relative frequencies for destination areas are shown in Figure 3. Reported travel destinations for each patient are shown in Table 1.

Almost half (n= 52, 43%) of the patients were observed in August and September (see Figure 4 for the distribution during the rest of the year).

In more than half of the patients (n=71; 58.2%) the onset of symptoms occurred from three days before to three days after the return from the endemic area. The median time interval between symptoms onset and hospitalization was 4 days (RIQ 3-6). Demographics and travel characteristics (compared with the ones observed in recent studies about imported Dengue in Europe) are shown in Table 2.

The majority of patients (n=115, 94.3%) had detectable IgM antibodies against DENV at the time of hospital presentation. More specifically, 35 patients (28,7%) had positive serology for both IgM

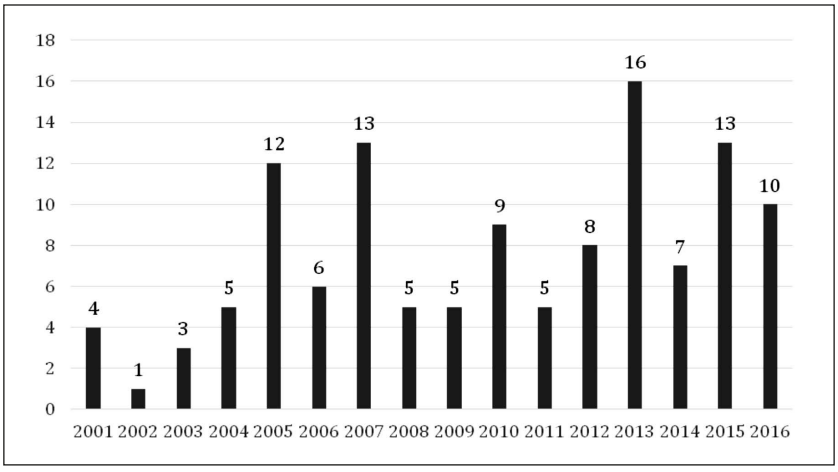

Figure 1 - Dengue Fever cases observed at Luigi Sacco Hospital's Department of Infectious Diseases, 2001-2016.

and IgG antibodies, 80 (65.6%) only for IgM class. Six (4.9%) had initially negative serology. In the latter group diagnosis was established either with PCR or serological follow-up. In one (0.8%) patient diagnosis was obtained by detection of NS1 antigen in a foreign hospital while abroad.

Overall, PCR for DENV was positive in 15 patients, with serotype identification in 7 cases: 4 DENV-1 (from Mexico, Thailand, Sri Lanka and Congo), 2 DENV-2 (from China and the Philippines) and 1 DENV-4 (from Thailand).

All patients were symptomatic at presentation, with

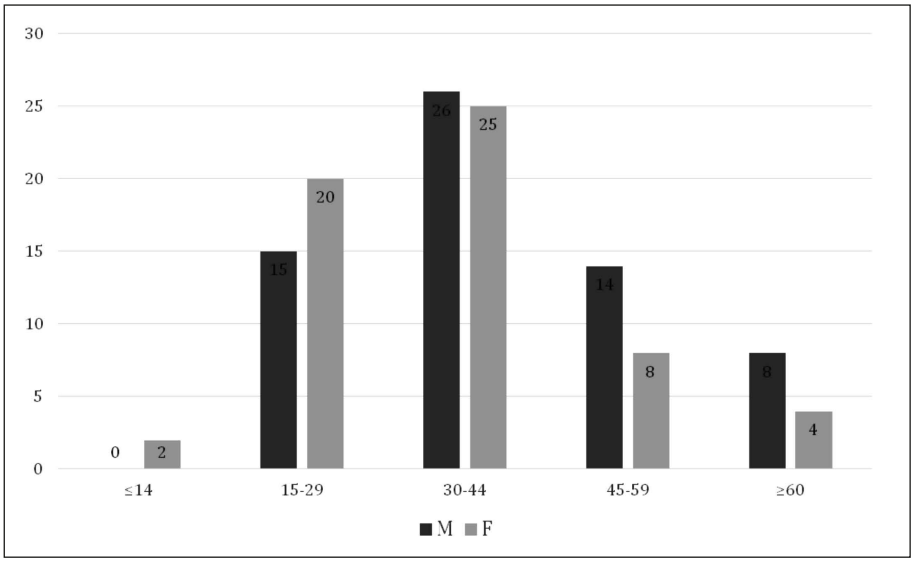

Figure 2 - Distribution of Dengue cases by age and gender.

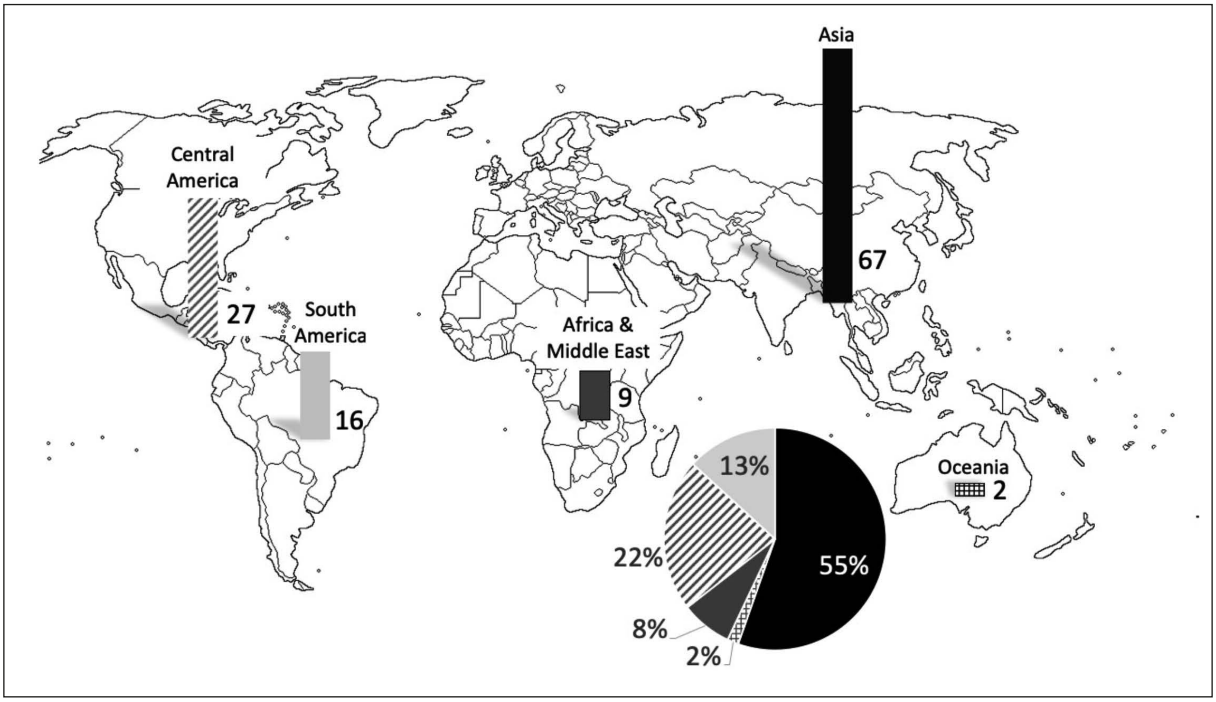

Figure 3 - Geographical distribution of imported Dengue Fever cases. The figure shows absolute number (columns) and percentage (pie-chart).

Table 1 - Reported travel destination(s) of patients affected by Dengue Fever.

Destination	*n*	*%*
ASIA	**67**	**55.74**
Thailand	22	18.85
India	12	9.84
Indonesia	5	4.10
Sri Lanka	4	3.28
Sri Lanka + Maldives	3	2.46
Philippines	3	2.46
Bali	2	1.64
Cambodia	2	1.64
Bangladesh	2	1.64
Laos + Cambodia + Thailand	2	1.64
Maldives	1	0.82
Thailand + Cambodia	1	0.82
Indonesia + Malesia + Singapore	1	0.82
Laos + Cambodia	1	0.82
Malesia	1	0.82
Myanmar	1	0.82
Myanmar + Cambodia + Thailand	1	0.82
Pakistan	1	0.82
Thailand + Malesia	1	0.82
Thailand + Singapore + Maldives	1	0.82
CENTRAL AMERICA and CARIBBEANS	**27**	**22.13**
Mexico	8	6.56
Dominican Republic	6	4.92
Cuba	5	4.10
Costa Rica	2	1.64
Haiti	2	1.64
Barbados	1	0.82
Cuba + Dominican Republic	1	0.82
Guadalupe	1	0.82
Grenada	1	0.82
SOUTH AMERICA	**16**	**13.11**
Brazil	8	6.56
Venezuela	3	2.46
Ecuador	3	2.46
Ecuador + Colombia	1	0.82
Colombia	1	0.82
AFRICA and MIDDLE EAST	**9**	**7.38**
Congo	1	0.82
Saudi Arabia	1	0.82
Cameroon	1	0.82
Eritrea	1	0.82
Mali	1	0.82
Nigeria	1	0.82
Kenya	1	0.82
Seychelles	1	0.82
Zanzibar (Tanzania)	1	0.82
OCEANIA	**2**	**1.64**
Polinesia	2	1.64
TOTAL	**121†**	

†: data based on 121 patients, no data available about travel destination in one patient

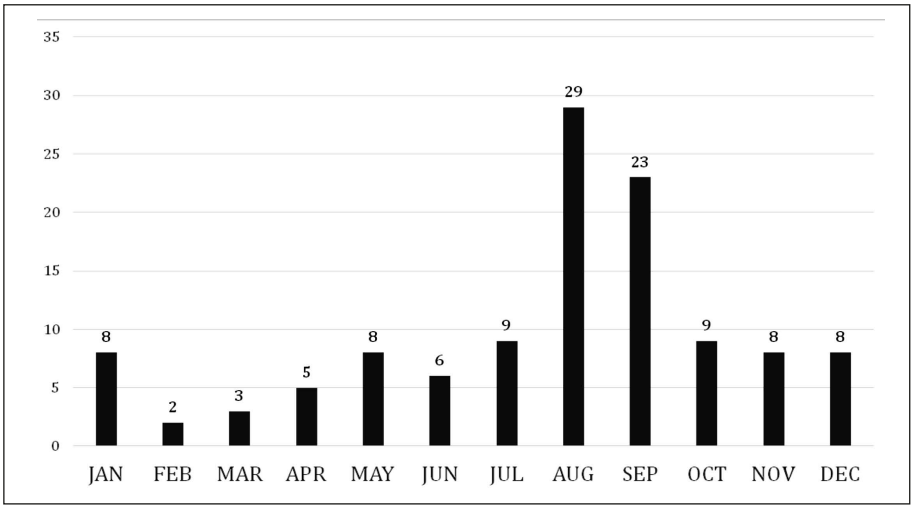

Figure 4 - Distribution of dengue fever cases per month, 2001-2016

Table 2 - European literature review: traveler's demographics and travel characteristics.

Author, reference	*Present work*	*Teichmann [20]*	*Laferl [21]*	*Vinner [22]*	*Lagi [28]*	*Trojanek [23]*	*Hoffmeister [24]*	*Tavakolipoor [25]*	*Calleri [26]*	*Kuna [27]*	*Riddell [29]*
Year of publication	2020	2004	2006	2012	2014	2015	2015	2016	2016	2016	2017
Country	IT	D	A	DK	IT	CZ	D	D	IT	PL	UK
Study period	2001-2016	1993-2001	1990-2005	2001-2009	2006-2012	2004-2013	1996-2010	2007-2011	2010-2015	2010-2015	2010-2015
PATIENTS (N)	122	71	93	95	36	132	56	119	113	65	44
Age, year (median)	35	34	32.5	35.5	39	33	35	35	34	38	34
M:F	0.93:1	1.37:1	1.16:1	1.18:1	4.14:1	1.69:1	0.8:1	1.28:1	0,94:1	2,42:1	2,14:1
Europeans: Extra-europ.	10.09:1	NA‡	9.33:1	NR	6.2:1	NR	3.67:1	NR	NR	NR	NR
Hospitalized (%)	71.3	81.6	68	NR	NR	38.6	NA§	17.8	57,5	NA§	NR
DESTINATION (%)											
Asia	54.9†	77.5	74	85	50	88	74[a]	73.1	49,6	44,6	70,4
Central America	22.1†	11.3	7.50	8.40	27.80	3.78	11[b]	4.2	39,8b	4,6	11,4
South America	13.1†	8.4	5.30	2.10	13.90	6	[b]	9.2	[b]	7,6	11,4
Africa	7.4†	1.4	10	4.20	2.80	0.80	9c	12	7,1	19	4,5
Oceania	1.6†	1.4	3.20	0	5.50	0	[a]	1	0	0	0
REASON FOR TRAVELLING (%)											
Tourism	80.1	95.8	NR	NR	63.90	71.20	NR	76.1	NR	26,1	NR
Work	9.9	4.2	NR	NR	13.90	25	NR	8.5	NR	35,3	NR
VFR	7.4d	0	10	NR	19.40	3.80	NR	0.8	NR	NR	NR
TRAVEL CHARACTERISTICS											
Duration (median days)	18	29	23.5	NR	NR	21	NR	21	NR	924#	NR
Ill before return (%)	36.8	28.2	58	NR	NR	50.80	NR	NR	NR	NR	NR

M: F: male to female ratio; VFR: visiting friends and relatives; IT: Italy; A: Austria; DK: Denmark; CZ: Czech Republic; D: Germany; UK: United Kingdom; [a]WHO "Western Pacific" + "Southeast Asia" regions; [b]WHO "Americas" Region; [c]WHO "African" region; [d]VFR + residents; ‡all patients were German; §only hospitalized patients included in the study; #"33 months" reported in the study; NA: not applicable; NR: Not Reported.
†percentages based on 121 patients, no data available about travel destination in one patient.

fever observed in almost everyone (121; 99%); associated symptoms were as follow: maculopapular rash (62; 50.8%), headache (62; 50.8%), arthralgia (62; 50.8%), myalgia (57; 46.7%), nausea (32; 26.2%), vomiting (18; 14.8%) and diarrhea (22; 18%).
Signs and symptoms are reported in Table 3, compared with the ones observed in recent studies about imported Dengue in Europe.
Laboratory findings are presented in Table 4; thrombocytopenia (99; 81%) and leukopenia (94; 77%) were the most common laboratory alterations observed on admission. Elevation of transaminases (AST>ALT) and lactate dehydrogenase were other notable features.
We divided the patients in two groups, according to the time elapsed between the onset of symptoms and hospitalization (equal or less than 4 days and more than 4 days) and compared laboratory values between the two groups. Statistically significant differences ($p<0.05$) were found between the two groups for absolute neutrophil count (1530 *vs* 1109/µL, p=0.026), absolute lymphocyte count (749 *vs* 1041/µL, p=0.018) and SGPT values (35 *vs* 61 UI, p=0.002).

DISCUSSION

Dengue fever represents a raising concern in Southern Europe as a consequence of increased tourism to endemic areas and the widespread dif-

fusion of a competent vector (*Aedes albopictus*) in several countries (Italy, southern France, Greece, Croatia, oriental part of Spain) [9, 11]. This is especially true for Milan city area, where both international airports and high competent vector density (*A. albopictus*) coexist.

Nevertheless, autochthonous cases of Dengue have been reported only in Croatia, France and Spain so far [12-16]. Between 2013 and 2018, 13.398 cases of imported DF were reported from 27 countries in the European Union, 90.2% of which from seven countries: Germany (4428; 36.6%), United Kingdom (2669; 22.1%), France (1507; 12.5%), Spain (1265; 10.5%), Sweden (935; 7.7%), Belgium (649; 5.4%) and Italy (633; 5.2%) [18, 19].

We present 16-years data about epidemiological and clinical features of 122 cases of imported dengue fever observed at the Department of Infectious Diseases of Luigi Sacco Hospital in Milan, Italy. The majority of patients were young tourists of both genders. This is consistent with other

Table 3 - European literature review - signs and symptoms.

Author, reference	*Present work*	*Teichmann [20]*	*Laferl [21]*	*Lagi [28]*	*Trojanek [23]*	*Hoffmeister [24]*	*Tavakolipoor [25]*	*Calleri [26]*	*Kuna [27]*	*Riddell [29]*
SIGNS/SYMPTOMS (%)										
Fever	99.2	100	100	100	100	100	97.5	~100[e]	43	95.4
Arthralgia	50.8	79	63	63.9[a]	59.8	21.4	42[a]	~70[e]	NR	27.3[a]
Rash	50.8	66	43	41.7	68.2	46.4	47.9	~50[e]	18.4	22.7
Headache	50.8	86	67	41.7	65.9	10.7[c]	49.6	~60[e]	21.5	18.2
Myalgia	46.7	48	63	[a]	62.1	62.5%	[a]	NR	12.3	[a]
Nausea	26.2	14	34	63.9[b]	NR	NR	NR	NR	7.6	NR
Diarrhea	18	9	30	NR	36.4	NR	42.9	NR	15.5	22.7
Vomit	14.7	7	19	[b]	15.9	3.6d	NR	NR	9.2	22.7
Severe Dengue	0	1.4	8[f]	0	1	10.7[g]	0	0.9	0	2.3

[a]arthralgia and/or myalgia; [b]nausea and/or vomit; [c]retro-ocular pain; [d]persistent vomiting; [e]precise numeric values not reported, percentages extrapolated from graphic; [f]Dengue Hemorragic Fever (DHF) reported for 7 patients, Dengue Shock Syndrome (DSS) reported for 1 patient; [g]DSS reported for 2 patient; NR: data not reported.

Table 4 - European literature review: laboratory values.

Author, reference	*Present work*	*Teichmann [20]*	*Laferl [21]*	*Lagi [28]*	*Trojanek [23]*	*Hoffmeister [24]*	*Tavakolipoor [25]*	*Kuna [27]*	*Riddell [29]*
LABORATORY VALUES									
Plt (median)	103 x103/µL	116 x103/µL	66 x103/µL	NR	118 x103/µL	NR	NR	190 x103/µL	93 x103/µL
WBC (median)	2685/µL	2630/µL	2280/µL	NR	3200/µL	NR	NR	5190/µL	4000/µL
Hct (median)	40.9%	43 U/L	43.9%	NR	43.3%	NR	NR	NR	41%
AST (median)	62 U/L	30 U/L	2.51xULN	NR	70 U/L	NR	NR	60.4 U/L	NR
ALT (median)	46 U/L	36 U/L	2.22xULN	NR	85.3 U/L	NR	NR	50.8 U/L	67 U/L
LDH (median)	304 U/L	NR	1.31xULN	NR	294 U/L	NR	NR	NR	NR
Thrombocytopenia (%)	81.2	70	72	72.2	12.9[a]	84	32.8	20	NR
Leukopenia (%)	77.1	72	89	66.7	14.4[b]	73.2	26.1	24.6	NR
AST > ULN (%)	54.1	45	79	NR	NR	NR	32.8	23	NR
ALT > ULN (%)	45.9	41	70	NR	NR	NR	34.5	14	NR
LDH > ULN (%)	60.7	44	73	NR	NR	NR	31.1	NR	NR

[a]<50x10^9/µL; [b]<2000/µL; Plt: platelets; WBC: white blood cells; AST: aspartate aminotrasferase; ALT: alanine aminotrasferase; LDH: lactate dehydrogenase; ULN: Upper Limit of Normality; NR: data not reported.

European experiences, except for the study published by Lagi et al. (Italy) and Riddell et al. (UK), who observed a higher male prevalence [20-29]. A large proportion of patients (77%) became infected with DENV in Asia (Indian subcontinent and South-East Asia) and Central American/Caribbean area (22%), in agreement with the results reported in a recently published systematic review [30].

Seasonality in case occurrence reflects holiday season, with 43% of patients observed during August and September. This is also in agreement to the seasonality reported in Europe during the same years, with the notable exception of 2018 (a year not reported in our study), when the number of cases peaked in November [19].

It is also worth noticing that almost two-thirds (62,3%) of patients were observed in the period between June and October, when *A. albopictus* reaches the peak of its activity in our country [31]. In most of the patients (58.2%) the onset of symptoms occurred between 3 days before and 3 days after their return to Italy, reflecting both the short duration of travel and the brief incubation period of DF, which is usually 4 to 7 days [23]. Moreover, this high proportion of patients presenting during the acute phase of infection (thus potentially viremic) may represent a potential risk for the development of an autochthonous transmission cycle in Europe, as suggested by similar results reported from a TropNet study [32]. The reasons why we didn't yet witness a large autochthonous outbreak of dengue in Southern Europe, where both permissive climatic factors and a competent vector are present, are still a matter of discussion, the main one possibly being that *A. albopictus* is a less competent DENV vector than *A. aegypti* (11). It must be noted, though, that both the introduction of a more competent vector and an improvement in efficiency of the present one (mainly through virus mutations) are concrete possibilities [33].

From a clinical point of view, DF must be differentiated form other febrile illnesses, first of all malaria, and, in second place, Chikungunya and Zika fever [34]. Clinical symptoms are however very similar between DF and the aforementioned conditions, with a possible exception related to the frequency of skin rash and arthralgia. In our study maculopapular rash was observed in nearly half of patients, a frequency similar to the one reported in the majority of studies conducted in Europe, although two studies reported higher percentages (66-68%), while two others reported lower frequencies (21-22%) [20, 21, 23-29]. Skin rash is very rarely (if ever) observed in malaria, but it is, on the contrary, reported in 60-70% of patients diagnosed with Chikungunya and in more than 90% of those affected by Zika virus infection [35-40].

Arthralgia is by far more frequently encountered among patients with Chikungunya infection (83-96%) and it is notably characterized by persistence for several weeks in more than 30% of the patients [36, 39, 41, 42].

No patients fulfilled the criteria for severe dengue, in accordance to the majority of studies on imported DF, reporting less than 1% to no cases of severe DF; however, two studies (one of which used the previous dengue haemorragic fever/dengue shock syndrome classification) reported severe dengue in 2.3% and 8% of cases, respectively [20, 21, 23-29].

Consistent with other studies, diagnosis of dengue was established predominantly by antibody testing alone, with the majority of patients showing positive IgM class antibodies at the time of first observation [25, 26, 28, 29]. It should be highlighted, however, that the emergence and widespread diffusion of other arboviruses (suck as Zika and Chikungunya viruses), which frequently co-circulate in the same areas together with Dengue virus, makes the use of more specific tests (*i.e.*, RT-PCR) recommended in those patients presenting during the first five days of illness, together with serologies directed towards the above-mentioned viruses [43, 44]. Nevertheless, sensitivity limitations of molecular tests and possible serological cross-reactivity might require additional evaluation by plaque reduction neutralization tests performed by reference laboratories to correctly diagnose some case [44, 45]. Our study presents several limitations, the retrospective single-center design being the most relevant. Another potential limitation is that the majority of our study subjects were diagnosed with "probable" DF, which could have led to false positive diagnosis because of the relatively low specificity of serology alone.

In conclusion, according to our study, the "typical patient" with dengue fever is a young tourist, returning from a tropical area (more often from South-East Asia or the Caribbean/Central Amer-

ican area), presenting with fever associated with thrombocytopenia and leukopenia.
Awareness, early recognition and notification are fundamental, since importation and local transmission in Italy are concrete possibilities due to the presence of a competent vector. A prompt diagnostic approach to dengue in settings of high clinical and epidemiological suspicion, as well as the improvement of surveillance programs should be carried out to properly identify a possible autochthonous outbreak.

Conflict of interest
The authors declare no conflict of interest.

Funding
None.

REFERENCES

[1] Bhatt S, Gething PW, Brady OJ, et al. The global distribution and burden of dengue. *Nature.* 2013; 496, 504-7.
[2] Shepard DS, Undurraga EA, Halasa YA. Economic and disease burden of Dengue in Southeast Asia. Gubler DJ, editor. *PLoS Negl Trop Dis*. 2013; 7, e2055.
[3] Shepard DS, Coudeville L, Halasa YA, Zambrano B, Dayan GH. Economic impact of Dengue illness in the Americas. *Am J Trop Med Hyg*. 2011; 84, 200-7.
[4] W.H.O. Dengue: guidelines for diagnosis, treatment, prevention, and control. *Spec Program Res Train Trop Dis*. 2009; 147.
[5] Torresi J, Leder K. Defining infections in international travellers through the GeoSentinel surveillance network. *Nat Rev Microbiol*. 2009; 7, 895-901.
[6] Rocklöv J, Lohr W, Hjertqvist M, Wilder-Smith A. Attack rates of dengue fever in Swedish travellers. *Scand J Infect Dis.* 2014; 46, 412-7.
[7] Verschueren J, Cnops L, van Esbroeck M. Twelve years of dengue surveillance in Belgian travellers and significant increases in the number of cases in 2010 and 2013. *Clin Microbiol Infect.* 2015; 21, 867-72.
[8] Allwinn R. Significant increase in travel-associated dengue fever in Germany. *Med Microbiol Immunol (Berl).* 2011; 200, 155-9.
[9] Semenza JC, Suk JE. Vector-borne diseases and climate change: a European perspective.
FEMS Microbiol Lett. 2018; 365, 2. doi: 10.1093/femsle/fnx244.
[10] Schaffner F, Mathis A. Dengue and dengue vectors in the WHO European region: past, present, and scenarios for the future. *Lancet Infect Dis*. 2014; 14, 1271-80.
[11] Semenza JC, Sudre B, Miniota J, et al. International dispersal of dengue through air travel: importation risk for Europe. Kasper M, editor. *PLoS Negl Trop Dis*. 2014; 8, e3278.
[12] Tomasello D, Schlagenhauf P. Chikungunya and dengue autochthonous cases in Europe, 2007-2012. *Travel Med Infect Dis*. 2013; 11, 274-84.
[13] Marchand E, Prat C, Jeannin C, et al. Autochthonous case of dengue in France, October 2013. *Eurosurveill*. 2013; 18, 50, 20661.
[14] Succo T, Leparc-Goffart I, Ferré J-B, et al. Autochthonous dengue outbreak in Nîmes, South of France, July to September 2015. *Eurosurveill*. 2016; 21, 30240.
[15] Autochthonous cases of dengue in Spain and France, 1 October 2019. ECDC. 2019; 8.
[16] Lourenço J, Recker M. The 2012 Madeira Dengue Outbreak: Epidemiological determinants and future epidemic potential. Scarpino S V., editor. *PLoS Negl Trop Dis.* 2014; 8, e3083.
[17] Dengue Virus Infections | 2015 Case Definition. Available at: /nndss/conditions/dengue-virus-infections/case-definition/2015/ [accessed 18 February, 2020].
[18] Dengue - Annual Epidemiological Report for 2017. European Centre for Disease Prevention and Control. 2019. Available at: https://www.ecdc.europa.eu/en/publications-data/dengue-annual-epidemiological-report-2017 [accessed 18 February, 2020].
[19] Dengue - Annual Epidemiological Report for 2018. European Centre for Disease Prevention and Control. 2019. Available at: https://www.ecdc.europa.eu/en/publications-data/dengue-annual-epidemiological-report-2018 [accessed 18 February, 2020].
[20] Teichmann D, Göbels K, Niedrig M, Grobusch MP. Dengue virus infection in travellers returning to Berlin, Germany: clinical, laboratory, and diagnostic aspects. *Acta Trop*. 2004; 90, 87-95.
[21] Laferl H, Szell M, Bischof E, Wenisch C. Imported dengue fever in Austria 1990-2005. *Travel Med Infect Dis*. 2006; 4, 319-23.
[22] Vinner L, Domingo C, Ostby A-CB, Rosenberg K, Fomsgaard A. Cases of travel-acquired dengue fever in Denmark 2001-2009. *Clin Microbiol Infect*. 2012; 18, 171-6.
[23] Trojánek M, Maixner J, Sojková N, et al. Dengue fever in Czech travellers: A 10-year retrospective study in a tertiary care centre. *Travel Med Infect Dis*. 2016; 14 (1), 32-8.
[24] Hoffmeister B, Suttorp N, Zoller T. The revised dengue fever classification in German travelers: clinical manifestations and indicators for severe disease. *Infection*. 2015; 43, 21-8.
[25] Tavakolipoor P, Schmidt-Chanasit J, Burchard GD, Jordan S. Clinical features and laboratory findings of dengue fever in German travellers: A single-centre, retrospective analysis. *Travel Med Infect Dis.* 2016; 14, 39-44.
[26] Calleri G, Torta I, Gobbi F, et al. Imported dengue in two tertiary Italian hospitals: Use of rapid diagnostic tests. *Bull Société Pathol Exot.* 2017; 110, 13-9.
[27] Kuna A, Bykowska M, Kulawiak N, et al. Clinico-laboratory profile of dengue patients returning from

tropical areas to Poland during 2010-15. *J Vector Borne Dis*. 2016; 53, 234-9.
[28] Lagi F, Zammarchi L, Strohmeyer M, et al. Imported dengue fever in Tuscany, Italy, in the period 2006 to 2012. *J Travel Med*. 2014; 21, 340-3.
[29] Riddell A, Babiker ZOE. Imported dengue fever in East London: a 6-year retrospective observational study. *J Travel Med.* 2017; 24, 3. doi: 10.1093/jtm/tax015.
[30] Ahmed AM, Mohammed AT, Vu TT, et al. Prevalence and burden of dengue infection in Europe: A systematic review and meta-analysis. *Rev Med Virol*. 2019; e2093.
[31] Ministero Della Salute. Sorveglianza dei casi umani di Chikungunya, Dengue, West Nile Disease ed altre arbovirosi e valutazione del rischio di trasmissione in Italia - 2015. http://fidas.it/wp/wp-content/uploads/2010/03/circolare_arbovirosi_2015.pdf
[32] Neumayr A, Muñoz J, Schunk M, et al. Sentinel surveillance of imported dengue via travellers to Europe 2012 to 2014: TropNet data from the DengueTools Research Initiative. *Eurosurveill*. 2017; 22, 30433.
[33] Rezza G. *Aedes albopictus* and the reemergence of Dengue. *BMC Public Health*. 2012; 12, 72.
[34] Gupta N, Nischal N. Management of acute febrile diseases in limited resource settings: a case-based approach. *Infez Med*. 2020; 28, 11-6.
[35] Antinori S, Napolitano M, Grande R, et al. Epidemiological and clinical characteristics of imported malaria in adults in Milan, Italy, 2010-2015. *Eur J Intern Med.* 2018; 57, e13-6.
3[6] Bocanegra C, Antón A, Sulleiro E, et al. Imported cases of Chikungunya in Barcelona in relation to the current American outbreak. *J Travel Med*. 2016 Mar 16; 23 (3), tav033.
[37] Rossini G, Gaibani P, Vocale C, Finarelli AC, Landini MP. Increased number of cases of Chikungunya virus (CHIKV) infection imported from the Caribbean and Central America to northern Italy, 2014. *Epidemiol Infect*. 2016; 144, 1912-6.
[38] Burdino E, Ruggiero T, Milia MG, et al. Travelers with Chikungunya virus infection returning to Northwest Italy from the Caribbean and Central America during June-November 2014. *J Travel Med.* 2015; 22, 341-4.
[39] Vasquez V, Haddad E, Perignon A et al. Dengue, chikungunya, and Zika virus infections imported to Paris between 2009 and 2016: Characteristics and correlation with outbreaks in the French overseas territories of Guadeloupe and Martinique. *Int J Infect.* 2018; 72, 34-9.
[40] Petridou C, Simpson A, Charlett A, Lyall H, Dhesi Z, Aarons E. Zika virus infection in travellers returning to the United Kingdom during the period of the outbreak in the Americas (2016-17): A retrospective analysis. *Travel Med Infect Dis*. 2019; 29, 21-7.
[41] Vairo F, Mammone A, Lanini S, et al. Local transmission of chikungunya in Rome and the Lazio region, Italy. *PloS One*. 2018; 13, e0208896.
[42] Díaz-Menéndez M, Esteban ET, Ujiie M, et al. Travel-associated chikungunya acquired in Myanmar in 2019. *Euro Surveill*. 2020; 25(1), 1900721.
[43] Ohst C, Saschenbrecker S, Stiba K, et al. Reliable Serological Testing for the Diagnosis of Emerging Infectious Diseases. *Adv Exp Med Biol*. 2018; 1062, 19-43.
[44] Fortuna C, Remoli ME, Rizzo C, et al. Imported arboviral infections in Italy, July 2014-October 2015: A National Reference Laboratory report. *BMC Infect Dis*. 2017; 17, 216.
[45] Antinori S, Morena V, Pagani G, et al. Dengue fever complicated by liver dysfunction due to possible co-infection with hepatitis E in a returning traveler from Cuba. *Infez Med*. 2020; 28, 98-103.