



Five years surveillance of Dengue virus imported cases in Northern Italy

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Introduction and Purpose: Dengue, the most rapidly spreading mosquito-borne viral disease in the world, has increased dramatically in recent years as a consequence of the spread of vectors across tropical and subtropical areas and it has been recognized as a potential hazard to travellers. The aim of this study was to evaluate the Dengue virus (DENV) epidemiology in Italian and foreign travellers residents in Italy returning to our country with a febrile syndrome over a five years period.

Methods: Blood samples from 254 travellers returning to Lombardia region (Northern Italy) from Dengue endemic areas and presenting with febrile syndrome during the period 2010-2014 were analyzed. All the samples were tested to evaluate the presence of virus specific IgM (Dengue virus IgM Capture DxSelect FOCUS Diagnostics, Valley View St, Cypress, CA 90630, USA) and IgG (Dengue virus IgG DxSelect FOCUS Diagnostics). Type specific neutralizing antibodies were detected with a plaque reduction neutralization test (PRNT) (Russell PK *et al.* 1967) on VERO E6 using the 4 Dengue serotypes. In addition, Dengue virus RNA was amplified with two methods: a specific Real-time RT-PCR targeting a conserved region in 3'UTR (Huhtamo *et al.* 2010) of all serotypes 1-4 and with a Pan-Flavivirus Nested RT-PCR (Sánchez-Seco *et al.* 2005). All the positive samples were confirmed by sequencing of the amplicons. Finally Dengue NS1 antigen detection was performed using PlateliaTM Dengue NS1 Ag (Bio-Rad Laboratories, Hercules, CA).

Results: During the study period, 72 (28%) patients over 254 analyzed were positive for DENV infection either by serology and or by molecular RNA detection. The majority of the patients contracted the disease in the Caribbean area or in South East Asia. All the four DENV serotypes were detected. The distribution of the 72 cases detected per years in the period 2010-2014 is reported in Fig.1. Patients were divided into 3 groups according to the results obtained (Tab.1). Dengue RNA was sequenced in 24 viremic patients, but in 5 the typing was impossible due the low amount of RNA detected (Tab.2). In 16 patients the combination of neutralizing antibodies titre along with the IgM/IgG ratio $\leq 1,4$ (Kuno G. *et al.* 1991) reflected a secondary infection due to a different serotype. The range of neutralizing antibodies in case of secondary infection was between 1:2560 and $\geq 1:80000$ and the range of IgM-to-IgG ratio was between 0,1 and 1,3. 12/16 cases (75%) of secondary infection were foreign residents in Italy and travelling to there original endemic tropical country.

	Number of patients	DENV-RNA	NS1 Ag	IgM+/-IgG	IgG	Nt abs
Primary acute infections	35	20	19	35*	0	32 ^a
Secondary acute infections	16	4	3	16**	0	16 ^b
Past infections	21	0	0	0	21	21 ^c
Total	72	24	22	51	21	69

*: IgM/IgG ratio 1,4-8,4; **:IgM/IgG ratio 0,1-1,3; ^a: Nt abs titer 1:5-1:2560; ^b: Nt abs titer 1:2560->1:80000; ^c: Nt abs titer 1:5-1:5000

Table 1: Identification of acute primary, secondary and past infections

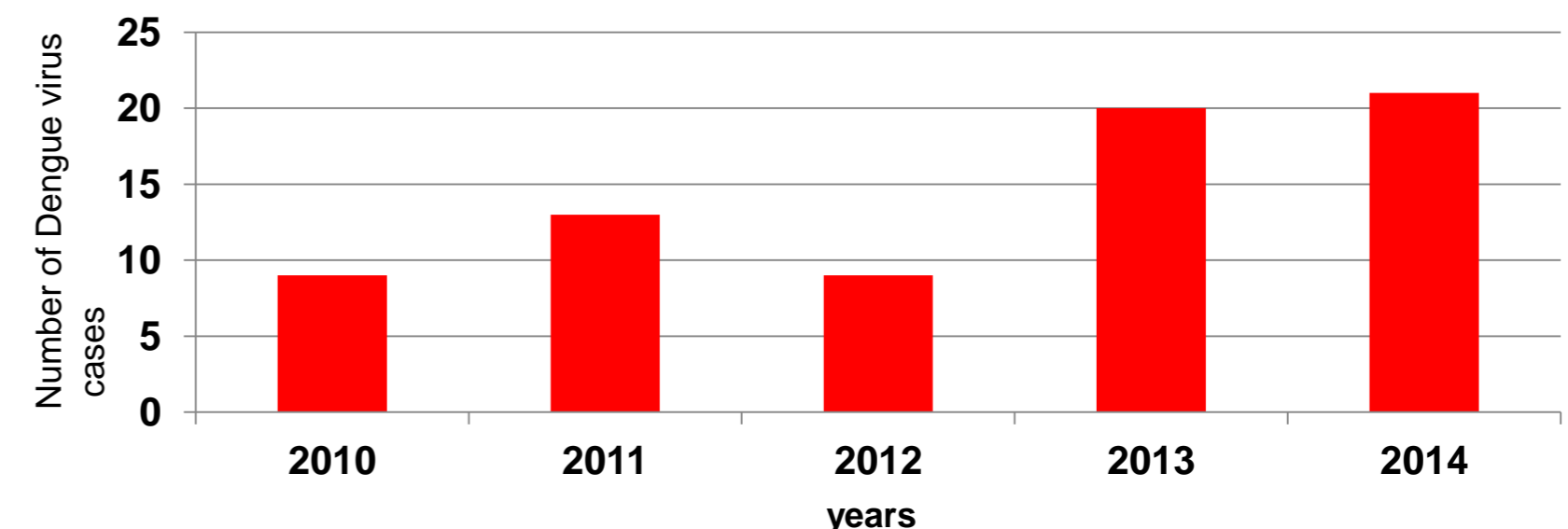


Figure 1: Number of Dengue imported cases in Lombardy region (Northern Italy) 2010-2014

Number of patients	DENV-1	DENV-2	DENV-3	DENV-4	Untyped
24	10	3	3	3	5

Table 2: Dengue virus typing

Conclusions:

- Pre-travel advice should be given to all travellers about Dengue endemic areas and the risk, in case of a secondary infection, to develop severe disease.
- Dengue fever must be included in the differential diagnosis for patients returning with a febrile syndrome from tropical areas.
- Imported Dengue virus infection bears the risk of autochthonous outbreaks during the vector season in Italy. Thus, containment of viremic patients is advised.
- Detection of neutralizing antibodies are mandatory for strain typing and for the diagnosis of a secondary infection due to a different strains.
- Neutralizing antibodies inhibit Dengue virus infection and can thus provide greater specificity in distinguishing antibodies to Dengue virus from other cross-reactive flavivirus antibodies.
- The combination of neutralizing antibodies and IgM/IgG ratio is useful for differentiating primary from secondary Dengue infections.