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Dengue fever as autochthonous infectious disease in Italy: Epidemiological, clinical and virological characteristics

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ABSTRACT

Background: Since August to November 2023, 82 cases of autochthonous or non-travel related Dengue virus (DENV) infection have been reported in Italy, highlighting a concerning trend of local transmission. We describe the clinical and laboratory findings of 10 autochthonous DENV in the metropolitan area of Rome admitted to the Lazzaro Spallanzani National Institute for Infectious Diseases.

Method and results: Ten patients (3 males, 7 females; median age: 51) with classic dengue fever symptoms were admitted between August and November 2023. Laboratory tests confirmed dengue infection through DENV non-structural protein 1 and/or immunoglobulins (IgM/IgG) positive tests, moreover leukopenia, thrombocytopenia, elevated transaminases were detected. A subset of patients underwent extensive biological sampling, including real-time RT-PCR and immunofluorescence, to monitor DENV-RNA and antibody levels over 30 days. DENV-1 was detected in 8 patients and DENV-3 in 2. Upon admission specific IgM antibodies were found in 7 patients while IgG antibodies in 4 patients. DENV RNA was consistently detected in blood within the first 8 days but was less common in saliva and urine. No DENV RNA was detected after day 24.

Conclusion: These findings contribute to the understanding of the clinical course of DENV infection in a non-endemic setting as integrated epidemiological and clinical model to increase syndromic surveillance and timely diagnosis of DENV infections.

1. Introduction

Since August 2024 to February 2024, 82 cases of autochthonous or not travel associated Dengue virus (DENV) infection have been reported in Italy, 41 in Lombardia and 41 in Lazio regions, respectively [1]. Nevertheless, Italy has not been the only European country experiencing autochthonous cases of DENV, other nations, including France, Spain, have also reported instances of autochthonous DENV transmission [1]. The surge of DENV cases in EU has raised significant concerns within the public health domain. Usually associated with tropical climates, the unexpected rise of DENV infection in these regions underscores the evolving nature of vector-borne diseases and the challenges posed by climate change [2,3]. The clinical manifestations of DENV encompass a spectrum ranging from mild flu-like symptoms to severe forms with alert signs or severe forms, characterized by plasma leakage, thrombocytopenia, and vascular instability [4,5]. De Carli et al., recently described three distinct dengue transmission events that occurred simultaneously in the Lazio region; the first event involved two DENV-3-infected cases; the second was sustained by DENV-1, involving 29 cases as of October 16. A third event, with one case of DENV-2, suggested a single intra-familial transmission [6].

Here, we describe the clinical and laboratory findings of 10 autochthons DENV in the metropolitan area of Rome admitted to the Lazzaro Spallanzani National Institute for Infectious Diseases.

2. Cases description

From August to November 2023, 10 patients (pts), 3 males and 7

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females, with a median age of 51 years old (IQR 41–76) with no history of recent international travel and no major concurrent illnesses were admitted to the Lazzaro Spallanzani Hospital in Rome, Italy. Nine patients (90%) presented with arthromyalgia and fever, 6/10 (60%) had skin rash followed by headaches and nausea in 4 of them (40%). Patients were admitted with a median of 6 days from a symptom onset (DSO) (IQR 5–7). Laboratory exams showed a median of 3.900/mm^3 WBC (IQR 2.3–4.6) with lymphopenia (median 890/mmˆ3, IQR 620-2.12), median value of platelets (PLT) of 134.000/mm^3 (IQR 85–211), median value of aspartate aminotransferase (AST) of 59 U/L (IQR 43–87), and alanine aminotransferase (ALT) of 48 U/L (27–72). Normal levels of haemoglobin and creatinine were observed. The PLT nadir was 35.000/mm^3 in pt 3 at 7 DSO while the maximum level of AST was 341 U/L in patient 6 at 12 DSO. At admission, we tested all 10 patients for DENV nonstructural protein 1 (NS1) and IgM and IgG by using fluorimetric rapid assays (Standard F Dengue NS1 Ag FIA and Standard F Dengue IgM/IgG FIA; SD Biosensor, https://www.sdbiosensor.com) [7]. In 9 patients (90%), rapid assay was positive for DENV NS1 antigen, an early marker of acute DENV infection [8]. Rapid IgM were positive in 7 (70%) patients, while IgG in 3 patients (30%). Tests for Chikungunya virus, HIV, hepatitis B virus, and hepatitis C virus were all negative.

Molecular and serologic analyses were longitudinally performed in 6 patients during a 30-day follow-up period. DENV-specific real-time RT-PCR on plasma and blood samples collected within 8 DSO yielded positive results, enabling us to identify in 8 cases a DENV-1 and in 2 cases a DENV-3.

Immunofluorescence assay (IFA) was performed to confirm specific DENV-IgM and IgG, also allowing to have a titre valuable during the follow-up, for both IgM and IgG.

At admission, 7 patients (70 %) had specific DENV IgM and 4 patients (40 %) had IgG only, at day 6 DSO (IQR 4–8).

The presence of DENV-RNA in serum, blood, urine pellet, and saliva was assessed in a subgroup of patients (Table 1). In the first 8 DSO, all patients had positive plasma and serum, 4 had positive saliva, and none had positive urine or eye swab. From 9 to 15 DSO, 2 patients had DENV positive in plasma and saliva, 2 had DENV on serum and urine and 1 on eye swab. From 16 to 23 DSO, no DENV detection was reported apart from one patient with positive urine. From 24 to 30 DSO, no DENV detection on body fluids was observed (data not shown).

3. Discussion

Substantial increase in dengue cases has been reported globally in the last five years, especially in Central and South Americas, where the number of cases has already exceeded eleven million by the end of August 2024, surpassing the peak of 4.6 million cases reported in 2023 [9]. Factors like population movement, climate change, and deforestation are increasing the risk of dengue outbreaks and spreading the disease to new areas [10,11]. Global warming with higher average temperatures, more precipitation and longer periods of drought, changing the distribution of the vectors, could lead to a record-breaking number of dengue infections worldwide. The impact of climate change on vector distribution, endemic areas, and outbreak frequency is evident for many arboviruses including dengue, chikungunya and zika, tick-borne encephalitis, Crimean-Congo haemorrhagic fever and West Nile virus [12].

Aedes aegypti and *Aedes albopictus*, the two main vectors of dengue fever, are both present in Europe [13]. *Aedes albopictus* reached Southern Europe more than 30 years ago and Italy was the first European country where the presence of the 'tiger' mosquito was recognized, in the early 90s [14]. *Aedes albopictus* is established in Italy, southern France, eastern Spain and on the east coast of the Adriatic Sea, and is gradually spreading to the northern latitudes of Europe. *Aedes albopictus* is able to settle in more temperate regions, can tolerate temperate winters and the eggs can survive temperatures of up to −10 °C [15,16]. This resilience has contributed to its northward expansion in Europe. In 2023, 45 cases were detected with self-limited DENV transmission in France [17,18] and 3 cases in Spain with no fatality [9]. Of them, many cases were reported along the coast of the Mediterranean basin, in Balearic Islands, and in major cities such as Paris and Madrid. The transmission can occur even in urban environments as *Aedes* thrives in cities and urban areas have large numbers of returning viraemic travellers who have visited dengue endemic regions.

In Italy, Dengue has always been considered an imported disease for travellers returning from endemic areas. As expected, the travel restrictions during the COVID-19 pandemic were associated to a limited number of DENV virus infections. However, in 2023, Italy reported over 250 DENV cases. As of early 2024, the total number of reported cases had already reached 324, fully reflecting the surging increasing of DENV worldwide infections [19]. Since August 2023, with the first cases detected in our non-endemic country, we have faced a new clinical

Table 1
Characteristics for DENVRNA cycle threshold(CT) in different body fluids during 30 day follow up from symptom onset.

Case	1	2	3	4	5	6
Days after symptom onset						
Within 8 days						
Plasma	22.4	21.03	25.3	26.9	19.6	NA
Serum	18.6	21.7	25.3	26.5	18.4	NA
Urine	Undetected	Undetected	Undetected	Undetected	Undetected	NA
Saliva	29.8	30.7	Undetected	23.8	33.8	NA
Eye swab	Undetected	Undetected	Undetected	Undetected	Undetected	NA
Vaginal swab	Undetected	NA	NA	NA	NA	NA
From day 9 to 15						
Plasma	33.2	38.5	Undetected	Undetected	NA	NA
Serum	35.0	35.3	Undetected	Undetected	NA	NA
Urine	Undetected	35.0	Undetected	Undetected	NA	25.2
Saliva	24.1	28.9	Undetected	Undetected	NA	27.9
Eye swab	Undetected	Undetected	Undetected	Undetected	NA	25.9
Vaginal swab	Undetected	NA	NA	NA	NA	Undetected
From day 16 to 23						
Plasma	Undetected	Undetected	Undetected	Undetected	NA	Undetected
Serum	Undetected	Undetected	Undetected	Undetected	NA	Undetected
Urine	28.9	Undetected	Undetected	Undetected	NA	Undetected
Saliva	Undetected	Undetected	Undetected	Undetected	NA	Undetected
Eye swab	Undetected	Undetected	Undetected	Undetected	NA	Undetected
Vaginal swab	Undetected	NA	NA	NA	NA	Undetected

Data from day 24 until day 30 were not reported since DENVRNA in all biological samples was undetectable. NA not available.

challenge considering the lack of a travel epidemiological link. Our case series provides insights into the clinical and diagnostic findings of the first autochthonous DENV infections in Italy, specifically in the metropolitan area of Rome. Half of the dengue patients were elderly, >70 years old, with no significant comorbidities or coinfections. None of our patients had a severe Dengue. Historically, clinical management strategies in non-endemic setting, were primarily targeted on younger travellers diagnosed with DENV. However, the evolving demographic scenario need to be considered reassessing public health strategies to identify a broader age spectrum of patients, moving from otherwise healthy young subjects to elderly individuals with likely multiples comorbidities.

On average, hospital admissions occurred six days after symptom onset, coinciding with the potential critical phase that occurs between day 3 and day 7 of illness when an increase of capillary permeability may occur. This delay in diagnosis of autochthonous dengue cases in a non-endemic setting, can be attributed to limited familiarity with DENV infections among primary care practitioners and emergency physicians.

We identified two types of Dengue, DENV-1, and DENV-3, linked to different transmission events [6]. Since the dynamic of DENV virus is not well known in the different body compartments, strict monitoring of DENV-RNA in whole blood, serum, urine, saliva, ocular and vaginal swab in a subgroup of patients until day 30 of follow-up was conducted. Moreover, at the same time points, serum samples were tested by IFA to evaluate the titre of specific IgM and IgG. The blood samples tested positive for DENV-RNA during the initial two weeks of DENV infection, while urine samples tested positive from day 9 of symptom onset until the third week.

These results indicate that the time frames for positive detection differ between urine and serum samples. DENV-RNA was detected in blood and serum from the first DSO, whereas in urine, detection was delayed until day 9. This is in line with other studies reporting detection rates of 50 % or higher in serum samples during the first week, while positive urine samples are typically reported from the second week onwards [20,21]. Indeed, DENV detection in urine can be used as a diagnostic tool for late dengue infection, when serum is often negative for DENV [21]. The detection of DENV genome within the first two weeks is not always associated with active viral replication. In a recent paper, we assessed the presence of antigenomic DENV RNA (negative-strand), marker of ongoing replication in blood, during prolonged viral shedding and we suggest that the 5-day isolation period is adequate to prevent secondary transmission cases after mosquito bites [22]. The detection of DENV genome in urine samples during the convalescent phase can be used to identify the viral strain, to characterize viral dynamics even in case of a delayed diagnosis, and to prompt wastewater surveillance programme in areas where the virus is not commonly found [23].The DENV detection in the eye was found during the third week of the disease in a single patient with no ocular symptoms. It is likely to be due to a blood spill during the plasma leakage phase [24,25]. The different DENV ocular manifestations can include uveitis, optic neuritis and even vision threatening complication [26]. The limited data on DENV in the eye needs further research to comprehensively understand its clinical impact.

Our findings highlight the dynamic nature of vector-borne diseases and the growing challenges posed by climate change. The emergence of DENV infections in regions typically associated with temperate mediterranean climates, such as Italy, is a significant concern. The epidemiological patterns and clinical presentations of DENV in non-endemic regions remain largely uncharted, presenting unique challenges in demographic and epidemiological contexts that differ from endemic countries. To effectively prevent and control DENV infections in these regions, increased vigilance, timely diagnosis, and appropriate management are essential.

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Ethical approval

Ethical Committee approval was not required due to the observational nature of the study. Patient’s written informed consent for publication was collected. The data were obtained from medical records and reviewed by a trained team of physicians.

CRediT authorship contribution statement

Serena Vita: Writing – original draft, Conceptualization. **Eleonora Lalle:** Writing – original draft, Formal analysis, Conceptualization. **Priscilla Caputi:** Data curation. **Francesca Faraglia:** Investigation. **Alessandra D’Abramo:** Writing – original draft, Conceptualization. **Licia Bordi:** Formal analysis. **Gabriella De Carli:** Investigation. **Giuseppe Sberna:** Formal analysis, Data curation. **Maria Letizia Giancola:** Writing – review & editing, Investigation. **Gaetano Maffongelli:** Investigation. **Cosmina Mija:** Writing – review & editing. **Andrea Antinori:** Writing – review & editing. **Stefania Cicalini:** Writing – review & editing. **Fabrizio Maggi:** Writing – review & editing, Supervision. **Enrico Girardi:** Writing – review & editing, Validation, Supervision. **Francesco Vairo:** Writing – review & editing, Supervision. **Emanuele Nicastri:** Writing – review & editing, Validation, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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