Rapid communication

Preliminary results on an autochthonous dengue outbreak in Lombardy Region, Italy, August 2023

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In August 2023, six locally acquired dengue virus 1 infections were detected in Lodi province, Lombardy Region, in northern Italy, where the vector Aedes albopictus is present. Four cases were hospitalised, none died. The viruses clustered with Peruvian and Brazilian strains collected between 2021 and 2023. This preliminary report highlights the importance of continued integrated surveillance of imported vector-borne virus infections and the potential for tropical disease outbreaks in highly populated regions of northern Italy where competent vectors are present.

Dengue virus (DENV) infection has become a growing health concern worldwide [1,2]. In the last decades, few autochthonous cases and limited outbreaks have been reported in Europe [3-7]. Here, we describe an outbreak of six autochthonous dengue cases occurring in Lodi province, Lombardy Region in northern Italy in August 2023.

Case description and sampling

On 3 August, an individual (Case 1) living in a village of ca 4,500 inhabitants in the Lodi province was admitted to the emergency department of the local hospital with fever (›39 °C), arthralgia, myalgia, maculopapular rash, confusion and headache. The patient was first discharged with supportive treatment. Six days after symptoms onset, the patient was hospitalised because symptoms persisted. The clinical samples were referred to the Microbiology and Virology Unit of Fondazione IRCCS Policlinico San Matteo, Pavia, for suspected infection with West Nile virus (WNV) on 9 August 2023. Samples were tested for WNV-specific antibodies using West Nile Virus VirClia IgM monotest and West Nile Virus VirClia IgG monotest (VirCell Microbiologists, Spain). Virological investigations were performed with a pan-flavivirus heminested RT-PCR [8] and a WNV-specific RT-PCR [9] on plasma and urine samples.

The pan-flavivirus heminested RT-PCR resulted positive in plasma and urine, while the WNV-specific antibody test and RT-PCR were both negative. A subsequent sequencing analysis revealed the presence of DENV serotype 1 RNA. The diagnosis of DENV infection was confirmed by the presence of viral RNA in plasma and urine by a DENV-specific RT-PCR [10] and detection of DENV IgM antibodies (dengue VirClia IgM monotest and dengue VirClia IgG monotest, VirCell Microbiologists).

Table

Clinical and virological data of dengue cases, Italy, August 2023 (n = 6)

Demographic and clinical characteristics				Antibody (index)		Pan-flavivirus PCR		DENV-specific RT-PCR (copies/mL)		Sequencing
Case	Hospitalisation	Days from symptom onset to sampling	Sample date	IgM	IgG	Plasma	Urine	Plasma	Urine	Typing
1	Yes	6	9 Aug	12.5	<0.9	Positive	Positive	3.5×10^5	2,025	DENV-1
		20	23 Aug	34.5	1.3	Negative	Positive	<45	<45	
2	No	18	22 Aug	32.2	1.6	Negative	Positive	<45	990	NA
3	Yes	6	22 Aug	12.9	<0.9	Positive	Negative	2.3×10^6	<45	DENV-1
4	Yes	2	23 Aug	<0.9	<0.9	Positive	Negative	15×10^6	<45	DENV-1
5	No	4	25 Aug	3.8	<0.9	Positive	Positive	6.4×10^5	630	DENV-1
6	Yes	6	25 Aug	25.7	<0.9	Positive	Positive	1×10^5	<45	DENV-1

DENV: dengue virus; NA: not available; RT-PCR: reverse transcription PCR.

Antibody index was considered negative when <0.9 and positive when >1.1; DENV-specific RT-PCR was considered negative when <45 copies/mL and positive when ≥45 copies/mL.

Further, IgG seroconversion was documented in a second sample, collected after 15 days (Table). Following the DENV diagnosis of Case 1, between 22 and 25 August, five additional people living in a neighbouring area of the same village of the first case were referred to the local hospital for suspected DENV infection and confirmed as for Case 1. Their details are summarised in the Table. The six cases were living at a maximum linear distance of 450 m from each other. The first case did not share outdoor activities with the other dengue cases. All six patients had symptoms including fever, arthralgia, myalgia and headache and note had travelled in the past months. At the moment of writing, all six cases were alive.

Sequencing

Whole genome sequencing (WGS) was performed using the metagenomic approach as previously described by Piralla et al. [11]. Reads were mapped to the genome ON123600 using the INSaFLU pipeline (https://insaflu.insa.pt) [12]. Phylogenetic analysis was performed on WGS obtained directly from clinical samples of five dengue cases (GenBank accession numbers: OR512925-OR512929) (Figure). A maximum likelihood phylogenetic tree was inferred using the IQ-TREE web server (v1.6.8) [13], the robustness of branches was evaluated using the Shimodaira–Hasegawa approximate likelihood-ratio test (SH-aLRT) and ultrafast bootstrap approximation tests. The Italian strains clustered with Peruvian and Brazilian strains collected in the period 2021 to 2023, with an average nucleotide identity of 98.6% (range: 95.1–99.9) between DENV-1 strains of genotype V.

Public health measures

When the first dengue case, defined according with the Commission Implementing Decision (EU) [14], was notified to the Local Health Authority (ATS) on 18 August 2023, ATS immediately carried out the epidemiological investigation. The patient reported that they had not travelled abroad in the weeks before the onset of symptoms and not had contact with people who had travelled to DENV-endemic areas. In addition, no travel-associated cases were reported in the area. Thus, Case 1 was classified as autochthonous and immediate notification was given by the ATS to the Regional and National Health Authorities. During the epidemiological investigations, Case 1 stated that they spent many hours a day outdoors, thus a careful mapping of the frequented places was carried out. Disinfestation activities for mosquito control (Cipermetrin and Permofen Forte; 1R-trans Phenothrin and Tetramethrin) were carried out around their home and in the areas they frequented. The neighbours of Case 1 and subsequent dengue cases were informed on mosquito prevention and control. Disinfestation was performed for each case as described above.

Hospitals and physicians in the Lodi province were notified to remain alert for patients with possible dengue symptoms. The population of the village where DENV cases were detected was informed about DENV.

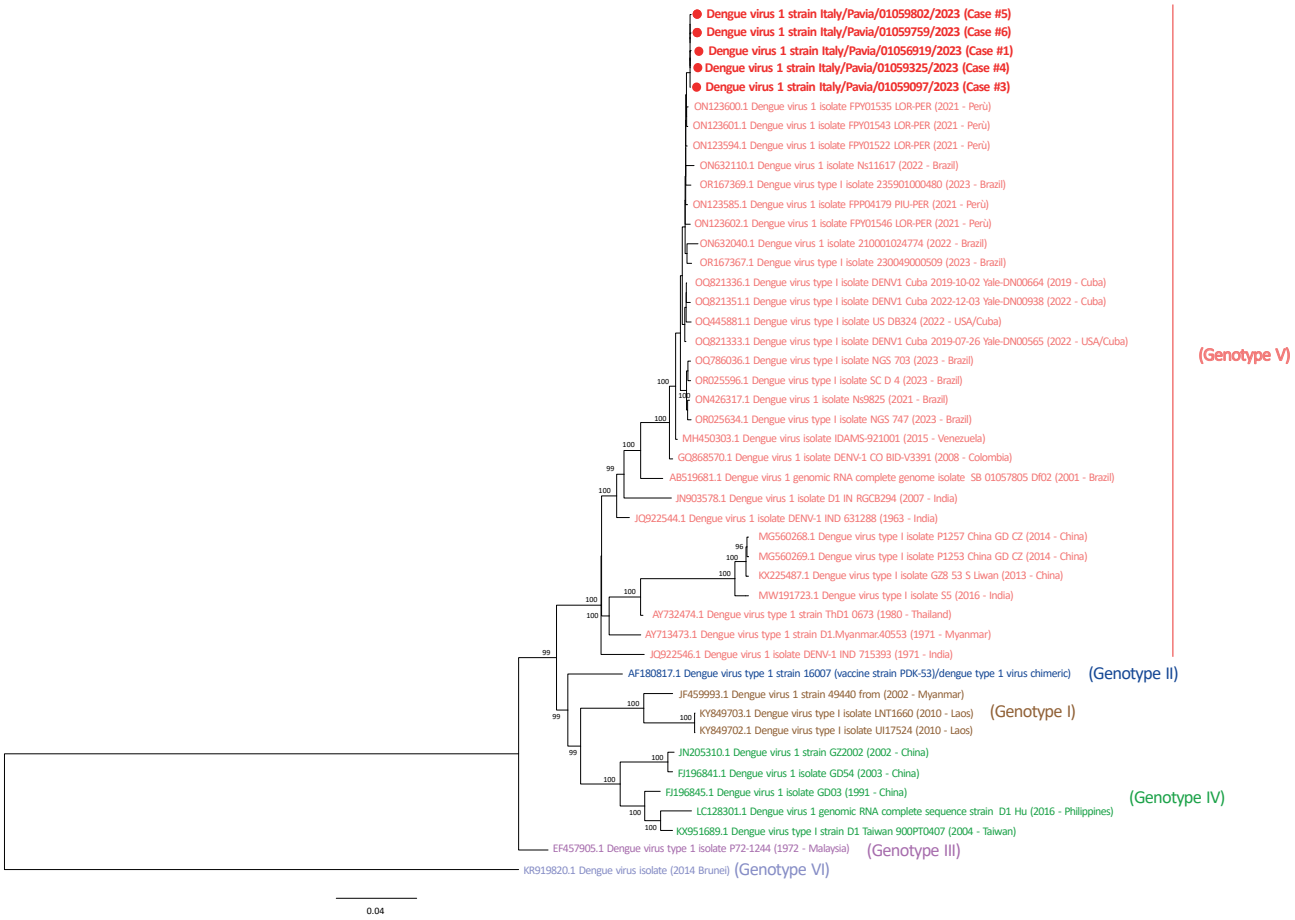
Entomological investigations

According to the Italian *National Plan for Prevention, Surveillance and Response to Arboviruses* [15], specific entomological inspections were carried out within a radius of 200 m around the homes and places frequented by the diagnosed dengue cases. Inspection of the home address was conducted within 2 days of the notification of each suspected or confirmed case. Adult mosquitoes were captured using different traps. The captured mosquitoes were immediately sent refrigerated to the laboratory at Istituto Zooprofilattico Sperimentale della Lombardia e Emilia-Romagna (IZSLER). Mosquitoes were morphologically identified using taxonomic keys [16] and pooled according to the date and site of collection.

These entomological inspections were performed 13 days after symptom onset of Case 1. By 30 August, 131 *Aedes albopictus* females had been sampled,

Figure

Phylogenetic tree of dengue virus serotype 1 complete genome sequences, Italy, August 2023 (n = 5 samples from the current outbreak)



DENV: dengue virus.

Sequences from the cases in this manuscript are shown in bold. Reference sequences were obtained from GenBank.

divided into eight pools and processed for flavivirus genome detection. The mosquito pools were homogenised, viral RNA was extracted and analysed with the same pan-flavivirus RT-PCR assay as used for the human samples [8]. To date, all tested mosquito pools analysed were negative. More samplings are scheduled at different sites until 7 September, close to the homes of the other five dengue cases.

Discussion

DENV infections have been reported in more than 100 countries [17], the vast majority in Asia, South Pacific Islands, the Carribbean and Latin America [18]. However, in the last decades, an increasing number of autochthonous cases have occurred in European countries were competent *Aedes* mosquitos are present. In 2022, 65 autochthonous dengue cases were reported in France [19]. In Italy, *Aedes albopictus* was reported for the first time in the 1990s [20] and it is likely that it has been present in the Lombardy Region in the Lodi province since the mid-1990s [21]. The vector is today present throughout Italy, and the first documented outbreak of autochthonous dengue occurred in the Veneto Region in 2020 [6,22]. Moreover, at the moment of writing, three autochthonous cases of DENV infection have been also reported in central Italy [23].

Effective surveillance systems for imported and autochthonous arboviruses infection as well as vector control programmes are active in Lombardy Region, in accordance with the Italian *National Plan for Prevention, Surveillance and Response to Arboviruses* [15]. Timely reporting of all suspected and confirmed cases to the local Public Health Unit is mandatory for consequent activation of vector control measures and active case finding. In addition, specific entomological surveillance is regularly performed when clinical cases of infection with DENV, Zika virus, chikungunya virus, West Nile virus or any other arboviruses are diagnosed.

Previous undiagnosed autochthonous dengue cases in northern Italy cannot be excluded, especially if asymptomatic. A seroprevalence screening of the inhabitants of the village in Lodi province is ongoing in order to identify potential asymptomatic cases or recently infected people who had not travelled abroad. The extent of the outbreak may be defined only after the population screening. This study will be useful to reveal the extent of the DENV outbreak in this area, as reported by another study in Madeira [24]. Active clinical, epidemiological, virological and entomological surveillance is still ongoing in the area involved in dengue outbreak aimed at detecting further secondary cases.

This report documented the first outbreak of DENV-1 in Lombardy region, highlighting the importance of continued integrated surveillance of imported virus infections and the potential for tropical disease outbreaks in the highly populated regions of northern Italy where *Ae. albopictus* has been present for many years [25]. Our findings suggest that the use of pan-flaviviruses PCR is fundamental for the differential diagnosis of the major pathogenic arboviruses belonging to the Flavivirus family. In parallel, virological surveillance of adult *Aedes* mosquitoes may be crucial for early identification of circulating arboviruses and rapid definition of local and regional public health measures that can control or prevent future outbreaks.

Conclusion

This preliminary report highlights the importance of continued integrated surveillance of imported vector-borne virus infections and the potential for tropical disease outbreaks in highly populated regions of northern Italy where competent vectors are present.

Ethical statement

Ethical approval was not needed for this study because human samples were collected as part of surveillance activities and the analysis was conducted as part of public health practice.

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Data availability

Sequence data generated in this study have been submitted to GenBank under accession numbers OR512925-OR512929.

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Conflict of interest

None declared.

Authors' contributions

Wrote the manuscript: IC, GF, FR, SS, MFac, FD, AL, AM, AP, MM; managed the patient: SPag, AR, Lombardy Dengue Network; performed laboratory investigations: GF, AP, IC, GC, AS, SPao, AM, DL, FD, Lombardy Dengue Network; collected samples: LC, SPag, AR, Lombardy Dengue Network; revised the manuscript: FR, FB, SS, MFac, AM, AL, FD, DC; coordinated the study: FB, DC, MFac, AL; performed and coordinated the entomological surveillance: AL, AM, FD, DL, MFar, Lombardy Dengue Network; coordinated and performed the public health interventions: MFac, SS, ER, MB, DC, LV, MT, Lombardy Dengue Network.

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