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Autochthonous dengue outbreak in Northern Italy, September 2024: epidemiological, microbiological, entomological investigation and public health response

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ABSTRACT

Background: Dengue is an emerging public health threat in temperate regions of Europe where *Aedes albopictus* is established. In 2024 Italy experienced an unprecedented increase in autochthonous dengue transmission. In September 2024, a cluster of locally acquired dengue cases was detected in Cavezzo, Emilia-Romagna, Northern Italy.

Methods: An outbreak investigation was initiated following confirmation of the first autochthonous dengue case on 17 September 2024. Enhanced surveillance was activated including retrospective case finding, targeted community screening, and strengthened laboratory diagnostics. Vector control measures were implemented starting within a 200m radius of confirmed cases and expanded based on epidemiological and entomological findings. Blood and organ donation safety measures were introduced, and whole genome sequencing were performed on human and mosquito samples.

Results: 44 autochthonous dengue cases were identified with symptom onset between 31 July and 23 September 2024; 68.2% were detected retrospectively. No epidemiologically linked cases were detected outside Cavezzo. Most cases occurred in older adults (median age 63 years); two patients were hospitalised and recovered fully. Dengue virus serotype 2 (DENV-2), genotype II, lineage F.1.1, was detected in both human and *Ae. albopictus* samples. Phylogenetic analysis identified a distinct cluster separate from sequences associated with the

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contemporaneous outbreak reported in Fano, Italy. Following vector-control interventions, mosquito density declined and no further virus-positive pools were detected.
Conclusion: This outbreak highlights the increasing vulnerability of temperate European regions to dengue transmission and underscores the importance of rapid case detection, community engagement, and targeted vector-control strategies in preventing wider spread.

1. Introduction

Dengue is a mosquito-borne viral infection transmitted by Aedes species and represents a growing global public health concern with an estimated 100–400 million infections annually, mainly in tropical and subtropical regions [1,2]. Although not endemic in mainland EU/EEA countries, the establishment of *Aedes albopictus* across much of Europe has enabled local transmission, and autochthonous cases have increasingly been reported since 2010 [3]. Climatic and environmental changes have contributed to expanding vector suitability, and in 2024 several European countries, including Italy, France and Spain, reported large autochthonous outbreaks with sustained local transmission [4–7].

In Italy previous autochthonous dengue transmission was limited to sporadic small outbreaks, including in Veneto (2020) and later in Lazio and Lombardy [8–10]. Recent risk assessments have identified Italy as among the European countries most vulnerable to local transmission of dengue and chikungunya [11]. Northern regions such as Emilia-Romagna are considered particularly suitable due to dense urbanisation, favourable climatic conditions, and widespread vector presence [12].

In September 2024 regional health authorities detected a cluster of locally acquired dengue cases in Cavezzo, a municipality of approximately 7,000 inhabitants in the province of Modena (Emilia-Romagna). Here we describe the epidemiological, microbiological and entomological investigations and public health measures implemented following identification of the first autochthonous case associated with this outbreak.

2. Methods

2.1. Public health measures

Dengue surveillance in Italy is conducted year-round and coordinated by the Istituto Superiore di Sanità (ISS) through a National Arbovirus Surveillance System, as defined by the National Arbovirus Plan 2020–2025 (PNA) [13]. The PNA provides a structured framework for surveillance and response measures aimed at preventing the introduction and local spread of arboviral diseases and ensuring timely public health action in response to changing epidemiological conditions in Europe. Within this framework, the surveillance system monitors both imported and autochthonous cases and is based on mandatory notification supported by laboratory confirmation.

The PNA case definition criteria were applied to assess all suspected cases in this dengue outbreak. A probable case was defined as an individual showing symptoms consistent with dengue (fever plus at least two of nausea and/or vomiting, rash, body aches, or headache) in addition to positive serology for IgM antibodies. A confirmed case required laboratory confirmation through either detection of viral RNA, dengue viral antigen (NS1), or the presence of dengue-specific IgM antibodies, with confirmation by neutralisation, seroconversion, or a four-fold increase in antibody titres in paired serum samples [14].

After the detection of the first dengue case on 17 September, general practitioners and physicians at the Emergency Departments in Carpi and Mirandola, the main referral hospitals for the municipality, were contacted and alerted to monitor patients with dengue-like symptoms, including both residents and visitors to the municipality (Fig. 1). They were asked to retrospectively search for and report patients seen in August or early September 2024 with symptoms suggestive of dengue, or

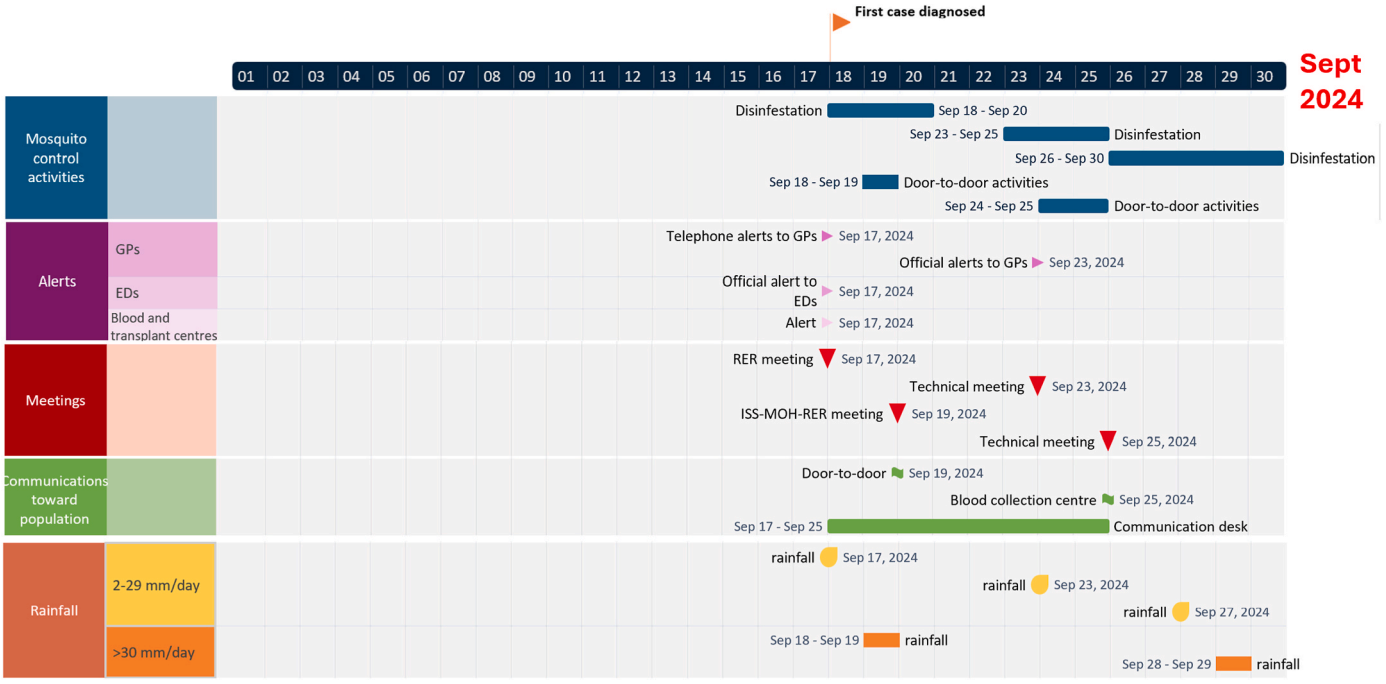

Fig. 1. Timeline of public health actions during the Cavezzo dengue outbreak, September 2024. Abbreviations**:** EDs, Emergency Departments; GPs, General Practitioners; ISS, Istituto Superiore di Sanità; MoH, Ministry of Health; RER, Regione Emilia-Romagna.

with history of travel to Fano municipality, where a recent autochthonous dengue outbreak had been also reported [6]. Subsequently, all emergency departments in the region were alerted via an official announcement on 21 September by the Emilia-Romagna Public Health Unit. This was also sent to all local hospitals and primary care centres, urgent care centres, community paediatric services and laboratories.

A communication desk operated from 17 to 25 September to encourage symptomatic individuals to seek care. From 26 September to 5 October, a dedicated blood collection centre was set up in Cavezzo, where anyone with symptoms or travel history to endemic areas were invited for testing. Samples were sent to the Regional Reference laboratory at the IRCCS Azienda Ospedaliero-Universitaria di Bologna for microbiological evaluation and positive results reported to the National Arbovirus Surveillance System.

From 17 September, specific measures to ensure blood and organ donation safety were introduced in the municipality of Cavezzo [15]. Screening using DENV NAT testing was performed on all blood donors in Cavezzo, as an alternative to temporary interruption of donations in the affected area. At the national level, all donors were requested to disclose any recent travel history to Cavezzo. Donors with a positive history were temporarily deferred or, alternatively, screened by DENV NAT molecular testing. All donors were instructed to inform their reference blood establishment in case of symptoms compatible with dengue infection, or in case of diagnosis of DENV infection, within 14 days following the donation. Screening was also extended for organ, tissue and hematopoietic stem cell donations throughout the province until 15 November.

A regional emergency group, comprising of representatives from local public health authorities and the municipality, was convened on 17 September and met regularly thereafter. This group monitored the epidemiological trend and coordinated containment measures, formally concluding emergency operations on 8 October.

2.2. *Vector control measures and entomological investigations*

Following notification of the first case on 17 September, the extraordinary operational vector control protocol of the Emilia-Romagna Arbovirus Surveillance and Control Plan (PSCA-ER) was applied from 18 to 20 September within a 200m radius of the patient's residence (Fig. 2, Site 1) [16]. The protocol comprises three coordinated actions: night-time adulticide treatments in public areas for three consecutive days; door-to-door adulticide and larvicide applications in private properties, including removal of movable breeding sites; and larvicide treatment of public road drains. For the adulticidal treatments, an authorised commercial product based on deltamethrin and prallethrin synergised with piperonyl butoxide was used, while larvicide treatments employed a silicone-based monomolecular film with larvicidal and pupicidal activity.

The protocol was subsequently implemented around the residences of two additional cases in the northern sector (Fig. 2, Sites 2 and 3). As the number of confirmed and suspected cases increased, on September 23 the regional emergency group implemented enhanced measures, extending disinfestation to the entire northern sector and, following further case notifications and four virus-positive mosquito pools (including one in the southern sector), to the remaining inhabited area (Fig. 2). Due to heavy rainfall, the final sector was completed on October 10. In order to ensure compliance with door-to-door adulticide and antilarval treatments, the municipal mayor issued an urgent directive 24 hours before the residence visits to explain the importance of treatment and request residents to permit access to their private courtyard and gardens. Together with the ordinance, citizens received the information necessary to mitigate the risks associated with adulticide treatments.

Independent entomologists conducted continuous surveillance in accordance with PSCA-ER, assessing intervention quality by comparing pre and post-treatment adult and larval densities in public and private areas. Adult mosquitoes were collected using Human Landing Catch (HLC). To minimize HLC-related risk, two expert entomologists performed standardized 5-minute sessions, exposing only a small portion of one leg and aspirating females upon landing. Female *Aedes albopictus* mosquitoes were collected, pooled (maximum of 10 specimens per pool), and tested by real-time PCR [17]. For every sampling 8 to 25 HLC sessions were performed in different shaded and vegetated sites in the same private and public area. Sampling was performed to evaluate abundance and infection rate of the vector near the newly reported cases

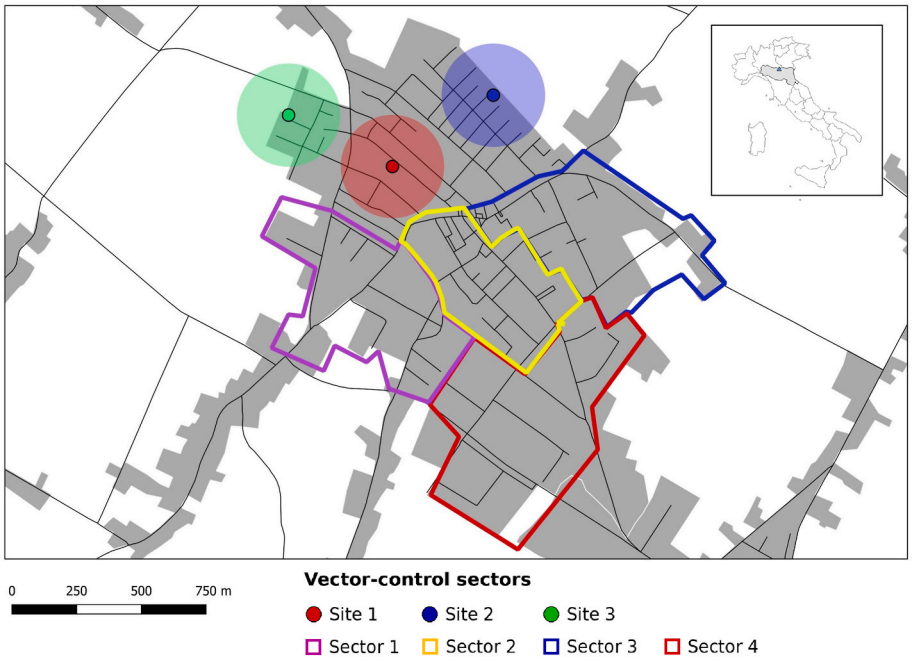

Fig. 2. Map of Cavezzo municipality. Locations of the first three notified autochthonous dengue cases (coloured circles indicating a 200m intervention radius then extended to the remaining parts of this northern sector) and four urban sectors targeted by successive enhanced vector-control interventions (outlined polygons). In the top-right corner, a map of Italy indicates the Cavezzo municipality with a blue triangle.

(pre-treatment samplings) and for evaluating the efficacy of insecticidal treatments (post-treatment sampling). Post-treatment results guided additional adulticide applications where residual densities remained above acceptable thresholds [18–20].

2.3. Laboratory investigations

2.3.1. Molecular and serological diagnostics

According to WHO diagnostic guidelines, dengue infection is confirmed based on at least one of the following test results: DENV detected by RT-PCR; DENV isolation by culture; four-fold increase in IgM or IgG titre between paired sera; or DENV antigen (NS1) detection [21]. RT-PCR is highly specific but viraemia declines rapidly within the first 5-7 days of infection, narrowing the window of molecular detection [22]. Demonstration of seroconversion or a four-fold increase in antibody titres requires paired blood samples collected 7-14 days apart, with confirmation by neutralisation assays because of cross-reactivity with other flaviviruses [23].

Molecular and serological diagnostics for both dengue virus (DENV) and West Nile virus (WNV) were performed on all suspected cases. Specifically, RT-PCR was conducted on whole blood, serum, plasma and urine samples using RealStar Dengue 3.0 and RealStar West Nile Virus 2.0 kits (Altona Diagnostics GmbH, Germany). IgM and IgG antibodies for DENV and WNV were tested using VirClia Monotest (VirCell Microbiologists, Spain) and indirect immunofluorescence assays (Euroimmun, Lübeck, Germany).

In our investigation, detection of dengue virus (DENV) RNA in blood or urine was used to confirm acute dengue infection. Serological evidence of recent infection was defined by the presence of anti-DENV IgM together with IgG seroconversion or a fourfold increase in antibody titre, consistent with the typical IgM peak approximately 1-2 weeks after symptom onset [20]. Detection of IgG in the absence of IgM was interpreted as evidence of past dengue or other flavivirus infection (Table 3).

2.3.2. Whole genome sequencing and genomic analysis of human and *Aedes albopictus.* samples

Total RNA extracted from DENV-positive human clinical samples and mosquito pools (cycle threshold < 30) was subjected to whole genome sequencing (WGS) using Illumina-based approaches. For human samples, WGS was performed using the Illumina Viral Surveillance Panel v2 [24] on an Illumina MiSeq platform (Illumina Inc., San Diego, United States), generating 2×150 bp paired-end reads. Consensus genome sequences and viral genotypes were determined using the Genome Detective DENV Typing Tool [25].

For mosquito pools, WGS was carried out following a protocol previously described by Sacco et al., 2024 [6]. Nearly complete consensus genome sequences and genotype/lineage assignments were obtained using the "DENV – serotype calculation and mapping" and "DENV-lineage identification" pipelines implemented in Genpat (https://genpat.izs.it/).

2.3.3. Phylogenetic inference

A maximum-likelihood phylogenetic tree was inferred using IQ-TREE with automatic model selection and 1000 ultrafast bootstrap replicates, based on a dataset of 498 sequences, including one outgroup sequence (2II); 486 global human 2II.F1.1 isolates (collected from 1 January 2023 to 31 December 2024 and retrieved from the GISAID repository (https://gisaid.org/publish/); six from the 2024 dengue outbreak in Fano, Italy [6]; and five from the outbreak investigated in this study. Tree topology and clade structure were inspected using MEGA version 12.0.11 (1993-2025). Additional details on the sequence selection, alignment, phylogenetic methods and model parameters are provided in the Supplementary Materials. Phylogenetic analysis was performed to assess genetic relatedness and clustering patterns. No phylogeographic reconstruction or molecular clock analysis was conducted.

2.3.4. Epidemiological methods

Epidemiological and clinical information was collected for all dengue cases through the National Arbovirus Surveillance System maintained by ISS. For each case, demographic variables (age and sex), date of symptom onset, clinical presentation, hospitalisation status, and place of likely exposure were recorded. Cases were classified by age group according to predefined categories (10–19, 20–39, 40–59, 60–79 and 80–99 years). Frequencies and proportions of each symptom were calculated overall and stratified by age group and sex. Median age and interquartile ranges (IQR) were computed for the study population. Hospitalised and non-hospitalised cases were compared descriptively.

Temporal trends were assessed using the date of symptom onset to construct the epidemic curve. Cases were classified as retrospectively detected if identified after outbreak recognition but with symptom onset before 10 September (the date of symptom onset of the index case), through retrospective record review or targeted community screening. Cases identified following activation of enhanced surveillance were classified as prospectively detected.

Spatial analyses were conducted by geolocating cases according to the place of likely exposure. To ensure confidentiality, all coordinates were anonymised by applying a 15m random white-noise displacement before mapping. All analyses were conducted using R (version 4.3).

3. Results

3.1. Initial detection and case finding

On 16 September 2024, a 63-year-old woman residing in Cavezzo presented to Mirandola Emergency Department with fever and joint pain, with symptom onset on 10 September. Initial laboratory tests were carried out for West Nile virus (WNV), as she lived in a high-risk area for WNV transmission. Following a negative result clinicians requested testing for dengue virus and on 17 September, this returned positive for dengue virus serotype 2 (DENV-2). In the absence of recent travel to endemic areas, the Local Health Authority of AUSL Modena (LHA) and the Regional Health Authority of Emilia-Romagna (RHA) met remotely to discuss an epidemiological investigation and control measures. The decision was made to initiate active public health surveillance to identify potential secondary cases, following recommendations set out in the PNA.

In total 90 people were tested for dengue, 9 at the Emergency Department of Mirandola Hospital and 81 at testing centres. A total of 44 autochthonous dengue cases were identified, including 35 classified as confirmed and 9 as probable. Based on symptom onset dates, a substantial proportion of cases were identified retrospectively through enhanced screening. Specifically, 30 cases (68.2%) had symptom onset before 10 September and were therefore detected retrospectively, while 14 cases (31.8%) were identified prospectively during active case finding (Fig. 3).

The date of first symptom onset was 31 July 2024, and the final date of symptom onset was 23 September 2024, as illustrated in Fig. 3. The first laboratory confirmed case was reported to the Italian National Health Institute (ISS) on 17 September 2024 and the last on 5 October 2024.

3.2. Epidemiological and clinical characteristics

Among the 44 cases, 25 (56.8%) were female, and the median age was 63 (IQR 42.8 – 73.5). Most cases (47.7%) were in the 60-79 age group. 97.7% of cases experienced fever (Table 1). Among other symptoms, the most commonly reported were systemic manifestations including asthenia (84.1%), arthralgia (70.5%), rash (50.0%), and myalgia (22.7%). Gastrointestinal upset was reported less frequently (nausea or vomiting in 13.6%, and diarrhoea in 11.4%). A small number of cases experienced retro-orbital pain (11.4%). There were no significant differences in frequency of symptoms reported between males and

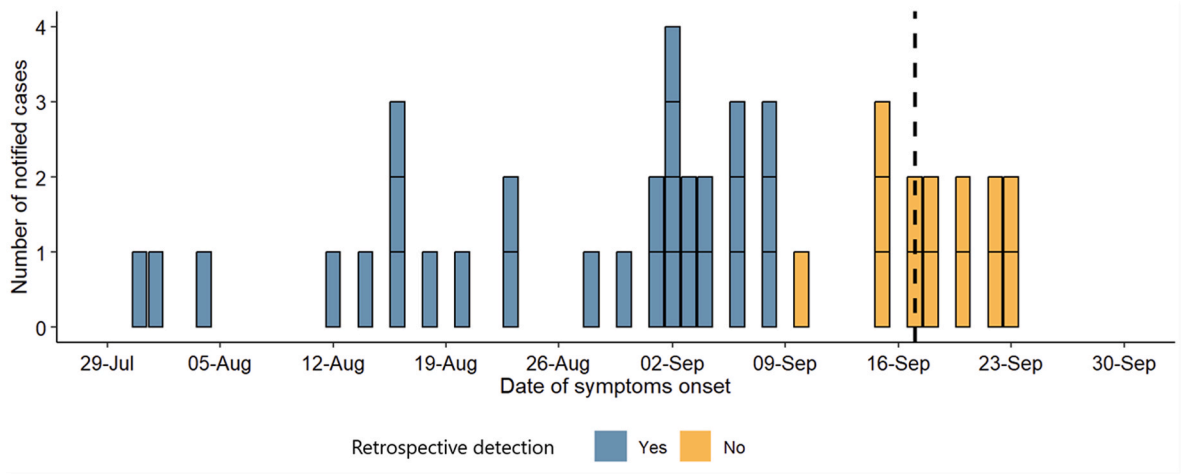

Fig. 3. Epidemic curve of autochthonous dengue cases in Cavezzo outbreak by date of symptom onset, July-September 2024 (n = 44). In blue cases identified retrospectively through enhanced screening; in yellow cases identified prospectively through surveillance activities. The dashed line refers to the date of notification of the first case.

Table 1
Demographic and clinical characteristics of cases in Cavezzo outbreak.

	Total (n = 44)		Hospitalised (n = 2)	
Age category	n	%	n	%
10-19	2	4.5	0	0
20-39	8	18.1	0	0
40-59	8	18.1	0	0
60-79	21	47.7	2	100
80-99	5	11.4	0	0
Sex				
Female	25	56.8	2	100
Male	19	43.2	0	0
Symptoms				
Fever	43	97.7	2	100
Asthenia	37	84.1	1	50
Arthralgia	31	70.5	1	50
Rash	22	50	0	0
Myalgia	10	22.7	0	0
Nausea/vomiting	6	13.6	0	0
Diarrhoea	5	11.4	0	0
Retro-orbital pain	5	11.4	0	0

females. When comparing the age distribution of participants experiencing or not experiencing each symptom, cases in the age group 60-79 experienced the majority of all symptoms.

Two cases were hospitalised, both women aged 60–79 years with comorbidities. They both required antibiotic therapy and one additionally received intravenous steroids and oxygen therapy. Both recovered fully. Symptom profiles for hospitalised and non-hospitalised cases are shown in Table 2.

3.3. Blood and transplant safety

During the screening of blood, organ, tissue and hematopoietic stem cell donations covering the period 17 September to 15 November 2024, no positive cases of dengue were detected. By 15 November over 2,400 blood donations were tested, all yielding negative results. On the same date, a total of 21 organ/tissue donors, 36 corneal donors, and 36 bone tissue donors underwent testing, confirming the absence of DENV infections.

Table 2
Proportion of cases in Cavezzo outbreak presenting with fever, asthenia, arthralgia and rash by age group and sex.

Age category	Fever (n = 43)		Asthenia (n = 37)		Arthralgia (n = 31)		Rash (n = 22)	
	n	%	n	%	n	%	n	%
10-19	2	4.7	2	5.4	1	3.2	1	4.5
20-39	8	18.6	7	18.9	8	25.8	8	36.4
40-59	8	18.6	8	21.6	6	19.4	6	27.3
60-79	21	48.8	15	40.5	15	48.4	15	68.2
80-99	4	9.3	5	13.5	1	3.2	1	4.5
Sex								
Female	25	58.1	21	56.8	16	51.6	14	63.6
Male	18	41.9	16	43.2	15	48.4	8	36.4

Table 3
Serology and virology results for DENV IgG, IgM and RNA (n = 44) in blood and/or urine samples.

	DENV IgG		DENV IgM		DENV RNA	
	n	%	n	%	n	%
Positive	36	81.8	38	86.4	35	79.5
Negative	8	18.2	6	13.6	9	20.5

3.4. Laboratory investigations

All 44 patients underwent molecular and serology testing for dengue infection. Serological analysis revealed that 38/44 patients (86.4%) tested positive for DENV IgM and 36 patients (81.8%) also tested positive for DENV IgG. Virological confirmation via the detection of DENV RNA in whole blood, serum, plasma and/or urine samples was achieved in 35 patients (79.5%), indicating a high prevalence of laboratory-confirmed cases. Dengue virus serotype 2 (DENV-2) was detected in all sequenced cases. As Modena province is endemic for WNV, a parallel testing protocol for WNV was implemented for all suspected cases. Notably, all nine probable cases (Dengue IgM positive, DENV-RNA negative) tested negative for WNV-IgM and WNV-RNA. This ruled out WNV infection and supported the dengue diagnosis, providing the necessary evidence to exclude cross-reactivity.

3.5. Genotype identification and phylogenetic clustering

Genome Detective Tool identified all five sequences outbreak isolates generated in this study - from both human and *Ae. albopictus*. pool samples - as DENV-2 genotype II, lineage F.1.1. This classification was also supported by maximum-likelihood phylogenetic analysis. All outbreak sequences were nearly identical, showing only rare single-nucleotide substitutions. Sequences clustered together, indicating circulation of the same virus in both human and mosquito hosts during the outbreak. The outbreak isolates formed a distinct cluster separate from the sequences associated with the dengue outbreak in Fano, Italy, which occurred during the same period as this outbreak. This was despite the Cavezzo isolates belonging to the same lineage, suggesting independent viral introductions into the two areas. Within the analysed dataset, the phylogenetically closest genomic sequences were individual strains of DENV-2 genotype II lineage F1.1. deposited in GISAID in 2023 (EPI_ISL_19132422, EPI_ISL_19081926, 28, 36, EPI_ISL_18510393), exhibiting the shortest genetic distances in the maximum-likelihood tree. The

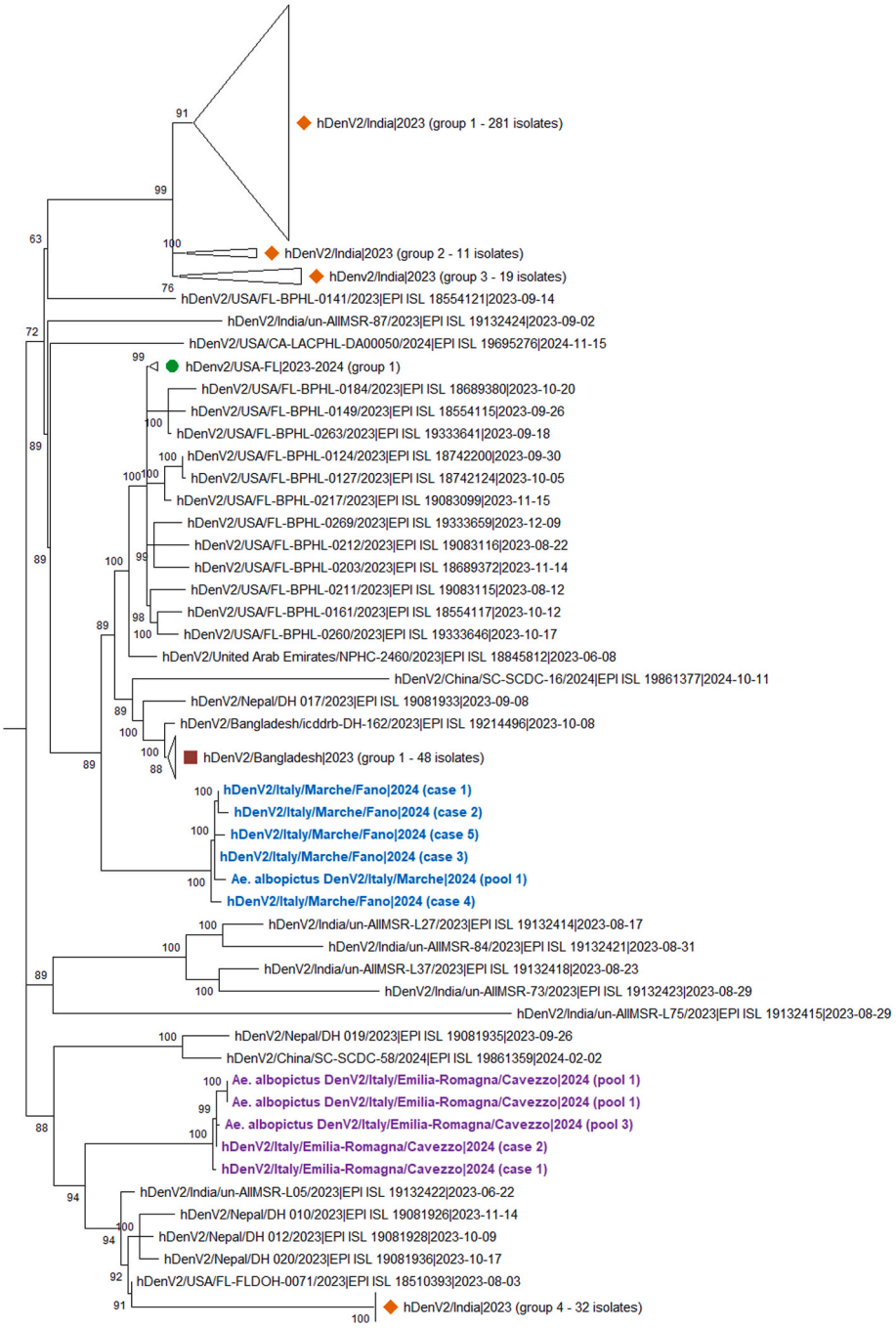

Fig. 4. Maximum-likelihood phylogenetic subtree of DENV-2 genotype II, lineage F.1.1 sequences, including Italian cases and mosquito pools, in relation to global isolates (2023-2024). The subtree was extracted from a full phylogeny of 498 isolates inferred under the GTR+F+G4 substitution model. Node labels indicate ultrafast bootstrap support (1000 replicates). Sequences included in the subtree show ≥98% nucleotide similarity to the five outbreak isolates and to sequences from the 2024 dengue outbreak in Fano, Italy. The Cavezzo outbreak cluster is highlighted in purple and Fano in blue. Closely related sequence groups were collapsed for clarity. Phylogenetic clustering reflects nucleotide similarity within the analysed dataset and does not imply specific geographic origin. The full phylogenetic tree, including the scale bar, is provided in Supplementary Material Fig. 5.

sequences were reported from India, Nepal and USA (Florida) (Fig. 4). A subtree is shown in Fig. 4, while the full phylogenetic tree is provided in Fig. 5, Supplementary Material.

3.6. Vector control measures and entomological investigations

Adult mosquitoes were sampled in two phases defined relative to the implementation of vector-control measures (pre- and post-intervention). Prior to insecticide application, 749 mosquitoes were collected across 60 sampling stations and screened in 83 pools (maximum 10 mosquitoes per pool). Four pools (4.8%) tested positive for DENV RNA by RT-PCR [26]. Three positive pools were detected on 20 September within 200m of the index case residence, and a fourth was identified on 25 September near the household of the fourth confirmed case in the southern sector of the municipality. Following the implementation of adulticide treatments, mosquito density decreased sharply. During the post-treatment samplings, 95 mosquitoes were collected in 75 sampling stations and tested in 12 pools, and no DENV-positive pools were detected.

The full set of entomological collections is shown in Table 4. Minimum infection rates (MIR) were 1.2% on 20 September and 0.5% on 25 September, corresponding to the sampling dates with detected positive pools. No infected mosquitoes were found after 25 September, suggesting that vector-control actions were effective in interrupting viral circulation in the local *Ae. albopictus* population.

4. Discussion

This outbreak represents a large cluster of autochthonous dengue transmission in northern Italy and highlights the increasing vulnerability of temperate regions in Europe to arbovirus spread. A total of 44 locally acquired cases were confirmed with symptom onset ranging from 31 July to 23 September 2024, indicating sustained prolonged transmission over nearly two months. The prolonged period of transmission in this cluster reflects both the capacity of *Ae. albopictus* to sustain virus circulation in favourable climatic conditions and the challenges of promptly detecting and containing dengue outbreaks in non-endemic settings. Notably, during the same period, a large autochthonous dengue outbreak was reported in Fano, in the neighbouring Marche region, underscoring a broader national context of increased dengue activity in Italy during the 2024 transmission season [6].

Whole genome sequencing enabled precise identification of outbreak isolates and confirmed the genomically quasi-identical virus between human and *Ae. albopictus* at this location. Phylogenetic analysis further showed that, although the Cavezzo and Fano outbreaks belonged to the same DENV-2 genotype and lineage, they formed distinct clusters within the global phylogeny, supporting the hypothesis of independent introductions rather than sustained spread between the two locations. The closest genomic neighbours identified in our sequences' dataset were sequences deposited in GISAID in 2023 from India, Nepal and the USA. However, given the widespread global circulation of DENV-2 genotype II [27], this phylogenetic proximity reflects overall nucleotide similarity within a globally circulating lineage and does not imply a specific geographic origin. The precise origin of the virus introduction cannot be determined based on phylogenetic inference alone.

The demographic profile of cases showed a predominance of older adults, with a median age of 63 years and nearly half of cases occurring in individuals aged 60–79. This distribution is likely driven by differences in clinical presentation and case detection rather than true age-related differences in susceptibility. Older adults may be more likely to develop symptomatic or severe disease and therefore present to healthcare services, although diagnosis may still be delayed due to the non-specific nature of symptoms. In contrast, infections in children may be milder and therefore under-detected.

Age-stratified symptom proportions (Table 2) indicated that older individuals more frequently presented with non-specific systemic manifestations, such as asthenia and generalised pain, rather than classical dengue features. This finding is consistent with growing evidence that dengue incidence and the risk of severe outcomes increase with age, likely due to age-related impaired immune function and atypical clinical presentation leading to delayed diagnosis [28–31]. In this outbreak, the high frequency of systemic symptoms suggests that classical dengue presentations may be less apparent in older individuals, potentially contributing to delays in clinical suspicion and diagnosis. Notably, both hospitalised cases occurred in elderly females with underlying comorbidities, and their full recovery following supportive therapy highlights the importance of early identification and prompt management in vulnerable populations.

Information on prior dengue infection or vaccination status was not available within the surveillance dataset and therefore the potential role of pre-existing immunity cannot be directly assessed. However, dengue is not endemic in Italy. While recent autochthonous outbreaks, including the contemporaneous outbreak in Fano, demonstrated that sustained transmission can occur at a local level, these events remain geographically confined and do not indicate endemic circulation. In addition, dengue vaccine QDENGA® was authorised in Italy only in 2023. It is not included in the national immunisation programme and its use remains limited. Taken together, these elements suggest that pre-existing immunity is unlikely to have substantially influenced the observed clinical or serological findings.

Importantly, more than two-thirds of cases were identified retrospectively through enhanced screening, indicating that transmission had been ongoing for several weeks before the outbreak was recognised. This highlights the challenges of early dengue detection in non-endemic settings, particularly in the absence of travel history. In such contexts surveillance relies heavily on clinical suspicion, and dengue is often considered only in patients with a history of international travel. Despite early-season communications from public health authorities recommending testing for arboviruses in patients with compatible symptoms regardless of travel history, this 'travel-history bias' may have delayed

Table 4
Adult mosquito collections, pool testing, and minimum infection rates (MIR) before and after vector-control interventions.

Sample date	Phase	Sector*	N stations	N mosquitoes collected	Pools tested	Positive pools	MIR (%)
20/09/2024	Pre-treatment	Site 1	25	243	23	3	1.2
21/09/2024	Post-treatment	Site 1	20	26	3		
25/09/2024	Pre-treatment	Sector 2	9	115	20	1	0.9
25/09/2024	Pre-treatment	Sector 3	6	99	10		
26/09/2024	Post-treatment	Site 2/Site 3	24	20	2		
27/09/2024	Pre-treatment	Sector 1	10	84	9		
27/09/2024	Pre-treatment	Sector 4	10	208	21		
07/10/2024	Post-treatment	Sector 2	7	5	1		
09/10/2024	Post-treatment	Sector 1	8	33	4		
10/10/2024	Post-treatment	Sector 3	8	5	1		
14/10/2024	Post-treatment	Sector 4	8	6	1		

*Sector refers to the intervention areas shown in Fig. 2, including Sites 1–3 (200m radius) and the four urban sectors.

notification and diagnostic testing. As a result, a period of silent transmission likely occurred, as reflected in the epidemic curve (Fig. 3). Delayed outbreak recognition may also have resulted in under-ascertainment of mild or pauci-symptomatic infections during the early stages of transmission, potentially leading to an underestimation of the true incidence.

At the same time, the retrospective identification of cases through enhanced screening demonstrates the added value of active case finding in reconstructing transmission dynamics and guiding timely public health interventions. Targeted screening was implemented among high-risk groups, including symptomatic individuals and asymptomatic individuals with recent travel to endemic areas, to maximise case detection.

Despite a delay in initial detection and sustained transmission, no epidemiologically linked cases were identified outside the municipality of Cavezzo. While this suggests successful containment at the municipal level, the possibility of limited spillover cannot be excluded, particularly given the delay in case detection and the reliance on passive surveillance in surrounding areas. Nonetheless, coordinated and comprehensive public health measures following confirmation of the first case likely contributed to limiting transmission. Proactive interventions including retrospective case-finding and intensive vector control, combined with strong community engagement supported by municipal ordinances, public communication, and police-assisted access to private properties, enabled high coverage of door-to-door adulticide and larvicide treatments. The area also routinely manages arboviral surveillance due to recurring WNV activity, contributing to a high level of awareness among healthcare staff and the public. Entomological monitoring demonstrated a marked decline in mosquito density and the absence of virus-positive pools following interventions, supporting the contribution of these measures in interrupting local transmission, although seasonal changes in vector dynamics and heavy rainfall during the intervention period may have also influenced this reduction.

Implementation of blood and organ donation restrictions in affected areas was a critical component of preventing secondary transmission, and no transfusion or transplant related dengue transmissions were identified during the enhanced screening period.

Overall, this outbreak highlights the need for continued preparedness activities in European regions where *Ae. albopictus* is established. As climate change continues to extend the geographic range and seasonal activity of competent vectors, the likelihood of future dengue outbreaks in temperate settings is expected to increase. Strengthening routine surveillance, maintaining rapid diagnostic capacity, and promoting early clinical suspicion are essential to minimise delays in outbreak detection. Equally important are community engagement and intersectoral collaboration, which proved critical to the successful implementation of control measures in this setting.

5. Conclusion

This autochthonous dengue outbreak in Northern Italy highlights the growing public health threat posed by arboviruses in temperate regions where *Ae. albopictus* is established. The identification of 44 locally transmitted cases over a two-month period, primarily affecting older adults, underscores the importance of clinical vigilance particularly as dengue symptoms in elderly individuals may be non-specific.

Delays in identifying suspected cases likely contributed to underdetection of early cases, emphasising the need for heightened awareness among healthcare providers during the mosquito season, even in the absence of travel to endemic areas. The rapid deployment of coordinated public health measures, including enhanced surveillance, targeted vector control, laboratory diagnostics, blood and organ donor screening and strong community engagement, likely contributed to limiting transmission within the municipality and further spread.

These findings reinforce the importance of maintaining robust surveillance systems, emergency preparedness plans, and cross-sector collaboration to respond effectively to emerging vector-borne disease threats in Europe, particularly in countries like Italy where the conditions for local transmission are increasingly met. As climate change continues to expand the geographic range and seasonality of vector activity, sustained investment in prevention, public awareness, and rapid response capacity will be essential to mitigate the impact of future outbreaks.

CRediT authorship contribution statement

Jasleen Singh: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Formal analysis. **Tiziana Lazzarotto:** Writing – review & editing, Validation, Project administration, Methodology, Investigation. **Christina Merakou:** Writing – review & editing, Visualization, Methodology, Investigation. **Giada Rossini:** Writing – review & editing, Methodology, Investigation. **Maurilia Marcacci:** Writing – review & editing, Methodology, Investigation. **Guido Di Donato:** Writing – review & editing, Methodology, Investigation. **Rodolfo Veronesi:** Writing – review & editing, Methodology, Investigation. **Antonino Bella:** Writing – review & editing, Project administration, Methodology. **Giulietta Venturi:** Writing – review & editing, Methodology, Investigation. **Marco Di Luca:** Writing – review & editing, Project administration, Investigation. **Arianna Puggioli:** Writing – review & editing, Methodology, Investigation. **Ilaria Pati:** Writing – review & editing, Project administration, Investigation. **Raffaele Donadio:** Writing – review & editing, Project administration, Investigation. **Rino Biguzzi:** Writing – review & editing, Project administration, Investigation. **Nicola Alvaro:** Writing – review & editing, Project administration, Investigation. **Alessandra Fantuzzi:** Writing – review & editing, Project administration, Investigation. **Flavia Riccardo:** Writing – review & editing, Project administration. **Giacomo Creola:** Writing – review & editing, Project administration, Investigation. **Paola Angelini:** Writing – review & editing, Project administration, Investigation. **Giulio Matteo:** Writing – review & editing, Project administration, Investigation. **Mattia Calzolari:** Writing – review & editing, Project administration, Investigation. **Michele Dottori:** Writing – review & editing, Project administration, Investigation. **Patrizio Pezzotti:** Writing – review & editing, Validation, Supervision, Project administration, Conceptualization. **Chiara Sacco:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Software, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Martina Del Manso:** Writing – review & editing, Writing – original draft, Supervision, Software, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Francesco Severini:** Investigation. **Luciano Toma:** Investigation. **Caterina Vocale:** Investigation. **Silvia Galli:** Investigation. **Liliana Gabrielli:** Investigation. **Greta Roncarati:** Investigation. **Giulia Piccirilli:** Investigation. **Simona Venturoli:** Investigation. **Evangelia Petrisli:** Investigation. **Giorgio Gallinella:** Investigation. **Luca Ruotolo:** Investigation. **Beatrice Mola:** Investigation. **Isabella Bon:** Investigation. **Margherita Ortalli:** Investigation. **Fabrizia Valleriani:** Investigation. **Angela Taraschi:** Investigation. **Chiara Pinoni:** Investigation. **Barbara Secondini:** Investigation. **Valeria Di Lollo:** Investigation. **Alessandro Albieri:** Investigation. **Romeo Bellini:** Investigation. **Marco Carrieri:** Investigation. **Roberta Colonna:** Investigation. **Luciano Donati:** Investigation. **Debora Ruggeri:** Investigation. **Sabina Sanseverinati:** Investigation. **Elisabetta Fioretti:** Investigation. **Barbara Mai:** Investigation. **Carmela Palomba:** Investigation. **Miriam Benatti:** Investigation. **Giuseppe Diegoli:** Investigation. **Giovanna Mattei:** Investigation. **Stefano Benedetti:** Investigation. **Cristina Liverani:** Investigation. **Monica Soracase:** Investigation. **Esther Rita De Gioia:** Investigation. **Claudio Gualanduzzi:** Investigation. **Cinzia Perilli:** Investigation. **Alessia Leucci:** Investigation. **Annalisa Grisendi:** Investigation. **Mara Scremin:** Investigation. **Katia Marzani:** Investigation. **Deborah Torri:** Investigation. **Letizia Lombardini:** Investigation.

Ethical statement

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

Declaration

The entomologists performing the HLC were employees of the Centro Agricoltura Ambiente 'G. Nicoli,' trained and qualified in mosquito collection with over 25 years of experience. Specifically, the HLC technique adopted was conducted based on a specific internal operational instruction that regulates the duration of the catch session and the extent of the skin area exposed to the necessary minimum, thereby limiting the risk of bites. The personnel involved were engaged through informed consent, being fully aware of the risks associated with this monitoring method, as well as their right to withdraw from the investigation at any time without any consequence.

Use of artificial intelligence tools

ChatGPT 5.2 (Open AI) was used to support with refining R code and grammar correction of the manuscript. The authors confirm the use of artificial intelligence tools did not contribute to the analysis or scientific content and the authors retain full responsibility for the content of the manuscript.

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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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tmaid.2026.102979.

Data availability

The data that support the findings of this study are available upon reasonable request in compliance with data protection regulations. All genome sequences were deposited in NCBI (https://submit.ncbi.nlm.nih.gov/) with the following accession identification codes: PX579132, PX579133, PX579134, PX578252, PX869148, PX869190.

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