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Molecular epidemiology and surveillance of imported dengue in travellers returning to Spain, 2022-2024

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ABSTRACT

Introduction: Dengue is the most significant vector-borne viral disease in global morbidity and mortality, and it is considered a re-emerging disease spreading into new regions. The risk of autochthonous dengue virus transmission in Spain remains high due to the increasing number of travellers returning from endemic areas and the presence of *Aedes albopictus* within our territory. Conducting epidemiological and molecular studies on returning travellers from endemic areas may be crucial to discern transmission patterns and track the global spread of the virus. This study focuses on the molecular characterization of suspected imported dengue cases from 2022 to 2024.

Methods: We analysed 600 samples from 539 suspected dengue-infected travellers between 2022 and 2024. All samples were tested by a quantitative RT-PCR, and PCR-positive cases were confirmed by performing a non-overlapping nested RT-PCR, going under subsequent sequencing to identify viral diversity.

Results: Of the 539 suspected cases, 183 were confirmed as DENV-positive, with Cuba as the most common travel origin associated with infections. Molecular analysis of positive samples identified all four DENV serotypes and ten genotype groups, with DENV-3 genotype III as the most predominant. Additionally, among confirmed cases reported from regions with *Aedes albopictus* presence, 73% (94/129) occurred during the vector's active season (May–November), increasing the risk of local transmission.

Conclusion: Our results highlight the importance of returning travellers as sentinels for ongoing dengue outbreaks and epidemiology in endemic regions. Additionally, these emphasize the importance of early case identification through sentinel surveillance to prevent potential autochthonous transmission.

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1. Introduction

Dengue is the most significant vector-borne viral disease in terms of global morbidity and mortality, with over 390 million new infections annually and currently prevalent in 128 countries [1–3].

Dengue virus (DENV) is classified into four serotypes (DENV-1 to DENV-4), further divided into 4-6 major lineages (genotypes) [4] that exhibit regional and temporal patterns. These changes may arise from chance or variations in the virus's adaptation to fit the vector and human reservoir to be effectively transmitted [5].

DENV is mainly transmitted by *Aedes* mosquitoes, especially *Aedes aegypti*. However, the spread and adaptation of *Aedes albopictus* to temperate climates has increased the risk of autochthonous dengue transmission in Europe [6], where imported cases can trigger local outbreaks under favourable environmental conditions.

In recent years, the Americas have experienced an unprecedented increase in dengue cases, with record transmission reported in 2024 and a particularly alarming increase in Brazil, Argentina and Peru [7]. European travellers frequently acquire dengue while visiting these endemic regions and may introduce the virus upon return. Once introduced, the risk of autochthonous transmission remains significant, as demonstrated by recent outbreaks in Europe. Since 2010, locally acquired cases have been reported in European countries, with an important increase since 2022 in countries such as France and Italy. In 2024, the EU/EEA reported 304 cases, three times more than in 2023, mainly in Italy (n = 213) and France (n = 83), with sporadic cases in Spain (n = 8) [8].

In this context, Spain represents a vulnerable setting for dengue due to the high travel volume to endemic regions and the widespread presence of *Ae. albopictus*. Imported DENV cases have increased, and autochthonous transmission has been reported since 2018 [8]. As international travel continues to rise, dengue-infected viremic travellers are expected to remain a key factor shaping the risk of local transmission.

Although several epidemiological studies have described imported dengue cases in Spain, updated information integrating post-pandemic surveillance and nationwide distribution of imported cases remains limited. Previous works have focused on specific regions, such as Catalonia [9,10], and no analyses have been conducted in this country since the COVID-19 pandemic, despite the global increase in dengue incidence.

Therefore, this study aimed to characterize imported dengue cases detected in Spain during 2022-2024, which will contribute to the understanding of global dengue circulation during this period while emphasizing the role of traveller-based surveillance in monitoring viral spread. Additionally, as samples were received from most Spanish regions, we aimed to describe the geographical distribution of imported cases and identify areas where the overlap with competent vector presence can increase the risk of autochthonous transmission.

2. Methods

2.1. Sample collection

Samples were obtained among those that were routinely received in the National Centre of Microbiology, Institute of Health Carlos III (NCM-ISCIII) from a broad network of Spanish hospitals.

A suspected dengue case is defined as a patient with residency or travel history in the previous 15 days to a dengue endemic area, or to a non-endemic dengue area but with vector's presence, who presented fever plus two of the following symptoms or haematological findings: myalgia, arthralgia, intense headache, retro-orbital pain, low back pain, rash, nausea, or vomiting [11].

A total of 600 samples from 539 patients collected between 2022 and 2024 were analysed. Among these, 71 patients provided two samples, while the remaining patients provided a single sample. Samples included five types: blood (n = 280), serum (n = 265), urine (n = 28), cerebrospinal fluid (CSF) (n = 14), and plasma (n = 10).

2.2. Samples data

Samples data were codified with a unique number to ensure the anonymity of patients. Epidemiological data collected were collection date, type of sample, symptoms onset and travel origin.

2.3. Ethical statement

The cases reported in this study were investigated with routine procedures according to the national surveillance plan for vector-borne diseases. A unique ID to ensure the anonymity of patients and no patient identifiers were included in the study.

2.4. Viral RNA extraction

Viral RNA was obtained using the QIAmp Viral RNA Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions.

2.5. Molecular diagnosis

Screening for DENV detection was performed using a quantitative reverse transcription-PCR (qRT-PCR) and positive results were subsequently confirmed by a non-overlapping specific nested RT-PCR. Patients were considered confirmed DENV cases when positive amplification was obtained with both methods.

When multiple samples were obtained from the same patient, all samples were independently tested by both amplification methods. In cases where more than one sample was positive, the sample with the strongest nested RT-PCR amplification product was selected for sequencing to ensure optimal template quality.

2.5.1. Quantitative RT-PCR

DENV genome was detected using a qRT-PCR which discriminates between Zika, Chikungunya and Dengue viruses, using a mix of Roche reagents: LightMix Modular CHKV 500, LightMix Modular DENV 580, LightMix Modular ZIKV 640, LightMix Modular EAV RNA Extraction Control and the LightCycler® Multiplex RNA Virus Master Kit. 10 μL of RNA was added to 15 μL of the master mix. Amplification was performed in the LightCycler®480 II (Roche): an initial reverse transcription step at 55 °C for 30 min, followed by a denaturation at 95 °C for 5 min and 45 cycles of amplification (95 °C for 5 s, 60 °C for 40 s and 72 °C for 15 s). A final cooling was carried out at 40 °C for 30 s.

2.5.2. Nested RT- PCR

Viral RNA from positive samples was subjected to a nested RT-PCR [12] to amplify a 328 nt fragment corresponding to the E-NS1 junction of the DENV genome.

2.6. DNA sequencing and phylogeny

Positive samples were sequenced from the obtained nested fragment by Sanger. Consensus sequences for each segment were assembled and analysed using the SeqMan program (DNASTAR, https://www.dnastar.com).

Phylogenetic trees were generated by using MEGA software (Molecular Evolutionary Genetics Analysis, Version 10) programme [13]. The phylogenetic trees were built using the Neighbor-Joining method based on partial sequences of the E-NS1 segment of the virus. The bootstrap consensus trees inferred from 1000 replicates and values < 50 are not shown. The evolutionary distances were computed using the p-distance method and are in the units of the number of base differences per site.

2.7. Statistical analysis

To determine statistical differences in the distribution of dengue serotypes by year and continent of travellers, we performed a chi-square analysis. Only sequenced cases with a known geographic origin were included. A p-value <0.05 was considered statistically significant. All analysis were conducted using Python (version 3.13).

3. Results

3.1. Imported dengue infections

From 539 suspected cases, 229 (42.5%) showed a positive result by real-time RT-PCR although only 183 (33.95%) were confirmed by nested-RT-PCR (2022, n = 60; 2023, n = 44; 2024, n = 79) (Fig. 1). The remaining 46 qRT-PCR positive samples were not confirmed by nested RT-PCR and were therefore excluded for further analyses.

The monthly distribution of imported cases throughout the study period shows that, across all three years, the number of cases reached its peak in August, with additional peaks in April and September of 2024.

The symptom onset date was available for 150 (82%) of the 183 confirmed DENV-positive patients. Samples from 131 patients were collected within the recommended period for viral RNA detection (from 0 to 7th days after symptom onset [11]), while the remaining samples were collected between the 8th and 20thday. Their positive cycle threshold (CT) values depend on the type of sample and the number of days post-symptom onset, setting blood and serum samples as the most reported ones in the early stages, while two urine samples tested positive at days 18 and 20 (Fig. 2).

3.2. Region of notification and presence of the vector

During the study period, 62 hospitals from several regions across Spain referred samples to the NCM. However, this represents only a subset of all hospitals in the country, meaning the data do not capture the full national case distribution. Among the 183 confirmed cases, Madrid had the highest number of notifications (75 cases (41%)).

A total of 129 cases (all of them viremic) were sent by hospitals located in regions where *Ae. albopictus* is established (Aragon, Basque Country, Castile-La Mancha, Catalonia, Extremadura, Galicia, Madrid, Murcia, Navarra and Valencian Community) [14] (Fig. 3). Ninety-four of the 129 cases detected in regions with *Ae. albopictus* presence (73%) were reported during the active period of the vector in Spain (May-November [15]).

3.3. Travellers' history

Of the 183 confirmed DENV-positive patients, travel origin was known for 138, who came (Table 1) from 33 countries across five continents.

Over the three-year period of our study, most of the infected travellers with a known geographic origin returned from the Americas (n = 106, 76.8%), followed by Asia (n = 27, 19.6%) and Africa (n = 3, 2.2%), with a marked seasonal peak occurring between July and September. In 2022, this peak was primarily associated with travellers returning from Cuba (n = 30, 54%), same as 2024 (n = 10, 37%) (Supplementary Table 1). In contrast, cases in 2023 were distributed throughout the year and involved travellers from 18 different countries. In 2024, the number of countries from which infected travellers returned increased to 19, including Italy [Table 1 near here].

3.4. Phylogenetic analysis

Among the confirmed DENV-positive patients, serotype was obtained from 150 individuals. All four serotypes were detected, with DENV-3 as the most prevalent (n = 75; 50%), followed by DENV-2 (n =

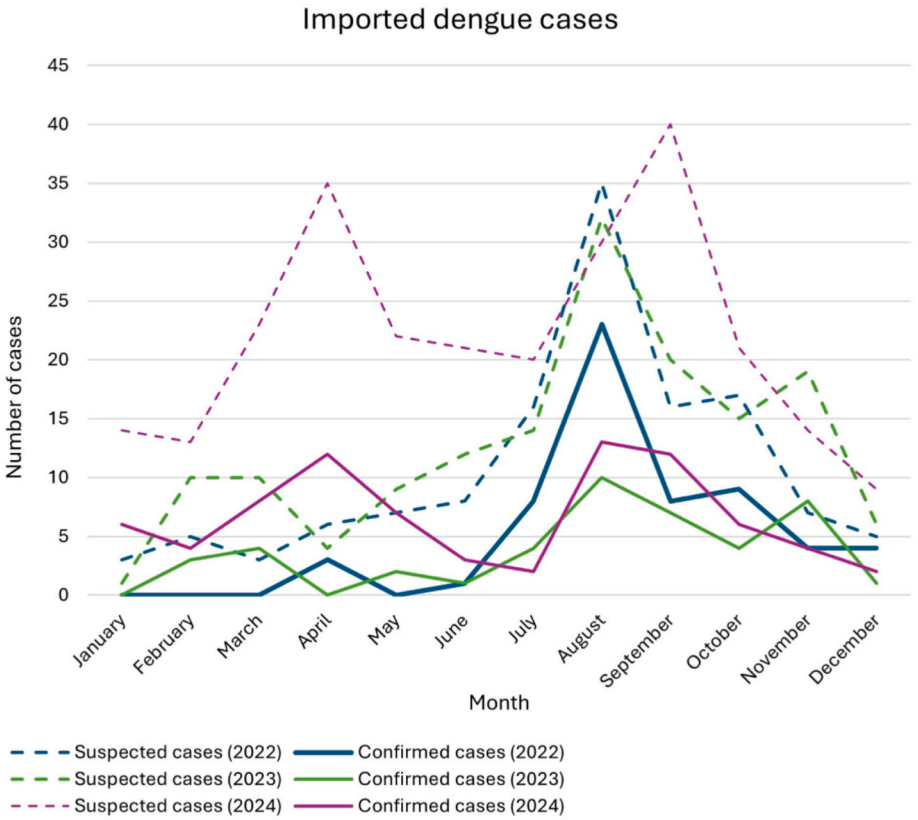

Fig. 1. Number of suspected and confirmed imported dengue cases by month, Spain, January 2022 – December 2024 (suspected n = 539; confirmed n = 183).

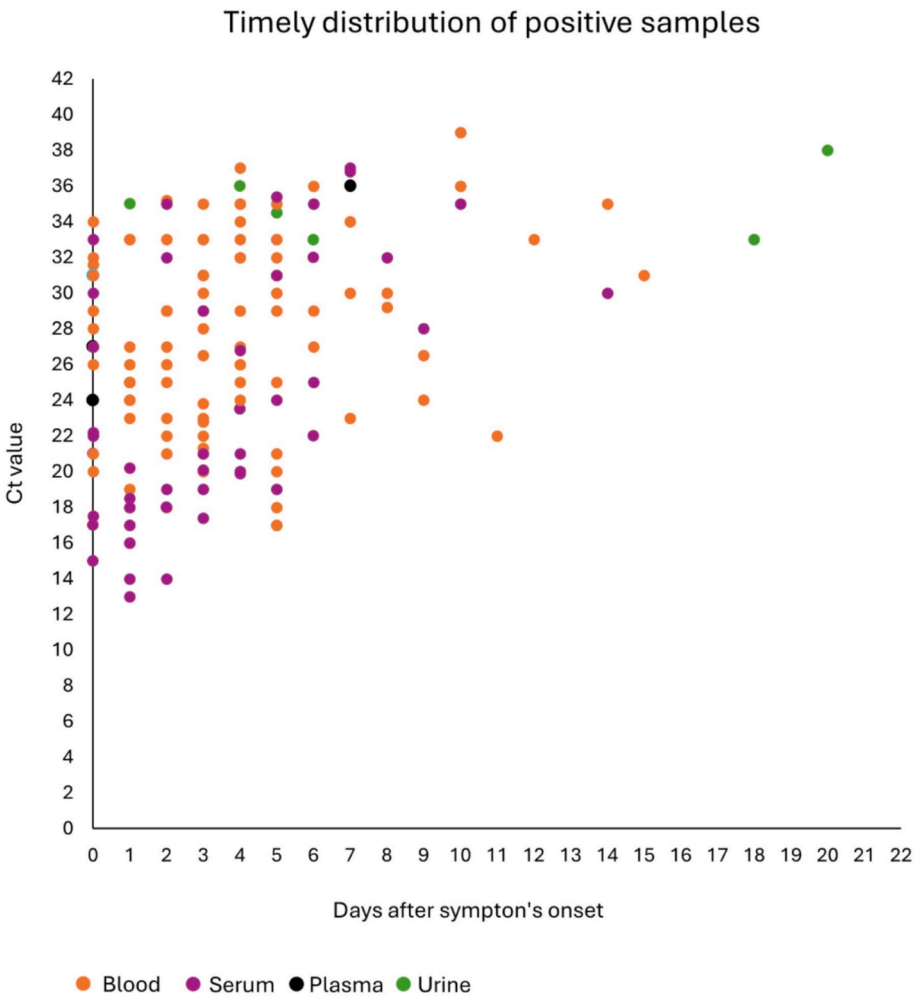

Fig. 2. CT values obtained from positive samples of 150 patients with a known date of symptom's onset by type of sample.

35; 23.3%), DENV-1 (n = 25; 16.7%) and DENV-4 (n = 15; 10%). The distribution of serotypes by year and continent is showed in Fig. 4.

DENV-3 was the most reported serotype throughout the study period, especially among travellers returning from the Americas. In 2022, the month-wise distribution of serotypes indicated that DENV-1 persisted throughout the whole year, while DENV-3 predominated from July to August and was exclusively associated with travellers from Cuba. In 2023, DENV-3 was consistently reported during the entire year and was identified in travellers from eight countries. Additionally, higher genetic variability was found within DENV strains detected from 2023 travellers. In 2024, the serotype distribution was more balanced among DENV-3 (n = 23), DENV-2 (n = 18), and DENV-1 (n = 11). DENV-4 was the least reported serotype (n = 8), but interestingly, it was the most frequently detected among Cuban travellers that year.

Analysis of serotype distribution by continent revealed that DENV-3 was the dominant serotype in the Americas, whereas DENV-2 was more commonly detected in our travellers from Asia. Only three cases were sequenced from Africa and one from Europe, limiting the statistical comparisons between continents. A chi-square test indicated no significant differences in serotype distribution across years of the study (χ^2 = 10.576; p = 0.1024), but significant differences were found across continents (χ^2 = 20.488; p = 0.0151).

3.4.1. *Dengue serotype 1*

Three DENV-1 genotypes were detected: I (n = 2), III (n = 5) and V (n = 18) (Supplementary Fig. 1a). Most genotype V strains were detected in travellers who returned from America during the whole period, whereas one sequence clustered with African strains. The rest of genotypes were only found in specific years: G.III (2022 and 2024); G.I (2023).

3.4.2. *Dengue serotype 2*

For DENV-2, we found three different genotypes: Asian-American (n = 9), Asia I (n = 1) and Cosmopolitan (n = 25) (Supplementary Fig. 1b). Although all genotypes were represented each year, most of the Cosmopolitan strains were detected in 2024.

3.4.3. *Dengue serotype 3*

DENV-3 was the most frequently detected serotype in our study population. Genotype III was the most prevalent lineage (n = 73), and nearly all G.III strains were detected on travellers returned from America, with a few exceptions: one case in 2023 from Thailand, the Italian case in 2024, and another case from the Philippines in 2024. Only two DENV-3 cases from the Asian continent were classified into another genotype (G.I) (Supplementary Fig. 1c).

3.4.4. *Dengue serotype 4*

Only genotype II was identified in our study (Supplementary Fig. 1d). Most cases were associated with travellers returning from the Americas. In 2022, these strains were exclusively detected in Cuba, whereas in 2023 and 2024, they were also reported in Nicaragua and Colombia. Within this genotype, two major clades were identified: one comprising all American strains that could be further subdivided in two subclades, and another linked to Asia.

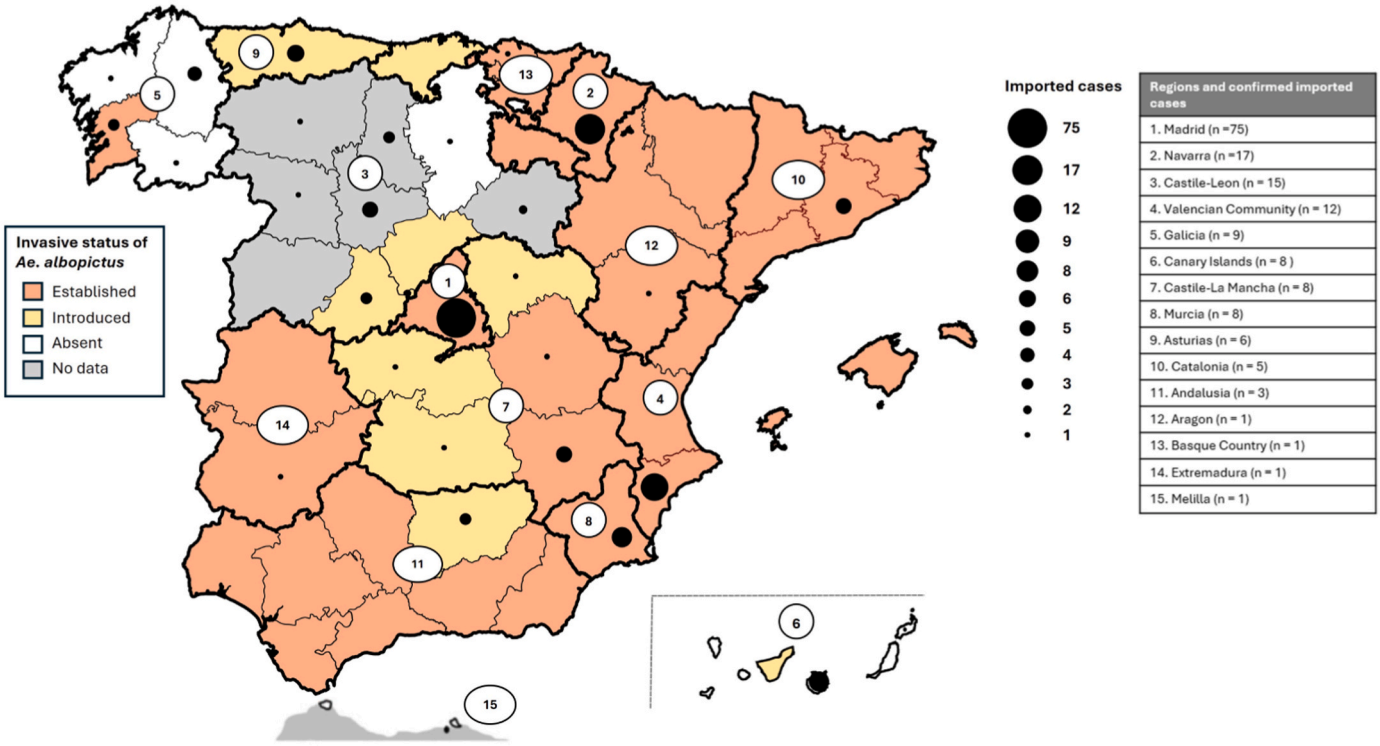

Fig. 3. Geographical location of confirmed imported dengue cases and presence of *Aedes albopictus* in provincial regions of Spain, 2022-2024.

4. Discussion

The drastic decline in dengue reporting during the COVID-19 pandemic, driven by lockdown measures and travel restrictions [16,17], was followed by a major rebound once international travel resumed, leading to an unprecedented increase in imported dengue cases in non-endemic countries. This global pattern was also observed in Spain, where imported dengue increased substantially in 2022 and 2023, with 358 and 398 reported cases [18,19], respectively.

In 2024, amid a global record of dengue cases of nearly 14 million cases [20], Spain confirmed its highest number of imported cases (n = 788) [21], of which 79 were diagnosed at the NCM. This rising trend was also evident in the autochthonous cases detected both in Spain and across Europe, underscoring the increasing risk of local transmission associated with infected travellers entering regions with established *Aedes* vectors.

All cases in this study were diagnosed and confirmed using molecular methods, which remain the gold standard for dengue diagnosis due to their speed, sensitivity and specificity. Only cases with positive results by both qRT-PCR and nested RT-PCR were included as confirmed DENV. The difference in sensitivity between the two assays explains the 46 discrepant cases (20% of all qRT-PCR positives). These samples showed high Ct values (mean Ct = 37.4), suggesting low viral load near the detection limit of the confirmatory assay.

Blood and serum were the most frequently collected sample types and showed the highest positivity during the early days of infection, whereas the persistence of viral RNA in urine supports its use as an additional sample for dengue diagnosis, even in travellers who attend the hospital during later phases of the disease, after clearance from blood or serum. Our findings are consistent with previous reports [22–25] and offer practical guidance for clinicians in the collection of samples for evaluating febrile travellers after their return from endemic regions.

Travel patterns influenced the epidemiological profile of our imported cases. Most infected travellers were diagnosed in August, probably coinciding with Spain's peak travel season. The predominance of cases from the Americas, particularly Cuba, followed by Asia, aligns with the data reported by the National Epidemiological Surveillance Network (RENAVE) [18,19,21] and reflects the global increase of dengue transmission.

In this study, we claim the value of traveller-based genomic surveillance. All four dengue serotypes were introduced into Spain during the study period, with DENV-3 being the most detected serotype, particularly among travellers from the Americas. The predominance of genotype III (97%) among DENV-3 cases is consistent with its reemergence in the region, including Cuba's largest outbreak in 15 years reported in 2022 [7,26]. Phylogenetic analysis suggests that this genotype was introduced from Southeast Asia around late 2020 [27] and quickly became the dominant lineage in the region.

Although our dataset only represents a subset of cases, our analysis of returning travellers revealed viral circulation patterns not reflected by official organisms at the time of data generation. For example, we detected DENV-3 and DENV-2 circulation in Ecuador in 2024 (Supplementary Fig. 1c) and 2023 (Supplementary Fig. 1b), respectively, and concurrent DENV-3 and DENV-4 circulation in the Philippines in 2024 (Supplementary Fig. 1d), information absent from PAHO and WHO reports. These findings demonstrate how data from returning travellers, generated in a short period of time, can provide complementary epidemiological information to fill surveillance gaps and anticipate outbreak trends.

The detection of DENV-3 in an Italian traveller shortly after Italy's first locally acquired case of this serotype further illustrates how imported infections can potentially start local transmission. Phylogenetically, this strain clustered within genotype III, closely related to 2023 Thai sequences and clearly separated from the 2023 Italian autochthonous outbreak cluster associated with American strains [28,29], suggesting a different introduction event. Similarly, a distinct DENV-2 Cosmopolitan cluster identified from our Vietnam and Indonesia travellers (Supplementary Fig. 1b) aligned with the newly emerged Vietnamese lineage detected during its 2022 outbreak [30], being consistent with the reports of the ongoing emergence of new lineages in Southeast Asia. These findings reinforce the need for continuous genomic

Table 1
Number of confirmed dengue cases (n = 183) by travel origin and sample collection year (2022 - 2024).
*: The traveller's itinerary included both places.

Travel destination – countries and territories	2022	2023	2024	Total number of cases
America				
Antilles	0	1	0	1
Argentina	0	0	4	4
Bolivia	0	1	0	1
Brazil	0	0	6	6
Colombia	0	2	2	4
Cuba	30	10	16	56
Dominican Republic	0	5	3	8
Ecuador	0	1	2	3
French Guiana	0	0	2	2
Guatemala	0	1	0	1
Honduras	1	0	4	5
Nicaragua	1	1	0	2
Nicaragua and Colombia *	0	1	0	1
Paraguay	0	0	2	2
Panama	1	0	0	1
Peru	0	3	1	4
Venezuela	0	1	4	5
Asia				
India	0	1	1	2
India and Nepal *	1	0	0	1
Indonesia	0	0	5	5
Malaysia	0	1	0	1
Malaysia and Qatar *	0	1	0	1
Maldives	0	1	3	4
Nepal and Tibet *	1	0	0	1
Philippines	0	0	2	2
Travel destination – countries and territories	2022	2023	2024	Total number of cases
Asia				
Thailand	0	4	0	4
Thailand and Japan *	0	1	0	1
Thailand and Vietnam *	0	1	0	1
Tibet	1	0	0	1
Vietnam	3	0	0	3
Africa				
Cape Verde	0	0	1	1
Democratic Republic of the Congo	0	0	1	1
Mali	0	0	1	1
Europe				
Italy	0	0	1	1
Oceania				
Australia	1	0	0	1
No travel destination specified				
Cases with missing data	20	7	18	45
Total number of cases	**60**	**44**	**79**	**183**

surveillance of travellers to detect introduction of new variants in Europe.

Beyond virological insights, our findings have relevant implications for clinical management of febrile patients. Awareness of circulating dengue serotypes in endemic regions can help clinicians infer the most likely infecting serotype in travellers returning from specific areas, supporting early risk evaluation of developing severe dengue. Disease severity varies by serotype and is influenced by whether the infection is primary or secondary, with some serotype-immune status combinations associated with more severe disease [31]. In addition, knowledge of serotype distribution can help in the selection of appropriate diagnostic tests, as the sensitivity of some methods, such as NS1 antigen detection, could vary depending on the infecting serotype [32]. Finally, these findings provide epidemiological context for public health preparedness, especially as differences in the vaccine efficacy have been described for Qdenga® [33]. However, vaccination strategies should better consider the serostatus and risk factors of each individual.

Moreover, 73% of confirmed cases from regions with *Aedes albopictus* presence were detected during its active season, which increases the risk of local transmission. Several European countries such as France, Italy, and Spain, have reported autochthonous transmission of DENV in recent years, representing a significant challenge to countries until now non-endemic. The situation in Spain is particularly concerning, given the observed pattern since 2018, when autochthonous cases were first reported. Since then, local transmission has been detected almost every year up to 2024, when eight cases were confirmed [8]. In this context, the effectiveness of vector control programmes will play a key role in limiting local transmission. However, experience from endemic regions shows that insecticide resistance, particularly knockdown resistance (*kdr*) associated mutations, can reduce the impact of control interventions [34]. Some of these *kdr* mutations have already been identified in *Aedes albopictus* across Mediterranean Europe, where the species continues to expand and has shown emerging pyrethroid resistance. In addition, the recent re-appearance of *Aedes aegypti* in Cyprus and Madeira raises concerns about the establishment of this highly competent vector in mainland Spain [35]. Combined with other invasive *Aedes* species, this could complicate control efforts. Taken together, these factors suggest that if local dengue transmission becomes sustained, Spain may face the same vector-control challenges observed in endemic regions, underscoring the need to strengthen control interventions and entomological surveillance.

However, this study has some limitations, including incomplete travel origin data for 25% of the travellers, and the limited number of samples submitted to the NCM. Additionally, most of our cases were detected in Madrid (41%) and Navarra (9.3%) while at national level Catalonia reports the highest number of dengue cases [18,19,21] and has detected most of the autochthonous cases (13 out of 24) in our country. These regional discrepancies highlight the importance of maintaining a continuous surveillance system that captures regional differences effectively. Despite these limitations, our study provides a nationwide overview of imported dengue case distribution in Spain and offers updated surveillance information that reflects global dengue patterns.

5. Conclusions

In conclusion, traveller surveillance, coupled with local vector monitoring, is essential for the detection, management and prevention of autochthonous transmission, as reflected in Spain's national protocols against *Aedes*-borne viruses [36]. Looking ahead to 2026, these integrated tools will be essential for the early detection of emerging dengue strains. Insights from the 2022–2024 cases should guide preparedness and genomic surveillance, which could provide information about emerging threats and improve the response to local outbreaks. This approach is not limited to dengue virus; it could also be applied to other arboviruses, such as Oropouche or chikungunya, whose current risk of local transmission is low but may change.

CRediT authorship contribution statement

Patricia Sánchez-Mora: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Francisca Molero:** Writing – review & editing, Investigation. **Ana Navascués:** Writing – review & editing, Resources. **Nelly Daniela Zurita:** Writing – review & editing, Resources. **Pilar Zamarrón:** Writing – review & editing, Resources. **Eduardo Lagarejos:** Writing – review & editing, Resources. **Sara Gómez:** Writing – review & editing, Resources. **Pere Joan Cardona:** Writing – review & editing, Resources. **Blanca Carrasco:** Writing – review & editing, Resources. **Marta López-Lomba:** Writing – review & editing, Resources. **Isabel Escribano:** Writing – review & editing, Resources. **Óscar Martínez-Expósito:** Writing – review & editing, Resources. **Teresa del Peso:** Writing – review & editing, Investigation. **Laura Herrero:** Writing – review & editing, Investigation. **Anabel Negredo:** Writing – review & editing, Investigation. **Laura Guillén-Calvo:** Writing – review & editing, Investigation. **Ana Peña:**

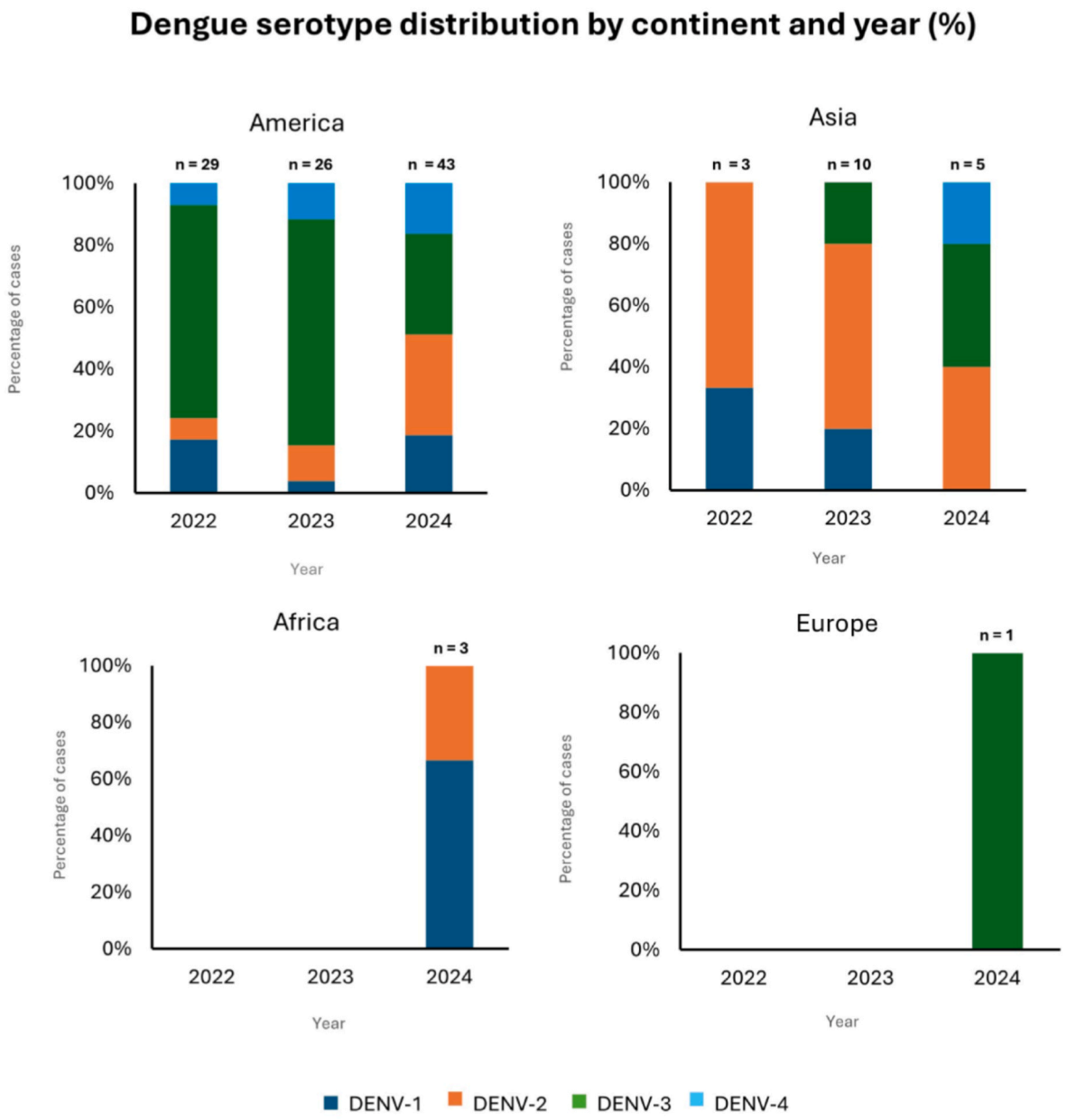

Fig. 4. DENV serotype distribution by year and continent, expressed as percentage of sequenced cases with a known geographic origin. Numbers above each bar indicate the total number of cases sequenced each year.

Writing – review & editing, Investigation. **Rafael Gutiérrez-López:** Writing – review & editing, Investigation. **Nuria Labiod:** Writing – review & editing, Investigation. **Ana Vázquez:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **María Paz Sánchez-Seco:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare no known conflict of interests, including 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.2026.102986.

Data availability

Nucleotide sequences obtained in this study that met the submission requirements have been deposited in GenBank. The accession numbers are: PX427357 - PX427465 and PX442426-PX442429.

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