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# Dengue Virus Dynamic and Persistence in Body Fluids of Infected Patients in Italy, 2018–2023

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**Keywords:** arboviruses | dengue virus | laboratory diagnosis | viral shedding | viremia duration

## ABSTRACT

Dengue, a mosquito-borne disease caused by the dengue virus (DENV), is constantly expanding worldwide. We investigated the presence and persistence of DENV RNA in the bloodstream and other body fluids to describe the viral kinetics in the human host. We longitudinally collected serum ($n = 118$), plasma ($n = 110$), whole blood ($n = 90$), urine ($n = 118$), oral swabs ($n = 68$), saliva ($n = 42$), semen ($n = 23$), and vaginal fluids ($n = 49$) from 42 DENV patients. We measured DENV RNA for a median of 28 (range 1–63) days from symptom onset (DSO). We estimated the probability of viral detection applying a generalized linear model, and the duration of viremia using Monte Carlo-Markov Chain approach. In the bloodstream, the highest rate of positivity, levels of DENV RNA, and persistence were observed in whole blood. The estimated probability of a positive test dropped below 5% after 12.5, 20.7, and 35.4 DSO for plasma, serum, and whole blood, respectively. The average duration of viremia was estimated to be 19.9 DSO. Saliva and oral swabs showed 76.2% and 58.8% of DENV RNA positivity during the first week of symptoms while the longest persistence was observed in urine (39 DSO). DENV was revealed in 20% cervicovaginal (up to 11 DSO) and 30% seminal (up to 35 DSO) fluids. Whole blood represents the preferential specimen for dengue molecular detection and the correct estimation of viremia duration which have clear implications for onward transmission and public health countermeasures. Blood, urine, and oral samples can be assayed according to time from disease onset, severity, and screening purposes.

Giulia Matusali and Mattia Manica shared co-first authorship.

Emanuele Nicastri and Fabrizio Maggi contributed equally to this work.

## 1 | Introduction

Dengue is a mosquito-borne viral disease caused by four distinct but closely related viral serotypes (DENV-1, -2, -3, and -4) primarily circulating in the tropics and subtropics. The geographical distribution and the global burden of DENV have increased over the last few decades due to the expansion of the *Aedes* vectors and the more frequent importation of cases into nonendemic areas. Approximately half of the world's population is at risk of DENV infection, with 100–400 million infections occurring annually [1]. In 2023 and 2024, an upsurge of DENV cases was globally observed [2]. The most affected countries included Brazil, Argentina, Bangladesh, Malaysia, Vietnam, and Burkina Faso. An unprecedented number of autochthonous cases was also reported in European countries such as Italy (83), France (77), and Spain (8) [3]. From January to July 2024, almost 5 million DENV cases have been confirmed in Brazil, marking a threefold increase compared to 2023 [4].

DENV infection is symptomatic in about 40% of cases [5]. Symptoms range from mild, self-limiting acute febrile illness without warning signs, to severe illness, resulting in hemorrhagic fever or shock syndrome [6].

Due to its nonspecific manifestations and the high percentage of asymptomatic infections, DENV can be misdiagnosed, and cases may be underreported [7, 8]. Proper laboratory diagnostic algorithms and surveillance activities are essential for clinical management, as well as for monitoring and controlling the DENV burden. DENV differential diagnosis is broad, and laboratory confirmation is needed. The latter relies on viral RNA or NS1 antigen detection in blood samples or serological detection of DENV-specific IgM and IgG in paired serum samples collected at least 1 week apart [9]. Moreover, the use of lateral flow rapid tests detecting the viral NS1 protein and IgM and IgG [10, 11] may be particularly useful, as a user-friendly first-line test, to facilitate timely DENV diagnosis and treatment decisions and to inform surveillance entities.

DENV viremia is usually reported for about 1 week from the onset of symptoms, but controversial results are presented in the literature [9, 12, 13]. Differences in the reported time of viremia duration may be due to the use of different blood-derived samples by different laboratories with longer detection reported in whole blood [14].

The duration of viremia (i.e., the viral persistence in the bloodstream) is an epidemiologically relevant metric as it defines the period during which a person can infect a mosquito, thus contributing to transmission chains. Moreover, this parameter contributes to determine the infection generation time.

Moreover, the dynamics and persistence of the virus may vary in different body fluids therefore triggering the investigation of samples alternative to blood such as urine and saliva. These samples require a noninvasive collection procedure which can be a particular advantage for testing infants and children, a population with a higher risk of developing DENV with warning signs [15–17], or for field screening. Therefore, understanding the kinetics of viral RNA or viral antigens in a range of blood-derived and nonblood specimens may be useful to correctly apply the available diagnostic tools.

Here, we report the analysis of DENV RNA in 42 patients who were referred and followed up at the National Institute for Infectious Diseases (INMI) "Lazzaro Spallanzani," Rome, Italy, from April 2018 to December 2023. The viral RNA presence and persistence were measured in three blood-derived samples—namely, serum, plasma, and whole blood—as well as in urine, oral swabs, saliva, and male and female genital fluids. These measures allowed to estimate the probability of viral detection in different compartments at different times from symptom onsets and some epidemiological parameters, such as the generation time.

## 2 | Material and Methods

### 2.1 | Study Samples

To study viral shedding, we tested a total of 618 samples from 42 DENV-confirmed cases who referred to INMI from April 2018 to November 2023 (2018 $n = 6$, 2019 $n = 22$, 2020 $n = 2$, 2023 $n = 12$). We collected 118 serum samples, 110 plasma samples (with EDTA as anticoagulant), 90 whole blood samples (with EDTA as anticoagulant), 118 urine samples, 68 oral swabs (collected in 1 mL of viral transport medium VTM), 42 saliva samples (collected by passive drooling), 49 vaginal swabs (VS, in 1 mL of VTM), and 23 seminal fluids (SF). The time of samples' collection varied from a minimum of 2 to a maximum of 63 days from symptom onset (DSO), the median time was 28 DSO. Patients' characteristics are described in the Section 3. The date of symptom onset was established at the time of the first visit.

### 2.2 | Laboratory Procedures

All cases were diagnosed by NS1 rapid test (STANDARD F Dengue NS1 Ag SD Biosensor, Relab, Genova, Italy) at the admission and confirmed by RT-PCR. The RNA was extracted by QIAsymphony as per manufacturer instructions (QIAgen, Hilden, Germany) and amplified for DENV RNA detection by a specific real-time RT-PCR (DENV-1-4 Real-Time RT-PCR Multiplex Assay, developed by Center for Disease Control and Prevention (CDC), as per manufacturer instructions). Negative and positive control of amplification were included in each run. Cycle threshold (Ct) values were provided as a proxy for viral RNA levels. Samples with a Ct value $> 42$ were considered negative. A qualitative, universal Pan-flavivirus nested PCR targeting the NS5 region of the viral genome (modified from Moureau et al. [18]) was also used for preliminary identification of flavivirus infections.

### 2.3 | Statistical Analysis

Absolute and relative frequencies (i.e., frequency of detection) were described as percentages, continuous variables (i.e., Ct values) using the median and interquartile range (IQR), non-continuous variables (i.e., antibody titers) as geometric mean,

and 95% confidence interval (95% CI). Differences in DENV RNA were assessed using the Mann–Whitney or Wilcoxon test as appropriate. The Fisher exact test was used to compare positivity rates and the Kruskal–Wallis test was used to compare antibody titers. GraphPad Prism Version 10 (GraphPad Software, La Jolla, California, USA) software was used; $p < 0.05$ was considered statistically significant.

Additionally, for each compartment, we investigated the probability of obtaining a positive test (i.e., Ct values < 42) conditional to the number of DSO by applying a generalized linear model (GLM) assuming a binomial distribution for the test result. This approach aims to estimate the relationship between test positivity and DSO despite the heterogeneous sampling across individuals and compartments, thereby enhancing the comparability of results across different compartments and diagnostic targets. The DENV serotype and its interaction with DSO were also considered in a separate analysis for sensitivity.

We estimated the distribution of DENV viremia duration from symptom onset using a Monte Carlo Markov Chain (MCMC) approach applied to the Gamma likelihood of viremia ending between the last positive sample and the following negative sample in the bloodstream (serum, plasma, and whole blood). Further details are available in the Supporting Information.

## 3 | Results

### 3.1 | Participants Characteristics

A total of 42 adult patients were enrolled with a median age of 38 years/old (IQR 30–52), 57% were female, all were symptomatic, and 93% were hospitalized (1 secondary DENV case, 1 requested Intensive Care Unit admission). The viral serotype was identified in 39 out of 42 cases (93%) including DENV-1 in 13 cases (31%), DENV-2 in 11 cases (26%), and DENV-3 in 15 cases (36%). In total, 22 (52%) individuals contracted the infection in Asia, 12 (29%) in the Americas, and 1 (2%) in Africa, while in 5 cases (12%) the infection occurred in the Lazio region, Italy. The characteristics of the enrolled patients are presented in Table 1.

### 3.2 | DENV RNA Detection in Blood, Urine, and Oral Samples

RT-PCR detected DENV RNA in all these specimen types with a variable load and rate of positivity depending on the DSO and body fluids. During the first week of symptoms, the highest viral RNA levels were observed in whole blood samples (median Ct value: 26.1, IQR: 22.4–31.7), followed by saliva (30.7, 27.3–39.9), serum (31.3, 24.6–35.4), plasma (32.4, 24.6–45), oral swabs (36.7, 31.9–45), and urine (median Ct below the detection limit) (Figure 1A). Similarly, the frequency of detection during the first week of symptoms was significantly higher in serum and whole blood than in plasma ($p = 0.0323$ and $p = 0.0197$, respectively), urine ($p < 0.0001$ and $p < 0.0001$), and oral swabs ($p = 0.0477$ and $p = 0.0311$) (Supporting Information S1: Table 1). During the second week, DENV RNA was still

**TABLE 1** | Demographical, epidemiological, and clinical characteristics and laboratory findings at diagnosis of the 42 enrolled DENV patients.

| Characteristics/findings | |
|---|---|
| *Demographical characteristics* | |
| Median age years [IQR] | 38 [30–52] |
| Men *n* (%) | 18 (43) |
| Women *n* (%) | 24 (57) |
| *Epidemiological characteristics* | |
| Origin of infection, *n* (%) | |
| Imported | 37 (88) |
| Asia | 22 |
| Americas | 12 |
| Africa | 1 |
| Autochthonous | 5 (12) |
| DENV-Serotype, *n* (%) | |
| DENV-1 | 13 (31) |
| DENV-2 | 11 (26) |
| DENV-3 | 15 (36) |
| Unknown | 3 (7) |
| *Clinical characteristics* | |
| Symptoms and sign at diagnosis, *n* | |
| Fever | 41 |
| Arthralgia | 29 |
| Rash | 27 |
| Asthenia | 26 |
| Headache | 25 |
| Myalgia | 19 |
| Ocular pain | 10 |
| Nausea | 6 |
| Diarrhea | 5 |
| Vomit | 3 |
| Inappetence | 2 |
| Hemorrhage | 2 |
| Cough | 2 |
| Petechiae | 1 |
| Hands edema | 1 |
| Comorbidities, *n* (%) | 10 (24) |
| Hospitalization, *n* (%) | 39 (93) |
| Days of hospitalization Median [range] | 5 [0–16] |
| Admission in intensive care unit, *n* (%) | 1 (2) |
| Secondary DENV, *n* (%) | 1 (2) |
| *Laboratory findings at diagnosis* | |
| White blood cells ($\times 10^3/mm^3$) median [range] | 3.015 [1.06–9.45] |
| Platelet ($\times 10^3/mm^3$) median [range] | 137.5 [16.0–291.0] |
| Hematocrit % [range] | 41.9 [35.1–51.3] |
| Positive DENV RT-PCR in blood samples, *n* (%) | 41 (98) |

Abbreviation: IQR, interquartile range.

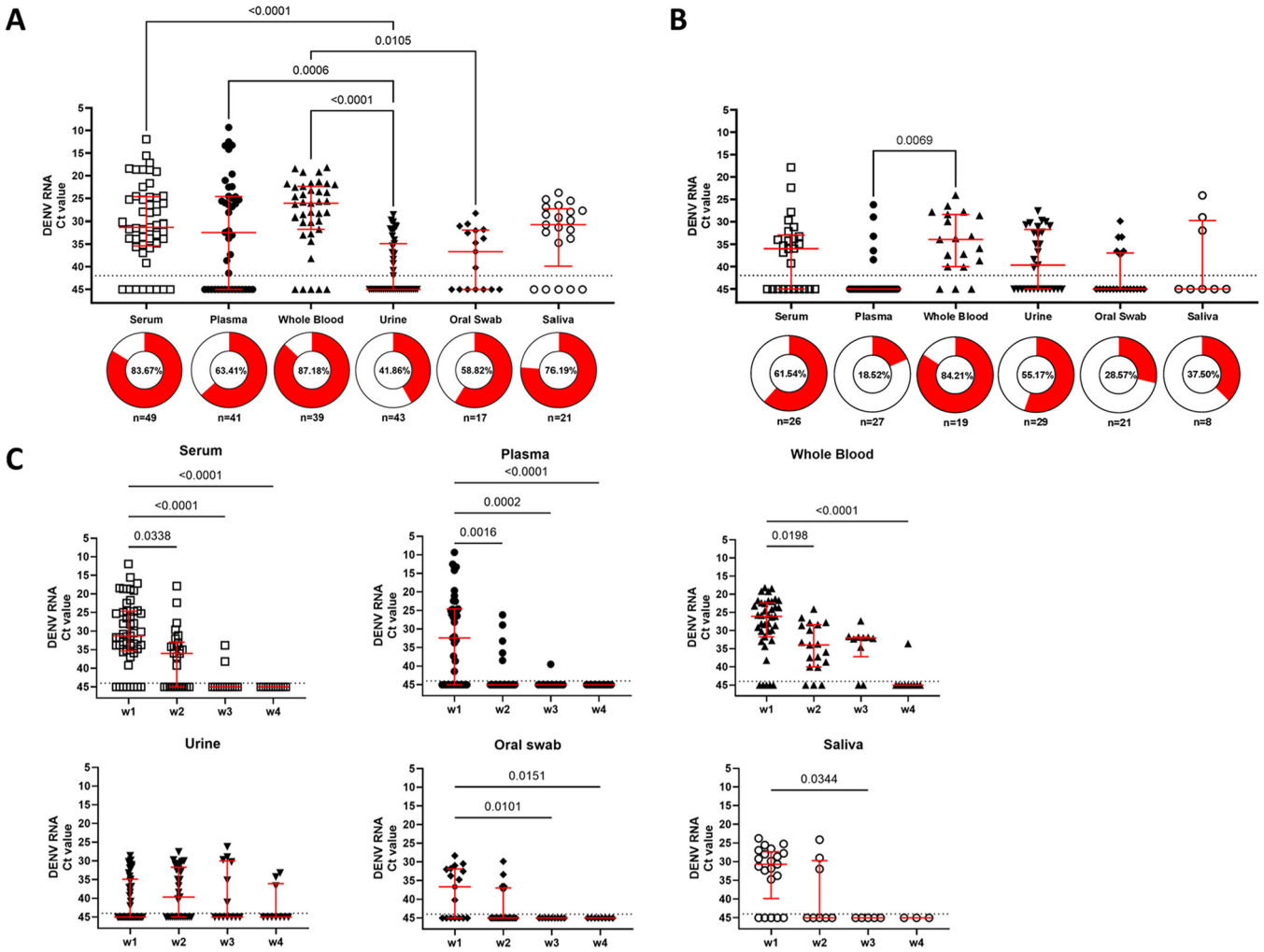


**FIGURE 1** | DENV-RNA shedding during the acute phase of infection. DENV RNA levels (Ct value) in serum, plasma, whole-blood, urine, oral swabs, and saliva during the first (A) and the second (B) week from symptom onset. The number of samples tested, and the percentage of positive samples are indicated in the donut graphs. (C) Kinetic of viral DENV RNA levels (Ct value) in serum, plasma, whole-blood, urine, oral swabs, and saliva during 4 weeks from symptom onset, w = week. The Kruskal–Wallis test was used to compare Ct values.

detectable in all tested samples, with whole blood and serum showing the lowest median Ct value (33.9 and 36.0, respectively) (Figure 1B). The viral loads in all the body fluids tended to decrease with time except urine, which exhibited higher DENV RNA levels at Week 2 compared to Week 1. However, this difference was not statistically significant (Figure 1C). We observed a significantly higher frequency of detection in whole blood compared to plasma ($p < 0.0001$), urine ($p = 0.0369$), oral swabs ($p = 0.0004$), and saliva ($p = 0.0152$) (Supporting Information S1: Table 1). Plasma also exhibited a significantly lower frequency of reactive samples compared to serum ($p = 0.002$), and urine ($p = 0.0046$) (Supporting Information S1: Table 1). During the third week, DENV RNA remained detectable in 80% of whole blood tested samples ($n = 10$) with a median Ct value of 32 (IQR: 31.8–34), in 46% of urine ($n = 13$), and a small percentage of serum (15%, $n = 13$) and plasma (7%, $n = 14$) samples (Figure 1C, Supporting Information S1: Table 1). At-Week 4, the viral RNA was detected in one whole blood ($n = 10$) and in three urine samples ($n = 10$) (Figure 1C, Supporting Information S1: Table 1). A higher frequency of detection in whole blood than in plasma ($p = 0.0004$) and serum ($p = 0.0186$) was further confirmed in 62 paired blood-derived samples showing DENV RNA detection in 35 whole blood, 21 serum, and 15 plasma samples.

Overall, the shortest viral persistence was observed in saliva (9 DSO) and oral swabs (14 DSO), DENV RNA was detected till 16 DSO in serum and plasma and 32 DSO in whole blood. Notably, DENV showed the longest persistence in urine with 3 positive samples out of 9 detected during the 5th week and viral RNA presence till 39 DSO.

Consequent estimates of the probability of obtaining positive test results conditional to the DSO were highly heterogeneous across compartments (Figure 2). At 7 DSO the highest probability of a positive test result was estimated in whole blood (88.9%, 95% CI: 77.8%–94.8%, $n = 39$), followed by serum (78.2%, 95% CI: 65.9%–86.9%), saliva (60.3%, 95% CI: 38.5%–78.7%), oral swabs (47.2%, 95% CI: 30.2%–64.8%), urine (46.3%, 95% CI: 35.7%–57.2%), and plasma (42.9%, 95% CI: 29.2%–57.8%) (Figure 2, Supporting Information S1: Table 2). By 14 DSO, the estimated probability of a positive test result was 69.9% (95%

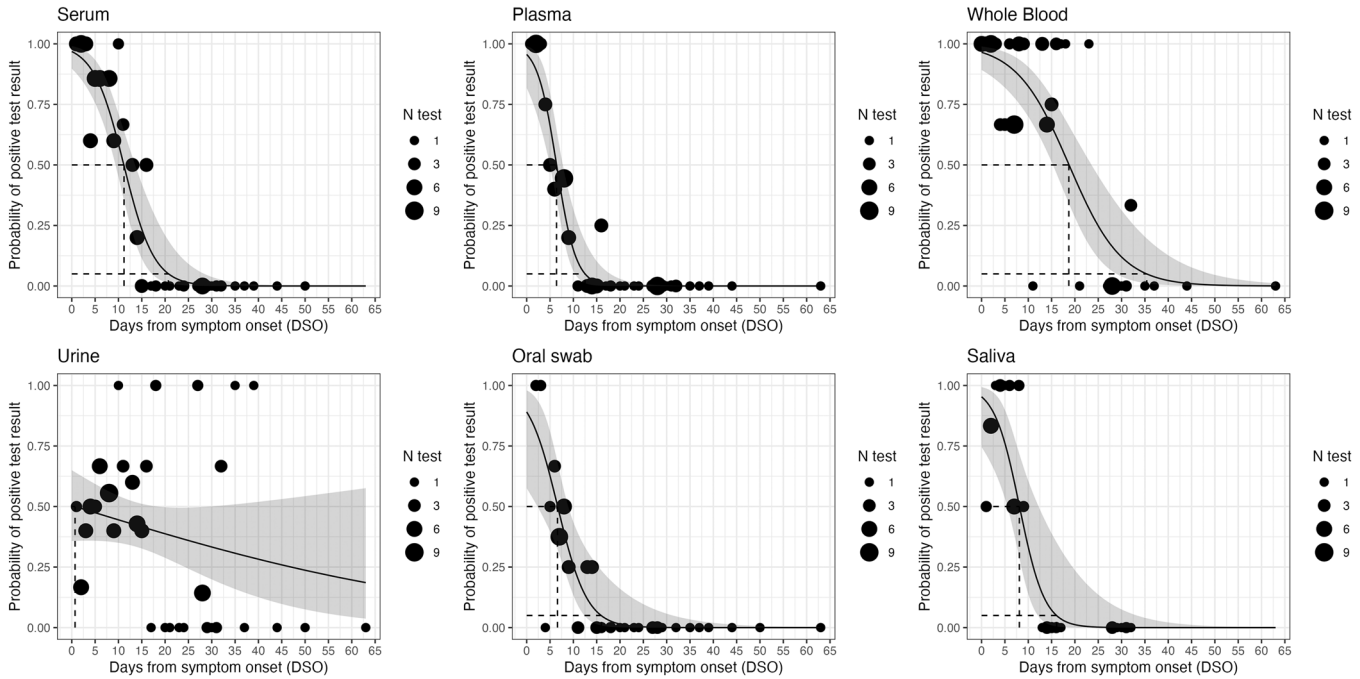


**FIGURE 2** | Estimated probability of a positive test result at different DSO. Each panel represents a different compartment. On the *x*-axis the DSO, on the *y*-axis the probability of a positive test result as obtained with the GLM model. The solid line represents mean estimates; the shaded area is the 95% confidence interval. Points represent the proportion of tests that resulted positive in the data. The dashed lines highlight the day when the estimated likelihood for a positive test result reaches 50% and 5%.

CI: 55.6%–81.1%) in whole blood, 42.2% (95% CI: 33.3%–51.6%) in urine, 29.4% (95% CI: 16.1%–47.6%) in serum samples, while dropping to 10.2% (95% CI: 1.7%–42.7%) and 2.5% (95% CI: 0.4%–13%) in saliva and plasma, respectively (Figure 2).

Among blood-derived samples, plasma was found to be associated with the steepest decrease in the probability of a positive test result, followed by serum and whole blood (Figure 2). On average, the estimated probability of a positive test dropped below 5% after 12.5, 20.7, and 35.4 DSO for plasma, serum, and whole blood, respectively. Urine showed a nonstatistically significant relationship between the occurrence of positive test results and DSO, starting with an uninformative probability (49.8%, 95% CI: 35.8%–63.8%) of positive test results in the first DSO, while maintaining a longer frequency of detection with the model estimating a 18.6% (95% CI: 3.7%–57.6%) probability of obtaining a positive test result at the end of the observed period (63 days). Oral swabs and saliva showed a pattern alike to plasma, with just a 5% estimated probability of a positive test result at 16 DSO.

In our cohort, no influence of DENV serotype on viral kinetics was observed when comparing samples derived from patients infected by DENV-1, DENV-2, or DENV-3 (Supporting Information S2: Figure 1 and Supporting Information S1: Table 3).

### 3.3 | Analysis of DENV Viremia Duration

Viremia duration was further investigated by fitting a Gamma distribution to longitudinal test results obtained from samples in the bloodstream. Model fitting was conducted by considering the likelihood of viremia ending between the last positive sample and the following negative sample. The mean duration of viremia was estimated to be 18.4 (95% CI: 14.7–22.8, percentile range of the distribution based on mean parameters' estimates: 3.5–45.4 days) DSO when considering all blood samples, 19.9 (95% CI: 1.5–24.4, percentile range of distribution based on mean parameters' estimates 3.2–51.3 days) DSO for whole blood, 12.3 (95% CI: 9.5–16.5, percentile range of the distribution based on mean parameters' estimates 2.1–31.4 days) DSO for serum, and 7 (95% CI: 5.7–8.8, percentile range of the distribution based on mean parameters' estimates 1.5–16.7 days) DSO for plasma (Figure 3, Supporting Information S1: Table 4).

### 3.4 | DENV RNA Detection in Genital Fluids

To investigate the presence and persistence of DENV in genital fluids, we collected 49 VS and 23 SF from 19 and 10 patients, respectively. The viral RNA was revealed in 10 (20%) VS and 7 (30%) SF, derived from 9 female and 5 male patients, respectively. The release of the virus was observed till 11 DSO in VS while longer persistence was observed in SF, where DENV RNA was detected till 35 DSO (Figure 4A). The median Ct values for both VS and SF were below detection from Week 1 to Week 4, with Ct values in positive samples always above Ct 25. Model estimates suggest that VS showed an uninformative probability (56.6% 95% CI: 22.9%–85.1%) of the positive test result in the first DSO, but at 14 DSO the estimated probability of a positive test decreased to 5.6% (95% CI: 1%–26.8%). Results for SF showed a very poor relationship with DSO (Figure 4B).

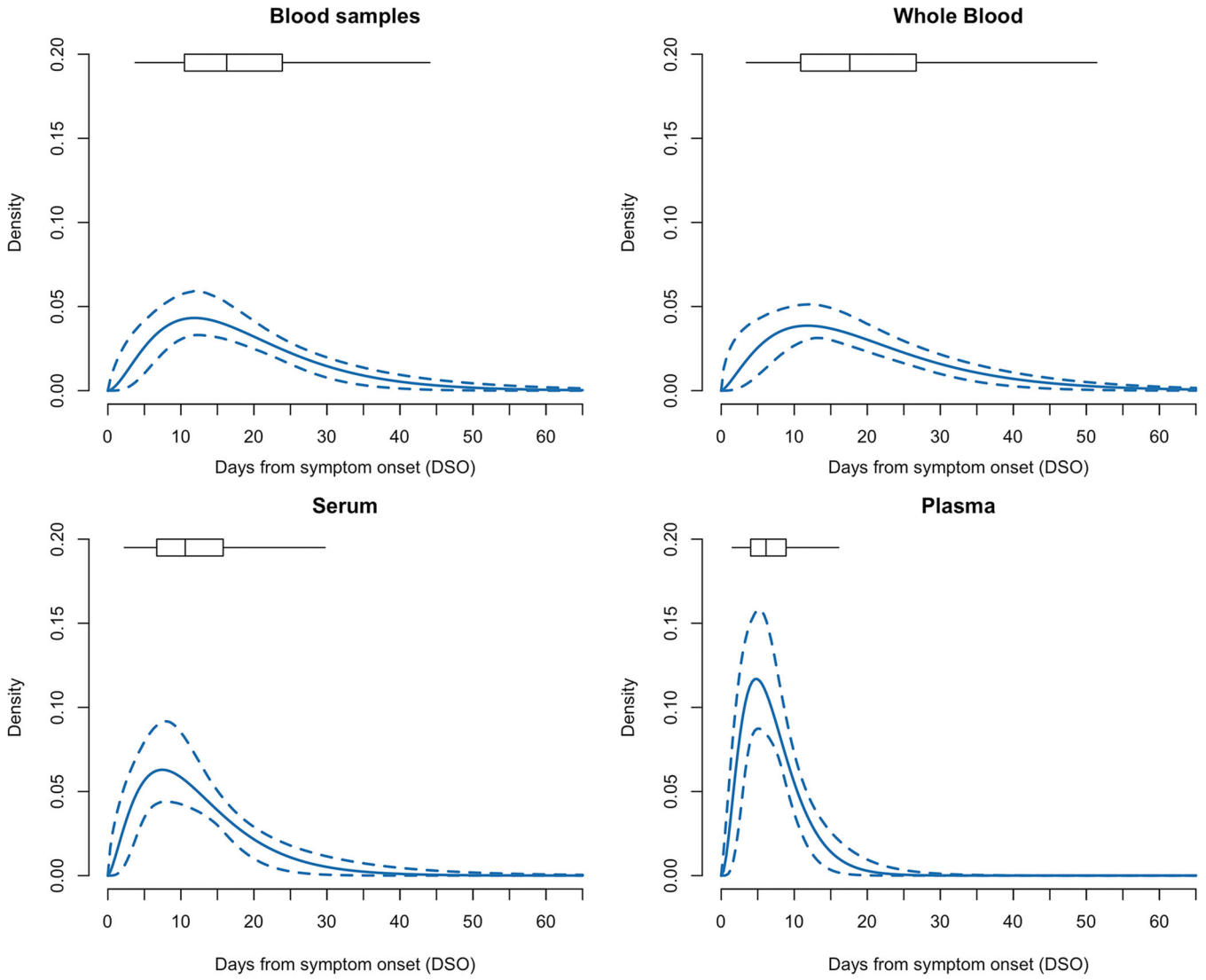


**FIGURE 3** | Distribution of DENV viremia duration from symptom onset in the blood compartments. The dark blue line represents the distribution of viremia duration as obtained by using the mean posterior values of the estimated parameters of the Gamma distribution. Dashed lines represent the 95% credible intervals. The horizontal boxplot summarizes the 2.5, 25, 50, 75, and 97.5 percentile of the estimated distribution.

## 4 | Discussion

This study on the persistence of DENV RNA in different body fluids provides information for the application of an optimal laboratory algorithm for dengue case definition, helping to choose the best sample to be analyzed according to the timing since the onset of symptoms. The study protocol, with a median of 4 weeks follow-up of patients from symptom onset, allowed the estimation of DENV persistence in different body compartments which may help understanding viral tropism and the dynamics of viral transmission.

We observed DENV RNA detection in the bloodstream, urine, oral, and genital fluids with frequency of detection and viral load varying with time of sampling and specimen types. As expected, the highest viral RNA level and positivity rate were observed in blood-derived samples.

Nevertheless, different blood specimens showed different yields of DENV RNA detection, with the latter showing lower frequency and shorter persistence in plasma than in serum and whole blood. A previous report [14] underlined the more sensitive and prolonged detection of DENV RNA in whole blood compared to plasma samples. Our findings indicate that DENV RNA is more persistently detectable in whole blood than in serum, suggesting that whole blood should be the first choice for molecular diagnosis of DENV, followed by serum and plasma. This information is also relevant when employing syndromic multiplex assays that have been validated using plasma samples.

We found evidence of DENV RNA in urine, oral swabs, and saliva samples. Previous reports investigated the use of saliva and urine for DENV RNA detection [19–21]. Our results confirm that these targets may enhance the diagnostic capability in subjects where noninvasive procedures would be preferred (e.g., newborns, children, and patients with hemorrhagic syndromes) [20, 22, 23]. The dynamics of viral RNA in these fluids are nevertheless different. Saliva and oral swabs have been found to be useful samples for

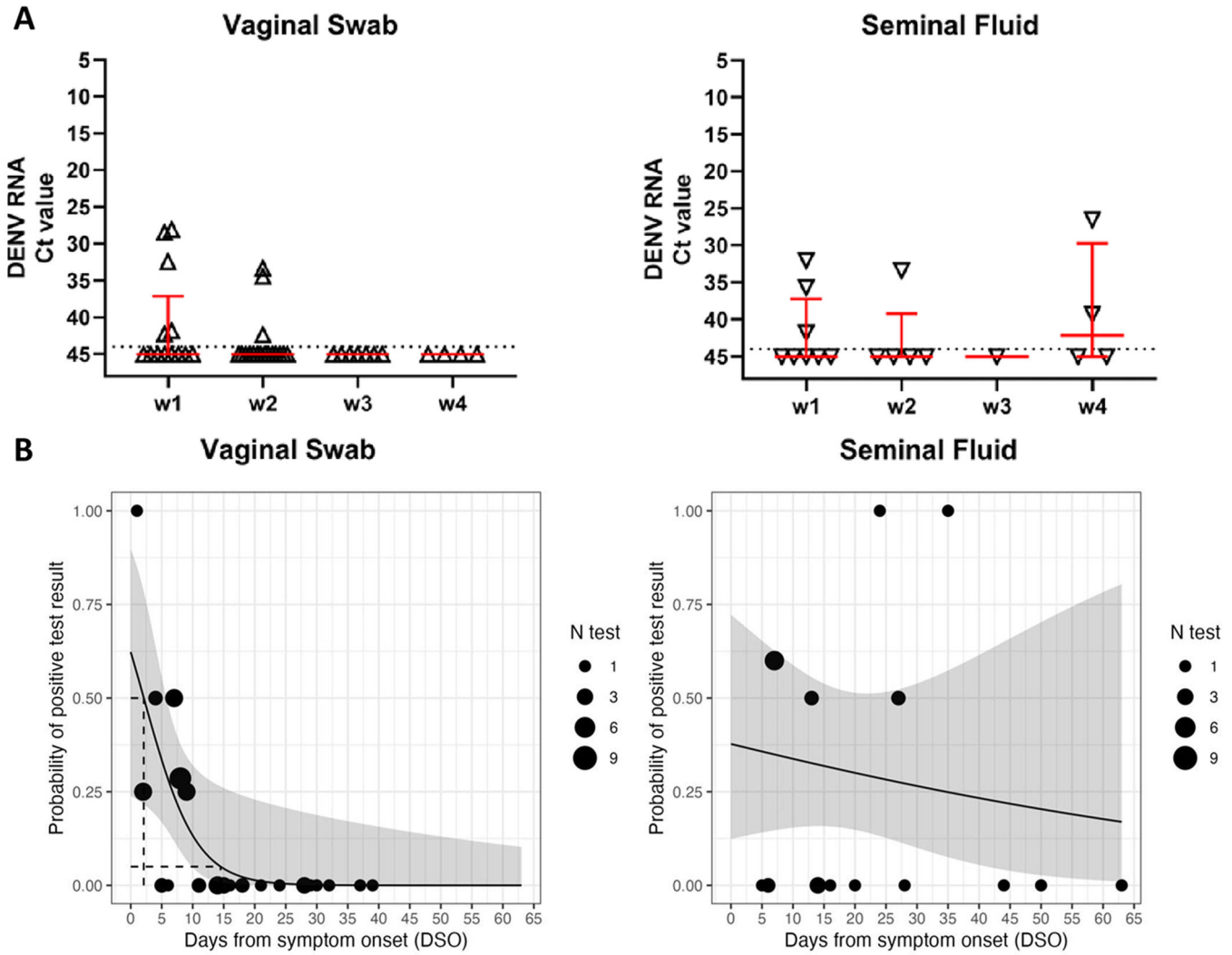


**FIGURE 4** | Kinetic of viral RNA in genital samples from DENV patients. (A) DENV RNA levels (Ct value) in vaginal swabs and seminal fluids during 4 weeks from symptom onset. w = week. (B) Estimated probability of a positive test result on different DSO. On the *x*-axis the DSO, on the *y*-axis the probability of a positive test result as obtained with the GLM model. The solid line represents mean estimates; the shaded area is the 95% confidence interval. Points represent the proportion of tests that resulted positive in the data. The dashed lines highlight the day when the estimated likelihood for a positive test result reaches 50% and 5%.

early diagnosis of infection, especially in the first week after the onset of symptoms, when they show a higher rate of positivity on molecular tests. The results suggest that urine may be a useful sample for laboratory diagnosis of DENV far after the onset of symptoms. Of note, DENV RNA persistence in urine did not show a clear temporal dynamic, with about 30% of subjects never showing detectable DENV RNA levels in this compartment, and most of the remaining subjects showing a decrease of Ct levels in the first 2 weeks after symptom onset followed by an increase of Ct values in the following week.

It must be mentioned that laboratory diagnosis of DENV also relies on serological analyses. Anti-DENV IgM and IgG levels were determined on serum samples from the 42 patients included but were not discussed because beyond the scope of this report. A role for the testing of IgA in saliva for case definition in large-scale screening through noninvasive procedures has been discussed by Yap et al. [24].

In our study, DENV was detectable up to 32 DSO in the bloodstream, and our estimates of 19.9 days of average duration of viremia in whole blood suggest that it lasts longer than usually reported (i.e., about 1 week from the onset of symptoms) [6], which may have key implication on the risk of onward transmission and the consequent design of public health policies.

This finding needs more studies to better define the timing of duration of the infectious period and the consequent timing for applying any isolation of bite protection measure, also considering the possible effect of previous infections.

To increase knowledge of the genital tropism of DENV, we tested both male and female genital fluids. Given the low rate of positivity, the low levels of viral RNA, and the unclear shedding kinetics observed, vaginal and seminal fluids are not appropriate specimens for DENV laboratory diagnosis. Nevertheless, we can conclude that DENV detection in the genital compartment

is not a rare event, and a longer persistence is observed in SF than in VS, in line with previous reports [19, 25, 26]. The probable sexual transmission of DENV infections has been described in two case reports, describing male-to-male [27] and female-to-male [28] transmission in nonendemic countries. Thus, sexual transmission is only anecdotally reported, despite the high incidence of DENV infections worldwide. Moreover, infectious virus was never recovered from vaginal fluids, while one case reported [19] DENV antigen staining upon semen inoculation on Vero E6 cells. Further studies are necessary to establish the origin of DENV in genital fluids and the potential role, if any, of sexual contact in viral transmission.

This study presents the following limitations. The testing timing and sampled compartment were heterogeneous across individuals even if regularly scheduled based on the day of diagnosis which occurred at variable DSO. The daily sampling would have been advantageous for the study but more invasive for the patients. Therefore, collected data did not allow to directly pinpoint the day when virus persistence in the sample was over. Moreover, the sample size was heterogeneous across DSO with decreasing number of samples during follow-up. The statistical approaches we employed aimed to overcome this limitation, by inferring the duration of viremia in the bloodstream and the probability of obtaining a positive test result conditional to the number of DSO. The presence of infectious particles by viral culture was attempted on a limited number of samples with a Ct value $< 25$ and depending on the availability of residual volume. Further investigations are needed to establish the infective potential of DENV detected at longer time from disease onset.

The Ct values were used to analyze viral persistence across compartments, but we lack a quantitative relationship to convert Ct values into DENV copies/mL, a more appropriate measure of viral load. Nevertheless, to the best of our knowledge, no specific study is yet available on the amount of viral load in the bloodstream needed to successfully infect a mosquito nor on the viral load needed in blood or other compartments to allow for alternative routes of transmission (e.g., blood transfusion, organ transplantation).

Finally, our estimates were derived from symptomatic cases, using the date of symptom onset as the reference point. This approach may lead to an underestimation of the duration of viremia. Conversely, our estimates rely on the assumption that the onset date of symptoms has been accurately identified during epidemiological investigations and case interviews.

The choice of the biological samples to be tested for DENV case confirmation should be carefully determined depending on clinical and epidemiological information. Blood, urine, and oral samples can be assayed according to time from disease onset, severity, and screening purposes. Whole blood represents the preferential specimen for DENV molecular detection and correct estimation of viremia duration which have clear implications on onward transmission and public health countermeasures. The long average of viremia duration in the whole blood needs further studies to assess the efficacy of transmission at different viral levels.

### Author Contributions

Conceptualization: Fabrizio Maggi, Emanuele Nicastri, Francesco Vairo, Giulia Matusali, Stefano Merler; data collection: Giulia Matusali, Francesco Vairo, Alessandra D'Abramo, Fabrizio Carletti, Francesca Colavita, Gaetano Maffongelli, Eleonora Lalle, Giuseppe Sberna, Eliana Specchiarello, Licia Bordi, Silvia Meschi, Gabriella De Carli, Martina Spaziante, Angela Corpolongo; data analysis: Giulia Matusali, Alessandra D'Abramo, Fabrizio Carletti, Francesca Colavita, Mattia Manica, Piero Poletti, Stefano Merler; manuscript preparation: Giulia Matusali, Mattia Manica; review and editing: Fabrizio Maggi, Emanuele Nicastri, Alessandra D'Abramo, Giulia Matusali, Francesco Vairo, Piero Poletti, Stefano Merler, Mattia Manica; Supervision: Fabrizio Maggi, Emanuele Nicastri, Enrico Girardi; funding: Fabrizio Maggi, Stefano Merler. All authors have read and agreed to the published version of the manuscript.


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### Study Group on Arboviruses

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### Ethics Statement

This study was conducted in accordance with the Declaration of Helsinki and upon approval by the Institutional Review Board of the National Institute for Infectious Diseases "Lazzaro Spallanzani" IRCCS (Issue No. 14/2015 and amendments).

### Consent

Patients provided written informed consent to participate in this study.

### Conflicts of Interest

The authors declare no conflicts of interest.

### Data Availability Statement

All data, materials, and methods are included in the article. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## Supporting Information

Additional supporting information can be found online in the Supporting Information section.