

Molecular Characterization of Dengue Virus Serotypes among Febrile Non-Malaria Patients in Three Hospitals in Southern Benin

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Abstract

In sub-Saharan Africa, malaria is commonly considered the leading cause of febrile illness. However, a substantial proportion of malaria-negative febrile patients suggests the involvement of other infectious agents, particularly arboviruses such as dengue. In Benin, data on circulating dengue virus serotypes in humans remain limited, especially among non-malarial febrile patients. **Objective:** The main objective of this study was to characterize the dengue virus serotypes circulating among malaria-negative febrile patients attending three hospitals in southern Benin (Abomey, Abomey-Calavi, and Lokossa). A cross-sectional descriptive study was conducted between June 2024 and January 2025 among 1490 febrile patients. After biological testing for malaria, 120 malaria-negative patients were selected for dengue virus detection using nested polymerase chain reaction (nested PCR). Positive samples were further analyzed for serotype identification. **Results:** Among the 1490 febrile patients, 288 (19.3%) had confirmed malaria, while 1202 (80.7%) tested negative. Of the 120 malaria-negative patients tested by nested PCR, 11 dengue cases were identified, corresponding to a prevalence of 9.16%. Cases were distributed across Abomey-Calavi (5 cases), Abomey (3 cases), and Lokossa (3 cases). Serotype



analysis revealed a marked predominance of DENV-2 (10 cases) and the presence of DENV-3 (1 case), indicating co-circulation of multiple serotypes. Young adults aged 20 - 30 years were the most affected group, with a female predominance. **Conclusion:** This study demonstrates active circulation of DENV-2 and DENV-3 among malaria-negative febrile patients in southern Benin. The predominance of DENV-2 and detection of DENV-3 confirm the co-circulation of dengue virus serotypes in the human population. These findings highlight the importance of integrating dengue testing into the diagnostic workup of non-malarial febrile illnesses and strengthening virological surveillance to prevent potential outbreaks and severe forms of the disease.

Keywords

Dengue, Serotypes, DENV-2, DENV-3, Fever, Nested PCR, Southern Benin

1. Introduction

Febrile illnesses remain one of the most common reasons for consultation in healthcare facilities in sub-Saharan Africa, representing a major challenge for both diagnosis and clinical management [1]. These syndromes encompass a wide range of infectious etiologies, including parasitic, bacterial, and viral infections, several of which are emerging or re-emerging pathogens [2]. Among these pathogens, some are responsible for severe diseases such as malaria, yellow fever, chikungunya, and dengue. Most of these infections are transmitted by mosquitoes, particularly species of the genera *Aedes* and *Anopheles*, making them an increasing global public health concern [3] [4]. The growing burden of mosquito-borne diseases has been documented in several African countries, including Burkina Faso, where significant dengue outbreaks have been reported in recent years [5]. In addition, environmental and climatic changes are increasingly recognized as important factors influencing the distribution of disease vectors and the emergence of vector-borne diseases worldwide [6].

In many African settings, malaria remains the primary suspected cause in cases of fever due to its high prevalence and the familiarity of clinicians with its clinical presentation. Consequently, febrile illnesses are frequently managed as malaria episodes, leading to the empirical and often systematic use of antimalarial treatments [1]. However, several arboviral diseases, particularly dengue, present clinical manifestations similar to those of malaria, including fever, headache, and myalgia. This symptomatic overlap significantly increases the risk of diagnostic errors and inappropriate treatments [7].

Dengue has been classified among the priority diseases requiring enhanced surveillance by the World Health Organization, a classification reaffirmed in 2023 by the Haut Conseil de la Santé Publique [8]. Globally, dengue has experienced unprecedented expansion over recent decades. It is estimated that approximately 390 million infections occur each year worldwide, of which nearly 100 million are

symptomatic, resulting in between 20,000 and 25,000 deaths annually [9]. The year 2019 was marked by one of the largest global dengue outbreaks, particularly in the Americas, where more than 3.14 million cases and 1538 deaths were reported [10]-[12].

The rapid global expansion of dengue is largely explained by several factors, including rapid urbanization, globalization, increased human mobility, and the geographic spread of its main vectors, *Aedes aegypti* and *Aedes albopictus*, now established in more than 129 countries [13].

In Africa, dengue has long been considered rare or sporadic. However, increasing evidence suggests that the disease is largely under-recognized and underreported. Several African countries have experienced significant outbreaks over the past decade. Burkina Faso reported major epidemics with 5094 cases in 2016 and 70,433 cases in 2023 [5]. In Côte d'Ivoire, 323 dengue cases were reported in 2023, while Nigeria shows evidence of endemic transmission, with dengue identified as one of the leading causes of non-malarial febrile illnesses in several states [12] [14].

Similarly, in Niger, the circulation of dengue virus serotype 3 (DENV-3) was confirmed in 2022, suggesting that dengue virus circulation in Africa may be more widespread than previously estimated [15].

Benin appears particularly vulnerable to the emergence of dengue due to its geographical proximity to countries that have recently reported epidemic outbreaks, notably Burkina Faso, Nigeria, Togo, and Niger. In addition, the country's tropical climate and environmental conditions favor the proliferation of mosquito vectors. Despite this context, the clinical management of febrile illnesses in Benin remains largely focused on malaria, while dengue and other arboviral diseases receive limited diagnostic attention.

Nevertheless, several studies have confirmed the presence of the main dengue vectors, *Aedes aegypti* and *Aedes albopictus*, in several regions of the country. Entomological investigations have also identified DENV-1 and DENV-3 serotypes in mosquito populations, suggesting local virus circulation [16]. Furthermore, confirmed human dengue cases have been reported in recent years. Infections were detected in 2019 in the Atlantique department, followed by new cases in 2020, including two deaths [17]. More recently, PCR analyses conducted in 2023 among suspected febrile patients confirmed the presence of dengue virus infections [18]. However, the circulation and distribution of other dengue virus serotypes in Benin remains poorly documented.

This diagnostic gap constitutes a major public health challenge in Benin. Indeed, febrile illnesses may be underdiagnosed or incorrectly attributed to malaria, leading not only to delays in appropriate patient management but also to an underestimation of the true burden of dengue in the country [19]. Consequently, it becomes essential to better document the circulation of the dengue virus and its different serotypes in order to improve epidemiological surveillance and guide diagnostic and control strategies.

In this context, the present study aims to determine the circulating serotypes of

the dengue virus among non-malarial febrile patients attending healthcare facilities in southern Benin. More specifically, it aims to detect dengue virus infection in these patients and identify circulating serotypes using molecular diagnostic techniques. The results of this study will contribute to improving the understanding of dengue epidemiology in Benin and provide essential data to strengthen surveillance, diagnostic, and management strategies for arboviral infections.

2. Material and Methods

2.1. Study Type and Duration

A cross-sectional descriptive study was conducted between Jun 2024 and January 2025.

2.2. Inclusion Criteria

All patients presenting with fever as the reason for consultation and for whom microscopic examination for malaria was requested.

2.3. Ethical Approval and Consent to Participate

This study was reviewed and approved by the Institutional Research Ethics Committee of CREC (No. 04/CEICREC/SA, approval dated June 1, 2024). Verbal informed consent was obtained from all adult participants after they had been fully informed about the objectives and potential risks of the study. The ethics committee approved the use of verbal consent, which was documented by the research team. For participants under 18 years of age, verbal consent was obtained from their parents or legal guardians.

2.4. Sampling

Blood samples were collected in the biomedical analysis laboratories of the district hospitals in each study site. Medical records were carefully reviewed, and only patients presenting with fever and confirmed negative for malaria by thick blood smear and parasite density were included.

A total of 1490 febrile patients were seen during the study period, of whom 1202 were confirmed malaria-negative. As the objective of this study was to investigate dengue virus circulation among non-malarial febrile patients, this group constituted the target population.

Ideally, the sample size would have been determined using statistical formulas based on an expected dengue prevalence. However, in the context of Benin, the lack of reliable epidemiological data on dengue prevalence did not allow accurate estimation of the parameters required for an a priori calculation. Furthermore, the exploratory nature of the study, combined with significant financial and logistical constraints related to the high cost of nested PCR analyses, precluded inclusion of all 1202 patients.

Therefore, a stratified proportional sampling strategy was adopted, consisting of selecting approximately 10% of malaria-negative patients from each study site

to preserve the initial geographic distribution. This resulted in the inclusion of 35 patients in Lokossa (out of 349), 50 patients in Abomey (out of 503), and 35 patients in Abomey-Calavi (out of 350), for a total of 120 samples.

This approach ensured proportional representation across study sites while accounting for operational constraints. However, it may limit the statistical representativeness of the findings, and this limitation has been acknowledged in the revised manuscript.

Figure 1 shows the geographical location of the communes where the hospitals involved in the study are located.

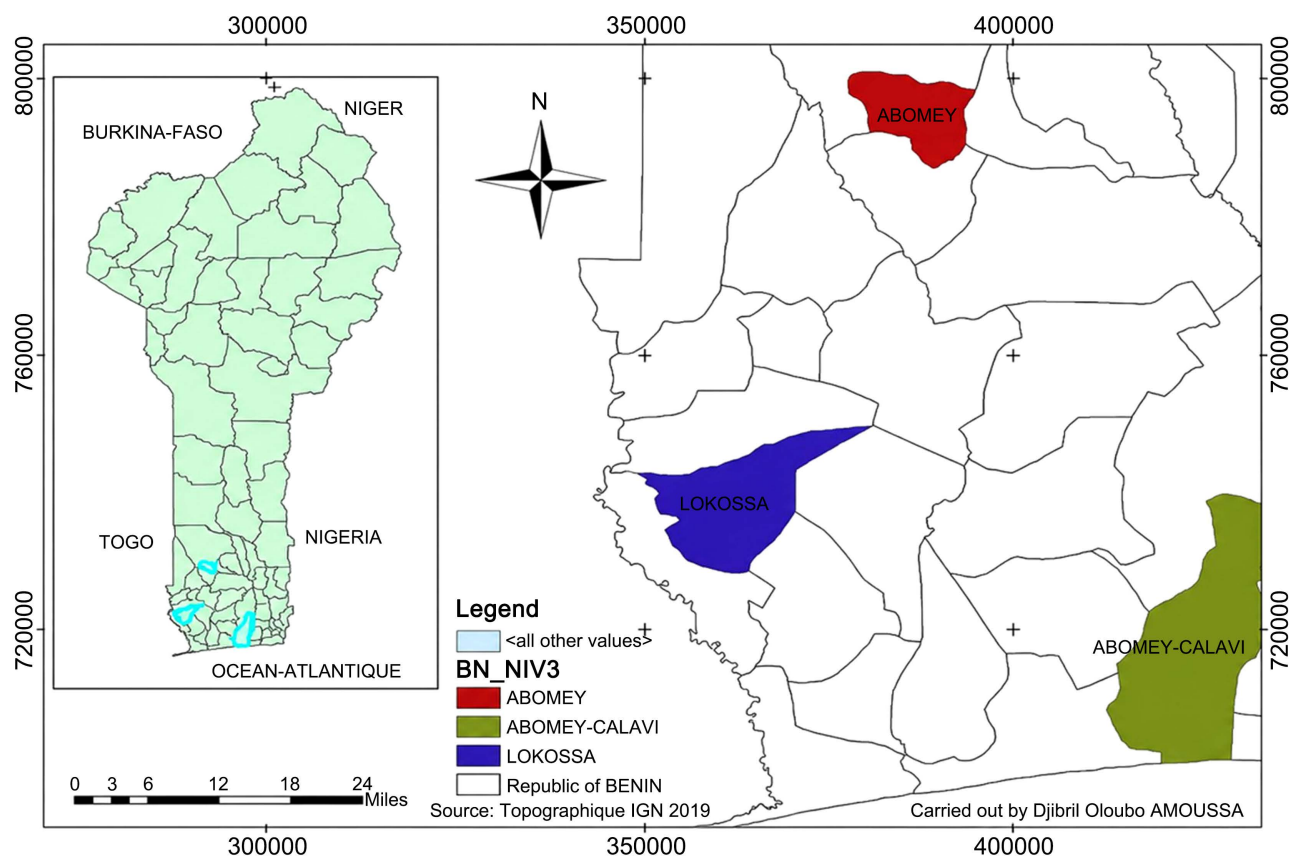


Figure 1. Map of the communes hosting the study.

2.5. Viral RNA Extraction

Viral RNA was extracted from 50 μL of whole blood using 200 μL of phenol and 200 μL of chloroform. Following centrifugation, the supernatant was collected and RNA was precipitated with isopropanol.

RNA quantification and purity assessment were performed using a NanoDrop spectrophotometer by measuring absorbance at 260 nm and 280 nm. The A260/A280 ratio was used as an indicator of RNA quality prior to PCR analysis.

All samples showed acceptable purity, with A260/A280 ratios ranging from 1.8 to 2.0.

Table 1 summarizes the results of RNA quantification and purity assessment.

Table 1. Summary of RNA quantification and purity assessment (n = 120).

Parameter	Value
Total number of samples	120
Minimum concentration (ng/μL)	≥10
Maximum concentration (ng/μL)	>200
Mean concentration (ng/μL)	68.7 ± 32.4
A260/A280 range	1.80 - 2.00

2.6. DNase Treatment

The extracted RNA underwent DNase treatment prior to complementary DNA synthesis. The purpose was to eliminate any DNA that could contaminate the RNA.

Table 2 presents the composition of the reagents used for the DNase procedure.

Table 2. Reagents used for DNase treatment.

Reagent	Volume
RNA: 10 μg	10 μL
DNase I Reaction Buffer (10X)	10 μL
DNase I (RNase-free)	1 μL
PCR water	10 μL

The mixture was then incubated at 37°C for 4 minutes, followed immediately by the addition of 1 μL of DNase RQ1 stop solution to terminate the reaction, and then incubated at 65°C for 10 minutes to inactivate the DNase [20].

2.7. Complementary DNA (cDNA) Synthesis

After the DNase treatment step, complementary DNA synthesis was performed using a cDNA reverse transcription kit.

Table 3 presents the reagents used for cDNA synthesis.

Table 3. Reagents used for cDNA synthesis.

Reagents	Volume (μL)
RT buffer 10X	2
dNTPs	0.8
10X RT	2
H ₂ O	6.4

A total of 10 µL of the master mix was added into the wells of a PCR plate for each sample, followed by the addition of 10 µL of DNase-treated RNA into each well, and mixed by pipetting. After sealing the plate with a film, it was centrifuged for 30 seconds.

Incubation was carried out in several steps: 25°C for 10 minutes for primer annealing, 37°C for 120 minutes for enzymatic polymerization, 85°C for 5 minutes to inactivate the reverse transcriptase, and finally 4°C for indefinite storage.

The concentration and purity of the synthesized cDNA were assessed using a NanoDrop spectrophotometer by measuring absorbance at 260 nm and 280 nm. The concentrations obtained were generally sufficient for molecular analyses, with a mean of 68.7 ± 32.4 ng/µL. The A260/A280 ratios ranged from 1.80 to 2.00, indicating acceptable cDNA quality and confirming the efficiency of the synthesis. These results demonstrate that the obtained genetic material was suitable for downstream amplification analyses. The samples were stored at -20°C until use for nested PCR amplification [20].

Table 4 summarizes the results of cDNA quantification and purity assessment.

Table 4. Summary of cDNA quantification and purity assessment (n = 120).

Parameter	Value
Number of samples	120
Minimum concentration (ng/µL)	≥10
Maximum concentration (ng/µL)	>200
Mean concentration (ng/µL)	68.7 ± 32.4
A260/A280 range	1.80 - 2.00

2.8. Determination of Dengue Serotypes

Positive and negative controls were included in each PCR run. Positive controls consisted of previously confirmed dengue virus RNA samples, while negative controls contained nuclease-free water to detect any potential contamination.

Strict contamination prevention measures were implemented, including physical separation of the extraction area, reaction mix preparation area, and amplification area, as well as the use of filter tips.

The validity of the results was confirmed by the presence of the expected bands in positive controls and the absence of bands in negative controls after gel electrophoresis.

For dengue virus serotyping, a nested PCR approach was used. This technique first detects the presence of dengue virus in positive samples and then identifies the specific serotype. The first amplification generates a larger fragment of the viral genome, while the second (inner) amplification targets a more specific region. This approach reduces non-specific amplification and allows more accurate

differentiation of dengue virus serotypes (DENV-1 to DENV-4).

Table 5 presents the reagents used for preparation of the mix.

Table 5. Reagents used for PCR1 mix preparation.

Reagents	Volume
10X buffer	2.5 µL
dNTPs	0.5 µL
mD1	2 µL
mD2	2 µL
Taq	0.1 µL

We used the Eppendorf Nexus thermocycler and the HotStarTaq Master Mix kit (Qiagen). The 25 µL reaction mixture contained 2 µL of cDNA, 2 µL of each primer (mD1 and D2), 2.5 µL of 10X buffer, 0.5 µL of dNTPs, 14.4 µL of H₂O, and 0.1 µL of HotStarTaq polymerase.

Denaturation at 95°C lasted 15 minutes, followed by 35 cycles of 15 seconds at 94°C, 15 seconds at 55°C for annealing, and 30 seconds at 72°C for extension. A final extension step of 10 minutes at 72°C was performed.

Table 6 presents the reagents used for preparation of the mix.

Table 6. Reagents used for the preparation of the PCR2 mix.

Reagents	Volume
10X buffer	2.5 µL
dNTPs	1 µL
mD1	2 µL
mD2	2 µL
mD3	2 µL
mD4	2 µL
Taq	0.1 µL
H ₂ O	6.4 µL

The 25 µL reaction mixture contained 5 µL of the product from the first PCR, 2 µL of each specific primer (mD1, rTS1, mTS2, TS3, and rTS4), 2.5 µL of 10X buffer, 1 µL of dNTPs, 0.1 µL of Taq polymerase, and 6.4 µL of distilled water. Denaturation at 95°C lasted 15 minutes, followed by 25 cycles of 15 seconds at 95°C, 15 seconds at 55°C for annealing, and 30 seconds at 72°C for extension.

2.9. Primers

The primers used for dengue virus detection and serotyping were those described by Lanciotti, a widely used method for dengue virus serotype identification using nested PCR. The sequences of the primers used for dengue virus detection and serotyping are presented in **Table 7**.

Table 7. Primers used for dengue virus detection and serotyping.

Primer	Sequence (5' → 3')
mD1	TCAATATGCTGAAACGCGAGAAACCG
D2	TTGCACCAACAGTCAATGTCTTCAGGTTC
rTS1	CCCGTAACACTTTGATCGCT
mTS2	CGCACAAGGGCATGAACAGTTT
TS3	TAACATCATCATGAGACAGAGC
TS4	TTCTCCCGTTCAGGATGTC

2.10. Data Entry and Analysis

Data were entered using Excel 2019 and analyzed using SPSS version 2021. Frequencies, proportions, and 95% confidence intervals were calculated.

Cross-tabulations were performed to explore associations between serotypes, age groups, sex, and geographical location.

3. Results

3.1. Sociodemographic Characteristics

Between July and August 2024, a total of 1490 patients presented for consultation and for whom microscopic examination for malaria was requested. Among them, 439 were from Abomey-Calavi, 601 from Abomey, and 450 from Lokossa.

In the overall study population, only 288 patients tested positive for malaria (19.3%), while 1202 patients tested negative for malaria (80.7%).

Table 8 presents the distribution of patients received in hospitals for TBS examination.

Table 8. Results of patients received in hospitals for TBS.

Hospitals	Total patients	TBS +	TBS –
Abomey-Calavi	439	89	350
Abomey	601	98	503
Lokossa	450	101	349
Total	1490	288	1202

3.1.1. Distribution of the Study Population by Age

Only febrile patients with negative TBS results were included for molecular detection of the dengue virus.

The study population consisted of 120 non-malarial febrile patients recruited from the three hospitals, namely: Abomey (n = 50), Lokossa (n = 35), and Abomey-Calavi (n = 35). Although the distribution by site was not homogeneous, the analysis of sociodemographic characteristics was conducted on all participants to provide an overall view of the study population.

The mean age of patients was 19 years, with extremes ranging from 0 to 80 years. **Figure 2** shows the distribution of the study population by age group.

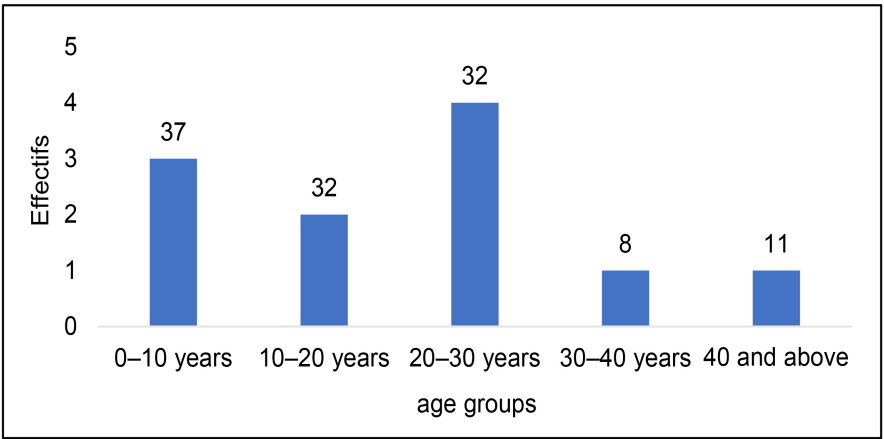


Figure 2. Distribution of the study population by age group.

3.1.2. Distribution of the Study Population by Sex

The distribution of the study population by sex showed a clear female predominance. Indeed, the male-to-female sex ratio was 0.52, indicating that there was approximately one man for every two women.

Table 9 summarizes the distribution of the study population by sex.

Table 9. Distribution of the target population by sex.

Sex	Number of individuals	Percentage
Male	41	34.17%
Female	79	65.83%
Total	120	100%

3.2. Determination of the Presence of Various Dengue Virus Serotypes in Febrile Patients

Electrophoresis of Serotype-Specific Amplicons (DENV-1 to DENV-4)

PCR products were visualized by electrophoresis on a 2% agarose gel stained with ethidium bromide. The size of the amplified fragments allowed the identification of each serotype.

From **Figure 3**, wells 1 to 12 correspond to clinical samples. Well 9: Negative control; DNA ladder M (bp) - 100 bp. Expected product sizes were: 208 bp for DENV-1, 119 bp for DENV-2, 288 bp for DENV-3, and 260 bp for DENV-4.

Wells 1, 3, 5, 8, and 12 corresponded to samples positive for DENV-2, showing a 119 bp band. Well 7 corresponded to a sample positive for DENV-3, showing a 288 bp band. Wells 2, 4, 6, 10, and 11 corresponded to negative samples.

Figure 3 shows an illustration of the migration of serotype-specific amplicons on an agarose gel.

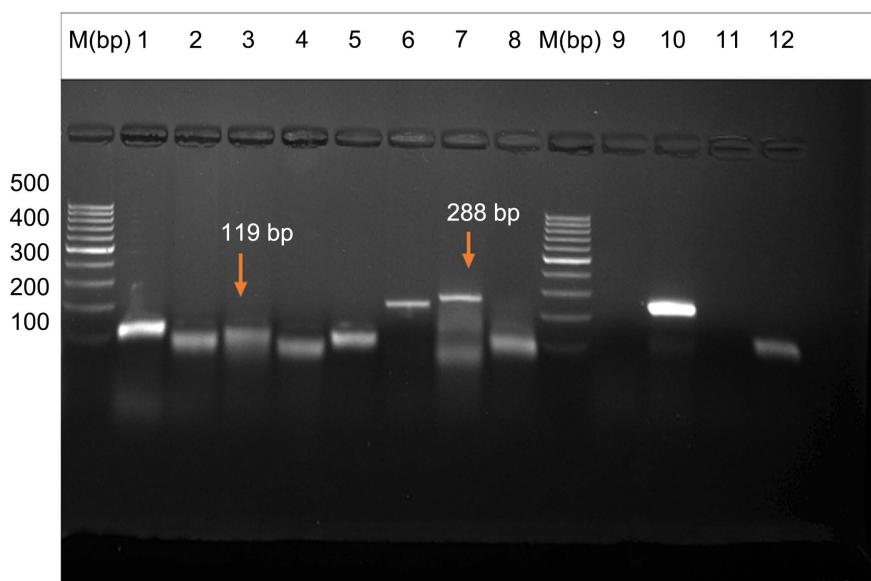


Figure 3. Illustrative image of a gel showing serotype-specific amplicons for dengue virus detection and typing. (Photo Fabrice C. GANDAHO, 2025).

3.3. Determination of Dengue Prevalence by Commune

Dengue infection prevalence was assessed in three hospitals: Abomey-Calavi, Abomey, and Lokossa. The results indicated the presence of positive cases in all study hospitals.

The highest prevalence was observed in Abomey-Calavi at 14.3% (95% CI: 4.8% - 30.3%). **Table 10** shows the prevalence of dengue by hospital.

Table 10. Dengue prevalence by hospital.

Commune	Number of patients	Number of positive cases	Prevalence (%)	95% CI
Abomey-Calavi	35	5	14.3	[4.8 - 30.3]
Abomey	50	3	6.0	[1.3 - 16.5]
Lokossa	35	3	8.6	[1.8 - 23.1]
Total	120	11	9.16	[4.7 - 15.8]

3.4. Prevalence of Dengue Serotypes by Commune

The prevalence of dengue infections was analyzed according to circulating serotypes in the population.

Out of 120 samples analyzed, the overall dengue prevalence was 9.16% ($n = 11$), DENV-2 accounted for 8.33% ($n = 10$), and DENV-3 accounted for 0.83% ($n = 1$). No cases of DENV-1 or DENV-4 were detected. Fisher's exact test showed that the prevalence of DENV-2 was statistically higher than DENV-3 in the study population.

Table 11 shows the prevalence of dengue serotypes in the hospitals.

Table 11. Prevalence of dengue serotypes by hospital.

Dengue Type	Abomey-Calavi	Abomey	Lokossa	Total positive cases	Prevalence (%)	95% CI	P value
DENV-2	4	3	3	10	8.33	[4.1 – 15.0]	0.009
DENV-3	1	0	0	1	0.83	[0 - 4.6]	
Total cases	5	3	3	11	9.16	[4 - 14.4]	

3.5. Distribution of Dengue by Age Group

20 - 30 years age group was predominantly infected by DENV-2, followed by the 0 - 10 years, 10 - 20 years, and ≥ 40 years age groups.

DENV-3 was found only in one patient aged 30 - 40 years. **Figure 4** illustrates the distribution of infection by age group.

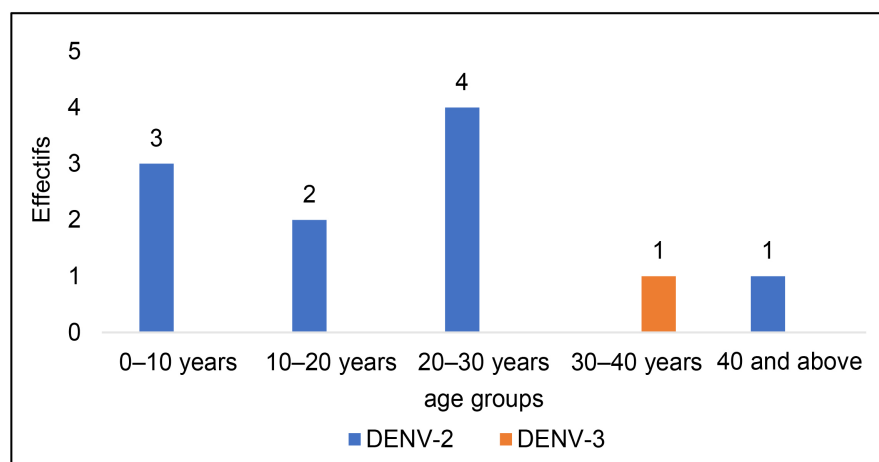


Figure 4. Distribution of dengue serotype infections by age group.

3.6. Distribution of Dengue by Sex

Among the two sexes in the study, females were more affected by dengue. DENV-3 was found only in a female patient. **Figure 5** shows the distribution of dengue serotype infections by sex.

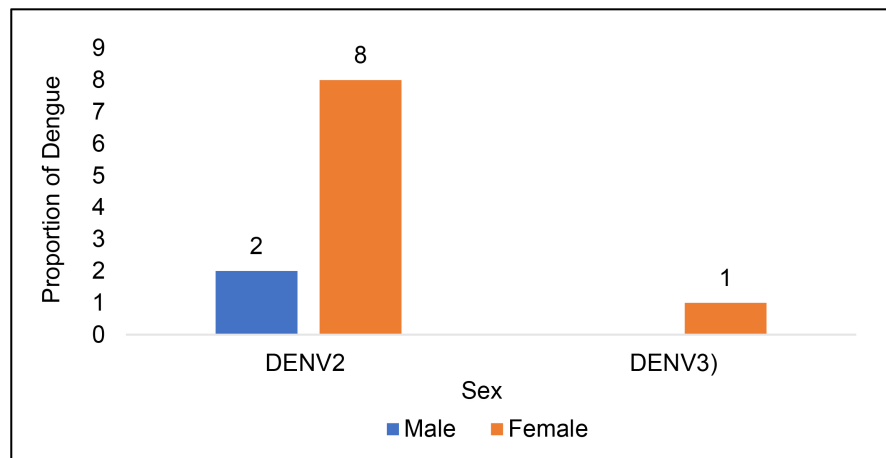


Figure 5. Distribution of dengue serotype infections by sex.

4. Discussion

This study highlights the circulation of dengue virus among malaria-negative febrile patients in southern Benin, with an overall prevalence of 9.16%. This finding confirms that dengue virus represents a non-negligible cause of febrile illness in Benin. Similar results have been reported in Nigeria, where dengue infection was identified among patients initially suspected of malaria, underscoring the risk of misdiagnosis in malaria-endemic settings. Likewise, a study conducted in Nigeria using NS1 antigen detection in febrile patients highlighted dengue as an important but often neglected cause of fever [21].

In other West African contexts, particularly in Burkina Faso, higher prevalence rates have been reported during epidemic situations, such as the 2016-2017 dengue outbreak, in which DENV-2 was the predominant serotype [22]. These differences in prevalence may be explained by several factors, including seasonal variation, epidemic contexts, and differences in diagnostic approaches. Indeed, studies combining serological assays (IgM/IgG, NS1) with molecular methods tend to report higher prevalence rates than those relying solely on PCR, which detects only acute infections.

Thus, although the prevalence observed in this study is moderate, it aligns with the regional epidemiological trend of dengue virus circulation in West Africa, where this infection is increasingly recognized as an important etiological agent of non-malarial febrile syndromes.

Among 1490 febrile patients who consulted during the study period, 288 (19.3%) had confirmed malaria infection, while 1202 (80.7%) tested negative for malaria. These results indicate that although malaria remains an important cause of fever in an endemic country like Benin, it is not the predominant etiology for most febrile episodes observed.

This proportion of malaria cases among febrile patients aligns with local and regional data. A study conducted at Dr. Pierre Boni Clinic in Cotonou reported a prevalence of approximately 31% among 731 patients analyzed during the rainy

season, with *Plasmodium falciparum* predominating among identified plasmodial species [23]. Another recent epidemiological study conducted in multiple hospitals in southern Benin emphasized that, despite malaria endemicity, it does not account for all febrile episodes, and other infectious agents contribute significantly to febrile clinical presentations [24].

Several factors may explain the predominance of non-malarial fevers observed in our cohort. On one hand, recent malaria control efforts in Benin, notably the introduction of the malaria vaccine in the Expanded Program on Immunization (EPI) and strengthened vector control strategies, contribute to reducing the clinical incidence of the disease, particularly among children. On the other hand, the epidemiology of febrile illnesses in tropical settings is multifactorial: various bacterial, viral, or parasitic infections can present with similar febrile symptoms and are increasingly recognized as common causes of fever in primary healthcare facilities. These observations highlight the need to strengthen differential diagnostic strategies for febrile syndromes in endemic contexts.

The high patient attendance in the three study centers, located in Abomey, Abomey-Calavi, and Lokossa, may be explained by population density and high utilization of hospital facilities in urban and peri-urban areas. Several studies have shown that urban areas in southern Benin have higher demand for malaria diagnostic tests, especially during the rainy season when febrile episodes increase [25].

For laboratory analyses, a selection of 120 febrile, non-malarial patients was conducted. Among them, 41 were men (34.16%), and 79 were women (65.83%), resulting in a male-to-female ratio of 0.52, indicating a clear female predominance. This overrepresentation of women aligns with observations by Ouédraogo [26], who also reported a high proportion of women in a study on dengue virus prevalence. Several factors may explain this female predominance: women tend to visit health centers more frequently than men, increasing their likelihood of testing and diagnosis; daily activities may expose them more to mosquito bites; and certain immunological differences between sexes may also contribute to this distribution.

PCR analysis identified 11 positive dengue cases across the three study locations: five (5) in Abomey-Calavi, three (3) in Abomey, and three (3) in Lokossa. These results confirm active circulation of the virus in these areas, consistent with previous reports [16] [18] [27]. The overall dengue prevalence in our study was 9.16%.

Serotype analysis revealed that among the 11 positive cases, ten (10) were DENV-2 and one (1) was DENV-3. The predominance of DENV-2 aligns with Allanonto [17], who reported this serotype as dominant in several southern Benin communes during investigations in the Atlantic, Littoral, and Ouémé departments. This observation is consistent with trends in several West African countries, where DENV-2 is frequently reported and sometimes associated with more severe disease forms [10].

Detection of DENV-3, although limited to a single case, is of particular interest. This serotype had previously been identified in *Aedes aegypti* mosquitoes in an

entomological study by several authors who reported DENV-3 detection in Beninese patients [16] [18]. The concordance between these studies and our results suggests that DENV-2 and DENV-3 are actively circulating in humans, supporting the hypothesis of local transmission sustained by infected vectors. These findings should alert healthcare professionals, as dengue testing should be considered when thick blood smear or rapid malaria tests are negative. It is also worth noting that other serotypes, including DENV-1 and DENV-4, have been detected in mosquitoes in Benin [27], though they were not identified in this study. This underscores the importance of continuous virological surveillance, as co-circulation of multiple serotypes can increase the risk of outbreaks and severe disease forms.

The detection of DENV-2 in Abomey and Lokossa is a significant contribution, as data describing virus circulation in humans in these areas remain limited. Virus presence may be linked to environmental and human factors, including artificial larval breeding sites, rapid urbanization, population density, and intercommunal mobility. An entomological study in Abomey-Calavi identified numerous artificial breeding sites facilitating vector proliferation [27]. Frequent movement between Abomey-Calavi, Abomey, and Lokossa could contribute to virus dissemination, supporting local transmission maintained by human mobility.

Analysis of positive cases showed that the 20 - 30 age group was the most affected, which is consistent with the results of a study conducted in Togo [11], where young adults were also predominantly affected. Their lifestyle, often characterized by high mobility and outdoor activities, may increase exposure to *Aedes aegypti*.

Furthermore, women were proportionally more infected than men [eight (8) women versus two (2) men for DENV-2, and one (1) woman for DENV-3]. This trend may relate to biological and immunological sex differences; women generally have stronger immune responses, which may facilitate more efficient virological detection during analysis [28] [29].

These findings indicate that multiple dengue virus serotypes circulate actively and affect diverse population groups, including in previously under-documented areas. Without systematic surveillance, these infections may go unnoticed, hindering appropriate prevention and control measures.

However, certain limitations should be considered. The relatively small sample size and short study duration may have limited detection of other circulating serotypes. Nonetheless, the data confirm the need to strengthen entomological and virological surveillance, integrate appropriate diagnostic tests into health facilities, and raise awareness among healthcare professionals about clinical suspicion of dengue in febrile syndromes.

5. Conclusions

This study aimed to determine circulating dengue virus serotypes among febrile, non-malarial patients in three southern Benin hospitals: Abomey-Calavi, Abomey, and Lokossa. It addresses a public health context marked by a high prevalence of

febrile illnesses of unknown origin, in a country where malaria remains the first diagnostic assumption and dengue, although present, is largely underestimated.

Analysis of 120 blood samples using molecular diagnostic methods provided new and essential data on this emerging virus in Benin. The results clearly indicate active dengue virus circulation in the three communes studied. Eleven positive cases were identified, ten of which were DENV-2 and one DENV-3, demonstrating the circulation of multiple serotypes and the notable predominance of DENV-2. Detection of DENV-3, though minor, reinforces the concept of serotype co-circulation and the potential risk of severe disease in secondary infections.

The geographic distribution of cases shows that dengue is not limited to major cities such as Abomey-Calavi but also affects less-documented communes like Abomey and Lokossa, suggesting that transmission may be more widespread than current data indicate.

A key takeaway is that despite confirmed virus presence and co-circulation of multiple serotypes, dengue is not yet systematically diagnosed in Beninese healthcare facilities. Febrile patients are often managed as malaria or bacterial infections, leading to delayed diagnosis, inappropriate care, and underestimation of the disease burden. These findings underscore the urgent need to strengthen diagnostic capacities, including reliable molecular and serological testing platforms in Beninese hospitals.

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Author Contributions

TFT, CFG, NK, MJA, LT, BA, and BAF designed the research; OST, ODA, MI, and IGY performed data collection; all authors contributed to data analysis. LM, RO, LBM, GL, DKG, and MA coded the data; TFT, CFG, MJA, and LT led the writing, with substantial contributions from ZSD, BM, and RO for the results section; all authors reviewed the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Abbreviations

cDNA: Complementary deoxyribonucleic acid

DENV1: Dengue virus serotype 1

DENV2: Dengue virus serotype 2

DENV3: Dengue virus serotype 3

DENV4: Dengue virus serotype 4

TBS: Thick blood smear