

Full Length Research Paper

# Prevalence of dengue fever in non-malaria fever syndromes at Angré University Hospital, Abidjan

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## Abstract

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**Introduction:** Côte d'Ivoire has an ecology favourable to arthropods and reports a high burden of acute febrile illnesses. However, the contribution of arbovirus infections to the burden of acute febrile illnesses is poorly understood. Given this situation, the present study was conducted to determine the level of dengue circulation in non-malaria febrile syndromes. **Method:** This prospective study was conducted from June to December 2021. Serum samples were collected from non-malaria fever patients after obtaining their consent at the Angré University Hospital laboratory. Dengue virus detection was performed using several biological diagnostic tools: serological tests, antigen tests and RT-PCR. **Results:** A total of 72 febrile patients agreed to participate in the study, with a majority of male patients (51%) and a predominance of patients aged between 21 and 31 (25%). There was a high level of ignorance about dengue fever (74%) and more than 98% had never seen a case among their friends and family. In addition, 78% said they did not use impregnated mosquito nets and 87.5% reported regular exposure to mosquito bites, indicating a high level of vulnerability. Biologically, a seroprevalence of 17% (12/72) was detected, solely through the presence of IgG antibodies, suggesting past infections. However, no NS1 antigen or IgM antibodies were detected, and RT-PCR was negative for all samples. **Conclusion:** Dengue circulates at low levels among non-malaria fever syndromes, with no viraemia detected at the time of the study.

**Keywords:** Dengue, febrile syndromes, thick smear negative, NS1 antigen, RT-PCR.

## INTRODUCTION

Dengue fever is an emerging arbovirus disease transmitted by daytime mosquitoes of the genus *Aedes*, mainly *Aedes aegypti* and *Aedes albopictus* (Schaefer et al. 2025), (Ahebwa et al. 2023). The dengue virus belongs to the genus *Flavivirus* of the Flaviviridae family. For several decades, dengue fever has been steadily

increasing worldwide. Before 1970, only nine countries had experienced severe dengue epidemics, compared to around 100 today. To date, there are five serotypes of the dengue virus: DEN-1, DEN-2, DEN-3, DEN-4 and DEN-5 (Whitehorn and Farrar 2010). Immunity to a given serotype is associated with a neutralising antibody response (WHO 2017). However, infection with one serotype only offers cross-immunity against other serotypes.

Clinically, dengue virus infection in humans causes a spectrum of diseases ranging from mild fever to severe and fatal haemorrhagic syndrome (Pourzangiabadi et al. 2025) , (Mallhi et al. 2015) . Given the similarity of its symptoms to those of other diseases, it is not uncommon for the symptoms of dengue fever to be clinically confused with other conditions such as: yellow fever, Japanese encephalitis, Zika virus infection, West Nile virus infection, alphavirus infections such as chikungunya and Sinbis, malaria, leptospirosis, typhoid, measles, enterovirus infection, influenza and other influenza-like syndromes, haemorrhagic fevers and acute HIV infection . Despite this picture, it has been observed that in regions where malaria is endemic, more than 70% of febrile illnesses are treated as suspected malaria, often without proper medical examination or laboratory diagnosis ( Amexo et al. 2004) ; (Mathison and Pritt 2017) ). According to the European GeoSentinel surveillance network, it is well documented that travellers suffering from fever are often misdiagnosed with malaria, with a misdiagnosis rate of up to 77% (Ndyomugenyi et al. 2007) . To date, many patients with fever in Africa are still classified as having fever of unknown origin or malaria and remain undiagnosed even if they do not respond to antimalarial drugs (Amexo et al. 2004) . In Côte d'Ivoire, malaria remains a major public health issue and the leading cause of consultations in health services. In the WHO's 2022 malaria report, morbidity and mortality rates in Côte d'Ivoire represented 3.1% and 2.5% respectively on a global scale (wmr-regional-briefing-kit-fre.pdf2022 .) At the same time, the number of dengue cases recorded was 360, including 3 deaths, according to the official website of the Government of Côte d'Ivoire in 2023( Health, Official portal of the Government of Côte d'Ivoire, 2023.) . To date, the diagnosis of dengue fever is based on three analytical techniques: molecular detection by RT-PCR, serum detection of the NS1 viral antigen, and serological tests (dengue IgG & IgM). Detection of the virus genome (RT-PCR) in patients' blood is the gold standard for early diagnosis used at the National Centre for Epidemiological Surveillance of Dengue at the Pasteur Institute of Côte d'Ivoire (IPCI). This technique is rapid and sensitive. However, it is expensive and is often unsuitable for field studies, as it requires qualified personnel and specialised laboratory equipment (Yang and Rothman 2004) . This constraint limits its large-scale use, particularly in resource-limited settings. As an alternative, rapid diagnostic tests (RDTs) are increasingly being used to facilitate early diagnosis, although their performance often requires confirmation by PCR in cases of doubtful results or in an epidemiological context suggestive of dengue.

In light of this, the present study was designed to assess the circulation of the dengue virus in non-malarial febrile patients in Abidjan. More specifically, it aims to describe the epidemiological characteristics of the study population, determine the seroprevalence of dengue using a rapid

diagnostic test, and perform genotyping of positive cases using molecular biology.

## METHOD

### • Study setting and type

The pre-analytical phase of this study, including patient selection and plasma collection from febrile patients with negative thick smears, was carried out at the medical biology department of the Angré University Hospital. Once the samples had been collected, serological and molecular analyses were carried out in the molecular biology unit of the Department of Epidemiological Virology (DEV) at the Pasteur Institute of Côte d'Ivoire (IPCI), using primer pairs specific to the dengue virus, in comparison with positive controls (Monica®). The cross-sectional, prospective study was conducted over a six-month period, from June to December 2021.

### • Study population

The target population included febrile patients without malaria. In practice, all patients presenting at the sampling room with a request for a thick smear were asked to complete a questionnaire, subject to their consent. Only patients whose thick smear test was negative were included. The plasma samples were then stored for subsequent serological and molecular analysis. A total of 72 patients were included in the study.

### • Selection criteria

- Inclusion criteria: Febrile patients with a negative thick smear during the study period, outpatients and inpatients, patients of all ages
- Exclusion criteria: Refusal to participate in the study and positive thick smear

### • Ethical considerations

Confidentiality and anonymity were guaranteed through the use of anonymised data collection forms, with patient identifiers replaced by codes during data entry. Samples were taken after obtaining informed consent from the parents of underage patients. Informed consent was also obtained from the administration of the Angré University Hospital.

### • Diagnosis of dengue virus infection

Three analytical techniques were used in this study: molecular detection by RT-PCR, serum detection of the NS1 viral antigen, and serological tests (dengue IgG & IgM).

- **Serum detection of antibodies (dengue IgG & IgM) and NS1 viral antigen of the dengue virus**

Serological analysis was performed using rapid diagnostic tests. The diagnostic cassettes used had the advantage of detecting both dengue seroprevalence through the detection of antibodies (IgG and IgM) and the dengue virus itself through the detection of the NS1 antigen as soon as the first clinical signs appeared. This test therefore offered the possibility of making a definitive diagnosis of DENV infection. As a result, the kinetics of the virus's appearance were covered by the use of this test. The tests used performed well, with a sensitivity of 92.4% for the NS1 antigen and 94.2% for IgG/IgM antibodies. Specificity was 98.4% (NS1) and 96.4% (IgG/IgM) respectively.

- **Molecular detection of dengue virus by RT-PCR.**

The real-time RT-PCR technique was used. Viral RNA extraction from plasma was performed according to the protocol of the QIAGEN Qiaamp RNA mini kit. This kit allowed the simultaneous processing of several samples and the isolation of a wide variety of viruses. Among the many protocols currently being developed, the analytical technique used in this study was that developed by Lanciotti *et al.* (Lanciotti *et al.* 1992) .

We used the RT-PCR protocol described by Lanciotti *et al.* (1992), recognised as a reference method due to its high sensitivity and ability to rapidly detect and type dengue viruses from clinical samples. This protocol, which has been widely validated in various epidemiological contexts, was chosen to ensure the comparability of results with previous studies. In our case, it was applied without modification. The principle of the technique consisted of performing a first reverse transcription step followed by a second amplification step on extracted total RNA. During the first amplification, a pair of oligonucleotides specific to conserved regions of the capsid and prM region allowed the amplification of a 511-base pair (bp) fragment. This amplification product is common to the five viral serotypes of dengue. The purpose of this first amplification with universal primers is to detect the presence of the dengue virus in the serum. If dengue virus is detected in the first RT-PCR, a second amplification step (semi-nested) is then performed to identify the viral serotype using five oligonucleotides, each specific to a serotype, generating PCR products of varying sizes depending on the serotype. The universal primers used for the first amplification were as follows (Table 1).

The reaction mixture contained 12.5 µl of 2X probe buffer (Monica), 8.25 µl nuclease-free water; 1.25 µl Primer F (Monica); 1.25 µl Primer R (Monica®); 0.5 µl Probe (Monica®); 0.25 µl Enzyme (Monica®) and 1 µl RNA. RT-PCR was performed with a reverse transcription step at 50°C for 30 min, followed by an initial activation at 95°C for 2 min and 40 cycles of 95°C for 15 s and 55°C for 120 s,

using DEN FP/DEN RP primers. (Table 1). At the end of the RT-PCR cycles, the tubes were incubated at 74°C for 5 min. Amplification was performed using a conventional thermal cycler. At the end of amplification, the amplicons were read using software (StepOne™) adapted to the microplate work surface.

- **Statistical analysis**

Statistical evaluation was performed using Epi/Info 6.2 (CDC) software to compare the independence of the means of the qualitative variables with the chi-square test ( $\chi^2$ ). Fisher's exact probability test was also used with this software to test the independence of the quantitative variables studied.

## RESULTS

- **Epidemiological data**

In a study population of 72 patients who agreed to participate in the survey, males (n=37) were in the majority, accounting for 51% of the sample, with a sex ratio (M/F) of 1.06 (Table 2). In addition to gender, the most represented age group was between 21 and 31 years old, with a proportion of 25%. With an average age of 22, the minimum and maximum ages were 9 days and 56 years, respectively. More than 74% (n=53) of the study population said they were unaware of the existence of dengue fever. However, 26% (n=19) of respondents acknowledged that they had been made aware of the existence of dengue fever through posters, television or radio. The coverage of impregnated mosquito nets was very low in the study population, at 22% (n=16). Finally, 87% (n=63) of the study population acknowledged having been exposed to mosquito bites (Table 3).

- **Serological, antigenic and molecular data from dengue diagnosis**

At the end of the investigation, the seroprevalence of non-malarial febrile subjects was 17% (n=12) with only IgG antibodies present, indicating that the infections were old (Table 2). At the same time, direct detection of the dengue virus by testing for the NS1 antigen proved negative. The RT-PCR results were negative for all 72 samples tested. (Table 2)

- **Seroprevalence of dengue and socio-demographic factors**

Regardless of age group, there was a heterogeneous distribution of dengue IgG serology (p= 0.13). Age was not a risk factor for the onset of dengue (Table 2).

The statistical evaluation of dengue seroprevalence and identified risk factors (gender, knowledge of dengue, use

**Table 1:** DEN RP/DEN FP primer pair and probe.

Probe	FAM: AAC-AGC-ATA-TTG-AGG-CTG-GGA-RAG-ACC- TAMRA	Nucleotide sequence
Primer F	AAG-GAC-TAG-AGG-TTA-KAG-GAG	511 base
Primer R	GGC-CYT-CTG-TGC-CTG-GAW-TGA	pairs (bp)

**Table 2:** Age groups and IgG seroprevalence.

Age group (years)	IgG seroprevalence	
	Positive	Negative
6 months–1 year (n=6)	1 (1%)	5 (7%)
]1–11] (n=14)	4 (6%)	10 (14%)
]11 – 21] (n=13)	2 (3%)	11 (15%)
]21 – 31] (n= 18)	2 (3%)	16 (22%)
]31–41] (n=12)	2 (3%)	10 (14%)
]41–51] (n=6)	1 (1%)	5
52 and over (n=3)	0	3 (4%)
<b>Total = 72</b>	<b>12 (17%)</b>	<b>60 (83%)</b>

**Table 3:** Dengue seroprevalence according to socio-demographic factors.

Socio-demographic factors	IgG detection		p
	Positive (12)	Negative (60)	
<b>Female (37)</b>	8 (11%)	29	P= 0.26
<b>Male (35)</b>	4 (6%)	31 (43%)	
<b>Sleeps under a mosquito net (n=16)</b>	3(4%)	13 (18%)	P= 0.50
<b>Does not use mosquito nets (n=56)</b>	9(13%)	47 (65%)	
<b>Aware of dengue fever (n=19)</b>	4 (6%)	15 (21%)	P= 0.06
<b>Unfamiliar with dengue fever (n=53)</b>	8 (11%)	45 (62%)	
<b>Exposed patients (n=63)</b>	11 (16%)	52 (72%)	P < 0,05
<b>Non-exposed patients (n=9)</b>	1 (1%)	8 (11%)	

of mosquito nets) did not reveal any correlation in most cases. However, unlike the previous risk factors, statistical analysis of the risk of exposure to mosquito bites indicates a strong correlation between seroprevalence and exposure to bites ( $P < 0,05$ ) (Table 3).

## DISCUSSION

At the end of the investigation, the serological results obtained indicated a variable distribution of

immunoglobulins (IgG) against dengue in subjects with febrile illnesses. The observed IgG seroprevalence was 17%. In this regard, it is important to note that the persistence of IgG-type anti-dengue antibodies has been widely reported in the literature. In fact, in 2018, a study conducted in Zhejiang Province, China, three (03) years after a dengue epidemic indicated a seroprevalence of 65% of IgG in the population, which showed that antibodies against dengue could persist long after infection (Luo et al. 2018). Furthermore, a 2007 study revealed that

serum samples from people who had contracted dengue-like illnesses during the Second World War were still positive for dengue-specific IgG antibodies more than 60 years later. (Imrie et al., 2007) . At the local level in Côte d'Ivoire, contrary to the results of the present study, in 2012, L'Azou et al. reported two IgM-positive cases (02/796), representing a seroprevalence of 0.4% among respondents from two centres (PISAM and Koumassi General Hospital) (L'Azou et al. 2015) . Recently, in 2021, a similar study conducted at the Naval Medical Centre on Victoria Island, Lagos by Ahmadu S.M. et al gave rates relatively similar to ours, with seroprevalence rates of 16.4% for IgG (50/305) and 6.9% (21/305 for IgM . We could also mention the results of the work by Soghaier et al. on dengue fever in Sudan, which showed a seroprevalence of 27.7%, which is higher than our results (Soghaier et al. 2013) . In Ethiopia in 2020, research into dengue seropositivity and associated risk factors in acute non-malarial febrile patients in the districts of Arba Minch, in southern Ethiopia, yielded IgG antibody seroprevalence rates of 25.1% (133/529) and IgM antibodies of 8.1% (43/529) (Eshetu et al. 2020) . The current result is also consistent with the findings of a study conducted in Djibouti (21.8%) (Andayi et al. 2014) . However, the seroprevalence rates obtained in Kenya and Brazil, which were 67.0% and 56% respectively, were significantly higher than our results (Sutherland et al. 2011) . Statistical evaluation of seroprevalence and identified risk factors did not reveal any correlation in most cases. In fact, regardless of age group, the distribution of dengue IgG serology in the study population was heterogeneous ( $p=0.13$ ), confirming that age was not a risk factor for the onset of dengue. In addition to age, the other risk factor explored was gender, with dengue seroprevalence being slightly higher in females. However, statistical analysis did not reveal a statistically significant association between the observed seroprevalence and gender in the study population ( $p = 0.26$ ). Thus, gender and age can be considered insignificant predictors of dengue IgG antibody seroprevalence during the study. This may be due to the fact that gender and age are not related to the duration of dengue IgG antibodies or to the small sample size of the study. Further research should be conducted to explore the relationship between the age of individuals infected with dengue and the duration of dengue IgG antibodies (Luo et al. 2018) . This lack of correlation between dengue seroprevalence and gender and age was also observed in studies conducted in Zhejiang Province, China, by Luo S et al (Luo et al. 2018) . In addition to gender and age, statistical analysis of mosquito net use in the survey showed no association between observed seroprevalence and mosquito net use. IgG seroprevalence was 19% in the study population that used mosquito nets ( $n=16$ ). In contrast, among patients who did not use impregnated mosquito nets ( $n=56$ ), seroprevalence was 16% ( $p=0.5$ ). This lack of association in the current study may be due to the fact that *Aedes aegypti* generally bites during the day

(Mazaba-Liwewe et al. 2014) , and therefore the use of mosquito nets may not help to provide a barrier between humans and the vector. Furthermore, consistent with the results of the Texas-Mexico study, this study showed no association between dengue exposure and the use of mosquito nets or mosquito repellents (Brunkard et al. 2007) . However, contrary to our results, a study in Haiti suggested that the use of mosquito nets could protect the population against dengue infection (Eshetu et al. 2020) , (Alcon et al. 2002) . Among the study population (74% ( $n=53$ )) who said they were unaware of the existence of dengue fever, 15% ( $n=8$ ) were IgG seropositive. Subsequently, statistical analysis did not reveal a correlation between the observed lack of awareness and IgG seroprevalence ( $p=0.06$ ). However, among the 87% ( $n=63$ ) of the study population who reported having been exposed to mosquito bites, 17% ( $n=11$ ) were IgG seropositive. Finally, unlike the previous risk factors, the statistical analysis performed on the risk of exposure to mosquito bites indicates a strong correlation between seroprevalence and said exposure to bites ( $P<0,05$ ). This demonstrates the role of the *Aedes* mosquito in the transmission of dengue fever (Daudé et al. 2015) .

At the same time, in terms of antigens, testing for the NS1 antigen in this study proved negative across the entire study population. It should be noted that the NS1 antigen is a non-structural protein of the dengue virus that is produced in excess and secreted during viral replication. It is detected in the serum of patients with dengue in the early stages (mainly from the 1<sup>st</sup> to the 5th day after the onset of fever) (Alcon et al. 2002) . The detection of this NS1 protein in serum is therefore used for the diagnosis of dengue in its early stages (WHO 2017) . These results show that the patients did not have dengue at the time of sampling. This is confirmed by a positive serology test for IgG only.

Ultimately, at the molecular level, RT-PCR did not detect any dengue virus in a sample of 72 febrile patients. It should be noted that this is the reference method for detecting the virus and identifying the viral genotype involved. It is a rapid technique that allows for early diagnosis, valid throughout the viremia phase. Plausible explanations here could be delays in hospital admission or a lack of knowledge about the disease, as evidenced by the high rate of fever of unknown origin. In this regard, it should be noted that in the present survey, only 26% (19/72) of the study population acknowledged having been made aware of dengue through posters or television. Of the 26%, only 21% (4/19) reported having had dengue patients in their circle of friends and family. In addition to the above arguments, we could mention traditional treatments and self-medication in our resource-limited countries, which delay hospital admissions (Hounsa et al. 2010) . Given this situation, the development and implementation of effective strategies to combat dengue fever is necessary. As dengue is a disease transmitted to humans by infected *Aedes* mosquitoes (Eshetu et al.

2020) , vector control measures need to be strengthened. In addition, both healthcare professionals and the general population need to be made aware of the circulation of dengue in the country. Furthermore, it is important to note that Côte d'Ivoire has a natural ecology favourable to arthropods and reports a high burden of acute febrile illnesses (Zahouli et al. 2017) . However, the contribution of arbovirus infections to the burden of acute febrile illnesses is poorly understood (L'Azou et al. 2015) , hence the interest in conducting further studies.

### Limitations

The main limitation of this study is the small sample size and the fact that it did not take into account previous treatment, which could have altered the course of the infection and complicated the diagnosis of recent cases through the detection of IgM and NS1 antigen. For future studies, it would be useful to increase the sample size and extend the inclusion period in order to improve the representativeness and robustness of the results.

### CONCLUSION

Dengue fever remains a significantly underdiagnosed and underreported disease, even in epidemic regions. In Côte d'Ivoire, most non-malaria febrile illnesses are classified as being of unknown origin. This is what motivated the present study to determine the level of dengue fever circulation in the study population. In terms of epidemiological, male subjects (51%, n=37) were in the majority. In addition to gender, the average age of the study population was between 21 and 31 years (25%). Most of the patients who were eligible and qualified for the study came from the community. Approximately 74% of patients surveyed responded that they were unaware of dengue fever. While 24% of patients acknowledged having heard of dengue fever through television or awareness posters, more than 98% of patients said they had never had any cases of dengue fever among their friends or family. It should also be noted that more than 78% of patients did not sleep under impregnated mosquito nets. Biologically, direct diagnosis through antigenic and molecular testing proved negative. However, for indirect diagnosis, 17% of respondents tested positive for IgG serology. Our study found a strong correlation between IgG seroprevalence and exposure to mosquito bites. Consequently, we recommend that the competent authorities strengthen vector control by organising awareness campaigns for the population on the need to destroy *Aedes* breeding sites.

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