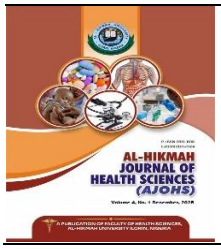
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Original Article

Serological and Molecular Detection of Dengue Virus Infection Among Febrile Patients Attending Healthcare Facilities in Ilorin, Nigeria

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ABSTRACT

Background: Dengue fever is an acute mosquito-borne viral disease posing significant public health challenge in tropical and subtropical regions, where presumptive treatment of all fevers as malaria in hyperendemic settings like Nigeria causes arboviral underdiagnosis.

Aim: This study evaluated serological and molecular prevalence of dengue virus infection among febrile patients in Ilorin, Kwara State.

Methods: Cross-sectional analytical study among 120 consented febrile patients from selected healthcare facilities in Ilorin. Five mL venous blood was collected into EDTA containers. Sera were screened for anti-DENV IgM and IgG using rapid immunochromatographic cassettes. Viral RNA extraction and RT-PCR targeting C-prM gene (512 bp) was performed only on IgM-positive acute samples (n=15). Data were analyzed with SPSS v27.0, $p \leq 0.05$ significant.

Results: Mean age 22.84±19.54 years, children 1-10 years 45.8%, females 66.7%, mean illness onset 3.46±1.79 days. Seroprevalence was 20.83% (25/120, 95% CI:14.43-29.11): IgM 12.50% acute, IgG 8.33% past. RT-PCR positivity was 6.67% (1/15, CI:1.19-29.82) among tested subgroup, equivalent to 0.83% (1/120, CI:0.15-4.57) of total cohort.

Conclusion: Findings confirm active dengue circulation among febrile patients in Ilorin, recommending routine arboviral screening alongside malaria testing to prevent misdiagnosis.

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Introduction

Dengue fever (DF) is one of the most rapidly expanding mosquito-borne viral infections globally, recognized by the World Health Organization as a major neglected tropical disease with significant epidemic potential (World Health Organization,

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2019). The illness is caused by the Dengue virus (DENV), a positive-sense, single-stranded enveloped RNA virus belonging to the genus Flavivirus and family Flaviviridae (Brady and Hay, 2020). DENV comprises four distinct but antigenically related serotypes (DENV-1, DENV-2, DENV-3, and DENV-4). Infection with one serotype confers lifelong homotypic immunity to that specific serotype, but only temporary cross-protective immunity to heterologous serotypes (Getachew *et al.,* 2015). Driven by rapid urbanization, climate change, international travel, and expanding habitats for competent mosquito vectors (Aedes aegypti and Aedes albopictus), global dengue incidence has increased dramatically over recent decades (Nwachukwu *et al.,* 2025).

In sub-Saharan Africa, including Nigeria, the epidemiology of dengue remains substantially underestimated and overshadowed by endemic parasitic infections such as malaria (Nwachukwu *et al.,* 2025). In primary and tertiary health institutions, acute non-specific febrile illness is routinely diagnosed and presumptively treated as malaria or typhoid fever (Kosasih *et al.,* 2016). However, an increasing proportion of febrile episodes test negative for malaria parasites on microscopy or rapid diagnostic tests, highlighting the necessity of investigating viral etiologies (Adesola *et al.,* 2024).

Laboratory diagnosis of dengue relies primarily on two complementary approaches: serological detection of host immune responses and molecular amplification of viral genomic RNA (Singh *et al.,* 2023). Serological assays detect Dengue-specific Immunoglobulin M (IgM) antibodies, which appear between days 3 and 5 post-symptom onset indicating acute infection, and Immunoglobulin G (IgG) antibodies, which reflect past exposure (Adesola *et al.,* 2024). Conversely, molecular diagnosis using Reverse Transcription-Polymerase Chain Reaction (RT-PCR) overcomes serological cross-reactivity constraints by amplifying conserved regions of DENV genomic RNA during the transient viremic window (Liang *et al.,* 2015).

Materials and Methods

Study Area

The study was conducted at designated healthcare facilities across Ilorin West, Ilorin South, and Ilorin East Local Government Areas in Ilorin, Kwara State. Blood samples were collected at the participating clinics. The serological procedure was performed at the Department of Medical Laboratory Science, Kwara State University Teaching Hospital, Ilorin. The molecular procedure was done at CERID Molecular Laboratory, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.

Study Design

This study was a cross-sectional analytical design conducted in Ilorin, Kwara State, Nigeria, among consented febrile patients presenting with acute febrile illness across all age groups.

Study Population and Selection Criteria

The study population comprised of consented male and female patients from all age groups presenting with ongoing acute febrile illness attending designated healthcare facilities across Ilorin West, Ilorin South, and Ilorin East Local Government Areas. Healthy individuals or patients presenting with fever who did not offer informed consent were excluded from the study.

Sample Size Determination

Sample size was calculated using the Cochrane formula $n = \frac{z^2 p(1-p)}{d^2}$

where: n = sample size, Z= the standard deviation at a 95% confidence interval (1.96), P = proportion of the population with the desired factor (adopted from dengue fever in Ilorin) = 8.2% = 0.082 as reported by (Sulaiman *et al.,* 2025), q = 1-p (0.918), while d is the maximum allowable error (5% = 0.05). Thus, a total of 120 estimated participants were recruited for this study.

Data Collection tools

Data were collected using well-structured, self-administered questionnaires which were specifically designed to obtain information that was used to either include or exclude individuals from this study. A questionnaire was given to the participants through an interviewer-assisted administration method, which served as the major source of primary data for this study. The participants were informed of the study's goals and objectives. The consent forms were submitted with the questionnaire, and informed consent from the participants was sought. The gathered data were kept on a safe, password-protected I-drive for future reference.

Sample collection

A volume of five milliliters (5 mL) of venous blood was collected aseptically from each participant into Ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes. Plasma was harvested following centrifugation at 3000 ×g for 5 minutes for serological procedures, while the remaining samples were stored at -80°C freezer until analyzed for molecular assays.

Laboratory Procedures and Protocols

Serological Detection of Anti-DENV IgM/IgG

Blood samples were screened for anti-DENV IgM and IgG antibodies using a commercial Dengue IgM/IgG Rapid Test Cassette (Nantong Diagnos Biotechnology Co., Ltd, China; Sensitivity: 96% and Specificity:

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98%), operating on immunochromatographic principles. Results were read and IgM positive samples were stored at -80ºC freezer for molecular procedures.

Molecular Characterization (RT-PCR)

Total viral RNA was extracted from plasma specimens using the QIAamp Mini RNA Extraction Kit (QIAGEN, Hilden, Germany). Reverse transcription and amplification targeting the C–prM gene junction were performed using sequence-specific primers (D1: 5'-TCAATATGCTGAAACGCGCGAGAAACCG-3' and D2: 5'-TTGCACCAACAGTCAATGTCTTCAGGTTC-3') following established protocols (Lanciotti *et al.,* 1992). PCR products were visualized by electrophoresis on a 1.2% agarose gel and visualized under a UV transilluminator, with the expected PCR product size of 512bp.

Statistical Analysis

Data were entered, cleaned, and analyzed using IBM SPSS Statistics for Windows, Version 27.0 (IBM Corp., Armonk, NY). Descriptive statistics were expressed as frequencies and percentages for categorical variables, and as Mean ± Standard Deviation (SD) for continuous parameters. Frequencies, percentages, and 95% confidence intervals were calculated, with statistical significance established at $p \leq 0.05$.

Ethical Considerations

Ethical approval for the study was granted by the Ethical Review Committee of Kwara State Ministry of Health (Approval Protocol Number: ERC/MOH/2025/09/520). Written informed consent was obtained from all adult participants or guardians of pediatric participants prior to recruitment. All procedures strictly adhered to the ethical standards outlined in the Declaration of Helsinki. Confidentiality and data security were strictly maintained.

Results

Table 1 shows the sociodemographic characteristics, revealing the majority of participants are children aged 0–10 years (45.8%), with females constituting 66.7%. Most have primary education (35.0%), and brick-built homes are predominant (90.8%). Over half do not have access to pipe water (57.5%), and over 90% benefit from window nets. Marital status is largely single (64.2%), while married individuals represent 34.2% and divorced 1.6%. Minority groups include those with Islamic education (6.7%) and residents aged over 60 years, who collectively account for less than 10% of the sample.

Table 1: Socio-demographic characteristics of the study Participants

Characteristic	Group	Frequency	Percentage
Age (years)	1 – 10	55	45.8
	11-20	20	16.7
	21 – 30	8	6.7
	31 – 40	12	10.0
	41 – 50	12	10.0
	51 – 60	4	3.3
	61 – 70	5	4.2
	71 – 80	4	3.3
	Mean (SD)	22.84	19.54
Gender	Female	80	66.7
	Male	40	33.3
Education	Nursery	16	13.3
	Primary	42	35.0
	Secondary	31	25.8
	Tertiary	23	19.2
	Islamic	8	6.7
Marital status	Single	77	64.2
	Married	41	34.2
	Divorced	2	1.6
Building type	Brick	109	90.8
	Mud	11	9.2
Pipe water	No	69	57.5
	Yes	51	42.5
Window net	Yes	113	94.2
	No	7	5.8

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Table 2 shows the clinical characteristics of the study population, focusing primarily on illness onset in days. The distribution shows that most participants (65.8%) experienced the onset of illness within one to three days: The mean onset was 3.46 days with a standard deviation of 1.79. Additionally, 97.5% (117 individuals) did not travel recently, while only 2.5% (3 individuals) had traveled. Regarding yellow fever vaccination, 99.2% (119 individuals) had not received it recently, with a very small proportion (0.8%, 1 individual) vaccinated. Vector control measures were not utilized by 82.5% (99 individuals), and only 17.5% (21 individuals) reported using them. Awareness of dengue fever was lacking, as 100% (120 individuals) had not heard of it. Overall, the data highlight rapid illness onset, limited travel and vaccination within study period, minimal vector control, and poor disease awareness within the population.

Table 2: Clinical Characteristics of the study population.

Characteristic	Group	Frequency	Percentage
Illness onset (days)	1-3	79	65.8
	4-6	32	26.7
	7-9	8	6.7
	10-12	1	0.8
	Mean ± SD	3.46	±1.8
Travel	No	117	97.5
	Yes	3	2.5
Daytime Mosquito bite	Yes	53	44.2
	Don't Know	45	37.5
	No	22	18.3
Yellow Fever Vaccination	No	119	99.2
	Yes	1	0.8
Vector control	No	99	82.5
	Yes	21	17.5
Heard of Dengue	No	120	100.00
	Yes	0	0.00

Table 3 shows the serological prevalence of dengue virus among febrile patients. Using immunoglobulin assays, IgM and IgG antibody responses were measured, revealing that 25 patients (20.83%) were positive for dengue virus antibodies, with a 95% confidence interval ranging from 14.43% to 29.11%. Of these, IgM positivity, indicative of recent or acute infection, was observed in 15 patients (12.5%), while IgG positivity, reflecting prior exposure, was detected in 10 patients (8.33%). Conversely, 95 patients (79.17%) were antibody-negative, with the confidence interval spanning from 70.89% to 85.57%. These results suggest that most febrile patients tested did not exhibit serological evidence of dengue infection, although both recent and past infections were identified among antibody-positive cases.

Table 3: The serological prevalence of dengue virus among febrile patients

	Frequency	Prevalence	Lower 95% CI	Upper 95% CI
Ig positive	25	20.83	14.43	29.11
Ig M	15	12.50		
Ig G	10	8.33		
Ig negative	95	79.17	70.89	85.57

Fig 1 shows the prevalence of dengue viral RNA among febrile patients, as determined by molecular techniques (RT-PCR), was notably low. Out of the 15 IgM seropositive cases, only one individual tested positive for dengue virus RNA, corresponding to a frequency of 6.67% with a 95% confidence interval ranging from 0.17% to 31.95%. In contrast, 14 patients were negative for dengue virus RNA, representing a frequency of 93.33% (95% CI: 68.05%–99.83%). These findings indicate that active dengue viral infection, as detectable by RT-PCR, was rare within this cohort of febrile patients.

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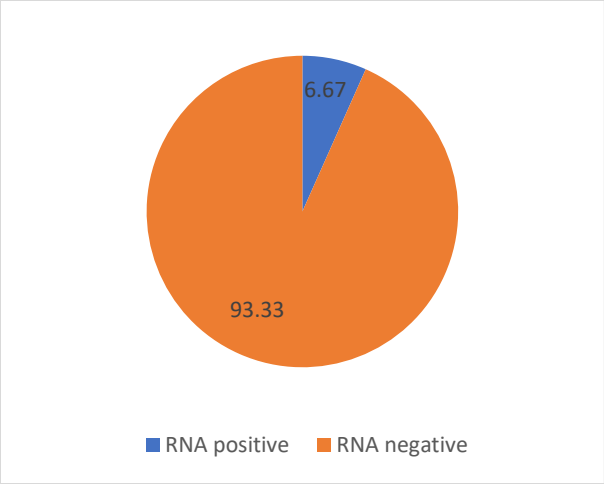


Fig 1: Prevalence of dengue viral RNA among febrile patients using molecular techniques

Discussion, Conclusion and Recommendations

This study evaluated patient age as the only sociodemographic factor significantly associated with dengue positivity ($p = 0.035$), with infections heavily concentrated among children aged 1–10 years and youths aged 18–44, whereas adults aged 45 and above were exclusively dengue-negative. This pattern aligns with global epidemiological findings where primary infections predominantly affect younger populations, while older adults are protected by acquired lifetime immunity. Nevertheless, this high concentration among children poses a critical public health vulnerability due to paediatric physiological traits, such as increased capillary fragility, which heighten their risk of progressing to Dengue Shock Syndrome (DSS) and multi-organ failure.

This study also revealed the serological and molecular prevalence of dengue virus infection among febrile patients in Ilorin, Kwara State. The findings establish an overall seroprevalence of 20.83%, with 12.50% demonstrating IgM positivity for acute infection and 8.33% showing IgG positivity for past exposure. This confirms that the dengue virus is actively circulating within the local population and contributes substantially to non-malarial acute febrile illnesses in Nigeria. The serological results align closely with regional epidemiological studies in Nigeria, such as Adeshina *et al.,* (2021) and Animasaun *et al.,* (2025), who documented considerable dengue antibody prevalence among febrile and healthy cohorts.

The presence of IgG antibodies (8.33%) highlights a sub-population with prior exposure, who theoretically face heightened susceptibility to antibody-dependent enhancement (ADE) upon secondary infection with a heterologous viral serotype (Guzman *et al.,* 2016). Molecular RT-PCR testing revealed a viral RNA positivity rate of 6.67% among tested acute cases. This disparity highlights the critical role of viremic kinetics in arboviral diagnostics. RT-PCR is highly specific but constrained to the early febrile window (days 1–5), whereas serological assays effectively capture patients presenting past the viremic peak during immune seroconversion (Wang, 2023). Integrating both serological screening and molecular confirmation provides a comprehensive diagnostic framework for arboviral surveillance in endemic regions.

This study concludes that dengue virus infection is actively prevalent among febrile patients in Ilorin, evidenced by a serological prevalence of 20.83% and molecular viral RNA detection. Routine arboviral screening alongside malaria diagnostics is imperative to prevent misdiagnosis in clinical management and enhance epidemiological surveillance.

This study recommends that healthcare practitioners in Ilorin and across hyperendemic regions must incorporate routine dengue diagnostic screening (specifically IgM/IgG rapid test assays) into standard paediatric fever management guidelines, given that children aged 1–10 bear the highest infection burden and are uniquely vulnerable to severe complications like Dengue Shock Syndrome.

Public health authorities and medical institutions should establish rigorous clinical guidelines to eradicate the presumptive management of all acute febrile illnesses as malaria. Clinicians must be educated to avoid the empirical administration of non-steroidal anti-inflammatory drugs (NSAIDs) in undifferentiated fevers until arboviral aetiologies are ruled out.

The State Ministry of health should commission structured, multi-centre surveillance systems that combine serological seroprevalence mapping with molecular RT-PCR testing during peak transmission seasons to continuously monitor viral circulation, track serotype shifts, and forestall antibody-dependent enhancement (ADE) outbreaks.

Contributions to Knowledge

1. This study provides definitive empirical evidence of active dengue virus transmission among febrile patients in Ilorin, Kwara State, establishing a total antibody seroprevalence of 20.83% (12.50% IgM acute and 8.33% IgG past exposure) alongside a 6.67% molecular RT-PCR positivity rate.
2. The research demonstrates that patient age is a statistically significant determinant of dengue positivity ($p = 0.035$), revealing that infections are predominantly concentrated among children aged 1–10 years (comprising 45.8% of the cohort) and youths, thereby flagging pediatric populations as high-risk groups requiring prioritized clinical monitoring.
3. By proving active viral circulation alongside endemic febrile illnesses, the study emphasizes

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the clinical hazard of presumptive malaria treatment and the inadvertent administration of non-steroidal anti-inflammatory drugs (NSAIDs), providing a critical rationale for integrating routine arboviral screening to prevent severe haemorrhagic complications.

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