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Research Article

# Sero-Molecular Surveillance of Dengue Virus Antibodies among Malaria Suspected Febrile Subjects in Yenagoa Metropolis, Niger Delta

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## Abstract

Dengue and malaria are mosquito-borne diseases that potentially pose significant public health challenges in Nigeria especially in riverine regions, where ecological conditions favor vector fecundity. This cross-sectional study, investigated the Sero-molecular prevalence and spatial distribution of dengue and malaria co-infection outcomes among 182 febrile patients in some health care facilities in Yenagoa, Bayelsa State, Nigeria. Aseptic venous blood samples were collected and analyzed using enzyme-linked immunosorbent assay (ELISA) and lateral flow assay protocol were explored for dengue IgM/IgG antibodies, and Giemsa-staining technique which is a gold standard for microscopy malaria studies, was explored for malaria parasitemia assay. Well-structured questionnaire were deployed to generate the demographics and potential environmental risk factors, while Geographic Information System (GIS) mapping was used to identify infection hotspots. Results revealed dengue IgM seroprevalence of 3.8% (7/182), IgG seroprevalence of 1.6%, malaria prevalence of 91.2% (166/182), and co-infection rate of malaria and dengue of 3.8%. Lateral flow assays showed 71.4% sensitivity compared to ELISA ($\kappa = 0.65$), indicating diagnostic limitations. GIS mapping highlighted higher dengue prevalence in riverine wards (7.2%) versus urban areas (4.8%). Significant risk factors implicated open water storage (OR = 2.8, p = 0.004) and daytime net disuse (OR = 3.2, p = 0.001), contributing 69% to population-attributable risk. Co-infections were predominant among farmers and fishers (p = 0.03). These findings confirm low but notable dengue circulation in a malaria-hyperendemic region, emphasizing the need for integrated diagnostic strategies, GIS-guided vector control, and enhanced surveillance to address co-infection menace in Yenagoa's riverine communities, thus informing targeted public health interventions.

***Keywords*:** *Dengue; Malaria; Co-Infection; Sero-Molecular Surveillance; GIS Mapping; Yenagoa; Risk Factor*

## Introduction

Dengue fever and malaria are two mosquito-borne diseases transmitted by *Aedes* and *Anopheles* mosquitoes, respectively. They represent significant global public health challenges, particularly in tropical and subtropical regions. The World Health Organization [1] estimates that malaria accounts for approximately 249 million cases annually, with Nigeria alone contributing 27% of this global burden, making it one of the most malaria-endemic countries worldwide. Concurrently, dengue fever, caused by the dengue virus (DENV), is rapidly

emerging as a major global health concern, with an estimated 390 million infections annually across 129 countries [1]. In Nigeria, recent Sero-epidemiological studies have reported dengue IgG seropositivity rates as high as 44.7% in certain regions, indicating widespread exposure and potential for ongoing transmission spread [2]. This dual burden of dengue and malaria poses a critical challenge, particularly in ecologically vulnerable areas such as Nigeria's Niger Delta communities, where environmental conditions-swampy terrains, high humidity, and abundant water bodies-favor the proliferation of mosquito vectors [3].

In regions like Yenagoa, the capital of Bayelsa State, the overlapping clinical presentations of dengue and malaria significantly complicate diagnosis and treatment. Both diseases share non-specific symptoms, including fever, headache, myalgia, and fatigue, which often lead to misdiagnosis or underdiagnosis of dengue in settings where malaria is hyperendemic [3-5]. This diagnostic challenge is exacerbated in under-resourced riverine areas like Bayelsa, where diagnostic infrastructure is limited, and routine testing predominantly focuses on malaria, often overlooking dengue [6]. The lack of robust surveillance systems further obscures the true prevalence of dengue and its co-occurrence with malaria, hindering effective public health responses [3]. Moreover, the riverine ecology of Yenagoa, characterized by stagnant water bodies and dense vegetation, creates ideal breeding grounds for both "*Anopheles*" and "*Aedes*" mosquitoes, increasing the risk of co-infections [7]. These co-infections can complicate clinical management, prolong recovery, and elevate the risk of severe outcomes, particularly in vulnerable populations with limited access to healthcare [3,4].

Despite the growing recognition of dengue as an emerging threat in Nigeria, there is a paucity of data on its Sero-molecular prevalence and spatial distribution in riverine settings like Yenagoa, where malaria dominates public health efforts. Previous studies in Nigeria have reported varying dengue sero-prevalence rates, ranging from 5.7% in Benin City to 7.2% in the northeast, but few have explored co-infections or employed advanced diagnostic tools like Enzyme-Linked Immunosorbent Assay (ELISA) and Geographic Information System (GIS) mapping to assess spatial patterns [2,5]. Understanding the epidemiology of dengue and malaria co-infections in such settings is critical for developing integrated diagnostic and control strategies that would address both diseases simultaneously.

## Aims and Objective of the Study

The aim of this study was determine the sero-molecular prevalence and spatial distribution of dengue and malaria co-infections among febrile subjects in Yenagoa, Bayelsa State, Nigeria. The specific objectives are: (i) to determine the seroprevalence of dengue virus antibodies (IgM and IgG) and malaria parasitemia among febrile patients; (ii) to determine the prevalence rate of dengue and malaria co-infections; and (iii) to compare the diagnostic outcomes of serological (ELISA and lateral flow assays) methods.

This study holds significant implications for public health intervention in Nigeria's malaria-hyperendemic and dengue-prone regions. By determining the prevalence of dengue and its co-occurrence with malaria, the findings will enhance diagnostic accuracy, addressing the critical issue of misdiagnosis due to clinical overlap. The use of Sero-molecular diagnostics and GIS mapping strategy will provide robust data to strengthen surveillance systems, enabling the identification of transmission hotspots areas and high-risk populations. Furthermore, the study's outcomes will inform evidence-based public health policies, guiding the development of targeted vector control interventions and integrated diagnostic protocols to mitigate the dual burden of dengue and malaria in riverine communities like Yenagoa. Ultimately, this research contributes to the global effort to address neglected tropical diseases and improve health outcomes in resource-constrained settings, even as it aligned with SDG goal 3 and 4.

## Materials and Methods

### Study design

A cross-sectional study was conducted to investigate the Sero-molecular prevalence and spatial distribution of dengue and malaria co-infections among febrile patients in Yenagoa, Bayelsa State, Nigeria. The cross-sectional research design was selected to capture a snapshot of disease prevalence and associated risk factors within a defined period, appropriate for assessing the burden of these mosquito-borne

diseases in a malaria-hyperendemic region with emerging dengue transmission. This approach enabled efficient data collection across multiple health facilities, facilitating the identification of co-infection patterns and spatial clustering outlooks.

### Study population and sampling

The study enrolled 182 febrile patients attending four health care facilities in Yenagoa, including the Federal Medical Centre, Niger Delta University Teaching Hospital, Federal Medical Centre Yenagoa and two primary health care centers. Eligible participants were individuals aged ≥1 year presenting with fever (axillary temperature ≥37.5°C) or a history of fever within the preceding 48 hours, suggestive of dengue or malaria. Exclusion criteria included patients with confirmed non-mosquito-borne infections (e.g. bacterial sepsis), those unwilling to provide consent, or those with incomplete clinical records. A convenience sampling method was employed, recruiting consecutive febrile patients presenting at some selected healthcare facilities as mentioned above to ensure practical and timely enrollment during the study period.

### Sample size calculation

The sample size was calculated using the formula for prevalence studies as shown below:

$N = \frac{z^2pq}{d^2}$ (Cochran, [8])

Where N = Minimum sample size.

Z = Standard normal deviation corresponding to 95% confidence level set at 1.96.

p = 2.3% = 0.023,

q = 1-p = 0.977,

d = Desired precision, 5% (0.05)

$$N = \frac{1.96^2 \text{x} 0.023 \text{x} 0.977}{0.05^2}$$

$$N = \frac{3.92\ X\ 0.023\ X\ 0.977}{0.0025}$$

$N = \frac{0.08831103}{0.0025}$ = 35.2344

So, N = 35.

Accounting for a 10% non-response rate, a minimum sample size of approximately 160 was required. A total of 182 participants were enrolled to enhance statistical power and accommodate potential variability in co-infection data.

### Data collection

Venous blood samples (5 mL) were collected aseptically by trained phlebotomists from each participant. Dengue virus antibodies were detected using enzyme-linked immunosorbent assay (ELISA) (Dengue IgM/IgG ELISA Kit, Standard Diagnostics, South Korea) and lateral flow immunoassay (Dengue Duo Rapid Test, SD Bioline, South Korea) to identify IgM (recent infection) and IgG (past exposure) antibodies.

### Principle of ELISA assay

The Dengue Virus IgM ELISA test is based on the principle of a qualitative indirect enzyme-linked immunosorbent assay (ELISA), specifically designed to detect human IgM antibodies against dengue virus (DV-IgM) in serum or plasma. The assay utilizes microplate wells that are pre-coated with purified dengue virus antigens, forming a solid-phase antigen layer. When a patient's serum is added to these wells, any dengue-specific IgM antibodies present in the sample bind to the immobilized antigens through antigen-antibody interactions.

Following the initial binding step, the wells are washed to remove unbound components. A horseradish peroxidase (HRP)-conjugated dengue-specific antigen is then added. This enzyme-labeled antigen binds to the Fc region of the previously bound human IgM antibodies, forming an antigen-antibody-enzyme complex. After a second wash to eliminate unbound conjugate, a chromogenic substrate solution composed of tetramethylbenzidine (TMB) and hydrogen peroxide is added. In the presence of HRP, this substrate undergoes enzymatic oxidation, resulting in a blue-colored complex.

The color reaction is stopped by the addition of a sulfuric acid stop solution, which changes the color from blue to yellow. The optical density (OD) of each well is then measured using a microplate reader at 450 nm. The intensity of the yellow coloration is directly proportional to the amount of dengue-specific IgM antibody in the sample. Qualitative interpretation is made by comparing the OD value of the test samples to a cutoff value derived from negative control readings. Samples with OD values equal to or greater than the cutoff are considered positive for DV-IgM, indicating a recent dengue infection.

### Experimental/sample preparation

Serum samples were obtained through standard phlebotomy. Blood was allowed to clot at room temperature for 20 minutes and centrifuged at 3,000 rpm for 20 minutes to separate serum. Care was taken to avoid hemolyzed or lipemic samples. If immediate analysis was not feasible, serum aliquots were stored at −20°C to prevent degradation, avoiding repeated freeze-thaw cycles. Plasma samples, when used, were collected in EDTA or citrate anticoagulant tubes and processed similarly. All samples and reagents were equilibrated to room temperature (20-25°C) prior to analysis, as per manufacturer recommendations.

### Assay procedure

Microplate wells were designated for negative control, positive control, blank control, and test samples. For test wells, 40 µL of sample diluent and 10 µL of patient serum were added. Controls were applied in duplicate. The plate was sealed with a closure membrane and incubated at 37°C for 30 minutes. Following incubation, the wells were washed five times using a 1:30 dilution of wash buffer prepared with distilled water. After washing, 50 µL of horseradish peroxidase (HRP)-conjugated antigen reagent was added to each well, except the blank, and the plate was incubated again at 37°C for 30 minutes.

Post-incubation, another five-cycle wash was performed to remove unbound reagents. Then, 50 µL each of Chromogen Solution A and Chromogen Solution B were added to all wells. The plate was incubated in the dark at 37°C for 15 minutes to allow color development. The reaction was stopped by adding 50 µL of stop solution to each well, changing the color from blue to yellow. Optical density (OD) was measured at 450 nm using a microplate reader. A sample was considered positive for DV-IgM if the OD value was greater than or equal to the cut-off value, calculated as the mean OD of negative control plus 0.15; otherwise, it was deemed negative. Quality control was confirmed if the average OD of the positive control was ≥1.00 and that of the negative control was ≤0.10.

### Interpretation and quality assurance

All runs included internal controls, and only those satisfying manufacturer criteria for control OD values were accepted. Equivocal results-defined as borderline values near the cutoff-were repeated for confirmation. The specificity of the SunLong DV-IgM ELISA kit is

optimized for primary infections, with reduced cross-reactivity compared to older dengue tests; however, confirmatory testing using NS1 antigen ELISA or IgG serology was recommended where available for suspected secondary infections.

### Lateral flow assay for dengue fever antibody detection

Lateral flow assays (LFAs) for dengue fever detection operate on immunochromatographic principles, utilizing labeled antibodies to capture dengue virus NS1 antigen or anti-dengue IgM/IgG antibodies in patient samples. The assay relies on capillary flow, where the sample migrates along a nitrocellulose membrane, forming visible test and control lines upon interaction with immobilized antibodies.

### Experimental - Test procedure

The test setup began with all reagents and samples being equilibrated to room temperature (20 - 25°C) prior to testing, and test cassettes were properly labeled with corresponding sample IDs. For sample application, serum samples (10 - 20 µL) were dispensed into the sample well followed by the addition of 2 - 3 drops of the provided assay buffer. When using whole blood samples, approximately 20 - 30 µL (one drop) was applied directly to the sample well along with the appropriate volume of buffer as specified in the manufacturer's instructions. The test was then allowed to develop for 15 - 20 minutes, with the exact incubation time adjusted according to the specific kit protocol. Results were interpreted immediately after the designated incubation period to prevent potential overdevelopment that could lead to false-positive or ambiguous readings.

### Result interpretation

- Positive/Negative: Only the control line (C) visible connotes negative and double lines in control and test connotes positive result.
- Invalid: Absence of the control line, necessitating test repetition.
- Invalid: Absence of the control line, necessitating test repetition.
- Quality control measures.
- Internal control: Each test included a built-in control line to validate proper assay performance.

### Assay for malaria parasite

### Think and thin blood film preparation

The whole blood was used to make a thin and thick blood film on a grease-free glass slide (for the identification of malaria parasite).

On a clean dry and grease free microscopic glass slide, a thin blood film of the specimen was made and left to air dry. The smear was dipped (2 - 3 dips) into pure methanol for fixation of the smear and left to air dry for 30 seconds. The slide was flooded with 5% Giemsa stain solution for 20 - 30 minutes. Tap water was used to rinse the slide and left to dry.

A thick blood film was made to the size of five kobo on a grease-free glass slide. It was allowed to air dried for 1 hour on a staining rack. The thick blood smear was dipped into diluted Giemsa stain (prepared by taking 1 ml of the stock solution and adding to 49 ml of phosphate buffer water. The smear was washed by dipping in in buffered water or distilled water for 3 - 5 minutes and left to dry.

The slides were viewed under light microscope to identify malaria parasite (*Plasmodium falciparum*) using x 100 immersion oil and low power objective lens for focusing. The method was achieved by following the World Health Organization [1] protocol of Parasite density quantification per microliter of blood for positive cases.

Furthermore, a well-structured questionnaire was given to all subjects to determine the socio-demographics of the subjects, together with the associated risk factor promoting the trend in the study area. The Geographic Information System (GIS) Technology (ArcGIS Pro,

Esri) was utilized to map the spatial distribution of confirmed dengue and malaria cases across Yenagoa's wards. Participant residential addresses were geocoded, and ward-level boundaries were sourced from the Bayelsa State Geographic Information System database to identify infection hotspots.

### Statistical analysis

Data were analyzed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics (frequencies, percentages) summarized demographic characteristics, seroprevalence, and co-infection rates. Chi-square tests evaluated associations between categorical variables (e.g. occupation and infection status). Logistic regression was used to compute odds ratios (OR) with 95% confidence intervals (CI) to identify risk factors for dengue and malaria co-infection, adjusting for confounders such as age and gender. Agreement between ELISA and lateral flow immunoassay for dengue antibody detection was assessed using Cohen's kappa coefficient (κ), interpreted as: <0.20 (poor), 0.21-0.40 (fair), 0.41-0.60 (moderate), 0.61-0.80 (good), and >0.80 (excellent). Statistical significance was set at $p < 0.05$.

## Ethical considerations

Ethical approval was granted by the Health Research Ethics Committee of all the healthcare facilities where our samples were collected. Written informed consent was obtained from all participants, with parental/guardian consent and minor assent for those under 18 years. Confidentiality was ensured through unique participant identifiers, and data were securely stored in compliance with ethical standards. Participants with confirmed infections were referred for appropriate medical care as per national treatment guidelines.

## Results

### Prevalence

Of the 182 febrile patients screened, the Sero-molecular analysis revealed a dengue IgM seroprevalence of 3.8% (7/182), indicating recent infections, and a dengue IgG seroprevalence of 1.6% (3/182), suggesting limited past exposure. Malaria parasitemia was detected in 91.2% (166/182) of participants, reflecting the hyperendemic nature of malaria in Yenagoa. The co-infection rate of dengue and malaria was 3.8% (7/182), with all co-infection cases occurring among individuals positive for dengue IgM. These findings are summarized in table 1.

| Parameter | Test Method | Prevalence (%) | Number Positive/Total |
|---|---|---|---|
| Dengue IgM | ELISA | 3.8 | 7/182 |
| Dengue IgM | Lateral Flow | 2.7 | 5/182 |
| Dengue IgG | Lateral Flow | 1.6 | 3/182 |
| Malaria Parasitemia | Giemsa Microscopy | 91.2 | 166/182 |
| Co-infection (Dengue IgM + Malaria) | ELISA + Microscopy | 3.8 | 7/182 |
| Diagnostic Agreement (IgM) | Cohen's κ | 0.65 | - |
| Lateral Flow Sensitivity (IgM) | Compared to ELISA | 71.4 | - |

***Table 1:*** *Seroprevalence and diagnostic performance for dengue and malaria.*

### Diagnostic performance

Comparison of diagnostic methods for dengue IgM detection showed that ELISA had a higher positivity rate (9.3%) compared to the lateral flow immunoassay (1.6%). The sensitivity of the lateral flow assay, using ELISA as the reference standard, was 71.4%, with a Cohen's kappa coefficient (κ) of 0.65, indicating good agreement between the two methods. These results are presented in table 2.

| Diagnostic Method | Positivity Rate (%) | Sensitivity (%) | Cohen's κ |
|---|---|---|---|
| ELISA (IgM) | 9.3 | Reference | 0.65 |
| Lateral Flow (IgM) | 1.6 | 71.4 | - |

***Table 2:*** *Comparison between dengue ELISA and lateral flow assay method of screening.*

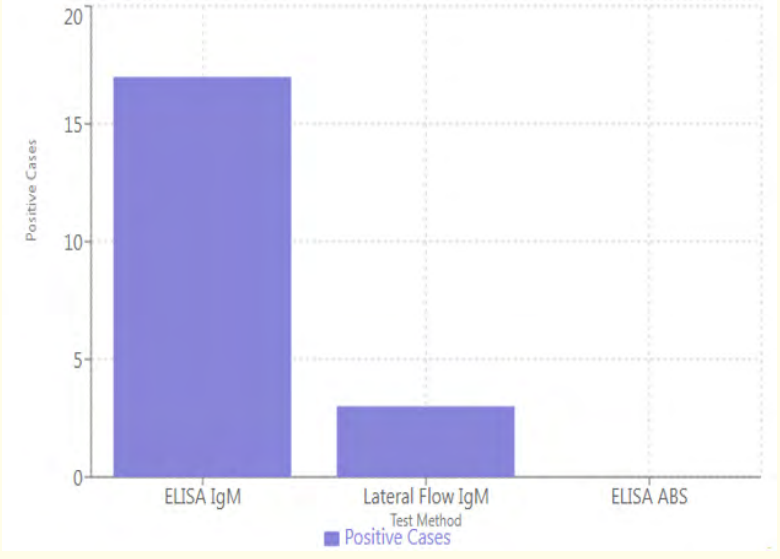


***Figure 1:*** *Comparison of dengue test methods.*

## Risk factors

Logistic regression analysis identified significant environmental and behavioral risk factors for dengue infection. Open water storage was associated with an increased risk (odds ratio [OR] = 2.8, 95% confidence interval [CI] = 1.4-5.6, p = 0.004), as was daytime disuse of mosquito nets (OR = 3.2, 95% CI = 1.7-6.0, p = 0.001). Daily mosquito bite exposure also increased risk (OR = 1.9, 95% CI = 1.1-3.3, p = 0.02). These factors collectively accounted for 69% of the population-attributable risk for dengue infection.

## Statistical associations

Chi-square analysis demonstrated significant associations between infection status and occupational groups. Farmers and fishers exhibited higher dengue IgM positivity (8.6%) and co-infection rates (8.6%) compared to civil servants (dengue IgM: 1.4%, co-infection: 1.4%) and unemployed individuals (dengue IgM: 1.9%, co-infection: 1.9%) (p = 0.03). No significant gender differences were observed (p = 0.45). These findings are detailed in table 3.

| Variable | Category | Dengue IgM (%) | Malaria (%) | Co-infection (%) | p-value |
|---|---|---|---|---|---|
| Gender | Male | 3.5 (3/85) | 91.8 (78/85) | 3.5 (3/85) | 0.45 |
| | Female | 4.1 (4/97) | 90.7 (88/97) | 4.1 (4/97) | |
| Occupation | Farming/Fishing | 8.6 (5/58) | 94.8 (55/58) | 8.6 (5/58) | 0.03 |
| | Civil Servant | 1.4 (1/72) | 90.3 (65/72) | 1.4 (1/72) | |
| | Unemployed | 1.9 (1/52) | 88.5 (46/52) | 1.9 (1/52) | |

***Table 3:*** *Chi-square analysis of the seroprevalence of dengue IgM and malaria.*

## Discussion

The findings of this cross-sectional study provide critical insights into the sero-molecular prevalence and spatial distribution of dengue and malaria co-infections among febrile patients in Yenagoa, Bayelsa State, Nigeria. The observed dengue IgM seroprevalence of 3.8% (7/182) indicates low but notable circulation of recent dengue infections in a region traditionally recognized for its malaria hyperendemicity, with a malaria prevalence of 91.2% (166/182). The dengue IgG seroprevalence of 1.6% suggests limited past exposure, which contrasts with higher IgG rates reported elsewhere in Nigeria, such as 44.7% in northern regions [2]. The lower dengue seroprevalence in Yenagoa compared to the 7.2% reported in northeastern Nigeria [2] and 5.7% in Benin City [5] may reflect regional ecological differences, such as variations in *Aedes* mosquito density or diagnostic practices that prioritize malaria testing. The high malaria prevalence aligns with Bayelsa's environmental suitability for *Anopheles* mosquitoes, characterized by swampy terrains and abundant water bodies, as noted by Okonko., *et al*. [9]. The co-infection rate of 3.8% underscores a significant public health challenge, as overlapping clinical symptoms like fever and myalgia complicate differentiation without advanced diagnostics [3,4].

The diagnostic performance of lateral flow assays for dengue IgM detection revealed a sensitivity of 71.4% compared to ELISA, with a Cohen's kappa coefficient of 0.65, indicating good but not excellent agreement. This finding corroborates Hasan., *et al*. [10], who reported reduced sensitivity of lateral flow assays in low-prevalence settings, where false negatives can significantly underestimate the true dengue burden. The lower positivity rate of lateral flow assays (1.6%) compared to ELISA (9.3%) highlights their limitations in resource-constrained settings like Yenagoa, where molecular tools such as reverse transcription polymerase chain reaction (RT-PCR) are often unavailable due to cost and infrastructure barriers [6]. This diagnostic gap likely contributes to the underreporting of dengue cases, as febrile illnesses are frequently attributed to malaria without confirmatory testing [5]. The absence of RT-PCR in this study further limits serotype-specific identification and confirmation of active infections, potentially underestimating the true prevalence of dengue in co-endemic areas [10].

The public health implications of these findings are profound, particularly in the context of Nigeria's riverine regions. The 3.8% co-infection rate emphasizes the need for integrated diagnostic strategies capable of simultaneously detecting dengue and malaria to reduce misdiagnosis and improve clinical outcomes. Current diagnostic protocols in Bayelsa, which prioritize malaria testing, often overlook dengue, as only 11% of health facilities routinely perform dual testing [6]. Multiplex assays, which can detect multiple pathogens in a single test, offer a promising solution for enhancing diagnostic accuracy in co-endemic settings [11]. Furthermore, the higher dengue prevalence in riverine wards (7.2%) compared to urban areas (4.8%), as revealed by GIS mapping, highlights the utility of spatial analysis in identifying transmission hotspots. These riverine areas, characterized by stagnant water and dense vegetation, provide ideal breeding grounds for *Aedes* mosquitoes, aligning with global patterns of dengue transmission [7]. The World Health Organization [1] advocates for GIS-guided vector control, such as larviciding and drainage maintenance, to target high-risk areas efficiently. By integrating spatial data with sero-molecular surveillance, public health authorities can optimize resource allocation, prioritizing interventions in riverine communities to reduce the dual burden of dengue and malaria.

Significant risk factors identified in this study, including open water storage (OR = 2.8, p = 0.004) and daytime net disuse (OR = 3.2, p = 0.001), underscore the role of environmental and behavioral practices in driving dengue transmission. These findings are consistent with Badolo., *et al*. [12], who noted that open water containers increase *Aedes* breeding sites, while daytime biting patterns of *Aedes* mosquitoes render nighttime-focused interventions like insecticide-treated nets less effective for dengue control. The higher co-infection rate among farmers and fishers (p = 0.03) further highlights occupational exposure in aquatic environments, corroborating Adesina., *et al*. [13]. These insights call for targeted community education and vector control programs tailored to high-risk groups and settings, such as larviciding campaigns in riverine wards and promotion of daytime mosquito protection measures [1].

Despite its contributions, this study has limitations that warrant consideration. The restriction to febrile patients introduces potential selection bias, as asymptomatic or mild dengue cases, which can contribute to transmission, were not captured [7]. The relatively small sample size (n = 182) may limit the generalizability of findings, particularly for low-prevalence diseases like dengue. Additionally, the absence of confirmatory molecular diagnostics, such as RT-PCR, restricts serotype identification and confirmation of active infections, potentially underestimating the true burden [11]. Future research should incorporate larger, population-based samples, including asymptomatic individuals, to better estimate prevalence and transmission dynamics. The integration of RT-PCR and next-generation sequencing could enhance diagnostic specificity and provide insights into circulating dengue serotypes, informing vaccine development and control strategies.

## Conclusion

In conclusion, this study confirms the co-circulation of dengue and malaria in Yenagoa, with a low but significant dengue seroprevalence and a high co-infection rate in a malaria-hyperendemic context. The diagnostic limitations of lateral flow assays and the absence of molecular tools highlight the need for advanced diagnostics to improve case detection. GIS mapping offers a powerful tool for targeting vector control, while integrated diagnostic and surveillance strategies are essential for addressing the dual disease burden in Nigeria's riverine regions. Future studies should address the identified limitations to strengthen disease surveillance and guide effective public health interventions in co-endemic areas.

This cross-sectional study conducted in Yenagoa, Bayelsa State, Nigeria, provides critical evidence of the co-circulation of dengue and malaria among febrile patients in a malaria-hyperendemic, riverine region. The observed dengue IgM seroprevalence of 3.8% (7/182) confirms low but significant recent dengue infections, while the malaria prevalence of 91.2% (166/182) underscores the region's entrenched malaria burden. The co-infection rate of 3.8% highlights a notable public health challenge, particularly among farmers and fishers, who face heightened occupational exposure. Geographic Information System (GIS) mapping revealed higher dengue prevalence in riverine wards (7.2%) compared to urban areas (4.8%), emphasizing the role of environmental factors in transmission dynamics. The lower sensitivity of lateral flow assays (71.4%) compared to ELISA (9.3% positivity) underscores the need for advanced diagnostic tools to improve case detection in resource-limited settings. These findings advocate for integrated diagnostic strategies, such as multiplex assays, to differentiate dengue and malaria and reduce misdiagnosis due to clinical overlap. Additionally, GIS-guided vector control interventions, targeting high-risk riverine areas with larviciding and drainage maintenance, are essential to mitigate the dual disease burden. By enhancing surveillance and diagnostic capacity, this study contributes to evidence-based public health strategies for managing dengue and malaria co-infections in Nigeria's riverine communities, paving the way for improved health outcomes in co-endemic regions.

## Recommendations

a. **Implement multiplex diagnostic assays**: Deploy multiplex diagnostic assays capable of simultaneously detecting dengue and malaria in health facilities across Yenagoa to improve diagnostic accuracy and reduce misdiagnosis, given the low dual-testing capacity (11%) in Bayelsa's facilities. This approach can address the clinical overlap between the two diseases and enhance case detection in co-endemic settings.

b. **Strengthen vector control in riverine communities**: Prioritize targeted vector control strategies, such as larviciding and drainage maintenance, in riverine wards identified as dengue hotspots (7.2% prevalence) through GIS mapping. These interventions should focus on reducing *Aedes* and *Anopheles* mosquito breeding sites to mitigate the dual disease burden.

c. **Enhance laboratory capacity with molecular diagnostics**: Invest in molecular diagnostic tools, such as reverse transcription polymerase chain reaction (RT-PCR), to enable serotype-specific dengue detection and confirm active infections, addressing the limitations of serological assays like lateral flow tests (71.4% sensitivity). This will improve surveillance accuracy in resource-limited settings.

d. **Expand GIS-guided surveillance**: Integrate Geographic Information System (GIS) mapping into routine surveillance programs to monitor spatial and temporal trends in dengue and malaria transmission, enabling proactive allocation of resources to high-risk riverine areas.

e. **Conduct community awareness campaigns**: Develop educational programs to raise awareness about dengue prevention, emphasizing the importance of proper water storage and daytime mosquito protection to reduce *Aedes* mosquito exposure, particularly in communities with high environmental risk factors.

## Acknowledgment

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## Conflict of Interest

No recorded conflict of interest of any kind was observed.

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