# Serological and Molecular Survey for Dengue Virus Infection in Febrile Suspected Patients in Selected Local Government Areas in Adamawa State, Nigeria

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**Research Article**

**Keywords:** Dengue, Virus, Febrile, Malaria, Serotyping, Phylogeny

**Posted Date:** March 17th, 2022

**DOI:** https://doi.org/10.21203/rs.3.rs-1395732/v1

# Abstract

Dengue is a disease caused by the dengue virus that is primarily transmitted by *Aedes aegypti* mosquito. Currently, the disease poses a threat to public health with about 390 million people reported to be infected annually across the endemic regions of the world. In Nigeria, the disease is underreported and often misdiagnosed as malaria. This study was designed to conduct a serological and molecular survey for dengue virus infection in febrile patients in three Local Government Areas (LGAs) in Adamawa State-Nigeria. Serum samples from 424 patients were analyzed by Enzyme-Linked Immunosorbent Assay (CALBIOTECH, Dengue Virus IgM ELISA). Thick and thin smear microscopic techniques were used to determine the presence of malaria parasites. Overall, 19.4% patients were sero-positive for dengue in the three study locations. A total of 11%, 14.5% and 12.3% participants were found to be co-infected with dengue and malaria in Mubi, Jimeta and Numan respectively. The CDC DENV1-4 Real-Time RT-PCR Assay reagent was used for serotype specific detection and identification of circulating serotypes. Only Dengue serotype 1 was found to be in circulation in all three study locations.

With an overall sero-prevalence of 19.4%, dengue virus infection may be one of the major causes of febrile illnesses across the study locations; hence, public healthcare professionals should not neglect other aetiologies of febrile illnesses and the need to conduct laboratory diagnosis to determine the possible causes of febrile illnesses.


# Introduction

Dengue virus is a positive-sense single-stranded RNA virus with a genome size of about 11 kb that belongs to the genus *Flavivirus* and family *Flaviviridae*, transmitted by the *Aedes aegypti* mosquitoes [1]. The virus causes dengue disease that is endemic in the urban and peri-urban cities across the tropics and subtropical regions of the world and currently poses a major threat to public health [2]. It has been reported that about 390 million cases occur annually worldwide [3, 4]. The virus is responsible for more morbidity and mortality compared to other vector-borne viral infectious diseases with an estimated mortality rate of 24,000 annually [5]. There are four known immunological serotypes in circulation that share antigenic relatedness (DENV1 - DENV4). Infection with any one of the serotypes which are usually asymptomatic with mild manifestations provides lifelong immunity against the same serotype and partial immunity against the other serotypes [6, 7]. Currently, dengue has been classified as dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) in increasing order of severity [8]. Even though there is no available effective treatment in place, early initiation of rehydration therapy of severe conditions can be successfully managed by careful monitoring and survey of the warning symptoms [9]. For the past ten years, commitments have been made to focus on three ground-laying areas: surveillance for planning and response, reducing the disease burden and consciously changing behaviour patterns to improve vector control [9]. There have been reported cases of dengue outbreaks in Nigeria that may be neglected due to under-reporting, misdiagnosis, and lack of awareness [10]. Recent reports indicate that the dengue virus is a major cause of febrile illness in Nigeria often misdiagnosed as malaria [11]. The majority of public health care institutions in Nigeria overlook the possibility of other

causes of febrile illness of viral and bacterial origin making greater emphasis on the parasitic source [12, 13, 14]. In Nigeria, the prevalence of dengue disease and circulating viral serotypes are not clear as the disease is under-diagnosed or misdiagnosed as malaria and misdiagnosis of dengue and other arboviral infections as malaria is probably going to have enormous results in the overall management of febrile infection in Nigeria [15]. Data on dengue occurrence and the circulating serotypes are critical for effective control, management, integrated surveillance and outbreak preparedness.

# Materials And Methods

### Study Design

This was a cross sectional study designed to carry out a serological and molecular survey for dengue virus infection in febrile patients in selected LGAs in Adamawa state, Nigeria. Adamawa State is made up of 21 LGAs and is divided into three (3) political zones; northern, central and southern senatorial districts. The three LGAs were randomly chosen by balloting from each zone. From each of the selected LGA, one secondary and primary healthcare facilities each were selected based on convenience and purposively. A structured questionnaire form was administered to establish an association between behaviour patterns and dengue morbidity.

### Data Analysis

Statistical package for social science (SPSS) version 24 was employed in the analysis of data. The Chi-square test was used to search for association between dengue virus infection and the socio-demographic information of the participants in the study locations. Level of significance was set at $P<0.05$. The degree of association between dengue morbidity and clinical manifestations was examined by calculating the odds ratio framed at 95% confidence intervals. The Maximum likelihood statistical algorithm evaluated with 1000 bootstrap replicates based on the Tamura-Nei model was used in the phylogenetic analysis.

### Ethical Clearance

Ethical clearance was obtained from the Adamawa State Ministry of Health, ethics committee (**S/MoH/1131/I**) and clearance from the respective hospitals board of ethics (General hospital and Kolere Primary Healthcare, Mubi; Specialist hospital and Jimeta Clinic & Maternity, Yola North; General hospital and Sabon Pege Primary Healthcare, Numan). Written informed consent of adult patients >18 years was obtained. For participants between 13 and 18 years, signed informed consent of both minor and guardian and or parent was obtained. For participants < 13 years, signed informed consent was obtained from the parent/guardian.

All experiments and methods were performed in accordance with relevant guidelines and regulations.

### Malaria Diagnosis

Blood samples from recruited participants were collected by venipuncture using 5ml EDTA coated tubes. Malaria parasitaemia was determined by Giemsa stained thick and thin film microscopy [16].

### Dengue Diagnosis

The samples were subjected to centrifugation at 10,000g for 5 minutes. Serum was used to screen for the presence of dengue virus antibody using Enzyme-Linked Immunosorbent Assay (ELISA) specific kit for dengue IgM according to the manufacturer's instructions (CALBIOTECH, Dengue Virus IgM ELISA). A positive ELISA result was defined as having an antibody index value >1.1

### Viral RNA Extraction

Viral RNA was extracted from human serum using DaAn Gene Co., Ltd (Spin Column) following the manufacturer's instruction. A final volume of 60µl of RNA was obtained and used as a template for cDNA synthesis and for the subsequent Real-Time PCR assay. The concentration (ng/µl) and purity of RNA was determined using Nanodrop spectrophotometer (Thermo Fisher Scientific) at 260/280 OD. The extracted RNAs were stored at -80$^{o}$C for further analysis.

### First Strand cDNA Synthesis

Following RNA extraction, ProtoScript II First Strand cDNA Synthesis Kit using standard protocol was used for cDNA synthesis; 2µl of 50ng/µl RNA sample was mixed with 2µl of Oligo d(T) 23VN to a total volume of 8µl in a nuclease free water in a sterile microfuge tube and incubated for 5min at 65$^{o}$C. The mixture was briefly spun and promptly put on ice. 10µl of ProtoScript II reaction mix and 2µl ProtoScript II enzyme mix were added to reaction tube. The cDNA synthesis reaction was incubated at 42$^{o}$C for 1 hour. The reaction was inactivated at 80$^{o}$C for 5 min and the cDNA products were quantified and stored at -20$^{o}$C.

### Real-Time RT-PCR Serotyping

Dengue virus serotyping was carried using Real-Time RT-PCR (BIO-RAD CFX96 Real-Time System) with CDC DENV1-4 Real-Time RT-PCR assay ancillary reagents. The optimized reaction conditions for PCR amplification involved initial denaturation at 95$^{o}$C for 2 min followed by 40 amplification cycles of denaturation and annealing at 95$^{o}$C for 15s and 60$^{o}$C for 1min. Briefly, the CDC DENV1-4 Real-Time RT-PCR Assay includes a set of oligonucleotide serotypes-specific primers and dual-labeled hydrolysis (TaqMan®) probes) for *in vitro* qualitative detection of DENV serotypes 1-4 from human serum with febrile illness consistent with dengue infection. The targeted regions (DENV1 NS5 gene 112 bp, DENV2 E gene 78 bp, DENV3 prM gene 74 bp, and DENV4 prM gene 89 bp) of the viral genome were amplified by the polymerase chain reaction (PCR). The fluorescently labeled probes anneal to amplified DNA fragments and the fluorescent signal intensity was monitored by BIO-RAD CFX 96 instrument during each PCR cycle. Target amplification was recorded as an increase and accumulation of fluorescence over time in contrast to the background signal. The assay was performed in a multiplex reaction targeting each

DENV serotype with a different coloured probe, i.e. each Taqman probe targets a single DENV serotype and is conjugated to a fluorophore that emits fluorescence at different excitation wavelengths (5'-FAM DENV-1, 5'-HEX DENV-2, 5'-Texas Red DENV-3, and 5'-Cy5 DENV-4) [17]. The optimized reaction mix is given in Table 1.1.

Table 1.1: Multiplex DENV1, 2, 3, and 4 reactions mix

| Reagent | Volume/rx | Total Volume |
|---|---|---|
| Nuclease-free Water | 3.2 µL x N | 3.2 µL x N |
| qPCR Universal mix | 10µL x N | 10µL x N |
| Primer D1-F | 0.5 µL x N | 0.5 µL x N |
| Primer D1-R | 0.5 µL x N | 0.5 µL x N |
| Primer D2-F | 0.25 µL x N | 0.25 µL x N |
| Primer D2-R | 0.25 µL x N | 0.25 µL x N |
| Primer D3-F | 0.5 µL x N | 0.5 µL x N |
| Primer D3-R | 0.5 µL x N | 0.5 µL x N |
| Primer D4-F | 0.25 µL x N | 0.25 µL x N |
| Primer D4-R | 0.25 µL x N | 0.25 µL x N |
| Probes (DENV1-4) | 0.45 µL x N | 0.45 µL x N |
| Total Volume | 20 µL x N | 20 µL x N |

## Sequencing and Phylogenetic Analysis

Sequencing was outsourced (Inqaba Biotec West Africa Ltd, Ibadan) and performed using Big Dye Terminator cycle sequencing (Applied Biosystems). Both forward and reverse strands were sequenced and the sequence chromatogram files were edited for bad calls using BioEdit. The sequences were compared with available sequences using the Basic Local Alignment Search Tool (nBLAST) and the GenBank database to validate the identities of viral serotypes.

The sequences were aligned using Muscle and Molecular Evolutionary Genetics Analysis Software (MEGA X) version 10.1.7 was used for phylogenetic analysis using the Maximum likelihood statistical method and the robustness of the phylogenetic tree was evaluated with 1000 bootstrap replicates based on the Tamura-Nei model. The phylogenetic tree was inferred in MEGA X version10.1.7.

# Results

Out of the 424 samples collected, 146 (34.4%) were collected from Mubi, 124 (29.4%) from Jimeta, Yola and 154 (36.2%) from Numan. An overall total of 82 (19.3%) patients were positive for dengue IgM from the three study locations. Out of the 82 patients, 50 (11.8%) were females and 32 (7.5%) were males. The age-specific sero-prevalence was found to be predominant in the younger age groups (0–30). Out of the 82 patients, 53 (12.5%) were found to be co-infected with dengue and malaria. Out of the 424 recruited participants, 341 (80.4%) admitted to have been exposed to mosquito bites in their homes and 82 (19.3%) of the patients were positive for dengue IgM. A total of 203 (47.9%) out of the recruited participants admitted to having some form of open water in and around their surroundings and 71 (16.7%) of the patients were positive for dengue IgM detection assay. A significant statistical association at p-value (0.01, 0.02 and 0.01) was observed between the behaviour patterns and dengue morbidity across the three study locations.

DENV1 isolates were detected across the three study locations with e-value of 3e-17 (less than 0), 100% query cover and 80% similarity with Brazilian isolates DENV1 NS5 gene coding sequence (cds) (KM085991.1).

Table 1.2 presents dengue virus antibody (IgM) detection assay using ELISA for Mubi. Twenty-nine (19.9%) of participants were positive for dengue IgM antibody detection. The age-specific range of dengue sero-prevalence was predominant in the age group 6–20 years as presented in Table 1.3.

Table 1.2
ELISA detection of dengue IgM

| IgM | Frequency | Valid Percent |
|---|---|---|
| + | 29 | 19.9 |
| - | 117 | 80.1 |
| Total | 146 | 100.0 |

Table 1.3
Dengue virus infection detection among patients in Mubi by age group

| Age Groups | IgM Results (+) | IgM Results (-) | Total Patients | Age groups Sero-prevalence (%) | P-value |
|---|---|---|---|---|---|
| 0–5 | 1 | 25 | 26 | 0.68 | |
| 6–10 | 9 | 9 | 18 | 6.16 | |
| 11–15 | 2 | 12 | 14 | 1.37 | |
| 16–20 | 10 | 17 | 27 | 6.85 | |
| 21–25 | 0 | 10 | 10 | 0.00 | |
| 26–30 | 3 | 11 | 14 | 2.05 | |
| 31–35 | 2 | 9 | 11 | 1.37 | |
| 36–40 | 0 | 7 | 7 | 0.00 | |
| 41–45 | 2 | 7 | 9 | 1.37 | |
| 46–50 | 0 | 1 | 1 | 0.00 | |
| 51–55 | 0 | 3 | 3 | 0.00 | |
| > 56 | 0 | 6 | 6 | 0.00 | |
| Total | 29 | 117 | 146 | 19.9 | 0.003 |

Table 1.4 presents data on dengue and malaria co-infection in the recruited subjects. Out of the 146 patients tested, 16 (11%) were found to be co-infected with dengue and malaria.

Table 1.4
Dengue and malaria co-infection among patients in Mubi

| Co-infection | Frequency | Valid Percent |
|---|---|---|
| Yes | 16 | 11.0 |
| No | 130 | 89.0 |
| Total | 146 | 100.0 |

Figure 1.1 is bar chart showing participants that were exposed to mosquito bites without control measures in place in Mubi. Out of the 146 participants, 118 (80.8%) admitted to have been exposed to mosquito bites in their homes and of whom 29 (19.9%) of the patients were positive for dengue IgM.

Figure 1.2 presents participants who had some form of open water around their dwelling place in Mubi. Out of the 146 participants, 73 (50.0%) had some form of open water in and around their surroundings

and of whom 26 (17.80%) of the patients were positive for dengue IgM.

Table 1.5 presents dengue virus antibody (IgM) detection assay using ELISA in Jimeta, Yola. Twenty-four (19.4%) participants were positive for dengue IgM antibody detection. The age-specific range of dengue sero-prevalence was predominant in the age group of 0–35 years as presented in Table 1.6.

Table 1.5
ELISA detection of dengue IgM

| IgM | Frequency | Valid Percent |
|---|---|---|
| + | 24 | 19.4 |
| - | 100 | 80.6 |
| Total | 124 | 100.0 |

Table 1.6
Dengue virus infection detection among patients in Jimeta, Yola by age group

| Age Groups | IgM Results (+) | IgM Results (-) | Total Patients | Age groups Sero-prevalence (%) | P-value |
|---|---|---|---|---|---|
| 0–5 | 2 | 6 | 8 | 1.61 | |
| 6–10 | 3 | 7 | 10 | 2.42 | |
| 11–15 | 4 | 9 | 13 | 3.23 | |
| 16–20 | 1 | 18 | 19 | 0.81 | |
| 21–25 | 1 | 12 | 13 | 0.81 | |
| 26–30 | 9 | 16 | 25 | 7.26 | |
| 31–35 | 1 | 14 | 15 | 0.81 | |
| 36–40 | 1 | 5 | 6 | 0.81 | |
| 41–45 | 0 | 7 | 7 | 0.00 | |
| 46–50 | 1 | 3 | 4 | 0.81 | |
| 51–55 | 0 | 1 | 1 | 0.00 | |
| > 56 | 1 | 2 | 3 | 0.81 | |
| Total | 24 | 100 | 124 | 19.4 | 0.238 |

Table 1.7 presents data on dengue and malaria co-infection in the recruited subjects.. Out of the 124 recruited patients tested, 18 (14.5%) were found to be co-infected with dengue and malaria.

Table 1.7
Dengue and malaria co-infection in Jimeta, Yola

| Co-infection | Frequency | Valid Percent |
|---|---|---|
| Yes | 18 | 14.5 |
| No | 106 | 85.5 |
| Total | 124 | 100.0 |

Figure 1.3 is a bar chart showing participants that were exposed to mosquito bites without control measures in place in Jimeta, Yola. Out of the 124 participants, 107 (86.3%) admitted to have been exposed to mosquito bites in their homes and of whom 24 (19.4%) of the patients were positive for dengue IgM.

Figure 1.4 presents participants who had some form of open water around their dwelling place in Jimeta, Yola. Out of the 124 participants, 55 (44.4%) had some form of open water in and around their surroundings and of 24 (19.4%) of the patients were positive for dengue IgM.

Table 1.8 presents dengue virus antibody (IgM) detection assay using ELISA in Numan. Twenty-nine (18.8%) participants were positive for dengue IgM antibody detection. The age-specific range of dengue sero-prevalence was predominant in the age group of 6–25 years as presented in Table 1.9.

Table 1.8
ELISA detection of dengue IgM

| IgM | Frequency | Valid Percent |
|---|---|---|
| + | 29 | 18.8 |
| - | 125 | 81.2 |
| Total | 154 | 100.0 |

Table 1.9
Dengue virus infection detection among patients in Numan by age group

| Age Groups | IgM Results (+) | IgM Results (-) | Total Patients | Age groups Sero-prevalence (%) | P-Value |
|---|---|---|---|---|---|
| 0–5 | 1 | 15 | 16 | 0.65 | |
| 6–10 | 4 | 8 | 12 | 2.60 | |
| 11–15 | 5 | 13 | 18 | 3.25 | |
| 16–20 | 6 | 19 | 25 | 3.90 | |
| 21–25 | 4 | 12 | 16 | 2.60 | |
| 26–30 | 2 | 12 | 14 | 1.30 | |
| 31–35 | 1 | 8 | 9 | 0.65 | |
| 36–40 | 1 | 9 | 10 | 0.65 | |
| 41–45 | 3 | 4 | 7 | 1.95 | |
| 46–50 | 1 | 9 | 10 | 0.65 | |
| 51–55 | 0 | 5 | 5 | 0.00 | |
| > 56 | 1 | 11 | 12 | 0.65 | |
| Total | 29 | 125 | 154 | 18.8 | 0.417 |

Table 1.10 presents data on dengue and malaria co-infection in the recruited subjects. Out of the 154 patients tested, 19 (12.3%) were found to be co-infected with dengue and malaria.

Table 1.10
Dengue and malaria co-infection in Numan

| Co-infection | Frequency | Valid Percent |
|---|---|---|
| Yes | 19 | 12.3 |
| No | 135 | 87.7 |
| Total | 154 | 100.0 |

Figure 1.5 is a bar chart showing participants that were exposed to mosquito bites without control measures in place in Numan. Out of the 154 participants, 116 (75.3%) admitted to have been exposed to mosquito bites and of whom 29 (18.8%) of the patients were positive for dengue IgM.

Figure 1.6 presents participants who had some form of open water around their dwelling place in Numan. Out of the 154 participants, 75 (48.7%) had some form of open water in and around their surroundings and of whom 29 (18.8%) of the patients were positive for dengue IgM.

The sequence generated from sequencing service was subjected to nucleotide sequence BLAST using NCBI to establish sequence similarity with relevant sequences in the GenBank. An e-value of 3e-17 (less than 0), 100% query cover and 80% similarity with Brazilian isolate DENV1 NS5 gene cds (KM085991.1) was generated. Sequences were aligned using MUSCLE in MEGA X.

Figure 1.7 presents the inferred phylogenetic tree of DENV1 isolates using Maximum likelihood statistical method and the robustness of the phylogenetic tree was evaluated with 1000 bootstrap replicates based on the Tamura-Nei model in MEGA X version 10. As can be seen from the tree, the isolates were all monophyletic but the isolates from the three study locations were more closely related to those from Brazil.

# Discussion

Dengue is a major arthropod-borne viral disease of humans, and its control and management remain the primary priority of public health for many endemic countries. Unlike the situation in the Asia-Pacific and Latin American regions, where dengue burden and epidemiology is well documented, the burden and epidemiology of dengue virus infection in Africa including Nigeria is not clear, even though there are reported cases.

In an attempt to improve surveillance and to track the viral burden and epidemiology in Nigeria, this population-based survey was conducted in secondary and primary healthcare facilities to determine the occurrence of dengue virus infection and serotypes in Mubi, Yola North (Jimeta) and Numan LGAs in Adamawa state, Nigeria. Febrile patients with clinical signs and symptoms compatible with dengue virus infection, which were referred to a laboratory to conduct malaria parasitaemia diagnosis and who consented to participate before sample collection, were recruited into this study. Despite being undetected prior to this study active transmission of dengue virus, dengue and malaria co-infection were detected across the three study locations. A sero-prevalence of 19.4% for dengue virus infection was established.

In Mubi, out of the 146 patients, 97 (66.4%) tested positive for malaria parasites and 49 (33.6%) were negative. Twenty-nine (19.9%) of the participants were positive for dengue antibody. The age-specific range of dengue sero-prevalence was observed to be significant in the younger participants aged 6–30 years with a p-value of 0.003. This could be plausible, since these age groups are known to be actively involved in outdoor activities where possible contact with vector is very likely. A sero-prevalence of 19.9% for dengue virus IgM was obtained in Mubi. This, therefore, means that younger people within the age range of 5–30 years should be the target age for the dengue vaccination programme, if the need becomes imperative. Out of the 146 patients, 16 (11%) were found to be co-infected with dengue and malaria. This observation has brought to light the fact that examination of febrile illnesses should not be limited to parasites and bacteria, but that flaviviruses can equally cause febrile illnesses.

In Jimeta, out of the 124 patients, 83 (66.9%) tested positive for malaria parasites and 41 (33.1%) were negative. Twenty-four (19.4%) of the participants were positive for dengue antibody. The age-specific range of dengue sero-prevalence was observed not to be statistically significant in the younger

participants aged 0–35 years with a p-value of 0.238. This suggests that the infection was not limited to the younger age group.

Out of the 124 patients, 18 (14.5%) were found to be co-infected with dengue and malaria. This again suggests that febrile illnesses should not be limited to malaria and typhoid but that flaviviruses infection can result in febrile illness; hence, proper laboratory diagnosis for febrile illnesses should be readily available and recommended before drugs prescription.

In Numan, out of the 154 patients, 97 (63.0%) tested positive for malaria parasites and 57 (37.0%) were negative. Twenty-nine (18.8%) of the participants were positive for dengue antibody. The age-specific range of dengue sero-prevalence was predominant in the age group of 6–25 years. This observation, however, was not statistically significant with a p-value of 0.417. Like Jimeta, dengue sero-prevalence was found across all the age groups but more pronounced in the younger age groups. Out of 154 patients, 19 (12.3%) were found to be co-infected with dengue and malaria. This further confirms that flaviviruses are capable of causing febrile illness as it was found to be present across all the study locations.

From the findings, malaria may not be the only mosquito-borne disease-causing febrile illness across the study locations. With dengue sero-prevalence rates of 19.9%, 19.4% and 18.8% in the three study locations respectively, there may be an active transmission of dengue across the entire state and possibly beyond, due to the fact that dengue is frequently being transported from one place to another by infected travelers coupled with inherent behavior pattern of the population that encourages vector breeding. The development implies that dengue may be endemic in these areas, but undetected or possibly misdiagnosed as malaria.

The co-infection of febrile patients with dengue and malaria in the study could suggest the possibility of mosquitoes carrying more than one pathogen or the presence of *Aedes* spp as a suitable vector across these locations. A similar scenario of dengue and malaria co-infection was reported by Ayukekbong [15] in Ibadan where 10% of malaria patients had active dengue infection. As it can be observed from the results of this study, dengue rate of occurrence was higher in Mubi (19.9%) and Jimeta (19.4%) than Numan (18.8%), even though more samples were collected from Numan than in each of these two locations. This may be so since Mubi and Jimeta are the economic hubs of the state with greater population density than Numan. The difference is not statistically significant and is only marginal.

In a recent serological survey of dengue IgM, Oladipo et al. [18] reported a 17.2% prevalence in seemingly healthy individuals in Ogbomosho, while Adesina and Adeniji [19] reported a sero-prevalence of dengue IgM of 25.7% among febrile volunteer subjects in Ile-Ife, western Nigeria.

In Jos and Ibadan, 2.2% and 35% of febrile subjects were reported to be positive for dengue nonstructural protein one (NS1) antigen among febrile subjects respectively [11]. In another separate study, Hamisu et al. [20] reported 37.4% positive for dengue IgM in Maiduguri and Bello et al. [21] in a study conducted in Kaduna reported 51.1% and 48.9% positive for dengue IgM in females and males respectively.

Only dengue virus serotype 1 was identified in the three study locations and this may highlight the active occurrence of dengue virus in the study areas. In a similar study in Ibadan, DENV serotypes 1 and 2 were detected while DENV serotype 1 was reported in Abeakuta [22]. In Lagos, Ayolabi et al. [23] reported the circulation of DENV serotypes 1 and 3 in febrile patients. In a study conducted by Yousseu et al. [24] in Douala, Cameroon, active circulation of DENV serotype 1 was reported. Parreira et al. [25] reported DENV serotype 1 to be responsible for the outbreak in Angola. However, other reports indicated that DENV serotypes 2 and 3 may cause more severe forms of dengue infection than the other serotypes [26].

The detection of DENV serotype 1 in this study could explain why severe cases of dengue virus infection due to secondary infection and cross-reactivity with other serotypes have not been reported in the three study areas. However, this does not rule out potential outbreaks and may not be the current situation as possible cases of importation of other serotypes cannot be overruled.

Currently, dengue prevention and control depends heavily on effective vector measures, hence, sustained local involvement can improve vector control efforts substantially as local risk to dengue outbreaks is linked to the population's knowledge, attitude as well as behavioural practices that encourage vector breeding. Communities are encouraged to avoid exposure to mosquito bites especially during the day time, rid their environment of possible mosquito breeding sites as proximity of breeding sites is a significant risk factor to dengue transmission, and active community engagement through sensitization and mobilization against mosquito-borne diseases.

# Conclusion

This study established the occurrence of dengue virus infection in human subjects tested in Mubi, Jimeta and Numan LGAs in Adamawa State, Nigeria. A sero-prevalence of 19.9%, 19.4% and 18.8% were generated respectively. Dengue and malaria co-infection was established; this suggests that febrile illness should not only be limited to malaria and typhoid. A significant association was established between behaviour patterns and dengue morbidity. As at the time this study was conducted, DENV serotype 1 isolates were found to be in circulation across the three study locations; therefore, the public healthcare professionals should not neglect other aetiologies of febrile illnesses and the need to conduct laboratory diagnosis to determine the possible causes of infection.

# Declarations


### Funding

This study was supported by the African Centre of Excellence for Neglected Tropical Diseases and Forensic Biotechnology (ACENTDFB), Ahmadu Bello University, Zaria-Nigeria.


### Competing Interest

The authors declared none

### Authors Contributions

D.T.T carried out the research work and wrote the manuscript while J.K.P.K and G.S.N.K supervised and proof read the manuscript.

### Data Availability

Data set supporting this article are available in DDBJ data repository (https://www.ddbj.nig.ac.jp/PRJDB13211).

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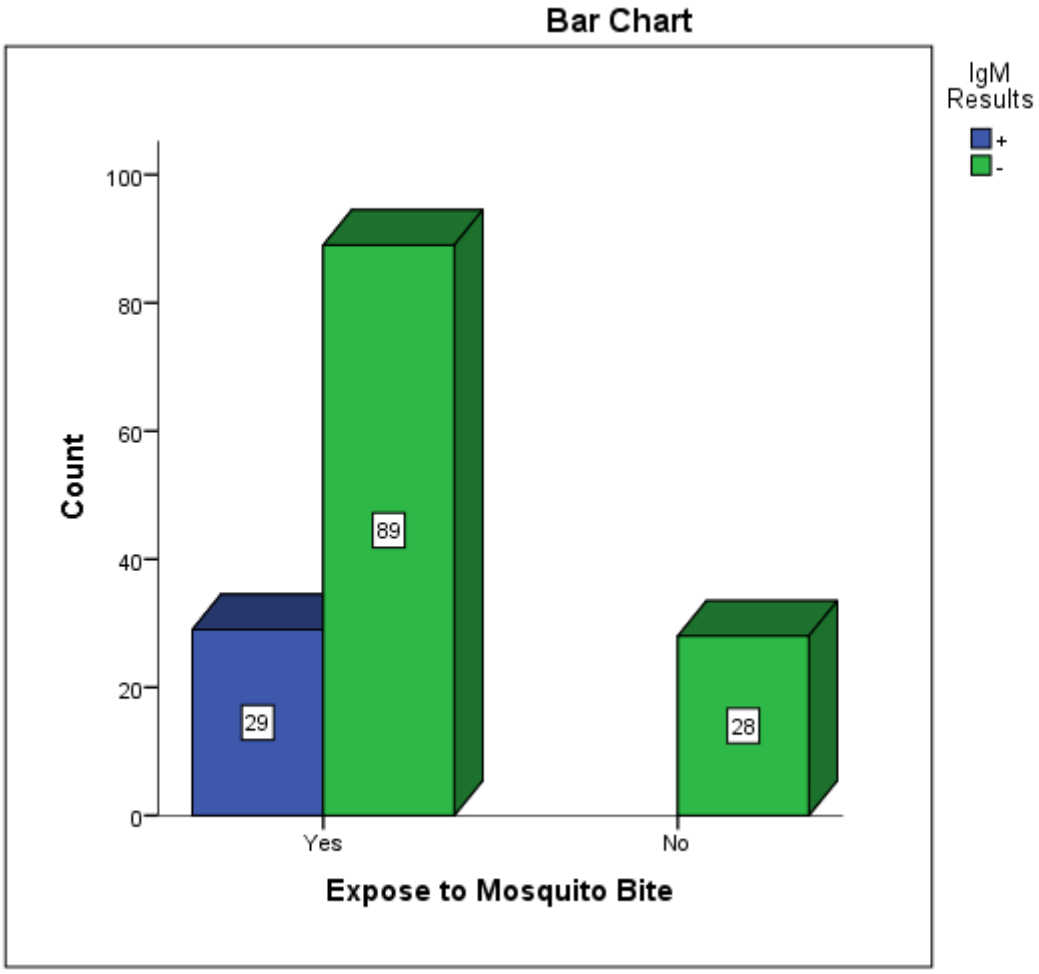
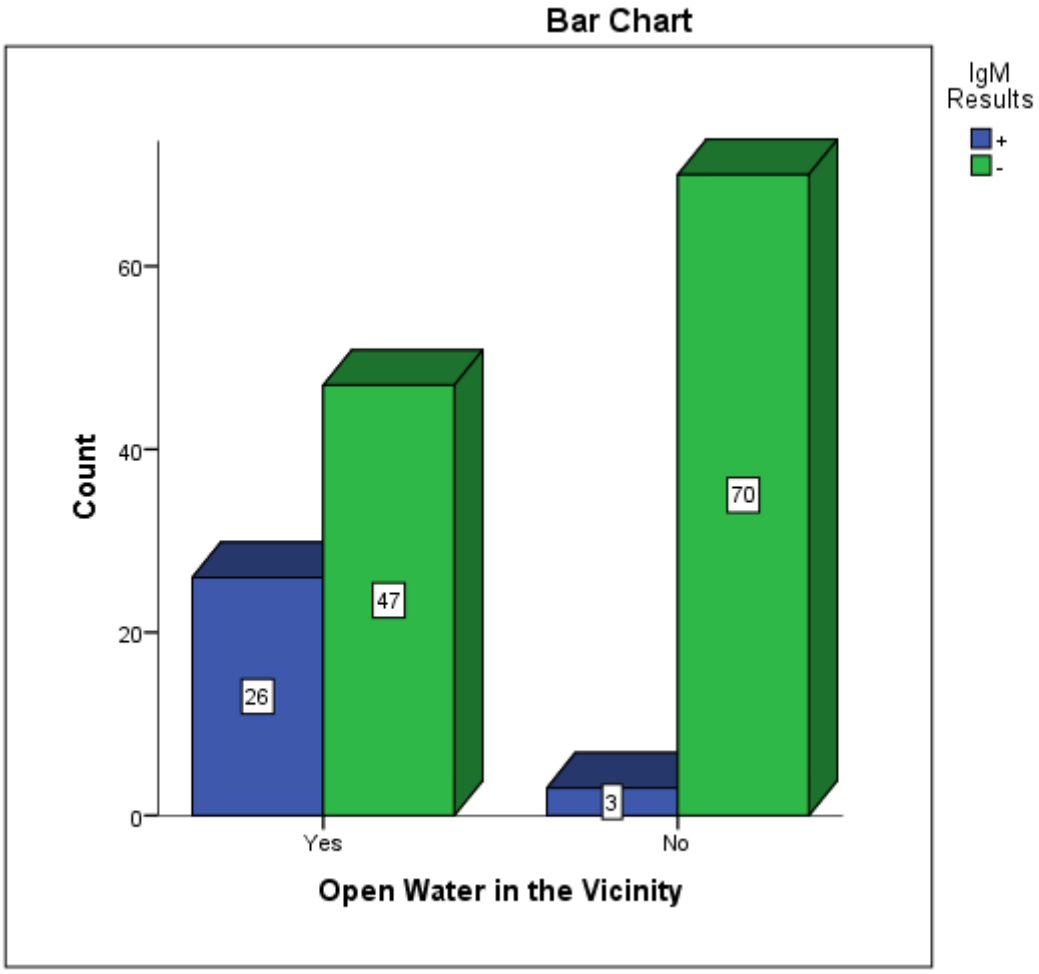
# Figures



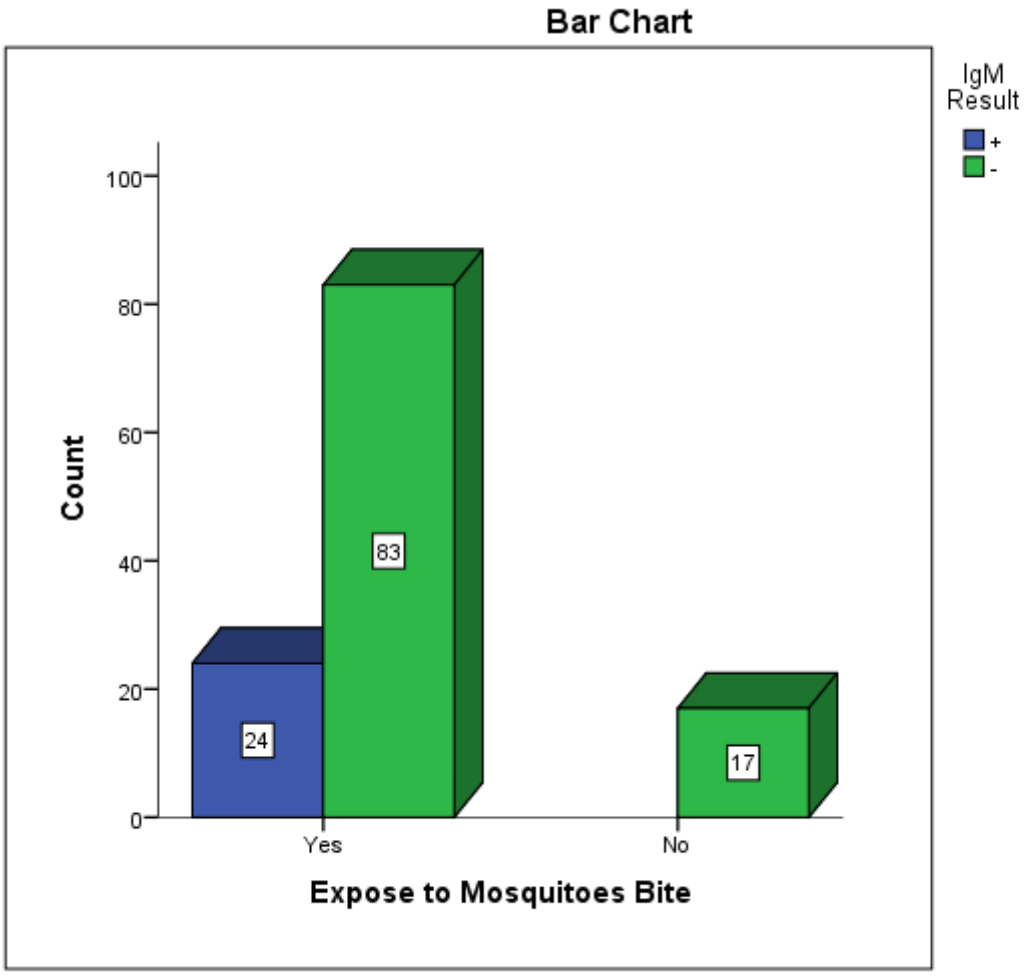

## Figure 1

Proportion of participants exposed to mosquito bites in Mubi



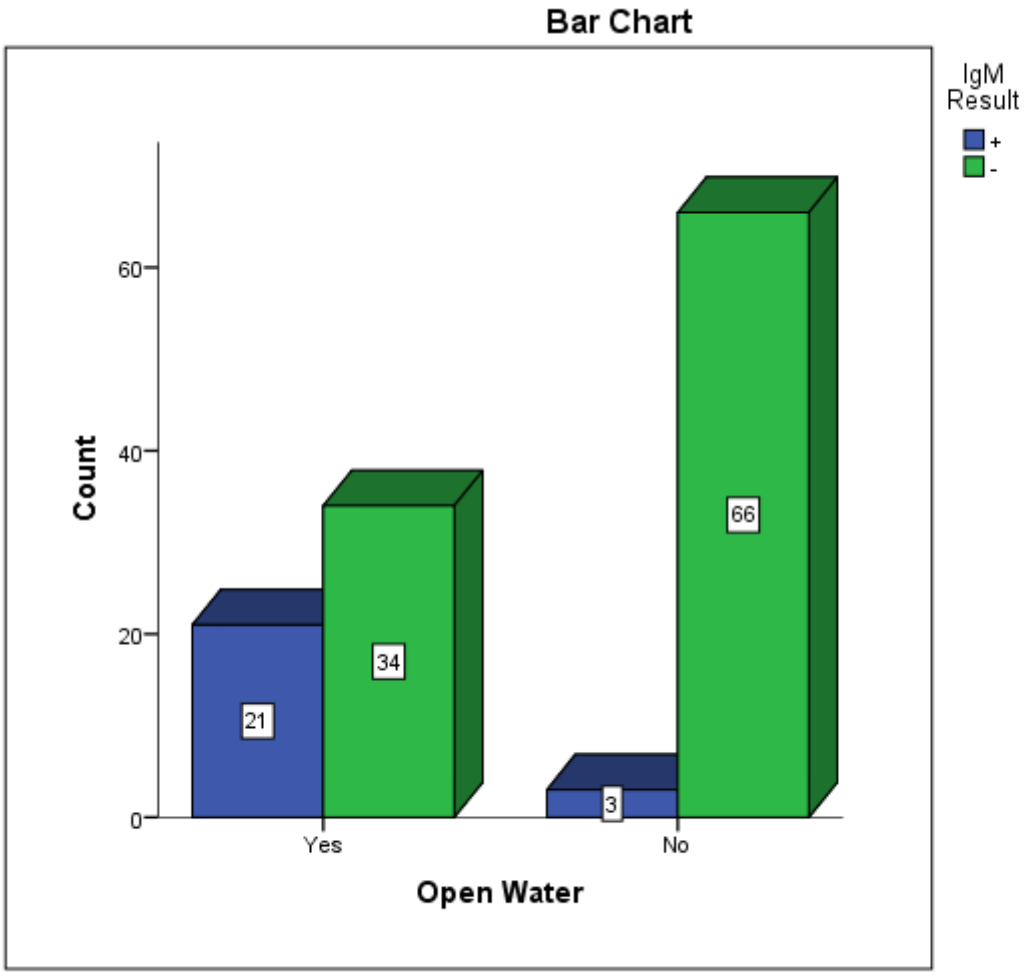

## Figure 2

Participants with open water in their surroundings in Mubi



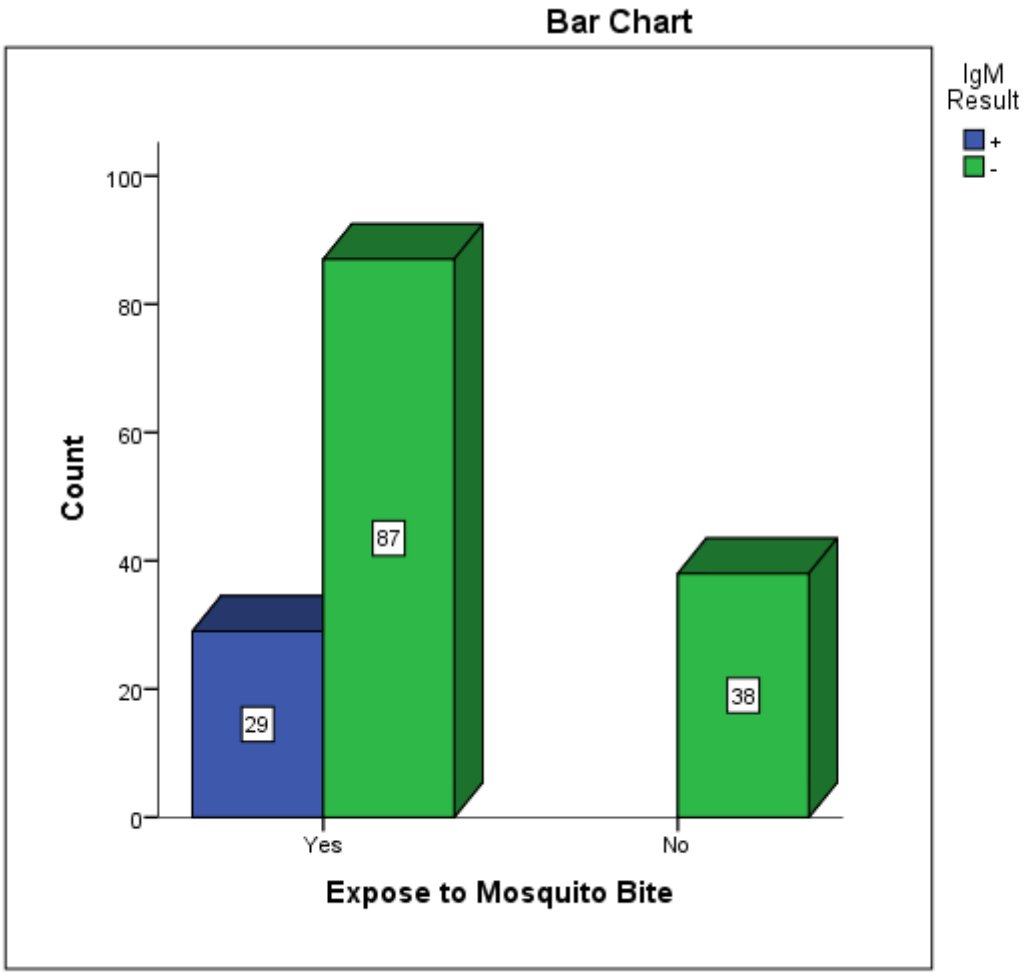

## Figure 3

Participants exposed to mosquito bites in Jimeta, Yola



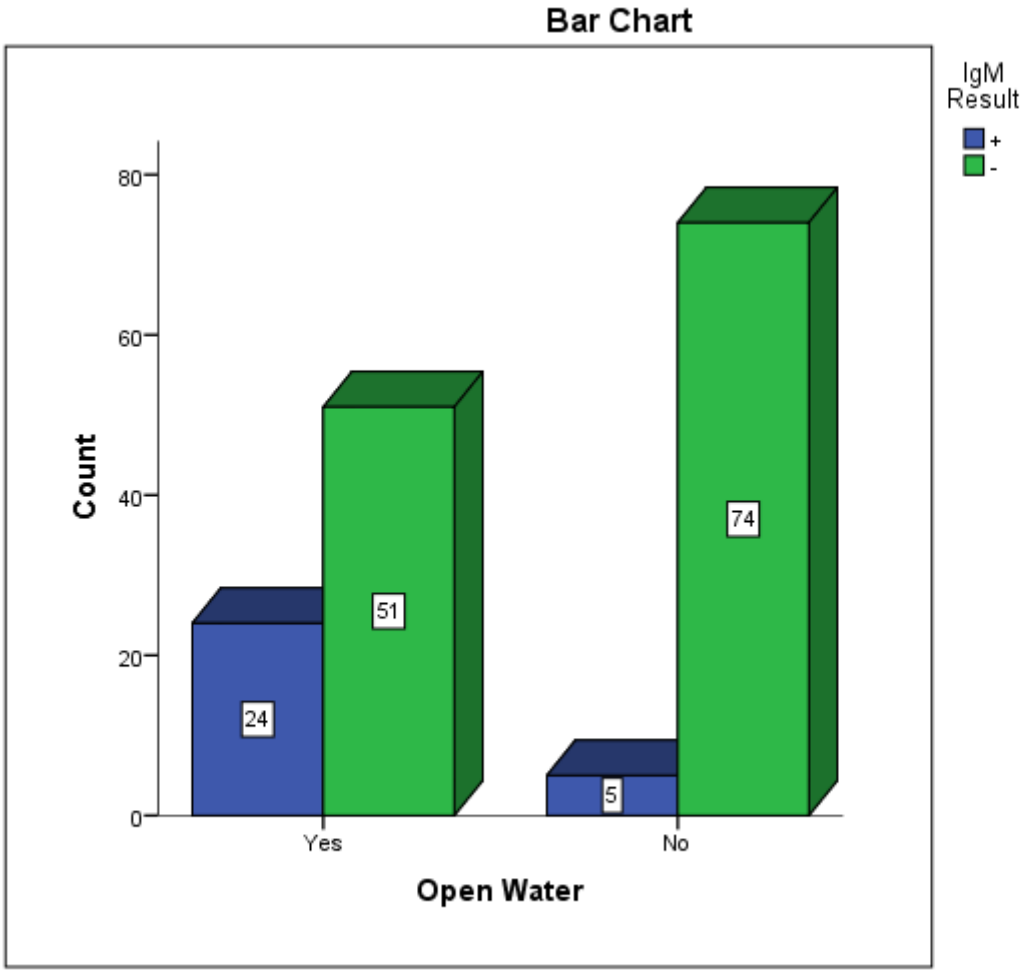

## Figure 4

Participants with open water in their surroundings in Jimeta, Yola



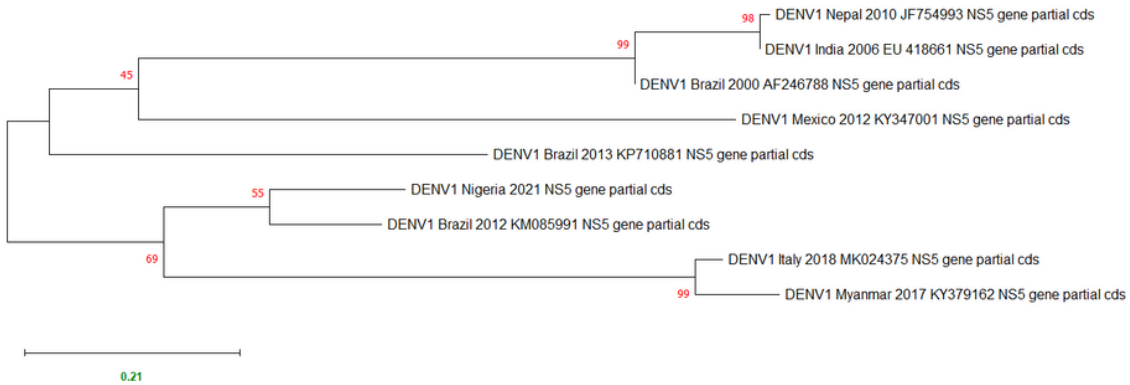

## Figure 5

Participants exposed to mosquito bites in Numan




## Figure 6

Participants with open water in their surroundings in Numan




## Figure 7

Maximum likelihood phylogenetic tree of DENV1 from the study locations based on NS5 partial coding gene sequence (110bp) and their most related sequences from GenBank repository