Genetic diversity of Dengue virus serotypes circulating among *Aedes* mosquitoes in selected regions of northeastern Nigeria

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A B S T R A C T

The flaviviruses are mosquito borne pathogens that continue to pose a considerable public health risk to animals and humans. The members of this group includes, Dengue virus (DENV), Yellow fever virus (YVF), Japanese encephalitis virus (JEV), West Nile virus (WEV) and Zika virus (ZKV). The DENV mosquito vector is endemic to tropical and subtropical climates, placing ~40% of the world's population at direct risk of dengue infection. Currently, in Nigeria the status of DENV serotypes circulating among mosquito vectors is unknown. Our study was designed to identify and characterize the DENV serotypes circulating in *Aedes* mosquito populations collected in selected sites in Nigeria. The mosquitoes were collected and identified morphologically to species level using colored identification keys of Rueda. Generally, each species identified was tested in pools of 20 individuals of each *Aedes* species. RT-PCR and semi nested PCR were used to detect DENV serotypes in mosquitoes and characterized using Sanger sequencing methods. The results showed that DENV serotypes were detected in 58.54% (24/41) of the pools of *Aedes* mosquitoes from Mubi, Numan and Yola screened. All DENV1-4 serotypes were detected in *Ae. aegypti*. While DENV 1, 2 and 4 were detected in *Ae. albopictus*. And only DENV 2 was detected in *Ae. galloisi* with DENV4 serotype being reported for the first time in Nigeria. DENV2 (37.8%) was the most detected serotypes, while double and triple co-infections of serotypes were detected in 24.4% of the pools. Phylogenetic analysis revealed a strong evolutionary relatedness of DENV serotypes in our study with that of South and Southeast Asia, North America, and other African countries. This is the first reports on the natural DENV serotypes co-infection among *Aedes* species pools in Nigeria, which can create possible interaction with other flaviviruses causing animal and human diseases. In addition, our study postulates the possible linkage between DENV serotypes infection and human febrile flu-like disease burden being experienced by host communities in northeastern Nigeria.

1. Introduction

Dengue is a mosquito-borne viral disease that has spread to almost all the regions of the world in recent years. Climate change and globalization has firmly established dengue as threat to public health systems [1]. DENV is transmitted by infected female mosquitoes mainly of the species *Aedes aegypti* and to a lesser extent, *Aedes albopictus*. These are also vectors of other members of the Flaviviridae and Togaviridae families [1]. The *Ae. aegypti* is recognized as the main vector of DENV worldwide, largely attributable to its vector competency and strong host preference for humans compared to *Ae. albopictus* [2]. However, *Ae. albopictus* has been known to be the driving force in the worldwide emergence of
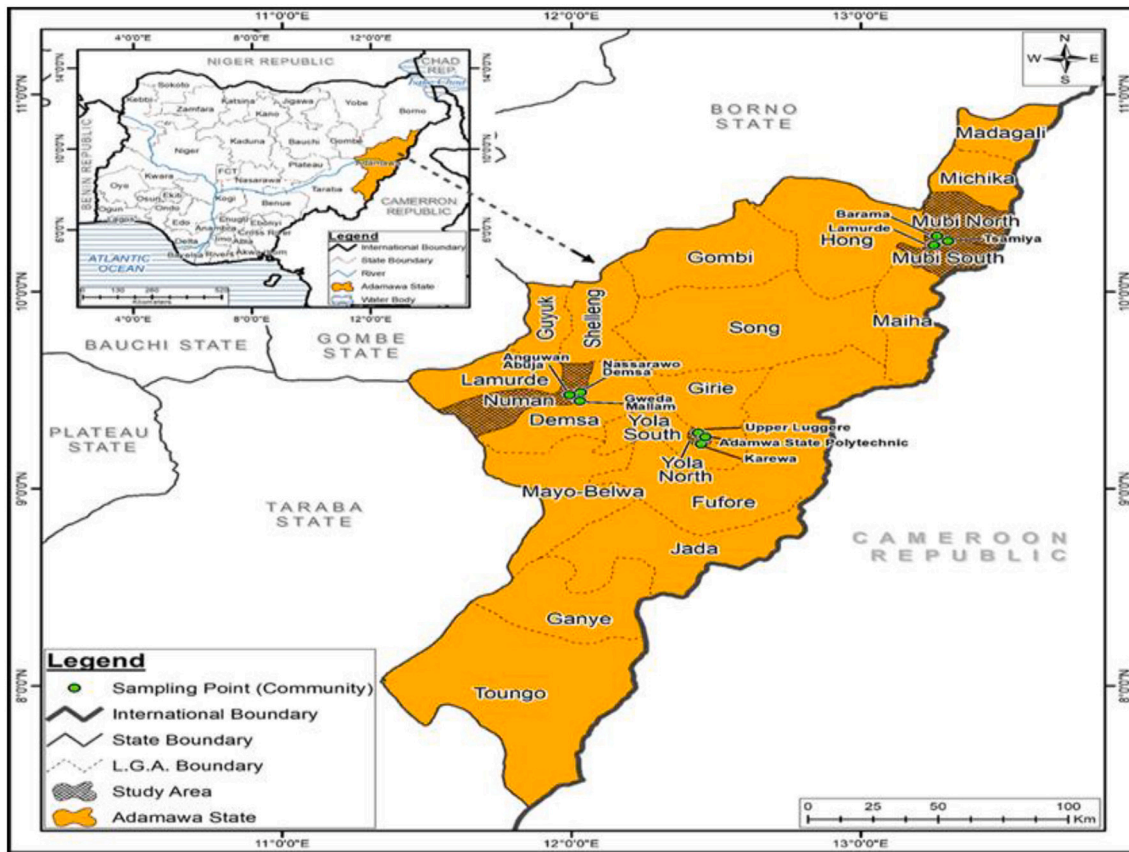

Fig. 1. Map of Adamawa State in northeastern Nigeria showing the study area. (Source: Modified from the administrative map of Adamawa State).

Table 1
Oligonucleotide sequences used for Cytochrome Oxidase 1, Dengue consensus and serotype-specific RT-PCR analysis.

| Primers	Oligonucleotide Sequences	Amplified size (bp)	References
OSK2499	5′-TTGATTTTTTGGTCATCCAGAAGT -3′	930	[42]
OSK2500	5′-TGAAGCTTAAATTCATTGCACTAATC -3′		
D1	5′-TCAATATGCTGAAACGCGCGAGAAACCG-3′	511 Dengue consensus sequence	[43]
D2	5′-TTGCACCAACAGTCAATGTCTTCAGGTTC-3′		
D1	5′-TCAATATGCTGAAACGCGCGAGAAACCG-3′	482 (Dl and TS1) DENV1	[43]
TS1	5′-CGTCTCAGTGATCCGGGGG-3′		
D1	5′-TCAATATGCTGAAACGCGCGAGAAACCG-3′	290 (TS3 and Dl) DENV3	[43]
TS3	5′-TAACATCATCATGAGACAGAGC-3′		
D1	5′-TCAATATGCTGAAACGCGCGAGAAACCG-3′	392 (Dl and TS4) DENV4	[43]
TS4	5′-CTCTGTTGTCTTAAACAAGAGA-3′		
DV1	5′-GGRACKTCAGGWTCTCC-3′	362 (DV1 and DSP2) DENV2	[18]
DSP2	5′-CCG GTGTGCTCRGCYCTGAT-3′		

Chikungunya virus (CHIKV) [3].

Over 3.9 billion people in 129 countries are at risk of DENV infection and 390 million dengue infections occur per year of which 96 million manifests clinically. In addition, the largest number of dengue cases was recorded in 2019 that affected most regions of the globe, and transmission was reported for the first time in some countries [1]. Despite its global public health significance, little attention has been given to DENVs or *Aedes*-borne arboviruses in Africa. These posed a greater public health threat to animals and immunologically naive African populations. The neglect of these viruses could be due to the high burden of malaria and other neglected tropical diseases in Africa, in addition to the poor- health resource settings for accurate identification of viral infections [4]. In Nigeria, most cases of dengue are undiagnosed or misdiagnosed as malaria or referred to as fever of unknown cause [5]. Nigeria's urban ecology, with limited sanitation infrastructure, heavy rainy seasons, pervasive household water storage, and with no public awareness of dengue, presents a similarly prime environment for breeding of *Aedes* mosquitoes and disease transmission. The vectorial capacity of *Aedes* species in Nigeria is still poorly studied [6]. A study conducted in Zaria, Kaduna Nigeria reported co-circulation of DENV and YVF in the *Aedes* populations [7]. A prevalence rate of 51.9% of IgM antibodies to DENV was reported in the northwest zone of Nigeria [8].

Table 2
Dengue virus positive pools of *Aedes* species collected from municipalities of Adamawa state in northeastern Nigeria.

Study locations	No. of pools screened	DENV positive pools	Percentage
Numan	16	7	43.75
Yola	17	9	52.94
Mubi	8	8	100
Total	41	24	58.54
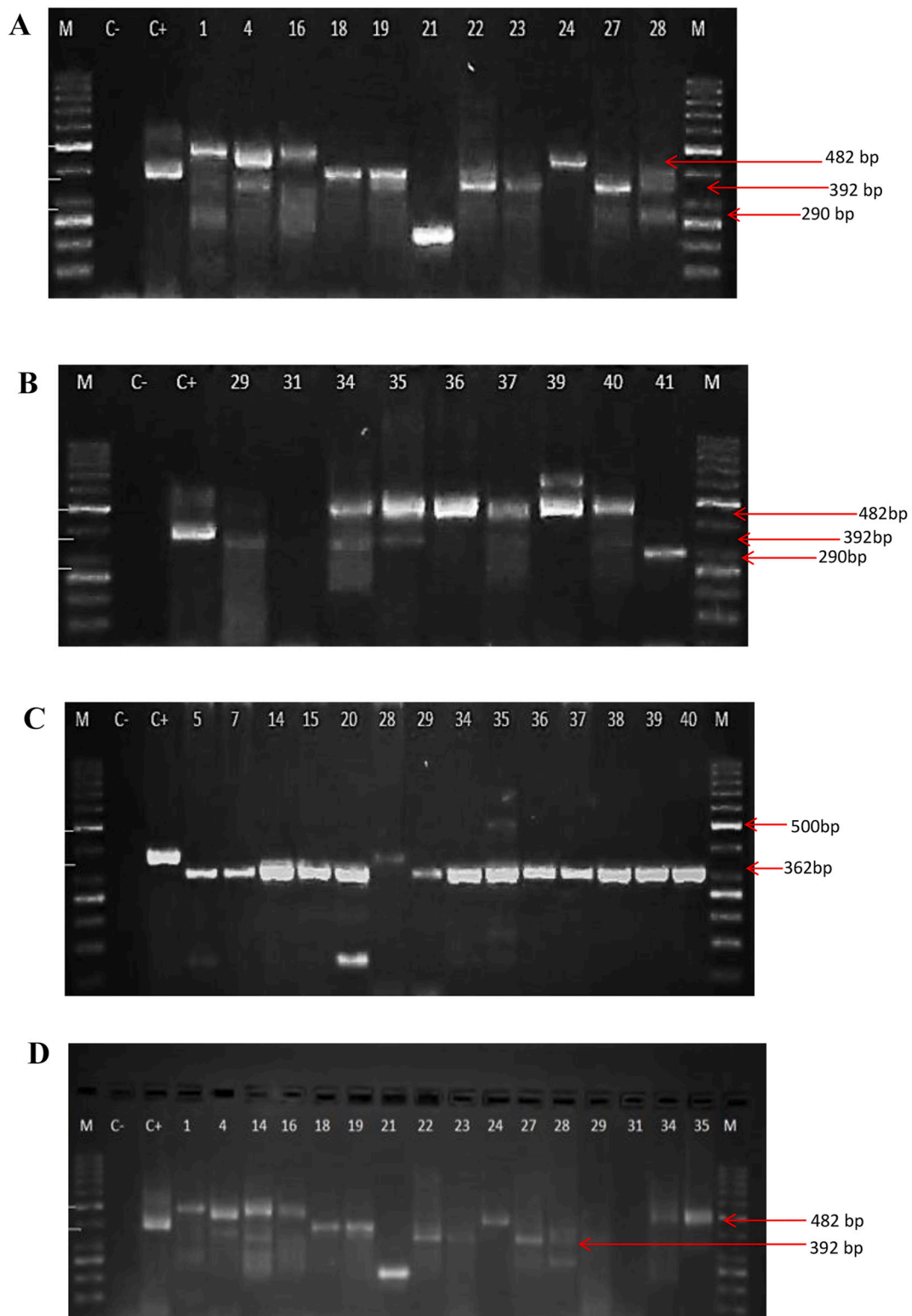
Fig. 2. A, B, C and D: The detection of DENV serotypes. Electrophoregram of amplified cDNA by RT-PCR. DENV 1, 2, 3 and 4 serotypes specific primer sets were used with specific amplicons of 482 bp, 290 bp, 392 bp and 362 bp respectively. Lane M: Standard size marker of 1000 bp, C- = negative control, C+ = positive control of DENV4 (392 bp) and DENV2 (362) from cell culture propagation in Vero cells. Lane 5 is amplicon for DENV2 infection in *Ae. galloisi* pool (Fig. 2C) while lanes 14, 15, 23,27,37 and 38 show DENV1, 2, 3 and 4 serotypes infection in *Ae. albopictus* pools (Fig. 2A, B, C and D).

DENV is a positive sense, single-stranded RNA virus of the family Flaviviridae which comprised of more than 70 viruses. The virus have four antigenically distinct serotypes, DENV1-4 [9]. These viruses have a wide geographic range, circulating on every continent except Antarctica [10]. The virus circulates in two distinct transmission cycles; the sylvatic cycle between the mosquito vector and non-human primates and the human transmission cycle which includes domestic and peri-domestic *Aedes* mosquitoes [11]. Limited available evidence suggests only *Ae.*

Table 3

Aedes species identified and Dengue virus serotypes detected circulating among mosquitos collected from municipalities of Adamawa state in northeastern Nigeria.

Study	Pools no.	Mosquito Species	Positive DENV	DENV serotypes			
				1(482 bp)	2(362 bp)	3(290 bp)	4(392 bp)
	1	*Aedes aegypti*	+	+	−	−	−
	2	*A. aegypti*	−	−	−	−	−
	3	*A. aegypti*	−	−	−	−	−
	4	*A. aegypti*	+	+	−	−	+
	5	*Aedes galloisi*	+	−	+	−	−
	6	*A. aegypti*	−	−	−	−	−
	7	*A. aegypti*	+	−	+	−	−
Numan	8	*A. aegypti*	−	−	−	−	−
	9	*A. aegypti*	−	−	−	−	−
	10	*A. aegypti*	−	−	−	−	−
	11	*A. aegypti*	−	−	−	−	−
	12	*Aedes albopictus*	−	−	−	−	−
	13	*A. aegypti*	−	−	−	−	−
	14	*A. albopictus*	+	+	+	−	−
	15	*A. albopictus*	+	−	+	−	−
	16	*A. aegypti*	+	+	−	−	−
	17	*Ochlerotatus vigilax*	−	−	−	−	−
	18	*A. aegypti*	+	−	−	−	+
	19	*A. aegypti*	+	−	−	−	+
	20	*A. aegypti*	+	−	+	−	−
	21	*A. albopictus*	−	−	−	−	−
	22	*A. aegypti*	+	−	−	−	+
	23	*A. albopictus*	+	−	−	−	+
	24	*A. aegypti*	+	+	−	−	−
Yola	25	*A. aegypti*	−	−	−	−	−
	26	*A. aegypti*	−	−	−	−	−
	27	*A. albopictus*	+	−	−	−	+
	28	*A. aegypti formosus*	+	−	+	+	+
	29	*A. aegypti*	+	−	+	−	+
	30	*A. aegypti*	−	−	−	−	−
	31	*A. aegypti*	−	−	−	−	−
	32	*A. aegypti formosus*	−	−	−	−	−
	33	*A. aegypti*	−	−	−	−	−
	34	*A. aegypti*	+	+	+	−	+
	35	*A. aegypti*	+	+	+	−	+
	36	*A. aegypti*	+	+	+	−	−
Mubi	37	*A. albopictus*	+	+	+	−	−
	38	*A. albopictus*	+	−	+	−	−
	39	*A. aegypti*	+	+	+	−	−
	40	*A. aegypti*	+	+	+	−	−
	41	*A. aegypti*	+	−	−	+	−

Key: DENV = Dengue virus, bp = base pair, + = positive, − = negative,

aegypti is involved in transmission of dengue in Nigeria. However, several isolates of DENV were obtained from this mosquito specie and none from *Ae. albopictus* and *Ae gaillosi* [12]. Also, despite the growing effect of DENV infections amongst human populations, there is no information on the natural presence of DENV serotypes circulating in adult field *Aedes* mosquito populations in north eastern region of Nigeria. Therefore, this study was designed to evaluate and characterized DENV serotypes circulating in wild caught *Aedes* mosquitoes in this area. Furthermore, to unravel the burden human febrile disease being experienced by host communities in northeastern Nigeria.

2. Materials and methods

2.1. Study area

The study was carried out in three purposively selected Local Government Area (LGAs) of Adamawa State, in northeastern Nigeria that is located between latitude 9^0 14′N and longitude 12^0 28′E (Fig. 1). It has an estimated area of 39,742,12sq. Km which accounts for 4.4% of the total landmass of Nigeria. The projected population as per 2006 population figure stands at 3,737,223 and it is traversed by mountainous landforms like the Mandara Mountains, Cameroon Mountains and Adamawa hills and large rivers: Benue, Gongola and Yadzarem. The mean annual rainfall ranges from 700 mm in the northeastern and of the 1600 mm in the southern part [13]. The study areas are also characterized with high population movement due upheavals in the northeast with many people storing water around their homes and dumping of solid waste in open gutter and close to water bodies, which provide suitable environment for breeding of *Aedes* species and interaction with humans [14].

2.2. Aedes mosquitos' sampling and preparation for detection of DENV serotypes

Aedes species were collected both indoors and outdoors from Mubi, Yola and Numan LGAs. By trained research assistance and volunteers for four months (July to October 2017). Collection was in the morning between 6:00–10:00 am); while the evening collection was from 3:00 -7:00 pm. Collection consisted of a combination of aspiration of indoor resting adults, human-landing techniques that attracted mosquitoes on exposed parts of human-bait and sweep nets [15,16]. All mosquitoes collected were sorted and *Aedes* mosquitoes were identified using colored identification /taxonomic keys by Rueda [17]. The *Aedes* species identified morphologically were segregated generally, in pools of 20 females labeled according to species, LGAs and communities. A total of 41 pools were used for the study, which comprises of 16 pools from 3
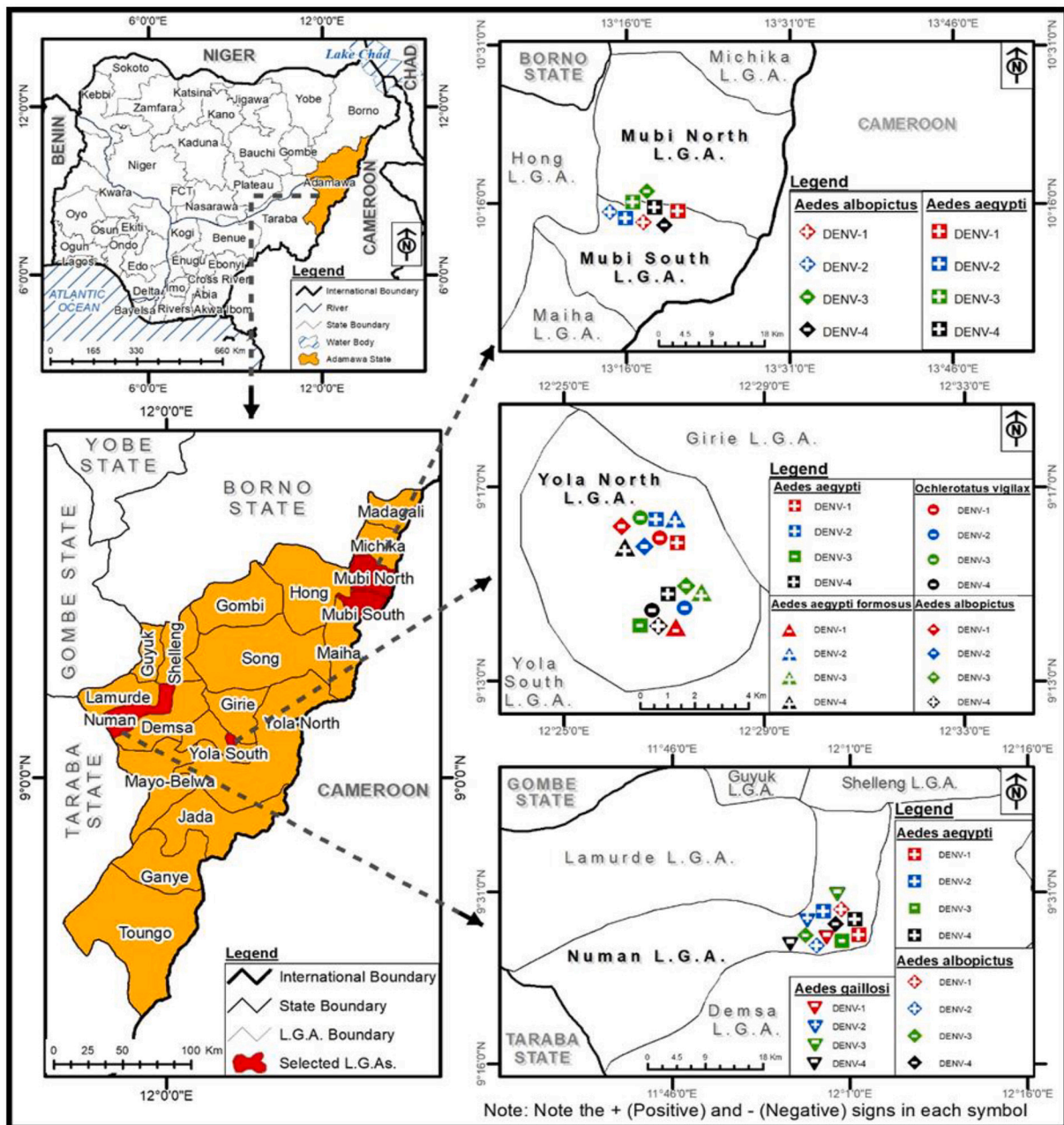
Fig. 3. Map of Adamawa State in northeastern Nigeria showing Local Government municipalities where DENV serotypes were detected in *Aedes* mosquito species.

communities in Numan, 17 pools from 3 communities in Yola and 8 pools from 3 communities in Mubi (Fig. 1). *Aedes* samples were stored in −80 °C for RNA extraction.

2.3. RNA extraction from pools of Aedes mosquito species

Briefly, DENV RNA was extracted with All Prep DNA/RNA Mini extraction kit (Qiagen, Germany, Cat. No. 80204) following SOPs of the Laboratory for Viral Research, University of Bremen, Germany, following manufacturer's instruction with little modification (www.giagen.com). Each pool of *Aedes* species was thoroughly homogenized with a mini handheld homogenizer and single-use microfuge pestle (Sigma Aldrich, Munich, Germany) in 350 μL buffer RLT (lysis buffer). The homogenate was centrifuged for 3 mins at 13000 rpm. The homogenized lysate was transferred to an AllPrep RNA spin column in a 2 mL collection tube and one volume (350 μL) of 70% ethanol was added to the flow-through and mixed thoroughly by pipetting repeatedly. Then

lysate was transferred to an RNAeasy spin column placed in a 2 mL collection tube and was centrifuged for 15 s at 13000 rpm. This was followed by the twice washing steps using RW1 and RPE buffers in spin columns and the flow-through were discarded. The column was dried by centrifuging for 1 min at 13000 rpm after which 30 μL elution buffer was used to elute the RNA into a 1.5 mL collection tube by centrifuging for 1 min at 13000 rpm. The RNA was quantified using Nanodrop 1000 apparatus (Thermo Scientific, Dreieich, Germany) at a wavelength of 260 nm. The RNA extracted was stored at −80 °C until used. Agarose gel electrophoresis was run to determine quality and integrity of RNA extract.

2.4. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) analysis

To perform RT-PCR, cDNA was synthesized using 80 ng of RNA. RNA extract were mixed with 1 μL (0.2 μg/μL) random hexamer primer and 1

A

B
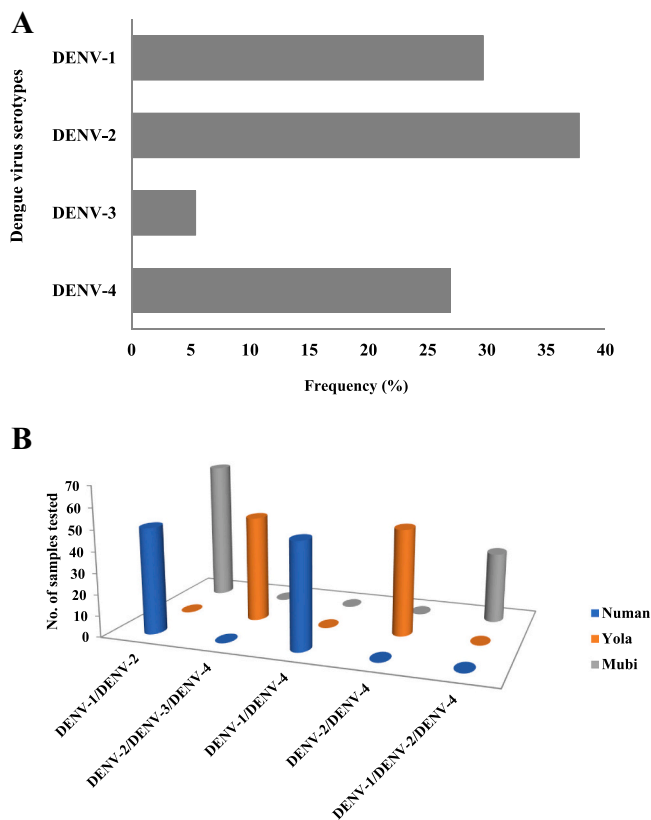
Fig. 4. The occurrence of DENV serotypes 4A and 4B, co-infections of DENV serotypes in pools of *Aedes* mosquitoes collected from Adamawa state of northeastern Nigeria.

μL (10 mM) dNTP mix in a 0.2 mL PCR tube. The mixture was made up to 15 μL with nuclease-free water on ice. The following component was then added to the PCR tube: 4 μL of 5× reaction buffer, 0.5 μL of Ribolock RNase inhibitor (40 U/μL) and 0.5 μL of Maxima H minus Reverse Transcriptase (10,000 U, 100 U/μL) making up to a total volume mixture of 20 μL. The reaction was vortexed, centrifuged and incubated in a thermocycler with the following conditions: 25 °C for 5 mins, 50 °C for 30 mins. The reaction was then terminated by heating at 85 °C for 5 mins (Thermo Scientific, Dreieich, Germany). The cDNA was amplified using DENV serotypes -specific consensus primers D1 and D2 that target the envelope and non-structural protein 1 (E/NS1) junction of the virus genome (Table 1). The RT-PCR and Multiplex semi nested PCR were carried out according to a previous protocol [18] with some adjustment. In 25 μL of final volume reaction mixture containing 2.5 μL of *Aedes* species cDNA product, 2.5 μL (10 x dreamTaq buffer containing 20 $MgCl_2$), 0.5 μL of dNTP Mix (10 mM, final concentration of 0.2 mM), 1 μL of 25 mM MgSO4 (final concentration 1.0 mM), 0.5 μL of dream Taq polymerase (final concentration 5 U/μL), 0.875 μL of forward D1 and reverse D2 primers (100 μM; final concentration 0.7 μM), which was designed against the consensus cDNA sequence of DENV1-4 serotypes that amplify a fragments of 511 base pair. The reaction volume was made up to 25 μL with double-distilled water (ddH20). The thermo cycling conditions are as follows: Initial denaturation at (94 °C, 3 mins), 35 cycles of denaturation (94 °C, 30 s), primers annealing (55 °C, 1 min), primer extension (72 °C, 2 mins) and final extension at 72 °C for 5 mins.

2.5. Genetic serotyping of DENV serotypes from Aedes mosquito species

The PCR product obtained from the RT-PCR amplification using D1 and D2 as primers was further used as the template for DENV1-4 different serotypes. Based on the above temperature conditions and

reagent volumes, 0.5 μL and 1 μL of the Nested 1 RT-PCR product (1:10 and 1:1000 in ddH20 for DENV serotypes 2 and DENV serotypes 1, 3 and 4 respectively) was used as template in the subsequent nested PCR reaction. A 25 μL reaction volume containing forward primer D1 and type-specific (TS) reverse primers: TS1, TS3 and TS4 (reverse primers for serotype 1, 3 and 4), and forward primer DV1 and DSP2 reverse primers for serotype 2 (Table 1) was further amplified by nested PCR step II. The entire PCR product was resolved on a 3% agarose gel containing 0.5 μg/mL ethidium bromide (SERVA, Heidelberg, Germany). The expected sizes of the amplified products were 482 for DENV1, 362 for DENV2, 290 for DENV3 and 392 for DENV4 [18].

2.6. Sequencing and phylogenetic analysis of DENV serotypes

The PCR amplicons were purified using GeneJet DNA purification kit (Thermo Scientific, Dreieich, Germany) following manufacturer's protocols and sequenced at Microsynth Sequencing Laboratories Göttingen, Germany. Forward and reverse strands were sequenced, and amplified target bands of Sanger sequence chromatograms were assembled and visualized using Geneious Pro Version 5.5.9. The sequences were compared with available sequences using Basic Local Alignment Search Tool (BLAST) for Virus Pathogen Resources (ViPR) (https://www.viprbrc.org/brc/blast.spg) and the Genbank data base to validate the identity of the virus isolate. Phylogenetic analysis was inferred using MEGAX [19]. Consensus sequences were aligned using Muscles alignment tool. The phylogenetic tree was reconstructed using a Maximum Likelihood (ML) method and estimated using best-fit General Time Reversible (GTR) model with gamma-distributed rate variation among sites. Other sequences like the study sequences in GenBank were obtained using the BLAST algorithm were also included in the analysis. Bootstrap replicates of 1000 were employed to assess the robustness of individual nodes of phylogeny. Complete gap deletion was employed for all the E gene sequences.

2.7. Data analyses

Descriptive statistics was used to analyse the distribution of DENV serotypes in *Aedes* species in Adamawa state. The proportion of DENV positive individuals True Infection Rate per 100 mosquitoes were calculated and the minimum infection rate (MIR) was also estimated per 1000 *Aedes* mosquitoes.

3. Results

3.1. Detection of DENV serotypes in Aedes species

A total of 706 *Aedes* mosquitoes were collected from the study locations. Samples were identified morphologically and confirmed molecularly targeting CO1 gene. Overall, 58.5% (24/41) pools were positive for DENV in the study locations (Table 2). Mubi had the highest 100% (08/08) pools positivity rate while Numan 43.75% (07/16) with the lowest DENV pools positivity rate.

Concurrent infection of *Ae. albopictus* pools with DENV1 and 2 amplicons in lanes 37 (Fig. 2B and 2C). DENV1-4 serotypes were positive in 24 pools of *Aedes* species. Seventeen of the positive pools was *A. aegypti,* 6 of the positive pools was *Ae. albopictus* while one positive pool was *Ae. galloisi* and none for *Ochlerotatus vigilax* (Table 3). All the four DENV1-4 serotypes were detected in Yola and Mubi while DENV3 was (0.0%0.0/09) not detected in Numan (Fig. 3).

The occurrence of DENV serotypes in the pools of species in the locations, shows that DENV2 (37.8%:14/37) was the most occurring serotypes circulating than DENV1, (29.7%; 11/37) and DENV4, (27.0%; 10/37) while DENV3 (5.4%; 02/37) was the least in Adamawa during the study (Fig. 4A). Accordingly, the distribution of DENV serotypes circulating in *Aedes* species collected from the pools in the locations indicated DENV4 (58.3%;07/12) as the highest DENV serotype
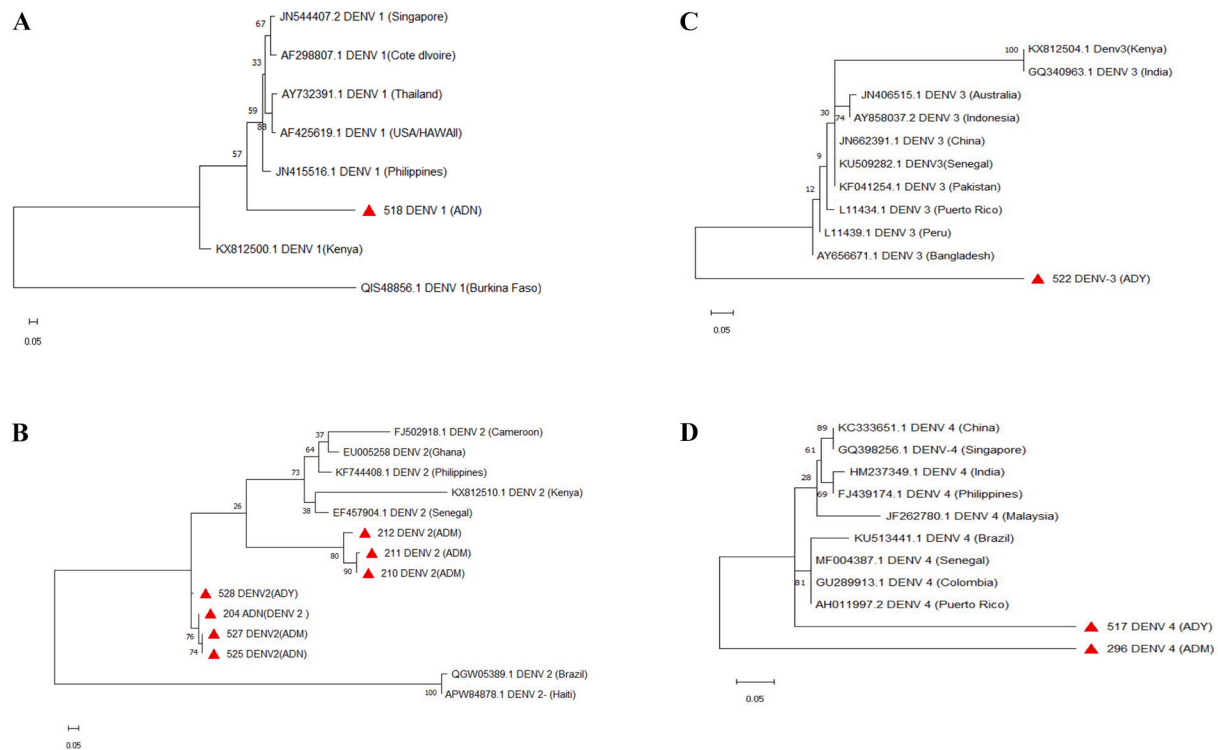
Fig. 5. A, B, C and D. Phylogenetic analysis tree of DENV1-4 Serotypes isolated from *Aedes* species in the study area with other referenced DENV1-4 serotypes. The evolutionary analyses are based on the partial E gene sequences of DENV serotypes and reconstructed using Maximum Likelihood method (MEGA X). Value at nodes indicates bootstrap support. Study samples are designated in solid triangle. Abbreviations: ADN, Adamawa Nigeria while taxon names for references are labelled by their Genbank accession number, strains, and country.

circulating in Yola, while DENV1 and DENV2 serotypes (44.4%, 04/09) in Numan and DENV2 (43.75%) in Mubi. Co-infections of DENV serotypes where two serotypes were detected in a pool of *Aedes* species in Adamawa state, with the highest double occurrence of DENV1/DENV2 (66.7%; 04/06) in Mubi. Furthermore, co-infection of DENV2/DENV3/DENV4 was detected in a pool from Yola 50.0% (01/02). Similarly, co-infection of DENV1/DENV2 and DENV4 was detected in Mubi (33.3%; 02/06). Both DENV1/DENV4 were detected in the same pool in Numan 50% (01/02) but none was detected in Yola and Mubi (Fig. 4B).

3.2. Phylogenetic analysis of DENV serotypes distribution in Aedes species and their relatedness to other global serotypes

To perform the phylogenetic analysis for DENV1-4 serotypes in the study area, the genetic relationship of DENV serotypes strain were analyzed targeting the E gene sequences of the virus. The tree was generated using E gene sequences for one DENV1 serotype and 7 reference strains (Fig. 5A). Notably, DENV1 from the study location form clade with that of South East Asia, West Africa, and North America. This study indicated diverse geographical distributions of DENV1 serotype in different continents. Also, 7 DENV2 E gene sequences were aligned with 7 reference strains from Genbank (Fig. 5B). The DENV2 serotype from Mubi clustered together with that of Cameroon, Ghana, Senegal, and Kenya (Africa continent) and Philippines in Asia continents. While four of the DENV2 serotype from Yola, Numan, and Mubi formed a separate clade.

Furthermore, one DENV3 serotype from Yola was determined with that of 10 reference serotypes from Genbank (Fig. 5C). DENV3 serotype from Adamawa formed a separate cluster which is distantly related with the referenced DENV3 serotype. However, DENV3 serotype from Kenya (East Africa) is closely related with DENV3 from India (South Asia). Only two DENV4 serotype were used from the study location with 9 referenced DENV4 (Fig. 5D). The result of the Phylogenetic analysis revealed

that DENV4 from Yola appears to be more closely related to viruses from Asia continents. While DENV4 from Mubi is distantly related to the reference viruses.

4. Discussion

DENV is considered endemic in Africa and with little to no information on its current status in Nigeria [20]. Available information relied hugely on serological surveys in humans and the circulating serotypes in field caught *Aedes* mosquitoes has not been fully described. Our study delineated the circulating DENV serotypes and the presence of *A. aegypti, Ae. galloisi, Ae. albopictus, Ochlerotatus vigilax* and subspecies of *Ae. aegypti formosus* in northeastern Nigeria (Table 3). The circulating DENV serotypes detected in our study was 58.54% (Table 2), which was analogous to the 62% detected in rural areas of Colombia [21]. In contrast, this was higher than the 33% and 11% obtained from Armero and Brazil respectively [22,23]. The high pools positivity rate obtained in our study could be due to increased interaction between *Aedes* species and humans. In addition, this could be as a result of the mosquitoes were collected during the rainy season (July-October), which is the peak period of their activities. Moreover, this concur with Halstead, [24] who reported that DENV peak transmission in most endemic areas is usually associated with periods of higher rainfall. On one hand, this might have been exacerbated due to changes in human behavior that favored the *Aedes* mosquito species expansion in the study locations, which is not unconnected to the population explosion and protracted humanitarian crisis being experience in the northeast of Nigeria (www.unocha.org). Furthermore, it could also be due to increase in international boundary cross-border socioeconomic activities between the study area and Cameroon. Thus, highlighting the possibility of cross-border importation of DENVs and vectors. The Minimum Infection Rate (MIR) was 33.99% (24 positive pools/706 total processed individual mosquitoes × 1000) while the estimated *Aedes* mosquito's individual's true infection

rate was calculated at 5.24%. This was slightly-higher when compared to an estimated *Ae. aegypti* infection rate of 4.12% and MIR of 33.3/1000 reported in Anapoima and La Mesa local municipalities of Colombia [22]. However, this was in contrast to high individuals TIR of 0.78% and 7.75% MIR of DENV infection reported for *M. Africana* mosquitoes collected in Bayelsa, Nigeria [25]. Furthermore, the MIR reported in our study is testament to the likelihood continual transmission of DENV in the study area. We detected DENV2 in *Ae. galloisi* and DENV1, 2 and 4 serotypes circulating in *Ae. albopictus* (Fig. 3). *Ae. albopictus* is known to be an invasive species with strong capabilities of ecological adaptability and geographical spread. In addition, DENV2 has been proven experimentally in *Ae. galloisi* and *Ae. albopictus* [26,27]. Similarly, *Ae. albopictus* has been shown to be a competent vector for DENV2 and CHIKV [27–29]. Thus, our study here corroborated the vector competency of *Ae. albopictus* to DENV1-4 serotypes. In addition, nearly all the *Aedes* mosquitoes' pools in Mubi were positive 100% (08/08) for DENV (Table 2). This showed the unique public health risk position of Mubi municipality in the study area compared to the other locations. Mubi is the commercial hub of Adamawa state and harbors large numbers of Internally Display Persons (IDPs) from Borno state. The population explosion could lead to increased human interaction with the mosquito vectors, which will aid the spread of DENVs in this area.

Changes usually occur in the epidemiology of arboviruses after an introduction of invasive species [30]. And in 2014 insurgents activities had led to the displacement of thousands of residents who took refuge in Cameroon. Thus, there may be the possibility of cross border transmission since there are reports of outbreaks associated with *Ae. albopictus* in Cameroon [28]. Furthermore, *Aedes galloisi* which is regarded as an invasive species was reported in our study [26]]. The introduction of *Aedes galloisi* vector into north eastern Nigeria could be possible via migration as a result of the humanitarian crisis where cross-border crossings are not monitored. Since DENV can replicate in *Ae. galloisi*, hence, conceivable be a potential vector for DENV transmission in this region. And the colonization of *Ae. galloisi* could occur, which will lead to the introduction of other flaviviruses in the study area. We detected DENV1-4 serotypes circulating in the pools of *Aedes* mosquitoes from Mubi and Yola, while DENV3 was not detected in Numan (Fig. 3). This is not surprising because Mubi and Yola share similar characteristics as the main entry points for travelers and visitors from different parts of the world. And many IDPs camps and humanitarian partners are situated in Yola and Mubi, accommodating thousands of refugees from different parts of northern Nigeria, affected by the insurgency. In addition, mosquitoes control activities are not practiced, which provides conducive atmosphere for the introduction and breeding of *Aedes* species thereby increasing the spread of DENV serotypes in the study area [5,31]. All DENV serotypes have been detected in Africa [20]. However, DENV1 and DENV3 was reported to be the circulating serotypes in Lagos, Nigeria [32] Similarly, DENV1 and DENV2 serotypes were detected in serum samples collected from Cross River, Nigeria [33]. In addition, DENV3 was detected in *Mansonia africana* in Bayelsa, Nigeria [25]. Interestingly, our study detected DENV4 serotype, which has not been previously reported in Nigeria. This may be due to vector evolutionary competence or introduction of other invasive arthropod species in the area.

The *Aedes* mosquitoes are usually infected for life and there is high possibility of vertical transmission [34]. Therefore, the circulation of DENV in humans and mosquito populations could be maintained for exceptionally long periods. Consequentially, being infected with one serotype does not provide immunity for another serotype. The circulation of multiple serotypes can lead to increased cases of severe form of DENV infections [35]. Overall these could be a potential public health threat, which is to be prepared for in order to avert an outbreak/epidemic of DENV. DENV2 was the most frequently detected serotype in our study followed by DENV1 (Fig. 4A). This acceded with previous report that suggested DENV2 as the cosmopolitan serotype causing most epidemics in Africa [20]. In contrast, DENV1 was the most common

serotype circulating in serum samples collected in selected regions of Kenya [36]. Our study, showed concurrent detection of more than one DENV serotype in single pools of *Aedes* species (Fig. 4B). Co-infection of individual mosquitoes is not common; however, Thavara [37] reported co-infections in adult *Ae. aegypti* and *Ae. albopictus* in Southern Thailand. Furthermore, double co-infection DENV serotypes were detected in five *Ae. aegypti* pools and two *Ae. albopictus* pools. And triple co-infection of DENV serotypes were detected in three *Ae. aegypti* pools. The highest co-infections (66.7%) occurred in Mubi municipality of the study area. This corroborated the report of Perez-Castro [22] on the detection of double and triple co-infection of DENV in one pool of *Ae. aegypti*. Similarly, co-infections of double DENV2/DENV4 and DENV3/DENV4 was reported in *Ae. albopictus* from Kuala Lumpur, Malaysia [38]. The infection of larvae with up to three DENV serotypes has been reported experimentally [39]. Although, there is lack of information on the natural infection of all four DENV serotypes in an individual mosquito or human. However, this can be possible during intense transmission in hyper-endemic areas or via transovarian transmission [21].

Our study investigated, variation in DENV serotypes using sequence analysis. DENV 1, 2 and 4 serotypes (Fig. 5A, B and C) respectively from the study locations formed clades together with that of South and Southeast Asia, North America and other African countries indicating strong evolutionary relatedness of the virus, signifying a common ancestor. Our study corroborates with that of Konongoi [36] who reported relatedness of DENV serotypes in Kenya (East Africa) with that of South and Southeast Asia. Therefore, these countries portend high-risk areas for DENV because of the possibility of exportation of the serotypes to other areas. Thus, our study reveals continued spread and wide geographic range of DENV serotypes. Interestingly, DENV2 in our study showed a variation in level of relatedness to other similar serotypes (Fig. 5A). Some of the DENV2 serotypes from our study location showed close relationship while others are distantly related with the referenced DENV sequences used. The differences observed could be due to possible genetic evolutionary trends, since evolution is a continuous process and genetic geographic variations are inevitable. Point mutation could occur in lineages over time which could lead to divergence [40]. The study also indicated DENV3 as distantly related with serotypes from Asia and African countries suggesting possible variation from the other serotypes. Our study further supported the reports that the major epidemics thought to be dengue, emanated from three continents: Asia, Africa, and North America [41]. There is a sudden increase in the number of imported DENV cases worldwide due to globalization [40]. The northeastern state of Adamawa Nigeria, shares a porous international borders with other African countries. It is imperative to note that travelers or animal movement may serve as vehicles for vector geographical expansion and DENV transmission, since 50% of the world's populations live in dengue endemic areas [36]. Thus, the identification of these DENV serotypes and other *Aedes* mosquito species can create evolutionary disease ecology for the emergence of other flaviviruses causing animal and human diseases in this area.

5. Conclusion

Our study detected all the DENV1-4 serotypes circulating in *Aedes* species in northeastern Nigeria and reported for the first time the occurrence of DENV4 serotype, as new serotype, which has not been previously described in Nigeria. Also, the isolation of DENV in *Ae. albopictus and Ae. galloisi* present a new threat of increased vector competency in the transmission cycle of DENV across animal species and to humans. There was strong evolutionary relatedness of DENV serotypes in our study with that of South and Southeast Asia, North America, and other African countries, which support the geographical spread of DENVs. Furthermore, our study postulates the possible linkage between the identification of DENV serotypes, *Aedes* mosquito vectors and the human febrile disease burden being experienced by host communities that are affected by the humanitarian crisis in the northeastern Nigeria.

It is pertinent to note that as part of the limitation of our study, we did not look into the contributing effect of climate change due to lack of funding. However, the effect climate change on the geographical spread of DENVs and mosquito vectors cannot be rule out. Therefore, to achieve the goals of One Health and address potential or existing global and transnational disease health risks, an intensified monitoring and long term surveillance studies of both the vectors and viruses are essential to elucidate interaction human, animal and environment interface.

Authors' contributions

II, ISN, MA and AYS conceived and designed the study. II conducted the field study. II performed field data collection under ISN and AYS supervision. II performed the laboratory and data analysis under AD's and GC supervision. II drafted the manuscript. GC, MA, ISN, AD, and AYS critically revised the manuscript. II and AYS wrote the final manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Data supporting the conclusions of this article are included within the article

Ethics approval and consent to participate

Ethical approval for the study was obtained from the Ethical Committee of the Adamawa State ministry of Health and from University Ethical Committee on the use of Human and Animal Subject for Research, ABU, Zaria (Ref. N0. S/MoH/1131/1).

Consent for publication

Not applicable

Authorship statement

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in the journal of One Health.

Declaration of Competing Interest

The authors declare that they have no competing interests

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