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Prevalence of malaria and dengue virus co-infection in Delta State, Nigeria

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ABSTRACT

Malaria and dengue virus are significant mosquito-borne infections in Nigeria, and their co-infection poses a growing public health concern in Delta State. This study was aimed at determining the prevalence of malaria and dengue virus co-infection in Delta State, Nigeria. The study adopted a longitudinal survey design among participants. A total of 400 blood samples were collected from the volunteered participants, fifty (50) samples from each of the eight hospitals selected for the study. Ethical approval was obtained, and informed consent was secured from all study participants. A questionnaire was administered to collect basic demographic information. Dengue virus was detected using Nexted Polymerase Chain Reaction techniques, while malaria parasitemia was identified through microscopy. Statistical analysis included ANOVA, t-tests and pearsons correlation to assess species association and seasonal trends. Out of the 800 participants examined, 243 (30.4%) were infected with malaria alone, with a higher proportion in males (35.7%) than females (26.8%). Among the 243 individuals tested for dengue, 18 (7.4%) were positive for dengue only, and an equal proportion (7.4%) had malaria–dengue co-infection, both showing higher infection rates in females (9.3%) compared to males (5.3%). Malaria infection was generally higher among males during the dry season (41.5%). In the rainy season, males had higher number of malaria infection rate (30.0%) when compared to females (27.9%) while female participants aged 21 - 39 years had the highest malaria infection rate of 40.8% when compared to other age groups. Highest malaria prevalence was recorded in Sapele (34%), followed closely by Abraka (32%) and Ughelli (32%). During the rainy season, 2 (4.2%) males and 7 (10.4%) females tested positive for dengue, while in the dry season, 4 (6.1%) males and 5 (8.1%) females tested positive. Ute-Okpu recorded the highest dengue infection rate of 16.1%, followed by Agbor (12.9%) and Ughelli (9.7%). Four serotypes of dengue D1 – D4 were detected both during the rainy and dry season. The study therefore highlights a notable prevalence of malaria, dengue, and their co-infection in Delta State, emphasizing the need for targeted public health interventions and enhanced vector control measures.

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INTRODUCTION

Malaria is one of the leading causes of acute febrile illness (AFI) in Africa and are transmitted by the bite of infected female anopheles mosquito (Agarwal *et al.*, 2017). The malaria parasite is primarily spread by nocturnal and indoor biters. *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae*, and *Plasmodium knowlesi* are some of these malarial parasites. *P. falciparum* causes more than 95% of malaria infections in Nigeria, with *P. ovale* and *P. malariae* having a modest contribution (Akanbi *et al.*, 2025).

Both vertebrates and invertebrates can contract arboviruses, which are viruses spread by arthropods. They are not the same as insect-specific viruses that can only reproduce in invertebrates. Arboviruses are a major cause of infections in humans and animals worldwide (Agarwal *et al.*, 2017). Due to their strikingly similar clinical manifestations, arboviral infections are frequently misdiagnosed and treated as malaria (Salam *et al.*, 2018). Co-infections with malaria parasites and arboviruses have been documented in Senegal, Nigeria (Ghazy *et al.*, 2025), and European tourists in Senegal, Guinea, and Sierra Leone (Cunnington *et al.*, 2024). Since Charrel *et al.*'s 2005 study, there have been several reports of concurrent dengue and malaria infections (Jones *et al.*, 2023). However, as it was a retrospective study, care should be taken when interpreting the results. According to Epelboin *et al.* (2012), concurrent dengue and malaria infections are typically more severe than single infections because they are marked by haematologic abnormalities such thrombocytopaenia and anaemia, which are recognised risk factors for severe dengue fever and/or malaria.

Dengue fever, often known as break bone fever, is an infectious tropical disease caused by the dengue virus. DENV-1, DENV-2, DENV-3, and DENV-4 are the four strains of the virus that are known to exist (Onoja and Fagbami, 2018). These fever-causing diseases (malaria and dengue) have become major global public health concerns, especially in Africa, because of their endemicity and similar symptoms, which include fever, severe joint and muscle pains, headache, sore throat, malaise, nausea, an irritating rash, etc (Lu *et al.,* 2022).

Individuals infected with any of these microbial organisms may be misdiagnosed when using clinical considerations alone, without the requirement for laboratory testing, due to these similarities in symptom presentations. Therefore, the aim of this study is to determine the prevalence of malaria and dengue virus co-infection among febrile patients attending hospitals in Delta State.

MATERIALS AND METHODS

Study area

Delta State is situated between latitudes 5.7040° N and longitudes 5.9339° E. 17,698 square kilometers make up the land area. Edo borders Delta State to the north (Erhenhi *et al.,* 2016; Lemy and Egwunyenga, 2017), Ondo borders it to the northwest; Anambra borders it to the east; and Bayelsa and the Rivers border it to the southeast. The southern side of Benin's 160 km of coastline is known as the Bight (Bay) (Orhewere *et al.,* 2023). A map of the study area and locations is shown in Fig. 1 below. This study was carried out in some major hospitals within Delta State.

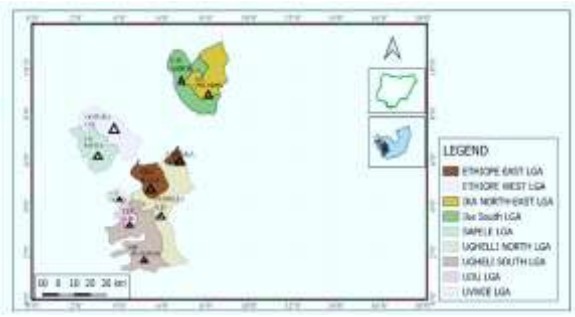

Fig. 1. Map of study (Developed using QGIS)

Study design

The study employed a longitudinal survey design involving patients attending Udu General Hospital, Abraka General Hospital, Ughelli General Hospital, Central Hospital Sapele, Central Hospital Agbor, General Hospital Ute-Okpu, and General Hospital Ekpan. Blood samples were collected at the

laboratory units of each hospital, which served as the designated sampling points. Demographic and other relevant information were obtained using a well-structured questionnaire administered to the participants. In total, 400 blood samples were collected from volunteers, with fifty (50) samples obtained from each of the eight selected hospitals (Taherdoost, 2022).

Ethical approval

Ethical approval was gotten from the Faculty of Science ethical committee, Delta State University, Abraka and Delta State Ministry of Health, Asaba. Secondly, an informed consent of the recruited patients was sought before sample collection. The intent of the study was clearly made known to the interested participants in the language he/she understands. In any situation where the interested participants cannot speak for him/herself, may be due to speech or ear defect, the patients' relative or any other person appointed by the participant was duly consented before sample collection.

Collection and analysis of blood samples

About 3 ml of whole blood was collected aseptically through venipuncture using sterile 5 ml syringe and transferred into a sterile EDTA container already properly labeled. The samples were taken to National Arbovirus and Vectors Research Centre (NAVRC) Enugu for sample processing and analysis. Microscopic analysis was done using a Pasteur pipete to withdraw a drop of the blood sample which was placed on a glass slide. The blood was spread in a circular form using MP tip. It was allowed to dry and was stained using Leishman stain. Leishman stain is basically used in peripheral blood film. For the staining, the slide was placed on a staining rack and flooded with 3% Giemsa stain and allowed to stay for 45 to 60 minutes. It was rinsed gently with slow running water, so that the excess stain could be washed off. It was placed vertically on a dry rack and allowed to dry, and was read using a x100 oil immersion microscope. Thereafter, the malaria parasite was identified (Kolluri *et al.*, 2018).

For the detection of dengue virus, Viral RNA was extracted using Qiamp RNA extraction (Qiagen) contents. AVL buffer containing carrier RNA was mixed thoroughly with the serum in a 1.5ml of clean eppendorf tube by vortexing. After brief centrifugation, absolute ethanol was added to the mixture and mixed by vortexing. The mixture was allowed to stand at room temperature for one minute and then transferred into a QiaAmp Mini spin column and centrifuged at 10000rpm for one minute, the filtrate was discarded. AW_1 and AW_2 were successively used to ensure complete washing off debris from the extract. AVE buffer was then used to elute 80ul of the RNA extract. The concentration of the extracted RNA was estimated using Nanodrop spectrophotometer (Mohanty *et al.,* 2009).

Statistical analysis

Data obtained from the study were summarized using descriptive statistics such as frequencies and percentages. The Chi-square test was employed to determine the level of association between categorical variables, and a *p*-value less than 0.05 was considered statistically significant.

RESULTS

Out of the 800 participants examined, 243 (30.4%) were infected with malaria alone, with a higher proportion in males (35.7%) than females (26.8%) (Table 1). Among the 243 individuals tested for dengue, 18 (7.4%) were positive for dengue only, and an equal proportion (7.4%) had malaria–dengue co-infection, both showing higher infection rates in females (9.3%) compared to males (5.3%). The *p*-value of 0.599 indicates that the observed difference in co-infection between sexes was not statistically significant.

The highest prevalence of malaria infection was observed in children aged 0–9 years (37.6%) and adults 21–39 years (35.9%), while the lowest was among those aged ≥60 years (17.9%), with no significant difference across age groups (p = 0.866) (Table 2). By occupation, drivers (60.0%), artisans (57.7%), and students (57.1%) had the highest

prevalence, whereas traders (15.2%) and civil servants (21.6%) had the lowest, though differences were not statistically significant (p= 0.205). Regarding educational level, primary school attendees had the highest prevalence (43.8%), followed by SSCE (32.1%), uneducated individuals (24.0%), and tertiary-educated individuals had the lowest prevalence (20.7%), with differences also not statistically significant (p = 0.419). Low and moderate parasitemia were most common across most demographic groups, while high parasitemia was relatively less frequent.

Table 1. Co-infection patterns of malaria and dengue fever

Infections	No. examined (%)			No. infected (%)			p-value
	Total	Males	Females	Total	Males	Females	
Malaria only	800	319 (39.9%)	481 (60.1%)	243 (30.4%)	114 (35.7%)	129 (26.8%)	–
Dengue only	243	114 (46.9%)	129 (53.1%)	18 (7.4%)	6 (5.3%)	12 (9.3%)	–
Malaria + Dengue	243	114 (46.9%)	129 (53.1%)	18 (7.4%)	6 (5.3%)	12 (9.3%)	0.599

p-value less than 0.05 is typically considered statistically significant.

Table 2. Prevalence of malaria according to demographic characteristics

Characteristics	No. examined (n = 800)	No. infected (n = 243)	Prevalence (%)	Level of parasitemia			p-value
				Low	Moderate	High	
Gender							
Males	319	114	35.7	28	26	11	0.188
Females	481	129	26.8	37	32	9	
Age group							
0–9	157	59	37.6	16	15	6	0.866
10–20	158	51	32.3	36	31	7	
21–39	237	85	35.9	10	9	5	
40–59	220	43	19.5	3	3	2	
≥60	28	5	17.9	0	0	0	
Occupation							
Students	98	56	57.1	10	8	3	0.205
Farmers	66	32	48.5	6	8	3	
Drivers	25	15	60.0	2	2	1	
Traders	230	35	15.2	14	12	4	
Civil servants	102	22	21.6	10	8	4	
Others	253	68	26.9	20	19	4	
Artisans	26	15	57.7	3	1	1	
Level of education							
Uneducated	50	12	24.0	8	7	1	0.419
Primary	210	92	43.8	15	12	4	
SSCE	240	77	32.1	22	20	9	
Tertiary	300	62	20.7	20	19	6	

p-value less than 0.05 is typically considered statistically significant.

Table 3. Seasonal distribution of malaria cases

Season	Age group	No. examined		No. infected (%)		Non-infected (%)		p-value
		Male	Female	Male	Female	Male	Female	
Rainy	0–9	30	48	10 (33.3%)	18 (37.5)	20 (66.7%)	30 (62.5%)	0.344
	10–20	32	47	11 (34.4%)	12 (25.5%)	21 (65.6%)	35 (74.5%)	
	21–39	47	71	13 (27.7%)	29 (40.8%)	34 (72.3%)	42 (59.2%)	
	40–59	44	66	12 (27.3%)	8 (12.1%)	32 (72.7%)	58 (87.9%)	
	≥60	6	8	2 (33.3%)	0 (0.0%)	4 (66.7%)	8 (100%)	
	Total	159	240	48 (30.0%)	67 (27.9%)	111 (69.8%)	173 (72.1%)	
Dry	0–9	30	49	14 (46.7%)	17 (34.7%)	16 (53.3%)	32 (65.3%)	
	10–20	32	47	19 (59.4%)	9 (19.1%)	13 (40.6%)	38 (80.9%)	
	21–39	48	71	17 (35.4%)	26 (36.6%)	31 (64.6%)	45 (63.4%)	
	40–59	44	66	16 (36.4%)	7 (10.6%)	28 (63.6%)	59 (89.4%)	
	≥60	6	8	0 (0.0%)	3 (37.5%)	6 (100%)	5 (62.5%)	
	Total	160	241	66 (41.5%)	62 (25.7%)	94 (58.8%)	179 (74.3%)	
Total		319	481	114 (46.9%)	129 (53.1%)	205 (64.3%)	352 (73.2%)	

p-value less than 0.05 is typically considered statistically significant.

The seasonal distribution of malaria cases by age group and sex showed that, among the 800 participants examined, 400 were surveyed in each season (Table 3). Malaria infection was generally higher among males during the dry season (41.5%). In the rainy season, males had higher number of malaria infection rate (30.0%) when compared to females (27.9%) while female participants aged 21 - 39 years had the highest malaria infection rate of 40.8% when compared to other age groups. During the dry season, males had higher malaria infection rate (41.5%) when compared to females (25.7%) while male participants 10 - 20 years had the highest malaria infection rate of 59.4% when compared to other age groups. Females ≥60 years in rainy season and males ≥60 years in dry season had the least malaria infection rate of 0%. Despite these differences, statistical analysis indicated that the observed variations in infection rates across seasons, sexes, and age groups were not significant (p = 0.344).

Table 4. Distribution of malaria cases by location

Location	No. examined	No. infected (%)	Non-infected (%)	p-value
Abraka	100	32 (32.0%)	68 (68.0%)	0.558
Otu Jeremi	100	29 (29.0%)	71 (71.0%)	
Ekpan	100	27 (27.0%)	73 (73.0%)	
Ute-Okpu	100	30 (30.0%)	70 (70.0%)	
Agbor	100	31 (31.0%)	69 (69.0%)	
Sapele	100	34 (34.0%)	66 (66.0%)	
Udu	100	28 (28.0%)	72 (72.0%)	
Ughelli	100	32 (32.0%)	68 (68.0%)	
Total	800	243 (30.4%)	557 (69.1%)	

p-value less than 0.05 is typically considered statistically significant.

Table 5. Prevalence of dengue according to demographic characteristics

Characteristics	No. examined (n = 243)	No. Infected (n = 18)	Prevalence (%)	p-value
Gender				
Males	114	6	5.3	0.042
Females	129	12	9.3	
Age group				
0–9	59	7	11.9	0.5523
10–20	51	6	11.8	
21–39	85	4	4.7	
40–59	43	1	2.3	
≥60	5	0	0.0	
Occupation				
Student	56	6	10.7	0.355
Farmer	32	5	15.6	
Driver	15	0	0.0	
Trader	35	3	8.6	
Civil servant	22	1	4.5	
Others	68	3	4.4	
Artisan	15	0	0.0	
Level of education				
Uneducated	12	3	25.0	0.401
Primary	92	7	7.6	
SSCE	77	5	6.5	
Tertiary	62	3	4.8	

p-value less than 0.05 is typically considered statistically significant.

According to location, highest malaria prevalence was recorded in Sapele (34%), followed closely by Abraka (32%) and Ughelli (32%), while the lowest was observed in Ekpan (27%) (Table 4). Other areas such as Otu Jeremi (29%) and Ute-Okpu (30%) showed comparable infection rates. The differences in malaria prevalence across the locations were not statistically significant (p = 0.558), indicating a relatively uniform distribution of malaria infection across the studied areas.

Out of the 243 individuals examined, 18 were infected with dengue, giving an overall prevalence of 7.4% (Table 5). Females had a higher prevalence (9.3%) than males (5.3%), and this difference was statistically significant (p = 0.042). Prevalence across age groups ranged from 11.9% in children aged 0–9 years to 0% in those aged ≥60 years, though the differences were not statistically significant (p= 0.5523). Among occupations, farmers (15.6%) and students (10.7%) had the highest prevalence, while drivers and artisans had no cases, with overall occupational differences not statistically significant (p = 0.355). For educational level, the uneducated group had the highest prevalence (25.0%) compared with tertiary-educated individuals (4.8%), but this variation was not statistically significant (p= 0.401).

Table 6. Seasonal distribution of dengue cases

Season	Age group	No. examined (243)		No. infected (%)		Non-infected (%)		p-value
		Male	Female	Male	Female	Male	Female	
Rainy	0–9	10	18	1 (10.0%)	3 (16.7%)	9 (90%)	15 (83.3%)	0.499
	10–20	11	12	1 (9.1%)	2 (16.7%)	10 (90.9%)	10 (83.3%)	
	21–39	13	29	0 (0.0%)	2 (6.9%)	13 (100%)	27 (93.1%)	
	40–59	12	8	0 (0.0%)	0 (0.0%)	12 (100%)	8 (100%)	
	≥60	2	0	0 (0.0%)	0 (0.0%)	2 (100%)	0 (0.0%)	
	Total	48	67	2 (4.2%)	7 (10.4%)	46 (95.8%)	60 (89.6)	
Dry	0–9	14	17	1 (7.1%)	2 (11.8%)	13 (92.9%)	15 (88.2%)	
	10–20	19	9	1 (5.3%)	2 (22.2%)	18 (94.7%)	7 (77.8%)	
	21–39	17	26	1 (5.9%)	1 (3.8%)	16 (94.1%)	25 (96.2%)	
	40–59	16	7	1 (6.3%)	0 (0.0%)	15 (93.7%)	7 (100%)	
	≥60	0	3	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (100%)	
	Total	66	62	4 (6.1%)	5 (8.1%)	62 (93.9%)	57 (91.9%)	
Total		114	129	6 (5.3%)	12 (9.3%)	108 (94.7%)	117 (90.7%)	

p-value less than 0.05 is typically considered statistically significant.

Table 7. Distribution of dengue cases by location

Location	No. examined	No. infected (%)	Non-infected (%)	p-value
Abraka	30	0 (0.0%)	30 (100.0%)	0.674
Otu Jeremi	30	1 (3.3%)	29 (96.7%)	
Ekpan	30	1 (3.3%)	29 (96.7%)	
Ute-Okpu	31	5 (16.1%)	26 (83.9%)	
Agbor	31	4 (12.9%)	27 (87.1%)	
Sapele	30	2 (6.7%)	28 (93.3%)	
Udu	30	2 (6.7%)	28 (93.3%)	
Ughelli	31	3 (9.7%)	28 (90.3%)	
Total	243	18 (7.4%)	225 (92.6%)	

p-value less than 0.05 is typically considered statistically significant.

During the rainy season, 48 males and 67 females were examined, with 2 (4.2%) male and 7 (10.4%) females testing positive for dengue, while 46 (95.8%) males and 60 (89.6%) females tested negative (Table 6). In the dry season, 66 males and 62 females were examined, with 4 (6.1%) males and 5 (8.1%) females testing positive, while 62 (93.9%) males and 57 (91.9%) females tested negative.

The distribution of dengue cases across the study locations showed varying prevalence rates, with Ute-Okpu recording the highest infection rate of 16.1%, followed by Agbor (12.9%) and Ughelli (9.7%), while Otu Jeremi, Ekpan, Sapele, and Udu each had low infection rates of 3.3–6.7% (Table 7). No dengue infection was detected in Abraka, where all participants tested negative. Overall, out of the 243 individuals examined, 18 (7.4%) were infected while 225 (92.6%) were not, and the difference in infection rates across locations was not statistically significant (p = 0.674).

Across both sexes, hemoglobin (Hb) and platelet counts decreased as parasitemia increased, while

white blood cell (WBC) counts increased (Table 8). In males, mean Hb droped from 12.6 g/dL at low parasitemia to 9.0 g/dL at severe parasitemia, platelets decreased from 210 ×10⁹/L to 120 ×10⁹/L, and WBC rises from 6.5 ×10⁹/L to 8.5 ×10⁹/L. A similar trend was observed in females, with mean Hb decreasing from 12.0 g/dL to 8.6 g/dL, platelets from 205 ×10⁹/L to 110 ×10⁹/L, and WBC increasing from 6.2 ×10⁹/L to 8.2 ×10⁹/L.

Table 8. Mean hematological parameters by level of parasitemia and sex

Level of parasitemia	Males				Females			
	No. examined	Mean Hb (11-16g/dl)	Mean WBC (4.0-11.0×10⁹/L)	Mean platelets (150-450×10⁹/L)	No. examined	Mean Hb (11-16g/dl)	Mean WBC (4.0-11.0×10⁹/L)	Mean platelets (150-450×10⁹/L)
Low (1-999)	28	12.6	6.5	210	37	12.0	6.2	205
Moderate (1,000-9,999)	26	11.0	7.0	170	32	10.4	6.8	160
Severe (≥ 10,000)	11	9.0	8.5	120	9	8.6	8.2	110

Key: Hb = Hemoglobin, WBC = White blood cel

Table 9. Knowledge, attitude and practice (KAP) towards *Plasmodium* infections

Variables	Categories	No. examined (n=800)	Malaria Infected (%) (n=243)	Malaria non-infected (%) (n=557)
Knowledge of dengue fever or dengue virus	Yes	312 (39.0)	60 (24.7%)	252 (45.2%)
	No	488 (61.0)	183 (75.3%)	305 (54.8%)
When last did you treat fever?	≤ 1 month	230 (28.8)	85 (35.0%)	145 (26.0%)
	≥ 2 months	180 (22.5)	65 (26.7%)	115 (20.6%)
	≥ 3 months	150 (18.8)	48 (19.8%)	102 (18.3%)
	Over 6 months	240 (30.0)	45 (18.5%)	195 (35.0%)
Before treatment, did you do test?	Yes	410 (51.3)	80 (32.9%)	330 (59.2%)
	No	390 (48.8)	163 (67.1%)	227 (40.8%)
What was the result of the test?	Positive	134 (16.8)	110 (45.3%)	24 (4.3%)
	Negative	560 (70.0)	90 (37.0%)	470 (84.4%)
	Undecided	106 (13.3)	43 (17.7%)	63 (11.3%)
Do you have recurrent fever?	Yes	290 (36.3)	140 (57.6%)	150 (26.9%)
	No	510 (63.8)	103 (42.4%)	407 (73.1%)
Living environment	Urban	340 (42.5)	70 (28.8%)	270 (48.5%)
	Rural	460 (57.5)	173 (71.2%)	287 (51.5%)
Type of housing	Flat	220 (27.5)	50 (20.6%)	170 (30.5%)
	1 Room	260 (32.5)	115 (47.3%)	145 (26.0%)
	Self Contain	150 (18.8)	40 (16.5%)	110 (19.7%)
	2 Bedroom	170 (21.3)	38 (15.6%)	132 (23.8%)
Number of occupants	Less than 5	310 (38.8)	75 (30.9%)	235 (42.2%)
	More than 5	490 (61.3)	168 (69.1%)	322 (57.8%)
Use of insecticide-treated nets (ITNs)	Yes	370 (46.3)	80 (32.9%)	290 (52.1%)
	No	430 (53.8)	163 (67.1%)	267 (47.9%)
Types of nets	ITNs	370 (46.3)	80 (32.9%)	290 (52.1%)
	Window nets	280 (35.0)	110 (45.3%)	170 (30.5%)

Among the malaria-infected group (n = 243), only 60 (24.7%) had knowledge of dengue fever, compared to 252 (45.2%) among the non-infected group (n = 557) (Table 9). Fewer infected participants 80 (32.9%) reported conducting a test before treatment and 80 (32.9%) used insecticide-treated nets, while higher proportions 163 (67.1%) and 163 (67.1%) respectively did not. A majority of infected individuals lived in rural areas 173 (71.2%), resided in one-room housing 115 (47.3%), and had more than five occupants per household 168 (69.1%), compared to 287 (51.5%), 145 (26.0%), and 322 (57.8%) among non-infected respondents.

PCR amplification confirmed the presence of Plasmodium falciparum DNA in positive samples, as evidenced by distinct bands at approximately 700 bp

following the second amplification. Lanes L1 and L5 contained the 100 bp DNA molecular weight marker, validating the expected fragment size and assay specificity (Fig. 2).

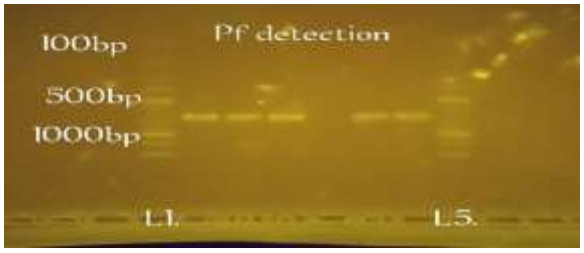

Fig. 2. Molecular detection of *Plasmodium falciparum* (Pf)
L1 and L5 are 100bp DNA molecular weight marker. The positive samples resolved at 700bp after the second amplification

RT-PCR analysis detected dengue fever virus serotypes in both rainy and dry season samples. Positive samples collected during the rainy season (L1–L7) and the dry season (L9–L16) showed distinct bands corresponding to DENV serotypes, while non-template controls (L3 and L12) showed no amplification. The four serotypes were identified by their expected amplicon sizes: D1 (492 bp), D2 (123 bp), D3 (246 bp), and D4 (369 bp), confirming successful molecular differentiation of dengue virus serotypes (Fig. 3).

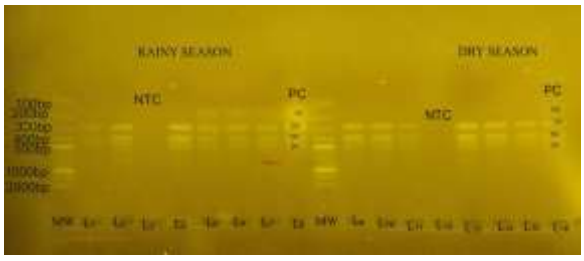

Fig. 3. Molecular detection of dengue fever virus serotypes
MW is the 100bp Molecular weight marker. NTC is the Non template control (L3 and L12), PC is the positive control (L8 and L16). L1 to L7 are positive samples collected during the rainy season, while L9 to L15 is the positive samples collected during the dry season. D1, D2, D3, and D4 resolved at 492bp, 123bp, 246bp and 369bp respectively.

DISCUSSION

The findings indicate that malaria remained the predominant infection among the study population, with 30.4% of participants infected, and a higher prevalence in males, likely due to increased outdoor exposure and greater contact with mosquito vectors. Dengue infection was comparatively lower (7.4%), and co-infection with malaria and dengue occurred in an equal proportion (7.4%), with females exhibiting slightly higher rates in both cases, although the difference was not statistically significant (p = 0.599). The higher female prevalence for dengue and co-infection may reflect differences in household or community exposure, vector behavior, or reporting patterns. The higher female co-infection proportion may reflect differing exposure patterns or health-seeking behaviours, though the p-value of 0.599 indicates no statistically significant sex-difference in co-infection rates, which is consistent with the findings of Michael *et al.* (2023) that gender alone may not reliably predict arbovirus–malaria co-infection in endemic settings.

The study revealed a malaria prevalence of 30.4% among the 800 individuals examined, with males (35.7%) having a higher prevalence than females (26.8%), though the difference was not statistically significant. Children aged 0–9 years and adults 21–39 years showed the highest prevalence, likely due to increased exposure to mosquito bites through outdoor activities and lower immunity in younger children. Occupations involving outdoor or field activities, such as drivers, artisans, and students, also recorded higher prevalence, reflecting occupational exposure as a risk factor. Similarly, individuals with lower levels of education, particularly primary school attendees, had higher prevalence, which may relate to limited knowledge and practice of preventive measures like bed nets. These findings are consistent with the report of Awosolu *et al.* (2021), who found higher malaria prevalence among males and individuals with lower education in Nigeria. Likewise, Kendie *et al.* (2021) observed that occupation and educational level were key determinants of malaria risk in Ethopia, supporting the patterns observed in

this study. In line with these results, Jenkins *et al.* (2015) reported higher prevalence among children under 10 years in Kenya, attributing this to incomplete immunity and higher susceptibility in younger age groups, rather than in the elderly.

The result showed that malaria infection was slightly higher among males during the dry season (41.5%), while in the rainy season, males (30%) recorded higher infection. Female participants aged 21 - 39 years had the highest malaria infection rate of 40.8%. Despite these variations, the differences were statistically insignificant (p = 0.344). The seasonal fluctuation in malaria prevalence can be attributed to vector breeding patterns, as *Anopheles* mosquitoes thrive better during periods of moderate rainfall and stagnant water availability. These findings align with the observations of Yusuf *et al.* (2024), who reported that malaria prevalence in rural communities of Southwestern Nigeria showed no significant seasonal difference due to perennial transmission. Similarly, Hussaini *et al.* (2025) noted that malaria burden persisted in both wet and dry seasons in Northern Nigeria, largely driven by environmental factors and vector adaptation. In contrast, Dakorah *et al.* (2022) found significantly higher malaria prevalence during the rainy season in Ghana, attributing it to increased mosquito breeding following heavy rainfall, which highlights how ecological variation influences seasonal malaria trends.

The findings showed that malaria prevalence varied slightly across the eight study locations, with Sapele recording the highest prevalence (34%), followed by Abraka (32%) and Ughelli (32%), while Ekpan had the lowest (27%). However, these differences were not statistically significant (p = 0.558), indicating a relatively uniform distribution of malaria across the surveyed areas. The similarity in infection rates may be due to comparable ecological and environmental conditions that favor mosquito breeding, such as stagnant water bodies, poor drainage, and humid tropical climate. The high prevalence in Sapele and Abraka may be associated with dense population and limited access to vector control measures. These results are in line with Awosolu *et al.* (2021), who reported relatively uniform malaria prevalence across rural and semi-urban communities in Southern Nigeria due to consistent ecological and socioeconomic exposures. Similarly, Uzochukwu *et al.* (2018) observed that malaria burden remained comparable across neighboring towns in Enugu State, underscoring the pervasive nature of transmission.

The study showed that dengue infection had an overall prevalence of 7.4% among the 243 individuals examined, with females significantly more affected than males (9.3% vs. 5.3%, p = 0.042), possibly due to differences in exposure patterns or time spent in mosquito-prone environments. Age-specific prevalence was highest in children aged 0–9 years (11.9%) and decreased with advancing age, though differences were not statistically significant, suggesting that younger individuals may be more susceptible due to lower immunity.

Occupation appeared to influence risk, as farmers and students had the highest prevalence, likely reflecting increased outdoor exposure to *Aedes* mosquitoes, whereas drivers and artisans recorded no cases. Educational level also showed a trend, with uneducated individuals having the highest prevalence (25.0%), possibly due to limited knowledge of preventive measures, though differences were not significant. These findings align with previous studies in similar endemic settings, which reported higher dengue prevalence among females, younger age groups, and individuals with occupations or education levels that increase exposure or reduce awareness of vector control (Adekola *et al.*, 2024).

During the rainy season, 10.4% of females and 4.2% of males were infected, while in the dry season, 8.1% of females and 6.1% of males were infected. The difference across seasons and sexes was not statistically significant (p = 0.499). The observed higher prevalence among females may be associated with greater exposure to mosquito bites within domestic environments where *Aedes aegypti* vectors thrive. The marginally higher infection rate during the dry season

may reflect persistence of breeding sites in stored water containers typical of water-scarce periods. These findings support the work of Oforka *et al.* (2024), who reported that dengue transmission in Lagos remained relatively stable across seasons due to urban environmental conditions. Likewise, Afolabi *et al.* (2006) highlighted that container breeding of *Aedes aegypti* sustained year-round transmission in Nigeria. Conversely, Owusu-Asenso *et al.* (2022) found a marked seasonal peak of dengue during the rainy season in Accra, Ghana, linking it to increased vector density and rainfall patterns, underscoring the variability of dengue ecology across different regions.

The study revealed that dengue infection was detected in nearly all sampled locations, with the highest prevalence recorded in Ute-Okpu (5.0%) and Agbor (4.0%), while Abraka showed no infection cases. The relatively low prevalence across Delta State (2.3%) may be attributed to factors such as ongoing vector control interventions, environmental differences, or limited viral circulation within the region. Additionally, improved awareness and use of preventive measures like insecticide-treated nets could have contributed to the reduced transmission (Ede *et al.,* 2025). This finding aligns with the report of Adesola *et al.* (2024), who recorded a similarly low prevalence of 2.8% in Southwestern Nigeria, This pattern is comparable to findings by Oyero and Ayukekbong (2014), who observed low but consistent dengue seroprevalence across several communities in southern Nigeria.

The result revealed that hemoglobin and platelet levels decreased progressively with increasing malaria parasitemia, while white blood cell counts slightly increased. This suggests that higher parasite loads contribute to anemia and thrombocytopenia, likely due to hemolysis, bone marrow suppression, and splenic sequestration caused by *Plasmodium* infection (Lemy *et al.,* 2022). The mild leukocytic rise reflects the immune response to infection. These findings align with those of Leticia *et al.* (2014), who reported significant reductions in hemoglobin and platelet counts among individuals with severe malaria in Ethiopia.

The results showed that malaria infection was significantly associated with respondents' knowledge, attitude, and practices (KAP) toward *Plasmodium* infection. Among the malaria-infected group, only 24.7% had knowledge of dengue fever, compared to 45.2% among the non-infected group. These results are consistent with the findings of Salami *et al.* (2025), who reported lack of awareness of dengue in rural communities in Uganda, attributing this to limited immunization programs. However, Atah *et al.* (2010) reported higher awareness of dengue fever in certain urban settings in Lagos, likely reflecting differences in media exposure and disease outbreaks.

The molecular analysis revealed that 7.4% of participants were seropositive for dengue immunoglobulins, confirming active circulation of dengue virus within the study area.

Dengue seropositivity was slightly higher among females (9.3%) compared to males (5.3%), which may reflect increased exposure to *Aedes* mosquito bites in peri-domestic settings where women spend more time. This finding is consistent with the report of Ahmed *et al.* (2025), who observed a higher prevalence of dengue infection among women in Southern Nigeria and attributed it to gender-related exposure risks. The higher prevalence during the dry season suggests that water storage practices and stagnant water sources during this period may serve as breeding sites for *Aedes* vectors, sustaining transmission despite lower rainfall.

This aligns with the findings of Sedda *et al.* (2018), who reported increased dengue transmission during the dry season in rural communities and highlighted poor water management as a major risk factor. The detection of all four dengue serotypes (D1–D4) in both seasons indicates hyperendemic transmission, a situation also reported by Emeribe *et al.* (2021) in Northern Nigeria, who warned of the increased risk of severe outcomes such as dengue hemorrhagic fever in areas with co-

circulating serotypes. By contrast, Asaga Mac *et al.* (2024) reported the predominance of only two serotypes (D1 and D2) in an urban surveillance study, concluding that serotype diversity may vary by geographical location and population density.

CONCLUSION

The study revealed that malaria remained the most prevalent infection in the study population, while dengue and malaria–dengue co-infections occur at lower rates. Malaria was more common in males, whereas dengue and co-infections showed higher prevalence in females, with dengue infection exhibiting a statistically significant sex difference. Younger age groups, certain occupations, and lower educational levels were associated with higher infection rates for both diseases, reflecting the influence of exposure, immunity, and awareness of preventive measures. Overall, these findings highlights the ongoing public health burden of malaria and dengue, the need for targeted interventions based on demographic risk factors, and the importance of community education to reduce vector-borne disease transmission.

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