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HIGH RATE OF UNRECOGNIZED DENGUE VIRUS INFECTION IN PARTS OF THE RAINFOREST REGION OF NIGERIA

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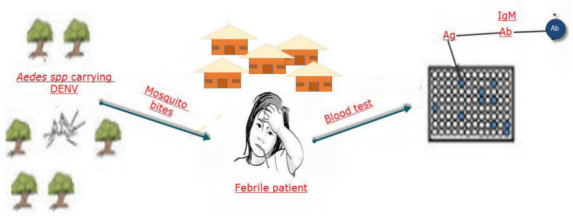
Abstract

Detection of DENV IgM in a cross-section of febrile patients using ELISA to determine sero-prevalence in the rain forest region in West Africa.



1.0. Introduction

The Centers for Disease Control and Prevention in 2014 estimated that about 50-100 million human dengue virus (DENV) infections occur annually, with nearly 2.5 billion people at risk. Over 30,000 deaths in children worldwide have been attributed to Dengue Hemorrhagic Fever and Dengue Shock Syndrome (Halstead, 2007). Presently, there is no effective antiviral drug against DENV (Harris et al., 2000). Several African countries continue to report dengue outbreaks and/or sporadic cases, while dengue is being diagnosed in travellers returning to Europe and North America from African countries (Amarasinghe et al., 2011). Although dengue exists in the World Health Organization Africa region, surveillance data is poor. It is not officially reported to the World Health Organization from countries in the region, where the burden of dengue is yet to be estimated (World Health Organization, 2009) despite increasing frequency of outbreaks (Nathan and Dayal, 2007). In many African countries, several cases of febrile illnesses are presumptively diagnosed as malaria. A study in Tanzania showed 14 laboratory-confirmed malaria cases, out of 528 patients with tentative

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Conflict of interest

We declare that there is no conflict of interest.

malaria diagnosis (Crump et al., 2013). In 2013 and 2014, over 50% of febrile patients visiting the University of Ibadan Health Services (Jaja Clinic) tested negative to malaria parasite count. Between 2011 and 2012 in Abidjan – Cote d'Ivoire, 0.4% patients had IgM antibody (L'Azou et al., 2015). During the 2013 dengue epidemic in Luanda, Angola Ministry of Health reported 811 positive dengue cases (Sharp et al., 2015). In 2014, 3.2% dengue IgM was reported in a study involving 218 children in Ghana (Stoler et al., 2015). Several other reports suggest people in some other West African countries are experiencing high rates of dengue infections (Bhatt et al., 2013). Stoler et al., (2014) highlighted the need for increased dengue surveillance in this part of Africa. Due to the dearth of relevant data on the epidemiology of dengue, we investigated the occurrence of dengue for eight months mainly during the rainy season in Nigeria.

2.0. Materials and methods

2.1. Study design

This cross-sectional study involved febrile patients from various urban centres in the rainforest region of Oyo State, Nigeria. Adeoyo Hospital Yemetu (7°24’14”N, 3°54’22”E) was selected as the study site because of the relatively high patient enrollment. Patients visiting the hospital were from 11 of the 33 Local Government Areas (LGAs) in Oyo State namely: Ibadan South East LGA, Ibadan North West LGA, Ibadan North LGA, Ibadan North East LGA, Egbeda LGA, Ona-Ara LGA, Oluyole LGA, Ido LGA, Ibadan South West LGA, Akinyele LGA and Lagelu LGA. Questionnaire was used to measure location of participants, clinical signs, socioeconomic status, whether or not they had been bitten by day-time-biting mosquitoes or if they took anti-malaria drugs before visiting the hospital. It is a modification of the enrolment questionnaire used by the Uganda People's Defense Forces, African Union Peace keeping troops and the Centres for Disease Control and Prevention USA. Informed consent was obtained and questionnaire interpreted in Yoruba for those who did not understand English Language. In the inclusion criteria, patients of all ages with the following symptoms were considered: temperatures ≥38°C for less than 10 days, headache, rash, fatigue, muscle ache, nausea, vomiting and diarrhea; while patients who tested positive to malaria parasite examination, those who had jaundice, fever of known bacterial and parasitic origin, fractures, complicated malaria according to the World Health Organization's definition were excluded (World Health Organization, 2001). In addition, patients who tested positive to typhoid fever, HIV, measles virus and other known infections were excluded.

2.2. Sample collection and processing

Plasma samples were collected from 274 febrile patients from 2 days old to 90 years of age. This was from April to December 2014. Three milliliter of blood was collected from each patient into sample bottle containing EDTA and transported in triple packaging to the Arbovirus Laboratory of the Department of Virology, University of Ibadan. After centrifugation for 5mins at 3,500 rpm (Microfuge™, Germany), the plasma was carefully transferred using Pasteur pipette in a Delta Series Biosafety Level II cabinet (Labconco Corp. Kansas City, Missouri), and preserved at −60°C until tested.

2.3. Serological assay

Sera were tested in 2014 using Dengue IgM (sandwich) ELISA kit commercially obtained from Diagnostic Automation/ Cortez Diagnostics Inc. Calabasas, CA, USA (ISO 13485:2003 and ISO 9001:2008). Assay was performed according to manufacturer's instructions after adding 40µl Rheumatoid Factor absorbent to each tube containing 100 µl of negative control, positive control or diluted samples. ELISA reader with SoftMax™ Pro software v5.4 1999-2009 (MDS Analytical Technologies Inc., USA) was set for bichromatic reading between 450 – 650nm. Negative control (NC) used was diluted negative human serum while positive control (PC) was diluted positive human serum provided in the kit. Expected values for negative control is 0.0 - 0.30 Optical density (OD) units while positive control value is ≥0.50 OD units. Results were interpreted based on these values. Assay is 97.8% specific and 93.5% sensitive.

2.4. Polymerase Chain Reaction detection of other flaviviruses

Conventional Polymerase Chain Reaction (PCR) was used to test for the 3' non-coding region of yellow fever virus according to Onyango et al., (2004), west nile virus according to Turrell et al., (2005) and Zika virus as used by Faye et al., (2008) in dengue IgM positive samples to rule out cross-reactivity by these flaviviruses.

2.5. Ethical consideration

Approval for this study was obtained from Institutional Review Board of the University of Ibadan/University College Hospital (UI/EC/13/0412) and the Ethics Board of Oyo State Ministry of Health (AD13/479/496).

2.6. Statistical analysis

Data analysis was done using SPSS version 16.0 software (IBM Corp. released 2011, IBM SPSS Statistics for Windows, Armonk, NY, USA). Chi square and Fischer's exact tests were used to determine association between IgM positivity and other variables in a univariate analysis. P value <0.05 was considered statistically significant.

3.0. Results

The number of patients tested monthly were as follows: April (n = 40), May (n = 41), June (n = 30), August (n = 30), September (n = 42), October (n = 33), November (n = 25) and December (n = 33). Of these, 82 (29.9%) were males and 192 (70.1%) females. No sample was collected in July due to strike action by resident doctors and allied health workers. Out of 274 people studied; 64 (23.3%) revealed evidence of recent dengue exposure in which 17 (26.6%) males and 47 (73.4%) females were IgM positive. There was a significant association between IgM antibody level and months with high prevalence (X^2=0.000; $p < 0.05$). Reliability of questionnaire from where clinical and socio-demographic data were obtained was found to be 72.1% with Cronbach alpha reliability statistics. Although 61 (95.3%) of those infected were low income earners and 3 (4.7%) in the middle class, no association was observed between IgM level and socioeconomic status. Eighty-four (30.7%) people took anti-malaria drugs before hospital visitation. No participant had all the clinical signs. Analysis showed no association between any of the clinical signs and recent dengue

exposure. One hundred and seven (39.1%) people reported to have been bitten by day-time mosquitoes, 92 (33.5%) were not while 75 (27.4%) were not sure if they had been bitten. Out of the 64 patients exposed to dengue virus, 23 (35.9%) had been bitten by daytime biting mosquitoes, 25 (39.0%) were not bitten and 16 (25%) were not sure if they had been bitten. There was no significant association between those who were bitten, those who were not and those who were not sure if they had been bitten (X^2=0.569; p>0.05). The participants in this study were not vaccinated against YFV. No study participant was positive for WNV, YFV and Zika virus by PCR.

4.0. Discussion

Although several studies on dengue have been reported in Nigeria and parts of west Africa, the present study highlights a high rate of recent dengue infection for eight months during the rainy season in Nigeria (Fig. 1). Most people exposed to dengue in this study are over 18.1 years old (Tables 1 and 2). This is consistent with studies suggesting that adults are more likely to have clinical dengue than young children (Simmons et al., 1931; Graham et al., 1999; Vaughn et al., 2001). A reason for this is because infections in older people are more likely to be due to secondary DENV infections which are associated with greater risks of symptomatic and severe disease (Nisalak et al., 2003; Cummings et al., 2009). However, it is not clear if more than one DENV serotype was circulating in the population during the study period hence the need for further studies to establish the molecular detail. Occurrence of high OD values in older individuals (those ≥18.1 years of age) in this study is an indication that they are more commonly affected (Tables 3). Studies in Latin America where several reports of increased number of adolescents with recent dengue infections have been made (Bhatt et al., 2013; Sharp et al., 2013; Teixeira et al., 2013; Villar et al., 2015) lends credence to the findings in our present study. A cross-sectional survey of febrile patients in Pemba Island, Zanzibar further supports our findings with a sero-prevalence of 15.4% in adults vs. 1.9% in patients under 15 years old (Vairo et al., 2012). However, reports from Asian countries where children under 5 years of age are particularly affected (World Health Organization, 2009) is at variance with the findings in this present study. The reason for this is not clearly understood. However, 15.6% prevalence among children under 5 years of age (Table 1) agrees with a study involving same age group of children in Ilorin, North central Nigeria where high prevalence was reported (Faneye et al., 2014), this underscores the importance of dengue in this age group. Although participants in this study were people visiting a hospital in Ibadan (Fig. 1), it is not certain if they were all bitten by mosquitoes in their abode or outside of their LGAs. Many residents in Ibadan store water in open containers in their houses which provides excellent breeding sites for *Aedes species*. It is also common sight to find indiscriminate disposal of hollow containers in most parts of Nigeria.

More DENV exposure occurred during the rainy season from April to October as demonstrated by higher seropositivity and IgM level (Tables 2 and 4). In two separate studies on recent dengue infection in different ecological zones in Nigeria, dengue was found to occur more in the rainforest region (Fagbami *et al.,* 1977; Baba *et al.,* 2009). This is due to abundant vegetation cover that is available in tropical rain forests region and the high relative humidity. This present study found monthly peaks in dengue prevalence during the

rainy season in April and August (Tables 2 and 4). A study in Selangor, Malaysia showed positive correlation between dengue outbreak and rainfall pattern with increase in number of breeding sites (Li et al., 1985). With reports of dengue outbreaks in other parts of Africa and importation of dengue into Europe from west Africa (Huhtamo et al., 2008; Institut de Veille Sanitaire, 2009; Raut et al., 2015) it is noteworthy that the incidence of dengue in this part of west Africa is very high. YFV, WNV and Zika virus were not detected in this present study but other flaviviruses such as Wesselsbron virus, Banzi, Uganda Z have been isolated in Nigeria and may cross-react in this assay. Fig 5 shows no association between clinical signs and sero-positivity which reinforces the need not to rely absolutely on clinical symptoms in the diagnosis of dengue in this part of the world as it will confound diagnosis.

Transmission of DENV in several urban LGAs (Fig. 1) is an indication that it is maintained in urban-human populations. Many of these areas are forested, with stagnant water and indiscriminate hollow containers that litter everywhere providing excellent breeding sites for increased vector activity. Mosquito-DENV-Human cycle has been reportedly found in nearly all urban and peri-urban environments throughout the tropics and subtropics (Rossi et al., 2012). Dengue in the tropical rain forest region has increased due to uncontrolled urbanization, lack of effective and sustainable vector control programs (Guzman et al., 2010). Although the serotype(s) involved in this epidemic is/are not known, all DENV serotypes involved in urban dengue cycle are transmitted by domestic and peridomestic *Ae. aegypti aegypti* and *Ae. albopictus* (Simmons et al., 1931; Sabin, 1952; Rosen et al., 1954; Halstead et al., 1964; Halstead, 1997). These *Aedes species* circulate steadily in Ibadan, south-west Nigeria (Onoja et al., 2016) and have been reported with other *Aedes species* in the rainforest region of south-east Nigeria (Anosike et al., 2007). Our findings extend the information on monthly dengue epidemiology in Nigeria and the observation of unrecognized recent dengue infection serves as an early warning of possible dengue outbreaks, as mosquito control efforts are not sustained to the peril of the inhabitants. A previous study investigating DENV serotypes in *Aedes aegypti* from different ecological zones in Nigeria lends credence to this assertion, with the detection of all serotypes in both male and female mosquitoes (Baba et al., 2009).

In conclusion, endemicity of dengue in the rain forest region of Nigeria has been brought to the fore. Hitherto, it was undiagnosed in most hospitals due to lack of routine screening. Blind treatment of dengue and other arboviral infections as malaria or typhoid fever was the practice. With this study, it has become more evident that routine dengue testing should be done for febrile illnesses. Active surveillance for DENV and other arboviruses is necessary to identify periods of increased virus transmission and to determine primary, secondary or tertiary DENV infections. Improved sanitary measures such as removing hollow containers from gutters and waterways will enhance environmental control strategy by dislodging or destroying mosquito larvae and controlling the vector.

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Highlights

- Prevalence of 23.4% recent dengue virus exposure was found in 8 months.
- Highest monthly prevalence of 40% dengue exposure occurred in April and August.
- People in 11 Local Government Areas were exposed to dengue virus.
- Routine dengue testing is recommended for febrile illnesses in Nigeria.

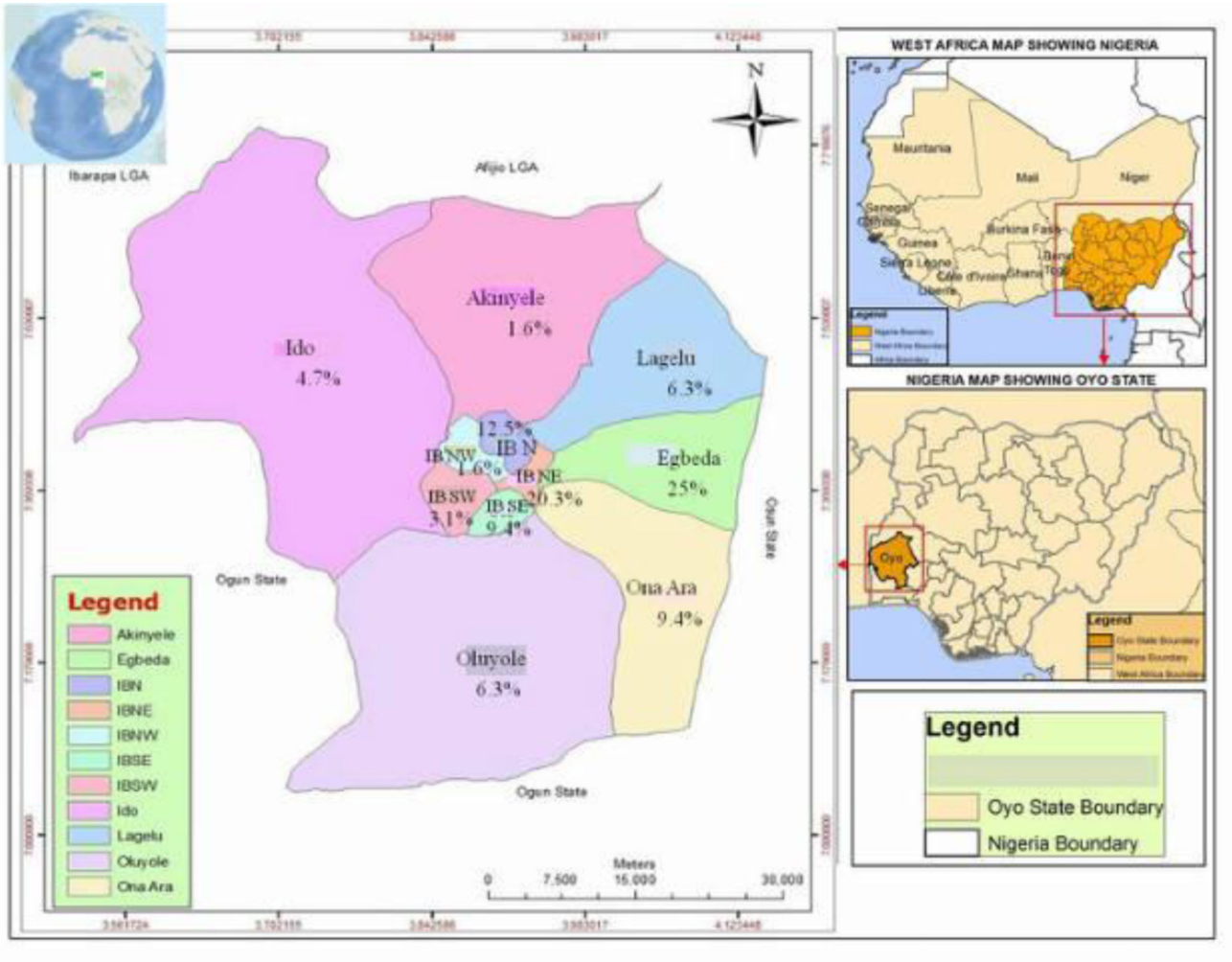

Figure 1.
Dengue prevalence in patients visiting Adeoyo Hospital from 11 LGAs in Ibadan, Nigeria.

Table 1

Age distribution of patients tested for dengue IgM in Adeoyo Hospital Yemetu - Ibadan.

Age	Number tested	Number infected	p value
≤ 1 year	27	2 (3.1%)	
1.1 - 5 years	35	8 (12.5%)	
5.1 - 10 years	13	4 (6.3%)	0.297
10.1 - 18 years	20	6 (9.4%)	
≥ 18.1 years	179	44 (68.8%)	
Total	274	64 (100%)	

Table 2

Monthly distribution of dengue in different age groups of people visiting Adeoyo Hospital Yemetu – Ibadan.

	Age group							
Month	**≤1 year**	**1.1-5 years**	**5.1-10 years**	**10.1-18 years**	**≥18.1 years**	**p value**	**Monthly prevalence**	**p value**
April	3.10%	4.70%	1.60%	4.70%	10.90%		40.00%	
May	0.00%	0.00%	1.60%	0.00%	7.80%		14.60%	
June	0.00%	1.60%	1.60%	0.00%	14.10%		36.60%	
July	Nil*	Nil*	Nil*	Nil*	Nil*	p=0.524	Nil*	p=0.000
August	0.00%	4.70%	0.00%	1.60%	12.50%		40.00%	
September	0.00%	0.00%	0.00%	1.60%	6.30%		11.90%	
October	0.00%	1.60%	0.00%	1.60%	12.50%		30.30%	
November	0.00%	0.00%	0.00%	0.00%	4.70%		12.00%	
December	0.00%	0.00%	1.60%	0.00%	0.00%		3.00%	

*The reason for no report of dengue in July was because nobody was sampled during the period.

Table 3

Distribution of Optical densities of dengue IgM ELISA across age groups

Age	Negative (≤0.30 OD)	Weakly reactive (0.31 - 0.49 OD)	Moderately reactive (0.50 - 0.99 OD)	Highly reactive (>1.0 - 3.55 OD)	p value
≤ 1 year	18 (6.6%)	7 (2.6%)	2 (0.7%)	0 (0.0%)	
1.1 - 5 years	14 (5.1%)	13 (4.7%)	7 (2.6%)	1(0.4%)	
5.1 - 10 years	3 (1.1%)	6 (2.2%)	2 (0.7%)	2 (0.7%)	p=0.126
10.1 - 18 years	8 (2.9%)	6 (2.2%)	5 (1.8%)	1 (0.4%)	
≥ 18.1 years	90 (32.8%)	45 (16.1%)	39 (14.2%)	5 (1.8%)	
Total	**133 (48.5%)**	**77 (28.1%)**	**55 (20.1%)**	**9 (3.3%)**	

Table 4

Monthly distribution of Optical densities of dengue IgM ELISA in study participants

Age	Negative (≤0.30 OD)	Weakly reactive (0.31 - 0.49 OD)	Moderately reactive (0.50 - 0.99 OD)	Highly reactive (>1.0 - 3.55 OD)
April	11 (4.0%)	13 (4.7%)	14 (5.1%)	2 (0.7%)
May	21 (7.7%)	14 (5.1%)	6 (2.2%)	0 (0.0%)
June	10 (3.6%)	8 (2.9%)	10 (3.6%)	2 (0.7%)
July	Nil*	Nil*	Nil*	Nil*
August	13 (4.8%)	6 (2.2%)	7 (2.6%)	4 (1.5%)
September	26 (9.5%)	11 (4.0%)	5 (1.8%)	0 (0.0%)
October	16 (5.8%)	7 (2.6%)	9 (3.3%)	1 (0.36%)
November	13 (4.7%)	9 (3.3%)	3 (1.1%)	0 (0.0%)
December	23 (8.3%)	9 (3.3%)	1 (0.4%)	0 (0.0%)
Total	**133 (48.5%)**	**77 (28.1%)**	**55 (20.1%)**	**9 (3.3%)**

*The reason for no report of dengue in July was because nobody was sampled during the period.

Table 5

Pattern of clinical signs among study participants

Clinical signs	No. of participants presenting with clinical signs	No. of participants exposed to dengue virus	p = value
Head ache	22 (24.7%)	23(35.9%)	0.586
Joint pain	7 (20%)	5 (7.8%)	0.352
Body pain	12 (33.3%)	9(14.1%)	0.129
Back ache	2 (20%)	0 (0.0%)	0.309
Vomiting	16 (31.4%)	0 (0.0%)	0.257
Rash	1 (10%)	0 (0.0%)	0.280
Fatigue	15 (28.3%)	0 (0.0%)	0.891