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High rate of unrecognized dengue virus infection in parts of the rainforest region of Nigeria

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ABSTRACT

Outbreaks and sporadic dengue virus infections continue to occur in Africa. Several reports of dengue among travellers returning from some African countries to Europe and North America have raised concerns about the epidemiological situation in Africa. We investigated recent dengue infections in febrile patients during the rainy season in various urban centres in the rainforest region of Nigeria, West Africa. This cross-sectional study was conducted for 8 months in 2014 with study participants from Adeoyo Hospital Yemetu – Ibadan, Nigeria. Plasma were collected from 274 febrile patients residing in 11 Local Government Areas of Oyo State. IgM antibodies were determined using semi-quantitative sandwich ELISA. Data was analyzed using Chi - Square and Fisher's exact test with SPSS 16.0. An overall prevalence of 23.4% dengue virus infection was found among study participants. Highest monthly prevalence of 40% was in April and August. The monthly distribution pattern of dengue virus infection indicates efficient virus transmission. Routine diagnosis will enhance dengue virus surveillance and improve patient care in West Africa.

1. 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-Drager, 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 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. 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 ($7^0 24' 14''$N, $3^0 54' 22''$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 Govern-

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ment 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 5 min at 3500 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 40 μl 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 and 650 nm. 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 for 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 Turell 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).

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%)	

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. 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 day-time 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. 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., 2000). 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). How-