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Co-circulation of dengue virus 1 and 2 several years after single serotype detection in Cross River State, Nigeria

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Abstract

Dengue is a mosquito-borne viral disease which affects over 390 million people annually around the world. High rates of unrecognized dengue infections have been observed in Nigeria through serological assays. This informed molecular investigation in some areas where previous reports were made. We screened individuals in a rural community in Nigeria for circulating dengue virus serotypes. Using a community based participatory approach, convenient sampling was employed to collect plasma from 17 febrile adults in Abia and Bendeghe wards within Etung Local Government Area of Cross River State, Nigeria. The area is forested with heavy mosquito presence. RNA extraction was carried out with viral RNA mini kit (QIAamp, QIAGEN GmbH, Hilden, Germany). Molecular detection of DENV serotypes was by Polymerase Chain Reaction using characterized DENV isolates as standard controls. Out of 17 participants, 3 (17.6%) tested positive to DENV. Two were infected with DENV-2 and one with DENV-1. All three were men in Abia ward while no person tested positive to the virus in Bendeghe. This present study highlights co-circulation of DENV-1 which demonstrates a change in the epidemiology of dengue in the area. It reinforces the need for public health professionals in the area to be vigilant, and signals the possibility of an increase in Dengue Hemorrhagic Fever. Sustained entomological surveillance is recommended.

Keywords: Dengue, Cross River State, Molecular detection, Nigeria, Rain forest

Introduction

Dengue virus (DENV) belongs to the family *Flaviviridae*, genus Flavivirus and occur as four distinct antigenic serotypes. Infection with any of them leads to a mild, self-limiting febrile illness (dengue fever) (Lanciotti *et al.,* 1992). DENV is widely distributed in tropical and subtropical countries. It is transmitted by day-biting mosquitoes of the genus *Aedes* and the infection often goes unrecognized in many African countries. It is transmitted between humans primarily by *Aedes aegypti* and *Aedes albopictus* (Gubler, 1988). A retrospective serological study suggests that DENV caused an epidemic in Durban, South Africa in 1927 (Kokernot *et al.,* 1956). DENVs have been reported to be circulating in Nigeria

since 1964 (Carey *et al.,* 1971). Recently, high rates of unrecognized dengue infection were reported in the tropical rain forest zone of Nigeria, West Africa (Onoja *et al.,* 2016) with an increase in incidence of Dengue hemorrhagic fever (DHF) (Fagbami & Onoja, 2018). The burden of DENV infection in Africa has not yet been estimated due to lack of sustained surveillance systems, or poor implementation of same (Sang, 2007). Only DENV-2 has been reported in the rain forest region of Cross River State (CRS) Nigeria (Baba *et al.,* 2009). With reports of importation of dengue into Europe and other parts of the world by travelers from West African countries (Franco *et al.,* 2010) including Nigeria, (Raut *et al.,* 2015). It is important to know current circulating DENV serotypes in parts of CRS which is an international tourist destination because of the Obudu cattle ranch and the annual December carnival. We therefore set out to investigate circulating DENV serotypes in rural communities in CRS where arboreal habitats are home to non-human primates.

Materials and Methods

Study design

This is a cross-sectional study carried out in 2016 from two wards namely Abia and Bendeghe Ekiem of Etung Local Government Area (LGA) of Cross River state, Nigeria. Convenient sampling was employed to obtain plasma samples from people. The areas are heavily forested with a lot of mosquitoes. Seventeen participants were recruited following a community based participatory approach, in which a community health practitioner informed member of the community with fever to come and be tested. Nine participants were from Abia and 8 from Bendeghe wards. Approval for this study was obtained from Institutional Review Board of University of Ibadan (UIEC/13/412) and Ethics Board of Cross River State (CRS/MH/HREC/017/VOL. VI/075). Demographic details such as age, gender, hospital visitation prior to sample collection, whether or not any medication was used prior to sample collection were obtained. Age distribution of participants was from 13 – 80 years. Inclusion criteria was febrile people living in Abia and Bendeghe wards. Individuals with underlying health conditions such as obesity and stroke were excluded from the study. Informed consent was sought, after which 3ml of blood was collected by venipuncture. Etung is located in the central part of CRS, in the tropical rainforest region with an annual rainfall ranging between 2,500 and 2,750 millimeters. There are two distinct seasons which are rainy or wet season (May to October) and dry season (November - April). Temperature ranges between 25^{o}C – 27^{o}C in January, and 31^{o}C in July. Humidity in January is about 75-95% and is relatively dryer towards the end of the year (Asuquo, 1987).

Molecular detection

RNA was extracted from 140µl plasma using viral RNA mini kit (QIAamp, QIAGEN GmbH, Hilden, Germany). All four DENV serotypes were tested by polymerase chain reaction (PCR). Positive controls used were inactivated tissue culture isolates of DENV-1 Hawaii strain, DENV-2 New Guinea C strain, DENV-3 H87 Philippines strain, DENV-4 H241 Philippines strain. PCR was carried out using SuperScript ® III One-Step RT-PCR Platinum® Taq Hifi containing 2× Invitrogen PCR mix, super script reverse transcriptase enzyme/Platinum Taq mix, 5mM MgSO4 in a reaction tube on Applied Biosystems GeneAmp 9700 thermalcycler. This assay was carried out in the molecular laboratory of the Department of Virology, College of Medicine, University of Ibadan Nigeria with protocol described by Maneekan *et al.* (2009). Primers used for detection of non-structural 5 (NS5) region are D1F: CAGGAAGAAGAAGCGTCTCAGG and D1R: AGGTTGTCCAAGGCATTCTGG (109bp), D2F: CAATGAGATGGTGTTGCTGCAG and D2R: GTCTCTTTCTGTATCCAATTTGACCC (139bp), D3F: CCAGGCATT TTCCAGACAACAAC and DV3R: GCGCTATTCCACTGACATAGCC (169bp), D4F: GACT GAAGCCAAGTCTGCCC and D4R: ACGTTCTTGAGTGTCGCCATG (199bp). Cycling condition was 50^{o}C 15mins, 95^{o}C 5mins, then 45 cycles of 95^{o}C 30s, 53^{o}C 30s, 72^{o}C 1min and final extension of 72^{o}C 7mins. Amplified product was resolved on 1.5% gel and visualized using Biorad® image reader. Size of amplified product for DENV-1 was 109bp and 139bp for DENV-2, respectively.

Results and Discussion

There were 6 males and 11 females. Nine were from Abia and 8 from Bendeghe Ekiem in Etung LGA of CRS. Out of 17 people, all three positive individuals were from Abia. Ages of those infected with dengue are 52, 32 and 13 years, respectively. Their occupations were farming, mechanic and student, respectively. Cross River State is a tourist hub in Nigeria, because of the December carnival which attracts people from several continents who come to watch the rich cultural display. This present study (Figure. 1) supports earlier report of circulating DENV-2 in CRS (Baba *et al.,* 2009). Several reasons are responsible for this, first, CRS is located in the coastal part of Nigeria with waterbodies that serve as breeding sites for vectors. Secondly, the dense forests and high relative humidity provides favourable climate for

breeding of Aedes *aegypti* and *Aedes albopictus*. Although the population studied is not large, two of the infected patients are adults which shows that occupational exposure during farming may have brought one of them in close contact with other people hence in this case, rural habitation is a factor that aids rural epidemics (Gubler, 2012). Autochthonous DENV may be maintained through vertical transmission and sustained in Abia. The mechanic was infected through mosquito bite and this will reduce his productivity due to malaise and other non-specific symptoms that will occur, it might become severe and lead to haemorrhagic manifestation. With 11.6% dengue infection found in this present study (Figure 2) which is higher than the earlier report of 0.1% in CRS (Baba *et al.,* 2009), it therefore follows that the rate of transmission on the whole will be higher in a typical rain forest vegetation zone hence the need for international travellers and tourists to be vaccinated, now that an effective dengue vaccine (denguevaxia) is available (Halstead, 2016). For many years, it was thought that only DENV-2 existed in CRS. This present study shows that DENV-1 also exists (Figure 1). It may have been present, but undetected all along because the first report of DENV-1 in Africa was made in Ibadan, south west Nigeria in 1964 (Carey *et al.,* 1971). If the theory of antibody-dependent immune enhancement (ADE) in which secondary, tertiary or quaternary DENV infection activates the complement cascade to cause increased permeability of blood vasculature (Halstead *et al.,* 1980) is to be considered, it implies that co-circulation of two DENV serotypes within close geographical range predispose residents to DHF. In current models of dengue immunopathogenesis, increase in virus load caused by ADE combined with strong anamnestic cross-reactive T cell responses are believed to result in cytokine storm that cause capillary leakage and symptoms of DHF/DSS (Mongkolsapaya *et al.,* 2003; Pang *et al.,* 2007; Rothman, 2011). If DENV-2 and -1 hitherto co-existed, one may wonder why incidence of DHF has not been reported in epidemic proportion especially in children (White & Fenner, 1994). It has been widely reported that several flaviviruses circulate in Africa. These flaviviruses contain common antigens which elicit cross-protective antibodies that are cross-protective and this may be the situation here (Rathore and St. John, 2020). Etung LGA was a hotbed for YFV during the epidemic in the 1980s in Nigeria. Dengue is a travel disease for those infected within the country, who are going to other countries or travelling back to their homeland. It is therefore important for health checks to be carried out on people travelling from Nigeria because of reports of importation of DENV into India (Raut *et al.,* 2015). The presence of DENV in a teenager in this present study underscores the vulnerability of young persons who have little or no protection, hence the need for effective vector control. DHF has been observed to be high among paediatric population in Nigeria (Fagbami & Onoja, 2018) as such, attention should be focused on this vulnerable group for better case management. This is because they are immunologically naïve hence the severe and sometimes fatal outcome of the disease among them.

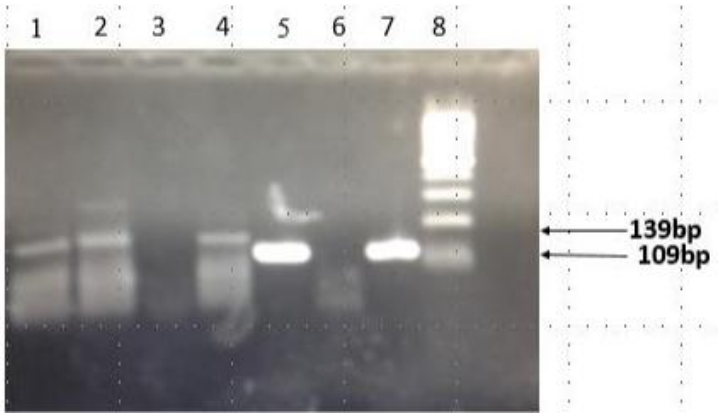

Figure 1. Human samples testing positive to DENV-1 and -2 by PCR in Etung LGA, Cross River State

Figure 1. Lane 1: Sample 5, lane 2: sample 10, lane 3: Negative Control, lane 4: DENV 2 New Guinea C strain as positive control, lane 5: sample 14, lane 6: Negative Control, lane 7: DENV 1 Hawaii strain as positive control, lane 8: 100bp DNA Ladder

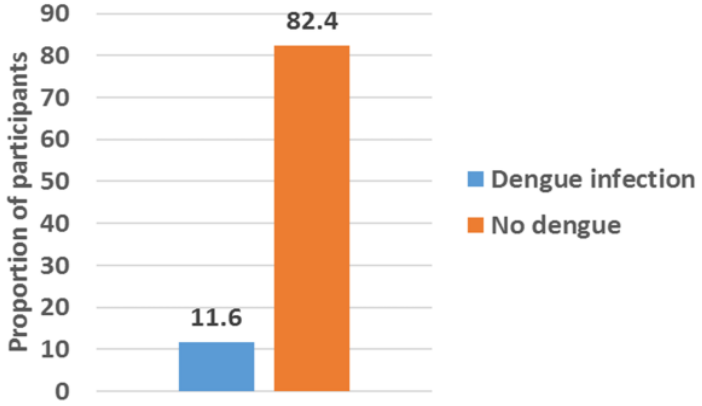

Figure 2: Proportion of participants screened for Dengue virus in Etung, Cross River State

In conclusion, we have reported the circulation of two DENV serotypes in Abia ward of Etung LGA in CRS.

There is the possibility of increased DHF. Young people are among the people affected. Public enlightenment on the increasing danger of dengue should be continuously made in all parts of the rain forest region of West Africa and other parts of the tropics. There is need for sustained vector control, construction of proper drainage and destruction of larvae. Effective and coordinated DENV surveillance is recommended. Further studies are required to determine the ancestry of DENVs and whether they are of sylvatic origin.

Conflicts of Interest

The authors declare no conflict of interest.

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