Erratum

Original Article

Seroprevalence and Associated Risk Factors of Dengue Virus Among Apparently Healthy Individuals in Karu Local Government Area, Nasarawa State, Nigeria

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Abstract

Introduction: Dengue virus (DENV) infection is a vector-borne disease with a diverse range of symptoms that mimic malaria and other febrile illnesses common in many African countries like Nigeria. **Aim:** Our study aimed to determine the seroprevalence and associated risk factors of DENV antibodies among apparently healthy individuals. **Materials and Methods**: From December 2015 to March 2016, a cross-sectional study was conducted among apparently healthy individuals in Karu Local Government Area, Nasarawa State, Nigeria, using a multistage sampling technique. A structured questionnaire was pretested and administered to the study participants, and blood samples were collected and tested for DENV virus antibody using the enzyme-linked immunosorbent assay technique. **Results**: Blood samples were collected from 354 participants, out of which 47.5% were women aged between 5 and 80 years (median: 23 years). Anti-dengue immunoglobulin G antibodies were found in 17 (4.8%) of the study participants, 7 (41.2%) of whom also tested positive for anti-dengue immunoglobulin M antibodies. Adults over 45 years of age (7; 41.2%) and farmers (8; 47.1%) had the highest seropositivity to dengue antibodies. People living in cleaner environments were 70% less likely to have dengue infection (odds ratio [OR]: 0.32, 95% confidence interval [CI]: 0.12–0.86, P = 0.03), and regular cleaning of gutters was found to be protective (OR: 0.03, 95% CI: 0.01–0.10, P < 0.001). **Conclusion**: Dengue infection is present among the study population and could be a cause of fever among them. Being 45 years and above, farming and lacking formal education were identified as factors associated with risk of DENV infection, while clean environment was protective. We recommend improved environmental sanitation and testing for dengue among febrile patients in health facilities.

Keywords: Asymptomatic infection, dengue fever, dengue immunoglobulin G, dengue immunoglobulin M, dengue infection, dengue virus, seroprevalence

INTRODUCTION

Dengue fever (DF) is an acute systemic viral infection caused by four serotypes of dengue virus (DENV).[1] It is an arthropod-borne disease transmitted through the bite of infected mosquito vectors *Aedes aegypti* and *Aedes albopictus,* which are found mainly in tropical and subtropical regions of the world.[2,3] Global transmission of DF has increased in recent years, primarily in urban and semi-urban areas, thus increasing public health concern.[4] DF is considered endemic in over 100 countries in many regions of the world including Asia, Latin America, the Pacific, and Africa. [4] With the re-emergence of the disease since the 1980s, the United States and other tropical regions have experienced major epidemics caused by DENV acquired locally or imported by travelers from other endemic regions.[5,6] In Africa, poor surveillance and limited access to testing have led to underdiagnosis of the disease, especially where differential diagnostic testing is not routinely done for patients with febrile illnesses who test negative for malaria.[7] The clinical outcomes of DENV infection vary from asymptomatic infection, through mild febrile DF, to severe and life-threatening dengue hemorrhagic fever and dengue shock syndrome. [7,8]

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DF symptoms may mimic other febrile illnesses such as malaria, typhoid, measles, and influenza,[9] all of which are endemic diseases in Nigeria, making the clinical diagnosis challenging. Due to similarities in clinical presentation, DF is often misdiagnosed as malaria, particularly in the early stages of illness, and several studies have indicated a high prevalence of dengue–malaria co-infection in some endemic settings.[10,11] Often, especially in resource-constrained settings, diagnostic capacity ranging from less expensive methods such as enzyme-linked immunosorbent assay (ELISA) to capital-intensive techniques such as polymerase chain reaction and cell culture are not readily available. As such, health-care providers, in a quest to save lives, resort to administering empirical treatment in the absence of definitive diagnosis when malaria and other available tests are negative. Most times, febrile cases are misclassified as malaria and treated without diagnostic confirmation. [12,13]

The four closely related but antigenically distinct DENV serotypes do not cross-protect but rather cross-react during testing. Therefore, infection with one serotype provides lifelong immunity to the infecting serotype only. Subsequent infection with a different serotype increases the likelihood of the patient developing more severe disease manifestations, such as dengue hemorrhagic fever.[11] Symptoms of infection usually occur 4–7 days after the mosquito bite and typically last 3–10 days.[7] Once a mosquito bites an infected human, the mosquito remains infected for the rest of its life.[3,14] Lack of efficient vector control and limited public health measures allow frequent contact between mosquitos and humans [15]; this is the case in our study area,[16] with limited infrastructural facilities and poor environmental management. Nevertheless, the WHO has provided a strategic approach with guidelines to prevent and control human–vector interactions in poor socioeconomic environments.[14] However, these are often not implemented by the local government authorities probably due to lack of funding and may often require a One Health approach involving the ministries of environment and health. Despite the increasing awareness about viral infections, health institutions in Nigeria lack diagnostic facilities for the detection of a variety of viruses, including arboviruses. Nigeria currently has a population of over 200 million,[17] and its prevailing factors such as increased urbanization, incursion of human activity into new ecosystems, climatic changes, and collapse of vector control and public health programs all favour the emergence of arbovirus infections. [15,18] There are several methods that can be used for the diagnosis of DENV infection which may depend on the time of patient presentation and the aim. This study goal was to determine the prevalence of the disease in the study area, and MAC ELISA IgG/IgM testing was conducted to measure the immune response to DENV both current and past infections. The main objective is to determine the seroprevalence and associated risk factors of DENV infection among healthy individuals in a discrete region of North Central Nigeria with a history of a DF outbreak in 2014. [19]

MATERIALS AND METHODS

Ethical Considerations

Ethical approval to conduct this research was obtained from the Nasarawa State Ministry of Health Ethics Committee, Lafia, Nasarawa State, Nigeria (reference number: S/MOH/843/ VOL1/XX). Before questionnaire administration and sample collection, adult participants, as well as parents or guardians of children participants, provided written informed consent. Data protection/confidentiality was assured to the study participants individually and during advocacy visits to the local government area (LGA) secretariat and community leaders.

Study Area and Study Design

A descriptive cross-sectional study was conducted between December 2015 and March 2016 on asymptomatic residents in rural and semi-urban settings in Karu LGA of Nasarawa State, which borders the Federal Capital Territory of Nigeria. Nasarawa State is made up of 13 LGAs, namely, Akwanga, Awe, Doma, Karu, Keana, Kokona, Lafia, Nasarawa, Nasarawa Eggon, Obi, Toto, Wamba, and Keffi [20] [Figure 1]. Karu was selected as the study area based on the incidence of a dengue hemorrhagic fever outbreak that occurred in a highly populated area of the state in March 2014.[20] Karu is located between latitudes 7° and 9° North and longitudes 7° and 10° East, covers a landmass of 27,117 km^2, and has 11 wards – Asokodape, Bagagi, Gitata, Gurku Kabusu, Karshi I, Karshi II, Karu, Keffin Shanu, Panda Kare, Tattare, and Uke. The state's primary industry is agriculture, with the production of a variety of cash crops throughout the year.[20]

Selection Criteria

Apparently healthy (asymptomatic) male and female individuals between ages 5 and 70 years who were resident in Karu LGA for at least 6 months prior to this study were included, while individuals with a current febrile illness were excluded. A sample size of 354 was estimated based on a prevalence of 0.36% in a previous study and a level of precision of 5%.[11] The questionnaire was pretested at Karshi II, which is one of the 11 wards in Karu LGA. Samples were collected from the remaining 10 wards. A list of the 39 settlements in the 10 wards was compiled and 14 were selected by simple random sampling; each settlement had approximately the same population size. Afterward, simple random sampling was used to select 25 participants from 10 of the settlements and 26 participants from 4 of the settlements.

A structured questionnaire with seven sections was used to collect data from participants. Collected data comprised sociodemographic data, personal knowledge of DENV infection, personal and household environmental factors, hygiene, and sanitation. Two

trained interviewers and two phlebotomists, who were also community health extension workers, administered the pretested and validated questionnaires and collected blood specimens from study participants who consented. Five milliliters of whole blood was collected aseptically and transferred into plain bottles. Collected blood samples were transported at 2°C–8°C using ice packs to the laboratory where they were immediately centrifuged using high-speed centrifuge TT-24B (Techmel and Techmel, United States) at 8000 rpm for 1 min. Separated sera were transferred into freshly labelled cryovials and stored at −20°C until all required samples were obtained.

Antibody Detection

Seroprevalence was determined using the ELISA IgG/IgM AccuDiag™ kit (Diagnostic Automation/Cortez Diagnostics, Inc., United States; Catalog Number: 8116-35; Lot Number: DA1290, DA1289, DA1289, DA1290) for primary testing, and determination of acute and convalescent dengue viral infection was used to detect anti-dengue immunoglobulin G (DENV IgG) antibodies. Samples reactive for IgG were further tested for anti-DENV IgM using the IgM kit of the AccuDiag™ ELISA to determine recent infection.[21] Antibody assays were read on the ELISA Microplate Reader – Thermo Scientific™ Multiskan FC Microplate Reader (Type: 357, Ref: 51119100, SN: 356-9066327, Thermo Fisher Scientific, Pudong, Shanghai 201206, China), which was set for readings on wavelengths of 450/620–650 nm using the automated washing option.

Data Management and Analysis

All data collected were cleaned and checked to facilitate data comparison and ensure accuracy. Descriptive statistics as well as univariate and bivariate analysis were performed using Epi Info™ version 7.0 statistical software for epidemiology developed by the United States of America Centers for disease control, Atlanta, Georgia, United States; https://www.cdc.gov/epiinfo/support/downloads.html) and Microsoft Excel (Version 2010) to compare proportions within and among groups (variables) for statistical significance ($P < 0.05$; 95% confidence interval [CI]).

RESULTS

Of the 354 participants recruited for the study, 168 (47.5%) were female and 186 (52.5%) were male [Table 1]. Participants' ages ranged from 5 to 70 years, and the median age was 23 years. The majority (250; 70.6%) of the participants were between 5 and 29 years old. More than half (57.1%) of the participants were

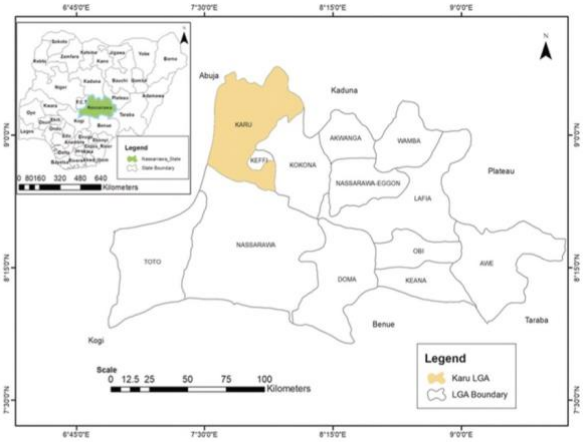

Figure 1: Map of Nigeria highligh/ng Nasarawa State Nigeria in yellow Study instruments

Table 1: Seroprevalence of dengue an/body by sociodemographic factors among apparently healthy par/cipants in Karu Local Government Area, Nasarawa State, Nigeria, December 2015 to March 2016

Socio-demographic characteristics	Number of participants (%)	IgG positive (%)
Age-group		
5-14	138 (39)	3 (2.2)
15-29	112 (31.6)	5 (4.5)
30-44	64 (18.1)	2 (3.1)
>45	40 (11.3)	7 (17.5)
Sex		
Male	186 (52.5)	9 (4.8)
Female	168 (47.5)	8 (4.8)
Occupation		
Business	80 (22.6)	3 (3.8)
Civil servant	18 (5.1)	2 (11.1)
Farmer	43 (12.1)	8 (18.6)
Private sector	11 (3.1)	1 (9)
Unemployed/Student	202 (57.1)	3 (1.5)
Level of education		
Primary	130 (36.7)	3 (2.3)
Secondary	123 (34.7)	5 (4.1)
Tertiary	33 (9.3)	2 (6.1)
Qur'anic/informal education	49 (13.8)	2 (4.1)
Adult education	19 (5.4)	5 (26.3)

either unemployed or students, and 130 participants (36.7%) had a primary school level of education. Anti-DENV IgG antibodies were detected in 17 (4.8%) samples. Seven (41.2%) of the 17 anti-DENV IgG-positive samples were also positive for anti-DENV IgM antibodies, indicative of recent infection. Seropositivity was higher among participants aged above 45 years (17.5%) and peasant farmers (18.6%) but lower among businessmen (3.8%). More participants (26.3%) with adult education were seropositive compared to those with tertiary education (6.1%). The absence of refuse and waste dumpsites around the house (odds ratio [OR]: 0.32; 95% CI: 0.12–0.86) and keeping gutters clean (OR: 0.03; 95% CI: 0.01–0.10) was found to reduce the odds of being seropositive for dengue infection [Table 2].

DISCUSSION

This study found an anti-DENV IgG seroprevalence of 4.8% among study participants, indicating previous infection. A study conducted in the Northeastern part of

Table 2: Risk factors for dengue seroposi/vity among apparently healthy par/cipants in Karu LGA, Nasarawa State, Nigeria, December 2015 to March 2016.

Risk factors	Number of responses N=337 †	IgG (%) positive	Odds ratio	(95%CI)	*p*-value
Engaged in farming activities					
Yes	126	9 (7.1)	1.88	(0.70–5.0)	0.30
No	211	8 (3.8)			
Absence of refuse and waste dumpsites around the house					
Yes	231	7 (3.0)	**0.32**	**(0.12–0.86)**	0.03
No	106	10 (9.4)			
Sleeping outside the house					
Yes	171	8 (4.7)	0.86	(0.32–2.29)	0.96
No	166	9(5.4)			
Presence of street gutters and drainages					
Yes	117	2 (1.7)	0.25	(0.05–1.11)	0.09
No	220	15(6.8)			
Storage of water in open containers					
Yes	82	2 (2.4)	0.41	(0.09-1.85)	
No	255	15 (5.8)			0.370
Presence of window nets					
Yes	141	3 (2.1)	0.30	(0.08–1.05)	0.08
No	196	14 (7.1)			
Use of mosquito nets at night					
Yes	125	10 (8.0)	2.42	(0.89–6.52)	
No	212	7 (3.3)			0.12
Presence of vegetation around the house					
Yes	56	0 (0)	0	(0.0–0.0)	0.0
No	281	17 (6.0)			
Spraying the environment with an insecticide daily					
Yes	61	0 (0)	0.0	(0.00–0-0)	0.0
No	276	17 (6.2)			
Frequency of cleaning surrounding gutters					
Biweekly/Daily	100	6 (6.0)	**0.09**	**(0.03–0.26)**	0.001
Never	16	11 (68.8)			

Note: † Total number of responses analyzed were 337 due to incomplete response from 17 participants. OR: Odds ratio CI: Confidence interval, IgG: Immunoglobulin G

Nigeria (Maiduguri State) in 2009 found a seroprevalence of 0.6% among febrile patients,[9] which is much lower than what we found in our study, while a more recent study conducted among febrile patients showed a higher seroprevalence of 28.9% in Plateau State, North Central Nigeria,[22] suggesting that dengue infection is on the increase while some of the febrile illnesses encountered in Nigeria may be DF. Our study was done among apparently healthy individuals, thus the presence of anti-DENV antibodies indicates previous exposure to DENV.[3,9] Of the 17 participants with IgG-positive samples, 7 (41.1%) were IgM positive, indicating recent past infection. This is similar to a previous finding where 27.9% of a sample population were positive for anti-DENV antibodies, and 11.0% of those were also positive for anti-DENV IgM.[22]

This is further corroborated by a global study which reported that 22 countries in Africa including Nigeria have outbreaks and sporadic cases, with a further 12 countries reporting cases in travelers.[9,23] Our findings are also similar to those of a study conducted in the neighboring Plateau State which found a 4.1% and 8.9% positivity rate for anti-DENV IgG and IgM, respectively.[24] Our study found a 2.2% positivity rate among asymptomatic children (aged between 5 and 14 years), consonant with the findings of a study that reported 5.6% of children with dengue infection, 86% of whom were asymptomatic or reported few symptoms, and a more recent study that reported 77.1% seroprevalence among children.[25,26] DENV seems to be circulating among the study population but is being under-reported, underdiagnosed, or not reported at all. The disease prevalence is higher in this setting compared to that found in other similar studies conducted between 2009 and 2014 in the country and within the continent. [9,11,26-31]

The exposure to DENV infection was found to be 4.7% and signifies the presence of the disease in the study area. The prevalence in this study is higher than previous in Nigeria although this was conducted among apparently healthy individuals) unlike two previous studies conducted among febrile (symptomatic) patients with suspected malaria and typhoid across the four geopolitical zones of Nigeria.[9] Earlier studies conducted in Maiduguri reported far much lower seroprevalence rates.[28] Findings from these studies indicate that DF may be an emerging and/or re-emerging infection and may be existing in different parts of the country world with potential to spread to other places and through international travel from endemic to nonendemic regions.[4,32] Our study set out to investigate the prevalence of DENV infection in Karu LGA of Nasarawa State. The decision was conceived after an incidence of DENV infection in the same LGA in 2014 which was later diagnosed to be dengue hemorrhagic fever Serotype 4.[19] Our study was a follow-up investigation to search and investigate the prevalence of this disease in this particular community. Our survey found the seroprevalence of antibodies to be higher in the older age group, 45 years and above (17.5%). This was in contrast to findings of a study conducted in Asia which found a higher prevalence in the much younger age group.[33] Idris *et al*. on the other hand documented a high seroprevalence of dengue antibodies among 30–39-year-olds in Maiduguri.[34] The higher seroprevalence in the older age group observed in our study could be associated with the fact that older people are engaged in farming activities and other businesses and tend to stay outside the house for longer periods during the day, and may thus be more exposed to the Aedes mosquito vector, which is a daytime-biting mosquito. [35,36] This was similar to the findings of a study conducted in Nigeria by Baba and Talle, [11] which found an increase in vector-borne infections with age.

The seroprevalence of anti-DENV IgG was found to be the same in both male and female participants in this study. However, another study by Sajid et al. reported that men were 1.2 times more likely to be exposed to DENV. [37] Meanwhile, other studies both in Asia (India) and Nigeria found the seroprevalence to be higher among women.[18,36,38] A study conducted in Ibadan, Nigeria, reported a similar finding to those of our study, with an equal female-to-male ratio in terms of disease seroprevalence [9] Depending on the geographic location and sociocultural engagements and activities, either men or women may engage more in outdoor activities such as farming and other related outdoor activities.

The presence of the developmental stage of the female mosquito in water habitats, mostly in artificial containers closely associated with human

dwellings around houses, makes it easier for human–vector interactions. Those who do not regularly clean gutters may be more at risk of mosquito bites and infection.[3 9] Nevertheless, people rather than mosquitos can also move the virus within and between communities. Like the findings of a study in Nigeria,[36] the seroprevalence of anti-DENV antibodies was higher (18.6%) among farmers in this study. Farmers have more frequent contact with the vector as they spend most of their days outside, especially during the farming/raining season.[3] Business professionals had a lower seroprevalence, further emphasizing the role of contact and increased occupational risk for farmers.

Our study indicated that participants with better sanitary standards such as regularly cleaning the gutters around their homes were 70% less likely to have been exposed to DENV. Other risk factors assessed included sleeping outside the house, storage of water in open containers, and the presence of gutters around the house, all of which were found to have no significant relationship with seroprevalence among study participants; other studies have, however, found an association between DENV seroprevalence and water storage in open containers [38] The use of mosquito nets during the night was not found to be protective, and this may be because dengue mosquitos are day-biting mosquitos.

Limitations

Flaviviruses (e.g., DENV, yellow fever virus, Chikungunya, and other flaviviruses) are prone to IgG antibody cross-reactivity, but this was not considered in the interpretation of the antibody assays in this study. The lack of a vector component (trapping and identification of Aedes mosquito vectors in the study area) in this study was also a limitation. Another limitation of this study was that the current infection was not determined. On the other hand, the strengths of this study are the community-based setting, robust sampling technique, detailed questionnaire, and complete participation in blood sampling.

CONCLUSION

This study found a high dengue seroprevalence among asymptomatic individuals in Karu LGA of Nasarawa State. The presence of refuse dumpsites around the house and lack of regular cleaning of surrounding gutters were found to be factors associated with dengue seroprevalence in this study. We recommend more investigative research to determine the actual burden of DENV infections in Nigeria, especially in areas where sporadic cases have been reported. Incorporation of routine diagnostics for DF during febrile illnesses in the country would help determine the disease prevalence. Proper waste disposal and environmental sanitation would aid in disease control.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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