

Seroprevalence of Neutralizing Antibodies to West Nile Virus (WNV) among Patients with Pyrexia of an Unknown Origin (PUO) In Maiduguri, Borno State, Nigeria

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ABSTRACT

The seroprevalence of West Nile Virus (WNV) infection was determined in 200 patients with pyrexia of an unknown origin using the virus micro-neutralization test (micro-VNT). The patients consisted of 104 (52%) males and 96 (48%) females attending clinics at the University of Maiduguri Teaching Hospital (UMTH) Maiduguri, Borno State. The patients are in the age range of 3 years to 70 years, with mean (\pm SD) age of 25.4 \pm 13.4 years. Of the 200 patients tested, 19 had neutralizing antibodies to WNV, giving an overall seroprevalence of 9.5% in the study area. The age distribution of positive sera were as follows: In the age group of 31-35 years, (25.0%), followed by 21-25 years, (13.9%), 6-10 and >50 years, with 11.8% and 11.1% respectively. However, no detectable neutralizing antibodies to the WNV were found in the age groups of 11-15 years and 36-50 years. The seroprevalence rates according to the different age groups did not differ significantly ($p=0.318$). Thirteen (12.5%) of the 19 positive sera were from males while 6 (6.3%) were from females, however, the difference in prevalence was not statistically significant ($p=0.153$) according to gender of the study population.

Keywords: West Nile Virus, Neutralizing Antibodies, Humans, Maiduguri, Nigeria;

INTRODUCTION

West Nile virus is an arthropod-borne flavivirus, first isolated in Uganda in 1937 (1). It affects a wide range of vertebrates, including birds and mammals (2). The natural cycle of infection involves birds and mosquitoes (mainly *Culex spp.*) (3). Although most WNV infection in humans remain subclinical, febrile illness develops in \approx 20% of infected persons and neuroinvasive disease in <1% (3, 4). The infection has been found in northern Africa, Israel, India and Australia, and has progressively spread to the Americas since 1999 (5 - 7). In the 1990s, several epidemics of neuroinvasive disease were reported in northern Africa, Eastern Europe and Russia. WNV strains are classified into at least 7 putative genetic lineages (8). Lineage1 are widespread in Africa, Europe, Asia, Australasia

and America (3). Lineage2 strains distributed mainly in sub-Saharan Africa and Madagascar (3). Lineage7 are also found in Africa, while other lineages occur in other areas of the world. In Nigeria, Olaleye *et al.* (9) detected anti-WNV antibodies in 71% of 62 adult male horses from two stables in Lagos in southwestern Nigeria. In 1990, a high (40%) prevalence of anti-WNV antibodies was detected among humans in Ibadan, southwestern, Nigeria (10). Also in southwestern Nigeria, Sule and co-workers (11) observed a high seroprevalence of anti-WNV antibodies (90.3%) among horses in 2015. Previous study conducted about ten years ago in Maiduguri, Borno state, Nigeria (a semi-arid area) reported a high prevalence of WNV IgG of 80.2 % among PUOs that also correlated significantly with seasonal pattern (12). In the recent past, Baba *et al.* (13) has reported a high (13.2%)

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serological activity of WNV among some animals, donkeys (8.6%), horses (11.5%) and Camels (17.7%) in the same study area. More, recent reports of studies conducted among humans indicated serological and virological evidence of other arthropod-borne viruses (arboviruses) such as Rift Valley fever (14) and Crimean Congo Haemorrhagic fever viruses (15) that was found to actively circulate in the

same study area, where their vectors (mosquitoes and ticks), animal and human reservoir hosts similar to WNV are known to abound. This study is aimed at determining the serological evidence of WNV activity in Borno state by assessing neutralizing antibodies to the virus in the different age and sex of patients presenting with febrile illnesses at one of the major tertiary health facilities in the northeast corner of Nigeria.

Table1. Age and Gender Distribution of WNV Neutralizing Antibody among the Study Population

Variables	Total No. tested	No. positive	Seroprevalence rate
Age group (Years)			
0-5	12	1	8.3
6-10	17	2	11.8
11-15	12	0	0.0
16-20	36	2	5.6
21-25	36	5	13.9
26-30	29	3	10.3
31-35	20	5	25.0
36-40	16	0	0.0
41-45	9	0	0.0
46-50	4	0	0.0
>50	9	1	11.1
Total	200	19	9.5
Gender			
Male	104	13	12.5
Female	96	6	6.3
Total	200	19	9.5

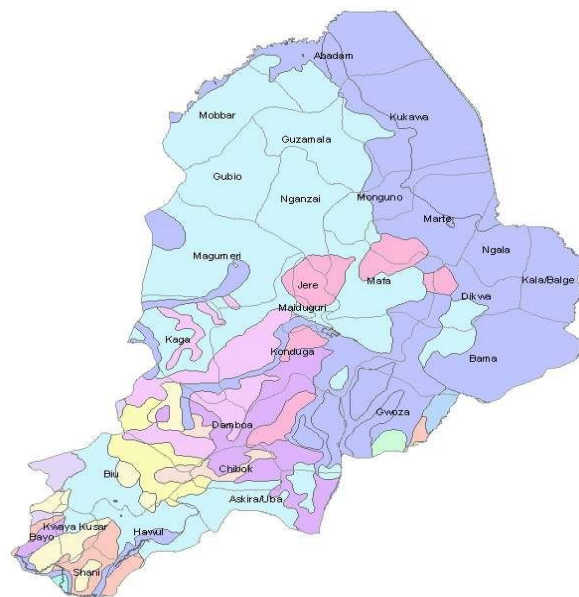


Figure1. Map of Borno State, Nigeria showing the 27 Local Government Areas.

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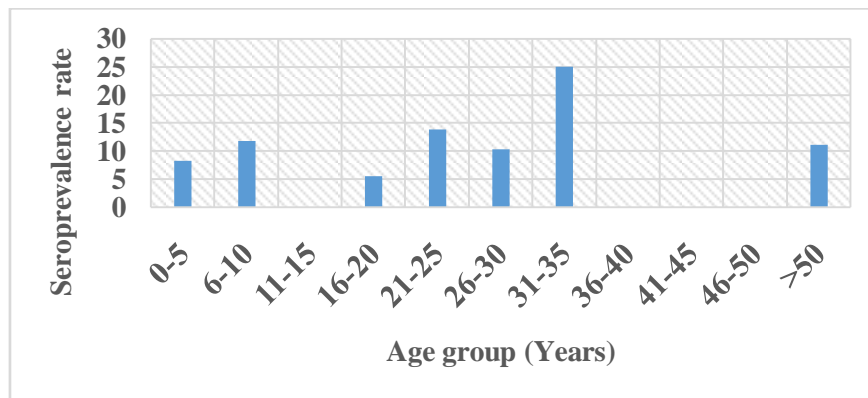


Figure2. Seroprevalence of WNV Neutralizing Antibody According to Age

MATERIALS AND METHODS

Study Area

This study was conducted in Maiduguri, also called Yerwa by its locals, is the capital of Borno State (Figure 1). It is located 11.85 latitude and 13.16 longitudes and it is situated at elevation 325 meters above sea level. Maiduguri has a population of 1,112,449 making it the biggest city in Borno. The city sits along the seasonal Ngadda River which disappears into the Firki swamps in the areas around Lake Chad. Borno State has an estimated area of 70,898 km² and a population of 2,596,589 (16). Borno State is located in the extreme Northeastern corner of Nigeria between latitude 10° 14' N and longitude 10° 11.8' E, and shares international boundaries with countries of Cameroon, Chad and Niger, and national borders with Nigerian states of Adamawa, Gombe and Yobe States. Majority of the inhabitants of Borno State are farmers, animal herders or fishermen. The state enjoys two distinct climatic conditions of a dry (November to May) and a rainy (June to October) seasons.

Study Population

A total of 200 consenting PUOs as assessed by the attending Physician were randomly selected. The patients were attendees of various clinics at the University of Maiduguri Teaching Hospital, the largest tertiary health facility in the northeast geopolitical zone. The patients were made up of 52% (104) males and 48% (96) females within the age range of 3 years to 70 years, with mean (\pm SD) age of 25.4 \pm 13.4 years. All patients (male and female) presenting to the clinic with high fever \geq 38°C, and gave informed consent to participate in the study were included in the study. Consent was obtained from the parents or guardians for those children under 12 years of age.

Sample Size Determination

The minimum sample size for the study was calculated using the formula developed by Cochran in 1963 (17). The prevalence of WNV of 25% was used based on a serosurvey study results by Baba *et al.* in 2013 (18). This was the most recent serosurvey for WNV infection among humans in Maiduguri.

Sample Collection and Processing

Following informed consent, between 3ml to 5ml venous blood was obtained from each participant. The blood was allowed to clot at room temperature and centrifuged at 2,500 rpm for 10mins, serum aspirated into nunc microvial tubes and stored frozen at -20°C until tested.

West Nile Virus

The WNV virus strain (Uganda/M12284) and positive control serum from ATCC used in a previous study (13) was adapted and used in this study.

Serology

All sera were inactivated by heating at 56°C for 30mins to remove inhibitors before testing. The presence of antibodies to WNV was determined in the sera of the study group using the standard serum virus micro-neutralization test (micro-VNT) as previously described (13, 19).

Statistical analysis

The data generated from the study was entered into Microsoft Excel 2016 spreadsheet. As appropriate, chi-square test or Fisher's exact test was used to compare the WNV antibody prevalence rates of all categorical variables, using GraphPad Prism version 5.01 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. The level of statistical significance was considered at a P-value \leq 0.05.

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RESULTS

Of the 200 patients tested, 19 had neutralizing antibodies to WNV, giving an overall seroprevalence of 9.5% in the study area. The seroprevalence of WNV neutralizing antibody according to age and gender of the study patients is as shown in Table 1 and Figure 2. The age distribution of positive sera from the highest prevalence are as follows: In the age group of 31-35 years, 5 (25.0%), followed by 21-25 years, 5 (13.9%), 6-10 and >50 years, with 2 (11.8%) and 1 (11.1%) respectively. This was followed by the age group of 26-30 years with 3 (10.3%), age group of 0-5 years and 16-20 years with 18.3(%) and 25.6 (%) each. However, no detectable neutralizing antibodies to the WNV were found in the age groups of 11-15 years and 36-50 years. Despite this, the variations in the seroprevalence rates for WNV antibodies among the different age groups did not differ significantly ($X^2=11.52$, $df=10$, $p=0.318$). Thirteen (12.5%) of the positive sera were from males while 6 (6.3%) were from females. The difference in the gender distribution of positive sera was found not to be statistically significant ($p=0.153$, Fisher's exact test).

DISCUSSION

An overall seroprevalence of 9.5% neutralizing antibodies to WNV is lower compared with 25% reported from a recent study (18) in the same area contrariwise for an area with high flavivirus activity as a result of the high presence of the mosquito vectors and amplifying hosts in the study area (14, 15).

This study indicates that there is no significant difference in the seroprevalence of WNV in the different age groups, although previous reports have shown that all age groups and both genders appear equally susceptible to WNV infection.

CONCLUSION

This study indicates that WNV is actively circulating in Maiduguri area of Borno state possibly due to presence of the mosquito vectors and amplifying or reservoir hosts in the study area. A detailed virological and vectoral study needs to be carried out in the area to elucidate the epidemiology of the virus in this ecological niche.

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