Trends in Medical Research

ISSN 1819-3587

Academic Journals Inc.

www.academicjournals.com

Trends in Medical Research 1 (1): 31-38, 2006
ISSN 1819-3587

The Seasonal Effect of West Nile Virus in a Semi-arid Zone, Nigeria

[1]M.M. Baba, [2]Marie-Francois Saron and [3]J.A. Adeniji
[1]Department of Medical Laboratory Science,
College of Medical Sciences, University of Maiduguri, Nigeria
[2]Department of Virology, Institut Pasteur De Dakar, Senegal
[3]Department of Virology, College of Basic Medical Sciences, University of Ibadan, Nigeria

Abstract: This study was designed to determine the season for the peak of WNV activity in a semi-arid zone Nigeria. A total of 973 sera from patients with febrile illness suspected of malaria/typhoid fever were used for the study. The sera were assessed for IgM and IgG antibodies to West Nile Virus (WNV) and Yellow Fever Virus (YFV) by IgM capture ELISA (MAC-ELISA). The IgM positive sera for WNV were also tested by Plaque Reduction Neutralization Test (PRNT) and RT-PCR. Twelve (1.2%) of the 973 sera tested had WNV IgM antibodies. Possible mixed infections of DEN-2 and WNV was observed in two samples. The prevalence of these antibodies during the cold harmattan period (IgM-2.9%) was significantly different from the dry (IgM-0.0%) and rainy season (0.1%). Overall, PRNT and ELISA results were in concordance The peak of WNV activity in the Semi arid Zone in Nigeria appeared to be November. A significant number of the patients tested (80.2%) had WNV IgG and the prevalence of WNV IgG was significantly different according to seasons. No YFV IgM was detected in 973 sera tested; nevertheless, a significant number of the patients (86.9%) had YFV IgG antibodies. Seasonal association with YF IgG antibody was observed. A positive correlation of seasons with incidence was found. To mitigate future impacts, both disease surveillance and control are necessary during the season with highest virus activity.

Key words: West Nile virus, seasons, febrile illness, Nigeria

Introduction

West Nile Virus (WNV) is an arbovirus belonging to the genus Flavivirus, family Flaviviridae. It is the causative agent of West Nile fever and WN encephalitis and belongs to the antigenic complex that includes St Louis, Murray Valley and Japanese encephalitis (Washington State Department of Health, 2005). Perdue News (2005) observed that the number of cases of WNV could vary due to temperatures, rainfall, regional seasonal differences and bird migratory patterns. Since mosquito density, activity and survival is related to various weather conditions, there may be a correlation between WNV infections and seasonal patterns. Koopman *et al*. (1991) reported that median temperature during the rainy season was the strongest predictor of arbovirus infections. Therefore, Jetten and Focks (1996) speculated that the duration of the transmission season could increase in temperate locations. Meanwhile, WNV is established as a seasonal epidemic in North America (Temperate location) that flares up in the summer (when mosquitoes are active) and continues into fall

Corresponding Author: M.M. Baba, Department of Medical Sciences, University of Maiduguri, P.M.B. 1069, Maiduguri, Borno State, Nigeria Tel/Fax: 234 76 235688

(CDC, 2005; California Department of Health Services, 2005). Yet there has been no study published of actual case data that seeks a statistical association between seasons and incidence of WNV in any part of Nigeria (Tropical location). This study is intended to determine seasonal distribution of WNV infections in a semi-arid zone in Nigeria.

Materials and Methods

Study Site

Serum samples were collected from patients who visited University of Maiduguri Teaching Hospital, Maiduguri, Nigeria for medical attention in 2001. The hospital is a tertiary Health Institution, located in Borno State, Nigeria and serves as a reference center for six States in Northeastern Nigeria and neighboring West African countries (Chad, Niger and Cameroon). The experiment was carried out at the Virology Department of Institut Pasteur de Dakar, Senegal in 2002/2003.

Climate of the Study Site

The climate of Maiduguri is typical of the dry tropical type with marked wet and dry seasons. Unlike some other parts of Nigeria, Maiduguri has three distinct seasons in a year namely, cold harmattan (September-December), dry hot (January-April) and rainy (May-August) seasons. It is worth noting that in some years, the seasons do overlap into each other. Rainfall in the study area varies from year to year. Available records spanning the period of 50 years (1951-2000) at the Airport station indicate that the area has a mean annual rainfall of 604.3 mm with a standard deviation of 147.6 mm. (Gimand Associates, 2002).

Study Population

Patients of both sexes whose ages ranged from less than 10 to above 60 years with fever ≥ 38°C and other clinical features suggestive of typhoid fever, sent to the Immunology laboratory of UMTH for Widal tests were used for the study. From the clinical records of these patients, other symptoms include headache, abdomiual discomfort and diarrhea.

Serum Samples Collection

A total of 973 sera were collected from these patients in 2001. About 5 mL of blood was collected by venu puncture from febrile patients. The blood was allowed to clot at room temperature and the serum was carefully collected after centrifugation at 8,000 rpm for 5 min and stored at -20°C until tested.

Standardization of Stock Antigens and Other Reactants

The stock antigens were prepared in mouse brain and titrated against the corresponding hyper immune mouse ascitic fluid by both Chessboard and ELISA methods using Maxisorp microtiter plates. WHO collaborating Center for Reference and Research on Arboviruses (CRORA), IPD, Senegal, kindly supplied the seed viruses [WNV Ref. 087074, YF Ref. (FNUZU) (094564) and DEN-2 MAF4 901317 including their corresponding hyper immune ascitic fluid. The reference strains were used in all control experiments as well as in the preparation of virus stock antigens. The batch of the coating antibody (the anti-human IgM) and the conjugates used were previously titrated by IPD before this project was executed there).

Serology

Stock antigens were prepared in mouse brain from stock viruses supplied by WHO Collaborating Centre for Reference and Research on Arboviruses, IPD, Senegal. All reactants were appropriately standardized.

Detection of IgM Antibodies

An IgM capture ELISA (MAC-ELISA) as previously described by Sathish *et al.* (2002) was adopted for the detection of IgM antibodies against WNV and YFV and for testing WNV IgM positive sera against dengue viruses 1-4. The virus with a higher Optical Density (OD) was considered the infectious agent as reported by CDC (2003).

Detection of IgG Antibodies

For the detection of IgG antibodies against WNV and YFV, an IgG capture ELISA was used as previously described by Chunge *et al.* (1989). Binding of the IgG antibodies was detected using goat anti-human IgG antibodies labeled horseradish peroxidase. Unfortunately these samples were not confirmed by Plaque Reduction Neutralization Technique (PRNT).

Interpretation of Results

The standard deviation of a battery of negative sera was calculated. A value of three standard deviations from the mean was used as the cut-off value to minimize false results as suggested by Innis *et al.*(1989)

Plaque Reduction Neutralization Test (PRNT)

This test was performed as previously described by Mangiafico *et al.* (1988) for the 12 WNV IgM positive samples.

RT-PCR on WNV IgM Positive Sera

The IgM positive sera for WNV were also tested by RT-PCR as described by Lanciotti *et al.* (1992).

Extraction of RNA and RT-PCR

Viral RNA was extracted using the QiAMP Viral RNA purification kit (Qiagen, Inc., Valencia, CA) as per the manufacturer's procedure. The RT-PCR was performed using Titan one Tube RT-PCR kit kindly supplied by IPD, Senegal. The primers used to detect WNV were

WN240: 5' GAG GTT CTT CAA ACT CCA T-3'
WN132: 5' GAA AAC ATC AAG TAT GAG G-3'

The RT-PCR running conditions were 1 cycle denaturation (95°C, 2 min) and 35 cycles (95°C, 40 sec), Hybridization (55°C, 40 sec), Polymerization (68°C, 40 sec) and Elongation (68°C, 7 min). Amplified products were separated by electrophoresis on I% agarose gels and visualized by staining with ethidium bromide and viewing under ultraviolet light. The WN240/WN132 primer set yielded a 330 bp product.

Results

IgM Capture ELISA for West Nile Virus

Twelve (1.2%) of the 973 sera tested had WNV IgM antibodies. Possible mixed infections of DEN-2 and WNV was observed in one sample (Fig. 1). Majority of cases had higher IgG than IgM antibodies. Samples 1 and 6 could probably be cases of anamnestic response for WNV.

The Seasonal Distribution of WNV IgM Antibodies

In Fig. 2, the seasonal distribution of WN IgM antibodies is presented. The prevalence of these antibodies during the cold harmattan period (IgM-2.9%) was significantly different ($\chi^2 = 11.38$, df = 2, p≥ 0 003388) from the dry (IgM-0.0%) and rainy season (0.1%). Table 1 shows the months during which sera of patients with WNV IgM antibodies were collected. Majority (66.7%) of these samples were collected in November during which the environmental temperature could be as low as 18°C or below in the study site.

Seasonal Distribution of WNV IgG Antibodies

In Fig. 3, the seasonal distribution of WNV IgG antibodies is presented. Generally, a significant number of the patients tested (80.2%) tested had WNV IgG and the prevalence of this antibody and the season of the year were significantly different ($\chi^2 = 12.79$, df = 2, p = 0.00166969).

Table 1: Monthly distribution of WNV IgM antibodies

Sample No.	Month of sample collection	Results
1243	19-5-01	Positive
3515	15-11-01	Positive
2172	6-8-01	Positive
3545	30-11-01	Positive
3494	6-11-01	Positive
3679	19-11-01	Positive
3322	25-10-01	Positive
2725	11-8-01	Positive
3460	2-11-01	Positive
3255	22-10-01	Positive
3481	5-11-01	Positive
3500	6-11-01	Positive
3562	9-11-01	Negative
3222	10-12-01	Boarderline

Table 2: Cross reactivity between DEN, WNV and YF by MAC-ELISA

Sample No.	DEN-2	WNV	YFV	RESULTS
1243	0.147	0.581	0.172	WNV
3515	0.352	0.254	0.192	D and WN
2172	0.112	0.302	0.215	WN
3545	0.131	0.277	0.158	WN
3494	0.121	0.29	0.114	WN
3679	0.198	0.763	0.295	WN
3322	0.160	0.427	0.227	WN
2725	0.23	0.32	0.113	D and WN
3460	0.215	0.478	0.108	WN
3255	0.189	0.49	0.109	WN
3481	0.242	0.25	0.087	WN
3500	0.257	0.276	0.202	WN
3562	0.149	0.150	0.089	Negative for DEN, WNV, YFV
3222	0.221	0.226	0.093	Border line for WNV

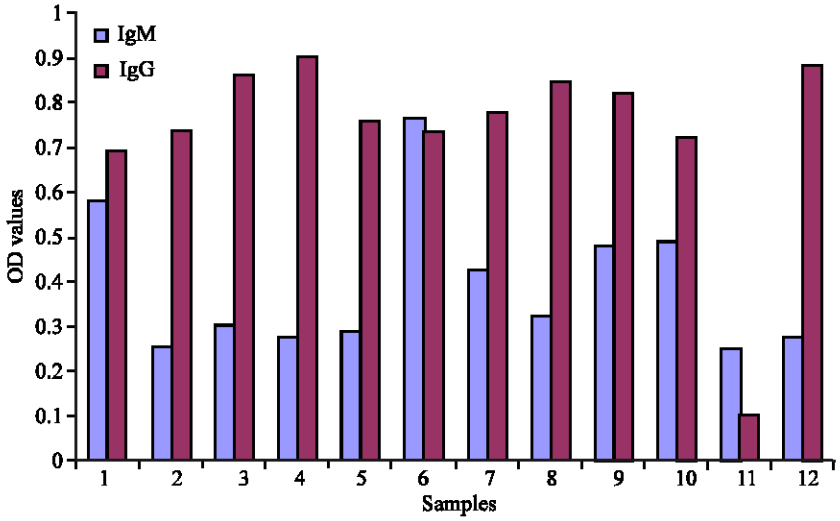

Fig. 1: West Nile IgM and the corresponding IgG antibodies

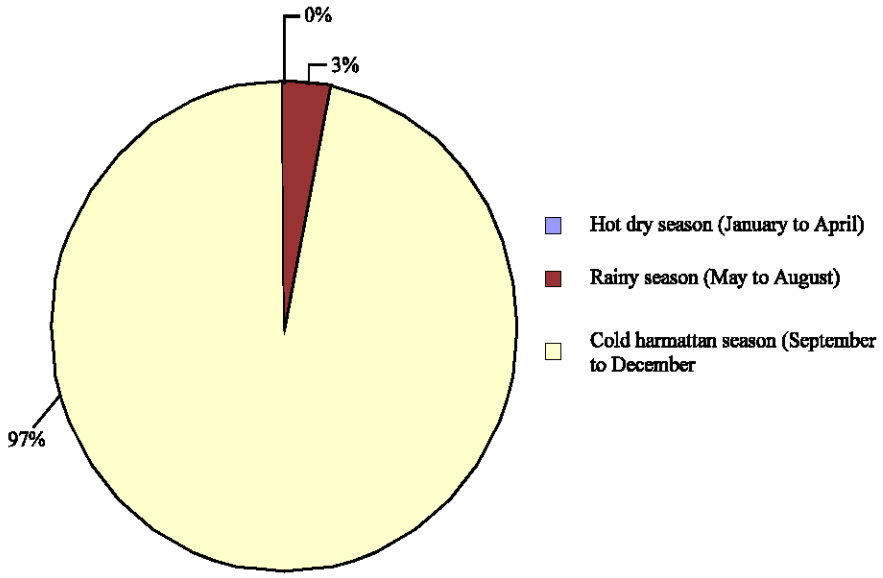

Fig. 2: Seasonal distribution of WN IgM antibodies

Plaque Reduction Neutralization Test on WNV IgM Positive Sera

Incidentally, all the sera that were positive by MAC-ELISA were found positive by PRNT probably because the IgG OD values at the time the sample of collection were higher than IgM Ods (Table 2).

Table 3: RT-PCR on WNV IgM positive sera

Sera Sample No.	RT-PCR	PT-PCR
1243	Positive	0.581
3515	Negative	0.254
2172	Negative	0.302
3545	Negative	0.277
3494	Negative	0.29
3679	Negative	0.763
3322	Positive	0.427
2725	Negative	0.32
3460	Positive	0.478
3255	Positive	0.49
3481	Negative	0.25
3500	Negative	0.276
3562	Positive	0.154 (Negative)
3222	Positive	0.226 (Boarder line)

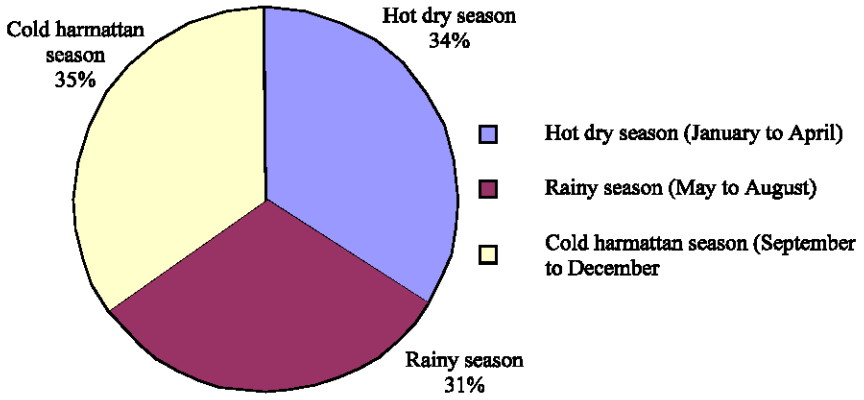

Fig. 3: Seasonal distribution of WNV IgG antibodies

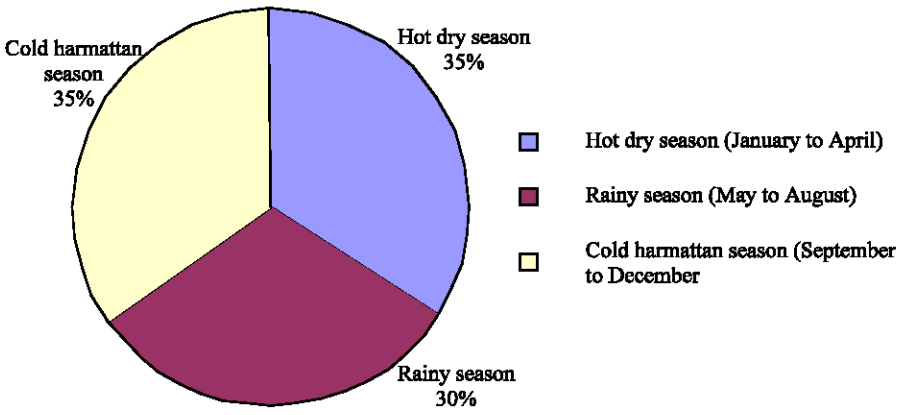

Fig. 4: Seasonal distribution of YFV IgG antibodies

The Prevalence of Yellow Fever IgM and IgG Antibodies

Surprisingly, no YFV IgM was detected in 973 sera tested. In Fig. 4, seasonal distribution of YFV IgG antibodies is presented. Like WNV, significant number of patients (86.9%), tested had YFV IgG antibodies. Seasonal association with IgG antibody was observed ($\chi^2 = 15.37$, df = 2, p = 0.00046022).

RT-PCR on WNV IgM positive samples

Out of the 12 WNV IgM positive sera, 4 (33.3%) were positive by RT-PCR (Table 3). In addition one borderline and one IgM negative sera had WNV RNA.

Discussion

Climate is one of the fundamental forces behind epidemics of arbovirus infections and its effects become evident if adaptive measures falter or cannot be extended to all populations at risk. Therefore a suitable climate is necessary for disease transmission. Other factors including a source of infection, a vector and susceptible human populations would need to be present for an epidemic of arbovirus infection to occur (Hales *et al*., 2002). The present findings confirm that the geographical limits of WNV transmission are strongly determined by the seasons of a place.

The endemicity of WNV infections in Nigeria (a tropical location) is eminent as evidence in this study. WNV infections were significantly higher during the cold harmattan (2.9%) than dry (0.0%) and rainy seasons (0.1%). This finding compared favorably with the previous report, which revealed that regional seasonal differences, temperatures and rainfall are factors that could affect WNV infections (Perdue News 2005). This implies that temperature induced variations in the Vectoral efficiency of mosquito vector may be significant determinant of the annnal pattern of these infections. The extreme lowest mean temperatures of 16.3 to 21.4°C and 21.0 to 23.3°C had been recorded during the rainy and dry seasons respectively. In this study extreme cool temperatures of 8.9 and 9.6°C (December to early January) appeared not to favor WNV transmission in agreement with California Department of Health Services (2005). These authors reported that cool, foggy and windy weather kept mosquito activity relatively low. The peak of WNV activity in temperate countries (CDC, 2005; Perdue News, 2005) occurs in summer/fall (August to October) but November [with extreme lowest and highest mean temperatures of 12.6 and 28°C (Gimand Associates 2002)] in this envirounent (tropical location) as revealed in this study. Like United States of America [where WNV infections peak in Northern part mainly during summer-fall but in the warmer southern regions, it is transmitted all year round (Nemours Foundation, 2005)]. The climatic conditions differ between Northern and southern parts of Nigeria. For instance, the harmattan and the dry seasons are not distinct in the South but the three seasons are very distinct in North where this study was carried out. Therefore there is need to carry out surveillance for this and other endemic arboviruses in different parts of Nigeria at different seasons to determine the precise relationship between these infections and the seasons of the year. It is hoped that the precise information on the seasonal distribution of WNV and other endemic arboviruses in Nigeria would suggest when disease prevention and surveillance measures should be focused with a view to determine the risk of increased transmission. As previously speculated by Koopman *et al*. (1991) in temperate locations, this study has demonstrated that season could be a strong predictor of WNV infections even in tropical settings.

The future implications of this study are more uncertain. Since a positive correlation of seasons with incidence was found, persistent changes in seasons is likely to have an impact on WN infections, increasing the disease's range and the number of infected individnals. Therefore, to mitigate future impacts, both disease surveillance and control are necessary. Surveillance serves as an early warning system for any impending outbreak of arbovirus disease.

Acknowledgements

I wish to express my profound gratitude to TWOWS (Third World Organization for Women in Science) and management of Institut Pasteur De Dakar, Senegal for sponsoring this project. Also, the technical support of Dr. M.M. Dura, the Management and staff of University of Maiduguri Teaching Hospital (UMTH) and Mr. David Bukbuk is highly appreciated.

References

California Department of Health Services, 2005. West Nile Virus Prevention and Mosquito Control, Environmental Health Section, Vector Control Program, pp: 1-3.

Centers for Disease Control, 2003. Instructions for sending diagnostic specimens for serological testing for Arbovirus, West Nile Home, pp: 1-3.

Centers for Disease Control, 2005. West Nile Virus: What you need to know? Fact sheet.

Chunge, E., J.P. Boutin and J. Roux, 1989. Significance of IgM titration by an immunoenzyme technique for the serodiagnosis and epidemiological surveillance of dengue in French Polynesia. Res. Virol., 140: 229-240.

Gimand Associates (Environmental and Human Resources Development Consultant, 2002. Enviroumental impact assessment of Nigerian Bottling Company PLC. Coca Cola Maiduguri Plant 'A' Wastewater discharge project: Draft Report, pp: 1-81.

Hales, S., N. Dewet, J. Maindonald and A. Woodward, 2002. Potential effect of population and climate changes on global distribution of dengue fever: An empirical model. Lancet, 360: 830-34.

Innis, B.L., A. Nisalak, S. Nimmannitya, S. Kusalerdchariya, V. Chongwasdi, S.P. Suntayakorn and C.H. Hoke, 1989. An enyme-liuked immunosorbent assay to characterize dengue infections where dengue and Japanese encephalitis co-circulate. Am. J. Trop. Med. Hyg., 40: 418-427.

Jetten, T.H. and D.A. Focks, 1996. Changes in the distribution of dengue transmission under climate warming scenarios. Am. J. Trop. Hyg, 47.

Koopman, J.S., D.R. Prevots and M.A.V. Marin *et al*., 1991. Determinants and predictors of dengue infections in Mexico. Am. J. Epidemiol., 133: 1168-1178.

Lanciotti, R.S., C.H. Calisher, D.J. Gubler, G.J. Chang and A.V. Vorndam, 1992. Rapid detection and Typing of Dengue Viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. J. Clin. Microbiol., 30: 545-551.

Nemours Foundation Kids Health, 2004. West Nile Virus, pp: 1-4.

Mangiafico, J.A., J.L. Sanchez, L.T. Figueiredo, J.W. LeDue and C.J. Peters, 1988. Isolation of a newly recognized Bunyamwera sero-group virus from a febrile human in Panama. Am. J. Trop. Med. Hyg., 39: 593-596.

Purdue News, 2005. Unpredictable Weather, Disease Carriers Make Protection Paramount., pp: 1-3.

Sathish, N., D.J. Managan, V. Shankar, M. Abraham, G. Nithyanandam and G. Sridharan, 2002. Comparison of IgM captures ELISA with a commercial rapid immunochronatographic card test and IgM microwell. ELISA for the detection of antibodies to dengue viruses. Indian J. Med. Res., 115: 31-36.

Washington State Department of Health, 2005. Notifiable Conditions-Surveillance and Reporting Guidelines for West Nile Virus. Commnnicable Disease Epidemiol., pp: 1-6.