

The Effect of Climate on Dengue Virus Infections in Nigeria

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ABSTRACT: Dengue viruses (serotypes 1-4), are the causative agents of dengue fever, dengue hemorrhagic fever and dengue shock syndrome in humans and are transmitted predominantly by the *Aedes* mosquitoes. Arbovirus infections are usually sensitive to changes in rainfall and temperature. Consequently, their transmission intensity may be regulated by weather and climate. This study was designed to determine the seasonal distribution of dengue virus infections among febrile patients in a semi-arid zone in Nigeria. 973 samples collected during the rainy, harmattan (cold) and dry seasons of the year in a semi-arid zone were tested for antibodies to dengue viruses by MAC ELISA. Den IgM positive samples were further tested by PRNT and RT-PCR. Five (0.5%) of the 973 sera were positive for DEN virus IgM antibodies (4 DEN-2 and 1 DEN-1). A patient had an acute DEN-2 virus infection as demonstrated by a very high OD value of 1.151. All the sera that were DEN IgM positive by MAC-ELISA were found positive by PRNT. Two sera which showed mixed infections of WNV and dengue by MAC-ELISA were later confirmed to be positive for WNV by PRNT. DEN antibodies were significantly higher during the rainy season (1.3%) than the cold harmattan period (0.3%). No IgM antibody to DEN virus was detected during the dry season. July may be the peak of dengue virus activities in a semi-arid zone in Nigeria. Disease surveillance and control are best exercised during the season and month with highest virus activity.

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INTRODUCTION

Dengue (DEN) viruses are the most widely distributed and damaging arthropod-borne viruses (arboviruses) affecting humans. These viruses are transmitted by mosquitoes that are sensitive to changes in rainfall and temperature. Consequently, their transmission intensity may be regulated by weather and climate. (Johanson et al 2009). DEN viruses cause Dengue fever (DF), dengue haemorrhagic fever (DHF)/ dengue shock syndrome (DSS). The transmission of these diseases is climate sensitive for several reasons: mosquitoes require standing water to breed and a warm ambient temperature is critical to adult feeding behavior, the mortality rate of larval development and speed of virus replication (Keirans and Fay 1968). In countries where transmission does routinely occur, short-term changes in weather, particularly temperature, precipitation, and humidity, are often correlated with dengue incidence. (CDC, 2010). DEN viruses have been reported to cause seasonal epidemics of varying sizes in tropical and subtropical regions of the world. (Christophers 1960, Keirans and Fay 1968, Pant and Yasuno 1973, Reuda et al 1990). Despite the public health importance of diseases caused by these viruses, surveillance for arbovirus activities in

Nigeria is generally poor probably due to lack of diagnostic reagents and facilities. This study seeks a possible association between seasons and incidence of dengue virus infections in Nigeria and to determine the period when DEN activities peak 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 (UMTH), Maiduguri, Nigeria for medical attention. 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 samples were collected from Nigeria and transported in isothermal flask by air to Dakar, Senegal where the experiment was carried out at the Virology Department of Institut Pasteur de Dakar, Senegal.

Study population:

Patients of both sexes whose ages ranged from less than 10 to above 60 years with fever $\geq 38^{\circ}\text{C}$ 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, abdominal discomfort, and diarrhea. Any patients without fever were excluded from the study.

Serum Samples Collection:

A total of 973 sera were collected from these patients in 2001. These samples were collected during the three seasons of the year which include: hot dry (January to April), Rainy (May to August) and cold harmattan (September to December) seasons. Occasionally the seasons overlap each other although they are very distinct in the semi arid zone (Sahel savanna) compared to the southern part of Nigeria. About 5ml of blood was collected by venipuncture from febrile patients. The blood was allowed to clot at room temperature and the serum was carefully collected after centrifugation at 8,000rpm 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 DEN 1-4 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.

SEROLOGY

The capture ELISA (MAC-ELISA-developed in WHO CRORA, IPD, Senegal) was used to assess all the sera for IgM and IgG antibodies for the four DEN viruses. IgM positive sera were retested against Yellow Fever Virus (YFV) and WNV for possible cross-reactions. Sera with higher OD value was considered the possible infecting virus.

Detection of IgM :

Briefly the anti-human IgM coated onto the plate captures human IgM from the test sera. Positive and negative human control serum was included in the test. The trapped human IgM was made to react with an appropriately tittered antigen in the next step. The complex was allowed to react with a monoclonal antibody from mouse ascitic fluid. The

final complex formed was detected by the signal generating system comprising the conjugate (peroxidase-conjugated Sheep IGG Fraction to mouse) and substrate before the reaction was stopped with 4N sulphuric acid.

Detection of IgG:

Monoclonal antibody from specific mouse ascitic fluid was used to capture appropriately tittered antigen which further reacted with specific antibody from the test serum. Positive and negative human control sera were included in the test. The complex formed was detected by signal generating system which consisted of conjugate [peroxidase-conjugated Goat F (AB)² Fragment to Human IgG] and substrate. The reaction was stopped using 4N sulphuric acid.

Calculation of Results and Interpretation.

The difference between the OD values of serum with positive antigen and serum with negative antigens were obtained for each patient to validate the test. Invalid tests were repeated. Three standard deviations from the mean of a battery of negative sera were used as the cut off value to minimize false results.

Plaque reduction neutralization test (PRNT):

This test was performed as previously described (7) for the DEN IgM positive samples. Briefly virus diluted to contain approximately 100 plaque-forming -units per 0.1 ml were mixed with an equal volume of serial four-fold serum. After incubation at 4°C , the mixture was inoculated in duplicate wells containing Vero cell in a 12 well-plate. The inoculated plates were incubated for one hour at 37°C before being overlaid with medium containing 1% nutrient agar. A second nutrient agar overlay containing 0.17% neutral red was added 48 hours later and plaques were counted the following day. The neutralizing antibody titer of the positive serum was expressed as the reciprocal of the highest dilution of serum that inhibited $\geq 80\%$ of the plaques compared with the virus control titration (PRNT₈₀).

RT-PCR with DEN IgM positive sera

The IgM positive sera for DEN viruses were also tested by RT-PCR as previously described by Laciotti et al (1992).

Comparative titration to differentiate the four serotypes of dengue viruses:

Each sample considered positive for DEN IgM was further diluted and treated against the four serotypes of DEN viruses to determine the infecting

agent. For example, sample VF 30 was diluted from 1:100 to 1: 102400.

RESULTS.

Five (0.5%) of the 973 sera were positive for DEN virus IgM antibodies (4 DEN-2 and 1 DEN-1). A patient had an acute DEN-2 virus infection as demonstrated by a very high OD value of 1.151 while another showed an anamnestic response.

Plaque Reduction Neutralization Test on DEN IgM Positive Sera:

All the sera that were DEN IgM positive by MAC-ELISA were found positive by PRNT. (Data not included). Two sera which showed mixed infections of WNV and dengue by MAC-ELISA were later confirmed to be positive for WNV by PRNT. Failure to carry out PRNT for all the samples limits this study from giving the precise status of these patients with regards to dengue virus infections in Nigeria. This is because a negative acute-phase specimen is inadequate for ruling out such an infection underscoring confirmation by demonstrating virus-specific serum IgG antibodies in the same or later specimen.

RT-PCR with DEN IgM positive sera is presented on table 3:

Out of 5 IgM positive sera, 3 showed detectable viral RNA. However, 1 DEN-2 IgM negative serum was found positive (DEN-3) by RT-PCR.

Seasonal distribution of dengue virus IgM antibodies

The seasonal distribution antibodies to dengue viruses are presented in figure 1. A significant association ($\chi^2 = 5.82$, $df = 2$, $P = 0.054$) was observed between the prevalence of dengue antibodies and the seasons of the year in Nigeria. DEN antibodies were significantly higher during the rainy season (1.3%) than the cold harmattan period (0.3%). No IgM antibody to DEN virus was detected during the dry season. When the previous work on den viruses (Baba et al 2010) and this study were compared, it was also observed that 2 (11.1%), 10 (55.6%), and 5 (27.8%) of the DEN IgM positive sera were collected in the month of June, July, and August respectively. This implies that July may be the peak of dengue virus activities in Nigeria.

Table 2 shows the seasonal distribution of IgG antibodies to dengue viruses

83.9%, 88.9% and 86.2% of DEN IgG were obtained during the dry, rainy and harmattan seasons respectively. No significant relationship between the prevalence of dengue IgG antibodies and the seasons of the year was observed.

Table 4 shows the cross reactivity between DEN, WNV, and YFV by MAC-ELISA

Although cross –reactions among flaviviruses render MAC-ELISA less specific, the difference in the OD values, to some extent could give a presumptive idea to the infecting flavivirus. Samples 3515 and 2575 were suspected cases of mixed infections of DEN and West Nile virus (WNV).

Comparative titration to differentiate the four serotypes of dengue viruses:

The dilution of sample VF 30 as an example showed that: DEN-1, DEN-2, DEN-3 and DEN-4 had OD values of 0.126, 0.329, 0.216 and 0.267 respectively at dilution 1: 3200. Therefore DEN-2 was implicated in that case.

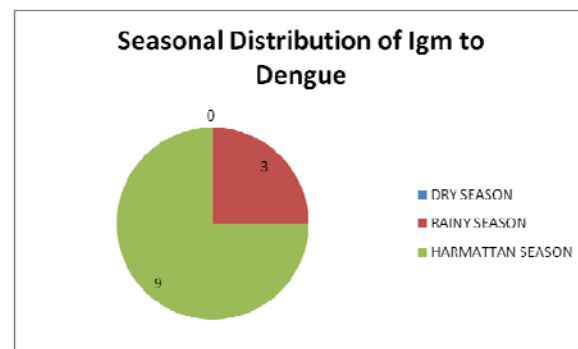


Figure 1: Seasonal Distribution of IgM to Dengue

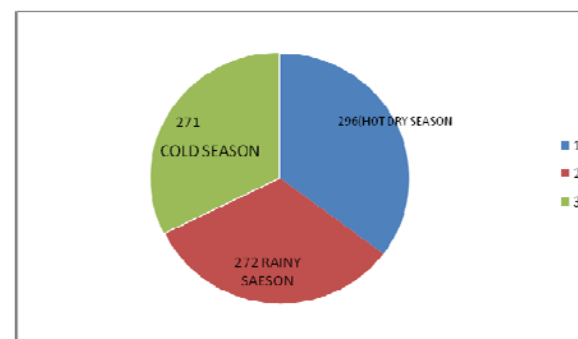


Figure 2: Seasonal Distribution of IgG to Dengue

TABLE 2: RT-PCR WITH DENS IGM POSITIVE SERA

S/NO	SAMPLE NO	RT-PCR	IgM POSITIVE SERA
1	VF 30	Negative	D2
2	VF32	positive	D2
3	VF 641	positive	D2
4	VF 334	D3	Negative
5	VF 2463	positive	D1
6	VF 667	Negative	D1

TABLE 3: CROSS REACTIVITY BETWEEN DEN, WNV AND YFV BY MAC-ELISA (Using the OD values)

Sample No	DEN	WNV	YFV	RESULTS
1243	0.001	0.581	0.172	WNV
3515	0.352	0.254	0.192	DEN & WNV
2172	0.112	0.302	0.215	WNV & YFV
3545	0.131	0.277	0.158	WNV
4394	0.121	0.29	0.114	WNV
3679	0.198	0.763	0.295	WNV
3322	0.116	0.427	0.227	WNV
2725	0.23	0.32	0.113	DEN & WNV
3460	0.118	0.478	0.108	WNV
3255	0.189	0.49	0.109	WNV
3481	0.119	0.25	0.087	WNV
3500	0.121	0.276	0.084	WNV

DISCUSSIONS

Temperature, precipitation, and humidity are critical to mosquito survival, reproduction, and development and can influence mosquito presence and abundance. Additionally, higher temperatures reduce the time required for the virus to replicate and disseminate in the mosquito (Johansson et al 2009, CDC 2010). In the latter report, the extrinsic incubation period must occur before the virus can reach the mosquito's salivary glands and be transmitted to humans. If the mosquito becomes infectious faster because temperatures are warmer, it has a greater chance of infecting a human before it dies. As important as the environmental factors may be, others such as presence of the virus, sufficient numbers of susceptible population, and mosquito vectors are critical to dengue transmission.

In this study, the prevalence of DEN IgM was significantly higher during the rainy season compared to harmattan and dry periods with a seasonal peak in July. In contrast to DEN, WNV was significantly higher during the harmattan period than rainy and dry season with a peak in November in the same environment as this study. This finding compared favorably with the report of Moore et al (1975) who revealed that arboviral activity was highest during the rainy season with peak in the month of June, July and August and lowest in the dry month of January and February in Nigeria. The results of this study imply that temperature induced variations in the Vectorial efficiency of mosquito vector may be significant determinant of annual pattern of DEN virus infections. A report showed that, during the dry and the rainy seasons, the extreme lowest temperature could vary from 16.3 °C to 21.4 °C and 21.0°C to 23.3°C respectively (Gimand Associates 2002).. It may be necessary to state that, the temperatures could be as high as 45-50°C during the dry season in Maiduguri where this study was carried out. This probably explains why no DEN activity was demonstrated during the dry season. The low DEN activity obtained in this study during the harmattan period (when the temperature could be as low as 11- 18oC) is supported by Hales et al (2002). These authors revealed that, if the climate is too cold, viral development is slow and mosquitoes are unlikely to survive long enough to become infectious. It has been determined that warmer temperatures reduce larval size of *Ae. Aegypti* (the principal vector of dengue viruses) which results in smaller adult size (Reuda, 1990). Smaller adult female mosquitoes have been found to feed more frequently to nourish their developing eggs which increases the possibility of transmission (Reita 1988). The positive relationship between biting rates and

temperatures have been reported in field studies in Bangkok by Pant 1973). In addition, extrinsic incubation period (EIP) for *Ae. Aegypti* decreased from twelve to seven days when mosquitoes were kept at 32-35°C instead of 30°C (Watts et al 1987). Several other reports also have shown that, temperature affects the rate of mosquito larval development, adult survival, vector size, gonotrophic cycle as well as the EIP of the virus in the vector (Focks et al 1993). Koopman et al (1991) therefore concluded that median temperatures during the rainy season were the strongest predictor of DEN infections. It is worth noting that the climatic conditions are different in other different parts of Nigeria. For instance, the harmattan and the dry seasons are not very distinct and the rainy season is longer in the Southern part of the country compared with the northeastern when the three seasons are distinct but with shorter duration of the rainy period.

In addition, Akhtar and Ebi (2001) reported that DEN virus transmission occurs all year round in the tropics but has seasonal peaks in most countries during the months with high rainfall and humidity. In accordance with that report, the bulk of the yearly rainfall (over 90%) in Maiduguri, is concentrated between the months of June and September (Gimand Associates 2002). That report further revealed that (i) the month of August marks the peak of the rainy season with the mean rainfall amount of 205.9mm and (ii) Months of July to September are associated with high relative humidity of more than 60%. This implies that the month of July during which the DEN virus activities peaked had high rainfall and relative humidity sufficient for the transmission of the virus.

Johnson et al (2009) revealed that, the strength of the association between monthly changes in temperature and precipitation and monthly changes in dengue transmission varies based on differences in local climate. These authors therefore concluded that, why DEN virus transmission may have a general system, its manifestation on a local scale may differ from global expectations. Therefore there is need for a more detailed study for endemic arboviruses in different parts of Nigeria at different seasons to determine the precise relationship between arboviral infections and the seasons of the year. Such information would suggest when disease prevention and surveillance measures should be focused to determine the risks of increased transmission.

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