Simultaneous Detection of Dengue and West Nile IgM Antibodies in Febrile Individuals in Ile-Ife, Nigeria

Adesina O. A.[1*] Amoo B. A.[2] Adeboje M. F.[3] Simeon O. N.[1] and Yusuf O. O.[1]
1. Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria.
2. African Field Epidemiology Network, Abuja, Nigeria.
3. Jaypecstar Academy, Abuja, Nigeria
* Corresponding author: adesinafat@yahoo.co.uk

Abstract: While there are over 80 members of the *Flaviviridae* Family, some of which represent a global threat, Dengue, and West Nile viruses represent two of the major human pathogens that produce life-threatening conditions and complications. These mosquito-borne viral infections have not so much been studied in rural and sub-urban areas of the sub-Saharan regions where the vectors thrive. This study seeks to know the burden of dengue and West Nile infections, and likely co-infection in Ile-Ife and its environs. Two hundred and fifty-five (255) participants from the Primary Healthcare facilities in Ile-Ife township and the surrounding Local Government Areas were involved in the study. Some subjects residing outside Ile-Ife who visited the facilities were also included. Venous blood samples collected from 255 participants were screened for Dengue and West Nile IgM antibodies using corresponding ELISA kits from Dia-Pro, Italy. Out of the 255 screened, positive for dengue IgM only were 74 (29.0%), positive only for West Nile IgM 125 (49.0%) and 46 (18.0%) were positive for both dengue and West Nile IgM. Ife Central Local Government Area has the highest prevalence (38.5%) of dengue while Ife Area Office (Modakeke) has the highest West Nile infection, 69.6%. More females, 36 (14.1%), out of 46 (18%) were co-infected with WNV and Dengue. Mostly affected were women, middle-aged individuals of ages 19 – 27 years, and traders. The environment, occupation, academic level, and availability of modern healthcare facilities for these sub-urban areas must be improved upon if these tropical arboviral diseases would be controlled.
Key word: Co-infection**,** Dengue**,** ELISA**,** IgM**,** West-Nile

INTRODUCTION

Arboviruses are known to cause disabling fever syndromes worldwide, yet the global burden and effect of their infections have not been systematically evaluated. Arbovirus-caused acute infections can result in encephalitis or hemorrhagic diathesis, which can cause long-term physical and cognitive impairment or even early death. They can also cause silent illness, severe undifferentiated fever, or more complex secondary diseases (CDC, 2010a, CDC, 2010b). Not less than 100 arboviruses are considered a global threat to humans as they have caused varying conditions from febrile illnesses to specific epidemics in sub-Saharan countries (Forshey *et al*., 2010; WHO, 2004). Three notable arboviral families, *Flaviviridae, Bunyaviridae,* and *Togaviridae,* which affect human health are transmitted in almost every part of the world. Arboviruses cause viremia in the blood of the host species, fever, and epidemics with a high record of fatalities that are difficult to control (Solignat *et al*., 2009). The illnesses caused by many arboviruses appear clinically similar with fever being common to all arboviral infections, hence the likelihood of its misdiagnosis as malaria.

Flaviviruses (Family *Flaviviridae*), contain many single-stranded RNA viruses that cause severe widespread infection and epidemics globally. Prominent members of this family and important to human health include West Nile virus (WNV), Japanese encephalitis virus (JEV), dengue virus (DENV), Zika virus (ZIKV), yellow fever virus (YFV), and tick-borne encephalitis virus (TBEV). Ticks and mosquitoes are the primary vectors of arboviruses. Three principal ecological families of flaviviruses have been identified: mosquito-borne, tick-borne, and unknown vector (Heinz *et al*., 2020). Further subdivision of the mosquito-borne flaviviruses according to vector genus has Culex-borne and Aedes-borne viruses (Gaunt *et al*., 2001) where West Nile viruses are transmitted by *Culex nigripalpus* and dengue transmitted by *Aedes aegypti*. Contrary to what is known with most arboviruses, a group of flaviviruses replicate

only in invertebrate cells and are referred to as an insect-specific group (Cook and Holmes 2006). Owing to the prevailing socioeconomic and ecologic factors that favour the propagation of arboviral vectors in most developing tropical countries, infections caused by mosquito-borne viruses remain prevalent and pose a global health challenge. Mosquitoes have been identified as the primary vector of viral pathogens including dengue virus (DENV serotype 1-4) and West Nile virus (Coatsworth *et al*., 2022). Humans either serve as the accidental or terminal hosts of many of these zoonotic viruses, even though animal species other than humans are essential to their preservation (Karabatsos, 2001).

While similar symptoms present in most arboviral infections (Mediannikov *et al*.,2013), dengue and West Nile viruses are known to cause Non-Malarial Febrile Illnesses (NMFIs) (Crumps *et al*., 2013). Arboviral coinfections namely of Chikungunya, west Nile; Chikungunya, and dengue exist in some African countries (Caron *et al*., 2012; Furuya-Kanamori *et al*., 2016) and more emphasis has been laid on malaria and dengue (Arya *et al*.,2005) in many African studies.

Although the mosquito species vectors that transmit arboviruses, especially dengue and West Nile viruses are well established in many parts of Nigeria (Baba *et al*., 2013) and arboviruses are known to cause widespread morbidity in Nigeria, there is little documented research on their burden and distribution. This study was therefore designed to carry out all-inclusive observation in Ile-Ife land and the surrounding Local Government Areas including their rural and sub-urban areas from where movement to and outside Ile-Ife is a regular practice.

MATERIALS AND METHODS

Study locations: The study was carried out in selected towns and areas from three (3) of the four Local Government Areas (LGA) in Ile-Ife. The LGAs are Ife Central, Ife East, Ife North, and an area office, Ife area office, Modakeke.

Enrolment of study participants: The cross-sectional laboratory-based study was designed to capture more of the people in the rural and suburban areas and those who visit Primary Health Care (PHC) facilities in the Local Government Areas of Ife land. The study participants therefore mostly included medium to low-income earners with minimal academic qualifications who are typically not resident in the heart of the town.

Sample collection: About 3ml of venous blood was collected from each consenting participant. The blood samples were then separated into the packed cell and serum and stored in appropriately labelled cryovials at -20^{o}C until analyzed.

Data collection: A paper-based structured questionnaire was administered to each participant to gather information on socio-demography, the environment they live in, symptoms, travel and medical histories, and the probable risk factors.

Laboratory procedure: The two hundred and fifty-five (255) samples were analyzed for the presence of West Nile IgM and Dengue IgM by ELISA (Dia-Pro, Diagnostic Bioprobes, Italy) following the manufacturer's instructions. It is an indirect sandwich ELISA technique.

Data analysis: Descriptive and inferential statistical analyses were achieved using the SPSS v20 software. Categorical variables were compared and cross-tabulated using Fisher's exact test at 95% Confidence interval (CI).

RESULTS

This cross-sectional study included all categories of febrile individuals visiting Primary Healthcare facilities in three of the four Local Government areas of Ile-Ife and the Modakeke Area Office. The participants cut across different categories of people as shown in table 1.

In all, 74 (29 %) and 125 (49 %) of the participants had detectable Dengue and West Nile IgM respectively. These are distributed

among the study areas and those who came to visit the areas fell ill and visited the health facility used for the study. Details about the distribution according to the locations and socio-demographic factors are shown in Table 2 and Table 3 respectively.

For DENV-WNV co-infection in this study, 46 (18.0%) were positive for both dengue and West Nile IgM with more females, 36 (14.1%), out of 46 (18%) being co-infected. People of ages 19 – 40 years have more co-infection cases as shown in Figure 1 and Table 4.

Table 1: Distribution of samples collected

		Local Government Areas (LGAs)					
		Ife Central	Ife East	Ife North	Ife Area office	*Outside Ife	Total
Age range	< 11	3	0	10	2	0	15
	11 – 18	2	3	7	1	1	14
	19 – 27	20	22	37	24	11	114
	28 – 40	17	8	22	22	6	75
	>=41	10	2	15	7	3	37
	Total	52	35	91	56	21	**255**
Gender	Female	28	23	61	52	12	176
	Male	24	12	30	4	9	79
	Total	52	35	91	56	21	**255**
Marital Status	Married	27	19	51	51	8	156
	Single	25	16	40	5	13	99
	Total	52	35	91	56	21	**255**

Key: * -- non-Ife residents who visited the health facility during the study

Table 2: Distribution of dengue and West Nile according to Local government areas (LGAs)

LGAs	**Dengue**			**WNV**		
	Positive (%)	Negative (%)	Total	Positive (%)	Negative (%)	Total
Ife Central	20 (38.5)	32 (61.5)	52	21 (40.4)	31 (59.6)	52
Ife East	7 (20.0)	28 (80.0)	35	13 (37.1)	22 (62.9)	35
Ife North	25 (27.5)	66 (72.5)	91	44 (48.4)	47 (51.6)	91
Area Office	19 (34.0)	37 (66.0)	56	39 (69.6)	17 (30.4)	56
Outside Ife	3 (14.3)	18 (85.7)	21	8 (38.1)	13 (61.9)	21
Total	**74** (29.0)	**181** (71.0)	**255**	**125** (49.0)	**130** (51.0)	**255**

Table 3: Distribution of Dengue and West Nile with sociodemographic factors

Factors		Dengue			West Nile		
		Positive (%)	Negative (%)	P value	Positive (%)	Negative (%)	P value
Age	< 11 years	5(1.9)	10(3.9)	0.901	8(3.1)	7(2.7)	0.001*
	11–18 years	3(1.2)	11(4.3)		5(2.0)	9(3.5)	
	19–27 years	34(13.3)	80(31.4)		41(16.1)	73(28.6)	
	28–40 years	23(9.0)	52(20.4)		47(18.4)	28(11)	
	>40 years	9(3.5)	28(11.0)		24(9.4)	13(5.1)	
	Total	74(29)	181(71)		125(49)	130(51)	
Gender	Female	54(21.2)	122(1.9)	0.236	94(36.9)	82(32.2)	0.025*
	Male	20(7.8)	59(1.9)		31(12.2)	48(18.8)	
	Total	74	181		125	130	
Occupation	Students	19(7.5)	64(25.1)	0.408	29(11.4)	54(21.2)	0.015*
	Civil servant	9(3.5)	26(10.2)		15(5.9)	20(7.8)	
	Trading	36(14.1)	76(29.8)		65(25.5)	47(18.4)	
	Retired	1(0.4)	1(0.4)		2(0.8)	0(0)	
	Housewife	2(0.8)	1(0.4)		2(0.8)	1(0.4)	
	None	7(2.7)	13(5.1)		12(4.7)	8(3.1)	
	Total	74	181		125	130	

Note: * statistically significant at 95% Confidence interval

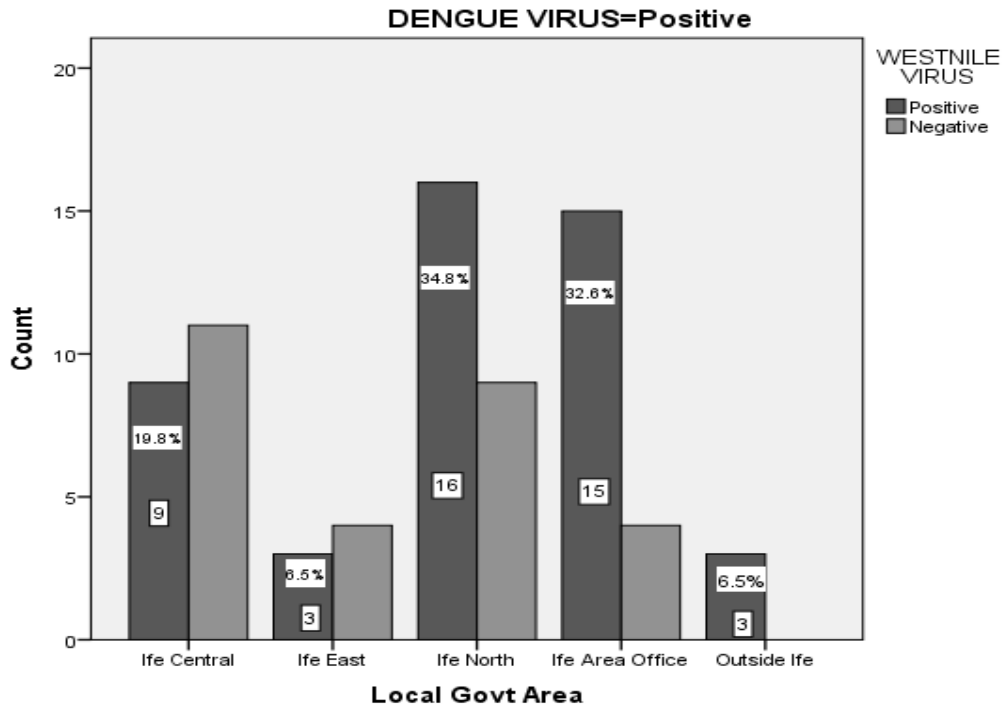

Figure 1: Distribution of West Nile – Dengue co-infections in the Local Government Area

Table 4: Distribution of dengue - west Nile Coinfection with age and gender

Dengue			Age category					Total	Gender		Total
			<11	11-18	19-27	28-40	>40		Female	Male	
Positive	WNV	Pos	4	2	20	15	5	46	36	10	46
		Neg	1	1	14	8	4	28	18	10	28
	Total		5	3	34	23	9	74	54	20	74
Negative	WNV	Pos	4	3	21	32	19	79	58	21	79
		Neg	6	8	59	20	9	102	64	38	102
	Total		10	11	80	52	28	181	122	59	181
Total	WNV	Pos	8	5	41	47	24	125	94	31	125
		Neg	7	9	73	28	13	130	82	48	139
	Total		15	14	114	75	37	255	176	79	255

DISCUSSION

The study covered representative towns in three Local Government areas in Ile-Ife namely Ife Central, Ife East, Ife North, and Ife Area office, Modakeke, Osun state, Nigeria. The overall dengue prevalence of 29% (N = 74/255) and WNV of 49% (N = 125/255) were observed in this study. The result supports the claim that the prevalence of Dengue is hyperendemic in Nigeria (Emeribe *et al.*, 2021) although in-depth studies of the virus are not common in the country. Apart from the general prevalence, Emeribe *et al.*, (2021), in their review, further reported that the South-west Geo-political zone is one of the zones with the highest number of dengue cases in Nigeria which could be due to the vegetation cover, annual rainfall, and temperature. West Nile virus has undergone substantial geographical spread around the world, after its discovery in 1937, through many activities including globalization, land use, and international travel. The spread has also affected Nigeria after its identification in the 1950s (Sule *et al.*, 2018; Bai *et al.*, 2019), a reflection of what is seen in this study where a prevalence of 49% (N = 125/255) was reported in rural,

suburban, and urban areas of Ile-Ife. The reason for this high prevalence may not be far from the conducive environment for the relative abundance of mosquitoes involved in its transmission. Ife Central has the highest prevalence of Dengue (38.5%) while the Modakeke Area office has 69.6% for WNV. The WNV prevalence has more significance with demographic values though not well studied most of the time and places in Nigeria probably because it is not seen to be in the country.

In all, 46 (18%) were positive for both Dengue and WNV with a Pearson Chi-square significant value of 0.007 at a 95% Confidence Interval. Out of the 46 coinfected, there were 36 females and 10 males: The age group 19 – 27 years was the most common group with coinfection. It is not unlikely that other routes of transmission of these viruses may become possible due to the many activities of the most affected group of people who are expected to be active in many aspects of life. This calls for more studies to control the spread of the diseases while vector control is intensified. More traders (21) who are mostly involved in traveling to the villages for their businesses and those who are often in a location where resident vectors have unrestricted access to them due to an unkempt environment and uncontrolled population were coinfected. The coinfection with dengue and WNV reported is calling for prompt action including enlightenment and better preventive measures as an individual infection with any flavivirus can produce any of the neurotropic, congenital, and visceral diseases thus making a disease syndrome possible in any of the persons coinfected with Dengue and WNV observed in this study. Different flaviviruses have different neurotropic properties. WNV, for example, is known to cause severe neurological syndromes like meningitis, encephalitis, and acute flaccid paralysis; DENV, on the other hand, can cause visceral disease that results in liver failure, hemorrhagic syndromes, and vascular compromise, which may ultimately cause the infected person to die or become severely disabled (Pierson & Diamond, 2020). DENV and WNV infection surveillance in Nigeria has not been as expected probably because they have not been listed as a public health priority, their often febrile presentations, insufficient studies and reportage of their pathogenesis, lack of public awareness of the viruses, and meagre understanding by healthcare professionals which is seen in their misdiagnosis and underdiagnosis in many uncategorized febrile illnesses in a country reported to be hyperendemic for Dengue and underreported for West Nile (Amarasinghe *et al.,* 2011; Nasir *et al.,* 2017). With case detection, management, and vector control, the prevention and control of WNV and DENV transmissions will be carried out more effectively. In the same way that environmental factors like climate and land cover have a positive impact on WNV transmission because they support mosquito population dynamics and ecology, some studies have found a strong correlation between annual WNV incidence in the "early season" model in Ontario, Canada, and climate indices like mean minimum temperature in February and higher winter minimum temperatures (Mallya et al., 2018). Consequently, there is a rise in mosquito abundance, mosquito biting frequency, virus replication, and transmission rates (Reisen *et al.,* 2006 & Chuang *et al.,* 2012). On the contrary, this study has found age, gender, and occupation significant in West Nile virus transmission. It is therefore important to understand our environment before agreeing to what other regions have found to apply to them. The same applies to the simultaneous infection of DENV and WNV as more females and individuals of ages 19 – 27 and 28 – 40 years are more affected by the viruses. Their occupation could be the major factor as many are either trading, traveling, or operating a business around the house where sanitation is not up to the standard. There is still much to do to get rid of the mosquito vectors thereby controlling the spread of arboviruses.

CONCLUSION

It is important to conclude by reiterating that studies related to arboviruses generally need to be improved upon and funded more so that these re-emerging infectious diseases can be controlled and hence the menace of the likely various complications they produce. It should also be noted that because these viruses and their vectors are with us does not make them part of us, they must be given more attention as to other "dreaded" viral infections.

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