

Co-Circulation Evidence of Dengue Virus Serotypes at the Kenyan Coast in 2014, 2015

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Abstract: Dengue virus is transmitted to humans by the *Aedes aegypti* and *Aedes albopictus* mosquitoes. These species of mosquito are ubiquitous in the Kenyan Coast. Dengue causes fever outbreaks and other fever associated symptoms that include Dengue fever (DF), Dengue Hemorrhagic fever's (DHF) and Dengue Shock Syndrome (DSS). Laboratory tests are almost always never done, and most patients are often given a course of antibiotics and painkillers after getting the diagnosis of fever of unknown origin. The study was done to determine the prevalence of DENV in the year of study and to provide evidence of co-circulation of DENV serotypes at the Kenyan Coast. Five hundred (500) samples were successfully collected from febrile patients at CPGH from January 2014 to March 2015 and tested. They were tested for Dengue virus using in house indirect Enzyme Linked Immunoassays (ELISA) and Focus Reduction Neutralization tests (FRNT). From the 500 samples collected, a total of 75 (15%) samples were positive for Dengue. FRNT was then performed on the samples that tested positive by IgM ELISA where 58 (11.6%) neutralized DENV1, DENV 2 which was predominant neutralized 73 (14.6%), 68 (13.6%) neutralized DENV 3 and 34 (6.8%) neutralized DENV4. Of the 75 samples positive by IgM, 4 (5.3%) neutralized one serotype, 14 (18.7%) neutralized 2 serotypes, 27 (36%) neutralized 3 serotypes and 30 (40%) samples neutralized all four DENV serotypes. In 2014, 2015, DENV accounted 15% of all the fevers presenting at CPGH. Dengue is therefore endemic and an important cause of morbidity at the Kenyan Coast. This calls for definitive public health interventions shown to work elsewhere.

I. Background

Dengue virus is a vector borne pathogen which belongs to the family Flaviviridae and genus Flavivirus. Four distinct serotypes of the Dengue virus (dengue-1, dengue-2, dengue-3, and dengue-4) exist, with numerous virus strains found worldwide [1]. Dengue virus is spread by the mosquito vector *Aedes aegypti* and *Aedes albopictus* [2]. During 1960–2010, a total of 22 countries in Africa reported sporadic cases or outbreaks of dengue; 12 other countries in Africa reported dengue only in travelers [3]. In 1982, an outbreak of dengue fever was reported at the Kenyan coastal towns of Malindi and Kilifi. Clinical presentation was consistent with classical dengue fever, with no severe dengue reported [4]. Co-infection with 2 dengue viruses (DENV-1 and DENV-4) was reported in Puerto Rico in 1982. Since then, many cases of concurrent infections with multiple DENV serotypes have been reported in many countries [5, 6]. In areas where multiple dengue serotypes are transmitted concurrently, clinical cases caused by more than 1 serotype of dengue virus may be more common than previously thought. The high attack rates of cases that occur during epidemics would likely result in many infections with multiple virus serotypes in humans (both clinical and sub-clinical), and also provide opportunities for mosquitoes to become infected with two or more serotypes. It has been suggested that concurrent infection by multiple dengue serotypes might influence clinical expression, and this was initially considered as one explanation for the emergence of DHF and DSS [5, 7]. In the 1960s, Dr. Scott Halstead and his colleagues were studying the dengue virus in Thailand. They noticed that people who had been exposed to dengue a second time had an increased risk of severe dengue compared with those who had not been previously exposed [8].

II. Materials and Methods

Ethics statement: Participants consented to participate in their own language. Written consent was obtained from all adults; children assented with parental written consent. The study was undertaken after approval from The Scientific Steering Committee (SSC) of the Kenya Medical Research Institute (KEMRI) and The National

Ethical Review Committee (ERC) which grants approval for research studies involving human subjects in compliance with the Helsinki declaration.

Study Samples: Samples used were selected from samples taken from the Coast Provincial General Hospital under the Protocol SSC 1981, collected in 2012. Samples were collected according to the inclusion criteria, where they were negative for fever caused by typhoid and malaria. Samples used for the study were then tested for DENV after which the serotyping was done. The samples used were anonymous having no names but contained sample identities which were similar to their corresponding questionnaires.

IgM capture ELISA

IgM capture ELISAs to identify primary exposure to Dengue were conducted using tetravalent Dengue Antigens by well described methods[9] and measured using the same approach. A Positive/Negative (P/N) ratio greater than two was considered positive for each of the viruses.

Focus Reduction Neutralization Test (FRNT₅₀)

Focus reduction neutralization tests were conducted to determine the presence of each Dengue virus serotype using well described methods without modification[10]. The samples used were those that tested positive for IgM ELISA. In all cases, absorbance was read at 492 nm using an ELISA plate reader.

Statistical analysis

Data were analysed using R statistic version 3.3.0 (2016-05-03). Fisher’s exact or *chi*-squared tests were used for categorical variables considered significant. Logistic regression analysis was used to determine risk factors associated with being positive for at least one dengue virus serotype. All tests were conducted at the 5% level of significance.

III. Results

Demographic data

Five hundred (500) samples were collected from febrile patients at CPGH from January 2014 to March 2015 and tested. Of these samples, 258 (51.6%) were male, 232(46.4%) female and 10 (2%) whose gender was unidentified. They were aged zero (0) to ninety four (94) years with 26 years being the median age. We categorised these ages in two ways, first children were categorised below 16 years and adults 16 and above. From this, children were 133(26.6%), adults 363 (72.6%) and 4(0.8%) had their ages none identified. Secondly, they were categorised in age groups from 0-5 to 61-65 where majority of the patients (60) were in the age group 16- 20. Of these 25(5%) were male and 35(7%) female. All the patients whose samples were collected came from Mombasa County, which consists of 45 districts and 122 villages. Majority of the patients 30 (6%) came from Bamburi village and 107(21.4%) from Mombasa District. From the samples collected, the common complaints from patients were fever, jaundice and rash. Of the 500, 97(19.4%) manifested with jaundice, 156(31.2%) manifested with rash and 2(0.8%) manifested with bleeding.

ELISA assay

By IgM ELISA, a total of 75(15%) samples were positive for Dengue. Age group 26-30 had the most number of DENV infections 12(2.4%). From this age group 54(10.8%) were male while 45(9.0%) were female. When the children are categorised below 16 years and adults above 16 years, the results show that 62(12.4%) of the DENV positive samples by IgM were from adults. From this category 193(38.6%) of the adults were male while 170(34%) were female. For the children 65(13.0%) were male while 61(12.2%) were female. Of the 75 patients in whom dengue virus was detected by ELISA, 75(100%) had fever, 12(16%) had jaundice, 1(1.3%) suffered bleeding and 24(32%) had rash.

FRNT assay

Of the samples that tested positive by IgM ELISA, 58 (11.6%) neutralized DENV1, DENV 2 which was predominant neutralized 73 (14.6%), 68 (13.6%) neutralized DENV 3 and 34(6.8%) neutralized DENV4 as shown in figure 1 below.

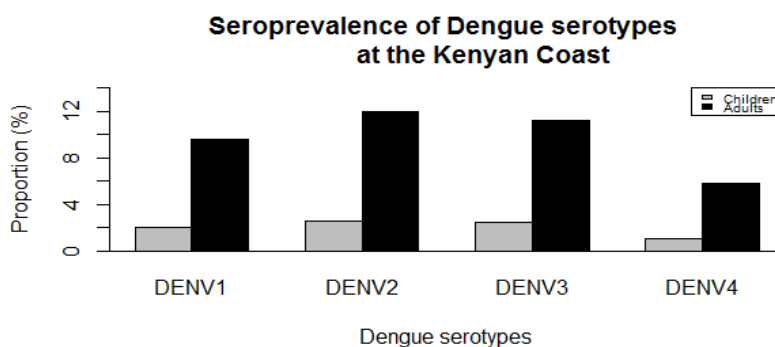


Figure 1. DENV serotypes in children and adults

Of the samples that neutralized DENV, 58 (11.6%) neutralized both DENV 1 and 2, 53(10.6%) Neutralized both DENV 1 and 3, 33(6.6%) neutralized DENV 1 and 4, 66(13.2%) samples neutralized DENV 2 and 3, 34(6.8%) neutralized DENV 2 and 4 while 31(6.2%) neutralized DENV 3 and 4. In figure 2 below, of the 75 samples positive by IgM, 4(5.3%) neutralized one serotype, 14(18.7%) neutralized 2 serotypes, 27(36%) neutralized 3 serotypes and 30(40%) samples neutralized all four DENV serotypes.

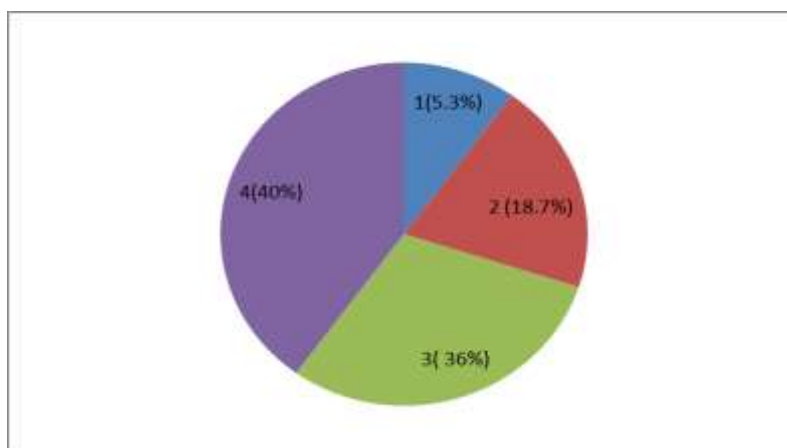


Figure 2. 1, exposure to one serotype; 2, exposure to two serotypes; 3, exposure to three serotypes; 4 exposure to all four serotypes.

In the univariate analysis done of the data as shown in table 1, age (OR 1.02, 95%CI 1.00-1.03, P 0.0103) was a significant factor in having DENV. Of this adults showed a more likely association in getting the virus (OR 1.90, 95%CI 1.04-3.73, P 0.047). Occupations that showed significant associations included, businessmen (OR 0.09, 95%CI 0.00-0.81 P 0.0493), pupils (OR 0.11, 95%CI 0.02-0.73 P 0.0183) this also included the children who have not yet begun going to school (OR 0.19, 95%CI 0.04-1.7, P 0.0447). On multivariate analysis done those who came from Mshomoroni District showed significance of 0.041 with being infected by the virus.

Table 1.Univariate Analysis of Socio-Demographic Factors

Covariate	OR	95% CI	P-value
Age (ungrouped)	1.02	(1.00-1.03)	0.0103
Age group			
Children	1		
Adults	1.90	(1.04-3.73)	0.047
SEX			
Female	1		
Male	1.03	(0.63-1.70)	0.898
OCCUPATION			
Accountant	1		
Business	0.09	(0.00-0.81)	0.0493
Child	0.19	(0.04-1.70)	0.0447
Pupil	0.11	(0.02-0.73)	0.0183

IV. Discussion

Although dengue epidemics are infrequent in eastern Africa when compared with south-east Asia, the Americas and the Caribbean, all four serotypes of dengue have caused outbreaks in this region. In 1982, an outbreak of dengue fever caused by dengue virus 2 (DENV 2) was reported in the Kenyan coastal towns of Malindi and Kilifi[11]; clinical presentation was consistent with classical dengue fever, with no severe dengue reported. Since then there have been sporadic cases of dengue reported in Kenya. Five hundred samples were selected from January 2014 to March 2015 for this study. The results show that adults are more likely to get the virus (OR 1.90, 95%CI 1.04-3.73, P 0.047). Age group 26-30 had the most number of DENV infections 12(2.4%). This group as observed comprises of adults. Many studies have proven that adults are more susceptible but no clear reason has been identified, one contributing factor though might be that the study had more adults as compared to any other age group,[12]. Despite this, findings from this study provide strong empirical evidence that age is an important factor in determining risk for DENV. When the children are categorised below 16 years and adults above 16 years, the results show that 62(12.4%) of the DENV positive samples by IgM were from adults. This study correlates with one done in Belém and Ananindeua in Pará State, Brazil[12-14]. Children are usually the most affected age group, but in recent decades, dengue and severe

dengue have become more common among adults[15]. From the results, all the patients came from Mombasa County there were no travellers at the time. Patients in this study came from 45 districts and 122 villages. Majority of the patients 30 (6%) came from Bamburi village and 107(21.4%) from Mombasa District. Those who came from Mshomoroni District showed significance of 0.041 being infected by the virus.

The principal symptoms of dengue are high fever, severe headache, rash, low white cell count and joint pain. DHF is characterized by a fever that lasts from 2 to 7 days, with general signs and symptoms consistent with dengue fever. The smallest blood vessels become excessively permeable (leaky) allowing the fluid content to escape from the blood vessels into the peritoneum and pleural cavity. This may lead to failure of the circulatory system and shock and possibly death. The patient with DHF has a low platelet count and haemorrhagic manifestations [16]. Symptoms covered in this study include fever which was a key criterion of sample selection rash, jaundice and bleeding. Of the 500, 97(19.4%) manifested with jaundice, 156(31.2%) manifested with rash and 2(0.8%) manifested with bleeding. The prevalence of Dengue virus at the Coast in 2014-2015 was 15%. All the four dengue virus serotypes were found to be co-circulating with DENV 2 being the predominant serotype. DENV 2 which was predominant neutralized 73 (14.6%). From a study conducted from February 2012 to July 2012 among patients visiting Coast Province General Hospital[17]. This shows that there is an increase in the prevalence of DENV at the Kenyan Coast.

There has not been any report of death this far even in the presence of multiple infections by the different serotypes. As it has been documented before, multiple infections with different serotypes has led to severe Dengue characterised by Dengue Shock Syndrome and Dengue Haemorrhagic Fever [18, 19], both of which have not been reported at the Kenyan Coast[20]. The results show that of the samples that tested positive by IgM ELISA, 58 (11.6%) neutralized DENV1, DENV 2 which was predominant neutralized 73 (14.6%), 68 (13.6%) neutralized DENV 3 and 34(6.8%) neutralized DENV4. From the samples that neutralized DENV, 58 (11.6%) neutralized both DENV 1 and 2, 53(10.6%) Neutralized both DENV 1 and 3, 33(6.6%) neutralized DENV 1 and 4, 66(13.2%) samples neutralized DENV 2 and 3, 34(6.8%) neutralized DENV 2 and 4 while 31(6.2%) neutralized DENV 3 and 4. Out of the 75 samples positive by IgM, 4(5.3%) neutralized one serotype, 14(18.7%) neutralized 2 serotypes, 27(36%) neutralized 3 serotypes and 30(40%) samples neutralized all four DENV serotypes. Dengue is an arthropod borne virus spread by the *aedes aegyptimosquito*[21]. A factor that has influenced the spread of the co-circulation is the geographic distribution and population density is the increased number of *A. aegypti* especially in urban areas of the tropics[2]. This is evidence enough to show that there is co-circulation of the DENV serotype at the Kenyan Coast. We observe a marked increase of temperature in all four serotypes co infection from 19 to 40%. Most of the patients experienced Dengue Fever with temperatures ranging from 38⁰ to 40⁰C. This result shows that it is more common to find individuals with a co infection of all four serotypes as opposed to the rest.

V. Conclusion

The virus continues to be present even after the outbreak that occurred in 2013. The study shows that DENV 2 was the predominant serotype. The prevalence continues to be in the rise. It is evident from this study that DENV serotypes 1-4 are co-circulating at the Kenyan Coast and the patients who are co infected with two or more of the serotypes are more as compared to those infected with a single serotype. The co-circulation of dengue virus serotypes suggests that dengue virus is endemic at the Kenyan coast. Even with this there is still no sign of DSS and DHF in the study area.

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