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Dengue and West Nile Virus Transmission in Children and Adults in Coastal Kenya

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Abstract. Dengue virus (DENV) and West Nile virus (WNV) are important reemerging arboviruses that are underrecognized in many parts of Africa due to lack of surveillance. As a part of a study on flavivirus, alphavirus, and parasite exposure in coastal Kenya, we measured neutralizing antibody against DENV and, to evaluate assay specificity, WNV in serum samples that tested positive for serum anti-DENV IgG by enzyme-linked immunosorbent assay. Of 830 anti-DENV IgG-positive samples that were tested for neutralizing activity, 488 (58.8%) neutralized DENV and 94 (11.3%) neutralized WNV. Of children ≤ 10 years of age, 23% and 17% had serum neutralizing antibody to DENV and WNV, respectively, indicating that DENV and WNV transmission has occurred in this region within the past decade. The results suggest that ongoing DENV and WNV transmission continues on the coast of Kenya and supports a need for routine arboviral surveillance in the area to detect and respond to future outbreaks.

Dengue virus (DENV) and West Nile virus (WNV) are flaviviruses that have been reemerging as important global health threats. It is estimated that DENV causes nearly 400 million infections per year.[1] WNV is one of the most important arboviral causes of encephalitis worldwide. It is known to cause disease on all continents, save Antarctica,[2] in the form of epidemics or endemic infections. Yet, in places such as Kenya and other African countries that lack routine arboviral surveillance programs, the transmission and overall burden of DENV and WNV infection is largely unknown. Without such information, the ability to detect and respond to outbreaks is severely hampered.

Epidemic DENV infection in Kenya has been reported previously,[3] with the most recent outbreak occurring in 2013 in Mombasa.[4] However, data on human WNV infections in Kenya are sparse.[5–7] In this report, we present evidence of transmission of DENV and WNV in residents living on the coast of Kenya within the past decade, which supports the need for greater surveillance of these emerging infections.

The main goal of this study was to investigate DENV seroprevalence, as an extension of a previously reported study of alphavirus seroprevalence among residents of two village clusters in coastal Kenya.[8] Participants were enrolled in protocols approved by the institutional review boards (IRBs) of Case Western Reserve University (protocol no. 11-07-42), Stanford University (IRB-31488), Children's Hospital and Research Center Oakland (IRB 2013-023), and the Kenya Medical Research Institute (SSC 2611) as part of a larger study of polyparasitism, conducted between 2009 and 2011.[9,10] For the original study, a census was performed for each of five village clusters. All residents ≥ 1 year of age were eligible to participate.[10] Consent from adult participants was obtained in the participant's native language, and child assent with parental consent was obtained for participants who were 7–17 years of age. For this study, stored serum samples from healthy participants from two of the five village clusters that were part of the original study were tested with approval from the Ethical Review Committee of the Kenya Medical Research Institute, and the IRBs of Children's Hospital and Research Center Oakland and Stanford University.

To investigate DENV seroprevalence, we assayed samples for anti-DENV IgG by indirect enzyme-linked immunosorbent assay (ELISA). In brief, serum samples, diluted 1:100, were tested for reactivity to pooled lysates of Vero cells infected with each of the four DENV serotypes, as previously described.[11–13] Samples were considered positive if they produced an optical density (OD_{405}) value against DENV-infected cell lysates that was ≥ 4-fold over the respective OD_{405} measured against lysates from uninfected cells. Because of the concern for potential cross-reactivity of antibodies against heterologous flaviviruses to the ELISA DENV antigen,[14–16] we used a plaque reduction neutralization test (PRNT) to assess specificity of serum antibodies for neutralizing DENV-2 (strain PR9-11). As the heterologous flavivirus control, we also performed PRNT against WNV (strain EG101). Briefly, dilutions of heat-inactivated serum samples (56°C, 30 minutes) were incubated with either DENV-2 or WNV, and the mixtures added to monolayers of Vero cells. After a binding period, cells were overlaid with 0.4% agarose in cell culture medium and incubated at 37°C for 4–5 days. The PRNT titer was defined as the reciprocal of the serum dilution that yielded an 80% decrease in virus plaque-forming units compared with a virus-only back-titration control.[8] Titers ≥ 10 were considered positive for virus-neutralizing activity. For samples that neutralized both DENV and WNV, a ≥ 4-fold difference in titer was interpreted as presence of neutralizing antibody directed against one virus with possible cross-reactivity against the other. If there was < 4-fold difference in titer, then neutralizing antibody specificity could not be determined, and the sample was interpreted as having neutralizing antibody against both viruses.

A total of 1,863 samples were available for testing, from participants 1–99 years of age, who resided in one of two village clusters in Kwale County, coastal Kenya: Vuga (4.19°S and 39.51°E) and Milalani-Nganja (4.47°S and 39.46°E). Of the participants, 850 were from the village of Vuga and 1,013 were from Milalani-Nganja. Vuga and Milalani-Nganja are separated by about 40 km. Elevation of Vuga is

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TABLE 1
Samples tested by PRNT

DENV IgG ELISA	*n* (%)	No. tested by PRNT	PRNT positive *n* (%)			PRNT negative *n* (%)
			DENV	WNV	Both	
Positive	895 (48)	830	488* (58.8)	94* (11.3)	48 (5.8)	296 (35.7)
Negative	968 (52)	107	5 (4.7)	10 (9.3)	1 (0.9)	93 (86.9)

DENV = dengue virus; ELISA = enzyme-linked immunosorbent assay; PRNT = plaque reduction neutralization test; WNV = West Nile virus.
*Data include samples that were positive for both DENV and WNV.

approximately 150 m, and Milalani-Nganja is at 25 m above sea level. Of the participants, 57.3% from Vuga and 55.7% from Milalani-Nganja were female. The median age of Vuga participants was 17 years (range: 2–88 years) and of Milalani-Nganja participants was 18 years (range: 1–99 years).

Of the 1,863 samples, 895 (48%, 95% confidence interval [CI]: 45.8–50.3%) were positive for IgG against DENV by ELISA. There were no significant differences in overall percentage of positive samples from each village. However, fewer samples from Nganja males were positive than were samples from Nganja females (40% versus 55%, respectively, $P = 0.0019$). Although this difference was not observed for other villages (49% males versus 53% females, $P = 0.23$ and 46% males versus 46% females, $P = 0.89$, for Milalani and Vuga, respectively), the lower percentage of samples that were ELISA IgG-positive from Nganja males affected the overall frequency of positive sera from all males versus all females (45% versus 50%, respectively, $P = 0.017$).

Of the IgG-positive samples, 830 had sufficient volumes available for testing by PRNT. Of these IgG-positive samples, 488 (58.8%) were positive for neutralizing activity against DENV (Table 1). Ninety-four samples (11.3%) were positive for neutralizing activity against WNV, including 48 (5.8%) samples that neutralized both DENV and WNV. Of particular interest, there were 46 (5.5%) samples that neutralized WNV, but not DENV, yet nevertheless were positive by DENV IgG ELISA.

There was no difference in PRNT positivity by gender for DENV (51.5% of males versus 53.3% of females positive, $P = 0.59$, Fisher's exact test) or WNV (10.8% of males versus 11.4% of females, $P = 0.83$). However, more subjects from Vuga had serum DENV-neutralizing activity than did subjects from Milalani-Nganja (56.6% versus 49.2%, respectively, $P < 0.03$). In contrast, more subjects from Milalani-Nganja had neutralizing activity against WNV than did Vuga subjects (18.3% versus 2.8%, respectively, $P < 0.001$).

When subjects were grouped into 5-year age groups, the proportion of samples that were positive for neutralizing antibody was different between the age groups for DENV (Figure 1, black bars; $P < 0.001$, analysis of variance), but not for WNV (gray bars, $P = 0.13$). However, we did observe that serum samples from subjects 71 years and older were more frequently positive for WNV-neutralizing activity than samples from subjects 70 years or younger (33.3% versus 10.3% positive, respectively, $P = 0.0005$, Fisher's exact test). The higher seroprevalence for WNV-neutralizing activity in the older age group could be consistent with a WNV outbreak among this population about 70 years ago. We observed serum-neutralizing activity against DENV in children as young as 3 years of age, and against WNV in children as young as 1 year. Overall, 23% and 17% of children aged $\leq$ 10 years had serum-neutralizing antibody to DENV and WNV, respectively, providing evidence that DENV and WNV transmission has been actively occurring within the past decade on the Kenyan coast.

Some individuals had neutralizing activity against both DENV and WNV. This may represent previous exposure to both viruses. Alternatively, different human serum antiflaviviral antibodies previously have been shown to bind and neutralize heterologous flaviviruses.[14–16] It is therefore possible that anti-DENV neutralizing antibodies cross-reacted and neutralized WNV, or vice versa. For example, an anti-DENV monoclonal antibody has been shown to neutralize both DENV and WNV.[17] However, others have reported that humans immunized with experimental DENV vaccines did not develop neutralizing activity against WNV.[18] Further, we are unable to exclude the possibility of cross-reacting antibodies elicited by other flaviviruses, such as yellow fever (YFV) or Zika virus (ZIKV) or an as-yet unidentified flavivirus, that may neutralize DENV or WNV. Although YFV has been observed in western Kenya, it has not been reported from the Kenyan coast, and YFV vaccine is not routinely administered in these areas.[19] ZIKV also has not been reported from the Kenyan coast. Although sera from patients acutely infected with DENV have been shown to neutralize ZIKV,[20] it remains unclear whether antibodies elicited by ZIKV can neutralize DENV or WNV.

Among the samples with WNV-neutralizing activity, 46 were positive by ELISA for DENV IgG yet did not neutralize DENV by PRNT. Further experiments outside the scope of this study are needed to determine whether these samples contained both non-neutralizing antibodies to DENV and neutralizing antibodies to WNV, or whether WNV-neutralizing antibodies cross-reacted with DENV on ELISA, but were unable to neutralize the DENV virus. The latter possibility

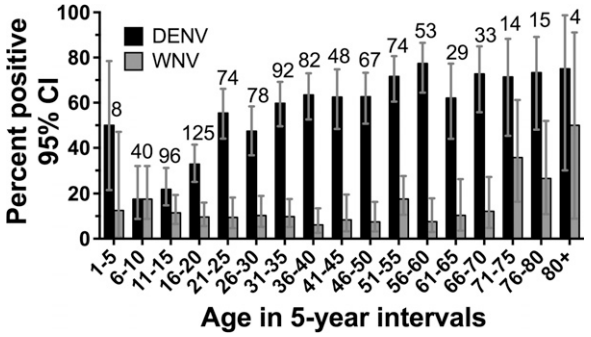

FIGURE 1. Percent of plaque reduction neutralization test tested samples that were positive, stratified by age in 5-year increments. The numbers above the bars are the number of samples tested per age group. Dengue virus (DENV) prevalence is represented in black bars, and West Nile virus (WNV) prevalence is represented in gray bars. Error bars represent 95% confidence interval (CI).

raises the question of whether antibodies against other flaviviruses that bind, but do not neutralize DENV, affect the risk of developing severe dengue disease via antibody-dependent enhancement of infection.

Overall, the finding of WNV and DENV exposure in children aged ≤ 5 years indicates recent transmission of these arboviral infections and supports the need for greater arboviral surveillance. The unanticipated emergence or reemergence of arboviral disease in recent years highlights the limits of our understanding of the dynamics that govern transmission of arboviruses. Without sufficient monitoring and surveillance programs to understand better the ecology of arboviruses, we will remain unprepared to prevent future epidemics from both unknown and known arboviruses.

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