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Short Report: Serologic Evidence of Arboviral Infections among Humans in Kenya

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Abstract. Outbreaks of arthropod-borne viral infections occur periodically across Kenya. However, limited surveillance takes place during interepidemic periods. Using serum samples obtained from asymptomatic persons across Kenya in 2000–2004, we assessed (by indirect immunofluorescent assay) prevalence of IgG against yellow fever virus (YFV), West Nile virus (WNV), tick-borne encephalitis virus (TBEV), dengue virus serotypes 1–4 (DENV1-4), and chikungunya virus (CHIKV). Older persons on the Indian Ocean coast were more likely to be seropositive than children inland: YFV = 42% versus 6%, WNV = 29% versus 6%, TBEV = 16% versus 6%, DENV-1 = 63% versus 9%, DENV-2 = 67% versus 7%, DENV-3 = 55% versus 6%, DENV-4 = 44% versus 8%, and CHIKV = 37% versus 20%. Among inland samples, children in lowlands were more likely to be seropositive for CHIKV (42% versus 0%) than children in highlands. In Kenya, transmission of arboviral infection continues between known epidemics and remains common across the country.

Arthropod-borne viral outbreaks have occurred sporadically across regions of Kenya but few surveillance efforts have taken place during interepidemic periods. Examples of recent documented outbreaks of arboviral diseases in Kenya include yellow fever in 1992[1,2] and 1994–1995,[3] chikungunya fever in 2004,[4] and Rift Valley Fever in 1997[5] and 2006.[6] Dengue virus serotype 2 was initially isolated from patients along the coast of Kenya in 1982 and from additional positive cases in subsequent years, suggestive of a more extensive circulation of dengue virus than commonly noted.[7] Limited surveillance efforts in the years between outbreaks suggest low-level endemic transmission even outside initial geographic outbreak boundaries.[2,7–9] Many more diseases of arboviral origin have likely occurred but have gone unrecognized, especially in more remote locations.

Medically important arthropod vectors flourish in many parts of Kenya.[7,10,11] Given the vectorial capacity for arboviral transmission in the region and the history of confirmed arboviral outbreaks, serosurveys are an important component for understanding the circulating, but perhaps rarely diagnosed, human arboviral transmission prevalence. Currently, there are no countrywide serosurvey data regarding arbovirus infection across Kenya or the types of human arboviral infections that are most prevalent.

Although emergent disease outbreaks in Kenya have confirmed the presence of a number of arboviral diseases within the human and animal populace, much still remains unknown regarding the true prevalence of arboviral infections, in part because of spotty surveillance and clinical misdiagnosis. Historic serosurveys in Kenya have documented a number of arboviruses including West Nile virus, o'nyong-nyong virus, dengue virus, chikungunya virus, and Kadam virus, even in the absence of clinical outbreaks.[12,13] Flaviviral infections, such as yellow fever, West Nile fever, and especially dengue fever, are among the most common arboviral infections worldwide, but the resulting symptoms are non-specific, leading to misdiagnosis.[14–16] For example, chikungunya fever, caused by the alphavirus chikungunya virus, is an emerging infectious disease in endemic and non-endemic regions and can be misdiagnosed as dengue fever, which is largely sympatric in the Old World.[16–21]

Because the clinical presentation of arboviral disease is often non-specific, reliance on diagnostic testing becomes necessary for accurate differentiation.[22,23] Current laboratory methods are limited by lack of specificity and make conclusive diagnosis difficult.[23,24] Accurate detection of arboviral circulation and clinical cases could lead to improved prevalence and incidence estimates with more tailored patient care, rapid control intervention, and direct public health benefit for disease-endemic communities. An accurate determination of baseline prevalence of arboviral diseases across Kenya could contribute to early detection of epidemics and enable rapid prevention of disease spread through targeted control measures, public health education, and early vaccination campaigns.

This study ascertained the presence of IgG in human serum samples against yellow fever virus (YFV), West Nile virus (WNV), tick-borne encephalitis virus (TBEV), dengue virus serotypes 1–4 (DENV1-4), and chikungunya virus (CHIKV) as an indication of the ongoing circulation of human arboviral disease in Kenya. Serum samples were tested from two accessible population-based studies: 1) a 2000–2003 study of 419 pregnant women (15–46 years of age) in villages of Msambweni District, Coast Province (4°27′49″S, 39°28′24″E), and 2) a 2004 study of 122 inland children (3–15 years of age) (Figure 1). Sixty-five of the pediatric samples were obtained in the highlands of Rift Valley Province, Kipsamoite Village, Nandi District (0°12′0″N, 35°6′0″E) and 57 samples were obtained from the western lowlands of Nyanza Province, Kanyawegi Village, Kisumu District (0°5′51″S, 34°45′16″E).[25]

Indirect immunofluorescent assays (IFAs) (EuroImmun AG, Lübeck, Germany) were performed for detection of antibodies against viral antigens. The flavivirus IFA protocol enables rapid interpretation of IgG results in human serum samples. Each IFA slide contained multiple fields consisting of biochips containing infected Vero E6 cells with known arboviral antigens and uninfected Vero E6 cells. Flavivirus Profile 2 slides were used to test WNV, DENV1-4, YFV, and TBEV simultaneously. These slides had specificities between 94% and 100% and sensitivities between 93% and 100% for each virus.

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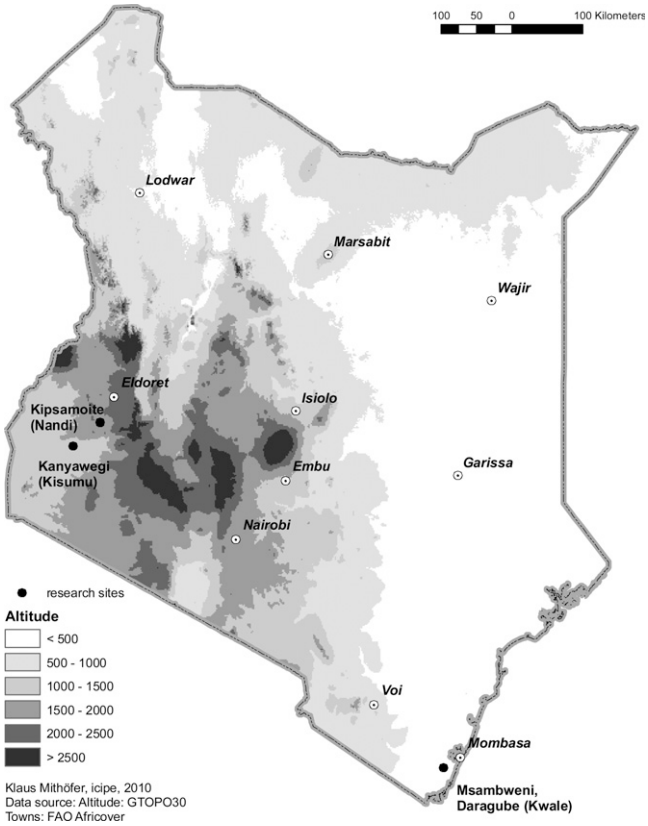

FIGURE 1. Map of study region, Kenya.

CHIKV antibodies were tested on a separate slide and had a sensitivity of 96.1% and a specificity of 100%. Diluted serum samples (1:10) were tested in accordance with the manufacturer's recommendations. If the serum was positive, human IgG in the sample bound to the viral antigens and was stained with fluorescein and visualized by using a fluorescence microscope. Fluorescence of each sample was compared with virus-specific positive and negative controls and evaluated on the basis of intensity as recommended by the manufacturer. Samples with greater intensity fluorescence than the positive control were considered positive for presence of the respective IgG.

Numerous IgG-positive samples were identified in all regions of our retrospective study of serum samples from Kenya, indicating a much greater prevalence of circulating arboviruses in Kenya than previously documented. Twenty percent (n = 25, 95% confidence interval [CI] = 13.99–29.23) of inland samples were positive for at least one flavivirus and 20% were positive for CHIKV (n = 24, 95% CI = 13.29–28.31). Seventy percent (n = 294, 95% CI = 67.84–76.81) of coast samples were positive for at least one flavivirus and 37% were positive for CHIKV (n = 153, 95% CI = 32.75–42.45). IgG against DENV-2 was most prevalent in Kenya overall (n = 289, 53%, 95% CI = 53.53–62.36) and on the coast (n = 281, 67%, 95% CI = 63.77–75.77), which is consistent with previously documented outbreaks of DENV-2 infection (Table 1).[6] DENV-1 was the most prevalent flavivirus inland (n = 11, 9%, 95% CI = 4.66–15.86), However, CHIKV IgG was the most commonly identified antibody overall (n = 24, 20%, 95% CI = 13.29–28.31) in inland samples. Forty-two percent (n = 24, 95% CI = 29.14–55.92) of the Kisumu (lowland) samples were positive for CHIKV IgG; these positive samples were evenly distributed between the sexes. There were no CHIKV IgG-positive samples identified in the Nandi (highlands) dataset. Subsequent to IFA testing, plaque reduction neutralization testing (PRNT) was performed on the IFA CHIKV-positive samples by using the 181/clone 25 vaccine strain[26] and standard methods.[27] All IFA CHIKV-positive samples were also positive by PRNT, with titers in the range of 1:40 to > 1:1,280.

The IFA study data demonstrate the high level of human infection by varied arboviruses in two regions of Kenya. DENV-2 is now believed to be endemic to Kenya and Somalia, where DENV-3 has also been identified.[7] In this study, evidence of DENV-2 was most common, followed by evidence of DENV-1, DENV-3, and DENV-4. Cross-reactivity among flaviviruses prevents conclusive determinations of which dengue serotypes are circulating in Kenya, but previous studies have identified DENV-1 and DENV-2 in human samples, and DENV-3 has been found in nearby Somalia.[7,12] Given the inter-continental trade routes between Kenya and other dengue-endemic countries, the circulation of multiple DENV serotypes is not unexpected.

Levels of IgG against arboviruses in coastal Kenya, the site of recent chikungunya fever outbreaks, indicates that additional arboviruses, including alphaviruses, circulate at low levels, often undetected by persons or their local health care providers. Given the transient nature of some arboviral infections and the wide variation in disease spectrum including asymptomatic infection, it is likely that many infected persons and circulating arboviruses are not captured by surveillance efforts. Of samples positive for at least one flavivirus, most were from persons ≥ 25 years of age (n = 166; $P = 0.0014$) suggesting that the chance for arboviral exposure continues over time, yielding cumulative increases in IgG seropositivity with age.

High levels of IgG against flaviviruses and alphaviruses in the pediatric population in Nyanza Province and Rift Valley Province were unexpected. Evidence of YFV and CHIKV exposure among inland children highlights ongoing low-level transmission because many of these children were born after the last documented outbreaks and are too old to have circulating maternal antibodies. YFV IgG may have been caused by vaccination (immunization status is unknown for these children), but is unlikely given that the respective villages were not included in any yellow fever vaccination campaigns. CHIKV IgG was present in a high proportion of samples on the coast and in Kisumu District, indicating continued circulation after

TABLE 1
Seroprevalence of IgG against arboviruses, by study site, Kenya*

Region and group	TBEV	WNV	YFV	DENV-1	DENV-2	DENV-3	DENV-4	CHIKV
Coastal, women (n = 419)	69 (16)	123 (29)	176 (42)	263 (63)	281 (67)	232 (55)	185 (44)	153 (37)
Inland, all (n = 122)	7 (6)	7 (6)	7 (6)	11 (9)	8 (7)	7 (6)	10 (8)	24 (20)
Kisumu, lowlands, children (n = 57)	5 (9)	5 (9)	3 (5)	6 (11)	5 (9)	4 (7)	4 (7)	24 (42)
Nandi, highlands, children (n = 65)	2 (4)	2 (4)	4 (7)	5 (9)	3 (5)	3 (5)	6 (11)	0 (0)

*Values are no. (%) reactive. TBEV = tick-borne encephalitis virus; WNV = West Nile virus; YFV = yellow fever virus; DENV = dengue virus; CHIKV = chikungunya virus.

the chikungunya fever outbreaks in 1970[21] but before the 2004 coastal epidemic.[28] Flaviviral antibodies were identified in both district sites, although Kisumu District (lowlands) appears to have a greater diversity of arboviruses infecting humans.

Alphaviral antibodies, specifically those against CHIKV, were found only in children from Kisumu District (lowlands) and not in children from Nandi District (highlands). The geographic and climatic differences between these two regions could provide evidence for varying environmental and social factors related to arbovirus transmission risk. Mosquito vectors are not as prolific in the colder climate of the Kenya highlands (Nandi, elevation = 6,522 feet) and may be less fit with regard to disease transmission. In contrast, the lowlands (Kisumu) offer warmer and wetter areas for mosquito development and could provide an appropriate environment for mosquito vectors and subsequent arboviral transmission.[29]

Given the parameters of this study, there were several limitations. Only previously obtained samples were used, which limited our geographic approach. Although the data demonstrate the presence of IgG against flaviviruses and alphaviruses on the coast and inland near Kisumu, they do not provide seroprevalence information for the remainder of the country. Additionally, our coastal serum samples were limited to female donors, and our inland serum samples were limited to children (< 16 years of age). These sampling constraints could have led to age and sex confounding and therefore restricted our analysis and limited broad application of our conclusions. The lower rates of arboviral disease that we found in inland samples were likely a result of the younger age of the persons tested. Antigenic similarity among alphaviruses and especially flaviviruses results in cross-reactivity among tested samples (Coldren RL and others, unpublished data).[23] The extremely high levels of IFA-identified YFV IgG in the coastal population and the TBEV-positive samples from across the country emphasize the inherent cross-reaction amongst related flaviviruses and alphaviruses, a known complication for arboviral IgG testing methods, including the IFA and the gold standard of PRNT.

Although we can conclude that flaviviruses and alphaviruses are present in Kenya, unequivocal diagnosis is difficult. Modern technologies continue to improve arboviral diagnostics during acute infections. However, definitive tests are still lacking. Because completely accurate diagnosis is not available, we lack a full appreciation of the range and distribution of arbovirus-related disease syndromes in at-risk populations. It would be greatly beneficial, especially for the communities studied, to further develop techniques that can conclusively determine past exposure to and the low-level circulation of precise arboviral infections during interepidemic periods, so that appropriate control measures can be guided by accurate diagnosis and targeted to the specific vector responsible for arboviral disease.

These results indicate continued arboviral circulation of alphaviruses and flaviviruses across Kenya and variable transmission patterns across different regions. Arboviruses continue to circulate even after outbreaks wane. Development of new accurate field testing that differentiates between viral species will enable researchers, physicians, and public health officials to detect the burden of arboviral disease, clarify disease spectra of these illnesses, and provide effective control measures targeted to appropriate vectors.

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