Seroprevalence of Chikungunya Virus (CHIKV) Infection on Lamu Island, Kenya, October 2004

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Abstract. An outbreak of Chikungunya virus (CHIKV) disease associated with high fever and severe protracted arthralgias was detected in Lamu, Kenya, peaking in July 2004. At least 1,300 cases were documented. We conducted a seroprevalence study to define the magnitude of transmission on Lamu Island. We conducted a systematic cross-sectional survey. We administered questionnaires and tested 288 sera from Lamu residents for IgM and IgG antibodies to CHIKV. Chikungunya virus infection (seropositivity) was defined as a person with IgG and/or IgM antibodies to CHIKV, IgM antibodies to CHIKV were detected in 18% (53/288) and IgG antibodies in 72% (206/288); IgM and/or IgG antibodies were present in 75% (218/288). The seroprevalence findings suggested that the outbreak was widespread, affecting 75% of the Lamu population; extrapolating the findings to the entire population, 13,500 (95% CI, 12,458–14,328) were affected. Vector control strategies are needed to control the spread of this mosquito-borne infection.

INTRODUCTION

Chikungunya virus (CHIKV) is a positive single-stranded RNA virus belonging to the family Togaviridae, genus Alphavirus, and the Semliki Forest virus antigenic complex.1–3 The Semliki forest complex constitutes CHIKV, O’nyong nyong virus, Ross River, and Barmah Forest viruses from the South Pacific, which are associated with similar clinical manifestations. O’nyong’nyong, Mayaro, and Ross River viruses are closely related to CHIKV antigenically.4 Chikungunya virus was first isolated from the blood of a febrile patient in Tanzania in 1953.5 “Chikungunya” in the local dialect in Tanzania means stooping or bending, describing the position assumed by patients with the illness.5,8

Chikungunya virus disease is associated with fever, severe arthralgias, rash, headache, and malaise. Other symptoms include muscle aches and retro-orbital pains. Arthralgia can be debilitating and prolonged.4,6,7 Chikungunya disease is rarely fatal but is associated with significant morbidity. Chikungunya illness has an approximate incubation period of 1–2 weeks.

The vectors principally responsible for transmission of the virus are Aedes mosquitoes.6–8 In Africa, CHIKV apparently is maintained in a sylvatic transmission cycle involving primates and forest-dwelling Aedes mosquitoes.9 Sylvatic vectors that have been implicated in transmission include Ae. africanus in East Africa,10 Ae. furcifer, Ae. taylori, Ae. deltsieli, and Ae. luteocephalus in West Africa,9,11 and Ae. taylori and Ae. codellieri in South Africa.12 In contrast, transmission of CHIKV in Asia has been documented to occur mainly in urban areas where Ae. aegypti and Ae. albopictus are the identified vectors.13–15

Chikungunya is a re-emerging disease of public health importance in both African and Southeast Asian countries, causing major outbreaks. Outbreaks in Democratic Republic of Congo,1 Malaysia,16 Indonesia,17 and Senegal18 were reported earlier. An outbreak of Chikungunya occurred on Lamu Island beginning May 2004 and peaked in July 2004. Illness caused by Chikungunya had not been recognized previously on Lamu Island. After that outbreak, other associated outbreaks occurred in Mombasa, Kenya, between November and December 2004, Comoros Islands from January to May 2005 (unpublished data), Reunion Island,19 other islands in the Indian Ocean,20 and in India21 in 2006.

The Ministry of Health, Lamu district, documented at least 1,300 patients meeting a clinical case definition of Chikungunya illness with no deaths. Of 130 specimens collected and sent to KEMRI for testing, IgM antibodies against CHIKV were detected in 60, and CHIKV was isolated by viral culture or detected by polymerase chain reaction in an additional 22 specimens. The extent and scope of CHIKV infection on Lamu Island was unknown. A seroprevalence study was carried out to define the magnitude of the outbreak and make recommendations for prevention and control interventions. This paper describes the findings of the serosurvey.

MATERIALS AND METHODS

Study design. We conducted a cross-sectional seroprevalence study during October 5–9, 2004 (9 weeks after the peak of the outbreak). Serum samples were collected from selected inhabitants whose residences were distributed throughout the entire Lamu Island. We sampled from all 30 preexisting census enumeration areas (EAs) on the island. Sample size in each EA was determined using probability proportional to size sampling (PPS), in which the probability that a particular sampling unit was to be selected in the sample is proportional to the population size of the sampling unit. In each EA, the first household was randomly selected; every subsequent eighth household was chosen for the survey. In each selected household, one resident, irrespective of whether ill or not, was chosen randomly as the survey participant for interview and blood collection, by blindly picking a numbered slip of paper from those placed within a container. Infants (< 1 year old) were excluded from the survey.

Standardized questionnaires were administered by field workers to collect data on demographics, symptoms, and treatment of members of each household. The questionnaires...
were translated into Kiswahili, the local dialect. With informed consent, questionnaires were administered and blood specimens were collected. The Kenya Ministry of Health determined that this study was part of its public health response to the outbreak and that review by an Ethical Review Committee was not required.

**Study site.** Lamu Island covers an area of 102.4 km$^2$ along the Kenyan coast in the Indian Ocean (Figure 1). The projected population for 2004 was 18,000 based on the 1999 census. The Lamu population is predominantly urban and is mostly composed of Swahili-speaking people who are Muslims of Arabic origin.

**Definition of CHIKV infection.** A person with CHIKV infection (seropositive) was defined as any person in whom IgM or IgG antibodies to CHIKV were detected in serum.

**Laboratory diagnosis.** Sera were separated from whole blood specimens and tested at Kenya Medical Research Institute (KEMRI) for IgM and IgG antibodies to CHIKV using antibody capture enzyme-linked immunosorbent assay (MAC ELISA). All sera were heat inactivated at 56°C for 30 minutes before testing for presence of CHIKV IgM using either indirect ELISA or IgG using direct ELISA. For both tests, we used a positive control serum specimen obtained from a previous Chikungunya virus outbreak in East Africa; a negative control serum specimen was from a person from a non-endemic region who tested negative for arbovirus infection. For CHIKV-specific IgM detection, 96-well polystyrene ELISA plates were coated with 1:1,000 dilution of anti-human IgM (Kirkegaard and Perry Laboratories, Gaithersburg, MD) overnight at 4°C. After five washes with phosphate-buffered saline (PBS) with 0.05% Tween-20 (PBS-T), non-specific binding was blocked by adding 5% non-fat dry milk in PBS with 0.5% Tween-20 and incubated for 30 minutes at room temperature. Test serum was added at 1:400 dilution and incubated for 60 minutes at 37°C. Each diluted test serum sample was added in quadruplicate, with two wells serving as positive control (with CHIKV antigen) and two wells serving as negative control. After adding a 1:40 dilution of CHIKV antigen (S-27; Centers for Disease Control and Prevention, Fort Collins, CO), plates were incubated overnight at 4°C before adding a horseradish peroxidase–conjugated alphavirus-specific monoclonal antibody 2A2C-3 (Centers for Disease Control and Prevention) at 1:6,000 dilution and further incubated for 30 minutes at 37°C. Presence of CHIKV antibodies was detected by adding ABTS [2,2’ amino-bis(3-ethylbenthiazoline-6-sulfonic acid)] substrate (Kirkegaard and Perry Laboratories), and the absorbance was read at 405 nm. Positive samples required a mean optical density (OD) value ≥ 0.2 above that of the negative control for each sample. The range of OD values for positive serum specimens in this study was 0.3–1.5 above that of the negative control.

For virus-specific IgG detection, plates were coated with 1:2,000 dilution of CHIKV antigen (United States’ Naval...

**FIGURE 1.** Lamu Island where CHIKV outbreak occurred in 2004 on the map of Kenya (Source of map: Central Bureau of Statistics, Kenya).

**FIGURE 2.** Scatter distribution of IgG and IgM antibodies from study participants.
TABLE 1
Demographic characteristic of participants of Chikungunya virus disease serosurvey, Lamu Island, Kenya, October 2004 (N = 288)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Seropositive (IgG or IgM positive) (n = 215)</th>
<th>Seronegative (IgG and IgM negative) (n = 73)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>34 (34%)</td>
<td>30 (37%)</td>
</tr>
<tr>
<td>Median</td>
<td>32 (32%)</td>
<td>25 (34%)</td>
</tr>
<tr>
<td>Range</td>
<td>1–80</td>
<td>1–80</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>73 (34%)</td>
<td>22 (30%)</td>
</tr>
<tr>
<td>Women</td>
<td>142 (66%)</td>
<td>51 (70%)</td>
</tr>
</tbody>
</table>

Medical Research Unit, Bethesda, MD) overnight at 4°C. Heat-inactivated serum was added at 1:100 dilution and incubated for 60 minutes at 37°C. Each diluted test serum sample was added in quadruplicate, with two wells serving as a positive control (with CHIKV antigen) and two wells serving as a negative control. After adding a 1:3,000 dilution of horseradish peroxidase-conjugated anti-human IgG (Kirkegaard Perry Laboratories), presence of CHIV-specific IgGs was detected by adding ABTS substrate, and the absorbance was read at 405 nm. Positive samples required a mean OD value ≥ 0.2 above that of the negative control for each sample.

Data analysis. Data collected from the questionnaires were entered and analyzed using Epi Info 2002 software (Centers for Disease Control and Prevention). Age, sex, and locality distribution of the seropositive and seronegative participants were compared. Locality was defined as urban (living within Lamu town) or rural (living away from Lamu town).

RESULTS

Among 445 households that were selected, 428 (96%) heads of household agreed to participate. Among the household members (one from each household was randomly selected for blood collection), 302 (71%) consented. Fourteen serum specimens had inadequate volume or were hemolyzed and were not tested; thus, 288 serum specimens were analyzed for IgG and IgM antibodies to CHIKV.

Among 288 serum specimens tested, CHIKV IgG or IgM antibodies were detected in 215 (75%); 206 (72%) had IgG antibodies and 53 (18%) had IgM antibodies. Nine serum specimens with antibodies detected had only IgM antibodies (no IgG detected). For patients with detectable IgG or IgM antibodies, levels of IgG antibodies were higher, as indicated by higher OD values compared with IgM antibodies (Figure 2). Given a population of 18,000 and an attack rate of 75%, we estimate that 13,500 people were infected on the island during the outbreak (95% CI, 12,458–14,328 persons).

Seropositive and seronegative participants were similar regarding sex distribution (Table 1). The highest seropositivity ratio was in the 1- to 4-year-old group (86%) (Table 2), although participants ≥ 15 years old were more likely to be seropositive than those < 15 years old (P = 0.04; Table 2). The proportion of participants who were IgG seropositive was higher than the proportion of participants who were IgM seropositive for all age groups. Among participants ≥ 15 years old, 192 of 257 had CHIKV IgG antibodies detected, which was significantly higher than the proportion of participants < 15 years of age with IgG antibodies (P = 0.002). However, there was no statistical difference in the presence of IgM antibodies among persons ≥ 15 years of age and the younger participants (19% versus 16%; P = 1.00).

Among symptomatic seropositives, 84% (97/115) reported missing work or school for a mean of 7 days (range, 1–90 days). Among asymptomatic seropositives, 84% (97/115) reported being hospitalized or confined to bed at home for a mean of 7 days (median, 7 days), with a range of 1–90 days.

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DISCUSSION

The findings of this serosurvey suggested that the magnitude of the CHIKV outbreak was substantially greater than what was predicted based on the number of cases detected in health facilities. The outbreak may have affected 75% of the population on Lamu Island. There is no documentation of previous outbreaks or cases of alphavirus infection on Lamu Island, although such occurrences would likely not have been studied.

We cannot be certain that all seropositives were infected during the course of this outbreak; however, the disproportionately high IgG, but not IgM, seropositivity of persons ≥ 15 years old compared with younger participants suggested that previous outbreaks of CHIKV or other alphaviruses (because antibodies to other alphaviruses may cross-react with CHIKV antibodies) or sporadic infections might have oc-
curred on Lamu Island. However, our survey was conducted after the peak of the outbreak; thus, it is possible that IgM antibodies had already waned in many participants exposed during the outbreak peak. This hypothesis is supported by data showing higher levels of IgG than IgM in most of the participants with detectable antibodies (Figure 2). In addition, it is possible that persons > 15 years old were more likely to have behaviors that increased the risk of exposure to CHIKV-infected *Aedes* mosquitoes. It is possible that older residents may have been previously infected with CHIKV or other alphaviruses and boosted their IgG antibodies on exposure to CHIKV during this outbreak. At the moment, little is known about the lifecycle of circulatory CHIKV specific antibodies, beyond what is generally known about the kinetics of IgG and IgM antibodies with other immune correlates.

Although persons with IgG or IgM antibodies to CHIKV (seropositives) were distributed throughout Lamu Island, infection seemed to occur more commonly in urban areas. As with dengue, CHIKV vector is most frequently transmitted by the *Ae. aegypti* mosquito,13–15 which tend to thrive in urban areas where more favorable conditions for breeding and transmission exist, including the presence of water storage containers, discarded water holding containers, and other debris in which stagnant water can accumulate.

Symptoms associated with CHIKV infection such as fever, joint pains, and myalgias are non-specific and could be mistakenly identified with a variety of other diseases including dengue, malaria, Rift Valley fever, and influenza. However, pronounced persistent severe joint pains that affect wrists, elbows, fingers, and knees in some patients should raise the suspicion of alphavirus infection, especially chikungunya disease or O’nyong-nyong fever, which also occurred in epidemic form in East Africa in the late 1990s.4,7,23

Among those who were symptomatic, a large proportion (84%) reported absence from work or school because of the illness for prolonged periods. An epidemic of O’nyong-nyong virus infection in East Africa in 1959–1962 caused at least one quarter of employed adults to miss at least 5 days of work.24 Although not commonly associated with mortality, epidemics of Chikungunya disease present public health threats because of substantial morbidity, associated suffering, and loss of economic productivity.

Participants were asked about exposures, which likely occurred months earlier, so there may have been problems with recall, resulting in the loss of important information about illnesses. The refusal rate (29.4%) for blood collection could have resulted in an overestimation of the magnitude of infection, if persons who had been ill or lived in close proximity to people who were ill (and were more aware and concerned about the outbreak) were more likely to agree to participate.

In summary, Chikungunya virus infection during the outbreak on Lamu Island was widespread with a high attack rate, affecting an estimated 75% of the Lamu population. In addition to substantial illness-associated discomfort and suffering, the outbreak most likely had a significant economic impact.

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