SEROPREVALENCE OF CHIKUNGUNYA VIRUS INFECTION ON GRANDE COMORE ISLAND, UNION OF THE COMOROS, 2005

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Abstract. An outbreak of Chikungunya virus (CHIKV) illness associated with high fever combined with prolonged and severe arthralgias occurred on Grande Comore Island from January through May 2005; 5,202 cases were reported. A seroprevalence study was conducted to define the extent of transmission on the island. We conducted a cross-sectional survey using a multistage sampling technique. A total of 481 households were sampled. In each household, one resident was selected randomly for interview and blood collection. We administered questionnaires and tested 331 sera for CHIKV-specific IgM and IgG antibodies by capture enzyme-linked immunosorbent assay. Infection with CHIKV infection (seropositivity) was defined as presence of IgG and/or IgM antibodies to CHIKV. A total of 331 (69%) of 481 survey participants consented to blood collection. Antibodies to CHIKV were detected in 63% of sera; IgM antibodies were found in 60% of specimens and IgG antibodies were detected in 27% of specimens. Extrapolation of the findings to the entire Grande Comore population suggested that nearly 215,000 people were infected with CHIKV during the outbreak. A total of 79% of the seropositive persons were hospitalized or stayed at home in bed for a mean of 6 days (range = 1–30 days); 52% missed work or school for a mean of 7 days (range = 1–40 days). The findings suggest that CHIKV was broadly transmitted during the outbreak with a high attack rate. Although not fatal during this outbreak, CHIKV infection caused significant morbidity and decreased economic productivity.

INTRODUCTION

Chikungunya virus (CHIKV) disease is a febrile, vectorborne viral illness associated with high morbidity. Chikungunya virus is an RNA virus belonging to the family Togaviridae, genus Alphavirus and the Semliki Forest virus antigenic complex.1–3 The complex consists of CHIKV (Africa, Asia), O’nyong-nyong virus (Africa), Ross River virus, Barmah Forest virus (Australia), and Mayaro virus (South America), which all cause similar clinical manifestations and are antigenically closely related.4 Patients with CHIKV illness have fever, severe arthralgias, rash, headache, malaise, muscle aches, and retroorbital pains. The most prominent clinical feature of CHIKV infection is arthralgia, which can be debilitating and prolonged.4–6 The incubation period for this disease is 1–2 weeks. Chikungunya virus was first isolated from the blood of a febrile patient in Tanzania (formerly Tanganyika) in 1953.7 Chikungunya in the local dialect (derived from Kiswahili) in Tanzania means stooping or bending, which describes the position often assumed by afflicted patients.2,7 Since then, CHIKV has caused major epidemics in both Africa and southeast Asia and is a re-emerging agent of public health importance. Recently, outbreaks in Democratic Republic of Congo,1 Malaysia,8 Indonesia,9 Senegal,10 and Kenya (Sergon K and others, unpublished data) have been reported. Illness caused by CHIKV is not typically fatal, but it is associated with significant morbidity with potential substantial effect on labor intensive industries, including agriculture and manufacturing, as well as tourism.

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An outbreak of CHIKV illness was reported on Comoros Island in February 2005, which peaked in late March 2005; 5,202 cases were reported with no deaths. Dengue fever was initially felt to be the cause of the outbreak because previous outbreaks of dengue fever in the Comoros had occurred in 1948, 1984, and 1993. However, serologic and genetic evidence from serum of affected patients confirmed that CHIKV was the etiologic agent in this outbreak. After the outbreak on Comoros Island, CHIKV outbreaks have been reported on other islands in the Indian Ocean (Mauritius, Reunion Island, Seychelles, and Madagascar) and in western India.11–14

The Comoros Islands are an archipelago of four islands and several islets located in the western Indian Ocean (Figure 1). The four major islands are Ngazidja (Grande Comore), Mwali (Moheli), Nzwani (Anjouan), and Maore (Mayotte) (Figure 2). Mayotte is under French administration. The archipelago arose from the seabed of the western Indian Ocean as the result of volcanic activity. The outbreak predominantly occurred on Ngazidja Island (Grande Comore). Grande Comore is the youngest and largest of the islands and is closest to Africa. It has a history of recent massive, volcanic activity and the land surface is completely covered with lava. Because the archipelago arose from the seabed of the western Indian Ocean as a result of volcanic activity, there are no rivers or underground sources of fresh water on Grande Comore. The island’s inhabitants must harvest rainwater, which is then stored in cisterns usually located at the residence. The projected population for Grande Comore Island for the year 2005 was 341,000 based on a census conducted in 2003 with an estimated annual population growth of 2.5%. Islam is the predominant religion. A seroprevalence study was carried out to define the magnitude of the outbreak and to further characterize the clinical spectrum of infection with CHIKV.
MATERIALS AND METHODS

Study design. A cross-sectional survey of the entire Grande Comore Island was conducted from March 18 to March 26, 2005 using a multistage sampling technique. The island was divided into 13 pre-existing administrative units (districts). Sample size for each administrative district was calculated using probability proportionate to size sampling. In each administrative district, 30% of the localities (villages) were selected randomly (for a total of 74 villages). To further scale the number of localities to a workable and appropriate size, 28 localities (30%) were selected randomly from the above 74 for sampling of households.

The number of households sampled in each of the 28 selected localities was calculated using probability proportionate to size sampling. In each selected household, one resident was selected randomly (by picking a number from a hat) irrespective of history of recent illness as the survey participant for interview and for blood collection. Children less than five years of age were excluded. With an alpha of 5%, study power of 80%, and confidence of 95%, the targeted sample size for the study was 461 households on the basis of the assumption of 50% prevalence of CHIKV infection. Informed consent was sought from each selected participant.

Data collection tools. Standardized structured questionnaires were administered by field workers to collect data on demographics, symptoms, and treatment given. The questionnaires were developed in English, translated into French, and administered in French.

Case definition. A case of CHIK virus infection (seropositive) was defined as a person with CHIKV-specific IgM and or IgG antibodies detected in sera by antibody capture enzyme-linked immunoassay.

Processing of laboratory specimens. Sera were separated from whole blood specimens and tested at the Kenya Medical Research Institute for IgM and IgG antibodies to CHIKV using an antibody enzyme-linked immunosorbent assay (ELISA). All sera were heat-inactivated at 56°C for 30 minutes before testing for CHIKV IgM using either an indirect ELISA or CHIKV IgG using a direct ELISA. For both tests, a positive control serum sample obtained from a previous Chikungunya virus outbreak in east Africa was used and a negative sample from a healthy person was used. Chikungunya virus was isolated from six patient sera by cell culture, which confirmed active infection.

For CHIKV-specific IgM detection, 96-well polystyrene ELISA plates were coated with a 1:1,000 dilution of anti-human IgM (Kirkegaard and Perry Laboratories, Gaithersburg, MD) overnight at 4°C. After the plates were washed five times 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 the plates were 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 controls (with CHIKV antigen) and two wells serving as negative controls. After adding a 1:40 dilution of CHIKV antigen (S-27; Centers for Disease Control and Prevention, Ft. Collins, CO), plates were incubated overnight at 4°C, a horseradish peroxidase–conjugated alphavirus-specific monoclonal antibody (2A2C-3; Centers for Disease Control and Prevention) at a 1:6,000 dilution was added, and the plates were incubated for 60 minutes at 37°C. Antibodies to CHIKV were detected by adding 2,2’ amino-bis(3-ethylbenthiazoline-6-sulfonic acid) (ABTS) substrate (Kirkegaard and Perry Laboratories) and the absorbance was read at 405 nm. Positive samples had a mean optical density (OD) value ≥ 0.2 above that of the negative control for each sample.

For virus-specific IgG detection, plates were coated with a 1:2,000 dilution of CHIKV antigen (United States Naval Medical Research Unit, Bethesda, MD) overnight at 4°C. Heat-inactivated serum was added at a 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 positive controls (with CHIKV antigen) and two wells serving as negative controls. After adding a 1:3,000 dilution of horse-radish peroxidase–conjugated anti-human IgG (Kirkegaard

Figure 1. Comoros Island.

Figure 2. Union of Comoros showing Grande Comore (Ngazidja) Island.
Perry Laboratories) CHIV-specific IgG was detected by adding ABTS substrate and the absorbance was read at 405 nm. Positive samples had a mean OD values ≥ 0.2 above that of the negative control for each sample.

Data analysis. Data were entered and analyzed using Epi Info 2002 statistical software (Centers for Disease Control and Prevention).

RESULTS

A total of 481 households were surveyed with 331 (69%) of the selected survey participants consenting to blood collection. Among the 331 serum specimens tested, IgM or IgG antibodies to CHIKV were detected in 209 (63%). IgM antibodies were detected in 198 (60%) of the sera and IgG antibodies were found in 89 (27%) (Table 1); only 11 seropositive sera had IgG, but no IgM antibodies. With an attack rate of 63% for CHIKV infection, (Table 1) and an estimated population of 341,000 people, extrapolation of the serosurvey data suggest that 214,830 persons (95% confidence interval [CI] = 196,757–233,244 persons) were infected on Grande Comore Island during the outbreak.

Seropositivity was 68% (139 of 204) among females compared with 55% (70 of 126) among males. Females were more likely to be infected (seropositive) than males (prevalence odds ratio = 1.71, 95% CI = 1.08–2.7, P = 0.029). The mean ages were similar for seropositive and seronegative participants (Table 2). Participants in all the age groups were more likely to be seropositive than seronegative. The highest seropositivity was in the 45–54-year age group (75%) (Table 3). However, there was no statistical difference in antibody prevalence when comparing young (≤ 15 years of age) and older (>15 years old) participants (P = 0.93). IgM seropositivity was higher than IgG seropositivity in most age groups (Table 4).

The predominant occupations of seropositive participants included housewife (47%), farmer (16%), and student (11%). There was a higher prevalence of seropositive persons in specific districts, especially Bambao (Table 5).

Of those persons who were seropositive, the mean duration of joint pains was 9 days (range = 1–60 days) with knee joints being the most frequently affected (Table 6). Leg muscles were commonly reported to be painful (42%) (Table 6). Paracetamol was taken during the course of illness by 90% of seropositive participants and non-steroidal anti-inflammatory drugs were taken by 26% (Table 7). A total of 30 (14%) seropositive participants did not report fever or joint pains.

Among 168 seropositive participants with fever and joint pains, 132 (79%) were hospitalized or stayed at home confined to bed for a mean of 6 days (range = 2–30 days, median = 5 days, information not available for 41 participants). Infection also affected productivity; among 152 seropositive persons with fever and joint pains, 79 (52%) missed work or were absent from school for a mean of 7 days (range = 2–40 days, median = 6 days, information was not available for 57 patients).

DISCUSSION

The prevalence of antibodies to CHIKV on Grande Comore Island during a recent CHIKV epidemic was 63%. This high prevalence is similar to the seroprevalence of CHIKV on the Kenyan Island of Lamu (75%) after an outbreak of CHIKV infection in 2004 (Sergon K and others, unpublished data). Additionally, a serosurvey conducted during a CHIKV outbreak in Senegal, which occurred in the late 1990s, found a prevalence of 35.3%. In contrast, seroprevalence of IgG antibodies to CHIKV during a non-outbreak period along the Kenyan coast in 1987 was 0.7%, which suggests that sporadically occurring CHIKV infection in the east African region is not a frequent occurrence.
Aedes mosquitoes may be one of the principal vectors of CHIKV. In east Africa, Aedes aegypti mosquitoes, which indicated that patterns of transmission in Asia may be quite distinct from those found in Africa.

Interestingly, in our study, females on Grande Comore Island were 1.7 times more likely to be infected with CHIKV than males. Increased exposure to the predominant vector for CHIKV, the peridomestic Aedes mosquitoes, may be one possible explanation. Aedes aegypti was the principal vector for CHIKV transmission on Comoros Island (Sang R and others, unpublished data). In east Africa, Aedes species mosquitoes feed most frequently from early afternoon to dusk, a time when women sit in and around their homes preparing their evening meals, perhaps placing them in close proximity to mosquito breeding and resting sites (e.g., water storage cisterns around the home) for longer periods than men.

Most seropositive participants had IgM antibodies, which supports the notion that we detected recent infections. The seroprevalence study was done during the peak incidence of the outbreak, which would be consistent with that finding and may have underestimated the seroprevalence of CHIKV because some infections may have occurred after the study was completed. Another recent seroprevalence study done in Lamu found similar overall infection rates with a higher proportion of participants having IgG antibodies to CHIKV (71%); however, that study was done nine weeks after the peak of that outbreak (Sergon K and others, unpublished data).

IgG seropositivity was not more common in older participants than younger participants, which is consistent with the notion that a CHIKV outbreak had not occurred for many years. Higher seroprevalence within some districts suggests that there were common risk factors in certain areas; however, the study did not identify factors associated with increased incidence in the various communities.

In our study, the mean duration of joint pains reported by seropositive subjects was 9 days with a range of 1–60 days. This value may have been greater if the serosurvey had been undertaken later in the outbreak or even after it had subsided. Joint pains associated with CHIKV infection may last for months, can be quite severe and debilitating, and may be a distinguishing clinical feature for CHIKV infection. Laboratory capacity to diagnose malaria, dengue, and CHIKV would be helpful in ecologic settings where all three diseases will be part of a differential diagnosis.

Most cases were treated with paracetamol and/or non-steroidal anti-inflammatory drugs. It is not possible to assess the relative merits of analgesic and anti-inflammatory therapeutic options from this study. Such data would be useful to guide clinical management. Other than effective symptomatic treatment, there is no definitive treatment option that can be provided for ill patients during CHIKV outbreaks.

The findings of this serosurvey suggested that a substantial number of Comoros Island residents (215,000 people) were infected. Although mortality is not typically linked to CHIKV infection, the severe morbidity certainly strained health resources and likely had significant impact on the workforce. At least half of the infected participants were absent from their work place for periods of time up to 40 days. This is even more substantial than an epidemic of O’nyong-nyong virus infection in east Africa in 1959–1962 that caused at least one-fourth of the workforce to miss at least five days of work. Infection with CHIKV is an emerging public health threat that should be addressed through prevention. Because there is no vaccine against CHIKV, the cornerstone of prevention is vector control strategies and environmental manipulation, as is done for dengue control. Key environmental interventions include covering fully all water storage containers, draining stagnant water, and proper waste disposal such as eliminating used tires and other containers that can collect water. Aggressive community education and mobilization is often needed for a control and prevention program to be successful. Vector control activities that have been used in dengue control programs include use of predatory fish in water ponds and cisterns, spraying with insecticide and larviciding.
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