Original Research Article

Seroprevalence of chikungunya fever virus and O’nyong Nyong fever virus among febrile patients visiting selected hospitals in 2011-2012

Trans Nzoia County, Kenya

Kevin K. Kamau1,2*, Gabriel Magoma3, Allan Ole Kwallah2, Charles K. Syengo3, Matilu Mwau2

1Department of Biomedical Sciences, Institute of Tropical Medicine and Infectious Diseases (ITROMID), Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya
2Centre for Infectious and Parasitic Diseases Control Research (CIPDCR), Kenya Medical Research Institute (KEMRI), Busia, Kenya
3Pan African Institute for Basic Sciences, Technology and Innovation, Juja, Kenya

Received: 13 April 2018
Accepted: 05 May 2018

*Correspondence:
Mr. Kevin K. Kamau,
E-mail: kimindiri.kamau@gmail.com

Copyright: © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT

Background: Chikungunya virus (CHIKV) is an alphavirus in the Semliki Forest complex, and is most closely related to O’Nyong Nyong virus (ONNV). CHIKV and ONNV are mosquito-borne alphaviruses endemic in East Africa that cause acute febrile illness and arthralgia. The objectives of this study were to measure seroprevalence of CHIKV and ONNV in selected health facilities in Western Kenya and link it to demographics and other risk factors.

Methods: The study design was cross sectional in selected health facilities. We tested for anti-CHIKV antibodies using In-house Indirect IgG Enzyme Linked Immunosorbent Assay (ELISA) and In-house IgM Capture ELISA and confirmed with Focus Reduction Neutralization Test (FRNT) for specific alphavirus neutralizing antibodies against CHIKV or ONNV. Mean, median and standard deviation were used to summarize the data. Comparisons of means and medians were done using Student’s t test. Prevalence rates were determined using descriptive statistics (e.g. proportions, rates).

Results: From the 382 samples that were successfully collected, 114 (29.84%) had anti-CHIKV antibodies by the ELISA test. Of these, 27 (7.1%) had CHIKV-specific neutralizing antibodies and 5 (1.3%) had ONNV-specific neutralizing antibodies. Age was significantly associated with seropositivity (OR=1.03; P=0.015, 95% C.I 1.01-1.06). Males were less likely to be seropositive (OR=0.67; P=0.358, 95% C.I 0.27-1.52). Risk factors associated with seropositivity included collecting firewood (OR=2.73 95% 1.13- 6.41) and walls with holes and cracks (OR=0.23 95% C.I 0.04 -0.86).

Conclusions: Both CHIKV and ONNV infections were confirmed in the participants’ homes in women and adults, demonstrating undocumented and ongoing transmission in Western Kenya. In 2011 and 2012 CHIKV and ONNV contributed 8.4% of fevers presented in the three selected health facilities in Western Kenya.

Keywords: Chikungunya virus, Focus reduction neutralization test, O’Nyong Nyong virus, Western Kenya

INTRODUCTION

Arthropod-borne viruses (arboviruses) are transmitted by arthropods such as mosquitoes, ticks, midges, and sandflies, and most of them belong to the Togaviridae, Bunyaviridae, Rhabdoviridae, Reoviridae, and Flaviviridae families.1,2 Arboviruses are a major cause of morbidity in sub-Saharan Africa.2 The past two decades have experienced epidemics of Alphaviruses of public health concern such as Chikungunya virus (CHIKV) and
O’Nyong Nyong virus (ONNV) which has resulted in high morbidity in humans. The actual burden of the diseases in human populations is not known. This may be attributed to the lack of sero-epidemiological studies and reliable diagnostic tools. In addition, the role of animals, birds, and arthropod vectors in the transmission and dissemination of these viruses across diverse geographical areas is unclear. Emphasis has been put on other arboviruses that present more severe symptoms such as encephalitis and/or haemorrhagic fevers, which result in high mortality with little attention on Alphaviruses causing arthralgia and fever, leading to high morbidity and long-lasting symptoms.

ONNV and CHIKV are closely related alphaviruses in the Semliki Forest antigenic complex. CHIKV was initially isolated from serum of a febrile male in Tanzania in 1953. Minor outbreaks periodically occurred over the next 30 years, but no major outbreaks were recorded until 2004, when a large epidemic started on the coast of Kenya. Recent studies conducted in Western Kenya show that Arboviruses are circulating. ONNV was initially isolated in Northern Uganda from anopheline mosquitoes and human serum during a 1959 epidemic. During 1959–1962, there was an outbreak of ONNV fever that began in northern Uganda and involved an estimated 2 million people in Kenya, Tanzania, and Uganda. After the epidemic results of seroprevalence studies suggested that sporadic human infections with ONNV virus continued to occur within the region, though no cases of ONNV fever were documented after 1962. High rates of CHIKV fever and ONNV fever have been reported in the Kenyan Coast. Unrecognized ONNV transmission, in particular, has been ongoing and underappreciated in this region.

ONNV causes a similar syndrome to CHIKV, Ross River virus (RRV), and other alphaviruses, characterized by fever, rash, debilitated arthralgia, and myalgia. In contrast to CHIKV, which has recently been shown to produce neurologic manifestations and death in some individuals, ONNV is not known to cause fatal disease. While there is growing research interest in CHIKV as it spreads within Europe and into the Americas, the role of ONNV in endemic regions, especially in sub-Saharan Africa, remains unclear despite its close relationship to CHIKV. A limited number of serosurveys in Western Kenya have availed information about CHIKV but largely ONNV has been left out. In this study, we aimed to determine whether CHIKV and ONNV co-circulate among selected health facilities in Western Kenya.

**METHODS**

**Study design**

The study design used was retrospective cross-sectional in a laboratory based setting using archived human biobanked sera. Samples for this work were collected from Kitale District Hospital, Andersen Medical Center and Endebess sub-district Hospital as part of the parent study protocol SSC 1698. Inclusion criteria included fever of unknown etiology (≥37.5°C) negative for typhoid and/or malaria.

**Study procedures**

After obtaining written informed consent from eligible volunteers, a trained clinician from each respective facility collected demographic information, obtained a standardized clinical history including onset of illness, symptoms and signs present at health care seeking. Five milliliters of human whole blood were collected by venipuncture in vacutainer tubes. The tubes were transported on dry ice to the infectious diseases research laboratory in KEMRI-Alupe where they were processed as soon as possible after collection. The total time taken to transport samples from the field to the laboratory did not exceed 48h. Once in the lab, the whole blood samples were centrifuged at 600xg for 10min to clarify the serum formed. 1ml for serology was aliquoted into two separate 1.5ml screw-cap cryotubes for storage at -80°C in well labelled cryovial boxes that indicated hospital code and position of each index sample. The samples used were anonymous having no names but contained sample identities which were similar to their corresponding questionnaires.

**Ethics statement**

All participants in this study consented to participation. Written consent was obtained from all adult participants; children provided assent with parental written consent. The study was undertaken after approval from the Kenya Medical Research Institute (KEMRI)/ Scientific and Ethics Review Unit (SERU) protocol number SSC 2875.

**Laboratory methods**

**Virus strains and cell cultures**

For Chikungunya, S27 prototype African strain and ONNV strain (SG650) obtained from KEMRI-Arbovirus laboratory, National reference laboratory. Both strains are Bio-Safety Level II containment pathogens in Kenya. The virus strains were propagated in vero (Biken) cells (African green monkey kidney cell line) maintained at 37°C in Eagle’s minimum essential medium (EMEM) (Life Technologies, New York, USA) supplemented with 2% fetal calf serum (FCS) (Life Technologies, New York, USA) and 0.2 mM nonessential amino acids (NEAA) (Life Technologies, New York, USA) and harvested after 3 days. A confluent monolayer of Vero cells was inoculated with the respective virus and then incubated at 37°C. Cells were observed daily for cytopathic effect (CPE); at 80% or more CPE, the infected culture fluid (ICF) was harvested by centrifugation at 600 x g for 10 min at room temperature and stored in 1.5ml cryotubes at -80°C.
**CHIKV indirect IgG ELISA**

The assay developed for this work used purified CHIKV in a standard In-house Indirect IgG ELISA. Positive samples required a titer $\geq 1:3,000$ above that of the negative control for each plate. The OD was read at 492 nm for each well and IgG titers were calculated using a standard curve.

**CHIKV IgM capture ELISA**

We conducted an IgM capture ELISA (MAC-ELISA), with modifications from Bundo and Igarashi, 1985. A sample was considered positive with an OD492/negative control OD492 ratio $\geq 2.0$

**Focus reduction virus neutralization test (FRNT<sub>30</sub>)**

All ELISA positive samples were subjected to FRNT to determine whether seropositivity was due to CHIKV or ONNV infection. Samples were considered CHIKV FRNT positive if the titer was $\geq 20$ and the ONNV titer was four-fold lower than the CHIKV titer. Because there is a unique one-way antigenic cross-reactivity between CHIKV and ONNV. A sample was designated ONNV positive if its titer was $\geq 20$ and four-fold greater than the CHIKV titer.

**Data analysis**

Data were analyzed using R statistic version 3.3.0. Children were defined as those being less than 16 years of age, while adults were those individuals that were 16 years of age or older. Analyses included means, measures of variability, proportions and confidence intervals at the 5% level. CHIKV and/or ONNV seropositivity was the principal outcome measure for the study. Categorical data was tabulated. Mean, median and standard deviation were used to summarize the data. Comparisons of means and medians were done using Student’s t test. Prevalence rates were determined using descriptive statistics (e.g. proportions, rates). Univariate logistic regression was initially carried out for selected variables, based on previous studies and biological plausibility. Odds ratio (OR) and 95% confidence intervals (CI) were calculated. Those variables significant at $p < 0.2$ were entered into the multivariate regression model and retained based on the likelihood ratio test. In Univariate analysis, variables that were associated with the outcome at a significance level of p-value $< 0.05$ were considered to be statistically significant while those with p-value $< 0.1$ were considered in the multivariate logistic regression model. This is helpful in identifying variables that, by themselves, are not significantly related to the outcome but make an important contribution in the presence of other variables. Traditional levels such as 0.05 can fail to identify variables known to be important and that’s why any variable that was significant at the 0.1 level in the multivariate analysis was put in the model.

**RESULTS**

**Characteristics of study participants**

Three hundred and eighty-two venous blood samples were collected successfully from three healthcare facilities in Trans Nzoia County namely; Andersen Medical Centre (AMC), endebess sub district hospital (END) and Kitale District Hospital (KDH). The total samples included 135 (35.3%) adult males, 226 (59.2%) adult females, 10 (2.6%) male children and 11 (2.9%) female children. The range of age in years was between 4 and 87 with a mean age of 32 years. END had the highest number of study participants 151 (39.5%) followed by KDH 131 (34.3%) and AMC 100 (26.2%). The largest age group was between 26-30 years 74 (19.4%). Majority of our study participants came from a rural setting 251 (65.7%) and 131 (34.3%) from an urban setting. Approximately 231 (60.5%) of the study participants were recruited during the long and short rainy seasons. The major symptoms reported were muscle pain 292 (76.4%) and rash 109 (28.5%).

**IgM capture ELISA and indirect IgG ELISA**

Recent CHIKV exposure was checked using an in-house IgM capture ELISA following Bundo and Igarashi, 1985, protocol. A P/N (positive control (or sample) OD492/negative control OD492) ratio $\geq 2.0$ was considered positive. Of the 382 samples, 3 (0.8%) tested seropositive for IgM antibodies. One was a female from KDH 33 years while the other two were male from KDH and END, 35 and 37 years respectively. Indirect IgG ELISA was used to quantify IgG antibodies for previous infection. Of the 382 samples 112 (29.3%) were seropositive, (cut-off point was set as $\geq 1:3,000$). One sample was seropositive for both IgM and IgG antibodies. Seropositivity for anti-CHIKV antibodies by ELISA was 114 (29.8%) while those negative were 268 (70.2%). The majority of CHIKV infections were observed during the long rains with peak transmission in July while still in the other months there was low silent circulation (Figure 1).

**Focus reduction neutralization test (FRNT)**

Comparative FRNTs used CHIKV and ONNV all which are known to have circulated in Western Kenya. Of the 114 samples that were seropositive by ELISA, seven were excluded because of specimen volume limitations that would have required for both CHIKV and ONNV FRNT. We tested 107 samples for neutralizing antibodies against CHIKV or ONNV. Of the three IgM positives, two had IgM neutralizing antibodies for CHIKV while one sample had both IgM and IgG neutralizing antibodies against ONNV. Of the 104 IgG, seropositive samples, 27 (7.1%) had IgG neutralizing antibodies against CHIKV and 4 (1.3%) had IgG neutralizing antibodies against ONNV (Table 1). We found no correlation between ELISA OD and neutralization titre. Antibodies detected
by ELISA cross-react, especially within a genus and particularly for antibodies elicited by alphaviruses and flaviviruses. Immunodiagnosis conventionally is confirmed by virus isolation or FRNT/PRNT.\textsuperscript{25}

![CHIKV Seroprevalence by Month](image)

**Figure 1:** CHIKV seroprevalence by month.

![Western Kenya CHIKV Seroprevalence](image)

**Figure 2:** Seroprevalence of CHIKV by age group as measured by FRNT.

**Univariate and multivariate analysis of demographic and risk factors associated with CHIKV and ONNV infection**

On the univariate analysis for CHIKV infection, males were less likely to be seropositive (P=0.358). Ungrouped age in years was significant in CHIKV seropositivity (OR=1.03; P=0.015, 95% C.I 1.01-1.06). Risk factors that showed significant association included collecting firewood (OR=2.80; P=0.013, 95% 1.21-6.25) and mosquito control activity (OR=4.02; P=0.011 95% 1.24-11.13). Those variables significant at p <0.2 were entered into the multivariate regression model.\textsuperscript{21} On multivariate analysis, risk factors associated with CHIKV seropositivity included collecting firewood (OR=2.73 95% 1.13-6.41), mosquito control activity (OR=3.32 95%
CI 0.97 -9.80), walls with holes and cracks (OR=0.23 95% CI 0.04 -0.86) (P<0.1 Table 2). On univariate analysis for ONNV infection (Table 3), age was the only significant variable (OR=1.08; P=0.002, 95% 1.03-1.14). Males were less likely to be seropositive (P<0.05). On multivariate analysis age was significantly associated with ONNV seropositivity (OR=1.09; P=0.002, 95% 1.04-1.17).

Table 1: Immunologic assays for serum samples testing IgM and IgG positive and their associated alphavirus FRNT Titres.

| Sample ID | CHIKV ELISA | Alphavirus FRNT |
|-----------|-------------|-----------------|
|           | IgM | IgG | CHIKV | ONNV |
| AMC/763/2011 | 0.621 | 8750 | 40 | <10 |
| AMC/823/2012 | 0.771 | 7500 | 40 | <10 |
| KDH/993/2012 | 0.338 | 10,000 | 80 | <10 |
| AMC/832/2012 | 0.658 | 6250 | 80 | <10 |
| AMC/838/2012 | 0.320 | 5625 | 80 | <10 |
| AMC/759/2011 | 0.468 | 32500 | 180 | <10 |
| END/96/2012 | 2.200 | 7500 | 40 | 640 |
| KDH/997/2012 | 0.330 | 25000 | 160 | <10 |
| KDH/984/2012 | 0.352 | 30000 | 160 | <10 |
| AMC/822/2012 | 0.421 | 15000 | 160 | <10 |
| END/10/2012 | 0.362 | 11125 | 160 | <10 |
| END/52/2011 | 0.460 | 7500 | 160 | 640 |
| MBPAMC/129/2011 | 0.318 | 20,000 | 160 | <10 |
| END/56/2012 | 0.372 | 18175 | 240 | <10 |
| END/11/2012 | 0.577 | 7500 | 320 | <10 |
| END/88/2012 | 0.255 | 8,750 | 320 | <10 |
| KDH/941/2011 | 0.759 | 8750 | 320 | <10 |
| KDH/963/2011 | 0.404 | 15,000 | 320 | <10 |
| END/51/2011 | 0.470 | 12,500 | <10 | 320 |
| AMC/842/2012 | 0.320 | 3750 | 160 | <10 |
| MBPAMC/132/2011 | 0.966 | 12,800 | 320 | <10 |
| KDH/972/2011 | 0.350 | 3,000 | 320 | <10 |
| KDH/936/2011 | 0.254 | 3,250 | 320 | <10 |
| MBPAMC/127/2011 | 0.239 | 4,000 | <10 | 640 |
| END/53/2011 | 0.241 | 18,750 | 480 | <10 |
| MBPKDH/140/2012 | 2.464 | 2500 | 640 | <10 |
| KDH/937/2011 | 0.287 | 12,500 | 640 | <10 |
| END/23/2012 | 0.526 | 13750 | 640 | <10 |
| KDH/985/2012 | 2.472 | 1250 | 640 | <10 |
| KDH/994/2012 | 0.340 | 4,000 | <10 | 160 |
| MBPAMC/119/2011 | 0.091 | 3250 | 20 | <10 |
| MBPKDH/135/2011 | 0.530 | 6,250 | 80 | <10 |

Grouping the ages using 5-year intervals the highest seroprevalence by FRNT was observed between 26-30 years and 46-50 years (Figure 2).

CHIKV seropositive cases mostly occurred in adults 26 (6.8%) and less common in children 1 (0.3%), while for ONNV that had 5 cases, two of them occurred in adults aged >65 years. Majority of CHIKV IgG positive cases was observed between the age bracket of 16-40 years 71 (18.6%).
antibodies. ONNV seropositive females were 1.3% (3/237) of which 67% (2/3) had anti-ONNV IgG antibodies. Women were more likely exposed to ONNV than males similarly to CHIKV in our study.

Table 2: Univariate and multivariate regression analysis of demographic characteristics and risk factors associated with chikungunya virus infection.

| Characteristics | CHIKV Univariate analysis | CHIKV Multivariate analysis |
|-----------------|---------------------------|-----------------------------|
| **Demographic characteristics** | **OR (95%C. I)** | **p-value** | **OR (95%C. I)** | **p-value** |
| **Gender** | | | | |
| Male | 0.67 (0.27-1.52) | 0.358 | - | - |
| Female | Ref | - | - | - |
| **Age years (Ungrouped)** | 1.03 (1.01-1.06) | 0.015 * | 1.02 (0.99-1.05) | 0.155 |
| **Age group (years)** | | | | |
| 1-15 years | Ref | - | - | - |
| 16 years and above | 1.55 (0.3-28.40) | 0.674 | - | - |
| **Facility code** | | | | |
| AMC | Ref | - | - | - |
| END | 0.37 (0.12-1.04) | 0.064 | - | - |
| KDH | 0.82 (0.33-2.06) | 0.675 | - | - |
| **Seropositivity** | | | | |
| IgM | 28.32 (2.63-621.84) | 0.007 * | 210.63 (7.58- 10653.79) | 0.002* |
| IgG | 14.12 (1.64-121.78) | 0.009 * | 66.23 (13.58-1195.4) | <0.001* |
| **Year of study** | | | | |
| 2011 | Ref | Ref | - | - |
| 2012 | 1.99 (0.91-4.46) | 0.087 | 1.55 (0.62-3.90) | 0.348 |
| **Risk factors** | | | | |
| Collect firewood | 2.80 (1.21-6.25) | 0.013* | - | 0.023 * |
| Walls with holes/cracks | 2.16 (0.98-4.80) | 0.055 | 1.95 (0.77-4.97) | 0.155 |
| Water bodies near | 0.29 (0.05-1.02) | 0.101 | 0.23 (0.04-0.86) | 0.059* |
| Broken tins and bottles | 1.71 (0.75-3.77) | 0.192 | 1.32 (0.50-3.38) | 0.564 |
| Mosquito control activity | 4.02 (1.24-11.13) | 0.011 * | 3.32 (0.97-9.80) | 0.038 * |

Table 3: Univariate and multivariate analysis of demographic characteristics and risk factors associated with O’Nyong-Nyong virus infection.

| Characteristics | ONNV Univariate analysis | ONNV Multivariate analysis |
|-----------------|---------------------------|-----------------------------|
| **Demographic characteristics** | **OR (95%C. I)** | **p-value** | **OR (95%C. I)** | **p-value** |
| **Gender** | | | | |
| Male | 1.09 (0.14-6.66) | 0.925 | - | - |
| Female | Ref | - | - | - |
| **Age years (Ungrouped)** | 1.08 (1.03-1.14) | 0.002* | 1.09 (1.04-1.17) | 0.002* |
| **Seropositivity** | | | | |
| IgM | 46.87 (1.95- 605.59) | 0.004* | 111.04 (3.92- 2347.44) | |
| IgG | - | - | - | - |
| **Year of study** | | | | |
| 2011 | Ref | Ref | - | - |
| 2012 | 1.01 (0.13- 6.16) | 0.992 | - | - |
| **Risk factors** | | | | |
| Walls with cracks | 0.47 (0.02-3.21) | 0.501 | - | - |
| Surrounding vegetation | 0.58 (0.08-3.52) | 0.550 | - | - |
| Near water bodies | 2.64 (0.34-16.20) | 0.292 | - | - |
| Dumping site near | 0.58 (0.03-3.95) | 0.624 | - | - |
| Broken tins and bottles | 0.59 (0.03-4.05) | 0.640 | - | - |
| Hunt for birds | 1.99 (0.1-13.87) | 0.541 | - | - |
Among the 27 CHIKV seropositive participants’ majority had the following symptoms: muscle pains 74.1% (20/27) and rash 51.9% (14/27) while for the 5 ONNV seropositive participants, two experienced fever (40%) and 80% (4/5) reported muscle pains and one case (20%) had Meningitis meningoencephalitis.

**DISCUSSION**

In Kenya, like many other countries in Africa, infectious disease is part of everyday life. The cause of disease is often unknown or incompletely understood because of nonspecific clinical features, lack of diagnostic laboratory support, or little or no knowledge about disease prevalence in a region. Evidence of transmission rates for ONNV was likely expected, given that the last known outbreak occurred in Rakai district of southwestern Uganda in 1996. Recently an ONNV infection was diagnosed in a German 60-year-old woman traveler who had gone to the lake shores in Kisumu in the year 2013. Serological results showed 1:5,560 for CHIKV IgG. Cross-neutralizing antibodies against CHIKV were detected also, but with a notably lower titer (1:80) when compared with the ONNV titer (1:1,280). Alphavirus exposure, particularly ONNV exposure, was found to be co-circulating with CHIKV in Western Kenya, despite little previous public health attention or research.

There was an overall CHIKV seroprevalence rate of 7.1% and 1.3% seroprevalence rate for ONNV in the present study, covering selected parts of Western Kenya, during the period 2011-2012. In an earlier study by Mwongula et al, 2010 they reported a prevalence of 11% for CHIKV among children aged 1-12 years in Busia county. This difference may be attributed to a lower number of children in our study. A recent study on CHIKV and ONNV conducted in Coastal Kenya reported CHIKV prevalence of 6% and high rate of ONNV transmission at a staggering 56%. Coastal parts of Kenya have reported higher transmission rates than studies done in Western Kenya due to several factors. One is due to the fact that CHIKV epidemic was first reported in 2004 where it caused a major outbreak in the Indian ocean region and has been circulating since and spreading to other parts of the country and continent. Two, evidence that the incubation temperatures of vector directly impact virus transmission by influencing the likelihood of infection and dissemination of CHIKV. Vector competence is higher in mosquito populations from high temperature which weakens the midgut infection barrier. Hence, suggesting Coastal Kenya population is more susceptible to Alphavirus infection therefore having a weaker midgut infection barrier than the Trans Nzoia population. Furthermore; humidity can also play a role, with increased humidity facilitating increased survival of mosquitoes.

Sera positive for anti-CHIKV antibodies by ELISA, most exhibited IgG antibodies suggesting late acute phase disease. IgM antibodies are usually produced within the first few days after onset of illness, while IgG antibodies appear within 7-14 days. One of the characteristic of arbovirus infection is the long-term persistence of IgM, commonly for many months, therefore unlike many other infections, detection of IgM is not, of itself, a completely reliable indicator of recent infection. FRNT is the laboratory standard for immunologic assays. It measures in vitro virus neutralization and is the most virus-specific serologic test to confirm immunologic test results. Testing CHIKV positive IgM sera, one sample had ONNV neutralizing antibodies with a titer of 1:640 as compared to CHIKV titer of 1:40. We demonstrated that the CHIKV ELISA we used can detect antibodies to both viruses and confirmed the results by FRNT (Table 1). Many of our samples had high titer (>20) to both ONNV and CHIKV, however, only 30% (32/107) of the CHIKV ELISA positives had neutralizing antibodies against CHIKV and ONNV. Of the remaining 70% (75/107) equivocal samples that didn’t neutralize, other alphaviruses circulating within the region might be the cause although the remaining Semliki Forest virus complex viruses are more distantly related genetically. The presence of antibody titer against both viruses could be due to several factors: one, both viruses are co-circulating in the region and two, continued evolution of ONNV has made it antigenically more similar to CHIKV than previously reported resulting in greater cross neutralization (a one-way cross reactivity between CHIKV and ONNV has previously been documented with antibodies generated against ONNV typically recognizing only ONNV and not CHIKV another Alphavirus that induces antibodies that cross-neutralize both CHIKV and ONNV could be circulating in the area.

The three sites in Trans Nzoia County offered a forested set up in END and AMC due to nearness to Mt. Elgon and the border to Uganda and KDH an urban set up. However, the seroprevalence rates did not significantly differ between the three health facilities. Collecting
firewood and nearness to a water body were risk factors associated with CHIKV infection (P<0.1).

Women were more exposed to alphavirus infection. It may be because of the cultural cues in the communities whereby they tend to homestay where Aedes mosquitoes are blood seeking as compared to men who are out in the farm fields or in urban towns earning for the family. Age groups 26-30 years and 46-50 years had the most number of CHIKV and ONNV infections 32 (8.4%); one contributing factor might be that the study had more adults as compared to any other age group.

Among the CHIKV and ONNV positive cases majority of the participants reported muscle pains/joint pains as a symptom 74.1% (20/27) and 80% (4/5) respectively. This symptom is an important hallmark of Alphaviral infection. A reason behind it is that while neutralizing antibodies are a good indication of protective immunity, antibodies that don’t neutralize virus in vitro may also provide protection in vivo via other immune mechanisms such as complement mediated cytolysis (CMC) or antibody-dependent cell-mediated cytotoxicity (ADCC). Non-neutralizing antibodies have also been implicated as a cause of more severe disease due to antibody-dependent enhancement (ADE). This process may also have a role in pathogenesis of joint pain following Alphavirus infections.33

Our study had several limitations. We used bio-banked sera taken as part of a separate parent study on rift valley fever virus, yellow fever virus and dengue virus in Western Kenya. Samples had been repeatedly frozen and thawed for investigations, this might have resulted into false negative IgM-capture ELISA results. The specificity of the in-house IgM-capture ELISA and in-house indirect IgG ELISA were limited because of cross-reactivity with other alphavirus-related infections. We were not able to trap vectors for viral testing; hence we cannot associate vector type abundance or mosquito infection rates with human data in this study.

Some ELISA positive individuals who were FRNT negative may not truly be false positives but may have been exposed but not mounted neutralizing antibodies responses. Furthermore, using only retrospective field-collected samples limited the analysis. In a prospective study, patients would be sampled during the acute phase and again during the convalescent phase of illness. Virus isolations, antigen-detection ELISAs, and/or reverse transcription PCR would be attempted on all acute-phase samples.

Testing acute- and convalescent-phase serum would enable both IgM and IgG testing and confirm positive results by a 4-fold increase in neutralizing titer. In this retrospective study, we had only acute-phase samples. However, because of the robust size of our sample, we feel that our conclusions are well supported.

CONCLUSION

In conclusion, CHIKV and ONNV infections were found to be common in Western Kenya. It is likely that both virus infections are causing human disease that is going undiagnosed in this area. Unrecognized ONNV transmission, in particular, has been ongoing and underappreciated in this region. Among local residents, women and adults were found to be more likely to have been exposed similarly reported in other studies. These continued studies will add to the body of knowledge for CHIKV and ONNV fever that occur naturally within Western Kenya.

ACKNOWLEDGEMENTS

Authors would like to thank the support from laboratory staff at the Production Department KEMRI, Nairobi and Center for Infectious and Parasitic Control Research (CIPDCR): Infectious Disease Research Laboratory (IDRL). KEMRI, and Alupe. We would also like to thank KEMRI-Arbovirus laboratory, National reference laboratory for providing the ONNV strain (SG650).

Funding: JICA/KEMRI JICA-JST-SATREPS projects Conflict of interest: None declared

Ethical approval: The study was approved by the KEMRI/Scientific and Ethics Review Unit

REFERENCES

1. Pfeffer M, Dobler G. Emergence of zoonotic arboviruses by animal trade and migration. Parasites & vectors. 2010 Dec 3(1):35.
2. Kuniholm MH, Wolfe ND, Huang CY, Mpoudi-Ngole E, Tamoufe U, Burke DS, et al. Seroprevalence and distribution of Flaviviridae, Togaviridae, and Bunyaviridae arboviral infections in rural Cameroonian adults. The American journal of tropical medicine and hygiene. 2006 Jun 1;74(6):1078-83.
3. Meltzer E. Arboviruses and viral hemorrhagic fevers (VHF). Infectious Disease Clinics. 2012 Jun 1;26(2):479-96.
4. Weaver SC, Rico-Hesse R, Scott TW. Genetic diversity and slow rates of evolution in New World alphaviruses. InGenetic Diversity of RNA Viruses. Springer, Berlin, Heidelberg. 1992:99-117.
5. Toivanen A. Alphaviruses: an emerging cause of arthritis?, Current opinion in rheumatology. 2008 Jul 1;20(4):486-90.
6. Powers AM, Brault AC, Tesh RB, Weaver SC. Re-emergence of Chikungunya and O’nyong-nyong viruses: evidence for distinct geographical lineages and distant evolutionary relationships. General Virology. 2000 Feb 1;81(2):471-9.
7. Robinson MC. An epidemic of virus disease in Southern Province, Tanganikya territory, in 1952–1953. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1955 Jan 1;49(1):28-32.
8. Lumsden WH. An epidemic of virus disease in Southern Province, Tanganikya territory, in 1952–1953 II. General description and epidemiology. Transactions
of the Royal Society of Tropical Medicine and Hygiene. 1955 Jan 1;49(1):33-57.
9. Powers AM, Brault AC, Shirako Y, Strauss EG, Kang W, Strauss JH, Weaver SC. Evolutionary relationships and systematics of the alphaviruses. J virology. 2001 Nov 1;75(21):10118-31.
10. ole Kwallah A, Inoue S, Thairu-Muigai AW, Kuttoh N, Morita K, Mwau M. Seroprevalence of yellow Fever virus in selected health facilities in western Kenya from 2010 to 2012. Japanese J infectious diseases. 2015;68(3):230-4.
11. Awando MM, Ongus JA, Ouma JR. Seroprevalence of dengue virus among febrile patients visiting selected hospitals in the western region of Kenya, 2010/2011. African J Health Sciences. 2013;26(4).
12. Mwongula AW, Mwamburi LA, Matuji M, Siamba DN, Wanyama FW. Seroprevalence of Chikungunya Infection in Pyretic Children Seeking Treatment in Alupe District Hospital, Busia County Kenya. Int J Curr Microbiol App Sci. 2013;2:130-9.
13. Hadlow AJ, Davies CW, Walker AJ. O'nyong-nyong fever: An epidemic virus disease in East Africa 1. Introduction. Transactions Royal Soci Trop Med Hyg. 1960 Nov 1;54(6):517-22.
14. Williams MC, Woodall JP, Corbet PS, Gillett JD. O'nyong-nyong fever: An epidemic virus disease in East Africa VIII. Virus isolations from anophelines mosquitoes. Transactions Royal Soci Trop Med Hyg. 1965 May 1;59(3):300-6.
15. Tsai TF, Sanders EJ, Hendricks E, Dougle M, Sluys D, Van den Born BJ, et al. Serological Evidence of O'Nyong Nyong Viral Infection in Kenya, 1994-95. American J Trop Med Hyg. 1997;57:365.
16. LaBeaud AD, Banda T, Brichtard J, Muchiri EM, Mungai PL, Mutuku FM, et al. High rates of o’nyong nyong and Chikungunya virus transmission in coastal Kenya. PLoS neglected tropical diseases. 2015 Feb 6;9(2):e0003436.
17. Robin S, Ramful D, Le Seach F, Jaffar-Bandjee MC, Rigou G, Alessandri JL. Neurologic manifestations of pediatric chikungunya infection. child neurology. 2008 Sep;23(9):1028-35.
18. Igarashi A. Technical Manual of Arbovirus study. Japan;2000.
19. Chanas AC, Hubalek Z, Johnson BK, Simpson DI. A comparative study of O’nyong nyong virus with Chikungunya virus and plaque variants. Archives of virology. 1979 Sep 1;59(3):231-8.
20. Schoepp RJ, Rossi CA, Khan SH, Goba A, Fair JN. Undiagnosed acute viral febrile illnesses, Sierra Leone. Emerging infectious diseases. 2014 Jul;20(7):1176.
21. Zim MM, Sam IC, Omar SS, Chan YF, AbuBakar S, Kamarulzaman A. Chikungunya infection in Malaysia: comparison with dengue infection in adults and predictors of persistent arthralgia. J Clinical Virology. 2013 Feb 1;56(2):141-5.
22. Bendel RB, Afifi AA. Comparison of stopping rules in forward “stepwise” regression. J American Statistical association. 1977 Mar 1;72(357):46-53.
23. Mickey RM, Greenland S. The impact of confounder selection criteria on effect estimation. American J epidemiology. 1989 Jan 1;129(1):125-37.
24. Ochieng C, Ahenda P, Vittor AY, Nyoka R, Gikunju S, Wachira C, et al. Seroprevalence of infections with dengue, rift valley fever and chikungunya viruses in Kenya, 2007. PLoS one. 2015 Jul 15;10(7):e0132645.
25. Beatty BJ, Calisher CH. Arboviruses. In: Lennette EH, Lennette ET, editors. Diagnostic procedures for viral, rickettsial, and chlamydial infections. Washington (DC). American Public Health Association. 1995:89-212.
26. Mwau M, Kubo T, Nzou S, Kageha S, Ngoi J, Demba N, Morita K. Seroprevalence of Chikungunya, West Nile Virus and Dengue Virus in Febrile Patients visiting selected health facilities in Trans Nzoia County, African J Pharmacol Therapeutics. 2012 Dec 31;1(4).
27. Lanciotti RS, Ludwig ML, Rwaguma EB, Lutwama JJ, Kram TM, Karabatsos N, et al. Emergence of epidemic O’nyong-nyong fever in Uganda after a 35-year absence: genetic characterization of the virus. Virology. 1998 Dec 5;252(1):258-68.
28. Tappe D, Kapaua A, Emmerich P, de Mendonca Campos R, Cadar D, Günther S, et al. O’nyong-nyong virus infection imported to Europe from Kenya by a traveler. Emerging infectious diseases. 2014 Oct;20(10):1766.
29. Schwartz O, Albert ML. Biology and pathogenesis of chikungunya virus. Nature Reviews Microbiology. 2010 Jul;8(7):491.
30. Mbaika S, Lutomiah J, Chepkorir E, Mulwa F, Khayeka-Wandawba C, Tigoi C, et al. Vector competence of Aedes aegypti in transmitting Chikungunya virus: effects and implications of extrinsic incubation temperature on dissemination and infection rates. Virology. J 2016 Dec;13(1):114.
31. Suhrbier A, Linn ML. Clinical and pathological aspects of arthritis due to ross river virus and other Alphaviruses. Curr Opin Rheumatol. 2004;16:374-9.
32. Staples JE, Breiman RF, Powers AM. Chikungunya fever: an epidemiological review of a re-emerging infectious disease. Clinical infectious diseases. 2009 Sep 15;49(6):942-8.
33. Hoarau JJ, Bandjee MC, Trotot PK, Das T, Li-Pat-Yuen G, Dassa B, et al. Persistent chronic inflammation and infection by Chikungunya arthriogenic alphavirus in spite of a robust host immune response. J Immunology. 2010 May 15;184(10):5914-27.