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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

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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' more so 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.[3] 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.[4] 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.[5]

ONNV and CHIKV are closely related alphaviruses in the Semliki Forest antigenic complex.[6] CHIKV was initially isolated from serum of a febrile male in Tanzania in 1953.[7,8] 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.[9] Recent studies conducted in Western Kenya show that Arboviruses are circulating.[10-12] ONNV was initially isolated in Northern Uganda from anopheline mosquitoes and human serum during a 1959 epidemic.[13] 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.[14] After the epidemic results of seroprevalence studies suggested that sporadic human infections with ONN virus continued to occur within the region, though no cases of ONN fever were documented after 1962.[15] 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.[16]

ONNV causes a similar syndrome to CHIKV, Ross River virus (RRV), and other alphaviruses, characterized by fever, rash, debilitating arthralgia, and myalgia.[13] 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.[17] 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.[6] 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 bio-banked 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 (≥37.5ºC) 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 600×g 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.[18] 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 × *g* for 10 min at room temperature and stored in 1.5ml cryotubes at -80ºC.

CHIKV indirect IgG ELISA

The assay developed for this work used purified CHIKV in a standard In-house Indirect IgG ELISA.[18] Positive samples required a titer ≥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.[18] A sample was considered positive with an OD492/negative control OD492 ratio ≥2.0

Focus reduction virus neutralization test ($FRNT_{50}$)

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 ≥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.[19] A sample was designated ONNV positive if its titer was ≥20 and four-fold greater than the CHIKV titer.[20]

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.[21] 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.[22-24]

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 ≥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 ≥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.[25]

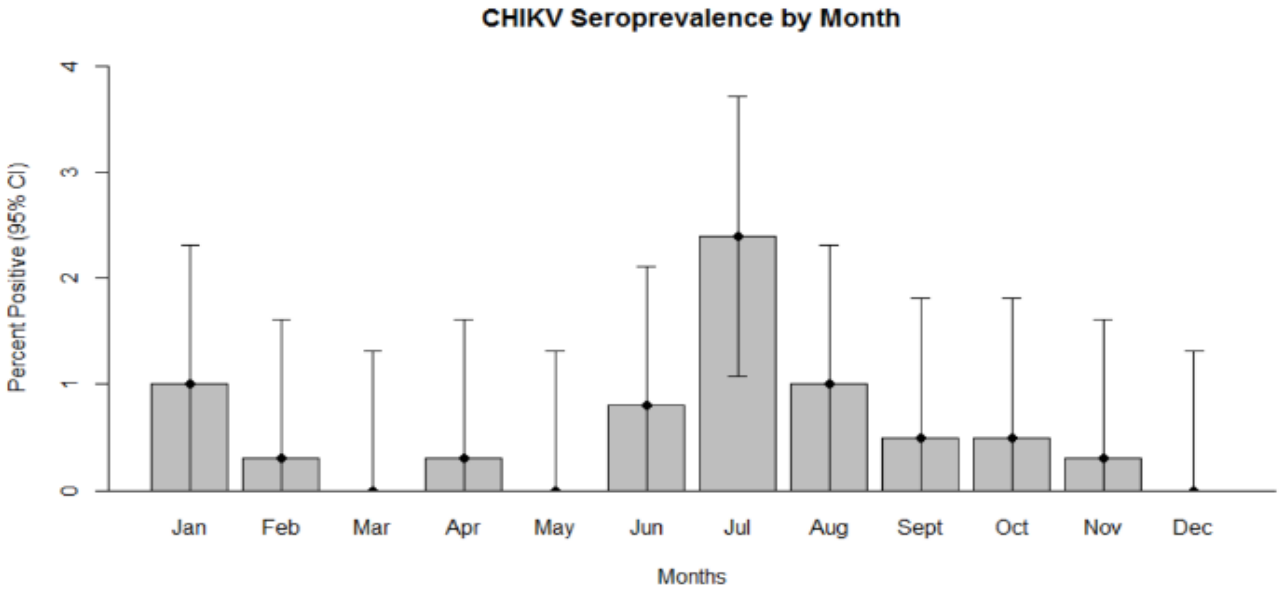

Figure 1: CHIKV seroprevalence by month.

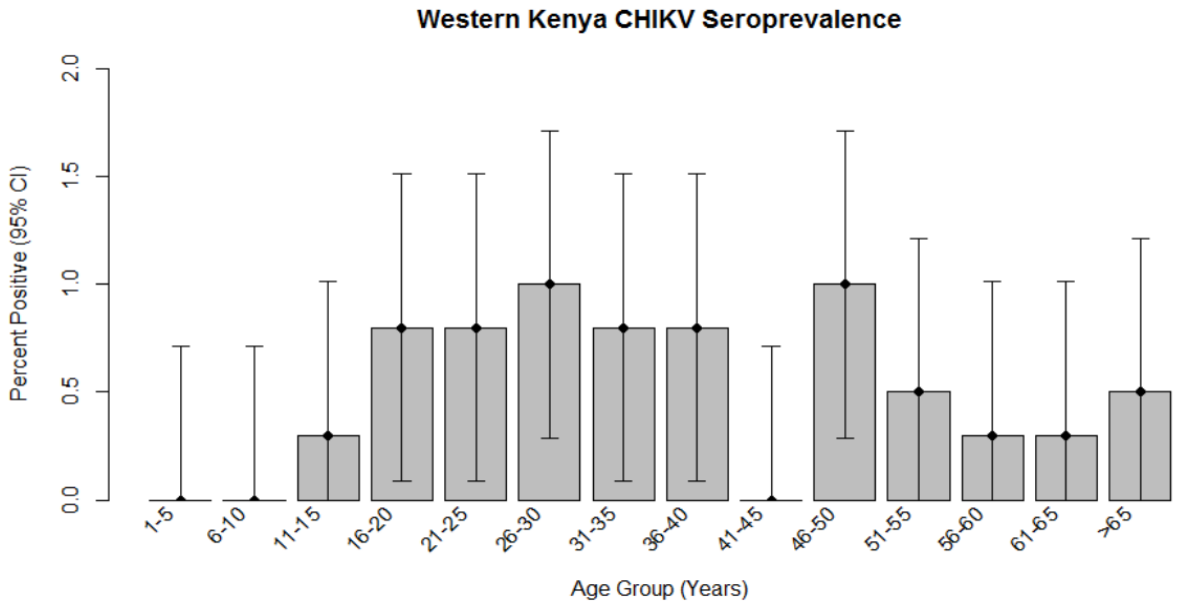

Figure 2: Seroprevalence of CHIKV by age group as measured by FRNT.

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.[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%

C.I 0.97 -9.80), walls with holes and cracks (OR=0.23 95% C.I 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%).

Males were 145 (38%) and females were 237 (62%). CHIKV seropositive males were 5.5% (8/145) of which 87.5% (7/8) had anti-CHIKV IgG and 12.5% (1/8) had anti-CHIKV IgM antibodies. CHIKV seropositive females were 8% (19/237) of which 94.7% (18/19) had anti-CHIKV IgG and 5.3% (1/19) had anti-CHIKV IgM antibodies. More women were likely exposed to CHIKV than males (Figure 3). ONNV seropositive males were 1.4% (2/145), of which one was positive for both IgG and IgM antibodies while the other was only positive for IgG

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.

	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			
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	-	-

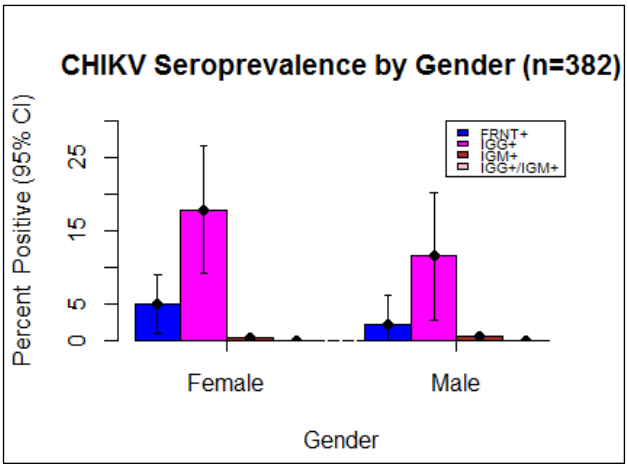

Figure 3: Seroprevalence of CHIKV across gender, as measured serologically by ELISA and FRNT with 95% C.I. indicated by bars.

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.[26] Evidence of transmission rates for ONNV was likely expected, given that the last known outbreak occurred in Rakai district of southwestern Uganda in 1996.[27] 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 1:2,560 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).[28] 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.[12] 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%.[16] 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.[29] 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 mid gut infection barrier than the Trans Nzoia population.[30] Furthermore; humidity can also play a role, with increased humidity facilitating increased survival of mosquitoes.[31]

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.[31] 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 titers 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.[16,19,32]

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.

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