EAHRC
EAST AFRICAN HEALTH RESEARCH COMMISSION
Research for Health and Prosperity

ORIGINAL ARTICLE

Concurrent Infection With Dengue and Chikungunya Viruses in Humans and Mosquitoes: A Field Survey in Lower Moshi, Tanzania

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ABSTRACT

Introduction: Dengue and Chikungunya have re-emerged as important diseases of global concern. Co-infections with Dengue virus (DENV) and Chikungunya virus (CHIKV) could have serious outcomes if not diagnosed and managed optimally. However, the key focal points for the maintenance of CHIKV and DENV infections and the extent of their co-infection remain poorly understood in many geo-ecologically distinct parts of Tanzania.

Objective: We aimed to comparatively examine the prevalence and factors for seropositivity to DENV and CHIKV and their infection rates in humans and mosquitoes

Methods: A cross-sectional study was performed in the Lower Moshi area of the Kilimanjaro region from April to July 2020. DENV and CHIKV exposure was determined by detecting IgM to the viruses using enzyme linked immunosorbent assay whereas infection was determined by real time quantitative polymerase chain reaction (RT-qPCR) assay.

Results: Insecticide Treated Bed Net (ITN) use ($\chi2=3.504$; $p< 0.05$), being ≥7 individuals living in the same household ($\chi2=4.655$; $p<0.05$) and a recent travel to an urban destination ($\chi2=3.39$; $p< 0.05$) were the only factors associated with CHIKV seropositivity. ITN use was the only factor associated with CHIKV infection ($\chi2=5.204$; $p<0.05$). A recent travel to an urban destination ($\chi2=4.401$; $p< 0.05$) was the only factor associated with DENV seropositivity. Five (1.5%) Ae. aegypti pools were positive for CHIKV whereas 1 (0.3%) was positive for DENV. Two Cx. pipiens, pools (1.9%) were positive for CHIKV. None of the Cx. pipiens mosquitoes was positive for DENV. No associations between DENV and CHIKV seropositivity was observed in humans but DENV infection was strongly associated with CHIKV infection ($\chi2 = 238.45$; $p<0.01$). CHIKV infection was observed to be consistently higher in both, humans and mosquitoes.

Conclusion: Detection of DENV and CHIKV in both humans and vector mosquitoes confirms that both viruses are actively circulating in the Lower Moshi area of Kilimanjaro region in Tanzania. Our findings point out the Lower Moshi area as a potential focal point for the maintenance of the two viruses and possibly other vector borne viruses. We call upon sustained active surveillance of arboviruses and other re-emerging infections to be better prepared for possible outbreaks by the viruses.

INTRODUCTION

Dengue and Chikungunya are vector borne diseases of public health and socioeconomic importance with shared endemic profiles and symptoms. Co-infections with Dengue virus (DENV) and Chikungunya virus (CHIKV) could have serious outcomes if not diagnosed and managed optimally.

In recent years, the spread of DENV and CHIKV has gained global concern, especially, in tropical and subtropical regions because of their recurring outbreaks[1]. Both DENV and CHIKV are spread by common mosquito vectors, mainly Aedes aegypt.i[2] Dengue is considered as the most important arbovirus disease compared to Chikungunya, mainly known from its epidemics in continental Africa and Asia. Chikungunya, on the other hand, has been prevalent in Africa and Asia for many years.[3,4] CHIKV was also detected in America in 2013, whereby more than 2 million cases have been reported.[5]

Although CHIKV and DENV belong to different genera of the Togaviridae family, ie, the alphavirus and the *flavivirus* genera respectively, both cause febrile syndromes that share many similar signs and symptoms including fever plus any two of the following: nausea, vomiting, rash and headaches that leads to a

high likelihood of misdiagnosis by clinicians.[6] A wide range of vector-borne and zoonotic pathogens exist in tropical Africa and elsewhere.[7] Most of these pathogens co-infect a significant proportion of inhabitants in a given setting.[8,9] Co-infections with both viruses may obscure clinical suspicion, as signs and symptoms for many of these pathogens overlap. In endemic areas, this becomes a particularly pressing issue that must be taken into account to ensure accurate diagnosis for optimal case management. Although, currently, there is no empirical evidence of a higher severity in these DENV-CHIKV co-infection cases, reports are available that report a more severe clinical disease in dual infection with arboviruses than mono infection.[10-12]

A recent study conducted in the same study area, reported an active transmission of Rift Valley Fever virus (RVFV) in Lower Moshi area of Kilimanjaro region, pointing to it as a potential hotspot for RVF.[13] The presence of vector mosquitoes for Dengue and Chikungunya viruses in the area,[14] prompted the design of this study to determine the prevalence of DENV and CHIKV in humans and vector mosquitoes (Aedes aegypti and Culex pipiens) in the absence of current outbreaks. Dengue and Chikungunya have re-emerged as important pathogens of global concern.[15,16] However, the key focal points for the maintenance of CHIKV and DENV infections remain poorly understood in many geo-ecologically distinct parts of Tanzania. Results from the current study will not only be useful in understanding the burden of the viruses and the extent of DENV-CHIKV co-infection in the area, but also inform health care providers and policy makers on potential unreported hotspots for DENV and CHIKV outbreaks and thus guide decision makers to implement integrated vectors interventions (IVM).

METHODOLOGY

Study Design and Site

This was a cross-sectional study conducted in lower Moshi area (37°20'E 3°21'S) of Moshi district, Kilimanjaro region of Tanzania between April and July 2020 involving 3 villages, namely Mikocheni, Chemchem, and Arusha Chini. Lower Moshi, as described previously[13], is an intensive rice irrigation area, located on the southern foothills of Mount Kilimanjaro[13] (Figure 1).

The population in the area is engaged in agriculture and livestock keeping. Two rivers, the Pangani and the Rau provide water for irrigation. The rice irrigation schemes have structured and unstructured canal networks covering an area of about 1,100 hectares. During the rainy season, temporary pools that serve as mosquito breeding sites are formed. Their persistence beyond the rains contributes to unremitting mosquito breeding. The area has two rainy seasons; the long rains which run from March to June and the short rainy season from November to January. The study was carried out during the rainy season when vector activity is at its peak in order to capture the highest possible transmission of the viruses studied.

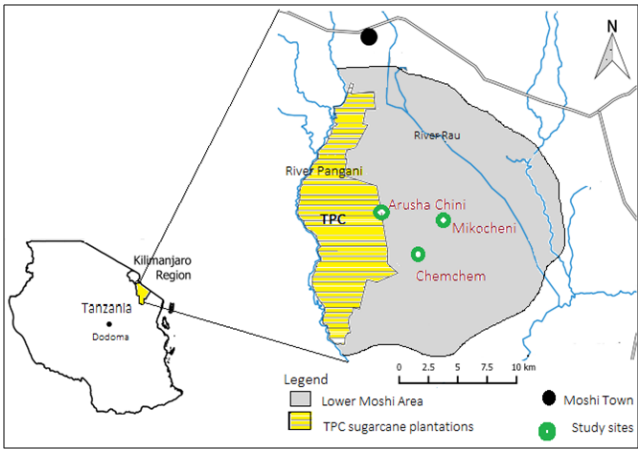
Participants and Sample Collection

Participants in this study were males and females aged between 10 and 70 years from 266 households. The main occupation of inhabitants in the area is agropastoralism. Consent to participate in the study was obtained from adults aged ≥18 years whereas parents or legal guardians for participants aged <18 years assented for participants aged 18 years or below. Sample size was estimated by "Epitools" online sample size (ss) calculator based on the formula ss= $Z^2(P)\ (1\text{-}P)/\varepsilon^2$, where, Z is the value (1.96 for 95% confidence level [CI]), P represents prevalence, and ε is the minimal tolerable error at 95% CI, expressed as a decimal (0.05). These estimations gave a minimum sample size of 183. However, in order to increase the power of the statistical analyses, the sample size was increased to 266.

Collection of Blood Samples

Blood sampling was performed by phlebotomists from the Kilimanjaro Christian Medical Center (KCMC). Three milliliters of blood were collected by venipuncture from each participant from the median cubital vein. Each sample was divided into two aliquots of 1.5 ml each, and aliquots placed into plain and EDTA vacutainer tubes, respectively. To each EDTA tube containing a sample, 4.5ml of Tri Reagent (Zymo Research, Irvine, CA, U.S.A.) were added. The mixture was gently mixed by shaking for 1 minute and immediately shipped to the KCRI biotechnology laboratory at 4°C, for RNA extraction and PCR analyses. Samples in plain tubes were allowed to clot for a maximum of 20 minutes at room temperature before they were centrifuged at 2,000 x g for 10 minutes at 4°C and serum transferred to clean sterile serum tubes. Serum samples were tested for presence of IgG/IgM to DENV and IgM to CHIKV. Blood samples that were positive by serology were subjected to PCR analysis. Demographic data from participants were collected using electronic forms designed using Open Data Kit (ODK) tools (https://opendatakit.org/) deployed in Android tablets.

FIGURE 1. Map of Tanzania Showing the Lower Moshi Area where the Study was Conducted



Mosquito Trapping

Mosquito trapping was performed from 8.00 am to 6.00 pm near sampled houses for 10 consecutive days as previously described by Kajeguka and colleagues.[17] Briefly, BG Sentinel trap (BGS) (Biogents AG, Regensburg, Germ-

any) to target outdoor host-seeking adult mosquitoes. The BGS-Trap, developed by BioGents GmbH (Regensburg, Germany), is made of an easy to transport, collapsible white bucket with gauze covering. Captured mosquitoes were immediately morphologically identified in the field and sorted according to their species using taxonomic keys.[18,19] Two key most abundant and known DENV and CHIKV vector species, Cx pipiens and Ae aegypti, were sorted for qPCR analysis of DENV and CHIKV RNA in pools of 50s.

Laboratory Procedures

DENV IgM and CHIKV IgM ELISAs

Enzyme Linked-Immunosorbent assays (ELISA) for antibodies to DENV and CHIKV were performed as previously described[20]. Briefly, serum from plain tubes was obtained by centrifugation at 2,000 rpm x g for 10 minutes and serum samples stored at −20°C until serological analyses were performed. For seropositivity of CHIKV, anti-CHIKV IgM was detected using indirect ELISA kit (SD, Gyeonggi-do, Korea and IBL international, Hamburg, Germany, respectively). Detection of DENV IgM antibodies was done using a direct ELISA kit (SDInc, Gyeonggi-do, Korea) as described by[21]. All assays were performed according to manufacturers' instructions.

Ribonucleic Acid (RNA) Isolation and Detection by PCR

For DENV and CHIKV, Blood samples kept in EDTA tubes were centrifuging at 1,000 rpm x g for 10 minutes in a refrigerated centrifuge to obtain buffy coat. Ribonucleic acid (RNA) was extracted from buffy coat samples using the Boom method[22] as described by[17]. Total RNA was extracted from 200 µl of homogenized individual Aedes and Culex mosquitoes using QIAGEN RNeasy Mini Kits according to the manufacturer's instructions. Using the real-time RT-PCR method, primers and probes[23] were followed to screen mosquito homogenates for evidence of Chikungunya and Dengue viral RNA.

RT-PCR for Detection of DENV and CHIKV in Human and Mosquito Samples

Both, blood samples and mosquito extracts were tested with the RealStar Dengue RT-PCR Kit 1.0 (Altona Diagnostics [Altona], Hamburg, Germany[15]; and the Tropical Fever Core Multiplex Real-time PCR (Fast Track Diagnostics [FTD], Luxembourg). All procedures were performed according to the manufacturer's protocols.

Data Analysis

Data analysis was performed using IBM SPSS Statistics for Windows version 26 (IBM Corp, Armonk, NY, USA). Descriptive data were presented as frequencies and percentages, means, and medians wherever it was applicable. Categorical data were reported as a tabulation of proportions and compared between humans and goats. Chi-squared statistic ($\chi 2$) was used to examine associations between seropositivity to DENV and CHIKV in humans and DENV and CHIKV infection in both humans and mosquitoes. Co infection and co- exposure data was reported as numbers and corresponding percentages. Associations between exposure and infection in humans and mosquitoes was determines by the $\chi 2$ test. In all cases, associations reaching a P value of .05 or less were considered as significant.

Ethical Issues

This study obtained approval by the College Research and Ethics Committee (CRERC) of the Kilimanjaro Christian Medical University College (KCMUCo) with approval certificate #2419. The study obtained permission from the Kilimanjaro Regional and District Administrative Secretaries, District Medical and Veterinary Officers, and local village and ward executive officers of respective villages. Participants were asked to voluntarily consent to participate in the study after an explanation about the study aims, procedures, risks and benefits was made to them.

Participants aged 18 years and above signed "informed consent" forms whereas parents and/or legal guardians of participants under 18 years and participants who could not read or write signed the "informed consent" on behalf. All authors hereby confirm that all procedures in this study were approved by CRERC and were performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki.

RESULTS

Demographic Characteristics of Participants

A total of 266 participants were involved in the study, fifty-two percent of which were aged between 21 and 50 years whereas 56.4% were females. Majority of participants (74.4%) lived in families of 4 individuals or above in the same household. Most participants had attained primary education (63.2%) and kept livestock (72.9%) (Table 1).

TABLE 1: Demographic Characteristics of Participants

Characteristics	n	%
Age group (years)		
≤20	28	10.5
21-50	140	52.7
>50	98	36.8
(Median, IQR) years	45(30-55)	
Sex		
Male	116	43.6
Female	150	56.4
Individuals living in a household		
<4	68	25.6
≥4	198	74.4
Highest education		
No formal	51	19.2
Primary	168	63.2
Tertiary	47	17.7
Type of animals kept by participant		
Animal Keeping	194	72.9
None	72	27.1

IQR, Interquartile Range

Prevalence and Factors Associated With Chikungunya Virus Seropositivity and Infection in humans

Results show that the use of Insecticide Treated Bed Nets (ITNs) (χ^2=3.504; P<.05), being more than 7 individuals

in the same household (χ^2=4.655; P<.05) and a recent travel to an urban destination (χ^2=3.39; P<.05) were the only factors associated with CHIKV seropositivity. For CHIKV infection, ITN use was the only factor that was associated with CHIKV infection (χ^2=5.204; P<.05). We observed a higher PCR positivity rate than seropositivity to DENV (Table 2).

Prevalence and Factors Associated With Dengue Virus Seropositivity and Infection in Humans

With regards to DENV, the only factor that was associated with DENV seropositivity was a recent travel to an urban destination (χ^2=4.401; P<.05). None of the studied factors was found to be associated with DENV infection (Table 3).

CHIKV and DENV Infection in Aedes aegypti and Culex pipiens

For Ae aegypti, 333 monospecific pools of mosquitoes were tested while 106 pools of Cx pipiens were tested for both CHIKV and DENV infections. Out of these, 5 (1.5%) Ae aegypti pools were PCR positive for CHIKV, while only 1 (0.3%) was positive for DENV. One hundred and six Cx pipiens complex pools were tested, of which 2 (1.9%) were PCR positive for CHIKV. None of the Culex mosquito pools was positive for DENV.

Association Between Chikungunya and Dengue Infection in Humans and Mosquitoes

When DENV and CHIKV infection in humans and mosquitoes were tested for any independent associations, no associations were detected by statistical analyses (Table 4). When we attempted to find out whether dual infection by DENV and CHIKV and seropositivity to the viruses were associated in humans, our analyses showed no associations between DENV and CHIKV seropositivity in humans. However, DENV infection (as determined by PCR), was found to be strongly associated with CHIKV infection (χ^2 = 238.45; P<.01) (Table 5). In humans, the prevalence of antibodies to CHIKV was higher than to DENV. Likewise, a marginally higher infection rate by CHIKV was recorded in humans. CHIKV infection was observed to be consistently higher in both, humans and mosquitoes (Aedes and Culex), whereas none of the Culex mosquitoes was found to be infected by DENV.

DISCUSSION

This study investigated the concurrent circulation of DENV and CHIKV viruses in humans and their designated vector mosquitoes it terms of their risk factors and comparative seropositivity and infection rates in an area intensively used for irrigation in the Lower Moshi area of Kilimanjaro region in Tanzania. Our study highlights the active circulation of DENV and CHIKV in both, humans and vector mosquitoes in the study area. From this study, individuals within a household who did not use ITNs, individuals who were more than 7 in the same household (sleeping under the same roof), and individuals who recently traveled to an urban destination were more seropositive to CHIKV. ITN use was associated with lower CHIKV infection.

Previous studies had reported a range of factors that increase the risk for infection by Dengue and Chikungunya viruses including older age and male sex.[24]

Our current study, however, reports no association of these factors with higher CHIKV or DENV seroprevalence and infection, contrary to what some previous studies had reported[25-28]. The absence of associations between older age and gender with CHIKV and DENV infection could be explained by the nature of the main economic activities in the study area, where, almost all tested individuals, young and old were engaged in livestock keeping and irrigated sugar cane farming and had reported mosquito bites. In an environment of intense transmission of arboviruses, factors such as sex and age may not be important to predispose to infection.

Studies have reported significant seroprevalence of CHIKV antibody with the agro-pastoralist lifestyle compared to pastoralist lifestyles.[29-31] Agro-pastoralism could be associated with higher infection risk including environmental suitability for vector survival and thus virus maintenance. Further, an agro-pastoralism lifestyle is more likely to offer an intimate allow close contact between humans and DENV and CHIKV vectors. Recent travel to the urban area has been consistently associated with both DENV and CHIKV seropositivity and infection.[25,32] Millions of susceptible people moving to the cities and living in shanty towns with inadequate housing and dilapidated or no basic services such as clean water, sewer and waste management is thought to results into crowded human communities and creation of large mosquito populations leading to formation of ideal conditions for arboviruses transmission.[25,32-37]

Consistently, we observed higher seroprevalence and infection rates of CHIKV than DENV, which indicates the former to be more prevalent than the latter, Although Dengue has emerged as one of the most important re-emerging diseases[16,37] that has caused six outbreaks in Tanzania over the past 10 years including thousands of reported cases and multiple deaths,[36,38,39] its seroprevalence and infection rates have been reported to be lower compared to the endemic Chikungunya virus.[27,34,36,38-40] Notwithstanding that we could not establish any associations between DENV and CHIKV seropositivity in humans, DENV infection (by qPCR) was found to be strongly associated with CHIKV infection, suggesting the presence of common factors for the transmission of the two viruses in the area.

Our study was able to detect DENV and CHIKV in vector mosquitoes collected. Generally, Ae. aegypti higher infection rate by CHIKV compared to DENV, which underscores the importance of this mosquito species in the transmission of arboviruses. Consistent to the observations that Culex is not an important vector for DENV, none of the Culex mosquitoes were positive for DENV infection. The current study shows that Ae Aegypti mosquitoes are the main vector mosquitoes for the transmission of not only DENV and CHIKV, but also other arboviruses such as Rift Valley Fever Virus in the same area[41]. This may also mean that the residents of the studied sites are at risk of being infected by multiple arboviruses. The detection of active infections of CHIKV and DENV in both humans and vector mosquitoes during silent, inter-epidemic periods, albeit at low rates for DENV, points out to the possibility that the Lower Moshi area is a potential hot spot for future DENV and CHIKV outbreaks.

TABLE 2: Prevalence and Factors Associated With Chikungunya Virus Seropositivity and Infection in Humans

	ELISA seropositivity test				PCR for infection detection			
	Positive	Negative	All	%	Positive	Negative	All	%
Sex								
Males	8	107	115	7.0	4	10	14	28.6
Females	16	135	151	10.6	5	20	25	20.0
Total	24	242	266	9.0	9	30	39	23.1
	Chi-square = 1.09; P value=.21				**Chi-square = 0.37; P value=.41**			
Age group								
11 – 20	2	26	28	7.1	0	3	3	0.0
21-30	7	33	40	17.5	3	5	8	37.5
31-40	2	43	45	4.4	1	4	5	20.0
41-50	4	51	55	7.3	2	8	10	20.0
>50	9	89	98	9.2	3	10	13	23.1
Total	24	242	266	9.0	9	30	39	23.1
	Chi-square = 4.98; P value=.29				**Chi-square = 1.918; P value=.80**			
ITNuse ¥								
Yes	3	45	48	6.3	3	0	3	100.0
No	14	132	146	9.6	4	10	14	28.6
Total	17	177	194	8.8	7	10	17	41.2
	Chi-square = 3.5; P value=.04				**Chi-square = 5.204; P value=.05**			
Individuals per HH#								
1 - 3	4	64	68	6.3	1	4	5	20.0
4 - 6	12	138	150	8.7	2	22	24	8.3
7 and more	8	39	47	20.5	6	4	10	60.0
Total	24	241	265	10.0	9	30	39	23.1
	Chi-square = 4.66; P value=.04				**Chi-square = 10.646; P value=.16**			
Types of animal@								
None	10	62	72	13.9	4	11	15	26.7
chicken	4	78	82	4.9	2	8	10	20.0
Goats/Sheep	7	47	54	13.0	2	5	7	28.6
Cattle	3	52	55	5.5	1	6	7	14.3
Goats/Sheep/Cattle	0	3	3	0.0				
Total	24	242	266	9.0	9	30	39	23.1
	Chi-square = 5.96; P value=.14				**Chi-square = 0.586; P value=.39**			
Recent travel								
Yes	6	95	101	5.9	1	11	12	8.3
No	18	147	165	10.9	8	19	27	29.6
	24	242	266	9.0	9	30	39	23.1
	Chi square = 1.884; P value=.12				**Chi-square = 2.123; P value=.15**			
Destination								
Rural	1	39	40	2.5	0	5	5	0.0
Peri-urban	0	13	13	0.0				
urban	5	43	48	10.4	1	6	7	14.3
Total	6	95	101	5.9	1	11	12	8.3
	Chi-square = 3.39; P value=.05				**Chi-square = 0.779; P value =.59**			
Education level								
No Formal education	2	49	51	3.9	0	2	2	0.0
Primary Education	16	15	31	51.6	6	22	28	21.4
Tertiary Education	6	41	47	12.8	3	6	9	33.3
Total	24	105	129	18.6	9	30	39	23.1
	Chi-square = 2.47; P value=.09				**Chi-square = 1.176; P value=.25**			

Key: ¥ Insecticide treated bed-nets; # Household; @Types of animals kept by participants

TABLE 3: Prevalence and Factors Associated With Dengue Virus Seropositivity and Infection in Humans

Variable	ELISA				PCR			
	Positive	Negative	All	%	Positive	Negative	All	%
Sex								
Male	1	114	115	0.9	2	6	8	25.0
Female	6	145	151	4.0	4	12	16	25.0
Total	7	259	266	2.6	6	18	24	25.0
	Chi-square = 2.48; P value=.01				**Chi-square = 0; P value=.68**			
Age								
11 – 20	0	28	28	0.0	0	2	2	0.0
21 – 30	2	38	40	5.0	3	4	7	42.9
31 – 40	2	43	45	4.4	0	2	2	0.0
41 – 50	1	54	55	1.8	1	3	4	25.0
>50	2	96	98	2.0	2	7	9	22.2
Total	7	259	266	2.6	6	18	24	25.0
	Chi-square = 2.485; P value=.26				**Chi-square =2.561; P value=.47**			
ITN use ¥								
Yes	0	48	48	0.0	1	2	3	33.3
No	6	140	146	4.1	3	11	14	21.4
Total	6	188	194	3.1	4	13	17	23.5
	Chi-square = 2.036; P value= .18				**Chi-square = 0.195; p value = 0.56**			
Individuals per HH#								
1 – 3	2	66	68	2.9	1	3	4	25.0
4 – 6	4	146	150	2.7	1	11	12	8.3
>/=7	1	47	48	2.1	4	4	8	50.0
Total	7	259	266	2.6	6	18	24	25.0
	Chi-square = 0.072; Pvalue=.51				**Chi-square = 4.444; P value=.16**			
Type of animals@								
None	3	69	72	4.2	1	9	10	10.0
chicken	2	80	82	2.4	2	2	4	50.0
Goats/Sheep	1	53	54	1.9	2	5	7	28.6
Cattle	1	54	55	1.8	1	2	3	33.3
Goats/Sheep/Cattle	0	3	3	0.0	0	0	0	0.0
Total	7	259	266	2.6	6	18	24	25.0
	Chi-square = 1.025;P value=.24				**Chi-square = 2.692; P value=.23**			
Recent travel								
Yes	0	101	101	0.0	1	5	6	16.7
No	7	158	165	4.2	5	13	18	27.8
Total	7	259	266	2.6	6	18	24	25.0
	Chi-square = 4.401; P value=.03				**Chi-square = 0.296; P value=.52**			
Destination								
Rural	0	40	40	0.0	0	1	1	0.0
Peri-urban	0	13	13	0.0	0	0	0	0.0
urban	0	48	48	0.0	1	4	5	20.0
Total	0	101	101	0.0	1	5	6	16.7
	Nil				**Chi-square = 0.24: P value=.83**			
Education level								
No education	2	49	51	3.9	0	2	2	0.0
Primary education	5	163	168	3.0	3	13	16	18.8
Tertiary education	0	47	47	0.0	3	3	6	50.0
Total	7	259	266	2.6	6	18	24	25.0
	Chi-square = 1.679; P value=.02				**Chi-square = 3; P value=.10**			

Key: ¥ Insecticide treated bed-nets; # Household; @Types of animals kept by participants

TABLE 4: Associations Between Chikungunya and Dengue Infection in Humans and Mosquitoes

		Culex-DENV-PCR		Aedes-DENV-PCR		Culex-CHIKV-PCR		Aedes-CHIKV-PCR	
		Positive n (%)	Negative n (%)	Positive n (%)	Negative n (%)	Positive n (%)	Negative n (%)	Positive n (%)	Negative n (%)
Human CHIKV-PCR	Positive	0(0.0)	10(3.8)	0(0)	10(3.8)	1(20.0)	9(90.0)	0(0.0)	10(100.0)
	Negative	0(0.0)	256(96.2)	1(100)	255(96.2)	4(80.0)	252(96.6)	4(100.0)	252(96.2)
Human DENV-PCR	Positive	0(0.0)	9(3.4)	0(0.0)	9(100.0)	1(11.1)	8(88.9)	0(0.0)	9(3.4)
	Negative	0(0.0)	257(96.6)	1(100.0)	256(96.6)	4(80.0)	253(96.9)	4(100.0)	253(96.6)

TABLE 5: Chikungunya and Dengue Co-exposure and Infection

	DENV-ELISA*		DENV-PCR#	
	Positive n(%)	Negative n(%)	Positive n(%)	Negative n(%)
CHIKV-ELISA*				
Positive	0(0.00)§	10(100.00)		
Negative	5(2.00)	251(98.00)		
CHIKV-PCR#				
Positive			9(90.00)§	1(10.00)
Negative			0(0.00)	256(100.00)

*Chi- squared (X2) = 0.199; P value=.0824; # Chi-squared (X2) = 238.45;P value=0.0001; §Dengue-Chikungunya Co-infection

The observation that almost all IgM and PCR CHIKV and DENV positive participants had subclinical infection, and that, mosquitoes carry the viruses, implies the possibility of long-term maintenance of the viruses across seasons without being diagnosed. In order to be better prepared to control possible outbreaks caused by arboviruses, extra effort in active surveillance of arboviruses across hosts is mandatory. Our public health systems need to be more vigilant in generating more information and take steps to prevent outbreaks before they occur. Notwithstanding the strength of our study findings, we acknowledge the limitation that our study could not collect and analyze other mosquito species other than Ae Aegypti and Cx pipiens which could have provided additional data on vector abundance possible infection by DENV and CHIKV viruses.

CONCLUSION

Collected during the dry season of the year, findings of the current study show that both DENV and CHIKV are actively circulating in the Lower Moshi area of Kilimanjaro region in Tanzania. These findings are evidenced by the detection of the viruses in both humans and vector mosquitoes. Ae. Aegypti is a key vector for the two viruses especially CHIKV for the transmission and possibly maintenance of the viruses. The detection of viral infections by PCR during the dry season points out to the Lower Moshi area as a potential focal point for the maintenance of the two viruses and other vector borne viruses such as RVFV. We call upon sustained active surveillance of arboviruses and other re-emerging infections for better preparedness and response to future DENV and CHIKV outbreaks and other emerging and re-emerging pantheons.

Acknowledgements: We acknowledge the logistical support provided by KCRI research administrators Ms. Elizabeth Kussaga, Ms. Tupokigwe Jana, and Ms. Zuhura Lintu. We also acknowledge the assistance of Mr. Rule M. Budodo for specimen collection and lab analyses, the Regional, District, and Field Executive Officers for providing permission to conduct this study. We thank all participants who consented to be part of this study.

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Peer Reviewed

Competing Interests: None declared.

Funding: This study was not funded

Received: 01 August 2021; **Accepted:** 18 January 2022

Cite this article as Chilongola OJ, Mwakapuja SR, Horumpende GP, Vianney J, Shabhay A, Mkumbaye IS, Semvua SH, Mmbaga TB. Concurrent Infection With Dengue and Chikungunya Viruses in Humans and Mosquitoes: A Field Survey in Lower Moshi, Tanzania. *East Afr Health Res J.* 2022;6(1):78-86. https://doi.org/10.24248/easci.v4i1.57