

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

Jaffu O Chilongola*^{a,b}, Richard S Mwakapuja^c, Pius G Horumpende^d, John-Mary Vianney^e, Ahmed Shabhay^d, Sixbert I Mkumbaye^b, Hadija S Semvua^b, Blandina T Mmbaga^{a,b}

^aDepartment of Medical Biochemistry and Molecular Biology, Kilimanjaro Christian Medical University College, Moshi Tanzania, ^bKilimanjaro Clinical Research Institute, Moshi, Tanzania, ^cTanzania Veterinary Laboratory Agency, Dar es Salaam, Tanzania, ^dDepartment of Public Health and Research, Lugalo Military College of Medical Sciences, Dar es Salaam, Tanzania, ^eDepartment of Global Health and Biomedical Sciences, Nelson Mandela Institution of Science and Technology, Arusha, Tanzania

Correspondence to Jaffu Chilongola (j.chilongola@kcri.ac.tz)

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 ($\chi^2=3.504$; $p<0.05$), being ≥ 7 individuals living in the same household ($\chi^2=4.655$; $p<0.05$) and a recent travel to an urban destination ($\chi^2=3.39$; $p<0.05$) were the only factors associated with CHIKV seropositivity. ITN use was the only factor associated with CHIKV infection ($\chi^2=5.204$; $p<0.05$). A recent travel to an urban destination ($\chi^2=4.401$; $p<0.05$) 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 ($\chi^2=238.45$; $p<0.01$). 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 outbreaks¹. Both DENV and CHIKV are spread by common mosquito vectors, mainly *Aedes aegypti*.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 previously¹³, is an intensive rice irrigation area, located on the southern foothills of Mount Kilimanjaro¹³ (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

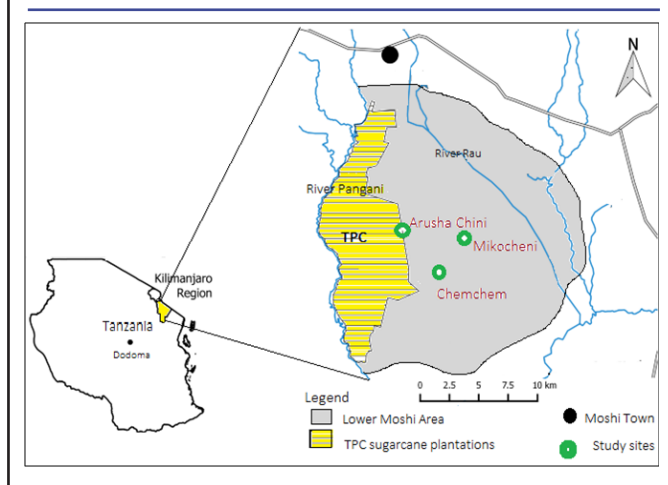
Participants in this study were males and females aged between 10 and 70 years from 266 households. The main occupation of inhabitants in the area is agropastoralism. Consent to participate in the study was obtained from ad-

ults 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 $ss = \frac{Z^2(P)(1-P)}{\epsilon^2}$, 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 described²⁰. 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 by²¹. 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 method²² as described by¹⁷. 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 probes²³ 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, Germany¹⁵; 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 (χ^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 χ^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) ($\chi^2=3.504$; $P<.05$), being more than 7 individuals

in the same household ($\chi^2=4.655$; $P<.05$) and a recent travel to an urban destination ($\chi^2=3.39$; $P<.05$) were the only factors associated with CHIKV seropositivity. For CHIKV infection, ITN use was the only factor that was associated with CHIKV infection ($\chi^2=5.204$; $P<.05$). 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 ($\chi^2=4.401$; $P<.05$). 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 ($\chi^2 = 238.45$; $P<.01$) (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 in 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 reported²⁵⁻²⁸. 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 area⁴¹. 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
Total	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	0(0.0)	10(3.8)	0(0)	10(3.8)	1(20.0)	9(90.0)	0(0.0)	10(100.0)
Human DENV-PCR	0(0.0)	256(96.2)	1(100)	255(96.2)	4(80.0)	252(96.6)	4(100.0)	252(96.2)
Human CHIKV-PCR	0(0.0)	9(3.4)	0(0.0)	9(100.0)	1(11.1)	8(88.9)	0(0.0)	9(3.4)
Human DENV-PCR	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 KCRl 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.

REFERENCES

1. Kilpatrick AM, Randolph SE. Drivers, dynamics, and control

- of emerging vector-borne zoonotic diseases. *The Lancet*. 2012;380:1946-1955.
2. Delatte H, Paupy C, Dehecq JS et al. *Aedes albopictus*, vector of chikungunya and dengue viruses in Reunion Island: biology and control. *Parasite (Paris, France)*. 2008;15:3-13.
 3. Salam N, Mustafa S, Hafiz A et al. Global prevalence and distribution of coinfection of malaria, dengue and chikungunya: a systematic review. *BMC Public Health*. 2018;18:710.
 4. Castellanos JE, Jaimes N, Coronel-Ruiz C et al. Dengue-chikungunya coinfection outbreak in children from Cali, Colombia, in 2018GÇô2019. *Int J Infect Dis* 2021;102:97-102.
 5. Yactayo S, Staples JE, Millot V et al. Epidemiology of Chikungunya in the Americas. *J Infect Dis*. 2016;214:S441-S445.
 6. Furuya-Kanamori L, Liang S, Milinovich G et al. Co-distribution and co-infection of chikungunya and dengue viruses. *BMC infectious diseases*. 2016;16:1-11.
 7. Rodriguez-Morales AJ, Paniz-Mondolfi AE, Faccini-Martínez A et al. The Constant Threat of Zoonotic and Vector-Borne Emerging Tropical Diseases: Living on the Edge. *Front Trop Dis*. 2021;2:3.
 8. Carrillo-Hernández MY, Ruiz-Saenz J, Villamizar LJ et al. Co-circulation and simultaneous co-infection of dengue, chikungunya, and zika viruses in patients with febrile syndrome at the Colombian-Venezuelan border. *BMC infectious diseases*. 2018;18:1-12.
 9. Villamil-Gómez WE, González-Camargo O, Rodríguez-Ayubi J et al. Dengue, chikungunya and Zika co-infection in a patient from Colombia. *J Infect Public Health*. 2016;9:684-686.
 10. Taraphdar D, Sarkar A, Mukhopadhyay BB et al. A comparative study of clinical features between monotypic and dual infection cases with Chikungunya virus and dengue virus in West Bengal, India. *Am J Trop Med Hyg*. 2012;86:720.
 11. Gandhi BS, Kulkarni K, Godbole M et al. Dengue and chikungunya co-infection associated with more severe clinical disease than mono-infection. *Int J Health Biomed Res*. 2015;3:117-123.
 12. Ferreira MLcB, de Brito CAA, de Oliveira Franço RF et al. Neurological disease in adults with Zika and chikungunya virus infection in Northeast Brazil: a prospective observational study. *The Lancet Neurology*. 2020;19:826-839.
 13. Kumaliya MS, Chilongola JO, Budodo RM et al. Detection of Rift Valley Fever virus inter-epidemic activity in Kilimanjaro Region, North Eastern Tanzania. *Global health action*. 2021;14:1957554.
 14. Medard S Kumaliya, Jaffu O Chilongola, Rule M Budodo et al. Detection of Rift Valley Fever virus inter-epidemic activity in Kilimanjaro Region, North Eastern Tanzania. *Global health action*. 2021;14:1957554.
 15. Weaver SC. Chikungunya in the New World: prospects for spread and health impact. *PloS Negl Trop Dis*. 2014; 8:e2921.
 16. World Health Organization. Global strategy for dengue prevention and control 2012-2020. 2012.
 17. Kajeguka DC, Kaaya RD, Desrochers R et al. Mapping clusters of chikungunya and dengue transmission in northern Tanzania using disease exposure and vector data. *Tanzania Journal of Health Research*. 2017;19.
 18. Huang YM, Ward RA. A pictorial key for the identification of the mosquitoes associated with yellow fever in Africa. 1981. SMITHSONIAN INSTITUTION WASHINGTON DC MEDICAL ENTOMOLOGY PROJECT.
 19. Gerberg EJ, Van Someren ECC. Pictorial key to the mosquitoes *Aedes (Stegomyia)* of East Africa. World Health Organization (Mimeographed document). 1970.
 20. Budodo RM, Horumpende PG, Mkumbaye SI et al. Serological evidence of exposure to Rift Valley, Dengue and Chikungunya Viruses among agropastoral communities in Manyara and Morogoro regions in Tanzania: A community survey. *PloS Neglected Tropical Diseases*. 2020;14:e0008061.
 21. Kajeguka DC, Kaaya RD, Mwakalinga S et al. Prevalence of dengue and chikungunya virus infections in north-eastern Tanzania: a cross sectional study among participants presenting with malaria-like symptoms. *BMC infectious diseases*. 2016; 16:183.
 22. Boom RCJA, Sol CJ, Salimans MM et al. Rapid and simple method for purification of nucleic acids. *Journal of clinical microbiology*. 1990;28:495-503.
 23. Pongsiri P, Praianantathavorn K, Theamboonlers A et al. Multiplex real-time RT-qPCR for detecting chikungunya virus and dengue virus. *Asian Pacific journal of tropical medicine*. 2012;5:342-346.
 24. Serگون K, Njuguna C, Kalani R et al. Seroprevalence of chikungunya virus (CHIKV) infection on Lamu Island, Kenya, October 2004. *Am J Trop Med Hyg*. 2008;78:333-337.
 25. Kajeguka DC, Msonga M, Schieler KL et al. Individual and environmental risk factors for dengue and chikungunya seropositivity in North-Eastern Tanzania. *Infection, disease & health*. 2017;22:65-76.
 26. Kinimi E, Shayo MJ, Patrick BN et al. Evidence of chikungunya virus infection among febrile patients seeking healthcare in selected districts of Tanzania. *Infection ecology & epidemiology*. 2018;8:1553460.
 27. Vairo F, Nicastrì E, Meschi S et al. Seroprevalence of dengue infection: a cross-sectional survey in mainland Tanzania and on Pemba Island, Zanzibar. *Int J Infect Dis*. 2012;16:e44-e46.
 28. Moro ML, Gagliotti C, Silvi G et al. Chikungunya virus in North-Eastern Italy: a seroprevalence survey. *Am J Trop Med Hyg*. 2010;82:508.
 29. Asebe G, Michlmayr D, Mamo G et al. Seroprevalence of Yellow fever, Chikungunya, and Zika virus at a community level in the Gambella Region, South West Ethiopia. *PloS one*. 2021;16:e0253953.
 30. Kawle AP, Nayak AR, Bhullar SS et al. Seroprevalence and clinical manifestations of chikungunya virus infection in rural areas of Chandrapur, Maharashtra, India. *J Vector*

- Borne Dis. 2017;54:35.
31. Mohanty I, Dash M, Sahu S et al. Seroprevalence of chikungunya in southern Odisha. *J Family Med Prim Care*. 2013;2:33.
 32. Gubler DJ. Dengue, urbanization and globalization: the unholy trinity of the 21st century. *Tropical medicine and health*. 2011;39:S3-S11.
 33. Agha SB, Tchouassi DP, Turell MJ et al. Entomological assessment of dengue virus transmission risk in three urban areas of Kenya. *PLoS neglected tropical diseases*. 2019;13:e0007686.
 34. Chipwaza B, Mugasa JP, Selemani M et al. Dengue and Chikungunya fever among viral diseases in outpatient febrile children in Kilosa district hospital, Tanzania. *PLoS neglected tropical diseases*. 2014;8:e3335.
 35. Gubler DJ, Clark GG. Dengue/dengue hemorrhagic fever: the emergence of a global health problem. *Emerging infectious diseases*. 1995;1:55.
 36. Mboera LE, Mweya CN, Rumisha SF et al. The risk of dengue virus transmission in Dar es Salaam, Tanzania during an epidemic period of 2014. *PLoS neglected tropical diseases*. 2016;10:e0004313.
 37. World Health Organization. Dengue and severe dengue. 2014. World Health Organization. Regional Office for the Eastern Mediterranean.
 38. Faustine NL, Sabuni EJ, Ndaro AJ et al. Chikungunya, Dengue and West Nile Virus Infections in Northern Tanzania. *Journal of Advances in Medicine and Medical Research*. 2017;1-7.
 39. Shauri HS, Ngadaya E, Senkoro M et al. Seroprevalence of Dengue and Chikungunya antibodies among blood donors in Dar es Salaam and Zanzibar, Tanzania: a cross-sectional study. *BMC infectious diseases*. 2021;21:1-6.
 40. Ward T, Samuel M, Maoz D et al. Dengue data and surveillance in Tanzania: a systematic literature review. *Tropical Medicine & International Health*. 2017;22:960-970.

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.v4i1.62>

© Chilongola et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are properly cited. To view a copy of the license, visit <http://creativecommons.org/licenses/by/4.0/>. When linking to this article, please use the following permanent link: <https://doi.org/10.24248/easci.v4i1.62>
