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Chikungunya, Dengue and West Nile Virus Infections in Northern Tanzania

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Authors' contributions

This work was carried out in collaboration between all authors. Author NLF designed the study, wrote the first draft of the manuscript. Authors EJS and EP performed the statistical analysis and wrote the protocol. Author AJN performed laboratory analysis of samples. Author JOC designed the study, managed the analyses of the study and made critical review of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: We aimed at determine the prevalence of Chikungunya virus (CHIKV), Dengue virus (DENV) and West Nile virus (WNV) in Bondo and Magugu wards in Handeni and Babati districts in Northern Tanzania, in a cross sectional analytical study.
Study Design: This was cross sectional, community based study involving individuals aged 1-80 years of age. Reverse transcription Polymerase Chain Reaction (RT-PCR) was used to detect arboviruses in whole blood samples. Data was analyzed using SPSS 22.0. Chi square and logistic regression analyses were used to determine associations of explanatory factors and arbovirus infection.
Results: Out of 128 participants recruited, 10 (7.8%) and 1 (0.8 %) were positive for CHIKV and DENV, respectively. None of the participants recruited was positive for WNV. Most cases of arboviruses were detected in Magugu with 8 (12.31%) and 1 (1.54%) individuals being positive for CHIKV and DENV respectively. Male sex was associated with CHIKV infection (χ2=9.126,

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p=0.003), with five times more odds of having CHIKV infection compared to females (OR: 5.30;(95%CI: 1.21-23.17), P=0.027). No WNV case was detected in any of the sites.
Conclusion: CHIKV is the most prevalent arbovirus in the Northern part of Tanzania. Magugu site has more arbovirus transmission than Bondo site. WNV could be a rare virus in the Northern part of Tanzania. Male sex is associated with higher CHIKV infection rates, with older children being more affected.

Keywords: West nile; chikungunya; dengue; arboviruses; Tanzania.

1. INTRODUCTION

Arboviruses are among the commonest causes of human febrile illnesses worldwide. Despite their public health relevance, very little is known about their geographical distribution, risk factors and impacts on the arbovirus infections in most regions of the world [1]. Arboviruses are a growing health threat globally and have been responsible for large epidemics in many sub-Saharan countries [1]. The viruses, which cause viraemia in the blood of the host species and fever, cause focal epidemics with high case fatalities that are difficult to predict and control [2]. Fever without specific cause is one of the most common presenting complaints among patients seeking healthcare in low income countries and poor communities. Etiologies of various febrile illnesses are difficult to distinguish from one another clinically; therefore clinical management is commonly driven by syndrome-based guidelines [3].

There is a reported shift in disease transmission patterns in Sub Saharan Africa (sSA), with regards to febrile illnesses whereby there is a decreasing number of malaria cases in many countries in sSA [4,5]. Despite the general drop in malaria incidence, the number of febrile cases due to other fever causing pathogens is still high [6]. This implies the presence of alternative causes of febrile illnesses including emerging and re-emerging infectious diseases including Dengue virus (DENV), Chikungunya virus (CHIKV) and West Nile virus (WNV) [7]. It is unfortunate that, apart from being known as a significant causes of Non Malarial Febrile Illnesses (NMFIs)[3], these infections are also known to share similar symptoms with other febrile infections including malaria [8,9]. They also display an overlap with malaria in terms of geographic and seasonal distribution [10]. The occurrence of arboviruses in Tanzania has been documented previously mainly by serological surveys for CHIKV [8,11,12], DENV [8,13,14] and others [12-15]. Recent studies in Tanzania have revealed that Dengue and CHIKV fever are commonly misdiagnosed and treated with either anti-malarials or antibiotics [15,16].

The Clinical presentation of arboviral infections is challenging since it is similar to many other causes of febrile illnesses such as malaria. Unfortunately, there is paucity of epidemiological data regarding the virological prevalence of arboviruses in many parts of Africa, including Tanzania. Such data could assist clinicians to make considerable and informed decisions when consulted with patients who present with symptoms that pose a diagnostic dilemma. We therefore designed this study to provide baseline epidemiological data on the virological prevalence of DENV, CHIKV and WNV infections in two sites in the Northern part of Tanzania that have different climatic characteristics. This data will provide useful baseline data that will inform on the magnitude of arbovirus infections and hence help clinical decisions and prescription of medicines.

2. MATERIALS AND METHODS

2.1 Study Design, Sites and Population

This was a cross sectional community based study that aimed at determining the prevalence of three arboviral infections that are thought to be important causative agents of fevers in two sites with different malaria transmission intensities, from November 2016 to May, 2017. The study included 128 participants from two wards, one from each, Handeni district of Tanga region and Babati Rural district of Manyara region in Northern Tanzania. These sites were selected due to the evidence of over diagnosis of malaria in patients with fever. The study enrolled children and adults aged 1 year and above who were residents in the study areas. Bondo is a coastal lowland rural area in Handeni, Tanga region located 309 m above sea level. Bondo is endemic to malaria with a perennial transmission. Magugu ward (S3099' S4001'; E35070' E35077') in Babati District is located in northern Tanzania along the rift valley, at

approximately 900m to 1200m. The ward has a total population of 26,131 people residing in seven villages. There is one government owned health centre which serves all the seven villages. Babati District, where Magugu site is located, is one of the eight national sentinel sites for neglected tropical diseases. The residents of Magugu areas are highly involved in paddy cultivation and livestock keeping with moderate human-animal integration. Magugu site experiences an average annual rainfall is about 650 mm [17] while Bondo site experiences an average annual precipitation of 921 mm [18]. Both areas have two rainy seasons per year, the long rainy season between February and May, while short rains span between October and December. The long rainy seasons are usually followed by high number of reported fever cases.

2.2 Sample Size and Sample Collection Procedures

The study sites were chosen conveniently as research field sites for the Kilimanjaro Clinical Research Institute and Kilimanjaro Christian Medical University College, in Moshi town in Kilimanjaro Region, Tanzania. Participants were selected randomly to represent an approximately equal number of participants from each site. A short questionnaire was used to obtain demographic information from participants. A blood sample of 0.5-1 mL was taken by venipuncture from all consenting participants. In order to manage participants with malaria, a thick and thin blood smears for malarial microscopy were prepared as described elsewhere [19] and about 10ul of whole blood was subjected to rapid diagnostic test for malaria diagnosis (SD BIOLINE® Malaria Ag P. f/Pan). Children under the age of 5 who were found to be malaria positive by rapid diagnostic test were immediately treated with anti-malarial according to national and WHO guidelines. Remaining whole blood was shipped at -4℃ till when viral RNA extraction was performed the next day. Adults with fever and positive by mRDT were treated with Arthemether-Lumefantrine (ALu), the first-line anti-malarial drug in Tanzania.

2.3 RNA Extraction and cDNA Synthesis

Viral RNA's were extracted from human whole blood using the QIAamp Viral RNA Mini kit (QIAGEN, Hilden, Germany) according to manufacturer's instructions. An aliquot of 200 µL from blood sample was lysed by QIAGEN protease and bound to QIAamp membrane by centrifugation as described by manufacturer's instructions. The wash buffers AW1 and AW2, each at a time, remove residual contaminants in order to improve the purity of the RNA. The purified RNA was then eluted from QIAamp membrane by Buffer AE and subjected to DNA by reverse transcription processes and then stored at –20℃ ready for PCR.

2.4 Multiplex Reverse Transcriptase Polymerase Chain Reaction

The PCR assay was carried out in an Applied Biosystems® ViiA™ 7 Real-Time PCR (Life Technologies Corporation, CA, USA). The Mastermix Kit (Fast-Track Diagnostics® (FTD), Luxembourg) for Tropical Fever Core was used to prepare the reaction mix. For single reaction, 12.5 µl of FTD buffer, 1.5 µl of Tropical Fever primers and probe mix (TF PP mix) and 1µl Enzyme mix were placed into a single MicroAmp® Optical 8-Tube compatible with the ViiA™ 7 RT PCR, followed by 10µl of sample. The same was done for all samples, positive control and extracted negative control. The detection of pathogens was done at wavelengths of 520 nm for Dengue virus (TF2 PP), 610 nm for West Nile virus (TF2 PP) and 550 nm for CHIKV virus (TF1 PP). The positive, negative and internal controls used in this assay were commercially prepared by Fast-Track Diagnostics (FTD), Luxembourg. The positive control contained plasmids for the detection of WNV, DENV and CHIKV. Negative control contained lysis buffer while the internal control contained Streptococcus equi (Sequi) which was also used as an extraction control. At the end of the run, amplification plots were reviewed in order to adjust the threshold line above all the background noise as instructed by the manufacturer.

2.5 Data Processing and Statistical Analysis

Data were listed, selected, sorted and manipulated using Microsoft Excel sheet and analyzed using SPSS 22.0 statistical software (IBM SPSS Inc., Chicago, IL, USA). Chi-square ($\chi 2$) was used to determine independent association of study factors. Risk factors for CHIKV, West Nile and Dengue viruses were analyzed by logistic regression. Odds ratios (ORs) and their confidence intervals (95% CIs) were calculated to determine the strength of association between outcome (arboviral positivity) and variables. p-value < 0.05

was considered the cut off for statistical significance.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Socio-demographic and clinical characteristics of the study participants

This study included 128 participants from Magugu and Bondo in Tanzania. Sixty three (49.2%) of the study participants were recruited from Magugu while sixty five (50.8%) participants were recruited from Bondo site. Nearly two-thirds (66.4%) of the study participants were females. Eighty three (64.8%) of the study participants were adolescents and adults (≥15 years). Out of the 128 participants recruited, 10 (8.7%) had fever at the time of survey with 3 missing fever cases (Table 1).

Table 1. Demographic and clinical characteristics of participants (n= 128)

Variable	Number	Percentage (%)
Sex		
Female	84	66.4
Male	43	33.6
Fever*		
No Fever	115	91.3
Fever	10	8.7
Age (years)		
<5	19	14.8
5-9	17	13.3
10-15	9	7
>15	83	64.8
Site		
Magugu	63	49.2
Bondo	65	50.8

**There were three missing fever cases*

3.1.2 Prevalence of viral infections

Out of 128 participants, 10 (7.8%) were CHIKV positive, 1 (0.8%) was DENV positive; there was no WNV positive cases detected. Also none of the participants recruited was found to have mixed or arbovirus co-infection. Since positivity for the tested pathogens was exclusively localized between the two sites, analysis for individual sites was not done. Taking study sites separately, there were 8 CHIKV positive cases in Magugu and only 2 from Bondo. The only DENV positive case was detected from Magugu (Table 2)

Table 2. Prevalence of arboviruses in Magugu and Bondo (n=128)

Virus	Bondo N (%)	Magugu N (%)
CHIKV	2 (3.17)	8 (12.31)
DENV	0 (0.00)	1 (1.54)
WNV	0 (0.00)	0 (0.00)

3.1.3 Association of age, fever and sex with CHIKV and DENV infections

Among the variables studied only sex was found to be associated with CHIKV positivity (χ^2=9.126, p=0.003), age and fever were not associated with CHIKV positivity. Further statistical analysis for DENV infection was limited due to the small number (only 1) of positive DENV cases (Table 3).

3.1.4 Risk factors for CHIKV infection

Gender was found to be a risk factor for CHIKV positivity by logistic regression. Male gender had 5 times higher odds of having CHIKV as compared to female gender (OR: 5.30; (95%CI: 1.21-23.17), P=0.027). This implies that being a male was a risk factor for of being positive for CHIKV (Table 4). Despite the absence of statistical evidence, this study has observed that Magugu residents were 11 times more likely to be CHIKV positive compared to Bondo residents (OR:11.31; (95%CI 0.70-18.31),p=0.087). Risk factors for DENV positivity, WNV positivity, and fever and arboviral co-infection were not analyzed due to the small number of DENV positive samples i.e. 1(0.8%) for DENV positive and none for WNV and arboviral co-infection (Table 3). Most of CHIKV positive cases had no fever whereby only one fever case was found to be infected with CHIKV, which could suggest the presence of other causes of fever apart from CHIKV.

3.2 Discussion

The current study was designed to assess the prevalence of CHIKV, WNV and DENV in both malaria endemic and non-endemic areas and assess the extent of co-infections between these viruses and their associated risk factors, in order to establish baseline epidemiological information in our setting. Analysis of blood samples by RT-PCR has shown that 10 (7.8%) of the individuals out of 128 individuals were infected with CHIKV while only 1 individual (0.8%) had DENV infection. None of the participants was found to be positive for WNV. Epidemiological data on

CHIKV and WNV in Tanzania has largely been reported in Dar-es Salaam, the capital city of Tanzania during dengue large-scale outbreak in 2010 [16] and Tanga region, both regions on the Tanzanian coast [18]. Most of such studies have used serological assays with only a few of them reporting virological prevalence the viruses. Our study is therefore among the few studies to provide a virological prevalence of the three arboviruses in the Northern part of Tanzania.

This study reports a virological CHIKV prevalence of 7.8%, suggesting an active CHIKV transmission in the Northern part of Tanzania. These results are in line with other studies which were done in Tanga, Moshi and Hai which reported a prevalence of 4.2% [18]. Differences in prevalence are logically attributable to the tests used in the diagnosis. While previous studies in North Tanzania had mainly used ELISA tests, our study has used RT-PCR, a highly sensitive molecular technique.

This study reports a higher CHIKV prevalence in Magugu than Bondo. Little data is available on the abundance of vector mosquitoes for CHIKV in Magugu and Bondo sites. However, our findings may suggest the possibility of higher abundance of *Aedes* spp mosquitoes in Magugu than Bondo, although this speculation needs to be ascertained. Current study reported 1 dengue positive case (0.8%), this indicates that dengue is a rare arboviral infection in Northern Tanzania [16,19] and is not associated with fever in the study community. This finding correlates with other studies in Handeni and in Northern Tanzania which reported the prevalence of 0.2% and 1.3% respectively. We did not detect any WNV in the current study, suggesting the absence of the virus in the Northern part of Tanzania.

Males had five times higher odds of being CHIKV positive. A likely explanation for this finding is due to the nature of outdoor activities that males are engaged with, such as agricultural activities, life style that make them stay late in evenings, when vector mosquitoes are active. This finding correlates with the study done in Handeni which reported females were at reduced risk for CHIKV infection by 28% as compared to men [16,19].

Table 3. Test results for associations between participant age categories, sex and fever status with Chikungunya virus infection in Boindo and Magugu sites (n=128)

Site	Variable	CHIKV positive	CHIKV negative	X^2 (p value)
Bondo	**Age**	**n(%)**	**n(%)**	
	<5	0(0.0)	14 (23.0)	(0.665)0.415
	5-9	2 (100)	14 (23.0)	
	10-15	0(0.0)	9 (14.3)	
	>15	0(0.0)	24 (39.3)	
	Sex			
	Female	2 (100)	43 (70.5)	(0.813)0.367
	Male	0 (0.0)	18(29.5)	
	Fever	1 (50.0)	9 (14.8)	(1.773)0.183
	Fever	1 (50.0)	52 (85.2)	
	No fever			
Magugu	**Age**			
	<5	1 (12.5)	4 (7.0)	(0.169)0.681
	5-9	0(0.0)	1 (1.8)	
	10-15	0(0.0)	9 (7.6)	
	>15	7 (87.5)	76(64.4)	
	Sex			
	Female	1 (12.5)	39 (68.4)	(9.126)0.003**
	Male	7 (87.5)	18 (31.6)	
	Fever			
	Fever	0 (0.0)	0 (0.0)	(0.059)0.809
	No fever	8 (100.0)	54 (100.0)	

***P<0.001*

Table 4. Risk factors associated with CHIKV positivity (n=128)

Variable	cOR (95% CI)	p-value	aOR (95% CI)	p-value
Site				
Bondo	1		1	
Magugu	4.28 (0.87 -21.01)	0.073	11.31 (0.70-18.31)	0.087
Sex				
Female	1		1	
Male	5.31 (1.30-21.73)	0.02	5.30 (1.21-23.17)	0.027*
Age(Years)				
<5	1		1	
5-9	2.67 (0.221-32.23)	0.44	11.54 (0.44-30.99)	0.142
10-15	0.00 (000)		0.00 (000)	
>15	1.84 (0.21-15.85)	0.578	1.21 (0.11-12.79)	0.874

*cOR= crude Odds Ratio, aOR= adjusted Odds Ratio, CI= Confidence Interval, *p <0 05.*

Furthermore according to recent report on prevalence of CHIKV and DENV fever among hospitalized febrile patients in Northern Tanzania [16], CHIKV was more common among infants and children than in adults and adolescences while the current study has shown that CHIKV was more common among adults and adolescences than infants and children. This may have been attributed by the fact that adults are more in contact with the mosquito bites than infants and children whom in most cases sleep under mosquito bed nets that are freely provided by various interventions and campaigns to infants and pregnant women [19]. The contradiction may have arisen due to differences in the study populations used. While the previous study involved hospitalized febrile patients, our study reports a community prevalence of the virus. The absence of co-infections in the current study may be explained by the sharp differences in prevalence of the viruses across the study sites

4. CONCLUSION

CHIKV is the most prevalent arbovirus in the Northern part of Tanzania while no WNV transmission is reported by the present study. Male sex was associated with CHIKV infection, with older children being more affected. Surveillance of arbovirus transmission combined with *Aedes* vector abundance would provide more precise transmission dynamics of arboviruses in affected areas.

CONSENT

All authors declare that written informed consent was obtained from the participants for publication of this manuscript.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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