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Mapping clusters of chikungunya and dengue transmission in northern Tanzania using disease exposure and vector data

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Abstract

Background: Dengue and chikungunya are mosquito-borne viral diseases that are of public health importance throughout the tropical and subtropical regions of the world. Seasonal variations in transmission of these viruses have been suggested owing to the ecology of their mosquito vectors. However, little is known about the epidemiology of the diseases Tanzania. To address this gap, seasonal community-based cross-sectional surveys were undertaken to identify potential clusters of transmission in Hai district in northern Tanzania.

Methods: Epidemiological and entomological data from two cross-sectional surveys were used to examine the spatial pattern of dengue and chikungunya transmission. Six villages namely, Boma Ng'ombe, Magadini, Rundugai, Nshara and Kware were involved in the study. Serological measures of dengue and chikungunya virus infections were derived using enzyme-linked immunosorbent assays, and all participants were geo-referenced to the household level using a global positioning system. Potential clusters of individual exposed to dengue and chikungunya virus , as well as clusters of *Aedes* mosquitoes in the wet and dry seasons were detected using SaTScan. All significant clusters (with $p \leq 0.05$) were mapped using ArcGIS.

Results: A large, widely dispersed cluster of chikungunya exposed individuals was detected spanning Rundugai and parts of Magadini villages (RR = 2.58, p= 0.01), while no significant clustering was observed in the dry season. Spatial clusters of *Aedes aegypti* were detected in Rundugai in both the wet and dry seasons (RR = 2.56, $p< 0.001$ and RR = 2.24, p=0.05, respectively). In the dry season a small cluster was also detected in Kware (RR = 2.25, p=0.05). No significant clusters of dengue were detected in both seasons.

Conclusion: Clusters of chikungunya-exposed individuals and *Aedes* mosquitoes indicate on-going transmission of chikungunya virus in Hai district of northern Tanzania.

Keywords: dengue, chikungunya, spatial, clusters, vector data, Tanzania

Introduction

Dengue and chikungunya are mosquito-borne viral diseases that are widely recognized as public health challenges in most tropical and sub-tropical parts of the world (Diop *et al.*, 2015). Usually, these infections go unnoticed in most countries in Sub-Saharan Africa (SSA). Therefore, diagnosis of patients during post symptomatic phase or asymptomatic (5-7 days after symptoms onset) needs to be supported by serological tests (Duong *et al.*, 2011; Neeraja *et al.*, 2013; CDC, 2016). The most commonly employed serological procedure is the immunoglobulin M (IgM) capture enzyme-linked immunosorbent assays (ELISA) (Chen & Wilson, 2010). In more than 50% of individuals, dengue IgM or chikungunya IgM antibodies are detectable 5

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or more days after onset of illness/symptoms in primary infection and may persist for 2 to 3 months (Peeling *et al.*, 2010). Hence, IgM-based assays can be used in clinical surveillance for viral illness or for random, population-based serological surveys, with the possibility that any positive results indicate a marker for recent infections although not necessarily acute infections (Peeling *et al.*, 2010; Blacksell, 2012).

Studies have reported that due to changes in environmental factors affecting vector populations, and human factors such as population density and immunological profiles, the transmission of dengue and chikungunya often shows dynamic spatial patterns in a given location (Louis *et al.*, 2014; Nsoesie *et al.*, 2015). There are various methods or techniques to uncover spatial patterns of disease including cluster detection, hotspot analysis, and regression models (Sugumaran *et al.*, 2009). Spatial analysis aiming at detecting dengue and chikungunya disease clusters has been widely used to generate risk maps of transmission (Flauzino *et al.*, 2009; Jeefoo *et al.*, 2011; de Melo *et al.*, 2012; Labeaud *et al.*, 2015; Nsoesie *et al.*, 2015). Thus, mapping populations exposed to dengue or chikungunya viruses can provide an early warning signal and to some extent facilitate the allocation of public health resources to prevent a possible epidemic before such occurs. Furthermore, the presence of vector in a given area is an important indicator for predicting transmission risk (Khormi *et al.*, 2011; Khormi & Kumar, 2012).

In epidemiological investigations, disease outbreaks are often preceded by research findings that demonstrate the presence of IgM markers of infection, hence indication of transmission (Mangu *et al.*, 2016). With increasing dengue and chikungunya activity in Tanzania, as suggested by sero-prevalence surveys conducted in different areas of the country (Heinrich *et al.*, 2012; Hertz *et al.*, 2012; Chipwaza *et al.*, 2014; Kajeguka *et al.*, 2016; Vairo *et al*., 2012) and recent dengue fever outbreaks in Dar es Salaam (Mboera et al., 2016; Baba *et al.*, 2016; Vairo *et al*., 2016), a clearer picture of the epidemiological clusters of transmission and areas at risk is required. Vector-borne disease transmission is often heterogeneous owing to the distribution of vectors and hosts, and underlying social and ecological determinants. Therefore, identifying clusters of disease transmission is useful for targeting strategies for surveillance, prevention and control, and as well focus future research.

Dengue and chikungunya transmission have been reported in urban settings, with social connections, movement of people among the homes of family and friends playing a key role in the spread of *Ae. aegypti* (Volk *et al.*, 2010; Weaver & Reisen, 2010; Stoddard *et al.*, 2013). Morover, dengue and chikungunya infections have been reported to circulate in rural communities (Demanou *et al.*, 2010; Vong *et al.*, 2010; Khatun *et al.*, 2015). This study presents a spatial analysis of dengue, chikungunya and mosquito occurrence data from a rural area of Tanzania to assess the presence of any significant spatial clusters of transmission during the wet and dry seasons.

Materials and Methods

Study setting, population sampling and design

This study was carried out in Hai District, Kilimanjaro region in northern Tanzania. The district has an area of 1,011 km^2 of tropical savannah with a population of about 210,000 (URT, 2013). There are two main rainy seasons, the long rainy season during March-June and the short rainy season during November-December. On average, the district receives 700 mm of rainfall in the lowlands, 1,250 mm of the rainfall in the mid zone and 1,750 mm of the rainfall in the upper zone (HDC, 2016). *Ae. aegypti* has been documented from several villages in Hai District (Hertz *et al.*, 2016).

In this study, five villages, namely, Boma Ng'ombe, Magadini, Rundungai, Kware and Nshare, were randomly selected (Figure 1). Boma Ng'ombe is a semi-urban township situated along the Moshi-Arusha highway, Rundugai and Magadini are rural villages situated in the lowland plains while Kware and Nshara are rural villages situated in the more dense vegetation-covered highland area. Two cross-sectional surveys were conducted during the wet and dry seasons in June 2015 and February 2016, respectively.

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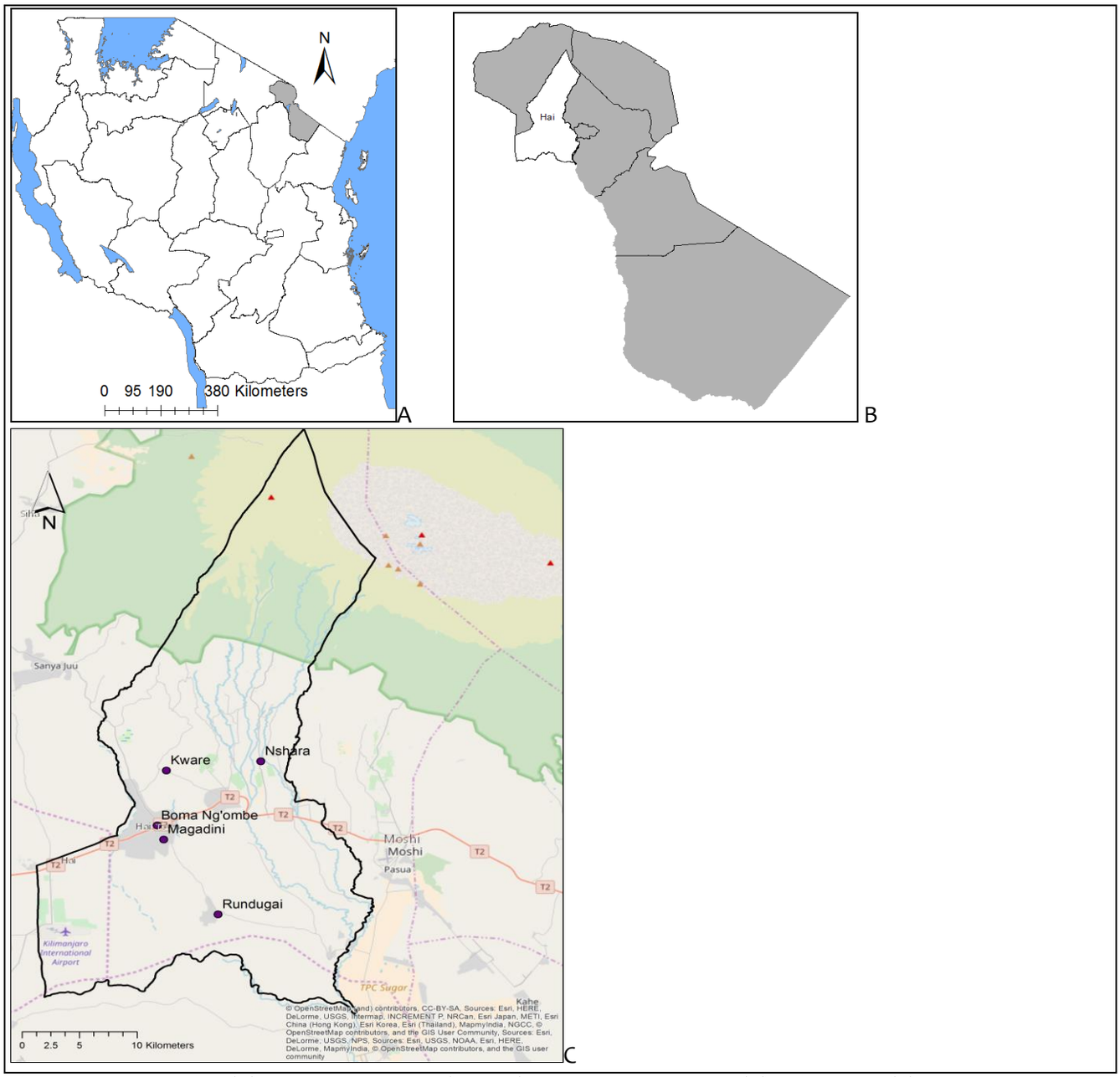

Figure 1: Map showing (A) Kilimanjaro region (in gray) in northern Tanzania, (B) Hai district; (C) study villages in Hai District (Source ESRI)

Demographic information and household geocordinates

Informations on all participants were collected using a standardized questionnaire, which covered population demographics (age, sex, level of education), symptoms (headache, joint or muscle pains) and geographical location. By using a Global Positioning System (GPS) receiver (Garmin Corporation, Taiwan), the geographical coordinates of the housheols were recorded.

Blood sample collection and serological analysis

A venous blood sample (1 ml) from each participant was collected into a 1.5 ml eppendorf tube containing ethylene diamine tetraacetic acid (EDTA) (Eppendorf, USA Scientific, Inc.). Samples were transported to the Kilimanjaro Christian Medical Centre Biotechnology laboratory within eight hours of collection for plasma separation and storage at -20°C. Serological analysis was conducted on all blood samples. Anti-dengue IgM

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antibodies were detected using a direct ELISA kit while anti-chikungunya IgM were analysed using an indirect ELISA kit (both from Standard Diagnostics, Gyeonggi-do, Korea). All assays were conducted according to manufacturer's instructions. Briefly, all plasma samples were diluted 1:100 with sample diluent provided with the kits. The optical density (OD) was measured at 450 nm and the units of antibody concentration and cut-off values calculated as described by the manufacturer. Briefly, for the anti-dengue IgM and anti-chikungunya IgM ELISAs the diagnostic cut-off value was calculated as the average OD of negative controls + 0.300.

Mosquito sampling and processing

Mosquito collection was carried out in the wet and dry seasons. In each village, two carbon-dioxide Mosquito Magnet® Liberty traps (American Biophysics Corporation) were set for two consecutive days to sample outdoor day-biting mosquitoes. The traps were placed at least 0.5 to 1 km apart and in proximity to potential *Aedes* breeding sites and nearby banana plantations or other vegetations and operated from 06:00 to 18:00 hours. Mosquitoes were transported to Kilimanjaro Christian Medical University College laboratory where they were freshly killed by placing them at -20 ºC for 20 minutes and then identified to species level using standard morphological identification keys (Huang, 2001). Mosquitoes were then preserved with RNAlater™ stabilization solution (Invitrogen, USA) and transported to the National Microbiology Laboratory (NML), Winnipeg, Canada for further analysis.

Molecular identification of *Aedes* mosquitoes was accomplished through sequence analysis of the CO1 gene, using a protocol developed at NML. Briefly, mosquito DNA was extracted using the QIAamp® DNA Mini Kit protocol (QIAGEN, Montreal, Quebec) and the CO1 gene was amplified using primers adopted from Folmer et al. (Folmer *et al.*, 1994): forward LCO1490 (5'GGTCAACAAATCATAAAGATATTGG-3'); reverse HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3'). For the polymerase chain reaction (PCR), 1 µl DNA template was added to 5 µl 10x buffer, 4 µl dNTP mix, 3 µl $MgCl_2$, 2 µl each primer at 10 µM and 0.5 µl Taq polymerase (all reagents from Invitrogen, USA). Thermocycling conditions consisted of; denaturation at 94°C for 2 minutes, 30 cycles of amplification at 94°C for 30 seconds, 53°C for 30 seconds and 72°C for 80 seconds, followed by a 5 minute extension at 72°C. Amplification products were analyzed by ethidium bromide-stained 2% agarose gels. The 710 bp products were purified using the Wizard SV GL and PCR clean up system (Promega) and sequenced on an ABI 3130xl Genetic Analyzer using BigDye™ Terminator version 3.1 cycle sequencing kits. Sequence data was analyzed using DNASTAR Laser gene 9 Software and compared to those in GenBank.

Molecular detection of dengue and chikungunya virus in the Aedes mosquitoes

Total RNA was extracted from 200 µl of homogenized individual *Aedes* mosquitoes using QIAGEN RNeasy Mini Kits according to the manufacturer's instructions. The real-time RT-PCR method, primers and probes (Pongsiri *et al.*, 2012) were followed to screen mosquito homogenates for evidence of Chikungunya virus. Amplification was performed using TaqMan Fast Virus 1 Step Master Mix (Applied Biosystems, Foster City, CA) where each 20 µl reaction contained 5 µl FastMix, 1µl of each forward and reverse primer (10 µM) and 0.5 µl of fluorogenic probe (10 µM). The reverse transcription was carried out at 50°C for 5 minutes, followed by enzyme activation at 95°C for 20 seconds and 40 amplification cycles at 95°C for 3 seconds and 60°C for 30 seconds. The ABI ViiA7 (Applied Biosystems, Foster City, CA) was used for the real time PCR reactions and the results were analyzed with the ViiA7 software.

The detection of dengue virus was carried out using a conventional PCR assay as previously described (Ayers *et al.*, 2006) with universal flavivirus primers FLAVI-1F and FLAVI-2. Briefly, 50 µl reaction mixtures were prepared using the QIAGEN OneStep RT-PCR kit to contain 10 µl 5x buffer, 2 µl dNTP mix, 3 µl each primer at 10 µM, 20 µl nuclease-free water, 2 µl enzyme mix (QIAGEN) and 10 µl of template RNA. Amplification was performed in an MJ Research PTC-200 Thermal Cycler with reverse transcription at 50°C for 30 minutes, followed by denaturation at 95°C for 15 minutes, and 45 cycles of amplification at 94°C for 1 minute, 58°C for 1 minute, and 72°C for 90 seconds. Amplification products were analyzed by ethidium bromide-stained 2% agarose gels. Seropositive/viral exposure/recent infections were defined as those who

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were positive for IgM ELISA tests. Dengue/chikungunya clusters were defined as group of individuals who were positive for dengue/chikungunya and had households positioned near to each other, in which rates of infection were significantly higher compared to the expected rates as determined by the spatial scan statistic.

Data analysis

Data analyses were performed using Statistical Package for Social Sciences (SPSS) software (version 22). Summary statistics were presented as means ±SDs for continuous data and frequencies and proportions for categorical data. Logistic regression analyses were used to calculate strength of statistical associations between predictors and dengue/chikungunya IgM seropositivity and presented as odds ratios (ORs) and their 95% confidence interval (CI). SaTScan™ v8 software (Kulldorff, 2009) was used to detect disease randomness for spatial clusters of dengue and chikungunya seropositive individuals as well as *Aedes* mosquitoes in the two seasons. Maps were created using ArcGIS software (version 10.5). Kulldorf's spatial scan statistic was applied for cluster detection (Kulldorff, 1999). The analyses were performed using 999 Monte Carlo replications to test for significance, with no geographical overlap allowed, and a maximum cluster size set to 50% of the population (Kulldorff, 2009; Toan *et al.*, 2013). A p-value of 0.05 was regarded as significant. All significant clusters (p≤0.05) were mapped using ArcGIS.

Ethical considerations

The study was approved by the Medical Research Coordinating Committee of the Tanzania National Institute for Medical Research (NIMR/HQ/R.8a.Vol. IX/1898). Additional ethical approval was obtained from the Ottawa Health Sciences Network Research Ethics Board (OHSN-REB Protocol No: 20150199-01H). A written informed consent was obtained from all participants. In the case of children under 18 years of age, a parent or caregiver responded.

Results

Demographic characteristics of the study population

A total of 598 participants from the five villages were enrolled (n=299 from each of the two cross sectional surveys), which included 28.1% (n=168) males and 71.9% (n=430) females in combining dry and wet seasons participants. The mean age of participants was 36.5 years (SD± 23.19 years) of which 32.4% (n=194) were aged ≤ 15 years. The majority of participants (61.2% n=366) reported to have primary school education followed by no formal education (20.9 %, n=125), pre-school attendance (9.4%, n=56) and secondary and higher education (8.5%, n=51). The majority of participants were from Rundugai (32.9%; n=197) while fewest participants were from Nshara (10.2%; n=61) (Table 1).

Table1: Demographic characteristics of the study population during wet and dry season

Variable	Response	Wet season % (n)	Dry season % (n)	Total
Sex	Male	29.1 (87)	27.1 (81)	28.1 (168)
	Female	70.9 (212)	72.9 (218)	71.9 (430)
Age (years)	≤15	28.1 (84)	36.8 (110)	32.4 (194)
	>15	71.9 (215)	63.2 (189)	67.6 (404)
Education level	None	14.7 (44)	27.1 (81)	20.9 (125)
	Pre-school	8.0 (24)	10.7 (32)	9.4 (56)
	Primary	68.6 (205)	53.8 (161)	61.2 (366)
	Secondary and higher	8.7 (26)	8.4 (25)	8.5 (51)
Village	Bomang'ombe	12.0 (36)	10.4 (31)	11.2 (67)
	Magadini	19.1 (57)	22.4 (67)	20.7 (124)
	Rundugai	20.4 (61)	45.5 (136)	32.9 (197)
	Nshara	15.1 (45)	5.4 (16)	10.2 (61)
	Kware	33.4 (100)	16.4 (49)	24.9 (149)

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Dengue and chikungunya seropositivity

Cross sectional surveys performed during the wet and dry season determined that 4.7% (n=14) and 2.7% (n=8) of the participants were dengue IgM seropositive, respectively. Combining both surveys, three (1.5%) participants aged ≤15 years were dengue IgM seropositive while among participants above 15 years of age, 4.7% (n=19) were IgM seropositive. In the wet and dry seasons, 11.7% (n=35) and 11.0% (n=33), respectively, were chikungunya IgM seropositive. In the adjusted analyses accounting for village-level clustering, odds of dengue seropositivity was lower among females (OR: 0.83, 95%CI 0.71-0.97) as compared to males. Odds of chikungunya seropositivity was higher among pre-school children (OR: 1.73, 95%CI 1.15-2.59) compared those with no formal education (Table 2).

Table 2: Sero-prevalence of recent dengue and chikungunya virus infection in dry and wet seasons in Hai district

Variable			Dengue IGM			Chikungunya IGM		
		Total (N)	% pos (n)	Adjusted OR**	95% CI	% positive (n)	Adjusted OR**	95% CI
Sex	Male	168	4.2 (7)	Ref		10.0 (17	Ref	
	Female	430	3.5 (15)	0.83	0.71, 0.97*	11.9 (51)	1.20	0.71, 2.00
Age (years)	≤15	194	1.5 (3)	Ref	0.66, 15.0	11.9 (23)	Ref	
	>15	404	4.7 (19)	3.14		11.1 (45)	0.93	0.54, 1.62
Education level	None	125	2.4 (3)	Ref		8.88 (11)	Ref	
	Pre-school	56	1.8 (1)	0.74	0.05, 11.0	14.3 (8)	1.73	1.15, 2.59*
	Primary	366	4.9 (18)	2.10	0.31, 14.2	12.0 (44)	1.42	0.84, 2.38
	Secondary and higher	51	0.0 (0)	-	-	9.8 (5)	1.13	0.57, 2.25
Season	Wet	299	4.7 (14)	Ref		11.7 (35)	Ref	
	Dry	299	2.7 (8)	0.56	0.31, 1.00	11.0 (33)	0.94	0.50, 1.74
Village	Bomangombe	67	4.5 93)	Ref		9.0 (6)	Ref	
	Magadini	124	4.8 (6)	1.08	0.26, 4.48	14.5 (18)	1.73	0.65, 4.58
	Rundugai	197	4.1 (6)	0.90	0.23, 3.51	13.2 (26)		
	Nshara	61	0.0 (0)	-	-	6.6 (4)	0.71	0.19, 2.66
	Kware	149	3.4 (5)	0.74	0.17, 3.19	9.4 (14)	1.05	0.39, 2.87
	Total	598	3.7 (22)			11.4 (68)		

*p-value<0.05; **unadjusted OR presented for village

Mosquito composition and distribution

Outdoor mosquito collections over a total of eight trap-days per village achieved a total of 43 *Ae. aegypti*, five *Anopheles gambie* s.l. and 96 *Culex* species across the five villages in both seasons. Mosquito densities varied by species, season and village. Rundugai village displayed the highest densities of collected mosquitoes for *Aedes* and *Culex* species during the wet season; this village also had the highest *Aedes* density in the dry season (Table 3). All 43 *Aedes* mosquito specimens were identified as *Ae. aegypti* by CO1 sequencing. No *Aedes* mosquitoes tested positive for chikungunya or dengue virus by RT-PCR analysis.

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Table 3. Adult mosquito species collected using Mosquito Magnet traps

Season	Village	Mean trap density of mosquitoes by genera		
		Aedes species	Anopheles	Culex species
Wet	Boma Ng'ombe	1.5	0.5	0.5
	Kware	0.0	0.0	1.0
	Nshara	3.0	0.0	3.5
	Magadini	0.0	0.0	0.0
	Rundugai	5.5	0.0	18.5
	Total	20	1	47
Dry	Boma Ng'ombe	1.0	0.0	1.5
	Kware	3.5	0.0	3.0
	Nshara	1.0	0.0	0.0
	Magadini	1.0	2.0	12.0
	Rundugai	5.0	0.0	8.0
	Total	23	4	19

Spatial analysis of dengue, chikungunya and Aedes mosquitoes clusters

No significant clusters of dengue were detected. Spatial analysis of chikungunya detected one significant cluster in the wet season (June 2015), while no significant clustering was observed in the dry season (February 2016) (Figure 2). A large, widely dispersed cluster of chikungunya was detected spanning Rundugai and parts of Magadini (RR = 2.58, p= 0.01). This cluster had a diameter of almost 19 km and contained 20% of all seropositive individuals. Spatial clusters of *Ae. aegypti* mosquitoes were detected in Rundugai in both the wet (RR = 2.56, p<0.001) and dry seasons (RR = 2.24, p=0.05), while in the dry season a small cluster was also detected in Kware (RR = 2.25, p=0.05) (Figure 2, Table 4).

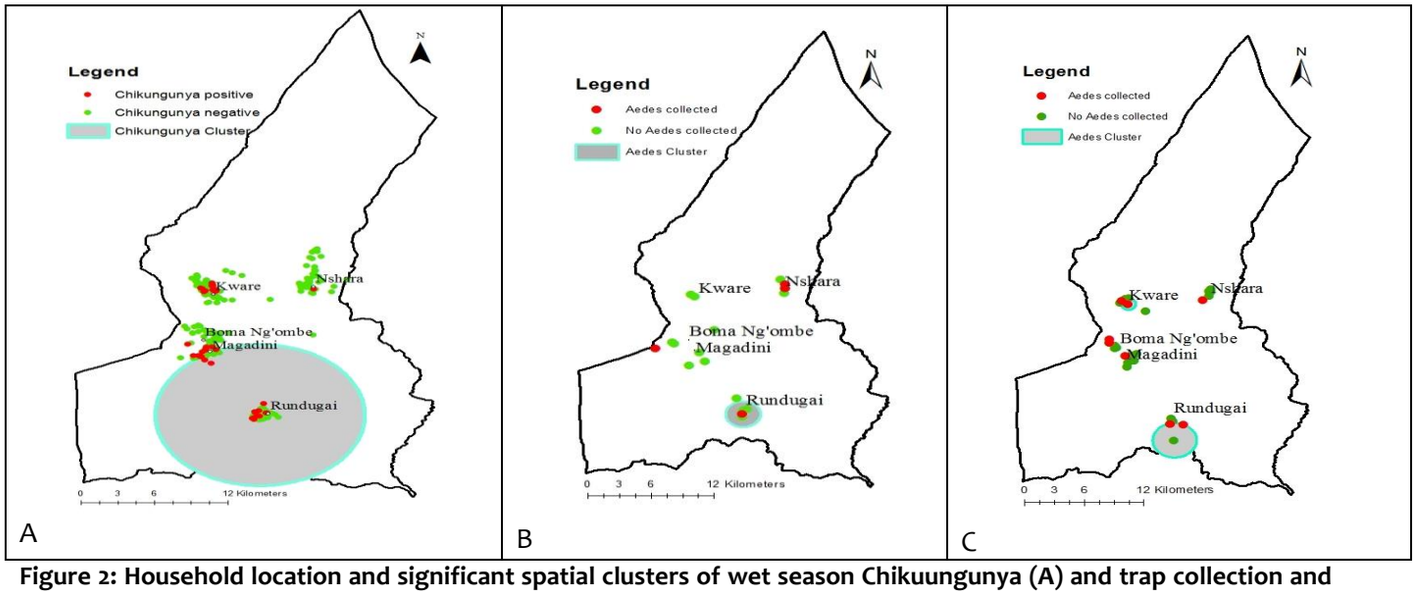

Figure 2: Household location and significant spatial clusters of wet season Chikuungunya (A) and trap collection and clusters of Aedes mosquitoes in wet season (B) and dry season (C)

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Table 4: Household clusters of chikungunya and trap clusters of *Aedes* mosquitoes detected using the Purely Spatial Analysis

Type	Season/Year	Village	Coordinates	Expected	Observed	RR	LLR	P-value
CHIKV	Wet/2015	Rundugai	-3.414142 S 37.189649 E	13.86	22	2.58	4.37	0.01
	Wet/2015	Rundugai	-3.418857 S 37.193744 E	6.47	11	2.56	7.64	<0.001
Ae. aegypti	Dry/2016	Rundugai	-3.440081 S 37.196983 E	5.88	10	2.24	4.73	0.05
		Kware	-3.282260 S 37.155210 E	3.74	7	2.25	4.96	0.05

Key: RR= Risk ration; LLR= Log likely ratio

Discussion

We applied a spatial scan technique (Kulldorff, 2001) using SaTScan software to study possible clustering of dengue and chikungunya infections (characterized as recent infections based on IgM seropositivity) as well as *Aedes* mosquitoes in Hai District, Tanzania. This method has previously been used to detect geographical clustering of dengue (Flauzino *et al.*, 2009; Jeefoo *et al.*, 2011; de Melo *et al.*, 2012) and chikungunya (Labeaud *et al.*, 2015; Nsoesie *et al.*, 2015), as well as other diseases outbreaks in order to identify risk factors for infection and guide control programmes (Elias *et al.*, 2006; Coleman *et al.*, 2009; Coulibaly *et al.*, 2013).

Serological data indicate that less than 5% of the population was recently exposed to dengue virus, with no significant seasonal variation although sero-prevalence was slightly higher during the wet season. The present dengue sero-prevalence is slightly higher as compared to the previously reported among febrile and afebrile individuals in the same district in 2014 (Kajeguka *et al.*, 2016), although direct comparisons are not possible due to differences in methodology. Chikungunya exposure was relatively similar in the two seasons and was not different from the previously reported study (Kajeguka *et al.*, 2016). Sensitivity and specificity of the IgM ELISA kits used in this study have been reported to be 97.1% and 98.9% according to the manufacturer's data (Standard-Diagnosis, 2017). Therefore, it is unlikely that the observed results are due to cross reactivity with other flaviviruses or alphaviruses.

Although findings of our present study did not detect dengue or chikungunya virus infection in the mosquitoes tested, the entomological survey data indicate that *Ae. aegypti* is likely to be the main local vector for the two infections in the area. This is in line with the study by Hertz et al. (Hertz *et al.*, 2016) which reported the occurrence of *Ae. aegypti* mosquitoes in Hai district. We observed that one village (Rundugai) had higher *Aedes* mosquito densities as compared to other villages in both seasons. This could be because of the presence of household water storage (data not shown) that may favor vector breeding, although our results are based on a limited number of trap-days. Other studies suggest that in the absence of a significant level of household water storage, dry season rainfall may be sufficiently frequent and abundant for vector populations and the risk of dengue transmission to be as high or higher at times than during the wet season (Lambdin *et al.*, 2009). The importance of rainfall in forming breeding sites for *Aedes* mosquitoes, and thus its association with arbovirus transmission is varied. In a study in the peri-and urban Asia social and environmental factors including the use of water storage containers and vector control measures were reported to largely determine the variation in dengue vector breeding in the dry and wet seasons (Wai *et al.*, 2012).

Identification of locations where diseases are clustered is useful for early evaluation of case distribution to provide an assessment of risk at a small geographical scale for targeted control. IgM can stay in the body for at least 2-3 months (Blacksell, 2012), and the participants from the two surveys were different, therefore it is unlikely that the measured IgM reflects carryover from the previous survey. We detected a large diffuse cluster of chikungunya-exposed individuals in rural lowland villages that coincided with clustering of *Ae. aegypti* mosquitoes. This supports the hypothesis of local chikungunya transmission in this area. *Aedes* mosquitoes were detected in the wet and dry seasons, highlighting the need to identify the

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importance of natural and peri-domestic breeding sites in order to inform appropriate risk messaging and vector control strategies. The detection of a chikungunya cluster in rural lowland villages is important and suggest for inclusion of chikungunya in the differentila diagnosis of febrile illness in this population.

Seasonal and geographic variations in population exposure to dengue and chikungunya viruses were observed in the Hai district of Tanzania. For chikungunya, a significant cluster of exposed individuals was observed in the wet season, which may represent the on-going activity, which may also indicate the risk area of transmission. While no detectable clusters of dengue-exposed individuals were observed, despite evidence of recent population exposure in some communities, clustering of chikungunya-exposed individuals was observed in a rural lowland area. While both dengue and chikungunya are likely to be transmitted principally by *Ae. aegypti* in this region, this may reflect differences in the transmission of these pathogens due to social and environmental factors and/or population mobility. Further characterization of the vector population and epidemiological and ecological risk factors for dengue and chikungunya infections would help to elucidate the patterns of disease transmission in this region. Given recent outbreaks of dengue in Tanzania, and the potential for international transport of arboviral pathogens stemming from such outbreaks, a better understanding of arbovirus epidemiology and ecology in this region is critically needed.

This study is subjected to some limitations, including low sample sizes for statistical analyses and mosquito collections. Sampling of participants during cross-sectional surveys was done using a community-sensitization and random selection approach; however, it is possible that selection bias may have occurred due to unavailability of certain population segments during the survey period. A census-based selection approach would have been preferable but this was beyond the scope of this study. Despite these limitations, the distribution of households across the study villages was relatively even and the application of spatial scan statistics enabled us to assess patterns in disease exposure and mosquito vector density with a high degree of spatial precision.

In conclusion, clusters of chikungunya exposed individuals and *Aedes* mosquitoes indicate on-going transmission of chikungunya virus in the area. No significant clustes of dengue were detected in both seasons. The application of spatial scan statistics enabled us to assess patterns in disease exposure and mosquito vector density with a high degree of spatial precision in small geographical scale.

Competing interests

The authors declare that they have no competing interest.

Author Contributions

DCK conceived the idea, designed the study, participated in data collection, and performed the experiments, data analysis and interpretation. RDK participated in data collection and performed the experiments. RD performed the data analysis and interpretation. MI performed the experiments and critical review of the manuscript. RAK designed the study and critical review of the manuscript. SM performed the data analysis and critical review of the manuscript. KLS participated in drafting and critical review of the manuscript. MA participated in drafting and critical review of the manuscript. RL participated in drafting and critical review of the manuscript. AD performed the experiments and critical review of the manuscript. FWM designed the study and critical review of the manuscript. MAK conceived the idea, designed the study, performed data analysis and interpretation.

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